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SCHOOL OF ADVANCED STUDIES

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Sustainable Approaches for the Synthesis of Nitrogen and Oxygen Functionalized Small Organic Molecules

Cycle XXXIV

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PREFACE

The term “Small Molecule”, within the fields of molecular biology and pharmacology, indicates a group of natural/synthetic compounds that possess a molecular weight that does not exceed about 900 daltons.[1] This type of molecules held great attention for the chemists and biochemists due to their potent biological activity, therapeutic applications and effectiveness on the functions of macromolecules in living systems,[2] employment as additives in photovoltaic panels,[3] for the crystallization of proteins[4] and as semiconductors in transistor systems;[5] bioimaging in complex systems;[6] anticancer,[7] and many others. In addition, small molecules as drugs offers several advantages over traditional protein design: (i) high practicability due to a detailed understanding and historical precedents of their clinical application and development; (ii) oral bioavailability (as opposed to infusion therapy); (iii) they can across physiological barriers (iv) access to intracellular disease targets when they cannot be tractable by protein therapeutic agents; (v) well-understood formulation and dosage; (vi) relative inexpensive treatment.[8] Three different sources can be distinguished for obtaining small molecules: (i) naturally available sources; (ii) commercial sources; (iii) synthetic pathways[9] and this last one is a very powerful instrument to replicate natural molecules and/or obtain new ones. Synthetic chemists can follow three routes to obtain these special targets. The first one exploits target-oriented synthesis (also called TOS) that consists, first, on discovery of natural molecules with specific properties, then, once identified, extracted, isolated and elucidated their structure by spectroscopic techniques, chemists develop new synthetic pathways for them. The second one is based on medicinal and/or combinatorial chemistry that leads to develop a new synthethical pathway of analogues from or a new rationally designed structured molecules, developed from a mechanistic hypothesis and/or a crystal structure of a macromolecule of interest. The last one is the DOS way (Diversity Orientated Synthesis) which aims to develop new pathways leading to the efficient (three- to five-step) synthesis of small molecules having skeletal and stereochemical diversity with defined coordinates in chemical space.[10]

During the last two decades, the scientist has focused their efforts on developing new sustainable synthetic approaches.[11] In fact, “*How getting there*” has become as important as the “*getting there”*, so chemists has begun to realize about the environmental impact that different chemical process can have.[12] For these reasons the

employment of microwaves irradiations (for saving energy), use of more environmental friendly and safer catalysts, reagents, solvents (or solventless reactions), phase-transfer catalysts and supported catalyst has begun more exploited for the development of new synthetic protocols.[13]

Among small molecules, O- and N- functionalized molecules play a prominent role. Such functionalized molecules are widely distributed in nature and are found in many natural compounds such as alkaloids, polyphenols, sugar unit of nucleotides, nucleobases, vitamins, and many others, and the biological properties and relevance of such compounds are well-known today.[14-17] The synthesis of these compounds has always been an important focus of synthetic chemistry and nowadays many strategies are reported in literature, also with the purpose to obtain them in a more sustainable way.[18], [19]

This work was carried out in the research group of Prof. Enrico Marcantoni from December 2018 to February 2022, at Univerisity of Camerino, with the goal to achieve new synthetic pathways for O- and N-functionalized small molecules with different biological and industrial applications.

The work is divided in two main macro-chapters in which the focus of the first one is the synthesis of Oxa-functionalized small molecules, while the second one focuses on the synthesis of Aza-heterocycles. Each chapter is introduced by a general abstract about the topic, then for each section a first part about the state of art of the topic is present and the second part reports about results obtained about it.

The first chapter is divided in two sections: (i) the first one is focused on the chemical modifications of Adenosine with the purpose to synthesize the LNA derivative by linear strategy; (ii) while the second section is focused in the synthesis a new phenolic molecule with potential antiviral activity against SARS-Cov-2.

The second chapter is divided in three sections: (i) the first focuses on the synthesis of 9-membered-triaza-macrocycle that possess industrial applications, restyling the method reported in the patents and literature; (ii) the second one focuses on the synthesis of aza-heterocycles starting from acyclic precursors, like primary amines and aldehydes, studying in deep mechanistic and thermodynamic aspects, evaluating the possible employment of new relative not toxic catalysts and reagents; (iii) the third section reports the results about the Brønsted and Lewis Acid promotion of Pictect-Spengler Reaction

for the synthesis of tetrahydro-β-carbolines, with the purpose to use not toxic and easy handle promoters.

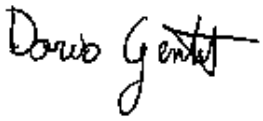
“Chemistry is the study of substances, but I prefer to see it as the study of changes. For example, think about this: electrons, they change their energy levels; the molecules ... the molecules change their bonds; elements ... combine and change into compounds. Well, this ... this is life, right? That is, it is just ... it is the constant, it is the cycle: creation and dissolution, then again creation then again dissolution, it is growth then decay, then transformation! And it's fascinating”

Walter White ~ Breaking Bad

AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the university's Regulation and Code of Practice for School of Advanced Studies, and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of others is indicated as such. Any views expressed in the dissertation are those of the author.

Signature of Candidate Date 27/02/2022



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Chapter 1

Synthesis of O-funcionalized Small Molecules with biological activity.

Abstract

Two important categories of O-functionalized compounds are O-heterocycles and polyphenols. All these two categories are widespread in nature and present as scaffold in many natural compounds. Well-known examples are sugar core of DNA and RNA, Quercitin, Curcumin, Vitamins (especially E and C), Taxol, Dynemicin, and many others; and their biological applications are well-known too (anti-inflammatory, anti-oxidant, antiviral, anticancer and others). The synthesis of O-functionalized compounds has been always an important and crucial research field of organic synthesis and nowadays many methods are reported in literature.[20 - 25]

This first chapter is focused on two different projects related together by the O-functionalization of final target products and by the very important biological, antiviral, therapeutical applications that they found or they could find.

The aim of first section is to synthesize LNA-Adenosine derivative by linear strategy. The LNA (Locked Nucleic Acid) are very important Nucleic Acid analogues, synthesized for the first time by Iminashi *et al.*[26] and Wengel *et al.*[27] respectively in 1997 and 1998, in an independently way. These analogues are very important due to the antisense, antigene, antiviral, and many others bio-applications, overcoming some problems related to employment of not-modified nucleic acids.

The topic of the second chapter is the synthesis of a new phenolic compound with potential antiviral activity against Sars-CoV-2. Our purpose is to obtain this new polyphenol through the combination of two intermediates, an alkyne and an aldehyde, obtained from two commercial, cheap and easy available starting material.

1.1 LNA: Synthesis of Nucleic Acid Analogue with potent biological activities

1.1a Improvement of Chemical Modification on Nucleic Acids

DNA and RNA are the building blocks of every known form of life. These natural polymeric macromolecules are composed by monomers called nucleotides, which are made up by three main parts: (i) the nitrogenous base (Thymine only for DNA), Guanine, Uracil (only for RNA), Adenine, Cytosine, (ii) β-D-ribose sugar core, which can be simple ribose (RNA) or deoxyribose (DNA), (iii) the triphosphate moiety which links all nucleotides togheter by a phosphodiester bond.

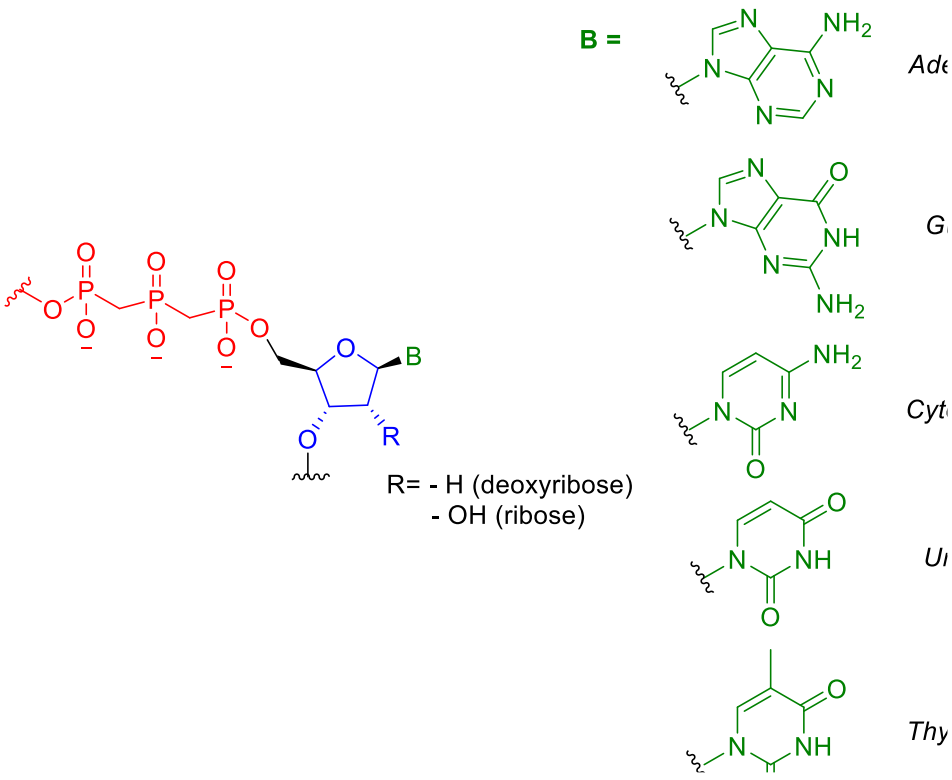


Figure 1.1.1. Structure of DNA and RNA Nucleotides

Nowadays, long-term storage role of DNA is well-known. In fact, all instructions for building all components of cells, proteins and "RNA" molecules are stored in very long DNA-filaments called chromosomes. DNA usually is compared to blueprint for this

reason. Meanwhile, even if there are many structure-similarities between DNA and RNA, they differ for some and important aspects:

- RNA is composed of ribose core nucleotides
- Uracil is present as nitrogenous base instead of Thymine
- RNA is present as single strand, whereas DNA is present in double strand

Unlike DNA, RNA is not involved in the long-term storage of genetic informations, but rather in their expression, actually it is involved in the processes of coding (rRNA), decoding, transportation (tRNA) and transmission of information to ribosomes (mRNA). The discovery of "nuclein" by Friederich Miescher in 1869 during his work on composition of lymphoid cells (white blood cells) gave the first impetus to the field of research about nucleic acids, named in this way by Richard Altmann in 1889.[28] Only nine years after from Miescher's discovery, Albert Kossel published a paper confirming the separation and the analysis of xanthine and hypoxanthine[29] (two natural analogues of guanine), and after more than sixty years, in 1929, Phoebus Levene defined the three main components that make up the DNA and the structure of nucleotides: sugar core, the four nitrogenous bases and the phosphate linkage, also revealing the order phosphate-sugar-base sequence, suggesting that phosphoester moiety links together all nucleotides together constituting the so-called "backbone".[30] However, it was not until 1953 Watson and Crick thanks to the crystallography work of Rosalind Franklin and Maurice Wikins, were able to set up the helical structure of DNA duplex, confirming the famous base pairing A:T and C:G and also the sugar-phosphate core.[31]

Since that time many other discoveries about DNA/RNA have been made. Because of the very important features of DNA and RNA, some scientists had supposed to use oligonucleotides in biomedical fields. In 1978 Zamecnik and Stephenson suggested the possible application of oligonucleotides on controlling of gene expression.[32] But unfortunately, the direct application of unmodified DNA/RNA oligonucleotides is unsuitable due to their intrinsic instability in biological media (attacked by nuclease) and for the poor affinity toward the complementary target sequences.[33]

Therefore many scientists focused their effort to chemically modify the nucleotides itself with the purpose to improve their physiological and biochemical properties, specificity and affinity toward to target sequences.[34] Nucleic acids can be modified at three different levels: phosphodiester backbone, nucleobases and the sugar core.

BACKBONE MODIFICATION

The backbone modification has been studied since 1966. At that time Eckstein reported a chemical modification of the phospho-interlinkage, in which the substitution of an oxygen atom by one sulphur atom leads to the formation of a phopshothioate nucleotides that was unexpectedly resistant to phosphatases.[35] Today this modification is widely used in biomedical field, and experimental data show higher cellular uptake and bioavability *in vivo* of this class of oligomers.[36] Many other interlinkage modifications have been reported in literature such as methylphosphonate,[37] borane phosphonate,[38] phosphoro amidates.[39]

PNAs (Peptide Nucleic Acid) are famous backbone modified oligonucleotides. They consist of a pseudo peptide skeleton (N-2-aminoethylglycine repeating units) to which nucleobases are attached. Despite the deep differences between DNA and PNA, this last one forms higher stable duplex due to the lack of electrostatic repulsions, undergoing also to Watson-Crick base pairing with DNA and RNA. Nowadays PNA-oligonucleotides are widely used in antisense and antigene therapy[40] and they show potent and selective gene inhibition.[41] "Antisense" refers to all interferences of processes in which RNA is involved. These include the inhibition and/or alteration of splicing, translational arrest and degradation of mRNA. Interference with dsDNA processes, on the other hand, is grouped under the name "antigene effect".Major drawbacks of PNA are due to lipophilic and neutral nature of backbone. The peptide skeleton makes the PNA oligomers only partially soluble in water, in addition, these oligomers cannot across the lipid membranes of cells, making the delivery to target a very difficult task, making it necessary the development of many delivery system.[42]

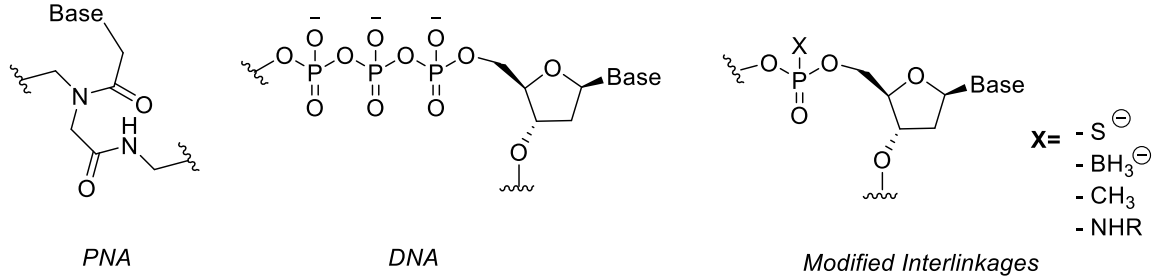


Figure 1.1.2. Backbone modified Oligonucleotides

NUCLEOBASE MODIFICATION

The modification of nitrogenous bases aims to expand the π-electron cloud of bases by increasing the affinity for the target sequence,[40] moreover, the dipole-dipole stacking

interactions enhances duplex stability.[41] By adding a propynyl moiety in position-7 of 7-deaza-2'-deoxyguanosine, Buhr *et al.* detected an improvement in the antisense activity of oligonucleotides containing these modified nucleotides; on the other hand, they detected a contrary result for 7-propynyl-7-deaza-2'-deoxyadenosine.[45] On the other hand, the addition of an amino group in position-2 of adenosine/7-deaza adenosine greatly enhance the affinity toward guanine.[46] The simultaneous modification of position-7 and addition of amino group in position-2 of 7-deaza adenosine increases the antisense activity, affinity and thermal stability of oligodeoxynucleotides, especially when a 7-propynyl or 7-iodo moiety were employed.[44]

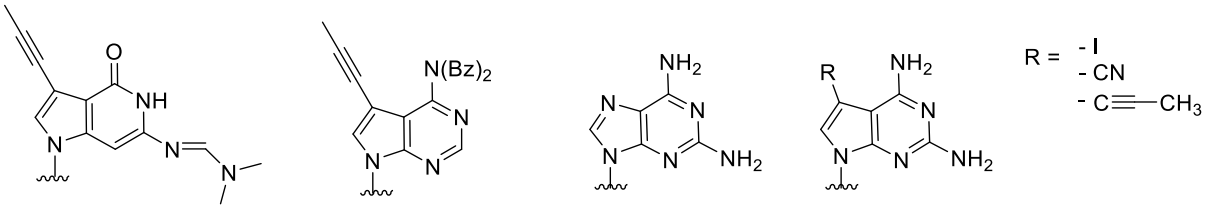


Figure 1.1.3. Some modified Nucleobases reported in literature

SUGAR MODIFICATION (LNA)

Nowadays many chemical modifications of the sugar core are reported in literature, especially on 2'-position of the furanose ring as which proven to be a very useful in enhancing the stability, pharmacokinetic and drugs-like properties.[47] The insertion of –OMe[48] and –F moiety[49] in position C-2' improved the stability and affinity toward RNA and DNA sequences. However, these modifications did not confer the necessary metabolic stability, and the size of 2'-substitutent destabilises the binding affinity toward the complementary RNA sequence.[50]

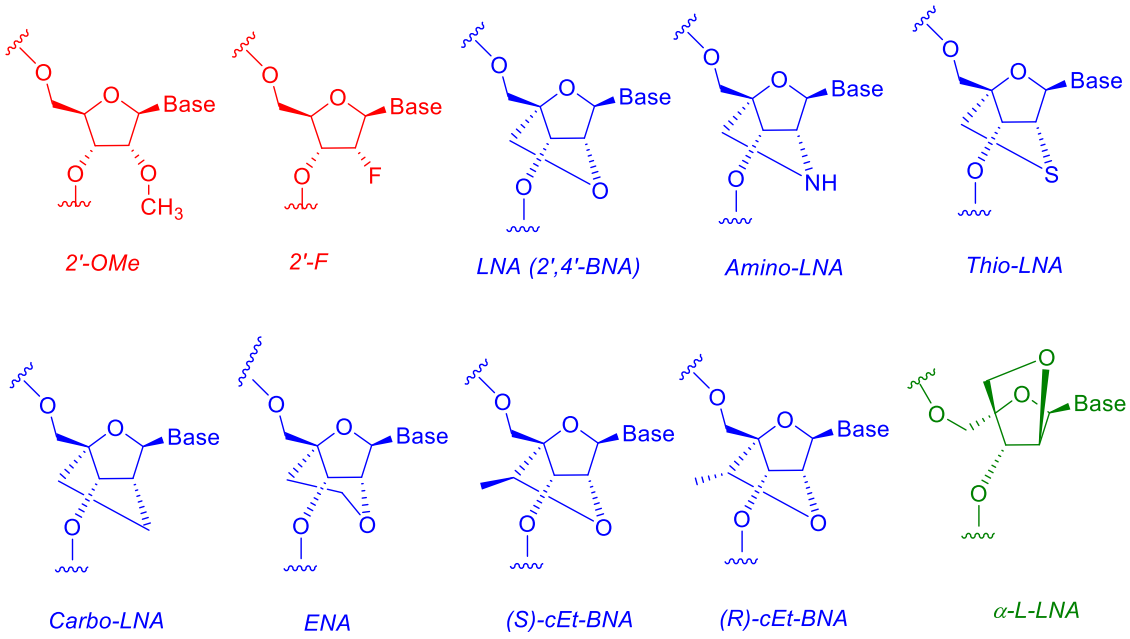
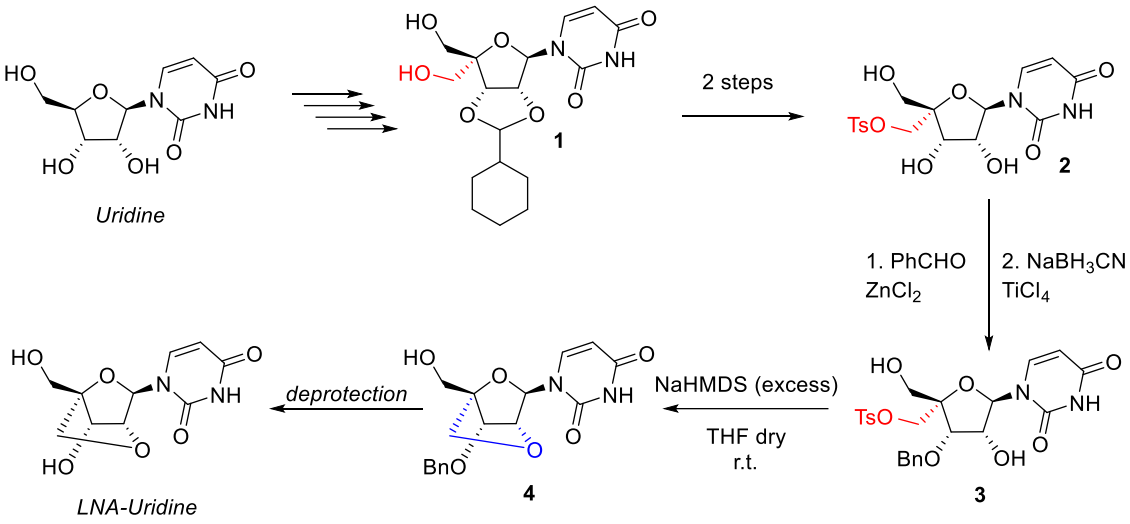


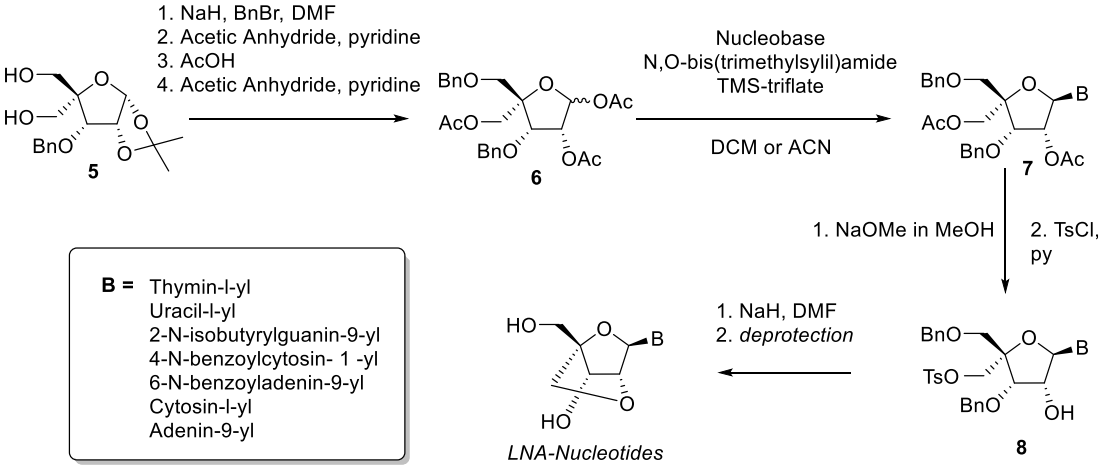
Figure 1.1.4. Some sugar-modified nucleotides reported in literature

In 1997 and in 1998 Iminashi[26] and Wengel[27] reported for the first time a synthetic methodology in which they synthesized a new bicyclic structure, analogue of ribose nucleotide that possess very high affinity and thermal stability. This new type of compound, called Locked Nucleic Acid (LNA, *figure 1.1.4.*), has a 2'-O,4'-methylene ring. This new class of compounds can be obtained by two possible strategy: (i) the first one is called "linear strategy", in which the chemical modifications are performed directly on nucleotides; (ii) the second is called "convergent strategy", in which the final analogue is achieved after the coupling between the modified sugar moiety and the nucleobase. Iminashi *et al.* reported a linear strategy for Uridine-LNA and Cytosine-LNA.[26] Starting from Uridine, they obtained the nucleotide **1** by protecting the two hydroxyl groups present in C-2' and C-3' and forming the $-CH_2OH$ by several steps. After the selective tosyl-protection of new $-CH_2OH$, the further steps were necessary to perform the selective protection of –OH present in C-3', performed by the reaction of **2** and benzaldehyde and a consecutive selective cleavage of C-O bond in 2'-position by sodium cyanoborohydride and $TiCl_4$. Then the LNA-Uridine was achieved in alkaline conditions using a large excess of NaHMDS (3 equivalents) in THF with a yield of 61% at room temperature starting from **3** and a consecutive deprotection of **4** (*Scheme 1.1.1.).*



Scheme 1.1.1. Linear Synthesis of Uridine-LNA by Iminashi and coworkers

Wengel *et al.*[27] have prepared various LNA-nucleotides starting from 4'-C-hydroxymethyl pentofuranose derivative **5** by convergent strategy. Their synthetic strategy foresees modification of **5** to give the intermediate **6** after the regioselective 5-O-benzylation, acetylation, and acetolysis followed by further acetylation. This intermediate **6** is the key to success of strategy. In fact, this one is employed for the stereoselective coupling with the bases, performed in presence of N,O-bis(triemthylsylil)amide and TMS-triflate, further, the nucleotide intermediate **7** is deacetylated and tosylated in selective way and the final LNA-nucleotide is obtained after reaction of **8** and NaH in DMF (*Scheme 1.1.2.*).



Scheme 1.1.2. Convergent synthesis for LNA-Nucleotides by Wengel and coworkers.

The term Locked Nucleic Acid refers to the lack of flexibility of furanose ring due to the new 2'-O,4'-methylene ring. This bicyclic structure favourites the N-type pucker sugar,

also called *C3'-endo* conformation and mimics the standard structure of RNA monomers.[51]

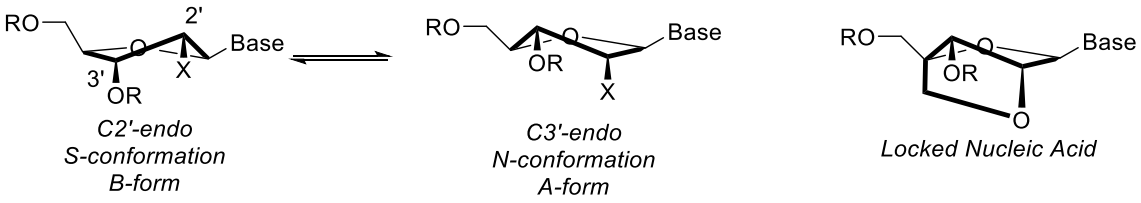


Figure 1.1.5. Pucker Sugar conformation of LNA

In addition to the numerous therapeutic applications (those will be discussed later), LNA-oligonucleotides display very high stability in serum medium, low toxicity, good solubility in water and Watson-Crick mode of binding.[33] Studies about thermal stability, containing a range of 6/14 of LNA-monomers, shown a remarkable increase of melting temperature: ~+1°C to +8°C against DNA and ~+2°C to +10°C against RNA per LNA-monomer.[52] The observed relative increase in thermal stability of LNA/DNA chimera, compared to native reference duplexes, reaches the maximum for chimera containing <50% LNA monomers, this fact is a direct consequence of the ability of LNA monomers to steer the conformation of neighboring DNA monomers into N-type conformations.[53] It is also very important to avoid a too large presence of LNA-monomers. For fully LNA-oligonucleotides, the very strong base pairing leads to the formation of LNA:LNA duplexes by self-assemblement, estimating a melting temperature of Tm>93°C.[54]

Oligonucleotides containing LNA-monomers have very high nuclease resistance and biostability in blood serum, which is necessary for antisense applications. In a study conducted by Wahlestedt *et al.* found a longer half-life of mix-mer containing LNA-monomers with respect to isosequential DNA and PS (phosphorothioate) segments. This fact could be explained by the high base pairing affinity of LNA-monomers that causes a block of RNA-processing enzyme.[51] LNA/DNA mixmers show a monomer position-dependence to nuclease resistance, i.e. oligomers with LNA-monomers stuck in 3' or 5' terminal positions are more resistant to hydrolysis of phosphoester bond by exonuclease.[34]

When compared to isosequential PS-oligonucleotides, that showed hypotension, fever, thrombocytopenia and other side effects,[55] no toxic effect of LNA-oligonucleotides were detected in concentrations up 1.0 μM in HeLa cellular system.[56] Studies about toxical issues of LNA in rodents showed that direct injection of fully modified LNA,

LNA/DNA mixmer, or LNA/DNA/LNA gapmer AONs (Antisense OlignoNucleotides) into striatum of rat brain do not elicit any histologically detectable toxicity or changes in body temperature.[51] While liver toxicity studies of LNA-PO (phosphodiester backbone), by controlling the serum level of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT), show no evidences of toxicity after 14 days of continuous treatment . However, at the highest dose of 5mg/kg/day an increment of ASAT and ALAT were detected, indicating some sort of liver toxicity, without detecting any changes in body temperature and histological parameters of liver and kidneys.[57]

1.1b Biological applications of LNA-Oligonucleotides

Given the very important features and characteristics of containing-LNA oligonucleotides, they have attracted the attention of the field of biomedicinal, biochemistry and therapeutic researchers. The automated synthesis of LNA-containing oligonucleotides is suitable for the conventional phophoroamidite chemistry, even in presence of DNA, RNA, 2'-OMe, phophorothioate and phophodiester linkages.[58] They can be positioned in some different ways: Gapmers (head and tail are made of three LNA-monomers separated by DNA/RNA monomers), Mixmers (LNA/DNA/RNA are mixed together without regular schemes), Headed (the LNA-monomers are present in the 5'-head of oligonucleotide), Tailed (LNA-monomers are present at 3'-end).[33]

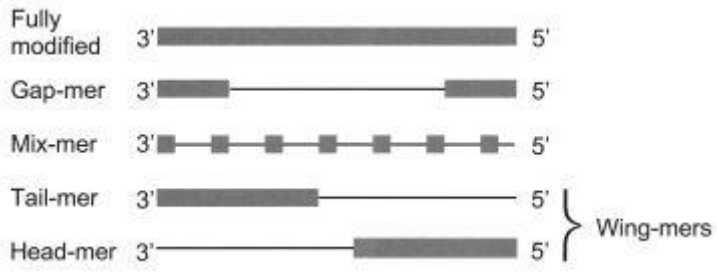


Figure 1.1.6. Possible combinational LNA-containing Olignonucleotides

The high binding affinity of LNA-oligonucleotides towards complementary RNA/DNA sequence provides the impetus for the development of new therapeutics by antisense and antigene mechanism.

