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Synthesis, reactivity and applications of 3,5-dimethyl-4-nitroisoxazole derivatives

Tesi di laurea sperimentale

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Abstract

3,5-dimethyl-4-nitroisoxazole derivatives are useful synthetic intermediates as the isoxazole nucleus chemically behaves as an ester, but establish better-defined interactions with chiral catalysts and lability of its N-O aromatic bond can unveil other groups such as 1,3-dicarbonyl compounds or carboxylic acids.

In the present work, these features are employed in a 3,5-dimethyl-4-nitroisoxazole based synthesis of the γ-amino acid pregabalin, a medication for the treatment of epilepsy and neuropatic pain, in which this moiety is fundamental for the enantioselective formation of a chiral center by interaction with doubly-quaternized *cinchona* phase-transfer catalysts, whose ability of asymmetric induction will be investigated.

Influence of this group in *cinchona*-derivatives catalysed stereoselective addition and Darzens reaction of a mono-chlorinated 3,5-dimethyl-4-nitroisoxazole and benzaldehyde will also be investigated.

Acronyms

A

ACS	American Chemical Society.

C

CD	Cinchonidine, *cinchona* alkaloid.
CN	Cinchonine, *cinchona* alkaloid.
CNB	Cinchonidinium bromide, cinchonidine-based phase-transfer catalyst.
CPD	Cupreidine, quinidine derivative.
CSP	Chiral stationary phase, for chromatography.

D

DCM	Dichloromethane, solvent.
DMF	Dimethylformamide, solvent.

E

e.g.	Exempli gratia, for example.
ee	Percent enantiomeric excess, absolute difference between the percent mole fraction of each enantiomer.
et al.	Et alii, and others/co-workers.

G

GABA	γ-amino-butyric acid, inhibitory neurotransmitter.

H

H-bond	Hydrogen bond, secondary interaction.

HPLC	High performance liquid chromatography, analytical technique.

I

IPA	Isopropyl alcohol, solvent.

N

NMR	Nuclear magnetic resonance spectroscopy, spectroscopic method.

P

PTC	Phase-transfer catalysis/catalyst.

Q

QD	Quinidine, *cinchona* alkaloid.
QDB	Quinidinium bromide, quinidine-based phase-transfer catalyst.
QN	Quinine, *cinchona* alkaloid.

R

Rf	Retention factor, ratio of the distance travelled by a spot and the one travelled by the solvent front in planar chromatography.
rt	Room temperature.

S

SQ	Squaramide-based organocatalysts.

T

TBAB	Tetrabutylammonium bromide, achiral phase-transfer catalyst.

THF	Tetrahydrofuran, solvent.
TK	Takemoto’s catalyst, thiourea organocatalysts.
TLC	Thin layer chromatography.

U

UV	Ultraviolet, range of electromagnetic radiation.

V

vic	Vicinal, functional groups placed on adjacent carbons.

Notations

syn	Synclinal, dihedral angle between planes of vicinal functional groups less than 90° (Synperiplanar = 0°).
anti	Anticlinal, dihedral angle between planes of vicinal functional groups more than 90° (Antiperiplanar = 180°).
cis	Orientation of functional groups within a molecule with restricted rotation: groups on same side of the reference plane.
trans	Orientation of functional groups within a molecule with restricted rotation: groups on opposite sides of the reference plane.
E	Orientation of substituents with highest priority on the two ends of an alkene: substituents on same side of the bond plane.
Z	Orientation of substituents with highest priority on the two ends of an alkene: substituents on same side of the bond plane.
re face	Prochiral face with priority of substituents decreasing in clockwise order.
si face	Prochiral face with priority of substituents decreasing in counter-clockwise order.
R	Absolute configuration for a chiral center: lowest priority group away from viewer, priority of substituents decreasing in clockwise order.
S	Absolute configuration for a chiral center: lowest priority group away from viewer, priority of substituents decreasing in counter-clockwise order.

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Chapter 1

Introduction

Although carbon-carbon bond formation is an essential feature in every organic synthesis, still only few chemical transformations succeed in extending a carbon backbone. Among the reactions that accomplish this rather complex task, one may find pericyclic reactions, transition metal mediated coupling reactions and the Wittig reaction.

One of the most important and broad category of carbon-carbon bond forming reactions is the addition of a nucleophilic carbon to an electrophilic one. This category includes many prominent reactions, like aldol reaction, Michael addition and Henry reaction.

An important feature of these organic transformations is the possibility to introduce asymmetric induction using mild reaction condition and inexpensive organic catalysts.

1.1 - Organocatalysis

Organocatalysis is the acceleration of chemical reactions with a substoichiometric amount of an organic compound that does not contain a metal ion.[1] The interest in this field raised in the last few years as it combines some interesting features: organocatalysts are generally stable, non-toxic and environmentally friendly. Moreover, catalysts for asymmetric catalysis are generally available in enantiomerically pure form directly from nature (*e.g.*, aminoacids, carbohydrates, aminoalcohols) and are therefore quite inexpensive.[2]

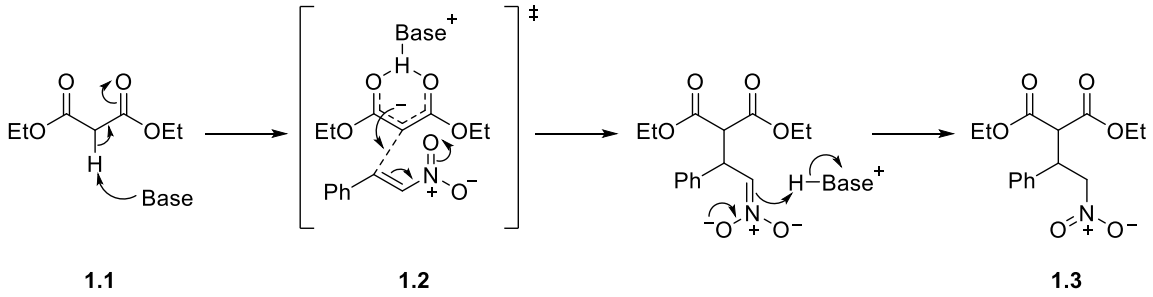
Indeed, the use of small organic molecules as catalysts has proven to be extraordinarily useful for asymmetric inductions. These molecular entities combine their functionalities with characteristic structural frameworks to catalyse an array of C-C and C-heteroatom bond forming reactions with high stereoselectivity on a broad range of substrates. In many cases, this strategy allowed unprecedented levels of control over the disposition of reactants in a given transition state, with benefits in term of both reaction rate and stereoselectivity.[3] Organocatalytic reactions proceed by either a much tighter or a much looser transition state than those mediated by chiral metal complexes. In the former case, organocatalysts act as covalently bonded reagents. In the latter case, organocatalysts induce a high level of enantioselectivity mainly through interactions as hydrogen bonding or ion pairing.[1]

1.2 - Bifunctional catalysis

Currently, bifunctional catalyst are one of the prominent classes in organocatalysis, increasing the strength and the directionality of the interactions.[4] One of the most attractive aspects of these catalysts is that they possess complementary functionalities capable of simultaneously activating two components of a chemical reaction through multiple interactions, typically weak ones.[5]

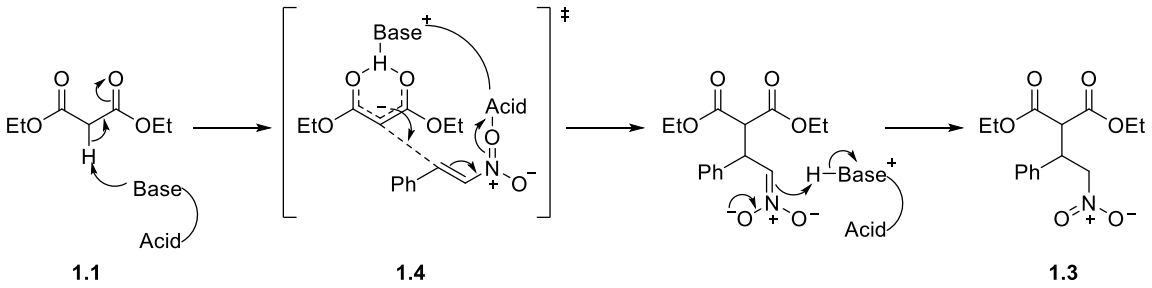
In the most common mode, bifunctional catalysts hold a site for the recognition of a nucleophile and one for the recognition of an electrophile.[6] Enantiopure agents like enzymes orient the reaction of a nucleophile and electrophile in highly stereoselective fashion. Bifunctional catalysts, in a similar way, activate simultaneously both the nucleophile and the electrophile by having a basic functional group connected with a chiral framework to an H-bond donor, allowing enantiocontrol and mild reaction conditions.

Scheme 1.1 shows a typical conjugate addition reaction, namely between diethyl malonate and (*E*)-nitrostyrene, using a simple base. The base deprotonates the acidic hydrogen on the malonate to produce enolate **1.1**, which can then react with nitrostyrene to form transition state **1.2**. During the reaction, a negative charge builds up on the nitro group of nitrostyrene, which then reestablish neutrality upon protonation.



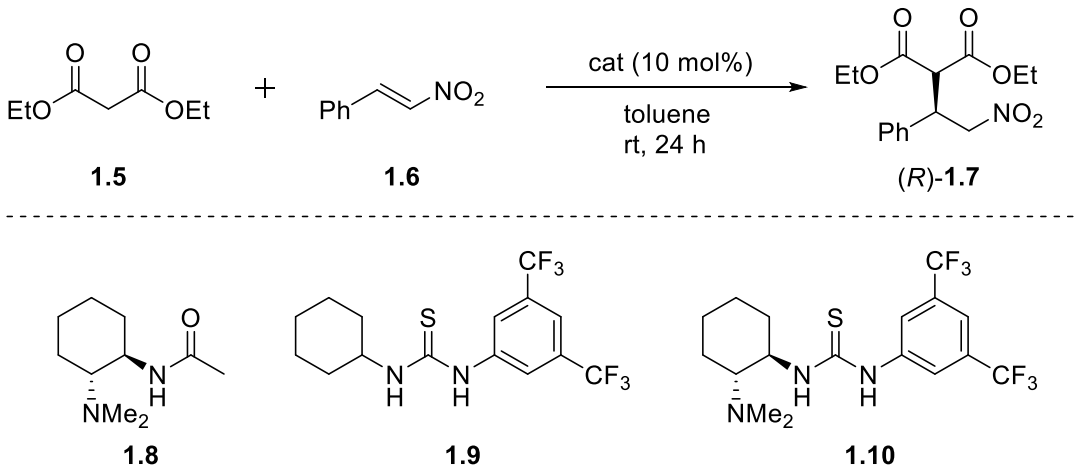
Scheme 1.1 - Base catalyzed diethyl malonate addition to (*E*)-nitrostyrene.

The advantage of using a bifunctional catalyst is shown in **Scheme 1.2**. The basic moiety of the bifunctional catalyst deprotonates the substrate same way as before, but in transition state **1.4** its acidic moiety stabilizes the negative charge on the nitro group, overall promoting the enolate attack to nitrostyrene. As long as a catalyst manages to hold the two components in close proximity to each other, its backbone can be modified to arrange the correct alignment of the two molecules, possibly resulting in faster reaction and, if chiral, higher enantioselectivity.



Scheme 1.2 - Diethyl malonate addition to (*E*)-nitrostyrene assisted by a bifunctional catalyst.

Research reported by Takemoto in 2003 showed the importance of both base and acid components and their effect on yield in a bifunctional reaction.[7] **Scheme 1.3** shows the same conjugate Michael addition between diethyl malonate **1.5** and (*E*)-nitrostyrene **1.6**. Et_3N and **1.8** are both bases capable of reaction catalysis, but give poor yields (17% and 14% respectively). **1.9** contains a thiourea group that acts as an H-bond donor and stabilizes the build-up of negative charge on the nitrostyrene through hydrogen bonding. When combined with Et_3N, yield improved (57%). An even better result was obtained using **1.10**, a bifunctional catalyst whose structure can be seen to be a combination of **1.8** and **1.9**, further increasing yield (86%). This shows how is not only important to have both acid and base moieties in the reaction, but that they should be part of the same molecule so that reactants can be held in closer area. Additionally, there was a marked improvement in enantioselectivity (93% *ee* over 35% *ee*) when **1.10** was use instead of **1.8**.



Scheme 1.3 - Diethyl malonate addition to (E)-nitrostyrene assisted by thiourea catalysts.

Bifunctional organocatalysis does not necessary include the exclusive establishment of weak interactions between catalyst and reactants. For example, mechanisms involving enamine or iminium ion catalysis can be considered bifunctional organocatalysis as well. This sort of catalysts have one amino group involved in the activation of the nucleophile (by means of either enamine or carbanion formation) and a second moiety that usually activates the electrophile (by means of hydrogen bonding).[8]

A noteworthy and historical example of this type of bifunctional catalysts is the cyclic aminoacid L-proline (**Figure 1.1**).[9] This molecule possesses a crucial combination of functional groups that in many cases grant high yield and selectivity. The amino group activates the nucleophile by either enamine or carbanion formation. An H-bond donor (carboxylic acid in the simpler case) is involved in the activation of the electrophile. In order to further improve reactivity, selectivity and range of substrates catalysed, many unnatural analogues have been developed by modifying the acidic moiety and therefore tuning the H-bond donating property.[10]

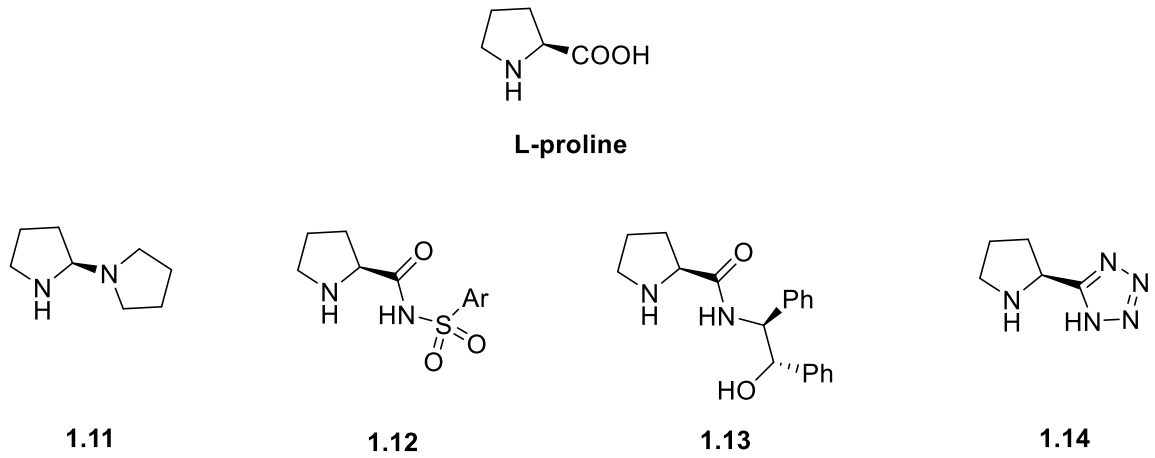


Figure 1.1 - L-proline and its derivatives.

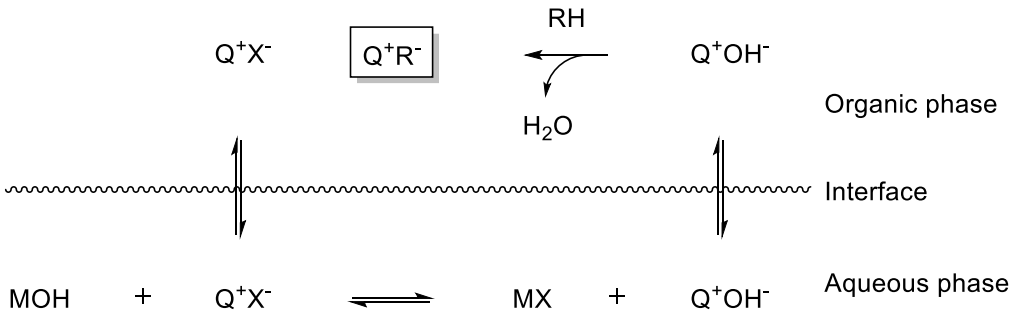
1.3 - Phase-transfer Catalysis (PTC)

In 1971, Starks introduced the term of "phase-transfer catalysis" to explain the critical role of tetraalkylammonium or phosphonium salt (Q^+X^-) in the reaction between two substrates located in different immiscible phases.[11] Since then, the chemical community has witnessed an exponential growth of phase-transfer catalysis as a practical methodology for organic synthesis. Advantages of this method lie in its simple experimental procedures,

mild reactions conditions, inexpensive and environmentally benign reagents and solvents employed, and the possibility of conducting large-scale preparations.[12]

The common reaction system in organic phase-transfer catalysis is a biphasic system made up by an organic phase containing an acidic methylene or methine compound (as latent nucleophile) and an electrophile, and an aqueous or solid phase containing an inorganic base such as alkaline metal (Na, K, Cs) hydroxide or carbonate. The key intermediate in this type of reaction is the onium carbanion species, mostly onium enolates or nitronates, which reacts with the electrophile in the organic phase to afford the product.

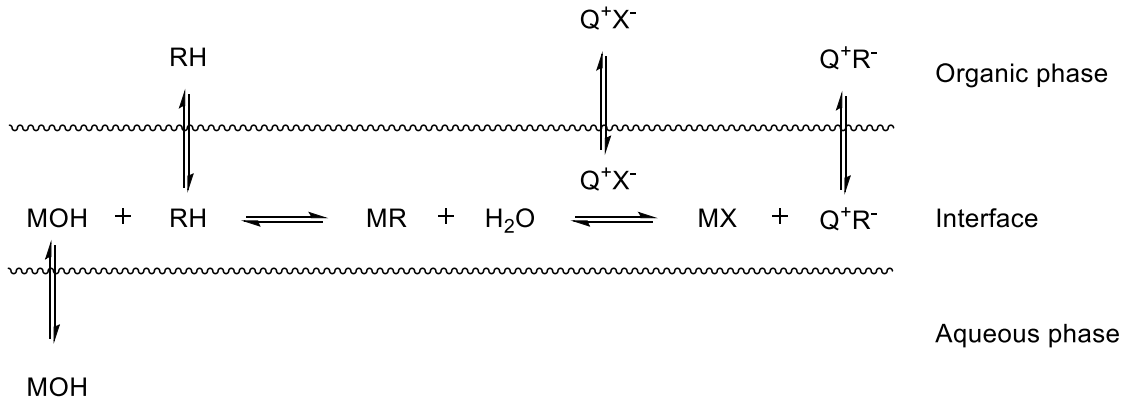
Despite the development of phase-transfer catalysis in organic synthesis, some mechanistic aspects of phase-transfer catalysis are still obscure, due mainly to the difficulty in investigating biphasic systems and the many complex parameters involved. Thus, the exact pathway for generating the reactive onium carbanion species remains the subject of controversy, typically between Starks extraction mechanism (**Scheme 1.4**) and the Makosza interfacial mechanism (**Scheme 1.5**).[13]



Scheme 1.4 - Starks extraction mechanism.

In Starks extraction mechanism (**Scheme 1.4**), the phase-transfer catalyst moves back and forth across the organic and aqueous phases. The onium salt equilibrates with the inorganic base (MOH) in the aqueous phase, and extracts hydroxide into the organic phase. The onium hydroxide then abstracts a hydrogen from the acidic organic compound to give the reactive intermediate Q^+R^-.

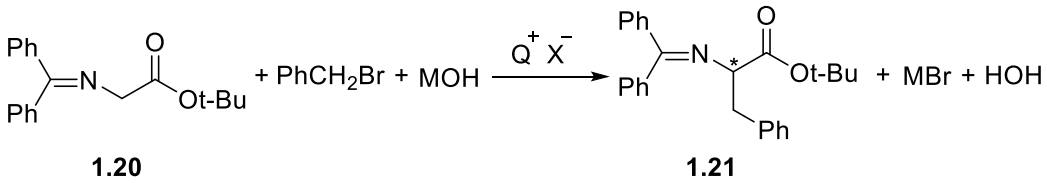
According to Makosza interfacial mechanism (**Scheme 1.5**), the formation of the metal carbanion occurs at the interface between the organic and aqueous phase in absence of phase-transfer catalyst, followed by extraction of the formed metal carbanion species from the interface into the organic phase by the action of phase-transfer catalyst.



Scheme 1.5 - Makosza interfacial mechanism.

Both transfer mechanisms are probably true depending on the nature of the onium cation, with the first more likely with small cations and the second with bigger ones. Thus, since catalysts involved in asymmetric organic PTC usually contain big, lipophilic chiral framework, the latter is the one that best describes the catalytic pathway of these phase-transfer catalysed reactions.[13]

Asymmetric benzylation of glycine Schiff base often serves as an example in PTC, and it is used to compare the activity of different catalysts (**Scheme 1.6**).[14]

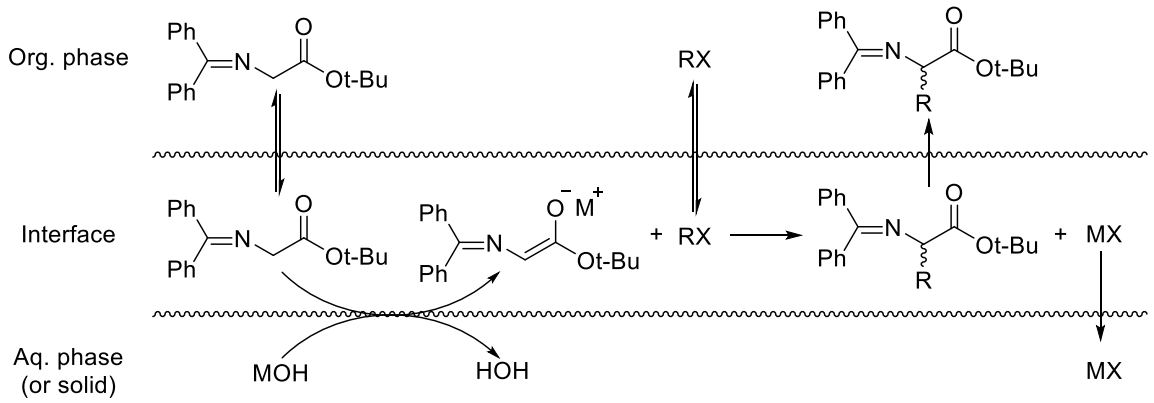


1.20 **1.21**

Scheme 1.6 - Asymmetric alkylation of glycine Schiff base.

Both reactants are in the organic phase, while the base stoichiometrically needed is in the aqueous phase (or even in a solid phase). The glycine Schiff base will distribute between the bulk and the interface. Here it will react with the base forming an intermediate (an enolate).

Two are now the pathways the reaction can take: in the first (**Scheme 1.7**), the alkyl halide from the bulk will diffuse to the interface as well in a relatively small quantity. It will react with the metal enolate affording the product, prone to move to the organic phase, and a salt, which will move out of the organic phase. This mechanism sees no enantioselection at all, so the product is a racemate.[13]



Scheme 1.7 - Mechanism for non-catalyzed glycine Shiff base alkylation.

The other mechanism involves the phase-transfer catalyst (**Figure 1.8**): the onium cation will swap the halide for the enolate. The new complex will be lipophilic enough to enter the organic phase and react with the alkyl halide there in an enantioselective fashion. At the same time, the halide will pass to the onium cation, regenerating the catalyst.

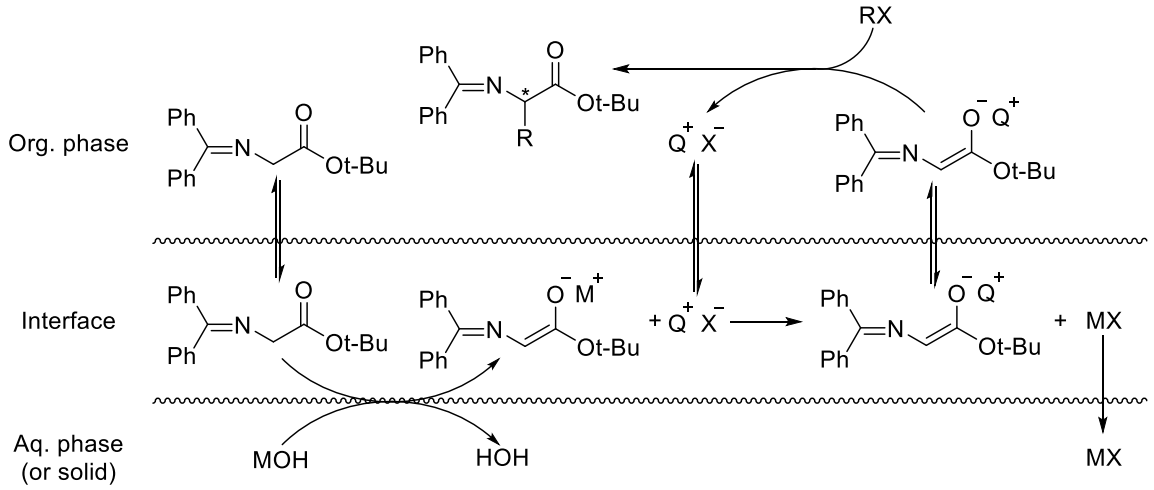
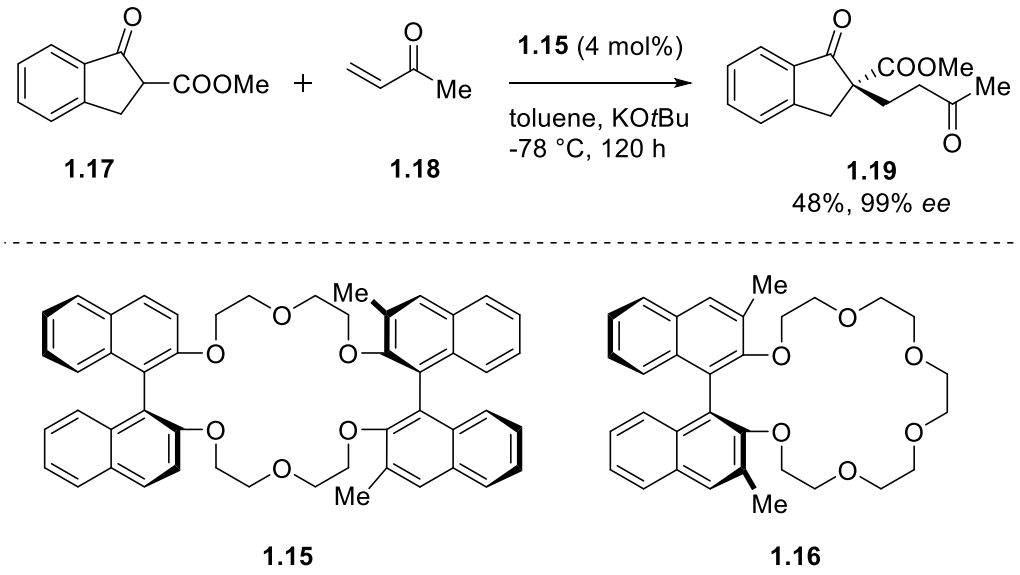


Figure 1.8 - Mechanism for catalyzed glycine Shiff base alkylation

This mechanism is more likely to happen, also because of the low affinity of the alkyl halide to the aqueous phase, but both mechanisms work simultaneously. Enantioselectivity depends on their relative proportion. Reaction conditions that best promote the catalyzed pathway will produce higher levels of stereocontrol.

Phase-transfer catalysis is not an exclusive of onium salts. Other classes served as catalysts, for example crown ethers and metal(salen) complexes. **Scheme 1.9** depicts the first highly successful Michael addition proceeding under phase-transfer catalysis reported by Cram

et al. in 1981.[15] The reaction made use of chiral crown ethers **1.15** and **1.16** as phase-transfer catalysts (**Scheme 1.7**). In presence of catalyst **1.15** and a base, β-keto ester **1.17** reacted with methyl vinyl ketone **1.18** giving product **1.19** in moderate yield but with complete stereoselectivity (over 99% *ee*).



Scheme 1.9 - Michael addition of a β-keto ester to methyl vinyl ketone

1.4 – *Cinchona* alkaloids

Cinchona alkaloids are a class of compounds extracted from the bark of homonym trees. In the extract, more than 30 alkaloids are present. Four of them represent 50% of all the alkaloids: quinine (**QN**), quinidine (**QD**), cinchonidine (**CD**) and cinchonine (**CN**). Quinine, the most known alkaloid, was isolated by Pelletier in 1820.[16] The early recognition of quinine as an antimalarial and antiarrhythmic agent had been later revaluated by countless applications, ranging from the use of quinine as a bitter flavouring agent in the food and beverage industry[17] to usage of *cinchona* bases as resolving agents.[18] L. Pasteur carried out the first resolution of a racemate in 1853 with a derivative of quinine.[19] *Cinchona* alkaloids emerged as versatile chiral basic catalysts,[20] ligands, NMR discriminating agents[21] and chromatographic selectors[22] in the past years. *Cinchona* alkaloids owe their importance as catalysts to the following characteristics: they are stable and recoverable, commercially available and cheap and the structure is easily tuneable to suit diverse catalytic applications.

The most important feature responsible for their success as catalysts is the pseudo-enantiomerism displayed by either the diastereoisomeric pairs. Quinine and quinidine, as well as cinchonidine and cinchonine, act in catalysis as if they were enantiomers, so almost any reaction catalysed by *cinchona* alkaloids can selectively afford both enantiomers (**Figure 1.2**).

