# SCHOOL OF ADVANCED STUDIES

***Doctorate Course in Chemical Science***

***PhD Thesis***

# *FUNCTIONALIZATION OF CYCLIC STRUCTURES FOR ADVANCED BIOLOGICAL AND PHARMACEUTICAL APPLICATIONS*

Cycle XXXI


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Academic Year 2015/2016 – 2018/2019

# Preface

The key components of the scaffold in pharmaceutical chemistry are ring systems, of different sizes, and are the fundamental factors of most of the drugs on the market today. Nowadays, the importance of cyclic structures is well understood by medicinal chemists, since they play a significant role in molecular properties such as the electronic distribution, three dimensionality, and molecule rigidity. They are often key factors in whole molecule properties such as lipophilicity or polarity and can determine molecular reactivity, metabolic stability, and toxicity. Cyclic structures have always fascinated organic and medicinal chemists, and many organic molecules form cycles with appealing biological properties. Research in cyclic chemistry continued to advance in synthetic methods development, conformational studies and investigation of their role for controlling biological functions.

This work, carried out in the Prof. Marcantoni's research group at the University of Camerino (Camerino, Italy) with the collaboration of Dompé S.p.A. (L'Aquila, Italy) from December 2015 to December 2018 and in the Prof. Poli's research group from January 2018 to July 2018 at the University Pierre and Marie Curie (Paris, France), had the objective to investigate new synthetic methodologies for the functionalization of cyclic compounds, as well as the formation of cyclic structures from acyclic precursor, for advanced biological purposes.

The first chapter focuses on the functionalization of the primary face of a β-cyclodextrin, in order to obtain a synthetic human receptor model, used for studying the possible interactions of this compound with a new class of biologically active compounds in development at Dompé S.p.A.

The second chapter, carried out in the Poli's research group, describes the selective C-3 functionalization trials of 2-furaldehyde and its derivatives by Directed *ortho* Metalation (DoM) chemistry in presence of organolithium compounds and focuses on the study of degradation products. The reaction of an alkyllithium compound with an aromatic structure bearing a Direct Metalation Group (DMG) normally leads to an *ortho*-metalated intermediate. Good DMGs are strong complexing or chelating groups that have the effect of increasing the kinetic acidity of protons in the *ortho* position.

The third chapter focuses on the in-depth study of the mechanistic aspect on the formation of 5-acylaminothiazoles starting from α-chloroglycinates, obtained by a new synthetic methodology developed in the Marcantoni's research group.

Finally, the fourth chapter focuses on the study of the role of Cerium trichloride in the formation of cyclic compounds via Nazarov cyclization.

# Chapter 1: Selective functionalization of the primary face of a β-cyclodextrin as CXCR1 receptor model

## 1.1. Importance of Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides biosynthesized from starch through an enzymatic process of intramolecular trans glycosylation by action of the enzyme cyclodextrin glycosyltransferase (CGTase).[1]

Three most common structures were isolated for the first time by Villiers (1891)[2] and subsequently classified in 1903 by Scardinger[3] and are the α-cyclodextrin (α-CD), the β-cyclodextrin (β-CD) and the γ-cyclodextrin (γ-CD), respectively constituted by 6, 7 or 8 α-glucopyranoside residues.

Thanks to their cyclic structure they present an apolar cavity, which is at the origin of their encapsulation properties towards the lipophilic part of a wide range of active organic molecules. Due to their inclusion properties, CDs have drawn the attention of academic and industrial researchers since the early 1980s with several hundreds of papers and patents, up to now with almost 20,000 published references. The field of applications of CDs is always increasing and concerns drug vectorization and drug delivery, pharmaceutical excipients, soil remediation, biotechnologies, biomaterials, catalysis, self-healing materials, wastewaters treatment, analytical chemistry, flavor and fragrance stabilization and controlled release. This is the primary reason why cyclodextrins have attracted great interest in a variety of industries, including those related to food, pharmaceuticals, cosmetics, chemicals, and agriculture.

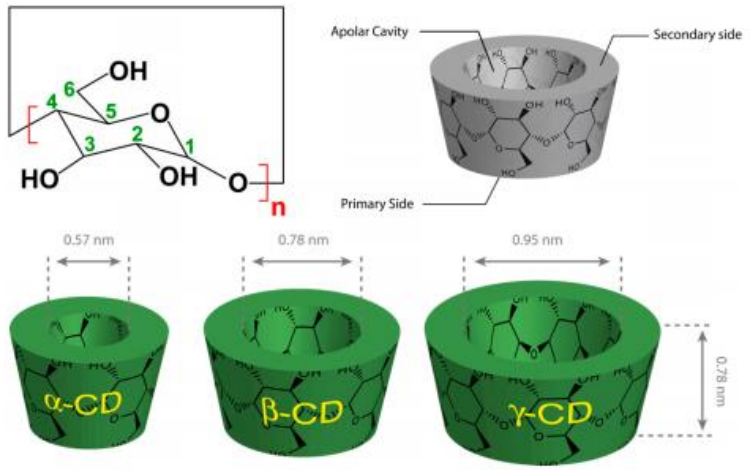



*Figure 1.1. Top: Functional structural scheme of α-CD (n = 6), β-CD (n = 7), and γ-CD (n = 8). Bottom: Geometric dimensions of cyclodextrins.*

## 1.2. Modified CDs as enzyme models

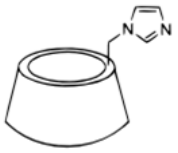
In their native forms, cyclodextrins show many features characteristic of enzymes: specificity, such as formation of catalyst-substrate complexes prior to chemical transformation and large accelerations of reactions in which they are involved.[4,5]

However, CDs as enzyme models suffer from a shortcoming, in fact their only catalytic group is the hydroxyl group, which restricts the scope of their applicability. Thus, many attempts to introduce other functional groups into cyclodextrins have been made. In these modified cyclodextrins, the introduced groups work as catalytic sites and the cavities of cyclodextrins act as the binding sites for the substrates.

In a direct study on facial selectivity about the possibility that the cyclodextrins have different catalytic activity according to the position of the substituent groups, catalysts were prepared with a phosphate group attached to either the primary or the secondary face of a β-CD, and it was found that both were effective.[6] Thus for many purposes either cyclodextrin face is suitable for catalytic group attachment,[7-9] but there are also examples of substrates that preferentially bind into the secondary face of β-CD, which is more open.[10-13] With such substrates, the facial placement of the catalytic group matters.

### 1.2.1. Chymotrypsin mimics

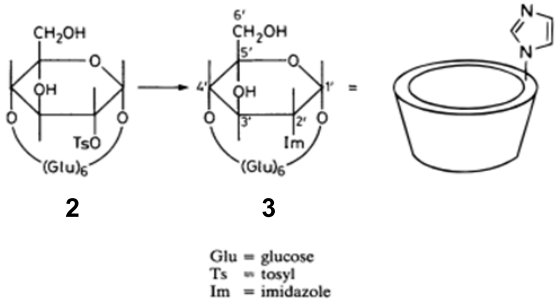
Attachment of a simple catalytic group to a cyclodextrin can afford interesting enzyme mimics. For example, Cramer and Mackersen[14] have introduced an imidazole group at C-6 of a β-CD (**1**) to mimic the enzymatic activity of chymotrypsin, a proteolytic enzyme that acts in the digestive system of many organisms by facilitating the cleavage of peptide bonds by a hydrolysis reaction. The reasoning behind this functionalization was that the enzyme has a optimum at pH of 7, indicating the partecipation of a catalytic group with pK ~ 7 in the rate determing step.




**1**

This system has shown only a slight rate enhancement compared to a “free” cyclodextrin since the catalytic group is attached to a primary side (C-6) of the cyclodextrin. Later efforts to attach an

imidazole group to a secondary side at C-2 or C-3 located on the more open face of cyclodextrin, to improve catalysis, have not been successful. The availability of the selective C-2 tosylate of β-CD (**2**), via an original procedure developed by Ueno and Breslow involving a tosyl transfer reaction,[9] allowed the reaction of **2** with imidazole to synthesise a catalytically efficient enzyme model of chymotrypsin (*Scheme 1.1*).




*Scheme 1.1.*

Preliminary kinetic results for the hydrolysis of *p*-nitrophenylacetate (*Table 1.1*) have shown that the chemical model **3** of the enzyme chymotrypsin with an imidazole on the secondary side of β-cyclodextrin has a rate constant 70 times higher than that of β-cyclodextrin with the imidazole substituent on the primary side.[15]

*Table 1.1. Pseudo-first-order rate constants[a] for the hydrolysis of p-nitrophenylacetate.*

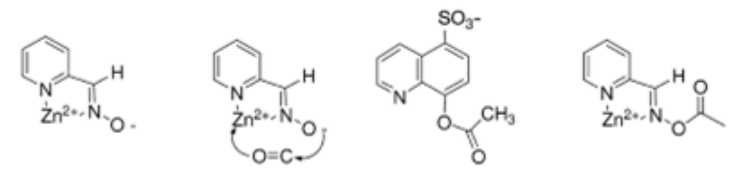
| **No.** | **Catalyst** | **$10^4 k$ ($s^{-1}$)[b]** |
|---|---|---|
| 1 | β-CD | 0.62 ± 0.01 |
| 2 | β-CD with imidazole on C-6 primary side | 12.20 ± 0.01 |
| 3 | β-CD with imidazole on C-2 secondary side (**3**) | 859.0 ± 2.5 |

[a] Rate of release of *p*-nitrophenol determined spectrometrically at 400 nm in Tris-HCl buffer (0.02 M, pH 7.5), catalyst (0.30 x $10^{-2}$ M), *p*-nitrophenylacetate (0.30 x $10^{-4}$ M) with 0.50% (v/v) added acetonitrile at 25°C.

[b] Average of three runs.

### 1.2.2. Metalloenzyme mimics

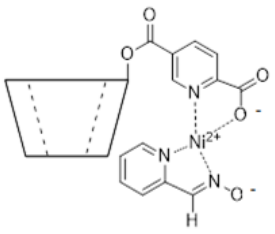
Chymotrypsin is only moderately effective as an enzyme, and much higher rates are seen with metalloenzymes. Zinc is particularly important in such hydrolytic enzymes. For example, the enzyme carboxypeptidase A uses zinc in a typical bifunctional role, at the same time activating the carbonyl towards addition by coordinating with its oxygen and activating water molecule to act as a nucleophile. Models for this type of process, that act by using metal complexing as the substrate binding force and a coordinated oxime as the nucleophile, have already been designed; one example is molecule **4**.[16] The structure of this compound points that the Lewis acidic Zinc and the basic oxime anion can co-exist without quenching each other; the electrons can flow from one to the other only through the bridging carbonyl group of the substrate (**5**). Consequently, the anion of **4** reacts with metal-bound substrate **6** to transfer the acetyl group to the oxime anionic oxygen, and then the intermediate **7** hydrolyses.



**4** **5** **6** **7**

In this process the metal ion serves multiple functions: it binds the substrate, acidifies the oxime, coordinates to the carbonyl oxygen of the transferring acetyl group, and then catalyzes hydrolysis of intermediate **7**.

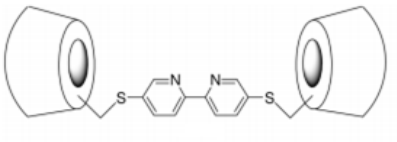
However, catalyst **4** is effective only with substrates that can bind to the metal ion. Researchers have developed new strategies in order to overcome this disadvantage by attaching this catalyst, coordinated as its $Ni^{2+}$ derivative, to the secondary face of α-cyclodextrin, obtaining molecule **8**.[17]



**8**

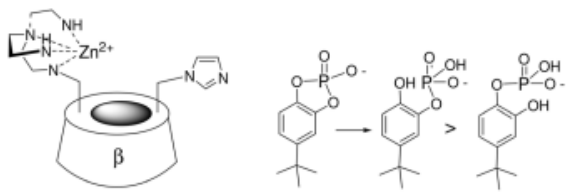
This was then able to use the metallo-oxime catalyst previously described, but with substrates that are not metal ligands, simply those that bind hydrophobically into the cyclodextrin cavity. Further kinetic studies showed a significantly increased rate of hydrolysis of *p*-nitrophenylacetate, much higher than that for hydrolysis without the catalyst or for simple acetyl transfer to the cyclodextrin itself.

Another very interesting example is provided by ligand **9**, in which the metal-coordinating group links two cyclodextrin rings.



**9**

Ligand **9**, as its $Cu^{2+}$ complex, gave as much as $10^5$-fold rate acceleration in the ester hydrolysis.[18,19] With an added nucleophile that also binds to the $Cu^{2+}$ ion, the reaction is accelerated by over $10^7$. The mechanism deduced involves the metal ion acting as a Lewis acid by coordination to the substrate carbonyl and delivering a bound hydroxide ion to the ester carbonyl group. With a cyclodextrin dimer related to **9** Breslow *et al.* managed to hydrolyze an ordinary doubly bound ester, not just the more reactive nitrophenyl esters, showing also catalytic turnovers.[20]As another example, disubstituted cyclodextrin **10** has been synthesized, in which one substituent is a metal-binding tris(2-aminoethyl)amine group while the other is an imidazole.[21] $Zn^{2+}$ complexed to the tris(2-aminoethyl)amine group gave good rate acceleration in the hydrolysis of bound catechol cyclic phosphate **11**, which was fastest when the two catalytic groups were attached to opposite sides of the cyclodextrin so they could not bind each other. The geometry of the complex showed also selectivity towards the formation of product **12** rather than **13** (*Figure 1.2*), while they both are formed equally by regular hydrolysis without the catalyst.



**10** **11** **12** **13**

*Figure 1.2. Disubstituted cyclodextrin as hydrolitic catalyst for catechol cyclic phosphate **11**.*

### 1.2.3. Bifunctional or multifunctional enzyme mimics

Enzymes often use acid and base catalysts derived from their amino acid side chains, and it is common for them to use more than one of such groups in simultaneous bifunctional or multifunctional catalysis. Thus, it is interesting to imitate this feature in artificial enzymes. For example, the enzyme ribonuclease A uses two imidazole groups, histidines 12 and 119, as its principal catalytic groups in the hydrolysis of RNA. To mimic this enzyme, two imidazole rings were attached to the primary face of β-CD.[22,23]

By the use of appropriate bridging groups, it is possible to make disulfonate esters of β-CD on adjacent glucose units (AB), on units one further apart (AC), or on units separated by two glucose residues (AD) (*Figure 1.3*).[24]

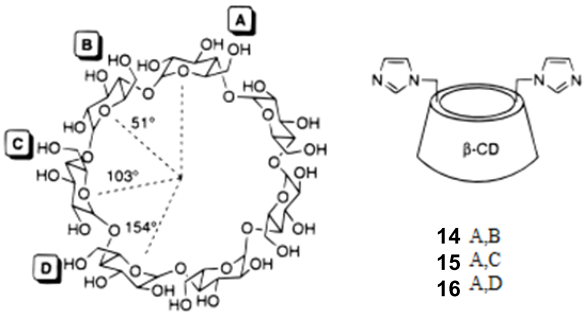



*Figure 1.3. The AB (**14**), AC (**15**), AD (**16**) isomers of a cyclodextrin carrying two imidazoles on the primary carbons.*

These bridged cyclodextrins were converted to the corresponding diiodides, and reaction with imidazole afforded catalysts **14-16**. All three of these enzyme mimics were able to catalyze the hydrolysis of a cyclic phosphate **17** that could bind well into the β-CD cavity, and all three showed a bell-shaped pH vs. rate profile with a rate maximum near pH 6.2. This is almost identical to the pH vs. rate profile for the enzyme ribonuclease itself and indicates that one imidazole works in its protonated form while the other is unprotonated (*Figure 1.4*). Isotope effect studies showed that the two catalytic groups were operating simultaneously.[25]

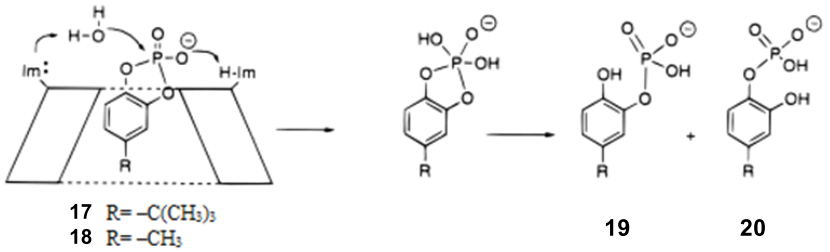



*Figure 1.4. Cyclodextrin bis(imidazoles) catalysing the hydrolysis of substrates **17** and **18.***

In the classical mechanism for the enzyme, the hydrolysis of the cyclic phosphate intermediate in RNA cleavage involves water delivery to the phosphorous atom by the unprotonated imidazole, while the leaving group is protonated by the imidazolium ion. If the enzyme mimics used a similar mechanism, the AD isomer **16** would be the most active, since it has the best geometry for this mechanism. However, it was found that the best catalyst for the hydrolysis of **17** was the AB isomer **14**. This indicated that the function of the imidazolium ion was to protonate the phosphate anionic oxygen, and it can be more easily reached by **14** than by other catalyst isomers.

A study was made on the importance of a tight fit of substrate into the binding cavity for such enzyme model systems.[26] The substrates were either the *tert*-butyl derivative **17** or an analogue **18** with a methyl group instead. The catalysts were all AB diimidazoles, but α, β, or γ-cyclodextrins were tested, investigating the binding of the substrate when increasing the cavity size. The strongest binding was seen with the *tert*-butylated substrate **17** into the β-CD derivative **14**, and this combination also gave the fastest rate of hydrolysis. It was also the most selective, since only product **19** could be detected; the other catalyst-substrate combination afforded mixtures of products **19** and **20**.

Being available a set of cyclodextrin catalysts carrying two imidazoles in different geometries, it is possible to investigate other reactions catalyzed by simultaneous acid-base proton transfers. One process studied was the enolization of a bound ketone, *p-tert*-butylacetophenone (**21**), which binds well into β-CD (*Figure 1.5*).[27,28]

The reaction showed a bell-shaped pH vs. rate curve, indicating that both the imidazole and imidazolium ion played a catalytic role. It was found that the best isomer for the enolization, monitored by deuterium exchange, was the AD isomer. This indicated what the preferred geometry is for proton abstraction from carbon, an important matter not easily determined without the geometric

information furnished by these bifunctional catalysts. The same catalyst set has also been examined, and found effective, in two intramolecular aldol condensations involving ketoaldehyde **22** and dialdehyde **23**.[29,30]

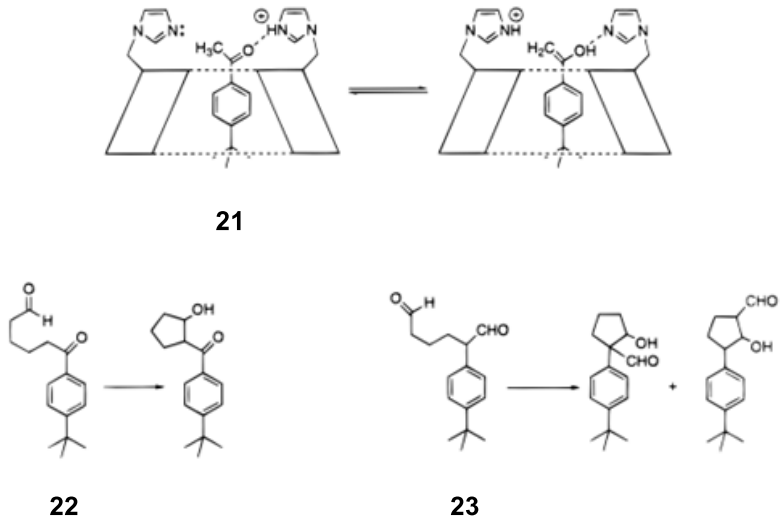



*Figure 1.5. Cyclodextrin bis(imidazoles) catalysing enolization of **21** and aldol condensation of **22** and **23**.*

Another very interesting example of bifunctional enzyme models is the one provided from the synthesis of a glycoside hydrolases enzyme mimicked by difunctionalized cyclodextrins. Glycoside hydrolases (also called glycosidases) assist in the hydrolysis of glycosidic bonds in sugars. The inspiration for the design of the CD glycosidases came from the world of natural glycosidases which in their catalysis typically surround two catalytically active carboxylate groups in their active site.[31] This acid/base-catalysis principle represents the basis for the invention of carboxylate cyclodextrin glycosidases, that were able to catalyze aryl glycoside bond hydrolysis reactions with rate enhancements of up to 1000 times, following the enzyme-characteristic Michaelis-Menten rate law pattern.[32,33] Their mechanism of catalysis is proposed to involve electrostatic stabilization of the transition state by the carboxylate groups, with subsequent nucleophilic substitution with phosphate (*Figure 1.6*).

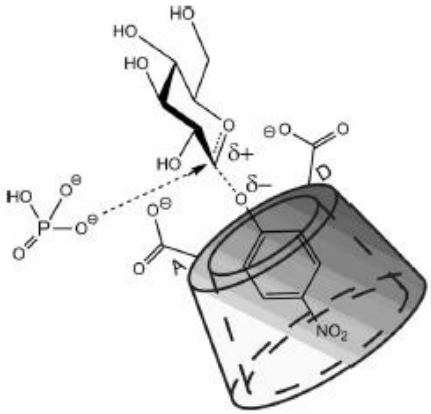


*Figure 1.6. Proposed mechanism of carboxylate CD hydrolysis of aryl glycosides in presence of phosphate buffer.*

Other cyclodextrin enzymes that act as artificial glycosidases are the trifluoromethyl alcohol CDs, but they do so only with moderate activity.[34] The greater breakthrough in CD artificial glycosides came with the discovery of the CD cyanohydrins, affording up to 8000 times increase in reaction rate.[35-37] Synthesis of $7^A,7^D$-dicyanohydrin-β-CD, in which the cyanohydrin functionality is positioned one carbon atom further away from the cavity than in the known $6^A,6^D$-dicyanohydrin CDs, offers a particularly efficient enzyme model.[38] In the proposed mechanism for the catalyzed reaction, the electron-withdrawing effect of the nitrile group acidifies the cyanohydrin hydroxy group, making easier the donation of this alcohol proton to the substrate glycosidic oxygen, thereby facilitating bond cleavage (*Figure 1.7).*

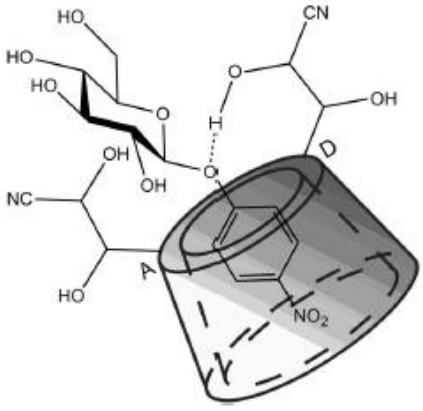
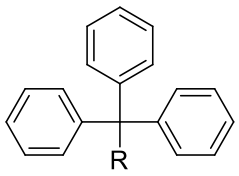


*Figure 1.7. Proposed mechanism of cyanohydrin CD catalysis.*

### 1.3. Primary face modifications of cyclodextrins

Primary hydroxyl groups of cyclodextrins are known to be more nucleophilic than their secondary counterparts, thus, they are easily modified into other functional groups. Selective permodification of all the primary hydroxyl groups is relatively easier than mono-, di-, or tri-substitution because symmetrical substitution is achieved when the reaction is allowed to run for a longer time with appropriate amount of reagents. Regioisomerism further complicates this situation when selective di- or tri-substituted cyclodextrins have to be prepared. Moreover, in most of the cases, these products require solvent and time-consuming chromatographic purification.

In principle, strong electrophiles such as alkyl, phosphoryl, silyl, sulfonyl, or carboxylic acid chlorides react with hydroxyl groups of CD to produce an alkylated, silylated, sulfonated, or acetylated product along with an acid, which is neutralized by using a basic solvent or a weak base. The reason for this is that cyclodextrins are stable under basic conditions while they decompose in the presence of a strong acid. All these reagents are very reactive and attack the hydroxyl groups of cyclodextrins indiscriminately producing mono-, di-, or tri-substituted products. Increasing the size of these reagents is not helpful in controlling the selectivity; even a bulky group like trityl (**24**) is not selective and gives a mixture of products which requires chromatographic separation.[39,40] However, in most of the described transformations we observe reaction mixtures from which it is very often difficult to separate the target molecule.

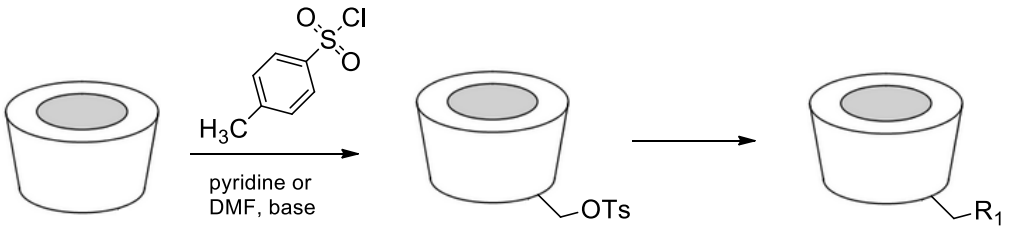



**24**

Therefore, from a synthetic point of view, among all the substituents, only the sulfonyl group acts as a good leaving group and can be displaced by nucleophiles to synthesize useful derivatives. The 6-sulfonates serve as precursor for the preparation of the 6-deoxycyclodextrin compounds. Many nucleophiles attack the carbon atom at the 6-position in these sulfonates to give the corresponding modified CD.

### 1.3.1. Monosubstitution at the 6-position of cyclodextrins

The most popular method for monomodifications at the 6-position of cyclodextrins is to convert the cyclodextrins into its mono-6-sulfonylcyclodextrin conjugate and then perform a nucleophilic attack of a reagent containing the appropriate group. These monosulfonates are prepared by reacting 1 equiv. of *p*-toluenesulfonyl chloride with cyclodextrin in pyridine or DMF containing a base (*Scheme 1.2*).

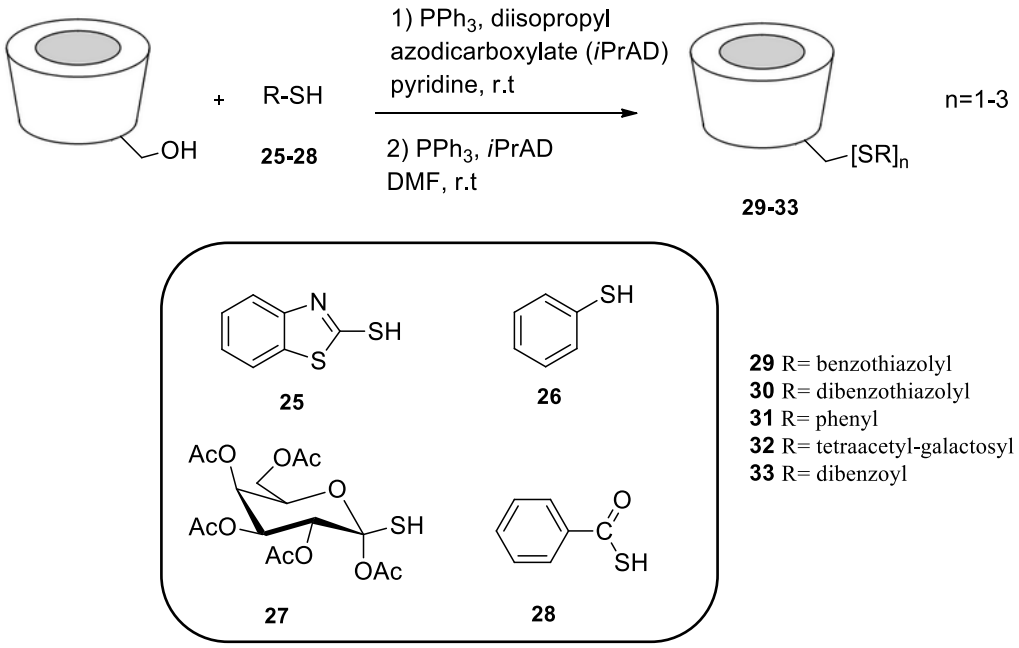



$R_1$ = $I^-$, $N_3^-$, -$SCOCH_3$, alkyl or polyalkyl amines, alkyl-thio

*Scheme 1.2*.

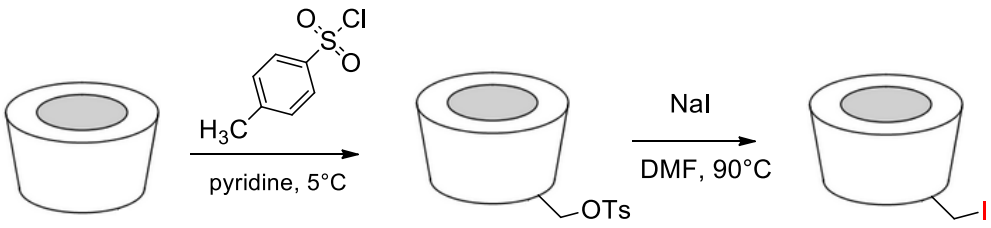
Monotosylation of cyclodextrin is often a nonselective process and produces a mixture of primary as well as seconday side tosylated products along with *di*- or *tri*-tosylated derivatives. Thus, depending on the desired purity of the final product, it requires extensive purification. The yield of the final product is often reduced because the tosylate can undergo exchange by chloride ions or an elimination process to give either a 3,6-anhydro compound or an alkene. Pyridine, a non-user-friendly solvent of choice for this reaction, forms a pyridinium complex with the cavity and complicates the workup process. However, the major advantage of this solvent is its ability to direct the reaction to the 6-position as compared to DMF where sulfonation occurs on both faces of cyclodextrin. Despite all these problems and drawbacks, monotosylates have been extensively studied.[41] One of the best developed methods for the synthesis of monotosylcyclodextrins is to react cyclodextrin with tosyl chloride in 1:1 equivalent ratio in aqueous alkaline medium for a short time, to give the mono-6-tosylate in good yield.

The direct synthesis of monothiocyclodextrins with aromatic thiol and unprotected cyclodextrin in DMF or pyridine can be performed by a thio-Mitsunobu reaction. This reaction gives a mixture of mono-, di-, and tri-substituted products which are purified by chromatography (*Scheme 1.3*).[42]



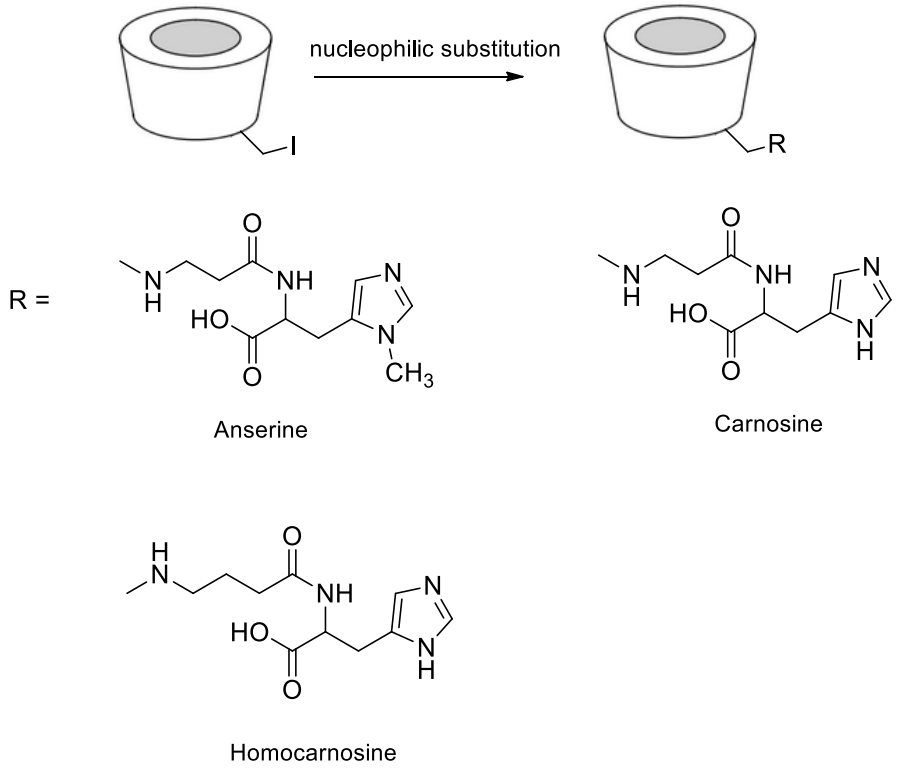

*Scheme 1.3.*

Mono-tosyl derivatives of cyclodextrins are particularly useful for the possibility to convert them into mono-deoxy-iodo-CDs (*Scheme 1.4*),[43] which can be readily reacted with nucleophile to obtain monofunctionalized cyclodextrins.



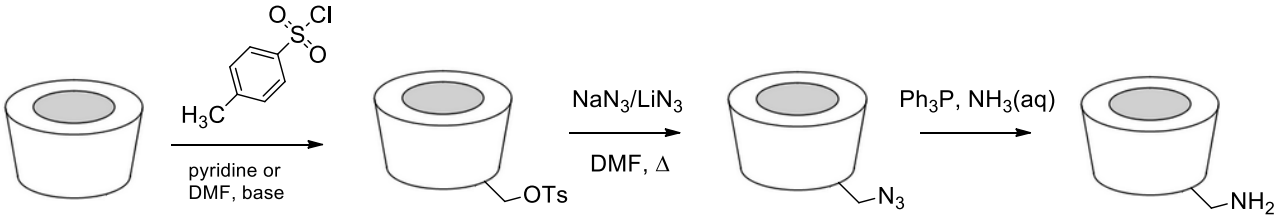

*Scheme 1.4.*

Thanks to the reactivity of these compounds, a group of Italian researchers have selectively functionalized a primary hydroxyl of a β-cyclodextrin with different substituents like Anserine (β-alanyl-3-methyl-*L*-histidine), Carnosine (β-alanyl-*L*-histidine) and Homocaronisine (γ-aminobutyryl-*L*-histidine) without using any protecting group (*Scheme 1.5*).[44]




*Scheme 1.5.*

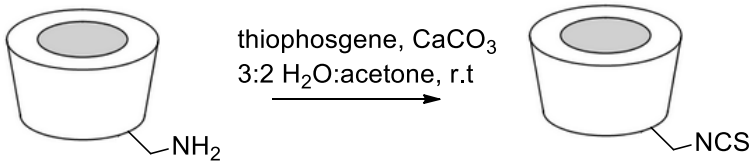
In addition, monoamino derivatives of cyclodextrin are particularly interesting. These are conveniently obtained from monoazides of cyclodextrin by reduction with triphenylphosphine in the presence of aqueous ammonia.[8] Monoazides of cyclodextrin are indirectly obtained by heating the monotosylate with sodium or lithium azide salt in DMF (*Scheme 1.6*).




*Scheme 1.6.*

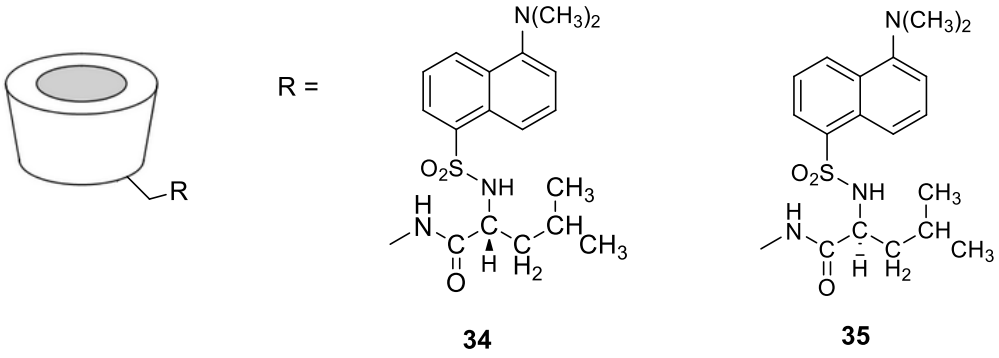
A direct approach to make monoazides is through Vilsmeier-Haack type reactions in which cyclodextrins are heated with $NaN_3$ containing triphenylphosphine in DMF.[45]

Monoamines show greater solubility and react with isocyanate without a need to protect the primary hydroxyl groups to produce isothiocyanatocyclodextrins (*Scheme 1.7).*[46]



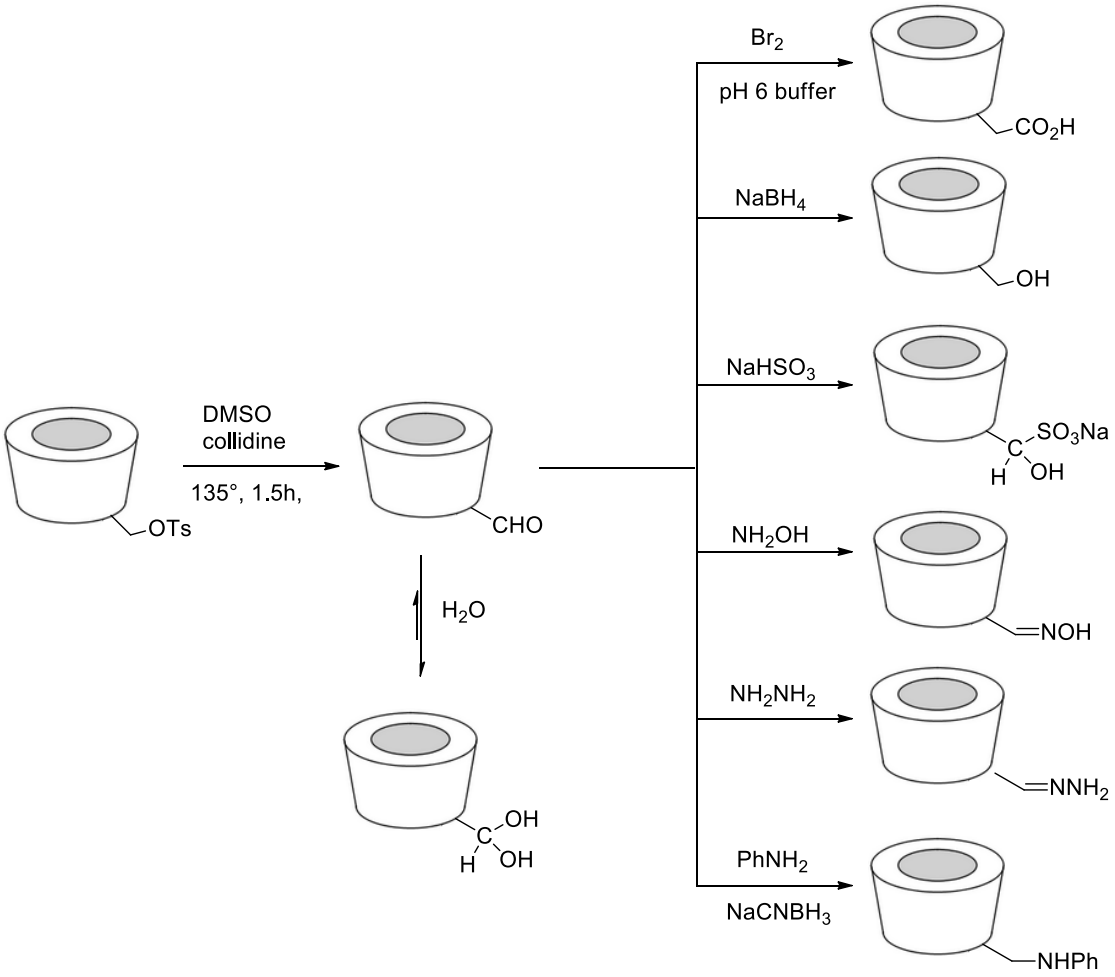

*Scheme 1.7.*

Monoamines are probably the best functionality used in attaching desired groups to the primary side of cyclodextrins via carbodiimide (DCC) coupling method. This strategy has been used to connect various sugar units such as β-*D*-glucose, β-*D*-galactose, α-*D*-mannose and β-*D*- and *L*-fructose to cyclodextrins through alkyl chains. Monoamines condense also with *D*- or *L*-*N*-dansylleucine and 1-hydroxybenzotriazole in DMF containing DCC at room temperature to form *D*- or *L*-mono-6-(*N*-dansylleucylamino)-6-deoxy- β-cyclodextrin in 50% yield (**34** and **35**).[47]



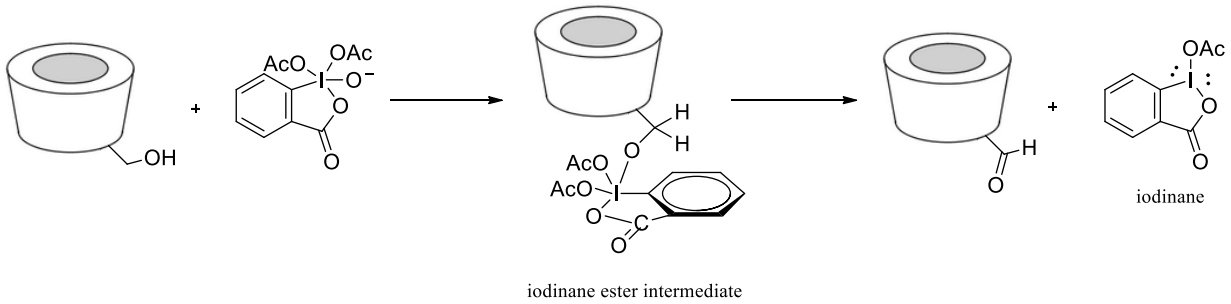

**34** **35**

Monoaldehydic cyclodextrins are an important class of derivatives because they provide a route for further modifications. The monoaldehyde has been synthesized by oxidizing 6-tosyl-β-cyclodextrin in DMSO with collidine added as a non-nucleophilic base. Further oxidation of the monoaldehyde leads to the corresponding carboxylic acid. If we react the monoaldehyde with hydroxylamine or hydrazine we can also produce a monooxime or a monohydrazone derivatives (*Scheme 1.8*).[48,49]




*Scheme 1.8.*

The monoaldehyde can be synthesized directly by reacting cyclodextrins with Dess-Martin periodinane (DMP) in 85-100% yield (*Scheme 1.9*).[50] This process avoids complications in the synthesis of monotosylcyclodextrin previously mentioned.




*Scheme 1.9.*

Alkyl ethers of cyclodextrins cannot be synthesized from tosylates because nucleophiles in this case (alkoxide ions) act as strong bases which pick up protons from hydroxyl groups at the 3-position and produce the 3,6-anhydro compound by ring inversion (*Figure 1.8*).

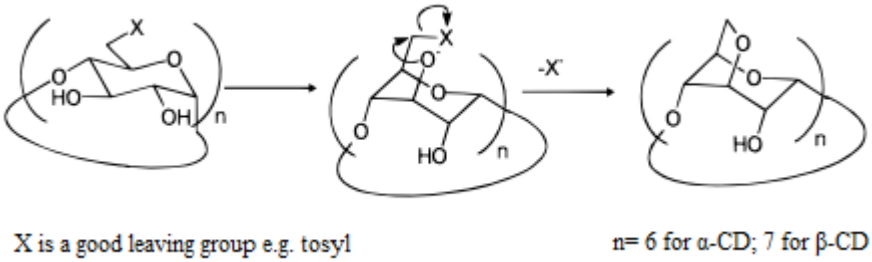
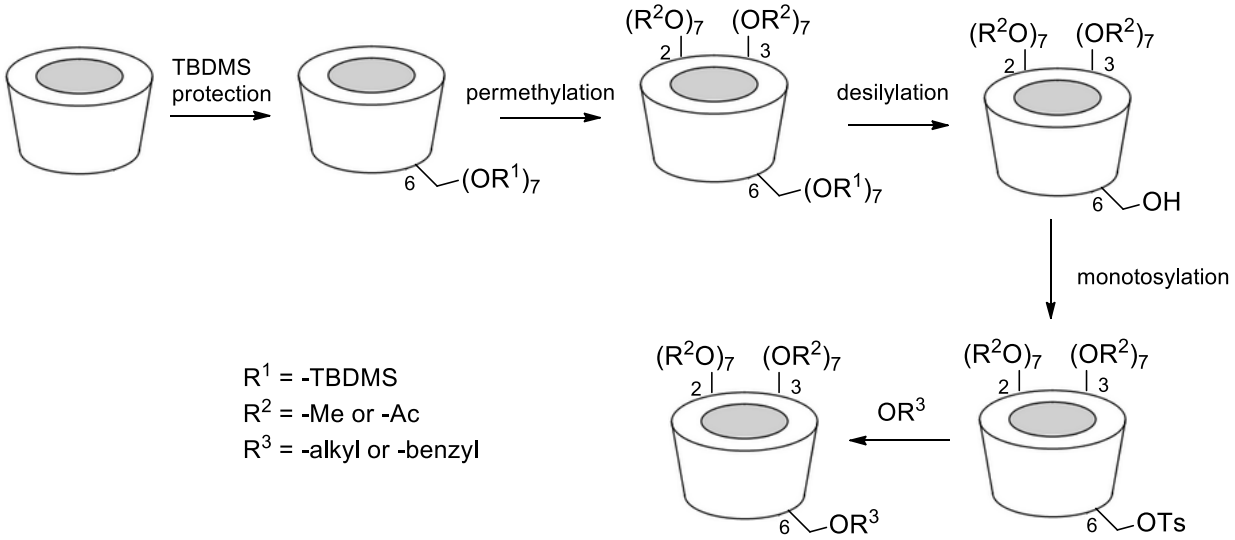



*Figure 1.8. Conversion of a 6-substituted cyclodextrin to a 3,6-anhydrocyclodextrin.*

Alkyl ethers of β-CD are obtained by a method that consists in a primary side protection with TBDMS. This is followed by permethylation of the secondary face (both 2- and 3-positions), desilylation of the primary side, and then monotosylation of the primary side. The reaction of an alkoxide ion with this protected tosylate gives the desired alkyl ether on the primary side without the formation of the 3,6-anhydro derivative (*Scheme 1.10*).[51]




*Scheme 1.10.*

The main problem with this approach is that methyl groups on the secondary side cannot be easily removed. This limitation can be overcome by using acetyl groups to protect the secondary side, which can be subsequently hydrolyzed under aqueous alkaline conditions to afford the final product. This

"long" method has been made shorter by directly protecting the secondary side using TBDMSCl without first protecting the primary side, a protection strategy that takes advantage of the acidity of the hydroxyl groups at the 2-position to selectively deprotonate them. In this way, their nucleophilicity is increased, as the reactivity towards TBDMSCl compared to that of the hydroxyl groups on the primary side. Then, the protected cyclodextrin can undergo selective alkylation of the primary face.[52] The advantage of this approach is that the protecting groups TBDMS is easily removed under mild conditions once the desired modification on the primary side are completed.

### 1.3.2. Disubstitution at the 6-position of cyclodextrins

Disubstituted cyclodextrins are obtained by using more than 1 equivalent of reagent with cyclodextrin under suitable conditions to give a mixture of products. It is a inconvenient process that affords the formation of positional isomers and regio-isomers that require extensive purification by HPLC. Statistical calculations suggest that disubstitution can produce 33 regioisomers in the case of β-cyclodextrins,[53] which indicates the enormous complexity of the process. As in the sulfonation reactions in general,[54] the situation can be further complicated by substitutions on the secondary side.

A particularly efficient method to obtain disulfonated cyclodextrins is by reaction of arenesulfonyl chlorides with cyclodextrins to give AB, AC, and AD isomers.[55] Although these disulfonyl chlorides give a mixture of regioisomers, they show a distinct regiospecificity based on their structures. An elegant method to control the regiospecificity to produce AB, AC, or AD isomers by the use of the geometry of the reagents has been described.[56] For example, as shown in *Figure 1.9*, *trans*-stilbene and biphenyl-based capping reagents preferentially give the AD isomers,[57] benzophenone-based reagents give AC isomers, and 1,3-benzenedisulfonyl chlorides (especially the electron-rich 4,6-dimethoxybenzene-1,3-disulfonyl chloride) give the AB isomers.[58]

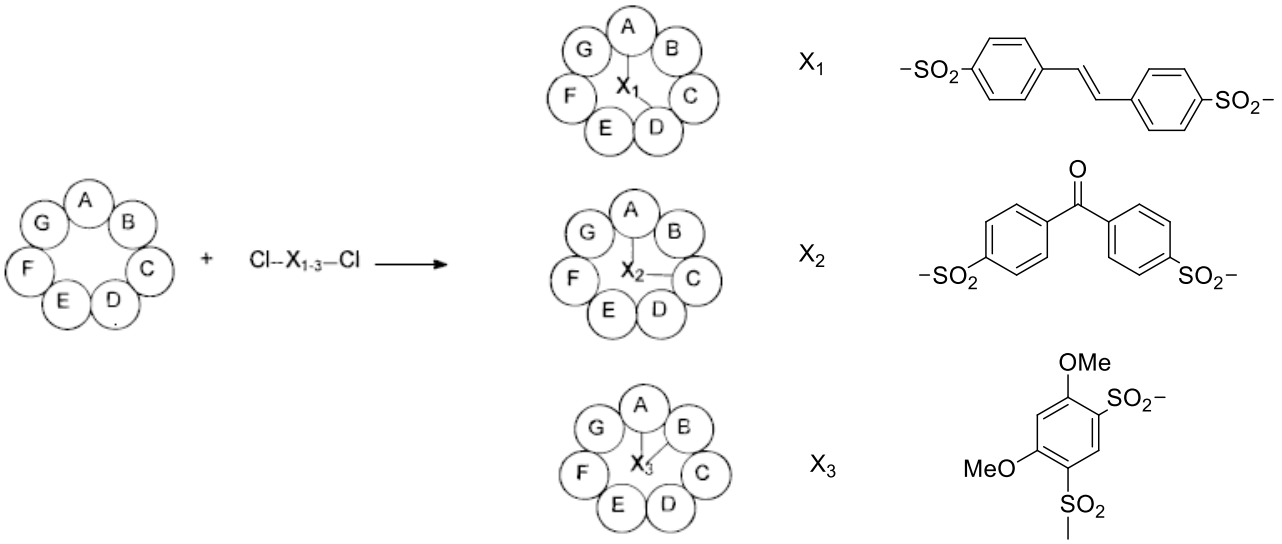
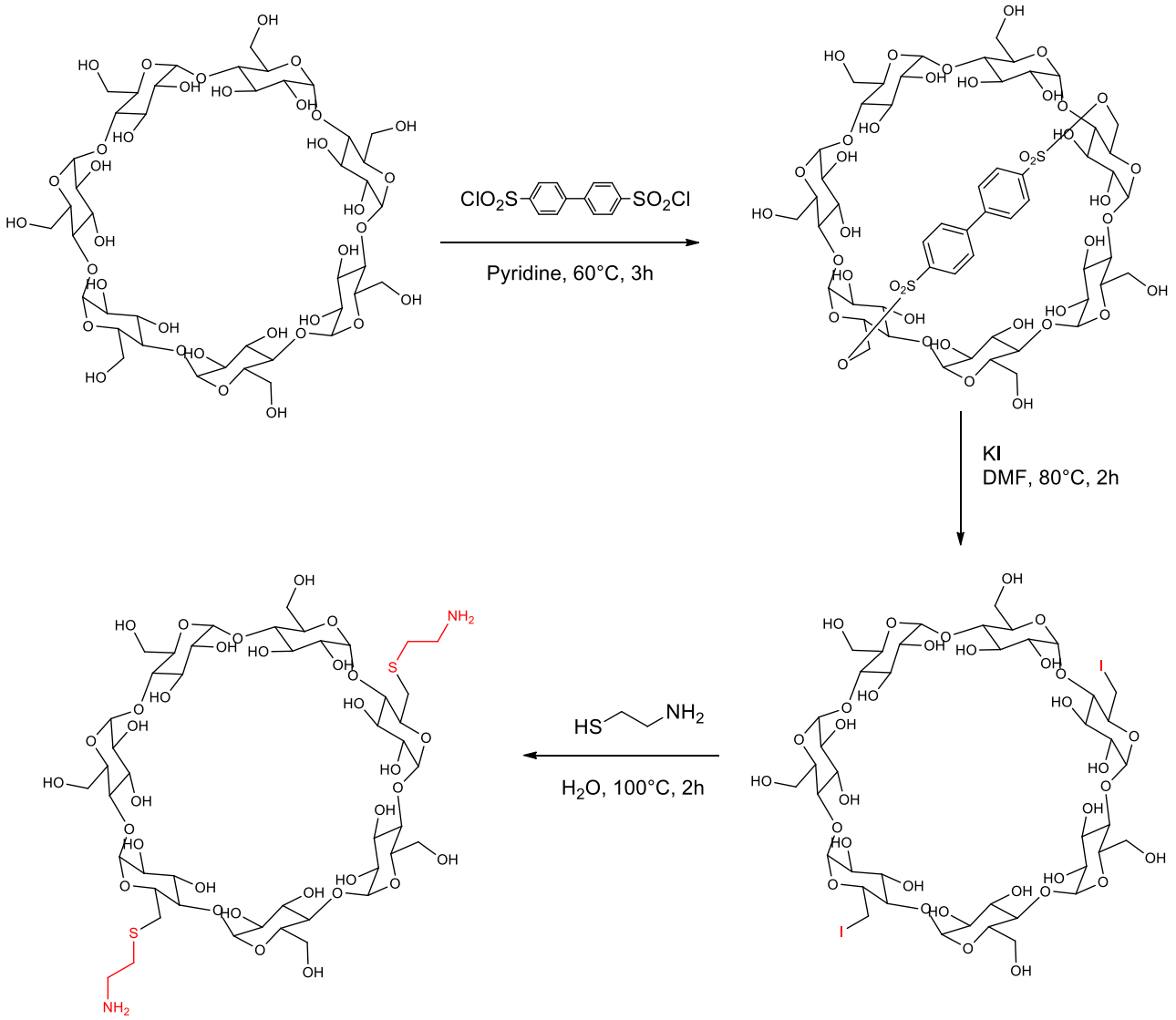



*Figure 1.9. Use of the geometry of reagents to direct the regiospecificity in disubstitution of cyclodextrins.*

Disulfonated cyclodextrins are important intermediates in the synthesis of disubstituted cyclodextrins. The strategy is to displace the ditosylates with nucleophiles in a manner similar to the reaction of monotosylates. It is important to note that the positional isomerism (AB, AC, AD) of the ditosylate is retained in these disubstituted derivatives.