Wahlestedt *et al.*[51] published encouraging results from isosequential DOR-AS-1 LNA/DNA mixmer and LNA/DNA/LNA gapmer in which these two oligonucleotides showed high antisense activity. These oligonucleotides exibited a higher serum stability

than the isosequential phosphorothioate sequences, a very high thermal stability, specify to target sequence of RNA and low toxicity. Unexpected results were observed for the activation of RNase H by these oligonucleotides. This suggestes that the activity of RNase H is not necessarily associated with a contiguous stretch of six DNA or phosphorothioate nucleotides when LNA is used as a component of oligonucleotides. It has been demonstrated that the position of target sequences is also important for the explication of antisense activity. In studies on the inhibition of Luciferase activity by LNA-AON (Antisense Oligonucleotides) in CV-1 cells, Braasch *et al.*[60] reported that some of LNA, LNA/DNA mixmers and gapmers explicate potent inhibitory effects when used to target the therminal 5'-UTR region of luciferase mRNA. Meanwhile, targeting downside the sequence from 5'-UTR, only modest or not significant inhibition were detected, even if the oligonucleotide-sequences possess complementary monomer and high termal stability.

LNA-oligonucleotides are very significant antiviral agents to their high affinity and selectivity toward the RNA filaments in antisense way.[61] It had been expected that the effect of such binding would result in steric blockage of important cellular functions of RNA such as translation. Taking advantage of this important feature, Arzumanov and co-workers developed a series of nucleotide mixmers, containing 12 residues of 2'-O-Me and LNAs, complementary to TAR (transactivation respons region) in HIV-1, that were able to effectively inhibit *in vitro* the Tat-protein-dependent in presence and/or in absence of HeLa nuclear cellular extract, indicating a very good selectivity for the sequence target.[56] Further studies showed that 12-16 mers sequences explicated the best inhibition activity, in which the 16-one was two/three fold more active than 12-one. The presence of at least 40-50% LNA monomers is essential for the inhibition since all 2'-O-Me modified oligonucleotide was ineffective. Dose-dependent of inhibition was also demonstrated (best dose with an IC_{50} was 120 ± 30 nM for 16-mer OMe/LNA mixmer containing 6 LNA units).[62] Similar results were obtained in the development of antiviral ASOs against HCV (Hepatiti C Virus). Laxton *et al.* studied forty-seven different mixmers/gapmers LNA-ASOs with the aim for targeting the 25- to 40-nt region of the HCV internal ribosome entry site (IRES) containing the distal and proximal miR-122 binding sites. Their results showed that only one oligonucleotide (seq132 and its analogues were mismatched were present) explicates a potent antiviral activity and low citoxicity and very high pharmacokinetics properties (long half life > 5 days).[63]

Due to the affinity toward RNA strands, LNA-oligonucleotides could be very important targeting probes for miRNA (microRNA). These ones are 20-23 nucleotides molecules that regulate many gene expression post-transcriptionally, such as insulin secretion, hematopoietic lineage differentiation; they can regulate brain morphogenesis in zebrafish, cardiogenesis in mice and altered miRNA expression were detected in many human cancers.[64] Studies of LNA-antimiR oligonucleotide in mice and not-human primates (African green monkeys) shown an effective (reduce of about 30/40% of cholesterol) and long-standing (the pre-treatment levels were reached again after three months) decrease of cholesterol in plasma by 5mg/kg day injections for mice and 3 or 10mg/kg in three injections per days for primates. Six weeks studies showed no elevations in hepatotoxicity or renal toxicity markers in the serum or in lipid accumulation in the liver (absence of increases in the levels of the plasma transaminases ALT and AST or of bilirubin, creatine phosphokinase or creatinine), no changes in blood coagulation were detected related to LNA-antimiRNA treatment.[65]

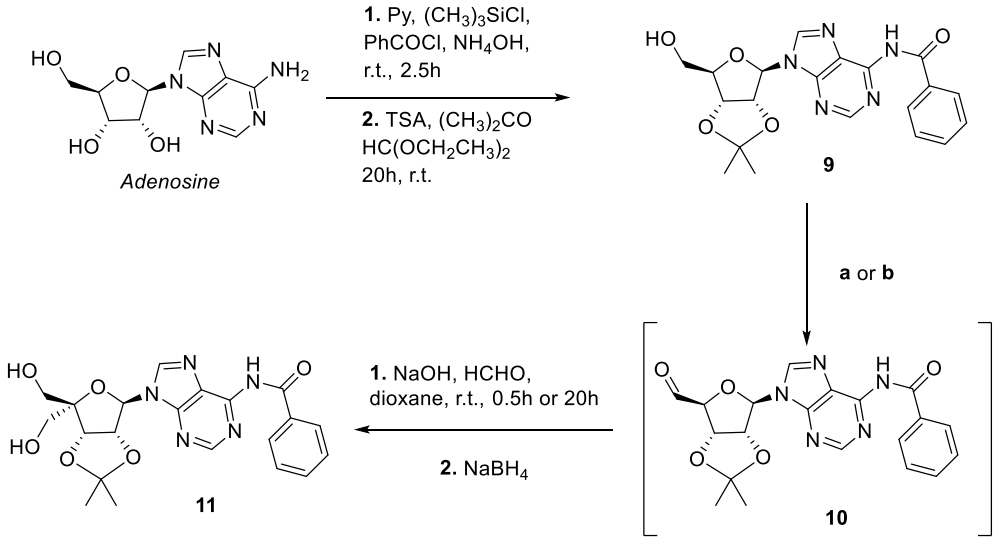
LNA-oligonucleotides could be very important therapeutic agents *in vivo* due to their high stability in serum media. A very interesting result was published by Fluiter and his collaborator in which they induced an inhibition of tumor growth *in vitro* and *in vivo*.[57] The LNA-PO (phosphodiester backbone) oligonucleotides were tested against POLAR2A (RNA polymerase II) in 15PC3 cells (prostate cancer cell line) in mice. Three different 16-mer oligonucleotides were examined: full matched-LNA PO (Cur616), and two in which 1 (Cur222) and 4 (Cur942) mismatched were present. At the beginning, they detected sequence and dose dependence. For what concern the sequence dependence, *in vitro* experiments shown that only full matched LNA-oligonucleotides explicates inhibition growth, while *in vitro* conditions also Cur222 and Cur942 inhibit the increment of tumor size; this fact can be explained by the thermal stabilization of LNA-monomers. For what concern the dose-dependence, between the four doses tested (0.5, 1.0, 2.5, 5.0 mg/Kg per day, for 14 days). Even if the best inhibition was detected at 5 mg/Kg dosage, a clear stop growing was also observed at 1 mg/Kg. At high dosage, the sequence dependence was also lost. In comparison to isosequential PS-olignonucleotides, LNA-PO showed an enhanced stability in serum and lower toxicity. This stabilization was also confirmed by MALDI-TOF analyses in which, analyzing the serum after 30 minutes from LNA-PO injection, only traces of 15-mer, 14 mer and 13-mer molecules derivatives were detected. A clear evidence of possible toxicity induced by LNA-POs treatment was detected only at highest dose of 5mg/Kg. In fact, a lower

dosage, such as 1.0 and 2.5 mg/kg, no detectable variation of ASAT and ALAT were observed. In addition, no changes in body temperature and liver and kidneys damages were detected by histological analyses.

1.1c Results and Discussion

AIM OF THE PROJECT AND PREVIOUS WORK

The focus of the project is the development of a synthetic pathway for the synthesis of Adenosine-LNA (β-D-Ribose sugar core) by linear strategy due to lack of method for this LNA-target. This topic has been already addressed in prof. Enrico Marcantoni's laboratory (Laura Marsili and Milind Jadhav's thesis). In previous works they tried to obtain the LNA-adduct through the synthesis of an aldehyde moiety in C-5' (**10**) and then perform an aldol condensation between this aldehyde intermediate and paraformaldehyde in presence of NaOH as base (*Scheme 1.1.3.*) in one pot approach, due to the instability of aldehyde intermediate.



Scheme 1.1.3. Marsili and Milind's aldehyde pathway. a) DMSO, DCM, Et_3N, Py^*SO_3, r.t., 3h b) DMP, DCM, $Na_2S_2O_3$, 6.5h

In the case of 20h reaction of aldol condensation (see *Scheme 1.1.3*) the diol target was isolated only in traces while a large amount of acid adduct was recovered. The formation of two products was confirmed by ESI-MS analysis. Instead, for 0.5h reaction poor results are due to low yield of final product (25% yield), and these results were due to N1-adenosine group reactivity.[66]

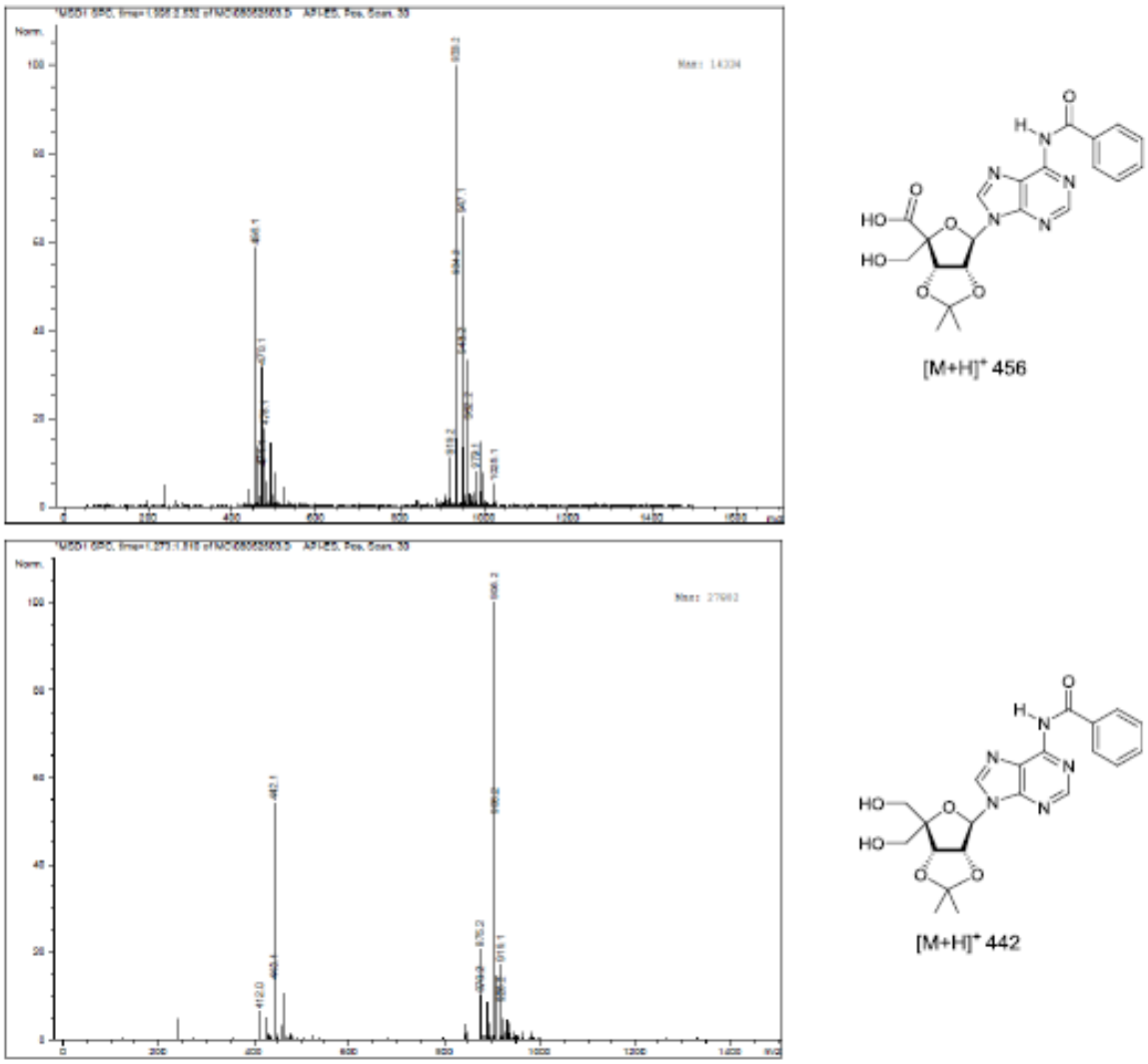
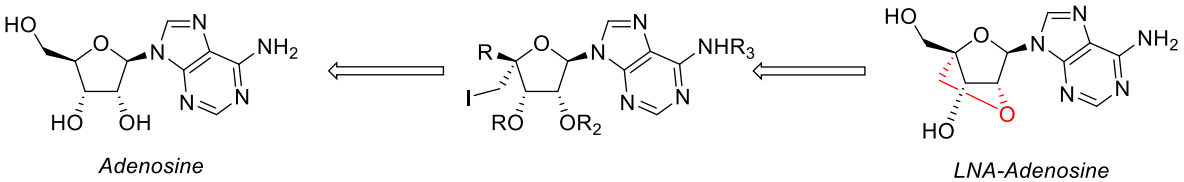


Figure 1.1.7. ESI-MS of Cannizaro's dismutation products

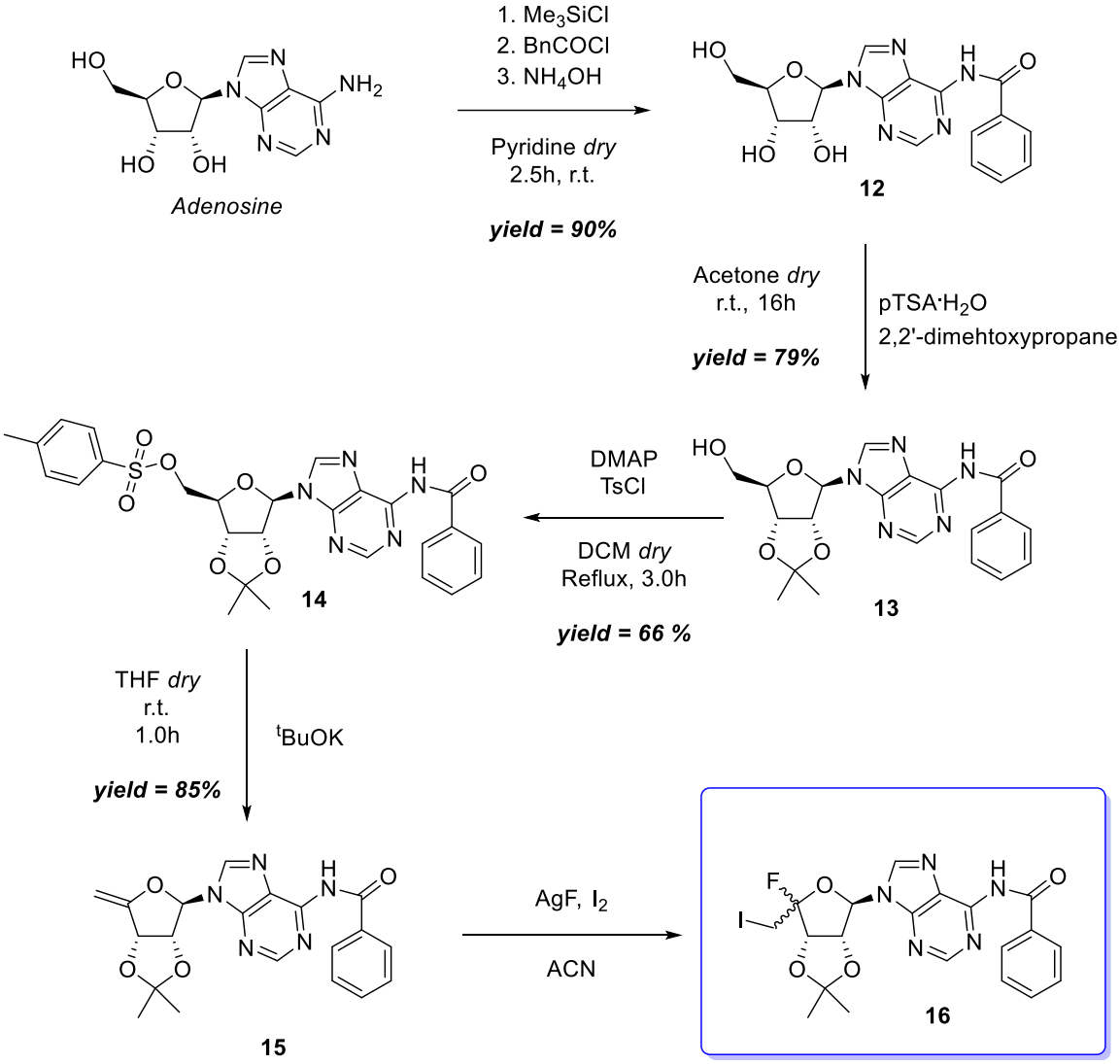
CURRENT WORK

Due to drawbacks of previous works, we tried to developed a new way for the synthesis of LNA-Adenosine in which we want to obtain a $–CH_2I$ moiety in C-5' position and then perform the ring-closing, passing through orthogonal protections of –OH and $–NH_2$ groups present in Adenosine.



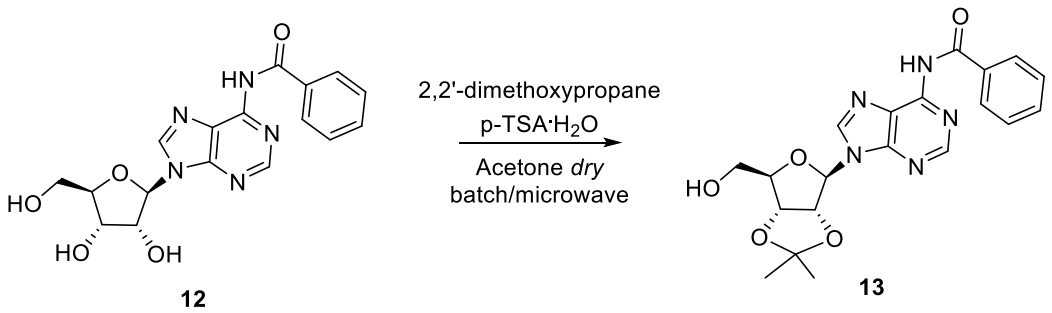
Scheme 1.1.4 New retro synthetic approach for LNA-Adenosine

As first approach we tried to synthesize a double bond moiety in C-4' in the sugar ring and then obtain the $–CH_2I$ moiety through the reaction between adduct X and I_2 in presence of AgF (*Scheme 1.1.5*).



Scheme 1.1.5. Silver Flouride synthetic approach

First, due to the high reactivity of amino group present in Adenosine and possible interference in further steps, we proceed to the protection of this functionality using benzoyl chloride, as protecting group, which proceed smoothly and in good yield. For the protection of two hydroxyl groups present in C-2' and C-3' different conditions were tested and are reported in the table below (*Table 1.1.1*).

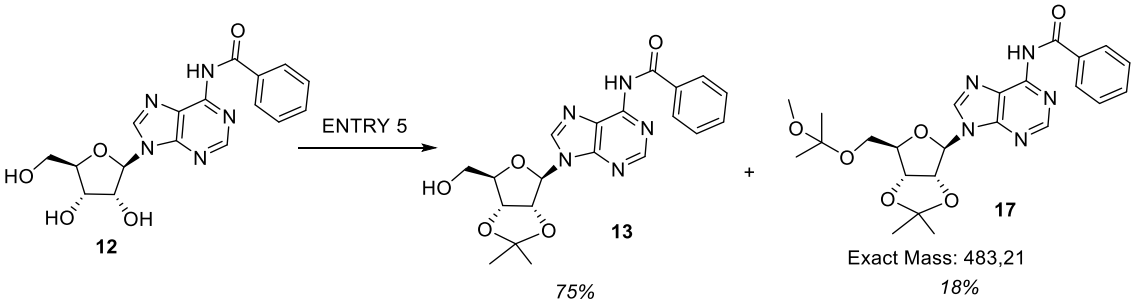


ENTRY[a]	2,2'-dimethoxyp.	pTSA·H_2O	Temperature	Time	Yield
1	9.5 eq.	0.2 eq.	Reflux	24h	58%
2	10 eq.	0.2 eq.	70°C M.W.	3h	73%
3	10 eq.	0.2 eq.	60°C M.W.	4h	49%
4	**9.0 eq.**	**0.6 eq.**	**r.t.**	**16h**	**79%**
5	9.0 eq.	0.5 eq.	r.t.	16h	73%
6	**10 eq.**	**0.1 eq.**	**70°C M.W.**	**3h**	**75%**

Table 1.1.1. Screening Conditions for –OH protecton.

[a]*All reactions were performed in Acetone dry*

The simultaneous protection of these two hydroxyl groups can be performed under two different conditions (Entry 4 and Entry 6, *Table 1.1.1*.), both with good and similar yields. Performing the reaction under microwave irradiation increases considerably the speed of reaction, in fact only 3 hrs are enough to isolate the product in good yield (Entry 2 and Entry 6, *Table 1.1.1*). Reducing the amount of acid promoter afforded similar yield for the desired product, but performing the reaction at lower temperature, resulted in a decrease of chemical yield, even if the reaction lasted for 4 hrs (Entry 3, *Table 1.1.1*). However, the major drawback of these conditions is the formation of total protection of –OH present in sugar core; in fact, the ESI-MS confirmed the formation of **17**.



Scheme 1.1.6. Formation of total protected subproduct.

Meanwhile performing the reaction at room temperature (Entry 4 and Entry 5, *Table 1.1.1*) leads to a slight higher yield, moreover it allows to recover of starting material **12**.

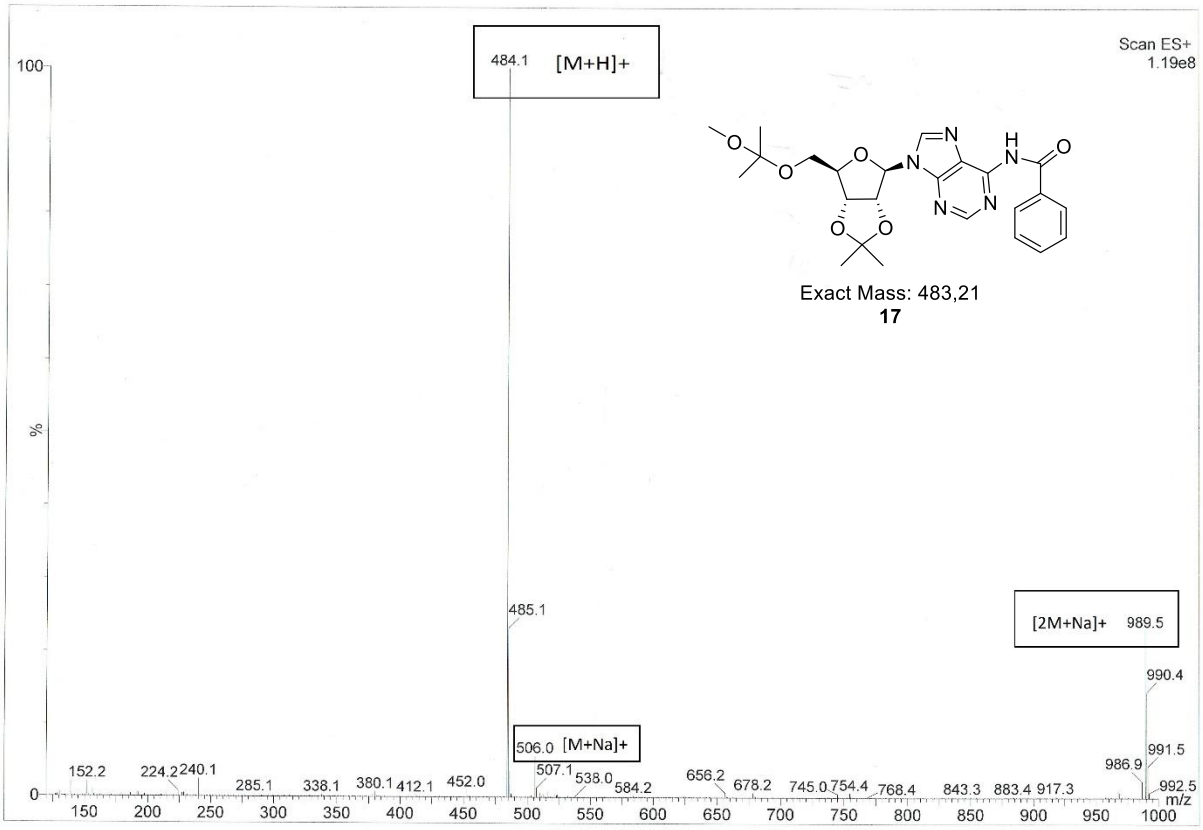
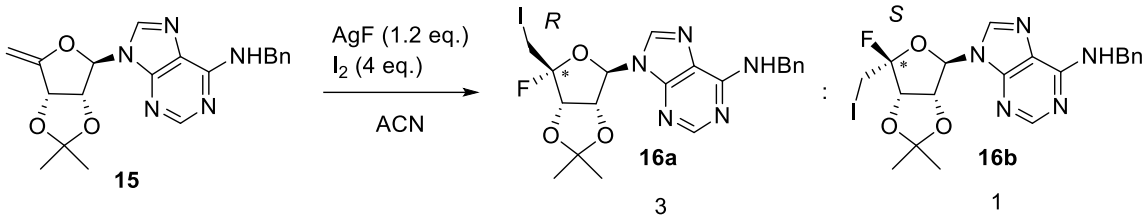


Figure 1.1.8. ESI-MS (+) of total protected adduct

Once achieved the protected diol **13**, the tosylation of –OH in C-5'was obtained between **13** and TsCl in presence of a large excess of DMAP in DCM *dry* at room temperature, a successive reaction of **14** and ^{t}BuOK gave the alkene moiety in a very good yield. At this point, we performed the reaction between intermediate **15** and AgF and I_2 and unfortunately, the target product **16** was not detected. Unlike what is reported in literature,[67] the authors confirmed that they separated the two epimers with carefulm chromatography obtaining a sufficient amount for NMR analysis. The strategy shows in addition to the (*R*)-epimer (**16a**) to be favorite over (*S*)-epimer (**16b**) (3:1 ratio); also the extreme lability of nucleoside containing the 4'-fluorine substituent because the presence at 4'-position makes the glycosidic linkeage to be easily hydrolyzed.

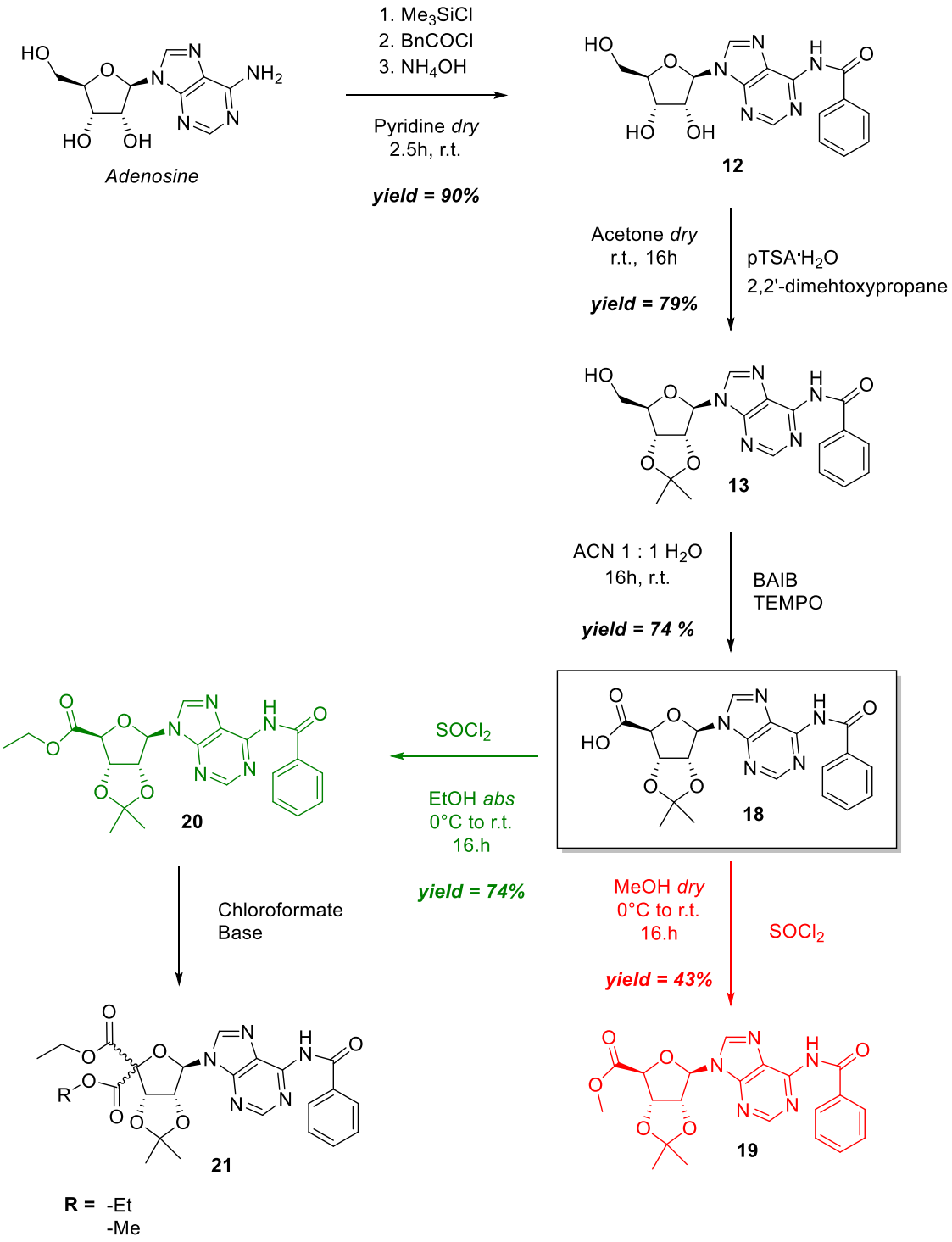
Moreover, further and deeper studies elucidated also that the stereoselectivity of the reaction is not favourable for our purpose since the R isomer-formation (**16a**) is favorite over the S-one (**16b**), with a ration of 3:1 between **16a/16b**.[66]



Scheme 1.1.7. Stereoselectivity reported in literature for AgF reaction

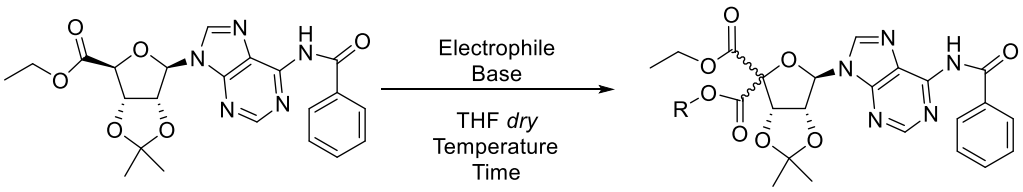
Due to unsuccessful results obtained with the previous synthetic way, we totally changed our approach. In this new synthetic pathway, our goal is to achieve an ester derivative in C-5', and then synthesize a diester moiety through the reaction between this intermediate and a formate or chloroformate. Further reduction of diester moieties and substitution of –OH with I, it will lead to the formation of two $–CH_2I$ moieties, in which –I is a good leaving group.