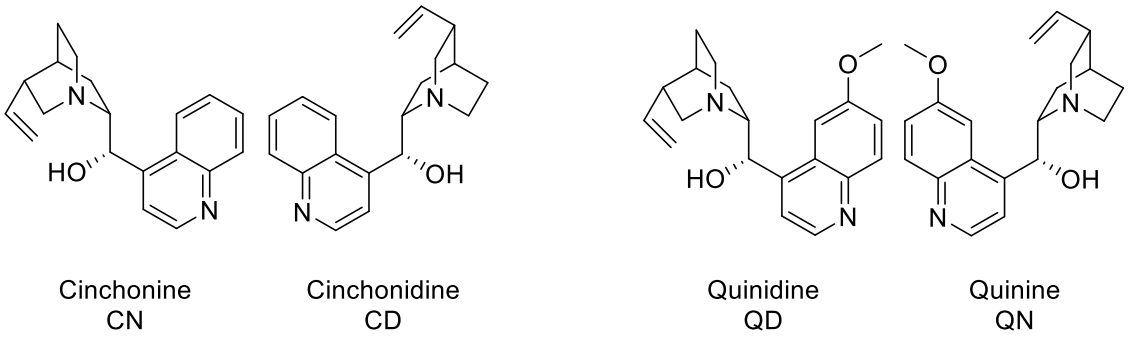


Figure 1.2 - Pseudoenantiomerism of *cinchona* alkaloids

These alkaloids' structures are composed by three different parts: the quinoline ring, the *vic*-amino function and the quinuclidine bicyclic moiety (**Figure 1.3**).

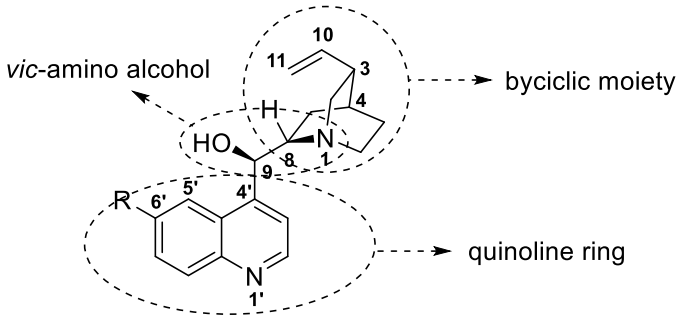


Figure 1.3 - *Cinchona* alkaloids structure.

The α,β-amino alcohol bridge is usually the core of the catalytic activity. The quinuclidine part of the structure is responsible for the catalytic action in base-catalyzed reactions (*e.g.* in the Michael-type addition to nucleophiles). These organic phase-soluble chiral bases catalyze an array of asymmetric phase-transfer reactions such as α-alkylation of carbonyl compounds, Michael addition and epoxidation of enones. [23]

In chiral discrimination, cooperative action may result from hydrogen bonding ability of the alkaloid C_9 hydroxyl group to form donor-acceptor complexes with electron-deficient molecules.

Many are the functional groups in *cinchona* alkaloids suitable for modifications (**Figure 1.4**) for design of more efficient ligands and catalysts for a specific application.

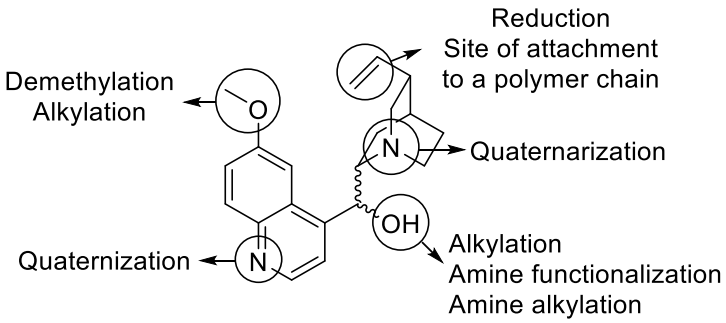


Figure 1.4 - Preferred sites of derivatizations.

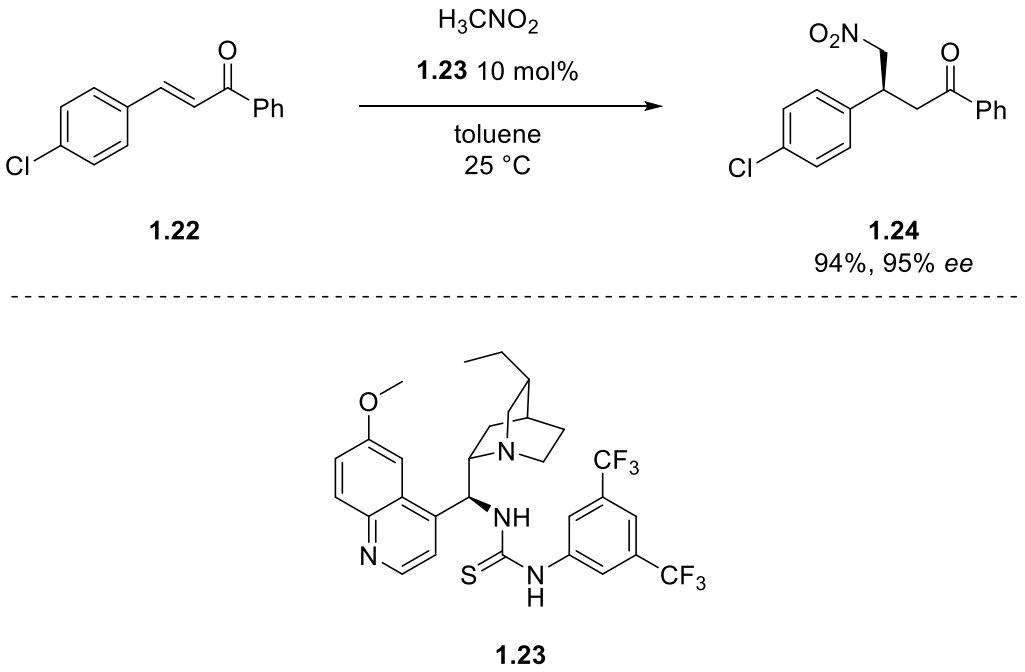
1.4.1 - Cinchona alkaloids and derivatives as bifunctional catalysts

Cinchona alkaloids have been applied extensively in asymmetric synthesis. They act properly as bifunctional catalysts mediating a number of nucleophile-electrophile reactions thanks the combined basic amine functionality and acidic hydroxyl one.[24]

In the view of Takemoto's catalyst results, the development of thiourea-substituted *Cinchona* alkaloid catalyst was the subsequent step. The C_9 stereocentre is a secondary alcohol, which can be transformed easily into a thiourea derivative via the corresponding primary amine.[25]

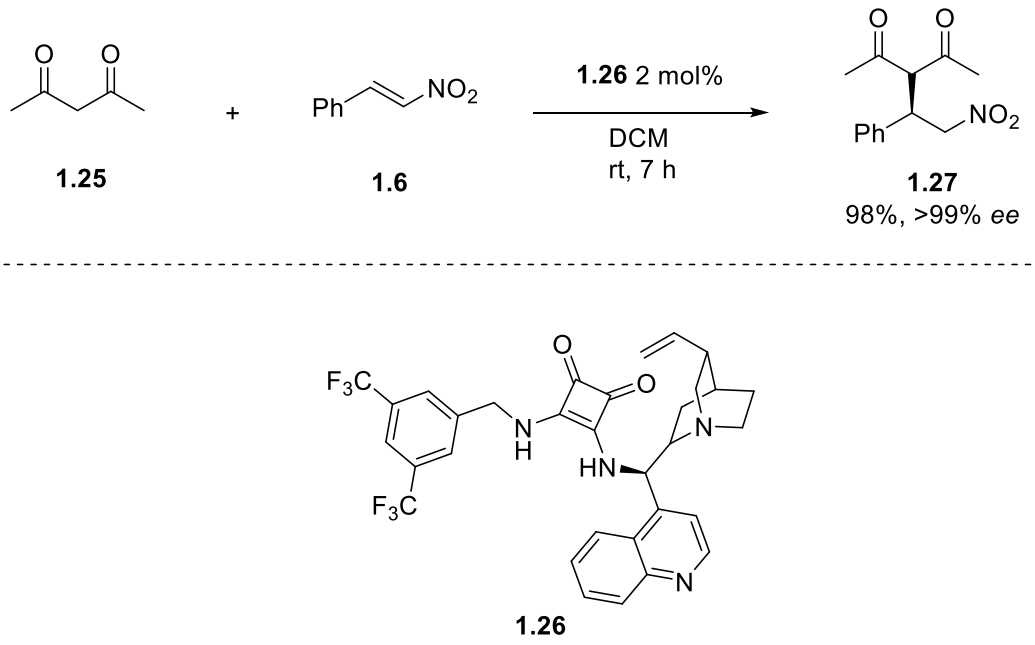
Soòs employed these thiourea-catalysts for the activation of carbonyl compounds. The new epiquinine-derived catalyst **1.23** mediated the asymmetric conjugate addition of nitromethane to chalcones (**Scheme 1.10**).[26] The analogous quinine-derived thiourea was inactive, highlighting the importance of correct relative orientation of acidic and basic moieties.

Essential to the overwhelming success of thiourea-based catalysts is its ability to form two hydrogen-bonds to a reactant. The second hydrogen bond not only further activates the reactant but also constrains it to a well-defined orientation, required for asymmetric induction.[27]



Scheme 1.10 - Catalysed nitromethane addition to calchones.

A new family of H-bonding catalysts based on the squaramide catalophore was also probed. The (-)-cinchonine-substituted derivative **2.13** was demonstrated to be an effective H-bond donor catalyst in the conjugate addition of 2,4-pentanedione **2.14** to β-nitrostyrene **2.8** affording the product in excellent yield and enantiomeric excess (**Scheme 1.11**).[28]

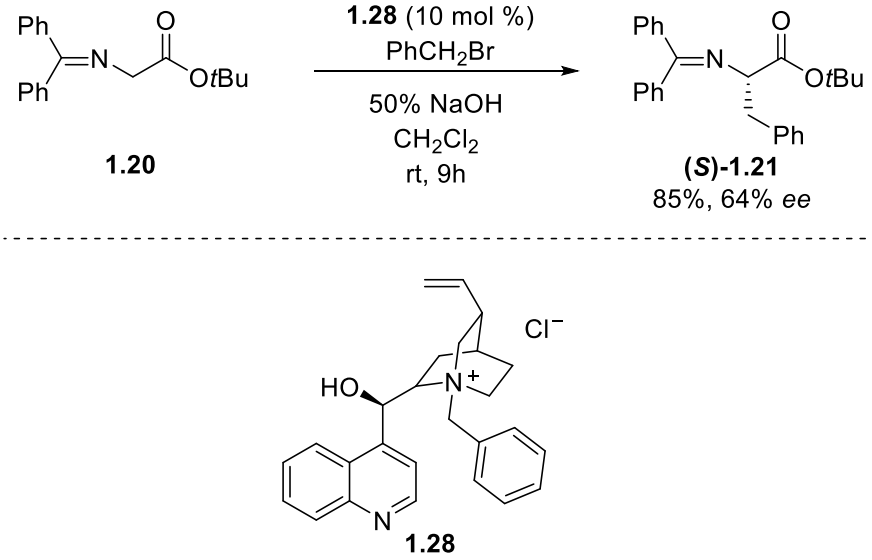


Scheme 1.11 - Catalysed acetylacetone addition to (*E*)-nitrostyrene.

1.4.2 - Cinchona derivatives as phase-transfer catalysts

Nowadays, *cinchona*-derived quaternary ammonium salts can be divided into four generations.[29]

First generation: *N*-benzyl-*cinchona* PTCs - The *N*-benzyl salts among the *N*-benzyl-*O*-protected derivatives belong to the first generation class. In 1984, Merck research group conducted the first successful application of *cinchona*-based quaternary ammonium salts as a chiral PTCs. [30] Five years later, O'Donnell *et al.* applied a similar *cinchona*-derived quaternary ammonium salt for the alkylation of *N*-(diphenylmethylene)glycine *tert*-butyl ester **1.20** and succeeded in obtaining alkylated product **1.21** with a certain degree of enantioselection (**Scheme 1.12**).[31]

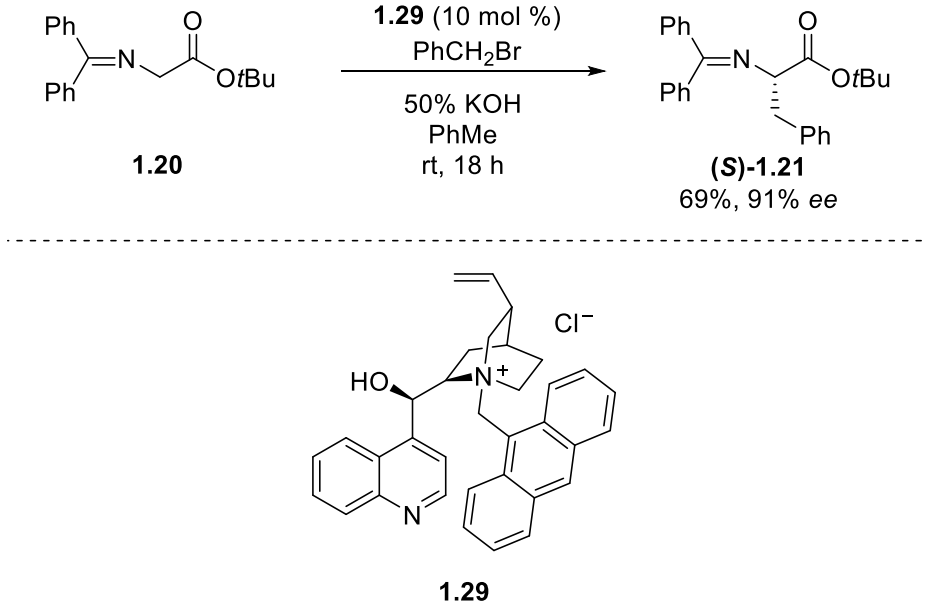


Scheme 1.12 - **1.28** catalyzed asymmetric alkylation of glycine Schiff base.

According to the classic model, the stereoselectivity in *cinchona* mediated enantioselective PTC reaction could be explained considering the tetrahedron identified by the four carbons bound to the quaternary nitrogen.[32] Cinchonidinium PTC provides effective steric shielding that can inhibit approach of the enolate from three faces (F1, occupied by quinuclidine ring, F2, by quinoline ring and C_9-O substituent, and F3, by benzyl) of this tetrahedron, leaving only one face (F4) sufficiently open to allow close contact between the enolate anion of **1.20** and the ammonium cation of the catalyst.

Second generation: *N*-9-Anthracenylmethyl-*cinchona* PTCs - After O'Donnell's successful asymmetric phase-transfer catalysis research was quite slow, until two

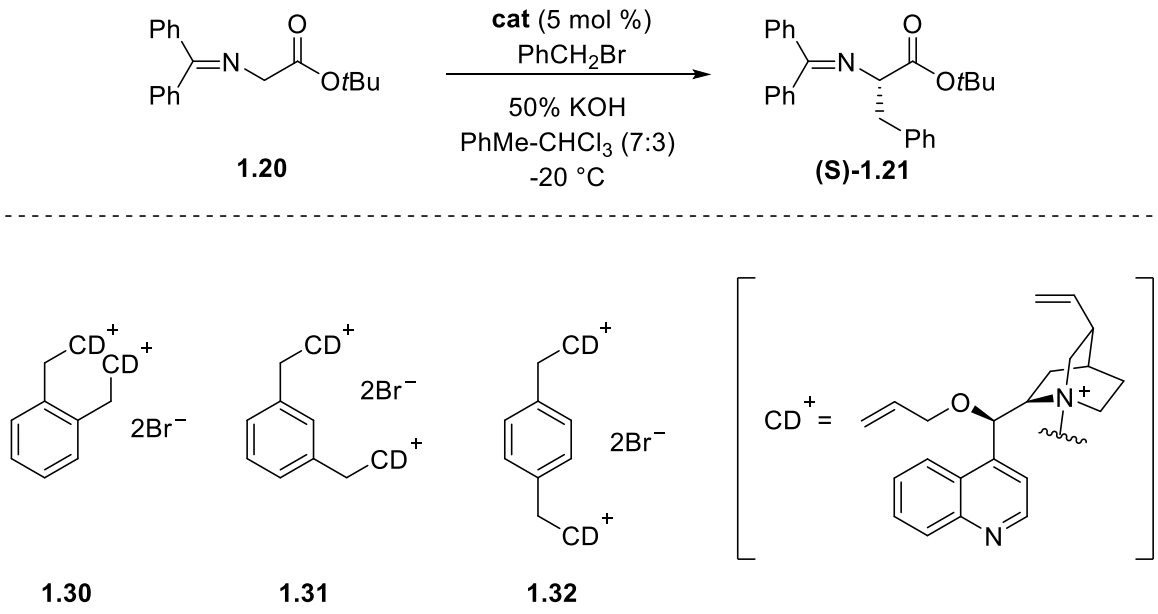
independent research groups developed a new class of *cinchona* PTC bearing the *N*-9-anthracenylmethyl group, which is bulkier compared to the *N*-benzyl group. The rationale behind this modification was the introduction of a larger flat substituent which would offer a better shielding of the F3 face. In 1997, Lygo and co-workers reported the development of the *N*-9-anthracenylmethyl-*cinchona* salt **1.29** and applied it to the phase-transfer alkylation of **1.20** with much higher enantioselectivities compared to that of *N*-benzyl analogue **1.28** (**Scheme 1.13**).[33]



Scheme 1.13 - **1.29** catalyzed asymmetric alkylation of glycine Schiff base.

N-9-anthracenylmethylcinchonidinium bromide **1.29** was also independently developed by Corey and co-workers.[32]

Third generation: polymeric *cinchona* PTCs - In 2001, as a part of a program for the discovery of new classes of *cinchona* PTCs, Jew *et al.* prepared the new dimeric cinchonidinium PTCs **1.30**, **1.31** and **1.32**, in which a phenyl is used as a spacer. Their enantioselective efficiencies were evaluated by phase-transfer benzylation of **1.20** using 5.0 mol% catalyst under phase-transfer conditions (**Scheme 1.14**). [34]



Scheme 1.14 - Phenyl ligand-based dimeric cinchonidinium PTCs.

Interestingly, the *meta*-dimeric catalyst **1.31** showed an enantioselectivity (95% *ee*) that was higher than the corresponding monomeric catalyst and two other dimeric catalysts **1.30** and **1.31**. The increase in enantioselectivity might be derived from the additional steric hindrance on cinchonidine unit, similar to the effect of the *N*-9-anthracenylmethyl on catalysts **1.29** (**Figure 1.5**).

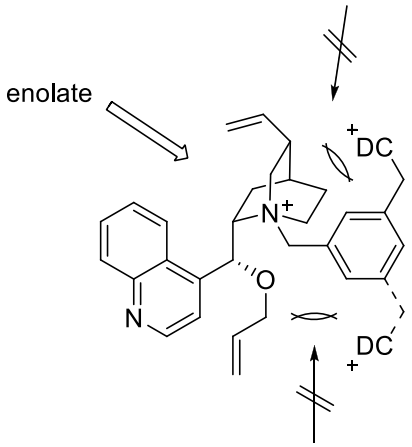
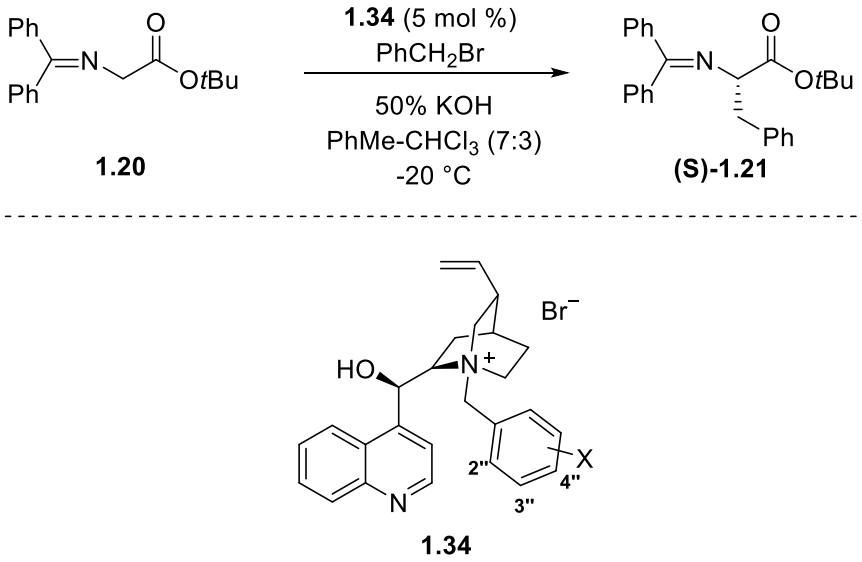


Figure 1.5 - Shielding effect of *meta*-dimeric cinchonidinium PTC.

Fourth generation: electronic factor-based *cinchona* PTCs - Several efficient catalysts were discovered based on the screening of steric factors; nevertheless electronic factors were not systematically studied. Since the ion-pair of the *cinchona* quaternary ammonium

cation and the anionic substrate is a very important intermediate for chiral induction, it was postulated that the presence of supplementary polar interactions established between the *N*-substituents and the substrate would have further stabilized the ion couple leading to an increase of the enantioselectivity observed. In 2002 Jew and co-workers began to investigate the role of the electronic factors in *cinchona* PTCs. A series of *N*-benzylcinchonidinium salts was prepared from cinchonidine and benzyl bromides containing various functional groups at the *ortho*-, *meta*-, and *para*- positions.[35] Their catalytic efficiencies were evaluated for the catalytic benzylation of **1.20** (**Scheme 1.15**).



Scheme 1.15 - Electronic functional groups incorporated into cinchonidinium PTCs.

The main objective of this study was to evaluate whether an electronic withdrawing functional group might increase enantioselectivity through formation of tighter binding ion pairs that can maintain a more rigid conformation. Data collected displayed that *meta*- and *para*-substituted derivatives did not show any significant difference in enantioselectivity in spite of their electronic properties. Interestingly, the 2''-F derivative (89% *ee*) showed enhanced enantioselectivity compared to 2''-H (74% *ee*). This was remarkable, considering that H and F have virtually the same steric properties. Further studies revealed that a monofluoride at the *ortho*- position was critical for the enhancement of enantioselectivity, and introduction of additional F at the 3''- and 4''- positions gave even higher enantioselectivity[35]. X-Ray crystallographic studies suggested that the role of 2''-F for the enhancement in enantioselectivity might be related to internal hydrogen bonding

exerted by the allylic oxygen and the F *via* a molecule of water that imposes a more rigid conformation (**Figure 1.6**).

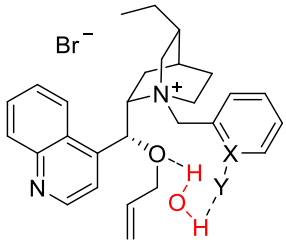
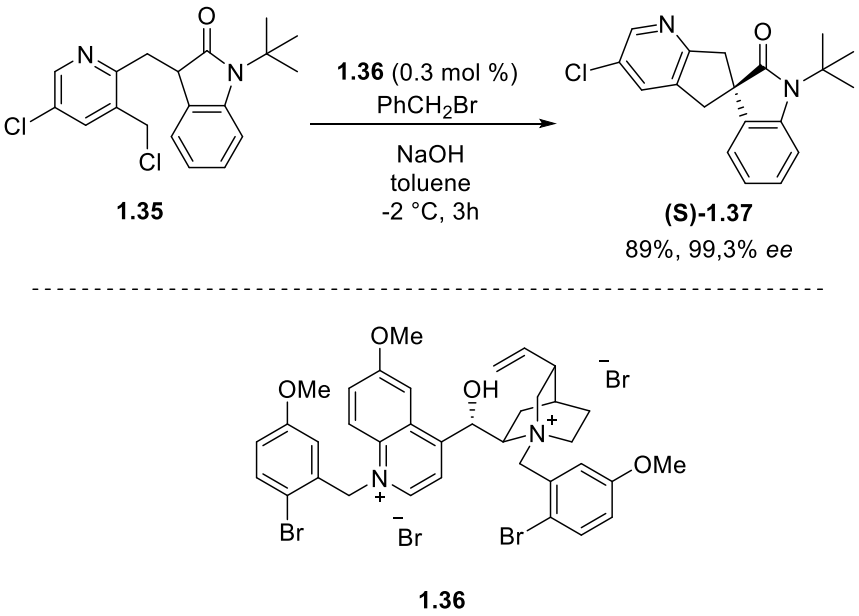


Figure 1.6 - Rigid conformation by coordination with water via hydrogen bonding.

New class: *N, N'*-disubstituted *cinchona* PTCs - Xiang *et al.* in 2014 reported the serendipitous discovery of a whole new class of PTCs, *N, N'*-disubstituted *cinchona* alkaloids, wherein both the classic quinuclidine nitrogen and the quinoline nitrogen are quaternized.[37] First observation of this new molecular entity occurred after achieving abnormal high results during an unsuccessful screening about spirocyclization of benzyl chloride **1.35** under phase-transfer catalysis. A survey on the effects of the newly introduced group led to similar and better results in comparison with regular *cinchona* derivatives catalyzed reactions. This showed how the outstanding outcome was not due to a lucky combination of factors, but instead to a more performing catalytic mechanism. Optimally tuned catalyst afforded the desired product in high yield and enantioselectivity with remarkably low catalyst load (**Scheme 1.16**).



Scheme 1.16 - **1.36** catalyzed spirocyclization of benzyl chloride **1.35**.

The possibility of an additional group bonded to quinoline nitrogen adds an important degree of freedom in *cinchona* PTCs properties tuning.

1.5 - Isoxazoles

Isoxazoles are an important class of five-membered aromatic heterocycles characterized by the presence of a nitrogen and an oxygen atom in adjacent position (**Figure 1.7**). They are a part of the bigger class of azoles, five-membered aromatic molecules containing one nitrogen atom plus at least one or more heteroatoms, like N, O, S.[38]

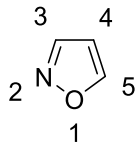
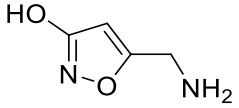


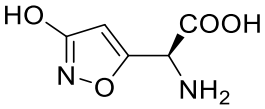
Figure 1.7 - Isoxazole structure and numeration.

They were discovered by Claisen in 1888, who synthesized the 3-methyl-5-phenylisoxazole.[39] The unsubstituted isoxazole, parent compound of the series, was first prepared in 1903 by oxymation of propargylaldehyde acetal. After that, the chemistry of isoxazoles remained latent until the 1950s, when different groups begun studying their properties.[40] In the last decades of XX century, the interest about their chemistry became more intense due to their versatility in the synthesis of natural products and different heterocycles, as well as the discovery of interesting biological activities displayed by isoxazole derivatives.[41]

Despite not being widespread in nature, different compounds containing the isoxazole core have been extracted from biological sources, like *algae* and *fungi*. Some examples are muscinol **1.38**, a potent CNS depressant and the natural occurring ibotenic acid **1.39**, a neurotoxin widely used for studies of glutamic acid receptors, extracted from *amanita muscaria* (**Figure 1.8**).[42]



1.38



1.39

Figure 1.8 - Natural occurring bioactive isoxazoles.

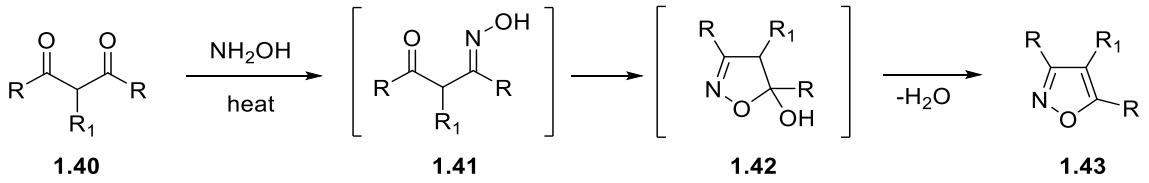
The number of pharmaceutical and synthetic applications in which they play a fundamental role due to their chemical-physical behavior demonstrates their importance. Several studies showed a wide range of biological activities like: selective agonist of the dopamine D4 receptor,[43] GABA antagonist,[44] analgesics, anti-inflammatories,[45] antimicrobial, antifungal, COX-2 inhibitors,[46] and anticancer agents.[47]

Moreover, from a synthetic point of view, isoxazoles are valuable intermediates in small molecules and natural products synthesis thanks to their unique chemical behavior. They can indeed act as masked 1,3-dicarbonyl compounds or carboxylic acids as well as precursors for a variety of others heterocyclic compounds.[48]

1.5.1 - Synthesis of isoxazoles

The isoxazole ring can be constructed by means of different synthetic methodologies. They are mainly four, and they can be classified based on the type of reaction occurring between the precursors: condensation, 1,3-dipolar cycloaddition, cycloisomerization and ring transformation reactions.[48]

The first synthesis of isoxazoles by condensation described by Claisen sees the reaction of a 1,3-dicarbonyl compound **1.40** with hydroxylamine to form an oxime **1.41**, followed by cyclization to isoxazoline **1.42**, which is not isolated and after dehydration produce the isoxazole **1.43** (**Scheme 1.17**).



