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ASYMMETRIC NEBER REACTION IN THE SYNTHESIS OF 2-(TETRAZOL-5-YL)-2*H*-AZIRINES

Dissertação de Mestrado em Química Medicinal,
orientada pela Professora Doutora Teresa Pinho e Melo
e apresentada ao Departamento de Química da Faculdade de Ciências e Tecnologia
da Universidade de Coimbra.

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Orientadora: Prof. Doutora Teresa M. V. D. de Pinho e Melo

Co-orientadora: Doutora Ana Lúcia Cardoso

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"Carry on my wayward son
There'll be peace when you are done
Lay your weary head to rest
Don't you cry no more"

Carry on my wayward son, Kansas

Agradecimentos

Durante estes últimos anos houve muitas perdas, mas também muitas conquistas. Muitas pedras foram também aparecendo pelo meu percurso, pedras das quais não me desviei, mas que em quais peguei para um dia construir algo não só meu, mas de todos nós. Pois quando se trabalha nesta área não estamos a trabalhar só para nós, mas sim pelos outros de forma a que estes também consigam remover as pedras que apareceram nos seus caminhos. Desta forma dedico este espaço a todas as pessoas que me deram todo o entusiasmo e conhecimento para que esta tese fosse realizada e, que de forma direta ou indireta, me ajudaram a pegar nas pedras e que me ajudaram na construção deste projeto. A todos eles deixo aqui o meu sincero agradecimento.

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Abbreviations

6-APA	6β-aminopenicilanic acid
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy
^{13}C NMR	Carbon-13 nuclear magnetic resonance spectroscopy
^{19}F NMR	Fluorine-19 nuclear magnetic resonance spectroscopy
BINOL	1,1'-Bi-2-naphtol
boc	tert-Butylcarbonyl
bs	broad singlet
cat	Catalyst
CD	Cinchonidine
CN	Cinchonine
d	Doublet
dd	Double doublet
(DHQD)$_2$PHAL	Hydroquinidine 1,4-phthalazinediyl diether
DMF	*N,N'*-Dimethyl formamide
DMSO	Dimethyl sulfoxide
dr	Diastereomeric ratio
ee	Enantiomeric excess
Equiv.	Equivalent
HPLC	High-performance liquid chromatography
Hz	Hertz
J	Nuclear Magnetic Resonance Coupling Constant
KO*t*-Amyl	Potassium tert-butoxide
LG	Leaving group
m	Multiplet
MHz	Megahertz
m.p.	Melting point
MsCl	Methanesulfonyl chloride
NMR	Nuclear Magnetic Resonance

NHC	*N*-Heterocyclic carbene
Nu	Nucleophile
PG	Protecting group
PNB	*p*-nitrobenzyl
PNBBr	*p*-nitro benzyl bromide
ppm	Parts per million
Py	Pyridine
IR	Infrared Spectroscopy
q	Quartet
QD	Quinidine
QN	Quinine
r.t.	Room temperature
s	Singlet
t	Triplet
TADDOL	α,α,α',α'-Tetraaryl-1,3-dioxolan-4,5-dimethanol
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
TMSCl	Chlorotrimethylsilane
TOF	Turnover Frequency

Resumo

O trabalho apresentado nesta dissertação tinha como objetivo desenvolvimento de uma metodologia assimétrica da reação de Neber na síntese de 2-(tetrazol-5-il)-2*H*-azirinas, recorrendo a organocatálise. Esta metodologia sintética irá ser aplicada em estudos futuros na síntese de bioisósteros de carboxilatos de 2*H*-azirinas biologicamente ativos ((-)-desidazirina e a azirinomicina).

Estudos previamente desenvolvidos no grupo de investigação onde este projeto foi desenvolvido, demonstraram que partindo de tosilatos de β-ceto-oximas era possível a síntese assimétrica 2-(tetrazol-5-il)-2*H*-azirinas através da reação de Neber mediada por organocatalisadores alcalóides quirais. Um dos objetivos principais nesta dissertação foi a exploração da síntese das 2*H*-azirinas num só passo, na qual ocorre primeiramente a tosilação *in situ* das β-ceto-oximas tetrazólicas, seguida da reação de Neber na presença de um catalisador quiral. Assim, a conversão de 2-(1-(4-nitrobenzil)-1*H*-tetrazol-5-il)-feniletanona-oxima na correspondente 2*H*-azirina, foi selecionada como reação modelo para os estudos de otimização da síntese assimétrica de Neber na síntese das 2-(tetrazol-5-il)-2*H*-azirinas. Foram realizados estudos da reação assimétrica de Neber no qual a quinidina foi utilizada como organocatalisador e, parâmetros tais como o tempo de reação, solvente, percentagem de catalisador e temperatura foram otimizados.

Nas condições de reação otimizadas com quinidina foram posteriormente estudados outros organocatalisadores, nomeadamente quinina, derivados sintéticos de alcalóides da cinchona contendo grupos aminoácidos e vários heterociclos e tioureias contendo grupos tiazolidínicos.

A síntese de novas tioureias derivadas do ácido 6β-penincilâmico, o (2*S*, 6*R*)-6-β-aminopenicilanato-(3,5-bis(trifluorometil)feniltioureído) de benzidrilo e o (2*S*, 6*R*)-6-β-aminopenicilanato-(feniltioureído) de benzidrilo, foi também conseguida e a sua aplicação como catalisador na reação assimétrica de Neber foi igualmente estudada.

Este projeto permitiu concluir que, dos derivados de alcalóides de cinchona estudados, a quinidina é o catalisador mais eficiente para obter o isómero *R* na síntese assimétrica de 3-fenil-2-(nitrobenziltetrazol-5-il)-2*H*-azirina, apresentando rendimento e excesso enantiomérico de 87% e 66%, respetivamente. Para o isómero *S,* os melhores

resultados, 66% de rendimento e 44% de excesso enantiomérico, foram alcançados quando a reação de Neber foi realizada na presença de quinina, conduzindo à formação do isómero desejado com 61% de rendimento e 44% de excesso enantiomérico.

Para além disso, os melhores catalisadores encontrados para a reação assimétrica de Neber foram as novas tioureias derivadas do ácido 6β-penicilâmico. Para o nosso agrado, estes catalisadores permitiram a síntese (2*R*)-3-fenil-2-(nitrobenziltetrazol-5-il)-2*H*-azirina, com excessos extraordinários superiores 99%.

Abstract

The main purpose of the work developed in this dissertation was to establish an asymmetric version of the Neber reaction for the synthesis of 2-(tetrazol-5-yl)-2*H*-azirines resorting on organocatalysis which could be applied to the synthesis of the bioisosteres of naturally occurring biological active 2*H*-azirine-2-carboxylates (azirinomycin, (-)-dysidazirine and antazirine).

Earlier studies developed in the group where this MSc project was carried out, already demonstrated that the alkaloid-mediated Neber reactions of β-ketoxime tosylates allows the asymmetric synthesis of 2-(tetrazol-5-yl)-2*H*-azirines. One of the main goals of this MSc project was to explore an one-pot procedure by carrying out the *in situ* tosylation of β-ketoxime tetrazoles followed by the Neber reaction in presence of chiral organocatalysts. Thus, the conversion of 2-(1-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone oxime into the corresponding 2*H*-azirine was selected as model reaction. An initial solvent screening using quinidine as organocatalyst was carried out and, within the studied solvents, the best results were obtained when the reactions were carried out in toluene. Other parameters such as temperature, catalyst loading, time reaction and co-base were also studied.

Under the optimized reaction conditions for quinidine, other organocatalysts, namely quinine, cinchona alkaloid derivatives bearing amino acids and several heterocycle moieties, as well as chiral bifunctional thioureas incorporating thiazolidine moieties were explored.

The synthesis of new 6β-aminopenicilanic acid derived thioureas, (2*S*,6*R*)-benzhydryl 6β-(3-(3,5-bis(trifluoromethyl)phenyl)thioureido)-aminopenicillanate and (2*S*,6*R*)-benzhydryl 6β-(3-(phenyl)thioureido)-aminopenicillanate, was also accomplished and their application as catalysts in the asymmetric Neber reaction was studied as well.

This study showed that within the screened cinchona alkaloid derivatives, quinidine is the most efficient catalyst to obtain the *R* isomer in the asymmetric synthesis of 2-(tetrazol-5-yl)-2*H*-azirines, since it presented the highest yields and *ee*, 87% and 66%, respectively. As for the *S* isomer the best results were achieved when the asymmetric

Neber reaction was carried out with quinine leading to the target isomer in 61% yield and 44% *ee*.

Moreover, our best catalyst for the asymmetric Neber reactions were the 6β-aminopenicilanic acid derived thioureas. To our delight, these new organocatalysts afforded the (2*R*)-3-phenyl-2-(1-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)-2*H*-azirine *R* isomer in very high enantiomeric excess (>99%).

Chapter 1

Introduction

"I could spend my life in this sweet surrender
I could stay lost in this moment forever
Every moment spent with you is a moment I treasure"

Don't wanna miss a thing, Aerosmith

1.1 Overview

In the past years, the work on the discovery and creation of new antibiotics by pharmaceutical companies have been reduced, due mainly to a perceived lack of return on investment.[1,2] But why is it so important to find new antibiotics?

Antibiotics are drugs used to prevent and treat bacterial infections. But, during the past years, antibiotic resistance has risen to highly dangerous levels all over the world. New antibiotic resistance mechanisms are emerging and spreading worldwide with an uncontrollable velocity, making it, with the passing of time, more and more difficult to treat bacterial infections. "A growing list of infections – such as pneumonia, tuberculosis, blood poisoning, gonorrhea, and foodborne diseases – are becoming harder, and sometimes impossible, to treat as antibiotics become less effective. (…)".[3] Antibiotic resistance occurs when bacteria change in response to the use of these drugs, this usually occurs because of the misuse or overuse of antibiotics. [4]

Drug resistance in bacteria is an example of a natural selection consequence. When an antibiotic is used on a specific bacteria species, those that can't resist to the drug die and don't reproduce, while those with a resistant gene survive and reproduce passing on the resistance gene to the next generations (vertical gene transmission). The resistance gene can be acquired through one of two mechanisms: with a sporadic mutation or can also be passed on to one bacterium by another of a different species (horizontal gene transmission). [5,6] With this, the drug resistance increases over generations. [7]

The widespread use of antibiotics, adequate or not, has led to a worldwide escalating antibiotic resistance. As already mentioned, the investment in the study and development of new antibiotics with new action mechanisms has dropped significantly, reaching a point where infections become harder and sometimes impossible, to treat due to the lack of new and innovative pharmaceuticals.[8] Poor hygiene conditions in hospital environments has also been proved to be an important factor for antibiotic resistance increase. Something as simple as poor hand hygiene between the patients can lead to the spread of infections and antibiotic resistance. In this sense, health care providers can minimize the growth of resistant infections by using appropriate sanitation and hygiene, including handwashing and disinfecting between each patient, and should also encourage the patients, visitors, and family members to do the same.[9]

Worldwide changes in antibiotic use are essential to stop this wide-spreading epidemic. Even with the creation of new drugs and mechanisms to contour the antibiotic resistance, a behavior change is needed, if not so, antibiotic resistance will remain a major threat. The major behavior changes that are needed are actions to reduce the growth of infections through vaccination, hand washing, good food hygiene and prescribing antibiotics only when they are needed.

Nature is a valuable source to find new active compounds. With this in mind, some academic research groups are joining their efforts to find new naturally active compound analogs as a solution for this epidemic.[10] For instance, Wuest and his group found analogs of naturally active carolacton[11] and Melander and his coworkers found Bromoageliferin analogues[12], having both antibiofilm properties.[10] Concerning this matter, this project is focused on the asymmetric synthesis of biologically active of naturally occurring 2*H*-azirines analogs.

1.2 Azirines

Azirines are small three-membered heterocycles containing two carbons, a nitrogen, and a double bond. There are two isomeric forms of azirines, the 1*H*-azirine, which has the double bond located between the two carbons, and the 2*H*-azirine isomer which has the double bond between the nitrogen and one of the carbons (Figure 1.1).[13–15] From both isomeric forms, the 1*H* isomer is the less stable and impossible to isolate at room temperature as result from its antiaromatic nature.[16] Since this project is about the asymmetric synthesis of 2*H*-azirine derivatives only this isomer will be discussed.

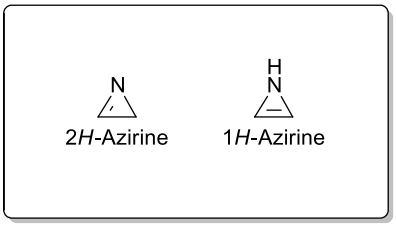


Figure 1. 1 Azirine isomeric forms.

The 2*H*-azirine ring was firstly reported by Neber *et al.* in 1926 as an intermediate in the synthesis of aminoketones by treatment of oxime *p*-toluenesulfonates with base,

transformation that nowadays is known as the Neber rearrangement.[17] Since then many azirine derivatives have been prepared using this approach. 2*H*-azirines can be synthesized through several main strategies (Figure 1.2): Neber rearrangement **A**, thermolysis or photolysis of vinyl azides **B**, Swern oxidation or elimination reactions of aziridine derivatives **C**, addition of nitrenes to acetylenes **D** or carbenes to nitriles **E**, ring contraction of isoxazoles or oxazaphospholes **F**, addition of silyldibromomethyllithium compounds to nitriles **G**, oxidation of enamines **H**, and catalytic decomposition of α-oximino diazo compounds **I**. Publications over the last years have demonstrated that the first three methods are the most reliable, flexible and therefore still the most commonly used for their preparation.[13,18]

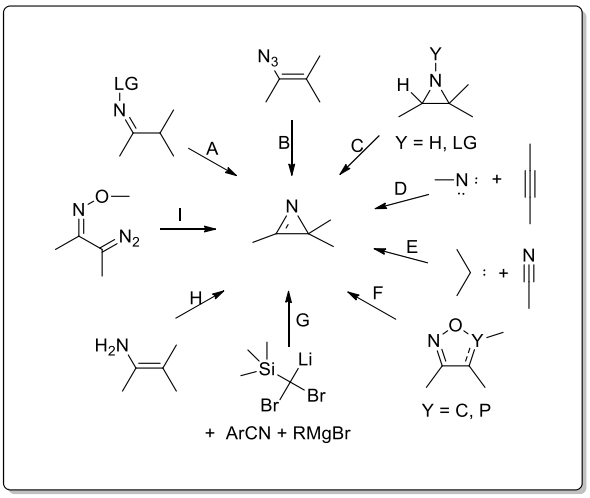


Figure 1. 2 2*H*-azirines synthetic strategies.[18]

Due to their availability and high chemical reactivity, 2*H*-azirines have been widely used as versatile building blocks of the synthesis of various nitrogen-containing compounds. The activated imine bond and the lone pair of electrons on the nitrogen allows the azirine to participate in several organic reactions, acting not only as nucleophiles and electrophiles but also as dienophiles and dipolarophiles in cycloaddition reactions. Furthermore, selective cleavage of each of the three bonds can be achieved, leading to highly reactive intermediates such as vinylnitrenes, nitrile ylides, and iminocarbenes (Figure 1.3).[13,18]

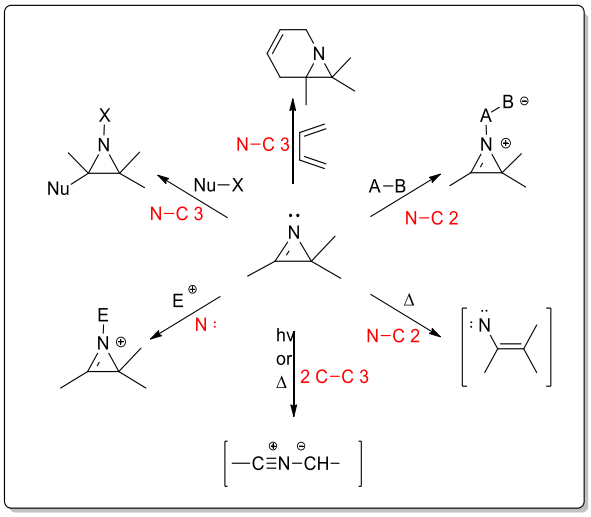


Figure 1. 3 Reactivity of 2*H*-azirines.[18]

Some of naturally occurring 2*H*-azirines are antibiotics and have been found in several natural products[19–22]. These biologically active chiral azirine derivatives bear a carboxylic acid or an ester group at C-2 and have aroused some interest due to their biological behavior. Azirinomycin, for example, was isolated from *Streptomyces aureus* and has a broad antibiotic spectrum activity against negative and positive gram bacteria.[19,23] Other azirine-containing natural products with antifungal activity against *Candida albicans* and *Saccharomyces cerevisiae* such as (-)-(*E*)-dysidazirine, (-)-(*Z*)-dysidazirine, (+)-(*Z*)-antazirine and (+)-(*E*)-antazirine, and corresponding chlorinated species were isolated from *Dysidea fragilis*. (-)-(*E*)-Antazirine and the corresponding acid (motualevic acid F) were recently isolated from the marine sponge *Siliquariaspongia sp.* (Figure 1.4).[18] Due to their enhanced properties as antimicrobial agents, the development of new asymmetric routes in the synthesis of 2*H*-azirine-2-carboxylates has attracted significant attention.

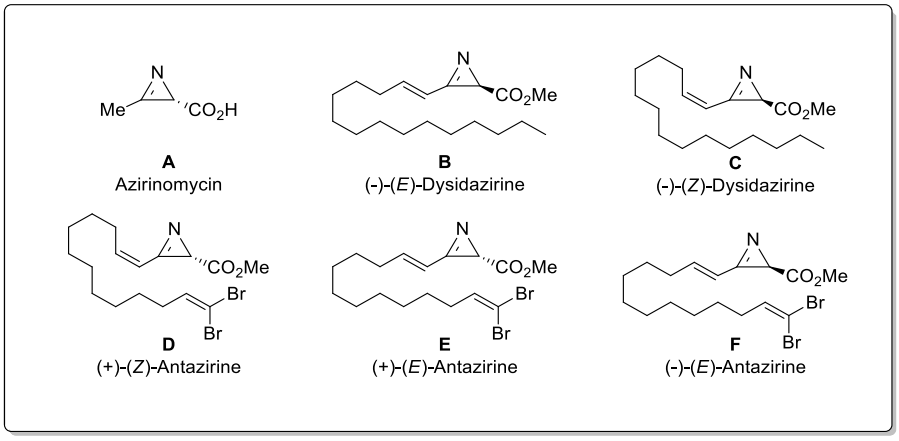


Figure 1. 4 Naturally occuring 2*H*-azirines.[18]

One of the main goals of the MSc project was the optimization of the asymmetric Neber reaction for further synthesis of 2-(tetrazol-5-yl)-2*H*-azirines, bioisosteres of the naturally occurring 2*H*-azirines showing biological activity.

1.3 Tetrazole as Carboxylic Acid Bioisostere

The isostere concept was introduced for the first time to the scientific community in 1919 by Languir, and in 1932 Hans Erlenmeyer applied this concept to biological systems. Isosteres are divided in two types of groups: the classical and the non-classical isosteres. Classical isosteres are atoms, ions and molecules that have identical outer shells of electrons. Non-classical isosteres don't follow these requirements, they must have similar atoms in their constitution, but their structures don't follow specific set of rules. Non-classical isosteres have, however, similar biological activities in living organisms.[1,24–26]

In medicinal chemistry the bioisosterism concept is one of the most used tools in drug design. Bioisosteres just as the isosteres can be divided in classic and non-classic bioisosteres. [1,24–26] Classical bioisosteres, are different atoms, ions and molecules with the same valence electron structure that have similar biological properties. For example, for hydrogen a classical bioisostere is fluorine. In some cases, the replacement of the hydrogen atom with fluorine prevents the metabolism of the active compound by restraining the metabolic oxidation, leading to a longer half-life. Fluorine is used as a bioisostere of hydrogen because of the similarities to the hydrogen in terms of size and electronic valence structure.[27]

Non-classical bioisosteres are structurally different, usually have different number of atoms and also have distinct steric and electronic properties (Figure 1.5).[24]

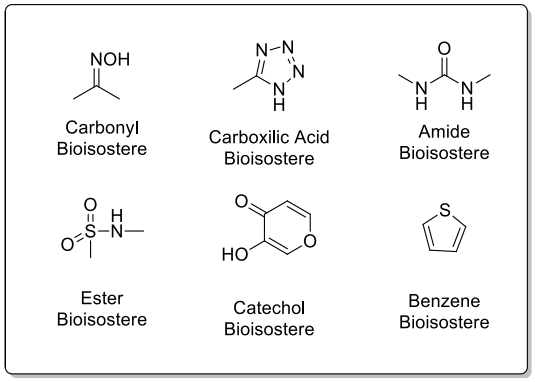


Figure 1. 5 Examples of some functional group bioisoteres.[24]

As previously mentioned one of the main goals of this MSc project was the synthesis of bioisosteres of the biologically active 2*H*-azirine carboxylates, namely the azirinomycin and dysidazirine bioisoteres. Since tetrazoles are one of the most used carboxylic acid derivative bioisosteres, this project was focused on the synthesis of tetrazoles, therefore only this type of bioisosterism will be discussed. Tetrazoles are five membered heterocycles containing a carbon, four nitrogen atoms and two double bonds. There are two tetrazole isomers, 1*H*-tetrazole and 2*H*-tetrazole, which are tautomeric forms of 5-substituted tetrazoles (Figure 1.6). Tetrazoles are uncommon in nature and in rare cases where they were found, they didn't show significant biological activities. The tetrazole moiety has been used in various pharmacophores as substituent of carboxylic acid groups, when unprotected, and of amide or ester groups, when protected.[24,27]

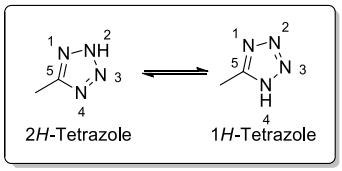


Figure 1. 6 Tetrazole tautomerism.

Tetrazoles, just as carboxylic acids, are ionized at physiological pH conditions. Nevertheless, the ionic form of the 5-substituted tetrazoles is approximately 10 times more lipophilic than the corresponding carboxylates. This property is extremely important for the pharmacokinetics of the tetrazole bioisosteres since a higher lipophilicity increases the permeability through the cell membane.[28] The tetrazole motif is such an interesting bioisostere motif since it demonstrated resistance to biological degradation, which can lead to a larger half-life of the active compound. Therefore, this

metabolism resistance makes them metabolically more stable than carboxylic acids. Other characteristics that make them appealing is the possibility of acting as acid or base simultaneously and the fact that they "biomimic" the carboxylic acid functional group. The charge delocalization in the tetrazole ring can lead either to interaction enhancement or decrease with a specific target, depending on the electronic distribution of the receptor. The size of this bioisostere can also lead to a decrease in affinity in comparison to carboxylic acids since a bigger functional group can lead to steric hindrance or to an inconvenient orientation of the functional groups.[29] The major difference between the carboxylate and the tetrazole anion stands out in the fact that all nitrogen atoms can act as acceptors of hydrogen bonds, which may increase the interaction with the target.

It was observed that the replacement of the carboxylic acid by a tetrazole can lead to increasing activity, or, in some cases, to the total disappearance of the activity. In addition to its biological and chemical advantages, tetrazole bioisosteres, are easily synthesized, for example, they can be obtained starting from sodium azide and nitriles or from diazotization of imidohydrazides yielding the 5-substituted tetrazoles in high yields.[29]

A successfull example of the use of tetrazole as a bioisostere is Losartan (**A**, Figure 1.7). Losartan is a selective antagonist of angiotensin II receptors, being an antihypertensive drug. This analogue presents higher activity than the compound with the carboxylic acid group (**B**, Figure 1.7). Losartan's story started during an investigation of a new series of analogues derived from a biphenyl scaffold. It was found that biphenyl carboxylic acid **B** was very active by intravenous injection into renal hypertensive rats. However, by administrating the drug orally the activity dropped significantly, and it was necessary to find a bioisostere to replace the carboxylic acid. When tetrazole was introduced at the C-2 position compound **A**, a dramatic enhancement in binding affinity was observed as well as a higher bioavailability leading to enhanced potency when the drug was administrated orally.

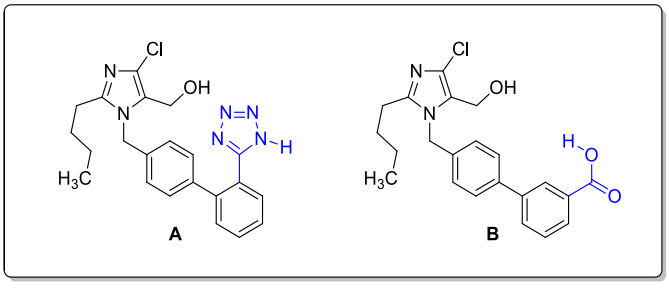


Figure 1. 7 Losartan (A) and its carboxylic acid analogue (B).[29]

In order to achieve a better understanding of the overall logic behind this dissertation organocatalysis and asymmetric synthesis will be discussed next.

1.4 Organocatalysis

In the last decades, enantioselective synthesis of compounds has been one of the central themes in research, since enantiomerically enriched products are considered as being the most valuable, due to the different properties presented by each enantiomer.[30– 34] In recent years, methods available for obtaining effective enantioselective transformations of organic compounds has increased exponentially.[35] Currently, the preparation of pure chiral compounds can be accomplished through three different methods – resolution of racemic mixtures, diastereoselective synthesis or asymmetric catalysis. Usually, the method used to obtain pure chiral compounds on industrial scale, with some exceptions, is resolution.[36] Nevertheless, this method presents some serious flaws since it is a time-consuming process and only 50% of the wanted enantiomer can be obtained leading to a tremendous waste of organic material.[30] However, if the conversion of the opposite enantiomer into the desired one is possible, the latter limitation can be reduced either by an alternative synthetic scheme or by a dynamic kinetic resolution, albeit increasing even more the time constraints.[37]

On the other hand, asymmetric synthesis is a method that leads to the formation of optically active compounds starting from symmetric molecules without requiring resolution of a racemic mixture. It was responsible for a revolution in organic chemistry in the second half of the 20th century and has assumed an increasingly prominent role in the preparation of pure chiral compounds.[38] It is also a prominent investigation area, since a small amount of chiral catalyst can lead to large quantities of enantiomerically enriched products leading to more economical synthetic strategies.[39] Owing to these advantages, the interest in this type of process has increased vastly over the last half century, and the development of efficient asymmetric synthesis for important organic reactions has been a main concern for organic chemists.[40]

One important milestone of asymmetric catalysis was when the Nobel Prize in Chemistry in 2001 was awarded to three chemists for their contributions in the catalysis

subject, namely to William R. Knowles[41] and Ryoji Navor[42] for their work on chirally catalyzed hydrogenation reactions and to K. Barry Sharpless[15] for his work on chirally catalyzed oxidation reaction. Since then, the interest in this area has increased considerably, and several catalysts for a large range of reactions have been developed.

Until recently, the catalysts used in asymmetric synthesis of organic compounds were categorized in two general groups: enzymes and transition metal complexes. However, during the past 20 years, this perspective has changed when simple chiral molecules, such as amino acids were introduced as enantioselective catalysts. This discovery led to the creation of a new approach in the catalytic synthesis of enantiomerically pure organic compounds, the asymmetric organocatalysis.[30,32,38]

Organocatalysis was introduced to the scientific community by two distinct research groups led by List and MacMillan. These authors described the asymmetric synthesis of chiral compounds using only small chiral molecules with low molecular weight. Benjamin List and his team[43] studied enantioselective aldol reactions where *L*-proline was used as organocatalyst, while David MacMillan and coworkers[44] used imidazolidinones as organocatalyst in the asymmetric version of Diels-Alder cycloaddition reactions. In both studies good enantioselectivities were observed and, since then, organocatalysis has only been increasing its popularity.

Organocatalysis decreases chemical reaction times using only substoichiometric amounts of organic metal-free chiral molecules.[38] Organocatalysts can be defined as organic molecules composed of carbon, hydrogen, nitrogen, sulfur, and phosphorus,[30] and they have great potential for costs, time and energy savings. Furthermore, they allow to reduce chemical waste and simplify experimental procedures. These lastly presented profits emerge from three factors:

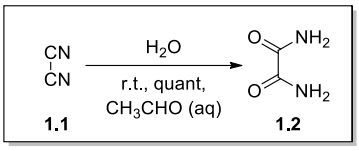
- the existing variety of naturally occurring and commercially available chiral molecules, such as amino acids, carbohydrates and hydroxy acids that can be used as catalysts.

- contrary to other types of catalysts, organocatalysts are usually less sensible to the atmospheric moisture and oxygen and don't need special storage conditions such as inert atmospheres.

- they also are more environment-friendly and non-toxic, since they are metal free. Moreover, this increases the safety in the catalysis process, on academic and industrial level.[45,46]

1.4.1 Historical Background

The past of organocatalytic reactions lays back much further than the year 2000. In fact, the first time that an organocatalyzed reaction was described was in 1859, when Justus von Liebig discovered by accident that dicyan **1.1** was transformed into oxamide **1.2** when it was placed in the presence of an aqueous solution of acetaldehyde (Scheme 1.1). [38]



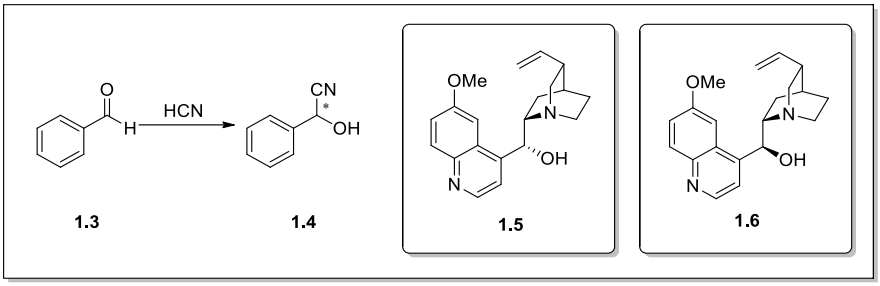
Scheme 1.1 Von Liebig's oxamide synthesis.[38]

Regarding asymmetric synthesis, the first references were made even before Von Liebig's discovery. In 1853, Louis Pasteur discovered the first asymmetric reaction - a decarboxylative kinetic resolution - introducing us to the term "dissymmetry", when he discovered that the organism *Penicillium glauca* destroyed more rapidly one of the enantiomers of a racemic resolution of ammonium tartrate. This discovery undoubtedly showed the importance of enzymes and their functions on the development of asymmetric catalytic reactions.[38]

In the beginning of the 20th century, based on Pasteur's discovery, Georg Bredig continued the studies to find the chemical origin of enzyme activity observed in living organisms. He studied asymmetric decarboxylation reactions under non-enzymatic conditions using chiral alkaloids, such as nicotine or quinine, as catalysts for the thermal decarboxylation of optically active camphorcarboxylic acid in *d* and *l* limonenes. [38]

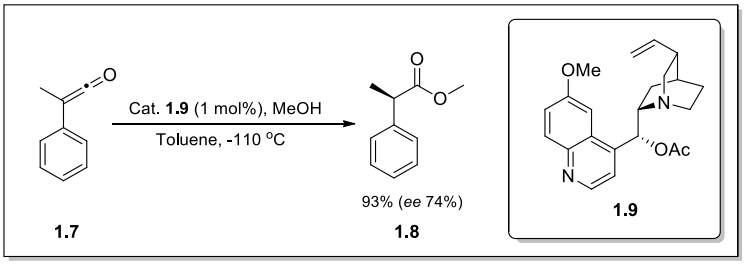
Bredig *et al.* also became pioneer by performing the first asymmetric C-C bond forming reaction. In 1912, they prepared mandelonitrile **1.4** by adding hydrogen cyanide to benzaldehyde **1.3** in the presence quinine **1.5** and quinidine **1.6**. This procedure was based on Rosenthaler's work, who synthesized the same product in the presence of

emulsin enzyme (Scheme 1.2). Although these studies were considered revolutionary, the enantiomeric excess obtained in these reactions was less than 10%.[38]



Scheme 1. 2 Mandelonitrile synthesis performed by Bredig and Fiske.[38]

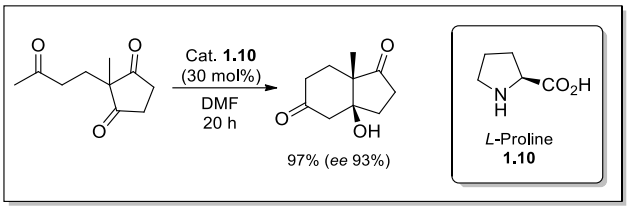
Significant levels of enantioselectivity were only achieved in 1960 by Pracejous when he demonstrated that (-)-α-phenyl methylpropionate **1.8** could be obtained from methyl phenyl ketene (**1.7**) in the presence of cinchona alkaloids, more specifically *O*-acetylquinine **1.9** (Scheme 1.3).[30] This remarkable result led to the beginning of the research on cinchona alkaloid assisted reactions.