At the beginning of this new approach, we proceeded with the protection of amino group of adenine and the two –OH in C-2' and C-3' as done in previous pathway. After that, the oxidation of primary alcohol to acid was performed by BAIB and TEMPO leading to the product with a good yield. This method has shown the most suitable, in fact, performing the oxidation with KOH and $KMnO_4$ in darkness conditions, the acid **18** was obtained with a lower yield (40%). The BAIB:TEMPO method avoids the employment of toxic reagents such as permanganate too.[68] To avoid the problem related to the aldehyde moiety this time we want to synthesize an ester moiety, a more stable compound with respect to the aldehyde. Two different derivatives were synthesized, a methyl ester and an ethyl ester. The methyl ester **19** was achieved with a poor yield (43%) using a little excess of $SOCl_2$ (1.2 eq.), even if it was the best choice due to the small size of methyl group. A better result was detected performing the reaction in EtOH abs (**20**) (yield of 74%). Although it is known that thionyl chloride is corrosive and with an unpleasant odor, in this step it is one of the most suitable methods as the use of Brønsted Acids could lead to the removal of acetal protection of hydroxyl groups.[69a, 69b, 69c]



Scheme 1.1.8. New synthetic pathway

The experiment for the synthesis of diester-intermediate are reported in the table below (*Table 1.1.2*), in which ester **20** is dissolved in THF *dry* (0.4M) and cooled. Then, the base (1 h reaction) and a solution of electrophile in THF *dry* (0.5M) were added at low temperature.



ENTRY	Base	Electrophile	Temp.	Time	Yield
1	KHMDS (1 eq)	Cl-$CO_2C_2H_5$ (1.5 eq.)	0°C to r.t.	16h	-
2	KHMDS (1 eq)	Cl-$CO_2C_2H_5$ (1.5 eq.)	0°C to Ref.	3h	--
3	KHMDS (2 eq)	Cl-$CO_2C_2H_5$ (1.5 eq.)	0°C to r.t.	16h	-
4[a]	KHMDS (2 eq)	Cl-$CO_2C_2H_5$ (1.5 eq.)	0°C to r.t.	8h	-
5[a]	KHMDS (2 eq)	H_2CO (1.5 eq)	0°C to r.t.	24h	-
6[a]	KHMDS (2 eq)	$HCO_2C_2H_5$ (1.5 eq.)	0°C to r.t.	24h	-
7	KHMDS (3 eq)	Cl-$CO_2C_1H_3$ (6.0 eq.)	-78°C to r.t.	16h	trace

Table 1.1.2. Reaction conditions for achivieng diesterintermediate, [a]The reaction was performed in presence of $CeCl_3$ dry (1.5 eq) as additive.

Many tentative for the synthesis of adduct **21** were performed as reported in table. As first electrophile, we chose ethyl chloroformate since the ethyl moiety avoids the formation of different diastereoisomers that could be a problem during the purification of the product. Performing the reaction at room temperature, in presence of a stoichiometric amount of KHMDS and excess of electrophile (Entry 1, *Table 1.1.2*), we recovered only starting material. Nevertheless, performing the reaction at reflux of THF leads to unsuccesfull results (Entry 2, *Table 1.1.2*). Due to the steric hindrance of α-H of ester moiety and constraint of molecule, we involved a large excess of base, but the product was not achieved (Entry 3, *Table 1.1.2*), and the addition of $CeCl_3$ *dry* as additive the product was not detected neither by ESI-MS. Switching to different electrophiles, such as ethyl formate and formaldehye, the target product was not observed again. The product was observed in trace at ESI-MS only performing the reaction in a very large excess of base (3 eq) and methylchloroformate (6 eq).

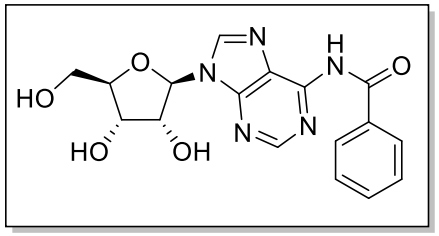
Due to the importance of LNA-derivatives and widespread applications in many different medicinal fields, further studies have still been carried out in the laboratory with the purpose to enhance the results obtained until now and achieve the LNA-adduct.

1.1d Experimental Section

MATERIALS & 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 120 °C for more than 2 hours prior to use. All solvents were dried (THF over metallic sodium, DCM over $CaCl_2$, ACN and Toluene over activated 4 Å M.S.) and freshly distilled (THF and DCM) or prelevated under nitrogen atmosphere (ACN and Toluene) 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, green bromochresol, potassium permanganate, cerium molybdate, 2,4-dinitrophenylhydrazine solutions. ^{1}H and ^{13}C spectra were recorded on a Varian Mercury 400 (400 MHz or 100 MHz respectively) or Varian Mercury 500 (500 MHz or 125 MHz). 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 ^{1}H and 77.2 ppm for ^{13}C) or dimethyl sulfoxide-d6 (DMSO-d6, 2.50 ppm for ^{1}H 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. GC-MS were recorded using Helwett-Packard GC 6850A series coupled with a MS 6890N (energy ionization 70 eV) using a capillary column of HP5-MS (5% fenilmetilpolisilossano, long 30 m, inner diameter of 0.25 mm and thick film of 0.25 µm). IR spectra were recorded with a Perkin-Elmer FT-IR spectrometer Spectrum Two UATR. ESI low-resolution mass spectra were recorded with an Agilent 1100 MSD ion-trap mass spectrometer equipped with a standard ESI source. Nitrogen served as dry gas.

*N-6-BENZOYL ADENOSINE **(12)***



Adenosine (500 mg, 1 eq., 1.87 mmol) was suspended in dry pyridine (10 mL) and stirred for thirty minutes and trimethylchlorosilane (1.28 mL, 5.5 eq., 9.35 mmol) is added to this solution to protect the hydroxyl groups. The mixture was stirred for one hour and benzoyl chloride (1.16 mL, 5.5 eq., 9.35 mmol) is added to protect the amine group. This reaction was maintained at room temperature for 2.5 hrs. The mixture was then cooled in ice-bath and washed with aqueous ammonia 33%. The mixture was stirred at room temperature for thirty minutes to deprotect the hydroxyl groups. The reaction was then evaporated to near dryness and the residue dissolved in a solution of water and Ethyl Acetate (3:1) and the precipitation of a white solid was observed after the separation of the layers. The solid was left to precipitate 16 hrs. Then the solution was filtered obtaining a white solid product with a yield of 90%.

Molecular Formula: $C_{17}H_{17}N_5O_5$

^{1}H-NMR (DMSO-d6, 400 MHz) δ = 8.72 (s, 1H), 8.69 (s, 1H), 8.05 – 8.01 (m, 2H), 7.62 (t, J = 7.4 Hz, 1H), 7.53 (t, J = 7.6 Hz, 2H), 6.02 (d, J = 5.8 Hz, 1H), 4.63 (t, J = 5.3 Hz, 1H), 4.19 – 4.16 (m, 1H), 3.96 (d, J = 3.6 Hz, 1H), 3.67 (dd, J = 11.9, 3.9 Hz, 1H), 3.56 (dd, J = 12.0, 4.0 Hz, 1H).

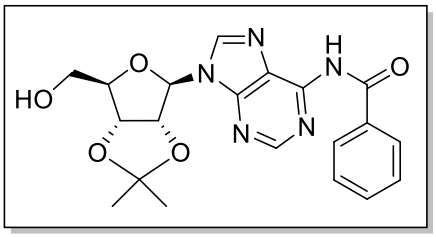
^{13}C-NMR (DMSO-d6, 100 MHz) δ = 166.45, 152.84, 152.27, 143.78, 134.15, 133.10, 129.18, 129.14, 125.56, 88.27, 86.41, 74.35, 71.06, 62.00.

ESI-MS (+): 372 $[M+H]^+$, 394 $[M+Na]^+$, 756 $[2M+Na]^+$ **ESI-MS** (-): 370 $[M-H]^-$, 406 $[M+Cl]^-$

IR (neat, cm^{-1}): 3324, 3116, 3077, 2921, 1695, 1613, 1591, 1461 1301, 1262, 1067, 1028, 1041, 703

m.p. (range in °C): 132 – 135 °C

*N-6-BENZOYL-2',3'-ISOPROPYLDENE ADENOSINE (**13**)*



Batch method: In a round bottom flask of 10 mL, 100 mg (0.269 mmol, 1.0 eq) of 6-N-Benzoyl Adenosine (**12**) were suspended in 1 mL of Acetone dry, then 296 µL of 2,2'-dimethoxypropane (2.42 mmol, 9.0 eq., d=0.85 g/mL) were added dropwise, after that 30 mg of p-toluenesulphonic acid (0.161 mmol, 0.6 eq.) were added to the solution. The resultant mixture is left to stir overnight at room temperature. The reaction is controlled by TLC (95 $CHCl_3$: 5 MeOH, Rf = 0.52). When no more starting material was observed, the solvent was evaporated by rotavapor and purified by filtration on buffered (1.0% v/v of Et_3N) Silica Gel (from 100 $CHCl_3$ to 98 $CHCl_3$:2 $^{i}PrOH$) obtaining a white solid with a yield of 79%.

Or

Microwave conditions: 100mg (0.269 mmol, 1.0 eq) of 6-N-Benzoyl Adenosine (**12**) were suspended in 1 mL of Acetone *dry* inside a microwave vial, then 329µL of 2,2'-dimethoxypropane (2.69 mmol, 10.0 eq., d=0.85 g/mL) were added dropwise, after that 5 mg of p-toluenesulphonic acid (0.028 mmol, 0.1 eq.) were added to the solution and stirred at 70°C under microwave irradiation. The reaction is controlled by TLC (95 $CHCl_3$: 5 MeOH, Rf = 0.52). When no more starting material was observed (3 hrs), the solvent was evaporated by rotavapor and purified by filtration on buffered (1.0% v/v of Et_3N) Silica Gel (from 100 $CHCl_3$ to 98 $CHCl_3$:2 $^{i}PrOH$) obtaining a white solid with a yield of 75%.

Molecular Formula: $C_{20}H_{21}N_5O_5$

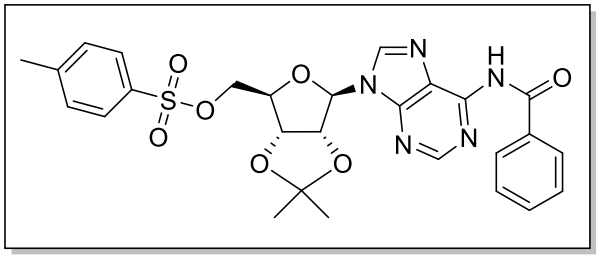
^{1}H-NMR (DMSO-d6, 400 MHz) δ = 11.22 (s, 1H), 8.76 (s, 1H), 8.66 (s, 1H), 8.11 – 7.94 (m, 2H), 7.62 (d, *J* = 7.7 Hz, 1H), 7.54 (t, *J* = 7.6 Hz, 2H), 6.26 (d, *J* = 2.8 Hz, 1H), 5.42 (dd, *J* = 6.2, 2.7 Hz, 1H), 5.14 (t, *J* = 5.3 Hz, 1H), 3.54 (dd, *J* = 9.8, 4.8 Hz, 2H), 1.55 (s, 3H), 1.33 (s, 3H).

^{13}C-NMR (DMSO-d6, 100 MHz) δ = 164.8, 152.6, 150.6, 150.5, 142.7, 133.6, 133.2, 129.1, 128.4, 124.4, 114.5, 94.4, 83.4, 83.2, 81.8, 63.4, 27.8, 25.4

ESI-MS (+) m/z: 412 $[M+H]^+$, 434 $[M+Na]^+$, 845 $[2M+Na]^+$ **ESI-MS** (-) m/z.: 410 $[M-H]^-$, 446 $[M+Cl]^-$

IR (neat, cm-1): 3354, 3202, 3060, 2990, 2938, 2865, 1712, 1608, 1591, 1461, 1058, 698.

*N-6-BENZOYL-2',3'-ISOPROPYLDENE-5'-O-TOSYL ADENOSINE (**14**)*



100 mg (0.243 mmol, 1.0 eq) of N-6-benzoyl-2',3'-O-isopropyldeneadenosine (**13**) was dissolved in 1 mL of DCM dry in 10 mL round bottom flask, then the solution is cool down to 0°C with an ice-water bath. Then 74 mg (0.608 mmol, 2.5 eq.) of DMAP were added to the solution and it is stirred for 30 minutes at 0°C and then 69 mg (0.364 mmol, 1.5 eq) of Tosyl chloride were added to reaction mixture and it is left to warm up to rt and stirred for 3 hrs. The reaction is monitored by TLC (100 EtOAc, Rf = 0.47). When no more evolution were observed, the reaction was cool down to 0°C and quenched with a saturated solution of $NaHCO_3$ (15 mL). Then the two phase were separated and the aqueous layer was extracted two times with dichloromethane (2x15mL) and the organic phase two times with brine (2x15 mL). Then collected organic phase were anhydrified with Na_2SO_4 anhydrous and the solvent was evaporated by Rotavapor. The crude was purified by Silica Gel (from 100 EtOAc to 95 EtOAc : 5 Hex) obtaining a yellow pale solid with a yield of 66%.

Molecular Formula: $C_{27}H_{27}N_5O_7S$

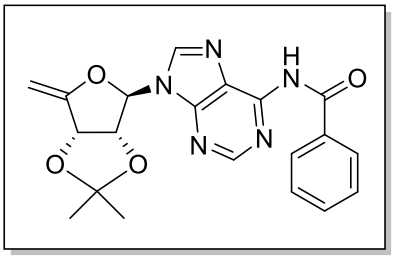
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 9.22 (s, 1H), 8.70 (s, 1H), 8.09 (d, J = 2.4 Hz, 1H), 8.06 – 8.03 (m, 2H), 7.66 – 7.60 (m, 3H), 7.53 (ddd, J = 6.6, 4.6, 1.3 Hz, 2H), 7.23 – 7.20 (m, 2H), 6.14 (d, J = 2.1 Hz, 1H), 5.36 (dd, J = 6.3, 2.3 Hz, 1H), 5.05 (dd, J = 6.0, 2.8 Hz, 1H), 4.54 – 4.50 (m, 1H), 4.25 (ddd, J = 16.4, 10.7, 5.0 Hz, 2H), 2.39 (d, J = 2.0 Hz, 3H), 1.60 (s, 3H), 1.38 (s, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 164.84, 152.87, 151.15, 149.99, 145.48, 142.25, 133.68, 133.14, 132.31, 130.00, 129.13, 128.17, 128.06, 123.64, 115.05, 91.38, 84.88, 84.38, 81.64, 69.12, 27.29, 25.49, 21.88.

ESI-MS (+) m/z: 566 $[M+H]^+$, 588 $[M+Na]^+$

IR (neat, cm^{-1}): 2925, 2856, 1695, 1608, 1578, 1457, 1171, 1071, 980, 794, 551.

*N-6-BENZOYL-2',3'-ISOPROPYLDENE-4'-METHYLENE ADENOSINE (**15**)*



100 mg (0.177 mmol, 1.0 eq) of N-6-benzoyl-2',3'-O-isopropyldene-5'-tosyl-O-adenosine (**14**) were dissolved in 1.0 mL of THF *dry* and then 129 mg (1.06 mmol, 6.0 eq) of ^{t}BuOK were added to the solution and the resulting mixture was stirred for one hour. The reaction was monitored by TLC (95 EtOAc : 5 Hex, Rf = 0.55), when no more starting material was observed, the reaction was dissolved with 15 mL of EtOAc and 15 mL of NH_4Cl saturated solution. Then the organic phase was recovered and the aqueous layer was extracted 2x15 mL with EtOAc, the organic phase was anhydrified with Na_2SO_4 anhydrous and the solvent evaporated by rotavapor. Then a pale yellow solid was obtained with a yield of 85%, the solid was used without further purification.

Molecular Formula: $C_{20}H_{19}N_5O_4$

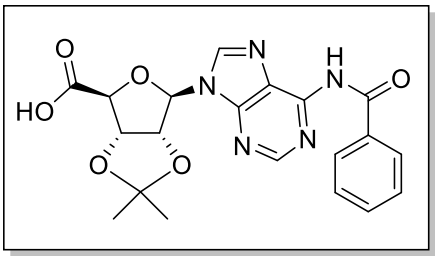
^{1}H-NMR ($CDCl_3$, 400 MHz) δ = 9.12 (s, 1H), 8.77 (s, 1H), 8.06 (s, 1H), 8.02 (d, J = 7.4 Hz, 2H), 7.60 (td, J = 7.0, 3.2 Hz, 1H), 7.52 (dd, J = 10.5, 4.9 Hz, 2H), 6.31 (s, 1H), 5.57 (d, J = 6.1 Hz, 1H), 5.34 (d, J = 6.1 Hz, 1H), 4.65 (d, J = 2.6 Hz, 1H), 1.59 (s, 3H), 1.43 (d, J = 9.5 Hz, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ = 161.63, 153.16, 151.40, 149.97, 141.84, 133.63, 133.13, 129.11, 128.15, 123.76, 114.60, 91.04, 89.33, 82.90, 79.80, 26.99, 25.85.

ESI-MS (+) m/z: 394 [M+H]+, 416 [M+Na]+, 809 [2M+Na]+

IR (neat, cm^{-1}): 3363, 2921, 2856, 1695, 1606, 1587, 1457, 1244, 1210, 1080, 876, 798.

*N-6-BENZOYL-2',3'-ISOPROPYLDENE ADENOSINE-5'-CARBOXYLIC ACID (**18**)*



100 mg (0.243 mmol, 1.0 eq.) of N-6-benzoyl-2',3'-O-isopropyldeneadenosine (**12**), 171 mg (0.535 mmol, 2.2 eq) of BAIB and 7.48 mg (0.048 mmol, 0.2 eq) of TEMPO were put in a 10 mL round bottom flask and 1 ml of a soltion H_2O:ACN (1:1) was added to the round bottom flask and the resulting mixture was stirred at room temperature for 16 hrs. After few hours a precipitation of a white solid was observed.

Then the reaction was monitored by TLC (95 $CHCl_3$: 5 MeOH) and when no more starting material was observed by TLC, The resulting precipitate was filtered, triturated sequentially with diethyl ether and acetone, and dried in vacuo, obtaining a white solid with a yield of 74%.

Molecular Formula: $C_{20}H_{19}N_5O_6$

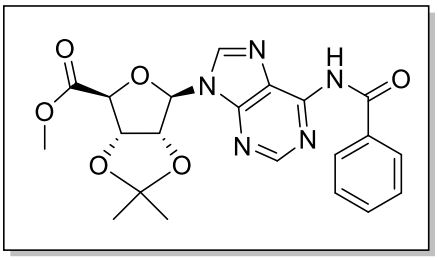
^{1}H-NMR (DMSO-d6, 400 MHz) δ = 12.90 (s, 1H), 11.18 (s, 1H), 8.67 (s, 1H), 8.58 (s, 1H), 8.03 (d, J = 8.1 Hz, 2H), 7.63 (t, J = 7.4 Hz, 1H), 7.57 – 7.49 (m, 2H), 6.46 (s, 1H), 5.57 (s, 2H), 4.76 (s, 1H), 1.52 (s, 3H), 1.36 (s, 3H).

^{13}C-NMR (DMSO-d6, 100 MHz) δ = 171.41, 166.22, 152.70, 152.02, 150.93, 144.79, 134.04, 133.13, 129.17, 129.14, 126.05, 113.38, 90.48, 86.38, 84.50, 84.08, 27.12, 25.57.

ESI-MS (+) m/z: 426 $[M+H]^+$, 448 $[M+Na]^+$, 873 $[2M+Na]^+$

IR (neat, cm^{-1}): 3350, 3094, 2995, 2943, 1717, 1691, 1517, 1491, 1335, 1257, 1188, 1071, 872, 794, 698.

*N-6-BENZOYL-2',3'-ISOPROPYLDENE ADENOSINE-5'-METHYL ESTER (**19**)*



100 mg (0.235 mmol 1.0 eq.) of N-6-benzoyl-2',3'-O-isopropyldeneadenosine-5'-carboxylic acid (**18**) were dissolved in 2 mL of methanol and then the solution was cooled down to 0°C with an ice-water bath and then 20.45 µL (0.282 mmol, 1.2 eq., d=1.64 mg/mL) of Thionyl chloride were added dropwise to the solution. Then the solution was left to warm up to room temperature and left stirring for 16 hrs. The reaction was monitored by TLC (95 $CHCl_3$: 5 MeOH), and when no more starting material were observed by TLC, the solution was diluted in $CHCl_3$ (15 mL) and a saturated solution of $NaHCO_3$ (15 mL), and then the organic phase was recovered and the aqueous layer was extracted with chloroform (2 x 15 mL) and the organic phase is dried with Na_2SO_4 anhydrous, the sodium sulphate is filtered and the solvent was evaporated by rotavapor. The resulting crude is purified by Siliga Gel obtaining a white solid with a yield of 43%.

Molecular Formula: $C_{21}H_{21}N_5O_6$

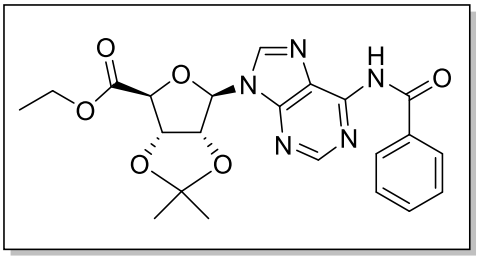
^{1}H-NMR (DMSO-d6, 400 MHz) δ = 11.21 (s, 1H), 8.66 (s, 1H), 8.58 (d, *J* = 0.8 Hz, 1H), 8.05 – 8.01 (m, 2H), 7.66 – 7.61 (m, 1H), 7.53 (dd, *J* = 10.4, 4.8 Hz, 2H), 6.51 (s,

1H), 5.63 – 5.61 (m, 1H), 5.55 (d, *J* = 5.7 Hz, 1H), 4.91 (d, *J* = 1.6 Hz, 1H), 3.28 (s, 3H), 1.52 (s, 3H), 1.36 (s, 3H).

^{13}C-NMR (DMSO-d6, 100 MHz) δ = 170.36, 166.28, 151.92, 151.07, 145.03, 134.01, 133.18, 129.79, 129.47, 129.17, 126.21, 113.40, 90.71, 86.68, 84.74, 84.08, 52.44, 27.03, 25.44.

ESI-MS (+) m/z: 440 $[M+H]^+$, 462 $[M+Na]^+$, 901 $[2M+Na]^+$

*N-6-BENZOYL-2',3'-ISOPROPYLDENE ADENOSINE-5'-ETHYL ESTER (**20**)*



100 mg (0.235 mmol 1.0 eq.) of N-6-benzoyl-2',3'-O-isopropyldeneadenosine-5'-carboxylic acid (**18**) were dissolved in 2 mL of absolute ethanol and then the solution was cooled down to 0°C with an ice-water bath and then 20.45 µL (0.282 mmol, 1.2 eq., d=1.64 mg/mL) of Thionyl chloride were added dropwise to the solution. Then the solution was left to warm up to room temperature and left stirring for 16 hrs. The reaction was monitored by TLC (95 $CHCl_3$: 5 MeOH, Rf = 0.41), and when no more starting material were observed by TLC, the solution was diluted in $CHCl_3$ (15 mL) and a saturated solution of $NaHCO_3$ (15 mL), and then the organic phase was recovered and the aqueous layer was extracted with chloroform (2 x 15 mL) and the organic phase is anhydrified with Na_2SO_4 anhydrous and the solvent was evaporated by rotavapor. The resulting crude is purified by Siliga Gel obtaining a white solid with a yield of 74%.

Molecular Formula: $C_{22}H_{23}N_5O_6$

1H-NMR ($CDCl_3$, 400 MHz) δ = 11.20 (s, 1H), 8.65 (s, 1H), 8.59 (s, 1H), 8.03 (d, *J* = 7.1 Hz, 2H), 7.62 (d, *J* = 7.3 Hz, 1H), 7.54 (t, *J* = 7.7 Hz, 2H), 6.51 (s, 1H), 5.62 (s, *J* = 16.1 Hz, 1H), 5.58 (s, 1H), 4.88 (s, 1H), 3.79 (d, *J* = 7.2 Hz, 1H), 3.59 (d, *J* = 7.4 Hz, 1H), 1.52 (s, 3H), 1.36 (s, 3H), 0.85 (t, *J* = 7.1 Hz, 3H)..

13C-NMR (DMSO-d6, 101 MHz) δ 169.93, 166.22, 152.56, 151.96, 151.06, 145.07, 133.97, 133.16, 129.17, 129.16, 126.21, 113.37, 90.64, 86.69, 84.81, 84.03, 61.50, 27.03, 25.49, 14.13..

ESI-MS (+) m/z: 454 $[M+H]^+$, 476 [M+Na] 929 $[2M+Na]^+$.

IR (neat, cm^{-1}): 3396, 3064, 2990, 2925, 1717, 1691, 1604, 1582, 1457, 1374, 1205, 1071, 707, 646.

1.1e Conclusions

From the first synthetic approaches of Locked Nucleic Acid (LNA) reported by Iminashi and Wengel, LNA-containing oligonucleotides (mixmers and gapmers) have found many applications in many different biomedical fields due to their important thermodynamic and structural characteristics. The 2'-O,4'-C methylene ring in ribose

moiety constrains the sugar structure to an N-type conformation, which allows LNA-monomers to mimic the RNA-conformation, making LNA-containing oligonucleotides very powerful tools for antisense and antigene applications.

In this work two different strategies were investigated. The first one, unfortunately, led to a bad result in which the target intermediate was not achieved. The second route explicates the very difficult task of synthesizing a bicarbonyl moiety in a satisfactory way, but some encouraging results were detected with the formation of a diester derivative. Nowadays, further studies hve been carried out to enhance the results a reaching the target goal.

1.2 Synthesis of a New Phenolic Derivative with Potential Antiviral Activity Against Sars-CoV-2.

1.2a Classification of Phenolic Compounds

Phenolic compounds are those molecules that contain, in their structure at least one phenolic moiety (an aromatic rings in which one o more hydroxyl groups are attached to ring). This class of compounds groups many molecules that possess a vast range of applications, structures and properties. They are largely distributed in plants kingdom, in fact they are also called phytochemicals (secondary metabolites of plants).[70] Classification and a correct definition of (poly)phenols has always been at the centre of many controversies and proposals, as given the vastness and diversity of chemical structures of these compounds. Theodore White, an industrial chemist of Forestal Land, Timber and Railway LTd., proposed, in 1957, to refer with the term "tannin" to all molecules that had a molecular weight between 500 and 3000 Dalton and a tannin-similar behaviour (possess a vast number of phenolic groups to be able to form hydrogen-bonds cross-linked structure with collagen molecules).[71] But some molecules, such as gallic acid and catechin, were not classfied as polyphenols by previous definition even if do not possess tanning actions but explicate some diagnostic phenolic reactions (complex with Fe(III) and oxidation by permanganate). Later, in 1962, Bate-Smith and Swain changed a little White's definition, adding some physical aspect (water solubility), eliminating the tannin-like properties, but specifying that those molecules must explicate "*usual phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins from solution, and possessing a molecular weight between 500-3000 Daltons."*[72] Subsequently, in 1994, Haslam added some more characteristics to this definition giving birth to well-known WBSSH definition, in which the polyphenols were defined as "*water-soluble plant phenolic compounds having molecular masses between 500-3000/4000 daltons and possessing from 12 to 16 phenolic groups on 5 to 7 aromaitc rings per 1000 Da of relative masses. In addition, they also explicate usual phenolic reactions and have the ability to precipitate some alkaloids, gelatin, and other proteins from solution*."[73] One feature common to all these definitions is the lower limit of molecular weight (500 Da). This limitation excluded many small phytochemical molecules that possess one or two phenolic moieties and explicate some phenolic-like properties (such as antioxidant one, solubility in water, etc.). For these reasons, Quideau proposed a new and exhaustive definition in 2011: "*The term "polyphenol" should be used to define plant secondary metabolites derived exclusively from the shikimate-derived phenylpropanoid and/or the*

polyketide pathway(s), featuring more than one phenolic ring and being devoid of any nitrogen-based functional group in their most basic structural expression."[71]

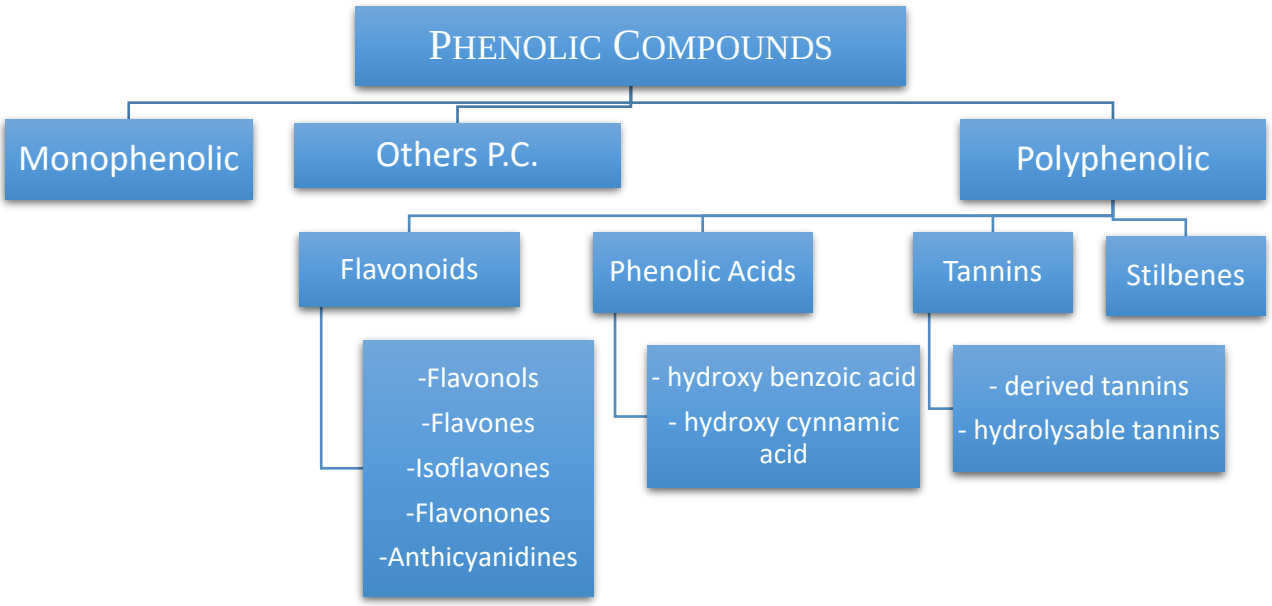


Figure 1.2.1 Classification of Phenolic Compounds

But this definition leave out, again, many monophenolic compounds (such as resorcinol, vanillin, cathechol, and many others), even if, in pharmaceutical, industrial, cosmetic, nutraceutic commercial advertisements fields are "vulgarly called" with the term polyphenols. Taking in mind chemistry and structurally aspects and literature notions, we summarized the classification of phenolic, dividing monophenolic, and polyphenols, and this last one groups many subcategories: Phenolic acids (Hydroxy benzoic and cynnamic acids), Flavonoids (Flavonols, Flavones, Flavonones, Anthicyanidines, Isoflavones), Tannins (derived tannins, hydrolysable tannins), Stillbenes; and finally other phenolic compounds (*Figure 1.2.1*) that groups other molecules such as curcuminoids, capsicains and others.