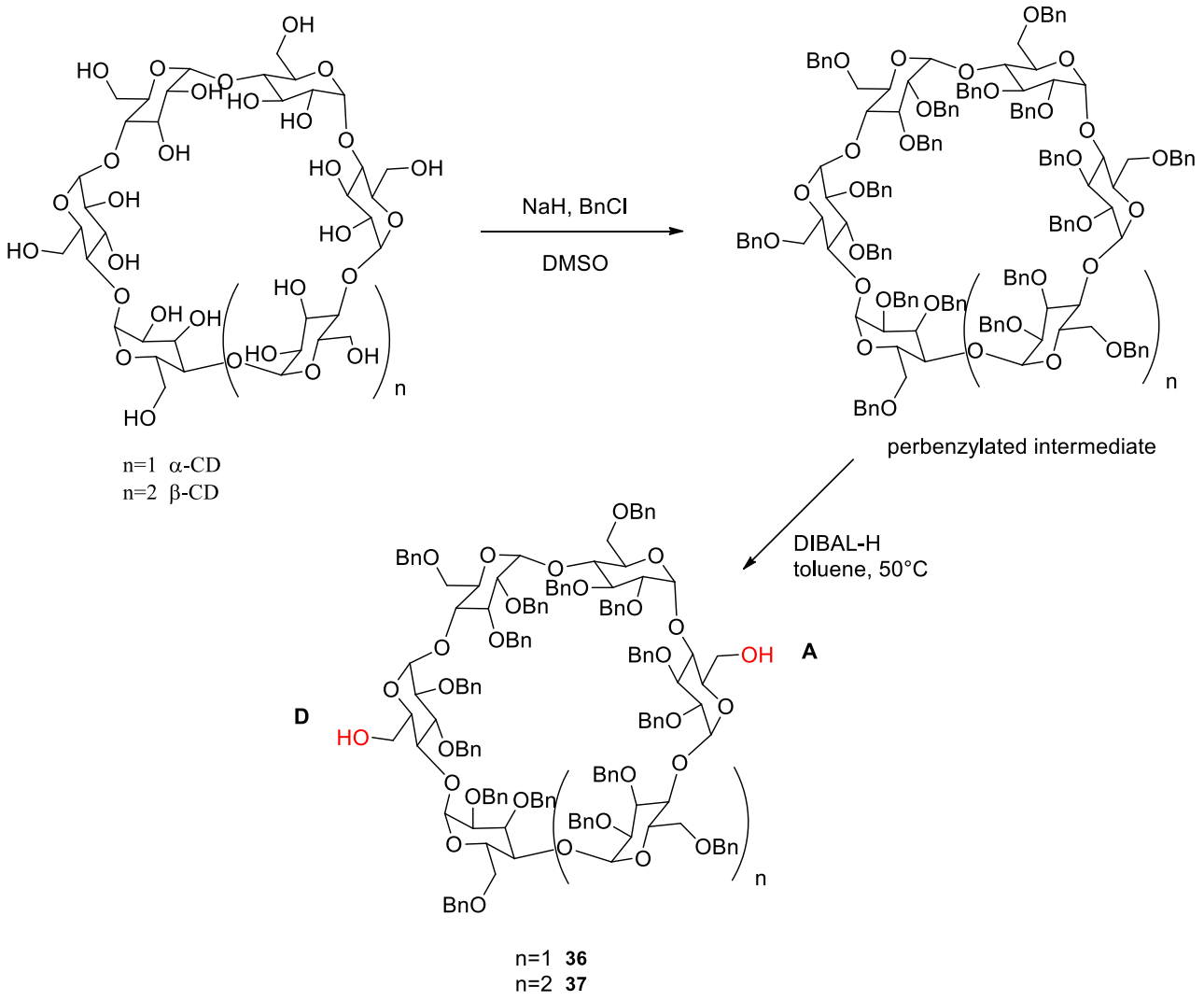
Another strategy for synthesis of substituted cyclodextrins is to convert the ditosylates to diiodo derivatives by a reaction with KI and then treat these diiodides with appropriate nucleophiles. An example is provided by the synthesis of the difunctionalized β-CD 6A,6D-di(2-aminoethanethio)-β-cyclodextrin (*Scheme 1.11*).[59]




*Scheme 1.11.*

This approach provides also the advantage that disulfonated or dihalogenated cyclodextrins react with alkanethiolates in aqueous or DMF medium to give thioethers of cyclodextrins.

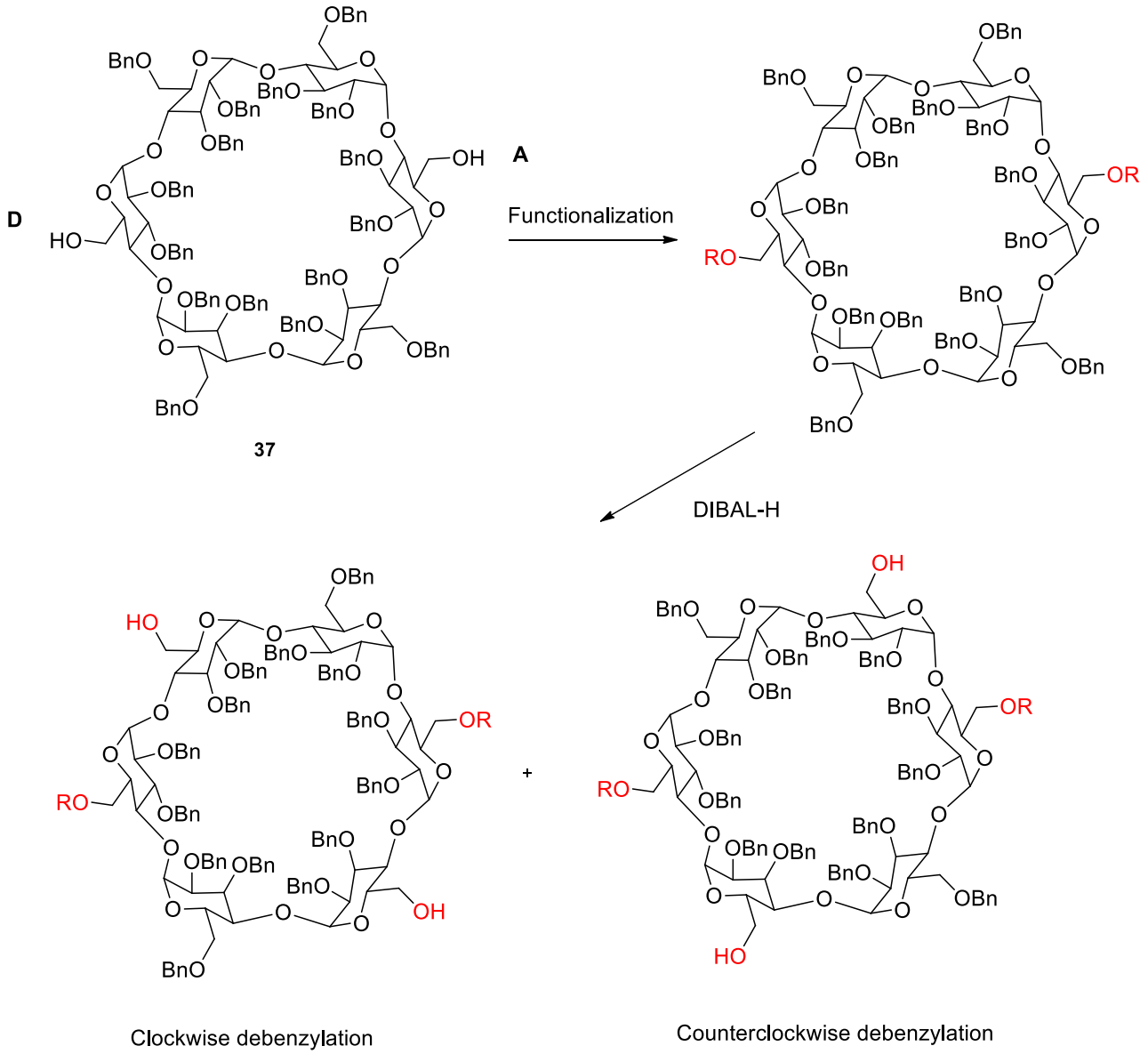
Another very particular and interesting approach is the one started from the discovery of an original strategy to regioselectively deprotect sugars[60] that allowed the possibility of differentiate cyclodextrins.[61] A fully benzyl-protected CD is selectively bis-deprotected by using diisobutylaluminium hydride (DIBAL-H) to afford $6^A$,$6^D$-diols **36** and **37** in 82 and 83% yield from α- and β-CDs, respectively (*Scheme 1.12*).




*Scheme 1.12.*

A remarkable feature of this reaction is that it is a rare example of selective CD bis-functionalization being as efficient on both α- and β-CDs.[62]

In order to better explore the potentiality of this approach, further studies on DIBAL-H promoted selective deprotection have been carried out. The first step was the functionalization of the α-CD **36** on positions $6^A$,$6^D$ by an appropriate OR group resistant to DIBAL-H. The aim of this work was to address if it was possible to duplicate the deprotection in a regioselective manner, and as result only two diametrically opposed hydroxyl groups were found to be deprotected, by analogy with the first deprotection process (*Scheme 1.13*).

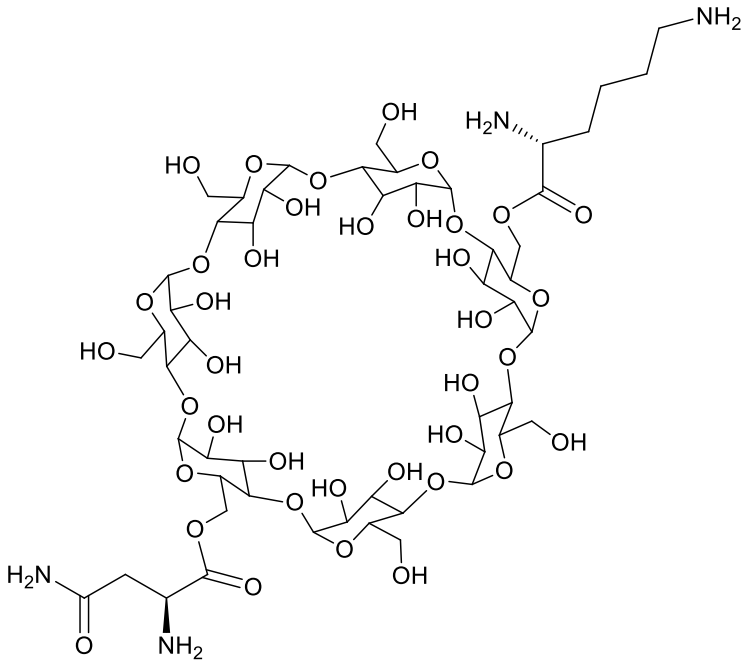



*Scheme 1.13.*

## 1.4. Results and discussion

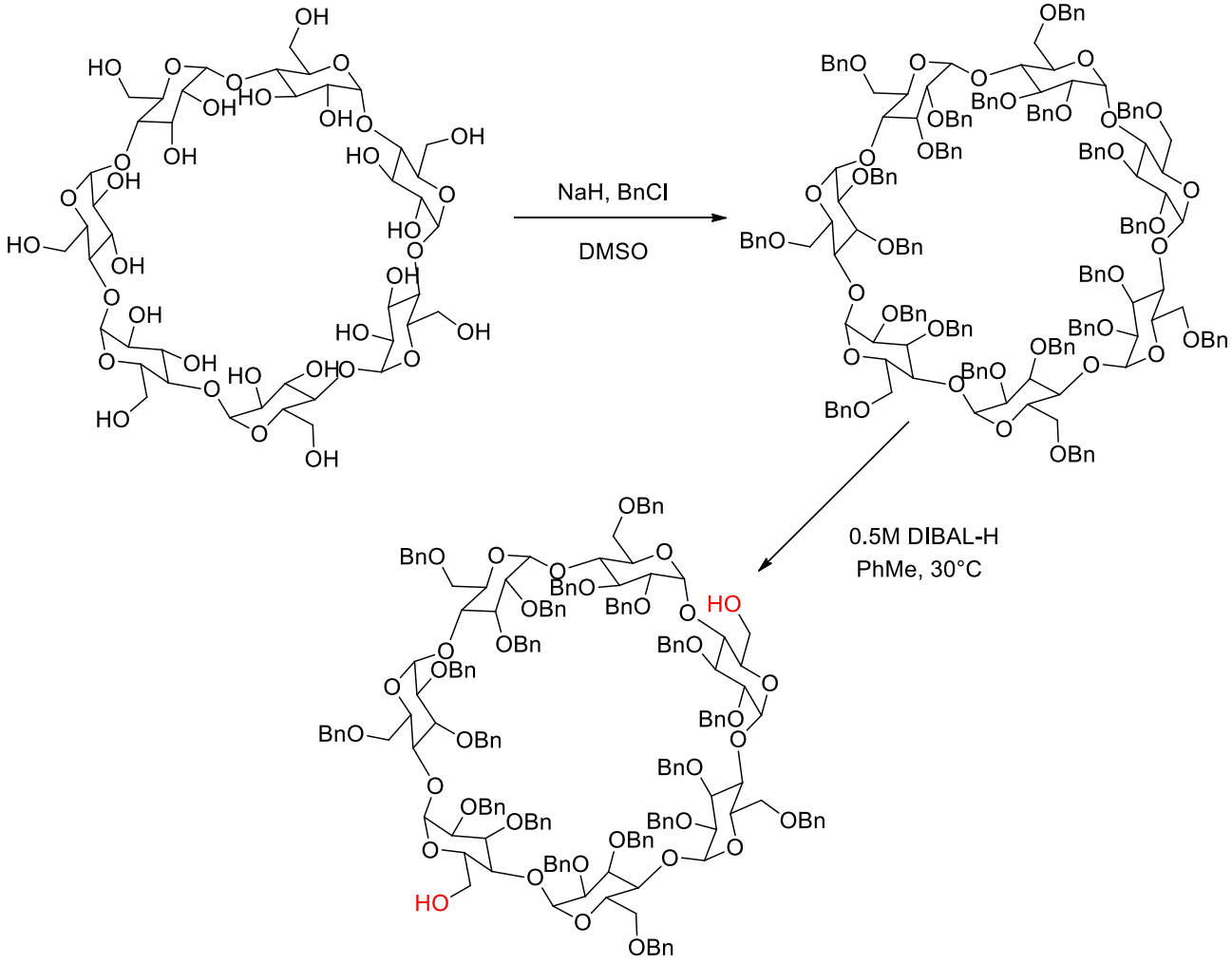
Computational studies performed by physical chemists at Dompé S.p.A. have shown that the distance between the helices 3,7 of the G-protein coupled receptor CXCR1, whose terminal amino acids are one unit of *L*-Lysine and one of *L*-Asparagine, can be considered comparable to the distance between the primary hydroxyls at position 1,4 ($6^A$, $6^D$) in a β-cyclodextrin. The possibility to build an enzymatic model of this kind is particularly useful for studying the interaction of this class of receptors with the already realized inhibitors and with new biologically active molecules.

The aim of this project was to develop a synthetic strategy to obtain the β-cyclodextrin **38** difunctionalized in positions $6^A$ and $6^D$ with one unit of *L*-Asparagine and one of *L*-lysine, respectively.



**38**

As already described in the previous chapter, some strategies to synthesize 1,4 ($6^A$–$6^D$) difunctionalized β-cyclodextrins are known in literature. One of them is based on the preparation of a perbenzylated cyclodextrin and subsequently, through the use of DIBAL-H, on the selective deprotection of the two desired position obtaining the diol **37**.

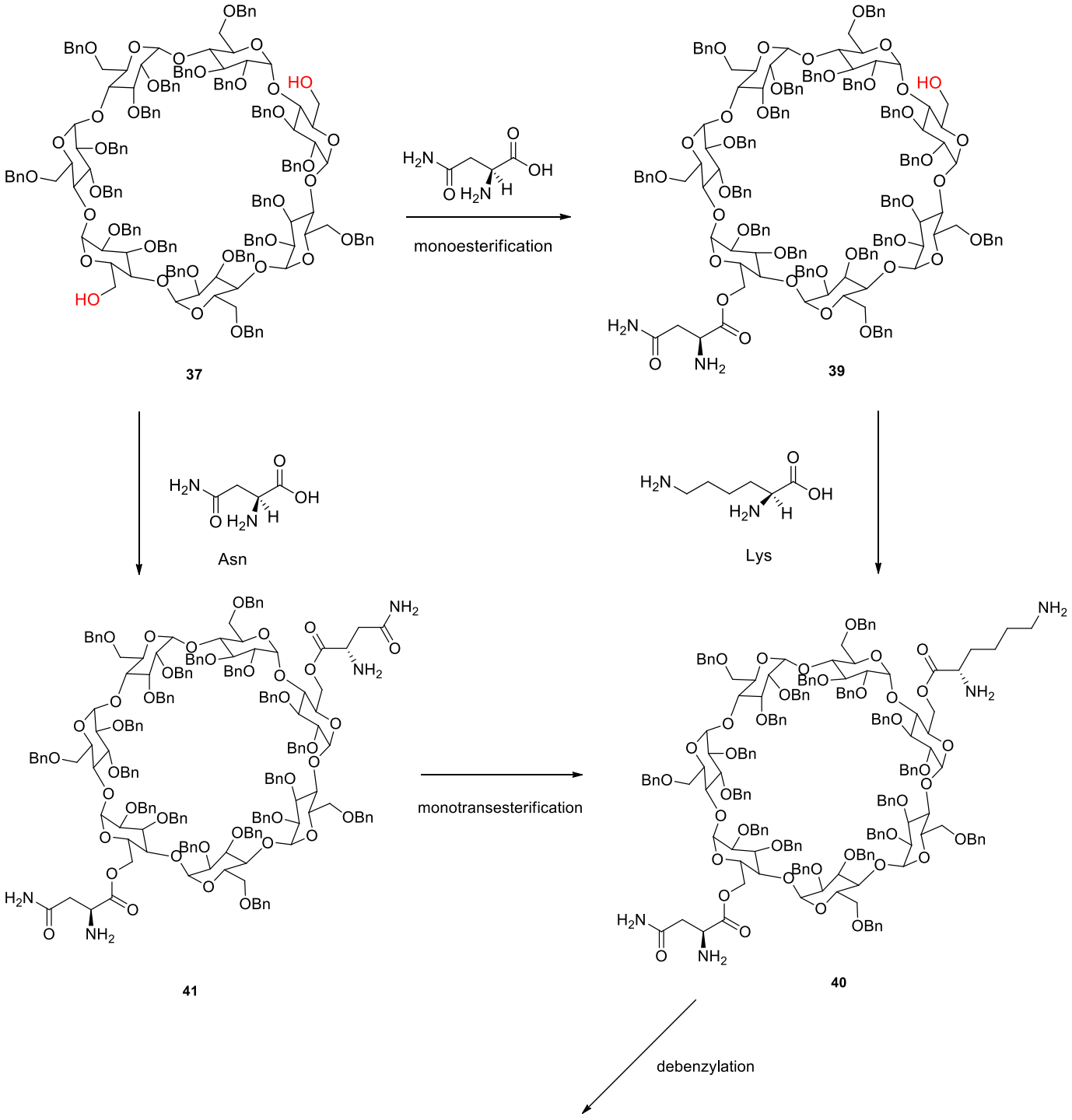



**37**

One might think to get to the target molecule **38** starting from **37** through chemical functionalization of cyclodextrins. However, it is clear that it remains difficult to functionalize selectively one of the hydroxyl group with a unit of Asparagine and the other with a unit of Lysine. At this point a possible strategy may be to functionalize the two free hydroxyl groups in two different steps. Some methods that allow to obtain monoesters from aliphatic diols,[63] although they have never been applied in the chemistry of carbohydrates, are present in literature.

This fact offers the possibility to proceed in two ways. The first consists in a monoesterification with an amino acid residue to obtain a monosubstituted cyclodextrin (**39**) and then proceed with a second monoesterification, making the second amino acid to react to obtain compound **40**. Another hypothesis is to synthesize a difunctionalized CD with the same amino acid (**41**) and, with a subsequent monotransesterifiction, achieve the derivative **40**. A final debenzylation would provide target cyclodextrin **37** (*Scheme 1.14*).

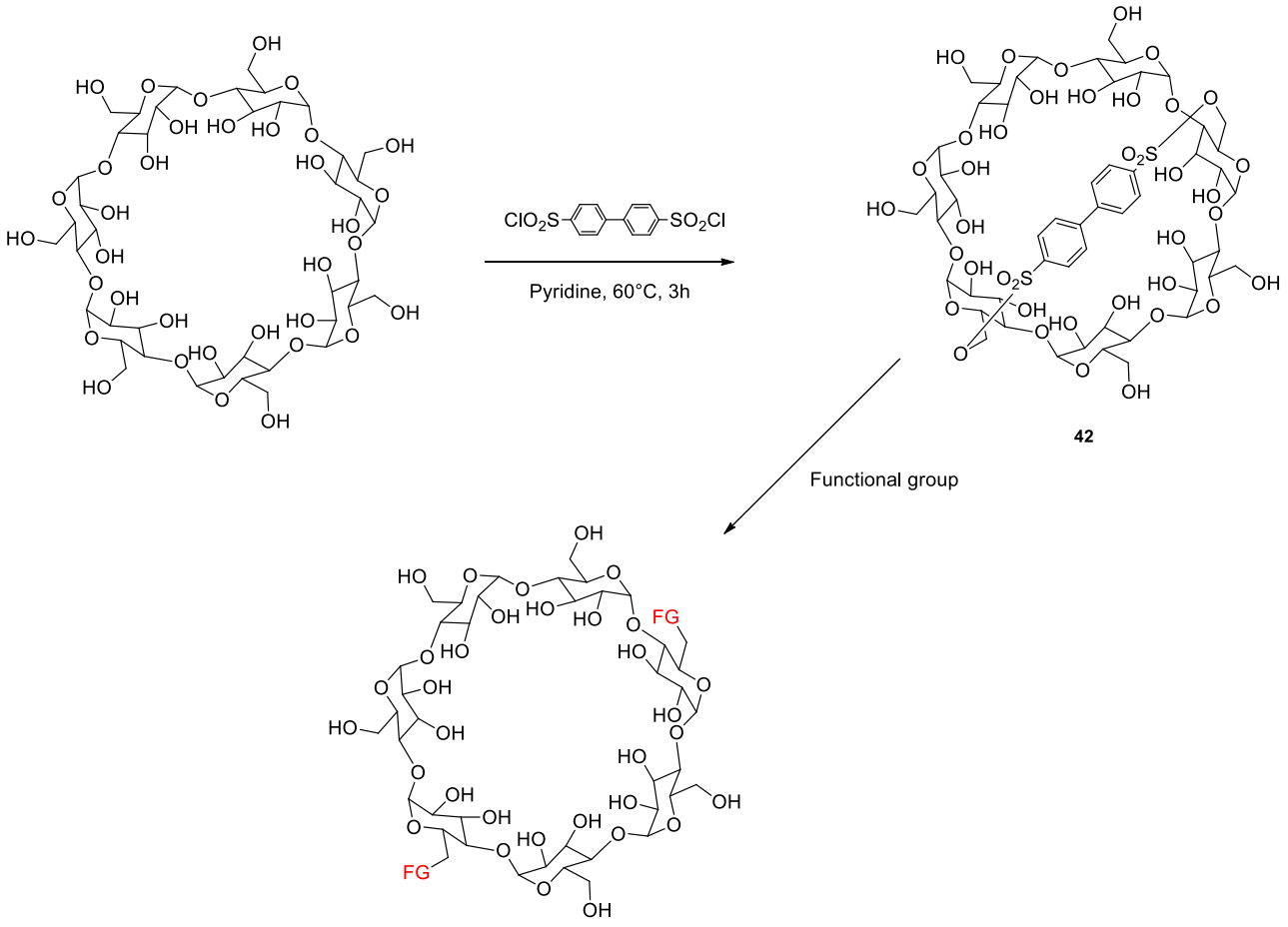
At this point all the possible isomers should be separated with a chiral chromatographic column. This strategy, as well as having the problem of a poor selectivity, has the disadvantage of the necessity to carry out the two steps of protection and deprotection, which would lengthen a synthesis already constituted by several steps and this could further affect the final yield.




*Scheme 1.14.*

Another route is the one that follows the capping mechanism of the position 1 and 4 of the cyclodextrin using arenesulfonyl chlorides. In particular, the use of a biphenyl-4,4'-disulfonyl chloride would lead to the synthesis of derivative **42** without the protection of other free hydroxyl groups (*Scheme 1.15*).[59]

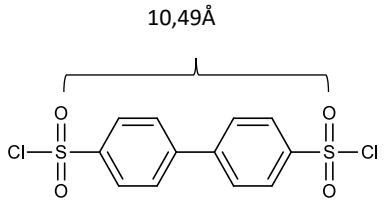
Certainly, the possibility to avoid the protection of the whole cyclodextrin allows to get to the difunctionalized β-CD through a more sustainable strategy. However, we must not underestimate that the unprotected β-CDs are not very soluble both in water and in most of the organic solvents.




*Scheme 1.15.*

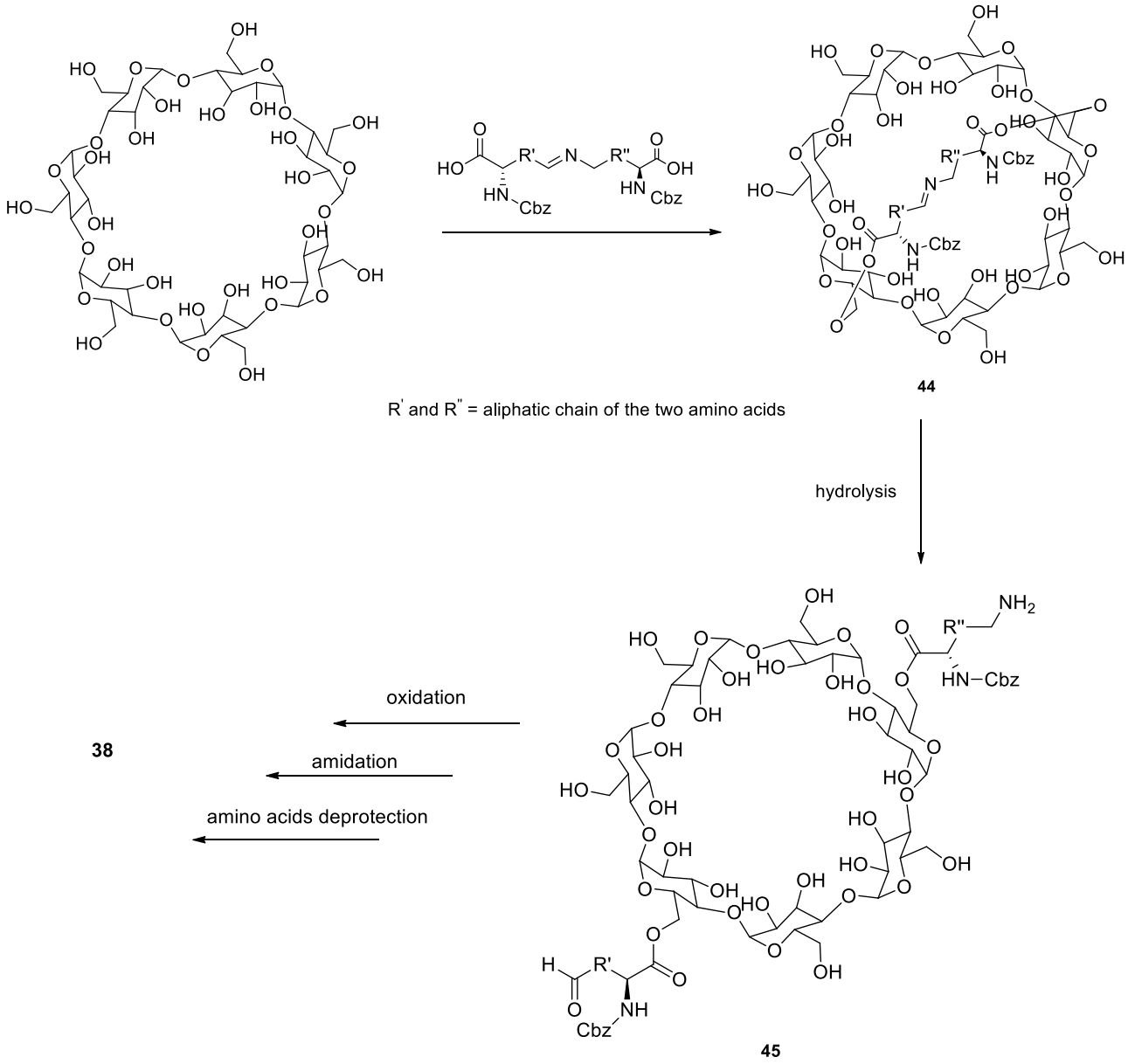
Through this strategy, however, we cannot overcome the problem of obtaining selectively a β-CD with two different functionalities as desired for the molecule **38**. In any case, the idea of a capped cyclodextrin has suggested a possible strategy to introduce a derivative, analogous to the biphenyl-4,4'-disulfonyl chloride, constituted by the two amino acids required for the functionalization of the β-cyclodextrin target, which, thanks to subsequent transformations, allow us to get molecule **38**.

Through very simplified computational calculations we found that the distance between two sulfur atoms in molecule **43** is equal to 10,49 Å.



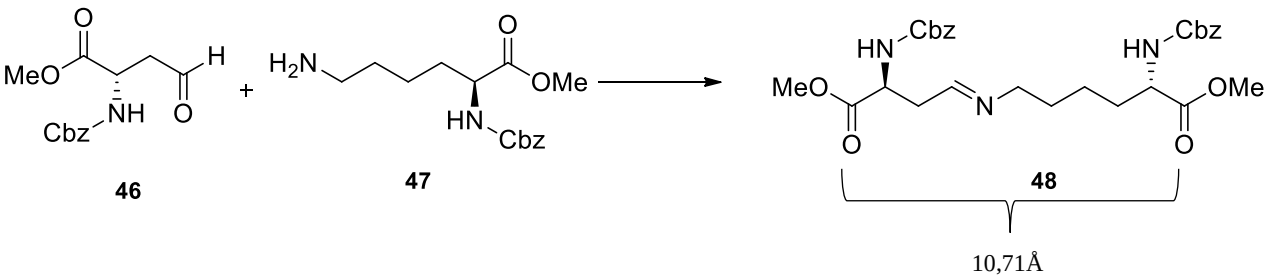

**43**

Therefore, through the formation of an imine deriving from the two amino acids, it is possible to think about getting a β-CD capped in both positions 1 and 4 as shown in structure **44**. Next, with the hydrolysis of the imine, it is possible to get the difunctional cyclodextrin **45**. With subsequent oxidation of the aldehyde functionality and amidation, we can obtain molecule **38** (*Scheme 1.16*).
This strategy allows us not only to avoid protection / deprotection steps of the entire cyclodextrin but also to have in the same step at the same time both the two different amino acids bound to the CD.



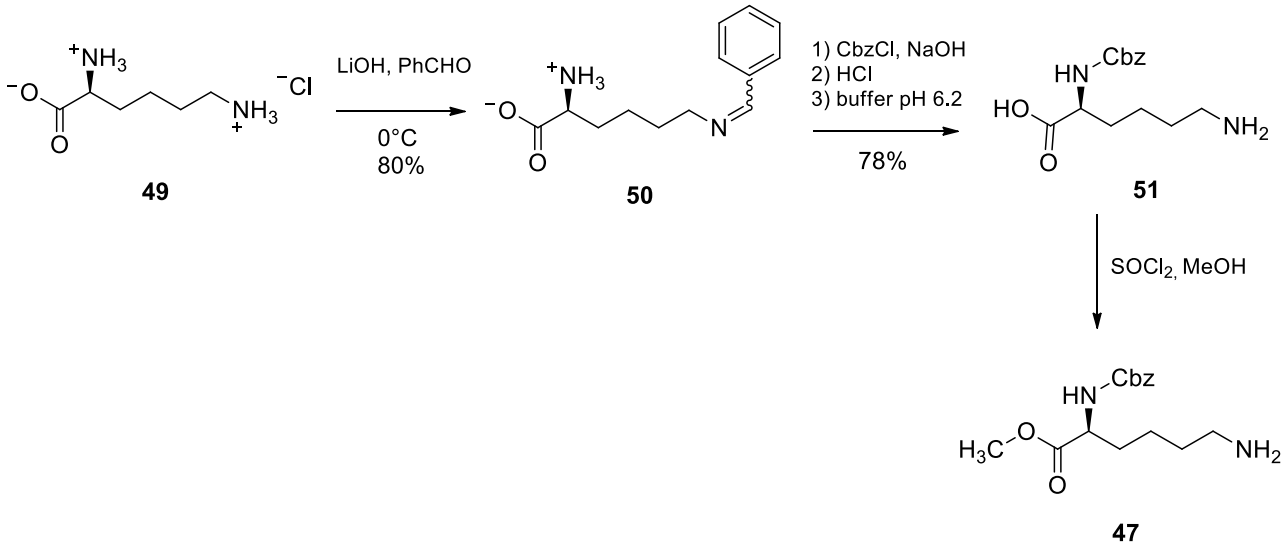

*Scheme 1.16.*

The dimer could be synthesized by reaction of the aldehyde **46**, resulting from the Aspartate,[64] with suitably protected Lysine **47**. By performing calculations similar to those conducted for the disulfonyl chloride **48**, we can derive the dimensions of this imine being 10,71Å, a value very close to that of the disulfonyl derivative. For this reason, this seemed a potential synthetic strategy (*Scheme 1.17*).




*Scheme 1.17.*

We started with the functionalization of *L*-Lysine following *Scheme 1.18.* Since this amino acid possesses two amino groups, in order to selectively protect the desired one we had to "lock" the terminal amine by reacting the aminoacid with benzaldehyde forming imine **50**. This reaction is almost quantitative since the terminal amino group reacts readily with benzaldehyde both for its position (less steric hindrance) and for its enhanced nucleophilicity with respect to the other $NH_2$ which is next to an electron withdrawing group. Once imine **50** was obtained, in a single step, we carried out the benzyl chloroformate (CbzCl) protection of the free amino group and, by adding concentrated HCl and heating the reaction mixture, we could hydrolize the imine unlocking the terminal $NH_2$.




*Scheme 1.18.*

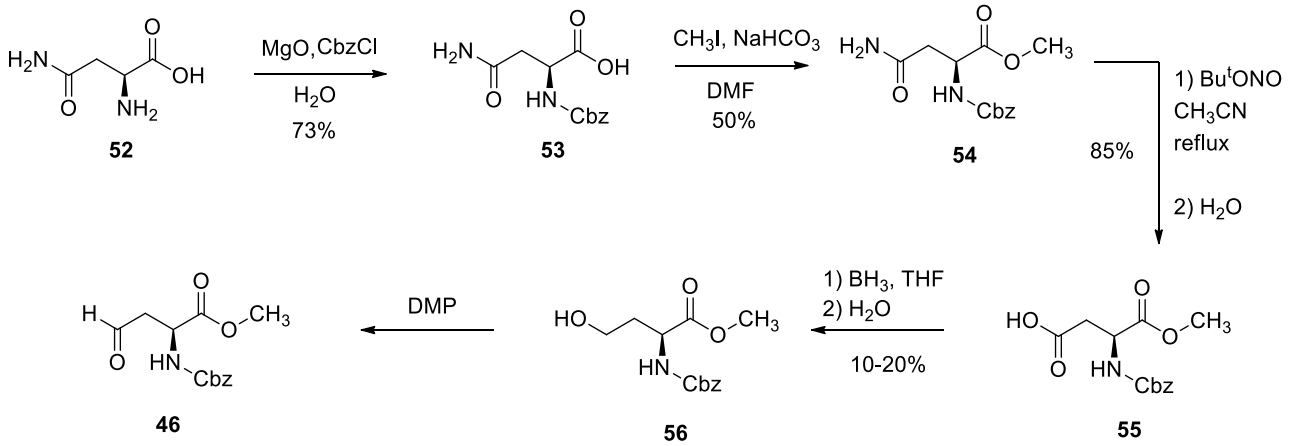
Since different reactions were carried out under acid o basic pH, we chose Cbz as protecting group because of its resistance to acidic and basic conditions and can be simply removed by Pd/C catalyzed hydrogenolysis. We also decided to convert the carboxylic acid to its methyl ester derivative in order to lower the possibility of side acid-base reactions during all the steps of the synthesis.

This step required more effort to find the right reaction conditions, reported in *Table 1.2*, until we could obtain a satisfying yield of 80% (*entry 8*).

*Table 1.2. Reaction conditions for the synthesis of methyl ester **47**.*

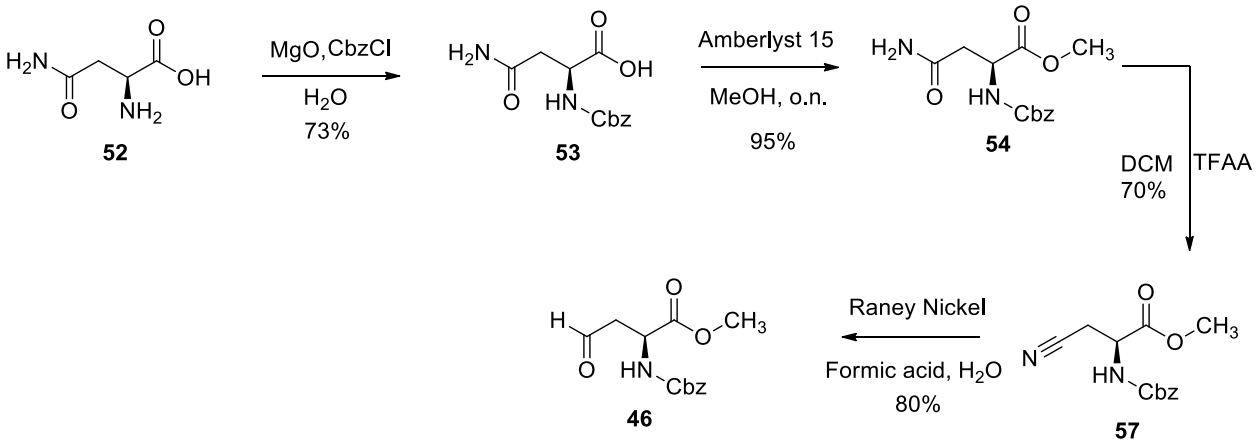
| N. | Solvent | Reactants | Temperature | Time | Yield |
|---|---|---|---|---|---|
| 1 | MeOH | $SOCl_2$ (2 eq.) | r.t | 5h | n/a |
| 2 | MeOH | $SOCl_2$ (2 eq.) | r.t. | Overnight | 15% |
| 3 | MeOH | $SOCl_2$ (2 eq.) + Amberlist 15 | r.t. | 5h | n/a |
| 4 | MeOH | $SOCl_2$ (2 eq.) + Amberlist 15 | r.t. | Overnight | 15% |
| 5 | MeOH | $SOCl_2$ (2 eq.) + $MgSO_4$ | r.t. | Overnight | 20% |
| 6 | MeOH | $SOCl_2$ (4eq) | r.t. | Overnight | 35% |
| 7 | MeOH | $SOCl_2$ | Reflux | Overnight | 50% |
| 8 | MeOH | $SOCl_2$ (3 eq.) | -80°C to r.t. | 20h | 80% |

At the same time, we proceeded also with the functionalization of the *L*-Asparagine. The aldehyde **46** was prepared according to *Scheme 1.19*. After a first benzyl chloroformate protection of the α-amino group to obtain compound **53**, we converted the caboxylic acid to the corresponding methyl ester **54** using methyl iodide in presence of $NaHCO_3$ in DMF. This substrate was then treated with *t*-BuONO to obtain the carboxylic acid derivative *N*-(benzyloxycarbonyl)-aspartic acid α-methyl ester (**55**) in a good yield.




*Scheme 1.19.*

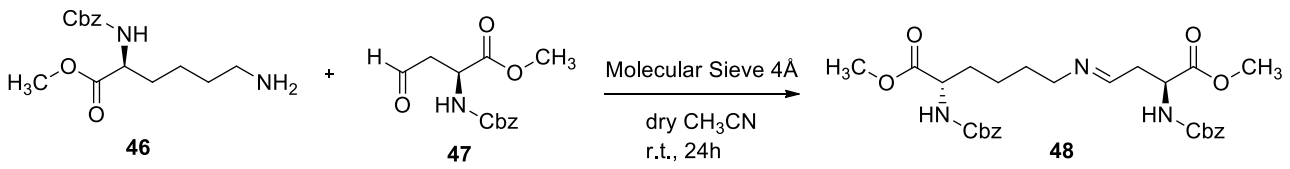
We found that the step to obtain the primary alcohol **56** by reduction of **55** with $BH_3$ in THF was a hard reaction to perform. In fact, despite it is reported that it is possible to obtain the desired compound in 30% yield,[65] we have never been able to obtain **56** with a yield higher than 20%. Moreover, the reaction itself was poorly reproducible, since other attempts to obtain the same compound under the same conditions resulted in a yield below 10%. For this reason, we considered this method to be unsatisfactory from a synthetic point of view and we decided to modify our synthetic strategy. We moved, then, to another synthetic pathway to achieve aldehyde **46** (*Scheme 1.20*).




*Scheme 1.20.*

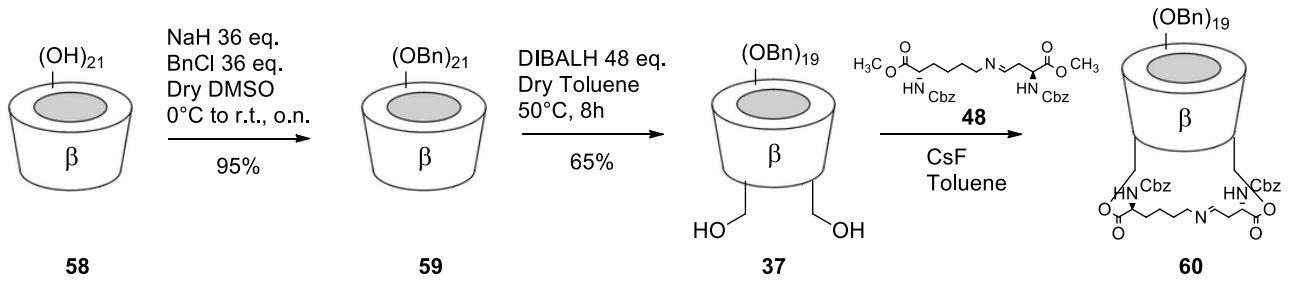
The first step of protection follows the same procedure presented in *Scheme 1.19.* The second step to obtain the methyl ester **53** was carried out, this time, with Amberlyst 15 in methanol, enhancing the yield of this step from 50% to 95%. Following a reported procedure,[66] this time we decided to convert the amide moiety of **54** to the corresponding nitrile **57**, treating the substrate with trifluoroacetic anhydride in $CH_2Cl_2$. A final reduction of **57** with Raney Nickel in formic acid and water, led to the target aldehyde **46**.

Once we had both building blocks in hand, a condensation reaction between the two in $CH_3CN$ in the presence of activated molecular sieves 4Å, allowed us to synthesize imine **48** (*Scheme 1.21).*




*Scheme 1.21.*

The imine **48** thus obtained was reacted with β-cyclodextrin **37**, firstly perbenzylated and then selectively deprotected in the two primary hydroxyl groups in position $6^A$ and $6^D$, following a reported procedure.[61]

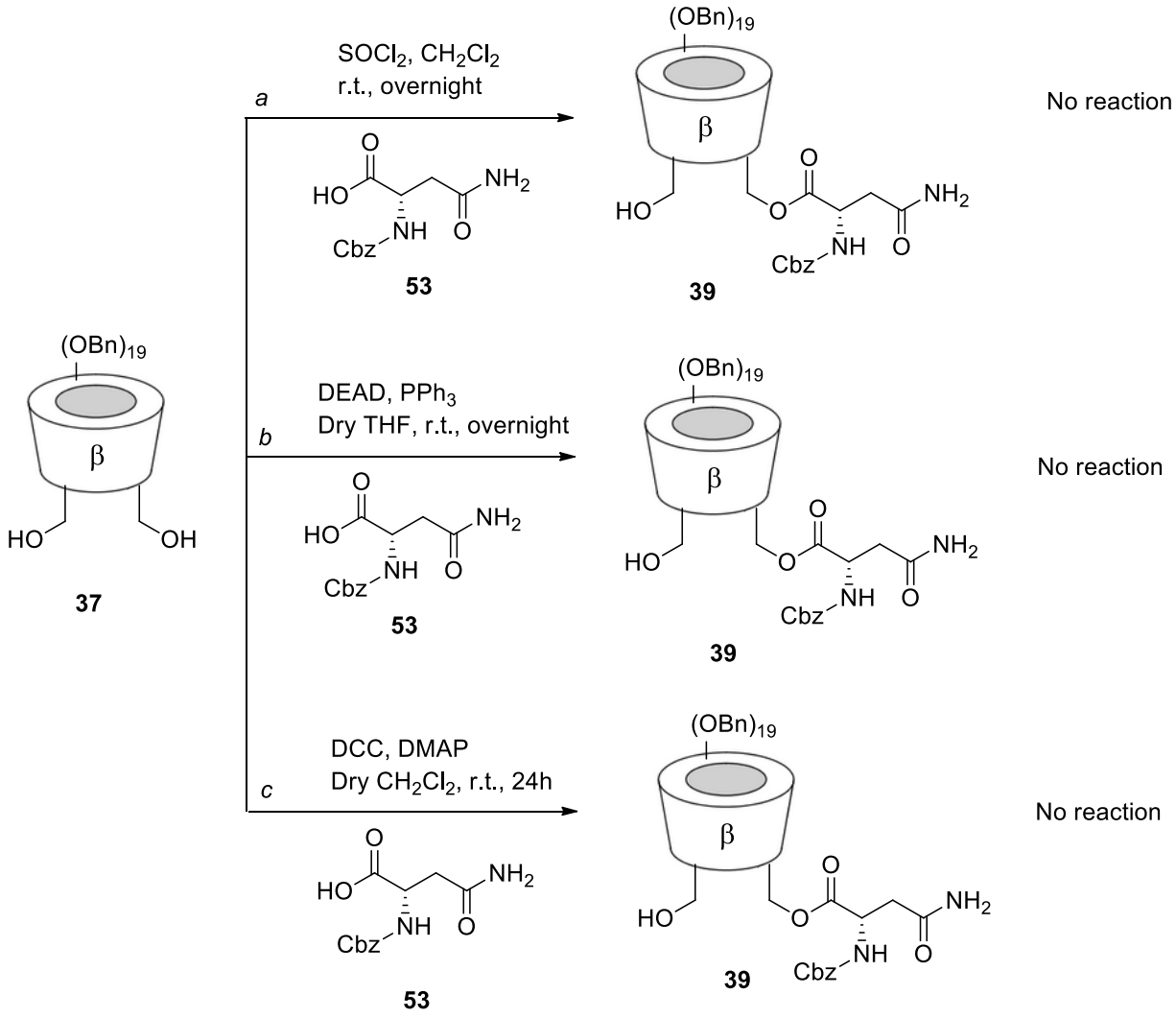



*Scheme 1.22.*

Unlike the capping with biphenyl-4,4'-disulfonyl chloride, which is performed on the unprotected cyclodextrin, we chose to work on the diol **37**. This provides two advantages, first, given that our imine is less rigid than the biphenyl-4,4'-disulfonyl chloride **43**, the selective deprotection of positions $6^A$ and $6^D$ gives us more chance to target the correct position of the cyclodextrin; and it also allows us to work in organic solvents and perform purification on normal phase silica gel chromatography. Coupling of imine **48** and CD **37** was performed following the procedure reported by Inahashi *et al.* that promotes transesterification reactions between methyl esters and primary alcohols in toluene in the presence of substoichiometric amount of CsF.[67]

TLC, ESI-MS and $^1$H-NMR of the crude of the reaction revealed a variety of different products and it was really difficult to recognize the product we were interested in, even if it is not to be excluded from our mixture. For the necessity of obtaining a clean product as soon as possible we have ruled out the path of an imminic capping due to the difficulties encountered in the synthesis of this imminic intermediate and the complexity of the mixture we obtained, and the need for an optimization of the reaction conditions could have taken too long.

With compound **37** in hands, we decided to explore the more classical ways to obtain an ester starting from a primary alcohol and the carboxylic acid of one of the two amino acids (*Scheme 1.23*). We performed these reactions via acyl chloride formation (*a*), under Mitsunobu conditions (*b*) and under Steglich conditions (*c*).



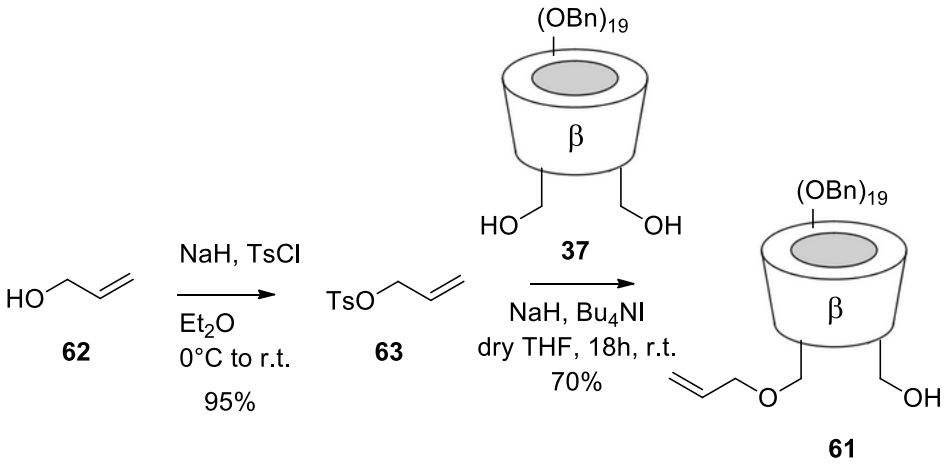

*Scheme 1.23.*

However, despite these reaction conditions are usually powerful tools to obtain esterification between a carboxylic acid and a primary alcohol, in all the three cases we could not observe any advancement of the reaction and we could only recover the starting materials unreacted. We recognized that the limits of our conditions were mainly the long reaction times required, which led to the decomposition of the acyl chloride, and the steric hindrance of the cyclodextrin moiety. Furthermore, one crucial factor to keep in mind in these types of reaction is that, most of the time, it is required an excess of the second reagent. In our case carboxylic acid, to obtain the desired functionalization, we instead had to work in a 1:1 ratio to avoid having a double functionalization in the two positions with the same amino acid.

At this point we have considered that the only possible strategy in order to reach the target molecule was through a mono-protection of one of the free hydroxyl groups in cyclodextrin **37**, while keeping

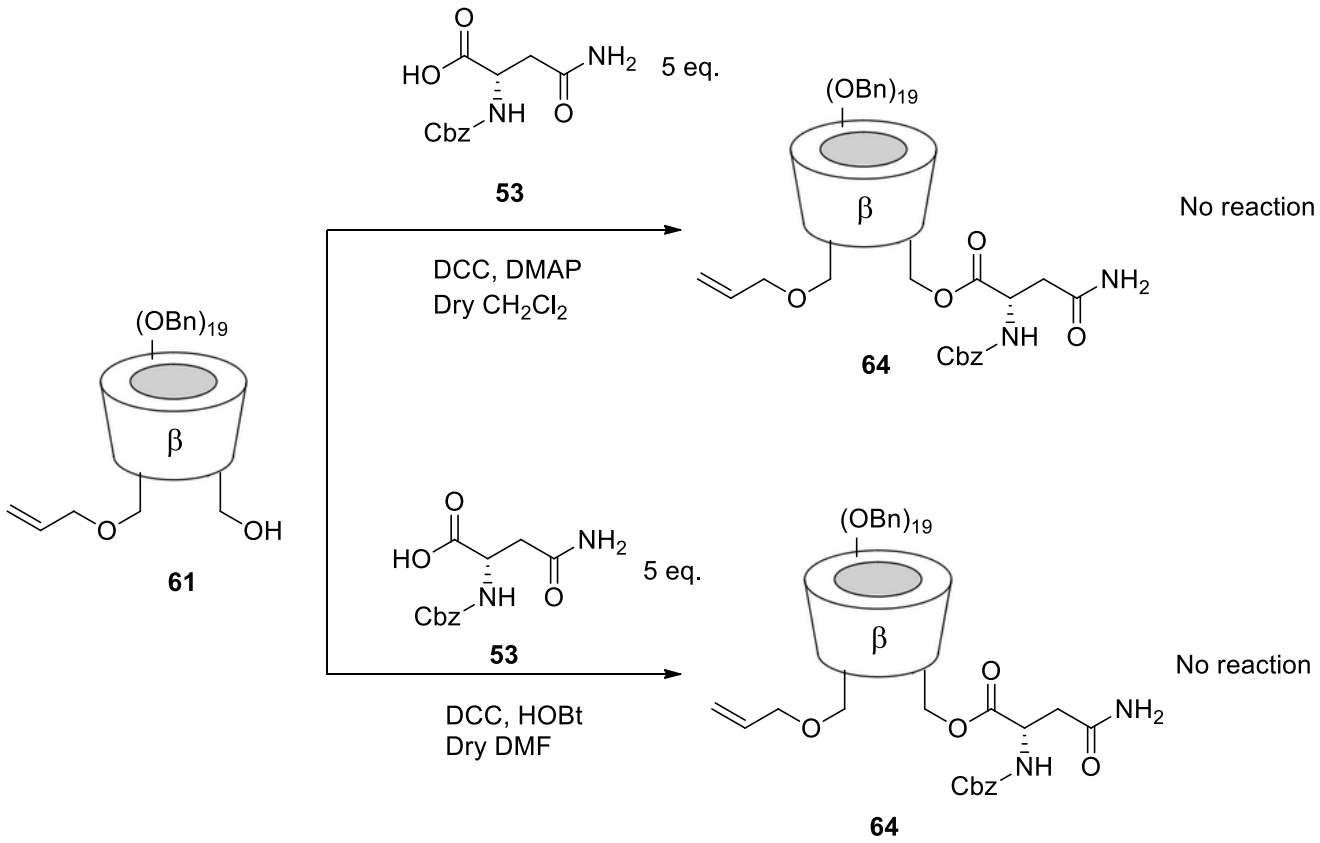
the other free for esterification with one of the two amino acids. Once this adduct is obtained, deprotection of the second OH can be carried out, followed by a second esterification of the free hydroxyl group with the second amino acid.

According to the procedure reported by O. Bistri *et al.*[68] it was possible to sinthesize the mono-allylated cyclodextrin **61** (*Scheme 1.24*).