Scheme 1.3 Pracejus's enantioselective ester synthesis from phenyl methyl ketene.[30]

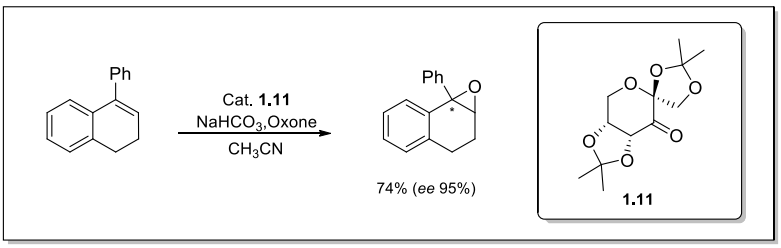
One of the most famous key events of organocatalysis history was reported in the beginning of the 1970s, when two independent research groups led by Zoltan G. Hajos, from Hoffmann-La Roche, and Rudolf Wiechert, from Schering AG, discovered an efficient *L*-proline-mediated asymmetric Robinson annulation. It all began in 1971 when Wiechert and his group described a Robison annulation assisted by a catalytic amount of *L*-proline **1.10**, which was enough to obtain the desired product in 83% yield and 71% enantiomeric excess.[47] Three years later, Hajos and his group reproduced a similar reaction by using lower temperatures, yielding the desired product with an impressive enantiomeric excess of 93%. This reaction became known as the Hajos-Parrish-Eder-Sauer-Wiechert reaction

(Scheme 1.4) which is an important reaction to obtain key building blocks for the synthesis of natural products.[30]



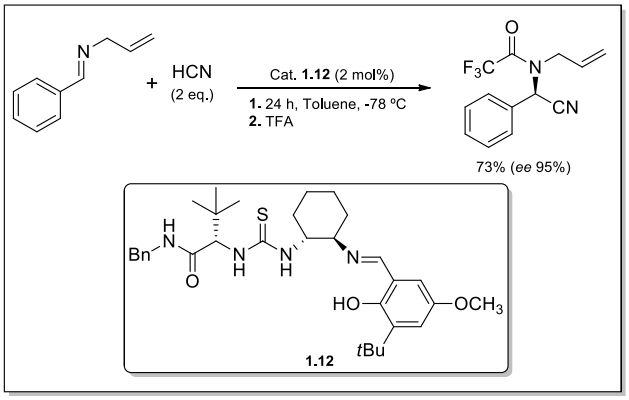
Scheme 1.4 *L*-proline-mediated asymmetric Robinson annulation.[30]

Although *L*-proline-mediated annulation received a considerable synthetic and mechanistic interest, studies in this field of chemistry were scarce for about 20 years. Organocatalysis only regained the attention of the scientific community in the late 1990's when Dan Yang,[48] Scott Denmark[49] and Yian Shi,[50] and their respective research groups, discovered that they could perform enantioselective epoxidations of simple alkenes using enantiomerically pure ketones as catalysts, in good yields and very high enantiomeric excesses (up to 95%) (Scheme 1.5).



Scheme 1.5 Olefin asymmetric epoxidation catalyzed by chiral ketones.[48]

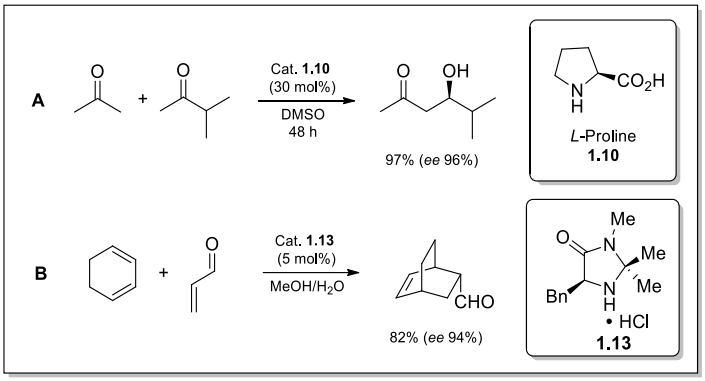
Thereafter, Miller[30] and his team reported the concept of kinetic resolution of alcohols with small peptides while three different research groups led by M. Lipton[51], Elias Corey[52] and Eric Jacobsen[53] described the first examples, namely in the asymmetrical Strecker reaction, where the catalysts mediated the reaction by hydrogen bonding (Scheme 1.6).



Scheme 1.6 Example of a Strecker reaction performed by Sigman and Jacobsen.[53]

Although all these studies didn't conceptualize organocatalysis as an efficient area of research, these examples showed for the first time the potential that organically pure compounds have in the synthesis of target chiral compounds and also in solving essential problems in chemical synthesis in general.[45] Only in 2000 the potential of organocatalysis was recognized and established as an indispensable branch of contemporary synthetic chemistry, resulting mainly from the contribution of the previous referred research groups.

Thus, by extending the studies of the Hajos-Parrish- Eder-Sauer-Wiechert reaction during the late 1990's, Carlos Barbas III, Richard Lerner and Benjamin List[43] discovered and reported that *L*-proline **1.10** could also catalyze intermolecular aldol reactions with different aldehydes (Scheme 1.7 A)[43]. Simultaneously, MacMillan described enantioselective organocatalytic Diels-Alder reaction mediated by a chiral imidazolidinone **1.13** derived from *L*-phenylalanine (Scheme 1.7 B)[44]. Furthermore, MacMillan defined and conceptualized organocatalysis as a new branch of asymmetric synthesis. Since then, there has been an exponential and extraordinary increase in research groups working in this area.



Scheme 1. 7 (A) Example of an intermolecular aldol reaction studied by Barbas III.[43] (B) Example of a Diels-Alder reaction performed by MacMillan.[44]

The excellent results obtained along the years inspired scientists to study and develop new classes of organocatalysts derived from privileged catalysts.[38,54–59] Privileged catalysts are structures with extraordinary ability to assist in an efficient way not only one, but a variety of chemical transformations.[60] Until now, there has been a steadily growing number of such organic compounds being found, of either natural or synthetic nature, that own this incredible chemical property. Chemical structures, such as cinchona alkaloids **1.5**, *L*-proline **1.10**, BINOL **1.14**, TADDOL **1.15** and carbohydrate **1.16** (Figure 1.8) are some of those privileged catalysts.[61,62]

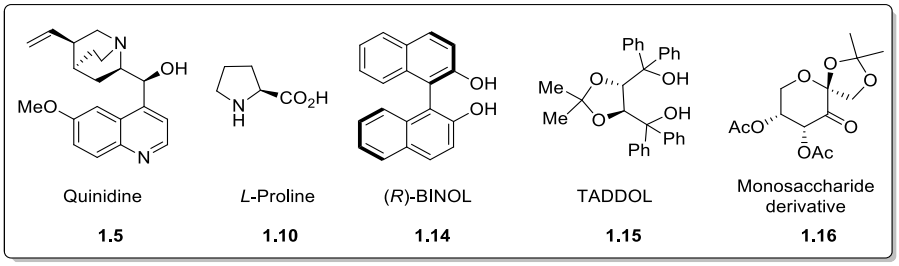


Figure 1. 8 Examples of chiral privileged catalysts scaffolds used in asymmetric catalysis.

In a short period, organocatalysis has found application in all branches of synthesis including the preparation of biologically relevant compounds. Moreover, all the information of the organocatalysts obtained until now, allowed the development of new tandem, cascade, domino and multicomponent reactions,[63] as well as reactions in aqueous medium and the immobilization of catalysts in solid supports.[64,65] Presently, reactions such as halogenations, aldol condensations, epoxidations, reductions, Mannich reactions, oxidations, Michael additions, alkylations, arylations, allylations, acylation, Diels-Alder

reactions, 1,3-dipolar cycloaddition reactions, among others, are possible to be catalyzed by organocatalysts.[66]

1.4.2 Why Organocatalysis?

Nowadays, organocatalysis has definitively been recognized as the third methodology available in asymmetric catalysis, parallel to organometallic catalysis and to biocatalysis (i.e. enzymatic catalysis).[67] Each of these methodologies owning advantages and disadvantages, as previously mentioned. Besides, there are also similarities between them, being the most obvious one that they are used to promote highly efficient chemical transformations. Nevertheless, there is a main difference between them, which is the way they promote chemical reactions. While an organocatalyst has a carbon structure with particular heteroatoms as a catalytic center, an organometallic catalyst has a transition metal as a catalytic center. Furthermore, the enzyme structures are some more complex, since they contain hundreds of amino acids in their structure, and only a small fraction of them belong to the catalytic center. Moreover, the catalytic center can also contain metals in its structure.[68]

In order to understand the importance of organocatalysis in asymmetric synthesis area, it's important to know the strong and the weak points of all the methodologies currently available. Starting with organometallic catalysis, which dominated for a long period of time this field, its main advantage relies on the ability of using different transition metals, thus allowing an increase of the ligand structure connected to the metal core and subsequently maximizing yields and enantioselectivity. On the other hand, very small amounts of catalysts are required (usually between 1 to 100 ppm), which is another significant benefit of this methodology.[69] The main disadvantages are related to the high costs of the different metals, the fact that the majority of metal catalysts are unstable in the presence of oxygen present in the atmosphere, as well as problems associated with purification processes.[70] Another weakness of this method, is that the amount of metal contamination allowed in pharmaceutical products is low, thus reducing the potential use of this methodology in the pharmaceutical and biological industries.[71]

Regarding biocatalysis, enzymes allow remarkably high enantioselective transformations due to their complex protein structure. The amount of catalyst necessary

is also very small and unlike metal catalysts, they don't present any toxicity problems. However, enzymes present major solubilization problems in organic solvents and, in addition, factors such as the temperature can easily lead to their denaturation. Furthermore, usually it is only possible to synthetize one of the target enantiomers with this type of asymmetric catalysis and these biomolecules only work with a very restricted range of structures, considering their high specificity.[62]

Considering the benefits and flaws of the asymmetric synthesis methodologies, we can easily conclude that organocatalysis offers the major advantages. Its operational simplicity, combined with the general stability of the organocatalysts under aerobic conditions and easy accessibility makes organocatalysis a strong method relatively to organometallic catalysis or biocatalysis, since the major weaknesses of the latter two methodologies are the strengths of organocatalysis.[72]

In organocatalysis it is relatively easy to obtain both enantiomers of a catalytic product, just like in organometallic catalysis.[73] Furthermore, there is no risk of metal contamination and non-expensive recovery processes for waste treatment. The only drawback of organocatalysis is the amount of catalyst necessary in the asymmetric reaction (usually between 1-20 mol%).[45]

Accordingly, studies and development of new organocatalysts are still underway for its application on industrial scale. Nowadays, the most promising applications of organocatalysis is in medicinal chemistry, since chiral organocatalysts have all the requirements needed for the synthesis of therapeutic agents enriched in one particular enantiomer.[32]

1.4.3 Classification of Modern Organocatalysis

One of the most important features leading to the success of organocatalysis during the last years has been the creation and identification of generic modes of catalyst activation, induction and reactivity. The generic activation modes describe how the reactive species can participate in the various reaction types with reliable high enantioselectivity. These reactive species are formed from the interaction of the chiral catalyst with the substrate.[45] The discovery of these organocatalytic reaction modes allowed authors to classify generally and universally the catalysts used in organocatalysis.

Generic activation modes are extremely valuable, because they can be used as template platform for designing new enantioselective reactions.[74,75] The main activation modes have been summarized briefly in the next sections and were divided into covalent and non-covalent catalysis, in accordance with the substrate activation.

Covalent catalysis

In this first class of catalysts are included those in which the substrate activation occurs through the formation of a covalent bond between the substrate and the organocatalyst, leading to an increase of the interaction between the substrates during the reaction. Many of the organocatalyzed reactions are of this type, which can be divided in two subgroups: Lewis base catalysis (or nucleophilic catalysis) and amine catalysis (Figure 1.9). [61,74–76]

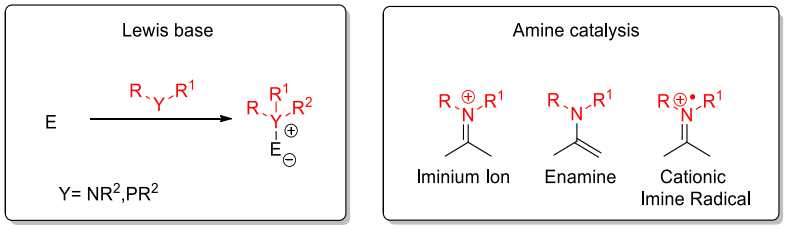


Figure 1.9 General activation mode observed in covalent catalysis and its subcategories (catalyst structures are represented in red).

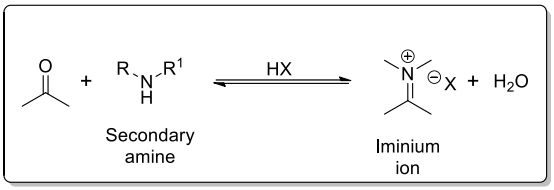
Alkaloids, amino acids, peptides and nitrogen-based synthetic molecules are part of this class of organocatalysis because they can establish covalent bonds with the substrates.[61,74–76]

Amine Catalysis

In recent years, nucleophilic amine-mediated catalysis has gained growing attention, and has been the target of worldwide researchers from a synthetic and mechanistic point of view.[76]

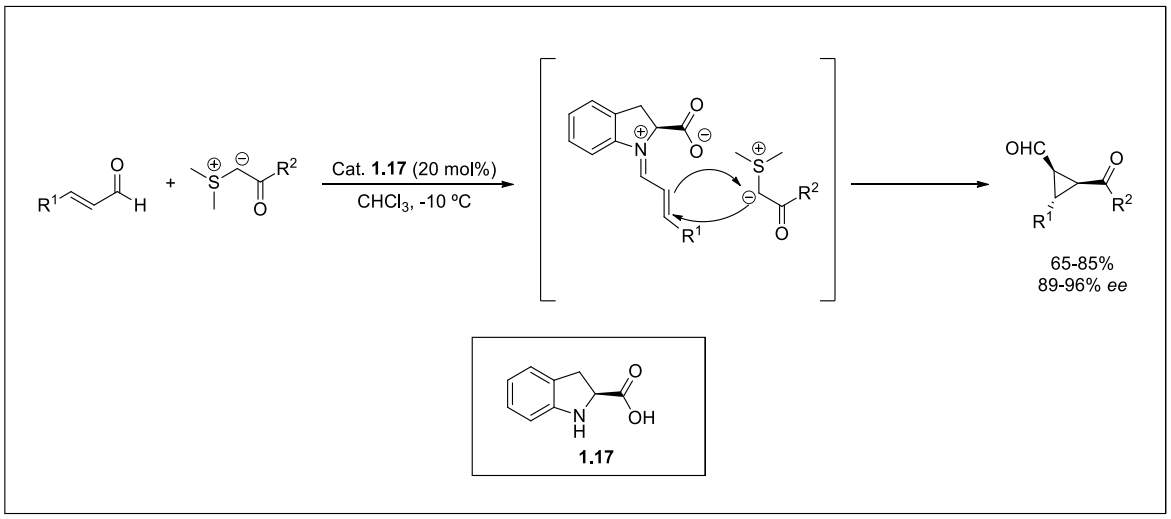
This type of catalysis is mainly used in the activation of carbonyl containing compounds and depending on the nature of the carbonyl substrate (saturated or unsaturated) and the reaction medium, the reaction will occur throughout enamine, iminium or cationic imine radical catalysis.

These activation modes differ in the reactive intermediates, nevertheless they are mechanistically tangled, and result from the condensation the amine (primary or secondary) of the catalysts with the carbonyl of the substrate, forming an iminium ion (Scheme 1.8).[77]



Scheme 1. 8 General formation of iminium ion through reversible condensation between a secondary amine and a ketone.

In scheme 1.9 is presented an example of an amine catalyzed cyclopropanation reaction of α,β-unsaturated aldehydes with sulfonium ylides performed by MacMillan and coworkers.[78]

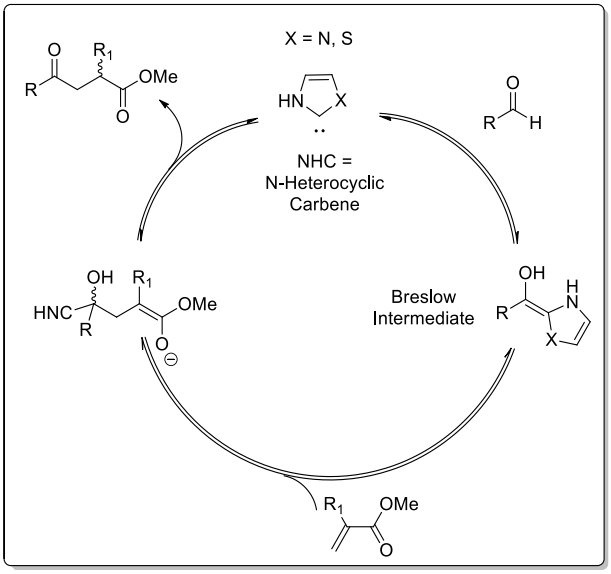


Scheme 1. 9 Cyclopropanation reaction of α,β-unsaturated aldehydes with sulfonium ylides catalyzed by (*S*)-2-carboxylic acid dihydroindole.[78]

Lewis base catalysis

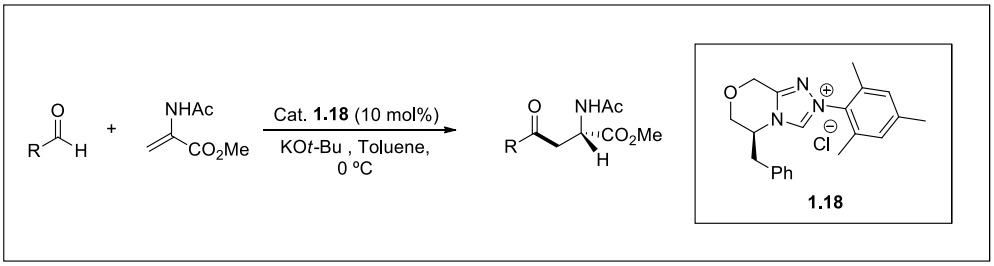
The second subclass of organocatalysts that uses covalent bonding in its mechanism of action for substrate activation is Lewis base. Usually, the catalysts included in this class contain an atom with nucleophilic character such as nitrogen, phosphorous, sulfur or even carbon atoms that bond covalently to the substrate.[30]

There are several examples of nucleophilic heteroatoms among which, the most interesting is an enzyme based carbene functionalized organocatalyst model. A good example is the *N*-heterocyclic Carbenes (NHCs), which have similar reactivities to coenzyme thiamine (vitamin B1).[79] NHC-mediated reactions form an acyl anion intermediate, known as the Breslow intermediate, and lead to optical inversion of the carbonyl group (Scheme 1.10).[80] These type of catalysts bring another type of diversity to nucleophilic organocatalysis since they mimic the active sites of proteins.



Scheme 1. 10 General organocatalytic cycle of a carbene reagent in nucleophilic catalysis.

This type of catalyst has already received some attention, and has been used in several types of reactions,[81] namely in the asymmetric Stetter reaction, which allows the synthesis of amino acid derivatives presented in scheme 1.11.[82]



Scheme 1. 11 Synthesis of α-amino acid derivatives via an NHC-catalyzed intermolecular Stetter reaction.[82]

Non-covalent catalysis

In non-covalent catalysis the activation mode occurs through weak, non-covalent, bonds between the substrates and the catalysts. This class of catalysis can be sub-divided into subcategories such as hydrogen bonding, phase transfer catalysis and Brønsted acids or bases (Figure 1.10).[61,74,75]

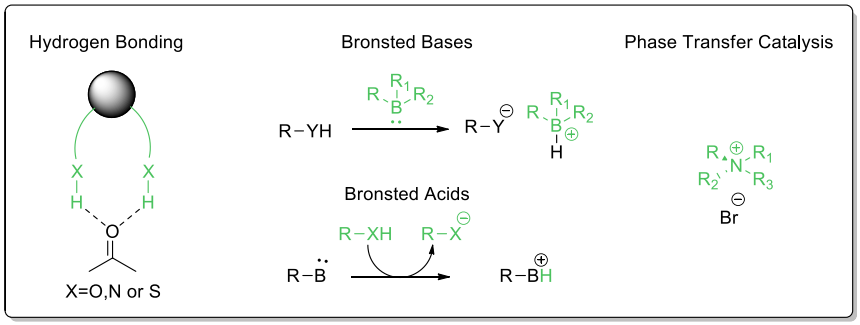


Figure 1. 10 General activation mode observed in non-covalent catalysis and its subcategories (catalyst structure in green).

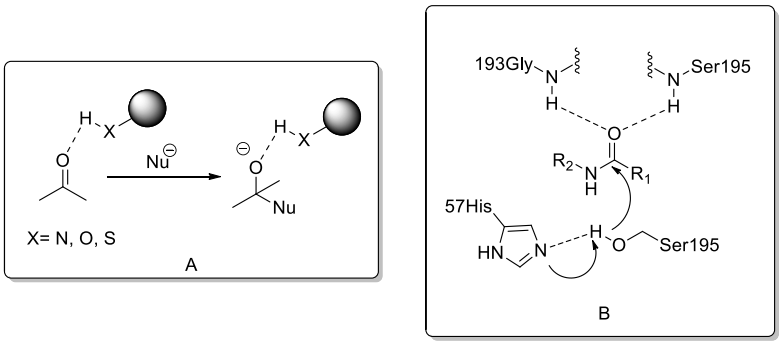
Hydrogen-bond catalysis

Hydrogen-bond catalysis is the type of catalysis that uses hydrogen bonding interactions to accelerate and control organic reactions. Hydrogen-bonds are weak electrostatic chemical bonds formed between covalently bonded hydrogen atoms and a strong electronegative atom with a lone pair of electrons. The hydrogen bond has a massive importance in biochemical processes and is also responsible for many of the structures that surround us, and life would be impossible without this type of bond.[83] In biochemical reactions, hydrogen bonding plays an important role in numerous enzymatic reactions, both in orienting the substrate molecules to the right position and lowering the reaction barriers, so that they can occur. In addition to their key role in numerous biological structures, hydrogen bonds have a crucial role in asymmetric catalysis.[84]

In hydrogen-bond-mediated catalysis, there is not only one but four different types of activation modes: stabilization of tetrahedral intermediates, anion binding, stabilization of anionic fragments and protonation. Details of these activation modes will be disclosed in the following sections.

Stabilization of tetrahedral intermediates

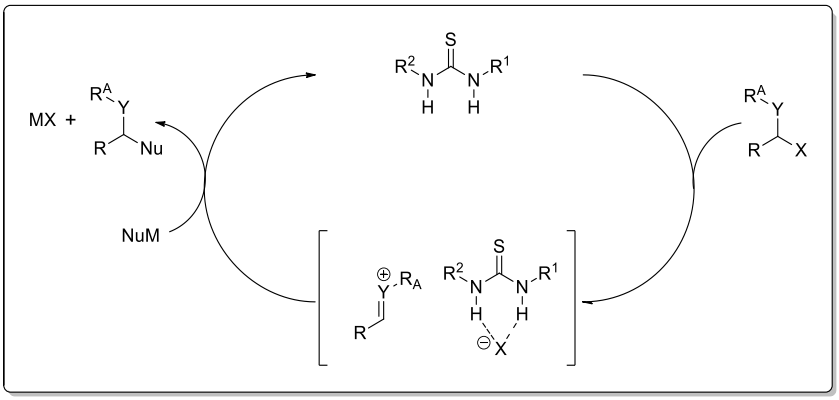
A great variety of organic reactions with functional groups such as aldehydes, amides or imines, occur through the formation of a tetrahedral intermediate after suffering a nucleophilic attack. In this type of reactions, the catalyst, which works as a hydrogen bond donor, stabilizes the tetrahedral anion intermediate formed. Using as example a nucleophilic reaction, the carbonyl coordinates with the catalyst through hydrogen bonds, making the carbonyl carbon into a more reactive electrophile. In this example, the attack of the nucleophile causes the displacement of the negative charge into the oxygen until the tetrahedral intermediate is formed. Consequently, the negatively charged oxygen atom forms a stronger hydrogen bond than the carbonyl oxygen, because of its higher negative charge. This bonding between the catalyst and the oxygen, lowers the energy of the intermediate and of the transition state, therefore accelerating the reaction (Scheme 1.12 A). This mode of catalysis activation can be found in enzymes, more specifically in their active sites, such as serine proteases (Scheme 1.12 B).[85,86]



Scheme 1. 12 (A) General example of tetrahedral intermediates stabilization (B) Active site mechanism of serine proteases.[85,86]

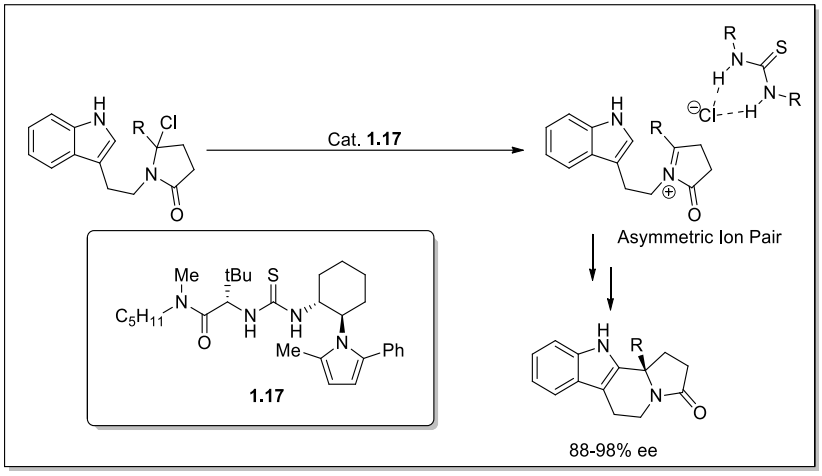
Anion Binding

Hydrogen-bond catalysts have also the capacity of assisting in the formation of electrophilic species by coordinating with anions such as halides. The most common class of catalyst used in this type of reactions are urea and thiourea catalysts. This is due to the fact that this type of molecules can easily bind to halides and other anions (Scheme 1.13).[87,88]



Scheme 1. 13 General reaction cycle for anion-binding thiourea catalysis.[84]

The use of chiral anion-binding catalysts enables the creation of an asymmetric ion pair inducing stereoselectivity. One of the first reactions using thiourea as anion-binding catalyst was the Pictet-Spengler-type cyclization of hydroxylactams with TMSCl (Scheme 1.14).[89]

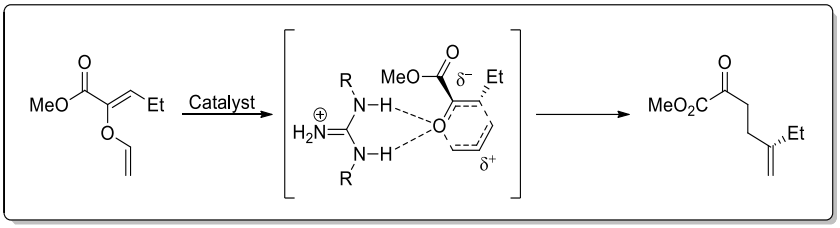


Scheme 1. 14 Thiourea catalyzed Pictet-Spengler-type cyclization of hydroxylactams. [89]

Stabilization of anionic fragments

Catalysts can also participate in the stabilization of reactions where partial negative charges are formed in the transition state. Reactions of concerted and pericyclic nature, where such catalysts have been applied, have shown good results. Thus, during the reaction, one of the reagents develops a partial negative charge and the latter can be stabilized by catalysts via hydrogen bonding. Eric N. Jacobsen and his research group, demonstrated that it is possible to catalyze Claisen rearrangements of ester-substituted

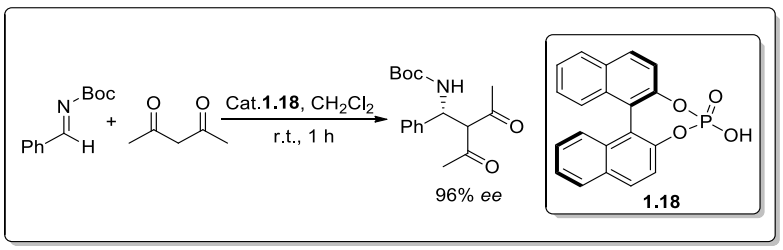
allyl vinyl ethers using this approach.[84,90] In this work, they found that a chiral guanidinium catalyst successfully catalyzed the reaction with high enantioselectivity at near room temperature conditions. In the transition state, the fragment that coordinates with the catalyst develops a partial anionic charge due to the electronegativity of the oxygen and the electron-withdrawing ester group present in the fragment. This partial anionic charge builds up the strength of hydrogen bond and lowers the transition state energy, yielding a much faster reaction. (Scheme 1.15) [84,90]



Scheme 1. 15 Claisen rearrangement performed by Jacobsen *et al.*[84,90]

Protonation

Generally, it's very difficult to differentiate between hydrogen-bond catalysis or general acid catalysis.[91] However, catalysis with strong acid catalysts as intervenient are often grouped in hydrogen-bond catalysis since they share catalytic similarities. This action mode mechanism starts with an initial protonation, which activates the electrophilic group and increases the electrophilicity of the substrate by creating an ion pair, allowing the transfer of stereochemical information. One example of this type of catalysis is the asymmetric Mannich reaction of aromatic aldimines with carbon nucleophiles, in which the protonation of the substrate has already demonstrated to be effective (Scheme 1.16).[85]

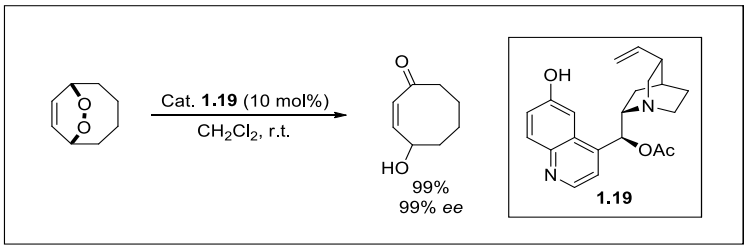


Scheme 1. 16 Mannich reactions of aromatic aldimines with carbon nucleophiles.[85]

Brønsted-Lowry acids and bases

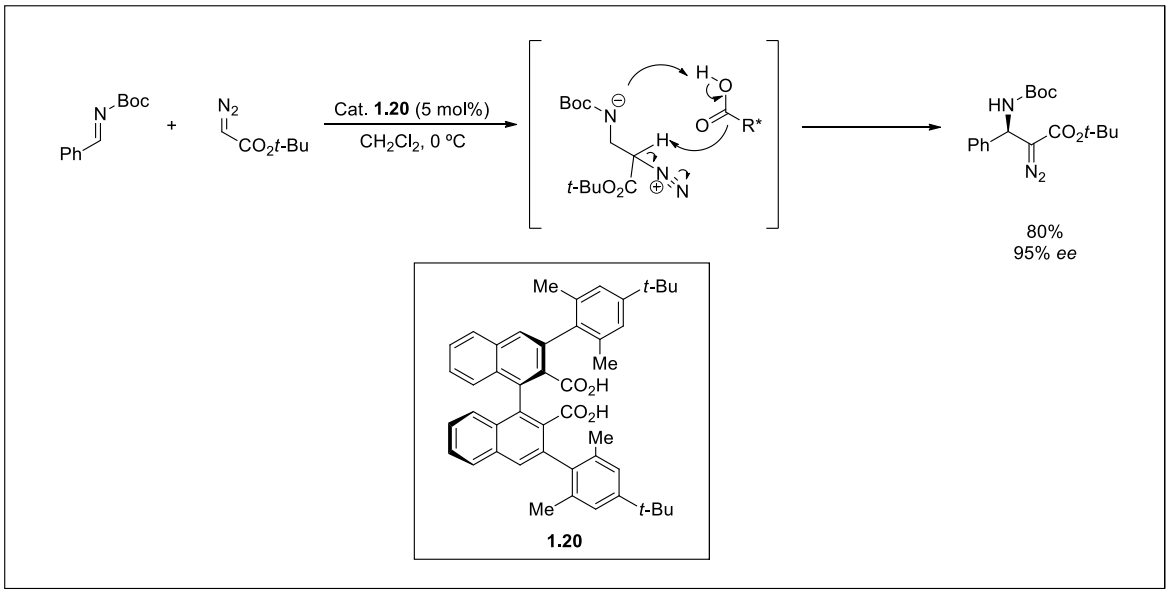
Brønsted acids and bases are remarkably important groups in the non-covalent catalysis class of organocatalysis, due not only to their many catalytic applications, as well as to the extraordinary results obtained in asymmetric synthesis.