In addition, polyphenolic resins deserve a special mentions as this type of phenolic compounds a polymers in which a phenolic unit is present as monomer. They can be synthesized starting from phenol(s) and aldehyde by several different conditions (i.e. Novalak, Resol) and they can be applied as moulding powders, adhesives, surface coatings, impregnant, laminating resins.[74] Just for example, Novalak is obtained by acid polycondensation between phenol and formaldehyde, and starting from this point Garcìa *et. al.* exploited the employment of tannins and different aldehydes for the synthesis of different polyphenolic resins obtaining a series of new novalac-similar resins with higher

decomposition temperature, fire resistance, elecotrolytic resistance to NaCl (1N); moreover, grafting, crossliniking, T_g, morphological characteristics were influenced by the type of aldehyde.[75]

Anyway, we leave out them from this classification due to their high molecular weight and polymeric nature as the focus of this thesis is on "small molecule".

MONOPHENOLIC COMPOUNDS

Molecules such as catechol, resorcinol, pyrogallol and many others polyhydroxy-benzene compounds are defined by IUPAC as "phenol" compounds.[76] Many others monophenolic molecules, such as phenolic acids, fall under this classification, but due to the phenols-like properties, people refer to them as "polyphenols".[71]

Here, we present as monophenolic compounds such molecules those compounds that are found as moiety in many phytochemicals.

Catechol, resorcinol and pyrogallol, for example, (*Figure 1.2.2*) are wide present scaffolds in many (poly)phenols and natural compound, i.e. quercetin, climacostol, ellagic acid, or in more simple compounds like caffeic acid and gallic acid.

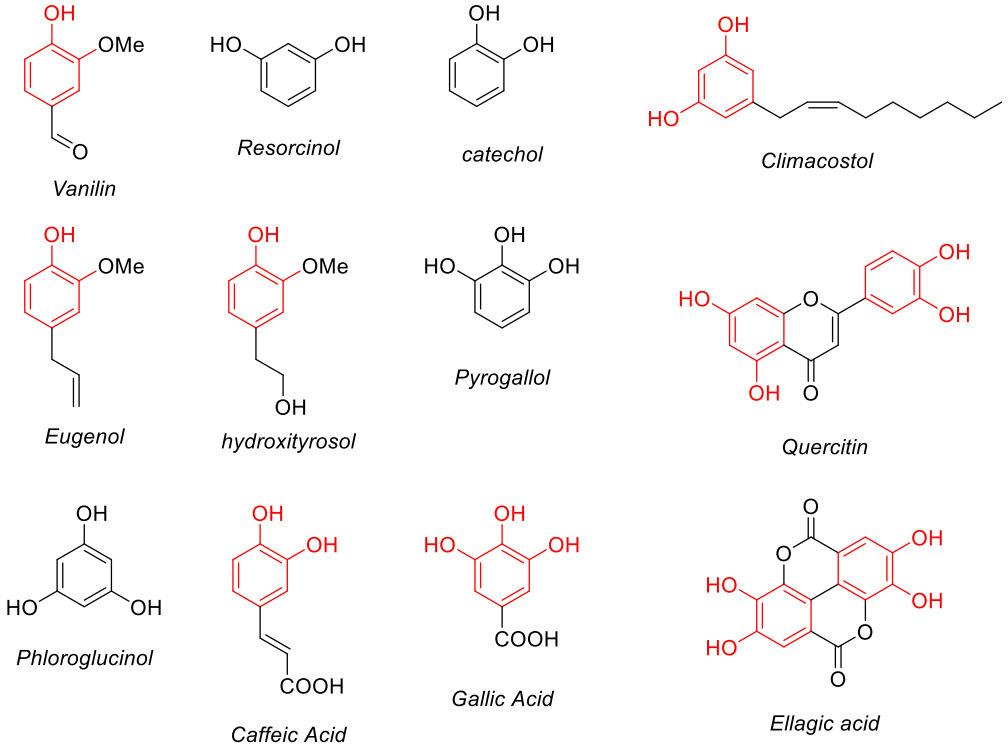


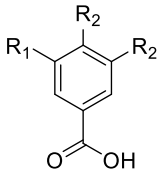
Figure 1.2.2. Structure of monophenolic compounds, not-phenolic acids, and molecules with monophenolic units (highlighted in red)

PHENOLIC ACIDS

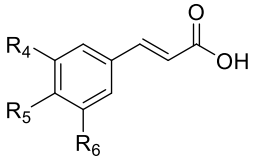
Phenolic acids is a class of compounds widespread in cereals, legumes, and many other seeds and they can be found in free form or linked to macromolecules such as cellulose, pectin, hemicellulose, forming bridges to build compact cell walls.[71], [77] They present a monophenylic structure, but usually they are grouped under "polyphenol" and here we leave this classification. Phenolic acids can be futher divide in two more subclasses: benzoic acids and cynnamic acids.

Benzoic acids present a backbone structure called C_6-C_1 and gallic, vanilic, protocatechuic and sytingic acid belong to this subclass.

Hydrocynnamic acids (C_6-C_3 backbone) is the major class of phenolic acids and phenolics in general, caffeic acid is the most representative molecules of this class, it exerts a very potent antioxidant activity, but p-coumaric, sinapic and ferulic acid have been under investigation by scientific community for their important bio-properties.[78]



Benzoic Acids



Cynnamic acids

Gallic Acid (R_1 = -OH, R_2 = -OH, R_3 = -OH)
Syring Acid (R_1 = -OMe, R_2 = -OH, R_3 = -OMe)
Vanillic Acid (R_1 = -H, R_2 = -OH, R_3 = -OMe)

Caffeic Acid (R_4 = -H, R_5 = -OH, R_6 = -OH)
Ferulic Acid (R_4 = -H, R_5 = -OH, R_6 = -OMe)
Sinapic Acid (R_4 = -OMe, R_5 = -OH, R_6 = -OMe)
p-Coumaric Acid (R_4 = -H, R_5 = -OH, R_6 = -H)

Figure 1.2.3. General structure of Phenolic acids

The major sources of these compounds blueberry, cranberry, pear, cherry (sweet), apple, orange, grapefruit, cherry juice, apple juice, lemon, peach, potato, lettuce, spinach, coffee beans, tea, coffee and cider.[79]

FLAVOINOIDS

Flavonoids are universally distributed in fruits and vegetables and they constitute about two-third of phenolic compounds in human dietary. Together the well-known bioactive properties of this class of compounds (discuss later), they present a typical backbone (C_6-C_3-C_6) in which a phenolic moiety (resorcinol, Ring A) is fused with a benzopyrane ring, and a second phenolic ring (catechol, Ring B) is linked to the byciclic structure.[79] Flavoinoids can be divided in six subcategories by the differences in pyrane ring and

linking position between fused structure and Ring B: Flavones, Isoflavones, Flavonols, Flavonones, Flavonol, Anthocyanidins.[80]

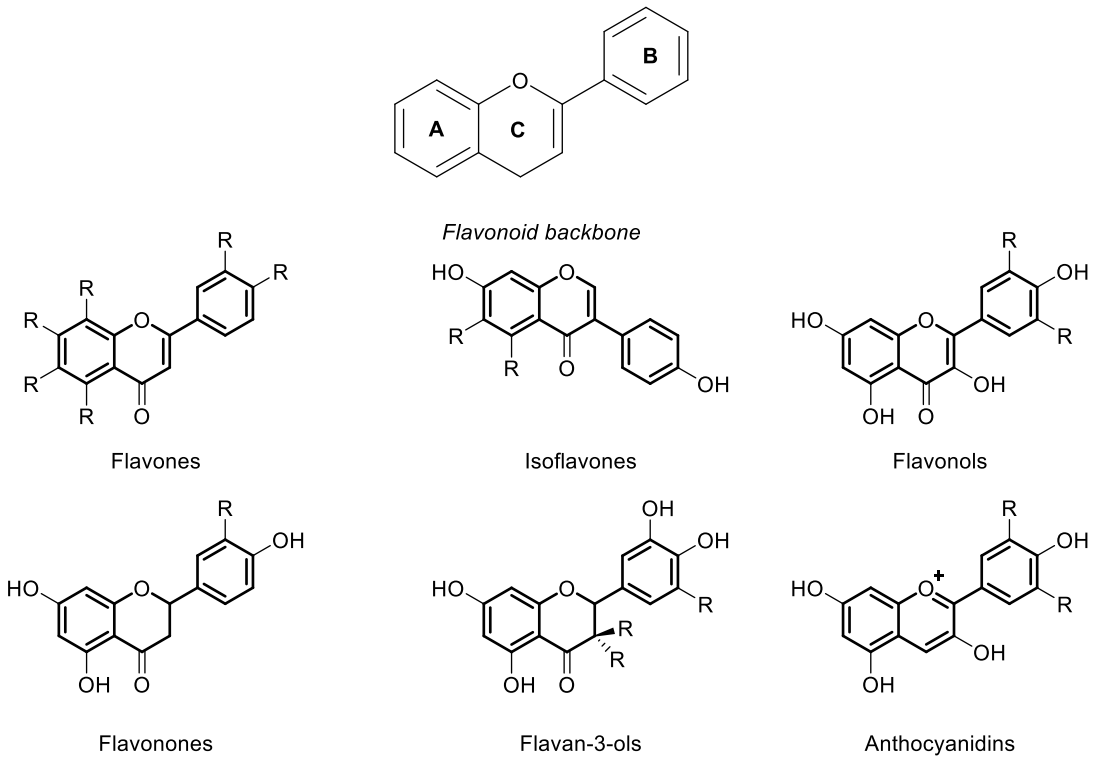


Figure 1.2.4. Structure of different Flavoinds classes

TANNINS

The term tannins were used to identify all secondary metabolites of plants that possess some specific characteristic and a determinate range of molecular weight.[71] Nowadays, this word indicates a class of relative high molecular weight and water-soluble molecules which are divided in two major classes: condensated and hydrolasyable tannins. Ester of gallic and ellagic acid constitute the first class, while in the second is made up by polymer of hydroxiflavan-3-ol as monomer. They are ubiquitous in natural plants (tea, coffee, wine, olive, plum etc.).[81]

STILLBENES

This class is composed by 1,2-diphenylethene derivatives that show good antibacterial properties and other biological properties.[82] Their core is represented by a structure C_6-C_2-C_6 in which the double bond can be present either *cis* or *trans*-configuration. (i.e. *trans*-resveratrol is the most abundant of two isomers, the most studied and the most

bioactive).[83] This type pf compounds are present in the grapes, wine, soy penauts and penaut products.[84]

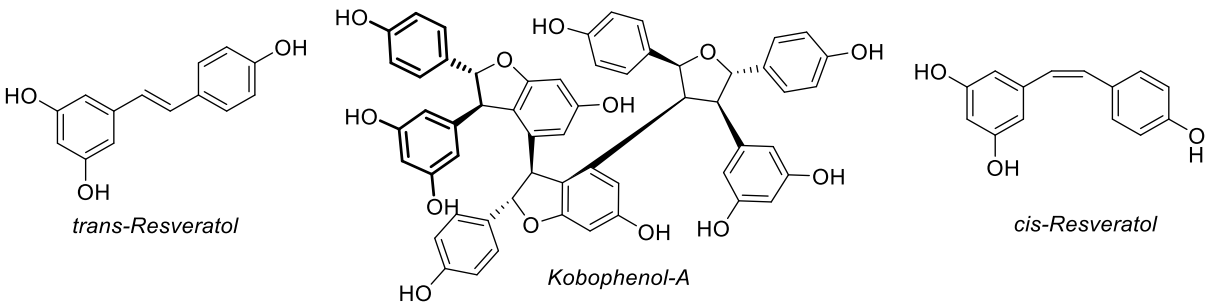


Figure 1.2.5. Structure of some stilbenoids

OTHER PHENOLIC COMPOUNDS

A satisfying, clear and precise classification of phenolic compounds is almost impossible due to the vast quantities of compounds that can be find in nature with at least a phenolic moiety in their structure. Molecule such as eugenol, vanillin and hydroxytyrosol present a phenolic moiety, but they cannot be classified as monophenolic reported above since there are not strictly phenolic subunits (see *Figure 1.2.3*). Eugenol presents a structure C_6-C_3 and it is extracted at industrial level from cloves and it constituted the main aroma of banana ripes, vanilin (C_6-C_1 structure) confers the good-taste vanilla aroma of vanilla beans, meanwhile hydroxityrol (C_6-C_2) is a potent antioxidant that is recovered from wastewaters of olive oil ill.[71]

A very important class of phenolic compounds are curcuminoid, of which major represent is Curcumin, extracted from *Curcuma lunga*, that has a β-diketones structure, conjugated with two double bonds and two phenolic structures (intense yellow colour of *Curcuma lunga*). Today biological properties of Curcumin are well-known (antioxidant, anticancer, anti-inflammatory, antiviral).[85]

Coumarins, Capsacinoids, Lignans and Chalones are other phenolic molecules.

Coumarins can be found in many plants (tonka bean, vanilla grass, sweet-clover) and as metabolites in some microorganism (*Streptomyces* and *Aspergillus* species, named novobiocin and coumermycin). They have a benzopyrone skeleton C_6-C_3.[86]

Capsacinoids is a class of compounds that present a vanillyl gropus linked to a fatty acid by amide functional groups, present only in fruits of *Capsicum* plants.

Lignans are present in low concentration but present in many plants as phytochemicals, they are formed by two units of phenylpropanoid forming a C_6-C_3-C_3-C_6 structure. Chalcones has phloglunicol moiety (see *Figure 1.2.3*) and a flavonoids similar structure C_6-C_3-C_6 in which there is not the pyran condensated ring, dihydrochalcones are most common molecules of this class, they are present in apple and apple-products.[80]

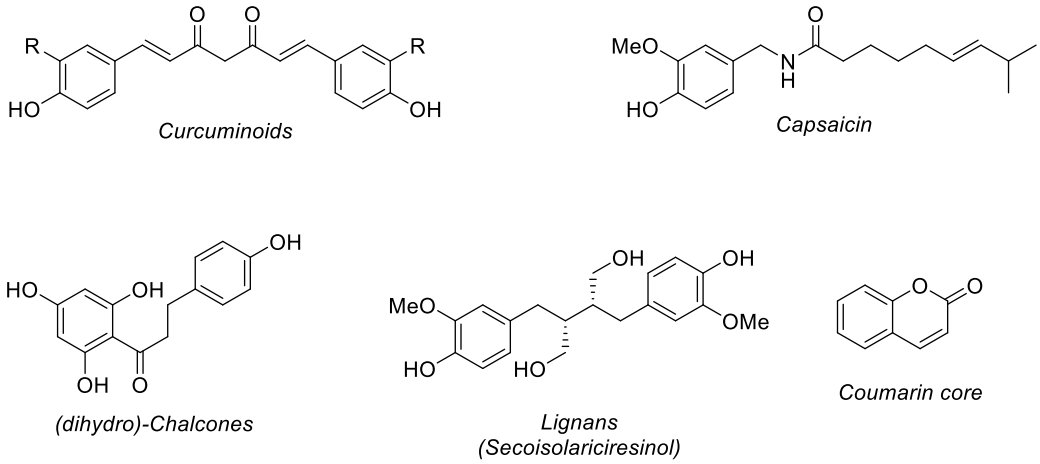
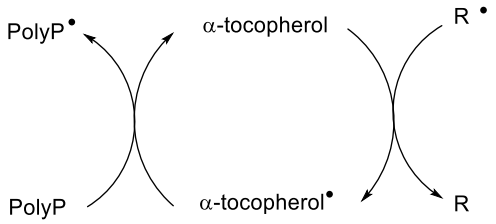


Figure 1.2.6. Structure of other phenolic compounds.

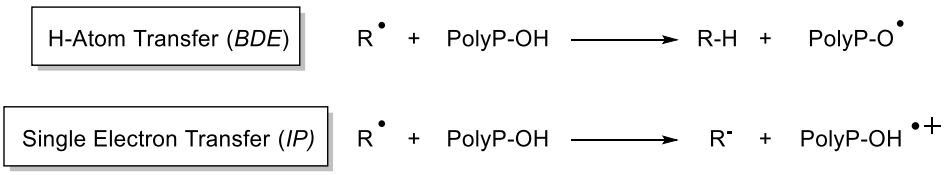
1.2b Antioxidant properties of (poly)phenols

Among the many important bioactive applications (those will discuss elsewhere), (poly)phenols are famous for their antioxidant activities. A high consumption of fruits and vegetables (polyphenols rich food) reduces the risk of many chronic disease like cancer, cardiovascular disease, chronic inflammation; that are related to a condition called oxidative stress.[87] This conditions come out when the cells were not more able to detoxify itself by ROS (reactive oxygen species, such as H_2O_2, $O_2^{\cdot-}$, $HO^{\cdot}$, $NO^{\cdot}$, HOCl, HOONO or RSNO) or the oxyradicals formed by damages of LDLs, proteins, DNA and RNA.[88a, 88b] The relationship between antioxidant activity and chemical structure of (poly)phenol compounds has been discussed, actually the type of compound, the number and position of hydroxy groups and degree of methylation are important parameters that discriminate the antioxidant activity of these molecules.[89] The (poly)phenols are able to neutralize these radicals species by two different mechanisms, or by a synergistic way with α-tocopherol regenerating the vitamin-E (α-tocopherol) starting a catalytic cycle. (*Scheme 1.2.1*).[90]



Scheme 1.2.1. Sinergystic antioxidant action Vitamin-E/Polyphenols

The first antioxidation mechanism is based on the ability to donate one hydrogen atom to a free radical (H-atom transfer, HAT). The relative position and the number of hydroxyl groups are crucial for the efficiency of the process. Species that contain catecholic and gallic moieties such as caffeic acid, gallic acid (see *Figure 1.2.3*) are very effective antioxidants since the extra vicinal –OH group(s) stabilize the out coming phenoxyl radical by hydrogen bonding.[91] From experimental data this stabilization is 4.4 kcal/mol for catechol and 7.5 kcal/mol for pyrogallol.[92] In addition, the presence of electrondonor and electronwithdrawing substituents in *ortho* and/or *para* position reduces the BDE (Bond Dissocation Energy, that this the driving force of the process): the ED-substituents compensate the electrovacancy of new radical by hyperconjugation (especially in the case of an *ortho*-alkyl substitution), the EW-substituents stabilize the radicals by delocalization of their unpaired electrons.[91] The second process, called also SET (Single Electron Transfer), bases on the ability of polyphenol to donate a single electron forming a stable radical cation $PolyPOH^{\cdot+}$, this process is described by IP (Ionization Potential).



Scheme 1.2.2. Two possible oxidation mechanism

In addition to ROS species, also metal cations such as Aluminum, Iron and Copper can generate radical species since they play an important role in free radicals formation and oxygen metabolism.[93] Particularly, Fe(II) and Cu(I) can react with H_2O_2 present beacause of oxidative stress, generating high reactive $OH^{\cdot}$ radical by the so-called Fenton Reaction, which can react immediately with DNA. The reaction between DNA and hydroxiradical leads to an abstraction of 4'-hydrogen atom, forming a radical that

rearranges cleaving the phosphodiester backbone and decomposing the strand; or damaging the nucleobases.[94] Polyphenols that present a 3',4'-dihydroxybenzene moiety (like catechol moiety) can chelate metals based at biological medium pH. Van Acker *et al.* tested different flavonoids on the ability to chelate the Fe(II) and they discovered that the catechol moiety was necessary for the complexation of metal core; and, in addition, the hydroxy group present in position 3 of heterocycle, in combination of double bond C2-C3 discriminate the ability to chelate the iron atom, by improving the scavenger ability. In fact, quercetin has shown a very good radical scavenger respect many other flavonoids.[95] It is known that also molecules that possess a gallol or cathecol moiety can chelate Fe(II) and Fe(III) in an octahedral geometry in 3:1 ratio, and by the Lewis acidity hardenss, the chelation of Fe(III) is stronger than Fe(II) due to its borderline lewis acidity (stability constant of Fe(II) complex is 7.95 and for Fe(III) is 20.01).[94]

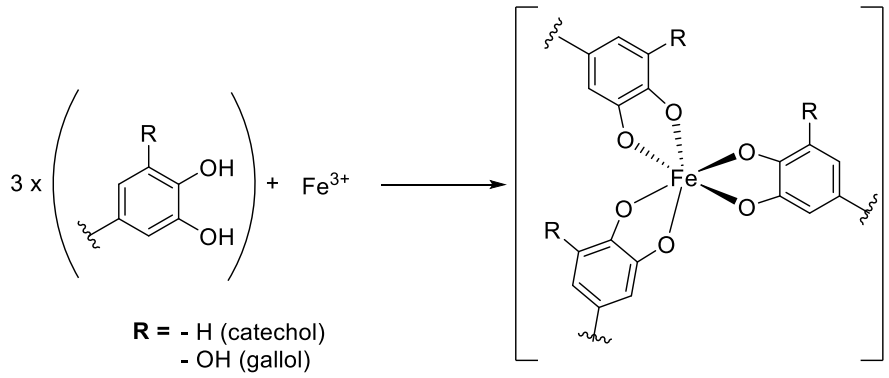
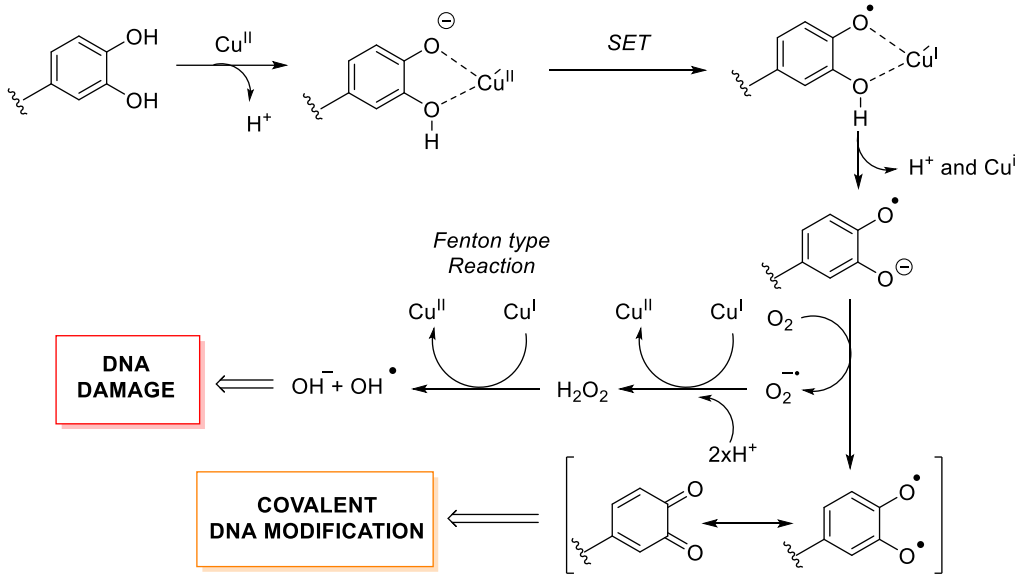


Figure 1.2.7. Octahedral geometry of catechol/gallol Iron complex

Unlike for Cu(I) which polyphenol has low affinity due to soft lewis acid nature, polyphenols can chelate easily Cu(II). This fact can be explained by results obtained by Perron and his coworkers in which they tested 13 different phenol compounds against H_2O_2-DNA damages induced by Cu(I) and Fe(II). In the case of Cu(I) they obtained an IC_{50} (Inhibition Concentrantion, value that indicates the concentration in which 50% of radicals were neutralized) raging from 10 to 480 µM, and for Fe(II) (lewis acidity hardness similar Cu(II)[96] from 1 to 59 µM. Moreover the result at EPR spectroscopy suggest that polyphenol can reduce easily Cu(II) to Cu(I). This fact can start an oxidant cycle in which Cu(I) is oxidized by H_2O_2 forming the hydroxyradical $OH\cdot$ (inducing DNA damages), the semiquinone formed after the reaction between (poly)phenol and Cu(II) can reduced the oxygen and forms H_2O_2 or it can react with ascorbate constituting the start (poly)phenol. This redox cycle can induce serious damages to DNA.[97] The

"ability" and "initiation" of the redox cycle described above is called "prooxidant effect". This prooxidant scenario is favourite in presence of copper atoms instead of iron atoms, because of the difference of potential reduction of copper couple is lower and easier to perform: Cu(II)/Cu(I) → 0.15 V and for Fe(III)/Fe(II) → 0.77 V.[98]



Scheme 1.2.3. Prooxidant mechanism of catechol moieties

1.2c Antiviral properties of (poly)phenols

Phenolic compounds explicate antiviral activity against a vast range of virus families: Rabies virus, Influenza virus, Polio virus, Coronavirus, Hepatitis virus, for instance (some other example are reported in *table 1.2.1*).[99] These compounds offer a valid alternative to "usual" virus treatment due to their wide availability, inexpensive production and low side effects.[100]

DESEASE	VIRUS	A.V.P. COMPOUNDS
Respiratory infection	Influenza (A,B,C)	2,3,4,6-Penta-O-galloyl-ß-D-glucose, Quercetin, Rutin
	Corona	Kaempferol, Chrysin
Hepatic infection	Hepatitis (A,B, C)	Curcumin, Ellagic Acid
	Eptsein-Barr	(−)-Epigallocatechin gallate
Auto-immune deficency	HIV	Quercetin, Myricetin, Gallic acid
Infantile paralisys	Polio	Quercetin
Gastrointestinal infection	Rotavirus	Licocoumarone, Glycyrin, Kaempferol, Chrysin
Deliurm and coma	Rabies	Quercetin, Qercetrin, Rutin

Table 1.2.1. Some examples of disease, virus family and polyphenols with antiviral action. Source reference[99 – 101].