*Scheme 1.24.*

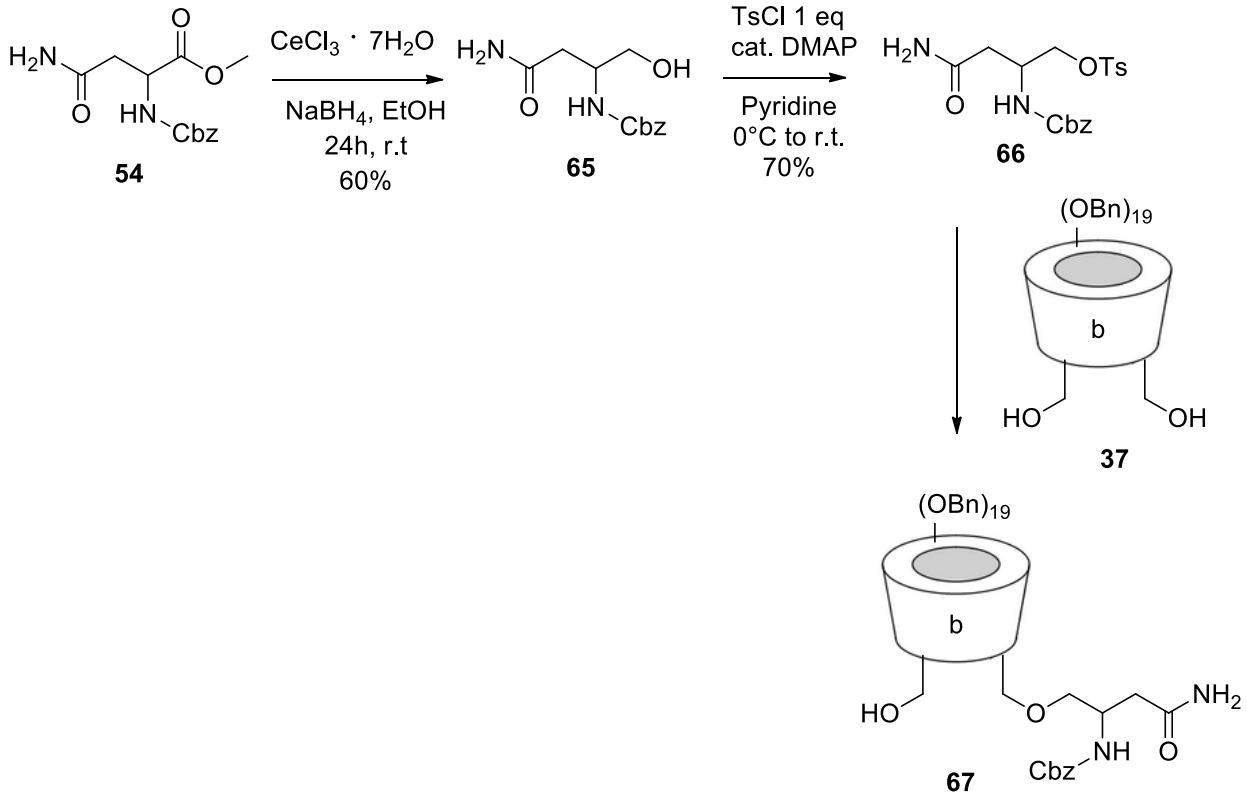
With monoalcohol **61**, again, we tried esterification of the primary hydroxy group with carboxylic acid **53**, using both DCC/DMAP and DCC/HOBt as promoters of the reaction (*Scheme 1.25*), this time using 5 equivalents of substrate **53**.




*Scheme 1.25.*

Once again, the reaction seemed not to proceed, and we could recover the unreacted starting materials. The lack of reactivity of these substrates is probably due to the fact that the steric encumbrance given by 19 benzyl as protecting groups is an undoubtedly limiting factor. Furthermore, systems such as DCC/DMAP and DCC/HOBt are likely not to provide the ideal conditions for activating the carboxylic acid.

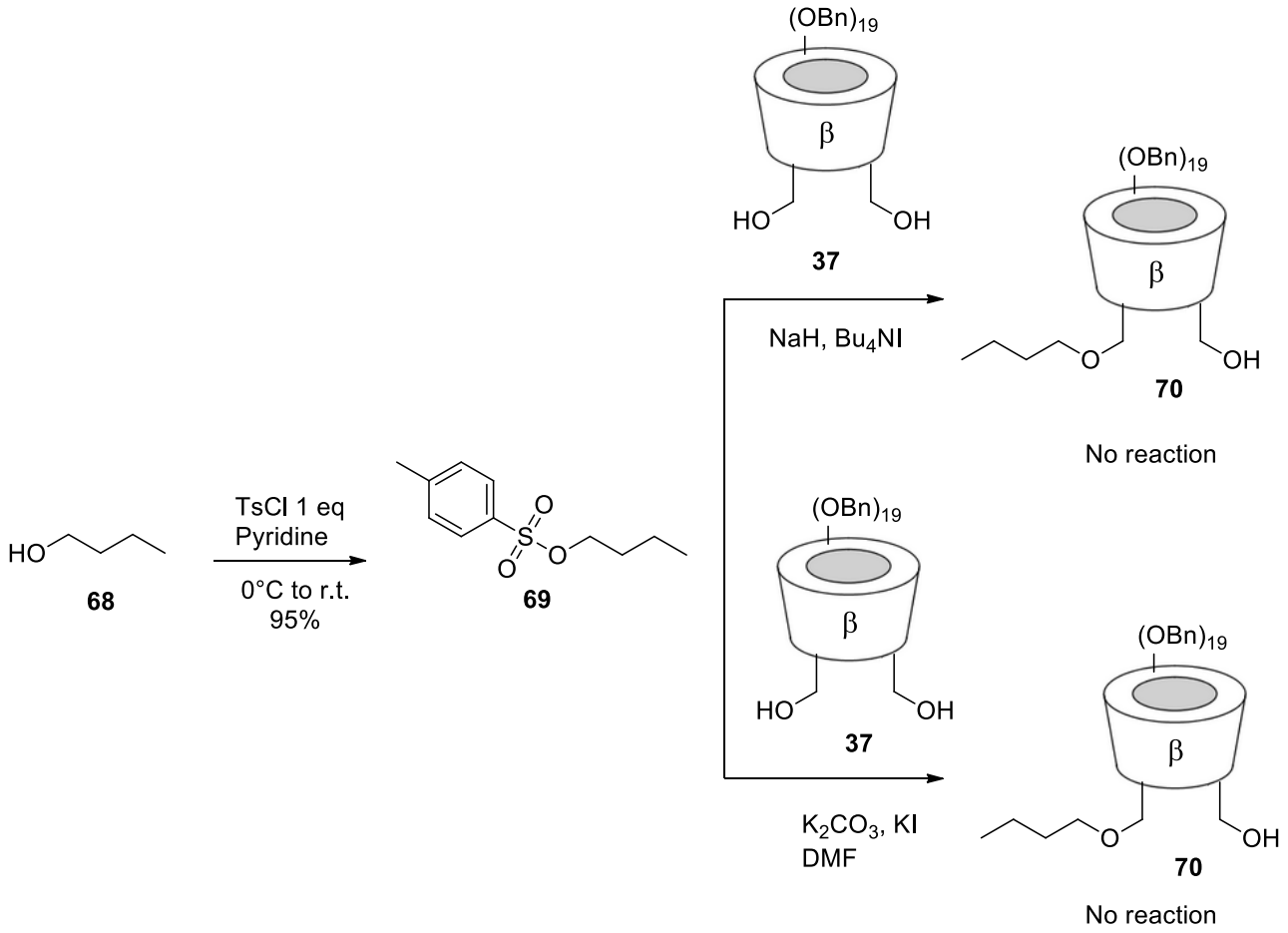
However, encouraged by the result obtained with the substitution reaction between the primary cyclodextrin hydroxyl group and the allyl 4-methylbenzenesulfonate derivative **63**, we have advanced the hypothesis of trying to react the same primary hydroxyl groups with a tosylated derivative of *L*-asparagine, previously reduced to the corresponding alcohol as shown in *Scheme 1.26.*




*Scheme 1.26.*

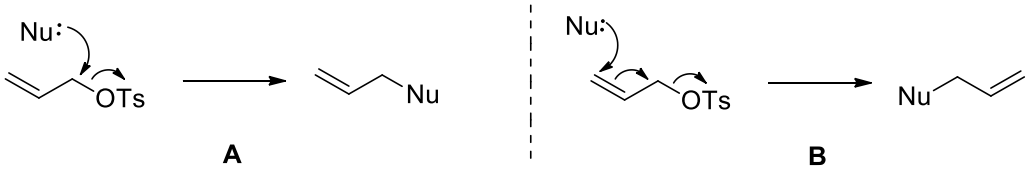
Following a reported procedure,[69] we carried out the first step of reduction of the methyl ester **54** under Luche conditions, to obtain alcohol **65**, which was consequently converted to the tosylated compound **66** in pyridine with catalytic amount of DMAP.

Before reacting the new synthesized compound with β-CD **37**, we decided to test the effectiveness of the reaction between the primary OH of the cyclodextrin diol, and a tosylated alcohol on a slightly simpler substrate, so we proposed to perform the tosylation of 1-butanol (also with 4 atoms of carbon such as *L*-asparagine) and try the reaction on these substrates (*Scheme 1.27*).




*Scheme 1.27.*

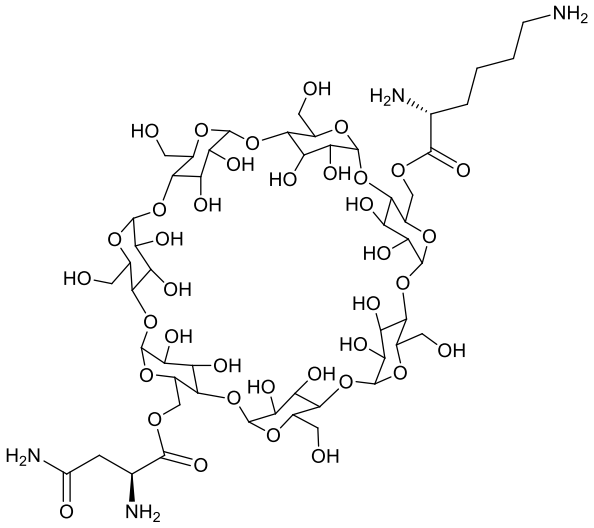
Even with a very simple substrate, not much different from allyl 4-methylbenzenesulfonate **63**, we couldn't obtain the target compound, suggesting that probably the mechanism of the reaction reported in *Scheme 1.24* doesn't follow a nucleophilic substitution on the carbon bearing the OTs group (*Scheme 1.28, A*), but on the terminal carbon atom (*Scheme 1.28, B*) confirming that hindered substrates cannot access the primary OH of the cyclodextrin.



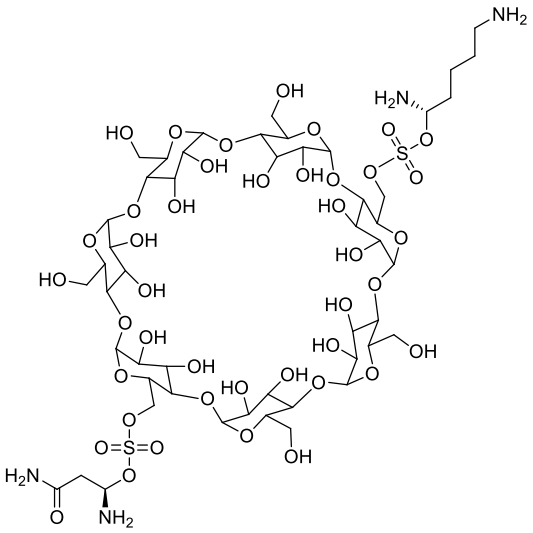

*Scheme 1.28.*

Given the difficulties encountered in the synthesis of the target molecule both by the formation of esters and ethers already described, we proceeded to perform new computational calculations and

studies and it was noticed that by replacing the ester functionality of the target compound **38** to a sulphate group (compound **71**) we could have a similar target with a reasonably similar distance between the two amino acid residues. Moreover, according to the studies carried out at Dompé s.p.a., we decided to focus primarily on the *L*-Lysine residue, because it seemed to play a more remarkable role in the interaction with the new class of compounds in question, compared to the *L*-Asparagine residue.

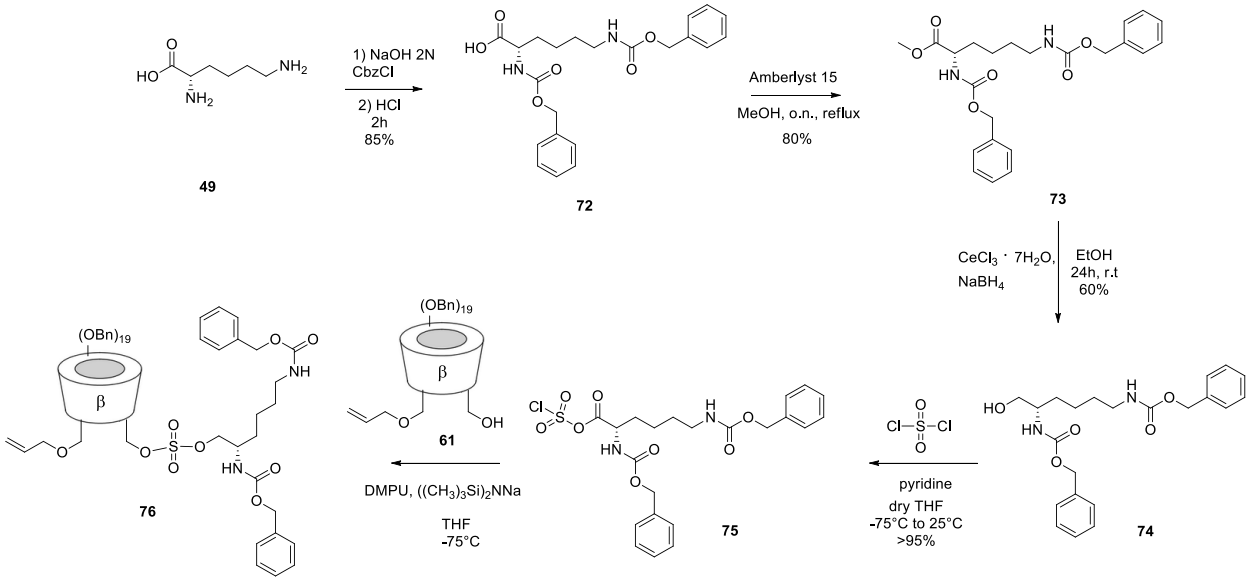


**38**



**71**

So, we decided to synthesize the (*S*)-2,6-bis(((benzyloxy)carbonyl)amino)hexyl sulfochloridate **75** according to *Scheme 1.29* in order to react it with cyclodextrin **61** to achieve the target compound **76**.

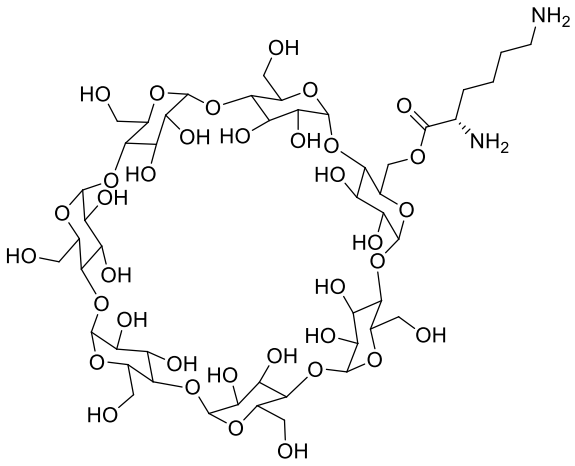



*Scheme 1.29.*

After protecting both the amino groups of the *L*-Lysine with benzylchloroformate, we obtained methyl ester **73** by refluxing the carboxylic acid **72** in methanol overnight, in the presence of Amberlyst 15. After reduction under Luche conditions, the alcohol **74** was converted to the chlorosulfate **75** by using sulfuryl chloride in dry THF and pyridine.[70] The reaction has been constantly monitored by HPLC and TLC and after the disappearance of the starting material, the solvent was removed under reduced pressure and the substrate **75** was immediately used in the next step.

Coupling with the functionalized β-cyclodextrin **61** has been carried out in THF and DMPU in presence of 1.5 eq of sodium bis trimethylsilyl amide and let the mixture stir for several hours, while constantly monitoring the reaction. TLC, Ion Trap-MS and $^{1}$H-NMR of the crude controls after 12, 24 and 48 hours underlined only the presence of the starting materials unreacted.

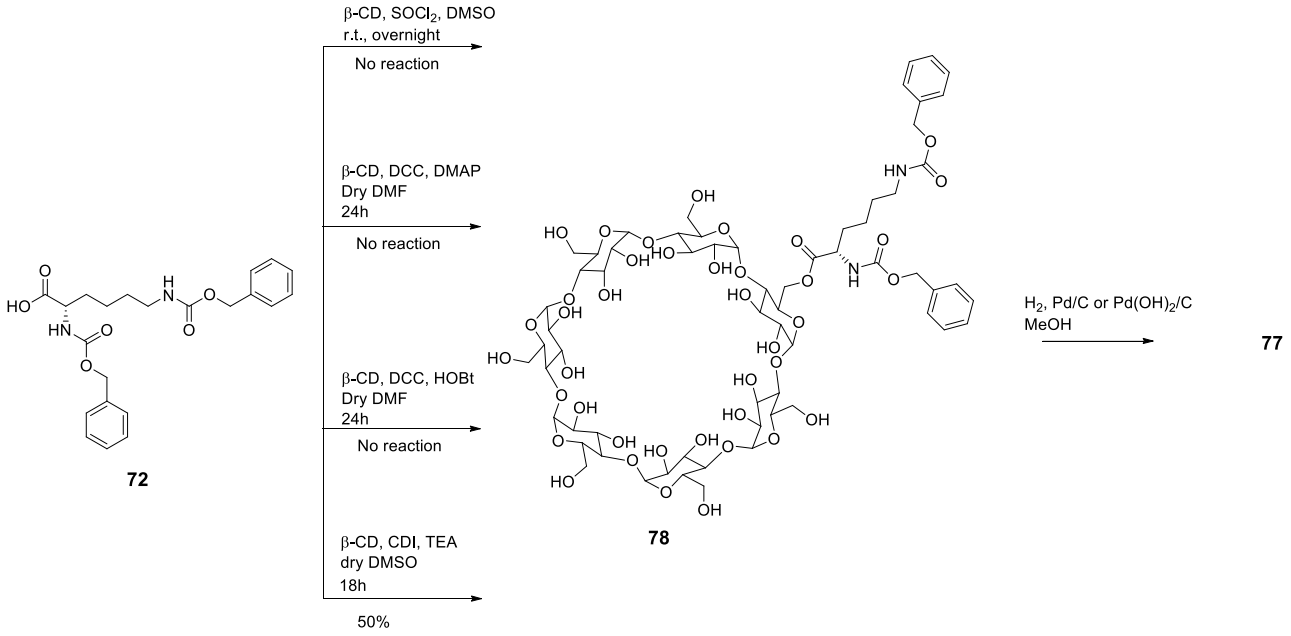
Given the extreme difficulty in obtaining the target difunctionalized β-cyclodextrin in the two positions $6^{A}$ and $6^{D}$ of the primary face, because of the absence of selectivity and handling difficulties due to poor solubility, in the case of completely deprotected cyclodextrin; and to the difficulty of reaching the free OH groups of diol **37** and alcohol **61** in the case of a protected cyclodextrin, due to the large steric hindrance, we decided to move to a simpler target. It has been already mentioned that some studies have shown that the *L*-Lysine residue was the most interesting in the target molecule, so we decided to synthesize the monofunctionalized cyclodextrin **77** in which one of the primary hydroxyl groups is replaced by one Lysine residue. Clearly, this change may weaken the initial enzymatic model efficiency but, for the preliminary mechanistic studies and interactions to be evaluated with this type of receptor, it seemed a good compromise.



**77**

The monofunctionalization avoids working with a perbenzylated cyclodextrin. There is still, however, the problem of tuning the reaction conditions to obtain one single functionalization of the primary face, while avoiding the functionalization of the hydroxyl groups of the secondary face.

Once again we have explored the different conditions for obtaining an esterification from an alcohol and a carboxylic acid (*Scheme 1.30*) and finally we obtained compound **78** by reacting the protected Lysine **72** with the β-cyclodextrin in DMSO in the presence of CDI.




*Scheme 1.30.*

Despite Yano *et al.* have reported similar reaction conditions for the coupling of a α-, β-, γ-cyclodextrin with a steroid, the prednisolone, obtaining a monosubstitution at the secondary face,[71] we obtained the monofunctionalization at the primary one. The structure has been confirmed by ESI-MS, $^{1}$H- and $^{13}$C-NMR. In fact, as Gao *et al.* reported,[72] the $^{13}$C-NMR of these compounds is crucial to understand precise information about the structure and the site of functionalization. When we have a primary face modification, we should observe two small peaks around 65 and 69 ppm, corresponding to a downfield shift of C-6' and upfield shift of C-5'. In our case at around 65 ppm we had peaks corresponding to the benzylic $CH_2$ of the Cbz protecting group, however a clear peak at 69.9 ppm appeared. Moreover, if we had a secondary face functionalization we should have notice a new small peak in the area of 73 ppm, corresponding to the shift of C-2',[71] signal that is not present in our spectrum, confirming that the amino acid residue is located at the primary face.

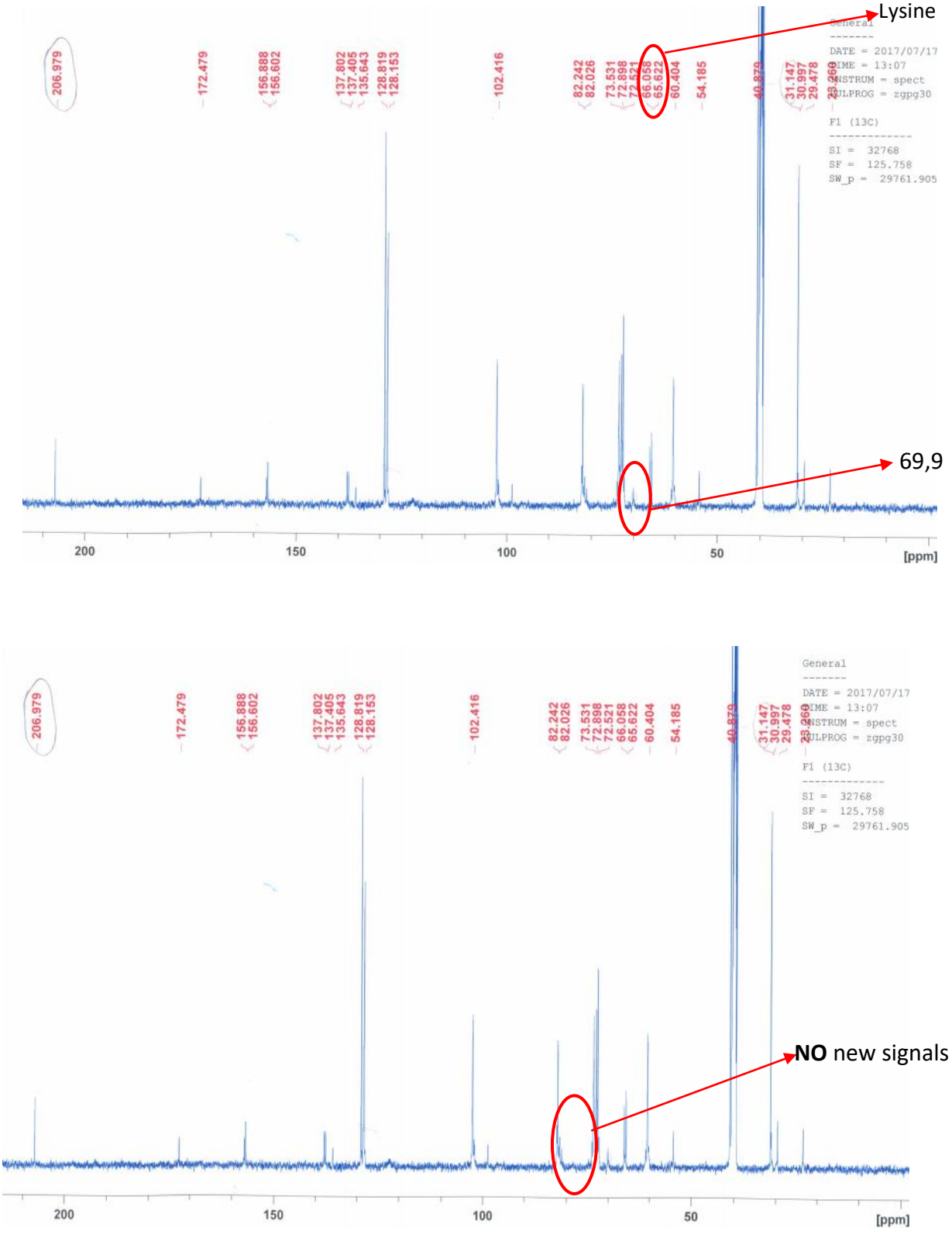


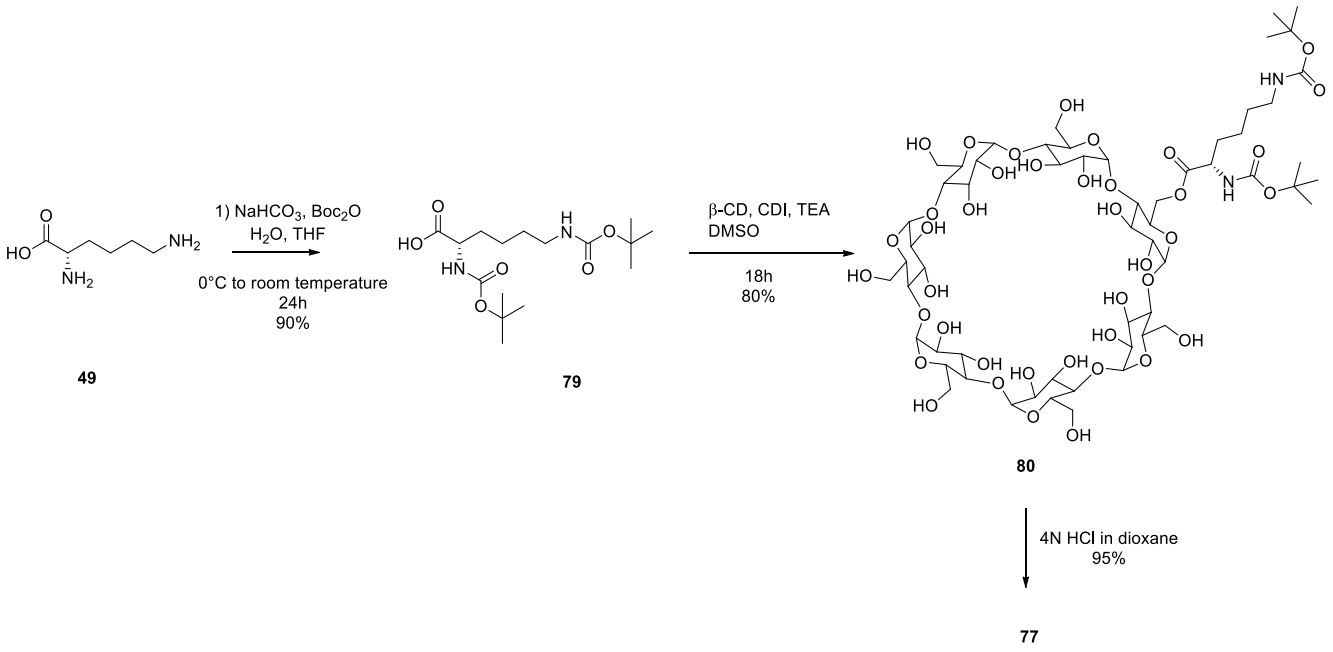

*Figure 1.10. $^{13}$C-NMR spectrum of compound **78**.*

After the coupling, we tried different conditions to perform the deprotection of the Cbz protecting group (*Table 1.3*), changing the catalyst, the quantity and even increasing the pressure of hydrogen but only the starting material was recovered in all the cases, even after several hours of reaction.

*Table 1.3. Reaction conditions for the deprotection step of **78**.*

| ***Entry*** | ***Catalyst*** | ***Temperature*** | ***Pressure*** | ***Yield%*** |
|---|---|---|---|---|
| 1 | Pd/C 5% | 25°C | 1 atm | / |
| 2 | $Pd(OH)_2$/C 30% | 25°C | 1 atm | / |
| 3 | Pd/C 30% | 25°C | 6 atm | / |

The lack of efficiency of these general deprotection methods using Pd-catalyzed hydrogenolysis,[73] has suggested to us to try the recent $Pd(OAc)_2$-catalyzed debenzylation with sodium hydride.[74] Unfortunately, the deprotection of the corresponding Cbz did not occur even after repeated attempts. It is interesting to note that the deprotection was not observed in any of the amino groups of the *L*-Lysine, and this is probably due to the fact that the Lysine residue is not necessary located outside the cyclodextrin cavity, but it is more probably located inside. For this reason, the lack of possibility to form substrate-catalyst complexes have stimulated us to move to another protecting group, so we proceeded with the synthesis of the boc-protected *L*-Lysine **79**, in order to exploit the host-guest inclusion complexes of β-cyclodextrin with an acid.




*Scheme 1.31.*

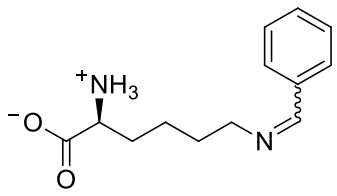
The coupling conditions were the same described for *Scheme 1.30* and the deprotection step was carried out with hydrochloric acid in dioxane. This time the reaction proceeded to finally give the target monofunctionalized cyclodextrin **77**. In this case, we didn't experience the same problem as before, probably because the Boc-protecting group is cleaved very simply under acid condition and, even if also in this case the aminoacid is inside the cavity, the hydrochloric acid in the solvent can easily reach the target, since these cyclodextrin are well-known to trap solvent molecules inside their cavity.

## 1.5. Experimental protocols

### *Materials and methods*

All reagents and solvents were purchased from commercial suppliers and used without further purification, unless mentioned otherwise. All reactions were performed under nitrogen atmosphere. All glassware was oven dried at 100 °C for more than 2 hours prior to use. All solvents were dried (THF over metallic sodium, DCM over $CaCl_2$) and freshly distilled prior to use. For thin-layer chromatography (TLC) analysis, Merck pre-coated TLC plates (silica gel 60 GF254 0.25mm) were used and products were observed under UV light or stain in iodine chamber or phosphomolybdic acid solution. $^1H$ and $^{13}C$ spectra were recorded on a Varian Mercury 400 (400 MHz or 100 MHz respectively). Chemical shifts are quoted in ppm and are referenced to residual protons in the deuterated solvent as the internal standard such as $CDCl_3$ (7.26 ppm for $^1H$ and 77.2 ppm for $^{13}C$) or dimethyl sulfoxide-d6 (DMSO-d6, 2.50 ppm for $^1H$ and 39.5 ppm for $^{13}C$). Coupling constants J are reported in hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were recorded with a Perkin-Elmer FT-IR spectrometer Spectrum Two UATR. ESI/APCI low-resolution mass spectra were recorded with an Agilent 1100 MSD ion-trap mass spectrometer equipped with a standard ESI/APCI source. Nitrogen served both as the nebulizer gas and the dry gas. HMRS were obtained using HPLC Ultimate 3000 (Thermofisher Scientific) coupled with high resolution Q Exactive Benchtop Quadrupole – Orbitrap Mass Spectrometer (Thermofisher Scientific).

*N$^{\varepsilon}$-Benzylidene-L-lysine* (**50**)

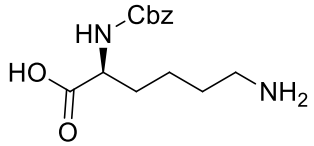



**50**

To a solution of *L*-lysine (1.46 g, 10 mmol) in 2N lithium hydroxide (4 ml) at 0°C was added benzaldehyde (0.84 ml, 12 mmol, 1.2 eq.). The reaction flask was stirred at the same temperature until the benzaldehyde had dissolved and a white solid precipitated. After standing in the refrigerator for several hours, the solid was filtered, washed with cold ethanol and dried over $CaCl_2$ under vacuo for one day to give N$^{\varepsilon}$-benzylidene-*L*-lysine as a white solid in 80% yield. It was used in the next step without further purification. m.p. = 187-189 °C. *$^1H$ NMR:* (400 MHz, $D_2O$) $\delta_H$ = 1.12-1.29 (m, 2H), 1.43-1.60 (m, 4H), 2.83 (t, 2H), 3.25 (m, 1H), 3.47

(m, 1H), 7.30-7.62 (m, 5H). The spectroscopic data of the product obtained are in accordance to the literature.[75]

*$N^{\alpha}$-Carbobenzyloxy-L-lysine* (**51**)

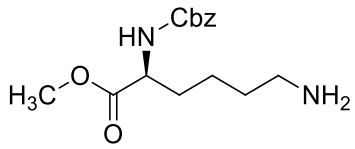



**51**

$N^{\varepsilon}$-benzylidene-*L*-lysine (1.868 g, 8 mmol) was dissolved in a mixture of 1N sodium hydroxide solution (7.8 ml) and ethanol (7.8 ml) below -5 °C. A cooled mixture of 1N sodium hydroxyde solution and ethanol (1:1, 31 ml) and benzyl chloroformate (1.52 ml, 10,4 mmol, 1.3 eq.) were added in two portions over 5 minutes at -10°C with vigorous stirring. The mixture was stirred at the same temperature for 10 min and then at room temperature for further 30 min.

Concentrated hydrochloric acid (2.34 ml) was added and the resulting mixture was heated at 50 °C for 30 min. The mixture was then extracted with diethyl ether (3x20 ml) and the aqueous layer was adjusted to pH 6.2 with a $NaOH/KH_2PO_4$ buffer. The resulting mixture was concentrated in vacuo to a volume approximately half of the starting volume. After standing in the refrigerator for one day, some white crystals precipitated. The remaining solvent was removed *in vacuo* and the solid was dried over $CaCl_2$ in vacuo for one day to give $N^{\alpha}$-carbobenzyloxy-*L*-lysine in 78% as a white solid, which was used in next step without purification. *$^{1}H$ NMR:* (400 MHz, $D_2O$) $\delta_H$ = 1.22-1.41 (m, 2H), 1.49-1.79 (m, 4H), 2.9 (t, 2H), 3.61 (m, 1H), 5.0 (m, 2H), 7.25-7.34 (m, 5H). *$^{13}C$ NMR* : (100MHz, $D_2O$) $\delta_C$ = 21.8, 26.0, 30.9, 39.0, 54.1, 66.4, 127.0, 127.8, 128.4, 135.8, 157.6, 178. The spectroscopic data of the product obtained are in accordance to the literature.[75]

*$N^{\alpha}$-Carbobenzoxy-L-lysine methyl ester* (**47**)

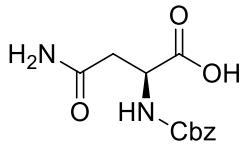



**47**

A solution of $N^{\alpha}$-Cbz-*L*-lysine (5 g, 0.017 mol). in 80 ml of MeOH were cooled to -80°C with an acetone bath and a cryostat. $SOCl_2$ (3.6 ml, 0.042 mol, 3 eq.) was added dropwise keeping the reaction at the same temperature and the solution was stirred for 20h at room temperature. After the reaction was completed (disappearing of the starting material by TLC Cyclohexane/EtOAc 70:30, bromocresol green stain) MeOH, excess of $SOCl_2$ and HCl formed during the reaction were removed by evaporation under reduced pressure to give **47** as a white solid (4g,

80%).*[1]H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.45-1.86 (m, 6H), 2,87 (t, 2H), 3.68 (s, 3H), 4.31 (m, 1H), 5.1 (s, 2H), 7.38-7.47 (m, 5H). *[13]C NMR* : (100MHz, $CDCl_3$) $\delta_C$ = 22.3, 26.7, 30.8, 40.4 , 51.9, 54.7, 66.5, 127.1, 128.1, 128.5, 157.4, 174.5. . The spectroscopic data of the product obtained are in accordance to the literature.[76]

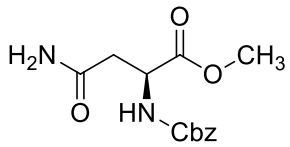
*N-Carbobenzyloxy-L-asparagine (***53**)



**53**

To a mixture of *L*-asparagine (1.32 g, 10 mmol) and MgO (0.86 g, 21 mmol, 2.1 eq.) in 10 ml of $H_2O$ was added in 4 portions at 5 °C benzyl chloroformate (1.713 ml, 12 mmol, 1.2 eq.). After stirring 15 min at the same temperature, the thick reaction mixture was stirred at r.t. for 3 hours. It was then acidified with 2N HCl to pH 1-2, filtered and the solid was washed with $H_2O$ and dried over $CaCl_2$ giving 2.1 g of product. The entire material was recrystallized from 40 ml of MeOH to yield 1.3 g of N-carbobenzyloxy-*L*-asparagine. Recrystallization in the same manner of the residue obtained from the concentration of mother liquors to dryness gave other 0.65 g of the product for a total yield of 73%. m.p. = 164-165 °C. *[1]H NMR:* (400 MHz, DMSO-d6) $\delta_H$ = 2.41-2.58 (m, 2H), 4.41-4.49 (m, 1H), 5.14 (s, 2H), 7.11-7.41 (m, 6H), 12.23 (br, 1H). The spectroscopic data of the product obtained are in accordance to the literature.[77]

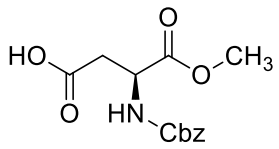
*N-Carbobenzyloxy-L-asparagine methyl ester* (**54**)



**54**

2g of N-Cbz-*L*-asparagine (7.5 mmol) were dissolved in 20 ml of MeOH. To this solution, 400 mg of Amberlyst 15 were added and the mixture was refluxed overnight. After this time, the reaction was complete (disappearing of the starting material by TLC Cyclohexane/EtOAc 70:30, bromocresol green stain). The reaction was filtered to remove Amberlyst and the solvent was evaporated under reduced pressure to obtain a white solid with 95% yield. m.p. = 152-153 °C. *[1]H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 2.41-2.59 (m, 2H), 3.61 (s, 3H), 4.38-4.45 (m, 1H), 5.02 (s, 2H) 6.91 (br s, 1H), 7.25-7.38 (m, 6H), 7.61 (d, J = 8.2 Hz, 1H). *[13]C NMR* : (100 MHz, $CDCl_3$) $\delta_C$ = 36.94, 50.68, 52.10, 65.6, 127.83, 127.95 , 128.48, 137.01, 155.9, 170.89, 172.31. The spectroscopic data of the product obtained are in accordance to the literature.[65]

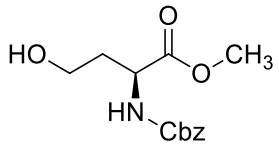
*N-Carbobenzyloxy-L-aspartic acid α-methyl ester* (**55**)



**55**

To a hot solution of N-carbobenzoxy-*L*-asparagine methyl ester (1.23 g, 4.3 mmol) in MeCN (13 ml) was added *tert*-butyl nitrite (1.051 ml, 8.6 mmol, 2 eq.) in one portion. The yellow solution was heated at reflux for several hours. After conversion of the starting material to its corresponding acid, checked by TLC; the solvent was removed under reduced pressure. The dark yellow oil was taken up with $NaHCO_3$ (5%, 30 ml) and washed with EtOAc (2x30 ml). After acidification with conc. HCl to pH 1-2, the product was extracted into $CH_2Cl_2$ (3x40 ml) and dried ($Na_2SO_4$). The solvent was removed *in vacuo* to obtain N-carbobenzyloxy-*L*-aspartic acid α-methyl ester in 83% yield. *$^1$H NMR:* (400 MHz, DMSO-d6) $\delta_H$ = 2.58 (dd, J = 16.45, 7.09 Hz, 1H), 2.71 (dd, J = 16.67, 5.55 Hz, 1H), 3.61 (s, 3H), 4.38-4.45 (m, 1H), 5.01 (s, 2H), 7.28-7.4 (m, 5H), 7.73 (d, J = 8.2 Hz, 1H), 12.49 (br s, 1H). *$^{13}$C NMR* (100 MHz, DMSO-d6) $\delta_C$ = 36.10, 50.68, 52.24, 65.76, 127.80, 127.92, 128.4, 137.01, 155.93, 171.53, 171.76. The spectroscopic data of the product obtained are in accordance to the literature.[65]

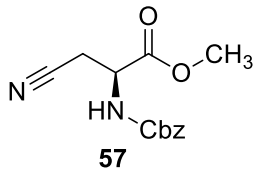
*(S)-Methyl 2-(((benzyloxy)carbonyl)amino)-4-hydroxybutanoate* (**56**)



**56**

N-carbobenzyloxy-*L*-aspartic acid α-methyl ester (0.2 g, 0.7 mmol) was dissolved in dry THF (1 ml) and cooled to -5°C with an ice-salt bath. Borane in THF (1 M, 1.422 ml, 2 eq.) was added dropwise over a period of 30 minutes. The solution was allowed to warm up to room temperature and stirring was continued overnight. After checking the reaction TLC, excess borane was quenched with citric acid (10% w/v, 40 ml) and the mixture was extracted with $Et_2O$ (4x50 ml). The combined organic layers were washed with brine, dried ($Na_2SO_4$) and the solvent was removed in vacuo to afford a yellow oily crude product, which was subject to column chromatography eluting with Exane:EtOAc 60:40 to obtain **56** as a yellow oil in 20% yield. *$^1$H NMR:* (400 MHz, DMSO-d6) $\delta_H$ = 1.65-1.7 (m, 1H), 1.79-1.85 (m, 1H), 3.36-3.46 (m, 2H), 3.61 (s, 3H), 4.18-4.21 (m, 1H), 4.54 (t, J = 5.1 Hz, 1H), 5.02 (s, 2H), 7.28-7.39 (m, 5H), 7.64 (d, J = 7.6 Hz, 1H). *$^{13}$C NMR* : (100MHz, DMSO-d6) $\delta_C$ = 33.9, 51.1, 51.9, 57.1, 65.6, 127.85, 127.93, 128.48, 137.01, 156.2, 173.35. The spectroscopic data of the product obtained are in accordance to the literature.[65]

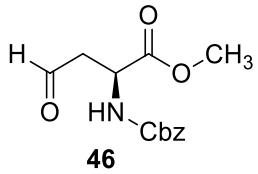
*(S)-Methyl 2-(((benzyloxy)carbonyl)amino)-3-cyanopropanoate* (**57**)



**57**

To a solution of N-carbobenzoxy-*L*-asparagine methyl ester (1 g, 3.5 mmol) in $CH_2Cl_2$ dry (30 ml) was added DMAP (0.61 g, 2.5 eq.) in 2 portions. The resulting mixture was cooled to 0°C in an ice-water bath and $(CF_3CO)_2O$ (1.26 ml, 2.5 eq.) was added dropwise. Then, the cooling bath was removed and the reaction mixture was stirred for 5h at room temperature. Then, the organic phase was washed with water (20ml) and the aqueous phase extracted with $CH_2Cl_2$ (3x15ml). The combined organic layer was dried over $Na_2SO_4$, filtered and the solvent removed under reduced pressure to obtain a white solid in a 70% yield. *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 2.95-3.15 (m, 2H), 3.78 (s, 3H), 4.67 (m, 1H), 5.05 (s, 2H), 7.38-7.47 (m, 5H), 7.70 (d, J = 8.2 Hz, 1H). *$^{13}$C NMR* : (100MHz, $CDCl_3$) $\delta_C$ = 20.38, 51.92, 56.72, 66.21, 117.78, 127.87, 127.93, 128.45, 136.74, 155.97, 172.35.

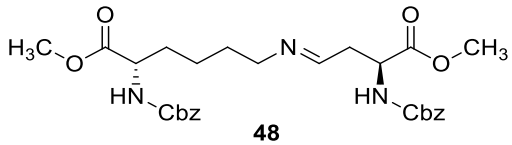
(*S)-Methyl 2-(((benzyloxy)carbonyl)amino)-4-oxobutanoate* (**46**)



**46**

To a Raney-Nickel water suspension (0.67 g) was added **57** (0.5 g, 1.9 mmol) in a mixture of formic acid (3ml) and water (0.5ml). After heating to 75°C for 5 hours, the nickel was filtered off, washed with 10ml of water and 5ml of $CH_2Cl_2$. The aqueous layer was then extracted with $CH_2Cl_2$ (3x20ml) neutralized with sat. $NaHCO_3$, washed with brine and dried over $Na_2SO_4$. Evaporation of the solvent under reduced pressure provided **46** as a yellow oil (80%). *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 2.77 (m, 1H,), 2.89 (dd, J = 17.2, 4.7 Hz, 1H), 3.63 (s, 3H), 4.57–4.61 (m, 1H), 5.04 (s, 2H), 7.29–7.38 (m, 5H), 7.77 (d, J = 7.9 Hz, 1H), 9.60 (s, 1H). *$^{13}$C NMR* : (100MHz, $CDCl_3$) $\delta_C$ = 44.38, 48.71, 52.30, 65.81, 127.88, 128.10, 128.50, 136.92, 155.97, 171.86, 199.89. The spectroscopic data of the product obtained are in accordance to the literature.[65]

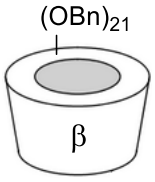
*(S)-Methyl 2-(((benzyloxy)carbonyl)amino)-6-((E)-((S)-3-(((benzyloxy)carbonyl)amino)-4-methoxy-4-oxobutylidene)amino)hexanoate* (**48**)



**48**

A mixture of the aldehyde **46** (100mg, 0.376mmol) and $N^{\alpha}$-Cbz-*L*-lysine methyl ester **47** (1 eq.) and activated 4Å molecular sieves in dry $CH_3CN$ was stirred at room temperature for 24h. After the removal of molecular sieves by filtration, the solvent was evaporated and the crude was subjected to ESI-MS analysis confirming the presence of the product **48**: MW:541.2, ESI-MS 563.8 $[M+Na]^+$, 1083.3 $[2M+H]^+$. The product thus obtained was used for the next step without further purification due to thermal and chromatographic instability.

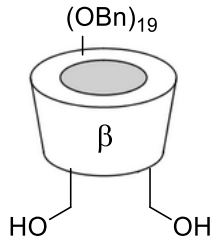
*Synthesis of perbenzylated* β-*Cyclodextrin* (**59**)



**59**

To a solution of β-CD (2.9 g, 2.55 mmol) in dry DMSO (40 ml) at 0°C was added NaH (2.5 g, 36 eq.) under nitrogen. Benzyl chloride (10.6 ml, 36 eq.) was slowly added dropwise at the same temperature in 1 hour. The reaction is allowed to warm up to room temperature and stirred overnight. After this time, it was checked by TLC (Cyclohexane/EtOAC 8:2, cerium molybdate stain) and was carefully quenched with MeOH (25 ml), diluted with water (100 ml) and extracted with $Et_2O$ (3x100 ml). The combined organic layers were washed with brine, dried over $Na_2SO_4$, filtered and concentrated. After purification by silica gel chromatography (Cyclohexane/EtOAc 8:2) perbenzylated β-CD was obtained as a white foam (6.1g, 80%). *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 3.51 (dd, J = 9.2, 3.3 Hz, 7H), 3.59 (d, J = 10.6 Hz, 7H), 3.95-4.10 (m, 28H), 4.37-4.40 (m, 14H), 4.50-4.55 (m, 14H), 4.80-5.15 (m,14H), 5.25 (d, J = 3.5 Hz, 7H), 7.16-7.38 (m, 105H). *$^{13}$C NMR* : (100MHz, $CDCl_3$) $\delta_C$ = 69.1, 71.3, 72.7, 73.2, 75.5, 78.7, 78.8, 80.5, 98.3, 126.8-128.3 (aromatic), 138.1, 138.4, 139.1. The spectroscopic data of the product obtained are in accordance to the literature.[61]

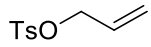
*Synthesis of* β-*Cyclodextrin diol (***37)**




**37**

DIBAL-H (1M in toluene, 25ml) was added dropwise to compound **59**(1.5 g, 0.5 mmol) at 0°C. The solution was stirred at 50°C for 8 hours. And monitored by TLC (Cyclohexane/EtOAc 8:2). The reaction was carefully quenched by dropwise addition of water. The aqueous phase was extracted with $CH_2Cl_2$ (3x40 ml) and the combined organic layer was washed with brine and dried over $Na_2SO_4$ and concentrated under reduced pressure. After purification by silica gel chromatography (Cyclohexane/EtOAc 8:2), **37** was obtained as a white foam (0.93 g, 65%). *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 2.70 (br, 2H), 3.42-3.53 (m, 5H), 3.60-4.10 (m, 37H), 4.45-4.88 (m, 33H), 4.90 (d, J = 3.3 Hz, 1H), 4.98-5.10 (m, 6H), 5.21-5.27 (m, 3H), 5.32 (d, J = 10.7 Hz, 1H), 5.60-5.65 (m, 4H), 7.12-7.33 (m, 95H). *$^{13}$C NMR* : (100MHz, $CDCl_3$) $\delta_C$ = 61.4, 68.6, 68.8, 69.0, 69.2, 69.3, 71.3, 71.6, 71.7, 71.8, 72.0, 72.4, 72.5, 72.6, 72.7, 72.8, 73.0, 73.1, 73.2, 73.3, 73.4, 74.3, 74.4, 74.5,74.8, 75.1, 75.6, 75.9, 76.1, 76.2, 78.5, 78.6, 78.7, 78.9, 79.4, 79.5, 79.6, 79.7, 79.9, 80.2, 80.5, 80.6, 80.7, 80.80, 80.81, 81.2, 81.3, 97.5, 97.6, 98.0, 98.3, 98.4, 99.0, 99.3, 126.6-128.2 (aromatic) 137.5, 137.8, 137.9, 138.1, 138.2, 138.3, 138.4, 138.5, 138.9, 139.0, 139.1, 139.2, 139.3, 139.4. The spectroscopic data of the product obtained are in accordance to the literature.[61]

*Allyl 4-methylbenzenesulfonate* (**63**)

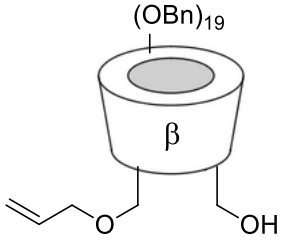



**63**

To a suspension of sodium hydride (1.56 g, 65 mmol, 1.5 eq) and $Et_2O$ (50 ml) under an atmosphere of argon was added allyl alcohol **62** (2.5 mL, 43 mmol) at 25 °C. After gas evolution ceased the reaction mixture was cooled to 0 °C. p-Toluenesulfonyl chloride (7.8 g, 43 mmol, 1 eq) was dissolved in $Et_2O$ (75 ml) and resulting solution was added slowly to reaction mixture. After the addition was complete, the reaction mixture was warmed to 25 °C and allowed to stir for 1 hour. The reaction mixture was added to a saturated solution of ammonium chloride (100 mL) in a separatory funnel. The mixture was extracted with $Et_2O$ (3x50 ml), dried ($Na_2SO_4$), filtered, and concentrated under reduced pressure. The resulting oil was washed with hexanes (2 x 15 ml) and concentrated under reduced pressure to furnish compound **63** (8.67 g, 41 mmol) in 95% yield as a clear oil. *$^1$H NMR:* (400 MHz, DMSO-d6) $\delta_H$ = 2.41 (s, 3H), 4.49 (m, 2H), 5. 19–5.31 (m, 2H), 5.78 (m, 1H), 7.31 (d, J = 7.9 Hz, 2H), 7.75 (d, J = 8.3 Hz, 2H). *$^{13}$C NMR* : (100MHz, DMSO-d6) $\delta_C$ =

21.6, 70.8, 120.2, 127.8, 129.9, 130.2, 133.1, 144.8. The spectroscopic data of the product obtained are in accordance to the literature.[78]

*Mono-allyl-mono-ol* β-*Cyclodextrin* (**61**)

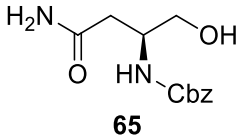


**61**

*n*$Bu_4NI$ (0.04 g, 0.3 eq.) and NaH (0.01 g, 1.3 eq.) were added to a solution of **37** (0.8 g, 0.281 mmol) in dry THF (30 ml) at 0°C under nitrogen atmosphere. The reaction mixture was stirred at 0°C for 30 minutes before adding **63** (0.07 g, 1.15 eq). The solution was stirred at room temperature for 18 h, then treated with MeOH (15 ml) and the solvent was evaporated under reduced pressure. The residue was diluted with $CH_2Cl_2$ (50 ml) and treated with a saturated solution of $NH_4Cl$ (20 ml). The layers were separated, and the aqueous layer was extracted with $CH_2Cl_2$ (3x15 ml). The organic layers were combined, washed with brine and dried with $Na_2SO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc 8:2) to give **61** (0.57 g) as a white foam in 70% yield. The double-substituted product can be observed only after prolonged reaction times.

*$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 2.70 (br, 2H), 3.42-3.53 (m, 5H), 3.60-4.10 (m, 37H), 4.45-4.88 (m, 35H), 4.90 (d, J = 3.3 Hz, 1H), 4.98-5.10 (m, 6H), 5.21-5.27 (m, 5H), 5.32 (d, J = 10.7 Hz, 1H), 5.60-5.65 (m, 4H), ), 5.80 (m, 1H), 7.12-7.33 (m, 95H). *$^{13}$C NMR* : (100MHz, $CDCl_3$) $\delta_C$ = 61.4, 68.6, 68.8, 69.0, 69.2, 69.3, 70.5, 71.3, 71.6, 71.7, 71.8, 72.0, 72.4, 72.5, 72.6, 72.7, 72.8, 73.0, 73.1, 73.2, 73.3, 73.4, 74.3, 74.4, 74.5,74.8, 75.1, 75.6, 75.9, 76.1, 76.2, 78.5, 78.6, 78.7, 78.9, 79.4, 79.5, 79.6, 79.7, 79.9, 80.2, 80.5, 80.6, 80.7, 80.80, 80.81, 81.2, 81.3, 97.5, 97.6, 98.0, 98.3, 98.4, 99.0, 99.3, 121.0, 126.6-128.2 (aromatic), 132.8, 137.5, 137.8, 137.9, 138.1, 138.2, 138.3, 138.4, 138.5, 138.9, 139.0, 139.1, 139.2, 139.3, 139.4. M.W.: 2887.4 ESI-MS $[M+Na]^+$ m/z: 2910.4.