When Brønsted bases are used as catalyst, an *in situ* nucleophile is formed by deprotonation of its precursor and the enantioselectivity mainly depends on the ionic interaction established between the catalyst and the substrate. This type of catalysts usually contain nitrogen functional groups, such as tertiary amines, guanidine or imidazole. One of the most important catalyst of this subclass are Cinchona alkaloids,[92] with a diversity of applications from which we can underline the Mannich, (aza) Henry and (hetero) Michael reactions along with kinetic resolution systems[93] and a few enantioselective rearrangements, namely the Kornblum-DeLaMare rearrangement presented in scheme 1.17.[94]



Scheme 1. 17 Cinchona alkaloid-mediated Kornblum-DeLaMare rearrangement.[94]

This subclass of catalysts also includes Brønsted acids, in which the activation process involves strong ionic interaction between the catalyst and the substrate (with basic character) which is essential to achieve enantioselectivity. In scheme 1.18 is also presented an example of an asymmetric Mannich reaction of arylaldehyde *N*-boc imines and diazo compounds catalyzed by a Brønsted acid. [95,96]



Scheme 1. 18 Brønsted acid-assisted Asymmetric Mannich Reaction.[96]

Phase transfer catalysis

Phase transfer catalysis resorts to chiral organic salts as catalysts for the enantioselective preparation of organic compounds. In this type of non-covalent catalysis, the activation mode is built on ionic pair interactions between the nucleophilic anions of the substrate and the positively charged catalysts, which usually is a quaternary ammonium salt.[64,97]Basically, phase transfer catalysis refers to reaction between two substances, located in different immiscible phases, in the presence of a catalyst. In this process the aqueous phase contains the reacting anions or nucleophiles (Cat^+X^-), and the organic phase, contains the organic reagents (RX) and the catalyst. The reagent nucleophile enters the organic phase by paring, via ionic pair interactions, with the catalyst cation. In the organic phase the nucleophiles react via nucleophilic substitution with alkyl halides (RX) (Figure 1.11). [98]

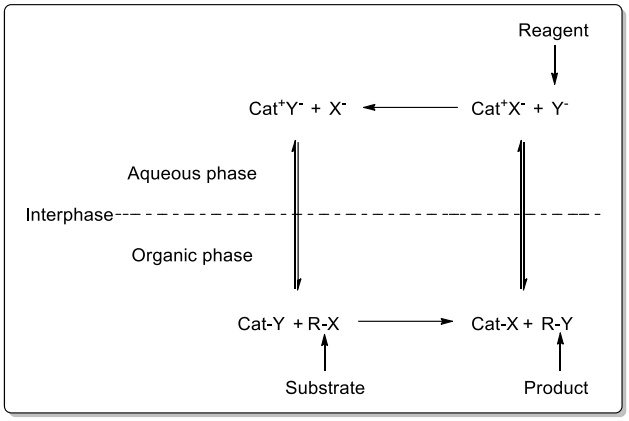


Figure 1. 11 Scheme of the general mechanism of a phase transfer catalysis.[98]

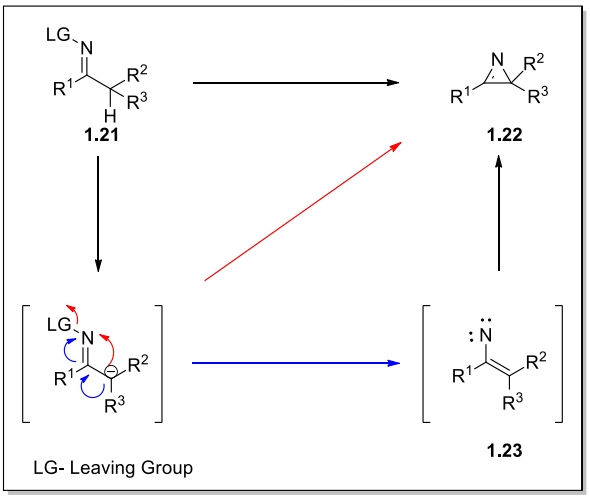
In this type of catalysis, the enantioselectivity is due to the stereochemical control of the chiral catalysts through interactions between the catalyst and substrate, which can be of electrostatic nature, via hydrogen bonds or π-π interactions.[99] During the last decades, the number of reactions performed via phase transfer catalysis has been increasing exponentially, in this sense, several asymmetric reactions such as aldol and redox reactions, epoxidations, asymmetric alkylations and Michael, Strecker and Mannich reactions have been developed for this type of catalysis. [99–103]

1.5 Asymmetric Neber Reaction Catalysts

Due to the essence of this work, it is important to discuss the type of catalysts used to achieve the main target, enantioselective synthesis of chiral 2*H*-azirine derivatives. In the next section the state of art concerning the asymmetric Neber reaction will be presented, namely the cinchona alkaloid and thiourea-mediated Neber reactions.

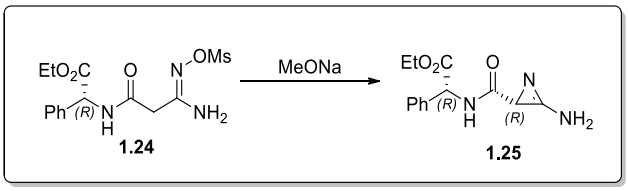
One important way to synthetize 2*H*-azirines is the Neber reaction. As mentioned, Neber *et al.* reported in 1926 for the first time the synthesis of 2*H*-azirines as intermediates in the synthesis of aminoketones by the treatment of oxime *p*-toluenesulfonates **1.21** with base. Several examples of the synthesis of 2*H*-azirine derivatives **1.22** through this method are known. Even though the reaction is known for such a long time, there is still uncertainty about the reaction mechanism. However, there are two proposed mechanisms for this type of reaction. In the first, the reaction occurs through an internal nucleophilic displacement while in, the second, the reaction occurs via an electrocyclization of a vinylnitrene **1.23** (Scheme 1.19). The presence of strong

electron withdrawing groups in the α-position relatively to the imine double bond, allows this rearrangement to occur in mild conditions since it increases the acidity of the α protons. [13,104]



Scheme 1. 19 Possible Neber reaction mechanisms.

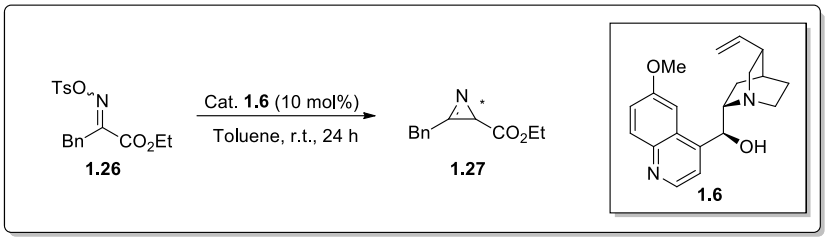
The first asymmetric version of this reaction was introduced in 1993 by Vosekalna *et al.* The reaction of chiral *O*-mesyl derivative of amidoxime **1.24** with NaOMe, yielded the respective optically active 2*H*-azirine **1.25** with 74% yield and 92% of diastereomeric excess (Scheme 1.20). This high diastereoselectivity without recurring to an organocatalyst can be explained by the presence of chiral auxiliary present in **1.24** which favored the formation of the (*R*,*R*) diastereoisomer.[105]



Scheme 1. 20 The first asymmetric Neber reaction by Vosekalna *et al.*[105]

In 1996, the first catalyst assisted asymmetric version of the Neber reaction was reported by Zwanenburg *et al.*. In this process, chiral cinchona alkaloid derivatives were used to induce the stereoselective formation of 2*H*-azirines. By treating ketoxime tosylates **1.26**, derived from 3-oxocarboxilic esters, with a large excess of potassium

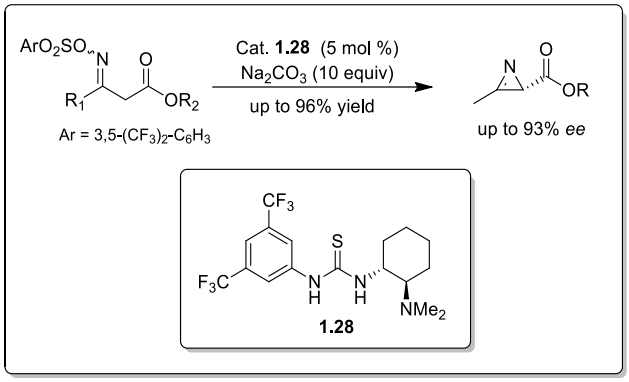
carbonate in the presence of catalytic amount of quinidine **1.6**, 2*H*-azirines **1.27** were obtained in yields ranging from between 29 to 85% and enantiomeric excesses up to 82% (Scheme 1.21). In this work, other organocatalysts were tested, such as sparteine, brucine, strychnine and other cinchona alkaloids, but quinidine was the catalyst that presented the best results. The results indicate that the alcohol function of the alkaloid base is prerequisite for the asymmetric Neber reaction. Furthermore, it was suggested that the alkaloid base formed a tight bonded complex with the ketoxime tosylate via hydrogen bonding through this hydroxyl group.[106]



Scheme 1. 21 First catalytic asymmetric Neber reaction by Zwanenburg *et al.*[104,106]

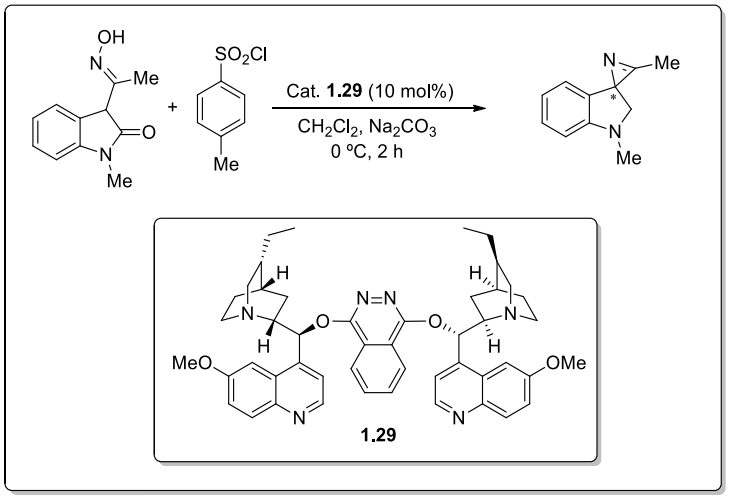
In the year 2000, Palacios *et al.* developed a similar strategy for the synthesis of 2*H*-azirines bearing a phosphonate group instead of the carboxylate group. In the following years, other research groups synthesized 2*H*-azirines via asymmetric Neber reaction. In 2002 Marouka *et al.* reported the synthesis of 2*H*-azirines using chiral transfer catalysis and in 2008, Molinski *et al.* reported the total synthesis of natural and biologically active (-)-(*Z*)-dysidazirine.[104]

More recently, in 2011, Takemoto *et al.* reported the efficient synthesis of chiral 2*H*-azirine carboxylic esters using bifunctional thioureas as catalyst. By treating β-ketoxime sulfonates with 10 equivalents of Na_2CO_3 and only 5 mol% of the thiourea catalyst the corresponding 2*H*-azirines were obtained with moderate to high yield and up to 93% of ee (Scheme 1.22).[107]



Scheme 1. 22 Asymmetric Neber reaction in the synthesis of 2*H*-azirine carboxylic esters using bifunctional thioureas as catalyst.[107]

Recently, in 2016, Yuan *et al.* also implemented the asymmetric Neber reaction in the synthesis of chiral spirooxindole 2*H*-azirines with a range of organocatalysts from which $(DHQD)_2PHAL$ **1.29** stood out. By treating the isatin ketoxime with tosylchloride in the presence of K_2CO_3 and 10 mol% of the catalyst they obtained the corresponding spiro-2*H*-azirines in yields up to 94% and enantiomeric ratios up to 92:8 (Scheme 1.23).



Scheme 1. 23 Asymmetric Neber reaction in the synthesis of chiral spirooxindole 2*H*-azirines.

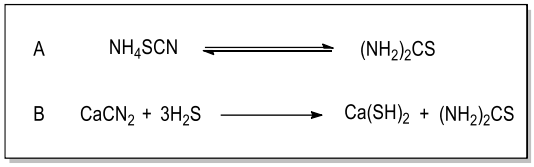
In the next section some of the Cinchona Alkaloids and Thiourea properties will be presented.

1.5.2 Thioureas

In 1828, Friedrich Wöhler, a young German chemist, described for the first time the synthesis of urea starting from inorganic raw materials. This discovery turned out to

be an important and revolutionary conceptual milestone in chemistry in the 19th century, since it showed for the first time that a natural organic molecule could be synthesized in laboratory without biological starting materials, contradicting the doctrine of vitalism that was held at the time. This finding led to a fast growth of organic chemistry and to the start of investigations on the synthesis and the properties of ureas, thioureas and guanidines. [108] Thioureas (also known as thiocarbamides or sulfoureas) belong to the class of organic compounds that contain sulphur in their structure, are structurally similar to ureas, with the exception that the carbonyl group of ureas is replaced by a thionyl group. Nevertheless, in terms of chemical and biological properties urea and thiourea differ significantly. [109]

The synthesis of thiourea was reported for the first time in 1869 by James Emerson Reynolds, a young physicist who had a great interest in chemistry. In his work, Reynolds succeeded to isolate thiourea by heating ammonium thiocyanate (Scheme 1.28 A). However, this type of reaction was an equilibrium reaction and the separation of the product from the reagent was rather difficult. For this reason, different synthetic methodologies were studied through the years. In 1909, the reaction between carbon disulfide and ammonia or ammonium carbonate was explored, but it wasn't appealing for the industry since it needed high pressures and high temperatures to obtain the desired product. Only in 1956, was found the method currently used in industry when Miller and Bann reacted calcium cyanamide with hydrogen sulfide (Scheme 1.28 B) obtaining the desired thiourea.[109]



Scheme 1. 24 Reaction equations of two diferent thiourea synthesis pathways.

In fact, thiourea have found their way into almost every branch of chemistry being an important raw material in industry with 5000 to 8000 tons of thiourea being produced annually. Thioureas are used in dyes, photographic films, plastics, textiles and many other applicatons.[109,110]Additionally, beyond the wide range of industrial applications of these compounds, they are also used for their biological activities such as antituberculous[111] **A**,

antithyroid[112] **B**, anthelmintic[113] **C**, rodenticidal[114] **D**, activitiy (Figure 1.13) and many others.[110]

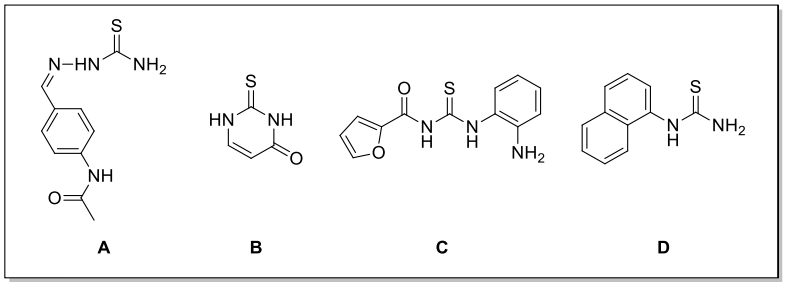


Figure 1. 12 Examples of thioureas with biological activity.

The successful story of thiourea catalysis started when Hine and coworkers discovered the double hydrogen bonding pattern in rigid 1,8-biphenylenediol (**1.32**). This molecule was able to establish two strong hydrogen bonds and catalyze aminolysis reactions of phenyl glycidyl ether. These results encouraged further studies and developments of 1,8-biphenylenediol derivatives. Then, following the concept of double hydrogen-bonding, Curran and Kuo presented in 1994 the first urea organocatalyst, the *N,N'*-diphenylurea **1.33**. This urea catalyzed successfully allylations of alfa-sulfonyl radicals with allyltributylstannane. In the following year, the same group, also reported the first application of a thiourea derivative (**1.34**) as catalyst in Claisen rearrangements. Still, after this encouraging findings, the studies and developments of new derivatives stopped until Schreiner's group, in 1997, continued the study of hydrogen bonding potential of thioureas in organocatalysis creating a new effective thiourea (**1.35**) for Diels-Alder reaction (Figure 1.14).[115]

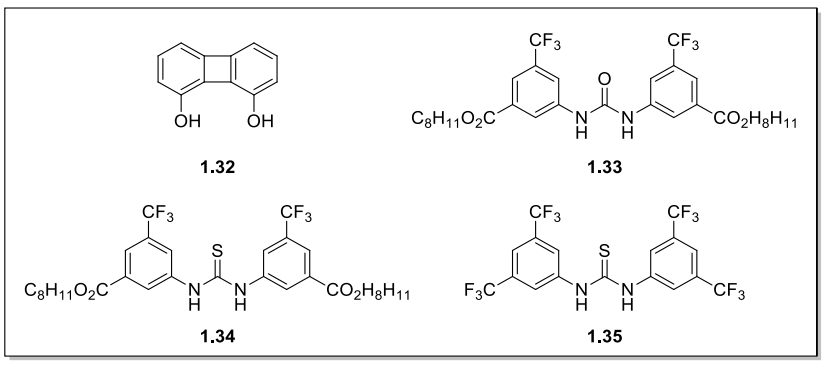


Figure 1. 13 Catalysts containing the double hydrogen bonding pattern.

Thioureas are valuable organocatalysts since they are green and sustainable. Moreover, they present numerous advantages such as their simple and inexpensive synthesis starting from primary amines and isothiocyanates, low catalyst-loading needs, high TOF (Turn-Over-Frequency) values and are easily recoverable and reusable. In addition, they are easy to handle and have versatile structure which allows further modifications for diverse applications, they can, for example, be immobilized on a solid phase. Other benefits of thioureas are their capacity to perform catalysis under almost neutral and mild conditions, even in water or aqueous media, and its nontoxicity in comparison to traditional metal-containing Lewis-acid catalysts. [116,117]

Thioureas can be classified in different categories based on the number of substituents bonded to the thiourea moiety. So, according to the number of substituents we can have mono *N*-substituted, disubstituted, trisubstituted thioureas or even tetrasubstituted thioureas[118] (Figure 1.15). In organocatalysis, usually only the *N,N'*-disubstituted thiourea derivatives are used, more specifically the 1,2-disubstituded.

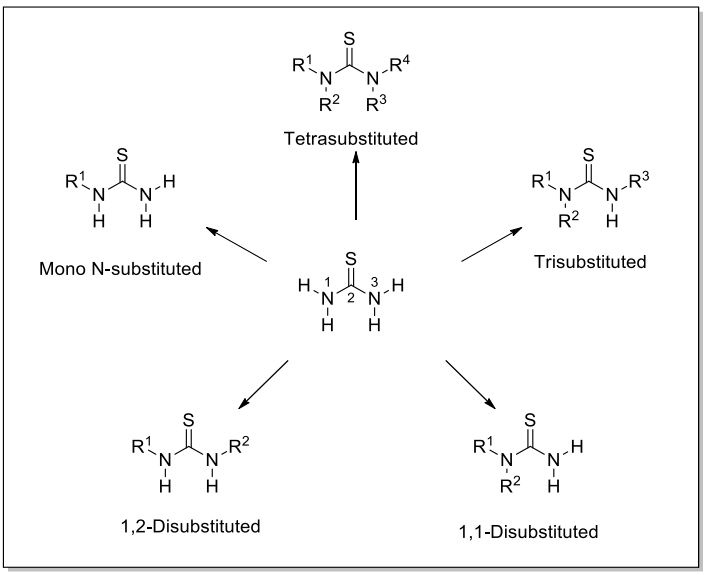


Figure 1. 14 Thiourea classification based on the number of substituents.[118]

To prove the importance of the N-H protons Curran *et al.* in the early years of thiourea organocatalysis, demonstrated that the catalytic activity disappears when substituting the protons with methyl groups.[119,120]

Schreiner and Wittkopp also found that the catalytic activity can be influenced by the rigidity of the catalyst. By applying different catalysts in the Diels alder reaction, they found that more flexible substituents on the thiourea can impair the interaction between catalyst and substrate. Thus, rigid thiourea catalysts form more stable complexes and its higher stability minimizes of "entropic penalty upon complexation" leading to more efficient catalysts. In this study they observed that if there were electron withdrawing groups (trifluoromethyl for example) present in the *para*- and *meta*- position of the aromatic substituents, the catalysts would be more rigid. The reason for this finding is that the hydrogen in the *ortho*- position becomes more positively polarized and this polarization leads to the formation of an intramolecular hydrogen bond between the *ortho*-hydrogens and the sulfur atom (Figure 1.16).[116] Furthermore, the electron withdrawing groups also increase the hydrogen bonding ability, as they decrease the pK_a of the N-H bonds of thiourea.[121]

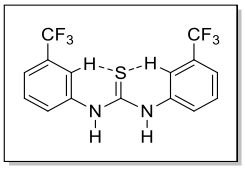
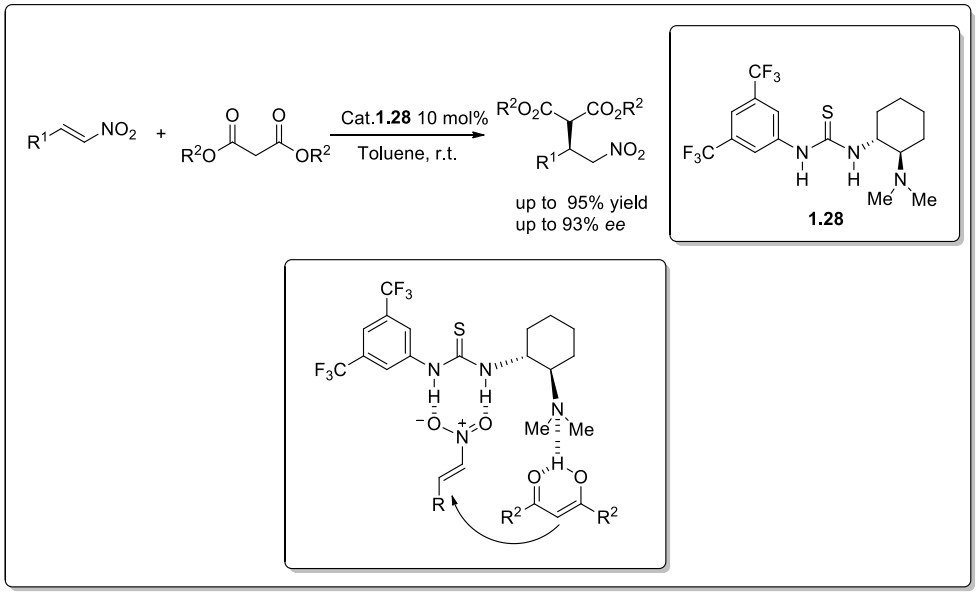


Figure 1. 15 Formation of an intramolecular hydrogen bond between the *ortho*- hydrogens and the sulfur atom.[116]

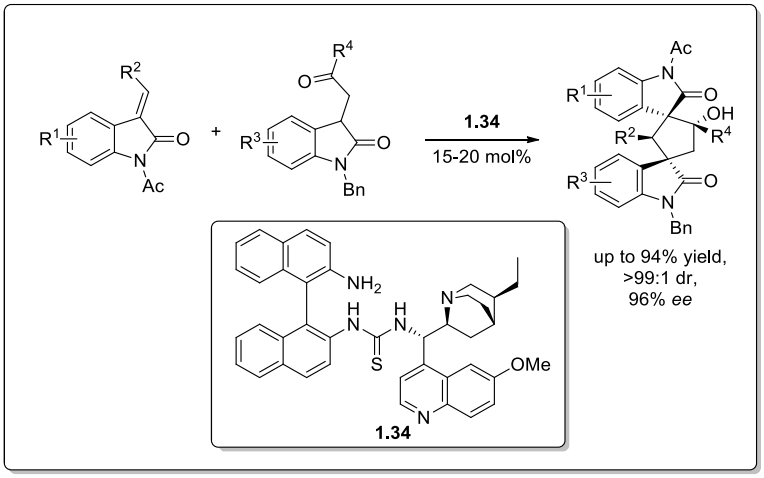
The earliest example where a thiourea derivative was used as an asymmetric organocatalyst, was the successful attempt of using a thiourea **1.12** for asymmetric Strecker reaction reported by Jacobsen et al, in 1998 (Scheme 1.7)[53]. In 2003, Takemoto and his group presented to the scientific community the first bifunctional thiourea **1.28**. This thiourea was capable to catalyze enantioselectively Michael addition of malonates to nitro-olefins providing the corresponding products in high yields and enantioselectivities. The proposed mechanism suggests a bifunctional transition state, where the nitro-olefin is activated by the thiourea group, while the malonate is activated by the tertiary amine (Scheme 1.29).[122]



Scheme 1. 25 Asymmetric Michael addition of malonates to nitroalkenes and the proposed mechanism by Takemoto and coworkers.[123]

Three years later, Takemoto also demonstrated that the same thiourea was capable of catalyzing the Michael reaction between benzimides and malononitrile and the aza-Henry reaction of nitroalkanes with *N*-Boc imines.[124]

During the last two decades, several chiral thiourea derivatives have been synthesized and applied in numerous asymmetric catalysis, namely in Baylis–Hillman reactions, Petasis reactions, Mannich-type reactions, Neber reaction and many others.[107,122] One quite interesting example that demonstrated the high potential of thioureas as organocatalyst was presented by Barbas and his coworkers. They described the use of a primary amine thiourea derivative **1.34** that catalyzed a domino Michael-aldol reaction leading to the synthesis of bispirooxindoles and forming new chiral carbons in high yields and excellent selectivities (Scheme 1.30).[122,125]



Scheme 1. 26 Synthesis of bispirooxindoles assisted by thioureas.[125]

1.5.1 Cinchona Alkaloids

Cinchona alkaloids are natural products obtained by extraction of the Cinchona tree, that makes part of the Rubiáces family, which are native of South America. Presently, they are mostly extracted from the bark of Cinchona ledgeriana from which are obtained about 30 different alkaloid molecules, including quinine, quinidine, cinchonidine and cinchonine which correspond to 50% of them.[126] Cinchona alkaloids have also a great medicinal history and are used in chemistry due to their interesting molecular structure.[60]

The interest to explore the catalytic features of these alkaloids arose nearly 50 years ago, and since then, these molecules gained a growing importance in organic and pharmaceutical chemistry, having a great impact in humanity in general.[127] It all started in the 17th century when it was found that the Cinchona bark had antimalaria properties and was used for medicinal purposes in Europe. Simultaneously South American natives also used the Cinchona bark to treat fevers showing their antipyretic properties.

Annually, are isolated approximately 700 tons of those cinchona alkaloids for several applications and industrial use. For instance, the pharmaceutical industry uses 40% of the extracted quinine, and the remaining 60% is used in food industry as a bitter aromatic agent in tonic water and other soft drinks. [128] Quinidine, for example, is used in the medicinal industry as an antiarrhythmic agent and cinchonidine in the racemic resolution of naproxen.[129]

The use of cinchona alkaloids as organocatalysts in asymmetric catalysis has gained increasing interest during the last decades. Following the actual interest in those alkaloids, their application in asymmetric synthesis has been extensively revised and assembled by several researchers such as Pracejus[130] in 1967, Morrison and Mosher[131] in 1971, Wynberg[132] in 1986, Kacprzak and Gowroński[133] in 2001, Kaufman e Rúveda[134] in 2005, Marcelli and Hiemstra[55]in 2010, Xi and Shi[135] in 2013, Zheng and Shienebeck[136] in 2014, as well as Song[126] in 2009 and many others.[61,137,138]

The earliest examples of the use of cinchona alkaloids in catalysis were in the addition of HCN to benzaldehyde reported by Bredig and Fiske[139] in 1912 (Scheme 1.2), the addition of methanol to phenyl methyl ketene described by Pracejus[140,141] in 1960 (Scheme 1.3) and the pioneering work in asymmetric thiol addition reactions, cyanohydrin reactions and Michael reactions done by Wynberg and his coworkers [132]. Those examples demonstrated that cinchona alkaloids had potential as catalysts for various enantioselective reactions. Until the 1990s, cinchona alkaloids and its derivatives were mostly used in phase transfer catalysis.[142–145] Subsequent to these studies, numerous reports have appeared concerning organocatalysis with cinchona alkaloids including the asymmetric Neber reaction as already mentioned.[106] Additionally, studies and reports of synthetic derivatives of cinchona alkaloids as organocatalysts have also appeared.

Nowadays, cinchona alkaloids achieved all the requirements that a privileged catalyst should have. Being economical and easily commercially available compounds, stable, having a robust and versatile structure, which allows modifications for diverse applications, makes them effective and attractive as catalysts. These features make cinchona alkaloids an interesting subject of study for the scientific community.[146]

The cinchona alkaloid family consists of two pairs of diastereomers – cinchonine (**CN**)/ cinchonidine (**CD**) and quinine (**QN**)/ quinidine (**QD**) (Figure 1.12).[146] Their uncommon and rich structure is the key factor that makes them of extreme importance in several areas, particularly in organocatalysis.

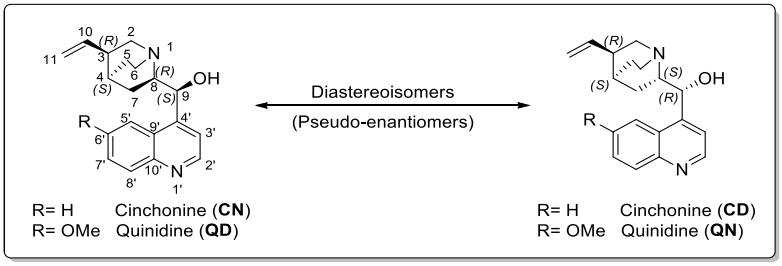


Figure 1. 16 Structure of the diastereomeric pairs of cinchona alkaloids and their chiral center absolute configuration.