Scheme 1.17 - Synthesis of isoxazoles *via* double condensation of hydroxylamine with 1,3-diketones.

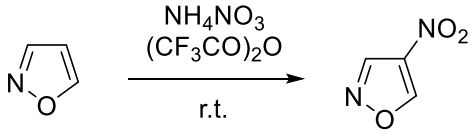
This methodology, although old, still has a great relevance for the synthesis of both 4-unsubstituted and 4-substituted isoxazoles bearing the same substituents at 3- and 5-positions.

Different kind of 1,3-dicarbonyl compounds may be used: 1,3-diketones, β-ketoesters, β-ketoaldehydes (often masked as acetals). Many examples can be found in literature.[49] The

major drawbacks of this method are the need of harsh reaction conditions and the low regioselectivity obtained when unsymmetrical dicarbonyl compounds are used.
To avoid these regioselectivity problems, different methodologies have been explored over the years.[50]

1.5.2 - Reactivity of isoxazoles

Electrophilic substitution - Direct functionalization of preformed mono-, di- and tri-substituted isoxazoles had been extensively studied as a method for the preparation of complex isoxazoles. Their reactivity can be rationalized considering that they exhibit the typical properties of an aromatic system which can be subjected to electrophilic substitution on the ring, especially at 4-position. The calculated π-electron density distribution of unsubstituted isoxazole shows that the electron density is higher at the 4-position, followed by 5- and 3-position on the ring. These calculations are consistent with the experimental observations showing the 4-position being the preferred site of attack for electrophilic substitution reactions.[51] Generally alkyl chains at the 3- and 5-positions increases the reactivity of the 4-position toward electrophiles, while the presence of aryl groups may lead to a mixed reactivity, with electrophilic substitution eventually occurring on both the isoxazole and the aryl nucleus.[38]
Isoxazoles can be readily nitrated and halogenated at 4-position of the ring, while sulfonation and acylation afford low yields and need drastic conditions. Moreover, chloromethylation, chlorobenzylation, hydroxymethylation and formylation have been reported.[49]
The nitration of unsubstituted and substituted isoxazoles can be achieved using various nitrating reagents, like mixed nitric and sulfuric acids, acetylnitrate and trifluoroacetylnitrate (**Scheme 1.18**).[52]



1.44 **1.45**

Scheme 1.18 - Nitration of isoxazole with ammonium nitrate and trifluoroacetic anhydride.

Ring cleavage - The presence of a relatively weak N-O bond make this site susceptible to reductive cleavage. Different computational calculations studies conducted by several groups proved the N-O bond having the lowest π-order compared to the other bonds of the molecule.[53] The N-O bond of the isoxazole ring can be cleaved *via* catalytic hydrogenation as well as reaction with strong bases.[41]

1.5.3 - 4-nitroisoxazoles

3,5-dimethyl-4-nitroisoxazole has emerged in the last decades as a powerful tool in organic chemistry.[48] His peculiar chemical behavior, with respect to other isoxazole derivatives, arises from the presence of the nitro group in 4-position (**Figure 1.9**).

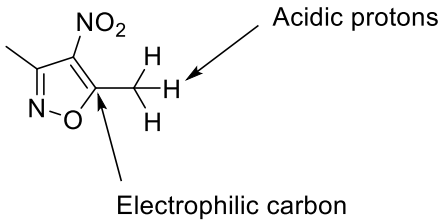
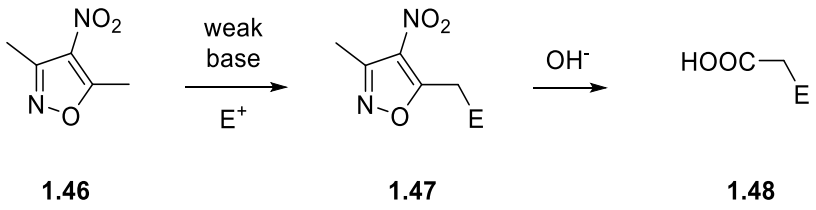


Figure 1.9 - Reactivity of 3,5-dimethyl-4-nitroisoxazole.

The electron-withdrawing nature of the nitro group deeply affects the electronic distribution on the isoxazole ring, modifying the usual reactivity of the isoxazole core.

Nucleophilic behavior - The methyl group at 5-position is conjugated with the nitro group in a 1,4-conjugated fashion, due to the low delocalization of the π-electrons on the ring. This feature results in an increase of the acidity of the protons in α-position that can undergo deprotonation by weak organic and inorganic bases. Thus, the deprotonated isoxazole can act as a soft nucleophile in different types of reactions with electrophiles.

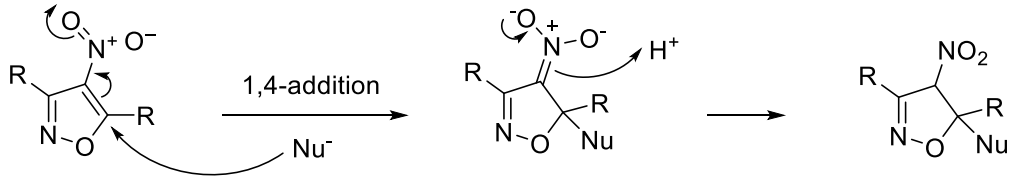
The conjugated system behaves as an ester moiety in the activation of the α-methyl group and C_5. Therefore, 3,5-dimethyl-4-nitroisoxazole can be considered as a masked carboxylic acid or ester, enhancing his usefulness in the preparation of valuable compounds (**Scheme 1.19**).[54]



1.46 **1.47** **1.48**

Scheme 1.19 General sequence for the use of 3,5-dimethyl-4-nitroisoxazole as a carboxylic acid synthon.

Electrophilic behavior - The presence of the nitro group in 4-position on the isoxazole ring, makes it susceptible of attack at C_5 by nucleophiles. Different kind of nucleophiles can react with the 3,5-disubstituted-4-nitroisoxazole core **1.49**, leading to the formation of isoxazolines **1.51** (**Scheme 1.20**).



1.49 **1.50** **1.51**

Scheme 1.20 - 1,4-addition of hard nucleophiles on the 4-nitroisoxazole core.

3-methyl-4-nitro-5-(alkylenethenyl/styryl) isoxazoles possess two electrophilic centers. They share with simple 3,5-dimethyl-4-nitroisoxazoles the typical reactivity at the C-5 of the isoxazole ring toward hard nucleophiles, like hydroxyl and trifluoromethyl anions, and thus can be considered α,β-unsaturated acids synthons. On the other hand, they present a second electrophilic center (**E_2**) at the β-position from the isoxazole ring (**Figure 1.10**).

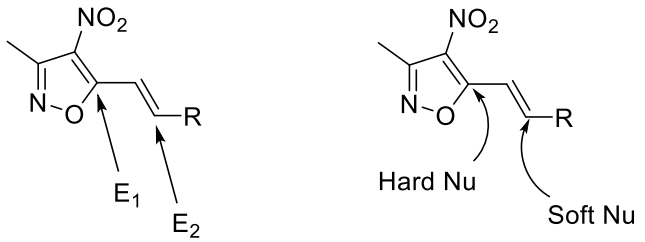
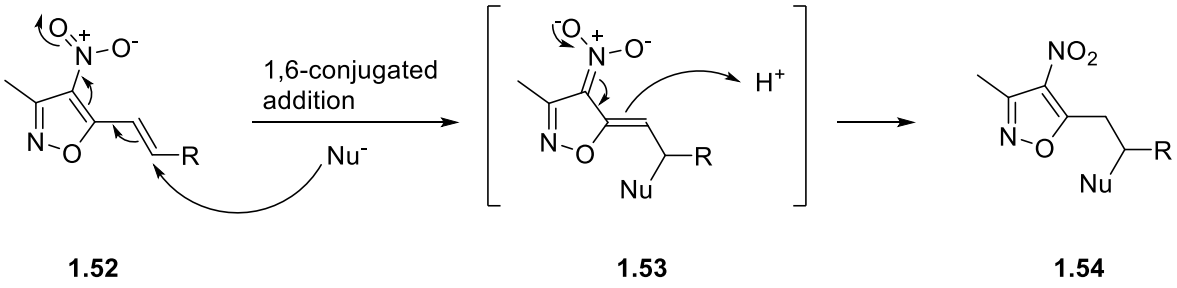


Figure 1.10 - Electrophilic behavior and reactivity of 3-methyl-4-nitro-5-styrylisoxazoles.

This position shows a typical Michael acceptor reactivity, reacting with soft, stabilized carbon, nitrogen and sulfur nucleophiles.[55] The 4-nitroisoxazole moiety activates the exocyclic double bond towards 1,6-conjugated addition and stabilizes the carbanion **1.53** formed after the nucleophile addition by delocalization of the electrons over the conjugated isoxazole system (**Scheme 1.21**).



1.52 **1.53** **1.54**

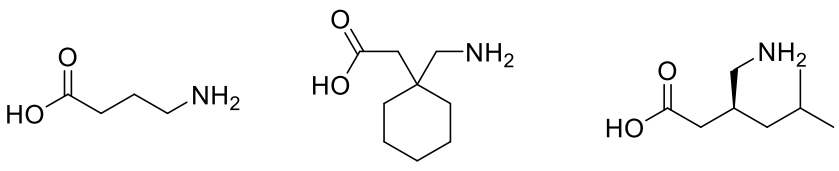
Scheme 1.21 - 1,6-Michael addition of soft nucleophiles on 3-methyl-4-nitro-5-(alkylenethenyl/styryl) isoxazoles.

1.6 – Pregabalin

Pregabalin is the common name for (S)-4-amino-3-(2-methylpropyl) butanoic acid [CAS: 148553-50-8], marketed by Pfizer under the trade name Lyrica.

Pregabalin is a medication used in the treatment of peripheral neuropathic pain and as an adjunctive therapy for partial seizures in patients with epilepsy.[56] It was invented by medicinal chemist Richard Bruce Silverman at Northwestern University in the United States, looking for an alkylated analogue of gabapentin with improved properties.[57]

Both pregabalin **1.57** and gabapentin **1.56** are synthetic analogues of GABA **1.55** (γ-amino-butyric acid), the main inhibitory neurotransmitter in the central nervous system (**Figure 1.11**).[58]



1.55 **1.56** **1.57**

Figure 1.11 - GABA, gabapentin and pregabalin.

Those compounds are supposed to exert their pharmacological activity through direct interaction with GABA receptors, placed in the voltage-gated calcium channels, reducing the release of excitatory neurotransmitters such as glutamate and noradrenaline.

The original pregabalin synthesis by Pfizer involved six steps, with the enantioselective one being a Rh(DuPhos) catalyzed hydrogenation.[59] Pfizer has recently developed a different synthetic route using ene-reductase enzymes to selectively generate the wanted S-isomers,[60] due to expensiveness of rhodium and chiral phosphines. Other processes to manufacture S-pregabalin have been published,[61] but none of them provided evidences for process scalability.

References

[1] P. I. Dalko, L. Moisan, *Angewandte Chemie International Edition* **2004**, *43*, 5138.

[2] a) P. R. Schreiner, *Chemical Society Reviews* **2003**, *32*, 289; b) A. Berkessel, H. Gröger, in *Asymmetric Organocatalysis*, Wiley-VCH Verlag GmbH & Co. KGaA, **2005**.

[3] A. G. Doyle, E. N. Jacobsen, *Chemical Reviews* **2007**, *107*, 5713.

[4] a) H. Yamamoto, K. Futatsugi, *Angewandte Chemie International Edition* **2005**, *44*, 1924; b) M. Kanai, N. Kato, E. Ichikawa, M. Shibasaki, *Synlett* **2005**, *2005*, 1491.

[5] S. J. Connon, *Chemical Communications* **2008**, 2499.

[6] L. Bernardi, F. Fini, M. Fochi, A. Ricci, *Chimia* **2007**, *61*, 224.

[7] T. Okino, Y. Hoashi, Y. Takemoto, *J. Am. Chem. Soc.* **2003**, *125*, 12672.

[8] a) U. Eder, G. Sauer, R. Wiechert, *Angew. Chem. Int. Ed.* **1971**, *10*, 496; b) Z. G. Hajos, D. R. Parrish, *J. Org. Chem.* **1974**, *39*, 1615; c) Y. Huang, A. K. Unni, A. N. Thadani, V. H. Rawal, *Nature* **2003**, *424*, 146.

[9] U. Eder, G. Sauer, R. Wiechert, *Angewandte Chemie International Edition in English* **1971**, *10*, 496.

[10] a) H. J. Martin, B. List, *Synlett* **2003**, *2003*, 1901; b) J. Kofoed, J. Nielsen, J.-L. Reymond, *Bioorganic & Medicinal Chemistry Letters* **2003**, *13*, 2445.

[11] C. M. Starks, *J. Am. Chem. Soc.* **1971**, *93*, 195.

[12] a) C. M. Starks, C. L. Liotta, M. E. Halpern, In *Phase-Transfer Catalysis: Fundamentals, Applications, and Industrial Perspectives*; Chapman & Hall, New York, 1994; b) Y. Sasson, R. Neumann, In *Handbook of Phase Transfer Catalysis*;

Blackie Academic & Professional, London, 1997; c) E. V. Dehmlow, S. S. Dehmlow, In *Phase Transfer Catalysis*, 3rd edn. VCH, Winheim; d) M. E. Halpern, In *Phase Transfer Catalysis*, ACS *Symposium Series* 659, American Chemical Society, Washington, DC.

[13] K. Maruoka, in *Asymmetric Phase Transfer Catalysis*, WILEY-VCH GmbH & Co. KGaA, **2008**, pp.1-8.

[14] Ooi, T., Kameda, M. and Maruoka, K. *J. Am. Chem. Soc.*, **2003**, *125*, 5139.

[15] D. J. Cram, G. D. Y. Sogah, *J. Chem. Soc., Chem. Commun.* **1981**, *13*, 625.

[16] J. Pelletier, J. B. Caventon, *Ann. Chim. Phys.* **1820**, *14*, 69.

[17] R. Verpoorte, J. Schripsema, T. van der Leer, In the *Alkaloids. Chemistry and Pharmacology*, Vol. 34, A. Brossi, Ed. Academic Press, New York, **1988**, *34*, 331.

[18] a) R. A. Sheldon, *Chirotechnology: Industrial Synthesis of Optically Active Compounds*, M. Dekker, New York, **1993**, chap. 6; b) P. Newman, *Optical Resolution Procedures for Chemical Compounds, Acids,* Vol.2*;* Optical Resolution Information Center, Manhattan College, Riverdale: New York, **1981**, 7; c) J. Jacques, A. Collet, S. H. Wilen, *Enantiomers, Racemates, and Resolutions*; John Wiley and Sons, New York, **1981**, 254; d) J. Jacques, A. Collet, S. H. Wilen, *Enantiomers, Racemates, and Resolutions*; John Wiley and Sons, New York, **1981**, 257; e) *Chirality in Industry*, Vol. 1 (Eds.: A. N. Collins, G. N. Sheldrake, J. Crosby), Wiley, Chichester, **1992**; f) *Chirality in Industry*, Vol. 2 (Eds.: A. N. Collins, G. N. Sheldrake, J. Crosby), Wiley, Chichester, **1997**.

[19] L. C. R. Pasteur, *Acad. Sci.* **1853**, *37*, 162.

[20] H. Wynberg, *Top. Stereochem.* **1986**, *16*, 87.

[21] M. Abid, B. Török, *Tetrahedron: Asymmetry* **2005**, *16*, 1547.

[22] a) P. Salvadori, D. Pini, C. Rosini, C. Bertucci, G. Uccello-Barretta, *Chirality* **1992**, *4*, 43; b) C. Rosini, C. Bertucci, D. Pini, P. Altemura, P. Salvadori, *Chromatographia* **1987**, *24*, 671; c) A. Mandl, L. Nicoletti, M. Lammerhofer, W. Lindner, *J. Chromatogr. A* **1999**, *858*, 1; d) W. Lindner, *Anal. Chem.* **2000**, *72*, 4623; e) W. Lindner, *Anal. Chem.* **2000**, *72*, 4614; f) P. Franco, M. Lammerhofer, P. M. Klaus, W. Lindner, *J Chromatogr A* **2000**, *869*, 111.

[23] a) M. J. O'Donnell, *Asymmetric Phase Transfer Reactions*, *In Catalytic Asymmetric Synthesis*, 2nd ed.; I. Ojima, Ed. Wiley-VCH, New York, **2000**, 727; b) A. Nelson, *Angew. Chem. Int. Ed.* **1999**, *111*, 1685; c) A. Nelson, *Angew. Chem. Int. Ed.* **1999**, *38*, 1583; d) D. Martyres, *Synlett* **1999**, 462.

[24] H. Li, Y. Wang, L. Tang, L. Deng, *Journal of the American Chemical Society* **2004**, *126*, 9906-9907.

[25] H. Martin R. Hoffmann, J. Frackenpohl, *European Journal of Organic Chemistry* **2004**, *2004*, 4293-4312.

[26] B. Vakulya, S. Varga, A. Csámpai, T. Soós, *Organic Letters* **2005**, *7*, 1967-1969.

[27] T. Okino, Y. Hoashi, T. Furukawa, X. Xu, Y. Takemoto, *Journal of the American Chemical Society* **2004**, *127*, 119-125.

[28] J. P. Malerich, K. Hagihara, V. H. Rawal, *Journal of the American Chemical Society* **2008**, *130*, 14416-14417.

[29] S.-s. Jew, H.-g. Park, *Chem. Commun.* **2009**, 7090.

[30] U. H. Dolling, P. Davis, E. J. J. Grabowski, *J. Am. Chem. Soc.* **1984**, *106*, 446.

[31] M. J. O'Donnell, W. D. Bennett, S. Wu, *J. Am. Chem. Soc.* **1989**, *111*, 2353.

[32] E. J. Corey, F. Xu, M. C. Noe, J. Am. Chem. Soc. 1997, 119, 12414.

[33] a) B. Lygo, J. Crosby, T. R. Lowdon, J. A. Peterson, P. G. Wainwright, *Tetrahedron* **2001**, *57*, 2403; b) B. Lygo, J. Crosby, T. R. Lowdon, P. G. Wainwright, *Tetrahedron* **2001**, *57*, 2391; c) B. Lygo, P. G. Wainwright, *Tetrahedron Lett.* **1997**, *38*, 8595.

[34] a) S.-s. Jew, B.-S. Jeong, M.-S. Yoo, H. Huh, H.-g. Park, *Chem. Commun.* **2001**, 1244; b) J.-H. Lee, M.-S. Yoo, J.-H. Jung, S.-S. Jew, H.-G. Park, B.-S. Jeong, *Tetrahedron* **2007**, *63*, 7906.

[35] S.-s. Jew, M.-S. Yoo, B.-S. Jeong, I. Y. Park, H.-g. Park, *Org. Lett.* **2002**, *4*, 4245.

[35] a) K. Julienne, P. Metzner, V. Henryon, *J. Chem. Soc. Perkin Trans. 1* **1999**, 731; b) K. Julienne, P. Metzner, V. Henryon, A. Greiner, *J. Org. Chem.* **1998**, *63*, 4532; c) J. Zanardi, C. Leriverend, D. Aubert, K. Julienne, P. Metzner, *J. Org. Chem.* **2001**, *66*, 5620.

[37] B. Xiang, K. M. Belyk, R. A. Reamer, N. Yasuda, *Angew. Chem. Int. Ed.* **2014**, 53, 8375.

[38] K. M. John A. Joule, *Heterocyclic Chemistry, 5th Edition*, **2010**.

[39] a) L. Claisen, O. Lowman, *Berichte der deutschen chemischen Gesellschaft* **1888**, *21*, 1149; b) L. Claisen, *Berichte der deutschen chemischen Gesellschaft* **1891**, *24*, 3900.

[40] A. Quilico, in *Chemistry of Heterocyclic Compounds*, John Wiley & Sons, Inc., **1962**, pp. 5-94.

[41] a) M. Sutharchanadevi, R. Murugan, in *Comprehensive Heterocyclic Chemistry II* (Eds.: A. R. Katritzky, C. W. Rees, E. F. V. Scriven), Pergamon, Oxford, **1996**, pp. 221-260; b) A. M. S. Silva, A. C. Tomé, T. M. V. D. Pinho e Melo, J. Elguero, in *Modern Heterocyclic Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, **2011**, pp. 727-808.

[42] a) K. Bowden, A. C. Drysdale, *Tetrahedron Letters* **1965**, *6*, 727-728; b) P. Krogsgaard-Larsen, T. Honore, J. J. Hansen, D. R. Curtis, D. Lodge, *Nature* **1980**, *284*, 64-66.

[43] M. Rowley, H. B. Broughton, I. Collins, R. Baker, F. Emms, R. Marwood, S. Patel, S. Patel, C. I. Ragan, S. B. Freedman, P. D. Leeson, *Journal of Medicinal Chemistry* **1996**, *39*, 1943-1945.

[44] D. Krehan, S. í Storustovu, T. Liljefors, B. Ebert, B. Nielsen, P. Krogsgaard-Larsen, B. Frølund, *Journal of Medicinal Chemistry* **2006**, *49*, 1388-1396.

[45] G. Daidone, D. Raffa, B. Maggio, F. Plescia, V. M. C. Cutuli, N. G. Mangano, A. Caruso, *Archiv der Pharmazie* **1999**, *332*, 50-54.

[46] J. J. Talley, D. L. Brown, J. S. Carter, M. J. Graneto, C. M. Koboldt, J. L. Masferrer, W. E. Perkins, R. S. Rogers, A. F. Shaffer, Y. Y. Zhang, B. S. Zweifel, K. Seibert, *Journal of Medicinal Chemistry* **2000**, *43*, 775-777.

[47] W.-T. Li, D.-R. Hwang, C.-P. Chen, C.-W. Shen, C.-L. Huang, T.-W. Chen, C.-H. Lin, Y.-L. Chang, Y.-Y. Chang, Y.-K. Lo, H.-Y. Tseng, C.-C. Lin, J.-S. Song, H.-C. Chen, S.-J. Chen, S. H. Wu, C.-T. Chen, *Journal of Medicinal Chemistry* **2003**, *46*, 1706-1715.

[48] F. Hu, M. Szostak, *Advanced Synthesis & Catalysis* **2015**, *357*, 2583-2614.

[49] P. a. V.-F. Grunanger, P., in *Chemistry of Heterocyclic Compounds*, John Wiley & Sons, Inc., **2008**, pp. 1-416.

[50] a) M. V. D. P. e. M. Teresa, *Current Organic Chemistry* **2005**, *9*, 925-958; b) Y.-i. Lin, S. A. Lang, *Journal of Heterocyclic Chemistry* **1977**, *14*, 345-347; c) R. G. Jones, C. W. Whitehead, *The Journal of Organic Chemistry* **1955**, *20*, 1342-1347.

[51] a) R. E. Wasylishen, J. B. Rowbotham, T. Schaefer, *Canadian Journal of Chemistry* **1974**, *52*, 833-837; b) G. Berthier, G. Del Re, *Journal of the Chemical Society (Resumed)* **1965**, 3109-3117; c) T.-K. Ha, *Journal of Molecular Structure* **1979**, *51*, 87-98.

[52] a) L. A. Reiter, *The Journal of Organic Chemistry* **1987**, *52*, 2714-2726; b) J. A. a. M. Joule, K., *Heterocyclic Chemistry, 5th Edition*, Wiley-Blackwell, **2010**.

[53] A. R. Katritzky, K. Jug, D. C. Oniciu, *Chemical Reviews* **2001**, *101*, 1421-1450.

[54] a) M. F. A. Adamo, V. R. Konda, D. Donati, P. Sarti-Fantoni, T. Torroba, *Tetrahedron* **2007**, *63*, 9741-9745; b) M. F. A. Adamo, S. Suresh, *Tetrahedron* **2009**, *65*, 990-997; c) R. Wells, M. Moccia, M. F. A. Adamo, *Tetrahedron Letters* **2014**, *55*, 803-805.

[55] M. F. A. Adamo, S. Chimichi, F. De Sio, D. Donati, P. Sarti-Fantoni, *Tetrahedron Lett.* **2002**, *43*, 4157-4160.

[56] J. E. Frampton, *CNS Drugs* **2014**, *28*, 835.

[57] R. B. Silverman, *Angewandte Chemie International Edition*, **2008**, *47*, 3500.

[58] R. H. Dworkin and P. Kirkpatrick, *Nature Reviews Drug Discovery*, **2005**, *4*, 455.

[59] Burk, M. J., de Koning, P.,D., Grote, T. M., Hoekstra, M. S., Hoge, G., Jennings, R. A., Kissel, W. S., Le, T. V., Lennon, I. C., Mulhern, T. A., Ramsden, J. A., and Wade, R. A. *J. Org. Chem*. **2003**, *68*, 5731.

[60] Debarge, S.; McDaid, P.; O' Neill, P.; Frahill, P.; Wong, J. W.; Carr, D.; Burrell, A.; Davies S.; Karmilowicz, M.; Steflik J. *Org. Process Res. Dev.*, **2014**, *18*, 109.

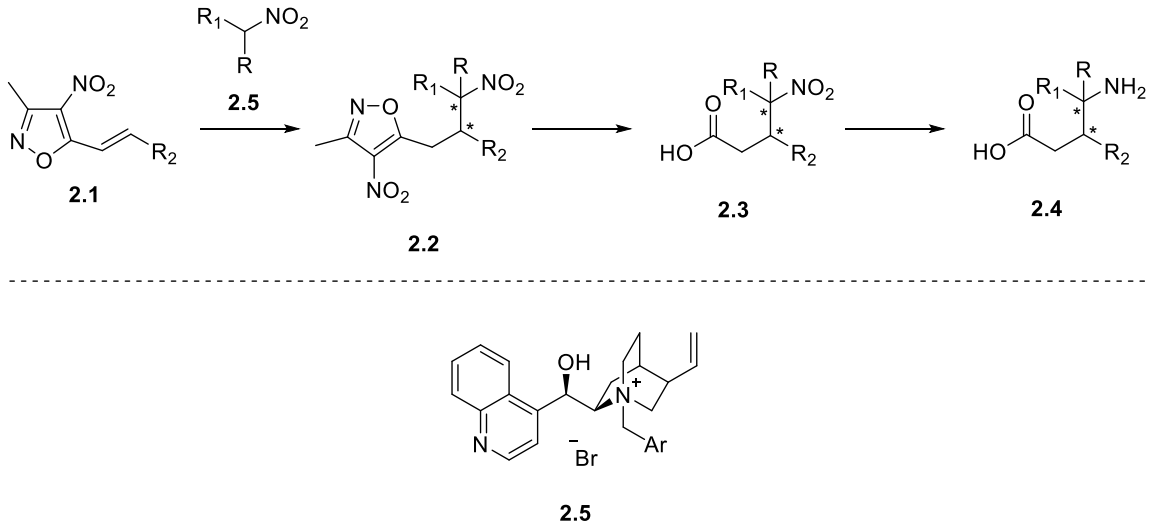
[61] a) Felluga, F.; Pitacco, G.; Valentin, E.; Venneri, C. D. *Tetrahedron: Asymmetry* **2008**, 19, 945; b) Izquierdoa, S.; Aguileraa, J.; Buschmann, H. H.; Garcia, M.; Torrens A.; Ortuno R. M. *Tetrahedron: Asymmetry* **2008**, *19*, 651; c) Gotoh, H.; Ishikawa, H.; Hayashi, Y. *Org. Lett.*, **2007**, *9*, 5307.

Chapter 2

N, N'-disubstituted *cinchona* alkaloids catalyzed enantioselective Michael addition of nitromethane to (E)-3-methyl-5-(4-methylpent-1-enyl)-4-nitroisoxazole for the synthesis of pregabalin

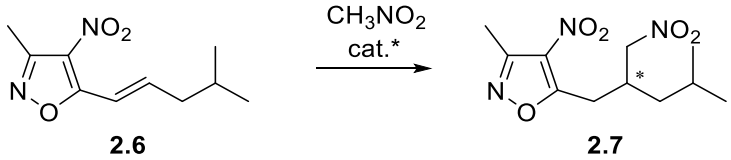
2.0 - Overview

Adamo *et al.* reported in 2009 the enantioselective Michael addition of nitroalkanes to 3-methyl-4-nitro-5-styrylisoxazoles **2.1** *via* phase-transfer conditions using cinchonidine-derived quaternary ammonium salts **2.5** for the synthesis of γ-nitroesters **2.3** and γ-amino acids **2.4**. (**Scheme 2.1**).[1]



Scheme 2.1 - Michael addition of nitroalkanes to **2.1** for the synthesis of γ-nitroesters and γ-amino acids.

This chapter deals with a synthesis of the γ-amino acid pregabalin in his S-enantiomer. Said synthesis builds on an analogous system (**Scheme 2.2**), 3-methyl-4-nitroalkylenethyl isoxazole **2.6** as equivalent of an α,β-unsaturated ester that similarly hydrolyzes affording a carboxylic acid but that, at the same time, is more reactive towards soft nucleophiles like nitroalkanes.[1] 4-nitroisoxazoles also establish better-defined interactions with chiral catalysts than esters, promoting the enantioselective addition of nitromethane to the double bond, the critical step of the whole synthesis.



Scheme 2.2 - Critical synthesis step: Michael addition of nitromethane to **2.6**.