*(S)-Benzyl (4-amino-1-hydroxy-4-oxobutan-2-yl)carbamate* (**65**)

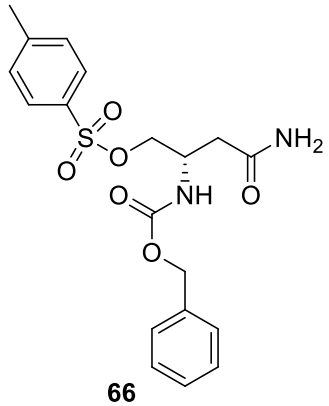


**65**

5 g of *N*-carbobenzyloxy-*L*-asparagine methyl ester **54** (0.0178 mol) were dissolved in 80 ml of EtOH and 700 mg of $CeCl_3$ * $7H_2O$ (0.00178, 0.1 eq.) were then added. To this solution was added $NaBH_4$ (1.36 g, 2 eq.). The obtained suspension was stirred at r.t. for 24h. After this time the solvent was evaporated under vacuum and

the residue obtained was treated with 100 ml of 1N HCl. The aqueous phases were extracted with EtOAc (3 x 80 ml) and the organic phase was washed with a saturated solution of $NaHCO_3$ and brine and then dried over $Na_2SO_4$. After filtration and evaporation of the solvent, the residue was recrystallized from hot/cold EtOAc to give a white solid (2.6 g, 60%). *$^1$H NMR:* (400 MHz, DMSO-d6) $\delta_H$ = 2.35-2.50 (m, 2H), 3.28-3.43 (m, 2H), 3.90 (m, 1H), 4.75 (br, 1H), 5.09 (s, 2H), 6.95 (br, 2H), 7.33-7.45 (m, 5H), 7.68 (d, J = 7.2 Hz, 1H). *$^{13}$C NMR* : (100MHz, DMSO-d6) $\delta_C$ = 33.1, 52.3, 65.4, 67.2, 127.3, 127.8, 128.5, 136.9, 155.6, 172.8.

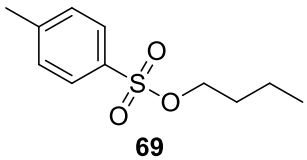
*(S)-4-Amino-2-(((benzyloxy)carbonyl)amino)-4-oxobutyl 4-methylbenzenesulfonate* (**66**)



**66**

Compound **65** (1 g, 0.0039 mol) was dissolved in dry pyridine (20 ml). The reaction was cooled to 0°C with an ice bath and TsCl (0.755 g, 1 eq.) was added at the same temperature. After stirring for 30 minutes, the solution is allowed to warm up to room temperature and left stirring overnight. After this time and after checking the reaction by TLC, HCl 1N was added to the solution and the aqueous phase extracted with $Et_2O$ (3x15 ml). The combined organic layers were washed with $H_2O$ (2x15 ml) and dried over $MgSO_4$. After filtration and concentration under reduced pressure, the residue was purified by column chromatography (n-hexane / EtOAc 7: 3) to obtain **66** in 70% yield. *$^1$H NMR:* (400 MHz, DMSO-d6) $\delta_H$ = 2.25-2.35 (m, 2H), 2.42 (s, 3H), 3.61-3.87 (m, 2H), 4.02 (m, 1H), 5.07 (s, 2H), 7.12 (br, 2H), 7.33-7.81 (m, 9H), 7.79 (d, J = 7.5 Hz, 1H). *$^{13}$C NMR* : (100MHz, DMSO-d6) $\delta_C$ = 20.8, 33.3, 50.8, 67.0, 70.3, 127.2, 127.5, 128.3, 128.7, 131.0, 136.4, 140.2, 145.1, 155.4, 173.1.

*Synthesis of butyl 4-methylbenzenesulfonate* (**30**):

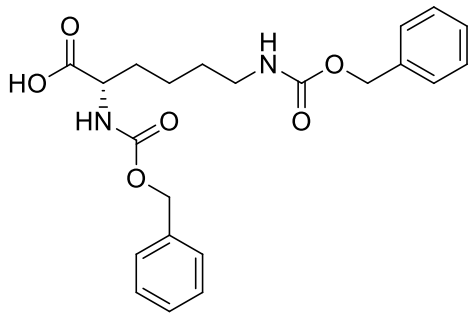


**69**

n-Butanol (0.5 g, 6.74 mmol) was dissolved in dry pyridine (10 ml). After cooling the reaction temperature to 0 ° C with an ice bath, TsCl (1.27 g, 1 eq.) was added. The solution was stirred overnight at room temperature. After this time, HCl 1N was added and the aqueous phase was extracted with $Et_2O$. The organic phase was washed two times with $H_2O$ and dried over $MgSO_4$. After filtration

and concentration under reduced pressure, the residue was characterized by $^{1}$H-NMR showing only peaks related to the desired product, in accordance to the literature.[79] So, it was used for the next step without further purification. *$^{1}$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 0.88 (t, J = 7.3 Hz, 3H), 1.31-1.40 (m, 2H), 1.59-1.64 (m, 2H), 2.47 (s, 3H), 4.05 (t, J = 6.3 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.81 (d, J = 8.2 Hz, 2H). *$^{13}$C NMR* : (100MHz, $CDCl_3$) $\delta_C$ = 13.2, 18.5, 21.7, 30.5, 70.2, 127.8, 129.9, 133.0, 144.5.

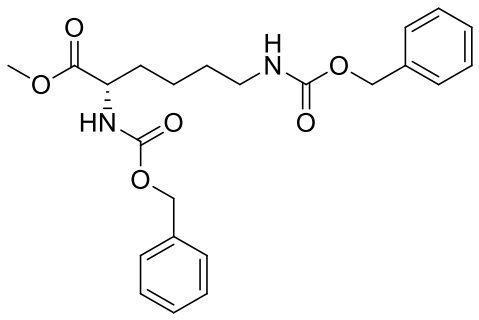
*2,6-bis(((Benzyloxy)carbonyl)amino)hexanoic acid* (**72**)



**72**

In a two-neck flask is dissolved *L*-Lysine*HCl (1 g, 6.84 mmol) in NaOH 2N (7 ml). While stirring vigorously, a solution of CbzCl (2 ml, 14.01 mmol, 2 eq.) in toluene/water 1:1 (8 ml) has been added dropwise. After 2 hours (TLC check) the reaction has been poured in a separating funnel and washed with diethyl ether (2x20 ml). The aqueous layer was acidified with HCl 6N until pH 1 and extracted with diethyl ether (3x30 ml), dried over $Na_2SO_4$ and the solvent removed in vacuo to obtain a thick colorless oil (2.4 g, 85%) which was pure enough and used without further purification in the next step. *$^{1}$H-NMR* (400 MHz, $CDCl_3$) $\delta_H$ = 1.30-1.40 (m, 4H), 1.51-1.55 (m, 1H), 1.55-1.60 (m, 1H), 2.95-3.05 (m, 2H), 3.80-3.95 (m, 1H), 5.10 (s, 2H), 5.20 (s, 2H), 7.30-7.52 (m, 10H), 12.55 (bs, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 23.34, 29.41, 30.87, 54.29, 65.57, 65.84, 128.19, 128.26, 128.81, 137.48, 137.74, 156.54, 156.64, 174.42. M.W.: 414.18. ESI-MS: $[M+H]^+$ m/z = 415, $[M+Na]^+$ m/z = 436.9, $[2M+Na]^+$ m/z = 851.1.

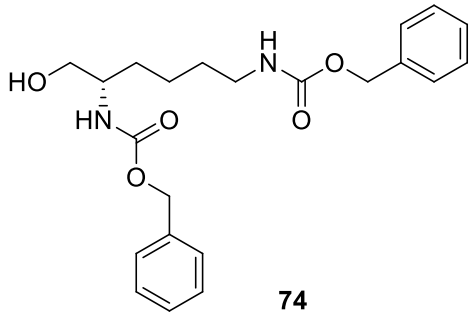
*Synthesis of (S)-methyl 2,6-bis(((benzyloxy)carbonyl)amino)hexanoate* (**73**)



**73**

2g of 2,6-bis(((benzyloxy)carbonyl)amino)hexanoic acid (4.83 mmol) were dissolved in 20 ml of MeOH. To this solution, 400 mg of Amberlyst 15were added and the mixture was refluxed overnight. After this time, the reaction was complete (disappearing of the starting material by TLC Cyclohexane/EtOAc 70:30, bromocresol green stain). The reaction was filtered to remove Amberlyst and the solvent was evaporated under reduced pressure to obtain **73** in 80% yield. *$^{1}$H-NMR* (400 MHz, $CDCl_3$) $\delta_H$ = 1.25-1.27 (m, 2H), 1.51-1.55 (m, 2H), 1.87 (m, 2H), 2.95-3.05 (m, 2H), 3.56 (s, 3H), 4.55 (m, 1H), 5.10 (s, 2H), 5.15 (s, 2H), 7.30-7.52 (m, 10H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 22.65, 29.6, 31.27, 40.3, 52.3, 58.29, 66.10, 127.7, 128.2, 128.9, 137.56, 137.61, 156.6, 156.7, 172.3. M.W.: 428.47. ESI-MS: $[M+H]^+$ m/z = 429.3, $[M+Na]^+$ m/z = 451.4.

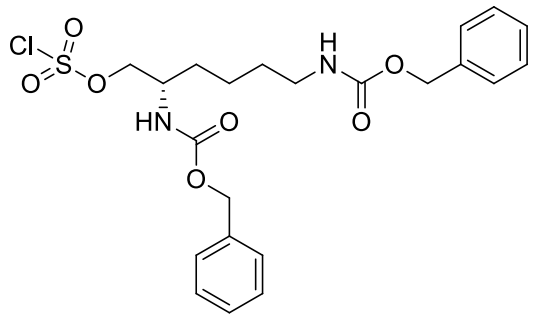
*(S)-Dibenzyl (6-hydroxyhexane-1,5-diyl)dicarbamate* (**74**)[12]



**74**

(S)-methyl 2,6-bis(((benzyloxy)carbonyl)amino)hexanoate **75** (1g, 2.33 mmol) were dissolved in 15 ml of EtOH and 87 mg of $CeCl_3$ * $7H_2O$ (0.1 eq.) were then added. To this solution was added $NaBH_4$ (176 mg, 2 eq.). The obtained suspension was stirred at r.t. for 24h. After this time the solvent was evaporated under reduced pressure and the residue obtained was treated with 30 ml of 1N HCl. The aqueous phases were extracted with EtOAc (3 x 20 ml) and the organic phase was washed with a saturated solution of $NaHCO_3$ and brine and then dried over $Na_2SO_4$. After filtration and evaporation of the solvent we obtained a white solid (933 mg, 60%). *$^{1}$H-NMR* (400 MHz, $CDCl_3$) $\delta_H$ =1.25-1.27 (m, 2H), 1.51-1.55 (m, 2H), 3.10-3.20 (m, 2H), 3.31-56 (m, 2H), 3.62 (bs, 1H), 3.94 (m, 1H), 5.10 (s, 2H), 5.15 (s, 2H), 7.40-7.58 (m, 10H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 22.4, 30.1, 31.0, 40.43, 56.64, 63.9, 66.10, 66.12, 127.3, 127.8, 128.5, 137.5, 137.58, 156.48, 156.56. M.W.: 400.46. ESI-MS: $[M+H]^+$ m/z = 401.3, $[M+Na]^+$ m/z = 423.4.

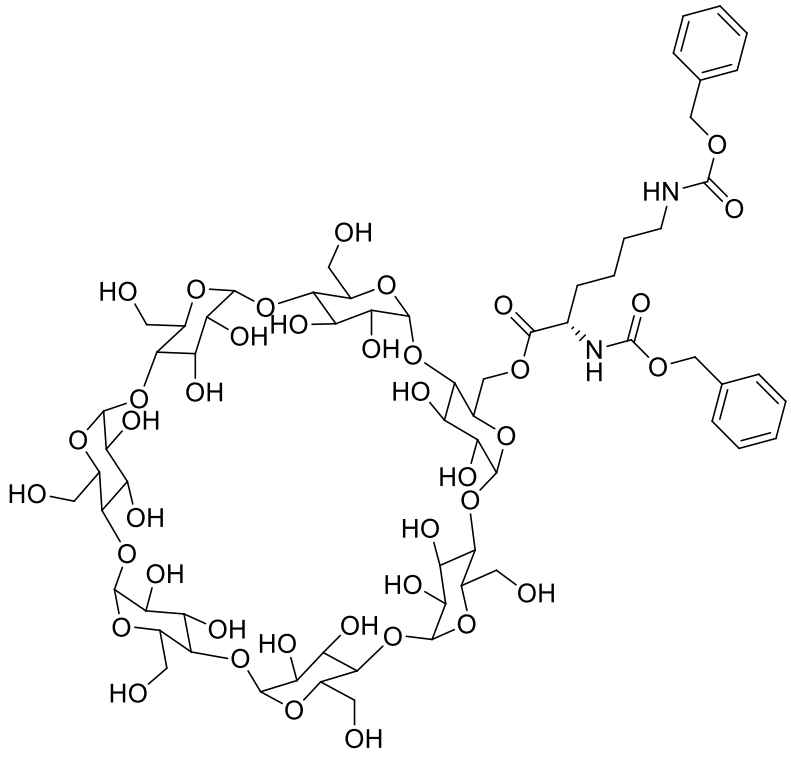
*(S)-2,6-bis(((Benzyloxy)carbonyl)amino)hexyl sulfochloridate* (**75**)



**75**

In a three-necked flask under nitrogen flow is added a solution of the amminoalcohol **36** (200 mg, 0.5 mmol) in dry THF (10 ml) and dry pyridine (120 µl, 3 eq) and the solution is cooled to -10 ° C . Under stirring, a solution of $SO_2Cl_2$ (84 µl, 2 eq.) in dry THF (5 ml) was slowly added, maintaining the temperature constant. Then, the mixture is gradually warmed up to room temperature. The reaction is constantly monitored by HPLC and TLC. After the disappearance of the starting material, the solvent is removed under reduced pressure and the chlorosulfate is immediately used in the next step.

*Mono-6'-N,N'-diCbz-L-lysine* β*-cyclodextrin* (**78**)



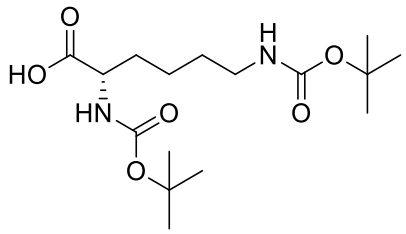
**78**

In a round-bottomed flask, N,N'-di-Cbz-*L*-lysine (1 g, 2.41 mmol) was dissolved in dry DMSO (12 ml) under nitrogen flow and carbonyldiimidazole (CDI, 703 mg, 1.8 eq.) was added. The solution

was stirred for 30 minutes at room temperature. After, β-cyclodextrin (2.18 g, 0.8 eq.) was dissolved in the solution, triethylamine (TEA, 15.4 ml) was added and the solution was stirred for 18 h at room temperature. The reaction was monitored by TLC, using a normal-phase plate precoated with silica gel $60F_{254}$ and an eluent of ethyl acetate/2-propanol/water = 3.5:3.5:2.5 v/v. The product is slightly UV visible (probably due to the two Cbz-protecting groups) and slightly visible with cerium molybdate stain.

After, the reaction mixture was poured dropwise in a large amount of acetone (100/120 ml) and immediately a white precipitate appears. It was left in the refrigerator for one night and the following day it was collected by filtration and washed with cold acetone to obtain a white powder (1.47 g, 50%). *$^{1}$H-NMR* (400 MHz, DMSO-d6) $\delta_H$ = 1.30-1.35 (m, 2H), 1.55-1.57 (m, 2H), 1.87-1.92 (m, 2H), 2.95-3.20 (m, 9H), 3.58-3.75 (m, 47H), 4.07-4.28 (m, 7H), 4.51-4.58 (m, 2H), 5.01-5.08 (m, 7H), 5.12 (s, 2H), 5.15 (s, 2H), 7.32-7.58 (m, 10H). *$^{13}$C-NMR* (100 MHz, DMSO-d6) $\delta_C$ = 23.26, 29.48, 31.05, 54.19, 60.41, 60.88 (CyD C-6'), 65.61, 66.06, 69.94 (CyD C-5'), 72.2 (CyD C-2'), 72.52, 72.90, 73.48, 81.57 (CyD C-4'), 82.03, 102.05 (CyD C-1'), 102.42, 128.15, 128.82, 137.41, 137.76, 156.60, 156.89, 172.60. M.W.: 1531.42. ESI-MS: $[M+H]^+$ m/z = 1532.3.

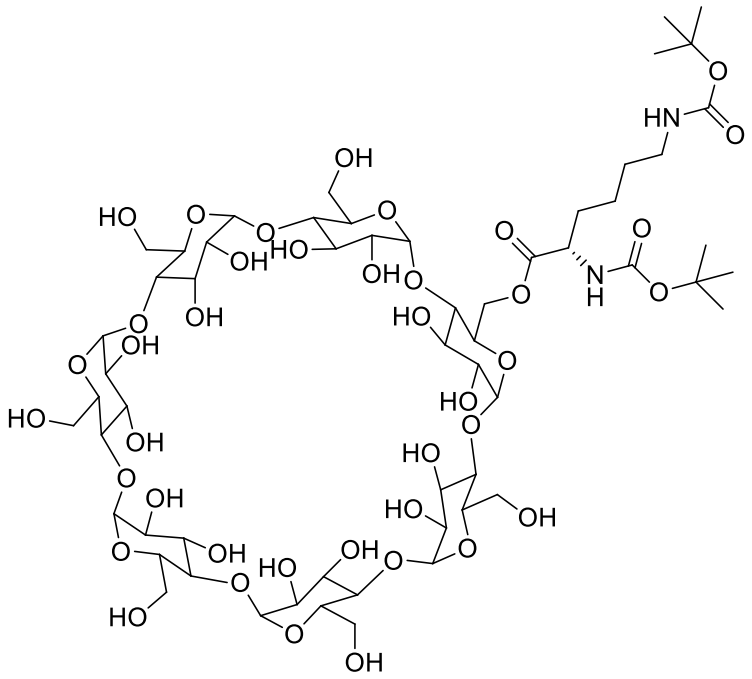
*2,6-bis((tert-Butoxycarbonyl)amino)hexanoic acid* (**79**)



**79**

*L*-lysine hydrochloride (1 g, 6.84 mmol) was dissolved in $H_2O$ (10ml) and to it $NaHCO_3$ (1.38 g, 3 eq.) was added and stirred. To this, di-*t*-butylpyrocarbonate ($Boc_2O$) (2.86 g, 4.8 eq.) in 10ml of THF was added at 0°C. Then the reaction mixture was stirred at room temperature for 24h. At the end of the reaction, THF was removed under reduced pressure, and the aqueous layer was washed with diethyl ether to remove organic impurities. Then, the aqueous layer was acidified to pH 4-5 using a solution of $CH_3COOH$ (10% v/v in water). The aqueous layer was then extracted with dichloromethane (3x20 ml). The organic layer was washed with brine and dried over $Na_2SO_4$ and the solvent was removed under reduced pressure to obtain the compound as a white solid (2.1 g, 90%). *$^{1}$H-NMR* (400 MHz, $CD_3OD$) $\delta_H$ = 1.35-1.50 (m, 20H), 1.59-1.69 (m, 2H), 1.75-1.85 (m, 2H), 2.95-3.05 (t, 2H), 4.00-4.07 (m, 1H), 4.9 (s, 1H), 5.5 (s, 1H). *$^{13}$C-NMR* (100 MHz, $CD_3OD$) $\delta_C$ = 22.76, 27.35, 27.41, 29.14, 31.07, 39.61, 53.44, 78.45. 79.05, 156.77, 157.18, 174.86. M.W.: 346.21. ESI-MS: $[M+Na]^+$ m/z = 369.1, $[M-H]^-$ m/z = 345.1.

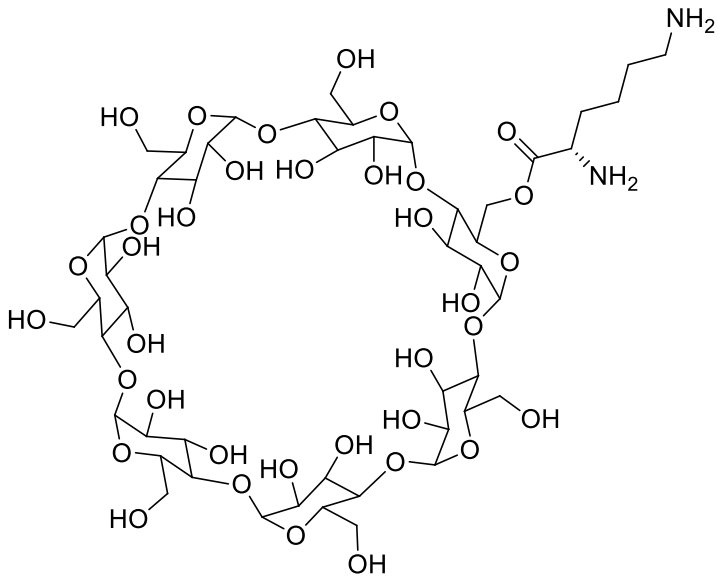
*Mono-6'-N,N'-diBoc-L-lysine* β*-cyclodextrin* (**80**)



80

In a round-bottomed flask, 2,6-bis((tert-butoxycarbonyl)amino)hexanoic acid (**79,** 834 mg, 2.41 mmol) was dissolved in dry DMSO (12 ml) under nitrogen flow and carbonyldiimidazole (CDI, 703 mg, 1.8 eq.) was added. The solution was stirred for 30 minutes at room temperature. After, β-cyclodextrin (2.18 g, 0.8 eq.) was dissolved in the solution, triethylamine (TEA, 15.4 ml) was added and the solution was stirred for 18 h at room temperature. After, the reaction mixture was poured dropwise in a large amount of acetone (100/120 ml) and immediately a white precipitate appears. It was left in the refrigerator for one night and the following day it was collected by filtration and washed with cold acetone to obtain compound **80** as a white powder (80%). *$^{1}$H-NMR* (400 MHz, DMSO-d6) $\delta_H$ = 1.30-1.40 (m, 20H), 1.55-1.57 (m, 2H), 1.78-1.85 (m, 2H), 3.02-3.20 (m, 9H), 3.58-3.75 (m, 47H), 4.07-4.28 (m, 7H), 4.48-4.54 (m, 2H), 5.01-5.08 (m, 7H). *$^{13}$C-NMR* (100 MHz, DMSO-d6) $\delta_C$ = 23.59, 28.91, 29.026, 29.82, 46.32, 54.10, 60.58, 61.10 (CyD C-6'), 70.05 (CyD C-5'), 72.70, 73.08, 73.62, 80.95 (CyD C-4'), 82.19, 102.07 (CyD C-1'), 102.61, 156.31, 156.78, 173.08. M.W.: 1462.57. ESI-MS: $[M-H]^-$ m/z =.1461.6.

*Synthesis of mono-6' -L-lysine* β*-cyclodextrin* (**77**)



**77**

In a round-bottomed flask, mono-6-N,N'-diBoc-*L*-lysine-β-cyclodextrin **80** (200 mg, 0.136 mmol) and 4N HCl in dioxane (1 ml) were stirred for 4 hours at room temperature. After, the solvent was removed in vacuo and the residue was resuspended in diethyl ether for 5-6 hours. The white precipitate was collected by filtration to obtain target molecule **77** as a white powder (155 mg, 95%). *$^{1}$H-NMR* (400 MHz, DMSO-d6) $\delta_H$ = 1.28 (m, 2H), 1.55-1.57 (m, 2H), 1.78-1.85 (m, 2H), 2.71 (m, 2H), 3.02-3.20 (m, 7H), 3.58-3.75 (m, 48H), 4.07-4.28 (m, 7H), 4.48-4.54 (m, 1H), 5.01-5.08 (m, 7H). *$^{13}$C-NMR* (100 MHz, DMSO-d6) $\delta_C$ = 21.64, 26.95, 29.93, 46.06, 52.36, 60.58, 61.85 (CyD C-6'), 70.55 (CyD C-5'), 72.71, 73.10, 73.73, 81.47 (CyD C-4'), 82.19, 101.56 (CyD C-1'),102.60, 170.46. M.W.: 1262.46. ESI-MS: $[M+H]^+$ m/z = 1263.3 $[M+Cl]^-$ m/z =.1297.3.

## 1.6. Conclusions

Since their discovery, cyclodextrins, thanks to their chemical and physical properties and their capacity to form inclusion complexes, have proved to be an important tool in pharmaceutical chemistry and drug delivery.

Furthermore, with the study and development of methods for their functionalization, it has been shown that they are fundamental for the synthesis of receptor models. This is due to the possibility of allocating molecules in their cavity, which makes them very similar to the binding site of an enzyme. Thanks to the appropriate functionalization it is also possible to study the interactions with different types of molecules, mimicking the structure of different receptors.

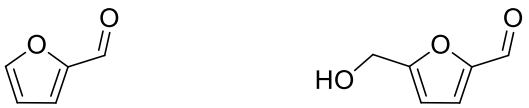
Although it was not possible, during this thesis work, to obtain molecule **38** as an accurate model of CXCR1 receptor, given the extreme synthetic difficulty due to the selectivity necessary to obtain the target molecule, we managed to synthesize a simplified analogue, compound **77**.

It is currently the subject of mechanistic studies at the pharmaceutical company Dompé S.p.A. to verify the interactions between these types of receptors, coupled with the G protein, and various inhibitors, both known and developing molecules.

# Chapter 2: Selective C-3 functionalization of furan ring in furfural and its derivatives via Directed ortho Metalation and study on the degradation of the substrate

## 2.1. Furfural and its derivatives

Furan-containing heterocycles like furfural (or 2-furaldehyde) (**81**) and the related 5-(hydroxymethyl)furfural (HMF) (**82**) represent an important renewable, non-petroleum based, chemical feedstock.

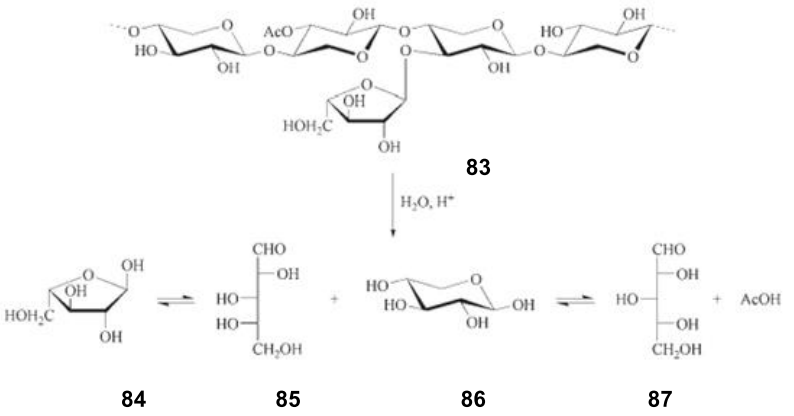



**81** **82**

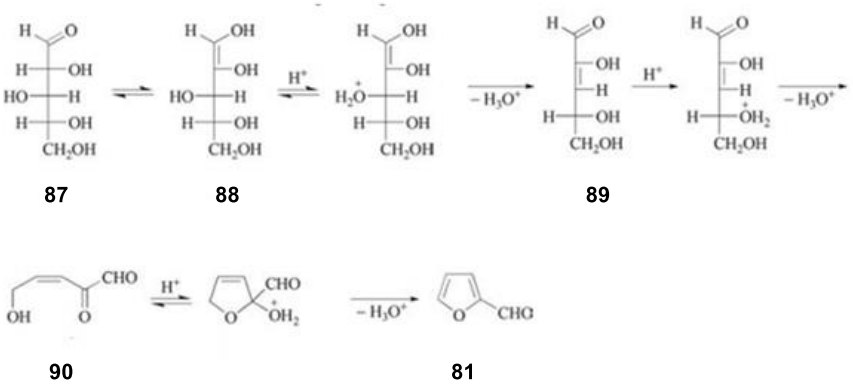
*Figure 2.1. Structures of furfural (**81**) and HMF (**82**)*

Furfural was first isolated in 1821 by the German chemist Johann Wolfgang Döbereiner, who produced a small sample as a byproduct of formic acid synthesis. At the time, formic acid was formed by the distillation of dead ants.

Nowadays, furfural is produced from the pentosan sugar fraction of biomass. This pentosan fraction consists of polysaccharides of C5 sugars and is generally built up from a backbone of 1,4-linked β-*D*-xylopyranosyl residues or xylan. The xylan backbone contains α-*L*-arabinofuranose groups attached at various positions. For this reason, these structures are called arabinoxylans (**83**). In the production of furfural, arabinoxylans are hydrolyzed by acids to the monosaccharides *L*-arabinofuranose (**84**) or *L*-arabinose (**85**) and *D*-xylopyranose (**86**) or *D*-xylose (**87**). Some hydroxyl groups of arabinoxylan (**83**) are acylated with short fatty acids like acetic acid. These esters are also hydrolyzed to the corresponding carboxylic acids.




In the second step monosaccharides are dehydrated to furfural. Here, the dehydration pattern is presented for *D*-xylose; but we can consider a similar pathway also for conversion of arabinose to furfural. Despite the mechanism of this step is still under debate, a generally accepted pathway for the dehydration occurs through the 1,2-enediol form (**88**) of xylose. A first water molecule is abstracted at the 3-position to produce 3-deoxy-*D*-xylosulose (**89**) in its enolic form. A further dehydration at the 4-position yields 3,4-dideoxy-*D*-xylo-3-pentenosulose (**90**), which is converted to the cyclic acetal and finally to furfural by loss of a third water molecule.[80]




Further chemical transformations of furfural lead to a number of useful compounds that can be used as bulk and fine chemicals and fuels relevant building blocks.[81-83] In fact, along with their derivatives, they have been recently emphasized as two of the top added-value chemicals derived from biomass.[84]

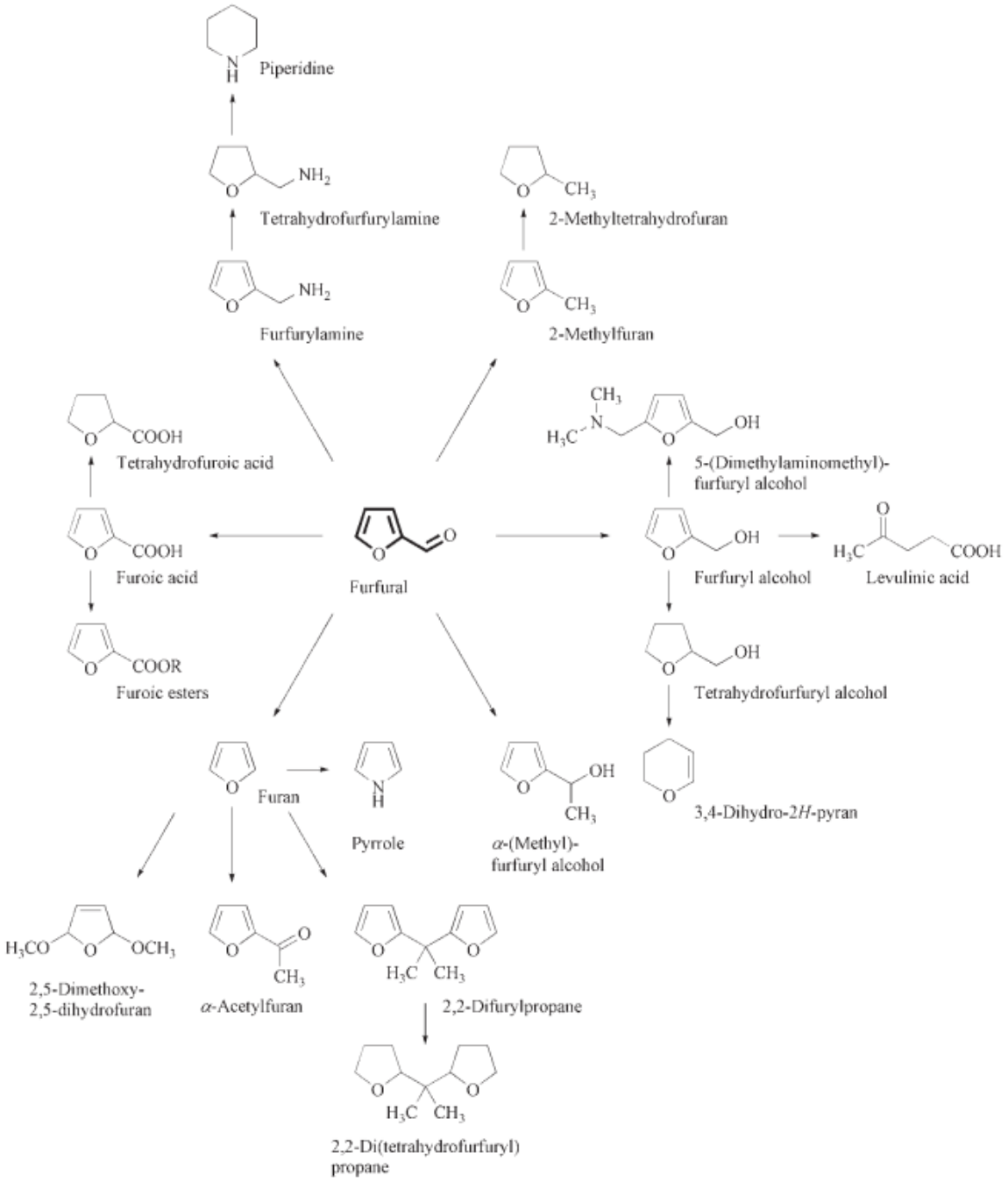



*Figure 2.2. Chemicals derived from furfural.*

Furfural and its derivatives have wide applications in many industries such as plastics, pharmaceuticals and agrochemicals, etc. Furfural itself is, for example, commonly used as a solvent; it is soluble in ethanol and diethyl ether and presents a certain solubility in water. Industrially, it is widely used for the extraction of aromatics from lubricating oils and diesel fuels or unsaturated compounds from vegetable oils and also in wax recovery. Synthetic rubber is made by the purification technology of butadiene or isoprene, and furfural plays an important role in the extractive distillation

of butadiene. It plays also an essential role in providing pharmaceutical building blocks. It is used in the manufacture of furan, an intermediate in the synthesis of pharmaceuticals, agricultural and fine chemicals as well as stabilizers. Furfural is also used in the formation of spandex, a synthetic fiber (polyurethane-polyurea copolymer), known for its exceptional elasticity. It is also used in formulations for rigid and flexible polyurethanes, particularly used in packaging and furniture industries as coatings, foams, adhesive and sealants.

Furfural is used in agriculture/horticulture as a weed killer.[85] It is also recognized as the active ingredient in several nematicides such as Crop guard and protected, which are currently used in parts of Africa. Furfural is generally recognized as a safe compound. It is a natural degradation product of vitamin C (ascorbic acid) and it is a significant component of wines and fruit juices. Even though furfural has an LD50 of 2330 mg $kg^{-1}$ for dogs, its toxicity to humans is relatively low.[86]

Hydrogenation of furfural provides furfuryl alcohol, which is used to produce furan resin, which is exploited in thermoset polymer matrix composites, cements, adhesives, casting resins and coatings. Further hydrogenation of furfuryl alcohol leads to tetrahydrofurfuryl alcohol, which is used as a solvent in agricultural formulations and as an adjuvant to help herbicides penetrate the leaf structure.

### 2.2. Direct C-3 functionalisation of furaldehydes

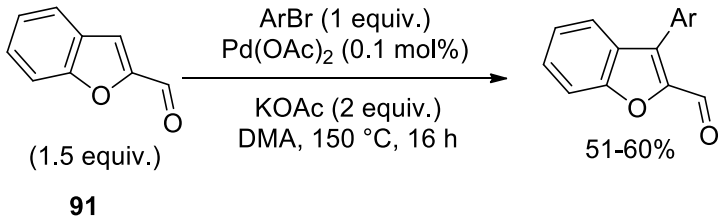
Given the importance of **81** and **82** as precursors for industrially relevant compounds, it would be highly desirable to achieve their functionalisation through the formation of new C-C bonds. This would allow the construction of more complex organic structures, to be used as building blocks, or even as new structural motifs for fine chemicals.

In this perspective, their direct functionalisation through a C-H activation process appears to be an attractive strategy to obtain more elaborated products.

As expected, the functionalization of the 2-furaldehyde **81** occurs more readily on the position 5 of the ring, due to the more marked acidity of the proton caused by the proximity effect of the furan oxygen. There are many papers published on the C-5 functionalization using both bases for deprotonation and catalysis mediated by transition metals.[87-90]

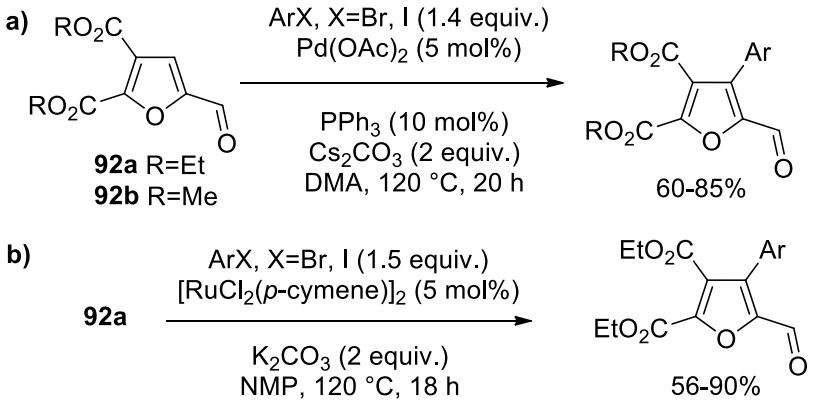
However, the functionalization of the C-3 of these compounds remains an ongoing challenge in organic chemistry. In fact, since this position is less reactive than position 5, a selective functionalization in C-3 of furfural is a more complex task, even more difficult if we do not have any substituent in C-5.

The first example of C-3 arylation of substituted furfurals has been reported by Doucet and co-workers in 2010. They describe the direct C3 arylation of different furan derivatives, and particularly of benzofuran-2-carbaldehyde (**91**), with electron-poor aryl bromides (*Scheme 2.1*), using $Pd(OAc)_2$ as the pre-catalyst, obtaining yields between 51-60% with three different aryl bromides.[91]



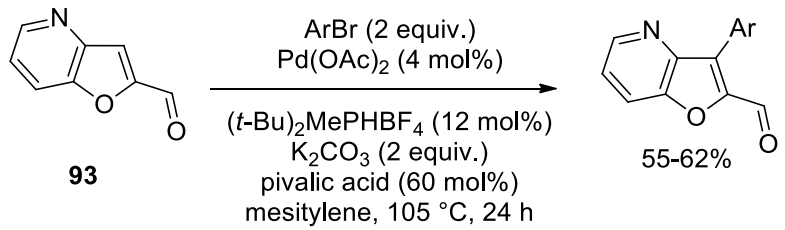

*Scheme 2.1.*

Cao and co-workers in the following year described the direct arylation of 4,5-disubstituted furfurals **92a** and **92b** with aryl bromides and iodides by Pd[92] or Ru[93] catalysis (*Scheme 2.2*). Yields were typically in the range 60-90%; slightly better results were obtained for aryl iodides than the corresponding bromides.




*Scheme 2.2.*

Similarly to **91**, furo[3,2-*b*]pyridine (**93**) was also arylated with aryl bromides in the presence of $Pd(OAc)_2$ as the pre-catalyst with 55-62% yields (*Scheme 2.3*).[94]

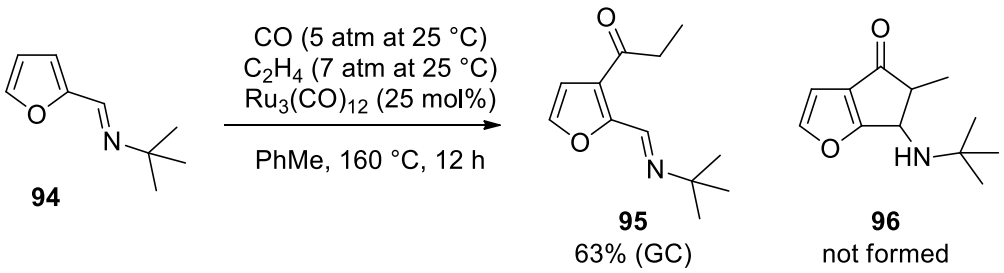



*Scheme 2.3.*

It must be noted that none of the described studies dealt with unsubstituted furfural, and in all these examples C-3 is the only available position for the direct arylation.

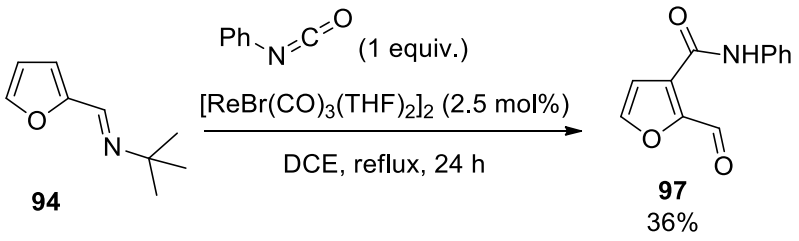
For their similarities with aldehydes, C-H activations reactions in C-3 of *N*-containing derivatives of furaldehydes should also be accounted. In this case, only derivatives at the same formal oxidation level of the aldehyde will be considered, such as imines and related compounds.

In 1997 Murai and co-workers reported the first example of this type. They described the C-3-selective propionylation of aromatic imines by treatment with CO and ethylene using $Ru_3(CO)_{12}$ as pre-catalyst.[95] Starting from the pre-formed imine **94** derived from furfural, the coupling product **95** was obtained in 63% GC yield (*Scheme 2.4*). While most substrates (imines of benzaldehydes and naphthaldehydes) underwent aldol-type cyclization with the newly formed ketone, in the case of **94** this did not occur and compound **96** was not detected, probably for the larger distance between the reactive centres in comparison with imines of six membered rings such as benzaldehydes.



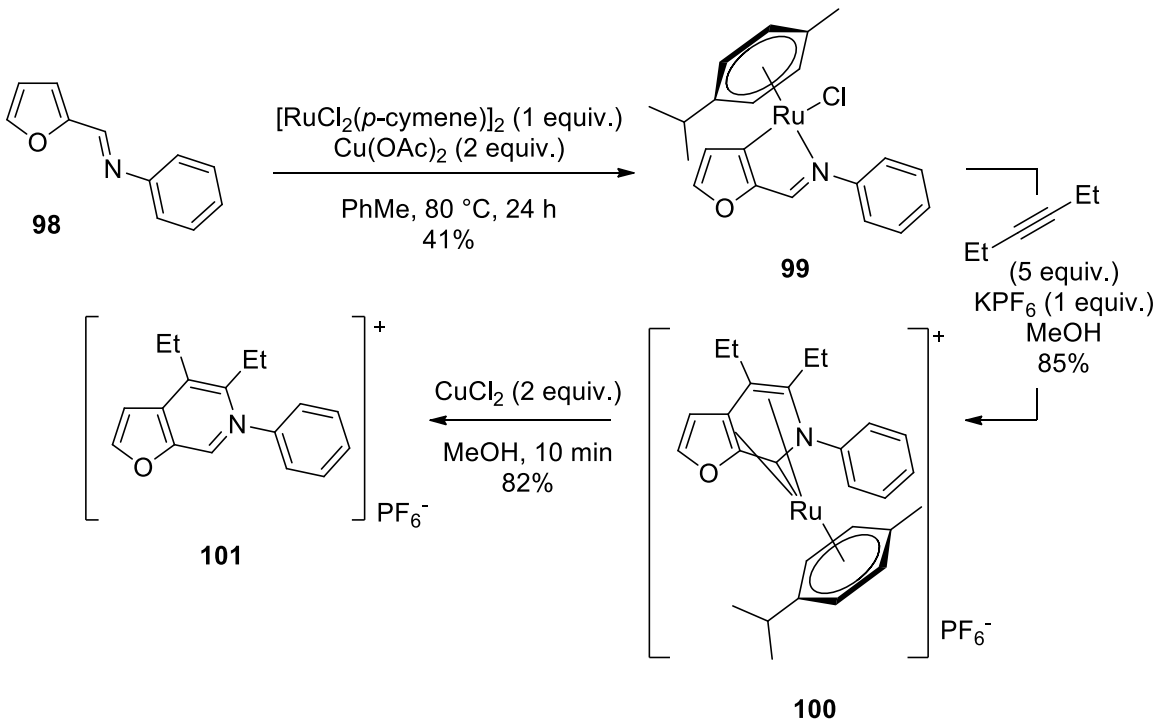

*Scheme 2.4.*

Another interesting example is provided by the hydroarylation of phenylisocyanate with **94** as aromatic partner.[96,97] This reaction consists in the formal insertion of the double C-N bond of the isocyanate into the C3-H bond of the imine promoted by a Re(I) bi-nuclear complex as pre-catalyst in 1,2-dichloroethane (DCE) as solvent to give, after silica gel chromatography, amide **97** in 36% yield (*Scheme 2.5*).




*Scheme 2.5*

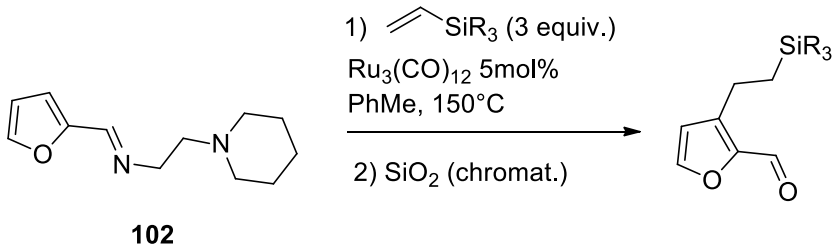
In 2012 Urriolabeitia *et al.* reported the reaction of heterocyclic imines with $[RuCl_2(p\text{-cymene})]_2$ and alkynes.[98] Imine **98** was reacted with the Ru(II) complex in the presence of $Cu(OAc)_2$ to afford complex **99**, a result of the activation of the C3-H bond. Regioselectivity is determined by the directing ability of the iminic group. This complex, when reacted with diphenylacetylene, provides compound **100**, as the result of the insertion of the acetylene in the C-Ru bond. In this complex the Ru atom is connected to the heterocyclic system by a combination of σ and π interactions. By further treatment of this substrate with $CuCl_2$, the product **101** was obtained (*Scheme 2.6*).




*Scheme 2.6.*

Recently, the group of G. Poli published a paper on the selective C-3 functionalization of a bidentate furfural-derived imine using $Ru_3(CO)_{12}$ as pre-catalyst and vinylsilanes as olefin partners, in toluene

at high temperatures, obtaining the aldehyde back from imine simply after chromatography on silica gel (*Scheme 2.7*).[99]




*Scheme 2.7.*

In their proposed mechanism, the *N,N'*-bidentate group chelates the Ru catalyst stabilizing the intermediates along the catalytic cycle, providing the first example of directed C3-alkylation of furfural (*Figure 2.3).*

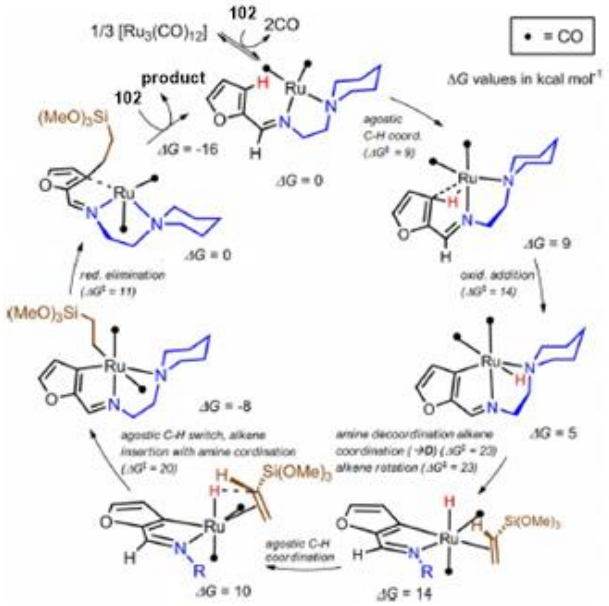
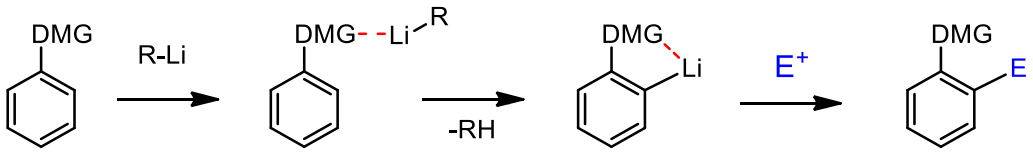



*Figure 2.3. Poli's proposed mechanism for C3-alkylation of furfural imine.*

## 2.3. The Directed *ortho* Metalation reaction

Directed *ortho* metalation (DoM) is an adaptation of the electrophilic aromatic substitution in which electrophiles attach themselves exclusively to the *ortho*-position of a Direct Metalation Group (DMG) through the intermediary of an aryllithium compound. It was first introduced by two independent discovery by Gilman and Bebb[100] and Witting and Fuhrman[101] in 1939 and 1940, respectively, thanks to their study on anisole *ortho* deprotonation by *n*-BuLi.




*Scheme 2.8.*

The DoM process can be described in a simplistic way as a three-step sequence (*Scheme 2.8*). After a first coordination of the alkyllithium, deprotonation occurs to give the coordinated *ortho*-lithiated species. A final reaction with the electrophile yields to the ortho-substituted product. The *ortho*-metalation followed by quench with an electrophile is favoured over traditional electrophilic substitutions due to the regioselective preference for the *ortho*-substitution as opposed to a mixture of *ortho*- and *para*-substitution showed by ordinary electrophilic substitution.

To have a successful deprotonation, the DMG must present a dual behaviour, it has to be a good coordinating site for the alkyllithium while showing poor electrophilic character. The DMG is a Lewis basic moiety that interacts with the Lewis acidic lithium cation allowing the deprotonation by the alkyllithium species from the nearest *ortho*-position on the arene. These groups should be able to effectively coordinate the alkyllithium species, making a heteroatom a necessity, in order to establish a complex-induced proximity effect (CIPE). Inductive effects may also play a role in some cases by lowering the pKa of the adjacent proton. It is important to be noted, however, that DMGs do not function alone in determining the site of metalation and steric hindrance and other functional groups on the arene also have a great influence in the outcome of the reaction.

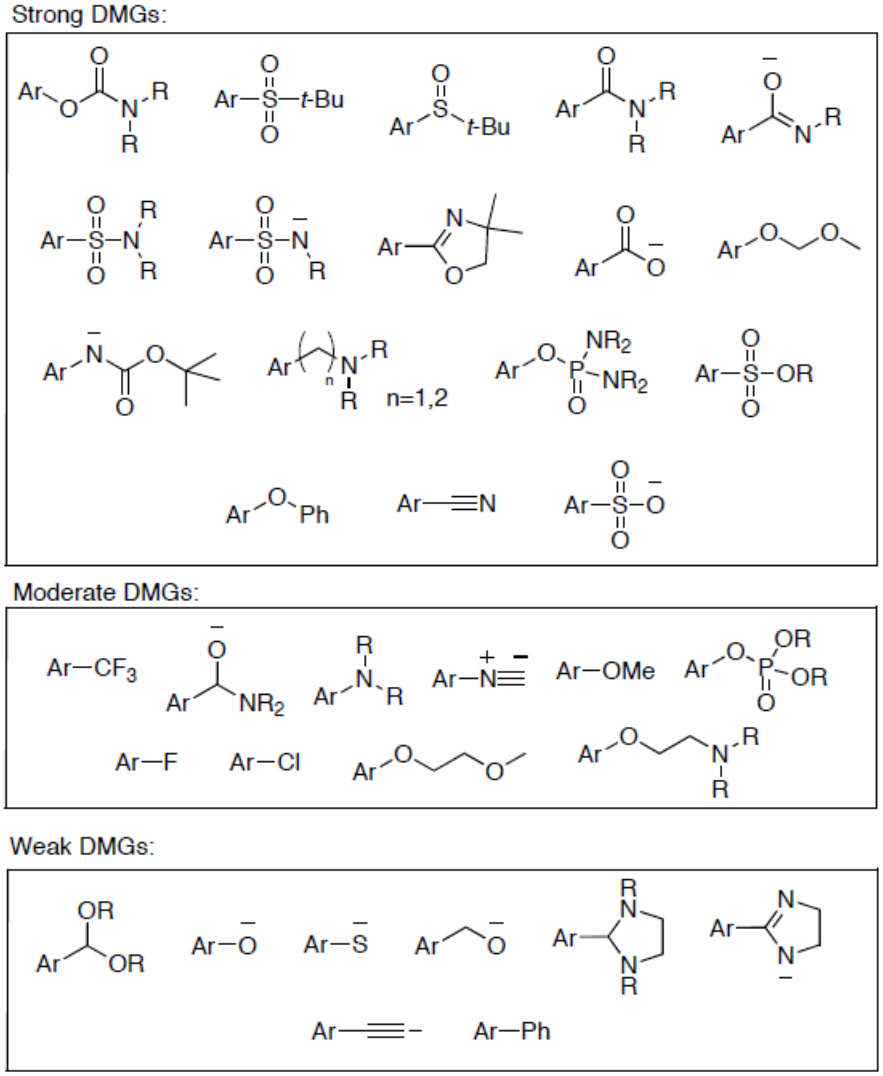
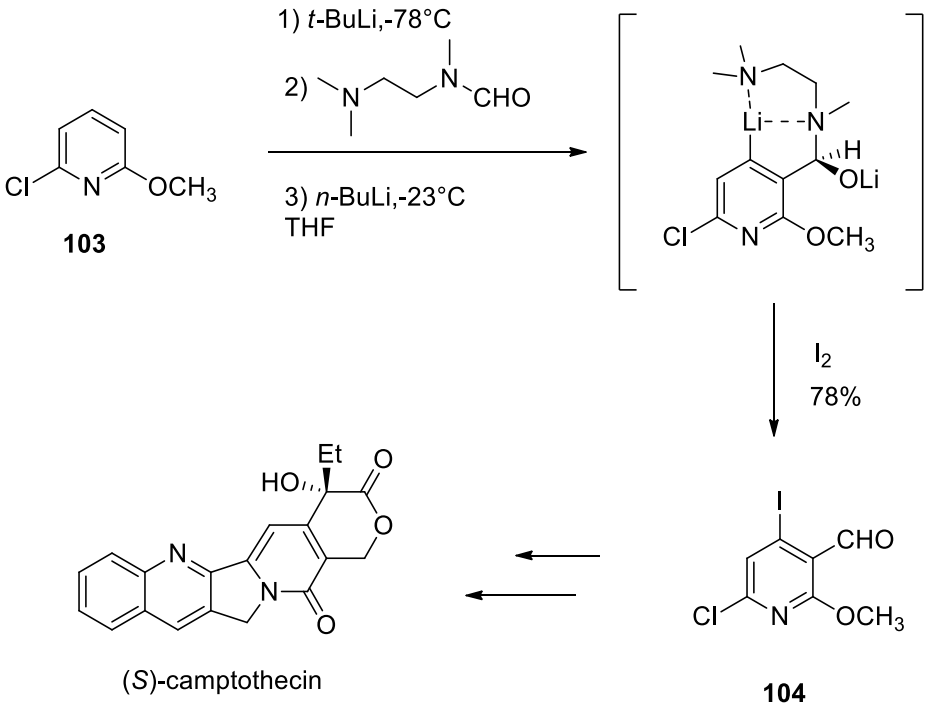


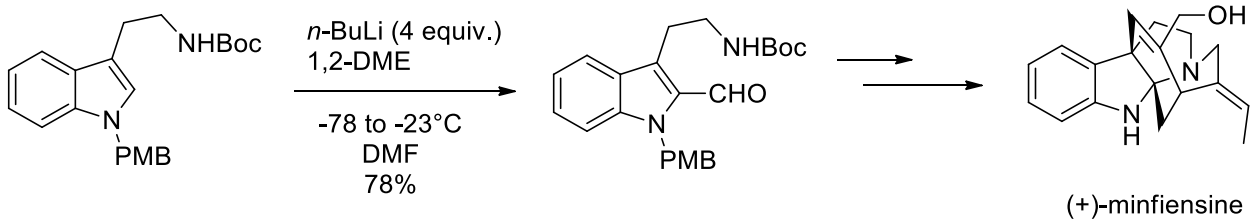

*Figure 2.4. List of common DMGs and their relative strength in directing metalation.*

In the last 30 years, this technique has found applications in many fields, namely the functionalization of aromatic,[102] heteroaromatic.[103-106] In 1992, Comins *et al.* reported an example in a 10-step asymmetric synthesis of (*S*)-Camptothecin (*Scheme 2.5*). Through a clever lithiation adjacent to the methoxyl group of the pyridine **103**, followed by trapping with the formamide, they were able to obtain an α-amino alkoxide to direct a second metalation reaction to obtain the iododerivative **104**.[107]



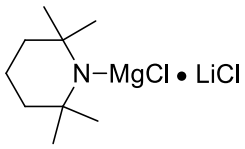

*Scheme 2.5.*

In 2009, MacMillan *et al.* used this type of reaction for a directed lithiation of a tryptamine derivative, which was then used in a cascade reaction in a nine-step enantioselective total synthesis of (+)-minfiensine (*Scheme 2.6*).[108]



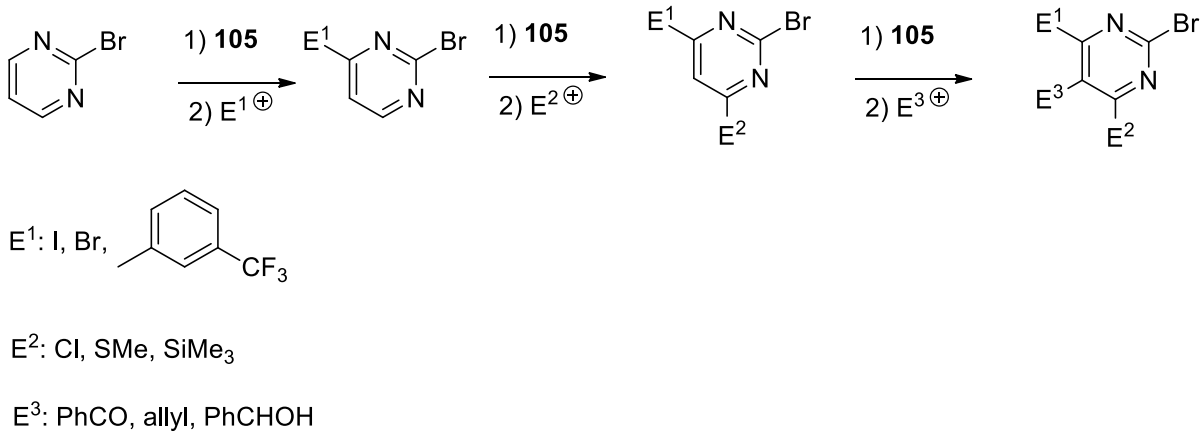

*Scheme 2.6.*

It is important to note that not only organolithium such as *n*-, *s*- or *t*-BuLi can be used in this type of *ortho*-metalation but also highly hindered amide base as TMPMgCl•LiCl (**105**) has been shown to effect efficient directed metallation of electron-poor heteroarenes and arenes containing sensitive functional groups.