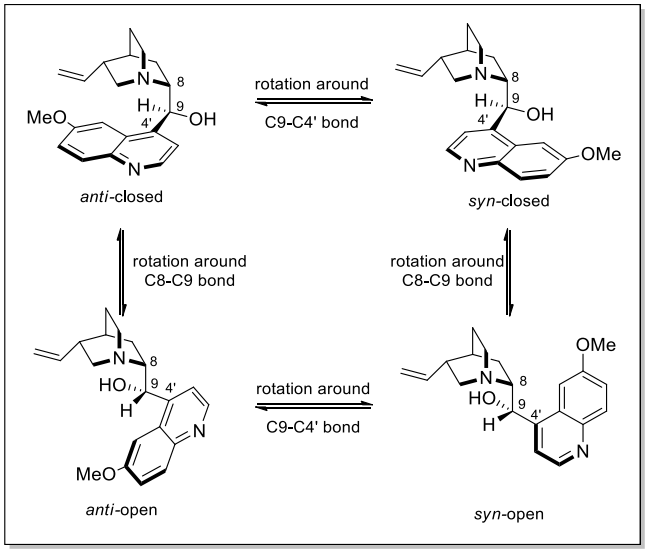
It wasn't easy to discover the structure of these alkaloids which took over 50 years to be determined. The currently known numbering used in cinchona alkaloids was initially proposed in 1907 by Rabe, a german chemist that dedicated great part of his life to the study of quinine. [147,148] Nevertheless, the known stereochemical assignment was only made in 1944, by Prelog and Zalan. [149,150]

The natural cinchona alkaloids have four different functional groups, the quinuclidine group (the bicyclic tertiary amine), a quinoline moiety (aromatic ring), a secondary alcohol group (or a β-hydroxylamine subunit) and a vinyl or ethyl group connected to the quinuclidine unit.

The diastereomeric pairs of the cinchona alkaloids have five stereogenic centers in their structure, being four of them chiral carbons (C3, C4, C8 and C9) and one chiral nitrogen of quinuclidine (N1).[55,60] The absolute configuration in the cinchona alkaloids only differs in the C8 and C9 carbons, with the other chiral carbons always presenting the same absolute configuration.[151] When used in asymmetric catalytic reactions, each pair of these catalysts originates a different chiral product with opposite absolute configuration (for example quinine and cinchonidine which are (-) induce selectivity for one enantiomer, while quinidine and cinchonidine which are (+) lead the other enantiomer), acting as enantiomeric pairs. Therefore, regardless of **CD**/**CN** and **QD**/**QN** being chemically diastereomers (i.e. they aren't mirror images of each other and aren't superimposable), they are known as pseudo-enantiomers.[55,60,146,152,153]

Cinchona alkaloids and their derivatives are bifunctional catalysts since they possess a secondary alcohol (Lewis acid site) and a nitrogen in the quinuclidine unit (Brønsted base site). They are considered to be Brønsted bases when the nitrogen moiety partially or fully activates a proton, leading to the formation of chiral intermediate species, which are generated selectively in the asymmetric catalysis.[154]

It is possible for cinchona alkaloids to rotate around the C4'-C9 and C9-C8 bonds, allowing it to adopt four different low energy conformations. Those conformers called anti-closed, syn-closed, anti-open and syn-open where identified via NMR spectroscopy and computational techniques (Scheme 1.24). They were labeled "open" because the quinuclidine nitrogen is distant from the quinoline group, thus being "exposed", meanwhile in the "closed" conformers the same nitrogen aligns with the quinoline, "blocking" it. The *syn* and *anti* terms are related the hydroxyl position in relation to C6' hydrogen: it is considered *syn* when both are on the same side and *anti* when they are on opposite sides.[151,155–159]



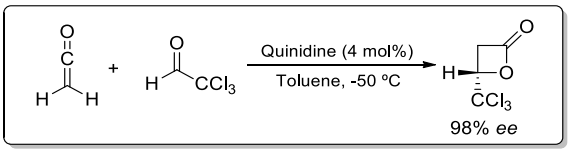
Scheme 1. 27 Four main low energy conformers of quinidine.[151]

Molecular modelling calculations also showed that quinidine and quinine prefer to adopt the *syn*-closed conformation in the gas phase. Nevertheless, those type of alkaloids assume preferentially an *anti*-open conformation in apolar solvents, while in polar solvents they assume both closed conformations, *anti*-closed and *syn*-closed. These studies have shown that the conformational behavior of the cinchona alkaloids can be influenced by many factors such as the nature of the solvent, intermolecular interactions or protonation. [160,161] It should also be pointed out that C9 substituents can also have effects on the conformation. For instance, if it's an ester substituent the conformation acquired in solution is *anti*-closed, while if the substituent is a methyl ether it assumes an *anti*-open conformation.[160]

As previously referred, cinchona alkaloids contain a versatile structure that allows the creation of simple synthetic derivatives making possible the maximization of the

catalytic activity for several asymmetric applications. Several structural modifications can be made through the C9 substituents, since it is the easiest way to derivatize the alkaloids by modifying or substituting the alcohol and changing it into amides, thioureas, ethers, esters free or substituted amines, guanidine and others. Many of these transformations lead to the inversion of the configuration of C9, forming the "*epi*-alkaloids". Also, the quinuclidine nitrogen can be transformed into a quaternary ammonium salt via alkylation, leading to catalysts which are commonly used in phase transfer catalysis. Another example of a possible derivatization is in the methoxy group of quinine and quinidine, which can be demethylated and then converted into a hydroxyl or substituted by an amine group, leading to intermediates that can be further derivatized.[62,133] The vinyl group connected to the quinuclidine group can also be modified allowing the immobilization of the catalyst on a variety of different solid supports. This, allows the recovery and recycle of the catalyst after the catalytic reaction, leading to economic advantages, especially on industrial scale. [162]

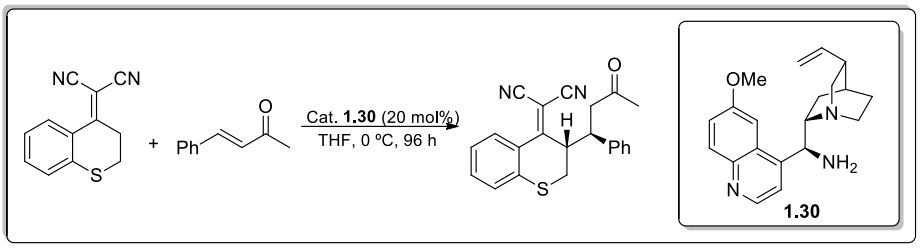
Nowadays, the most significant use of these alkaloids is in asymmetric organocatalysis. For example, Wynberg and collaborators demonstrated that the cinchona alkaloids can be used in a large range of different reactions. In 1981, they reported that cinchonidine was the most suitable cinchona alkaloid organocatalyst in the asymmetric conjugated addition of aromatic thiols to α, β-unsaturated cyclohexanones.[92] One year later, Wynberg described the synthesis of β-lactones through the reaction between ketenes and chloral using quinidine as catalyst (Scheme 1.25). The reaction afforded the desired product in very high enantiomeric excess and quantitative yield.[163]



Scheme 1. 28 Synthesis of β-lactones using quinidine as catalyst described by Wynberg.[163]

In 1995, Brunner reported the synthesis of cinchona alkaloid derivatives, 9-amino-(9-desoxy)-epi-cinchona derivatives.[164] In 2007, the enantioselective catalytic potential of these molecules was revealed in its use in Michael additions described by Cheng and

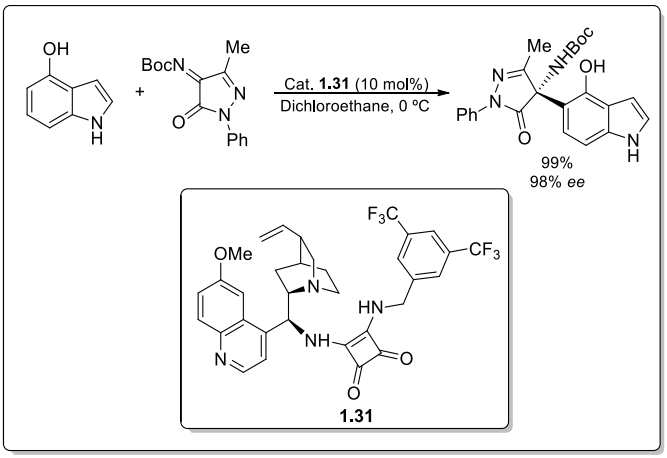
Deng[165] (Scheme 1.26) and in Friedel-Crafts alkylation reactions reported by Melchiorre[166] both obtained in good yields and enantioselectivities.



Scheme 1. 29 Asymmetric Michael addition reported by Cheng and Deng.[165]

Several years after, the cinchona alkaloid derivatives are still used in multiple catalytic reactions, such as rearrangements, epoxidation reactions, Diels-Alder reactions, substitution reactions and aza-Michael addition reactions.[167–176]

The beginning of organocatalysis "golden age"[177] led to the expansion of this field and brought with it innovation, creativity and enthusiasm for new studies and development of new classes of relatively simple, inexpensive, effective and versatile cinchona alkaloids, not only to obtain enantiomerically pure products but also to perform new catalytic reactions (Scheme 1.27).[178–190]



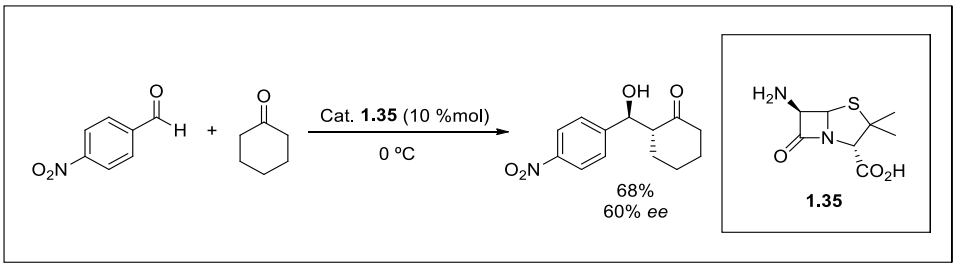
Scheme 1. 30 Organocatalytic enantioselective aza-Friedel-Crafts reactions of pyrazolinone ketimines with hydroxyindoles catalyzed by a squaramide derived cinchona alkaloid reported by Yang and Deng.[182]

1.5.3 6β-Aminopenicillanic Acid

In the work developed in this MSc project, 6β-aminopenicilanic acid and its benzhydryl derivative were also used as catalysts in the asymmetric synthesis of the 2*H*-azirine derivatives, therefore, some aspects concerning these compounds will also be discussed.

6β-aminopenicilanic acid (6-APA) is a natural occurring chiral compound with antibiotic activity against *Streptococcus* and some *Staphylococcus* species and its precursor, Penicillin G, is produced by several species of *Penicillium* and *Aspergillus*. 6-APA is the nucleus common to all penicillins and it is also a very special kind of amino acid. It was firstly discovered in 1957 by researchers of the Beecham Research Laboratories, Opening the way for the synthesis of several new penicillins. The first synthetic approach towards 6-APA was described by John C. Sheehan, in 1958, but the methodology wasn't appealing for mass production.[191] Instead, a semi-synthetic methodology where penicillins are converted to 6-APA through chemical or enzymatic processes was adopted. Actually, 6-APA is majorly obtained by penicillin acylase-assisted hydrolysis of Penicillin G. As mentioned, 6-APA is the main precursor in the production of antibiotic penicillins, therefore, its annual production reaches about 10,000 tons.[192]

In the catalysis field, 6-APA and its derivatives were only studied as catalysts in a crossed-aldol reaction in 2009, presented in scheme 1.31. In these studies, the products were obtained in good overall yield (up to 87%) albeit with low diastereoselectivity.[193]



Scheme 1. 31 6-APA mediated Cross-aldol reaction.[193]

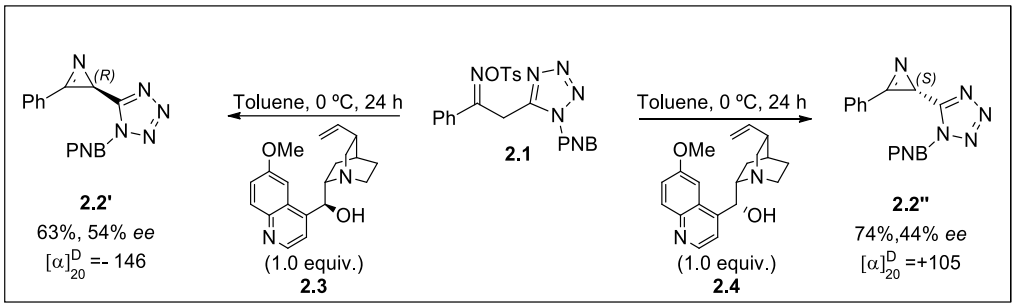
Chapter 2

Results and Discussion

"I hear my battle symphony
All the world in front of me
If my armor breaks
I'll fuse it back together"

Battle Symphoniy, Linkin Park

One of the research areas of the group where this work was developed, is the chemistry of small ring heterocycles, namely the synthesis and reactivity of 2*H*-azirines. The latest example is the application of the Neber reaction in the synthesis of a range of new 2-(tetrazol-5-yl)-2*H*-azirines bearing different substituents at C-3. The *in situ* tosylation of β-ketoxime-tetrazoles in the presence of base affords the target three-membered heterocycles. Firstly, the achiral version of the reaction of the β-ketoxime tosylates in the presence of triethylamine was developed. Then, preliminary studies on the asymmetric synthesis of 2-(tetrazol-5-yl)-2*H*-azirines were carried out which demonstrated that the Neber reaction assisted by quinidine and quinine leads to enantioselectivity, being the major enantiomer dependent on the alkaloid used (Scheme 2.1).



Scheme 2. 1 Quinidine and quinine assisted asymmetric Neber reaction in the synthesis 2-(tetrazol-5-yl)-2*H*-azirine derivatives.

Based on the work developed in the group, this MSc project was designed to continue the previous studies by optimizing the asymmetric reaction conditions for the synthesis of the 2-(tetrazol-5-yl)-2*H*-azirines for further application on the synthesis of medicinal chemistry relevant molecules, namely azirinomycin and dysidazirine bioisosteres (Figure 2.1).

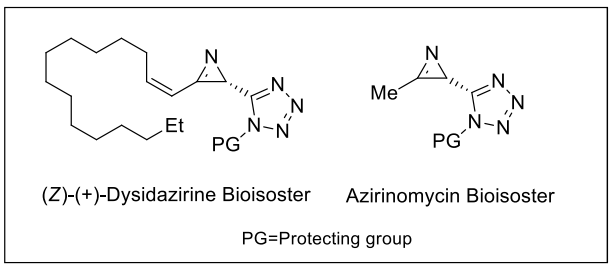


Figure 2. 1 Azirinomycin and (*Z*)-(-)-dysidazirine bioisosteres.

2.1 Synthesis of 2-(1-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone oxime

The project started by defining a model reaction for the asymmetric Neber reaction optimization studies. The chosen β-ketoxime was 2-(1-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone oxime (**2.5**) (Figure 2.2).

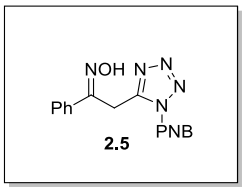
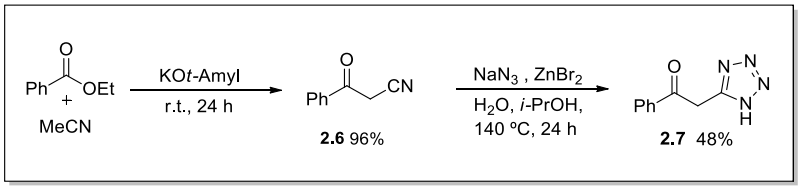


Figure 2. 2 2-(1-(4-Nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone oxime.

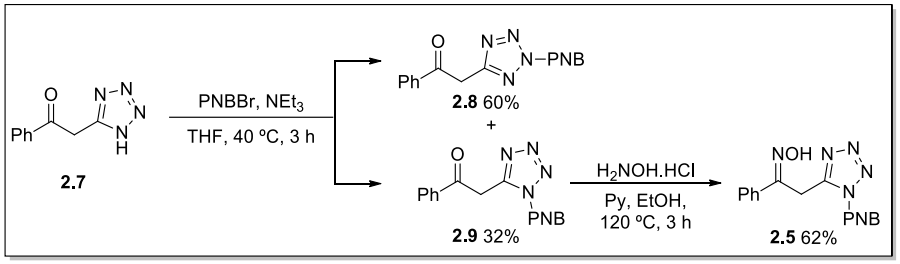
The synthetic strategy towards oxime **2.5** precursor is outlined in scheme 2.2. Thus, 3-oxo-3-phenylpropanenitrile (**2.6**), was obtained from the reaction of ethyl benzoate with acetonitrile in the presence of potassium tert-butoxide in 96% yield. Then, using Sharpless' method for the synthesis of tetrazoles, compound **2.7** was obtained by reacting **2.6** with sodium azide and zinc bromide in a sealed tube at high temperature.[194] The click reaction between nitrile **2.6** and the in situ generated zinc azide yielded the desired tetrazole in 48% yield (Scheme 2.2).



Scheme 2. 2 Synthesis of compounds **2.6** and **2.7**.

Next, tetrazole **2.7** was protected with *p*-nitrobenzyl bromide (PNBBr) leading to two different isomers: the 1,5-substituted tetrazole isomer **2.9** (32%) and the 2,5-substituted tetrazole isomer **2.8** (60%) which were separated by column chromatography. The synthetic methodology proceeded with regioisomer **2.9** since it is known that the 5-substituted 1*H*-tetrazoles are the effective carboxylic acid bioisosteres. [27] Therefore, in

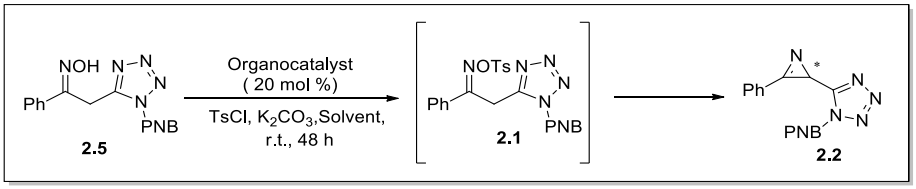
the final step of the synthetic route **2.9** was converted into the corresponding β-ketoxime by reaction with hydroxylamine hydrochloride in the presence of pyridine (Scheme 2.3). Although in the synthesis of the β-ketoximes two different isomers (*E* or *Z*) could in principle be obtained, only one isomer is formed.



Scheme 2. 3 Synthesis of compounds **2.8**, **2.9** and **2.5**.

2.2 Asymmetric Neber Reaction

One of the first optimization goals was to explore the one-pot version by carrying out the *in situ* tosylation of the β-ketoxime tetrazole **2.5** followed by the Neber reaction in the presence of an organocatalyst. In previous work, the alkaloid-mediated Neber reaction was carried out starting from β-ketoxime tosylate (**2.1**). In Scheme 2.4 the general reaction scheme for the one-pot procedure of chiral 3-phenyl-2-(1-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)-2*H*-azirine **2.2** is presented.



Scheme 2. 4 General reaction scheme of the one-pot synthesis of 2*H*-azirine **2.2**.

It was also the aim of this work to find the best organocatalyst. Therefore, a range of different organocatalysts were tested. As they can be subdivided in different categories (cinchona alkaloids, thioureas and 6β-aminopenicilanic acid derivatives) the results obtained will be presented in separated sections.

2.2.1 Cinchona Alkaloid Derivatives Mediated Asymmetric Neber Reaction

For this project different cinchona alkaloid derived organocatalysts such as quinidine, quinine and 9-amino-(9-deoxi)-*epi*-cinchonidine derivatives were tested in the asymmetric Neber reaction of β-ketoxime **2.5**. In figure 2.3 are represented the structures of the studied catalysts and as shown, all catalysts have a quinuclidine, a quinoline and a vinyl group linked to the quinuclidine unit in common. Quinidine **2.3** and quinine **2.4** have an additional methoxy group connected to the quinoline group, but the major differences between the catalysts are located at the C9 carbon which has different substituents (**2.10**-**2.19**).

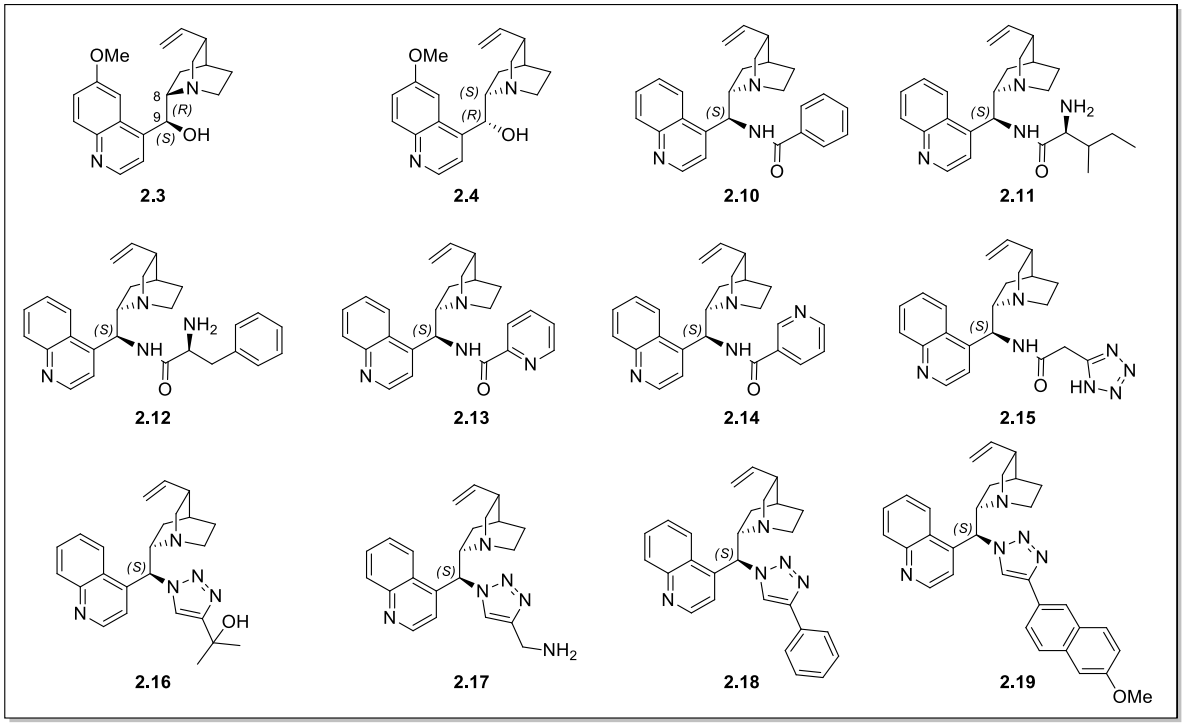
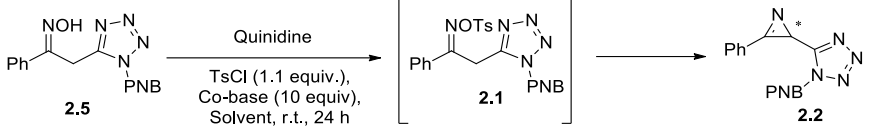


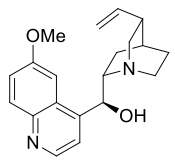
Figure 2. 3 Range of cinchona alkaloid derived organocatalysts tested in the asymmetric Neber reaction.

Quinidine as organocatalyst

As mentioned before, the preliminary studies on the asymmetric synthesis of 2-(tetrazol-5-yl)-2*H*-azirines done by the group demonstrated that quinidine and quinine can be used as organocatalysts in the Neber reactions leading to the target compounds in an enantioselective fashion. After these first findings, the group continued the studies in order to improve the observed selectivity. An initial solvent screening using quinidine as organocatalyst was carried out. The influence of Na_2CO_3 as co-base instead of K_2CO_3 was also studied and the results are presented in table 2.1.

Table 2.1 Solvent screening of the asymmetric Neber reaction of β-ketoxime **2.5** with quinidine as organocatalyst.



Organocatalyst	Entry	Solvent	Co-base	Catalyst Equivalents	Yield (%)	ee (%)[a]
 2.3	1	Acetonitrile	K_2CO_3	1	*	-
	2	Dichloromethane	K_2CO_3	20 mol%	42	45 (*R*)
	3	Diethyl ether	K_2CO_3	20 mol%	<40	n.d.
	4	Toluene	K_2CO_3	20 mol%	67	60 (*R*)
	5	Toluene	Na_2CO_3	20 mol%	19	55 (*R*)

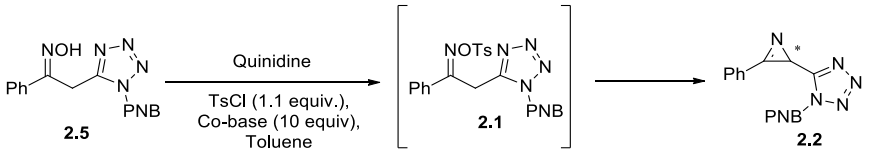
[a] Determined by chiral HPLC, mobile phase: water/ acetonitrile (65:35), 1 mL/min; *No reaction; n.d. Not determined.

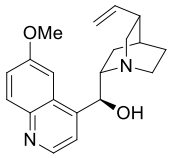
It was found that the desired product **2.2** was obtained when the reactions were performed in dichloromethane, diethyl ether or toluene (entries 2, 3 and 4, respectively), whereas no reaction occurred using acetonitrile (entry 1). Moreover, the reaction carried out in toluene allowed the synthesis of the target 2*H*-azirine (**2.2**) in higher yield (67%) and higher ee (60%, entry 4). It is also possible to afford **2.2** using Na_2CO_3 as co-base (19%, entry 5). Nevertheless, the yield was significantly lower than by carrying out the reaction in the presence of K_2CO_3 (67%, entry 4).

Based on these initial results, toluene and K_2CO_3 were selected for further studies. As the main goal was to improve the efficiency and selectivity of this reaction, the studies in this MSc thesis began by optimizing the conditions for the quinidine (**2.3**) mediated

asymmetric Neber reaction. Taking into account that in the initial study performed by the group a stoichiometric amount of quinidine was used, the first step was to decrease the amount of organocatalyst to catalytic amounts. Furthermore, the amount of the co-base K_2CO_3 was increased considering the work of Zwanenburg on the asymmetric Neber reaction of 2*H*-azirines carboxylic esters.[106] Therefore, quinidine was then explored under different reaction conditions as shown in Table 2.2.

Table 2.2 Asymmetric Neber reaction of β-ketoxime **2.5** with quinidine (**2.3**) as organocatalyst.

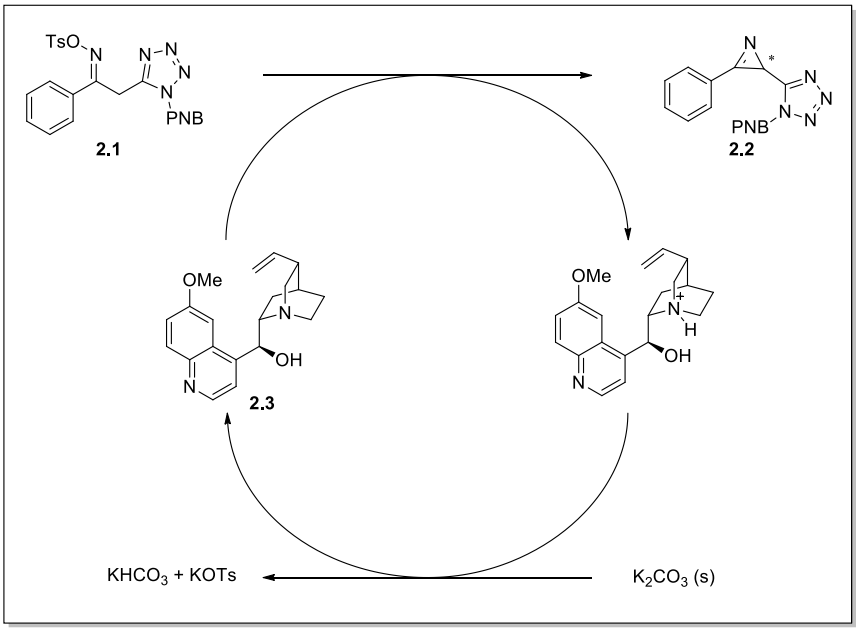


Organocatalyst	Entry	Temperature (°C)	Catalyst Equivalents	Time (h)	Yield (%)	ee (%)[a]
 2.3	1	0 °C	20 mol%	48	24	55 (*R*)
	2	r.t	20 mol%	24	45	50 (*R*)
	3	r.t	20 mol%	48	87	66 (*R*)
	4	r.t.	1	72	7	68 (*R*)
	5	r.t.	10 mol%	48	66	63 (*R*)

[a] Determined by chiral HPLC, mobile phase: water/ acetonitrile (65:35), 1 mL/min.

To our delight, the decrease of the catalyst amount from 1 equivalent to only 20 mol% allowed the synthesis of the target compound **2.2** in 87% yield and 66% ee (entry 3). Analyzing the results, we can observe that by decreasing the temperature neither improved the enantioselectivity of the reaction nor its efficiency (ee 55% and 24% yield, entry 1). In fact, reactions at room temperature proved to be more efficient (entry 1 vs. entry 3). By increasing the temperature from 0 °C to room temperature the yield increased to 45% only after 24 hours (entry 2). Furthermore, by increasing the reaction time to 48 hours it was possible to isolate **2.2** in excellent yield (87%) although with moderate ee (66%, entry 3). A longer reaction time, 72 h, was also studied using 1 equivalent of quinidine but unfortunately, the yield decreased drastically (7%, entry 4). This result may be justified by the degradation of the product. Lastly, the amount of **2.3** was decreased to 10 mol% (entry 5) and led to a lower yield (66% vs. 87%) although the ee was nearly the same (63% vs. 66%).

Based on Zwanenburg's investigations, a representation of the catalytic cycle for the asymmetric Neber reaction of compound **2.2** is presented in scheme 2.5. The quinuclidine unit from quinidine plays an important role in the abstraction of the methylene protons of the *in situ* generated β-ketoxime tosylate **2.1**. While the alcohol at C9 is responsible for stablishing a hydrogen-bond complex with one of the thionyl groups present in the tosylate. By removing one of the α-protons a carbanion is formed, which will lead to the formation of the 2*H*-azirine via one of the two previously mentioned mechanisms proposed for the Neber reaction. Another important reagent used is the co-base K_2CO_3, which assumes the role of regenerating the alkaloid base.



Scheme 2. 5 Catalytic cycle of quinidine in the asymmetric Neber reaction.

In figure 2.4 are presented chromatogram expansions of the racemic 2*H*-azirine **2.2** (**A**) and of the 2*H*-azirine product of the quinidine-mediated asymmetric Neber reaction (**B**) (entry 3, table 2.2).

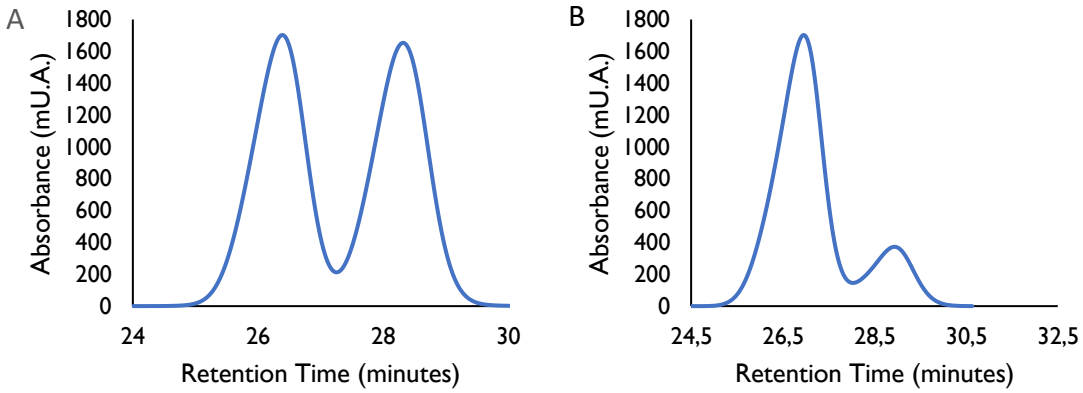


Figure 2. 4 (A) Chromatogram expansion of the racemic mixture (B) Chromatogram expansion of quinidine-mediated Neber reaction (entry 3, table 2.2) (small deviations regarding the minute values of the peak maximum, which can be justified by small pressure variations in the HPLC instrument).