Polyphenols can explicate the antiviral activity through several and different, and sometimes, overlapped mechanisms. The most important targets for antiviral action are viral envolpe, viral nucleic acid and viral replication.[99]

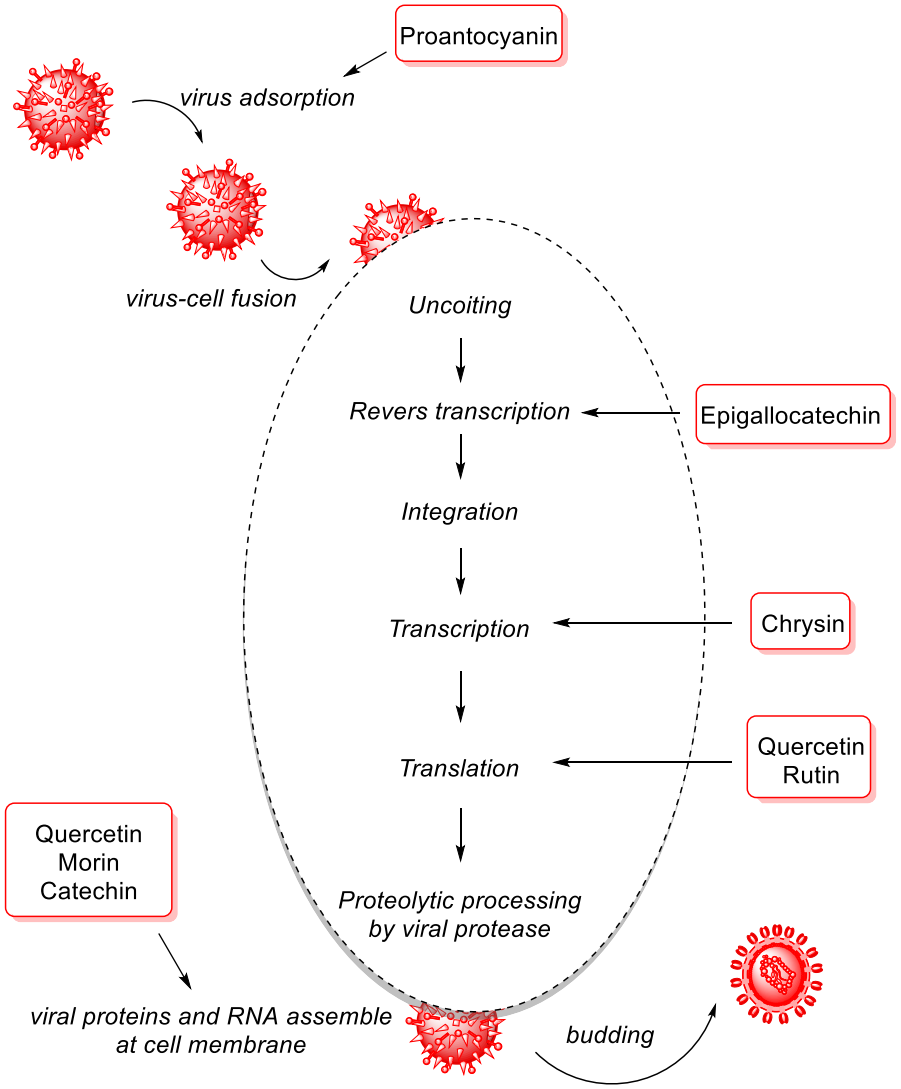


Figure 1.2.8. Viral cycle life and antiviral targets of phenolic compounds, adaptaion from reference[102]

Virus envelope (outer lipoprotein bilayer membrane) is a good target for antiviral drugs, in fact the destruction of this one makes genome virus exposed to degradation (similar action of detergent). For example, proanthocyanidin-enriched extract of Myrothamnus flabellifolia inhibits the viral adsorption and penetration of herpes simplex virus type-1 by this action.[99] Another important target for antiviral action is the genetic code. Once that virus enters in host cell, DNA/RNA polymerases of cells catalyse the replication of genetic codes and viral specific proteins (RNA). Inhibition and/or altering of viral

RNA/DNA replication, formation, and transcription is an important tool to fight the spread of virus. Important examples of inhibition of DNA replication has reported by Smith *et al*.[103] in which robinetin, myricetin, and (-)-epigallocatechin inhibits RNA replication of *S. Aureus* and DNA replication in *Proteus vulgaris*. Moreover, viral RNA acts as instrument for replication of viral proteins using enzymes of host cell or their own ones, in fact, most of developed antiviral drugs act as inhibitors of virus polymerases.[99] Quercetin, morin, rutin and catechin can inhibit polymerases of different viruses like herpes simplex virus (HSV), respiratory syncytial virus, poliovirus and Sindbis virus.[104] Finally it is reported that (poly)phenols can explicate antiviral action in further stages of viral cell life cycle. For example, SP-303 (oligomeric form of proanthocyanidin) affects the adsorption and penetration of HSV and HRV though the plasma membrane, or disrupting the nucleo-capsid and blocking infection or stimulating the defence mechanism of affected cells.[105a, 105b]

Some studies about the relationship between structure and activity (called also SAR, Structure Activity Relantioship) suggest that presence of hydroxy groups, ether and ester moieties influence the biological action of (poly)phenolic compounds by influencing the viral absorption and penetration.[99] In fact studies about SAR of 25 different flavonoids against three different influenza viruses (H1N1, H3N2, B/Jiangshu/10/2003) showed that, the 4'-OH, 7-OH, C4=O, and C2=C3 functionalities were essential to explicate good antiviral activity *in vivo*, detecting also a very different behaviour among the same class of flavonoids. In molecules such as Apigenin, Leutolin, Dinatin (each three flavones) and daidzein (isoflavones), in which this moieties are present, explicated good antiviral activity (IC_{50} about 30/40 μM), quercetin, myricetin (each two flavonols) and chrysin (flavones), for example, explicate moderate activity (IC_{50} ranged 40/80 μM), phenols like in hesperidin (flavononoles), rutin (flavonol), formononetin (isoflavone) exhibit very low antiviral activity (IC_{50} > 100μM).[106]

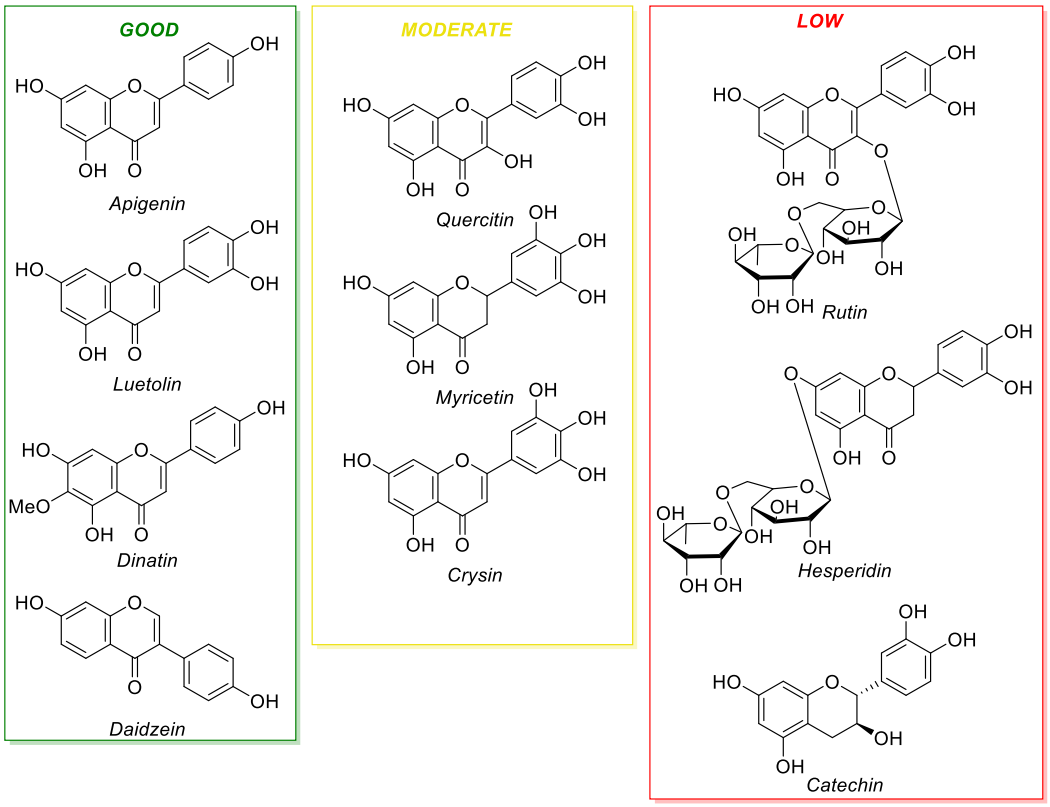


Figure 1.2.9. SAR of some of flavonoids studied by Liu and his coworkers.

1.2d Relationship between antioxidant and antiviral activities

Although a precise correlation between antiviral and antioxidant activity has not yet been found, it is known that some type of viruses (i.e. HIV, SARS-CoV-2, influenza viruses) can induce an alteration of oxidative metabolism (oxidative stress) and subsequently leads to cell death, helping the viral life cycle.[107] Studies about viral replication and oxidative damages accumulated by time on two different aged monkeys population affected by SARS-CoV (10-19 years old and 3-5 years old young adult) has shown that the age is an important factor for resistance to virus infections: the elder affected monkeys had more severe diseases with the same replication levels of younger ones.[108] Similar situation has observed in affected aged mice, in which a high proinflammatory status was observed.[109] These results can be explained by a weaker defence to alteration of oxidative metabolism from aged subjects, then this imbalance induces the production of redox-sensitive transcription factors, like (NF)-κB, inducing a proinflammatory status and a further amplified immune response and in worse cases in lung-injuries.[110] For example, *in vivo* studies about antiviral action of Quercetin against influenza virus A/Udorn/317/72 (H3N2) in affected mice showed that after 5 days from the infection, a

significant decrease of antioxidants like Vitamin C, Gluthathione, Vitamin E and concentration of SOD (superoxide dismuthases) were observed; inducing a high inflammatory status and lung injuries by oxidative stress. Treatment with bioflavonoid restored the normal level of antioxidants and enzymes, exception for Vitamin E, concluding that its antioxidant activity protect the tissues from injuries due to oxidative stress induced by influenza virus.[111] Also novel SARS-CoV-2 breaks the balance of oxidative mechanism too, by dysregulating the iron metabolism (free heme and hyperferritinemia), mithocondrial metabolism, NADPH oxidase; inducing a strong oxidative stress, serious damages in cellular and extracellular environment amplifying the injuries.[107] This inflammation status favourites the production of cytokines (i.e., IFNɤ, IL-1ß, IL-6, TNFα), that are signalling proteins those induct immunocells to inflammatory situs, vascular likeage, exudation, and stimulate the production of proteases and free radicals.[112] In this sense, hesperidin, a strong antioxidant flavonoid compound, could be an effective antiviral drugs against covid-19 due to combination of its scavenger ability (defence against oxidative damages) and inhibition of viral replication.[113a, 113b] From computational analyses it emerges that hesperidin possesses lower binding affinity toward 3CLpro (enzyme responsible for viral replication process, -10.1 kcal/mol for Chain A and -8.3 kcal/mol for Chain B) than other phenolic compounds like lopinavir (-8.0 and -6.8), ritonavir (-7.9 and -6.9).[114]

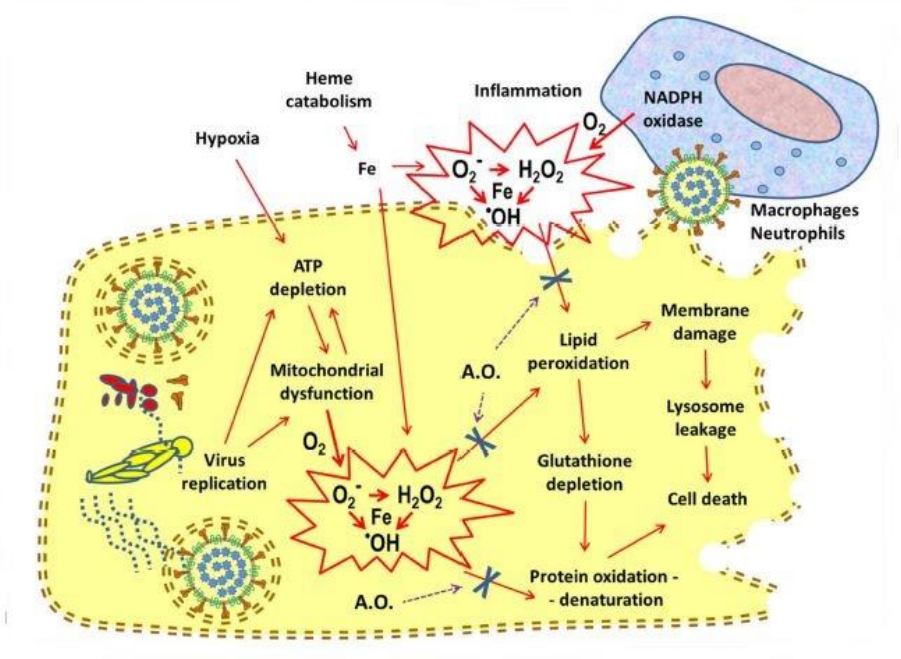


Figure 1.2.10. Oxidative stress induced by SARS-CoV-2 and possible antioxidants actions indicated by the "X". Image was taken in [107]

Therefore, antioxidants activity of polyphenols could become a potent tool to protect people from virus affections and treat already affected people.

1.2e Other biological activities of (poly)phenols

In addition to antioxidant and antiviral properties, polyphenols, but phenols in general, exert a spread range of biological properties.[70, 115a - 115d] These properties are due to the ability of (poly)phenols to modulate oxidative metabolism of cells, to scavenger activity of ROS and RNS and to chelate Fe(III), Fe(II), Cu(II).

ANTICANCER

Polyphenols are prominent anticancer-agent due to their ROS/RNS scavenger ability, in fact ROS/RNS play important role in cancer development, as these reactive species induce DNA damages by oxidative process.[116] Quercitin has shown a powerful anticancer agent. Iacopetta *et al.* evaluated the anticancer activity of Quercitin and its eight analogues (in which the –OH groups were eterified or esterified or protected). In this studies the **Q2** (total acetylated quercetin) and **Q5** (total ethyl-etherified quercetin) showed enhanced properties respect to not functionalized quercetin. In fact, these molecules were very effective at much lower concentration then Quercetin against breast cancer cell lines MCF-7 and MDA-MB-231(14.6 µM/11 µM for **Q2** and 42µM/16.6 µM for **Q5**, against 137 µM/40 µM for quercetin). Other two analogues, **Q3** and **Q4**, those present a bulky ketal cyclic moiety in Ring B, showed the highest anticancer activity, but they also explicate cytotoxic activity toward the healthy cells.[117] Climacostol (alkenyl resorcinol produced by *Climacostomum virens*) showed selectivity toward tumour cells respect to non-tumour ones. In addition, it explicates a persistent inhibition of tumour growth by injection of 600 µM of Climacostol in B16-F10 melanoma cell lines in mice, applying it as therapeutical agent for cancers.[118] A study conducted on pancreatic cancer, that is one of most difficult to treat, has shown that ferulic acid (it belongs to hydrocynnamic acids family) show potent anti-cancer action *in vitro* against MIA PaCa-2 human pancreatic cells. Fahrioglu and his coworkers has demonstrated that ferulic acid explicates its anti-cancer property by affecting the cell proliferation by stimulating apoptotic process; detecting the IC_{50} at 500 µM after 72hrs.[119] Meanwhile, the glycolysated form of ferulic acid with L-arabinose is an alternative way to

chemotherapy for cancer-lung treatment. Ferulate-L-arabinose shown scavenger activity comparable to ferulic acid, even slighter high at high concentration (<150 μM), determining a strong influence of ROS proliferation in H1299 lung cancer cells, without changing the morphology and proliferation of cells.[120]

ANTIBACTERIAL

The (poly)phenols play important roles in defence activity against microorganism such as bacterial, virus and fungi. In fact flavan-3-ols and flavonols show strong anti-bacterial (i.e. *S. aureus, Lacidophilus, F.nucleatum*), anti-viral (i.e. Adenovirus, Flu virus) and anti-fungal (i.e. *Candida albicans, microsporum gypseum*) acitivity; hydrosable tannins possess similar activities against different strain of bacterial (*Salmonella*, *Helycobacter*, *E.Coli*) virus (Herpes, HSV-1 and-2) and fungi. Finally, the antibacterial activities of phenolic acids are more notable.[114d] Certain class of polyphenols were under investigation to be used as preservative stuff for food due to their antimicrobial action and for their natural distribution and safeness. In 2008 Vaquero *et al*.[121] published a paper in which they tested different polyphenols against proliferaton and growth inhibition against *E.Coli* (common responsible for foodborne illness). From these studies it turned out that the number of hydroxyl groups was a determinant factor for antibacterial action: among gallic acid (three hydroxy groups), protocatechuic acid (two hydroxyl groups), caffeic acid (two hydroxy gropus) and vanillic acid (one hydroxy group and one methyl ether moiety), this last one possess the lowest inhibition activity (vanyllic acid started to explicate its antibacterial action at 100mg/L, unlike other phenolic compounds that show inhibition properties at lowest concentration, about 25 mg/L). Moreover, rutin, catechin and quercetin shown an inhibition in each concentration (25, 50, 100, 200 and 500 mg/L) and quercetin inhibit totally the growth of bacteria at highest concentration. Further studies demonstrated that combination of flavonoids and non-flavonoids phenols could inhibit the growth of *E.Coli* in meal at 20°C, after 21 days of incubation. Couples of gallic-protocatechuic acids, gallic-caffeic acids, and rutin-quercetin decreased the growth of bacteria of 30.2, 57.7, and 59.3%, respectively at 100 mg/L (total concentration of two, ratio 1:1), while 42.3, 86.0, and 100% of inhibition were detected at 200 mg/L. On the other hand, storaging at 4°C, the combinations of gallic-caffeic acids and rutin-quercetin were the most effective since no bacterial viable cells were dected after 14 and 21 days of incubations.[122]

ANTI-INFLAMMATORY

The anti-inflammatory properties of plant-products is well-known from centuries, phenolic compounds is one of the reasons of this plant properties. Inflammation status is a response of biological system to a tissue damages that lead to a respond off immune system producing pro-inflammatory cytokines (interleukin (IL)-1b, IL-6, tumor necrosis TNF-α). The overproduction of these cytokines can lead to outbreak diseases such as arthereoslerosis, allergy, arthritis, cancer.[70] In fact, for example, p-coumaric can explicate its anti-inflammatory properties by lowering TNF-α expression with an uptake of 100mg/kg in mice.[123] Chao and his coworkers[124] demonstrated that the assumption of ellagic and caffeic acid, by mice, avoided the inflammatory progression by decreasing glycative biomarkers (CML, pentosidine), suppressing AR (Aldose reductase) activity and lowering the release of cytokines IL-1β and IL-6; impacting also on mRNA expression of renal TNF-α, MCP-1 and AR.

Curcumin is one of most famous anti-inflammatory compounds, it inhibits the induction of COX-2, LOX, iNOS, production of cytokines such as interferon- and tumor necrosis factor, and activation of transcription factors like NF-κB, and AP-1.[125]

PARKINSON / ALZHEIMER

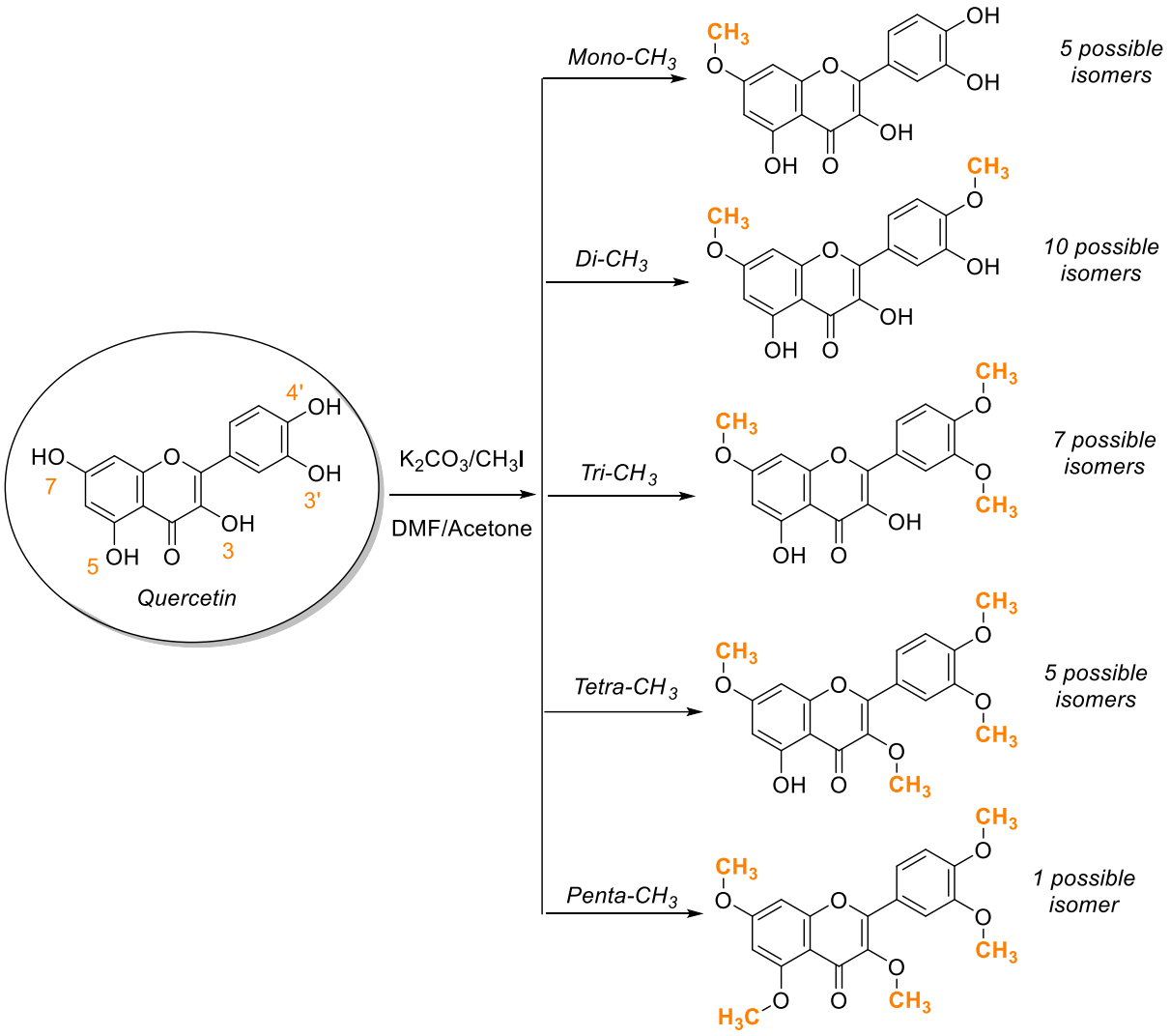
Parkinson (PD) and Alzheimer's diseases (AD) are the most common and notable neurodegenerative disorder.[126] Oxidative stress in brain caused by ROS species formed by mithocondrial methabolism, metal atoms like Fe(II) and Cu(II) and the excessive production of superoxide and nitric oxide leads to the outcome of AD. These species react with DNA, RNA, lipidic proteins, damaging them and leading to mild cognitive impairment brains. The scavenger properties of polyphenols are well-known, so the employment of this class of compounds and a rich-phenols diet are strong tools to prevent the outbreak of this disease.[127] For what concern the Parkinson's diseases, it is characterized by typical lesion called Lewy Bodies. These lesions are caused by the progressive accumulation of protein containing α-synuclein and ubiquitin in the cytoplasm of neurons, which leads to their apoptosis and/or necrosis. The ethiology of PD is still poorly understood, even if the numerous studies conducted on. Different substances that exhibits anti-inflammatory, anti-oxidant, metal chelating in central nervous system have been tested for PD treatment, included polyphenols. In addition to these properties, phenol compounds inhibit the formation of α-synuclein misfolded

aggregates and reduce mitochondrial dysfunction-induced by oxidative stress and inflammatory responses. They can also activate extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPK), serine/threonine protein kinase (AKT), protein kinase C (PKC) or inhibit a series of proteins (NF-κB). Many *in vitro* studies have been conducted.[126]

1.2f Chemical modification and synthesis of (poly)phenol-analogues

As reported in the previous section, (poly)phenol compounds possess and explicate a large range of bioactivities. However, the bioavailability, low water solubility and structure-action-relation are important factor to bring in mind for their applications. Even if nowadays the extraction, purification and characterization process have undergone of an important improvement, during recent years, scientists focused their effort to enhance natural polyphenols by chemical modifications and overcome some problems related to their employment.[128] In this section chemical modification of Quercetin, Climacostol, Curcumin will be more highlighted, respect to others, for the chemical structure analogies of target product of this section (see Result and Discussion).

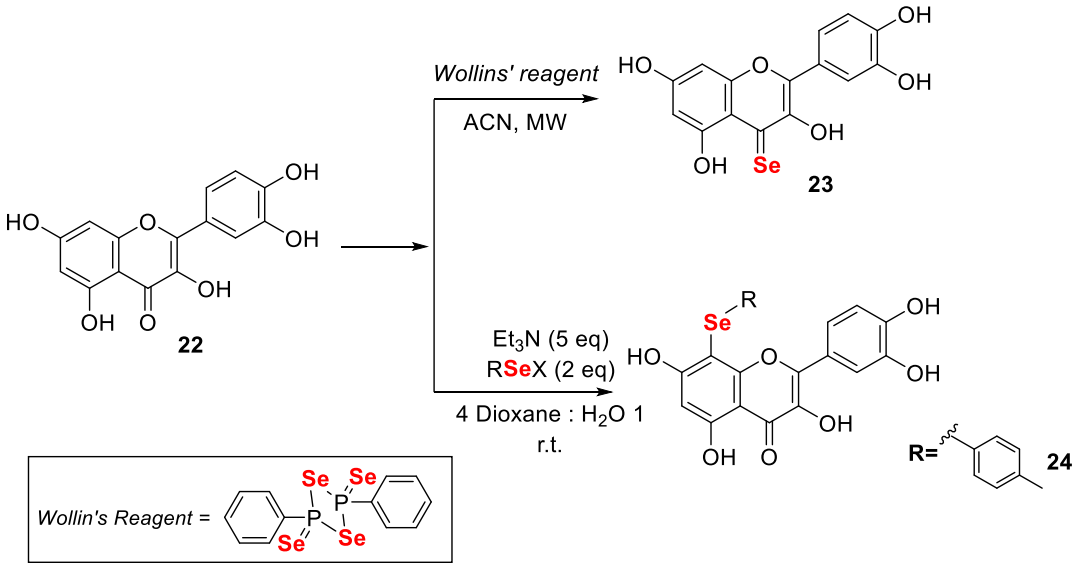
Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of most known (poly)phenols and it is present in plants, food, beverages. It can be employed as anti-cancer, anti-inflammatory, anti-oxidant, and it can form complexes with Cu(II). For these useful activities, chemical modification of this compound are aimed to overcome problems related to low solubility, fast degradation inside the body, low bioavailability. The most simple chemical modification is on hydroxy-groups present in quercetin moiety, even if there is not a clear conclusion of hydroxy substitution according to SAR, substitutions such as 5,7-dihydroxy; 5,7-dihydroxy-6-methoxy or 5,6,7-trihydroxy in ring A and 3', 4'-dihydroxy or 3',4'-dihydroxy-5'-methoxy in ring B were considered useful to take in consideration.[129] The O-methylation of –OH moieties (mono-, di-, tri-, tetra-, penta-) can be performed by CH_3-I/K_2CO_3 in DMF by a selective protection/deprotection (involving benzyl, MOM, ketal protected intermediates) way for mono-methylated compounds, while the other O-methylated products can be obtained in the same way, performing the reaction in DMF or acetone.[130, 131]



Scheme 1.2.4. O-methylated Quercetin reported in literature.

This simple modification greatly enhances properties of "original" quercetin, it allows overcoming problems related to solubility that is increased, same as for bioavailability. Methylation reduces toxicity by reducing side effects, and it improves cancer cell antiproliferative activity.[132] Even if mono-,di- and tri-methylations show potent inhibition growth of 16 different human tumor cells in which 3',4',7-O-methylated was the more potent;[131] penta-O-methylated- and tetra-O-methylated-(3,3',4',7)-Quercetin could be used as anti-multidrug resistance since it was demonstrated that they can influence breast cancer resistance protein, allowing a more potent treatment. The 3',4'-O methylation and 5-OH free is an important SAR pattern since this substitution explicates the most powerful inhibition of breast cancer-resistant protein, that it is decreased by 5-O-methylation.[130, 133] Introduction of bulky substituents like ethyl, n-butyl, benzyl, geranyl, pivaloxymethl, i.e., leads to improve the properties of quercetin

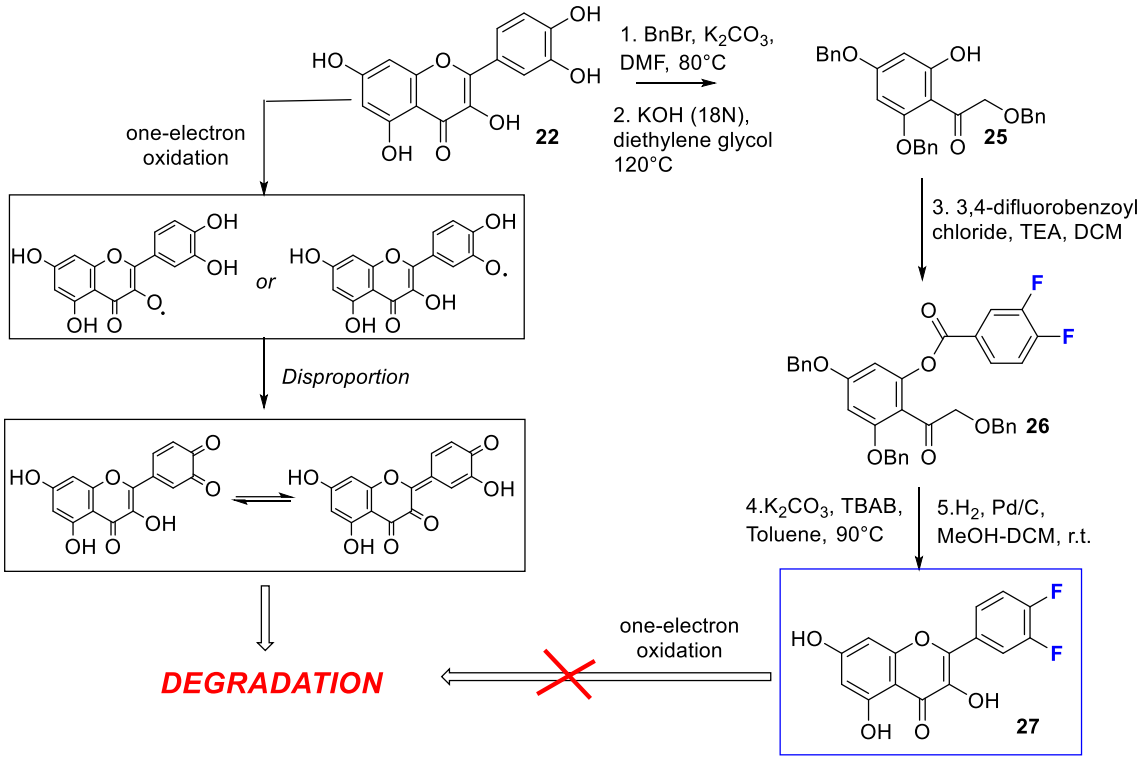
and showing good anticancer/inhibition growth properties.[134] Chemical functionalizations of flavonoid ring is another way to enhance properties of Quercetin **22**. Seleno-functionalization by Woolins'reagent (as source of Selenium) under microwave irradiation lead to 4-quercitin functionalization in which the carbonyl moiety is substituted by Selenyl one (**23**). This functionalization enhances the anticancer activity by increasing the radical scavenger of original flavonoid.[135] C-8 selective Se-funcationalization of Quercetin, performed in H_2O/Dioxane (1/4) in presence of Et_3N (excess) using a (p-methyl)phenylSe-Cl at r.t., leads to formation of a new phenolic compounds. The molecule **24** can block SARS-CoV-2 replication in infected cells with an IC_{50} much lower than quercetin (8 µM and 192 µM respectively). This improvement of antiviral activity is due, by docking experiments, by a formation of a hydrogen bond between the selenium atom and Gln189 residue, anchoring the quercetin scaffold and blocking the catalytic dyad His41/Cys145 of M^{pro} (protease involved in virus replication), stopping the replication process.[136]



Scheme 1.2.5. Selenium-functionalization of Quercetin.