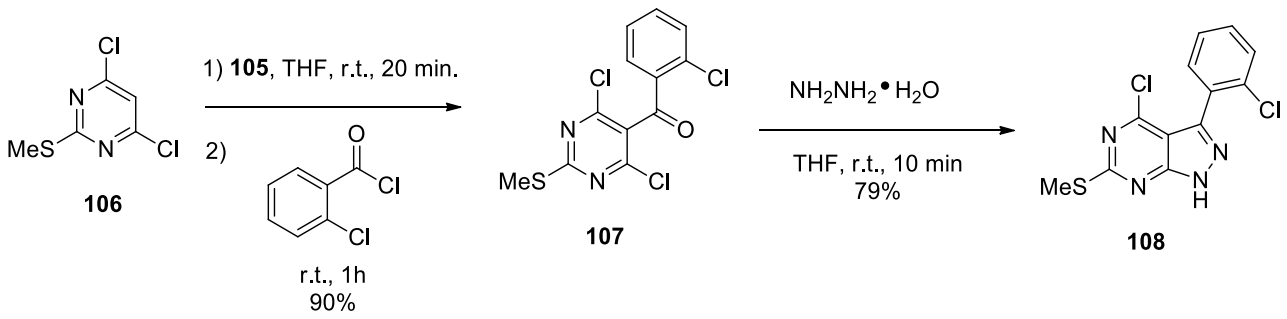

**105**

This metalation technology has been employed in 2008 by Knochel *et al.* in the regio- and chemoselective multiple functionalization of pyrimidine derivatives (*Scheme 2.7*).[109]




*Scheme 2.7.*

They broadened the scope of this method by synthesizing a p38 Kinase inhibitor (**108**) useful as an anti-inflammatory and antiviral agent (*Scheme 2.8).*[109]



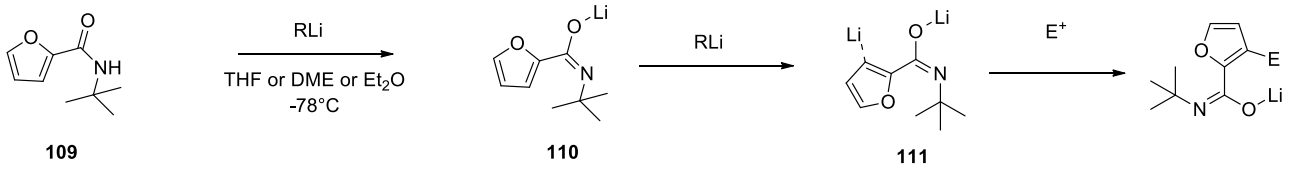

*Scheme 2.8.*

## 2.4. Results and discussion.

Directed metalation using lithium, magnesium or zinc amides is a common functionalization mode of heterocycles.[110] This type of reaction allows the selective activation of C-H bonds leading to the formation of organometallic intermediates which can then take part in C-C bond formation processes by electrophilic substitution.

Some procedures and conditions to achieve C-3 functionalized furan rings starting from furan-2-carboxylic acid derivatives have been already reported.

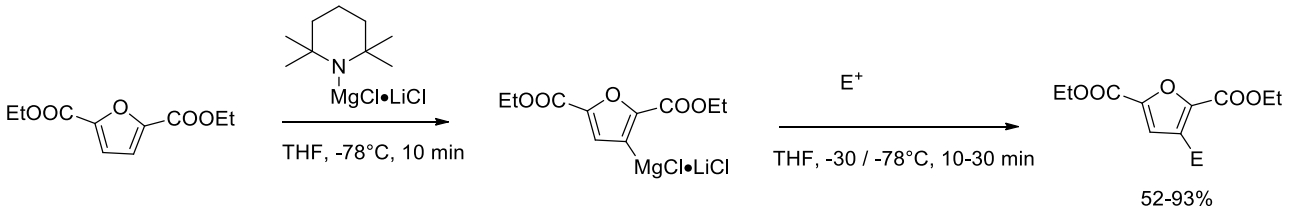
In 1985, Carpenter and Chadwick,[111] reported the *β*-directed metalation of 2-substituted furans. They achieved the C-3 functionalization starting from the *tert*-butyl amide **109** derived from the furfural, providing many different examples and expanding their study on the use of different solvents reporting that not only THF but also DME or $Et_2O$ are suitable solvents for this kind of reaction, and evaluating the efficiency of both *n*- and *sec*-BuLi as organolithium partner (*Scheme 2.9*).




*Scheme 2.9.*

The *tert*-butyl substituent was chosen, accordingly to the authors, to minimize the possibility of nucleophilic substitution on the carbonyl group and to help increasing the solubility of the derived anions in ethereal solvents. Furthermore, it is believed to play also a key role in providing a particular conformation to the system able to stabilize the lithium intermediates. After the functionalization, and acidic work-up, it is possible to recover the amide moiety intact.

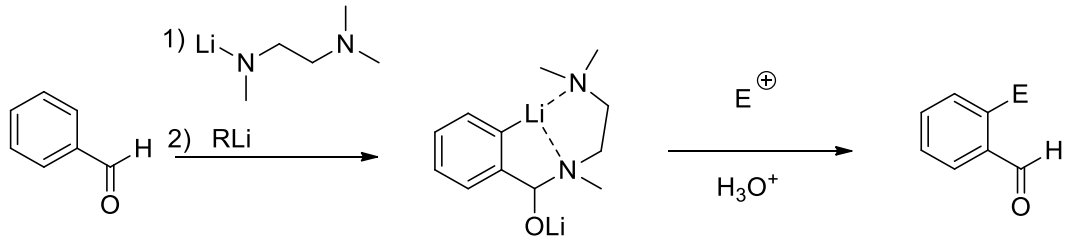
Piller and Knochel in 2011 reported the regioselective C-3 metalation of furfural-derived diesters by direct C-H activation by using magnesium amide bases, such as (tetramethylpiperidyl)magnesium chloride-lithium chloride (TMPMgCl•LiCl, **105**) or bis(tetramethylpiperidyl)magnesium-bis(lithium chloride) ($TMP_2Mg$•2LiCl).[112]




*Scheme 2.10.*

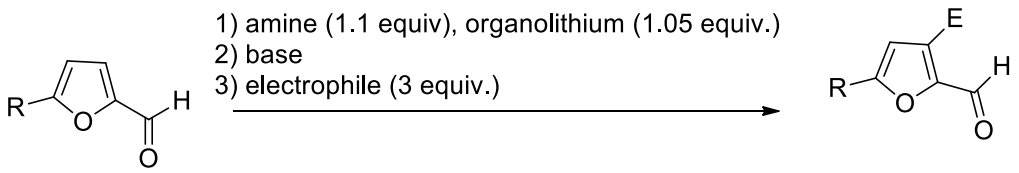
Despite a few methodologies to functionalize the C-3 position of furan rings in furfural derivatives have been developed already, the quest for a less-step-demanding synthesis still remains a challenge for organic chemists, and nowadays the need to develop new strategies for a direct C-3 functionalization of the furfural, keeping its oxidation state, is crucial.

The approach we adopted in the laboratory of Prof. G. Poli at the University Pierre et Marie Curie in Paris, was inspired by the work of Comins *et al.*,[113] which involves the treatment of an aromatic aldehyde (benzaldehyde and its derivatives) by an excess of lithium amide to achieve a selective *ortho*-metalation of the substrate. In this reaction, the alfa amino alkoxide formed allows both the protection of the aldehyde and the introduction of an *ortho*-directing group. After electrophilic substitution and hydrolysis, the functionalized aldehydes are obtained (*Scheme 2.11*).



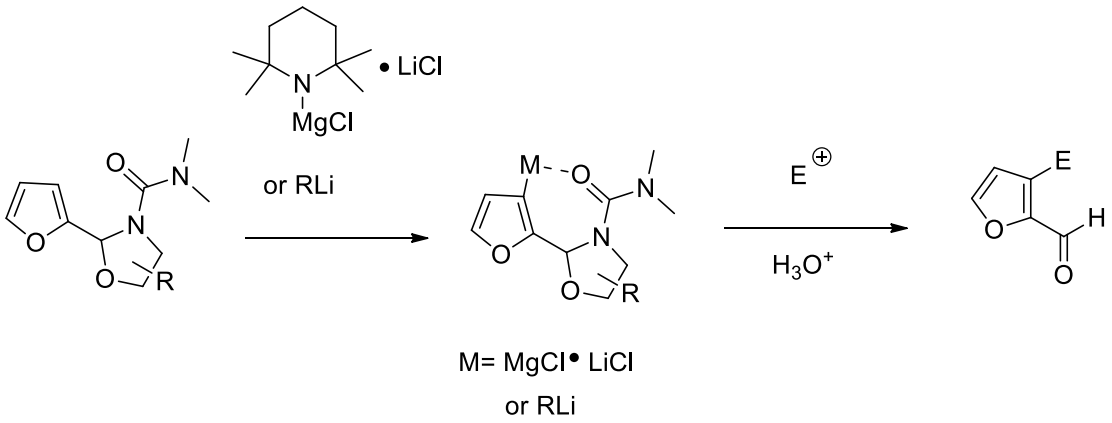

*Scheme 2.11.*

Thus, we decided to inspect this same procedure on our substrates, furfural and its derivatives, to achieve the C3-functionalization of them, by exploring both different lithium amides and organolithium or organomagnesium bases (*Scheme 2.12).*




*Scheme 2.12.*

We also decided to explore the use of other substrates like oxazolidines derived from 2-furaldehyde derivatives, whose synthesis was mastered in the team of Prof. G. Poli (*Scheme 2.13*).

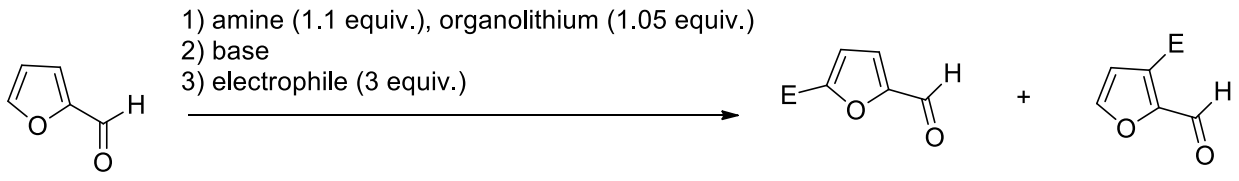



*Scheme 2.13.*

From here on, tables summarizing the reactions tested on the various substrates will be presented, reporting all the reaction conditions used, listed according to the different substrates.

### 2.4.1. Furfural C-3 metalation attempts.

The first substrate we have considered was 2-furaldehyde (*Scheme 2.14*). Many reaction conditions have been explored, changing the temperature at which the reaction was carried and the electrophiles and switching between *n*-BuLi, *s*-BuLi or TMPMgCl•LiCl to evaluate if the strength of the base used could change the outcome of the reaction. Also a screening of solvents was performed, choosing between the most suitable according to the literature,[111] namely THF, DME or diethyl ether.




*Scheme 2.14.*

Generally, results using furfural as starting material were not satisfying. This, above all, is due to the tendency of these substrates (especially of the furan ring) to degradation. Furthermore, due to the

competition with the C-5 position which has a more acidic and more reactive hydrogen, no selectivity towards C-3 could be observed. In fact, as it is shown in entries *2*, *3*, *4*, *5* and *15* (*Table 2.1*), we never managed to get more than 10% selectivity at C-3.

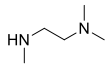
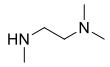
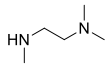
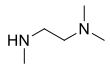
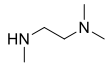
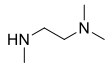
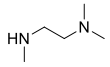
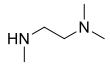
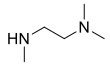
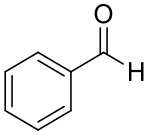
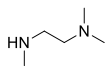
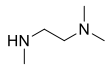
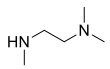
However, from NMR analysis, we could never recognize any structure for the products of degradation.

The use of different organolithium bases did not modify the selectivity, while with TMPMgCl•LiCl we did not observe conversion at all. Furthermore, the lower amount of degradation, underlined by a higher amount of recovery, suggested that probably deprotonation of the substrate does not occur.

The effect of the raising temperature, apparently, influences both the amount of degradation of the product and the selectivity towards the C-5 metalation.

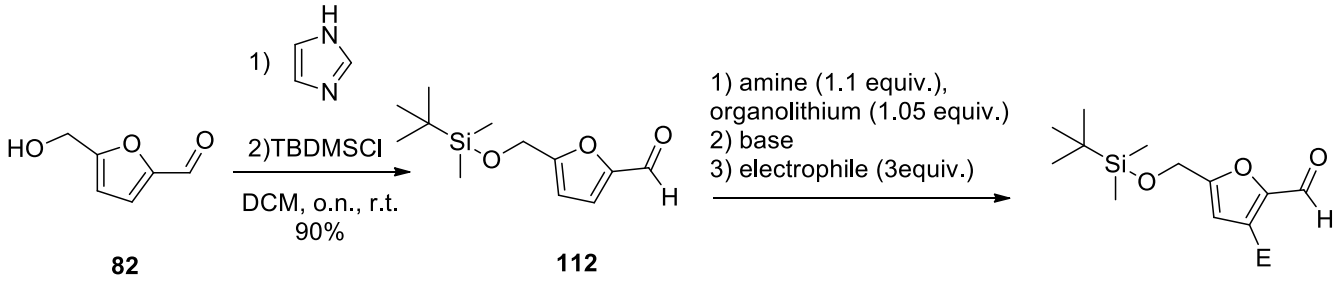
Best results overall were obtained using $Et_2O$ as solvent with *s*-BuLi (2 equiv.) as base for 1 hour at -78°C (*entry 16*) with 40% of recovered substrates as a mixture of C-5 and C-3 deuterated products.

*Table 2.1. Reaction conditions for furfural (**81**) C-3 metalation attempts. [a] Base equivalents are referred to the deprotonation step only and not for the formation of lithium amide. [b] Recovery calculated referring to butadiene sulfone as internal standard.*

| Entry | Solvent | Amine | Base (equiv.) [a] | T for deprotonation step (°C) | Time for deprotonation step | Electrophile | Crude NMR results |
|---|---|---|---|---|---|---|---|
| **1** | THF |  | *n*-BuLi (3) | -20 | 24 hours | $CH_3I$ | Degradation and **99%** of 5-methyl furfural |
| **2** | THF |  | *n*-BuLi (3) | -20 | 5 minutes | $CH_3I$ | Mixture of 3-methylfurfural, 5-methylfurfural and starting material. 3-methylfurfural is **23%** respect to 5-methyl. High amount of degradation is observed. |
| **3** | THF |  | *n*-BuLi (3) | -20 | 15 minutes | $CH_3I$ | Mixture of 3-methylfurfural, 5-methylfurfural and starting material. 3-methylfurfural is **10%** respect to 5-methyl. High amount of degradation is observed. |
| **4** | THF |  | *n*-BuLi (3) | -20 | 30 minutes | $CH_3I$ | Mixture of 3-methylfurfural, 5-methylfurfural and starting material. 3-methylfurfural is **12%** respect to 5-methyl. High amount of degradation is observed. |
| **5** | THF |  | *n*-BuLi (3) | -20 | 1 hour | $CH_3I$ | Mixture of 3-methylfurfural, 5-methylfurfural and starting material. 3-methylfurfural is **14%** respect to 5-methyl. High amount of degradation is observed. |
| **6** | THF |  | *n*-BuLi (3) | -78 | 15 minutes | $CH_3I$ | No conversion. Only starting material. High amount of degradation is observed. |
| **7** | THF |  | *n*-BuLi (3) | -78 | 1 hour | $CH_3I$ | Mixture of 3-methylfurfural, 5-methylfurfural and starting material. High amount of degradation is observed. |
| **8** | DME |  | *s*-BuLi (3) | -45 | 1 hour | $CH_3I$ | Mixture of 5-methylfurfural and starting material. High amount of degradation is observed. |
| **9** | DME |  | *s*-BuLi (3) | -45 | 1.5 hour | $CH_3I$ | Mixture of 5-methylfurfural and starting material. High amount of degradation is observed. |
| **10** | THF | | TMPMgCl•LiCl (1.1) | -78 | 30 minutes | $CH_3I$ | Only starting material. **Recovery: 50%.** [b] |
| **11** | THF | | TMPMgCl•LiCl (1.1) | r.t. | 30 minutes | $CD_3OD$ | Only starting material. **Recovery: 10%.** [b] |
| **12** | THF | | TMPMgCl •LiCl (1.1) | -40 | 15 minutes |  | Only starting material. **Recovery: 70%.** [b] |
| **13** | THF | | TMPMgCl•LiCl (1.1) | -40 | 15 minutes | $CD_3OD$ | Only starting material. **Recovery: 90%.** [b] |
| **14** | $Et_2O$ |  | *s*-BuLi (1.2) | -78 | 1 hour | $CD_3OD$ | **40%** of C-5 deuterated and **10%** of C-3 deuterated |
| **15** | $Et_2O$ |  | *s*-BuLi (2) | -78 | 1 hour | $CD_3OD$ | **Recovery: 40%.** [b] **60%** of C-5 deuterated and **10%** of C-3 deuterated |
| **16** | $Et_2O$ |  | *s*-BuLi (1.2) | -78 | 1 hour |  | Only starting material. **Recovery: 20%.** [b] |

### 2.4.2. 5-(((*tert*-Butyldimethylsilyl)oxy)methyl)furan-2-carbaldehyde C-3 metalation attempts.

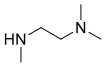
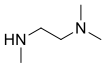
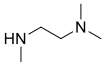
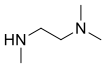
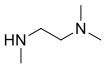
The next substrate that was evaluated was 5-(((*tert*-butyldimethylsilyl)oxy)methyl)furan-2-carbaldehyde (**112**), prepared according to a reported procedure,[114] by reacting HMF **82** with TBDMSCl in dichloromethane in the presence of imidazole to obtain **112** in 90% yield. The substrate thus obtained was used in the subsequent metalation reactions (*Scheme 2.15*).




*Scheme 2.15.*

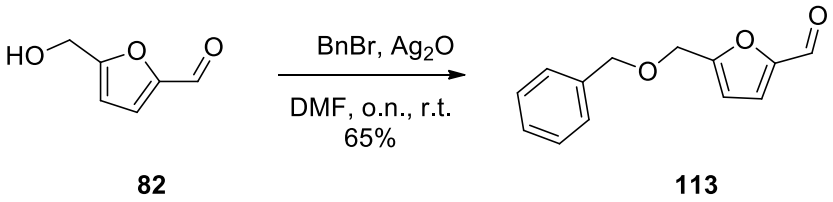
In this case, we managed to observe a higher amount of metalated compound, starting from 10% of entry *5* (T*able 2.2*) to 25% of entry *1*. However, also in this case, a very high amount of degradation was detected in every experiment, due to both the degradation of the furan ring and the silyl group.

*Table 2.2. Reaction conditions for 5-(((tert-butyldimethylsilyl)oxy)methyl)furan-2-carbaldehyde (**112**) C-3 metalation attempts. [a] Base equivalents are referred to the deprotonation step only. [b] Ratio between products formed and amount of starting material left.*

| Entry | Solvent | Amine | Base (equiv.) [a] | T for deprotonation step (°C) | Time for deprotonation step | Electrophile | Product vs starting material ratio by crude NMR analysis [b] | Crude NMR results |
|---|---|---|---|---|---|---|---|---|
| **1** | THF |  | *n*-BuLi (3) | -20 | 15 minutes | $CH_3I$ | 25% | Mixture of starting material and 3-metalated product. However, a large amount of degradation is observed. |
| **2** | THF |  | *n*-BuLi (3) | -20 | 24 hours | $CH_3I$ | 0% | No conversion, only starting material and degradation is observed. |
| **3** | THF |  | *n*-BuLi (3) | -20 | 12 hours | $CH_3I$ | 0% | No conversion, only starting material and degradation is observed. |
| **4** | THF |  | *n*-BuLi (3) | -20 | 1 hour | $ND_4Cl$ in $D_2O$ (1M) | 20% | 20% of 3-deuterated product is detected. However, a lot of degradation is observed. |
| **5** | THF |  | *n*-BuLi (3) | -78 | 1 hour | $CH_3I$ | 10% | Mixture of 3-methylfurfural and starting material. High amount of degradation is observed. |

### 2.4.3. 5-((Benzyloxy)methyl)furan-2-carbaldehyde C-3 metalation attempts.

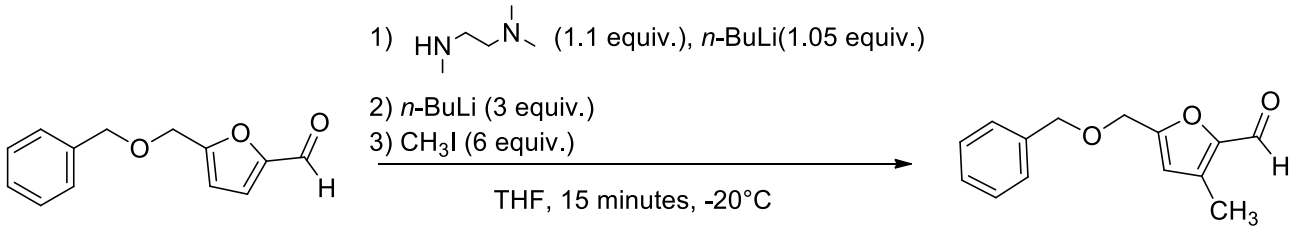
Following a reported procedure,[115] we managed to synthesize 5-((benzyloxy)methyl)furan-2-carbaldehyde **113**, starting from HMF and benzyl bromide in DMF using stoichiometric amount of silver oxide (*Scheme 2.16*).




**82** **113**

*Scheme 2.16.*

Again, substrate **113** was used in a metalation reaction using the best conditions found for the metalation of **112** (*Table 2.2, entry 1*) in order to check if, avoiding the degradation of the silyl group, we could manage to increase the recovery of the product.




**113**

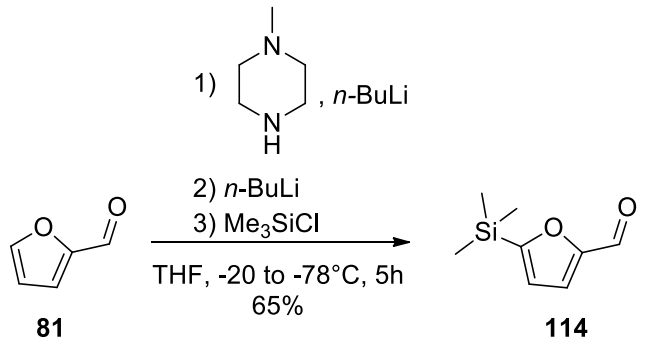
*Scheme 2.17.*

In this case, which is the only one tested with this substrate, we found 0% conversion, and only starting material was recovered. Also, almost no degradation was observed, which may be a signal that the deprotonation did not occur.

### 2.4.4. 5-(Trimethylsilyl)furan-2-carbaldehyde C-3 metalation attempts.

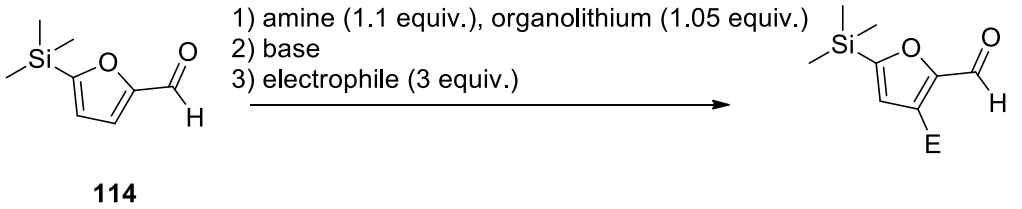
Substrate **114** was chosen next for C-3 metalation attempts of furan ring of furfural derivatives. It was synthesized by following a reported procedure starting from 2-furaldehyde **81** using lithium *N*-methylpiperazide as a temporary hindered protective group of the carbonyl moiety. A subsequent

deprotonation with *n*-BuLi at -78°C, reaction of the anion thus formed with a 1.5M solution of $(CH_3)_3SiCl$ in THF and acid work-up to restore the aldehyde from the imine formed, substrate **114** was obtained (*Scheme 2.18*). [116]




*Scheme 2.18.*

Metalation reactions performed on substrate **114** are reported in *Table 2.3*, following the general scheme:

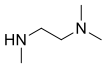
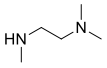
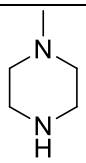
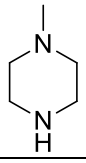
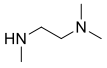



*Scheme 2.19.*

With a C-5 position blocked by a trimethylsilyl group, we managed to obtain a satisfying percentage of 3-substituted product. Moreover, with this substrate we found a strong correlation between the different organolithium used. Reactions with *n*-BuLi (*entries 2, 3 and 4*) gave no conversion, while those with *s*-BuLi (*entries 1 and 5)* gave 50-60% of 3-substituted compound, which dropped to 20% after chromatographic purification. However, as we usually experienced, a very high amount of degradation has been observed.

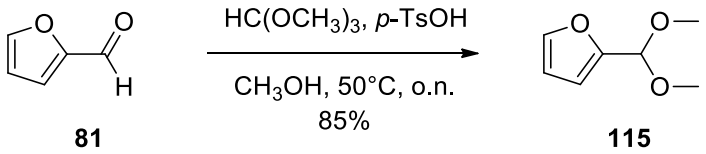
Since the synthesis of **114** was achieved with the use of lithium *N*-methyl piperazide without much degradation observed, few attempts were tested with it, in comparison to the lithium *N,N,N'*-trimethylethylene diamine. However, no conversion and only the presence of the starting material could be observed by the analysis of crude $^1$H-NMR.

*Table 2.3. Reaction conditions for 5-(trimethylsilyl)furan-2-carbaldehyde (**114**) C-3 metalation attempts. [a] Base equivalents are referred to the deprotonation step only. [b] Ratio between products formed and amount of starting material left. [c] Recovery calculated referring to butadiene sulfone as internal standard.*

| Entry | Solvent | Amine | Base (equiv) [a] | T for deprotonation step (°C) | Time for deprotonation step | Electrophile | Product vs starting material ratio by crude NMR analysis [b] | Crude NMR results |
|---|---|---|---|---|---|---|---|---|
| **1** | THF |  | *s*-BuLi (3) | -78 | 1 hour | $CH_3I$ | 50% | **Recovery: 1%.** [c] Mixture of starting material and 3-metalated product. However, a large amount of degradation is observed. |
| **2** | THF |  | *n*-BuLi (3) | -78 | 1 hour | $CH_3I$ | / | No conversion, only starting material and degradation is observed. |
| **3** | THF |  | *n*-BuLi (2) | -78 | 12 hours | $CH_3I$ | / | No conversion, only starting material and degradation is observed. |
| **4** | THF |  | *n*-BuLi (2) | -20 | 1 hour | $CH_3I$ | / | No conversion, only starting material and degradation is observed. |
| **5** | $Et_2O$ |  | *s*-BuLi (1.2) | -78 | 1 hour | $CD_3OD$ | | **Recovery: 20%.** [c] **60%** of C-3 deuterated compound. **20%** yield after chromatography. |

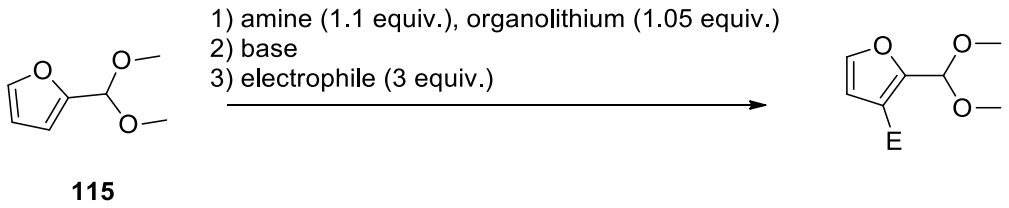
### 2.4.5. 2-(Dimethoxymethyl)furan C-3 metalation attempts.

After, 2-(dimethoxymethyl)furan (**115**) was synthesized. Starting from 2-furaldehyde and trimethyl orthoformate in the presence of a catalytic amount of *p*-toluensulfonic acid at 50°C overnight, compound **115** was obtained in 85% yield.




*Scheme 2.20.*

Despite the dimethyl acetal functionality is only a weak *ortho*-metalating group, we decided to test the behaviour of this substrate under our reaction conditions. Metalation reactions performed on substrate **115** are reported in *Table 2.4*, according to *Scheme 2.21*.




**115**

*Scheme 2.21.*

Similar results to the furfural can be observed with this substrate. In fact, the reaction either did not proceed at all, or we found a mixture of compounds, including the starting material, furfural deriving from the hydrolysis of the acetal or the C-5 deuterated product. In fact, being dimethyl acetal a weak *ortho*-directing group and without the help of the lithium amide as DoM group, we have not observed the C-3 metalation under any circumstances.

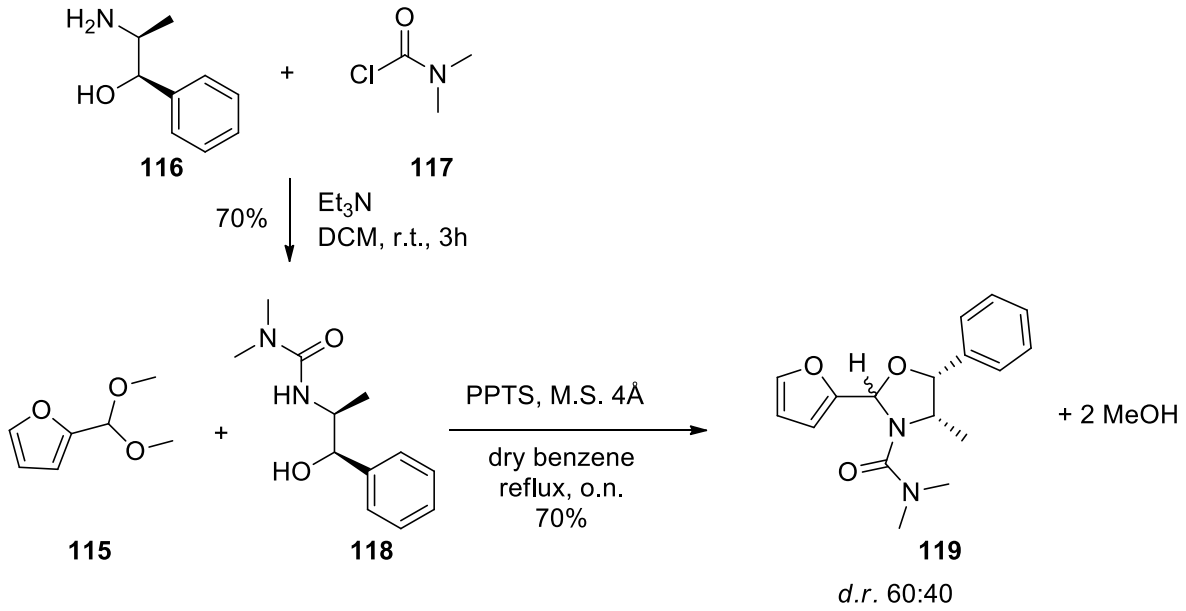
Almost in any case, it was not possible to recover the totality of the starting material, and degradation and hydrolysis have always been observed. Again, as reported for 2-furaldehyde in *Table 2.1*, the use of TMPMgCl•LiCl did not lead to any conversion and only unreacted starting material could be recovered.

*Table 2.4. Reaction conditions for 2-(dimethoxymethyl)furan (**115**) C-3 metalation attempts. [a] Base equivalents are referred to the deprotonation step only. [b] Recovery calculated referring to butadiene sulfone as internal standard.*

| Entry | Solvent | Amine | Base (equiv.) [a] | T for deprotonation step (°C) | Time for deprotonation step | Electrophile | Crude NMR results |
|---|---|---|---|---|---|---|---|
| **1** | THF | / | *s*-BuLi (3) | -78 | 1 hour | $CH_3I$ | **Recovery: 10%.** [b] Mixture of starting material, 5-metalated product and furfural. However, a large amount of degradation is observed. |
| **2** | THF | / | *n*-BuLi (1.2 ) | -78 | 1 hour | $CH_3I$ | **Recovery: 10%.** [b] Mixture of starting material, 5-metalated product and furfural. However, a large amount of degradation is observed. |
| **3** | THF | / | TMPMgCl•LiCl (1.1) | -78 | 30 minutes | $CH_3I$ | **Recovery: 60%.** [b] No conversion, only starting material and degradation is observed. |
| **4** | THF | / | TMPMgCl•LiCl (1.1) | r.t. | 30 minutes | $CD_3OD$ | **Recovery: 90%.** [b] No conversion, only starting material. |
| **5** | $Et_2O$ | / | *n*-BuLi (1.3) | -78 | 1 hour | $CD_3OD$ | **Recovery: 100%.** [b] No conversion, only starting material. |
| **6** | $Et_2O$ | / | *s*-BuLi (1.3) | -78 | 1 hour | $CD_3OD$ | **Recovery: 40%.** [b] No conversion, only starting material and degradation. |
| **7** | $Et_2O$ | / | *n*-BuLi (1.3) | r.t. | 1 hour | $CD_3OD$ | **Recovery: 40%.** [b] Mixture of starting material and 5-deuterated product. |

### 2.4.6. (*4S,5R*)-2-(Furan-2-yl)-*N,N*-4-trimethyl-5-phenyloxazolidine-3-carboxamide C-3 metalation attempts.

Next, we decided to move to another class of substrate, the oxazolidine **119** bearing a dimethylurea moiety which should act as a powerful DMG. Its synthesis has been achieved as reported in *Scheme 2.22.*




*Scheme 2.22.*

A first reaction between commercially available norephedrine **116** and dimethylcarbamoyl chloride in DCM with $Et_3N$ afforded urea **118** in fairly good yield, which was used in the next step with 2-(dimethoxymethyl)furan **115**. The reaction was carried out in the presence of catalytic amount of pyridinium *p*-toluenesulfonate, in refluxing benzene, in an apparatus like the one showed in *Figure 2.5.* The reaction was also performed both in toluene at 90°C or at refluxing temperature but it did not provide the expected product.

By using this apparatus, it is possible to heat the reaction mixture charged in the flask. After the vapours made of methanol and benzene condense in the overlying condenser, the drops pass through the addition funnel charged with activated molecular sieves capable of trapping methanol before flowing back in the flask, shifting the equilibrium of the reaction towards the formation of the product.

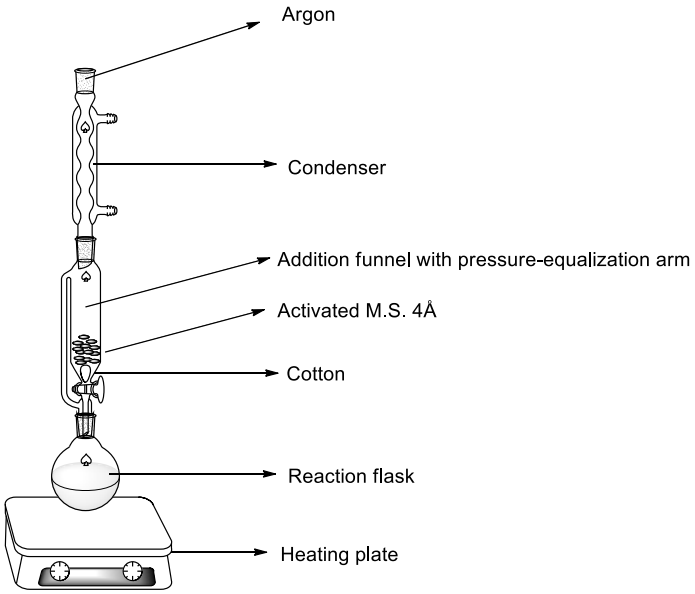
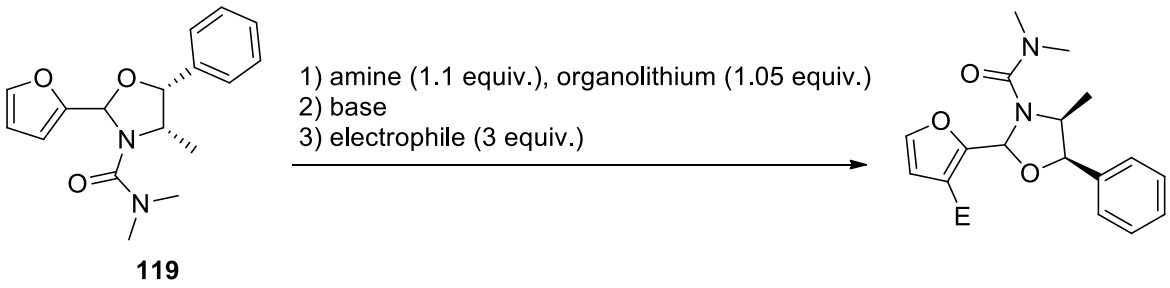



*Figure 2.5. Apparatus for the synthesis of **119**.*

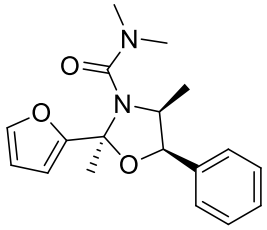
On the newly synthesized substrate, we carried out C-3 metalation reactions, according to the scheme:




**119**

*Scheme 2.23.*

Substrate **119** immediately showed different results from those expected. After the treatment with *s*-BuLi (3 eq.) in the presence of the lithium amide (*Table 2.5, entry 1*), we observed a total conversion (no more starting material) to product **120**. However, only 30% yield could be obtained after silica gel purification, the remaining part was degradation.



**120**

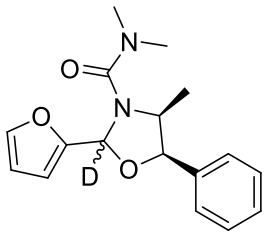
Very similar results were obtained also repeating the same reaction with *n*-BuLi (*Table 2.5, entry 2*) and *s*-BuLi without adding the lithium amide (*Table 2.5, entry 3*).

Interestingly, only one of the two diasteroisomers was selectively obtained in every case.

Tests with less equivalents of organolithium showed no conversion (*entry 4*) or only partial conversion (*entry 5*).

Unfortunately, tests with benzaldehyde as electrophile gave no results, probably due to the high steric hindrance of the two substrates (*entries 7 and 8*).

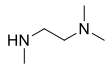
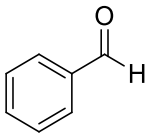
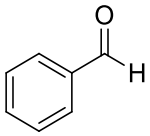
Deuterium incorporation led to product **121** with a d.r. of 65:35.




**121**

Overall, the reactivity shown by the substrate **119** was very interesting, and the same carbon with an electrophilic character in furfural (**81**) was converted to a carbon with nucleophilic character in the oxazolidine **119**. However, as we always experience in these substituted furan ring molecules, the drawback of degradation is always present.

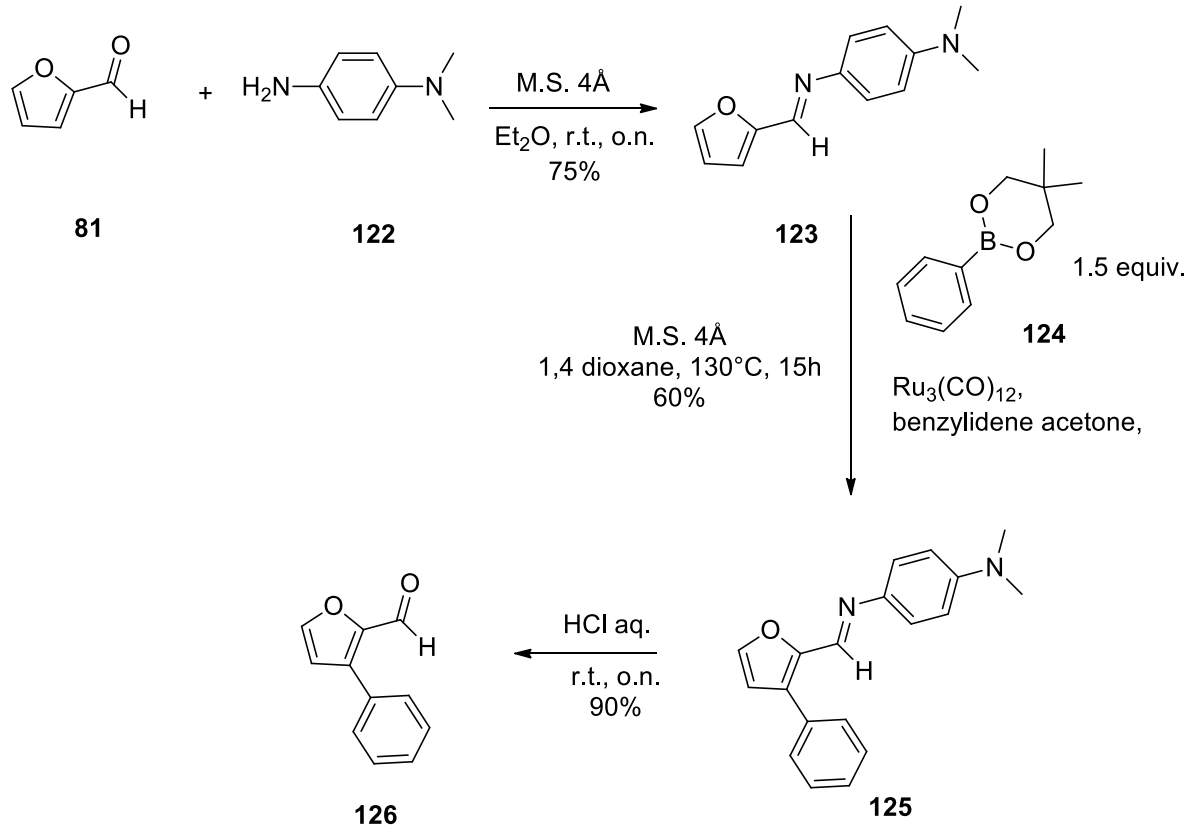
*Table 2.5. Reaction conditions for (4S,5R)-2-(furan-2-yl)-N,N,4-trimethyl-5-phenyloxazolidine-3-carboxamide (**119**) C-3 metalation attempts. [a] Base equivalents are referred to the deprotonation step only. [b] Ratio between products formed and amount of starting material left. [c] Recovery calculated referring to butadiene sulfone as internal standard.*

| Entry | Solvent | Amine | Base (equiv.) [a] | T for deprotonation step (°C) | Time for deprotonation step | Electrophile | Product vs starting material ratio by crude NMR analysis [b] | Crude NMR results |
|---|---|---|---|---|---|---|---|---|
| **1** | THF |  | *s*-BuLi (3) | -78 | 1 hour | $CH_3I$ | 100% | Some degradation observed. The only product observed is **11**. **Yield: 30%** after silica gel chromatography (d.r. 100:0 anti:syn) |
| **2** | THF | | *n*-BuLi (2) | -78 | 1 hour | $CH_3I$ | 100% | Some degradation observed. The only product observed is **11** (d.r. 100:0 anti:syn). |
| **3** | THF | | *s*-BuLi (3) | -78 | 1 hour | $CH_3I$ | 100% | **Recovery:30%.** [c] Some degradation observed. The only product observed is **11** (d.r. 100:0 anti:syn). |
| **4** | THF | | *s*-BuLi (1.2) | -78. | 1 hour | $CH_3I$ | 0% | **Recovery: 50%.** [c] No conversion, only starting material. |
| **5** | THF | | *s*-BuLi (2) | -78. | 1 hour | $CH_3I$ | 40% | Mixture of starting material and **11**.. |
| **6** | THF | | TMPMgCl• LiCl (1.1) | r.t. | 30 minutes | $CD_3OD$ | | **Recovery: 20%.** [c] No conversion, only starting material and degradation. |
| **7** | THF | | TMPMgCl• LiCl (1.1) | -40. | 15 minutes |  | 0% | **Benzaldehyde Recovery: 100%. Oxazolidine recovery: 30%** [c] Mixture of starting materials and degradation. |
| **8** | THF | | *s*-BuLi (3) | -78 | 30 minutes |  | 0% | **Benzaldehyde Recovery: 100%. Oxazolidine recovery: 10%** [c] Mixture of starting materials and degradation |
| **9** | THF | | *s*-BuLi (3) | -78 | 30 minutes | $CD_3OD$ | | **Recovery: 40%.** [c] **60%** of deuterated compound **12**.(d.r. 65:35 anti:syn) |

### 2.4.7. 3-Phenylfuran-2-carbaldehyde degradation study.

At this point, we thought that the high amount of decomposition in every test, was due to the fact that actually we could metalate the carbon on the C-3 of the furan ring, but, in our conditions, it was not stable enough to proceed to any kind of reaction before degradation occurs. For this reason, we

decided to synthesize a C-3 blocked substrate (**126**) to perform the same reactions on it and see whether we had the same amount of decomposition or not.

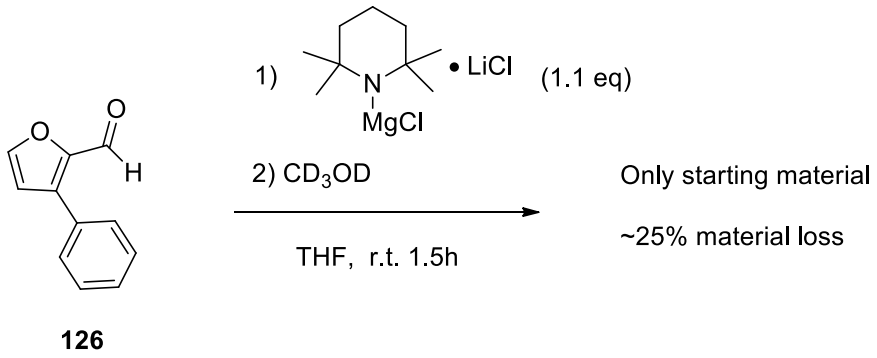



*Scheme 2.24.*

Following a previously reported procedure,[117] imine **123** was synthesized starting from furfural **81** and 4-(dimethylamino)aniline in diethyl ether in the presence of activated 4 Å molecular sieves. The substrate thus obtained was used in the following Ruthenium catalyzed C-H activation step, developed in prof. G. Poli's laboratory,[118] with boronic ester **124** to obtain the 2,3 disubstituted furan ring **125**. A final imine hydrolysis in aqueous hydrochloric acid led to the desired 3-phenylfuran-2-carbaldehyde **126**.[118,119]

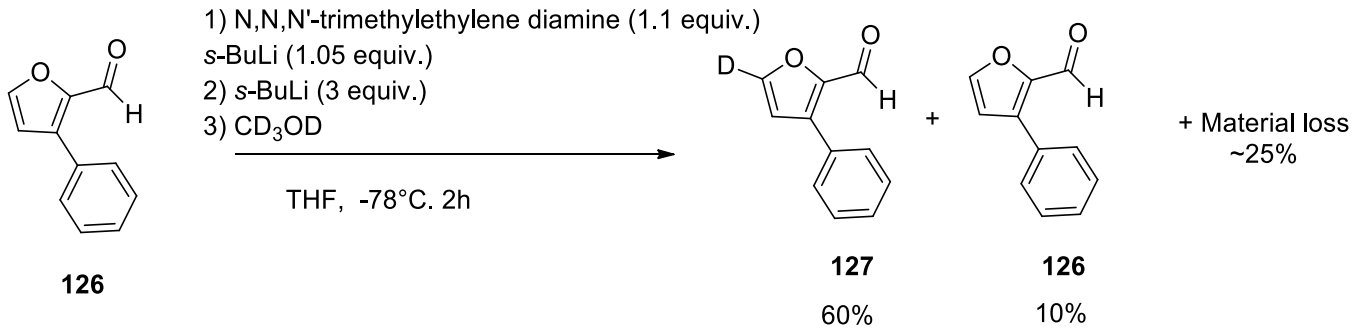
To prove the fact that degradation occurs when C-3 position undergoes deprotonation, we chose to react substrate **126** under the reaction condition that gave us a very high amount of degradation with furfural (**81**) both with TMPMgCl•LiCl (*Table 2.1, entry 11*) and *s*-BuLi (*Table 2.1, entry 15*, using 3 equivalents of organolithium instead of 2).

Reaction of **126** with the magnesium amide showed no conversion but only approximately 25% of degradation was observed, meaning that we have more than 75% recovery with respect to the 10% recovery on the same reaction performed on furfural and no reaction as well (*Scheme 2.25*).




*Scheme 2.25.*

Reaction of the same substrate with lithium amide and 3 equivalents of *s*-BuLi in THF at -78°C showed a 60% conversion to the 5-deuterated compound **127**. Also in this case, only 25% of meterial loss was observed (*Scheme 2.26*). Similar reaction with furfural with 2 equivalents of *s*-BuLi gave only 40% recovery (60% loss of material).

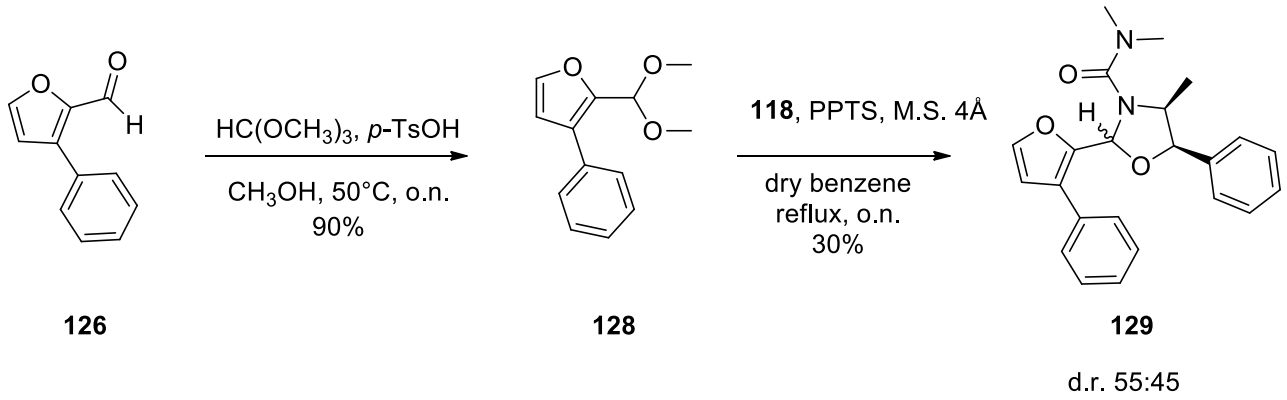



*Scheme 2.26.*

These experiments helped us to understand that most probably our hypothesis, according to which these molecules undergo degradation after the deprotonation in C-3, was reasonable.