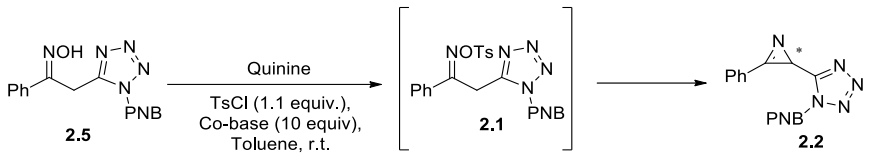
In the chromatograms the two peaks corresponding to the presence of both enantiomers can be clearly observed. In the optimized method, the typical retention times of the *R* isomer is near 27 minutes and for the *S* isomer is near 29 minutes. In chromatogram **A** it can be further observed that the proportion between both peaks is equal and the ratio between both isomers observed in chromatogram **B** is approximately 83 to 17 for the *R* isomer. The enantiomeric excess is then obtained with the following formula:

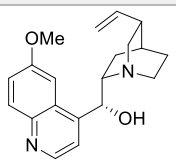
$$ee = \frac{|\text{Area of } R \text{ isomer} - \text{Area } S \text{ isomer}|}{\text{Sum of the area of both isomers}} \times 100$$

Quinine as organocatalyst

Quinine (**2.4**) the pseudoenantiomer of quinidine (**2.3**), was also evaluated as organocatalyst of the Neber approach in the synthesis of 1*H*-tetrazolyl-2*H*-azirines and different reaction conditions were studied, and the results are presented in table 2.3.

Table 2. 3 Asymmetric Neber reaction of β-ketoxime **2.5** with quinine (**2.4**) as organocatalyst.



Organocatalyst	Entry	Organocatalyst Equivalents	Time (h)	Yield (%)	ee (%)[a]
 2.4	1	60 mol%	48	52	51 (*S*)
	2	20 mol%	24	48	41 (*S*)
	3	20 mol%	48	61	44 (*S*)
	4	20 mol%	72	15	7 (*S*)

[a] Determined by chiral HPLC, mobile phase: water/ acetonitrile (65:35), 1 mL/min.

It should be highlighted that, as expected, quinine led to the formation of the *S* isomer whereas quinidine afforded its enantiomer. Carrying out the reaction in the presence of 60 mol% of quinidine a moderate yield and enantiomeric excess were obtained, 52% and 51%, respectively (entry 1). Other observation that can be made is that using 20 mol% of quinine, with either 24 or 48 hours of reaction time (entry 2 and 3), led to lower catalytic activity than quinidine (entry 2 and 3 of table 2.3). This result is in agreement with the outcome observed in Zwanenburg's work for the synthesis of 2*H*-azirine carboxylic esters.[106] Increasing the reaction time to 72 hours resulted in lower yield and ee (entry 4). The chromatogram expansions of the best result of quinine-mediated Neber reaction (**A**) (entry 3), and the best result using quinidine (**B**) are shown in figure 2.5.

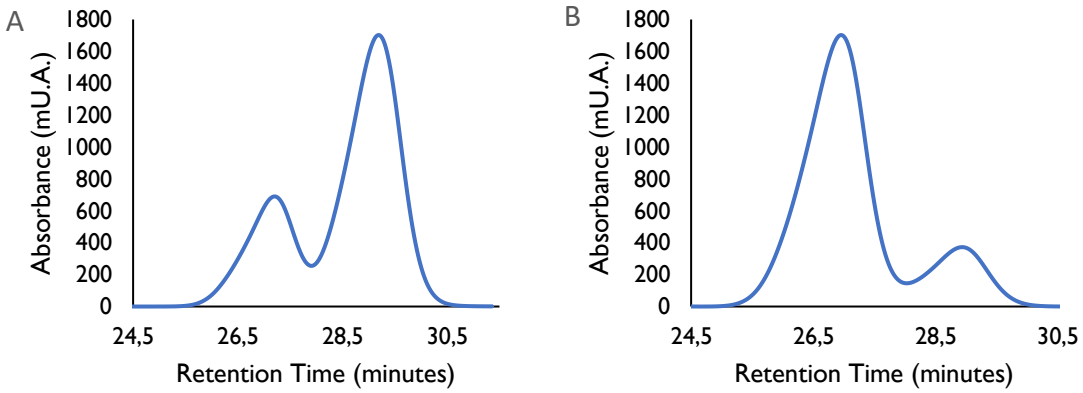


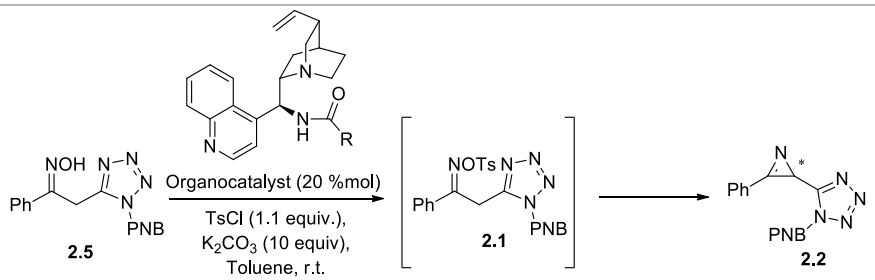
Figure 2. 5 (A) Chromatogram expansion of quinine-mediated Neber reaction (entry 3, table 2.4) (B) Chromatogram expansion of quinidine-mediated Neber reaction (entry 3, table 2.2).

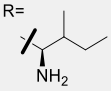
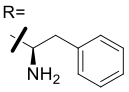
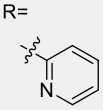
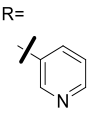
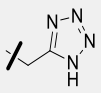
Since the studied organocatalysts are pseudoenantiomers, the chromatograms of quinine **A** and quinidine **B** gave opposite ratios of the *R* and *S* 2*H*-azirines enantiomers. Therefore, as expected the quinine catalyzed reaction gave the *S* isomer as major product (chromatogram expansion **A**) while in the quinidine-mediated reaction the major product was the R isomer (chromatogram expansion **B**).

Epi-cinchonidine derivative-assisted asymmetric Neber reaction

The results of the asymmetric Neber reaction catalyzed by *epi*-cinchonidine derivatives bearing amino acid or additional heterocycle moieties, are presented in table 2.4.

Table 2. 4 *Epi*-cinchonidine derivative-assisted asymmetric Neber reaction of β-ketoxime **2.5**.



Organocatalyst	Entry	Time (h)	Yield (%)	ee (%)[a]
2.10 R= 	1	24	22	49 (*R*)
	2	48	55	56 (*R*)
2.11 R= 	3	48	30	24 (*R*)
2.12 R= 	4	48	32	21 (*R*)
2.13 R= 	5	48	52	18 (*R*)
2.14 R= 	6	48	64	24 (*R*)
2.15 R= 	7	48	35	9 (*R*)

[a] Determined by chiral HPLC, mobile phase: water/ acetonitrile (65:35), 1 mL/min.

Reactions carried out in the presence of these *epi*-cinchonidine derivatives gave the *R* enantiomer as the major product. Within the presented catalysts, **2.10**, was the most promising showing a similar catalytic performance to quinidine in 24 hours reaction time (entry 1). The chromatogram expansion corresponding to the best result of the **2.10**-mediated (**A**) and quinidine-mediated (**B**) Neber reaction are presented in figure 2.6. By increasing the reaction time to 48 hours, an improvement in the yield and *ee* was observed (59% and 56% respectively, entry 2). The catalysts **2.11** and **2.12** presented rather disappointing results with yields up to 31% and enantiomeric excesses up to 24%. While *epi*-cinchonidine derivatives **2.13**-**2.15** bearing an additional heterocycle moiety afforded the product **2.2** was obtained in moderate yields. On the other hand, the enantiomeric excesses obtained were relatively low. These catalysts also presented some difficulty regarding the solubilization in the reaction solvent which could be the reason for these disappointing results. The best results in this serie were obtained with catalyst **2.14** (entry 7) which led to the formation of the desired product in 64% yield and 24% of enantiomeric excess.

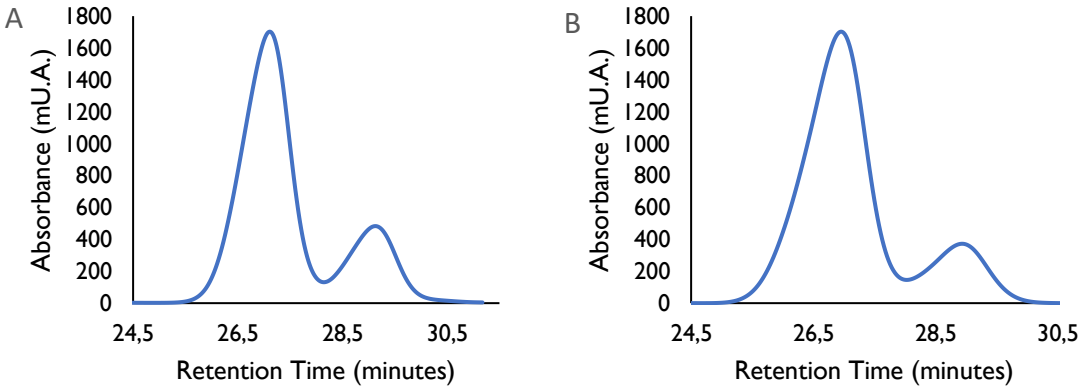
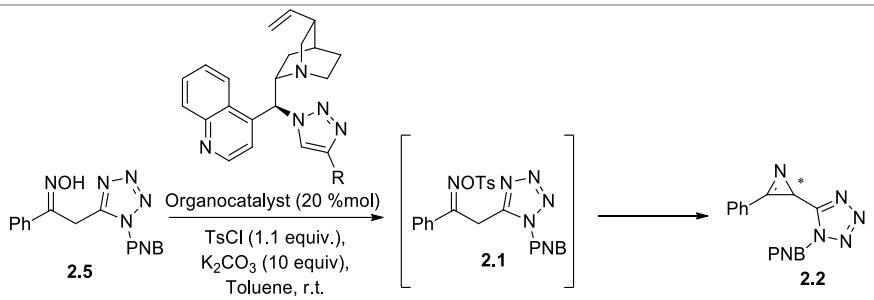
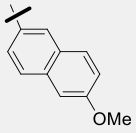


Figure 2. 6 (A) Chromatogram expansion of **2.10**-mediated Neber reaction (entry 2, table 2.4) (B) Chromatogram expansion of quinidine-mediated Neber reaction (entry 3, table 2.2).

The asymmetric Neber reaction catalyzed by *epi*-cinchonidine derivatives bearing a triazolyl moiety under the optimized reaction conditions was also studied (table 2.5).

Table 2. 5 Asymmetric Neber reaction of β-ketoxime **2.5** in the presence of *epi*-cinchonidine derivatives bearing triazolyl moieties as organocatalyst.



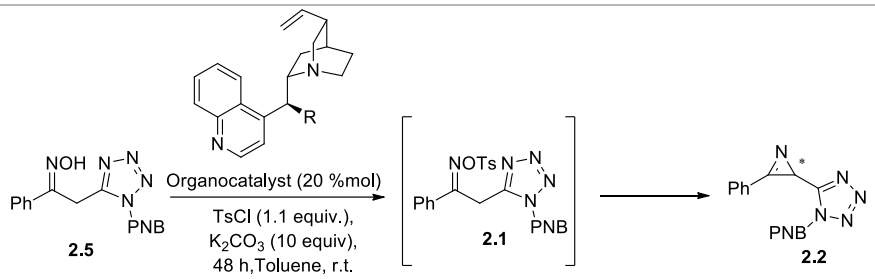
Organocatalyst	Entry	Time (h)	Yield (%)	ee (%)[a]
2.16 R= 	1	48	34	18 (*R*)
2.17 R= 	2	48	<1	n.d.
2.18 R= 	3	48	10	27 (*R*)
2.19 R= 	4	24	4	14 (*R*)

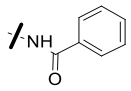
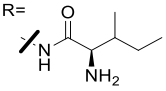
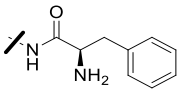
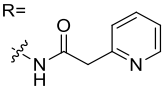
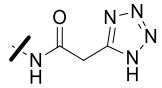
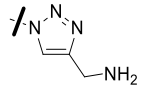
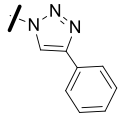
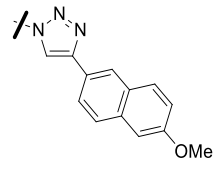
[a] Determined by chiral HPLC, mobile phase: water/ acetonitrile (65:35), 1 mL/min, n.d. not determined.

However, the results were rather disappointing. Nevertheless, it should be mentioned that in this serie all the catalysts were insoluble in toluene, which could have been one of the factors leading to these bad results. In this serie the higher yield was 34%, when catalyst **2.16** bearing a hydroxyl moiety was used (entry 1). Regarding the ee the best result was obtained, when using catalyst **2.18** bearing a phenyl group (27%, entry 3).

After these disappointing first results, the reactions were repeated in a different solvent in order to solubilize the catalysts that were insoluble in toluene. Therefore, dichloromethane was the chosen solvent and in table 2.6 both results obtained with dichloromethane and toluene in the asymmetric Neber reaction of β-ketoxime **2.5** are presented.

Table 2. 6 Asymmetric Neber reaction of β-ketoxime **2.5** with *epi*-cinchonidine derivatives using toluene and dichloromethane as solvent.



Organocatalyst	Entry	Solvent	Yield (%)	ee (%)[a]
2.10 R= 	1	Toluene	55	56 (*R*)
	2	CH_2Cl_2	41	43 (*R*)
2.11 R= 	3	Toluene	30	24 (*R*)
	4	CH_2Cl_2	21	16 (*R*)
2.12 R= 	5	Toluene	32	21 (*R*)
	6	CH_2Cl_2	19	21 (*R*)
2.13 R= 	7	Toluene	52	18 (*R*)
	8	CH_2Cl_2	13	15(*R*)
2.15 R= 	9	Toluene	35	9 (*R*)
	10	CH_2Cl_2	20	16 (*R*)
2.17 R= 	11	Toluene	<1	*
	12	CH_2Cl_2	20	6 (*R*)
2.18 R= 	13	Toluene	10	27 (*R*)
	14	CH_2Cl_2	21	6 (*R*)
2.19 R= 	15	Toluene	4	14 (*R*)
	16	CH_2Cl_2	19	6 (*R*)

[a] Determined by chiral HPLC, mobile phase: water/acetonitrile (65:35), 1 mL/min.

It can be observed that for both catalysts **2.10** and **2.11** the use of dichloromethane didn't improve the yield nor the enantiomeric (entries 2 and 4), as for catalyst **2.12** the yield also decreased but the *ee* remained the same (entry 6). For both catalysts in the heterocycle series, **2.13** and **2.15,** the yield values dropped (entries 8 and 10), but while for **2.13** the *ee* decreased for **2.15** it increased. Lastly, for the triazolyl moiety series the yield augmented in all reactions (entries 12, 14 and 16), but sadly, the enantiomeric excess diminished.

These results indicate that the solubility of the catalysts had some influence, but it isn't the major obstacle in this study since with dichloromethane all catalysts were solubilized.

2.2.2 Thiourea-Mediated Asymmetric Neber Reaction

Takemoto *et al.*, as mentioned in the previous chapter, had successfully applied bifunctional thioureas as organocatalysts in the asymmetric Neber reaction for the synthesis of 2*H*-azirine carboxylic esters.[107] Thus, based on his work, the research group where this MSc was developed, also performed the asymmetric Neber reaction in the presence of thioureas. The catalysts used were chiral thiazolidine-derived thioureas and are presented below in figure 2.7.

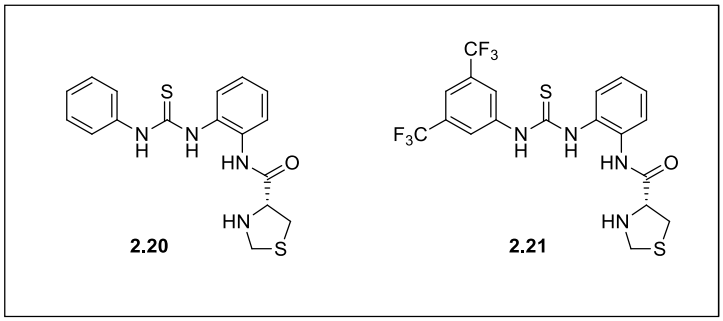
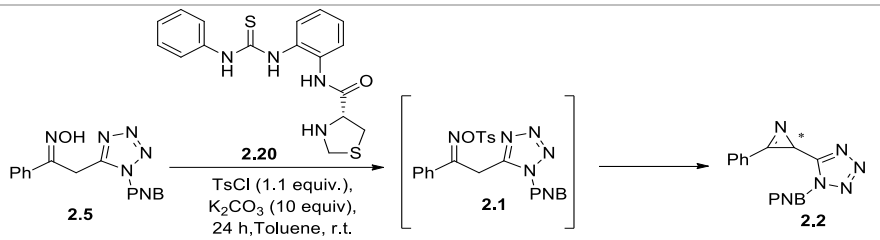


Figure 2. 7 Chiral thiazolidine derived thioureas.

In table 2.7 are presented the first results of the use of thiourea **2.20** as catalyst in the asymmetric Neber reaction.

Table 2. 7 Asymmetric Neber reaction of β-ketoxime **2.5** with chiral thiazolidine derived thiourea **2.20**.



Entry	Catalyst Equivalents	Yield (%)	ee (%)[a]
1	1	<30	74 (*S*)
2	20 mol%	*	-

[a] Determined by chiral HPLC, mobile phase: water/ acetonitrile (65:35), 1 mL/min * No reaction.

The reaction performed with thiourea **2.20** unfortunately only afforded azirine **2.2** in the presence of stoichiometric amounts of the catalyst (entry 1). Surprisingly, the enantiomeric excess obtained under these reaction conditions was very interesting 74% for the *S* isomer, although the yield was rather low (30%). Sadly, by reducing the amount of catalyst to 20 mol%, the catalytic activity of **2.20** was lost and no reaction occurred. In the chromatogram expansion of the product of the reaction catalyzed by one equivalent of **2.20** is shown in figure 2.8 confirming that the *S* isomer was the major product.

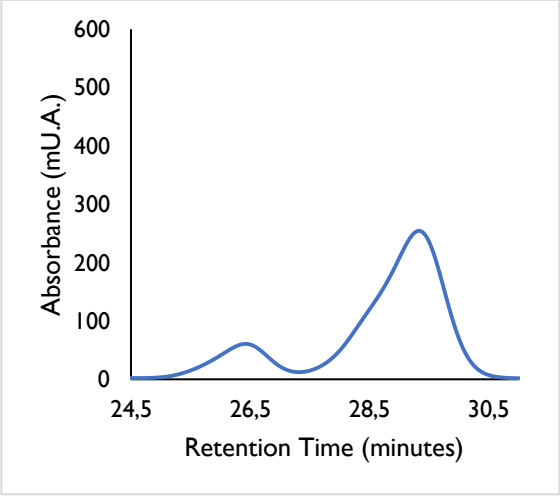
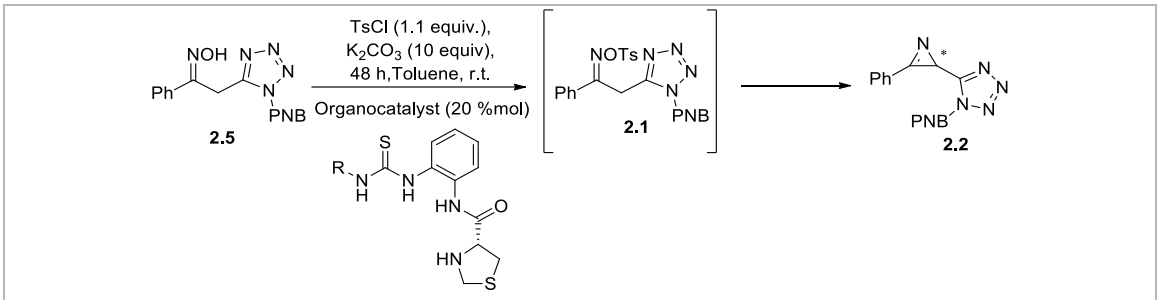
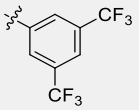


Figure 2. 8 Chromatogram expansion of **2.20**-mediated asymmetric Neber reaction (entry 1, Table 2.7)

Despite these results, in this MSc project thiourea **2.20** was studied as well as, thiourea **2.21** bearing a 3,5-bis(trifluoromethyl)phenyl group (Table 2.8). In these procedures the reaction time was increased from 24 to 72 hours and 10 equivalents of co-base were used.

Table 2. 8 Asymmetric Neber reaction of β-ketoxime **2.5** with chiral thiazolidine derived thioureas.



Organocatalyst	Entry	Time (h)	Solvent	Yield (%)	ee (%)[a]
2.20 R= 	1	72	Toluene	*	-
2.21 R= 	2	72	Toluene	6	9 (*R*)
	3	48	CH_2Cl_2	*	-

[a] Determined by chiral HPLC, mobile phase: water/ acetonitrile (65:35), 1 mL/min *No reaction.

The results for the thiourea derivatives bearing chiral thiazolidines, **2.20** and **2.21**, were once again very disappointing. The reaction carried out in the presence of 20 mol% of catalyst **2.21** for 72 hours, for example, didn't afford azirine **2.2**. In the case of catalyst **2.21**, two different solvents were tested (entries 2 and 3), whereas the reaction performed in toluene for 72 hours led to 2*H*-azirine **2.2** in only a yield of 6% and 9% *ee* (entry 2), while in dichloromethane the reaction didn't occur at all (entry 3).

The best results achieved so far were with catalysts **2.3** and **2.10** for the *R* isomer and catalyst **2.4** for the *S* isomer, whose structures are shown in figure 2.9. One of the reasons for the unsatisfactory results displayed by catalysts **2.13**, **2.14**, **2.17**, **2.20** and **2.21** may be due to the fact that the synthetic methodology involves an *in situ* tosylation.

Having tosyl chloride present in the reaction medium can lead to the tosylation of the primary and secondary amines present in some of these catalyst structures.

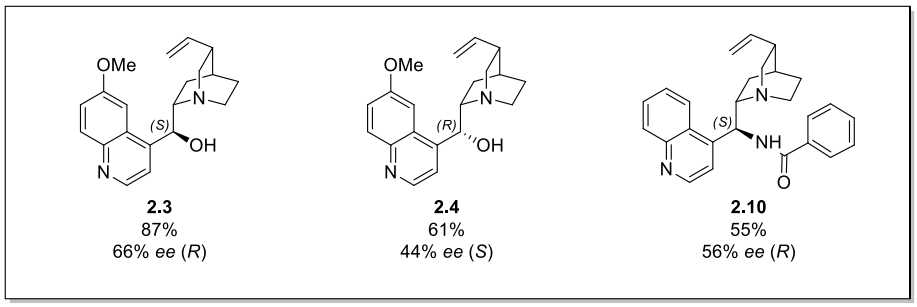


Figure 2. 9 Representation of the best catalysts.

Furthermore, the poorer results presented by the *epi*-cinchonidine derivatives bearing triazolyl moieties can be justified by the absence of a hydrogen bond donor at C9 carbon, which is crucial to catalyze the asymmetric Neber reaction (Figure 2.10). [106]

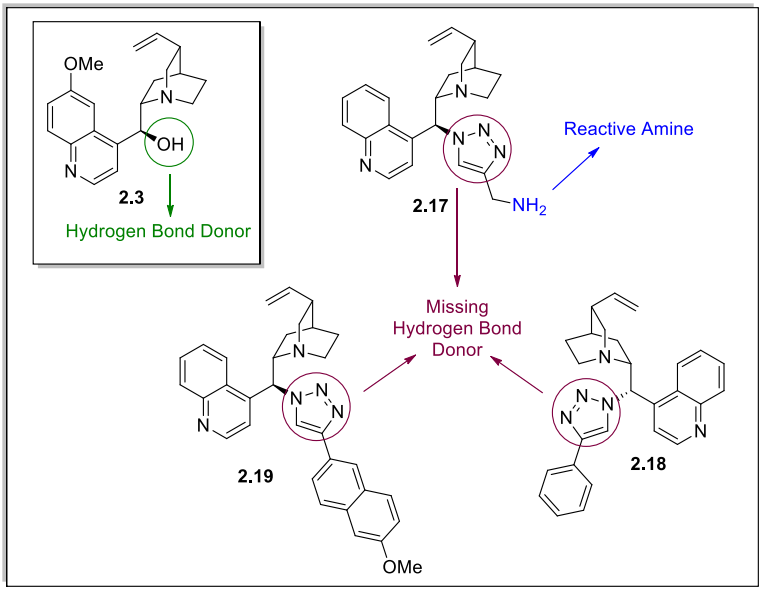
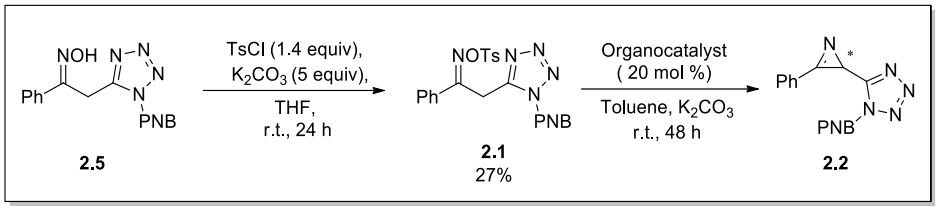


Figure 2. 10 Representation of the 9-amino-(9-deoxi)-*epi*-cinchonidine derivatives bearing triazolyl moieties catalysts.

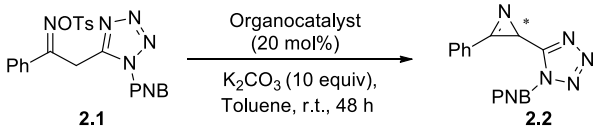
In order to avoid the reaction between the catalysts amines and tosyl chloride, it was necessary to take a step back and firstly perform the reaction between the β-ketoxime **2.5** and tosyl chloride instead of performing the one-pot asymmetric Neber reaction (Scheme 2.6).

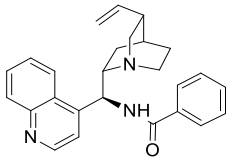
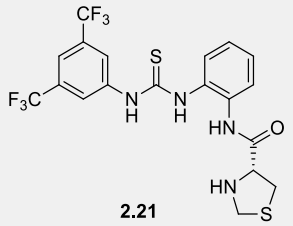


Scheme 2. 6 β-Ketoxime tosylation followed by the asymmetric Neber reaction.

The yields obtained for the tosylation reaction were rather low (27%) and this is due to the fact that it is difficult to stop the reaction in the β-ketoxime tosylate stage, since the conversion into the corresponding azirine is very favorable and usually mixture of these two compounds is obtained. Therefore, the synthesis of **2.1** can be considered an inefficient and a time-consuming process, since the yields are low, and the purification process is also difficult. Nevertheless, two catalysts were tested in the asymmetric Neber reaction of the β-ketoxime tosylate **2.1** (Table 2.9).

Table 2. 9 Asymmetric Neber reaction with the β-ketoxime tosylate **2.1**.



Organocatalyst	Entry	Yield (%)	ee (%) [a]
 2.10	1	30	49 (R)
 2.21	2	30	2 (*R*)

[a] Determined by chiral HPLC, mobile phase: water/ acetonitrile (65:35), 1 mL/min.

Analyzing the results, the conclusion that can be made is that there was no improvement in the asymmetric Neber reaction when starting from the β-ketoxime tosylate. Comparing the results with the one-pot procedure result for **2.10**, under the same reaction conditions (entry 2, table 2.4), the 55% yield and 56% *ee* also dropped to

30% and 49%, respectively, which only corroborates this observation. Even for thiourea **2.21**, where an improvement was expected, the result was rather disappointing, with a yield of 30% and ee of 2%.

2.3 Synthesis of New Catalysts

2.3.1 6β-Aminopenicillanic Acid-Mediated Asymmetric Neber Reaction

As mentioned in the previous chapter, 6β-aminopenicilanic acid (6-APA) is a natural occurring chiral compound. Since it is an easily affordable compound and it also successfully catalyzed a cross-aldol reactions,[193] 6-APA (**2.22**) and its ester (**2.23**) were also studied as organocatalyst in the asymmetric Neber reaction of β-ketoxime **2.5** (Figure 2.11, Table 2.10).

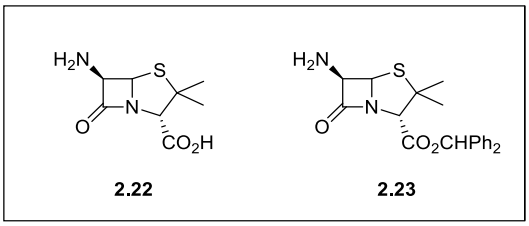
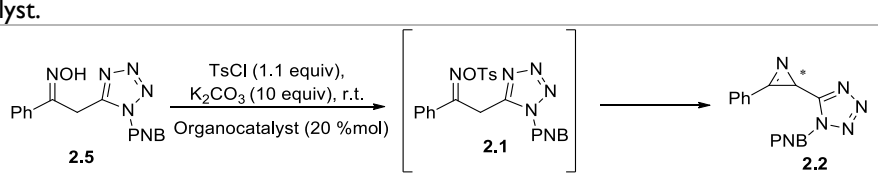


Figure 2. 11 Structure of 6-APA and its benzhydryl ester

Table 2. 10 Asymmetric Neber reaction of β-ketoxime **2.5** with 6β-aminopenicilanic acid and its benzhydryl ester as catalyst.



Organocatalyst	Entry	Time (h)	Solvent	Yield (%)	ee (%) [a]
2.22	1	24	Toluene	15	52 (*R*)
	2	48	Toluene	<5	5 (*R*)
	3	72	Toluene	5	11 (*R*)
2.23	4	48	Toluene	19	13 (*R*)
	5	48	CH_2Cl_2	*	-

[a] Determined by chiral HPLC, mobile phase: water/ acetonitrile (65:35), 1 mL/min; *No reaction.

The first reaction performed for 24 hours with 6-APA (**2.22**) as catalyst, presented a low yield but a quite promising *ee* of 52 % for the *R* isomer (entry 1). Encouraged by these promising results, the reaction time was increased in an attempt to increase the yield, but instead, a lower yield and *ee* was observed (entries 2 and 3). By carrying out the reaction in toluene in the presence of 6-APA benzhydryl ester **2.23**, low conversions and *ee*'s (19% and 13%, respectively, entry 3) were also observed. Using dichloromethane as solvent even worsened the results, since the reaction didn't occur (entry 4). The disappointing results obtained with these two catalysts, **2.22** and **2.23**, can be rationalized by its low solubility together with the presence of the amine group.

2.3.2 Synthesis of 6β-Aminopenicilanic Acid Derived Thiourea Organocatalysts

In this MSc project new 6-APA derived thiourea organocatalysts **2.24** and **2.25**, were also developed for the first time (Figure 2.12). Since 6-APA is a very cheap, easily available, enantiopure chiral compound it was chosen to integrate the new thioureas. Thus, firstly the synthesis of these new compounds will be described.