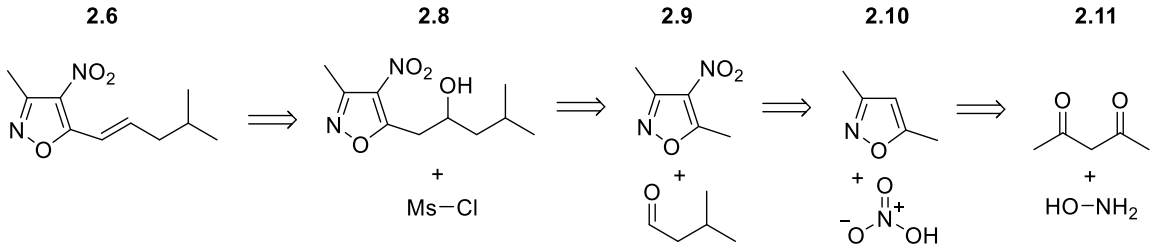
Quaternized *cinchona* derivatives showed good enantioselective catalysis of the reaction for both R-enantiomer (with cinchonidine-derived PTCs)[2] and S-enantiomer (with quinidine-derived ones),[3] which is the one of real interest as it leads to pregabalin.

Xiang *et al.* reported in 2014 the discovery of a new class of PTCs, *N, N'*-disubstituted *cinchona* alkaloids, wherein both the classic quinuclidine nitrogen and the quinoline nitrogen are quaternized.[4]

This chapter reports the study on the capability of the new class of PTCs to enantioselectively promote 1,6-conjugate addition of nitromethane to compound **2.6**.

2.1 – Synthesis of 1,6-conjugate addition starting material

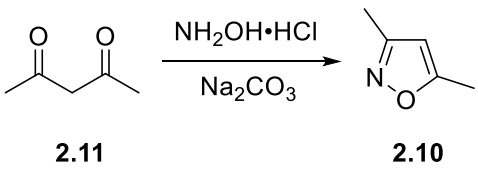
The various synthetic steps leading to starting material for 1,6-conjugate addition are reported in **Scheme 2.3**.



Scheme 2.3 - Retrosynthesis of compound **2.6**.

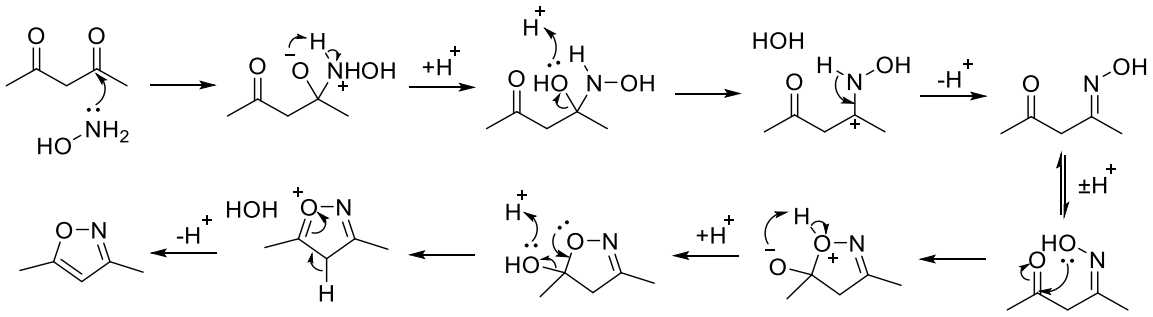
2.1.1 – Preparation of 3,5-dimethyl-isoxazole (2.10)

The isoxazole ring was built *via* condensation of acetyl acetone **2.11** and hydroxylamine, in the form of hydrochloride salt in the presence of sodium carbonate. (**Scheme 2.4**).



Scheme 2.4 - Preparation of **2.10**.

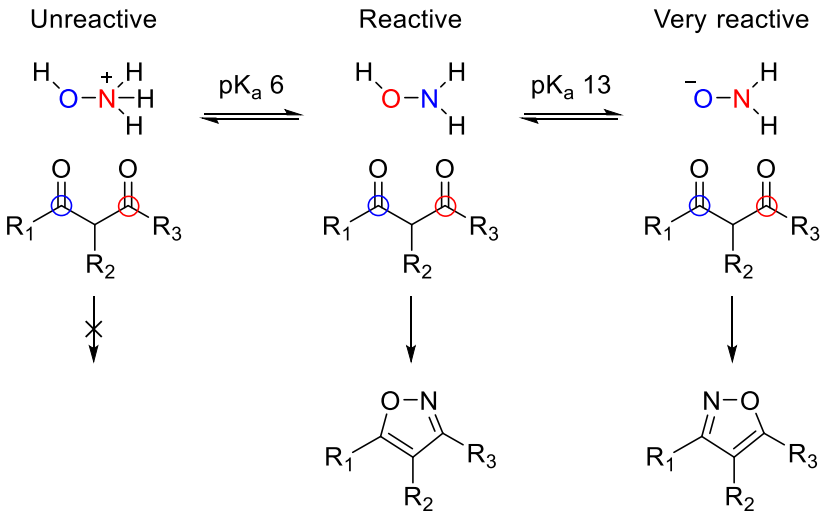
Scheme 2.5 depicts reaction mechanism.



Scheme 2.5 - Mechanism for preparation of **2.10**.

The formation of a thermodynamic stable aromatic ring drives the reaction and since the dicarbonyl compound employed is symmetric, no regioselectivity problems arise.

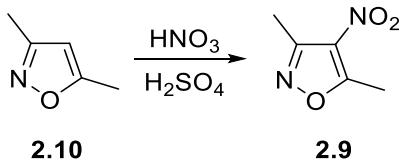
Hydroxylamine hydrochloride is employed as it is solid and more stable than an aqueous solution of hydroxylamine. The reason a base is needed is that it increases the nucleophilicity of the reactant, promoting its attack to carbonyl (**Scheme 2.6**). pH also influences the relative nucleophilicity of nitrogen and oxygen, modifying the geometry of the final product when the dicarbonyl is asymmetric.



Scheme 2.6 - Dependence of hydroxylamines's reactivity on pH.

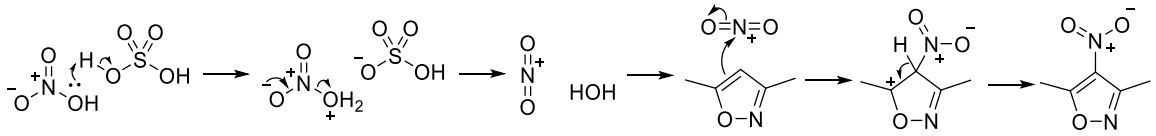
2.1.2 –Preparation of 3,5-dimethyl -4-nitroisoxazole (2.9)

The reaction is an electrophilic nitration, working through electrophilic aromatic substitution on isoxazole **2.10** with a mixture of concentrated nitric and sulfuric acids at high temperature (**Scheme 2.7**).



Scheme 2.7 - Preparation of **2.9**.

Scheme 2.8 depicts reaction mechanism.

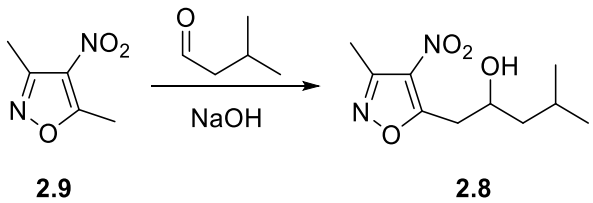


Scheme 2.8 - Mechanism for synthesis of **2.9**.

The only available position is at C_4, which is also the most reactive toward electrophilic aromatic substitution, so no byproducts are formed.

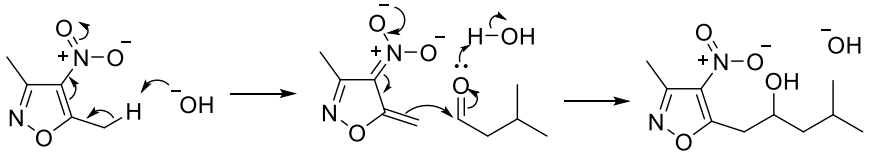
2.1.3 –Preparation of 4-methyl-1-(3-methyl-4-nitroisoxazol-5-yl)-pentan-2-ol (2.8)

Exploiting the acidity of its protons, **2.9** can undergo vinylogous nitro-aldol reaction with an electrophilic partner (**Scheme 2.9**). **2.9** is reacted with isovaleraldehyde in the presence of solid sodium hydroxide affording the alcohol **2.8**.



Scheme 2.9 - Preparation of **2.8**.

Scheme 2.10 depicts reaction mechanism.



Scheme 2.10 - Mechanism for preparation of **2.8**.

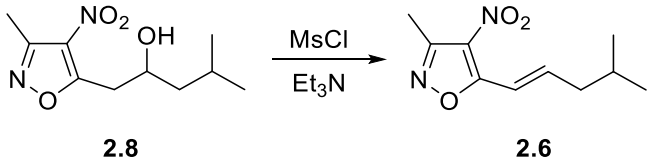
Under these reaction conditions **2.9** is a much better nucleophile than isovaleraldehyde, thus self-condensation of the aldehyde does not take place.

The product is formed as a racemate considering that in the subsequent step the hydroxyl group will be eliminated affording alkene **2.6**, some of which by the way is already formed in this step by direct dehydration of **2.8**.

The catalytic amount of sodium hydroxide is not enough for ring cleavage.

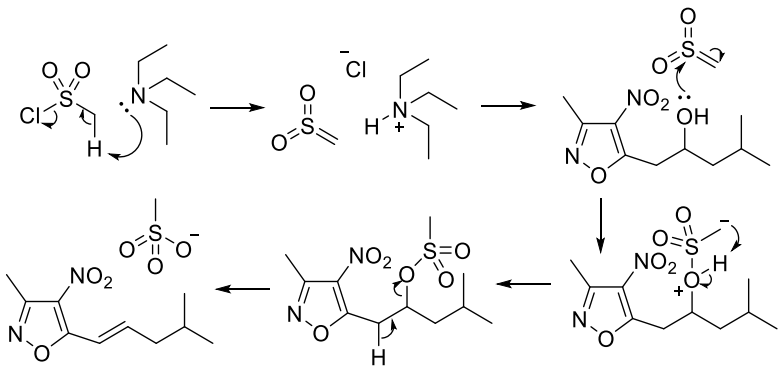
2.1.4 –Preparation of (E)-3-methyl-5-(4-methylpent-1-enyl)-4-nitroisoxazole (2.6)

Dehydration of **2.8** is accomplished by mesylation of the alcohol, promoting the formation of alkene **2.6** by monomolecular elimination of a stable mesylate anion (**Scheme 2.11**).



Scheme 2.11 - Preparation of **2.6**.

Scheme 2.12 depicts reaction mechanism.



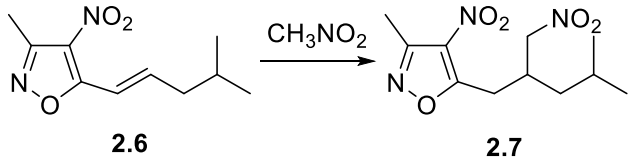
Scheme 2.12 - Mechanism for preparation of **2.6**.

Steric hindrance drives the reaction toward the formation of the sole E- isomer of **2.6**. It is important in this step to obtain only one isomer because this will influence the enantioselective outcome of the asymmetric addition of nitromethane.

2.2 - Mono-quaternized *cinchona* alkaloids catalyzed enantioselective 1,6-conjugate addition

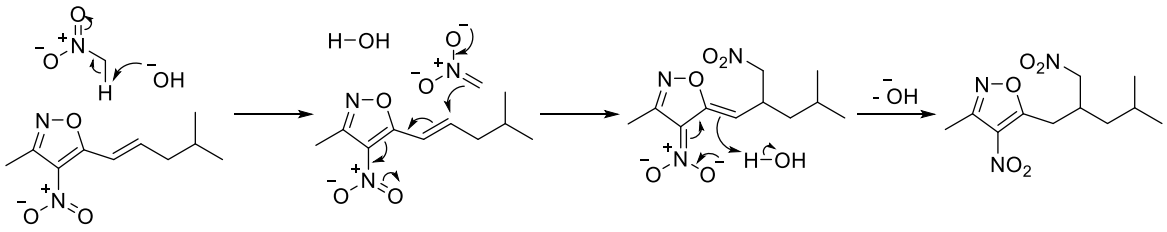
2.2.1 - 1,6-conjugate addition: preparation of 3-methyl-5-(4-methyl-2-(nitromethyl)pentyl)-4-nitroisoxazole (2.7)

As previously mentioned, compound **2.6** can successfully undergo Michael addition of nitromethane at the β-carbon due to the electron-withdrawing effect of the 4-nitro-isoxazole moiety that activates **2.6** towards 1,6-conjugate additions of soft nucleophiles (**Scheme 2.13**).



Scheme 2.13 - 1,6-conjugate addition of nitrometane to **2.6**.

Scheme 2.14 depicts reaction mechanism.



Scheme 2.14 - 1,6-conjugate addition mechanism.

The reaction produces a chiral center on the β-carbon. When the reaction is not under stereocontrol, the outcome is a racemate.

2.2.2 - Enantioselective catalysis of 1,6-conjugate addition

Nitromethane does not contain any chiral center that can bring stereochemical induction on 1,6-conjugate addition and **2.6** is prochiral. The three-point interaction model says that for an asymmetric catalyst to be of any use, it must interact with (at least) three positions of the transition state (**Figure 2.1**).

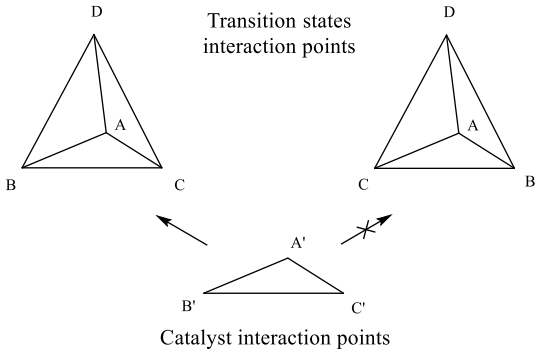
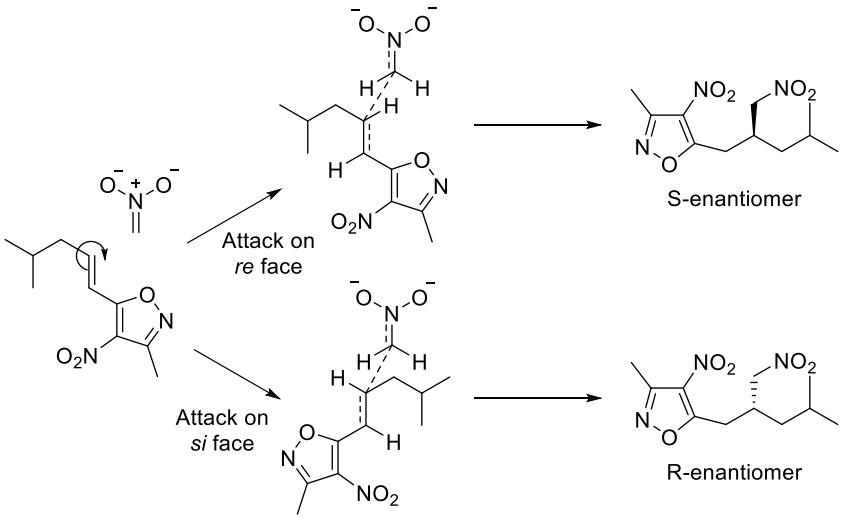


Figure 2.1 - Three-point interaction model for asymmetric catalysis.

Interactions can be of any kind, and either attractive (*e.g.* hydrogen bonds) or repulsive (*e.g.* steric repulsions), but at least one attractive interaction is needed for catalysis to take place.



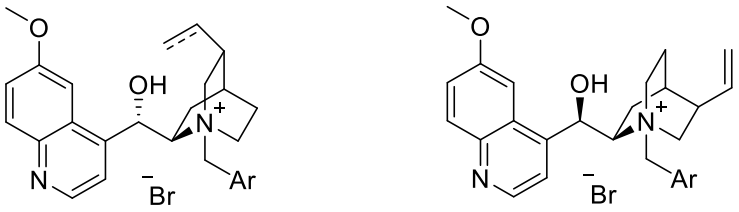
Scheme 2.15 - Stereoconfiguration of 1,6-conjugate addition transition states.

The generation of the chiral center arises from attack of nitronate to the two enantiotopic faces of prochiral **2.6**. **Scheme 2.15** shows the two possible enantiomeric transition states of the reaction. The stronger the catalyst interacts with the substrate, the more the catalytic

mechanism will prevail over the non-catalytic one and so the higher the enantiomeric excess. The more a chiral catalyst manages to differentiate with his interaction the two transition states, the higher the enantiomeric excess, again.

2.2.3 - Mono-quaternized cinchona alkaloids catalyzed enantioselective 1,6-conjugate addition

The classes of catalysts in **Figure 2.2** showed good enantioselection over 1,6-conjugate addition.

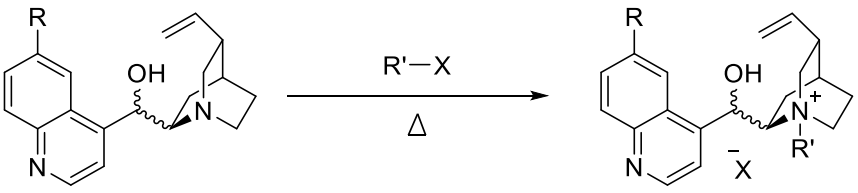


2.12 **2.13**

Figure 2.2 - Generic mono-quaternized *cinchona* alkaloids for asymmetric catalysis of 1,6-conjugate addition.

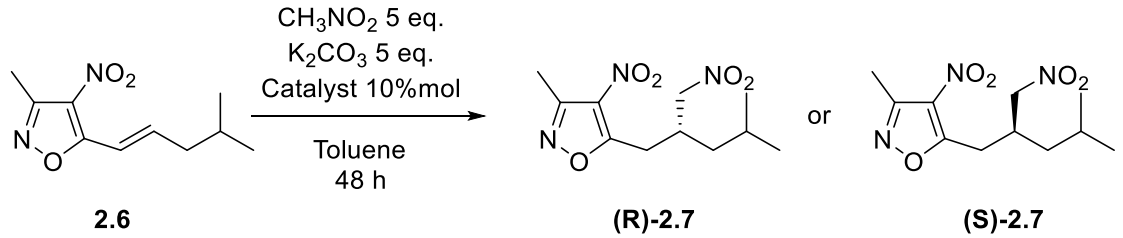
Catalysts **2.12** and **2.13** are mono-quaternized *cinchona* alkaloids and act as phase-transfer catalysts: their ability to interact with the substrates is due to electrostatic interaction of the quaternary ammonium salt as well as H-bonding interaction through the hydroxyl and benzyl groups.

Mono-quaternized *cinchona* salts are obtained from reaction of the respective *cinchona* alkaloid and a benzyl halide (**Scheme 2.16**). Reactants are dissolved in neutral conditions at high temperature to give the salt.



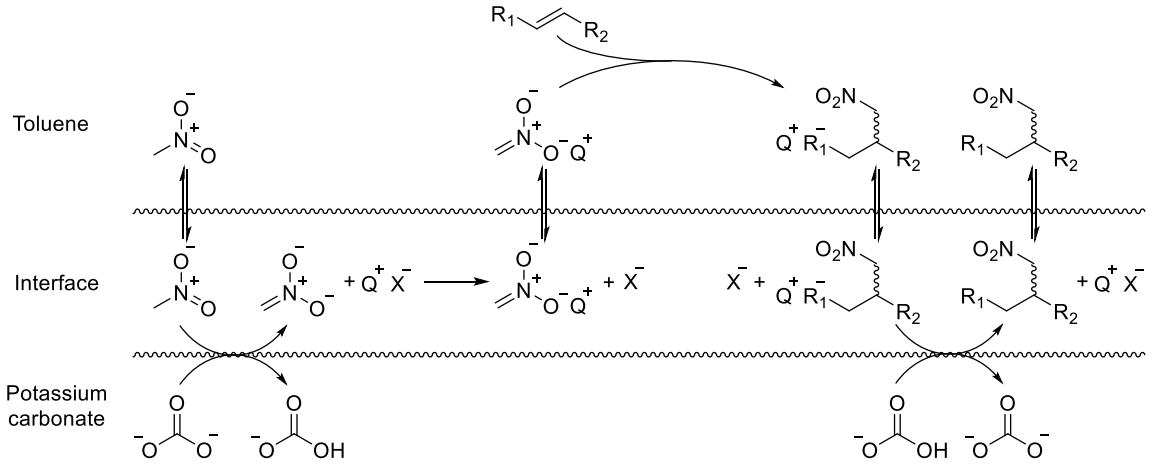
Scheme 2.16 - Preparation of mono-quaternized *cinchona* alkaloids.

For the Michael addition were followed the reaction conditions already reported, reacting **2.6** with nitromethane and potassium carbonate in toluene (**Scheme 2.17**).



Scheme 2.17 - Catalytic enantioselective 1,6-conjugate addition.

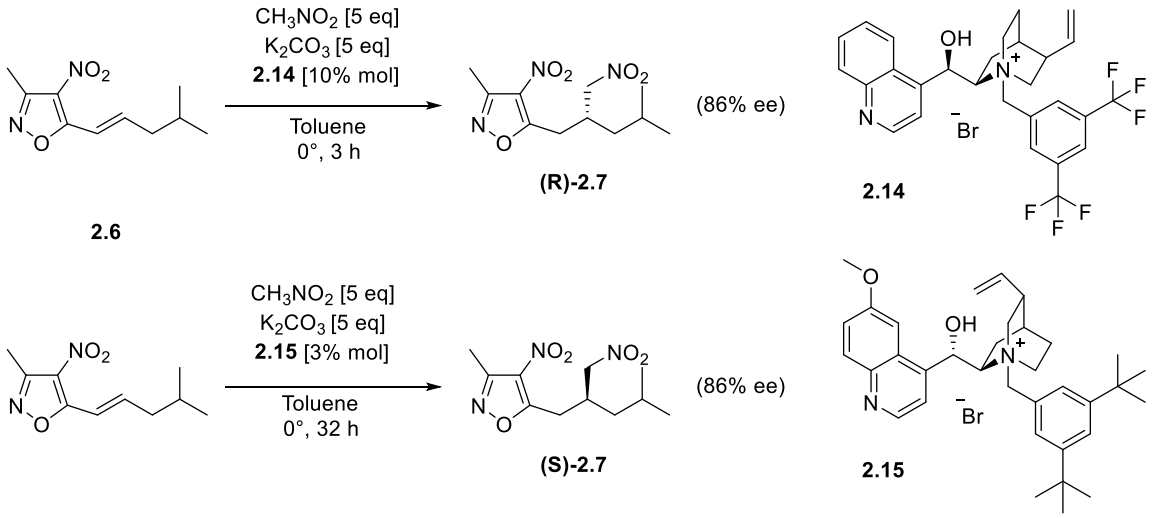
Under phase-transfer catalysis, 1,6-conjugate addition follows the mechanism in **Scheme 2.18**.



Scheme 2.18 - Mechanism of catalytic enantioselective 1,6-conjugate addition.

2.2.4 - Results

Scheme 2.19 reports the best results obtained from catalysts surveys on 1,6-conjugate addition.



Scheme 2.19 - Best results for enantioselective 1,6-conjugate addition.

In particular, it was demonstrated that the enantioselective step for obtaining the S-enantiomer could be successfully scaled-up affording the product on a 10 kg scale, with **2.15** immobilized on sulfonated polystyrene beads to improve their recyclability, keeping a 72% *ee*.[3]

2.3 - Doubly-quaternized *cinchona* alkaloids catalyzed enantioselective 1,6-conjugate addition

2.3.1 - Doubly-quaternized cinchona alkaloids

There are two different approaches to the synthesis of doubly-quaternized *cinchona* alkaloids:

1 - The quaternization reaction is repeated on a mono-quaternized catalyst, this time heating with 2 equivalents of alkyl halide in a more polar solvent, which will lead to quaternization of quinoline nitrogen.

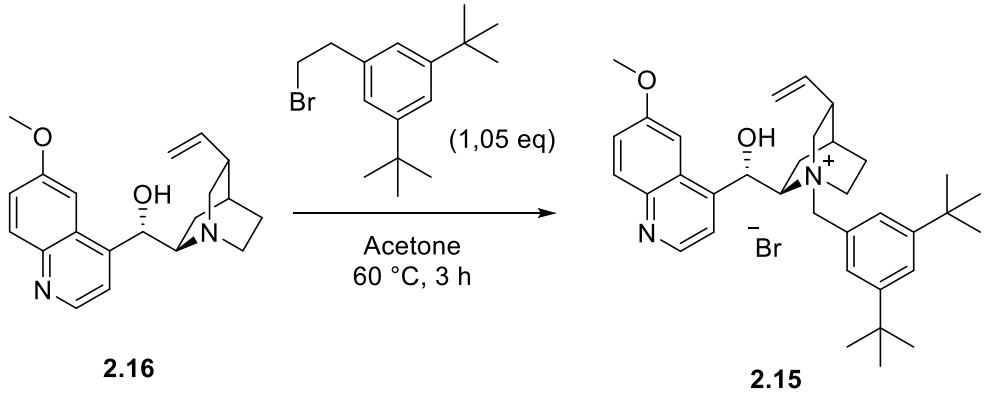
2 - In case the attached groups are the exact same, the first quaternization can be skipped. Running the reaction with 2.5 equivalents of alkyl halide in a more polar solvent and for a longer time affords directly the doubly quaternized catalyst.

As catalysts, they are used in pretty much the same way as mono-quaternized *cinchona* alkaloids, but there is reason to think that mechanism of action of these salts is quite different from the one of the mono-quaternized counterparts, as results obtained for the same substrates are so different.

2.3.2 - Doubly-quaternized cinchona alkaloids catalyzed enantioselective 1,6-conjugate addition

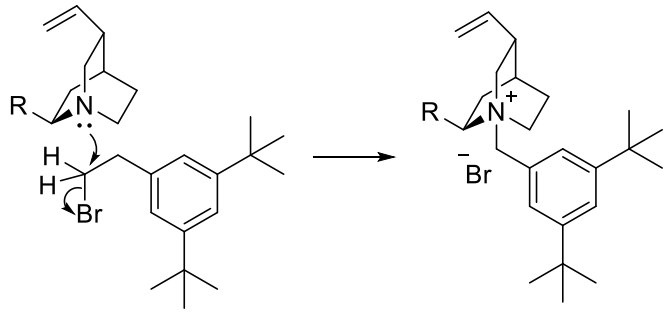
This section reports the route of doubly-quaternized *cinchona* alkaloids catalyzed enantioselective 1,6-conjugate addition testing. As mono-quaternized quinidines are the ones that catalyze the formation of **(S)-2.7**, quinidine **2.16** was the alkaloid employed; as **2.15** was the mono-quaternized quinidine with best *ee*s, that was used as a basis for doubly-quaternized catalysts.

Preparation of catalyst 2.15 - The synthesis of quinidinium bromide **2.15** was accomplished following the procedure reported in the literature,[2] which starts from quinidine **2.16** involving acetone as a solvent and an operating temperature of 60 °C held for 3 hours (**Scheme 2.20**).



Scheme 2.20 - Preparation of **2.15**.

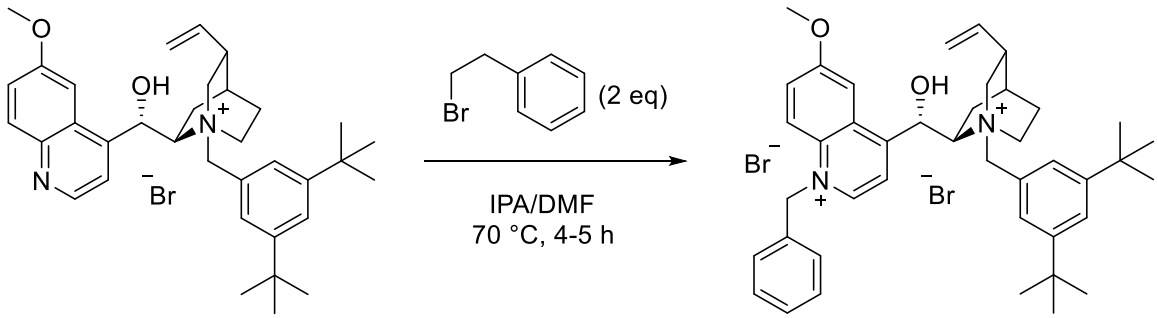
Scheme 2.21 depicts the simple reaction mechanism.



Scheme 2.21 - Mechanism for **2.15** preparation.

Crude obtained from solvent evaporation was then purified by silica gel column chromatography, eluting with a DCM:MeOH mobile phase.

Preparation of catalyst 2.17 - The first catalyst tested was the one obtained by adding a simple benzyl to **2.16**. Catalyst **2.17** was prepared following Xiang's procedure, using a mixture of isopropylalcohol and dimethylformamide as a solvent, heating to 70 °C for 4-5 hours (**Scheme 2.22**).