### 2.4.8. (*4S,5R*)-*N,N*-4-Trimethyl-5-phenyl-2-(3-phenylfuran-2-yl)oxazolidine-3-carboxamide degradation study.

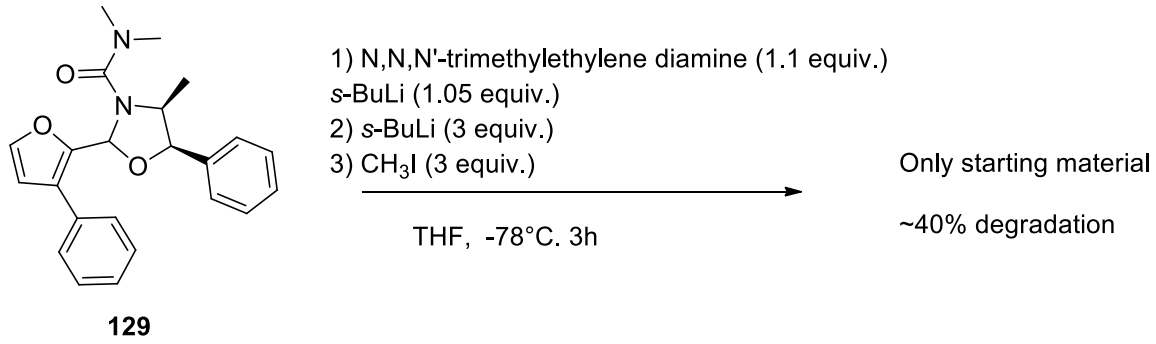
The other substrate that showed a high amount of loss due to degradation was the oxazolidine **119** (*Table 2.*5). To check if we could control the degradation of the substrate with a C-3 blocked position we decided to synthesize oxazolidine **129** starting from the aldehyde **126**.




*Scheme 2.27.*

The synthetic pattern followed to get to the target compound is the same already described in paragraph 2.4.6.

Reaction on this substrate was carried out following the conditions described in *Table 2.5, entry 3.*

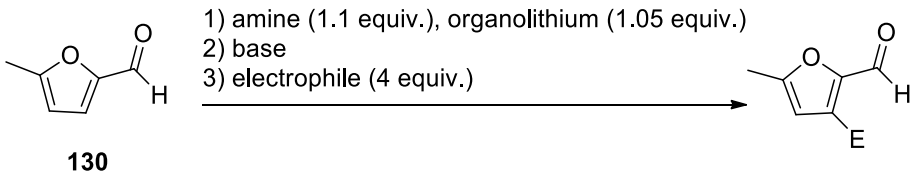



*Scheme 2.28.*

No conversion was detected and only the presence of starting material was observed by $^{1}$H-NMR analysis. In this case a recovery of 60% was detected, while the same reaction on **119** gave only 30% recovery.

### 2.4.9. 5-Methylfuran-2-carbaldehyde C-3 metalation attempts.

We also tried to explore the 5-methylfuran-2-carbaldehyde (**130**) as substrate for our reactions. This substrate is one of the best candidates for these kinds of transformation because it has both the substituted C-5 position, avoiding the competing reaction on that site, and it is more stable than the silylated derivatives.



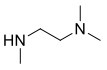
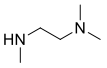
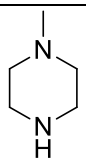
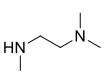
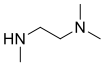

130

*Scheme 2.29.*

In fact, it showed C-3 metalation in almost every condition we tried (*table 2.6*). The only reaction in which we could not find the desired product was when it was reacted without the lithium amide (*entry 4*). However, the overall conversion, which goes from 10 to 30%, is still not satisfying, and the small quantity of compound recovered due to the problem of degradation suggest that these are not yet the right reaction conditions.

In this case, both the use of a different amine (*entry 3*) and the use of *t*-BuLi (*entry 6*) were studied, but they did not improve the outcome of the reaction.

*Table 2.6. Reaction conditions for 5-methylfuran-2-carbaldehyde (**130**) C-3 metalation attempts. [a] Base equivalents are referred to the deprotonation step only. [b] Ratio between products formed and amount of starting material left. [c] Recovery calculated referring to butadiene sulfone as internal standard.*

| Entry | Solvent | Amine | Base (equiv.) [a] | T for deprotonation step (°C) | Time for deproton ation step | Electro phile | Product vs starting material ratio by crude NMR analysis [b] | Crude NMR results |
|---|---|---|---|---|---|---|---|---|
| **1** | $Et_2O$ |  | *s*-BuLi (1.2) | -78 | 1 hour | $CD_3OD$ | | **Recovery: 30%.** [c] **25%** of C-3 deuterated product. |
| **2** | $Et_2O$ |  | *s*-BuLi (1.2) | -90 | 1 hour | $CD_3OD$ | | **Recovery: 30%.** [c] **20%** of C-3 deuterated product |
| **3** | $Et_2O$ |  | *s*-BuLi (1.2) | -78 | 1 hour | $CD_3OD$ | | **Recovery: 35%.** [c] **5%** of C-3 deuterated product. |
| **4** | $Et_2O$ | | *s*-BuLi (1) | -78. | 1 hour | $CD_3OD$ | | **Recovery: 10%.** [c] No reaction, only starting material. |
| **5** | $Et_2O$ |  | *s*-BuLi (1.2) | -78. | 1 hour |  | 40% | **Recovery: 30%.** [c] Mixture of starting material and 3-substituted product.. |
| **6** | $Et_2O$ |  | *t*-BuLi (1) | -78 | 1 hour | $CD_3OD$ | | **Recovery: 60%.** [c] **10%** of C-3 deuterated product. |

### 2.4.10. IR *in situ* monitoring

To truly understand what is happening in the reaction mixture and to understand which is the step that leads to the degradation of the substrate, we decided to carry out reactions monitored *in situ* by infrared technique. This method has also been chosen because, as previously mentioned, we have never been able to recognize, from the analysis of the NMR of the crude material, any peak attributable to any degradation product.

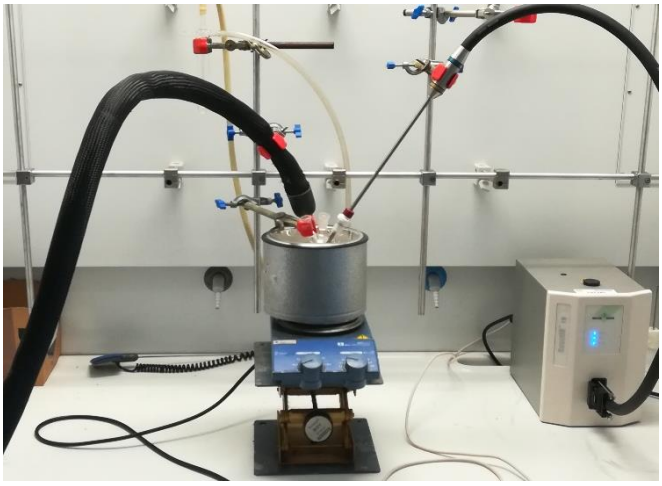
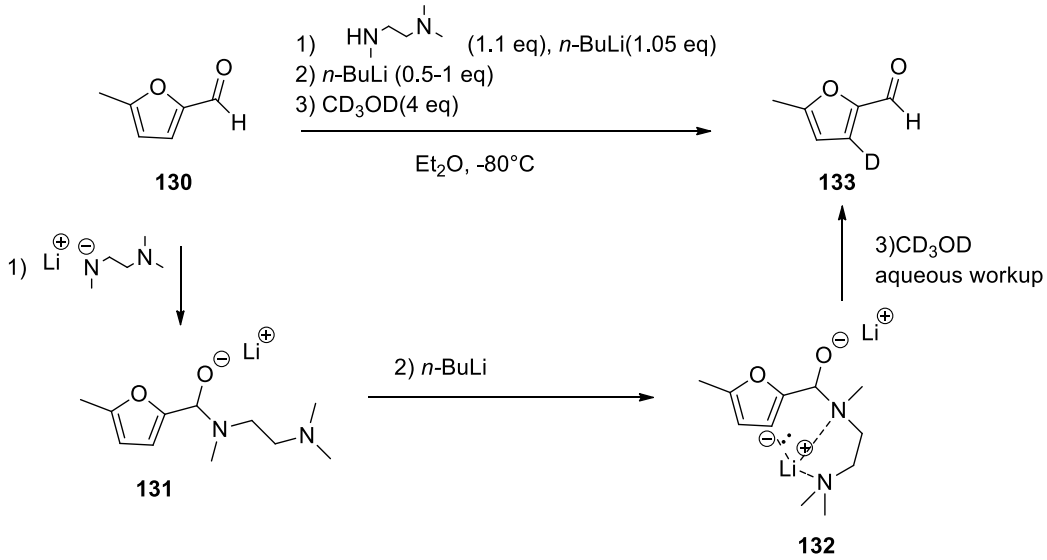


*Figure 2.6. IR apparatus for in situ monitoring.*

The chosen reaction conditions were the one that gave us the best results, by using diethyl ether as solvent, 5-methylfuran-2-carbaldehyde (**130**) as substrate, *N,N,N′*-trimethylethylenediamine and *n*-Buli as organolithium reagent.




*Scheme 2.30.*

We started acquiring spectra for all the species participating in the reaction: the background, the diethyl ether and the 5-methylfuran-2-carbaldehyde. We also acquired spectra for the amine and for the lithium amide that is formed after it reacts with 1.05 eq of *n*-BuLi.

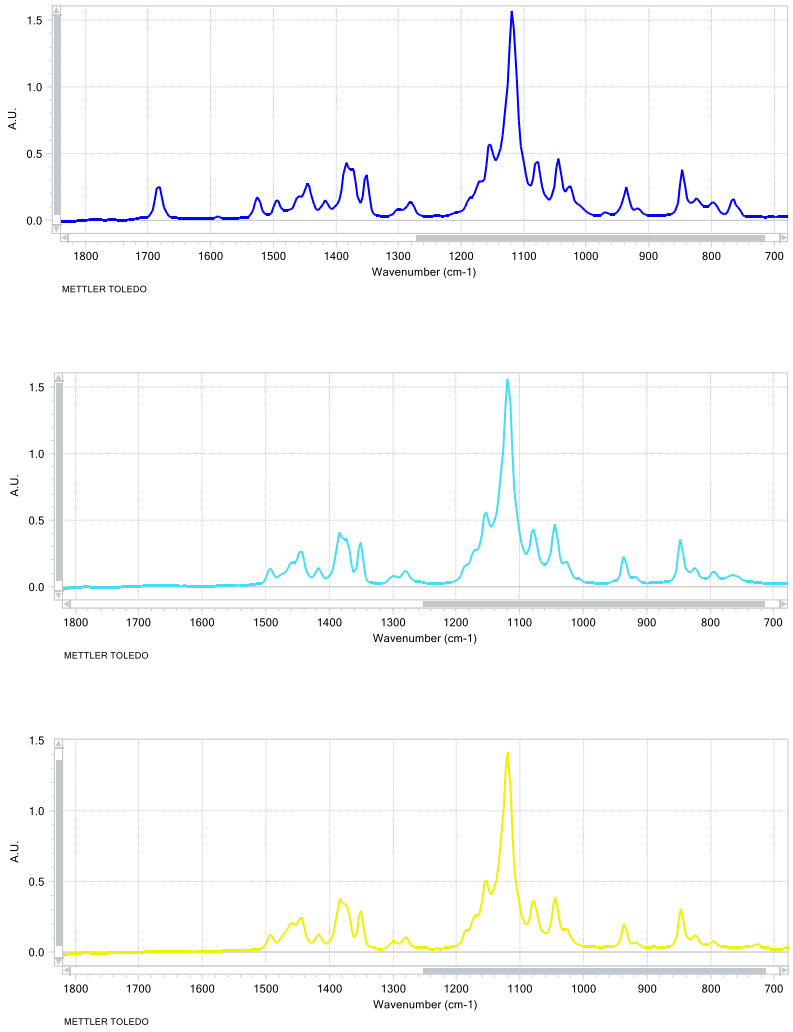



*Figure 2.7. IR of 5-methyl furfural (top), N,N,N'-trimethylethylenediamine (centre) and lithium (2-(dimethylamino)ethyl)(methyl)amide (bottom).*

We discovered that after the lithium amide reacts with **130**, we have a very fast reaction leading to the formation of the first intermediate (**131**), proved by the almost immediate disappearance of the signals referred to the carbonyl group of the aldehyde (1670-1700 $cm^{-1}$) as shown in *Figure 2.8*.

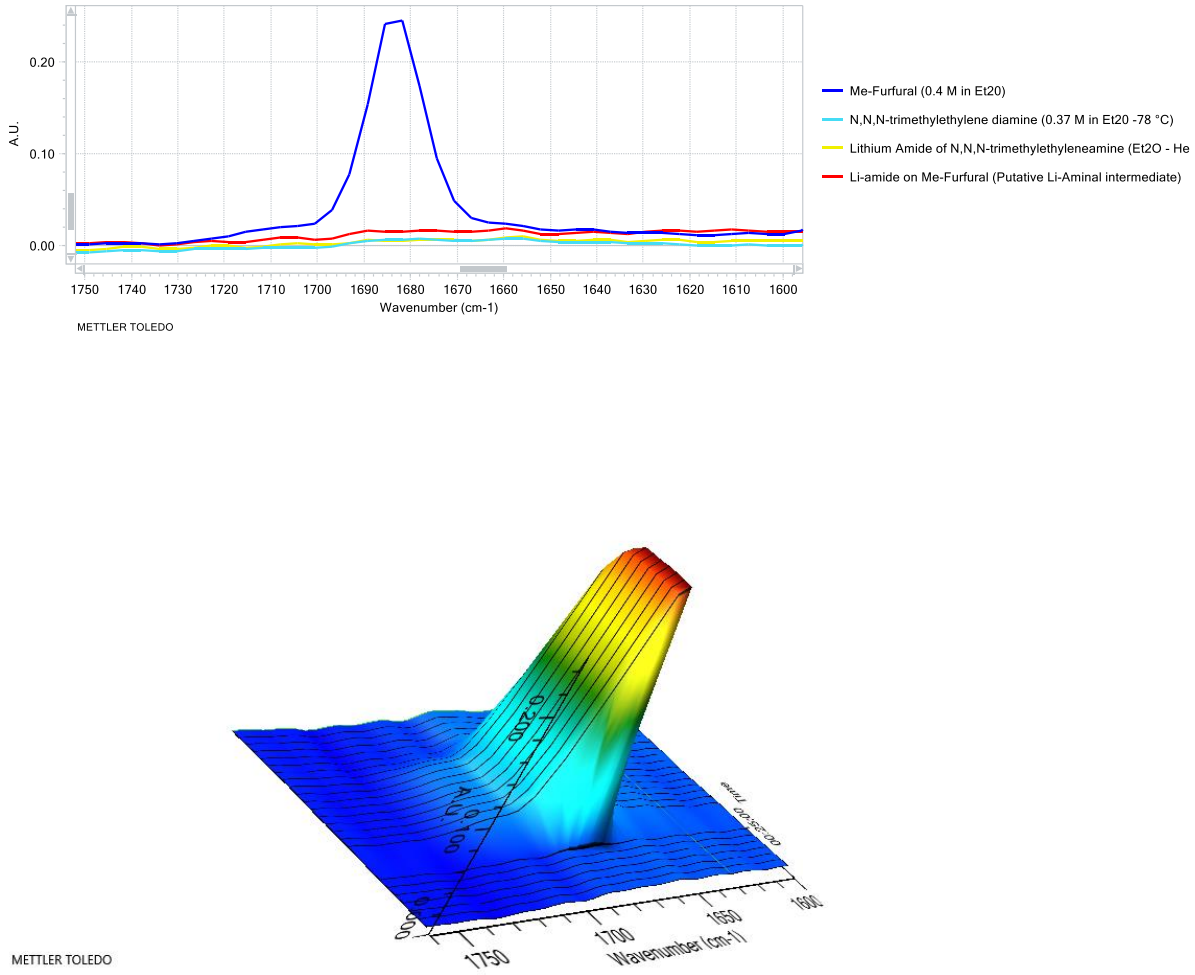
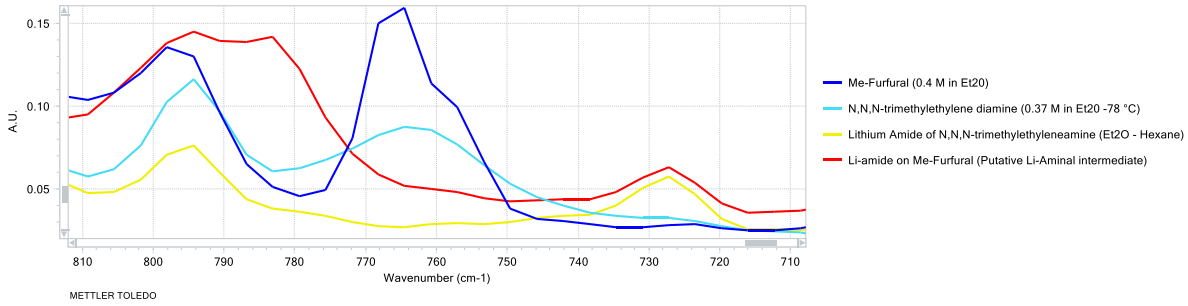



*Figure 2.8. IR spectrum and profile of carbonyl of the aldehyde signal disappearing after the addition of lithium amide.*

The formation of **131** is also proved by the change in shift and intensity of some of the peaks referred to the lithium amide in the region between 770 and 820 $cm^{-1}$(*Figure 2.9*).



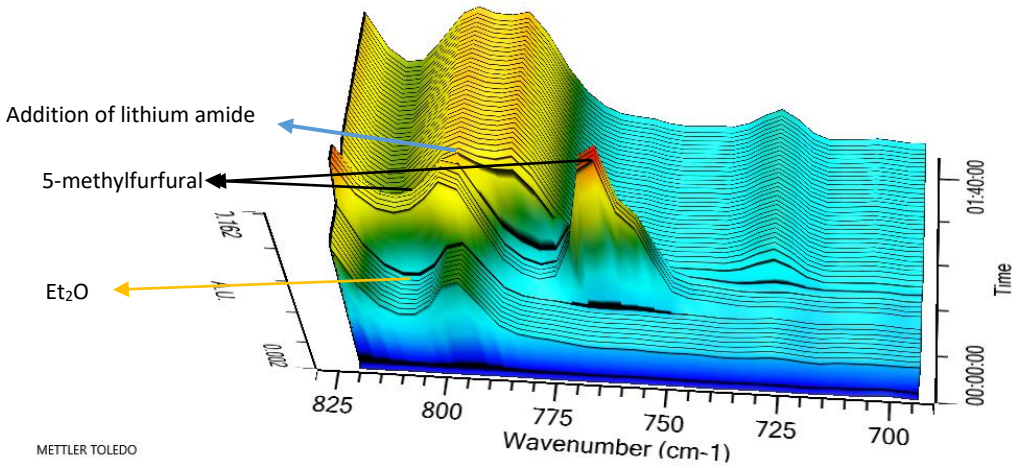



*Figure 2.9. Zoom of the IR spectrum and profile of the formation of intermediate **131**.*

For what concerns the second and the third steps, after addition of *n*-BuLi, we could observe changes in shift and shape for some of the peaks in the region between 770 and 820 $cm^{-1}$, suggesting that we have a deprotonation reaction. However, after the addition of $CD_3OD$ we could never observe the increasing of a specific peak that could be referred to the formation of the product. What is interesting is that when we used 0.5 equivalents of organolithium, no product was detected but we had a 100% recovery of our starting material (by crude $^1$H-NMR analysis), while when we used 1 equivalent of *s*-BuLi, only 60% of **130** was recovered, still without any conversion to the deuterated product **133.**

We thought that it could have been a problem due to the very low temperature of the reaction that did not allow the deprotonation of the substrate. Thus, we carried out the same reaction, with 1 equivalent of *s*-BuLi at -20°C, but also in this case no product was detected, and the recovery was the 65% of the total amount.

To be sure that the degradation occurs after the deprotonation step, we also carried out a third experiment in which the reaction was quenched right after the first step (after the addition of lithium amide) and, after the usual work-up, we could recover more than 95% of the substrate, confirming that it is the reaction of R-Li with intermediate **131** which leads to the decomposition of the substrate.

However, by carefully studying the IR spectra of both the reactions with 0.5 equivalents of *n*-BuLi and the one with 1 equivalent of organolithium, in which we had 100% and 60% recovery respectively, we could not find any differences, neither in the shape nor in the intensity of the peaks. This means that what we are losing is not detected by NMR or IR spectroscopy, so that is not due polymerization or intermolecular nucleophilic addition side reactions, suggesting finally that they could be volatile products.

Our hypothesis is that degradation occurs upon formation of the C-3 anion. Unfortunately, no information is available in literature regarding chemical degradation of furfural. On the other hand, very detailed information can be found on degradation of the furan ring. It is reported that furan ring decomposition leads to the formation of volatile compounds (carbon monoxide and methylacetylene)[120,121] as shown in *Figure 2.10*. It must be noted that this pathway is referred to pyrolysis of the furan ring. However, as shown in the figure, we can observe that the highest energy demanding step is the formation of the β-carbene which requires reasonably high temperature to be generated. In our case, it is more easily generated by our reaction conditions, suggesting that the furfural moiety probably follows a similar degradation pattern.

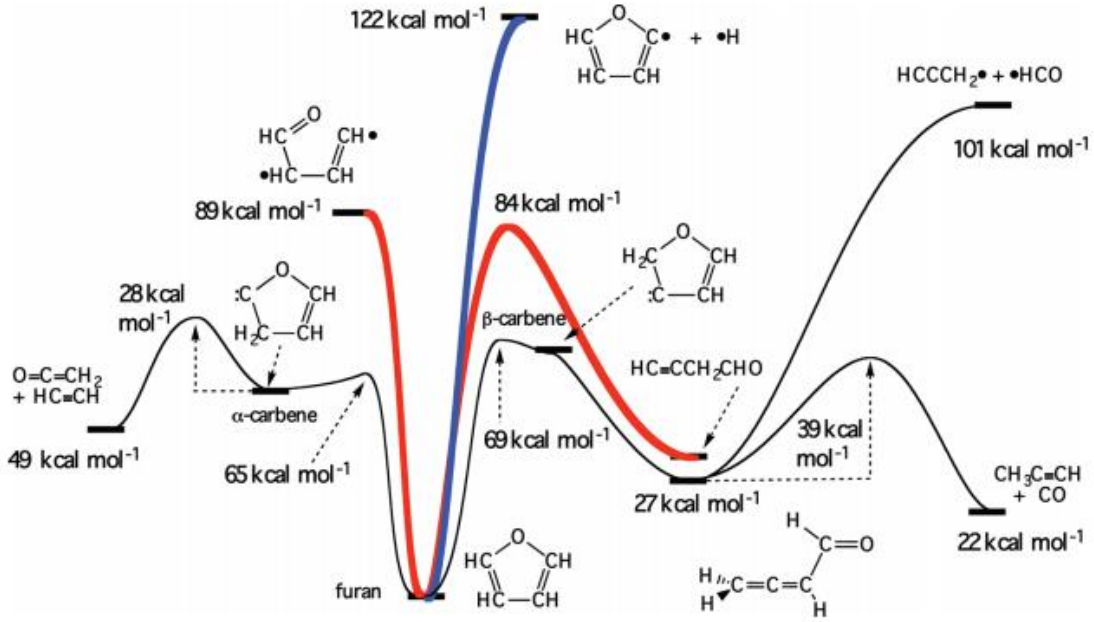



*Figure 2.10. Potential energy surface for the decomposition of furan at 0 K.*[119]

In fact, after addition of the organolithium to deprotonate the C-3 of the furan ring, our substrates can follow three main patterns shown in *Figure 2.11*

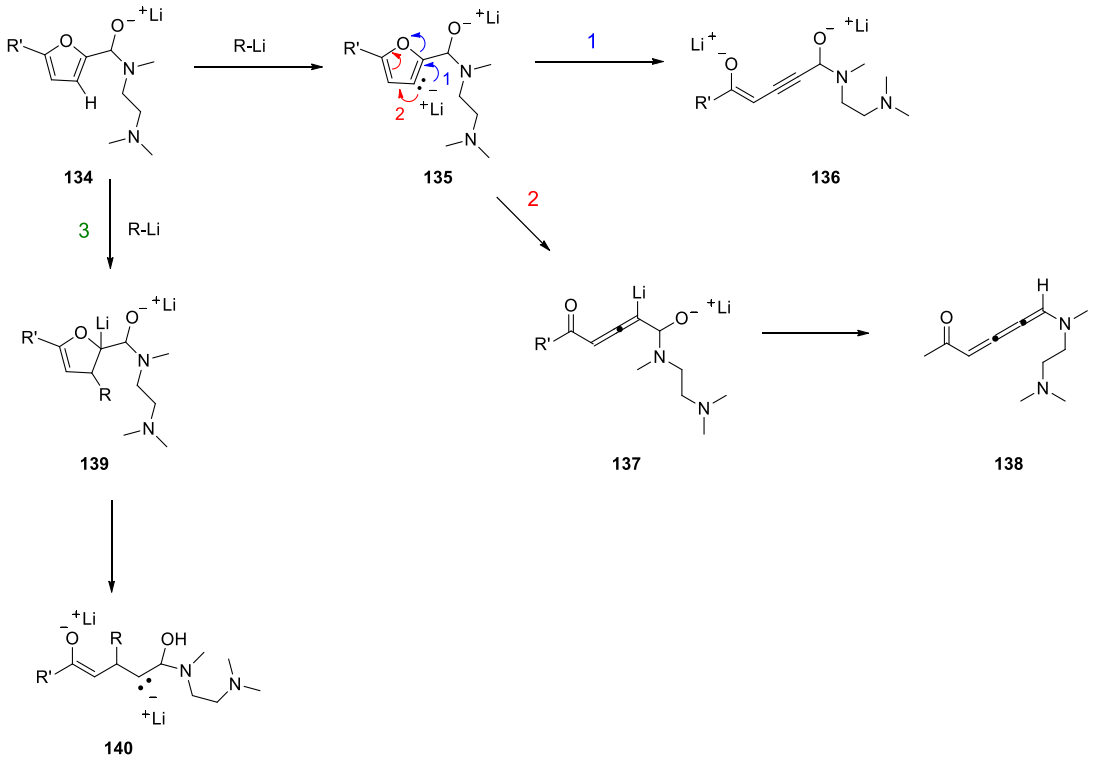
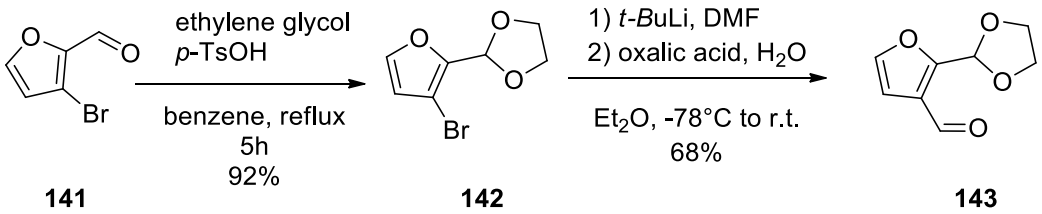



*Figure 2.11. Degradation pattern hypothesis for furfural derivatives under our conditions.*

The first two, leading to compounds **136** and **138**, are a consequence of the deprotonation of the C-3 carbon of the furan ring, followed by ring opening, while the third follows an addition-dearomatization pathway to compound **140**. However, we have never been able to detect any of those structure and we are not sure about the fate of those compounds. According to the scheme in *Figure 2.10* they could degrade further to obtain gaseous compounds; or after aqueous workup, they can form sufficiently polar species that remain in the aqueous phase after extraction with organic solvents. What can be very useful to understand more about the stability of the carbene and to achieve C-3 functionalization of furfural derivatives is the use of 3-bromo-2-furaldehyde derivatives. It has been reported[122] that functionalization of 2-furaldehyde bearing a bromine atom on carbon 3, leads to product **143** in 68% yield (*Scheme 2.31*) after treating it with *t*-BuLi, suggesting that the bromine atom in that position could be crucial for the improvement in stability of the carbene.



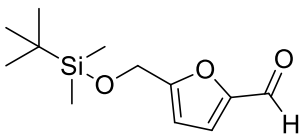

*Scheme 2.31.*

## 2.5. Experimental protocols

*General procedure for C-3 metalation of furfural and its derivatives.*

All the C-3 metalation attempts reported in *Tables 2.1-2..6* have been performed following the general procedure: in a 3-neck flask equipped with a thermometer and purged with Argon, was added solvent (THF or DME or $Et_2O$, 0.3M) distilled amine (1.1 equiv.) and the mixture was cooled to -20°C. Organolithium (1.05 equiv.) was then added dropwise to the reaction mixture. After stirring at the same temperature for 15 minutes, furfural derivative (1 equiv.) was added and after further 15 minutes, base (organolithium or TMPMgCl•LiCl, 1-3 equiv) was introduced dropwise. When it is reported that the temperature for the deprotonation step is lower than -20°C, the mixture was cooled before the addition of the base. The reaction was stirred for the indicated time (5 min – 24 hours) and then the electrophile was added. The mixture was then left stirring for the indicated times and quenched by addition of cold water. After extraction in $Et_2O$, drying over $MgSO_4$, and filtration, the solvent was removed under reduced pressure and the crude product was analysed by $^1$H-NMR. When referred to an internal standard, 1 equiv. of butadiene sulfone was added during the workup step.

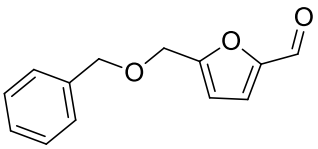
*5-(((tert-Butyldimethylsilyl)oxy)methyl)furan-2-carbaldehyde* (**112**)




**112**

Following a reported procedure,[112] in a round-bottomed flask equipped with a magnetic stirred, imidazole (1.1 equiv.) was added to a solution of HMF (**82**, 1 equiv.) in DCM (0.5M) and allowed to stir at room temperature for 15 minutes. TBDMSCl (1.1 equiv.) was then added. The flask was covered with aluminium foil to exclude light and the reaction mixture was let stir overnight at room temperature. The completion of the reaction was checked by TLC (cyclohexane: EtOAc, 6:4 as eluent). The mixture was diluted with water and extracted in DCM. The collected organic phases were dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure to give **112** as a yellow oil (90%). The spectral properties are in good agreement with those previously reported.[114] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 0.10 (s, 6H), 0.91 (s, 9H), 4.73 (s, 2H), 6.45 (d, J = 3.5 Hz, 1H), 7.19 (d, J = 3.5 Hz, 1H), 9.58 (s, 1H).

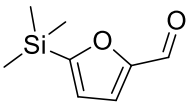
*5-((Benzyloxy)methyl)furan-2-carbaldehyde* (**113**)



**113**

Following a reported procedure,[115] a round bottomed flask equipped with a magnetic stirred was charged with HMF (1 equiv.). DMF (1M), benzyl bromide (1.5 equiv.) and silver oxide (1 equiv.) were added. The flask was covered with aluminium foil to exclude light and the reaction mixture was stirred overnight at room temperature. The completion of the reaction was checked by TLC (cyclohexane: EtOAc 6:4 as eluent). The mixture was diluted with ethyl acetate, filtered through Celite and the solvent removed under reduced pressure. The residue was diluted with water and extracted in $Et_2O$. The collected organic phases were dried over $MgSO_4$, filtered and the solvent removed under vacuum. The crude product was purified by silica gel chromatography (cyclohexane: ethylacetate 7:3 as eluent) to give **113** (65%). Spectral data are in good agreement with those previously reported.[115] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 4.57 (s, 2H), 4.61 (s, 2H), 6.53 (d, J = 3.5 Hz, 1H), 7.31 (d, J = 3.5 Hz, 1H), 7.36-7.30 (m, 5H), 9.62 (s, 1H).

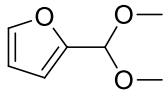
*5-(Trimethylsilyl)furan-2-carbaldehyde* (**114**)



**114**

Following a previously reported procedure,[116] a 3-neck flask equipped with thermometer and purged with Argon, was charged with THF (0.4M) and N-methylpiperazine (1.15 equiv.). The reaction mixture was cooled to -78°C and *n*-BuLi (1.15 equiv.) was added dropwise. After stirring 15 minutes at the same temperature, furfural (1 equiv.) was added and the reaction was let stirring for further 15 minutes. *n*-BuLi (1.15 equiv.) was then added dropwise and the reaction was stirred for 5 hours at -20°C. Finally, a 1.5M solution of $(CH_3)_3SiCl$ (1.15 equiv.) in THF was added dropwise and the mixture was allowed to warm to room temperature and stirred overnight. The completion of the reaction was checked by TLC (cyclohexane:EtOAc, 98:2 as eluent). After adding HCl (1N) at 0°C, the reaction was stirred for 10 minutes. It was then neutralized with sat. $NaHCO_3$ and extracted in $Et_2O$, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (cyclohexane:EtOAc, 98:2 as eluent) to give 65% of **114**. Spectral data are in good agreement with those previously reported.[116] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 0.28 (s, 9H), 6.72 (dd, J = 3.5 Hz, 1H), 7.15 (d, J = 3.5 Hz, 1H), 9.63 (s, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 1.2, 120.9, 121.4, 156.2, 167.1, 178.2.

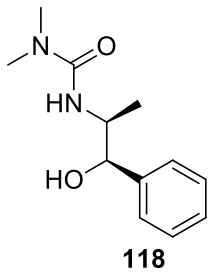
*2-(Dimethoxymethyl)furan* (**115**)



**115**

To a solution of methanol (0.5M) and furfural (1 equiv.) was added trimethyl orthoformate (4 equiv.) and *p*-toluensulfonic acid (0.011 equiv.). The mixture was stirred at 50°C overnight. After the starting material was totally consumed (by TLC monitoring, using cyclohexane:EtOAc 9:1 as eluent) the reaction was quenched with sat. $NaHCO_3$, extracted in $Et_2O$ and the organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (cyclohexane:EtOAc 85:15 as eluent) to obtain **115** in 88% yield. Spectral data are in good agreement with those previously reported.[123] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = d = 3.26 (s, 6H), 5.38 (s, 1H), 6.32 (m, 1H), 6.35 (m, 1H), 7.37 (m, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 53.1, 98.6, 108.7, 110.5, 143.0, 151.5.

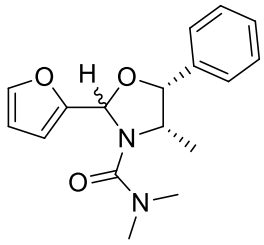
*3-((1R,2S)-1-Hydroxy-1-phenylpropan-2-yl)-1,1-dimethylurea* (**118**)



**118**

A round-bottomed flask purged with Argon was charged with norephedrine (**116**, 1 equiv.) in DCM (0.3M). Dimethylcarbamoyl chloride (**117**, 1.1equiv.) was added to the solution. After, freshly distilled $Et_3N$ (1.1 equiv.) was added dropwise and the reaction mixture was stirred 3 hours at room temperature. The reaction was monitored by TLC (DCM:acetone, 8:2 as eluent) and quenched by addition of a saturated solution of $NaHCO_3$. After the extraction in DCM, the organic phases were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (DCM: acetone, 8:2 as eluent) to give **118** with 70% yield. *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 0.96 (d, J = 7.0 Hz, 1H), 2.85 (s, 6H), 4.18 (m, 1H), 4.55 (d, J = 7.8 Hz, 1H), 4.78 (bs, 1H), 4.95 (bs, 1H), 7.21-7.31 (m, 5H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 15.55, 36.26, 52.01, 77.15, 126.59, 127.19, 127.91, 141.20, 159.04.

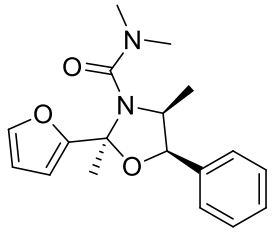
*(4S,5R)-2-(Furan-2-yl)-N,N-4-trimethyl-5-phenyloxazolidine-3-carboxamide* (**119**)



**119**

A round-bottomed flask which is part of an apparatus like the one in *Figure 2.5*, was charged with a 0.15M solution of dry benzene and 2-(dimethoxymethyl)furan (**115**). After addition of 3-((*1R,2S*)-1-hydroxy-1-phenylpropan-2-yl)-1,1-dimethylurea (**118**) and pyridinium p-toluenesulfonate (0.2 equiv.), the reaction was refluxed overnight. After the completion of the reaction, checked by TLC (cyclohexane:EtOAc, 7:3 as eluent), the mixture was quenched with a saturated solution of $NaHCO_3$. It was then extracted in $Et_2O$, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was purified by silica gel chromatography (cyclohexane: EtOAc, 7:3 as eluent) to give **119** with 70% yield, as a mixture of the two diasteromers (60:40). *$^1$H NMR:* (400 MHz, $CDCl_3$, major) $\delta_H$ = 0.79 (d, J = 6.5 Hz, 3H), 2.90 (s, 6H), 4.13 (q, J = 7.2 Hz, 1H), 5.56 (d, J = 5.6 Hz, 1H), 6.33 (dd, J = 1.8, 3.3, Hz, 1H), 6.50 (d, *J* = 3.2 Hz, 1H), 6.60 (s, 1H), 7.25-7.35 (m, 5H), 7.37 (dd, J = 0.8, 1.8 Hz, 1H). *$^1$H NMR:* (400 MHz, $CDCl_3$, minor) $\delta_H$ = 1.06 (d, J = 6.8 Hz, 3H), 2.91, (s, 6H), 4.37-4.46 (m, 1H), 5.06 (d, J = 4.9 Hz, 1H), 6.37 (dd, J ) 1.8, 3.2 Hz, 1H), 6.47 (d, J = 3.1 Hz, 1H), 6.68 (s, 1H), 7.22-7.31(m, 5H), 7.42 (dd, J = 0.9, 1.8 Hz, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$, major and minor) $\delta_C$ = 13.92, 15.47, 37.48, 38.30, 56.78, 59.21, 80.91, 81.83, 83.42, 84.38, 108.19, 108.52, 110.02, 110.10, 126.17, 126.19, 127.54, 127.70, 128.14, 128.17, 136.51, 136.58, 142.65, 142.70, 152.86, 153.0, 159.65, 162.93.

*(2S,4S,5R)-2-(Furan-2-yl)-N,N-2,4-tetramethyl-5-phenyloxazolidine-3-carboxamide* (**120**)

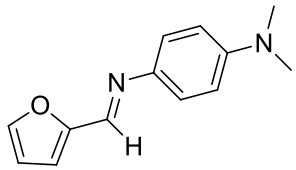


**120**

In a 3-neck flask equipped with a thermometer and purged with Argon, was added THF (0.3M), *N,N,N'*-trimethylethylene diamine (1.1 equiv.) and the mixture was cooled to -20°C. *s*-BuLi (1.05 equiv.) was then added dropwise to the reaction mixture. After stirring at the same temperature for 15 minutes, **119** (1 equiv.) was added and after further 15 minutes, the reactios mixture was cooled to -78°C and *s*-BuLi (3 equiv.) was introduced dropwise. The reaction was stirred for 1 hour and then $CH_3I$ (3 equiv.) was added. The mixture was then left stirring for 2 hours and quenched by addition of cold water. After extraction in $Et_2O$, drying over $MgSO_4$, and filtration, the solvent was removed under reduced pressure and the crude product was purified by

silica gel chromatography (cyclohexane: EtOAc, 7:3 as eluent) to give **120** in 30% yield. *$^{1}$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 0.85 (d, J = 6.6 Hz, 1H), 2.23 (s, 3H), 2.83 (s, 6H), 4.45 (m, 1H), 5.48 (d, J = 5.4 Hz, 1H), 6.29 (dd, J = 3.3, 1.8 Hz, 1H), 6.36 (dd, J = 3.3, 0.8 Hz, 1H), 7.26-7.39 (m, 6H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 16.06, 21.01, 38.9, 60.3, 79.1, 91.07, 107.08, 110.07, 126.2, 127.6, 128.2, 136.7, 141.9, 155.4, 160.5.

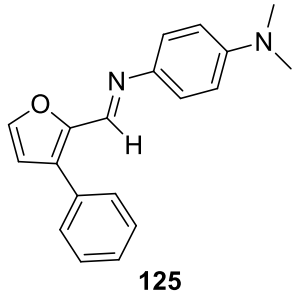
*(E)-4-((Furan-2-ylmethylene)amino)-N,N-dimethylaniline* (**123**)



**123**

Following a reported procedure,[118] furan-2-carbaldehyde (**81**, 1 equiv.) was added to a solution of 4-(dimethylamino)aniline **122** (1.1 equiv.) in anhydrous $Et_2O$ (0.2 M) and molecular sieves (4 Å 1.6 mm pellets). The flask was covered with aluminium foil to exclude light and the reaction mixture was stirred overnight at room temperature. The reaction completion was followed by $^{1}$H-NMR spectroscopy; if the reaction was not complete, one of the reagents was added, and the reaction mixture was let stir until completion. The mixture was then filtered over celite and washed with $Et_2O$. The solvent was evaporated under reduced pressure to give the corresponding imine **123** as a yellow solid (75% yield). Spectral data are in good agreement with those previously reported.[118] *$^{1}$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 2.98 (s, 6H), 6.53 (dd, *J* = 3.4, 1.8 Hz, 1H), 6.76-6.73 (m, 2H), 6.85 (d, *J* = 2.9 Hz, 1H), 7.30-7.27 (m, 2H), 7.57 (d, *J* = 1.7 Hz, 1H), 8.34 (s, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 40.8, 112.1, 112.9, 114.6, 122.5, 140.3, 143.5, 144.9, 149.8, 152.9.

*(E)-N,N-Dimethyl-4-(((3-phenylfuran-2-yl)methylene)amino)aniline* (**125**)

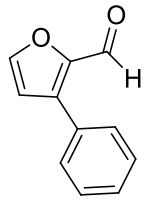


**125**

In a sealed tube equipped with a stir bar and purged under Argon atmosphere, a solution of $Ru_3(CO)_{12}$ (5 mol%), (*E*)-4-((furan-2-ylmethylene)amino)-N,N-dimethylaniline (**123**, 1.0 equiv.), 5,5-dimethyl-2-phenyl-1,3,2-dioxaborinane (**124**, 1.5 equiv.), benzylideneacetone (1.1 equiv.) and molecular sieves 4Å in 1,4-dioxane (0.5 M) was heated at 130 °C for 15 h. The reaction mixture was let cool to room temperature, diluted with $CH_2Cl_2$ and filtered through Celite. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (cyclohexane:EtOAc, 8.2 as eluent) affording **125** in 60% yield as a brown solid. Spectral data are in good agreement with those

previously reported.[118] *$^{1}$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 3.00 (s, 6H), 6.70 (d, *J* = 1.8 Hz, 1H), 6.75 (d, *J* = 9.0 Hz, 2H), 7.32-7.28 (m, 2H), 7.51-7.43 (m, 5H), 7.63 (dd, *J* = 1.8, 0.5 Hz, 1H), 8.47 (s, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 40.8, 112.9, 113.2, 122.6, 128.1, 128.9, 129.0, 131.9, 132.6, 140.7, 142.8, 144.7, 147.7, 149.8,

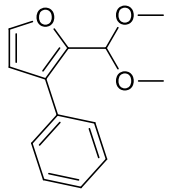
*3-Phenylfuran-2-carbaldehyde* (**126**)



**126**

Following a reported procedure,[118,119] to a solution of **125** (1 equiv.) in diethyl ether (0.02 M) was added dropwise HCl (aq, 1 N, 2.5 mL every 0.05 mmol). The mixture was stirred overnight at room temperature and diluted with diethyl ether. The aqueous layer was extracted with diethyl ether and the organic layers were dried over $MgSO_4$, filtered and concentrated affording the desired product **126** in 90% yield. *$^{1}$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 6.74 (d, *J* = 1.7 Hz, 1H), 7.58-7.45 (m, 5H), 7.70 (dd, *J* = 1.7, 0.8 Hz, 1H), 9.75 (s, 1H).

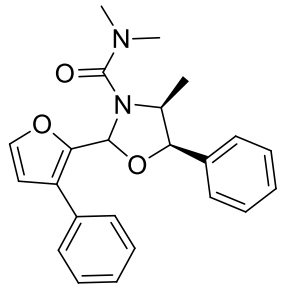
*2-(Dimethoxymethyl)-3-phenylfuran (***128***)*



**128**

To a solution of methanol (0.5M) and 3-Phenylfuran-2-carbaldehyde **126** (1 eq) was added trimethyl orthoformate (4 eq) and *p*-toluensulfonic acid (0.011 eq). The mixture was stirred at 50°C overnight. After the starting material was totally consumed (by TLC monitoring, using cyclohexane:EtOAc 9:1 as eluent) the reaction was quenched with sat. $NaHCO_3$, extracted in $Et_2O$ and the organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (cyclohexane:EtOAc 85:15 as eluent) to obtain **128** in 90% yield. *$^{1}$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 3.44 (s, 6H), 5.49 (s, 1H), 6.60 (d, J = 1.9 Hz, 1H), 7.34-7.52 (m, 6H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 53.5, 97.04, 111.4, 124.9, 127.2, 128.3, 128.6, 132.5, 142.2, 145.8.

*(4S,5R)-N,N,4-Trimethyl-5-phenyl-2-(3-phenylfuran-2-yl)oxazolidine-3-carboxamide* (**129**)



**129**

A round-bottomed flask which is part of an apparatus like the one in *Figure 2.5*, was charged with a 0.15M solution of dry benzene and 2-(dimethoxymethyl)-3-phenylfuran (**128**). After addition of 3-((*1R,2S*)-1-hydroxy-1-phenylpropan-2-yl)-1,1-dimethylurea (**118**) and pyridinium p-toluenesulfonate (0.2 equiv.), the reaction was refluxed overnight. After the completion of the reaction, checked by TLC (cyclohexane:EtOAc, 7:3 as eluent), the mixture was quenched with a saturated solution of $NaHCO_3$. It was then extracted in $Et_2O$, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was purified by silica gel chromatography (cyclohexane: EtOAc, 7:3 as eluent) to give **129** (30% yield) as a mixture of the two diasteromers (55:45). . *$^1$H NMR:* (400 MHz, $CDCl_3$, major) $\delta_H$ = 0.80 (d, J = 6.5 Hz, 3H), 2.81 (s, 6H), 4.49-4.61 (m, 1H), 5.74 (d, J = 5.7 Hz, 1H), 6.53 (d, *J* = 1.8 Hz, 1H), 6.67 (s, 1H), 7.31- 7.40 (m, 8H), 7.44 (d, J = 1.7 Hz, 1H), 7.62-7.68 (m, 2H). *$^1$H NMR:* (400 MHz, $CDCl_3$, minor) $\delta_H$ = 1.17 (d, J = 6.7 Hz, 3H), 2.83 (s, 6H), 4.30 (dd, J = 5.0, 6.7 Hz, 1H), 5.09 (d, J = 4.9 Hz, 1H), 6.59 (d, J = 1.8 Hz, 1H), 6.70 (s, 1H), 7.31- 7.40 (m, 8H), 7.50 (d, J = 1.8 Hz, 1H), 7.62-7.68 (m, 2H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$, major and minor) $\delta_C$ = 14.34, 15.32, 37.66, 38.39, 56.73, 59.04, 81.27, 82.21, 95.92, 98.0, 108.43, 110.05, 125.81, 126.31, 126.35, 127.25, 127.47, 127.74, 128.17, 128.22, 128.43, 128.50, 128.64, 128.68, 136.50, 136.91, 142.13, 142.47, 146.38, 147.95, 148.05, 150.87, 159.83, 162.99.

## 2.6. Conclusions

Given the importance of furfural and its derivatives, such as HMF and 5-methyl furfural, as important renewable, non-petroleum based, chemical feedstock with wide applications in many industries, a study was conducted in the laboratory of prof. Poli at the University Pierre et Marie Curie in Paris on the possibility of selectively functionalizing the position C-3 of the furan ring by exploring the possibility of a C-H activation with direct *ortho*-metalation using lithium and magnesium amides.

In the case of furfural, selective functionalization at this position was found to be almost completely absent in any test conducted because of competition with the C-5 position, bearing a more acidic hydrogen. The best result was obtained with 5-methyl furfural with 25% of the C-3 substituted product. Tests in which magnesium amide (TMPMgCl•LiCl) has been used showed no conversion, and only starting material could be recovered at the end of the reaction. Only in a few cases have we noticed a correlation between the outcome of the reaction and the use of different organolithium (*n*-, *s*- or *tert*-BuLi).

Substrates such as oxazolidines derived from the furfural have also been explored, which have led to umpolung, providing to the carbonyl carbon in the furfural a nucleophilic character in the oxazolidine, making it capable of reacting with electrophiles such as $CH_3I$ to obtain the product **120**.

The drawback we experience in each of the tests performed was the constant degradation of the substrates, for this reason, a study was conducted to understand more about the fate of the various molecules in our reaction conditions, and a degradation pattern was hypothesized. Unfortunately, however, more in-depth studies must be conducted to provide certain experimental evidence regarding this hypothesis.

Work is still in progress in Poli's research group in order to refine the the best conditions to obtain a C-3 metalation of the furfural and its derivatives, avoiding the degradation pattern of the substrates.

# Chapter 3: Elucidation of the cyclization mechanism to 5-acylamino-1,3-thiazoles from acyclic precursors

## 3.1. Importance of Thiazoles

Thiazoles represent one of the most important entities in the family of heterocyclic compounds exhibiting remarkable pharmacological activities. The knowledge of various synthetic pathways and different physicochemical parameters of such compounds have drawn the attention of medicinal chemists over the years to produce combinatorial library and carry out exhaustive efforts in the search of lead molecules. Thiazole was first described by Hantzsch in 1887 and its structure was confirmed by Popp in 1889. In pharmaceutical chemistry, substituted thiazoles compounds offer a number of advantages such as relative stability, enhanced lipid solubility with hydrophilicity and easy metabolism of compounds.[124] Thiazole core is found in many natural compounds like vitamin B1 (Thiamin) **144** or thiamine pyrophosphate (TPP, an important cofactor in all living systems) as well as in many potent biologically active compounds, such as a the antimicrobial agent, Sulfathiazole **145**, antifungal Ravuconazole **146**, antidepressant Pramipexole **147**, antineoplastic agent Tiazofurin **148**, anti-HIV drug Ritonavir **149** and the anti-inflammatory drug Meloxicam **150** (*Figure 3.1*).[125-127] Thiazole ring also finds applications in other fields, such as polymers,[128] liquid crystals,[129] fluorescent dyes,[130] and insecticides.[131]

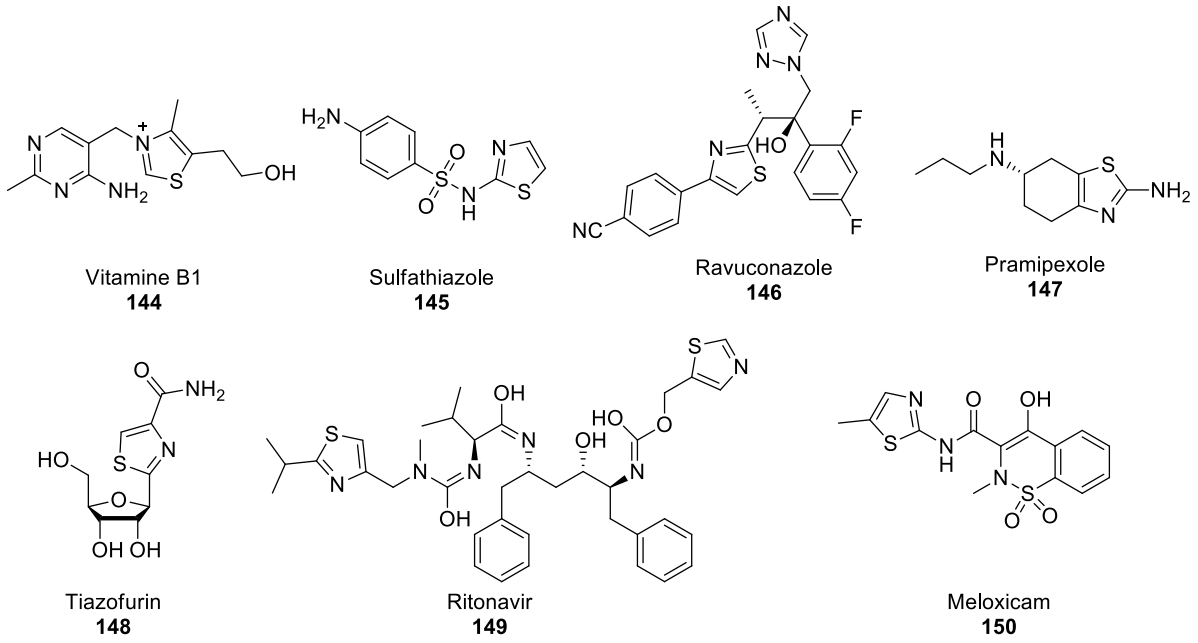
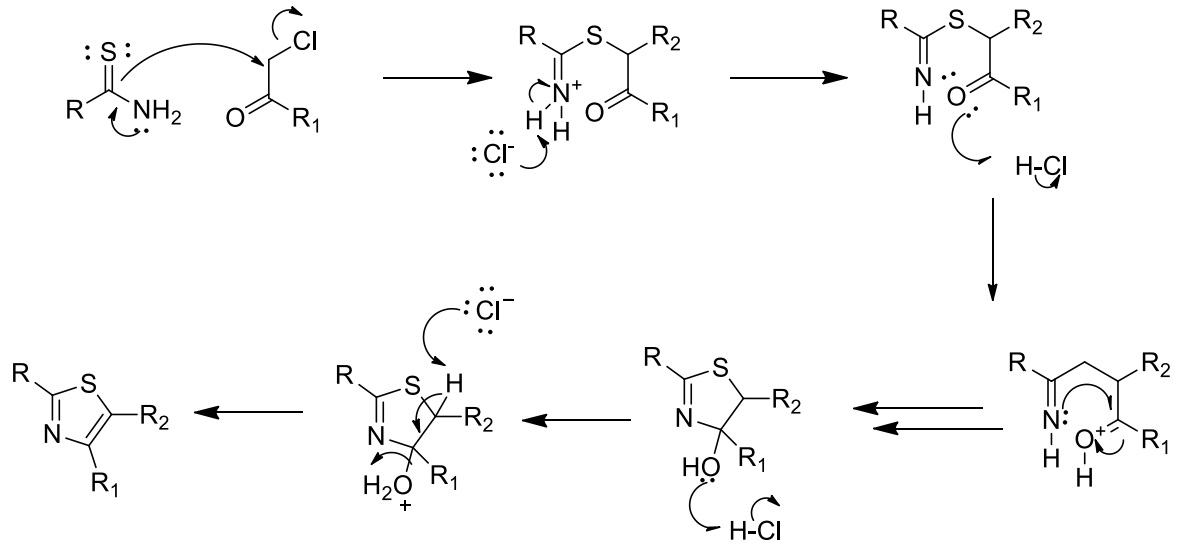



*Figure 3.1. Vitamin B1 and main thiazole-core containing drugs.*

### 3.2. Thiazole ring formation from acyclic α-chloroglycinates

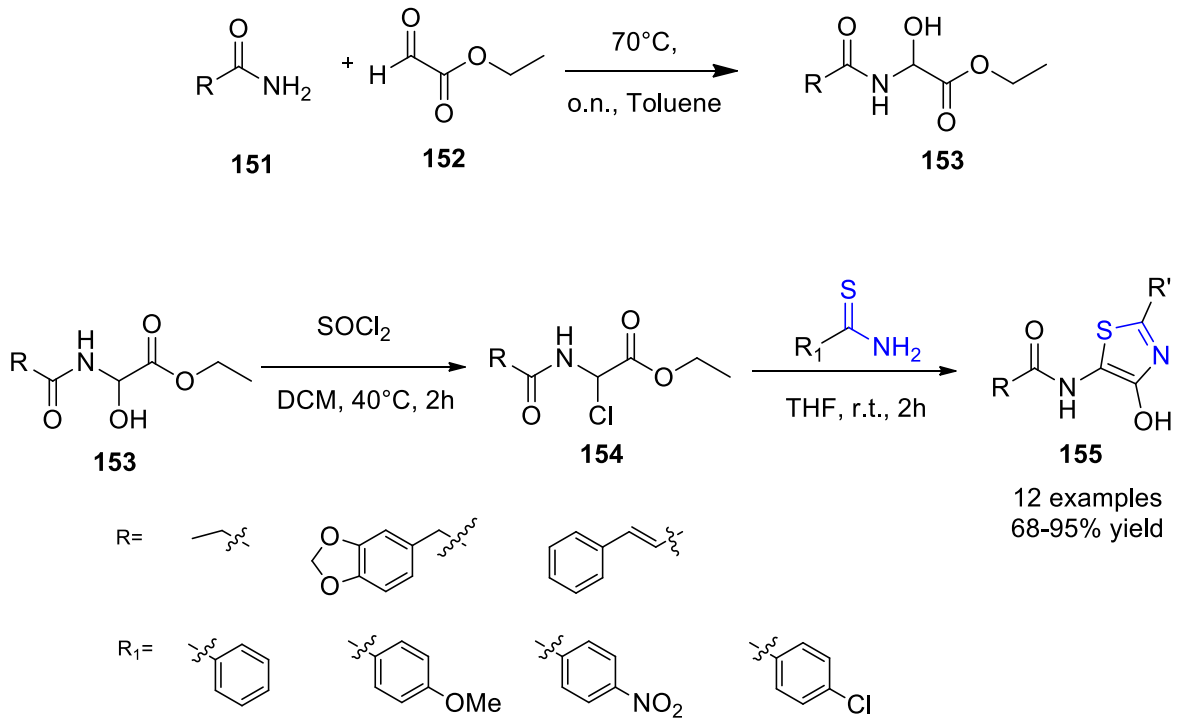
Although several synthetic procedures for thiazoles have been developed,[132] the most widespread methods are inspired by the pioneering work of the German chemist Hantzsch, who designed it in 1887.[133] This methodology involves the condensation between a compound having a carbon bearing two heteroatoms and α-halocarbonyl compounds, in order to synthesize 1,3-thiazoles (*Scheme 3.1*). Different compounds may serve as nucleophilic reagent in this reaction, such as thioamides, thioureas, ammonium thiocarbamates or dithiocarbamates, or other derivatives.