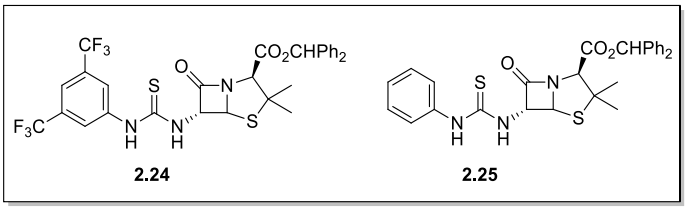
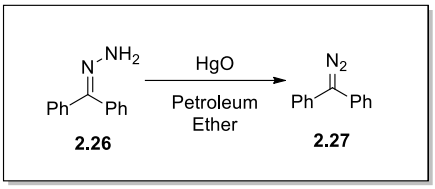


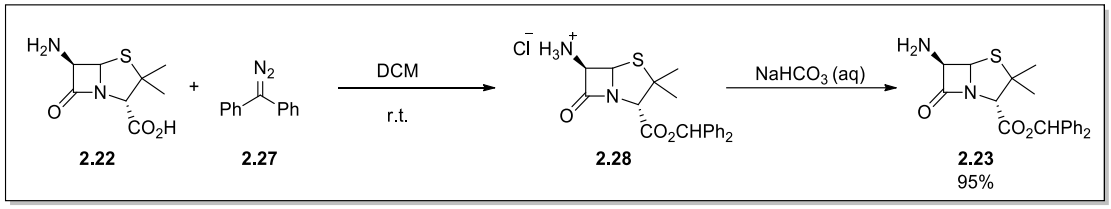
Figure 2. 12 New 6-APA derived thiourea organocatalysts

The first step in the synthesis of both thioureas was the protection of the 6-APA carboxylic acid with diphenyldiazomethane (**2.27**). For the synthesis of diphenyldiazomethane (**2.27**), a literature procedure was used,[195] where benzophenone hydrazone **2.26** was treated with HgO in a sealed round bottom flask, affording **2.27** in quantitative yield (Scheme 2.7).



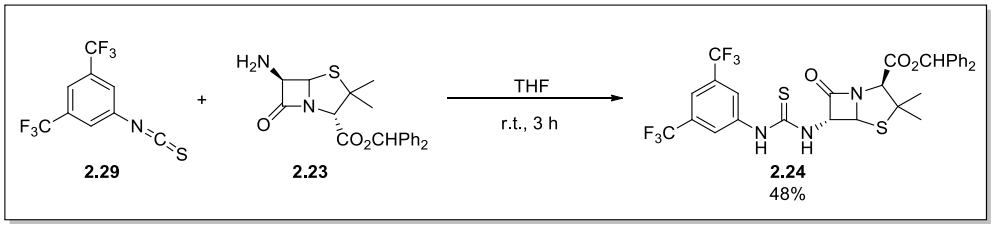
Scheme 2. 7 Synthesis of diphenyldiazomethane **2.27**.

Then, the 6-APA carboxylic group was protected by treatment with diphenyldiazomethane in dichloromethane at room temperature. The hydrochloride form of the 6-APA benzhydryl ester (**2.28**) is obtained, and simply by treating **2.28** with a $NaHCO_3$ saturated aqueous solution, compound **2.23** is isolated in high yield (95%, Scheme 2.8).



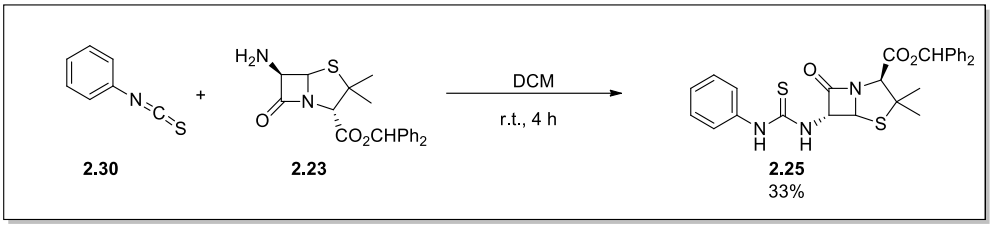
Scheme 2. 8 Synthesis of 6-APA benzhydryl ester **2.23**.

The final step of thiourea **2.24** synthesis, is the reaction of **2.23** with 3,5-bis(trifluoromethyl)phenyl isothiocyanate **2.29**. In a first approach the solvent used was dichloromethane and the reaction mixture was left stirring for 23 hours at room temperature. The reaction afforded the target thiourea **2.24** however, the isolation wasn't possible because of the presence of numerous degradation products that couldn't be removed. Then, it was explored a procedure based on Takemoto's work in the synthesis of bifunctional thiourea.[196] By reducing the reaction time to 3 hours and changing the solvent to tetrahydrofuran, thiourea **2.24** could be obtained in moderate yield (48%, Scheme 2.9).



Scheme 2. 9 Synthesis of thiourea **2.24**.

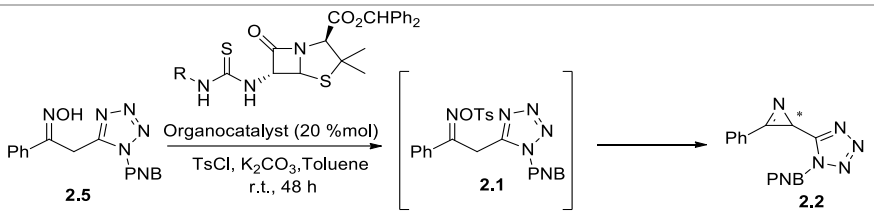
For the synthesis of thiourea **2.25** two approaches were also studied. In the first approach the phenyl isothiocyanate **2.30** was added to a solution of **2.23** in dichloromethane and left to stir for 24 hours. Thiourea **2.25** presented the same isolation difficulties than **2.24** and, for this reason, in a second approach the reaction time was decreased to 4 hours allowing the synthesis of the product in 33% yield (Scheme 2.10).

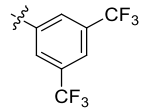


Scheme 2. 10 Second approach in the synthesis of thiourea **2.25**.

After successfully synthesizing both thioureas, they were both used as organocatalysts in our model reaction with β-ketoxime **2.5** and the results are presented in the following table 2.11.

Table 2. 11 Asymmetric Neber reaction of β-ketoxime **2.5** with 6-APA derived thioureas.

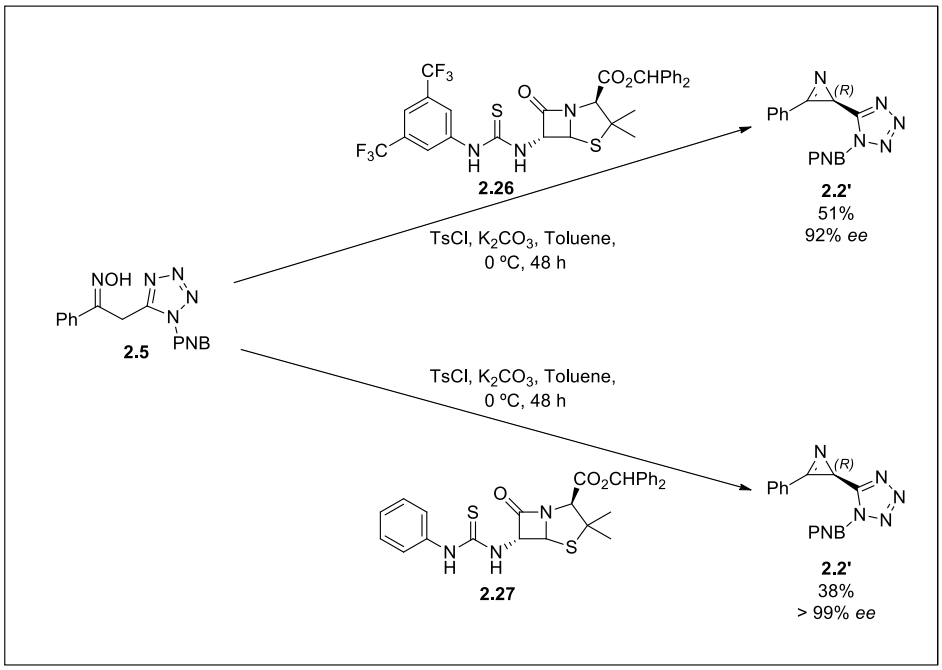


Organocatalyst	Entry	Yield (%)	ee (%)[a]
2.26 R= 	1	34	5 (*R*)
2.27 R= 	2	51	7 (*R*)

[a] Determined by chiral HPLC, mobile phase: water/acetonitrile (65:35), 1 mL/min.

The results obtained in our initial screening were once again disappointing for both catalysts in terms of enantiomeric excess. In an attempt to improve the results, the

catalysts were also evaluated in the reaction with β-ketoxime tosylate **2.1** but by performing the reaction with β-ketoxime tosylate **2.1** the results worsened, since none of the reactions afforded the desired 2*H*-azirine **2.2.** So, in a last attempt to improve the results, the reaction was performed at 0 °C, even knowing that the reaction efficiency usually doesn't proceed as well as at room temperature (Scheme 2.11).



Scheme 2. 11 6-APA derived thiourea-mediated asymmetric Neber reaction.

To our delight, the reactions performed at 0 °C showed excellent enantioselectivities although in moderate yields. Thus, the results with the new thiourea catalysts are the best results so far in the asymmetric Neber reaction for the synthesis of the *R* isomer, 92% *ee* for catalyst **2.24** and an outstanding >99% *ee* for catalyst **2.25**. These results also lead to the conclusion that these catalysts are not efficient at room temperature due to their lack of stability. The following chromatogram expansions presented in figure 2.13 correspond to the products from both thiourea-mediated reactions. As can be seen in chromatogram **A**, there is still some evidence of the presence of the *S* isomer whereas in chromatogram **B** there isn't any residual peak correspondent to the *S* isomer.

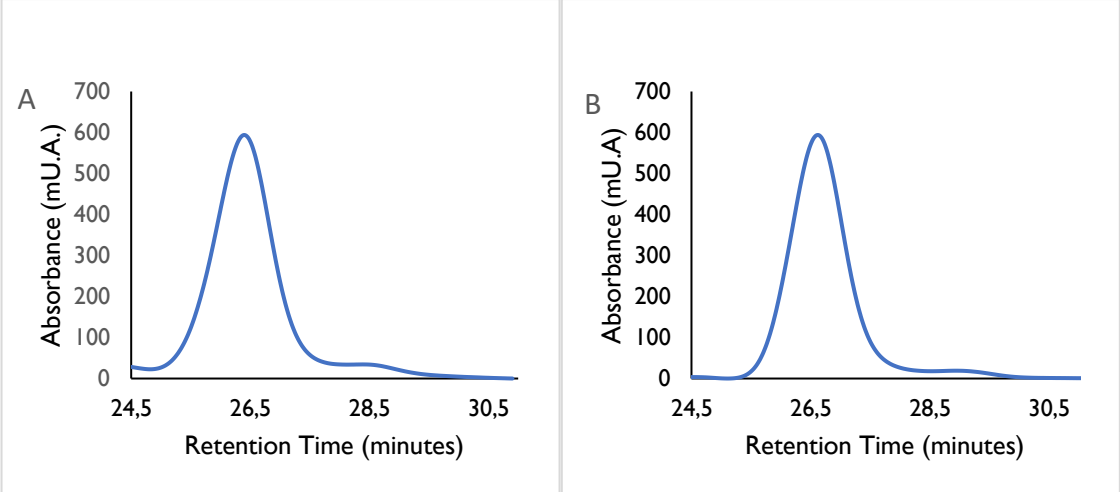


Figure 2. 13 (A) Chromatogram expansion of **2.24**-mediated asymmetric Neber reaction (B) Chromatogram expansion of **2.25**-mediated asymmetric Neber reaction.

As it has been proved that both thioureas (**2.24** and **2.25**) favored the formation of the *R* isomer, it can be concluded that they can be further applied in the asymmetric Neber reaction in the synthesis of (-)-(*E*)-dysidazirine, (-)-(*E*)-antazirine and (-)-(*Z*)-antazirine bioisosteres (Figure 2.14).

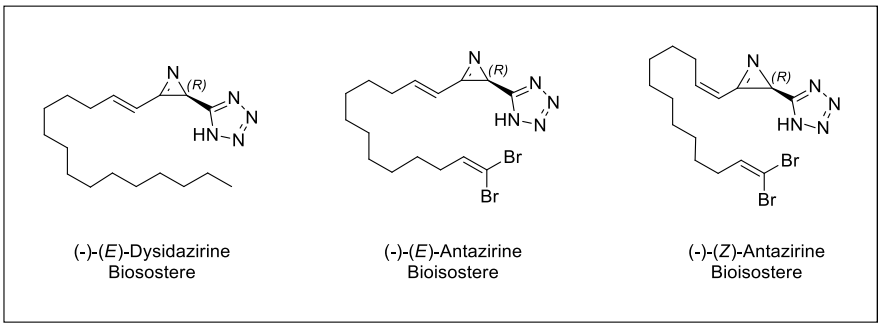


Figure 2. 14 (-)-(*E*)-dysidazirine, (-)-(*E*)-antazirine (-)-(*Z*)-antazirine bioisosteres.

These thiourea catalysts, just as Takemoto's thiourea catalysts, can also be considered as bifunctional thioureas since they allow a dual activation mechanism via triple hydrogen-bonding interaction, two as hydrogen-bond donor (marked blue) and another as hydrogen bond acceptor (marked pink) (Figure 2.15).

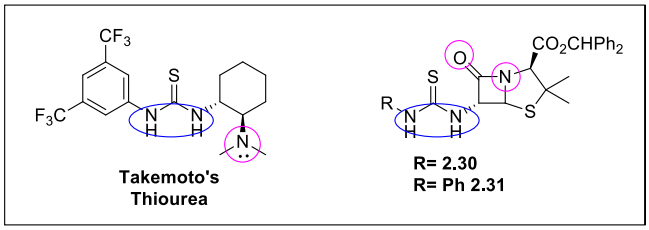
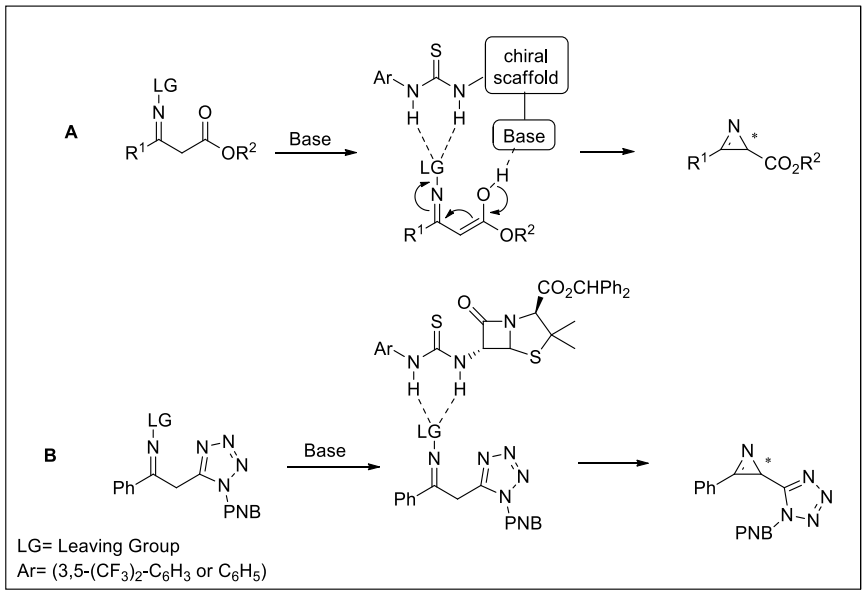


Figure 2. 15 Bifunctional thioureas.

In the dual activation mechanism proposed by Takemoto *et al.*, the thiourea protons of the catalyst lead to the formation of a stable complex between the catalyst and the substrate. Meanwhile the chiral base removes a proton of the enolic form of the carboxylic group which will lead to the formation of the *2H*-azirine. The asymmetric Neber reaction substrates of this work are however different than the ones used in Takemoto's work (Scheme 2.12 A). Thus, the reaction mechanism proposed for bifunctional thioureas can't be applied to our reaction, since there aren't any carboxylic group moieties in the substrates. In the reaction mechanism for the 6-APA derived thiourea catalyst it can however be assumed that there can also occur the formation of a complex between the catalyst's N-H protons and the leaving group (Scheme 2.12 B). Furthermore, other possible interactions between the 6-APA moiety and the substrate may contribute to the enantioselective transformation of the β-ketoxime **2.5** into the 2*H*-azirine **2.2**. Further studies are required in order to gain an insight into the interaction involved in these enantioselective transformations.



Scheme 2. 12 (A) Dual activation mechanism proposed by Takemoto *et al.* (B) Possible complex formed between 6-APA derived thiourea and the 2-(tetrazol-5-yl)-2*H*-azirine.

Chapter 3

Conclusion and Further Perspectives

"But life still goes on

I can't get used to living without, living without

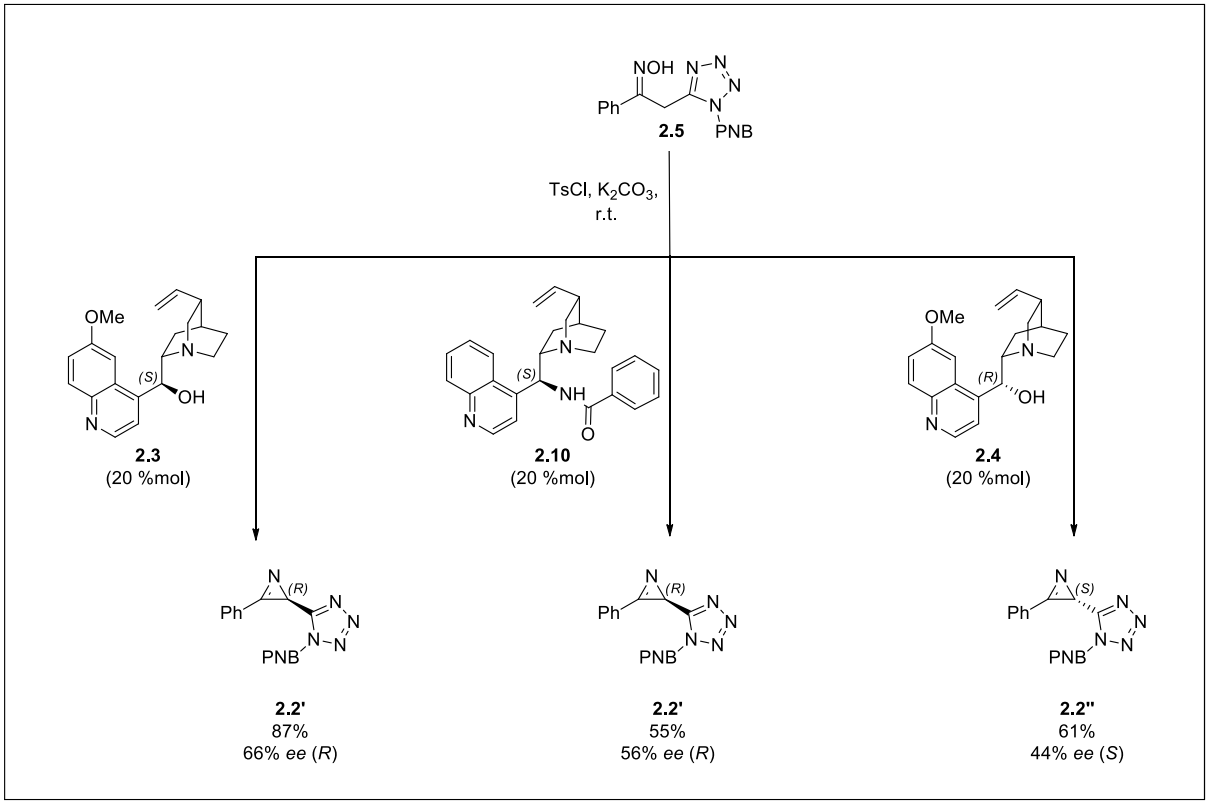
Living without you by my side"

I want to break free, Queen

3.1 Conclusion

This project's goal consisted in the development of the asymmetric Neber reaction for the synthesis of 2-(tetrazol-5-yl)-2*H*-azirines, bioisosteres of the biologically active and naturally occurring 2*H*-azirines.

To find the ideal catalyst for the asymmetric Neber approach to 2-(tetrazol-5-yl)-2*H*-azirines, a range of cinchona alkaloid catalysts were evaluated by reacting 2-(1-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone oxime (**2.5**) with tosyl chloride and base used as model reaction. Within the studied catalysts, quinidine (**2.3**) and *epi*-cinchonidine derivative **2.10** were the best catalysts to obtain the *R* isomer of 2-(tetrazol-5-yl)-2*H*-azirine **2.2** in yields of 87% and 55% and *ee* of 66% and 55% respectively. Regarding the synthesis of the *S* isomer the best results obtained until now were using quinine **2.4**, which afforded the target 2*H*-azirine **2.2** in 61% yield and 44% enantiomeric excess (Scheme 3.1).



Scheme 3. 1 Best results obtained with the studied cinchona alkaloids.

Results obtained with the *epi*-cinchonidine derivatives bearing triazolyl moieties also showed the importance of the presence of hydrogen bond donors in the catalyst structure since the catalytic activity of these catalysts dropped drastically (Figure 3.1).

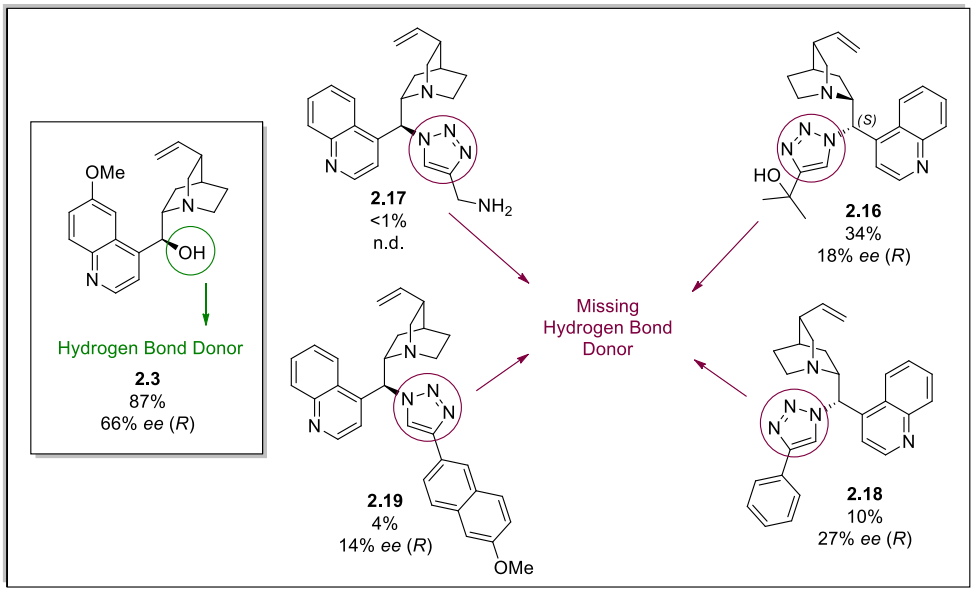
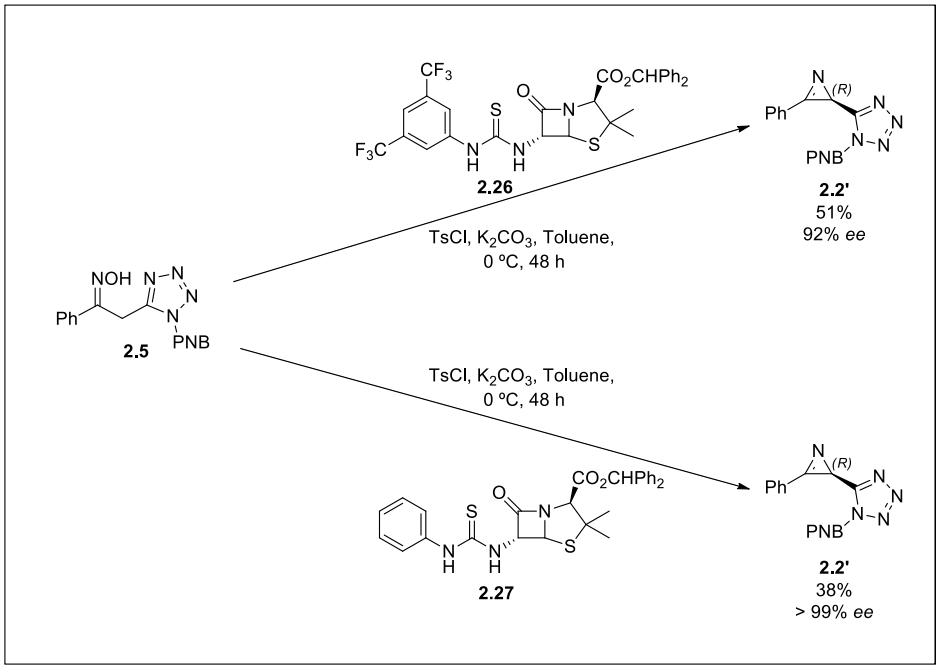


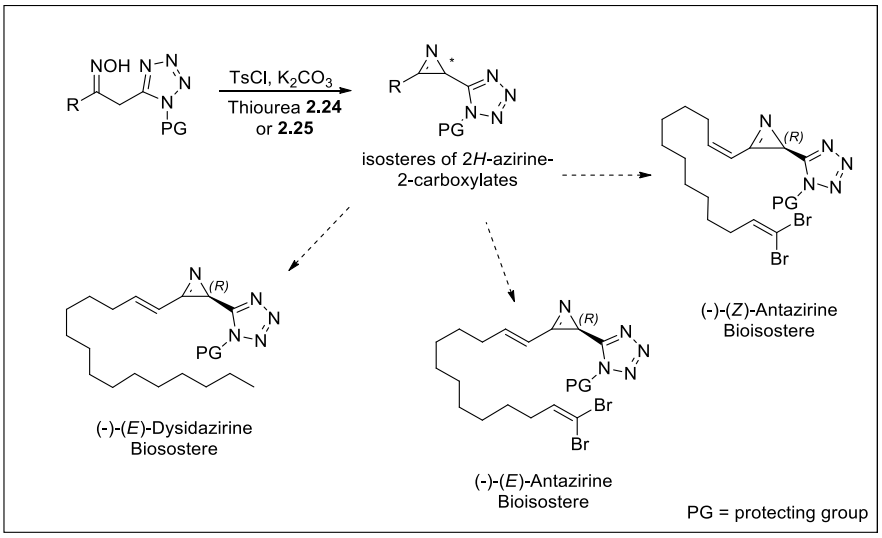
Figure 3. 1 9-Amino-(9-deoxi)-*epi*-cinchonidine derivatives bearing triazolyl moieties catalysts.

The synthesis of both new 6-APA derived thioureas **2.24** and **2.25** was successful (Scheme 3.2), and the preliminary results of the asymmetric Neber reaction of the 2-(tetrazol-5-yl)-2*H*-azirines showed the great potential of these compounds as organocatalysts, since they led to extraordinary enantiomeric excesses of 92% for thiourea **2.24** and over 99% for **2.25**. Thus, the ideal catalysts to optimize the asymmetric Neber reaction conditions in the synthesis of the *R* isomer of the 2-(tetrazol-5-yl)-2*H*-azirines were found.



Scheme 3. 2 Thioureas-mediated asymmetric Neber reactions.

Since both thioureas, **2.24** and **2.25**, favor the formation of the *R* isomer, they can be further applied in the asymmetric Neber reaction leading to the synthesis of (-)-(*E*)-dysidazirine, (-)-(*E*)-antazirine and (-)-(*Z*)-antazirine bioisosteres (Scheme 3.3).



Scheme 3. 3 (-)-(*E*)-dysidazirine, (-)-(*E*)-antazirine (-)-(*Z*)-antazirine bioisosteres.

3.2 Future Perspectives

As previously mentioned, the ultimate goal of this project is the application of the asymmetric Neber approach to the synthesis of bioisosteres of the biologically active and naturally occurring 2*H*-azirines-2-carboxylates, namely azirinomycin, (-)-(*E*)-dysidazirine, (-)-(*Z*)-dysidazirine, (+)-(*Z*)-antazirine, (+)-(*E*)-antazirine derivatives and (-)-(*E*)-Antazirine derivatives (Figure 3.2).

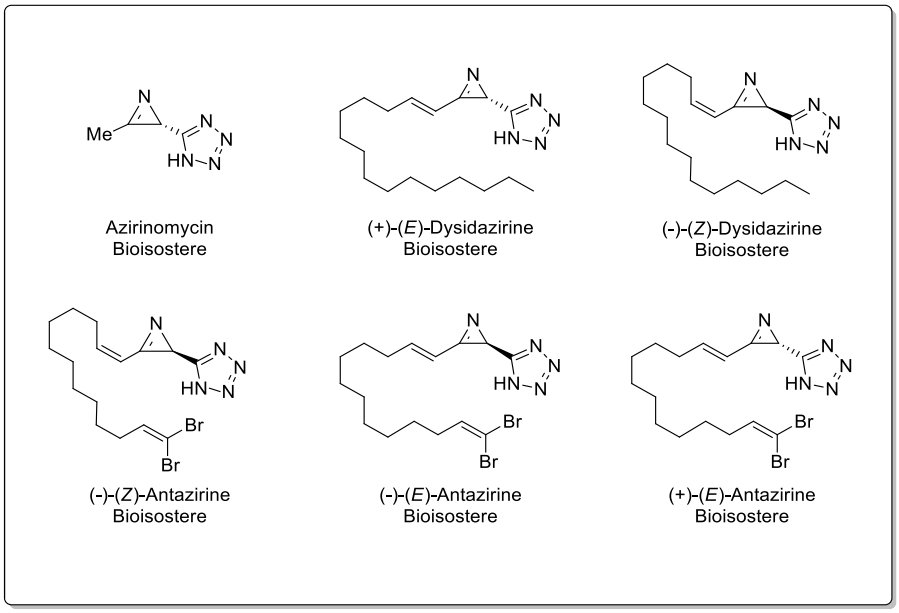
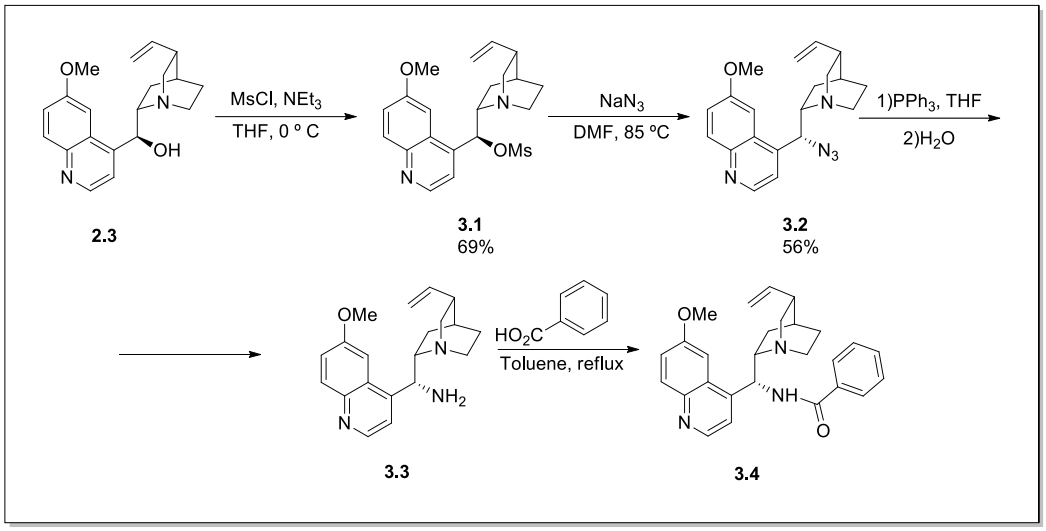


Figure 3. 2 Bioisosteres of the naturally occurring 2*H*-azirines-2-carboxylates.

The optimal reaction conditions of this synthetic methodology to obtain the *R* isomer were already reached. The next step will be to find the best reaction conditions with new catalysts to obtain the *S* isomer. In order to achieve a synthetic approach towards the preparation of 9-amino-(9-deoxi)-*epi*-quinidine derivatives, inspired by the best result obtained with *epi*-cinchonidine derivative **2.10**, was already started (Scheme 3.4).



Scheme 3. 4 Synthetic route for 9-amino-(9-deoxi)-*epi*-quinidine derivative **3.4**.

One of the targets is the synthesis of compound **3.4** outlined in scheme 3.4, but we also intend to explore the reactivity of compound **3.3** and create new catalysts for further application in the asymmetric Neber reaction in the synthesis of the *S* isomer of 2-(tetrazol-5-yl)-2*H*-azirines. The presence of the primary amine in **3.3** allows further derivatization and several functional groups can be attached. One interesting proposal would be an *epi*-quinidine derived thiourea with a 6-APA moiety (Figure 3.3).