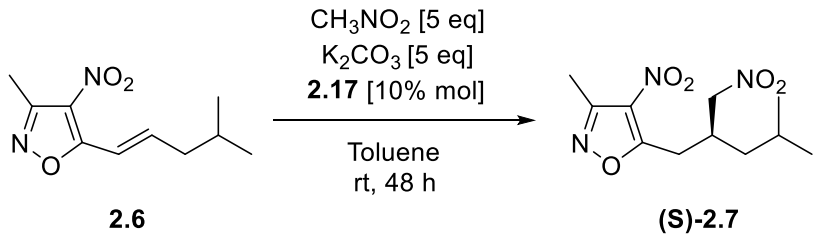
2.15 **2.17**

Scheme 2.22 - Synthesis of **2.17**.

As TLC analysis after said time showed low conversion of starting material, the reaction was left overnight.

Work up involved addition of the reaction mixture to ethyl acetate, with theoretical formation of a slurry to be filtered, but an oil formed instead. Addition of hexane fastened the product, which was recovered in moderate yield (59%).

2.17 catalyzed enantioselective 1,6-conjugate addition – After evaporation a solid was obtained, which was tested as a catalyst in 1,6-conjugate addition at room temperature for 2 days (**Scheme 2.23**).



2.6 **(S)-2.7**

Scheme 2.23 - **2.17** catalyzed enantioselective 1,6-conjugate addition.

The reaction was run in three variants:

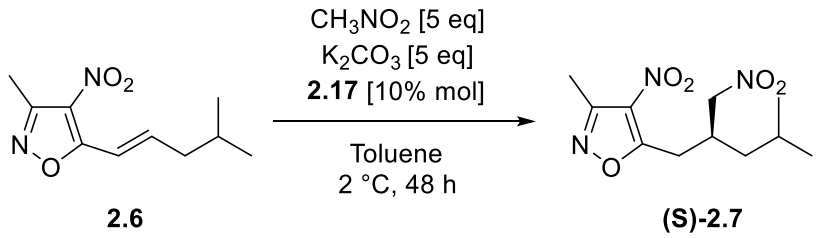
1 - 0,03 M: conversion extremely low after two days, inapplicable.

2 - 0,1 M: moderate conversion, moderate yield (59%), moderate *ee* (54% calculated in comparison of a racemic sample, obtained with achiral TBAB catalysis).

3 - 0,1 M in DCM: moderate conversion, moderate yield (52%), low *ee* (10%).

For the next tests, conditions number 2 were taken as a standard.

2.17 catalyzed enantioselective 1,6-conjugate addition at low temperature - The same reaction was repeated at low temperature, in order to see if in a less energetic system **2.17** was able to better discriminate between the two enantiomeric transition states (**Scheme 2.24**).

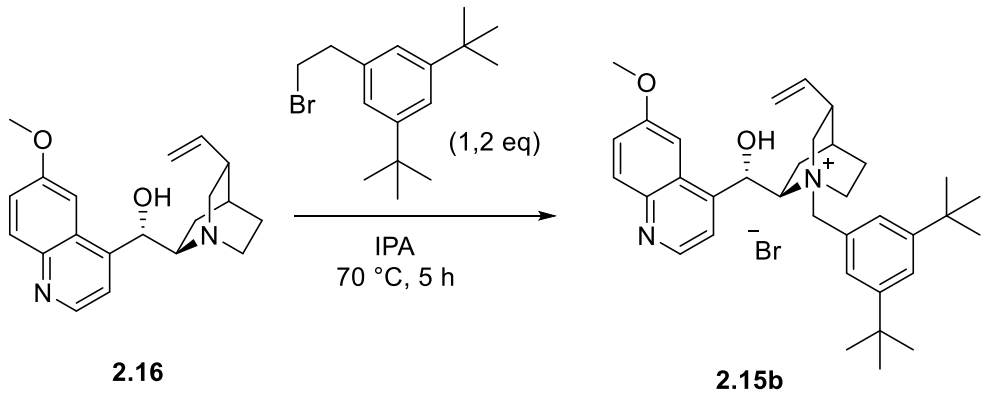


2.6 **(S)-2.7**

Scheme 2.24 - **2.17** catalyzed enantioselective 1,6-conjugate addition at low temperature.

Results obtained did not significantly differ from the previous ones.

Alternative synthesis of catalyst 2.15 (2.15b) - Possible effect of reaction impurities was tested by following Xiang's procedure for both mono- and bis-quaternization.[4] **2.15** was so resynthesized in IPA (**Scheme 2.25**).



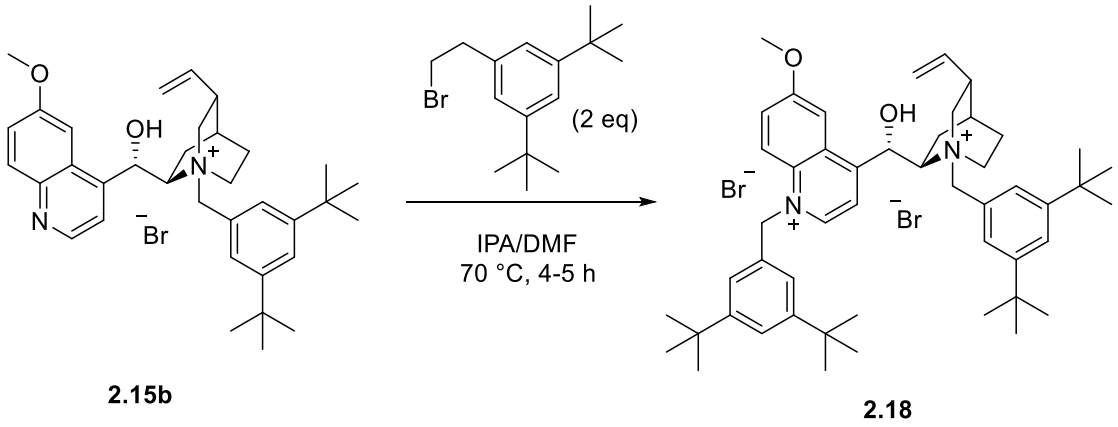
2.16 **2.15b**

Scheme 2.25 - Alternative synthesis of **2.15**.

This **2.15b** obtained (76%) was quaternized again same way as before, leading to **2.17b**. Work up again lowered yield (61%). Solid obtained was more crystalline with a slightly brighter color.

2.17b catalyzed enantioselective 1,6-conjugate addition - 2.17b obtained from alternative synthesis was tested on 1,6-conjugate addition, at both room and low temperatures; the outcome did not differ that much from previous tests. Therefore, no relevant impurity effect exist.
Results show how **2.17** is not a good catalyst for 1,6-conjugate addition, as low conversion indicates that it is not a good phase-transfer catalyst for this reaction, and it also has not a good enantiocontrol over the reaction.

Preparation of 2.18 - 2.15b was then used as a starting material for preparation of **2.18**, which has the same attached groups on both nitrogens. Reaction conditions are identical to the ones used in **2.18** preparation (**Scheme 2.26**).



Scheme 2.26 - Synthesis of **2.18**.

The reaction again was lengthen and let overnight, and same work up problems were observed (55%).

2.18 catalyzed enantioselective 1,6-conjugate addition - 1,6-conjugate addition was repeated again using **2.18** as a catalyst at both room and low temperatures.

This time yields were comparable to the previous ones, but pleasingly the increase in *ee* was remarkable: at room temperature *ee* raised to 70%, whereas at low temperature arrived to 77%. However, those results are just average results for a mono-quaternized catalyst on 1,6-conjugate addition.

In order to better understand the mechanism of catalysis, the effect of an additional bulky substituent on the quinoline-linked oxygen was tested, so the target molecule was **2.19** (**Figure 2.3**).

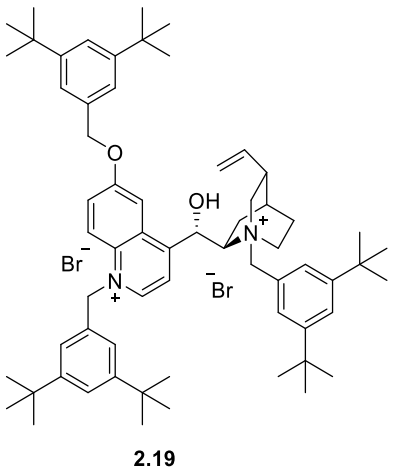


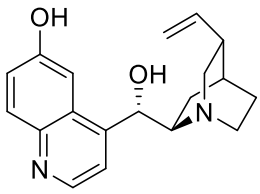
Figure 2.3 - Target molecule **2.19**.

2.19 has the same group on the oxygen as the ammonium and pyridinium nitrogens.

Protection of 2.16 - To prevent alkylation of oxygen on the bridge between quinuclidine and quinoline, protection of quinidine's hydroxyl was considered. Protection is usually accomplished by conversion to silyl ether (TBDMSO-).

As this would lower overall yield, considering also the deprotection step, and as literature reported that O-alkylation occurs at the aromatic hydroxyl quantitatively before the alkyl one (mechanism of alkylation involves the formation of an alkoxide, and an aromatic alcohol is usually quite more acidic than an alkyl one), protection of quinidine was not brought about. Moreover, drastic demethylation condition might deprotect the hydroxyl. After unprotected reaction results, it could be possibly taken in consideration and optimized in case of bad O-alkylation results and good 1,6-conjugate addition results.

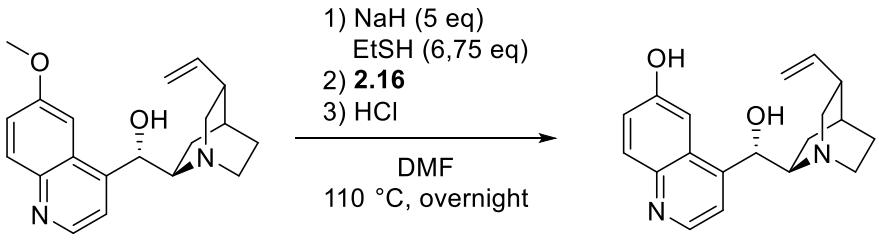
Synthesis of 2.20 - The first step in unprotected synthesis is O-demethylation (cleavage of a methyl ether) of quinidine to afford a *cinchona* derivative commonly known as cupreidine **2.20** (**Figure 2.4**).



2.20

Figure 2.4 - Cupreidine.

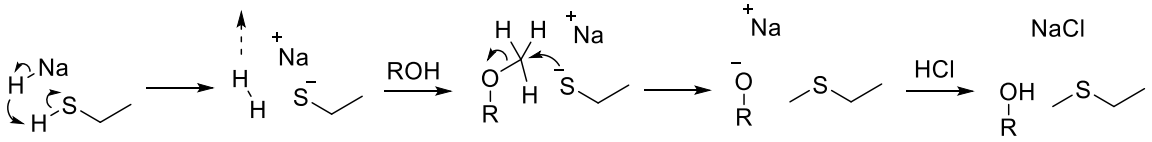
Demethylation reaction was run following Deng's procedure.[5] At first NaSEt is produced in situ, then quinidine is added in dry DMF at 110 °C (**Scheme 2.27**).



2.16 **2.20**

Scheme 2.27 - **2.20** preparation.

Scheme 2.28 depicts reaction mechanism.

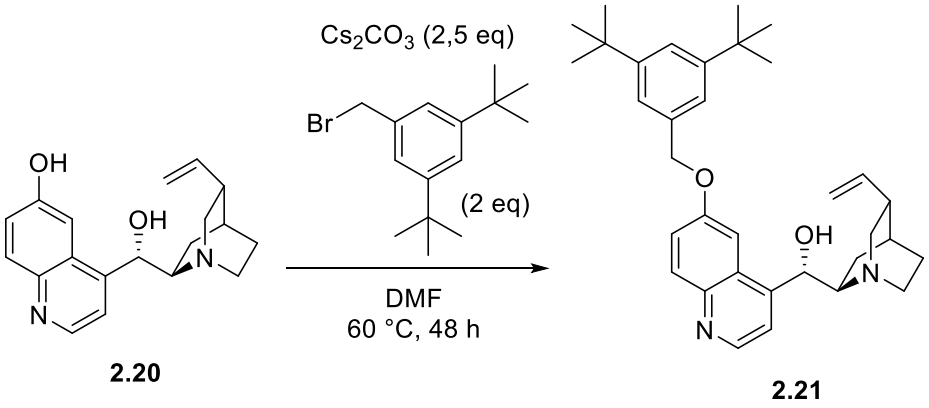


Scheme 2.28 - Mechanism for **CPD** preparation.

The reaction is favored in that a thiolate is a better nucleophile than an alkoxide. The reaction proceeded in good yield (75%).

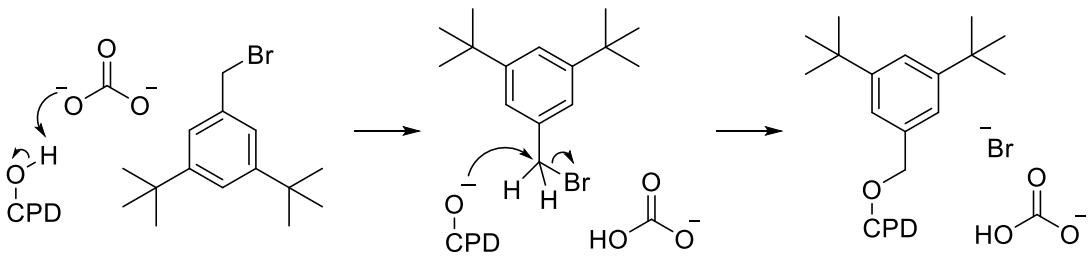
Synthesis of 2.21 - Cupreidine **2.20** was then selectively alkylated according to the procedure of Albrecht Berkessel *et al.*.[6]

To deprotonate the aromatic hydroxyl, cesium carbonate was used and the reaction was left in DMF at 60 °C for two days (**Scheme 2.29**).



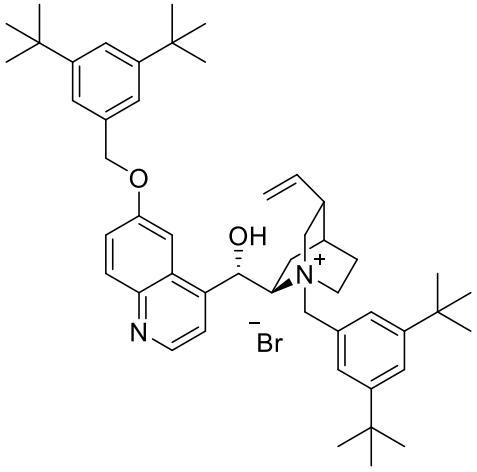
Scheme 2.29 - 2.20 alkylation.

Scheme 2.30 depicts reaction mechanism.



Scheme 2.30 - Mechanism for **2.20** alkylation.

The reaction was not clean: TLC analysis showed a mixture products, mostly superimposed. The major product was not the expected **2.21** but instead its ammonium salt **2.22**.



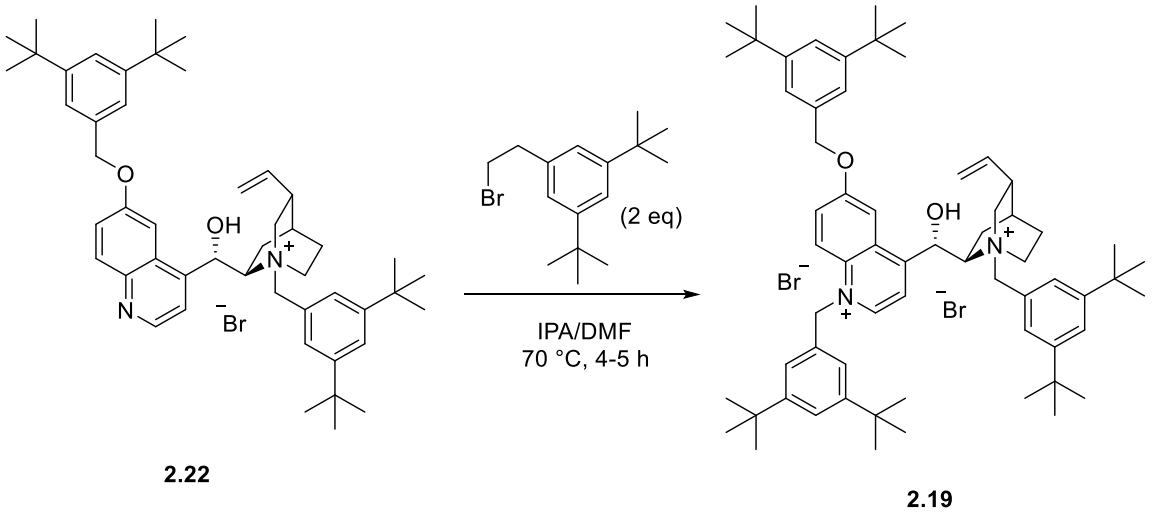
2.22

Figure 2.5 - Catalyst **2.22**.

This is because operating conditions were similar to the ones for quaternization, so the reaction did not stop at alkylation but carried on to the mono-quaternized product **2.22**. For the two consecutive reaction and harsh separation, the yield was low (42%, and only 2 equivalents of alkyl bromide were added).

2.22 catalyzed enantioselective 1,6-conjugate addition - **2.22** obtained was directly tested as a catalyst in 1,6-conjugate addition. The reaction was run at 2 °C and afforded the product in 91% yield and 78% *ee*, comparable to the results of the other mono-quaternized quinidinium catalysts.

Synthesis of 2.19 - Catalyst **2.22** was then subjected to a second quaternization following the same procedure as before. (**Scheme 2.31**).



2.22 **2.19**

Scheme 2.31 - Synthesis of **2.19**.

The reaction produced some by-products, and the desired product **2.19** was obtained in low yield (51%).

2.19 catalyzed enantioselective 1,6-conjugate addition - The reaction once again was run only at low temperature. The yield dropped to a comparable yield to the ones obtained with a doubly-quaternized catalyst **2.19** (62%), while the *ee* was 65%.

2.3.3 - Recap

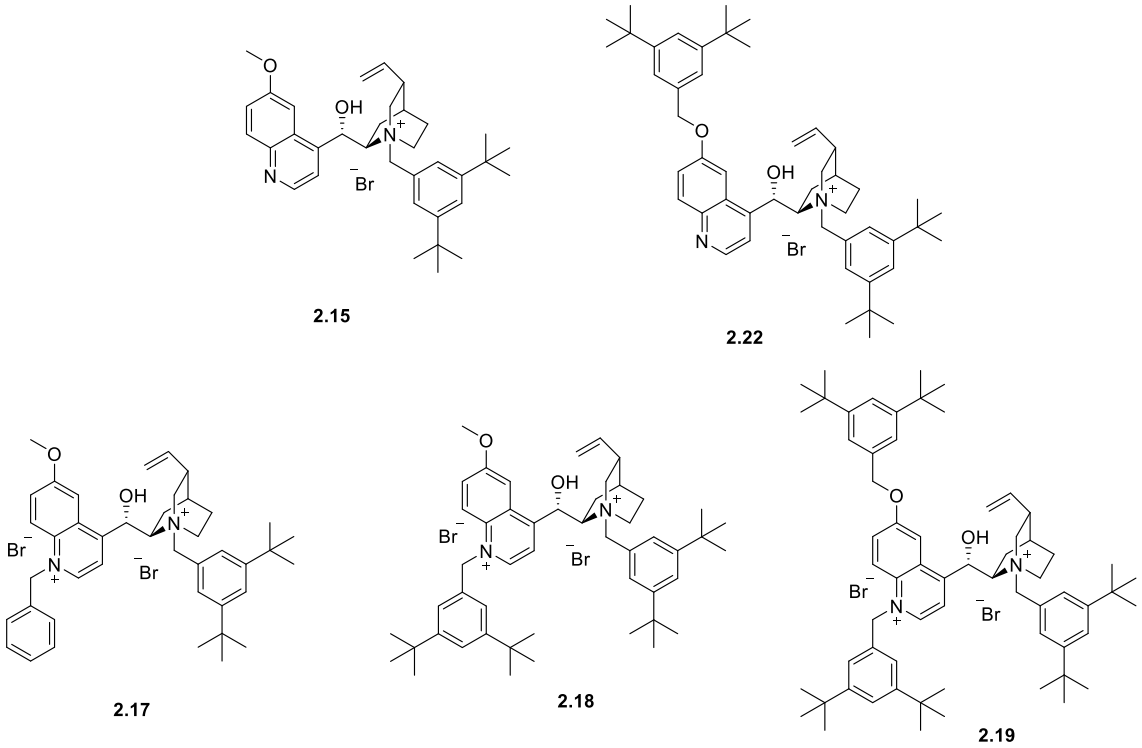


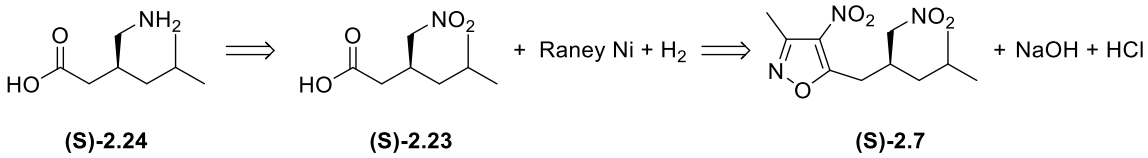
Figure 2.6 - Catalysts employed in 1,6-conjugate addition.

Entry	Catalyst	Degree of quaternization	Temperature [°C]	Yield [%]	ee [%]
1	**2.15**	Mono	2	97	86
2	**2.22**	Mono	2	91	78
3	**2.17**	Bis	RT	59	54
4	**2.17**	Bis	2	56	56
5	**2.17b**	Bis	RT	57	54
6	**2.17b**	Bis	2	59	57
7	**2.18**	Bis	RT	55	71
8	**2.18**	Bis	2	54	77
9	**2.19**	Bis	2	62	65

Table 2.1 - Data collected after the investigations.

2.4 - Later synthetic steps

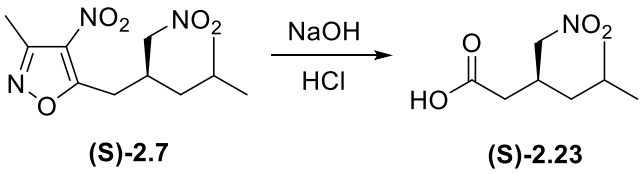
The remaining part of (S)-pregabalin retrosynthetic analysis is shown in **Scheme 2.32**.



Scheme 2.32 - (S)-Pregabalin retrosynthesis from recrystallized **(S)-2.7**.

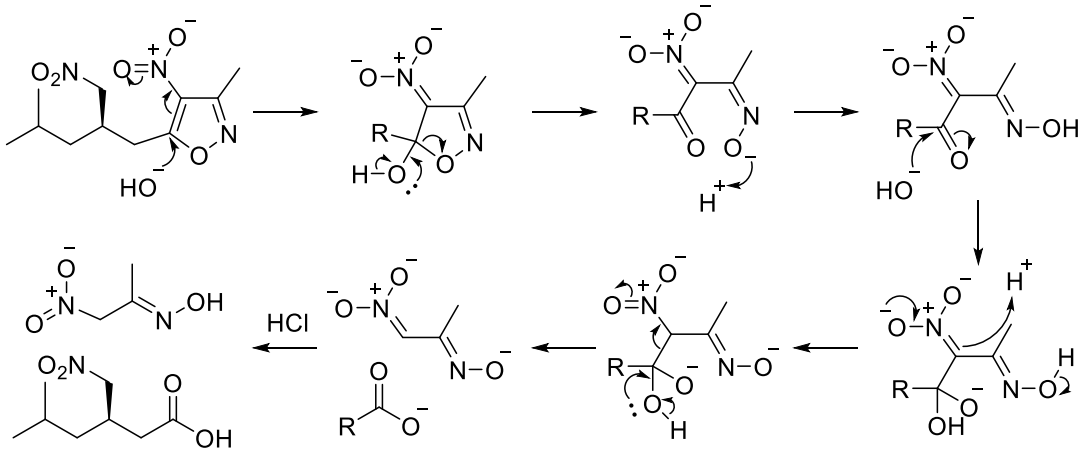
2.4.1 - Preparation of (S)-3-methyl-5-(4-methyl-2-(nitromethyl)pentyl)-4-nitroisoxazole ((S)-2.23)

Isoxazole nucleus can be cleaved *via* Sarti-Fantoni reaction, specific for 4-nitroisoxazoles.[7] Hydrolysis by soda followed by neutralization with hydrochloric acid affords carboxylic acid **(S)-2.23** (**Scheme 2.33**).



Scheme 2.33 - Synthesis of **(S)-2.23**.

Scheme 2.34 depicts reaction mechanism.



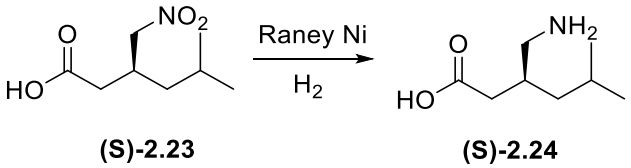
Scheme 2.34 - Mechanism for synthesis of **(S)-2.23**.

Evidence of this mechanism is the presence of ^{18}O in both carboxylic oxygens when labelled $Na^{18}OH$ is used instead of NaOH.[7]

The reaction does not involve the chiral center, so the enantiopurity is retained in the product.

2.4.3 - Preparation of (S)-pregabalin ((S)-2.24)

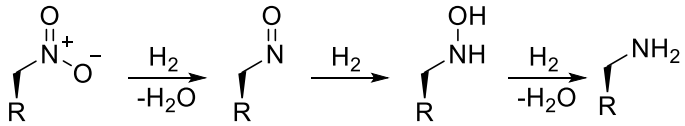
Hydrogenation of nitro group by Raney Ni leads progressively to primary amine **(S)-2.24** (**Scheme 2.35**).



(S)-2.23 **(S)-2.24**

Scheme 2.35 - Preparation of (S)-pregabalin.

Scheme 2.36 depicts reaction stages.



Scheme 2.36 - Stages in **(S)-2.23** reduction.

2.5 - Conclusions

Yields with doubly-quaternized catalysts are always lower than the ones with mono-quaternized catalysts.

When for the same reaction time the yield is lower, it means that the reaction is slower, which may imply that doubly-quaternized catalysts kinetically promotes the reaction less than mono-quaternized catalysts do. The reaction is catalyzed anyway, as the obtainment of an enantiomeric excess demonstrates, but modifications brought about by the second quaternization influence negatively the reaction, or better, doubly-quaternized do not influence the reaction as good as mono-quaternized catalysts do. Different explanation can be found for this:

A - The presence of an additional charged nitrogen might add a new interaction that creates a distortion in the transition state, increasing its energy and slowing down the reaction. In addition, the enantiomeric transition states may have more similar energies, so enantioselection is lower. The newly charged nitrogen might even create an ion pair with the nitronate itself, starting a parallel catalysis pathway, with lower or no enantioselection;
B - The presence of the newly attached group might warp the transition states for steric reasons, with same effects as A;
C - The presence of the newly attached group might hinder the approach of nitronate to the catalyst, or of **2.6** to nitronate already bind to the catalysts.
Moreover, catalysts employed are not simple catalysts, but phase-transfer ones. This means that the overall reaction speed depends not only on the reaction rate itself but also on mass transfer rates, which should be influenced by the second quaternization.
To conclude, the doubly-quaternized catalysts synthesized are not suitable for the 1,6-conjugate addition in exam, as yields are low and *ee*s good in some cases but not outstanding.
Further studies on doubly-quaternized catalysts must be carried on to better understand their mechanism of action and apply rational modification to quinidine structure for improving yields and enantioselections.
The presence of an additional attached group implies an additional synthetic step, which is not that attractive from an industrial point of view, considering also the complex purification of those compounds. Nevertheless, doubly-quaternized catalysts could be a good alternative catalysts in reactions in which the classical quaternary ammonium salts do not produce satisfying results.

References

[1] M. F. A. Adamo, A. Baschieri, L. Bernardi, A. Ricci and S. Suresh, *Angew. Chem. Int. Ed.* **2009**, 48, 9342.

[2] M. F. A. Adamo, M. Moccia and R. J. Wells, *Org. Biomol. Chem*., **2015**, 13, 2192.

[3] M. F. A. Adamo, M. Moccia, M. Cortigiani, C. Monasterolo, F. Torri, C. Del Fiandra, G. Fuller and B. Kelly *Org. Process Res. Dev.* **2015**, 19, 1274.

[4] B. Xiang, K. M. Belyk, R. A. Reamer, N. Yasuda, *Angew. Chem. Int. Ed.* **2014**, 53, 8375.

[5] Y. Liu, B. Sun, B. Wang, M. Wakem and L. Deng *J. Am. Chem. Soc.*, **2009**, *131*, 418.

[6] A. Berkessel, M. Guixa, F. Schmidt, J. M. Neudorfl, J. Lex, *Chem-Eur. J.* **2007**, 13, 4483.

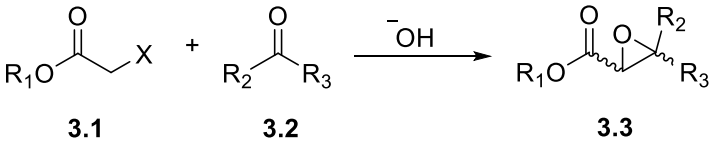
[7] P. Sarti-Fantoni, D. Donati, F. De Sio and G. Moneti, *J. Heterocyclic Chem.*, **1980** 17, 1643.