*Scheme 3.1.*

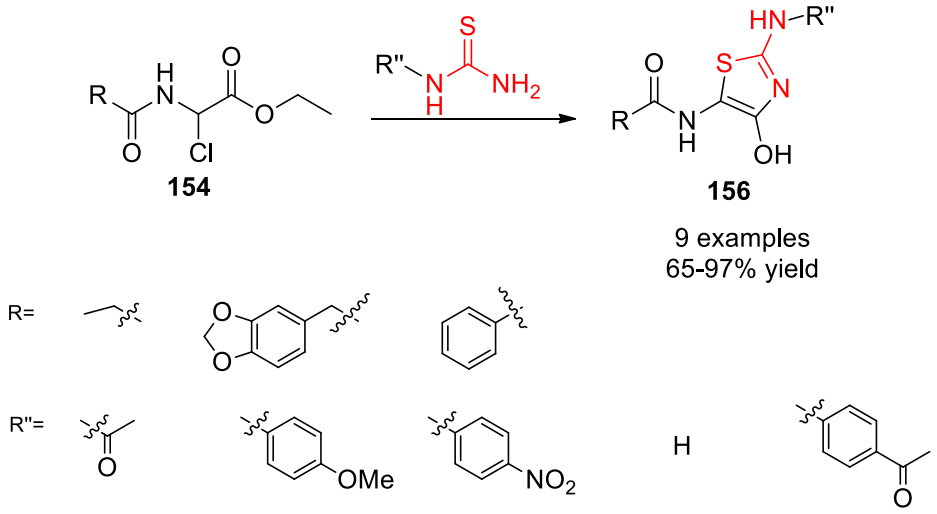
Inspired by this synthesis, in collaboration with other colleagues both from Marcantoni's research group and Dompé S.p.A., we recently developed a strategy to synthesize 5-acylamino-1,3-thiazoles from α-chloroglycinates without adding any catalyst or promoter, using mild conditions without need for chromatography in the whole procedure.

The synthesis starts with the formation of a α-hydroxyglycinate (**153**) by reaction of aliphatic or aromatic amides (**151**) and the commercially available ethyl glyoxaldehyde (**152**). The product is obtained in yields higher than 95% simply by trituration in $Et_2O$ and filtration. Compound **153** is then treated with excess of $SOCl_2$ in dry DCM at 40°C. No workup is required for this step and after evaporation of the solvent under reduced pressure, the product **154** is recovered in quantitative yield. By reaction of the α-chloroglycinate with differently substituted thioamides to obtain the corresponding 5-acylamino-1,3-thiazoles (*Scheme 3.2*).



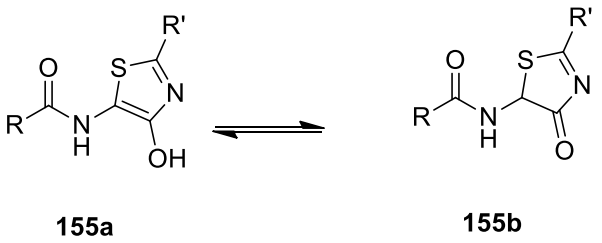

*Scheme 3.2.*

By extending the scope of the reaction switching from thioamides to thioureas, it was possible to synthesize a scaffold of 5-acylamino-2-amino-1,3-thiazoles (*Scheme 3.3*).




*Scheme 3.3*

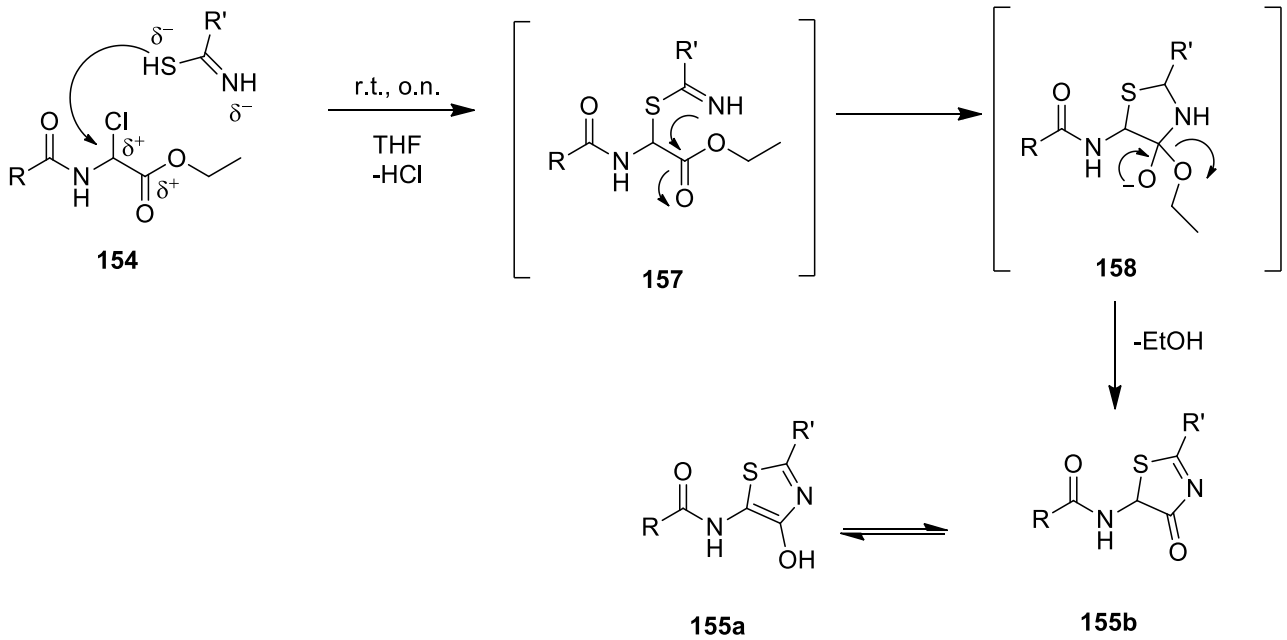
It must be noted, however, that since the final thiazole has a hydroxy group in position 4, the product is always observed in tautomeric equilibrium with its keto form (*Scheme 3.4*). The two tautomeric form cannot be isolated by chromatography.




*Scheme 3.4.*

### 3.3. Mechanism insight – results and discussion

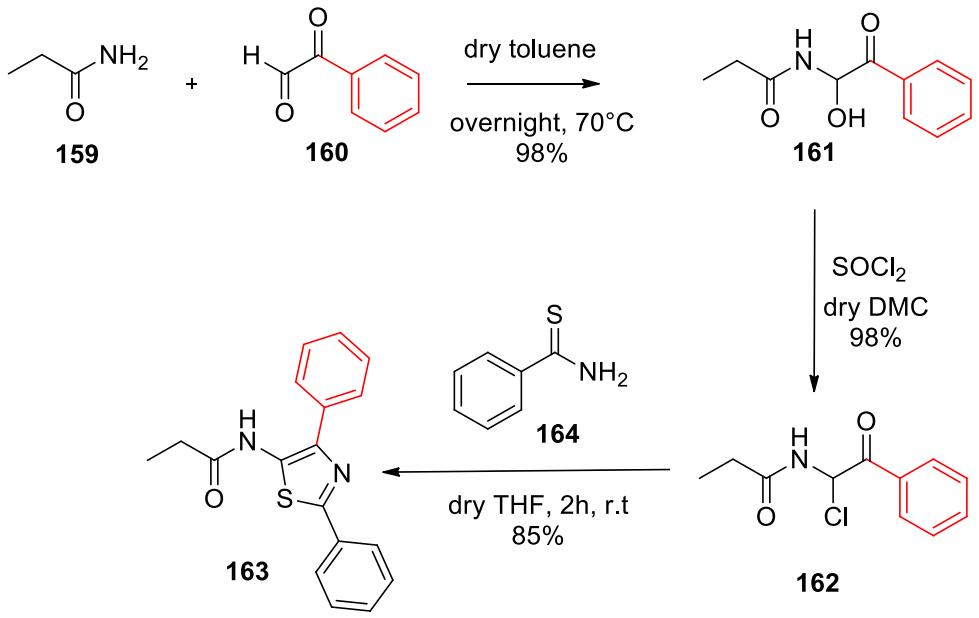
Like in the Hantzsch synthesis of thiazoles, our synthetic methodology was based on the presence of a 1,3-dinucleophilic compound, a thioamide or a thiourea, that react with a α-halo compound, possessing two electron deficient positions. What it is known is that carbonyl groups are hard electrophiles, so they should react with hard nucleophiles (basic nucleophiles), instead alkyl halides are soft electrophiles, so they react under frontier orbital control and with large uncharged nucleophiles. This means that the sulfur atom should attack the alkyl halide and the nitrogen the carbonyl group with the alkoxy group removal (*Scheme 3.5*).




*Scheme 3.5.*

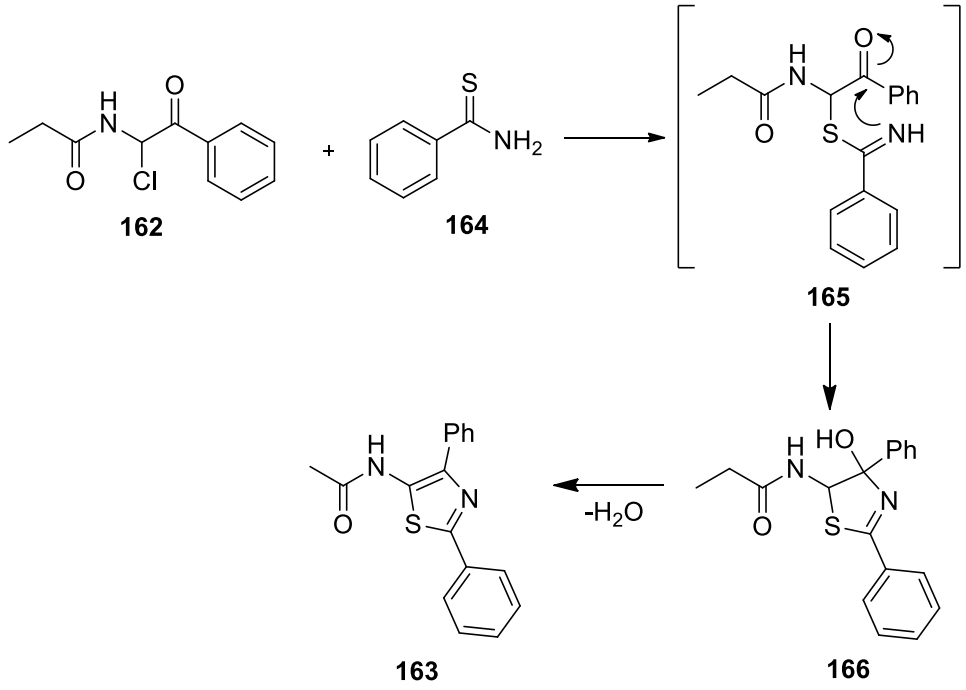
Although the mechanism of Hantzsch-type reactions has been postulated to be stepwise, it has always been infrequent to isolate the intermediates.[134] Even more difficult task is the isolation of intermediates **157** and **158** with an ester as a leaving group.

With the purpose of a more in depth study regarding the mechanism, we decided to synthesize the 4-alkythiazole **163** using phenylglyoxalate **160** to construct the α-chloroglycinate, instead of ethylglyoxalate (*Scheme 3.6*).



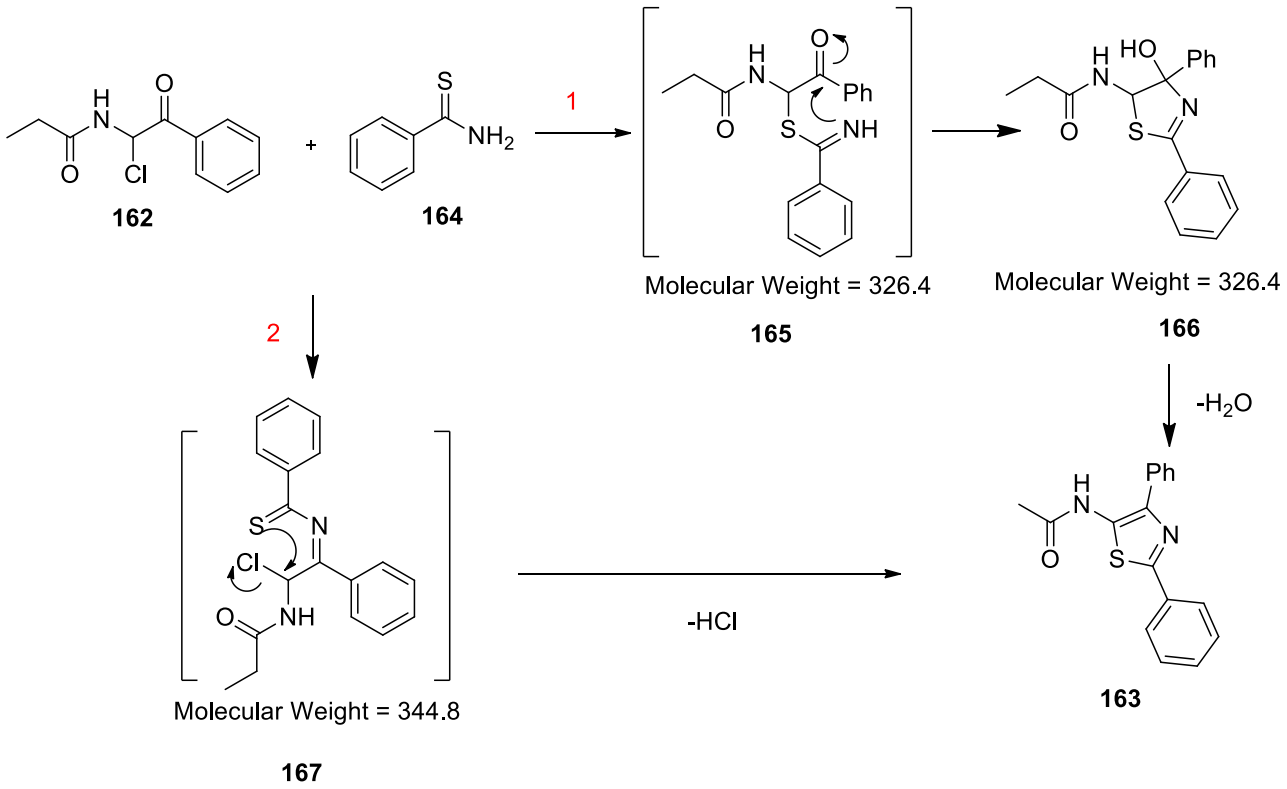

*Scheme 3.6.*

The mechanism of this last reactions should be only slightly different from the previous ones. After a first nucleophilic attack by sulfur of the thioamide on the alkyl chloride and a subsequent attack of nitrogen to the carbonyl group, we should obtain the 4-hydroxy-4-alkylthiazole **166**. At this point we should have the dehydration of this intermediate should produce the final 4-alkylthiazole **163** (*Scheme 3.7*)




*Scheme 3.7.*

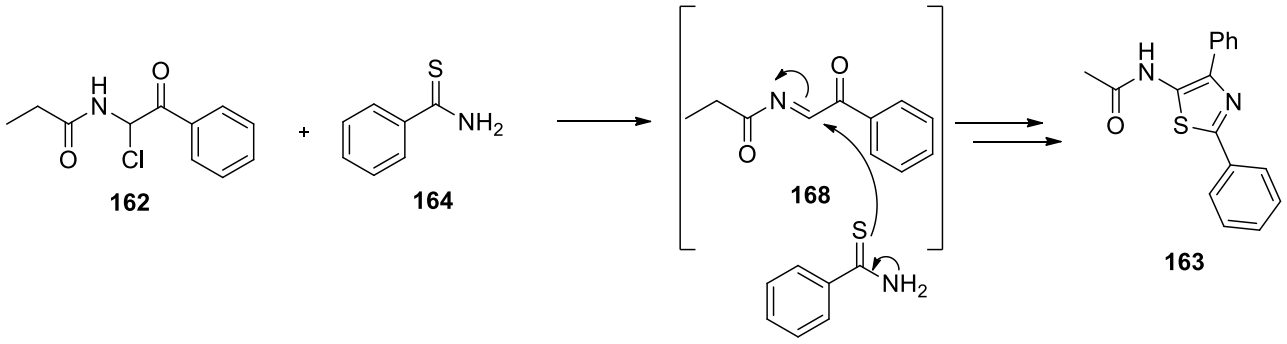
One may think that nitrogen is the first to attack the carbonyl forming an imine intermediate, followed by a subsequent sulfur attack on the alkyl halide. It is therefore possible to outline two different mechanisms (*Scheme 3.8*).




*Scheme 3.8.*

The reaction intermediates proved to be non-isolable. However, by withdrawing an aliquot of the crude reaction after 15 minutes of stirring and analysing it by ESI-MS spectrometry, it was possible to observe the presence of an ion of m/z 327; that could be actually the $[M+H]^+$ ion deriving either from intermediate **165** or **166** (molecular weight 326). No evidence of intermediates attributable to the formation of the imine intermediate **167** was observed. We can therefore state that the reaction proceeds with a first nucleophilic attack by sulfur on the alkyl halide followed by the closure of the cycle by the attack of the nitrogen atom to the carbonyl group (*pathway 1*, *Scheme 3.8*).

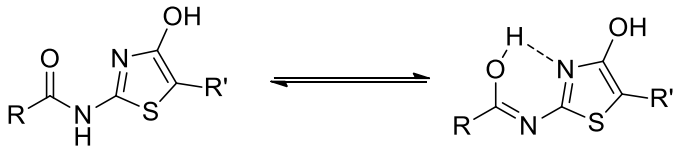
We then questioned ourselves if the reaction could proceed following a dehydrohalogenation pathway to form the imine intermediate **168**, rather than following a $S_N2$ mechanism (*Scheme 3.9*).[135]




*Scheme 3.9.*

To test this hypothesis, we studied the reaction through HPLC analysis. After checking the retention times of the two starting materials, we carefully checked the progress after 15, 30, 60 and 120 minutes. The same study was carried out by adding the 1,8-bis-(dimethylamino)naphthalene as a proton sponge base. Interestingly, the addition of a stoichiometric amount of the base to a solution of α-chloroglycinate **162** and subsequent addition of the thiobenzamide, does not show any improvement in yield of the corresponding thiazole and, above all, no changes in the speed of its formation at any time. Therefore, we can state that the cyclization reaction does not advance through an initial dehydrohalogenation of the substrate to form the corresponding imine, followed by nucleophilic addition to give the final thiazole product.

As already reported in *Scheme 3.4*, these 5-acylamino-4-hydroxy-1,3-thiazoles are often found in equilibrium with the tautomeric keto form. It should be noted that tautomeric equilibrium in acylamino-1,3-thiazoles could affect also the amide group by proton transfer between the amide N-H and C=O, as it has been observed for 2-acylamino-4-hydroxy-1,3-thiazoles (*Scheme 3.10*).[136]



*Scheme 3.10.*

However, we can exclude the presence of this tautomeric form in 5-acylamino-1,3-thiazoles synthesized in Marcantoni's research group. In fact, by carefully studying the $^{1}$H-NMR spectra of these products, we can extract the vicinal spin-spin coupling constants between amide proton and α-proton, $^{3}J_{HN\alpha}$, and it has a value reported to be typical for these system as a keto form (**155b**).[137]

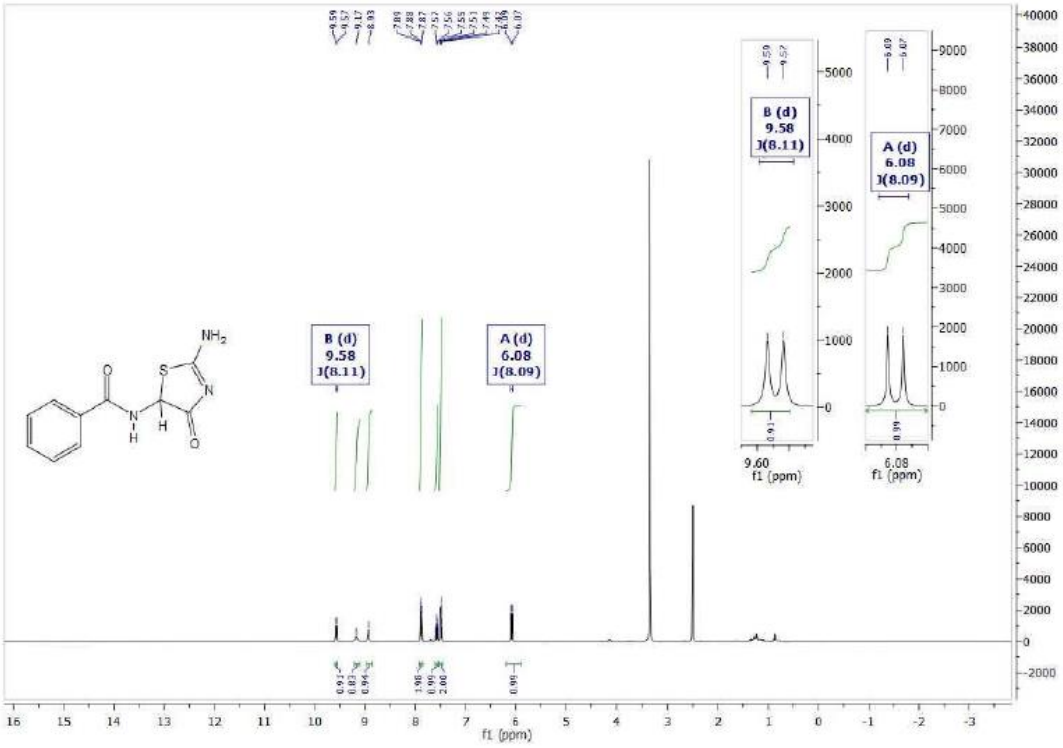



*Figure 3.2. Vicinal spin-spin coupling constants extracted from the $^{1}$H-NMR of N-(2-amino-4-oxo-1,3-thiazol-5-yl)benzamide.*

### 3.4. Experimental protocols

*General procedure for the synthesis of α-hydroxy Glycinates.*[138]

To a solution of ethyl glyoxylate (technical solution 50% m/m in toluene, 1.2 eq) in toluene (1 ml) was added the amide (1.0 mmol) and the reaction was left to run overnight at 70°C. The next morning a white precipitate appeared, and the solvent was removed under reduced pressure and it was suspended in $Et_2O$. The precipitated was filtered off and the solid collected was pure enough for the next step. Yields are generally quantitative.

*General procedure for the synthesis of α-chloro Glycinates.* [138]

To a suspension of hydroxy glycinate (1 mmol) in DCM dry (1 ml) under nitrogen was added dropwise thionyl chloride (10eq) and the reaction was gently warmed to 40°C and it was left to run for 3h. The reaction was checked directly by NMR until full conversion. After the reaction is complete the excess of thionyl chloride was removed under reduced pressure using high vacuum pump to obtain a yellowish solid which was used without further purification. Yields are considered quantitative. The chloride was immediately used in the next coupling reaction to avoid degradation.

*General procedure for the synthesis of 5-acylamino-4-hydroxy thiazoles.*

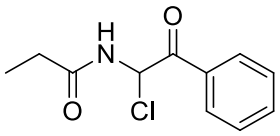
To a solution of chloroglycinate (1.0 mmol) in dry THF (2 ml) under nitrogen was added the thioamide (1.0 mmol) and the reaction was left to stir at room temperature for 2 hours. After this time, a precipitated appeared. The solvent was removed under reduced pressure and the crude was re-suspended in $Et_2O$ and the suspension was left to stir for 1 hour. The solid was collected to obtain the product.

*General procedure for the synthesis of 5-acylamino-2-amino thiazoles.*

To a solution of chloroglycinate (1.0 mmol) in dry THF (2 ml) under nitrogen was added the thiourea (1 mmol) and the reaction was left to stir at room temperature for 2 hours. After this time, a precipitated appeared. The solvent was removed under reduced pressure and the crude was re-

suspended in $Et_2O$ and the suspension was left to stir for 1 hour. The solid was collected to obtain a thiazole pure enough.

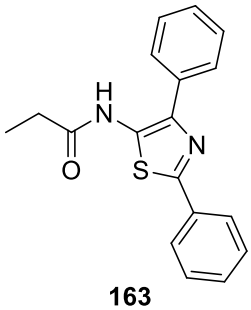
*N-(1-Chloro-2-oxo-2-phenylethyl)propenamide* **(162).**



**162**

To a suspension *N*-(1-hydroxy-2-oxo-2-phenylethyl)propionamide (1 mmol) in dry DCM (1 ml) under nitrogen was added dropwise thionyl chloride (10 equiv.) and the reaction was gently warmed until 40°C and it was left to run for 3h. The reaction was checked directly by NMR until full conversion. After the reaction is complete the excess of thionyl chloride was removed under reduced pressure using high vacuum pump to obtain a yellowish solid which was used without further purification. Yield: 99%. **$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.10 – 8.06 (m, 2H), 7.69 – 7.63 (m, 1H), 7.56 – 7.50 (m, 2H), 7.18 (d, *J* = 9.2 Hz, 1H), 2.39 (q, *J* = 7.6 Hz, 2H), 1.22 (td, *J* = 7.6, 3.6 Hz, 3H). **$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 194.67, 174.39, 134.39, 133.07, 129.51, 128.83, 77.41, 77.16, 76.91, 72.32, 29.65, 9.40.

*N-(2,4-Diphenyl-1,3-thiazol-5-yl)propanamide* (**163**)



**163**

To a solution of *N*-(1-chloro-2-oxo-2-phenylethyl)propionamide (**162**, 1.0 mmol) in dry THF (2 ml) under nitrogen was added benzothioamide (1.0 mmol) and the reaction was left to stir at room temperature for 2 hours. . After the reaction was complete (no more chloride is visible on TLC, DCM/MeOH 95/5) the solvent was removed under reduced pressure. The solid was re-suspended in $Et_2O$ and let it wash for several hours. The solid thus obtained was collected to give **163** with 85% as a non-crystalline amorphous yellow compound. **FTIR (neat, cm$^{-1}$):**3226, 1650, 1595, 1536.**$^1$H NMR (400 MHz, DMSO):** δ 10.65 (s, 1H), 7.96 – 7.91 (m, 2H), 7.81 (d, *J* = 7.3 Hz, 2H), 7.50 (dt, *J* = 6.3, 5.4 Hz, 5H), 7.40 (s, 1H), 2.48 – 2.43 (m, 2H), 1.11 (t, *J* = 7.5 Hz, 3H). **$^{13}$C NMR (100 MHz, DMSO):** δ 172.37, 158.76, 141.32, 134.07, 133.43, 129.77, 129.22, 128.64, 128.14, 127.76, 125.60, 40.15, 39.94, 39. 73, 39.52, 39.31, 39.10, 38.89, 28.25, 9.54. **M.W.:** 308.10, **ESI-MS:** $[M-H]^-$ m/z = 307.0. **HRMS** calcd for $C_{18}H_{16}N_2OS$**:** $[M-H]^-$:307.0911, found 307.0911.

## 3.5. Conclusions

Thiazoles represent an important class of heterocycles in pharmaceutical chemistry and their core is present in many drugs currently on the market.

Although there are several synthetic strategies for obtaining thiazoles, the majority of which inspired by Hantzsch's synthesis, in the laboratory of prof. Marcantoni it has been developed a new method to obtain 5-acylamminothiazoles from acyclic precursors.

After the optimization of the synthesis, the next step was to question ourselves about the possible mechanism of formation of the heterocycle. Several possible patterns have been supposed, based on possible routes in theoretical line. However, the experimental evidences have led us to formulate a mechanism that proceeds through a first nucleophilic attack by sulphur on the alkyl halide, followed by the closure of the cycle by the attack of the nitrogen atom to the carbonyl group, thus elucidating which of the 2 nucleophiles attacks first.

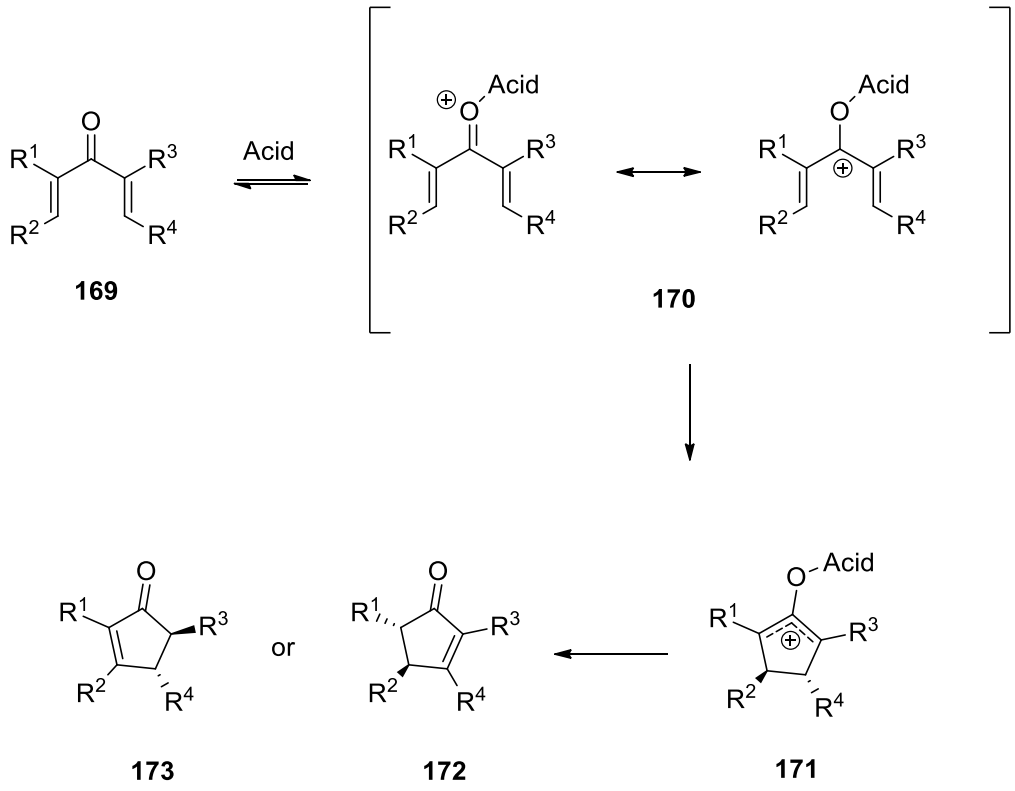
Morever, by following the reaction with HPLC analysis with and without the addition of a proton sponge, we have also ruled out that the mechanism passes through a dehydrohalogenation of the alpha chloroglycinate.

# Chapter 4: $CeCl_3$ promoted Nazarov cyclization as a powerful approach to form carbocyclic structures

## 4.1. The Nazarov cyclization

The Nazarov reaction, also known as Narazov cyclization, was first reported by a Russian scientist, Ivan Nikolaevich Nazarov, in 1942.[139] It represents the cyclization of cross-conjugated linear dienones to synthesize cyclopentenones through a 4π electrocyclization, in the presence of Brønsted or Lewis acids as catalysts or promoters. It is a powerful way to construct five membered rings that are part of many biologically active natural products. The recent raise of interest in the total synthesis of natural products with five-membered carbocycles led to a deeper exploration of the generality and versatility of the Nazarov cyclization.

The mechanism of the reaction begins with the coordination of either a Brønsted or Lewis acid to the dienone **169** to form the pentadienyl cation **170**. The main step of the reaction involves a cationic 4π electrocyclization to form the oxyallyl cation **171**, followed by a loss of a proton to form either cyclopentenone **172** or **173**, or a mixture of the two (*Scheme 4.1*).




*Scheme 4.1.*

As a result of the conrotatory cyclization, a *trans* relationship between $R^2$ and $R^4$ is observed in the intermediate **171**. The diastereospecificity of the reaction is due to an antarafacial overlap of the two cyclization termini in the transition state, which results in a stereospecific conrotatory cyclization. This leads to the formation of new stereocenters in the oxyallyl cation **171** that can be maintained upon the formation of the two pentacyclic products.[140]

The Nazarov reaction is tolerant to various substituent in the initial dienones, allowing the synthesis of highly functionalized 5-membered carbocycles and cyclopentenones derivatives fused to non-aromatic, aromatic or heteroaromatic rings.

Different transition metals such as Au(I),[141-144] Au(III),[145-148] Ag(I),[149-151] Pd(0),[152] V(IV),[153] Re(0),[154], Pt(II),[155] and Ir(III)[156] can catalyze this process. Also, in some cases, the cyclization can be promoted by UV light irradiation.[157]

Recently, it has been identified that the allylic cation like the one in the intermediate **170** can be generated not only from divinyl ketones but also from unsaturated carbonols,[158] oxiranes[159] and imines (*Figure 4.1*).[160]

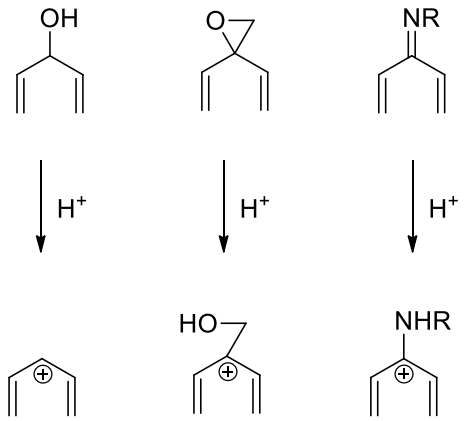



*Figure 4.1. Additional substrates for the Nazarov cyclization.*

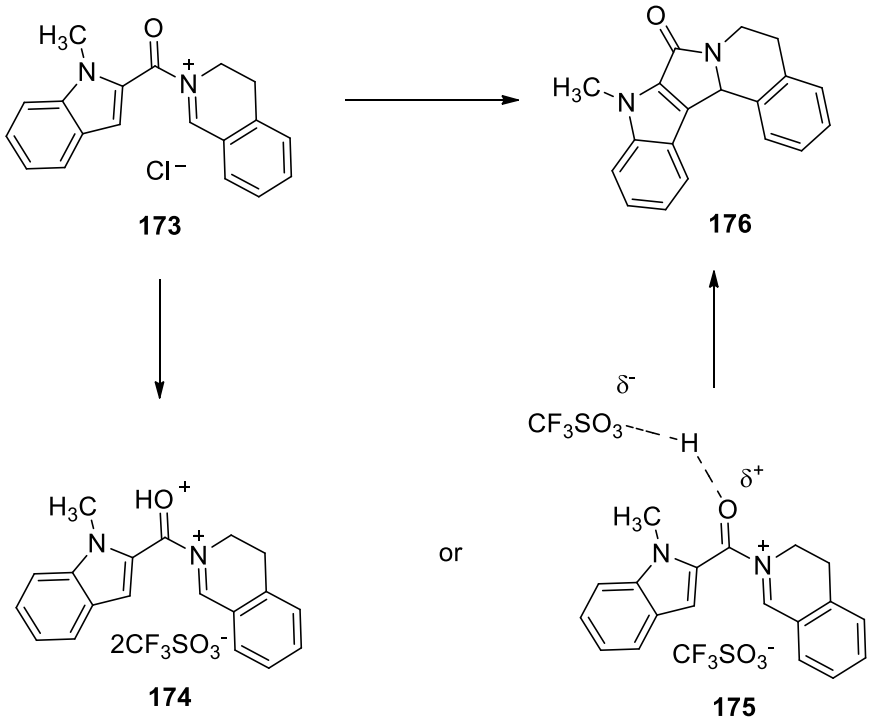
### 4.2. Brønsted and Lewis acid promoted Nazarov cyclization

Since its initial discovery in 1942, the Nazarov cyclization has received tremendous attention in the field of organic synthesis due to its ability to form synthetically versatile cyclopentenones. However, since 2005, this reaction has grown in interest for organic chemists and many different conditions, synthetic strategies and catalysts have been studied and developed, primarily with the aim of natural products synthesis.[161]

### 4.2.1. Brønsted acid promoted Nazarov cyclization

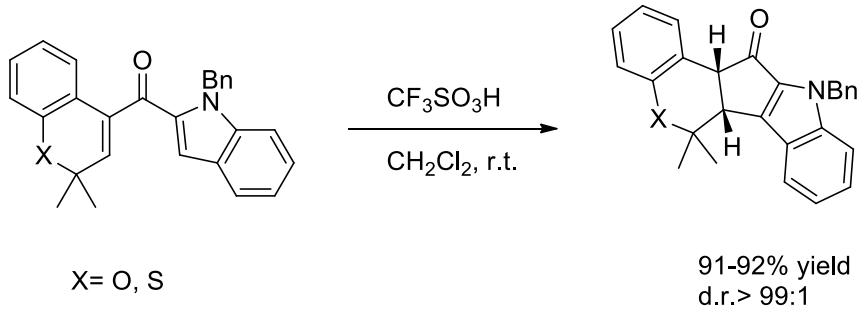
Nazarov cyclizations are often described as promoted by a stoichiometric amount of Brønsted acids. However, in some cases examples have been reported in which these acids can effectively catalyze certain transformations. Given the great interest for this reaction in organic synthesis and the will to expand the its generality, several examples are reported in literature in which Brønsted acids are used to promote the electrocylization.

In 2007, Klumpp and coworkers reported an example of aza-Nazarov that involves a catalytic amount of triflic acid (HOTf) promoting the cyclization of *N*-acyliminium salts. Authors reported that the presence of this superacid was required to support the formation of dicationic intermediates (**174** or **175**) that could not be obtained with weaker acids (*Scheme 4.2*).[162]



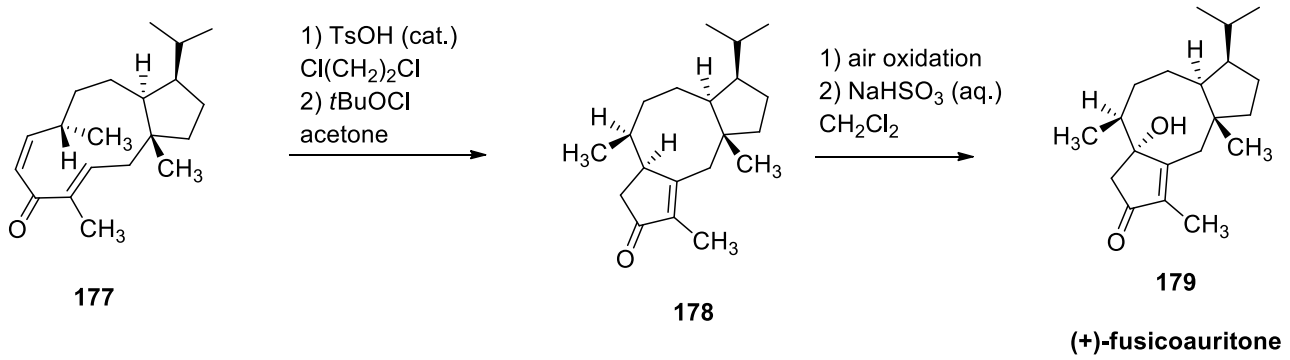

*Scheme 4.2.*

Panda *et al.* reported a synthetic strategy to obtain fused aromatic or heteroaromatic [6-5-6] tricyclic cores with high diastereoselectivity, screening different Brønsted and Lewis acids. The best results were obtained by using stoichiometric amount of triflic acid in dichloromethane (*Scheme 4.3*).[163]




*Scheme 4.3.*

It has been found that a catalytic amount of *p*-toluenesulfonic acid (TsOH, 5 mol%) is able to effectively promote the electrocyclization of *α*-alkoxy dienones under relatively mild conditions.[164] Williams and coworkers reported an example of Nazarov reaction in the total synthesis of (+)-fusicoauritone (**179**). They were able to achieve a ring construction of an eleven-membered dolabelladienone intermediate (**177**) to obtain a [5-8-5] diterpene skeleton (**178**, *Scheme 4.4*).[165]

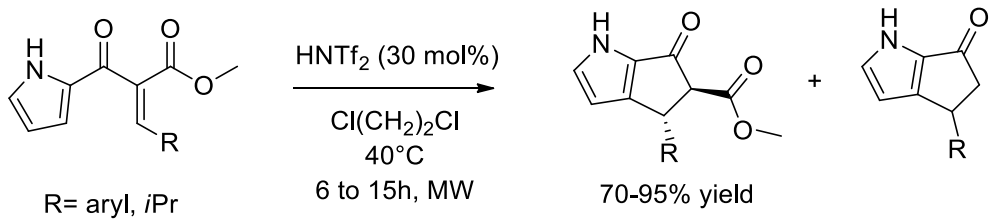



**177** **178** **179**

**(+)-fusicoauritone**

*Scheme 4.4.*

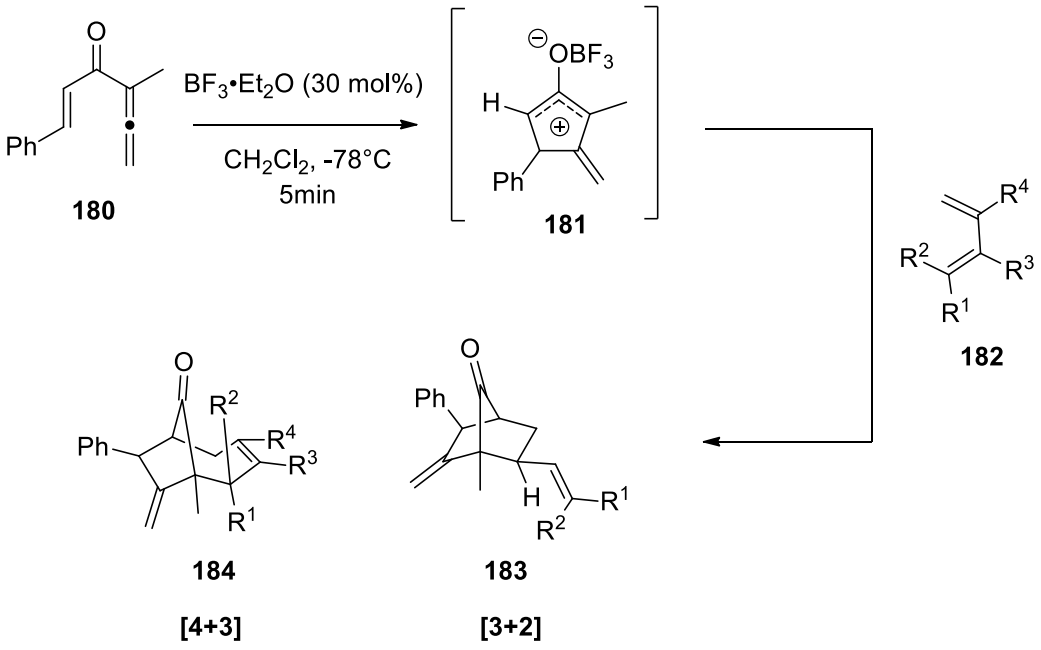
In 2009, Akiyama and Bachu reported a microwave assisted Nazarov cyclization starting from 2-substituted pyrroles derivatives with either aromatic or isopropyl side chains using a catalytic amount of triflimide ($HNTf_2$). By finely tuning the reaction conditions (lower temperatures and shorter reaction times) they were also able to obtain the desired products in high yield, inhibiting the formation of the decarboxylated side-product (*Scheme 4.5*).[166]




*Scheme 4.5.*

### 4.2.2. Lewis acid promoted Nazarov cyclization

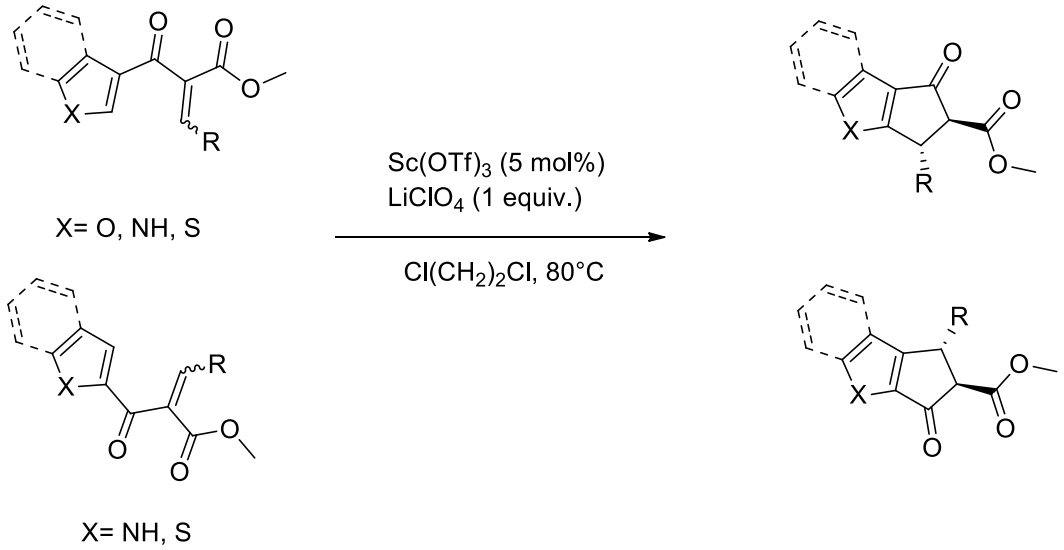
A very high number of Lewis acid have been developed and described in the recent years as promoters of the Nazarov reaction, in either stoichiometric or catalytic amount. In 2010, Burnell and Marx discovered that by treating allenyl vinyl ketone **180** with a catalytic amount of $BF_3$•$Et_2O$ they could generate the oxyallyl cation **181**, which, after reacting with various butadienes, allowed them to synthesize differently substituted [4+3] or [3+2] products in good regio and stereoselectivity (*Scheme 4.6*).[167]




*Scheme 4.6.*

The authors reported that the ratio between products **183** and **184** depended on the different substituents present in the acyclic diene **182**. They also managed to extend the reaction scope to cyclic dienes, but in this case a stoichiometric amount of the Lewis acid promoter was needed.

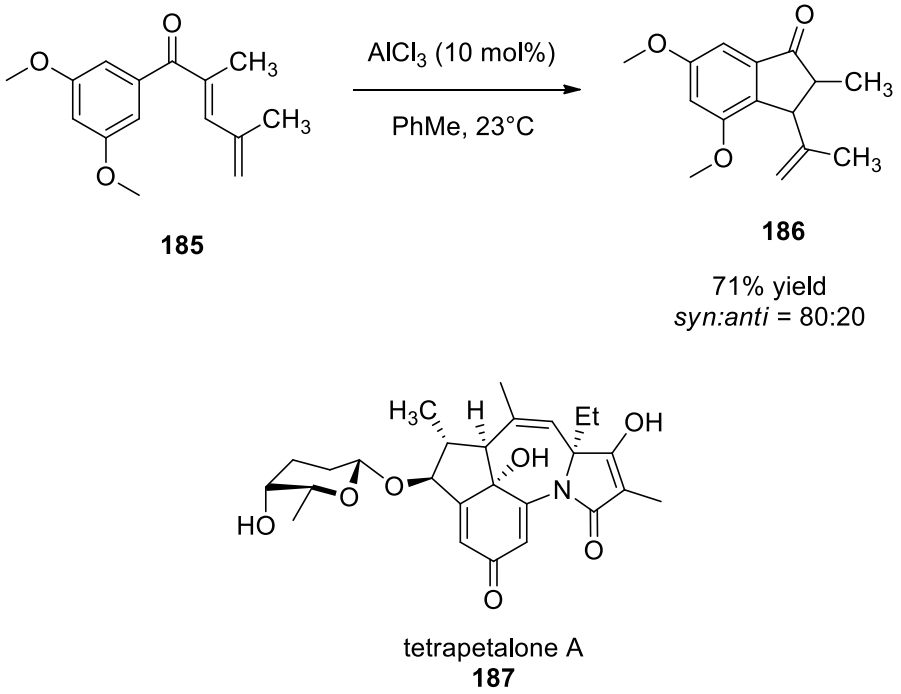
Frontier *et al.* reported in 2006 a general protocol to promote Nazarov cyclizations of 2- and 3-substituted heteroaryl vinyl ketones, using catalytic amounts of $Sc(OTf)_3$ or $In(OTf)_3$ and one equivalent of $LiClO_4$ (*Scheme 4.7*).[168] Although most of the heteroaromatic enones (pyrrole,furyl, thiophenyl, indole and benzofuryl) tested were converted to the corresponding fused cyclopentanones in 36-97% yield, the authors reported that 2- and 3-substituted furyl and benzofuryl did not show any conversion. Subsequent investigations demonstrated that these substrates undergo a different reaction involving a 1,2-hydride migration and Friedel-Crafts alkylation promoted by $Ir(III)^{3+}$ cation.[169]




*Scheme 4.7.*

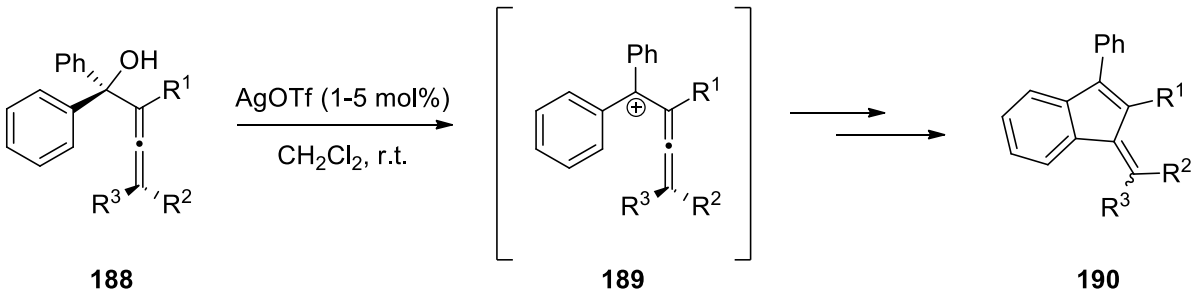
The system $Sc(OTf)_3/LiClO_4$ was also used in the synthesis of a cytotoxin agent, the roseophilin, to promote a Nazarov cyclization of a 2,5-disubstituted *N*-tosylpirrole intermediate,[170] and scandium triflate itself was also used to catalyze a Nazarov reaction in the synthesis of strigolactone analogues of an indole intermediate.[171]

In 2008, Sarpong and coworkers studied the cyclization of an aryl vinyl intermediate (**185**) in the synthesis of tetrapetalone A catalyzed by $AlCl_3$ (*Scheme 4.8*).[172]



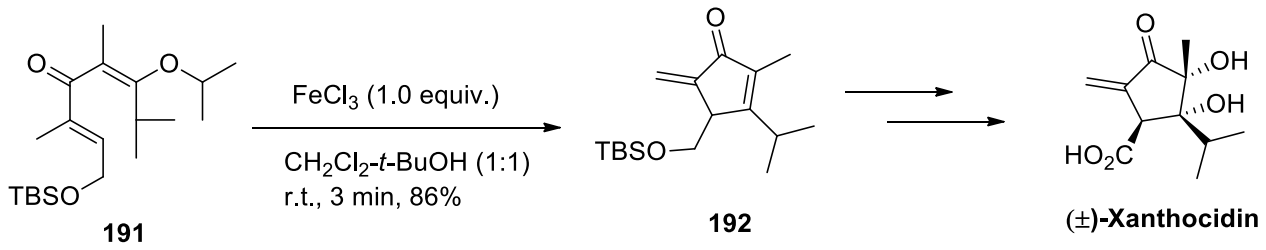

*Scheme 4.8.*

Also transition-metal based Lewis acids have been exploited for this kind of reaction. Catalytic amount of AgOTf were used in a variant of the Nazarov reaction starting from allenylic alcohols (**188**) to obtain benzofulvenes (**190**) after elimination of water (*Scheme 4.9*).[173]



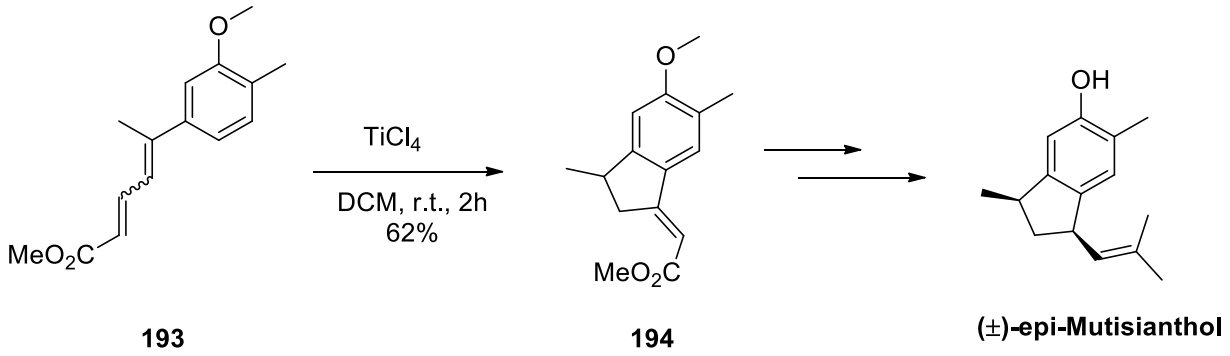

*Scheme 4.9.*

Yaji and Shindo reported an example of Nazarov reaction in the total synthesis of (±)-xanthocidin starting from the β-alkoxy divinyl ketone **191** in the presence of one equivalent of $FeCl_3$ to provide the α-*exo*-methylene cyclopentadienone **192** after β-elimination (*Scheme 4.10*).[173]




*Scheme 4.10.*

In 2015, a $TiCl_4$ promoted cyclization was reported as a key step for the synthesis of a substituted indane framework in the total synthesis of (±)-mutisianthol, starting from the α,β-unsaturated ester **193**. Both *cis* and *trans* isomers were independently treated with $TiCl_4$ in DCM to afford the indane **194** in 62% yield. Interestingly, by reaction of a mixture of the two isomers under the same conditions, compound **194** was obtained in the same yield (*Scheme 4.11*).[174]




*Scheme 4.11.*

Many others transition-metal Lewis acid have been studied in this kind of transformations, including copper, cobalt, vanadium, platinum and palladium complexes. In the last two decades, a great improvement in the outcome of the reaction was obtained thanks to the use of chiral metal catalysts to obtain Nazarov cyclization products with high diastereoselectivity, which however will not be described in this context.[161]

### 4.3. Cerium trichloride in organic chemistry

Lanthanides have been ignored for a long time in organic chemistry, however, in the last three decades, their use in organic transformations has grown enormously.[175] Cerium is the most abundant

element in the series of lanthanides and it has been extensively used in its stable +3 and +4 oxidation states. The more frequently used Ce(IV) salt is Cerium ammonium nitrate (CAN), which is used as a single-electron oxidant for a variety of chemical transformations.[176] However, the most stable salts belong to the trivalent oxidation state and, among these, cerium(III) trichloride heptahydrate is the most abundant and commercially available source of $Ce^{3+}$. It has proved to be a powerful Lewis acid catalyst in carbon-carbon and carbon-heteroatom bond formation reactions.