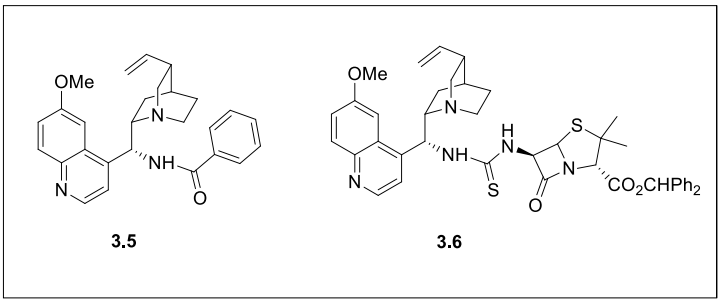


Figure 3. 3 Proposed e*pi*-quinidine derivatives for further application in the asymmetric Neber reaction.

In a moment in time when antibiotic resistance has only been growing stronger as one of the main world health concerns, the synthesis optimization of 2-(tetrazol-5-yl)-2*H*-azirines is of great interest not only in the academical sense but also as a possible solution for this problem since it allows the further generation of a new class of drugs to fight this epidemic.

Chapter 4

Experimental Section

"A thousand times I've tempted fate

A thousand times I've played this game

A thousand times that I have said today, today, today"

Up in the Air, Thirty Seconds To Mars

4.1 Laboratorial Equipment

Melting Points

Melting points were determined with a Falc Melting Point heated plate microscope, with the use of open capillaries. Raw results.

Infrared Spectroscopy

Infrared spectra were obtained with an Agilent Technologies Cary 630 FTIR spectrometer through the Attenuated Total Reflectance method (ATR).

Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectra were obtained with Brucker Avance III spectrometers, operating at 400 MHz (^{1}H NMR, ^{19}F NMR) and 100 MHz (^{13}C NMR). Deuterated chloroform and dimethyl sulfoxide were the solvents used. Chemical shift values are presented in parts per million (ppm), relative to tetramethyl silane (TMS), and the coupling constants (*J*) are expressed in Hz.

Chromatography

For the analysis of chemical reaction's evolution thin layer chromatography plaques with aluminium support (60 F254) from Merck were used. Most compounds were purified using column chromatography, using Merck's, Macherey-Nagel's or Fluka's silica gel (0.040-0.063 mm).

High Performance Liquid Chromatography

The enantiomeric excess determinations were performed by High Performance Liquid Chromatography (HPLC) in an Agilent Technologies instrument, equipped with a system with a Diode Array (model G1315D from Agilent, USA). The chromatographic separation of all compounds was performed in a CHIRALPAK IB (150x4.6 mm, 5 μm) chiral column.

Pressure Tube

The preparation of %-substituted tetrazole was carried out in an Ace pressure tube, Bushing type, B (volume ~ 35 mL)

4.2 Solvents and Reagents

All the solvents and reagents not mentioned below were obtained from Aldrich, Merck or Fluka, and were used directly, without additional purification.

Acetonitrile

This solvent was distilled after being refluxed during 3 hours in the presence of phosphorus pentoxide.

Dichloromethane and Chloroform

Distilled and stored on 4Å molecular sieves, after being refluxed for 3 hours with calcium chloride.

Ethanol and Methanol

Distilled from sodium alkoxide and stored on 3 Å molecular sieves, after being refluxed for 2 hours with magnesium (5 g/L) with iodine scraps (0.5 g/L).

Ethyl Acetate

Distilled, after being refluxed for 3 hours with potassium carbonate.

Diethyl Ether and Toluene

Distilled and stored on 4 Å molecular sieves, after being refluxed with sodium scraps, using benzophenone as an indicator.

Tosyl Chloride

An Erlenmeyer with hexane (200 mL) and tosyl chloride (30 g) was heated to 100 °C. The mixture was left stirring for approximately 20 minutes until it started to boil. Then, the solvent was decanted into another Erlenmeyer and was allowed to cool and rest. The formed precipitate was filtered off and left to dry. After being dry, the tosyl chloride crystals were stored for further use.

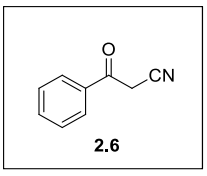
Organocatalysts

From the studied catalysts, quinidine and quinine were obtained from Sigma, the Cinchonidine derivatives were obtained in a collaboration work with the Organic Synthesis Group from Evora University leaded by Professor Anthony J. Burke[197] and the thiazolidine derived thioureas were synthesized in a previous work in our laboratory. [198]

4.3 Experimental Procedures

4.3.1 Synthesis of 3-Phenyl-2-(1-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)-2*H*-azirine (2.2)

Synthesis of 3-oxo-3-phenyl-propionitrile (2.6)



2.6

Nitrile **2.6** was prepared following the reported procedure[199] with a slight modification. To a solution of acetonitrile (38.30 mmol, 2 mL) in THF (36 mL) stirred at room temperature was added dropwise a solution of KO*t*-Amyl (55.80 mmol, 12 mL, 25% w/w in toluene), followed by dropwise addition of ethyl benzoate (153.20 mmol, 12 mL). After stirring for 24 h at room temperature, the reaction mixture was diluted with 0.25 M HCl (120 mL) and ethyl acetate (120 mL). The layers were separated, and the organic layer was washed sequentially with H_2O (2 × 50 mL) and brine (2 × 50 mL), the organic phase was dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated off,

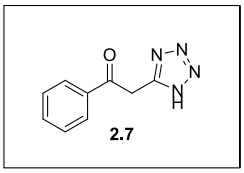
and the crude product was purified by flash chromatography [ethyl acetate/hexane (1:4), (1:3) and (1:2)] affording the product as white solid crystals (4.99 g, 90%).

m.p. = 81 °C (from literature)[199]

^{1}H NMR ($CDCl_3$): δ 7.92-7.91 (m, 2H), 7.68-7.64 (m, 1H), 7.54-7.51 (m, 2H), 4.12 (s, 2H).

^{13}C NMR ($CDCl_3$): δ 187.2, 134.8, 134.3, 129.2, 128.5, 113.9, 29.4.

Preparation of 1-Phenyl-2(1*H*-tetrazol-5-yl)ethanone (2.7)



2.7

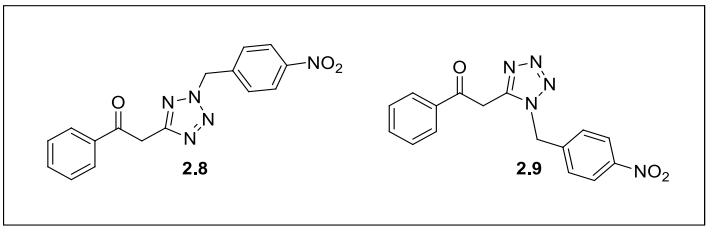
Tetrazole **2.7** was prepared using a general procedure described in the literature.[200] To a pressure tube was added the nitrile **2.6** (5.17 mmol, 0,075 g), sodium azide (5.69 mmol, 0.37 g), zinc bromide (5.17 mmol, 1.16 g), water (12 mL) and *i*-PrOH (1.5 mL). The sealed tube was then submerged in an oil bath at 140 °C and left stirring for 24 hours. After cooling the tube to room temperature HCl (3 N, 8 mL) and ethyl acetate (25 mL) were added. The mixture was stirred until all solids were dissolved. After, the organic layer was isolated, and the aqueous layer was extracted with ethyl acetate (2 × 25 mL). The combined organic layers were evaporated off under reduced pressure in a rotary evaporator. Then, 50 mL of 0.25 N NaOH was added, and the mixture was stirred for approximately 30 min, until the original precipitate was dissolved, and a suspension of zinc hydroxide was formed. The suspension was filtered through Celite, and the Celite pad was washed with 5 mL of 1 N NaOH. To the filtrate was added 10 mL of 3 N HCl with vigorous stirring, causing the tetrazole to precipitate. The tetrazole was filtered and dried. The desired product was obtained as a light-yellow solid (0.47 g, 48%).

m.p 178−180 °C (from literature)[200]

^{1}H NMR (DMSO-d_6): δ 8.10 (d, *J* = 7.6 Hz, 2H), 7.71−7.73 (m, 1H), 7.60 (pseudo t, *J* = 7.6 Hz, 2H), 4.96 (s, 2H).

^{13}C NMR (DMSO-d_6): δ 193.9, 150.8, 135.4, 134.0,128.9, 128.4, 34.0.

Synthesis of 2-(2-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-1-phenylethanone (2.8) and 2-(1-(4-Nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone (2.9)



The *p*-nitrobenzyl protected tetrazoles **2.8** and **2.9** were prepared following a reported procedure.[200] In a round-bottom flask the 5-substituted-1*H*-tetrazole **2.7** (12.25 mmol, 2.30 g) was slurried in THF (14 mL), and triethylamine (12.25 mmol, 1.57 mL) was added. The temperature of the solution was increased to 40 °C, and *p*-nitrobenzyl bromide (12.25 mmol, 2.65 g) in THF (7 mL) was added slowly. The solution was stirred at 40 °C for 3 hours, whereupon the mixture was left cooling and the triethylammonium bromide precipitate was filtered and washed with cold THF. The solvent was evaporated under reduced pressure, affording the crude product as a mixture of the 1,5- and 2,5-disubstituted isomers, which were separated by flash chromatography [ethyl acetate/hexane (1:2) and (1:1)]. Compound **2.8** was obtained as a light yellow solid (2.38 g, 60%) and **2.9** was obtained as a light yellow solid (1.27 g, 32%).

2-(2-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-1-phenylethanone (2.8):

m.p.=112-114 °C (from literature)[200]

^{1}H NMR (DMSO-d_6) δ 8.27 (d, *J* = 8.4 Hz, 2H), 8.05 (d, *J* = 7.6 Hz, 2H), 7.68−7.71 (m, 1H), 7.55−7.61 (m, 4H), 6.15 (s, 2H), 4.83 (s, 2H).

^{13}C NMR (DMSO-d_6) δ 194.6, 161.3, 147.5, 135.6, 133.8, 129.8, 128.8 ,124.0, 54.9, 36.6.

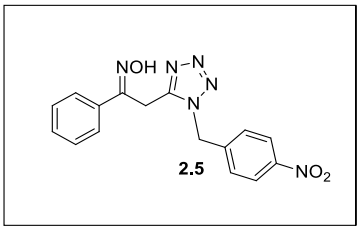
2-(1-(4-Nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone (2.9):

m.p. = 192-194 °C (from literature)[200]

^{1}H NMR (DMSO-d_6) δ 8.23 (d, *J* = 8.8 Hz, 2H), 8.03 (d, *J* = 7.2 Hz, 2H), 7.71−7.73 (m, 1H), 7.54−7.61 (m, 4H), 5.83 (s, 2H), 5.13 (s, 2H).

^{13}C NMR (DMSO-d_6) δ 193.5, 151.2, 147.3, 141.9, 135.3, 134.1, 129.4, 128.8, 128.5, 123.7, 49.1, 33.7.

Preparation of 2-(1-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone oxime (2.5)



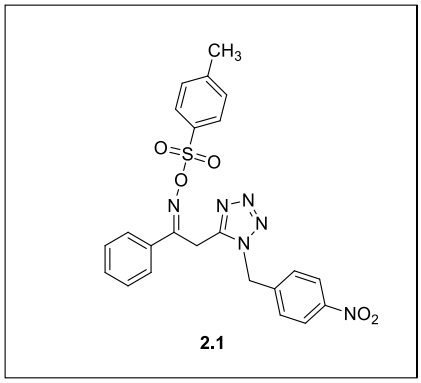
The β-ketoxime **2.5** was prepared following a reported procedure[200] with a slight modification. The protected tetrazole **2.9** (4.1 mmol, 1.32 g) was dissolved in a mixture of ethanol/pyridine (1:1) (7 mL), and hydroxylamine hydrochloride (12.30 mmol, 0.85 g) was added. The reaction mixture was refluxed for 3 h then, after cooling, the solvent was evaporated under reduced pressure. The crude substrate was dissolved in cold water (20 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered, and the solvent was evaporated off, affording the corresponding oxime, which was recrystallized in diethyl ether. The wanted product **2.5** was obtained as a light brown solid (0.86 g, 62%).

m.p.= 172−174 °C (from literature)[200]

^{1}H NMR (DMSO-d_6) δ 11.76 (bs, 1H, OH), 8.25-8.23 (d, *J* = 8.4 Hz, 2H), 7.66-7.64 (m, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.39-7.37 (m, 3H), 5.91(s, 2H), 4.43 (s, 2H).

^{13}C NMR (DMSO-d_6) δ 152.6, 150.4, 147.3, 141.8, 134.9, 129.1, 129.0, 128.4, 126.0, 123.8, 49.0, 19.9.

Synthesis of 2-(1-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone *O*-tosyl oxime (2.1)



2.1

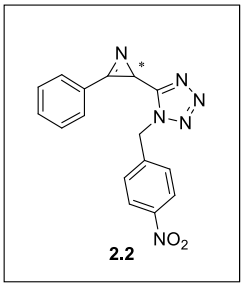
The β-ketoxime tosylate **2.1** was prepared based on a reported procedure in literature with a slight modification.[200] To a solution of β-ketoxime **2.5** (0.15 mmol) in THF (5 mL) was added tosyl chloride (0.18 mmol, 0.031 g) and potassium carbonate (0.30 mmol, 0.041 g). After stirring for 24 h at room temperature, the excess of potassium carbonate was filtered and washed with THF. The solvent was evaporated under reduced pressure, affording the crude product which was purified by flash chromatography [ethyl acetate/hexane (1:1)]. The desired product **2.1** was obtained as a white solid (0.020 g, 27%).

m.p.= 141−143 °C (from literature)[200]

^{1}H NMR ($CDCl_3$) δ 8.14 (d, *J* = 8.2, 2H), 7.80 (d, *J* = 7.8 Hz, 2H), 7.56 (d, *J* = 7.6 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.30-7.37 (m, 5H), 5.74 (s, 2H), 4.35 (s, 2H), 2.45 (s, 3H).

^{13}C NMR ($CDCl_3$) δ 158.9, 150.1, 148.2, 146.2, 139.7, 131.4, 130.8, 130.0, 129.0, 128.9, 128.3, 124.4, 50.1, 22.3, 21.8.

Preparation of 3-Phenyl-2-(1-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)-2*H*-azirine (2.2)



2.2

2*H*-azirine **2.2** was obtained via asymmetric Neber reaction by starting from β-ketoxime **2.5** or β-ketoxime tosylate **2.1** by following methods described in literature[200], which will be presented next:

Synthesis of 2*H*-azirine **2.2** starting from β-ketoxime **2.5**:

To a solution of β-ketoxime **2.5** (0.15 mmol, 0.05 g), K_2CO_3 (1.50 mmol, 0.207 g), tosyl chloride (0.17 mmol, 0.03 g) in toluene (4 mL) under a nitrogen atmosphere, was added the organocatalyst (0.03 mmol) in toluene (1 mL). The mixture was stirred for 48 h. The solvent was evaporated under reduced pressure, and the crude reaction was dissolved in ethyl acetate (20 mL) and washed with water (3 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered, and the solvent was evaporated under vacuum. The crude product was purified by flash chromatography [ethyl acetate/hexane (1:1)].

Synthesis of 2*H*-azirine **2.2** starting from β-ketoxime tosylate **2.1**:

To a solution of β-ketoxime tosylate **2.1** (0.15 mmol, 0.075 g), K_2CO_3 (1.5 mmol, 0.207 g), in toluene (4 mL) under a nitrogen atmosphere, was added the organocatalyst (0.03 mmol) in toluene (1 mL). The mixture was stirred for 48 h. The solvent was evaporated under reduced pressure, and the crude reaction was dissolved in ethyl acetate (20 mL) and washed with water (3 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered, and the solvent was evaporated under vacuum. The crude product was purified by flash chromatography [ethyl acetate/hexane (1:1)].

In both procedures the 2*H*-azirine **2.2** was obtained as a white solid.

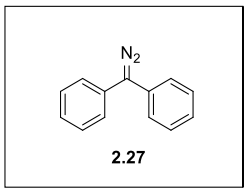
m.p.= 130−132 °C (from literature)[200]

^{1}H NMR ($CDCl_3$) δ 8.09 (d, *J* = 8.0 Hz, 2H), 7.75-7.76 (m, 2H), 7.61-7.65 (m, 1H), 7.50-7.52 (t, *J* = 8.0 Hz, 2H), 7.26-7.30 (m, 3H), 5.72 (s, 2H), 3.40 (s, 1H).

^{13}C NMR ($CDCl_3$) δ 162.5 153.9, 148.0, 140.4, 134.6, 130.4, 129.5, 128.3, 124.3, 121.9, 50.3, 22.7.

4.3.2 Organocatalysts Synthesis

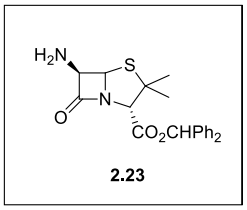
Synthesis of diphenyldiazomethane 2.27



2.27

Diphenyldiazomethane **2.27** was prepared following a known procedure.[195] In this procedure all the weighings were made in the laboratory fume hood and a protective gas mask were used. In a sealed tube with HgO (20.00 mmol, 4.38 g) and petroleum ether (30 mL), benzophenone hydrazone (20.00 mmol, 3.97 g) was added. The tube was closed and was wrapped in a humid cloth. The reaction mixture was left stirring at room temperature for 6 hours whereupon the reaction mixture was filtered through celite to remove the mercury and the benzophenone azine which was washed with more petroleum ether. The solvent of was evaporated under reduced pressure and the product was used without further purification. The product was obtained as a violet oil (3.88 g, quantitative yield).

Preparation of (2*S*,6*R*)-benzhydryl 6-β-aminopenicillanate



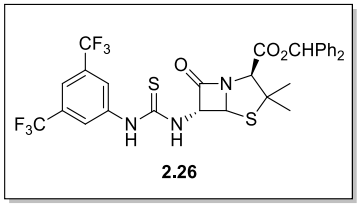
2.23

(2*S*,6*R*)-benzhydryl 6-β-aminopenicillanate **2.23** was prepared following a known method.[201] A solution of diphenyldiazomethane (9.25 mmol, 1.79 g) in dichloromethane (28 mL) was added dropwise, with a dropping funnel, to a suspension of 6-β-aminopenicilanic acid in methanol (9.25 mmol, 2.00 g, 10 mL). The reaction mixture was left stirring for 44 hours at room temperature. After the first 24 hours, another equivalent of the diphenyldiazomethane solution was added. At the end of reaction time the excess of 6-APA was filtered off, and the solvent was evaporated under reduced pressure. The crude product was then dissolved in ethyl ether and the mixture was acidified with 1 M HCl solution in an ice bath. The formed precipitate was filtered and dried. The obtained solid is the hydrochloride form of the desired product and to obtain the desired product is necessary to carry out the neutralization of the hydrochloride form. Thus, the solid was then transferred into an extraction funnel to which dichloromethane (2 x 25 mL) and a saturated $NaHCO_3$ (2 x 25 mL) solution were added. The layers were separated, and the organic layer was dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated off and the desired product was obtained as a yellow oil (3.18 g, 95%).

^{1}H NMR ($CDCl_3$) δ 7.35-7.30 (m, 10H), 6.94 (s, 1H), 5.51 (d, *J* = 4.0 Hz, 1H), 5.28 (s, 1H), 4.55 (d, *J* = 4.0 Hz, 1H), 4.50 (s, 1H), 1.80 (bs, 2H), 1.62 (s, 3H), 1.27 (s, 3H).

^{13}C NMR ($CDCl_3$) δ 177.8, 167.2, 139.2, 139.1, 128.6, 128.6, 128.4, 128.2, 127.7, 127.0, 78.3, 70.1, 70.0, 64.2, 32.0, 26.7.

Synthesis of (2*S*,6*R*)-benzhydryl 6-β-(3-(3,5-bis(trifluoromethyl)phenyl) thioureido)-aminopenicillanate (2.26)



2.26

Thiourea **2.26** was prepared based on a reported procedure in literature with some modifications.[196] To a solution of benzhydryl 6-β-aminopenicillanate (1.94 mmol, 0.74 g), under inert atmosphere in dry THF (3.5 mL) was added dropwise 3,5-bis(trifluoromethyl)phenyl isothiocyanate (1.94 mmol, 0.35 mL). After stirring for 3 h, the solvent was evaporated off and the crude product was purified by column chromatography on silica gel [ethyl acetate/hexane (1:2)] and recrystallized with diethyl ether/hexane. The product was obtained as a pink solid (2.64 g, 48%).

m.p. = 77.0-78.0 °C

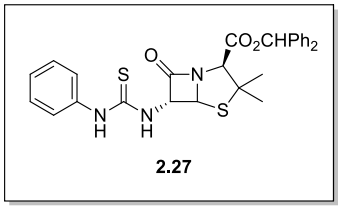
IR 682, 697, 1128, 1251, 1175, 1276, 1491, 1735, 2968, 2933, 3032, 3066, 3271 and 3291 cm^{-1}.

^{1}H NMR ($CDCl_3$) δ 7.93 (s, 1H), 7.84 (s, 2H), 7.35-7.31 (m, 13H), 6.95 (s, 1H), 5.18 (dd, *J* = 9.0 Hz and *J* =3.2 Hz, 1H), 4.26 (d, *J*= 3.6 Hz, 1H), 3.91 (d, *J*= 11.6 Hz, 1H), 1.64 (s, 3H), 1.00 (s, 3H).

^{13}C NMR ($CDCl_3$) δ 182.8, 170.6, 167.3, 139.1, 138.9, 133.9, 132.4 (q, *J* = 34.0 Hz, 2C), 128.8, 128.9, 128.6, 128.5, 128.2, 127.9, 127.7, 126.9, 126.8, 126.6, 124.1, 122.9 (m, 1C), 121.4, 78.7, 73.3, 66.5, 65.5, 65.1, 60.6, 60.0, 26.3, 26.1.

^{19}F NMR ($CDCl_3$) δ -62.8 (s, 6F).

Synthesis of (2*S*,6*R*)-benzhydryl 6-β-(3-(3phenyl) thioureido)-aminopenicillanate (2.27)



2.27

The thiourea **2.27** was prepared based on a reported procedure in literature with some modifications.[196] Under inert atmosphere, to a solution of benzhydryl 6-β-aminopenicillanate (1.94 mmol, 0.74 g) in dry dichloromethane (3.5 ml) was added dropwise phenyl isothiocyanate (1.94 mmol, 0.35 ml). After stirring for 4 h, the solvent was evaporated off and the crude product was purified by column chromatography on silica gel [ethyl acetate/hexane (1:4)] and recrystallized with diethyl ether/hexane. The product was obtained as a white solid (0.33 g, 33%).

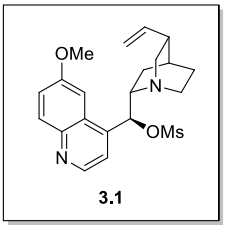
m.p. = 120.5-121.0 °C

IR 692, 742, 1182, 1251, 1492, 1735, 2963, 3033, 3063, 3292 and 3320 cm^{-1}.

^1H NMR ($CDCl_3$) δ 7.37-7.32 (m, 15H), 6.95 (s, 1H), 5.19-5.16 (dd, *J* = 8.8 Hz and *J* =3.2 Hz, 1H), 4.20 (d, *J* = 3.2 Hz, 1H), 3.94 (d, *J*= 11.6 Hz, 1H), 1.64 (s, 3H), 1.01 (s, 3H).

^{13}C NMR ($CDCl_3$) δ 184.6, 171.5, 168.0, 139.2, 139.0, 129.3, 129.2, 129.2, 128.7, 128.6, 128.6, 128.6, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.0, 126.7, 78.5, 73.2, 65.7, 65.0, 60.57, 59.9, 26.4, 26.1.

Synthesis of (8*R*,9*S*)-9-*O*-mesylquinidine (3.1)



3.1

(8*R*,9*S*)-9-*O*-Mesylquinidine was prepared following a known method with some slight modifications.[202] Quinidine (3.1 mmol, 1 g) was dissolved in 12.5 mL of anhydrous THF, to which was added triethylamine (9.6 mmol, 1.33 mL). The reaction mixture was cooled to 0 °C in an ice bath and methanesulfonyl chloride (6.2 mmol, 0.48 mL) was added dropwise. After the addition was completed, the mixture was stirred for 2 h at room

temperature. Then, 10 mL of a saturated solution of sodium bicarbonate was added to the crude mixture and extracted with CH_2Cl_2 (2 x 20 mL). The organic phase was dried with anhydrous Na_2SO4, filtered and the solvent was evaporated off under reduced pressure in a rotary evaporator. The crude product was purified by silica gel column chromatography [initially eluted with AcOEt followed by a mixture of CH_2Cl_2/MeOH (90:10)], affording the desired product as a pale-yellow solid (0.86 g, 69%).

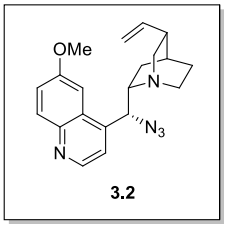
m.p. = 130.0-131.0 °C

IR 849, 877, 1166, 1223, 1353, 1618, 2861 and 2939 cm^{-1}.

^1H NMR ($CDCl_3$) δ 8.81 (d, *J* = 4.4 Hz, 1H), 8.01 (d, *J* = 9.2 Hz, 1H), 7.46 (bs, 1H) 7.42 (dd, *J* = 9.2 Hz and *J* = 2.8 Hz, 1H), 7.35 (bs, 1H), 6.08-5.99 (m, 1H), 5.15-5.09 (m, 2H), 3.97 (s, 3H), 3.33 (bs, 1H), 2.90 (d, *J* = 8.8, 2H) 2.32-2.25 (m, 1H), 2.00-1.94 (m, 1H), 1.86 (bs, 1H), 1.68 (bs, 1H), 1.59-1.54 (m, 2H).

^{13}C NMR ($CDCl_3$) δ 158.4, 147.5, 144.9, 139.9, 132.2, 126.5, 122.2, 115.2, 59.9, 55.7, 49.9, 48.9, 39.7, 39.1, 27.7, 26.3.

Synthesis of (8*R*,9*R*)-9-azido(9-deoxi)-*epi*-quinidine (3.2)



3.2

(8*R*,9*R*)-9-Azido(9-deoxi)-*epi*-quinidine was prepared following a known method.[203] Compound **3.1** (2.13 mmol, 0.86 g) was dissolved in 20 mL of anhydrous DMF at room temperature and 2 equivalents of NaN_3 (0.28 g, 4.26 mmol) were added. The mixture was stirred for 24 h at 80-85°C. After the 24 hours, the reaction solvent was evaporated off. Then, 15 mL of water was added to the crude mixture and extracted with dichloromethane (3 x 20 mL), dried with anhydrous Na_2SO4 and filtered. The solvent was concentrated on a rotary evaporator and the crude product purified by silica gel column

chromatography [CH_2Cl_2/MeOH (90:10)]. The desired product was obtained as a thick orange oil (56%).

IR 827, 849, 1028, 1224, 1507, 1619, 1671, 2095, 2867 and 2934 cm^{-1}.

^1H NMR ($CDCl_3$) δ 8.79 (d, *J* = 4.8 Hz, 1H), 8.06 (d, *J* = 9.2 Hz, 1H), 7.45 (bs, 1H), 7.43 (dd, *J* = 9.0 Hz and 2.6 Hz, 1H), 7.37 (d, *J* = 4.4 Hz, 1H), 5.91-5.83 (m, 1H), 5.12-5.07 (m, 3H), 3.98 (s, 3H), 3.30-2.99 (m, 5H), 2.34-2.28 (m, 1H), 2.09 (bs, 1H), 1.65 (bs, 1H), 1.59-1.52 (m, 2H), 1.59-1.52 (m, 2H), 1.16-1.10 (m, 1H), 0.98-0.94 (m, 1H).

^{13}C NMR ($CDCl_3$) δ 162.5, 158.2, 147.5, 145.0, 141.0, 140.4, 132.1, 127.6, 122.1, 120.7, 114.8, 101.4, 59.9, 56.6, 49.4, 47.4, 39.2, 36.5, 31.4, 27.2, 26.4, 25.2.

4.3.3 HPLC method

The enantiomeric excesses were obtained resorting on HPLC using a chiral column, CHIRALPAK IB (150x4.6 mm, 5 μm) from Daicel Corporation. Before the study sample injections, a control test was carried out by testing a standard sample of a racemic mixture of 2(tetrazol-5-yl)-2*H*-azirine (**2.2**), with already known values from previous work, in order to confirm that the HPLC instrument was working on the desired standards in which the separation of the two enantiomers was succeeded. The HPLC method was the following one:

Eluent	water/acetonitrile (35/65)
Flow Rate	1 mL/min
Temperature	25 °C
t1	≈ 26.5 min
t2	≈ 28.5 min
Analytical Injection	50 μL
Absorption Wavelength	250 nm
Sample Concentration	2 mg/mL

The enantiomeric excesses were calculated using the following formula:

$$ee = \frac{|\text{Area of } R \text{ isomer} - \text{Area } S \text{ isomer}|}{\text{Sum of the area of both isomers}} \times 100$$

Formula 4.1 Enantiomeric excess determination formula

The areas are obtained by integration of the signals and in figure 4.1 are demonstrated examples of typical chromatograms obtained in the HPLC studies. Where **A** is a sample of a racemic mixture of 2*H*-azirine **2.2**, **B** is a sample of the quinidine-mediated Neber reaction product and **C** is a sample of the quinine-mediated Neber reaction product.

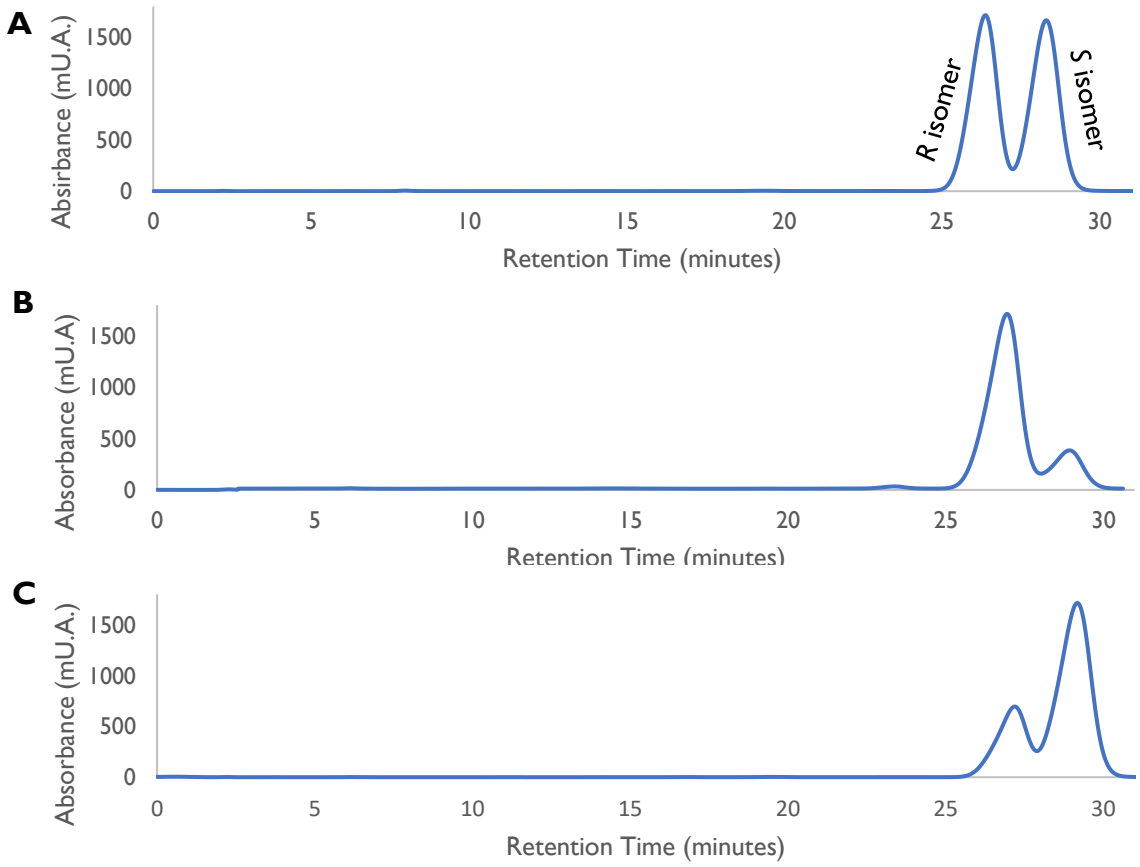


Figure 4. 1 Typical chromatograms of (A) Racemic Mixture (B) Quinidine-mediated Neber reaction (C) Quinine-mediated Neber reaction

Furthermore, in table 4.1 are presented the area values obtained for each enantiomer, under the different reaction conditions and the respective enantiomeric excess calculated with formula 4.1.