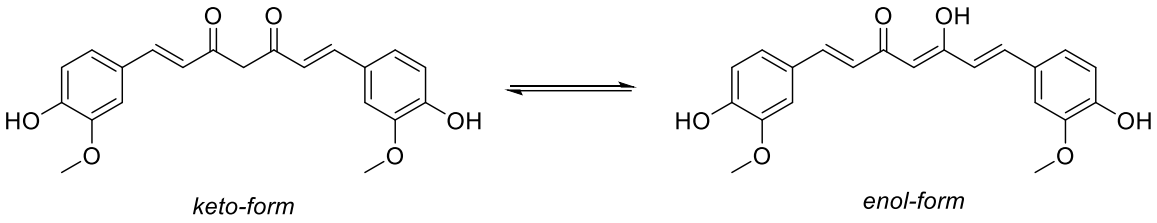
The substitution of two hydroxy groups in catechol moiety of quercetin with two fluorine atom enhances the stability of quercetin fluorine adduct, that is a "bioisostere" (chemical compound with some different structural features but with similar chemical, physical and biological properties to another chemical compound) of original flavonoid. The 3',4'-flourine substitution is obtained through a complex synthesis in which, after Bn-protection of –OH groups, denaturating of flavonoid core, then after the reaction between adduct **25** and 3,4-difluorobenzoyl chloride in presence of TEA lead to the formation of

intermediate **26** that after two more step is transformed to 3',4'-difluorine quercetin (*scheme 1.2.6.*). This adduct **27** does not undergo disproportionation to which this compound could be subjected during one-electron oxidation greatly improving biological activity of quercetin; in fact, 3',4'-difluoroquercetin **27** show $t_{1/2}$ >24h. It exhibits better anti-bacterial activity against the Gram-positive *S. Epidermidis* and *M. Luteus*, elucidating that anti-bacterial properties of quercetin might not be directly connected to its anti-oxidant feature; and the anti-cancer activity was similar to quercetin for breast cancer cells MCF-7 and slight lower for hepatoma (Huh-7). 3-aminoquercetin (quercetin analogue in which amino group is present instead of a -OH) showed a good oxidative stability, but low biological properties.[137]



Scheme 1.2.6. Synthesis and stability of 3',4'-difluoroquercetin

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is one of major extracted phytochemical from *Curcuma longa*. The biological properties of Curcumin today are well-know and studied, they include antioxidant, anti-inflammatory, and anti-atherosclerotic, anti-cancer, anti viral (HIV for example), it inhibits scarring, it can prevent Alzheimer's, it explicates many other biological properties.[138]



Scheme 1.2.7. Keto-enolic equilibrium of Curcumin

During these last years, the synthesis of analogues and chemical modifications of Curcumin has been at the centre of many researches with the purpose to improve water solubility, oral absorbability, metabolic stability (quickly degraded).[138] Preliminary observations about SAR-curcuminoids displayed that feruloyl moiety is crucial for explication of many biological activities. However the instability of this moiety at pH above 6.5. Similar argumentation can be done for β-diketone moiety since it is crucial for biological explication properties, but unstable at physiological conditions.[140]

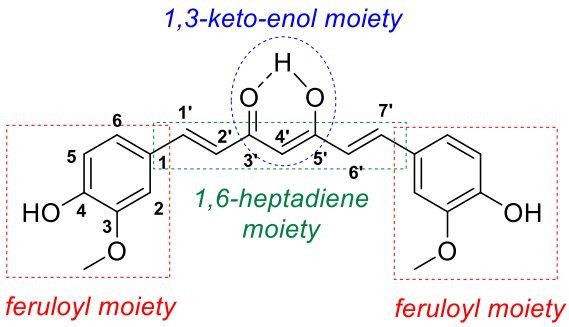
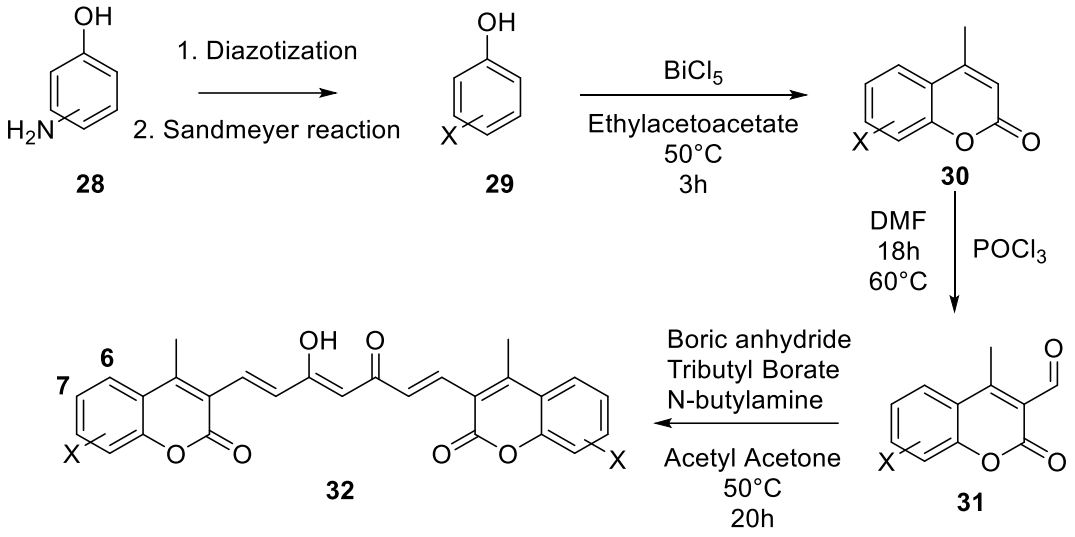


Figure 1.2.11 Structural features and chemical modification targets of curcumin analogues

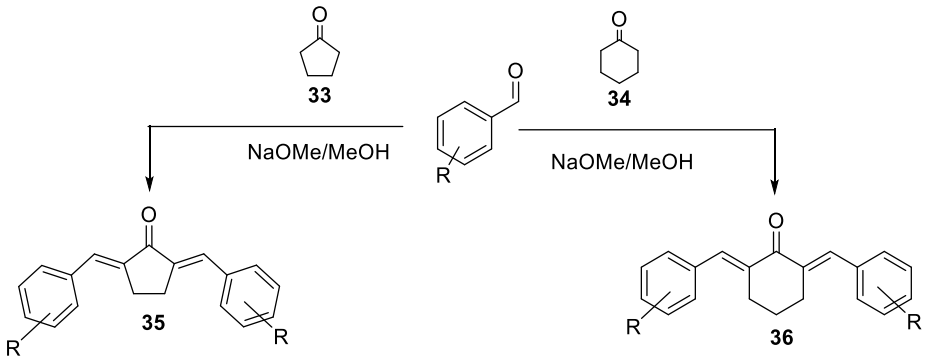
The structural differences of curcumin analogues mainly concern three different levels: the different substituents in feruloyl moiety and different rings in place of phenolic moiety; structural changes of 1,6-heptadiene core; modification of β-ketomoiety.[128], [140] Little changes on substituents and O-substitutions can greatly modify anti-oxidant activity since the 4-OH/3-OMe is involved in anti-oxidation pathways. For example, addition of one –OH on phenol moiety in position-5 leads to the formation of polyhydroxycurcumins that are more potent radical scavenger, while addition of other methoxy groups does not change the activity or reduces it. Diacetyl derivative of curcumin shows better antioxidant activity, as the introduction of bulky substituents. On the other hand, the replacing of methoxy moiety with one F-atom or replacing of methoxy and hydroxyl groups with two F-atoms enhance the selectivity toward COX-2, inhibiting their activity and improving anti-inflammatory action.[140] Synthesis of

curcumin analogues that possess coumarin-moieties instead the phenolic one showed similar antioxidant properties to original curcumin (probably due to high conjugation system that makes very easy to abstract the hydrogen of enol, very stable radicals). Preliminary investigations about anticancer activity of these analogues shown that 7-F-substituted and 6-F-substituted on coumarin ring of these new analogues (**32**) explicate better activity against MCF-7 cells and HeLA cells respectively. This fact can explained by an improved water solubility that leads to a better stability and uptake by the cells.[139]



Scheme 1.2.8. Synthesis of Curcumin analogues by Olgah et al.

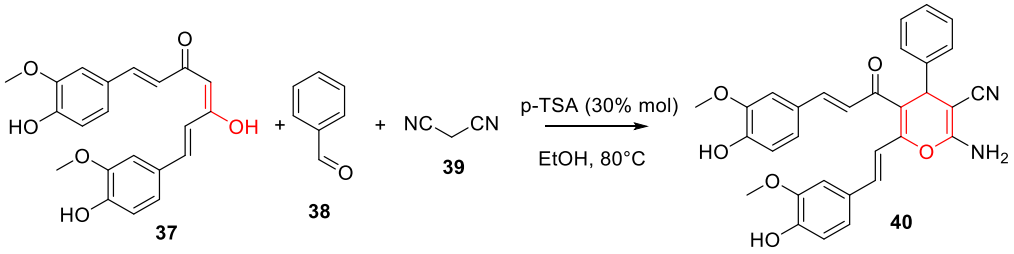
By exploiting the stability of cyclopentenones **33** and cyclohexanones **34**, it is possible to obtain curcumin analogues by the partial replacing of β-dicarbonyl moiety (**35**, **36**). The reaction between of cyclopentenone/cyclohexanone and several substituted benzaldehydes (or thiophene, pyrrole, furan, naphthalene aldehydes) in presence of NaOMe/MeOH system allow to obtain an impressive number curcumin of new analogues through an easy way. The conditions allow to obtain monocarbonyl curcumin derivatives also using acetone instead cycloketones. This mono-carbonyl analogues showed high stability in a phosphate buffer (pH 7.4) at 37°C for 75h (simulation biological conditions), especially the cyclopentenones derivatives those had one or more methoxy moiety linked to benzene ring.



Scheme 1.2.9. Synthesis of mono-carbonyl curcumin analogues by Liang et al.

Cytotoxicity on seven different tumor cell-lines shown that cyclohexanone-curcumin analogues possess higher biological activity than cyclopentenone and acetone derivatives as the cyclohexane moiety, in accordance to author, mimics better the 6-ring that come out in enol-form of curcumin thanks by the establishment of hydrogen bond between enol and second carbonyl group. From the SAR analyses, the 4'-weak electrodonating substitution and 2'-electrowithdrawing are the position in which the cytotoxicity is improved.[141]

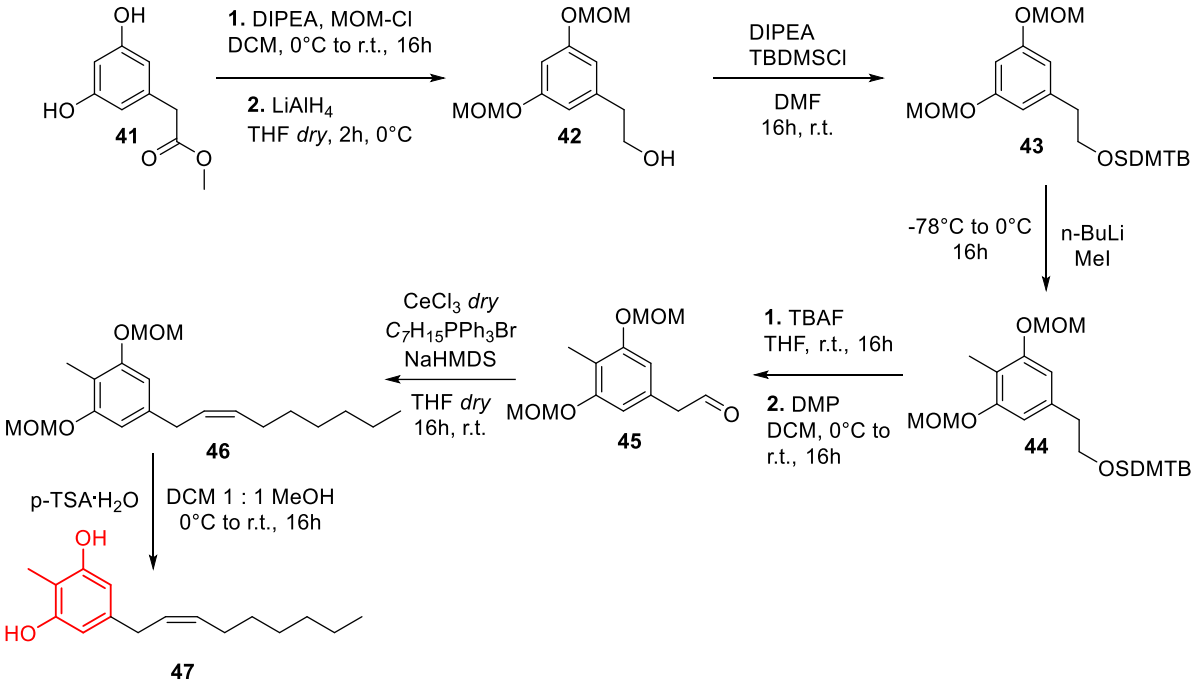
The synthesis of pyrane moiety **40** obtained through a condensation between curcumin **37**, benzaldehydes **38**, malonitrile **39** in presence of catalytic amount of p-TSA, exploiting one of carbonyl of β-diketone moiety, leads to the formation of curcumin-analogues **40** that can positively inhibit α-Gls enzyme (antidiabetic applications), preserve the antioxidant activity, without toxicity toward two common intestine bacterial flora (*Escherichia coli* (PTCC 1058), and *Lactobacillus plantarum* (PTCC 1553).[142]



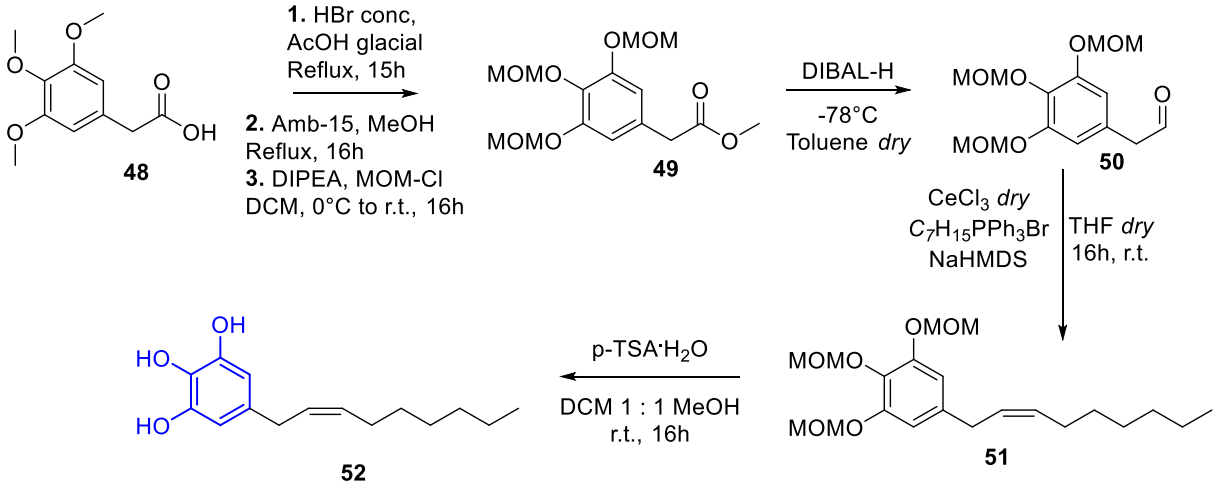
Scheme 1.2.10. Synthesis of pyrane-curcumin analogues by Zohrer et al.

Climacostol (1,3-dihydroxy-5-[(Z)-non-2-enyl]benzene) belongs to the family of resorcinolic lipids (or alkylresorcinols); it is a toxin produced by the ciliated protozoan *Climacostomum virens* as defence against unicellular and multicellular predators. As many other phenols compounds, it can be oxidised by Cu(II) and explicate prooxidant action and induces DNA damages.[144] Moreover, Climacostol can explicate a vast range

of biological activities such as antimicrobial and antifungi (*Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Candida*), cytotoxicity, inhibition of hunman/rat tumour growth.[144 - 146] Climacostol can exist as two isomer, (*E*)- or (*Z*)- due to the presence of double bond, in which the (*Z*)-is the more active of two. Synthesis of Climacostol has been an important target of prof. Marcantoni's laboratory. The selective synthesis of (*Z*)-Climacostol isomer has achieved through a selective Wittig reaction performed in presence of stoichiometric amount of $CeCl_3$ *dry* without detecting any trace of (*E*)-isomer at NMR and HPLC.[147] Further, two analogues were synthesized by the same Wittig conditions. The first one present a methyl group in position 4 of resorcinol ring (**47**), it was obtained starting from methyl-3,5,-dihydroxiphenylacetate **41** since the 4-methylated resorcinol was not commercially aviable. The methylation was obtained between the reaction of **43** in presence of n-BuLi and MeI, and after a selective Wittig reaction, followed by the deprotection, allowed to obtain a new Climacostol analogue **47** (see *Scheme 1.2.11*). In similar way, starting from 3,4,5-O-methyl-gallic acid **48**, it was possible to obtain a new analogue (**52**) in which a third –OH group was present in position 4 (see *Scheme 1.2.12*).[148]



Scheme 1.2.11. Total synthesis of 4-methyl analogue.



Scheme 1.2.12. Total synthesis of 4-hydroxy analogue.

The introduction of methyl (**47**) and hydroxy groups (**52**) in resorcinol ring modulate the potency and mechanism of action of "original" Climacostol. These modifications do not improve the anticancer activity in mammalian cells of native Climacostol, but they exert an appreciable antimicrobial activity, except against of *Escherichia coli* and *Pseudomonas aeruginosa* as probably these two analogues cannot overcome the lipid barrier of bacterial. Among them, the methylated analogue has proven to be more toxic than hydroxy-one against *S. aureus*, *E. faecalis* and *C. albicans*; and it has showed the higher toxicity (0.64 µg/mL < LC_{50} < 2.15 µg/mL) against four ciliates microorganism (*B. japonicum*, *P. multimicronucleatum*, *S. ambiguum*, and *S. teres*). On the other hand, hydroxy-analogue **52** can induce apoptosis in unicellular eukaryotes.[148]

1.2g Results and Discussion

Before starting, I want to thank Prof. Emilio Clementi (Pharmacologist – University of Milan); Prof. Claudio Ortenzi (Biologist – University of Macerata); Prof. Davide Cervia (Fisiologist – University of Viterbo); Dr. Elisabetta Catalani (Biologist – University of Viterbo); Prof. Anna Maria Fausto (Biologist – University of Viterbo); Prof. Simona Picchietti (Biologist – University of Viterbo); Prof. Cristiana Perrotta (Farmacologist – University of Milan); Prof. Federico Buonanno (Biologist – University of Macerata) for their indispensable contribution with docking calculations, consultancy and biological analyses that helped to give life to this project.

BACKGROUND OF THE PROJECT

Virus infection is one of the most important diseases that afflict human living all over the world, especially, during the last 20 years CoVs-affections became a very important issue that have caused several pandemic situations like severe acute respiratory syndrome coronavirus (SARS-CoV, 2002-2003); H1N1 influenza in 2009; Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012; and the last one the pandemic caused by COVID-19 (SARS-CoV-2, 2019 -).[149] This novel virus belongs to the large family of single-stranded RNA viruses (+ssRNA). Studies about the amino acid sequences of seven nonstructural protein and genome of SARS-Cov-2 has shown, respectively, a similarity of 94.6% of fees proteins and ≈80% of genome with SARS-CoV, suggesting a similar origin of these two viruses.[150, 151] In addition, a 96% similarity of genome between SARS-CoV-2 and bat sarbecovirus sampled from Rhinolophus affinis horseshoe bat in Yunnan province, called Bat-CoV (RaTG13), could indicate a bat origin of this virus.[152] After the outbreak of COVID-19 pandemic situation, scientist developed several vaccines to stop the spread of virus but, despite the high rate of protection, this remain a prevention treatment, while there is still the restless need for a novel therapy to fight the virus, especially for people already affected by Sars-CoV-2.[153] For this reason many scientists focus their effort to offer alternative ways to fight the virus such as: the employment of metal complexes of Au(I), Au(III), Bi(I), Re(I) o Se(I)-compound;[154] possible inhalation of low molecular weight and volatile organic molecule that possess antiviral and anti-inflammatory actions like 1,8-cineleone;[154] employment of nanoparticles since they could avoid the problem of resistance-drug

development by microorganism;[155] or employment of biologically active small molecules, some of which are currently under clinical trials.[156] The 3CLprotease ($3CL^{pro}$) is a valid target for small molecule drugs since this protein is involved in virus replication. Once that genome virus is inside the host cells, it is translated in two polyproteins pp1a and pp1ab (790 kDa), then, the cleavage of these two proteins, by collaboration between $3CL^{pro}$ and papain-like protease, leads to the generation of 16 functional non-structural proteins (nsps); those proteins assemble the viral replication transcription complex (RTC), initiating the replication of virus. $3CL^{pro}$ cleaves 11 sites of polyprotein1ab.[157] Quercetin emerges a good candidate as potential drug for inhibition of 3CLprotease by docking analyses and screening of small chemical library made up of 150 small molecules. Interaction between Quercetin and $3CL^{pro}$ alters the thermal stability of the protein, destabilizing and inhibiting the activity of protease. Moreover, Quercetin exhibits a higher BEI (Binding Efficiency Index, BEI = pKi/MW with pKi = -Log[Ki]) respect to already known $3CL^{pro}$ inhibitors alpha-ketoamide 13b. Even if Quercetin has a Ki (inhibitory constant) much lower than ketoamide (7.4µM and 0.19 µM respectively), that implicates a higher BEI, its lower molecular weight (0.302 kDa and 0.598 kDa respectively) makes quercetin more suitable for binding with protease and explicates a better inhibition activity than ketoamide 13b.[158]

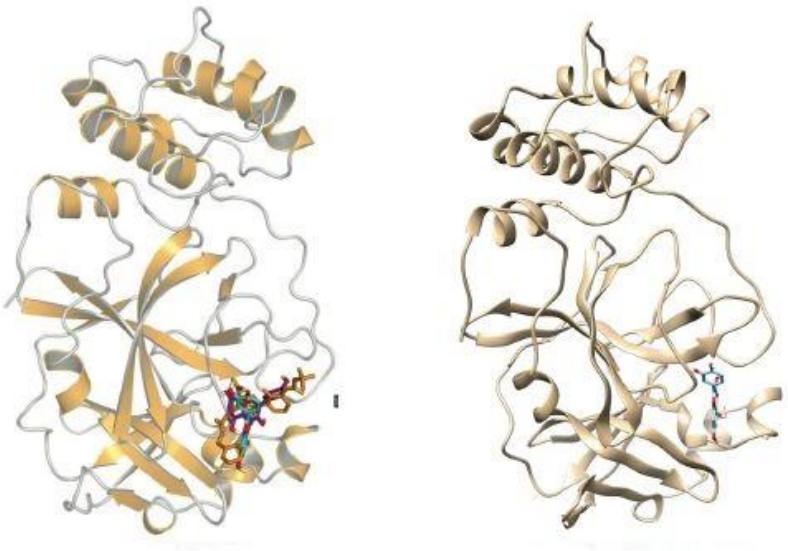


Figure 1.2.12. Complexation between 3CLprotease with ketoamide-13b (on the left) and Quercetin (on the right)[158]

Inspired by these results, we investigated the possible inhibition action of Climacostol, already synthesized in prof. Enrico Marcantoni's lab, and other possible analogues not

synthesized yet, using the affinity value for 3CLprotease of Quercetin as reference. The affinity values are reported in the table below.

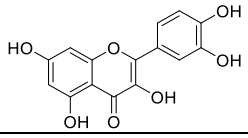
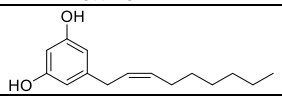
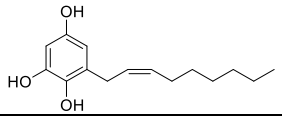
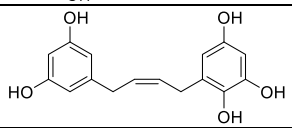
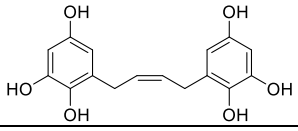
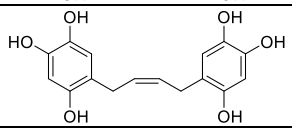
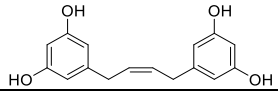
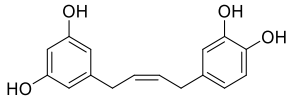
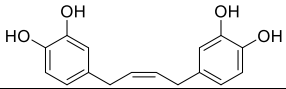
COMPOUND NAME	STRUCTURE	AFFINITY (kcal/mol)
Quercetin		- 7.2
Climacostol		- 5.0
New analogue 1		- 5.4
New analogue 2		- 6.5
New analogue 3		- 6.5
New analogue 4		- 6.9
New analogue 5		- 6.8
New analogue 6		- 6.7
New analogue 7		- 6.6

Table 1.2.2. Affinity values for CL3^{pro}

Among all analysed compounds, Quercetin is the molecule that has the major affinity toward the protease. The long alkenyl chains of Climacostol and its analogue 1 influence negatively the affinity for protease. The other new theoretical analogues registered good affinity results, although lower than result of quercetin. However, the two phenols-structure and (Z)-double bond configuration it seems to be a valid structurally choice as the affinity values of new analogues are quite closer to quercetin reference. The position of hydroxy groups linked to aromatics core do not change the affinity at all, the combination of resorcinol/catechol moieties (like in quercetin) is a good compromise between activity and commercially available molecules.

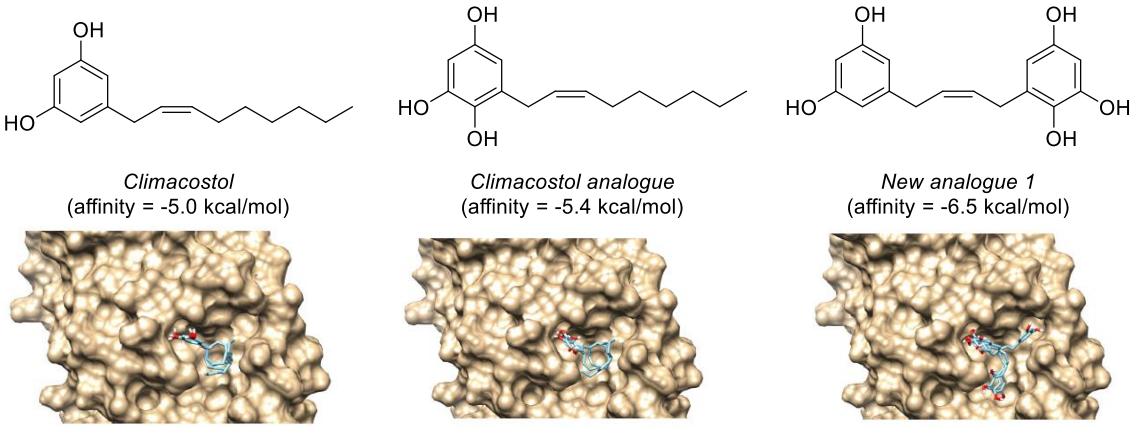


Figure 1.2.13. Simulation of complexation of some molecule reported in Table 1.2.1. with its affinity value

Keeping in mind all these informations obtained from the docking analyses, we decided to develop the synthesis of a new (poly)phenolic compound that had structural similarities, with the molecules shown in the table and with other natural molecules, and that could possess anti-viral action against SARS-CoV-2. Moreover, regarding the Lipinski's Rules of Five (LogP solubility <5, molecular weight < 500 Dalton, <5 H-bond donor, <10 H-bond-acceptors)[158] this new analogue respect the three of four parameters for oral drugs absorption and permeation (solubility water/n-octanol is still unknown): it weight 300 daltons, has 5 oxygen atoms (respected H-bond donors and acceptors).