According to Pearson's HSAB terminology, $Ce^{3+}$ is a "hard cation" and it shows strong affinity towards "hard bases" such as oxygen and nitrogen donor compounds. However, since the Lewis acidity is dependent on the charge density (Z/r) and consequently in the ionic radius, complexes and salts of $Ce^{3+}$ are considered mild Lewis acids. Combining these features with the low toxicity, ease of handling and low cost, the use of $CeCl_3$ has been particularly attractive for organic chemistry application, both in its anhydrous and hydrated forms.[177] It must be also noted that cerium trichloride is a water tolerant compound, greatly enhancing its applicability unlike most of the commonly used Lewis acids that undergo deactivation or decomposition in presence of water.

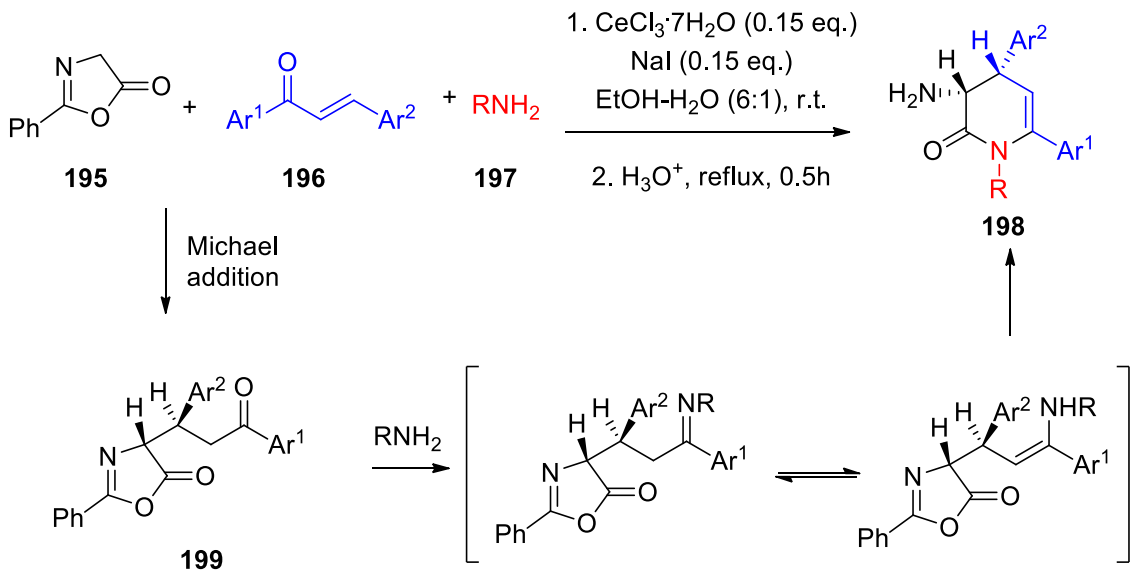
However, it has been proved that the hydrated $CeCl_3$ is sometimes only modestly active.[178] In this scenario, the Marcantoni's research group observed that the activity of $CeCl_3 \bullet 7H_2O$ can be enhanced by combination with an iodide source, thus generating a more powerful Lewis acidic system.[179] In fact, the addition of NaI together with $CeCl_3 \bullet 7H_2O$ often resulted in decreasing reaction times, reducing the formation of undesired byproducts and improving the final yield of the products.

Several explanations have already been considered regarding the mechanism of activation by the iodide anion, however, no one if them have a strong experimental evidence. The most valuable hypothesis is that the iodide ion can break the weakly active chlorine-bridged oligomeric structure of $CeCl_3$ leading to a more active monomeric array. What is clear, by XPS analysis (X-ray Photoelectron Spectroscopy), is that no direct interaction between the cerium (III) internal core and the iodide anion was observed, thus excluding an increasing of the activity due to a process of trans-halogenation.[175]

In recent years, $CeCl_3 \bullet 7H_2O$/NaI Lewis acid system has been used as promoter in several organic reactions, and it has shown particularly interesting activity in the assembly of 5-,6- and 7-membered heterocyclic compounds through cyclization reactions.

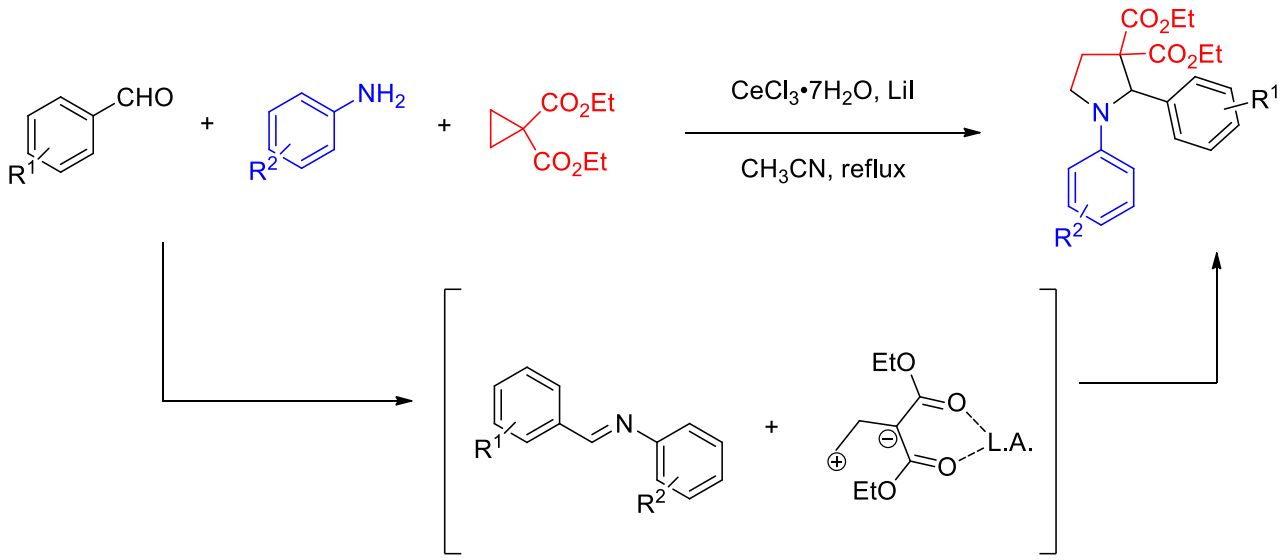
In 2008, Yadav and coworkers reported the stereoselective multicomponent synthesis of 3-amino-2(*H*)-pyridinones **198** from 2-phenyl-1,3-oxazolin-5-one **195**, α,β-unsaturated ketones and primary amines using $CeCl_3 \bullet 7H_2O$/NaI promoting system.[180] Thanks to the use of only 15 mol% $CeCl_3 \bullet 7H_2O$/NaI they were able to dramatically increase the *anti* diastereoselectivity of the reaction,

probably to the large size of $CeCl_3$ taking part in the first Michael addition step, leading to intermediate **199** (*Scheme 4.12*).




*Scheme 4.12.*

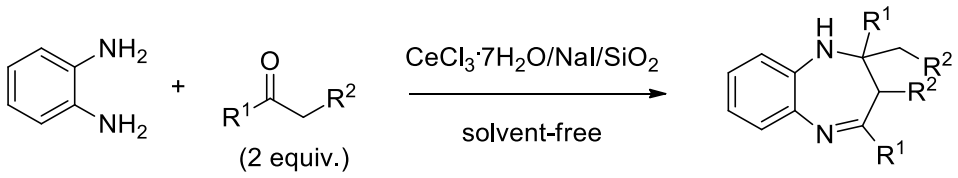
In 2009 Reddy e*t al.* used $CeCl_3 \cdot 7H_2O$ in the three-component reaction of differently substituted benzaldehydes, anilines and cyclopropanes for the preparation of pyrrolidine derivatives through annulation reaction (*Scheme 4.13*).[181] Authors reported that LiI acted as a more performing activator than NaI, allowing the ring expansion of the strained cyclopropane.




*Scheme 4.13.*

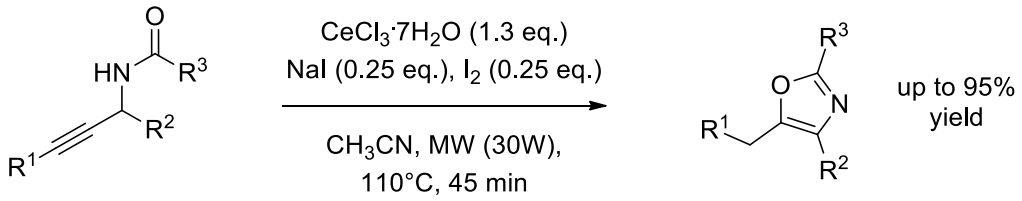
The $CeCl_3 \cdot 7H_2O$/NaI system was used also as a $SiO_2$- or $Al_2O_3$-supported catalyst in the synthesis and functionalization of heterocyclic scaffolds. The presence of silica or alumina can both enhance the activity of catalyst and make the process more environmentally friendly, removing the need for the extraction step from the synthesis. In fact, the reaction can just be filtered over a pad of celite, or directly loaded on chromatographic column.

In 2003, the group of prof. Marcantoni developed this method for the conjugate additions on α,β-enones,[182] and in 2004 Sabitha and coworkers studied the synthesis of 1,5-benzodiazepines from *o*-phenylenediamines and ketones promoted by $CeCl_3 \cdot 7H_2O$/NaI/$SiO_2$ in solventless conditions (*Scheme 4.14*).[183]




*Scheme 4.14.*

Further developments were recently reported by Marcantoni *et al*. in the cyclization of propargyl amides to polysubstituted oxazoles in moderate to good yields and without the need of expensive catalysts (Pd and Au)[184] using $CeCl_3 \cdot 7H_2O$/NaI and iodine under microwave irradiation (*Scheme 4.15*).[185]

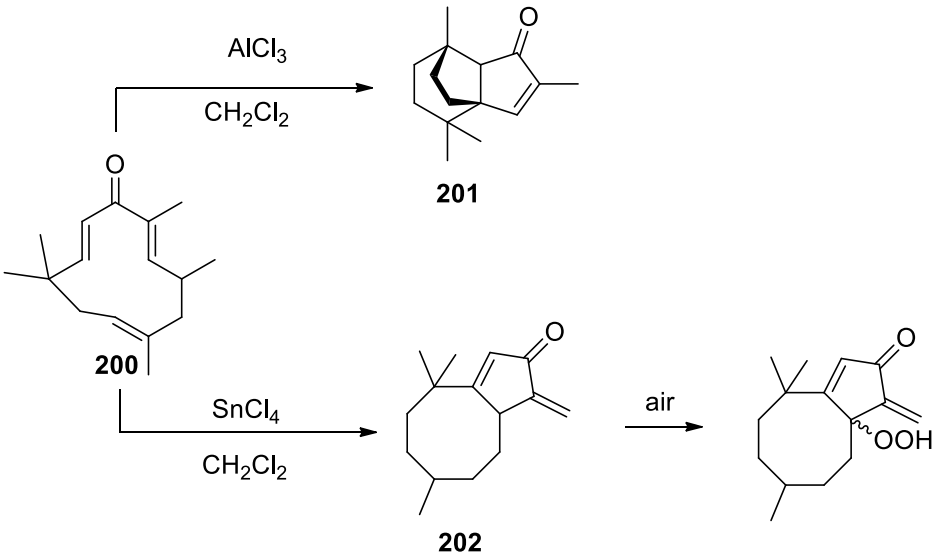



*Scheme 4.15.*

## 4.4. Results and discussion

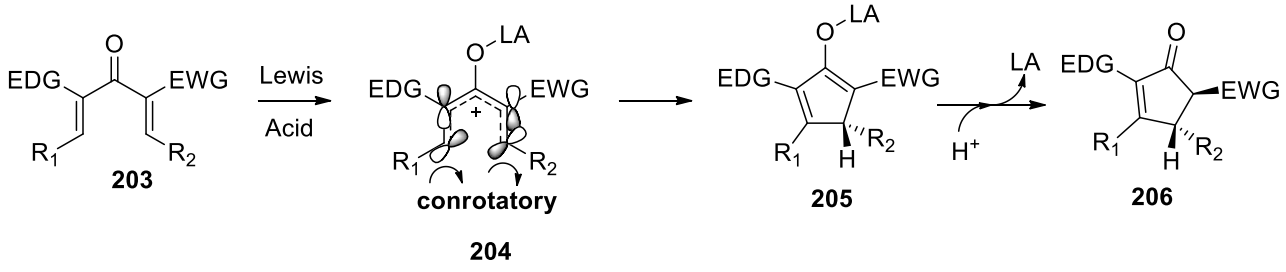
As already described in paragraph 4.2, Nazarov cyclizations can be promoted by Lewis acids. Surprisingly, in literature there are almost no examples of these reactions catalyzed or promoted by cerium salts as Lewis acid activators. One of the few tests reported is by Minassi and coworkers in

the attempt to perform the cyclization of zerumbone (**200**). They reported that by promoting the reaction with $AlCl_3$ or $SnCl_4$ they were able to obtain the tricyclic enone **201** and the bicyclic dienone **202**, respectively. Further tests with different promoters like $ZnCl_2$ or $CeCl_3$ did not provide any conversion.[186]




*Scheme 4.16.*

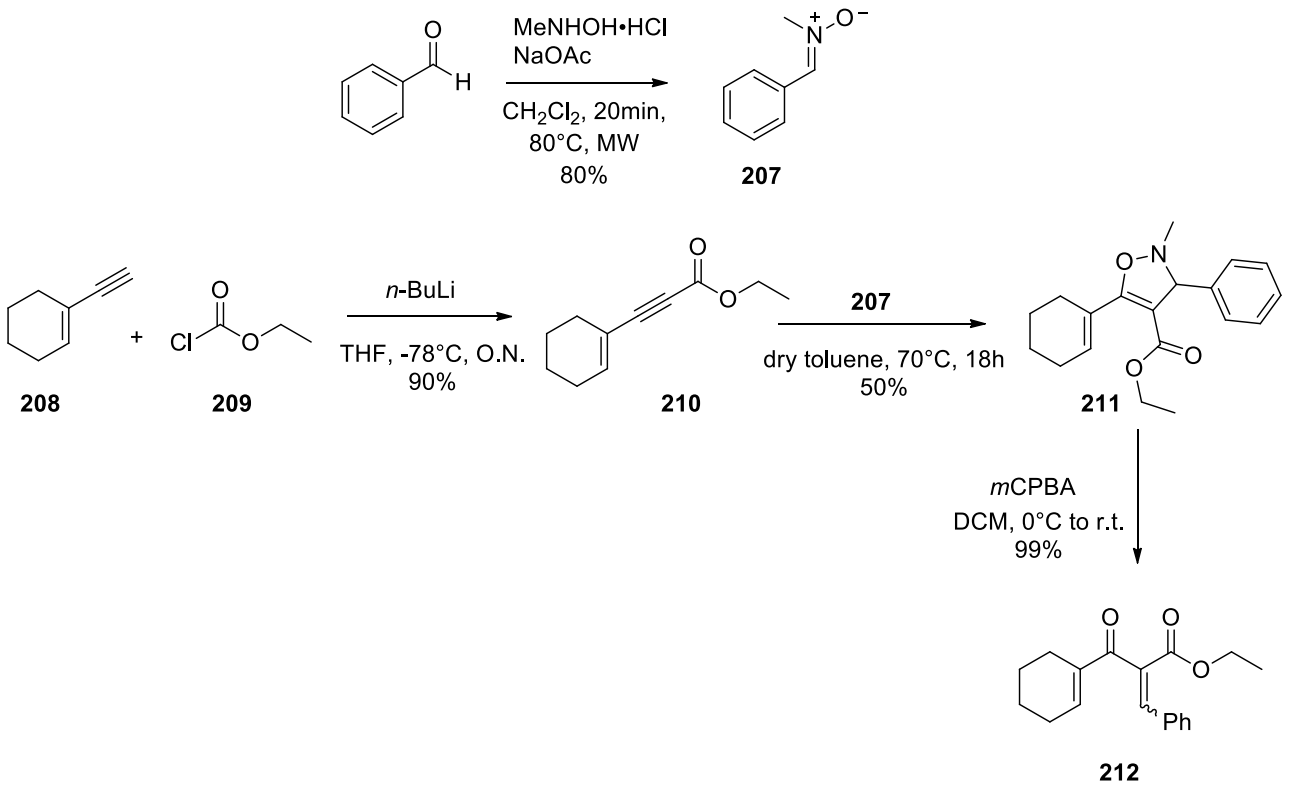
Given the fifteen-year experience of the group of prof. Marcantoni on the use of $CeCl_3$ as promoting system, we decided to study more in dept the power of this Lewis acid in the Nazarov reaction. We started our project from desymmetrized dienones (**203**) with properly positioned electron-donating (EDG) and electron-withdrawing (EWG) groups, that allow to lower the activation barrier for the orbital reorganization (*Scheme 4.17*).[171]




*Scheme 4.17.*

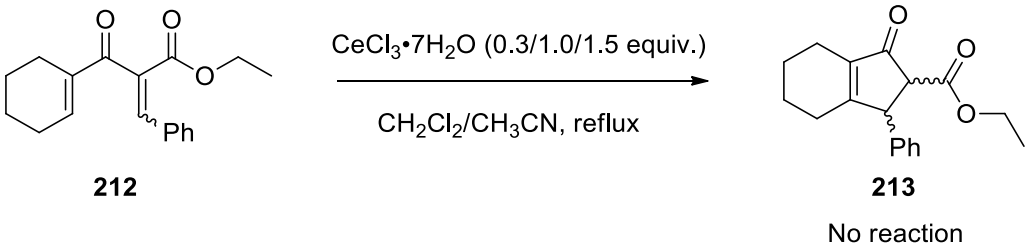
Following a reported procedure,[187] the synthesis of our template starting material is the result of a convergent synthesis between nitrone **207** and the electron deficient alkyne **210**, to afford the stable

isoxazoline **211**, which undergoes oxidation and extrusion of nitrosomethane to give the Nazarov precursor **212** (*Scheme 4.18*) as a mixture of the two diastereoisomers.



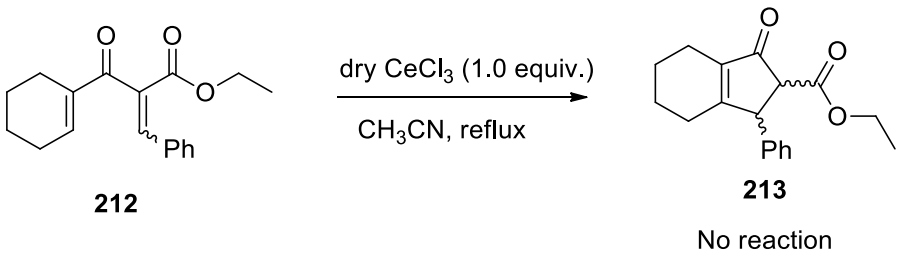

*Scheme 4.18.*

Initial tests done in different conditions of solvents ($CH_2Cl_2$ or ACN) and amount of promoter (0.3, 1.0 and 1.5 equiv.) at reflux did not show any conversion of the starting material (*Scheme 4.19*).



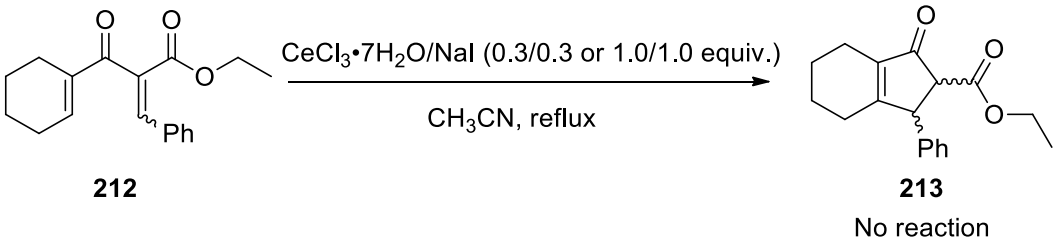

*Scheme 4.19.*

Questioning ourselves if it could be a problem due to the crystallization water of cerium trichloride heptahydrate, we also decided to do a test with dry $CeCl_3$ (dried at 140°C under vacuum) but also in this case no conversion was detected.



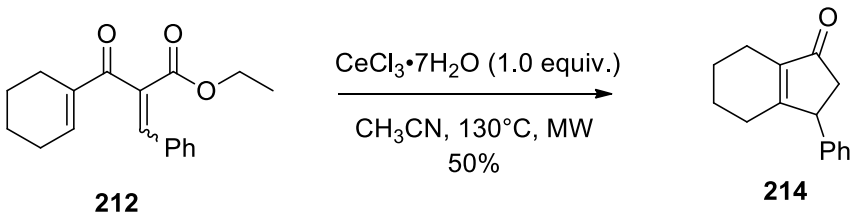

*Scheme 4.20.*

Based on the experiences described in section 4.3 we decided to add NaI to the reaction mixture. In presence of the promoting system $CeCl_3 \bullet 7H_2O$/NaI 0.3equiv./0.3equiv. or 1 equiv./1equiv. in acetonitrile at reflux, only starting material has been spotted in the reaction mixture even after several hours.




*Scheme 4.21*.

We were pleased to see a good amount of conversion when moving from batch to microwave assisted synthesis, in acetonitrile at 130°C, using 1 equivalent of $CeCl_3 \bullet 7H_2O$. However, the only product observed was the decarboxylated fused ring **214** (*Scheme 4.22*).

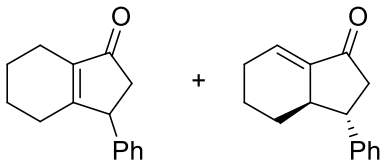



*Scheme 4.22.*

Different conditions were then tested to find the right ones to have a better conversion and yield, as described in *Table 4.1.* Switching from $CH_3CN$ to toluene, which is another solvent that allow us to reach 130°C in microwave apparatus, resulted in a slightly decrease of the yield (*entry 2*). We then tested the reactivity with the addition of both NaI and $CeCl_3 \bullet 7H_2O$ to the substrate, but no changes

or improvement have been observed, suggesting that sodium iodide in this case may not have any role in the "activation" of cerium trichloride. The use of a Brønsted cocatalyst (Benzoic acid, *entry 4*) to have a further activation of the two carbonyl moieties did not result in any kind of improvement once again. Based on our experience, we decided in the end to try also solid supported (on silica and acidic alumina) promoting system.

To our delight the reaction had a remarkable improvement with 0.35 equivalents of $CeCl_3 \bullet 7H_2O$ supported on silica, with an almost equal results with or without NaI (*entries 5* and *6*) up to 88% yield in 3 hours of microwave irradiaton. Almost full conversion has been obtained with cerium trichloride supported on acidic alumina (*entry 7*) and the same result has been obtained with only 0.1 equivalent of $CeCl_3 \bullet 7H_2O$ in 6 hours, same conditions (*entry 10*). In all the cases in which the promoting system is supported on silica or alumina (*entries 6, 7* and *10*) products were obtained as a mixture of two regioisomers **214** and **215** in 55-45 ratio.




**214** **215**

55% 45%

This type of regioisomerism has been observed also by Amere and coworkers in the Nazarov cyclization of similar dienones promoted by 4-toluenesulfonic acid. According to the conrotatory mode of cyclization and based on their work, we attributed a 3,4-trans relative stereochemistry to the substituents of cyclopentenone **215**.[188]

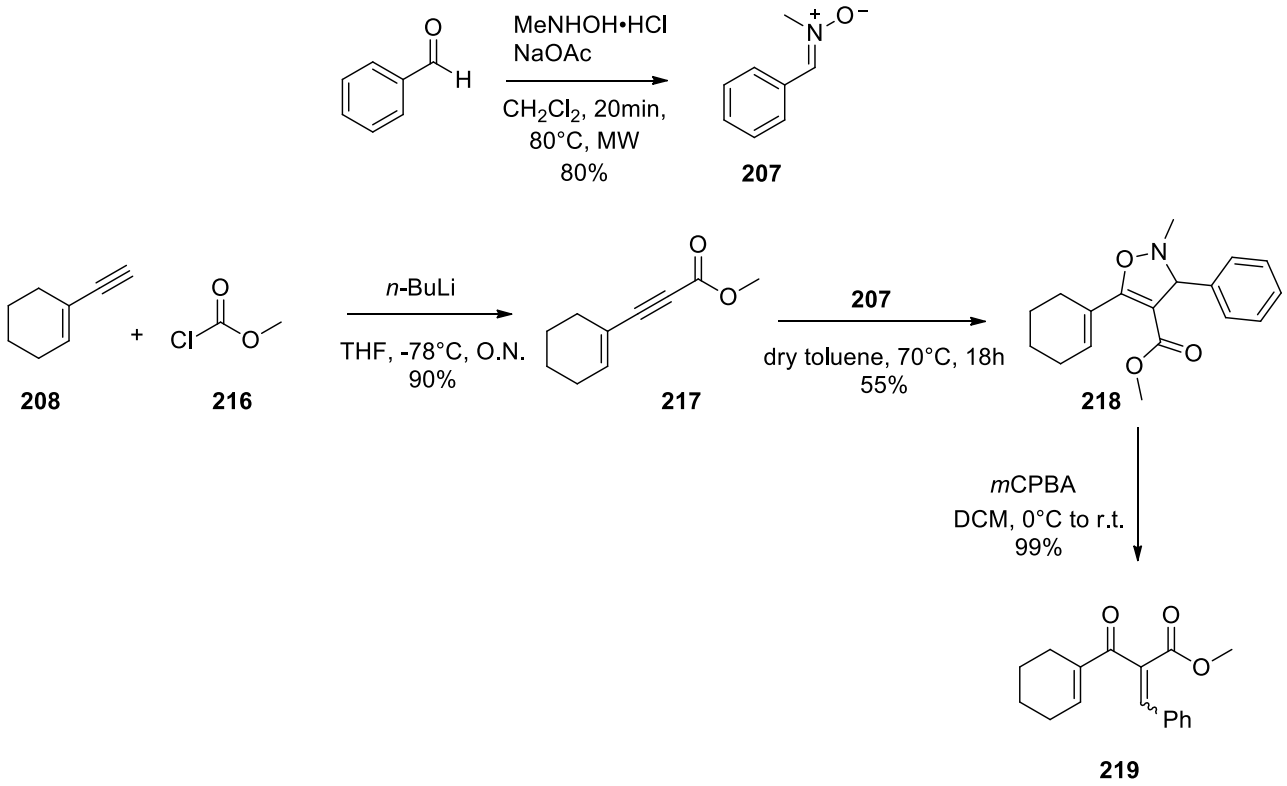
Also "blank" tests have been performed with $SiO_2$ and acidic $Al_2O_3$ and no Lewis acid supported catalyst (*entries 8* and *9*) resulting in no conversion of the starting material. Reactions under the described conditions are generally rather clean, and spots attributable either to the starting materials or to product **214** can be observed by TLC control, and no evidence of non-decarboxylated product **213** has been detected by TLC, NMR or GC-MS analysis.

*Table 4.1. Reaction conditions: in a vial for microwave the specified equivalents of promoting system were dissolved in the solvent and stirred for 15 minutes. After, 0.1 mmol of **212** were added and the reaction was left for 3 hours at 130°C in a microwave apparatus. [a]When inorganic support is used, the loading was 0.5g per 0.35 equivalents of $CeCl_3 \cdot 7H_2O$. [b]Isolated silica gel chromatography yield. [c] Reaction left for 6 hours instead of 3 hours.*

| Entry | Solvent | Promoting System [a] | Yield [b] |
|---|---|---|---|
| **1** | $CH_3CN$ | $CeCl_3 \cdot 7H_2O$ (1 equiv.) | 50% |
| **2** | Toluene | $CeCl_3 \cdot 7H_2O$ (1 equiv.) | 45% |
| **3** | $CH_3CN$ | $CeCl_3 \cdot 7H_2O$/NaI (1/1 equiv.) | 50% |
| **4** | $CH_3CN$ | $CeCl_3 \cdot 7H_2O$/NaI/Benzoic acid (0.3/0.3/0.3 equiv.) | 50% |
| **5** | $CH_3CN$ | $CeCl_3 \cdot 7H_2O$/NaI supported on $SiO_2$ (0.3/0.1 equiv.) | 85% |
| **6** | $CH_3CN$ | $CeCl_3 \cdot 7H_2O$ supported on $SiO_2$ (0.35 equiv.) | 88% as a mixture of **214** and **215** (55:45) |
| **7** | $CH_3CN$ | $CeCl_3 \cdot 7H_2O$ supported on acidic $Al_2O_3$ (0.35 equiv.) | 95% as a mixture of **214** and **215** (55:45) |
| **8** | $CH_3CN$ | $SiO_2$ | 0% |
| **9** | $CH_3CN$ | Acidic $Al_2O_3$ | 0% |
| **10** [c] | $CH_3CN$ | $CeCl_3 \cdot 7H_2O$ supported on $Al_2O_3$ (0.1 equiv.) | 95% as a mixture of **214** and **215** (55:45) |

As reported in paragraph 4.2.1, Akiyama and Bachu were able to tune the reaction condition of Nazarov cyclization on pyrrole derivatives by microwave irradiation to obtain the non-decarboxylated product, by lowering the temperature below 60°C and shortening the reaction times down to 6 hours.[166] However, by testing our substrate with $CeCl_3 \cdot 7H_2O$ supported on $Al_2O_3$ at 40, 60 and 80°C for several hours, we have not been able to promote the formation of product **213** and only unreacted starting material has been recovered from the reaction mixture.

To fully understand if the decarboxylation of the product was related to the nature of the ester group, we also tested the reaction condition (*entry 7*, *Table 4.1*) on the methyl ester **219**, synthesized following *Scheme 4.18*, starting from 1-ethynylcyclohexene **208** and methyl chloroformate **216** (*Scheme 4.23*).

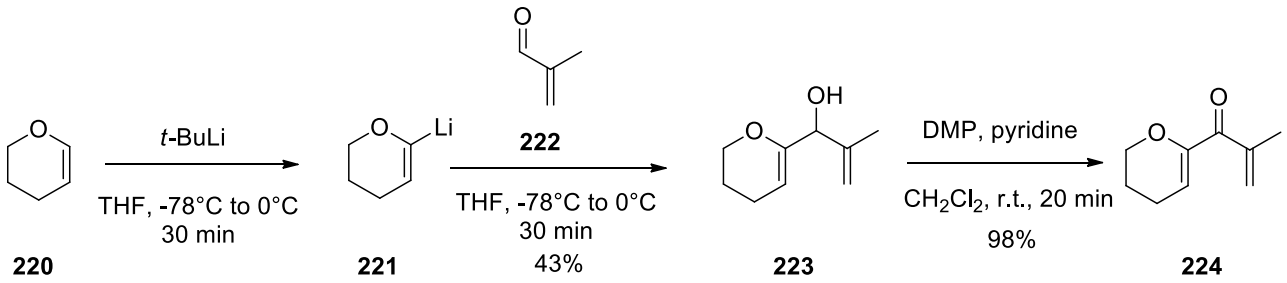



*Scheme 4.23.*

Using $CeCl_3 \cdot 7H_2O$ (0.35 equiv.) supported on acidic $Al_2O_3$ we had full conversion of the starting material into the two products **214** and **215** with the same ratio obtained with the ethyl ester (55%-45%). In order to conclude that the decarboxylation process is independent from the type of the ester moiety, few more tests also with bulkier groups should be carried out. However, it seems that the nature of the ester does not influence neither the outcome of the reaction, nor the ratio of the obtained mixture of products.

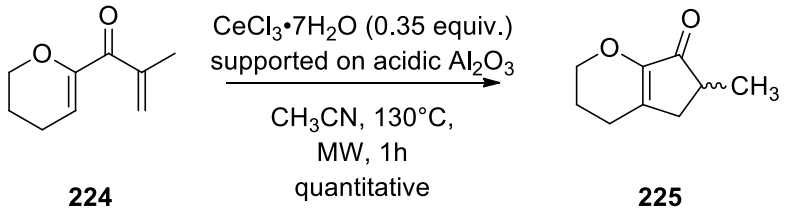
To check if our system was able to catalyze Nazarov cyclizations on less active substrates, namely without the presence of an ester moiety as EWG, we tested our conditions on 2-alkoxydienone **224**,

obtained following a reported procedure from 3,4-dihydro-2*H*-pyran **220** and *t*-BuLi to form the lithiated enol ether **221** followed by addition of 2-methylpropenal **222**, and subsequent oxidation of the resulting divinylcarbinol **223** using Dess-Marting periodinane (DMP) (*Scheme 4.24*).[189]




*Scheme 4.24.*

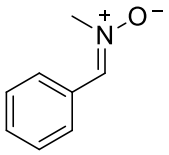
By treating substrate **224** with $CeCl_3 \bullet 7H_2O$ (0.35 equiv.) supported on acidic $Al_2O_3$ under microwave irradiation at 130°C, the reaction was completed within 1 hour, obtaining full conversion to product **225**.




*Scheme 4.25.*

## 4.5. Experimental protocols

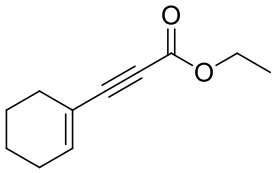
*N-benzylidenemethanamine oxide* (**207**)



**207**

Following a reported procedure,[190] a microwave vial has been charged with methylhydroxylamine hydrochloride (1 g, 12 mmol), $CH_2Cl_2$ (18 ml), NaOAc (1.178 g, 14.3 mmol, 1.2 equiv.). The mixture has been stirred at room temperature for 15 minutes. Benzaldehyde (0.990 ml, 14.3 mmol, 1.2 equiv.) was then added dropwise and the vial has been placed in a microwave apparatus at 80°C for 20 minutes. After the completion of the reaction checked by TLC (EtOAc:MeOH, 98:2) it has been filtered through a pad of celite and the solvent removed under reduced pressure. The crude product has been purified by silica gel chromatography (EtOAc:MeOH, 98:2 as eluent) to give product **207** in 80% yield. Spectral data are in good agreement with those previously reported.[190] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 3.86 (s, 3H), 7.35 (s, 1H), 7.45 (d, J = 2.4 Hz, 2H), 7.46 (d, J = 0.8 Hz, 1H), 8.20 (dd, J = 3.0, 6.5 Hz, 2H) . *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 54.2, 128.6, 128.7, 130.4, 135.5.

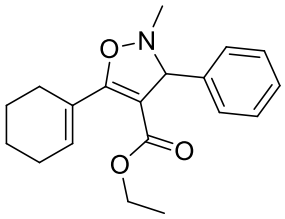
*Ethyl 3-(cyclohex-1-en-1-yl)propiolate* (**210**)



**210**

Following a reported procedure,[191] a 3-neck round bottomed flask under nitrogen atmosphere was charged with dry THF (14 ml) and 1-ethynylcyclohexene (1.5 g, 1.875 ml, 0.014 mol). After cooling to -78°C, *n*-BuLi in hexanes (2.5M, 1.32 ml, 1.05 equiv.) was added dropwise and the reaction mixture was stirred for 30 minutes at the same temperature. After, ethyl chloroformate (1.512 g, 1.32 ml, 1.0 equiv.) was added. The reaction was left at -78°C for 2 hours and allowed to warm to room temperature overnight. After quenching with saturated aqueous ammonium chloride (50 ml) it was extracted in dichloromethane (3 x 20 ml) and the combined organic phase dried over $Na_2SO_4$, filtered and concentrated under vacuum. Silica gel chromatography purification of the crude product (hexanes:EtOAc, 95:5 as eluent) afforded compound **210** in 90% yield. Spectral data are in good agreement with those previously reported.[192] ] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.28 (t, J = 7.1 Hz, 3H); 1.61-1.73 (m, 4H),2.10- 2.19 (m, 4H), 4.35 (q, J = 7.1 Hz), 6.54–6.65 (m, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 13.9, 21.5, 22.1, 26.4, 27.8, 62.0, 78.9, 88.7, 118.9, 142.3, 155.5.

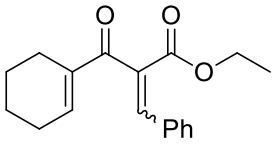
*5-Cyclohex-1-enyl-2-methyl-3-phenyl-2,3-dihydro-isoxazole-4-carboxylic acid ethyl ester* (**211**)



**211**

Following a reported procedure,[193] compound **210** (780 mg, 4.37 mmol) was dissolved in toluene (3.6 ml) in a flask under nitrogen atmosphere. Nitrone **207** (1.18 g, 8.74 mmol, 2 equiv.) was added and the mixture was heated to 75°C and left stirring for 18h. After completion, indicated by TLC (hexanes: EtOAc, 95:5 as eluent), the solvent was removed under vacuum, and the crude product purified by silica gel chromatography (hexanes: EtOAc, 95:5 as eluent) to give compound **211** in 50% yield. Spectral data are in good agreement with those previously reported.[193] *$^{1}$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.18 (t, J = 7.2 Hz, 3H), 1.63-1.70 (m, 4H), 2.27-2.55 (m, 4H), 2.99 (s, 3H), 5.01 (s, 1H), 6.43 (m, 1H), 7.31-7.38 (m, 5H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 14.1, 21.7, 22.4, 25.8, 26.4, 47.2, 59.9, 76.6, 102.6, 126.5, 127.4, 127.9, 128.6, 136.3, 141.9, 164.4.

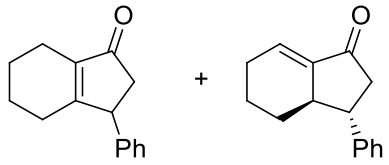
*2-(Cyclohex-1-enecarbonyl)-3-phenyl-acrylic ethyl ester* (**212**)



**212**

Following a reported procedure,[187] *m*-CPBA (148 mg, 0.858 mmol, 1.4 equiv.) was added to a solution of isooxazoline **211** (153 mg, 0.613 mmol) in dry DCM (5 ml) at 0°C. The reaction was stirred at the same temperature until complete by TLC (hexanes:EtOAc, 90:10 as eluent), about 10 minutes. The reaction was diluted with diethyl ether, washed with 1M NaOH, and the organic layer was dried over $Na_2SO_4$ and concentrated. Silica gel column chromatography (hexanes:EtOAc, 95:5 as eluent) gave the desired product in 99% yield. Spectral data are in good agreement with those previously reported.[187] *$^{1}$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.26 (t, J = 7.2 Hz, 3H), 1.55-1.66 (m, 4H), 2.10 (m, 2H), 2.33 (m, 2H), 4.35 (q, J = 7.2 Hz, 2H), 6.88 (m, 1H), 7.34-7.37 (m, 5H), 7.83 (s, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 14.3, 21.5, 21.7, 22.6, 25.9, 61.4, 128.5, 129.6, 129.8, 131.9, 133.0, 139.4, 141.3, 144.5, 166.1, 197.2.

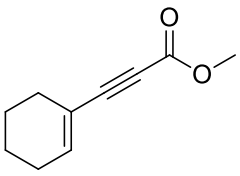
*3-Phenyl-2,3,4,5,6,7-hexahydro-1H-inden-1-one* (**214**) and *2,3,3a,4,5,6-hexahydro-3-phenylinden-1-one* (**215**)




**214** **215**

The solid supported promoting system was obtained by adding to a mixture of $CeCl_3 \bullet 7H_2O$ (130 mg, 0.35 mmol) in acetonitrile (15 ml) the inorganic support (acidic $Al_2O_3$ Fluka, activated acidic, Brockman activity, grade I, 150 mesh, 0.5g). The suspension was stirred overnight at room temperature and then the solvent removed under vacuum. The powder thus obtained (55 mg, 0.3 equiv.) was added in a microwave vial to a solution of dienone **212** (30 mg, 0.11 mmol) in $CH_3CN$ (2 ml) and left in a microwave apparatus for 3 hours at 130°C. After completion by TLC (hexanes:EtOAc, 9:1 as eluent), the reaction was filtered through a pad of celite, washed with DCM and the solvent removed under reduced pressure. The crude product was purified by silica gel chromatography (hexanes:EtOAc, 9:1 as eluent) to give **214** in 52% yield and **215** in 42% yield. **214**: spectral data are in good agreement with those previously reported.[194] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.65 – 1.71 (m, 4H), 2.01 – 2.10 (m, 2H), 2.17 – 2.25 (m, 2H), 2.41 (dd, J = 2.2, 18.7 Hz, 1H), 2.92 (dd, J = 6.8, 18.7 Hz, 1H), 3.81– 3.82 (m, 1H), 7.08 – 7.11 (m, 2H), 7.24 – 7.27 (m, 1H), 7.29 – 7.33 (m, 2H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 20.5, 21.9, 22.3, 27.0, 45.4, 47.9, 127.2, 127.5, 1298.8, 139.4, 142.5, 175.0, 208.2. **215**: spectral data are in good agreement with those previously reported.[195] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.08-1.16 (m, 1H), 1.45-1.55 (m, 1H), 1.86-1.91 (m, 1H), 2.04 (m, 1H), 2.15-2.84 (m, 1H), 2.30-2.38 (m, 1H), 2.61 (dd, J = 12.5, 17.7 Hz, 1H), 2.70 (dd, J = 7.4, 17.7, 1H), 2.75 (m, 1H), 2.86 (m, 1H), 6.78 (m, 1H), 7.26-7.41 (m, 5H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 21.5, 25.6, 27.3, 45.8, 46.2, 48.0, 127.3, 127.5, 129.0, 133.2, 140.9, 141.9, 205.1.

*Methyl 3-cyclohexenylpropiolate* (**217**)

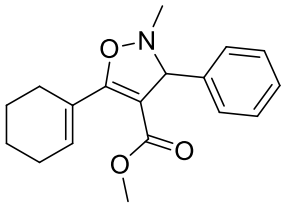



**217**

A 3-neck round bottomed flask under nitrogen atmosphere was charged with dry THF (14 ml) and 1-ethynylcyclohexene (1.5 g, 1.875 ml, 0.014 mol). After cooling to -78°C, *n*-BuLi in hexanes (2.5M, 1.32 ml, 1.05 equiv.) was added dropwise and the reaction mixture was stirred for 30 minutes at the same temperature. After, methyl chloroformate (1.323 g, 1.08 ml, 1.0 equiv.) was added. The reaction was left at -78°C for 2 hours and allowed to warm to room temperature overnight.

After quenching with saturated aqueous ammonium chloride (50 ml) it was extracted in dichloromethane (3 x 20 ml) and the combined organic phase dried over $Na_2SO_4$, filtered and concentrated under vacuum. Silica gel chromatography purification of the crude product (hexanes:EtOAc, 95:5 as eluent) afforded compound **217** in 90% yield. Spectral data are in good agreement with those previously reported.[196] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.53-1.70 (m, 4H), 2.10 (m, 4H), 3.81 (s, 3H), 6.35 (m, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 21.3, 21.9, 26.2, 28.2, 52.8, 80.1, 88.5, 119.5, 139.2, 156.3.

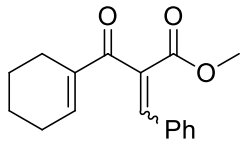
*Methyl 5-cyclohexenyl-2-methyl-3-phenyl-2,3-dihydroisoxazole-4-carboxylate* (**218**)



**218**

Following a reported procedure,[193] compound **217** (500 mg, 3 mmol) was dissolved in toluene (2.5 ml) in a flask under nitrogen atmosphere. Nitrone **207** (810 mg, 6 mmol, 2 equiv.) was added and the mixture was heated to 75°C and left stirring for 18h. After completion, indicated by TLC (hexanes: EtOAc, 95:5 as eluent), the solvent was removed under vacuum, and the crude product purified by silica gel chromatography (hexanes: EtOAc, 95:5 as eluent) to give compound **218** in 55% yield. *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.26-1.31 (m, 2H), 1.67-1.72 (m, 4H), 2.22-2.25 (m, 2H), 2.91 (s, 3H), 3.60 (s, 3H), 4.94 (s, 1H), 6.52-6.54 (m, 1H), 7.26-7.34 (m, 5H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 22.1, 22.7, 25.9, 26.8, 47.3, 52.2, 74.2, 101.8, 126.6, 127.3, 127.9, 128.4, 136.7, 138.2, 143.1, 165.0

*Methyl 2-(cyclohex-1-enecarbonyl)-3-phenylacrylate* (**219**)

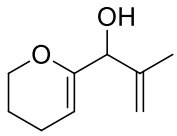


**219**

Following a reported procedure,[187] *m*-CPBA (97 mg, 0.56 mmol, 1.4 equiv.) was added to a solution of isooxazoline **218** (120 mg, 0.4 mmol) in dry DCM (4 ml) at 0°C. The reaction was stirred at the same temperature until complete by TLC (hexanes:EtOAc, 90:10 as eluent), about 10 minutes. The reaction was diluted with diethyl ether, washed with 1M NaOH, and the organic layer was dried over $Na_2SO_4$ and concentrated. Silica gel column chromatography (hexanes:EtOAc, 95:5 as eluent) gave the desired product in 99% yield. Spectral data are in good agreement with those previously reported.[197] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.55-1.70 (m, 4H), 2.07-2.14 (m, 2H), 2.31-2.40 (m, 2H), 3.81 (s,

3H), 6.82-6.84 (m, 1H), 7.30-7.35 (m, 5H), 7.80 (s, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 21.1, 21.8, 23.1, 26.7, 54.2, 128.6, 130.1, 130.8, 131.8, 133.5, 139.3, 142.0, 145.9, 166.0, 196.6.

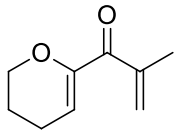
*1-(5,6-Dihydro-4H-pyran-2-yl)-2-methyl-prop-2-en-1-ol* (**223**)



**223**

Following a reported procedure,[189] to 0.500 g (5.94 mmol) of dihydropyrane in THF (0.3 ml) was added a solution of *t*-BuLi (1.7M in pentane, 3.84 ml) dropwise at –78 ºC. The reaction mixture was warmed to 0 ºC. After the reaction mixture was stirred for 30 min at 0 ºC and treated with further 0.2 mL of THF, the reaction mixture was cooled back to –78 ºC and 2-methylpropenal (0.480 g, 6.53 mmol, 1.1 equiv) was added dropwise. The reaction mixture was allowed to warm to 0 ºC. Upon reaching 0 ºC, the reaction was quenched with water (50 mL) and diluted with EtOAc (100 mL). The two layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried, filtered and concentrated in vacuo. The product was purified by column chromatography (hexanes:EtOAc, 6:1 as eluent) to afford compound **223** in 43% yield. Spectral data are in good agreement with those previously reported.[189] *$^{1}$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.65 (s, 3H), 1.78-1.85 (m, 2H), 2.01-2.06 (m, 2H), ), 2.31 (d, J = 6.1 Hz, 1H), 4.05-4.12 (m, 2H), 4.4 (d, J = 6.0 Hz, 1H), 5.01 (t, J = 3.8 Hz, 1H), 4.98-5.02 (m, 1H), 5.18 (s, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 19.2, 20.8, 22.58, 66.34, 78.2, 98.6, 111.4, 145.3, 156.5.

*1-(5,6-Dihydro-4H-pyran-2-yl)-2-methyl-propenone* (**224**)

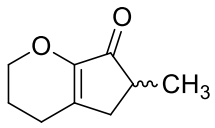


**224**

Following a reported procedure,[189] to a solution of **223** (0.2 g, 1.31 mmol) and pyridine (1 ml) in $CH_2Cl_2$ (25 ml) was added Dess Martin periodinane (0.676 g, 1.38 mmol, 1.05 equiv.) at 23 °C. After 20 min, the reaction was quenched with a solution of water and aqueous 6N NaOH (1:1, 20 ml). The mixture was stirred vigorously for further 10 min. The two layers were separated, and the aqueous layer was extracted with $CH_2Cl_2$. The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated in vacuo. The crude product was purified by column chromatography (hexanes, EtOAc, 6:1 as eluent) to afford **224** in 98% yield. Spectral data are in good agreement with those previously reported.[189] *$^{1}$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.88-1.95 (m, 2H), 2.01 (t, J = 1.1 Hz, 3H), 2.25-2.29 (m, 2H),

4.28 (t, J = 5.1 Hz, 2H), 5.63 (t, J = 1.5 Hz, 1H), 5.85 (s, 1H), 6.12 (t, J = 4.0 Hz, 1H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 17.1, 20.8, 22.5, 66.4, 116.2, 127.6, 141.6, 150.9, 193.1.

*6-Methyl-3,4,5,6-tetrahydro-2H-cyclopenta[b]pyran-7-one* (**225**)



**225**

The solid supported $CeCl_3$•$7H_2O$ over acidic $Al_2O_3$ (55 mg, 0.35 equiv.) was added in a microwave vial to a solution of dienone **224** (30 mg, 0.2 mmol) in $CH_3CN$ (2 ml) and left in a microwave apparatus for 3 hours at 130°C. After completion by TLC (hexanes:EtOAc, 6:1 as eluent), the reaction was filtered through a pad of celite, washed with DCM and the solvent removed under reduced pressure. The crude product was purified by silica gel chromatography (hexanes:EtOAc, 6:1 as eluent) to give **225** in 99% yield. Spectral data are in good agreement with those previously reported.[189] *$^1$H NMR:* (400 MHz, $CDCl_3$) $\delta_H$ = 1.18 (d, J = 7.3 Hz, 3H), 1.92-1.98 (m, 2H), 2.1 (m, 1H), 2.35 (t, J = 6.2 Hz, 2H), 2.51 (m, 1H), 2.70 (m, 1H), 4.28 (m, 2H). *$^{13}$C-NMR* (100 MHz, $CDCl_3$) $\delta_C$ = 16.4, 20.8, 22.9, 32.4, 37.5, 66.8, 141.1, 155.2, 204.3.

## 4.6. Conclusions

The Nazarov cyclization is a powerful way to construct five membered ring motifs through a 4π electrocyclization of cross-conjugated linear dienones to synthesize cyclopentenones. In the last thirty years, this transformation has gained great importance in organic synthesis because it combines the recent raise of interest in the total synthesis of natural products with a remarkable ability in building rings that are part of many biologically active natural products.

The Nazarov reaction can be efficiently promoted by either stoichiometric or catalytic amount of Brønsted or Lewis acids.

Therefore, many methods and promoting systems of this reaction have been developed during the years, with different Lewis acids such as $BF_3$ or scandium and silver triflates. Although in the literature it is reported the use many of these acids as their chlorine salts, such as $AlCl_3$, and even with transition metal cations, like $FeCl_3$ or $TiCl_4$, data using $CeCl_3$ as Lewis acid are almost non-existent, many of which stating a scarce activity of this specie in promoting the Nazarov transformations.

In the laboratory of prof. Marcantoni we have developed and optimized the reaction conditions to promote a Nazarov reaction using $CeCl_3 \bullet 7H_2O$ supported on acidic $Al_2O_3$ as promoting system, obtaining good preliminary results with conversions up to 95%. Some examples have been reported by varying the starting material from ethyl to methyl ester and an excellent conversion has also been observed with less activated dienones.

In the research group we are still working to expand the scaffold of products that can be obtained, also testing aromatic and heteroaromatic substrates and trying to apply these conditions to obtain useful intermediates in the synthesis of pharmaceutical relevant products.

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