Chapter 3

Stereoselective Darzens reaction and addition of benzaldehyde to 5-chloromethyl-3-methyl-4-nitroisoxazole

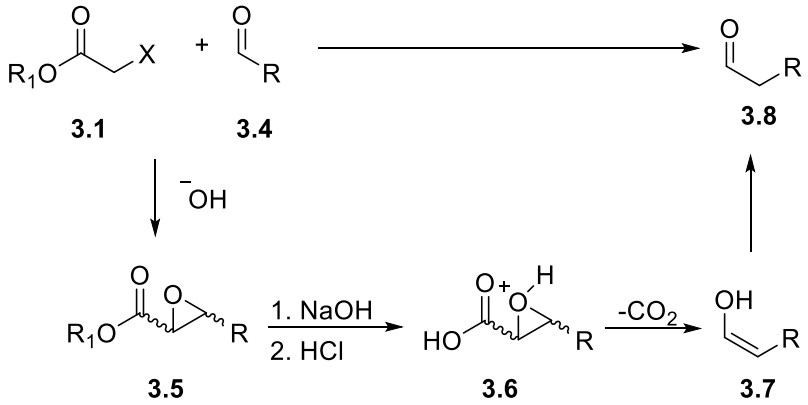
3.0 - Overview

The Darzens reaction, that takes its name from the French chemist who first discover it in 1904,[1] is a method to synthesize α,β-epoxy esters, also called glycidic esters.
This reaction is a particular case of an aldol reaction running under basic conditions between an α-haloester **3.1** as the nucleophilic carbonyl and an aldehyde or a ketone (**Scheme 3.1**). After nucleophilic attack of the enolate to the electrophile **3.2**, an alternative pathway is taken: the oxyanion formed attacks the adjacent carbon, displacing the halogen in an intramolecular S_N2. The result is α,β-epoxy ester **3.3**.



Scheme 3.1 - Darzens reaction.

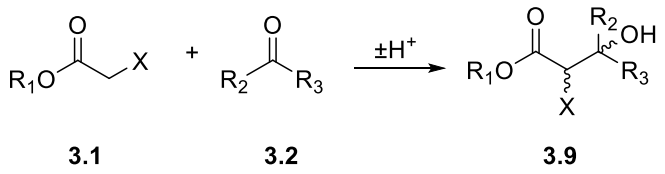
Darzens reaction is often used as homologation reaction for extending carbon chain of one unity from **3.4** to **3.8** (**Scheme 3.2**).



Scheme 3.2 - Darzens reaction as homologation reaction of aldehydes.

Darzens reaction was found out to work also on different substrates. It generally works on molecules containing electron withdrawing groups with good leaving groups on the α-position.

As previously said, the reaction works only under basic conditions (the reaction consumes a base equivalent, so there is no catalysis). Acid-catalysis of the same substrate proceeds without the formation the oxyanion intermediate and the final product is the aldol product, the halohydrin **3.9** (**Scheme 3.3**).

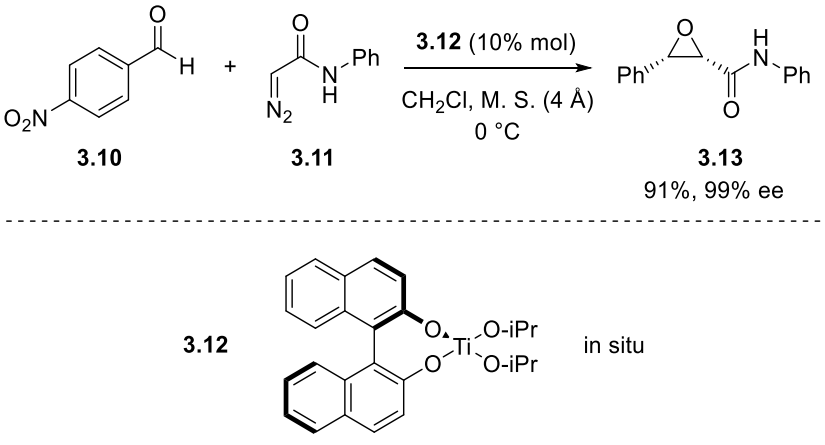


Scheme 3.3 - Acid catalyzed aldol reaction of an α-haloester and an electrophilic carbonyl.

A weak base again affords the halohydrin product, so a strong enough one is needed for the epoxidation.

Dependently on the substrate, in the product up to two chiral centers are formed, more specifically in the α and in the β position with respect to the carbonyl. Stereocontrol can theoretically be applied, although is difficult to obtain high levels of asymmetric induction, as demonstrated by the only few asymmetric Darzens reaction repoted in the literature.[2]

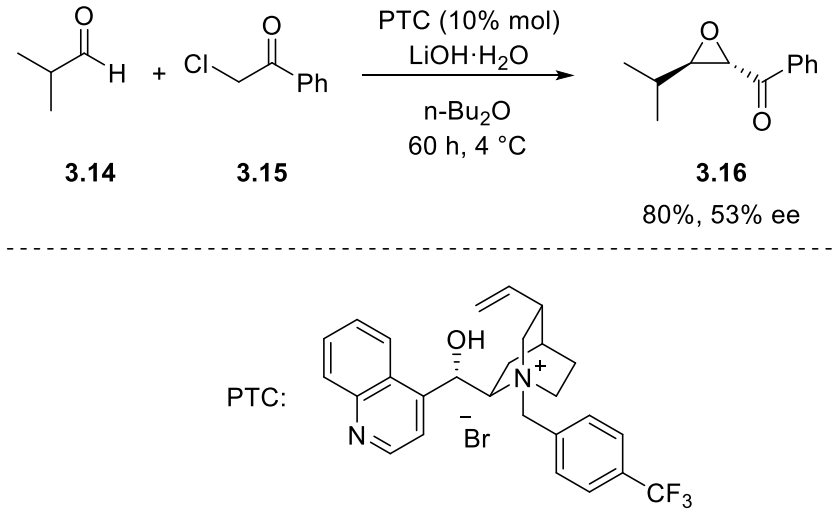
The main reason is that a stoichiometric amount of strongly acidic hydrogen halide is formed during the reaction, and this could both catalyze racemization and neutralize the catalyst.[3] So homogeneous catalysis with a chiral acid or Lewis base is challenging: the only successful example of highly enantioselective asymmetric Darzens reaction of this type is a chiral metal complex-promoted reaction of diazoacetamide with aldehydes (**Scheme 3.4**).[4]



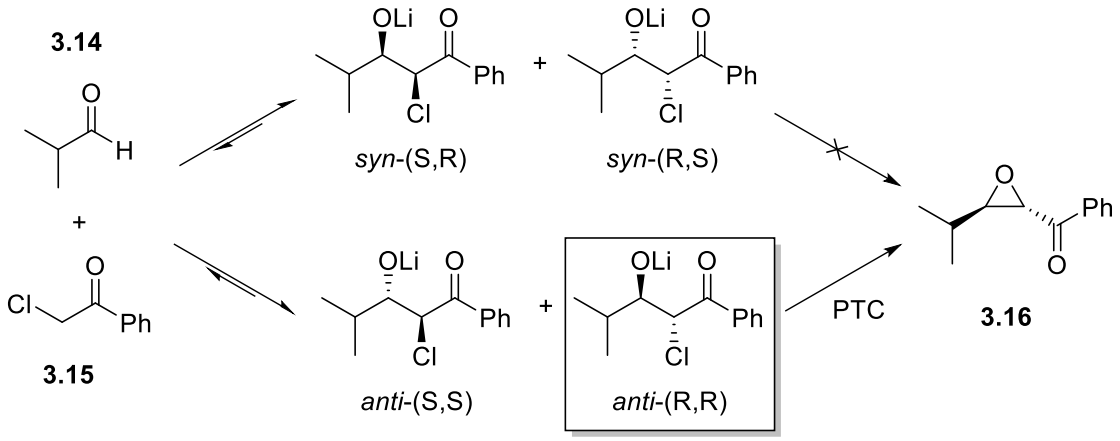
Scheme 3.4 - Asymmetric catalytic Darzens reaction between a diazoacetamide and an aldehyde.

Asymmetric PTC with quaternary onium catalysts proved to be a good method for asymmetric Darzens reactions.[5]

In this case the main challenge is that the stereocontrol in PTC is not applied in the fast carbon-carbon bond forming step, but instead in the kinetic resolution of the racemic mixture of the aldolate, as demonstrated by Arai and co-workers (**Scheme 3.5** and **3.6**).[6]



Scheme 3.5 - Asymmetric catalytic Darzens reaction between α-chloroketone and an aldehyde.

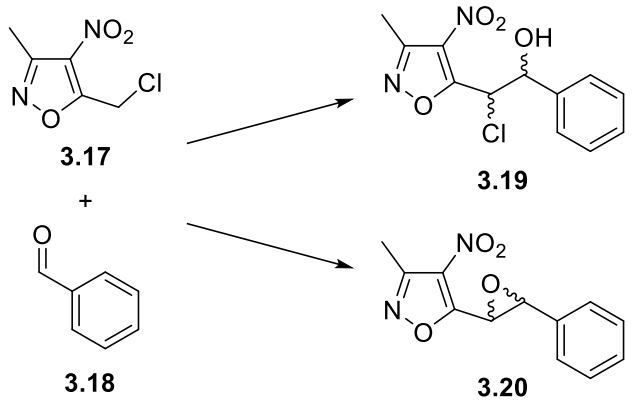


Scheme 3.6 - Kinetic resolution operated by PTC.

The *syn* intermediates cannot result in an epoxide for steric reason. After being formed, they will slowly epimerize by means of retroaldol. On the other hand, both the *anti* intermediates can undergo slow intramolecular carbon-oxygen bond formation, but the

chiral cinchonine-derived catalyst is able to recognize one aldolate over the other and promotes the final cyclization.

This chapter reports the feasibility study of the reaction of 3-methyl-5-chloromethyl-4-nitroisoxazole **3.17** with an aromatic aldehyde like benzaldehyde **3.18** taken as a model, to obtain both the chlorohydrine product **3.19**, and the Darzens product, α,β-epoxide **3.20** (**Scheme 3.7**).

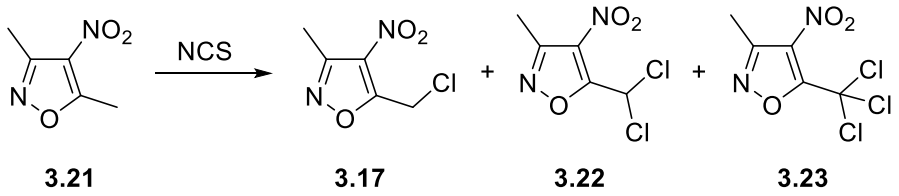


Scheme 3.7 - Reactions of 3.17 and benzaldehyde.

The reactions will be assisted by bifunctional catalysts in the first case and PTCs in the latter in order to check the possibility of stereocontrol.

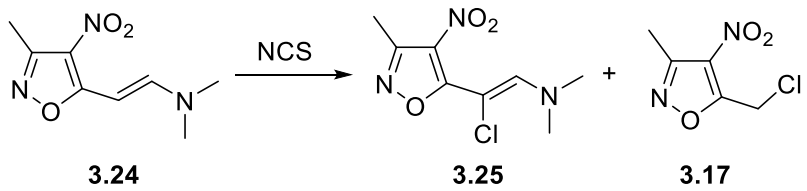
3.1 - Preparation of starting material

The base of the synthesis is the building block 3,5-dimethyl-4-nitroisoxazole, whose preparation had been already described in chapter 2. Monochlorinated compound **3.17** cannot be obtained by direct electrophilic chlorination of **3.21** with N-chlorosuccinimide (NCS) because poly-chlorinated products **3.22** and **3.23** are produced also when substoichiometric amounts of NCS are used (**Scheme 3.8**).



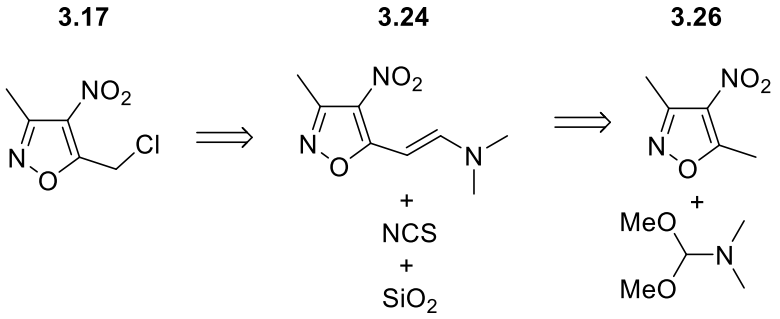
Scheme 3.8 - Direct chlorination of 3,5-dimethyl-4-nitroisoxazole **3.21**.

Thus, compound **3.17** was prepared *via* electrophilic chlorination of the enaminoisoxazole **3.24**, followed by silica-promoted decarbonylation of **3.25**, as reported by Adamo and co-workers (**Scheme 3.9**).[7]



Scheme 3.9 - Degradation of compound **3.25** obtained from enamine **3.24**.

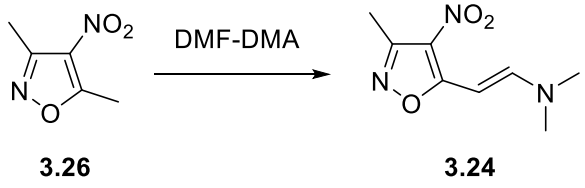
Hence, the synthetic steps leading to starting material for **3.17** are the ones reported in **Scheme 3.10**.



Scheme 3.10 - Retrosynthesis of compound **3.17**.

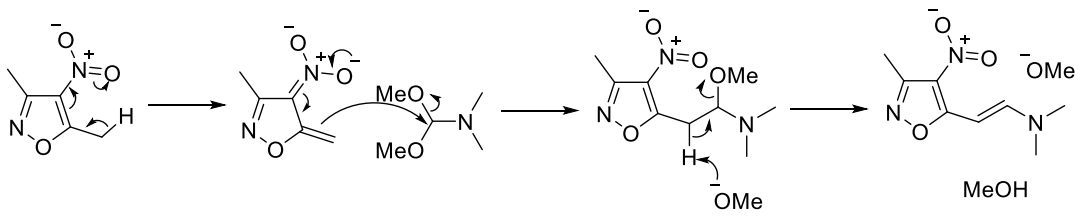
3.1.1 - Preparation of (E)-N,N-dimethyl-2-(3-methyl-4-nitroisoxazol-5-yl)ethen-1-amine (3.24)

The reaction is a nucleophilic attack of **3.26** to dimethylformamide dimethyl acetal to afford enamine **3.24** (**Scheme 3.11**).



Scheme 3.11 - Preparation of **3.24**.

Scheme 3.12 depicts reaction mechanism.

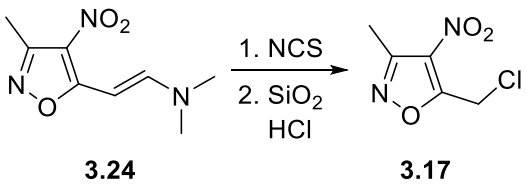


Scheme 3.12 - Mechanism for preparation of **3.24**.

Steric hindrance drives the reaction towards the formation of the only *trans*-isomer, although the presence of any amount of the *cis* isomer is not a problem for the following transformation.

3.1.2 - Preparation of 5-chloromethyl-3-methyl-4-nitroisoxazole (3.17)

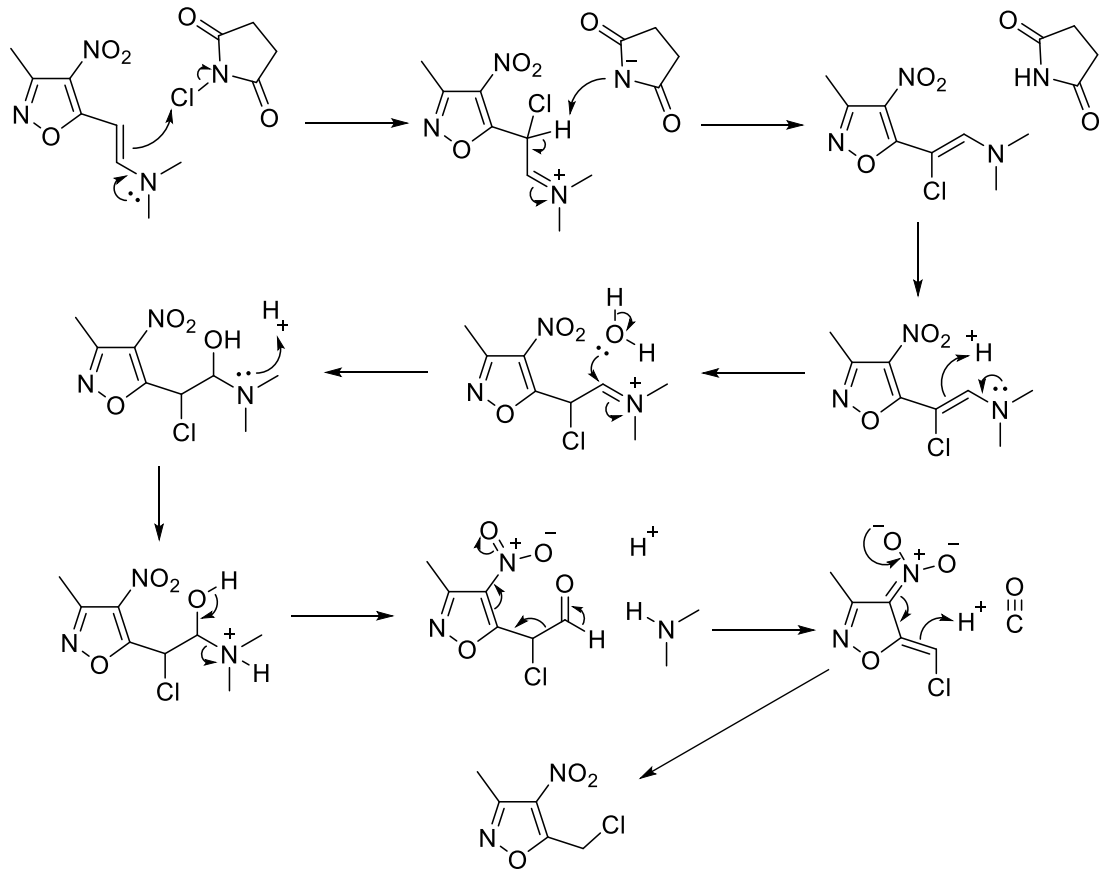
The first part of the reaction involves electrophilic chlorination of enamine **3.24**, while the second is a degradation assisted by a catalytic quantity of hydrochloric acid to afford monochlorinated product **3.17** in one pot (**Scheme 3.13**).



Scheme 3.13 - Preparation of **3.17**.

Scheme 3.14 depicts the proposed reaction mechanism.

In the second part, the acidic environment probably promotes formation of an aldehydic intermediate. After that, heating and the formation of a stable anion promotes decarbonylation of the aldehyde, affording the desired product **3.17**. The reaction indeed takes place over silica gel with a catalytic quantity of hydrochloric acid in a heated rotary evaporator.

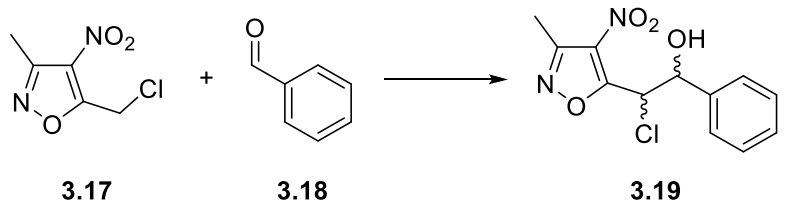


Scheme 3.14 - Proposed mechanism for preparation of **3.17**.

3.2 - Preparation of the addition product

3.2.1 - Preparation of 2-chloro-2-(3-methyl-4-nitroisoxazol-5-yl)-1-phenylethan-1-ol (3.19)

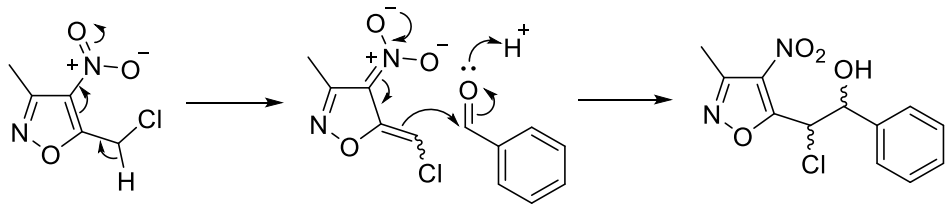
The chlorohydrine was synthesized screening solvents and bifunctional catalysts to promote the reaction. Compound **3.19** was produced by room temperature reaction of **3.17** and benzaldehyde **3.18** in homogeneous system (**Scheme 3.15**).



3.17 **3.18** **3.19**

Scheme 3.15 - Preparation of 3.19.

Scheme 3.16 depicts reaction mechanism.



Scheme 3.16 - Mechanism for preparation of **3.19**.

3.2.2 - Stereoselective catalysis of the reaction

Two new chiral centers are formed, so four are the possible outcomes (**Figure 3.1**).

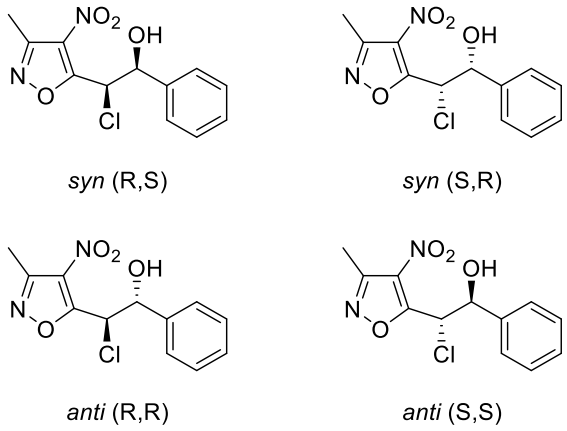
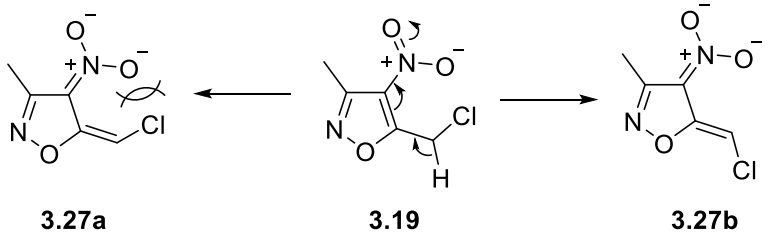


Figure 3.1 - Possible stereoisomers of **3.19**.

The four sites of substitution on the alkene of nitronate are occupied by four different substituents. This means that that nitronate **3.27** can exist in two different forms (**Scheme 3.17**).

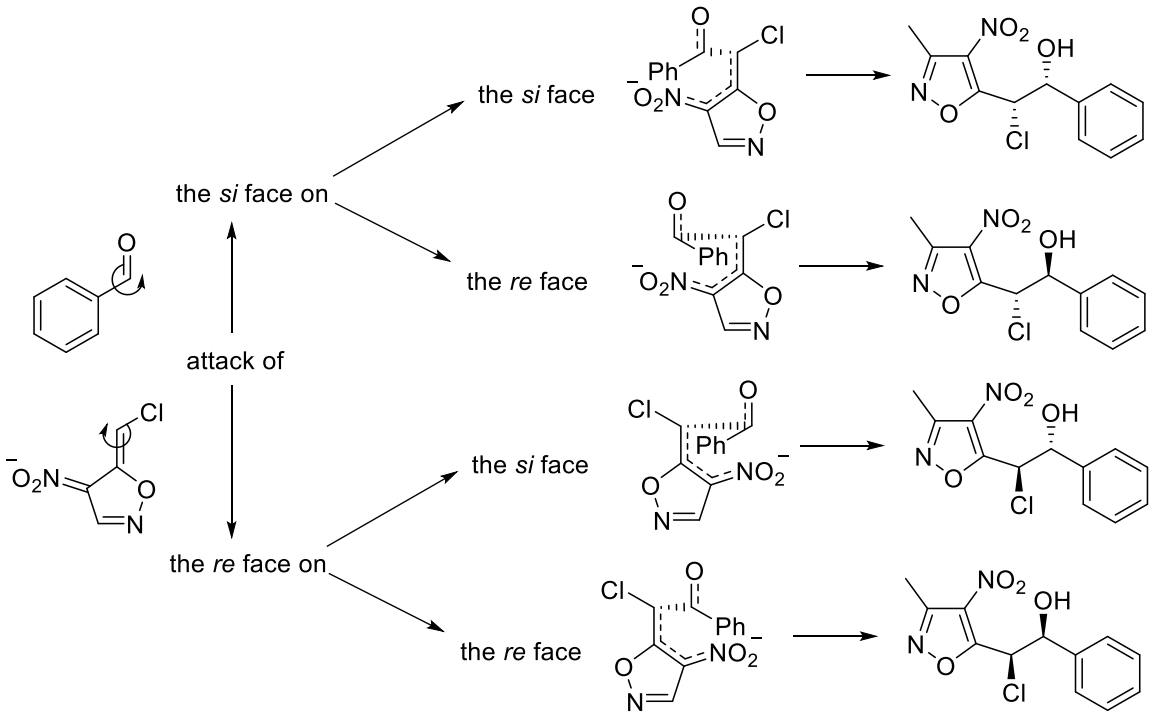


Scheme 3.17 - Nitronates of **3.19**.

As the whole structure of the nitronate **3.27** can be considered as planar, for electronic and steric reasons **3.27b** should be quite favored over **3.27a**. Nevertheless, this statement is backed up by no experimental evidence or computational studies whatsoever.

This could be a problem for stereoselectivity: if a catalyst stereoselectively promotes the attack of a form of the nitronate to benzaldehyde, the attack of the other form could be promoted with lower stereoselection or no stereoselection at all, or even selection towards another stereoisomer.

Assuming that **3.27b** as the major isomer, the four different approaches to benzaldehyde give 4 stereoisomers (**Scheme 3.18**).



Scheme 3.18 - Combination of attacks leading to the 4 stereoisomers of **3.19**.

For nitronate **3.27a**, for same attack as **3.27b** provides the diastereoisomer with opposite chlorine configuration.

The *anti* diastereoisomers should be the prevailing form as the most stable conformation is the one with the two bulky substituents antiperiplanar, and the other substituents in the *anti* diastereoisomers in this conformation are facing opposite ways (**Figure 3.2**).

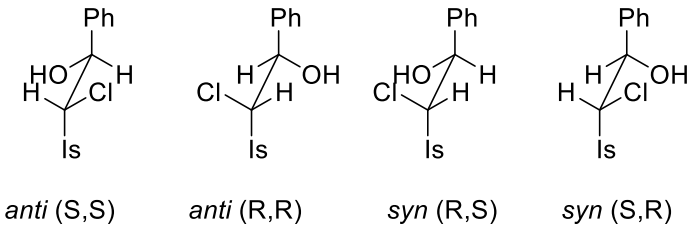
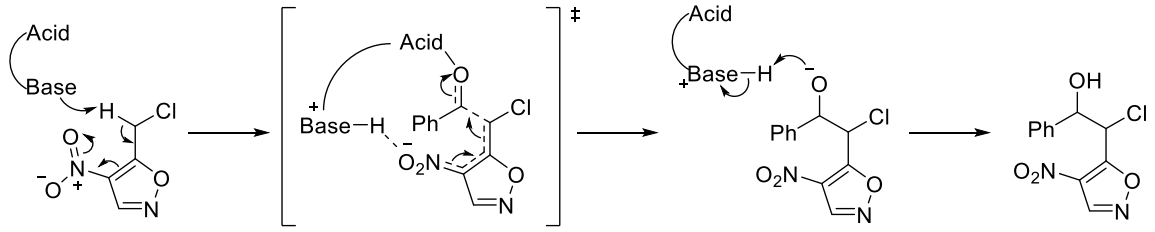


Figure 3.2 - Most stable conformations for stereoisomers of 3.19.

A chiral bifunctional catalyst can promote an approach by binding the nitro group with its basic moiety and the aldehyde with its hydrogen bond donor (**Scheme 3.19**).



Scheme 3.19 Reaction assisted by a bifunctional catalyst.

This specific system has another problem for stereoselectivity, because the α-proton with respect to the isoxazole ring is acidic. Chlorine in α-position even increases its acidity. Therefore, when the product is formed, loss and regain of hydrogen may lead to epimerization. As the time passes, the distribution of the two stereoisomers will tend towards the non-catalyzed one (between diastereoisomers, the hydroxyl's carbon does not change configuration over time) even with successful asymmetric induction from the catalyst.

3.2.3 - Results

The reaction was repeated in three different solvents and six bifunctional catalysts were employed (**Figure 3.3**).