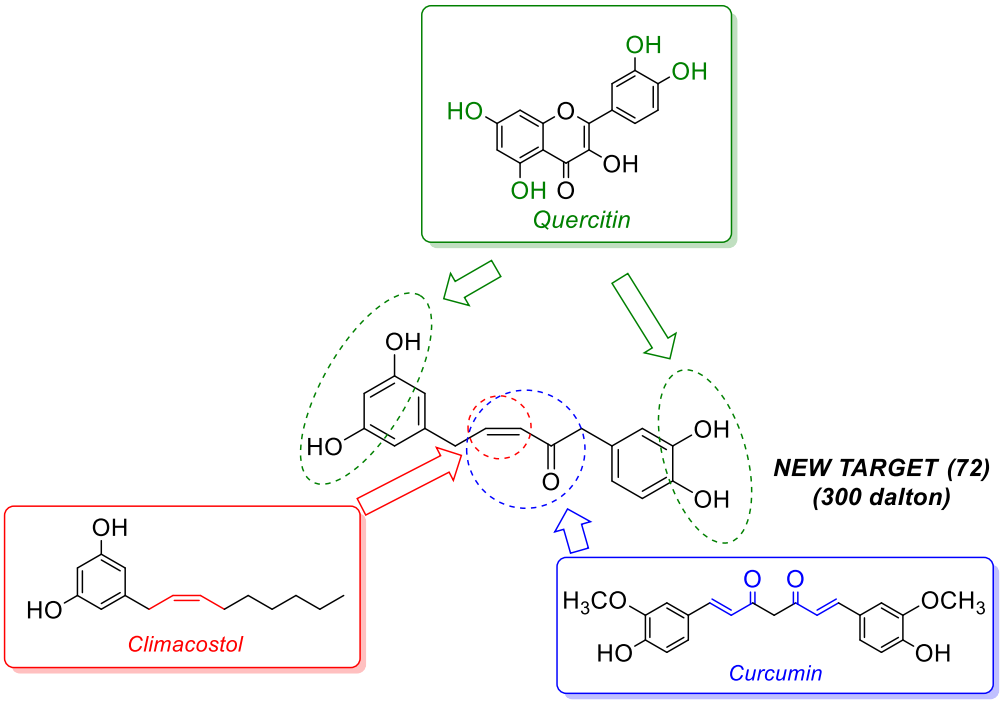


Figure 1.2.14. Structure of new polyphenol target

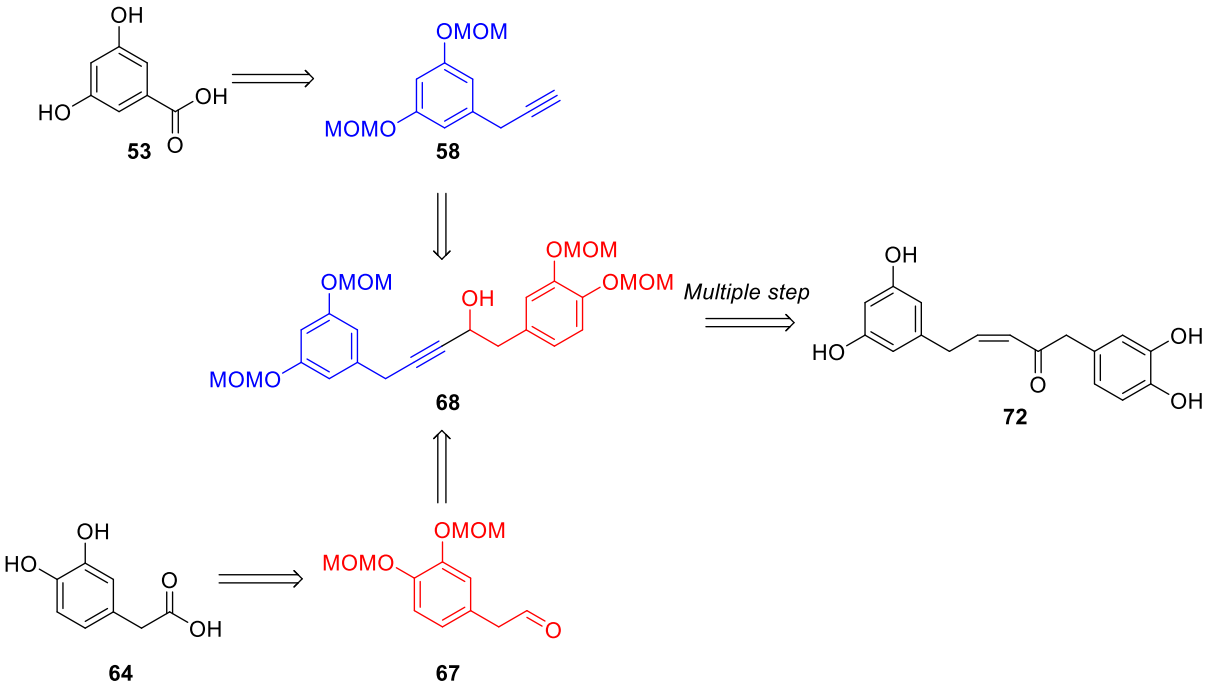
The principle structure analogies are related to:

- Climacostol, (*Z*) configuration of double bond and resorcinol moiety
- Quercetin, resorcinol/catechol moieties present together a double bond/carbonyl conjugation
- Curcumin, conjugation of double bond with and the presence of two phenol-moieties.

The biological properties of these three compounds are well-know and reported in previous sections.[117, 138, 144] Due to analogies of new target, it is possible that this molecule could possess and exhibit inhibition of tumour growth (like climacostol), anti-inflammatory and antioxidant activity (like curcumin), anti-cancer and prevention of Alzheimer and Parkison's diseases[160] (like quercetin), just to name few, in addition to potential antiviral activity that is the priority goal in this moment.

RETHRO SYNTHETICAL ANALYSES

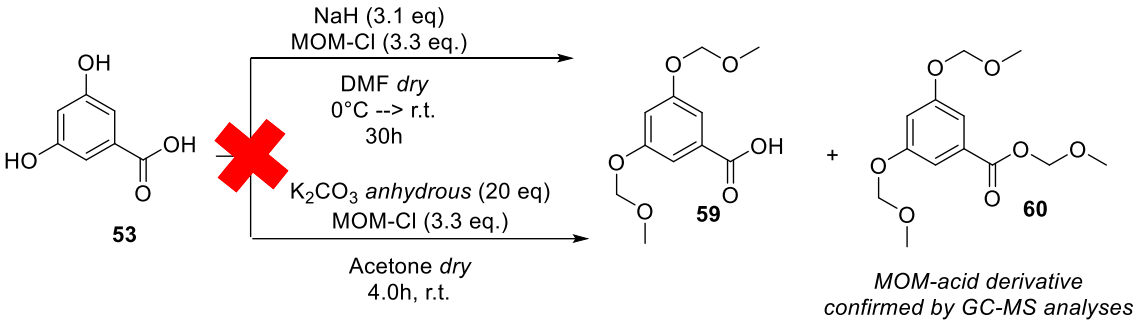
We have developed a synthetic strategy for this compound **72** which involves the synthesis of a propargyl alcohol intermediate **68** obtained by the reaction between an aldehyde **67** and an alkyne **58**, which are in turn obtained from two commercially available and inexpensive starting materials such as 3,4-dihydroxyphenyl acetic acid **53** and 3,5-dihydroxybenzoic acid **64**. In addition, these two phenols possess already the configuration of resorcinol and catechol moiety present in the final product, making them very suitable for the synthesis. As protecting group for –OH moieties we choose to use MOM-protection, since *in vivo* studies about anti-tumour of MOM-protected Climacostol showed good activity and selectivity,[161] leaving open the possibility of using the MOM-protected new target for biological applications.



*Scheme 1.2.13. Rethro synthetical analyses for new target **72.***

*SYNTHESIS OF ALKYNE **58**.*

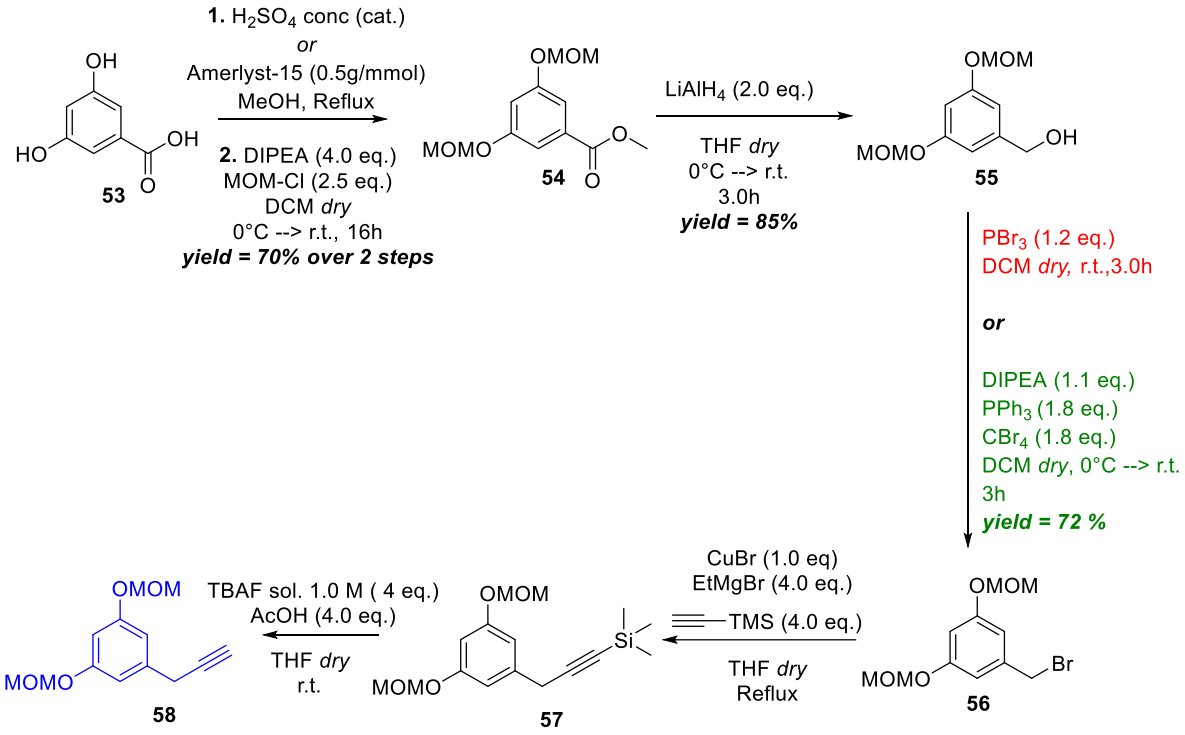
First, we tried to protect the two hydroxy groups present on 3,5-dihydroxybenzoic acid **53** with the free acid moiety performing the protection in two different condition: (i) DMF *dry* and NaH as base;[162] ii) using K_2CO_3 *anhydrous* as base in acetone;[163] but in these conditions we detected a low yield of product **59** and a huge formation of MOM-acid derivative **60**, confirmed by GC-MS analyses.



Scheme 1.2.14. MOM-protection of hydroxy groups with free acid moiety.

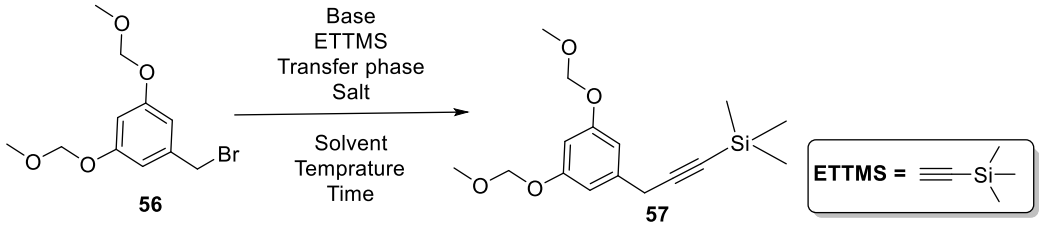
Therefore, due to the problems related to the free moiety acid, we decided to esterify the acid moiety using common Brønsted acids like H_2SO_4 conc. in catalytic amount or Amberlyst-15® in conditions reported by Petrini *et al.*[164] refluxing methanol. In each

condition it is possible to obtain the ester with a high yield (92% in case of sulphuric acid and almost quantitative for Amberlyst-15®), but although the Amberlyst-15® reaction is slower (16 hrs reaction against 3 hrs), the easy handling, no harmful, reactivation and possible reuse of Amberlyst-15®,[165] make this way more suitable, green and safe from synthesis at industrial level point of view. At this point, the MOM-protection of –OH is achieved just using DIPEA as base, performing the reaction in DCM *dry* at room temperature for 16 hrs, with an overall yield of about 70% over 2 steps (**54**). The successive reduction of ester moiety (yield = 85%) and reaction of alcohol **55** with PPh_3 and CBr_4 in presence of DIPEA leads the formation of bromide derivative **56** (yield = 72%). The halo substitution of –OH in **55** by PBr_3 did not result in any formation of the product.



*Scheme 1.2.15. First synthetic approach for alkyne **58***

The most difficult step for synthesis of alkyne **58** has been the achievement of silane **57**. Many experiments have been conducted and they are reported in the table below.

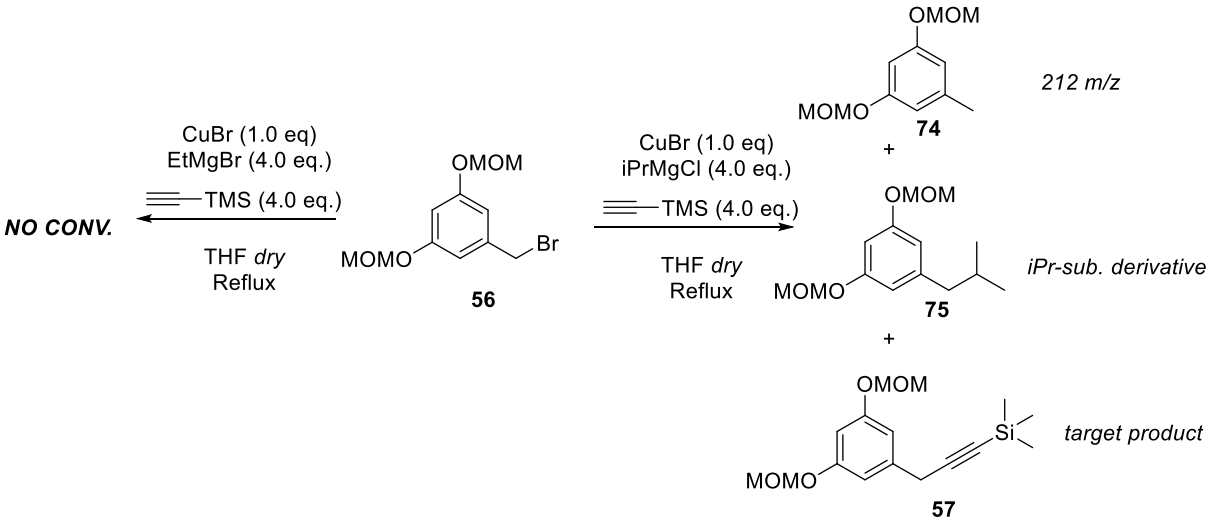


56 57

ENTRY	Base	ETTMS	Salt	Temp.	Time	GC conv.
1[a]	EtMgBr (4.0 eq.)	4.0	CuBr (1.0 eq)	Reflux	40h	-
2[a]	iPrMgCl (4.0 eq.)	4.0	CuBr (1.0 eq)	Reflux	40h	5%
3[b,c]	K_2CO_3 *anhyd.* (2.0 eq.)	1.3	CuI (1.0 eq)	60 °C	18h	50% (separated as mixture)
4[a]	nBuLi (2.0 eq.)	2.0	CuI (1.0 eq)	r. t.	20h	3%
5[a]	KHMDS (2.0 eq.)	2.0	CuI (1.0 eq)	r. t.	20h	SM and I- deriv.
6[b,c]	K_2CO_3 *anhyd.* (2.0 eq.)	1.3	CuBr (1.0 eq)	60 °C	16h	20%
7[b,d]	K_2CO_3 *anhyd.* (2.0 eq.)	1.3	CuBr (1.0 eq)	60 °C	24h	17%
8[a]	nBuLi (4.0 eq.)	5.0	LiI (1.0 eq)	Reflux	24h	SM and I- deriv.
9[b,c]	K_2CO_3 *anhyd.* (2.0 eq.)	1.3	LiI (1.0 eq)	45 °C	40h	9%

*Table 1.2.3. Reaction conditions for the synthesis of Silane **57**; a) reaction performed in THF dry; b) reaction performed in CAN dry; c) reaction performed in presence of transfer phase tetrabutyl ammonium iodide (TBAI = 1.0 eq.); d) reaction performed in presence of transfer phase tetrabutyl ammonium bromide (TBAB = 1.0 eq.)*

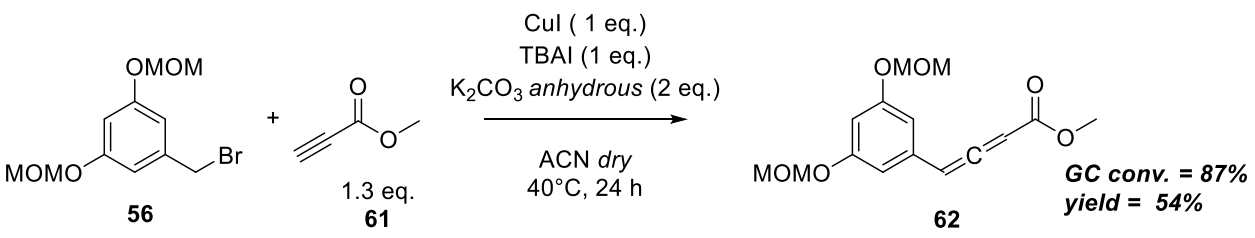
Many conditions for the synthesis of sylane **57** were tested. First, we tried to perform the coupling in presence of Grignard (EtMgBr Entry 1 *table 1.2.3.*, i-PrMgCl Entry 2 *table 1.2.3.*) and CuBr. In these conditions, the product was not detected or detected in very low conversion (5% in case of i-PrMgCl), for the EtMgBr reaction, a huge amount of starting material was recovered. Meanwhile, the i-PrMgCl reaction leads to formation of a little more complex mixture in which starting material was almost consumed and a the i-Pr-substituted derivative **75** was detected with a high conversion) and a possible abstraction of halogen could take place as a 212 m/z compound **74** was detected at GC-MS, consuming starting material for the good success of reaction.



Scheme 1.2.16. Grignard Conditions

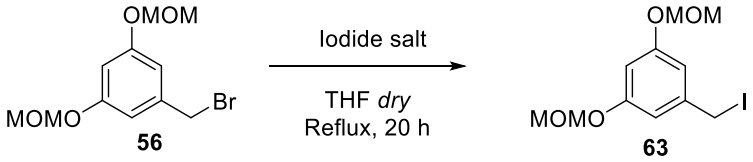
Changing drastically the approach slightly better results were obtained. Performing the reaction in the presence of a phase transfer salt/compound (Entry 3, *table 1.2.3*),[166] a higher conversion of the product was detected by GC-MS analyses but unfortunately the product was separated as mixture in which –I derivative was present in not negligible amount. Changing copper salt (Entry 6, *table 1.2.3*), transfer phase (Entry 7, *table 1.2.3*) and metal core (Entry 9, *table 1.2.3*) leads only to worse result, confirming that the combination of TBAI, K_2CO_3 *anhydrous*, CuI is the best reagents combination. Entry 4 is a test to avoid the formation of Grignard byproduct, but using n-BuLi as base, only a 3% of GC conversion of the product was observed, while using LiI instead of Copper iodide (Entry 8, *table 1.2.3*) starting material and iodide derivative were recovered. Finally, using a less strong base such as KHMDS, SM and –I derivative were collected again. At this point, questioning about these unsuccessful results, we decide to test a different alkyne and a different halo-derivative.

Using methyl propiolate **61** as alkyne under the conditions already described in *table 1.2.3* (entry 3), this time the product **62** was isolated in 54% of yield, even if in its allene form (GC-MS, FT-IR ^{1}H and ^{13}C-NMR characterized).



Scheme 1.2.17. Alkyne screening

On the other hand, we substituted the bromide with an iodide moiety as the –I is a better leaving group. A little screening of iodide salts and it showed that NaI (5 eq.) in *dry* THF resulted as the best reaction conditions. Screening of iodide salts is reported in the table below.



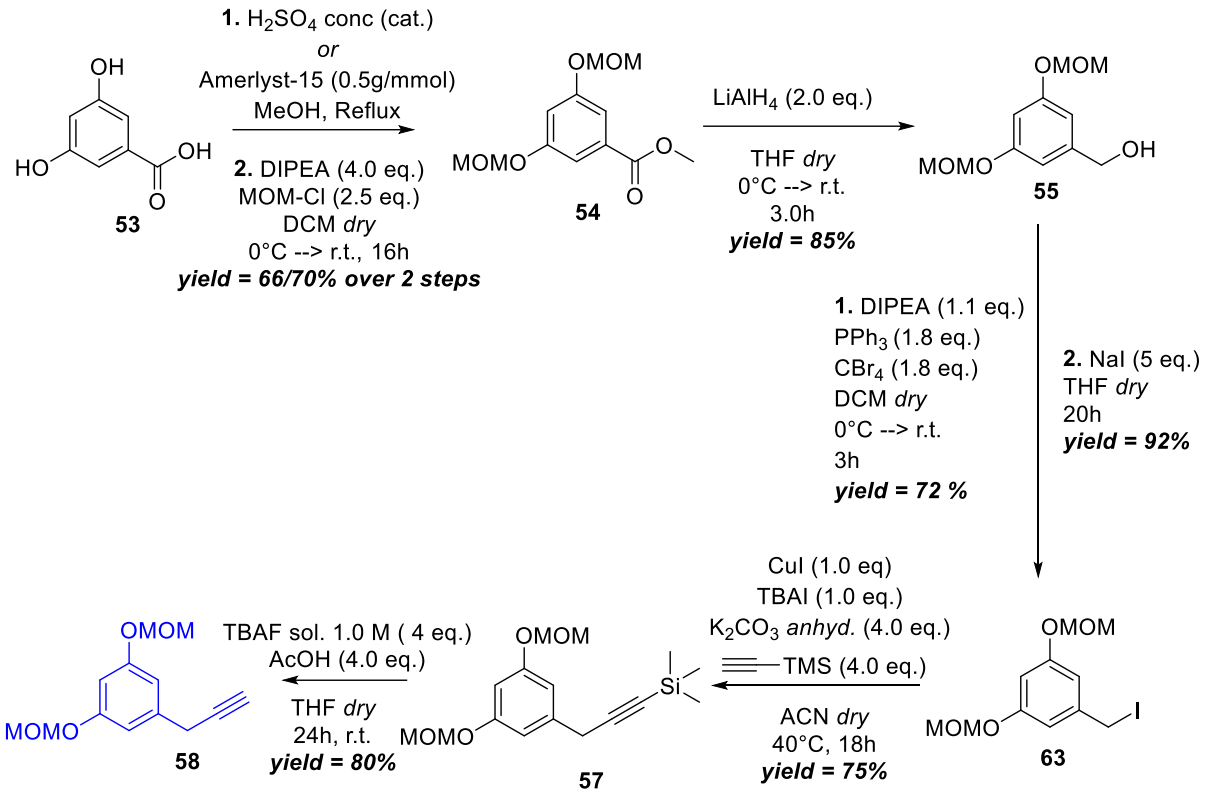
ENTRY	IODISED SALT	YIELD
1	NaI (4.0 eq.)	78%
2	LiI (4.0 eq.)	50%
3	CuI (4.0 eq.)	30%
4	**NaI (5.0 eq.)**	**92%**

Table 1.2.4. Screening reaction conditions for halo substitution

Then, performing the coupling with compound **63,** the silane **57** was achieved with a good yield of 75%. These tests confirmed, in our opinion, the low reactivity of silane-alkyne as nucleophile towards this transformation. In fact, when performing the same reaction using propiolate bearing an ester moiety conjugated with the alkyne, it proceeds more smoothly, thanks to the higher acidity of the proton resulting in an enhanced nucleophilicity of the compound.

Further silane-removal was performed with an excess of fluoride source (TBAF) in presence of acetic acid, achieving the target alkyne **58** with a yield of 80%.

In conclusion, the total synthesis of alkyne **58** is summarized in the scheme below (*scheme 1.2.9.*), it is obtained with an overall yield of 22% over 7 steps.



*Scheme 1.2.18. Final synthetic pathway for the alkyne **58.***

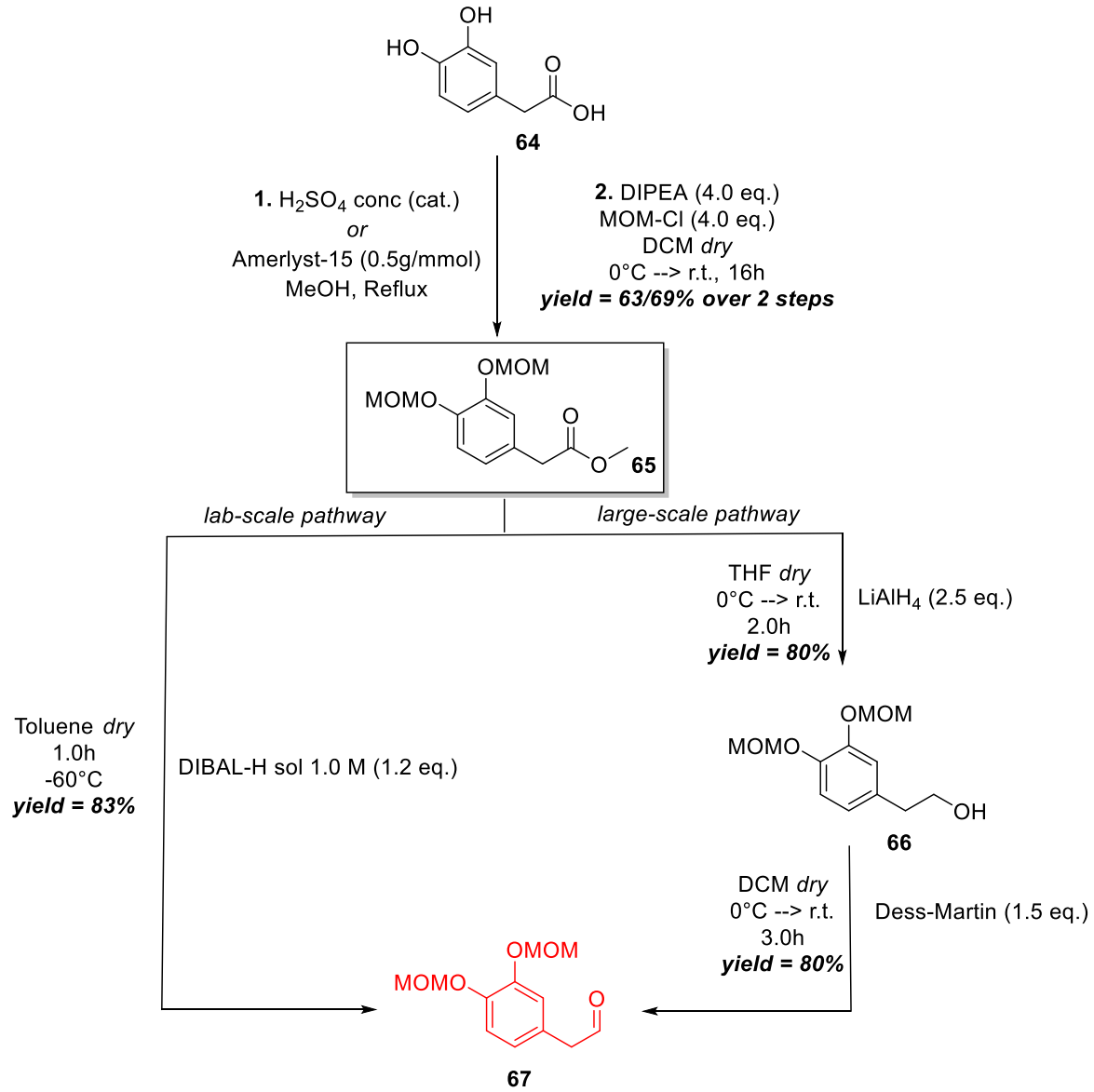
*SYNTHESIS OF ALDEHYDE **67.***

We developed two synthetic strategies for the aldehyde **67**: one of the two is designed for large-scale synthesis, possibly at industrial level, while the other one is more functional and more suitable for the scale-laboratory synthetic level (*scheme 1.2.19*). Following the industrial-pathway, four steps are necessary to obtain the aldehyde, to pass from protected ester to aldehyde it is necessary a sequence reduction-oxidation that involves mild conditions and not so harmful reagents. On the other hand, the ester can easily reduce to aldehyde DIBAL-H solution increasing the overall yield of intermediate **67**, but this way requires cryogenic conditions (-60°C) which are not exactly feasible at an industrial level and for large-scale production.

Keeping in mind the problems met during the protection of –OHs with free acidic moiety, we proceed directly with the esterification moiety of 3,4-dihydroxyphenil acetic acid **64** (as 3,5-dihydroxybenzoic acid, it is possible use H_2SO_4 conc. or Amberlyst-15®), and the successive reaction of ester with DIPEA and MOM-Cl leads to the formation of protected-ester **65**. The major problem related to these steps is the purification of compound **65** from the mono protected compounds, arising as a side products of the reaction. Purification by Silica gel (isocratic and gradient) were not able to separate with a satisfying purity di-protected product

from the two mono-protected isomers. This problem can be overcome in two ways: performing the purification by Al_2O_3 basic pad using dichloromethane as eluent or using a large excess of MOM-Cl and avoiding the formation of mono-protected side products for Le Châtelier-principle. However, this last choice implies a big amount of dangerous reagent, large waste-production and money consuming, making it not suitable with the green principles that rule the world today. From this point two possible ways can be followed. The first one, large-scale pathway, conducts to the synthesis of the aldehyde **67** through a reduction from ester to alcohol ($LiAlH_4$ as reducing agent) and a subsequent oxidation with Dess-Martin periodinane to the target **67** with an overall yield of 45% in four steps.

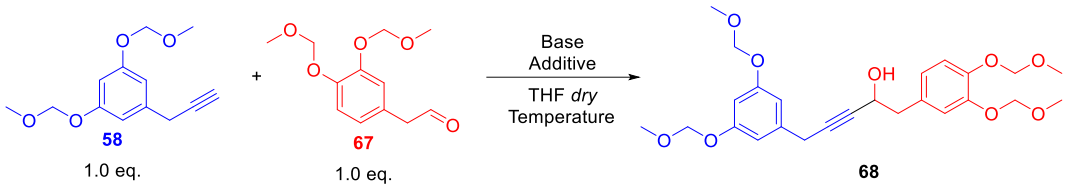
The second one, lab-scale pathway, leads to aldehyde target just using a little excess of DIBAL-H with an overall yield of 55% over three steps.