Table 4. 1 Area values obtained for each enantiomer under the organocatalyst-mediated Neber reaction and the respective enantiomeric excess.

Organocatalyst[a]	*R* isomer peak area	*S* isomer peak area	Enantiomeric Excess[b] (%)
2.3	82,237	16,933	66
2.4	28,1007	71,8796	44
2.10	37,1623	10,5499	56
2.11	44,0237	27,0095	24
2.12	57,428	37,4117	21
2.13	57,4112	39,5823	18
2.14	60,5630	37,2999	24
2.15	52,8955	43,7482	9
2.16	35,7002	24,9197	18
2.18	12,3427	7,0447	27
2.19	56,0771	42,3326	14
2.22	32,4064	29,1320	5
2.23	22,1118	16,9398	13
2.24	48,8956	1,9174	92
2.25	44,6806	0,0000	>99

[a]Reaction Conditions: β-ketoxime **2.5** (0.15 mmol, 0.05 g), K_2CO_3 (1.50 mmol, 0.207 g), tosyl chloride (0.17 mmol, 0.03 g), organocatalyst (0.03 mmol), 48 h at room temperature.
[b]Determined using formula 4.1.

Chapter 5

References

"Du musst auf dein Herz hör'n
Hör wie es schlägt, wie es fleht, wie es schreit
Hör wie es lebt, wie es lacht, wie es weint
Auch wenn du's willst, da misch' ich mich nicht ein
Wie du es machst, wird es schon richtig sein"

Herz, Sido

(1) Georges Wermuth, C.; Aldous, D.; Raboisson, P.; Rognan, D. *The Practice of Medicinal Chemistry*; 2015.

(2) Gualerzi, C. O.; Brandi, L. *Antibiotics: Targets,Targets, Mechanisms and Resistance*; 2013.

(3) Antibiotic resistance http://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance (accessed Jul 4, 2018).

(4) Pechère, J. C. *Clinical Infectious Diseases* **2001**, *33*, S170–S173.

(5) Cirz, R. T.; Chin, J. K.; Andes, D. R.; De Crécy-Lagard, V.; Craig, W. A.; Romesberg, F. E. *PLoS Biology* **2005**, *3*, 1024–1033.

(6) Gyles, C.; Boerlin, P. *Veterinary Pathology* **2014**, *51*, 328–340.

(7) Laxminarayan, R.; Duse, A.; Wattal, C.; Zaidi, A. K. M.; Wertheim, H. F. L.; Sumpradit, N.; Vlieghe, E.; Hara, G. L.; Gould, I. M.; Goossens, H.; et al. *The Lancet Infectious Diseases* **2013**, *13*, 1057–1098.

(8) Public Health Agency of Sweden. *Swedish Work on Containment of Antibiotic Resistance: Tools, Methods and Experiences.*; 2014.

(9) Girou, E.; Legrand, P.; Soing-Altrach, S.; Lemire, A.; Poulain, C.; Allaire, A.; Tkoub-Scheirlinck, L.; Chai, S. H. T.; Dupeyron, C.; Loche, C.-M. *Infection Control & Hospital Epidemiology* **2006**, *27*, 1128–1130.

(10) Rossiter, S. E.; Fletcher, M. H.; Wuest, W. M. *Chemical Reviews* **2017**, *117*, 12415–12474.

(11) Solinski, A. E.; Koval, A. B.; Brzozowski, R. S.; Morrison, K. R.; Fraboni, A. J.; Carson, C. E.; Eshraghi, A. R.; Zhou, G.; Quivey, R. G.; Voelz, V. A.; et al. *Journal of the American Chemical Society* **2017**, *139*, 7188–7191.

(12) Stowe, S. D.; Richards, J. J.; Tucker, A. T.; Thompson, R.; Melander, C.; Cavanagh, J. *Marine drugs* **2011**, *9*, 2010–2035.

(13) Palacios, F.; de Retana, A. M. O.; Martínez de Marigorta, E.; Manuel de los Santos, J. *Organic Preparations and Procedures International* **2002**, *34*, 219–269.

(14) Palacios, F.; de Retana, A. M. O.; de Marigorta, E. M.; de los Santos, J. M. *European Journal of Organic Chemistry* **2001**, *2001*, 2401–2414.

(15) Katritzky, A. R.; Wang, M.; Wilkerson, C. R.; Hongfang, Y. *Journal of Organic Chemistry* **2003**, *68*, 9105–9108.

(16) Banert, K.; Hagedorn, M.; Peisker, H. *Synlett* **2012**, *23*, 2943–2946.

(17) Neber, P. W.; Friedolsheim, A. V. *Justus Liebig's Annalen der Chemie* **1926**, *449*, 109–134.

(18) Khlebnikov, A. F.; Novikov, M. S. *Tetrahedron* **2013**, *69*, 3363–3401.

(19) Miller, T. W.; Tristram, E. W.; Wolf, F. J. *The Journal of Antibiotics* **1971**, *24*, 48–50.

(20) Molinski, T. F.; Ireland, C. M. *The Journal of Organic Chemistry* **1988**, *53*, 2103–2105.

(21) Salomon, C. E.; Williams, D. H.; Faulkner, D. J. *Journal of Natural Products* **1995**, *58*,

1463–1466.

(22) Keffer, J. L.; Plaza, A.; Bewley, C. A. *Organic Letters* **2009**, *11*, 1087–1090.

(23) Stapley, E. O.; Hendlin, D.; Jackson, M.; Miller, A. K.; Hernandez, S.; Mata, J. M. *The Journal of antibiotics* **1971**, *24*, 42–47.

(24) Brown, N.; Mannhold, R.; Kubinyi, H.; Folkers, G. *Bioisosteres in Medicinal Chemistry*; John Wiley & Sons: Weinheim, Germany, 2012.

(25) Silverman, R. B.; Holladay, M. W. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: Amsterdam, 2014.

(26) Lima, L. M.; Barreiro, E. J. *Current medicinal chemistry* **2005**, *12*, 23–49.

(27) Malik, M. A.; Wani, M. Y.; Al-Thabaiti, S. A.; Shiekh, R. A. *Journal of Inclusion Phenomena and Macrocyclic Chemistry* **2014**, *78*, 15–37.

(28) Kokate, A.; Li, X.; Jasti, B. *AAPS PharmSciTech* **2008**, *9*, 501–504.

(29) Roh, J.; Vávrová, K.; Hrabálek, A. *European Journal of Organic Chemistry* **2012**, *2012*, 6101–6118.

(30) Berkessel, A.; Gröger, H. *Asymmetric Organocatalysis : From Biomimetic Concepts to Applications in Asymmetric Synthesis*; Wiley-VCH, 2005.

(31) Brenna, E.; Fuganti, C.; Serra, S. *Tetrahedron: Asymmetry* **2003**, *14*, 1–42.

(32) Pellissier, H. *Recent Developments in Asymmetric Organocatalysis*; Royal Society of Chemistry: Cambridge, 2010.

(33) Mohr, J. T.; Krout, M. R.; Stoltz, B. M. *Nature* **2008**, *455*, 323–332.

(34) Agranat, I.; Caner, H.; Caldwell, J. *Nature Reviews Drug Discovery* **2002**, *1*, 753–768.

(35) Perdicchia, D.; Jørgensen, K. A. *The Journal of Organic Chemistry* **2007**, *72*, 3565–3568.

(36) Gaunt, M. J.; Johansson, C. C. C.; McNally, A.; Vo, N. T. *Drug Discovery Today* **2007**, *12*, 8–27.

(37) Trost, B. M. *Proceedings of the National Academy of Sciences of the United States of America* **2004**, *101*, 5348–5355.

(38) Dalko, P. I.; Moisan, L. *Angewandte Chemie International Edition* **2001**, *40*, 3726–3748.

(39) Ma, J.-A. *Chem. Soc. Rev.* **2006**, *35*, 630–636.

(40) Hong, L.; Sun, W.; Yang, D.; Li, G.; Wang, R. *Chemical Reviews* **2016**, *116*, 4006–4123.

(41) Knowles, W. S. *Angewandte Chemie International Edition* **2002**, *41*, 1998–2007.

(42) Noyori, R. *Angewandte Chemie International Edition* **2002**, *41*, 2008–2022.

(43) List, B.; Lerner, R. A.; Barbas III, C. F. *Journal of the American Chemical Society* **2000**, *122*, 2395–2396.

(44) Kateri A. Ahrendt; Christopher J. Borths, A.; MacMillan, D. W. C. *Journal of the American Chemical Society* **2000**, *122*, 4243–4244.

(45) MacMillan, D. W. C. *Nature* **2008**, *455*, 304–308.

(46) Siew, W. E.; Ates, C.; Merschaert, A.; Livingston, A. G. *Green Chemistry* **2013**, *15*, 663–674.

(47) Eder, U.; Sauer, G.; Wiechert, R. *Angewandte Chemie International Edition in English* **1971**, *10*, 496–497.

(48) Yang, D.; Yip, Y.-C.; Tang, M.-W.; Wong, M.-K.; Zheng, J.-H.; Cheung, K.-K. *Journal of the American Chemical Society* **1996**, *118*, 491–492.

(49) Denmark, S. E.; Wu, Z.; M. Crudden, C.; Matsuhashi, H. *The Journal of Organic Chemistry* **1997**, *62*, 8288–8289.

(50) Tu, Y.; Wang, Z.-X.; Shi, Y. *Journal of the American Chemical SocietyAmeri* **1996**, *118*, 9806–9807.

(51) Iyer, M. S.; Gigstad, K. M.; Namdev, N. D.; Lipton, M. *Journal of the American Chemical Society* **1996**, *118*, 4910–4911.

(52) Corey, E. J.; Grogan, M. J. *Organic Letters* **1999**, *1*, 157–160.

(53) Sigman, M. S.; Jacobsen, E. N. *Journal of the American Chemical Society* **1998**, *120*, 4901–4902.

(54) Lu, L.-Q.; Cao, Y.-J.; Liu, X.-P.; An, J.; Yao, C.-J.; Ming, Z.-H.; Xiao, W.-J. *Journal of the American Chemical Society* **2008**, *130*, 6946–6948.

(55) Marcelli, T.; Hiemstra, H. *Synthesis* **2010**, *2010*, 1229–1279.

(56) Akiyama, T.; Itoh, J.; Fuchibe, K. *Advanced Synthesis & Catalysis* **2006**, *348*, 999–1010.

(57) Akiyama, T. *Chemical Reviews* **2007**, *107*, 5744–5758.

(58) Shaw, S. A.; Aleman, P.; Vedejs, E. *Journal of the American Chemical Society* **2003**, *125*, 13368–13369.

(59) List, B. *Tetrahedron* **2002**, *58*, 5573–5590.

(60) Dalko, P. I. *Comprehensive Enantioselective Organocatalysis*; Wiley-VCH: Weinheim, Germany, 2013.

(61) Dalko, P. I. In *Enantioselective Organocatalysis*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany; pp 1–17.

(62) Yoon, T. P.; Jacobsen, E. N. *Science* **2003**, *299*, 1691–1693.

(63) Volla, C. M. R.; Atodiresei, I.; Rueping, M. *Chemical Reviews* **2014**, *114*, 2390–2431.

(64) Novacek, J.; Waser, M. *European Journal of Organic Chemistry* **2013**, *2013*, 637–648.

(65) Franzén, J. *Angewandte Chemie International Edition* **2013**, *52*, 12222–12222.

(66) Marson, C. M. *Chemical Society Reviews* **2012**, *41*, 7712.

(67) Chauhan, P.; Kaur, J.; Chimni, S. S. *Chemistry - An Asian Journal* **2013**, *8*, 328–346.

(68) Kumar, I.; Ramaraju, P.; Mir, N. A. *Org. Biomol. Chem.* **2013**, *11*, 709–716.

(69) Busacca, C. A.; Fandrick, D. R.; Song, J. J.; Senanayake, C. H. *Advanced Synthesis*

& *Catalysis.*; Wiley-VCH Verlag, 2001; Vol. 353.

(70) Hack, D.; Chauhan, P.; Deckers, K.; Hermann, G. N.; Mertens, L.; Raabe, G.; Enders, D. *Organic letters* **2014**, *16*, 5188–5191.

(71) Du, Z.; Shao, Z. *Chem. Soc. Rev.* **2013**, *42*, 1337–1378.

(72) Atodiresei, I.; Vila, C.; Rueping, M. *ACS Catalysis* **2015**, *5*, 1972–1985.

(73) Pellissier, H. *Tetrahedron* **2007**, *63*, 9267–9331.

(74) Alemán, J.; Cabrera, S. *Chem. Soc. Rev.* **2013**, *42*, 774–793.

(75) Brière, J.-F.; Oudeyer, S.; Dalla, V.; Levacher, V. *Chemical Society reviews* **2012**, *41*, 1696–1707.

(76) Holland, M. C.; Gilmour, R. *Angewandte Chemie International Edition* **2015**, *54*, 3862–3871.

(77) Buckley, B. R. *Annual Reports Section 'B' (Organic Chemistry)* **2013**, *109*, 189–206.

(78) Kunz, R. K.; MacMillan, D. W. C. *Journal of the American Chemical Society* **2005**, *127*, 3240–3241.

(79) Enders, D.; Balensiefer, T. *Accounts of Chemical Research* **2004**, *37*, 534–541.

(80) Seayad, J.; List, B. *Organic & Biomolecular Chemistry* **2005**, *3*, 719–724.

(81) Bugaut, X.; Glorius, F. *Chemical Society Reviews* **2012**, *41*, 3511–3522.

(82) Jousseaume, T.; Wurz, N. E.; Glorius, F. *Angewandte Chemie International Edition* **2011**, *50*, 1410–1414.

(83) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: Berlin, 1994.

(84) Knowles, R. R.; Jacobsen, E. N. *Proceedings of the National Academy of Sciences* **2010**, *107*, 20678–20685.

(85) Uraguchi, D.; Terada, M. *Journal of the American Chemical Society* **2004**, *126*, 5356–5357.

(86) Petrillo, T.; O'Donohoe, C. A.; Howe, N.; Malthouse, J. P. G. *Biochemistry* **2012**, *51*, 6164–6170.

(87) Schmidtchen, F. P.; Berger, M. *Chemical Reviews* **1997**, *97*, 1609–1646.

(88) Reisman, S. E.; Doyle, A. G.; Jacobsen, E. N. *Journal of the American Chemical Society* **2008**, *130*, 7198–7199.

(89) Raheem, I. T.; Thiara, P. S.; Peterson, E. A.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2007**, *129*, 13404–13405.

(90) Uyeda, C.; Jacobsen, E. N. *Journal of the American Chemical Society* **2008**, *130*, 9228–9229.

(91) Doyle, A. G.; Jacobsen, E. N. *Chemical Reviews* **2007**, *107*, 5713–5743.

(92) Hiemstra, H.; Wynberg, H. *Journal of the American Chemical Society* **1981**, *103*, 417–430.

(93) Palomo, C.; Oiarbide, M.; López, R. *Chem. Soc. Rev.* **2009**, *38*, 632–653.

(94) Staben, S. T.; Linghu, X.; Toste, F. D. *Journal of the American Chemical Society* **2006**, *128*, 12658–12659.

(95) Jacobsen, E. N.; MacMillan, D. W. C. *Proceedings of the National Academy of Sciences of the United States of America* **2010**, *107*, 20618–20619.

(96) Hashimoto, T.; Maruoka*, K. *Journal of the American Chemical Society* **2007**, *129*, 10054–10055.

(97) Starks, C. M. *Journal of the American Chemical Society* **1971**, *93*, 195–199.

(98) Selvi, S.; Nanthini; Sukanyaa. *International Journal of Scientific and Technolof Research* **2012**, *1*, 61–63.

(99) de Freitas Martins, E.; Pliego, J. R. *ACS Catalysis* **2013**, *3*, 613–616.

(100) Hashimoto, T.; Sakata, K.; Tamakuni, F.; Dutton, M. J.; Maruoka, K. *Nature chemistry* **2013**, *5*, 240–244.

(101) Hashimoto, T.; Maruoka, K. *Chemical Reviews* **2007**, *107*, 5656–5682.

(102) Maruoka, K. *Organic Process Research & Development* **2008**, *12*, 679–697.

(103) Ooi, T.; Maruoka, K. *Angewandte Chemie International Edition* **2007**, *46*, 4222–4266.

(104) Pellissier, H.; Lattanzi, A.; Dalpozzo, R. *Asymmetric Synthesis of Three-Membered Rings*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2017.

(105) Piskunova, I. .; Eremeev, A. .; Mishnev, A. .; Vosekalna, I. . *Tetrahedron* **1993**, *49*, 4671–4676.

(106) Verstappen, M. H.; Ariaans, G. J. A.; Zwanenburg, B. *J. Am. Chem. Soc.* **1996**, *118*, 8491–8492.

(107) Sakamoto, S.; Inokuma, T.; Takemoto, Y. *Organic Letters* **2011**, *13*, 6374–6377.

(108) Štrukil, V. *Beilstein Journal of Organic Chemistry* **2017**, *13*, 1828–1849.

(109) Mertschenk, B.; Beck, F.; Bauer, W. *Ullmann's Encyclopedia of Industrial Chemistry* **2011**, 1–15.

(110) Schroeder, D. C. *Chemical Reviews* **1955**, *55*, 181–228.

(111) Nishida, C. R.; Ortiz De Montellano, P. R. *Chemico-Biological Interactions* **2011**, *192*, 21–25.

(112) ASTWOOD, E. B. *Journal of the American Medical Association* **1943**, *122*, 78.

(113) Duan, L. P.; Xue, J.; Xu, L. L.; Zhang, H. B. *Molecules* **2010**, *15*, 6941–6947.

(114) RICHTER, C. P. *Journal of the American Medical Association* **1945**, *129*, 927.

(115) Pihko, P. M. *Hydrogen Bonding in Organic Synthesis*; Wiley-VCH: Weinheim, Germany, 2009.

(116) Wittkopp, A.; Schreiner, P. R. *Chemistry - A European Journal* **2003**, *9*, 407–414.

(117) Kotke, M.; Schreiner, P. R. *Synthesis* **2007**, *12*, 779–790.

(118) Shakeel, A. *Journal of Drug Design and Medicinal Chemistry* **2016**, *2*, 10–20.

(119) Curran, D. P.; Kuo, L. H. *Tetrahedron Letters* **1995**, *36*, 6647–6650.

(120) Connon, S. J. *Chemistry - A European Journal* **2006**, *12*, 5418–5427.

(121) Taylor, M. S.; Jacobsen, E. N. *Angewandte Chemie International Edition* **2006**, *45*, 1520–1543.

(122) Nord, M. *Sustainable Catalysis Without Metals or Other Endangered Elements Part 2*; The Royal Society of Chemistry: Cambridge, 2016.

(123) García Mancheño, O. *Angewandte Chemie International Edition* **2016**, *55*, 15955–15955.

(124) Okino, T.; Nakamura, S.; Furukawa, T.; Takemoto, Y. *Organic Letters* **2004**, *6*, 625–627.

(125) Tan, B.; Candeias, N. R.; Barbas, C. F. *Nature Chemistry* **2011**, *3*, 473–477.

(126) Song, C. E. *Cinchona Alkaloids in Synthesis and Catalysis : Ligands, Immobilization and Organocatalysis*; Wiley-VCH: Weinheim, Germany, 2009.

(127) Ishikawa, H.; Shiomi, S. *Organic & Biomolecular Chemistry* **2016**, *14*, 409–424.

(128) Brossi, A. *The Alkaloids Chemistry and Pharmacology. Volume 34*; Academic Press: San Diego, 1988.

(129) Collins, A. N.; Sheldrake, G. N.; Crosby, J. *Chirality in Industry*; Wiley: Chichester, 1995.

(130) Tille, A.; Pracejus, H. *Chemische Berichte* **1967**, *100*, 196–210.

(131) Morrison, J. D.; Mosher, H. S. *Asymmetric Organic Reactions*; American Chemical Society: Washington, 1986.

(132) Wynberg, H. Wiley-Blackwell, 2007; pp 87–129.

(133) Kacprzak, K.; Gawroński, J. *Synthesis* **2001**, *7*, 961–998.

(134) Kaufman, T. S.; Rúveda, E. A. *Angewandte Chemie International Edition* **2005**, *44*, 854–885.

(135) Xi, Y.; Shi, X. *Chemical Communications* **2013**, *49*, 8583–8585.

(136) Zheng, S.; Schienebeck, C. M.; Zhang, W.; Wang, H.-Y.; Tang, W. *Asian Journal of Organic Chemistry* **2014**, *3*, 366–376.

(137) Nielsen, M.; Worgull, D.; Zweifel, T.; Gschwend, B.; Bertelsen, S.; Jørgensen, K. A. *Chem. Commun.* **2011**, *47*, 632–649.

(138) Bhanushali, M.; Zhao, C.-G. *Synthesis* **2011**, *2011*, 1815–1830.

(139) Bredig, G.; Fiske, P. S. *Biochemische Zeitschrift* **1912**, No. 46, 7–13.

(140) Pracejus, H. *Justus Liebigs Annalen der Chemie* **1960**, *634*, 9–22.

(141) Pracejus, H. *Justus Liebigs Annalen der Chemie* **1960**, *634*, 23–29.

(142) O'Donnell, M. J. *Accounts of Chemical Research* **2004**, *37*, 506–517.

(143) Lygo, B.; Andrews, B. I. *Accounts of Chemical Research* **2004**, *37*, 518–525.

(144) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chemical Reviews* **1994**, *94*, 2483–2547.

(145) Xiang, B.; Belyk, K. M.; Reamer, R. A.; Yasuda, N. *Angewandte Chemie International Edition* **2014**, *53*, 8375–8378.

(146) Kacprzak, K.; Gawroński, J. *Synthesis* **2001**, No. 07, 961–998.

(147) Rabe, P. *Berichte der deutschen chemischen Gesellschaft* **1907**, *40*, 3655–3658.

(148) Rabe, P. *Berichte der deutschen chemischen Gesellschaft* **1908**, *41*, 62–70.

(149) Prelog, V.; Zalán, E. *Helvetica Chimica Acta* **1944**, *27*, 535–545.

(150) Prelog, V.; Zalán, E. *Helvetica Chimica Acta* **1944**, *27*, 545–547.

(151) Caner, H.; Ulrich Biedermann, P.; Agranat, I. *Chirality* **2003**, *15*, 637–645.

(152) Braje, W. M.; Wartchow, R.; Hoffmann, H. M. R. *Angewandte Chemie International Edition* **1999**, *38*, 2539–2543.

(153) France, S.; Guerin, D. J.; Miller, S. J.; Lectka, T. *Chemical Reviews* **2003**, *103*, 2985–3012.

(154) Boratyński, P. J.; Skarżewski, J. *The Journal of Organic Chemistry* **2013**, *78*, 4473–4482.

(155) Bürgi, T.; Baiker, A. *Journal of the American Chemical Society* **1998**, *120*, 12920–12926.

(156) Dijkstra, G. D. H.; Kellogg, R. M.; Wynberg, H.; Svendsen, J. S.; Marko, I.; Sharpless, K. B. *Journal of the American Chemical Society* **1989**, *111*, 8069–8076.

(157) Dijkstra, G. D. H.; Kellogg, R. M.; Wynberg, H. *The Journal of Organic Chemistry* **1990**, *55*, 6121–6131.

(158) Dijkstra, G. D. H.; Kellogg, R. M.; Wynberg, H. *Recueil des Travaux Chimiques des Pays-Bas* **2010**, *108*, 195–204.

(159) Olsen, R. A.; Borchardt, D.; Mink, L.; Agarwal, A.; Mueller, L. J.; Zaera, F. *Journal of the American Chemical Society* **2006**, *128*, 15594–15595.

(160) Prakash, G. K. S.; Wang, F.; Ni, C.; Shen, J.; Haiges, R.; Yudin, A. K.; Mathew, T.; Olah, G. A. *Journal of the American Chemical Society* **2011**, *133*, 9992–9995.

(161) Cheong, P. H.-Y.; Legault, C. Y.; Um, J. M.; Çelebi-Ölçüm, N.; Houk, K. N. *Chemical Reviews* **2011**, *111*, 5042–5137.

(162) Fredriksen, K. A.; Kristensen, T. E.; Hansen, T. *Beilstein journal of organic chemistry* **2012**, *8*, 1126–1133.

(163) Wynberg, H.; Staring, E. G. J. *Journal of the American Chemical Society* **1982**, *104*, 166–168.

(164) Brunner, H.; Bügler, J.; Nuber, B. *Tetrahedron: Asymmetry* **1995**, *6*, 1699–1702.

(165) Xie, J.-W.; Chen, W.; Li, R.; Zeng, M.; Du, W.; Yue, L.; Chen, Y.-C.; Wu, Y.; Zhu,

J.; Deng, J.-G. *Angewandte Chemie International Edition* **2007**, *46*, 389–392.

(166) Bartoli, G.; Bosco, M.; Carlone, A.; Pesciaioli, F.; Sambri, L.; Melchiorre, P. *Organic Letters* **2007**, *9*, 1403–1405.

(167) Russo, A.; De Fusco, C.; Lattanzi, A. *ChemCatChem* **2012**, *4*, 901–916.

(168) Cassani, C.; Martín-Rapún, R.; Arceo, E.; Bravo, F.; Melchiorre, P. *Nature Protocols* **2013**, *8*, 325–344.

(169) Liu, W.; Mei, D.; Wang, W.; Duan, W. *Tetrahedron Letters* **2013**, *54*, 3791–3793.

(170) Melchiorre, P. *Angewandte Chemie International Edition* **2012**, *51*, 9748–9770.

(171) Jew, S.; Lee, J.-H.; Jeong, B.-S.; Yoo, M.-S.; Kim, M.-J.; Lee, Y.-J.; Lee, J.; Choi, S.; Lee, K.; Lah, M. S.; et al. *Angewandte Chemie* **2005**, *117*, 1407–1409.

(172) Sánchez-Roselló, M.; Aceña, J. L.; Simón-Fuentes, A.; del Pozo, C. *Chem. Soc. Rev.* **2014**, *43*, 7430–7453.

(173) Su, Z.; Lee, H. W.; Kim, C. K. *European Journal of Organic Chemistry* **2013**, *2013*, 1706–1715.

(174) Grayson, M. N.; Houk, K. N. *Journal of the American Chemical Society* **2016**, *138*, 1170–1173.

(175) Liu, Y.; Wang, X.; Wang, X.; He, W. *Org. Biomol. Chem.* **2014**, *12*, 3163–3166.

(176) Castro-Osma, J. A.; Comerford, J. W.; Heath, S.; Jones, O.; Morcillo, M.; North, M. *RSC Advances* **2015**, *5*, 3678–3685.

(177) Dalko, P. I.; Moisan, L. *Angewandte Chemie International Edition* **2004**, *43*, 5138–5175.

(178) Reddy, B. V. S.; Reddy, S. M.; Swain, M. *RSC Adv.* **2013**, *3*, 930–936.

(179) Kacprzak, K.; Gierczyk, B. *Tetrahedron: Asymmetry* **2010**, *21*, 2740–2745.

(180) Madhusudhan Reddy, G.; Ko, C.-T.; Hsieh, K.-H.; Lee, C.-J.; Das, U.; Lin, W. *The Journal of Organic Chemistry* **2016**, *81*, 2420–2431.

(181) Lam, Y.; Grayson, M. N.; Holland, M. C.; Simon, A.; Houk, K. N. *Accounts of Chemical Research* **2016**, *49*, 750–762.

(182) Yang, Z.-T.; Yang, W.-L.; Chen, L.; Sun, H.; Deng, W.-P. *Advanced Synthesis & Catalysis* **2018**, *360*, 2049–2054.

(183) Kong, S.; Fan, W.; Wu, G.; Miao, Z. *Angewandte Chemie International Edition* **2012**, *51*, 8864–8867.

(184) Lee, H.-J.; Cho, C.-W. *The Journal of Organic Chemistry* **2013**, *78*, 3306–3312.

(185) Wang, D.; Liang, J.; Feng, J.; Wang, K.; Sun, Q.; Zhao, L.; Li, D.; Yan, W.; Wang, R. *Advanced Synthesis & Catalysis* **2013**, *355*, 548–558.

(186) Xie, H.; Song, A.; Zhang, X.; Chen, X.; Li, H.; Sheng, C.; Wang, W. *Chem. Commun.* **2013**, *49*, 928–930.

(187) Claraz, A.; Landelle, G.; Oudeyer, S.; Levacher, V. *European Journal of Organic Chemistry* **2013**, *2013*, 7693–7696.

(188) Nakamura, S.; Takahashi, S.; Nakane, D.; Masuda, H. *Organic Letters* **2015**, *17*, 106–109.

(189) Díaz-de-Villegas, M. D.; Gálvez, J. A.; Badorrey, R.; López-Ram-de-Víu, P. *Advanced Synthesis & Catalysis* **2014**, *356*, 3261–3288.

(190) Lam, Y.; Houk, K. N. *Journal of the American Chemical Society* **2015**, *137*, 2116–2127.

(191) Sheehan, J. C.; Henery-Logan, K. R. *Journal of the American Chemical Society* **1962**, *84*, 2983–2990.

(192) Nandi, A.; Pan, S.; Potumarthi, R.; Danquah, M. K.; Sarethy, I. P. *Journal of analytical methods in chemistry* **2014**, *2014*, 1–10.

(193) Emer, E.; Galletti, P.; Giacomini, D. *European Journal of Organic Chemistry* **2009**, *2009*, 3155–3160.

(194) P. Demko, Z.; Sharpless, K. B. *Journal of Organic Chemistry* **2001**, *66*, 7945–7950.

(195) Staudinger, H.; Anthes, E.; Pfenninger, F. *Berichte der deutschen chemischen Gesellschaft* **1916**, *49*, 1928–1941.

(196) Okino, T.; Hoashi, Y.; Takemoto, Y. *Journal of the American Chemical Society* **2003**, *125*, 12672–12673.

(197) Barrulas, P. Universidade de Évora, 2014.

(198) Félix, A. R. Universidade de Coimbra, 2014.

(199) Ji, Y.; Trenkle, W. C.; Vowles, J. V. *Organic Letters* **2006**, *8*, 1161–1163.

(200) Cardoso, A. L.; Gimeno, L.; Lemos, A.; Palacios, F.; Pinho e Melo, T. M. V. D. *The Journal of Organic Chemistry* **2013**, *78*, 6983–6991.

(201) Sheehan, J. C.; Commons, T. J. *The Journal of Organic Chemistry* **1978**, *43*, 2203–2208.

(202) Röper, S.; Franz, M. H.; Wartchow, R.; Hoffmann, H. M. R. *The Journal of Organic Chemistry* **2003**, *68*, 4944–4946.

(203) He, W.; Liu, P.; Le Zhang, B.; Li Sun, X.; Zhang, S. Y. *Applied Organometallic Chemistry* **2006**, *20*, 328–334.