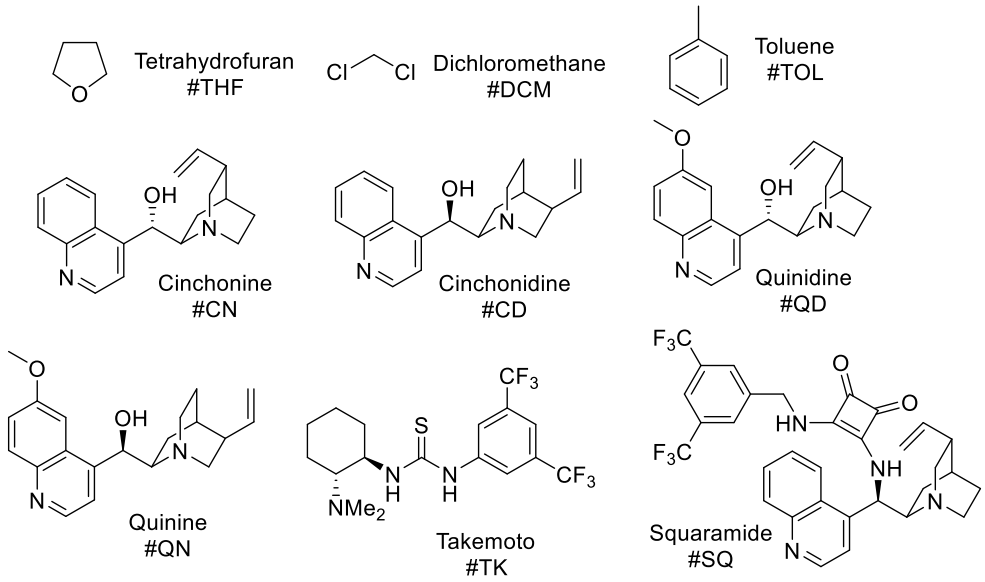
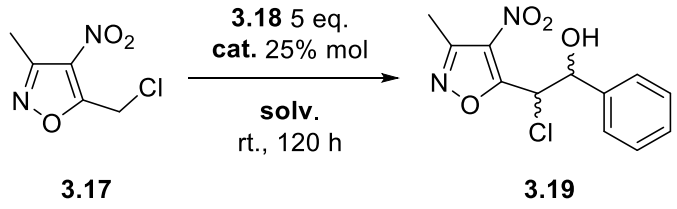


Figure 3.3 - Solvents and catalysts employed.

Scheme 3.20 shows reaction conditions.



Scheme 3.20 - Reaction condition for preparation of 3.19.

Table 3.1 reports the results of the screening.

The reaction proved to be very slow, probably because both of the reactants are bulky and so their approach is not kinetically easy. For this reason, every reaction was let for 5 days. Even after this time, yields were modest, and generally lower when the reaction was in toluene.

Entry	Catalyst	Solvent	Yield	1%	2%	3%	4%
1	None	DCM	/	29,5	20,9	20,9	28,7
2	CN	DCM	0,75	31,2	19,0	19,0	30,9
3		TOL	0,68	30,1	20,2	20,2	29,5
4		THF	0,61	31,8	18,3	18,1	31,8
5	CD	DCM	0,79	26,9	23,4	23,1	26,7
6		TOL	0,63	30,0	19,8	19,2	31,0
7		THF	0,85	31,8	18,3	18,1	31,8
8	QD	DCM	0,74	29,9	20,1	20,3	29,7
9		TOL	0,49	29,5	21,0	21,0	28,5
10		THF	0,74	30,9	19,2	19,2	30,6
11	QN	DCM	0,74	29,3	20,7	20,4	29,6
12		TOL	0,58	29,6	20,2	19,5	30,7
13		THF	0,84	31,3	18,9	18,7	31,1
14	TK	DCM	0,80	27,6	21,4	19,9	31,1
15		TOL	0,68	28,2	21,8	20,9	29,1
16		THF	0,92	25,1	23,2	19,8	31,9
17	SQ	DCM	0,74	34,4	23,9	20,9	20,9
18		TOL	0,58	32,4	27,4	17,2	23,1
19		THF	0,84	32,5	24,9	18,0	24,7

Table 3.1 - Results of the chlorohydrin screening.

A reaction with no catalyst was also run as a reference. A CSP-HPLC method for the determination of relative quantities of each stereoisomer was developed. The four peaks of the adduct from the non-catalyzed reaction can be divided in two groups with similar area, approximatively 29% of the total (1 and 4) and 21% (2 and 3). The two groups should be the two couples of enantiomers, with the major stereoisomers being the *anti* products for the reasons already explained.

The correct approach to the calculation of distribution of stereoisomers takes in account the fact that diasteroisomers of the same molecule may have a different molar attenuation coefficient as the atoms disposition influences the energy of electronic states. A correction can be introduced by calculating the relative distribution of diastereoisomers from NMR

spectra. Ratio between areas of NMR peaks is not affected by the structure, but only by the distribution of diastereoisomers. Enantiomers, on the other hand, in a non chiral environment have the same molar attenuation, so the proportion between their areas in the chromatogram is the proportion of their distribution.

There is no particular good result in asymmetric induction, and there does not seem to be a particular trend in stereoselectivity. Anyway, this result was somehow already expected, mainly because of the acidity of the product that under basic catalysis and over long reaction times probably brings to epimerization. Only catalyst SQ seems to create an unbalance, though not marked. Other possible causes may be:

- Inefficient catalysis, the catalysts are not able to promote the reaction enough to make the reaction happen through them. The distribution of stereoisomers will tend to the non-catalyzed one.

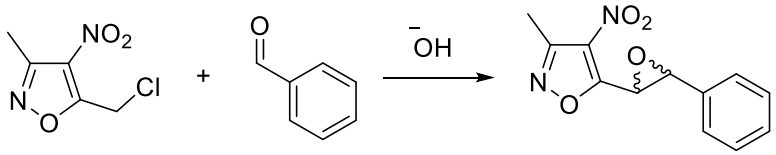
-Undifferentiated catalysis, the catalysts are not able to effectively induct asymmetry by interaction with their chiral elements. So, again, although the reaction is catalyzed, the distribution of stereoisomers will tend to the non-catalyzed one.

-Alternative form of nitronate, the catalyst present a different behavior with a form of the nitronate than the other, with the effects already explained.

3.3 - Preparation of the Darzens product

3.3.1 - Preparation of 3-methyl-4-nitro-5-(3-phenyloxiran-2-yl)isoxazole (3.20)

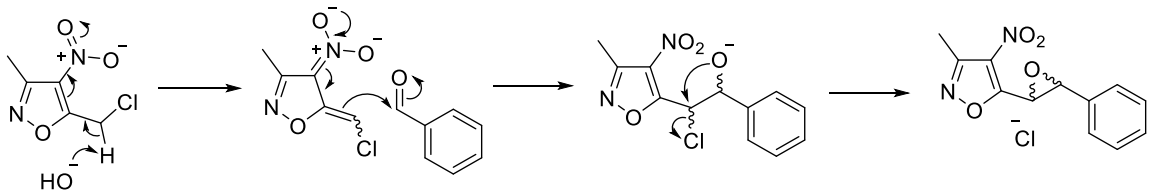
The reaction of **3.17** and **3.18** in presence of solid inorganic bases afforded epoxide **3.20** (**Scheme 3.21**).



3.17 **3.18** **3.20**

Scheme 3.21 - Preparation of **3.20**.

Scheme 3.22 depicts reaction mechanism.



Scheme 3.22 - Mechanism for preparation of **3.20**.

3.3.2 - Stereoselective catalysis of the reaction

Two chiral centers are formed, so once again four are the possible products (**Figure 3.4**).

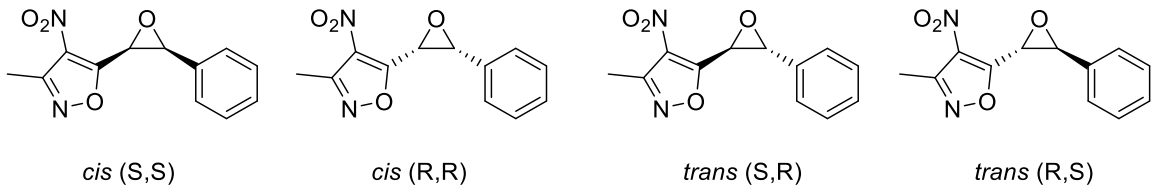
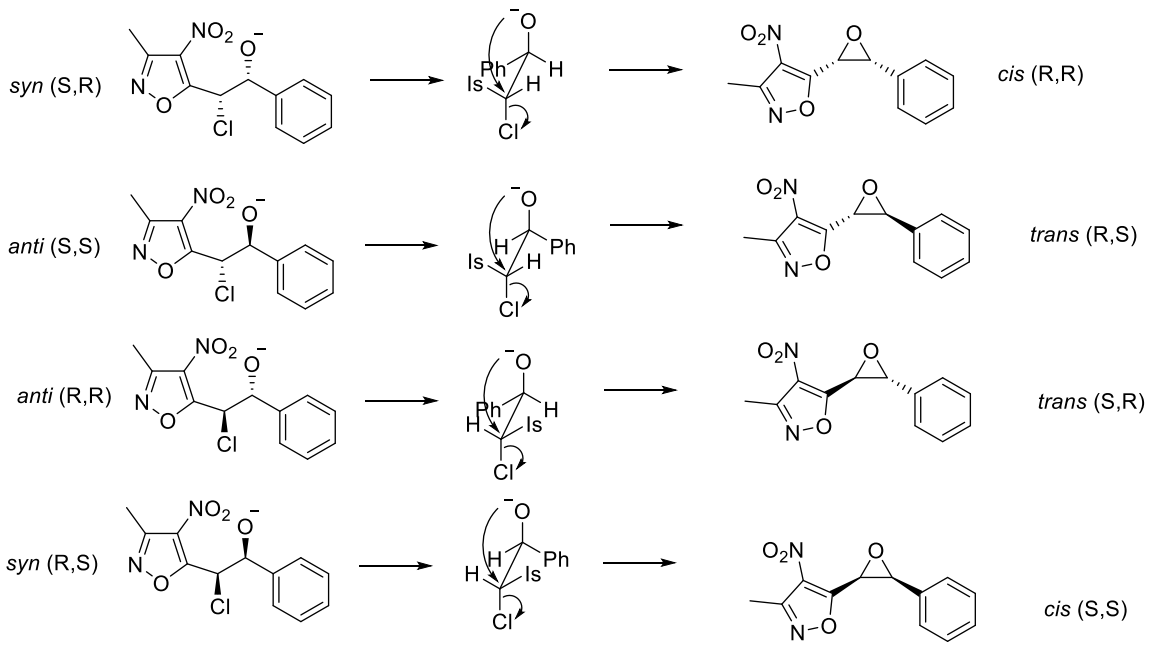


Figure 3.4 - Stereoisomers of **3.20**.

The two chiral centers are decided with addition reaction in the same way as chlorohydrine. The configuration is kept in the Darzens product as the reaction is stereospecific. This is because for the intramolecular cyclization to take place, oxygen and chlorine require an antiperiplanar conformation. Every isomer has only one antiperiplanar conformation for these two atoms, so each stereoisomer of the Darzens products comes out from only and only one approach of the two reactant (**Scheme 3.23**).

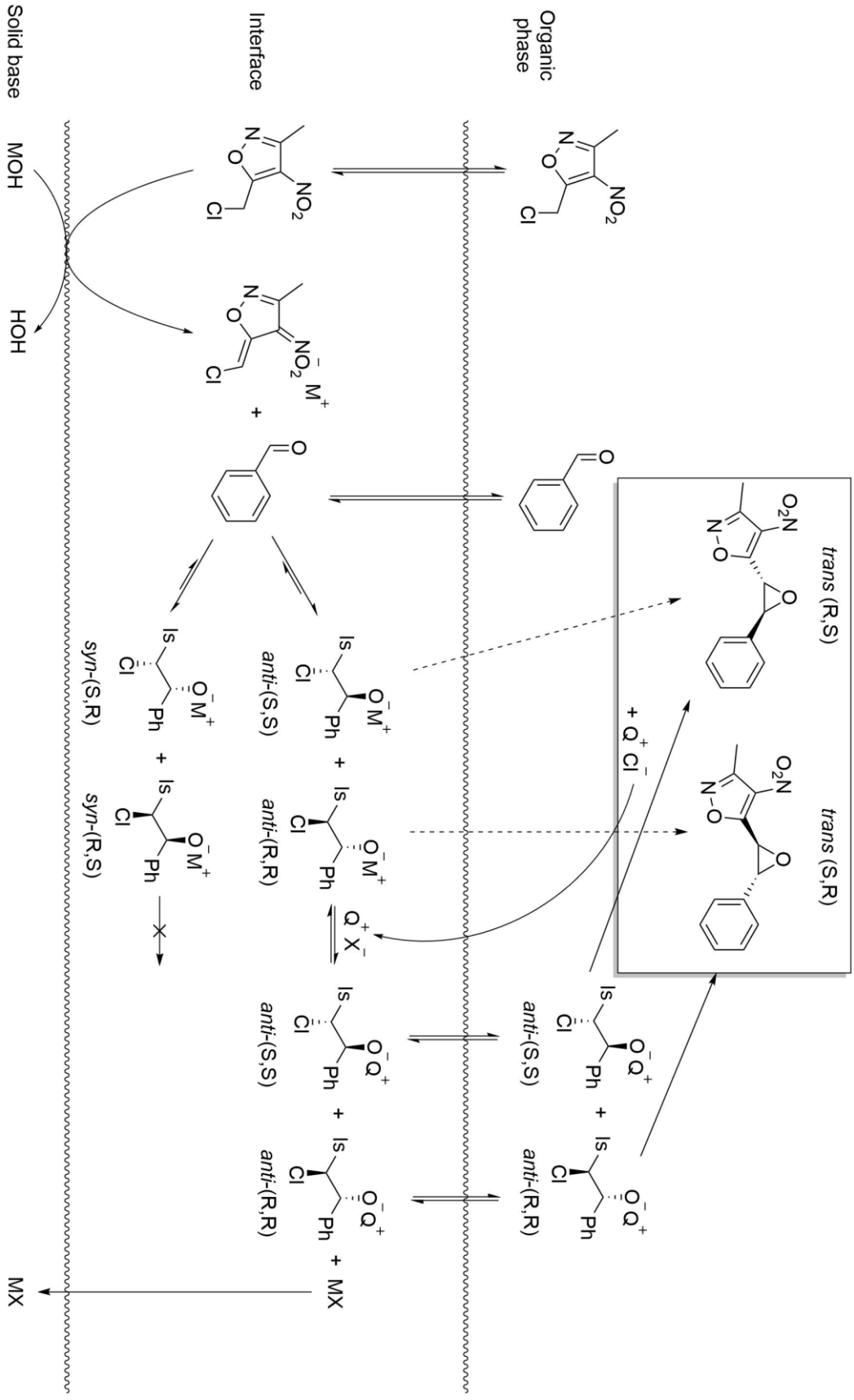


Scheme 3.23 - Correlation between conformations of intermediates and products.

Of course, the configuration of the intermediate is not the same as the product as there is inversion of configuration with the chlorine displacement, but to one configuration of the intermediate corresponds one final configuration.

Scheme 3.23 also explains why the cis products are not formed: in the transition state the isoxazole and phenyl ring are close to each other. Due to steric hindrance the antiperiplanar conformation in the *syn* adducts is unfavored, and epoxidation does not take place. Therefore, after *syn* intermediates form, they keep on reequilibrating with reactants by retro aldol. The equilibrium is shifted towards the reactants because of subsequent formation of *anti* intermediates that can go on and afford the Darzens products (**Scheme 3.24**).

Building on Arai's results, the relatively long lifetime of the metal alkoxide should let the ion exchange process between these molecules and chiral quaternary ammonium catalysts to happen. An efficient PTC with its structure should now allow only one enantiomer to cyclize.



Scheme 3.24 - Mechanism of catalysis for preparation of 3.20.

3.3.3 - Results

The reaction was repeated in three different solvents, with two phase transfer catalysts and four bases employed (**Figure 3.4**).

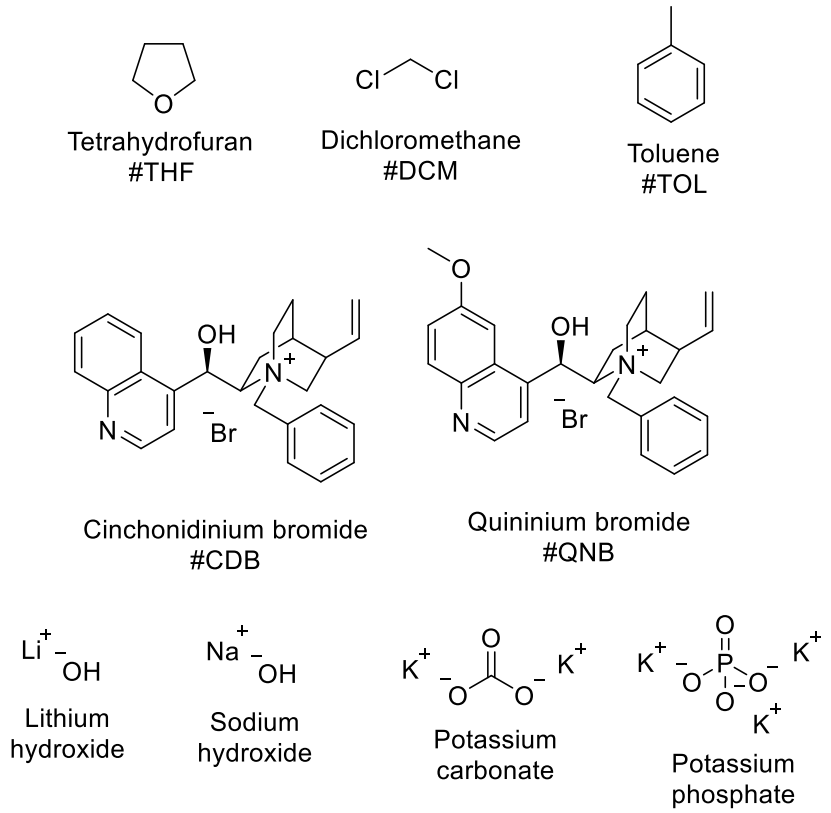
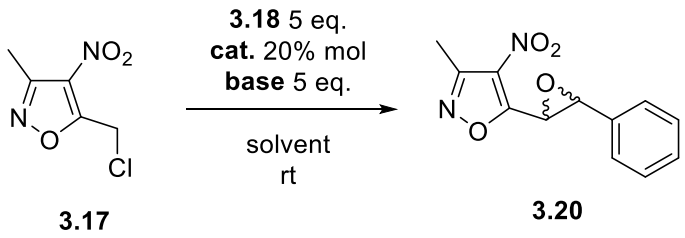


Figure 3.4 - Solvents, catalysts and bases employed.

Scheme 3.25 shows reaction conditions.



Scheme 3.25 - Reaction condition for preparation of 3.19.

Table 3.2 reports the results of the screening.

Entry	Catalyst	Solvent	Base	Time [h]	Yield	1%	2%
1	CDB	DCM	K_2CO_3	24	0,36	48,8	51,2
2			LiOH	24	0,16	48,8	51,2
3			K_3PO_4	48	0,39	48,7	51,3
4			NaOH 1 M (1 eq.)	24	/	/	/
5			NaOH 0,1 M (1 eq.)	24	/	/	/
6		TOL	K_2CO_3	120	0,47	53,5	46,5
7			LiOH	120	0,15	52,7	47,3
8			K_3PO_4	120	0,32	50,6	49,4
9			NaOH 1 M (1 eq.)	24	/	/	/
10			NaOH 0,1 M (1 eq.)	24	/	/	/
11		THF	K_2CO_3	24	/	/	/
12			LiOH	24	/	/	/
13			K_3PO_4	24	/	/	/
14			NaOH 1 M (1 eq.)	24	/	/	/
15			NaOH 0,1 M (1 eq.)	24	/	/	/
16	QDB	DCM	K_2CO_3	48	0,52	47,8	52,2
17			LiOH	24	0,47	46,5	53,5
18			K_3PO_4	72	0,54	45,1	54,9
19			NaOH 1 M (1 eq.)	24	/	/	/
20			NaOH 0,1 M (1 eq.)	24	/	/	/
21		TOL	K_2CO_3	168	0,37	42,8	57,2
22			LiOH	168	0,47	46,4	53,6
23			K_3PO_4	168	0,63	40,4	59,6
24			NaOH 1 M (1 eq.)	24	/	/	/
25			NaOH 0,1 M (1 eq.)	24	/	/	/
26		THF	K_2CO_3	24	/	/	/
27			LiOH	24	/	/	/
28			K_3PO_4	24	/	/	/
29			NaOH 1 M (1 eq.)	24	/	/	/
30			NaOH 0,1 M (1 eq.)	24	/	/	/

Table 3.2 - Results of the epoxide screening.

This time the reaction was slow as well, especially in toluene, while DCM gave moderate yields in relatively short time. This again may have happened because of the difficult approach between the two bulky reactants: indeed the epoxidation is a consecutive reaction to the one previously examined. Liquid NaOH at any concentration caused degradation even using 1 equivalent against 5, and therefore the product was not observed. Epoxidation was also unsuccessful in reaction with THF.

Lithium hydroxide lowered yield employing CNB, while this effect is not marked with QDB. This may be due to inefficiency of the cinchoninium to displace lithium cation for alkoxide ion pairing, and this result seems to be proved by average lower yield with CNB than QDB.

A CSP-HPLC method for the determination of relative quantities of each stereoisomer was developed. Two main peaks are displayed in the chromatogram of purified product, corresponding to the two enantiomers of the *trans* product. NMR spectra revealed that a negligible amount of cis product is formed (2% at most).

Anyway, catalysis of the reaction did not provide substantial enantioselectivity, with the highest enantiomeric excess being around 20% using QDB and potassium phosphate in toluene. For QDB in toluene *ee*s obtained are always higher than the analogues in DCM.

Oddly, *ee*s with CNB in toluene are of opposite sign than the same in QDB (considering that they share the same configuration), as well as with CNB itself in DCM. This is quite surprising, even though the *ee*s are not much high. This may be caused from a different disposition of the quinolinic aromatic system in this solvent with and without the methoxyl that tips over selectivity.

In this system, there is no epimerization due to acidic protons so this cannot contribute to the low enantioselectivities. Some other causes may be:

-Problematic ion exchange process, if the ion exchange process does not proceed smoothly, epoxidation can occur only from metal alkoxides, and the product is a racemate.

-Catalyst structure does not allow epoxidation, if the chiral complex can be formed but it does not allow any enantiomer to epoxidize, again epoxidation can occur only from metal alkoxides, and the product is a racemate.

-Catalyst structure allows epoxidation of both enantiomer, also in this case the product is a racemate.

3.4 - Conclusions

Both the simple addition product and Darzens product were successfully synthesized. Unfortunately, the reactions were slow and the yields low. In addition, stereoselectivities for both the transformations were low as well.

Future work for the simple adduct may be investigation of the reaction by using different reactants (different halide, different aldehydes) for times and yields, while effect of temperature on stereocontrol may be checked by operating at low temperatures. The effect of epimerization may be eliminated by fluorination.

The Darzens product, of higher synthetic relevance, deserves further studies. Here again the effect of different reactants may be tested. Different reaction condition should be probed, for example lower temperatures, newer generations PTCs, or different bases.

Synthesized Darzens products, thanks to the special reactivity of the new epoxidic group implanted, can become a useful intermediate for the 4-nitroisoxazoles chemistry. Enantioselective synthesis of these products would improve their usefulness by complete control of the upcoming stereocenters.

References

[1] G. Darzens, *Compt. Rend.*, **1904**, 139, 1214.

[2] S. Shirakawa and K. Maruoka, *Angew. Chem. Int. Ed.*, 2013, 52, 4312.

[3] Y. Liu, B. A. Provencher, K. J. Bartelsona and L. Deng, *Chem. Sci.*, **2011**, 2, 1301.

[4] W.-J. Liu, B.-D. Lv and L.-Z. Gong, *Angew. Chem. Int. Ed.* **2009**, 48, 6503.

[5] S. Arai, K. Tokumaru and T. Aoyama, *Tetrahedron Letters* **2004**, 45, 1845.

[6] S. Arai, Y. Shirai, T. Ishida, and T. Shioiri, *Tetrahedron* **1999**, 55, 6375.

[7] R. Dere, C. Monasterolo, M. Moccia, M. F. A. Adamo, *Tetrahedron Letters* **2015**, 56, 7168.

Chapter 4

Experimental section

4.1 - General experimental details

^{1}H NMR spectra were recorded on a Bruker 400 spectrometer. Chemical shifts (δ) are reported in ppm relative to residual solvent signals for ^{1}H (^{1}H NMR: 7.26 ppm for CDCl3 and 4.87 ppm for MeOD). Coupling constants (*J*) are in Hz. Multiplicities are reported as follows: s, singlet, d, doublet, dd, doublets of doublets, t, triplet, q, quartet, m, multiplet, c, complex, and br, broad.

Reactions were checked for completion by TLC (EM Science, silica gel 60 F254) which were visualized by quenching of UV fluorescence (λ_{max} = 254nm) or by staining with either 10% w/v ammonium molybdate in 2M sulphuric acid or basic potassium permanganate solution (followed by heat) as appropriate.

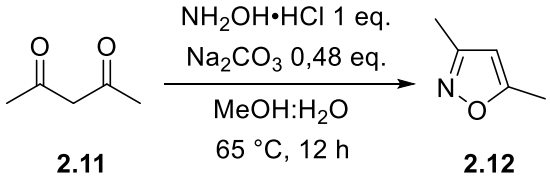
Flash chromatography was performed using silica gel 60 (0.040-0.063 mm, 230-400 mesh).

The enantiomeric excess (*ee*) of the products was determined by chiral stationary phase HPLC (Daicel Chiralpak AD, Chiracel OJ, Chiracel OD, Chiralpak AS, Chiralpak IB columns), using a UV detector operating at 254 nm. Retention factors (Rf) are reported to ±0.05.

4.2 - Experimental section for chapter 2

4.2.1 - Synthesis of pregabalin

*Preparation of 3,5-dimethyl-isoxazole (**2.10**)*

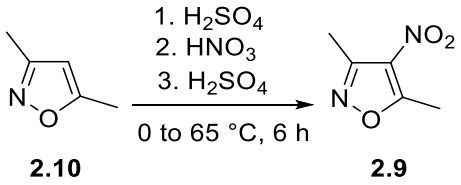


Scheme 4.1 - Preparation of **2.10**.

In a round bottomed flask fitted with a magnetic stirrer were put 75 mL of a solution 1:2 MeOH:water, followed by NH_2OH•HCl (3.475 g, 50 mmol). Small portions of Na_2CO_3 (2,544 g, 24 mmol) were slowly added.

Acetylacetone (5,13 mL, 50 mmol) was added in one portion and the reaction mixture heated at 65°C for 12h. At the end of this time, the reaction mixture was cooled to rt. An extraction was performed using Et_2O (2x100 mL) and brine (25 mL). The organic phase was then dried with Na_2SO_4 and filtered under vacuum. The solvent was evaporated in rotavapor and used in next step without further purification. Yellow oil, 3.76 g; 99% yield; δH (400 MHz, CDCl3) 5.75 (1H, s), 2.31 (3H, s), 2.19 (3H, s).

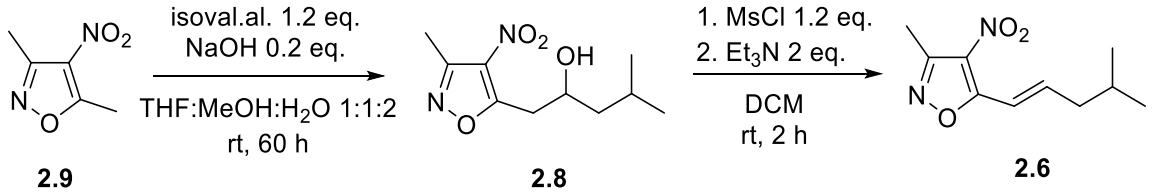
*Preparation of 3,5-dimethyl -4-nitroisoxazole (**2.9**)*



Scheme 4.2 - Preparation of **2.9**.

In a round bottomed flask fitted with a magnetic stirrer and kept at -5 to 0°C by an ice salt bath, was put H_2SO_4 (95-98% ACS reagent, 2.7 mL; d=1.83) then 3,5-dimethylisoxazole (1 g, 10.3 mmol) was added slowly to ensure that the temperature stays close to 0°C. Then HNO_3 conc. (1 mL; d=1.413) and additional H_2SO_4 conc. (4.2 mL) were added in small portions with similar precautions. The solution was then heated at 60-65°C for 6 hours, then allowed to reach room temperature and poured dropwise in a flask containing ice and water under vigorous stirring. A fine precipitate was obtained then filtered, washed and dried . This product could be used in next step without further purification. Yellowish solid, 1,45 g; 99% yield; δH (400 MHz, CDCl3) 2.82 (3H, s), 2.56 (3H, s).

*Preparation of (E)-3-methyl-5-(4-methylpent-1-enyl)-4-nitroisoxazole (**2.6**)*



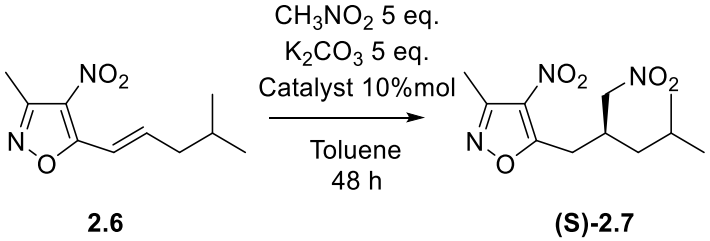
Scheme 4.3 - One pot preparation of **2.6**.

Since in preparation of **2.8** some of **2.6** was already formed, the two steps were performed in one pot.

In a round bottomed flask fitted with a magnetic stirrer 3,5-dimethyl-4-nitroisoxazole (710 mg, 5 mmol) was dissolved in THF (4mL) then a H_2O:MeOH mixture (3:7, 12mL) was added. To the cloudy solution NaOH powder (40 mg, 1 mmol) was added. The solution turned deep yellow and was stirred at room temperature for 30 minutes then isovaleraldehyde (6 mmol, 658 μl) were added dropwise over 30 minutes. The reaction mixture was stirred at room temperature for 60 h, then THF was removed under vacuum, the mixture extracted with dichloromethane (x3) dried over sodium sulphate and the solvent removed under reduced pressure.

To the crude, dry DCM (35 mL) was added. The solution was cooled down to 0°C and then methanesulfonyl chloride (137.5 mg, 93 μl) was added. The mixture was allowed to stir for 30 minutes and then trimethylamine (203 mg, 280 μl) was added drop wise at 0°C. The mixture was left stirring for 2 hours at RT. The reaction mixture was extracted with DCM (x3). The organic phase was recovered, dehydrated with Na_2SO_4, filtered off and the solvent removed under vacuum. The crude product obtained was subjected to column chromatography (SiO_2, Petroleum ether:Ethyl acetate 97:3) to provide the desired alkene. Pale yellow oil, 194 mg, 85% overall yield**;** R*f* = 0.2 (Petroleum ether:Ethyl acetate 90:10); δH (400 MHz, CDCl3) 7.12-7.00 (m, 2H), 2.55 (s, 3H), 2.27 (t, 2H, J = 6) 1.89-1.82 (m, 1H), 0.97 (d, 6H, J = 7).

*Preparation of (S)-3-methyl-5-(4-methyl-2-(nitromethyl)pentyl)-4-nitroisoxazole (**(S)-2.7**)*



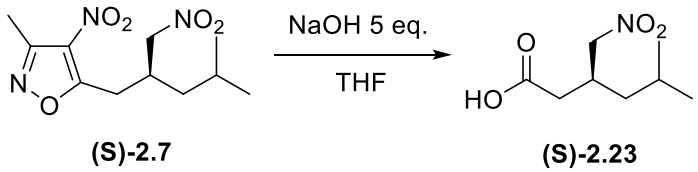
2.6 **(S)-2.7**

Scheme 4.4 - Preparation of **(S)-2.7**.

In a test tube equipped with a magnetic stirring bar were sequentially added (E)-3-methyl-5-(4-methylpent-1-enyl)-4-nitroisoxazole (42 mg, 0.2 mmol), catalyst (0.02 mmol), nitromethane (1 mmol) and toluene (1 mL). Finely ground K_2CO_3 (1 mmol) was added in one portion. The mixture was then vigorously stirred at the reported temperature, with no

precautions to exclude moisture or air. After 48 h, the reaction was filtered using cotton wool and rinsing with DCM. The solvent was evaporated and the crude was subjected to column chromatography (SiO_2, Petroleum ether:Ethyl acetate 95:5) to provide pure **2.7** as a yellow oil in the reported yields and enantiomeric excesses**.** The *ee* of the product was determined by CSP-HPLC using a Chiralcel OD column (*n*-hexane/IPA 95:5, flow rate 1 mL/min, t_{maj} = 19 min, t_{min} = 24 min); R*f* = 0.6 (Petroleum ether:Ethyl acetate, 80:20); δH (400 MHz, CDCl3) 4.38 (d, 2H, *J* = 6), 3.35 (dd, 1H, *J* = 15, *J* = 6), 3.28 (dd, 1H, *J* = 15, *J* = 7), 2.87 (sept, 1H, *J* = 7), 2.56 (s, 3H), 1.68 (sept, 1H, *J* = 7), 1.36-1.23 (m, 2H), 0.92 (d, 3H, *J* = 4), 0.90 (d, 3H, *J* = 4).

Preparation of (S)-3-methyl-5-(4-methyl-2-(nitromethyl)pentyl)-4-nitroisoxazole ***((S)-2.23)***

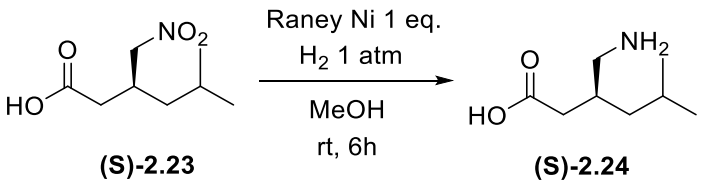


(S)-2.7 **(S)-2.23**

Scheme 4.5 - Preparation of **(S)-2.23**.

A solution of adduct (S)-3-methyl-5-(4-methyl-2-(nitromethyl)pentyl)-4-nitroisoxazole (68 mg, 0.25 mmol) in THF (0.5 mL) was charged in a round bottomed flask and treated with an aqueous solution of NaOH (1N, 1.25 mL). The resulting deep yellow solution was refluxed for 6h, then allowed to reach room temperature, the THF was evaporated in *vacuo*; to the aqueous solution ethyl acetate was added and the mixture brought to 0°C. The pH was adjusted to 3 by slow addition of 3N aqueous HCl. The mixture was extracted three times with ethyl acetate, dried over Na_2SO_4 and the solvent removed under vacuum. The crude mixture was submitted to column chromatography (Petroleum ether:Ethyl acetate 8:2) and compound **(S)-2.23** was obtained as yellow oil 44 mg, 94% yield; R*f* = 0.5 (Petroleum ether:Ethyl acetate, 3:7); δH (400 MHz, MeOD) 9,27 (bs, 1H, OH), 4.46 (dd, *J* = 6.4, *J* = 12.4, 1H), 4.40 (dd, *J* = 5.6, *J* = 12.4, 1H), 2.67-2.61 (m, 1H), 2.46 (d, *J* =6.4, 2H) 1.65- 1.59 (m, 1H), 1.26-1.19 (m, 2H), 0.89-0.86 (m, 6H).

*Preparation of (S)-pregabalin (**(S)-2.24**)*

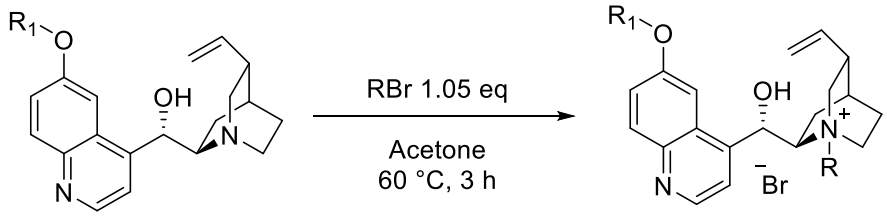


Scheme 4.6 - Synthesis of (S)-pregabalin.

In a round bottomed flask were charged Raney-Ni (1g), methanol (2.5 mL) and nitroacid (S)-3-methyl-5-(4-methyl-2-(nitromethyl)pentyl)-4-nitroisoxazole (0.5g). The suspension obtained was stirred at room temperature under H_2 (1 atm) for 6h, then the liquid phase decanted. The methanolic solution was evaporated to give pregabalin (0.394g, 95% yield) as a colorless solid. Pregabalin was identified with published data. [H. Gotoh, H. Ishikawa, Y. Hayashi, *Org. Lett*., 2007, **9**, 25, 5307]

4.2.2 - Preparation of catalysts

Preparation of N-substituted cinchonium bromide (MQs)



Scheme 4.7 - Preparation of mono-quaternized catalysts.

In a round bottomed flask fitted with a magnetic stirrer were put sequentially quinidine or derivative (9,25 mmol), acetone (5,2 mL) and finally 3,5-ditertbutylbenzyl bromide (2,8 g, 9,7 mmol). The resulting solution was heated at 60 °C for 3h. The reaction mixture was then allowed to reach room temperature and the solvent evaporated. The crude was submitted to column chromatography (DCM:MeOH 98:2) to afford the product in reported yields.

*N-(3,5-di-tert-butylbenzyl) quinidinium bromide (**2.15**)*

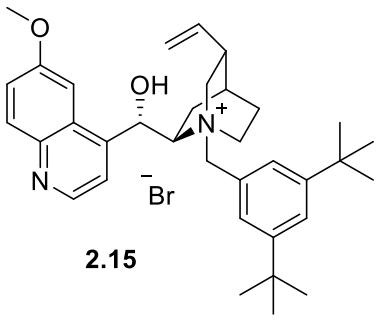
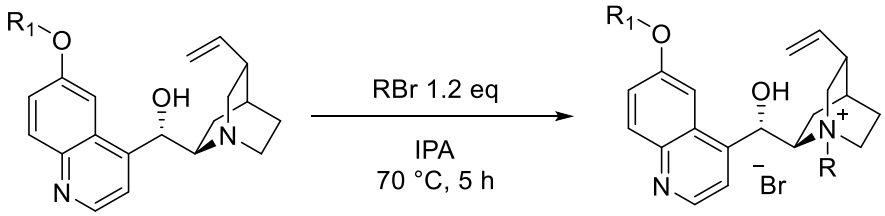


Figure 4.1 - 2.15.

Yellowish powder, 4.6 g, 82% yield. δH (400 MHz, CDCl3) 8.61-8.59 (1H, m), 7.97-7.94 (1H, m), 7.68-7.67 (1H, m), 7.60-7.59 (1H, m), 7.53-7.51 (1H, m), 7.3-7.26 (2H, m), 6.79-6.77 (1H, m), 6.58-6.55 (1H, m), 6.00-5.92 (1H, m), 5.83-5.80 (1H, m), 5.19-5.15 (2H, m), 4.85-4.76 (1H, m), 4.62-4.57 (1H, m), 4.10-4.04 (1H, m), 3.93 (3H, s), 3.88-3.83 (1H, m), 3.50-3.44 (1H, m), 3.07-3.02 (1H, m), 1.89-1.85 (1H, m), 1.80-1.75 (1H, m), 1.08-1.01 (1H, m).

Alternative preparation of N-substituted cinchonium bromides (MQs)



Scheme 4.8 - Alternative preparation of mono-quaternized catalysts.

A degassed slurry of quinidine or derivative (0.5 mmol) and 3,5-ditertbutylbenzyl bromide (0.12 g, 0.6 mmol) in IPA (1.5 mL) was warmed to 70 °C under nitrogen atmosphere and held overnight. The reaction mixture was cooled to room temperature and ~1.2 mL of solvent were removed under reduced pressure. The residue was added to EtOAc (5 mL) over 5 to 10 min with vigorous stirring. The resulting slurry was aged for 1-2 h at rt, filtered, rinsed with IPA:Petroleum ether 1:1 and dried under vacuum to give solid mono-quaternized products in reported yields.

*N-(3,5-di-tert-butylbenzyl) quinidinium bromide (**2.15b**)*

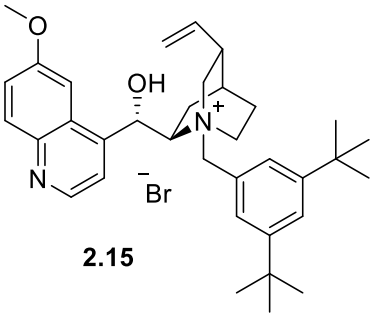


Figure 4.2 - 2.15b.

Yellow powder, 0.23 g, 76% yield. δH (400 MHz, CDCl3) 8.61-8.59 (1H, m), 7.97-7.94 (1H, m), 7.68-7.67 (1H, m), 7.60-7.59 (1H, m), 7.53-7.51 (1H, m), 7.3-7.26 (2H, m), 6.79-6.77 (1H, m), 6.58-6.55 (1H, m), 6.00-5.92 (1H, m), 5.83-5.80 (1H, m), 5.19-5.15 (2H, m), 4.85-4.76 (1H, m), 4.62-4.57 (1H, m), 4.10-4.04 (1H, m), 3.93 (3H, s), 3.88-3.83 (1H, m), 3.50-3.44 (1H, m), 3.07-3.02 (1H, m), 1.89-1.85 (1H, m), 1.80-1.75 (1H, m), 1.08-1.01 (1H, m).

*O'-(3,5-di-tert-butylbenzyl)-N-(3,5-di-tert-butylbenzyl) quinidinium bromide (**2.22**)**

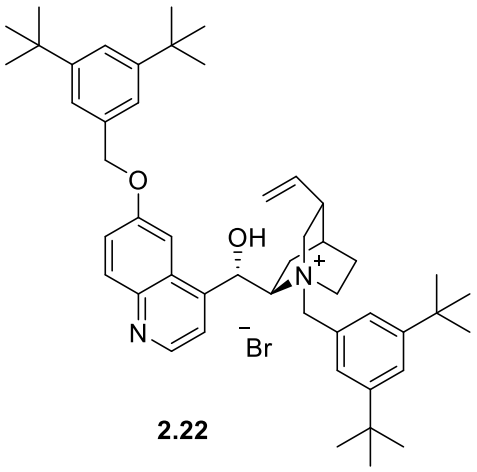
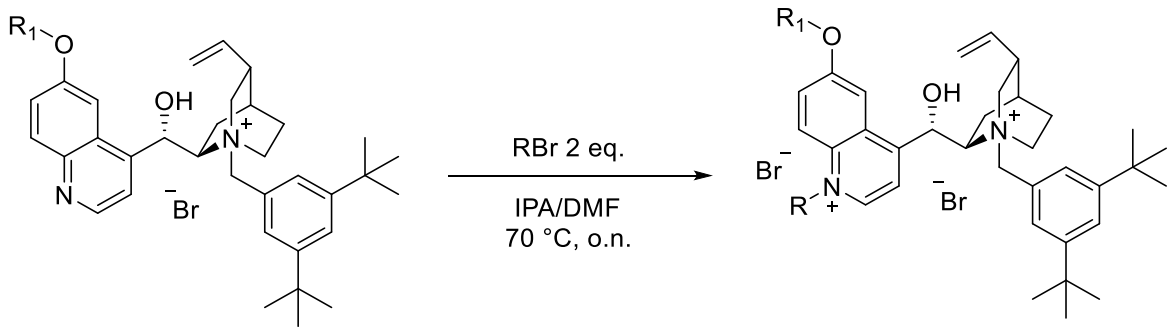


Figure 4.3 - 2.22.

*Obtained directly from alkylation of CPD, yield referred to CPD.

Greenish powder, 0.53 g, 42% yield. δH (400 MHz, CDCl3) 8.82-876 (1H, m), 8.16-8.10 (1H, m), 7.95-7.89 (1H, m), 6.81-6.72 (1H, m), 6.07-5.98 (1H, m), 5.79-70 (1H, m).

Preparation of N, N'-disubstituted cinchonium bromides (DQs)



Scheme 4.9 - Synthesis of doubly-quaternized catalysts.

A degassed slurry of mono-quaternized catalyst (0.31 mmol) and bromoalkane (0.61 mmol) in IPA (0.075 mL) and DMF (0.53 mL) was warmed to 70 °C and held overnight. The reaction mixture was cooled and added to EtOAc (6 mL) over 5-10 min. The resulting slurry was aged for 1-2 h at 22 °C, filtered, rinsed with EtOAc (twice, 5 mL each) and dried under vacuum to give the product in reported yields.

*N-(3,5-di-tert-butylbenzyl)-N'-benzyl quinidinium bromide (**2.17**)*

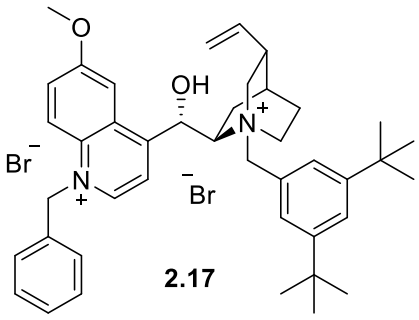
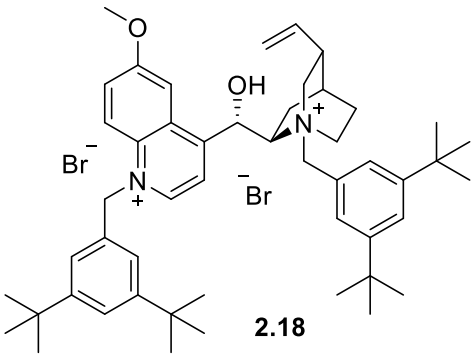


Figure 4.4 - **2.17**.

Yellowish powder, 59% yield. δ_H (400 MHz, CDCl3) 9.36-9.28 (1H, m), 9.16-9.04 (1H, m), 8.52-8.44 (1H, m), 8.30-8.24 (1H, m), 8.12-8.08 (1H, m), 8.07-8.04 (1H, m), 7.55-7.51 (1H, m), 6.90-6.84 (1H, m), 6.40-6.18 (2H, m), 6.05-5.94 (1H, m), 5.67-5.60 (1H, m), 5.27-5.19 (2H, m), 5.11-5.04 (1H, m), 2.22-2.12 (2H, m), 1.82-1.73 (1H, m).

*N- N'- (3,5-di-tert-butylbenzyl) quinidinium bromide (**2.18**)*

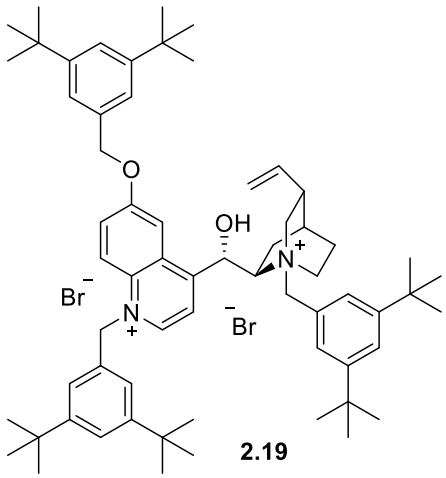


2.18

Figure 4.5 - 2.18.

Yellowish powder, 55% yield. δ_H (400 MHz, CDCl3) 8.85-8.80 (1H, m), 8.48-8.44 (1H, m), 8.35-8.30 (1H, m), 8.07-8.04 (1H, m), 7.75-7.69 (1H, m), 7.56-7.51 (1H, m), 6.97-6.92 (1H, m), 6.20-6.08 (2H, m), 6.07-5.97 (1H, m), 5.76-5.69 (1H, m), 4.99-4.93 (1H, m), 4.93-4.84 (1H, m), 4.82-4.74 (1H, m), 4.59-4.50 (1H, m), 3.49-3.40 (1H, m), 2.62-2.54 (2H, m), 2.22-2.11 (1H, m).

*O'-(3,5-di-tert-butylbenzyl)-N- N'- (3,5-di-tert-butylbenzyl) quinidinium bromide (**2.19**)*

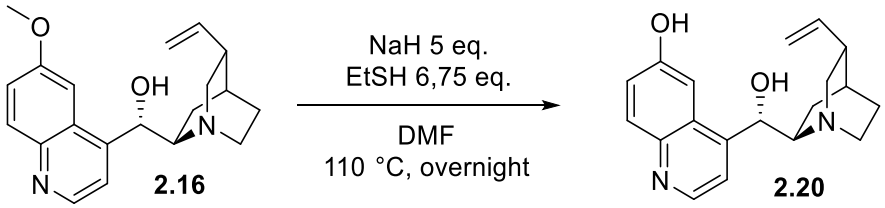


2.19

Figure 4.6 - 2.19.

Greenish powder, 51% yield. δ_H (400 MHz, CDCl3) 8.87-8.83 (1H, m), 8.15-8.11 (1H, m), 7.99-7.93 (1H, m), 6.14-6.07 (1H, m), 5.97-5.90 (1H, m), 4.55-4.47 (1H, m).

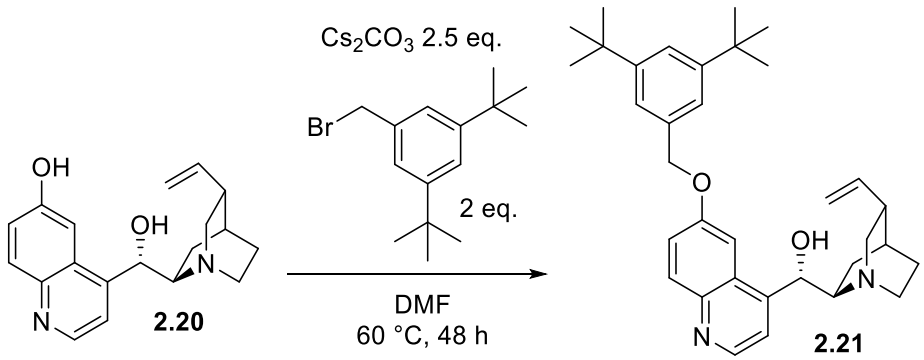
*Preparation of **2.20***



Scheme 4.10 - **2.20** preparation.

To a suspension of NaH (0.92 g, 40% suspension in mineral oil, 15 mmol) in dry DMF (20 mL) was added EtSH (1.6 mL, 21 mmol) dropwise at 0 °C in inert atmosphere over 20 min. After the reaction mixture was stirred at rt for 2h, quinine (1 g, 3 mmol) in DMF (7.7 mL) was added and the resulting mixture was stirred at 110 °C for 16 h. The mixture was extracted with ethyl acetate (2x60 mL). The combined organic phase was washed with aqueous HCl (2 N, 2x20 mL) and the combined aqueous phase was treated with ammonium hydroxide until the pH of the aqueous phase was 9-10. Then the mixture was extracted with ethyl acetate (3x60 mL). The organic phase was washed with brine (4x80 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was used without any purification. White solid, 0.69 g, 75% yield; δ_H (400 MHz, MeOD) 8.67-8.65 (1H, d), 7.95-7.90 (1H, m), 7.75-7.72 (1H, m), 7.42-7.39 (1H, m), 7.39-7.35 (1H, m), 6.79-6.77 (1H, m), 6.15-6.06 (1H, m), 5.31-5.22 (2H, m), 4.32-4.23 (1H, m), 2.80-2.71 (1H, m), 2.50-2.42 (1H, m), 1.27-1.17 (1H, m).

*O'-(3,5-di-tert-butylbenzyl)-quinidine (**2.21**)**



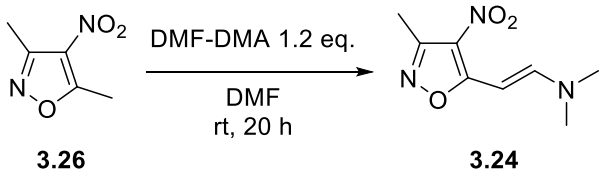
Scheme 4.11 - **2.20** alkylation.

*The product was not observed as it underwent directly quaternization, affording **2.22**.

Cesium carbonate (1.3 g, 4 mmol) was added to a stirred solution of cupreidine (0.5 g, 1.6 mmol) in dry DMF (7 mL). The bromoalkane (3.2 mmol) was added, and the reaction mixture was stirred for 48 h at 60 °C. The reaction mixture was diluted with DCM (50 mL) and the organic phase was washed with brine (4x15 mL), dried over Na2SO4 and concentrated in *vacuo*. The result in solid was purified by flash chromatography to afford the desired product.

4.3 - Experimental section for chapter 3

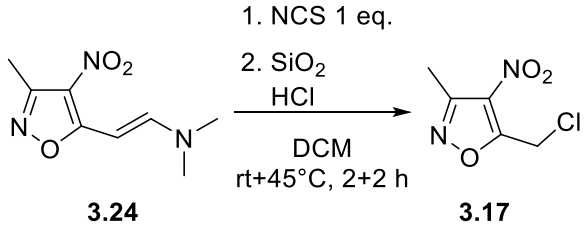
*Preparation of (E)-N,N-dimethyl-2-(3-methyl-4-nitroisoxazol-5-yl)ethen-1-amine (**3.24**)*



Scheme 4.12 - Preparation of **3.24**.

To a solution of 3,5-dimethyl-4-nitroisoxazole (8g, 0.06 mol) in dry DMF (30 mL) was added diethoxymethyldimethylamine (12.4 ml, 0.07 mol) and the resulting reaction mixture refluxed over 20h. The dark reaction mixture so obtained was allowed to reach room temperature, the solvent removed under reduced pressure. The crude dark residue was re-crystallized from methanol to give pure dimethyl-[2-(3-methyl-4-nitro-isoxazol-5-yl)-vinyl]-amine (7.93 g, 66% yield) as a yellow-green solid; δ_H (400 MHz, CDCl3) 7.60 (1H, d, *J* = 12), 5.82 (1H, d, *J* = 12), 3.15 (3H, s), 2.93 (3H, s), 2.41 (3H, s).

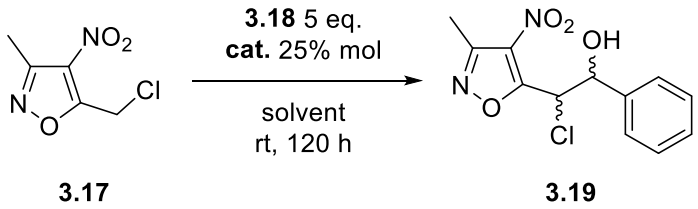
*Preparation of 5-chloromethyl-3-methyl-4-nitroisoxazole (**3.17**)*



Scheme 4.13 - Preparation of **3.17**.

To a solution of **3.24** (400 mg, 2.030 mmol) in DCM (3 mL), *N*-chloro succinimide (270 mg, 2.030 mmol) was added portion wise and the resulting solution stirred for two hours at room temperature. Then, silica gel (2.5 g) and HCl 1M (0.25 mL) were consecutively added and the solvent was evaporated under reduced pressure. The mixture was kept for two hours at 45 °C in the rotary evaporator and purified by column chromatography, using Petroleum ether:Ethyl acetate 9:1 as eluent, to give compound **3.26** (337 mg, 94% yield) as a pale brown oil; δ_H (400 MHz, CDCl3) 4.97 (2H, s), 2.60 (3H, s).

*Preparation of 2-chloro-2-(3-methyl-4-nitroisoxazol-5-yl)-1-phenylethan-1-ol (**3.19**)*

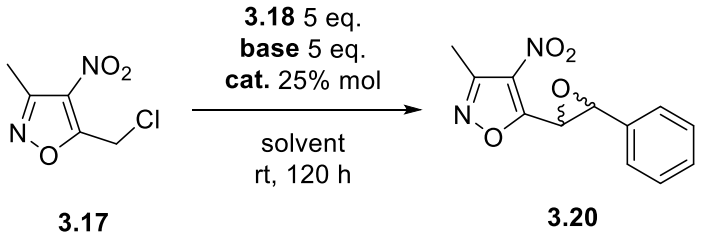


3.17 **3.19**

Scheme 4.14 - Preparation of **3.19**.

In a test tube fitted with a magnetic stirrer 5-chloromethyl-3-methyl-4-nitroisoxazole (44 mg, 0,25 mmol) was dissolved in solvent (0,5 mL) then benzaldehyde (127 uL, 1,25 mmol) and catalyst (0,0625 mmol) were added. The reaction mixture was stirred for 120 h at room temperature. The crude was loaded on a silica chromatographic column, using Petroleum ether:Etyl acetate 9:1 as eluent. The product was provided as a brownish oil in reported yields and distribution of stereoisomers. The distribution of stereoisomers was determined by CSP-HPLC using a Chiralpack IB column (*n*-hexane/IPA 97.2:2.8, flow rate 1 mL/min, t_1 = 22 min, t_2 = 25 min, t_3 = 27 min, t_4 = 29 min); δ_H (400 MHz, CDCl3) [D_{min}] 7.30-7.26 (5H, m), 5.91 (1H, d, J=8), 5.26 (1H, d, J=8), 3.38 (1H, s, OH), 2.44 (3H, s); [D_{maj}] 7.44-7.35 (5H, m), 5.85 (1H, d, J=8), 5.26 (1H, d, J=8), 3.13 (1H, s, OH), 2.56 (3H, s).

*Preparation of 3-methyl-4-nitro-5-(3-phenyloxiran-2-yl)isoxazole (**3.20**)*



Scheme 4.15 - Preparation of **3.20**.

In a test tube fitted with a magnetic stirrer 5-chloromethyl-3-methyl-4-nitroisoxazole (44 mg, 0,25 mmol) was dissolved in solvent (1 mL) then benzaldehyde (127 uL, 1,25 mmol), base (1,25 mmol) and catalyst (0,05 mmol) were added. The reaction mixture was stirred for reported time at room temperature. The reaction was quenched with HCl 1 N (3 mL), extracted with DCM (3x10 mL), anhydrified on Na_2SO_4 and evaporated. The crude obtained was loaded on a silica chromatographic column, using Petroleum ether:DCM 7:3 as eluent. The product was provided as a yellowish oil in reported yields and distribution of stereoisomers. The *ee* of the product was determined by CSP-HPLC using a Chiralcel AS column (*n*-hexane/EtOH 99:1, flow rate 0.5 mL/min, t_1 = 20 min, t_2 = 21 min); δ_H (400 MHz, CDCl3) [D_{maj}] 7.45-7.35 (5H, m), 4.74 (1H, d, J=4), 4.48 (1H, d, J=4), 2.61 (3H, s).

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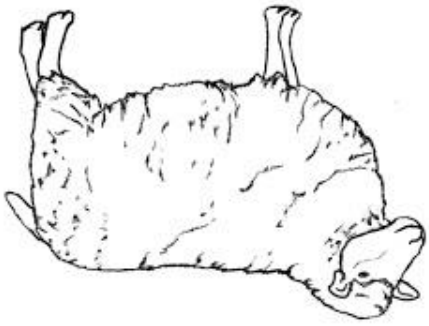

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