*Scheme 1.2.19. Two possible pathways for the synthesis of aldehyde **67***

*SYNTHESIS OF PROPARGYL ALCOHOL INTERMEDIATE **68.***

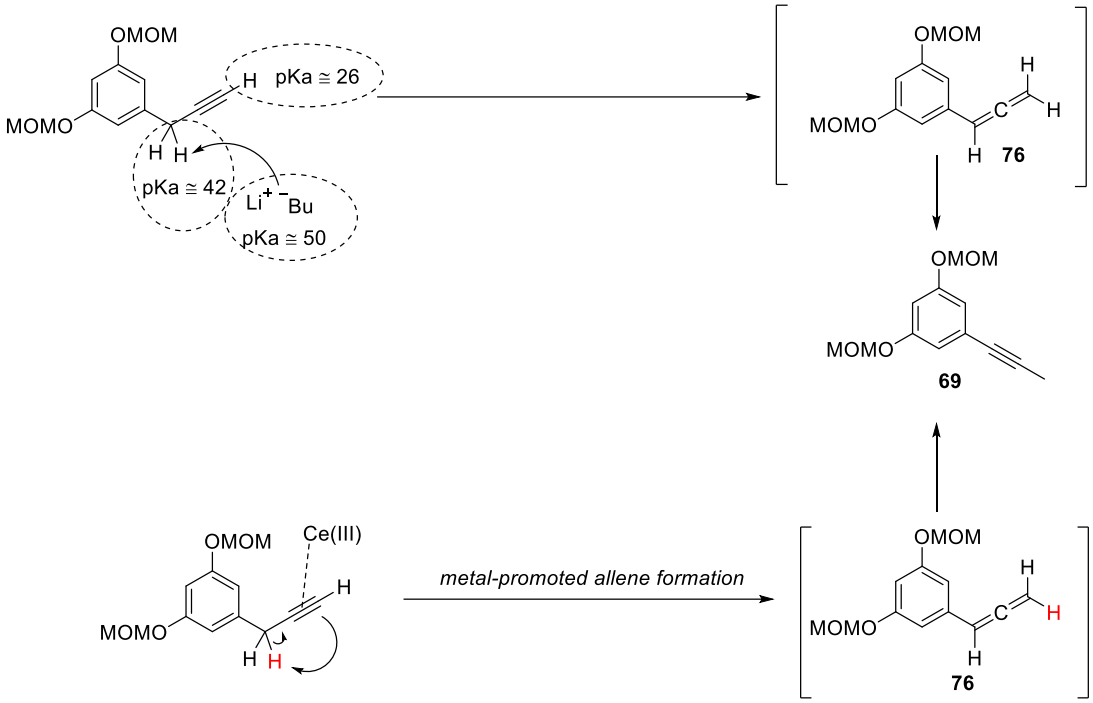
Once that the two intermediates were obtained, we tried to synthesise the intermediate **68**. All the experiments conducted are reported in the table below.



ENTRY	Base	Additive	Temp. (time)	Yield
1	nBuLi (2.2 eq.)	$CeCl_3$ *dry* (1.0 eq.)	-40°C → r.t. (24 h)	-
2	nBuLi (1.0 eq.)	-	-10 °C → r.t. (16 h)	35%
3	nBuLi (1.0 eq.)	-	-78°C (16h) → r.t. (7.0 h)	10%
4	nBuLi (1.0 eq.)	$CeCl_3$ *dry* (1.0 eq.)	-78°C (16h) → r.t. (7.0 h)	15%
5	nBuLi (1.0 eq.)	-	-10 °C (5h) → r.t. (16 h)	38%
6	nBuLi (1.0 eq.)	-	-20 °C → r.t. (16 h)	29%
7[a]	LiHMDS (1.2 eq.)	-	-78°C → r.t. (16 h)	-
8[a]	KHMDS (1.2 eq.)	-	-78°C → r.t. (16 h)	-
9[a,b]	Et_3N (1.2 eq.)	$Ce(OTf)_3$ *dry* (1.1 eq.)	r.t. (24 h)	-
10	EtMgBr (1.0 eq.)	-	r.t. (16 h)	18 %
11	-	Zn activated/allyl bromide (1.0 eq./1.0 eq.)	r.t. (48 h)	trace
12[c]	nBuLi (1.0 eq.)	-	-78°C → r.t. (16 h)	71%

Table 1.2.5. Screening conditions for alkynaltion of aldehyde; a) reaction performed with 1.2 eq. of aldehyde; b) reaction performed in Toluene dry instead THF dry; c) addition of aldehyde solution done after 5 minutes base-alkyne reaction

Substantially five different methods were tested as reported in *table 1.2.5*. Applying the conditions reported by Principala *et al.*[167] the product was not achieved, but this result is explained by the isomerization of triple bond from benzylic to phenylic position (isomer GC-MS and ^{1}H-NMR characterized, see experimental section). This isomerization can be promoted by two possible and hypothetical way. The first one is due to the slight acidity of benzylic protons; in fact Jeanbourquin *et al.*[168] reported the isomerization of 1-aryl-3,3-azatrienes induced by ^{t}BuOK, in their work this reaction leads to the formation of allene intermediate; in our case, probably the formed allene isomerizes another time to alkyne isomer **69**. The second possibility is an allene **76** isomerization promoted by Ce(III) followed by rearrangement to internal alkyne; in fact, in literature it is known that transition metals can promote the formation of allene from alkyne.[169]



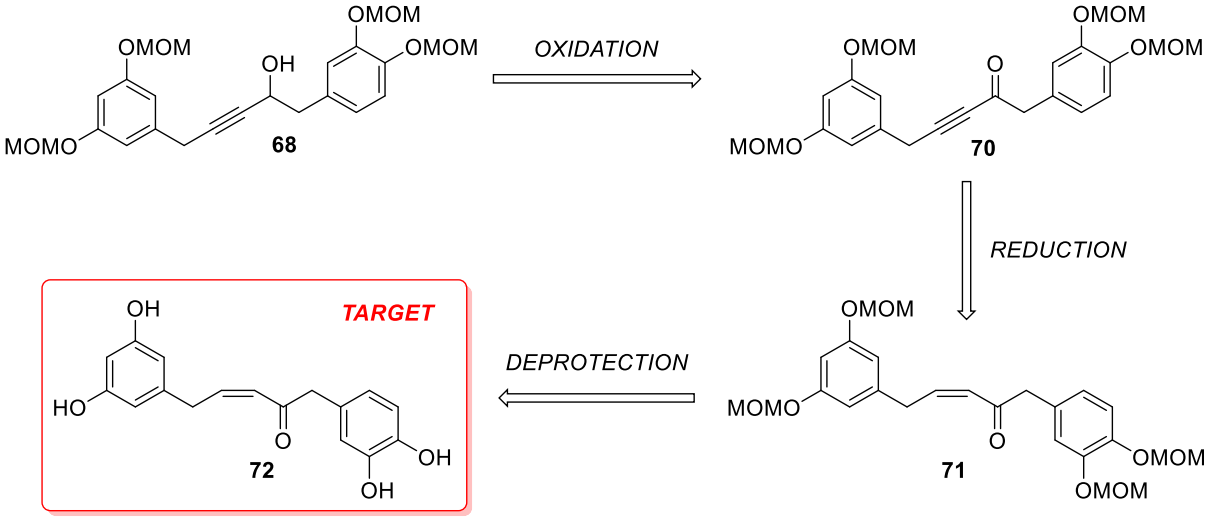
Scheme 1.2.20. Two possible pathways for isomerization triple bond.

Starting from this result, we tried to change the temperature addition, the amount of the base and the time reaction at low temperature (entry 2-6 of *table 1.2.5*). In all experiments the formation of alkyne isomer **69** was detected. Good temperature for the addition was -10°C as in entry 2 and entry 5 conditions quite good yields were obtained. It can be noticed that with decreasing temperature there is a progressive decrease in yield, as the isomerization process is probably favoured at low temperatures, and the addition of $CeCl_3$ *dry* (entry 4, *table 1.2.5*) does not so positively influence the trend of the reaction, since it can favourite also the formation of alkyne isomer. The detection of alkyne isomer **69** in each reaction could be a confirm of base-promoted isomerization of starting alkyne **58**. For this reason, we employed a less strong and not-nucleophilic bases like LiHMDS[170] (entry 7, *table 1.2.5*) and KHMDS (entry 8, *table 1.2.5*) but no target product was not detected while a little formation of isomer was observed in TLC. We changed totally approach, but also in these attempts, the product was never achieved satisfactorily. Trying a $Et_3N/Ce(OTf)_3$ system, inspired by literature (entry 9, *table 1.2.5*)[171] the product was neither detected, while trying to form an organomagnesium by using a Grignard entry 10, *table 1.2.5*)[172] the product was isolated in low yield. Trace of product were detected in a Zn (acid activated)/allyl bromide system (entry 11, *table 1.2.5*)[173] and in this condition the isomerization of alkyne was not observed by TLC or GC-MS. Finally, questioning about the reaction time between alkyne and base could be the problem; we performed another experiment in which aldehyde solution was added after only 5 minutes (entry 12, *table 1.2.5*) reaction

between base and alkyne. In fact, a good yield was registered in this conditions because probably, once formed the alkyne-anion, it reacts suddenly with the aldehyde and it has no time to isomerize to compound **69**.

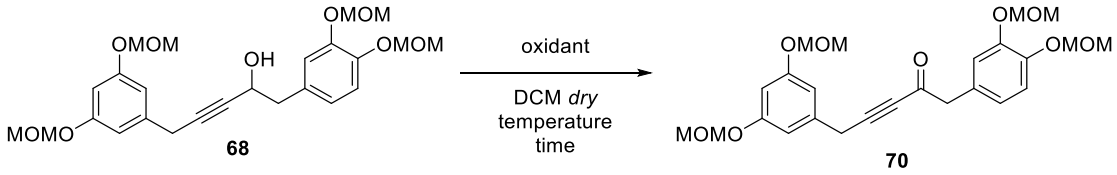
*FINAL STEPS FOR SYNTHESIS OF NEW TARGET PHENOLIC **73***

Once obtained the propargylic alcohol intermediate **68**, other three steps were left to achieve the final target phenol compounds: an oxidation, reduction and deprotection, as reported in scheme below.



Scheme 1.2.21. Final three steps for the synthesis of new target phenols

First, we tried several oxidation methods, reported in literature, to achieve the ketone **70**.

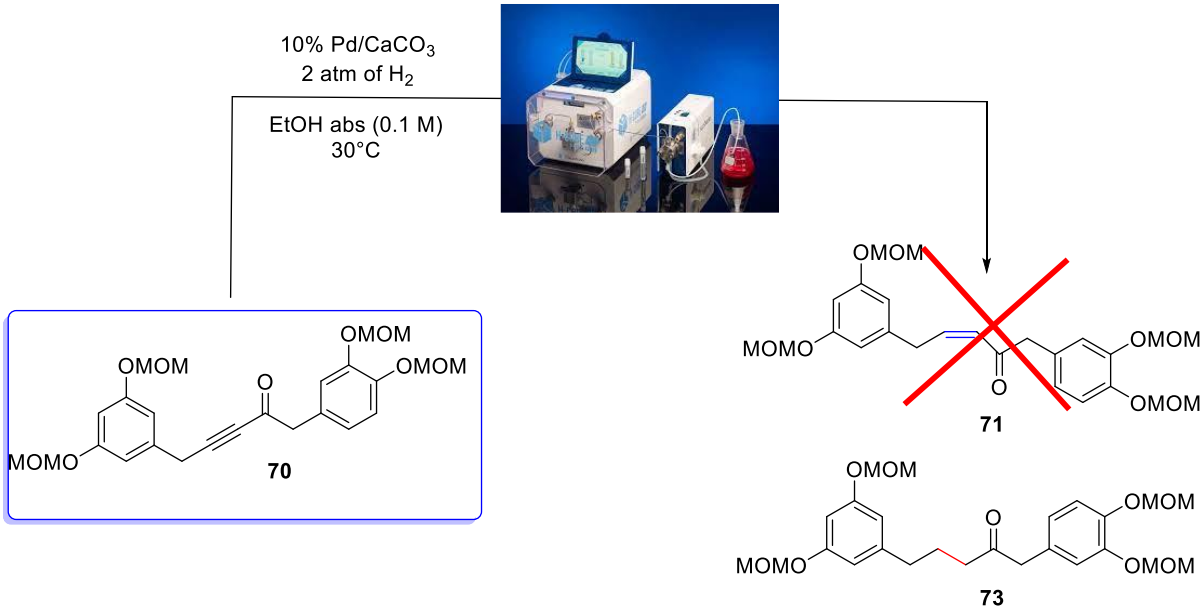


ENTRY	Oxidant	Time	Temp.	Yield
1	DMP (1.5 eq.)	7 h	r.t.	50%
2	DMP (1.5 eq.)	16 h	r.t.	42%
3[a]	DMP (1.5 eq.)	8 h	r.t.	40%
4	PDC (1.5 eq)	8 h	r.t.	-
5[b]	PCC (1.5 eq)	16 h	r.t.	38%
6[c]	PCC (1.5 eq)	16 h	Reflux	-

*Table 1.2.6. Screenig oxidant for synthesis of ketone **70**; a) reaction was performed in presence of 3.0 eq of $NaHCO_3$; b) reaction was performed in presence of 0.3 eq of NaOAc; c) reaction was performed in presence of Al_2O_3 neutral (1.6g/mmol)*

Unfortunately, the oxidants tested until now lead to not so good results. Dess-Martin Periodinane has shown the best oxidant, since in all three experiments (Entry 1-3, *table 1.2.6.*) the product was obtained in yield range of 40 to 50%. Lasting the reaction 16 hrs or adding 3.0 equivalents of sodium bicarbonate does not change at all the outcome of the reaction, even a lower yield has detected. Oxidation performed with pyridinium dichromate (PDC, entry 4, *table 1.2.6.*) did not produce any results. On the other hand, using a different pyridium chromate salt, PCC (entries 5-6, *table 1.2.6*) two different results have detected. In fact, performing the oxidation with PCC in presence of a catalytic amount sodium acetate, the product was achieved and isolated with a yield of 38%, while, using neutral alumina as additive, the product was not observed.

The project has stopped at reduction of which only a tentative was performed. Since a (*Z*)-configuration of final double bond was request, the classical reduction by Lindar catalyst is the most suitable method for our purpose. During these last years, H-Cube® has becoming a green alternative route for reduction.[174a, 174b] Using a cartridge Lindar catalyst-analogue (10% $Pd/CaCO_3$) and performing the reaction at 30°C, 2 atm of hydrogen pressure, in EtOH abs (0.1 M) at flow of 1 mL/min, the starting alkyne moiety was totally reduced, obtaining an alkane chain confirmed by ESI-MS. The carbonyl moiety was not reduced in these conditions since a peak at 1722 cm^{-1} was detected.



Scheme 1.2.22. Reduction experiment conducted with H-cube®

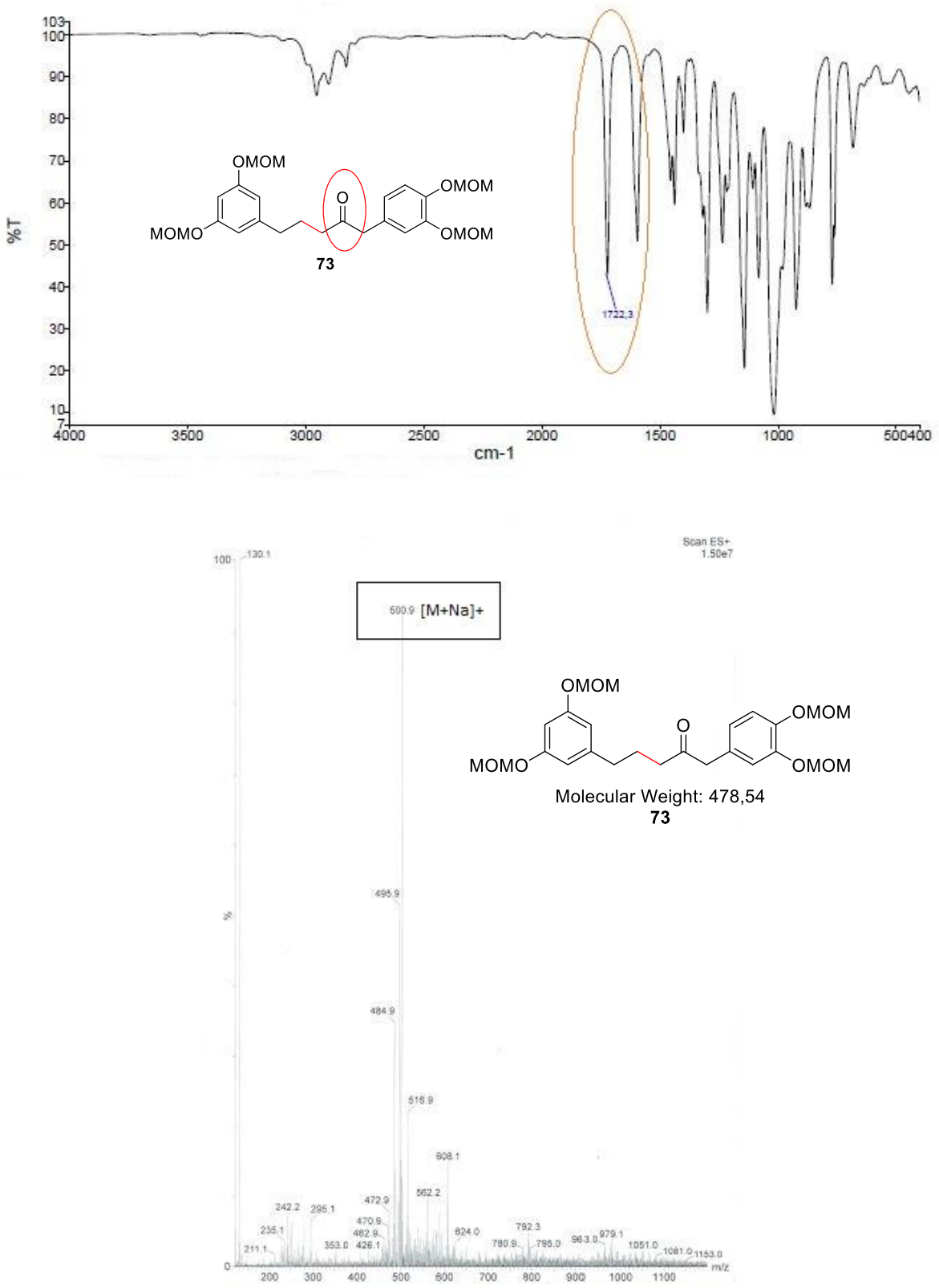


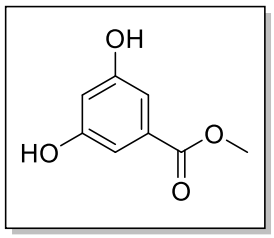
*Figure 1.2.15. FT-IR above and ESI-MS of compound **73**.*

As told before the project stopped at this point, but due to problem related to Covid-19 and the necessity of new therapeutic treatments and other potential biological applications that this new

phenolic compound can explicate, further studies and improvements for the synthesis are still carried out in our laboratory.

1.2h Experimental Section

METHYL 3,5-DIHYDROXYBENZOATE (77)



Sulfuric acid method: In a 25 mL round bottom flask, 3,5-dihydroxybenzoic acid **53** (200 mg, 1.3 mmol, 1.0 eq) is dissolved in 6 mL MeOH dry and 76 µL of H_2SO_4 was added dropwise. The solution was refluxed for 3 hrs. The reaction was monitored by TLC (4 Hex : 6 EtOAc Rf = 0.48)/GC-MS. Once the reaction finished, methanol was evaporated and the residue was partitioned between Ethyl acetate and saturated solution of $NaHCO_3$ and aqueous layer was washed 2 times with fresh EtOAc, the obtained organic phase dried over Na_2SO_4 and solvent evaporated. The final product obtained (beige solid) with a yield of 92%.

Or

Amberlyst-15 method: In a 25 mL round bottom flask, 3,5-dihydroxybenzoic acid **53** (200 mg, 1.3 mmol, 1.0 eq) is dissolved in 6 mL MeOH dry and 650 mg of Aberlyst-15® (500 mg/mmol) were added. The solution was refluxed for 16 hrs. The reaction was monitored by TLC (4 Hex : 6 EtOAc Rf = 0.48)/GCMS. Once the reaction finished, the solution is filtered by paper filter and the amberlyst-15® was washed three times with fresh methanol (3x25 mL), the amberlyst is recovered and reactivated, the organic solution is evaporated by rotavapor to give a beige solid in quantitative way.

Molecular Formula: $C_8H_8O_4$

^{1}H-NMR (400 MHz, DMSO-d6) δ = 9.62 (s, 2H), 6.79 (d, 2H, J=2.24 Hz), 6.42 (t, 1H, J=2.27 Hz), 3.77 (s, 3H).

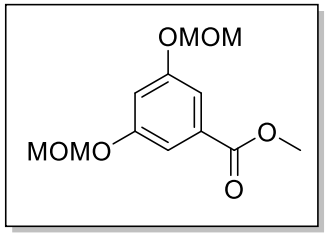
^{13}C-NMR (100 MHz, DMSO-d6) δ = 166.92, 159.21, 131.95, 107.75, 52.73.

GC-MS $C_8H_8O_4$ (70eV; EI): 168 ($M^{\cdot+}$), 137 (100), 109, 81, 69, 53.1, 39.1, 29.1

FT-IR (cm^{-1}): 3231, 2955, 2856, 1689, 1598, 1488, 1441, 1161, 873, 766.

m.p. (range in °C): 159 – 162 °C

*METHYL 3,5-BIS(METHOXYMETHOYX)BENZOATE (**54**)*



To a solution of methyl 3,5-dihydroxybenzoate **77** (200 mg, 1.22 mmol, 1.0 eq) is suspended in DCM *dry* (4 mL). DIPEA (N,N-Diisopropylethylamine) (820 µL, 4.88 mmol, 4.0 eq) was added dropwise and stirred 30 minutes at 0°C. To this solution, MOM-Cl (238 µL, 3 mmol, 2.5 eq) was added dropwise and left to warm up to room temperature. The reaction was monitored by TLC/GC-MS. Once the reaction completed, reaction mixture washed with saturated solution of $NaHCO_3$ and the DCM and separated the phases. Aqueous layer was washed 2 times with DCM, the obtained organic phase is dried over Na_2SO_4 and the solvent evaporated. The obtained crude purified by silica column chromatography (7 Hexane: 3 EtOAc Rf = 0.43). The final product (yellow bright oil) is obtained with 75 % yield.

Molecular weight: $C_{12}H_{16}O_6$

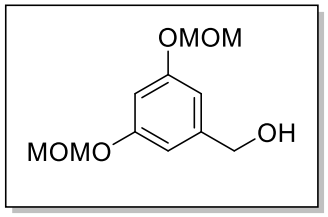
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.35 (d, 2H, J=2.08 Hz), 6.90 (s, 1H), 5.17 (s, 4H), 3.88 (s, 3H), 3.46 (s, 6H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 166.73, 158.3, 132.34, 110.84, 110.78, 94.62, 56.38, 56.33, 52.49

GC-MS (70eV; EI) = 256 ($M^{\cdot+}$), 225, 210, 196, 153, 139, 63, 45 (100).

FT-IR (cm^{-1}) = 2958, 2907, 2823, 1722, 1592, 1453, 1437, 1303, 1235, 1140, 1017, 926, 768.

*(3,5-BIS(METHOXYMETHOYX)PHENYL)METHANOL (**55**)*



$LiAlH_4$ (60 mg, 1.6 mmol, 2.0 eq) was put in an oven dried 3-neck round bottom flask of 25 mL, suspended in THF *dry* (1.5 mL) and cooled down to 0° C by an ice water bath. A solution of methyl 3,5-bis (methoxy methoxy) benzoate **54** (200 mg, 0.78 mmol, 1.0 eq) in THF *dry* (0.5 mL) was added dropwise to the first solution. The final mixture was diluted with THF *dry* (1 mL) and

left stirred at r.t. The reaction was monitored by TLC (6 Hexane: 4 EtOAc Rf = 0.38)/GC-MS. Once the reaction completed, the reaction mixture was cooled down to 0° c with an ice water bath, quenched with Brine and diluted with EtOAc. The two phases separated, aqueous layer washed 3 times with EtOAc, the obtained organic phase dried over Na_2SO_4 and solvent evaporated. The final product obtained as a colourless oil with 85 % yield.

Molecular Formula: $C_{12}H_{16}O_5$

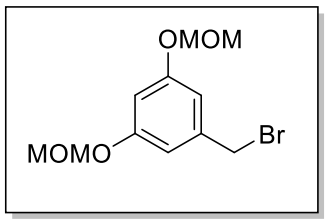
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.66 (d, 2H, J=2.24Hz), 6.60 (t, 1H, J= 2.25 Hz), 5.11 (s, 4H), 4.55 (s, 1H), 3.43 (s, 6H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 158.51, 143.97, 108.05, 104.11, 94.52, 64.99, 56.21

GC-MS (70eV; EI) = 228 ($M^{\cdot+}$), 198, 183, 168, 152, 137, 122, 77, 63, 45.1 (100)

FT-IR (cm^{-1}) = 3408, 2931, 2899, 1598, 1456, 1287, 1137, 1074, 1015, 916, 845.

*1-(BROMOMETHYL)-3,5-BIS (METHOXYMETHOXY)BENZENE (**56**)*



To a solution of (3,5-bis(methoxymethoxy)phenyl) methanol **55** (200 mg, 0.88 mmol, 1.0 eq) in DCM dry (2 mL), add DIPEA (170 µL, 0.97 mmol, 1.1 eq), CBr_4 (526 mg, 1.58 mmol, 1.8 eq) and PPh_3 (415 mg, 1.58 mmol, 1.8 eq) at 0° C. Stir this solution at 0° C for 0.5 hr and then warm to r.t. Allow to stir at r.t for 3 hrs. Solution turned to yellow after 0.5 hr. The reaction was monitored by TLC/GC-MS. Once the reaction completed (3 hr), the reaction mixture was quenched with water and diluted with EtOAc. The two phases separated, aqueous layer washed 3 times with EtOAc, the obtained organic phase dried over Na_2SO_4 and solvent evaporated. The obtained crude purified by silica column chromatography (8 Hexane: 2 EtOAc Rf = 0.57) The final product obtained as a yellow oil with 72% yield.

Molecular Formula: $C_{11}H_{15}O_4Br$

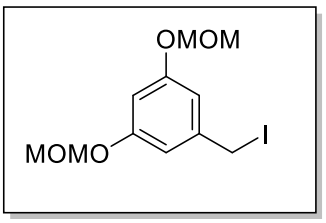
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.73 (m, 2H), 6.67 (m, 1H), 5.15 (m, 4 H), 4.41 (s, 2H), 3.47 (s, 6H)

^{13}C-NMR (100 MHz, $CDCl_3$) δ = : 158.61, 140.07, 110.52, 105.13, 94.69, 56.35, 33.55

GC-MS (70eV; EI) = 289.9 ($M^{\cdot+}$), 259.9, 181, 151, 107, 91, 77, 45 (100).

FT-IR (cm^{-1}) = 2958, 2895, 2827, 1592, 1457, 1295, 1215, 1140, 1081, 1021, 918, 847, 697.

*1-(IODOMETHYL)-3,5-BIS (METHOXYMETHOXY)BENZENE (**63**)*



In an oven dried round bottom flask, 1-(bromomethyl)-3,5-bis (methoxymethoxy)benzene **56** (200 mg, 0.7 mmol, 1.0eq) dissolved in THF dry (4mL) and NaI (456 mg, 5 mmol, 5.0 eq) was added. The reaction mixture was refluxed and stirred overnight. Once the reaction finished (20 hrs), the reaction mixture was quenched with $Na_2S_2O_3$ sat. solution and diluted with EtOAc. The two phases separated, aqueous layer washed 3 times with EtOAc, the obtained organic phase dried over Na_2SO_4 and solvent evaporated. The obtained crude purified by silica column chromatography (8 Hexane: 2 EtOAc Rf = 0.58). The final product obtained with 92 % yield as a yellow oil.

Molecular Formula: $C_{11}H_{15}O_4I$

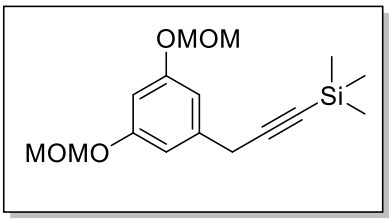
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.71 (m, 2H), 6.62 (m, 1H), 5.14 (s, 4H), 4.36 (s, 2H), 3.47 (s, 6H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 158.53, 141.56, 110.28, 104.69, 94.68, 56.35, 5.56

GC-MS (70eV; EI) = 337 ($M^{\cdot+}$), 211(100), 167, 121, 107, 91, 77, 45.1.

FT-IR (cm^{-1}) = 2957, 2896, 2823, 1593, 1455, 1292, 1207, 1142, 1073, 1021, 919, 850, 692.

*(3-(3,5-BIS(METHOXYMETHOXY)PHENYL) PROP-1-YN-1-YL) TRIMETHYLSILANE (**57**)*



To a solution of 1-(iodomethyl)3,5-bis(methoxymethoxy)benzene **63** (200 mg, 0.6 mmol, 1.0 eq) in acetonitrile *dry* (6 mL), CuI (115 mg, 0.6 mmol, 1.0 eq), TBAI (222 mg, 0.6 mmol, 1.0 eq) and K_2CO_3 *anhydrous* (330 mg, 2.4 mmol, 4 eq) was added. To this stirred solution, Ethynyl trimethyl silane (330 µL, 2.4 mmol, 4 eq) was added

dropwise, and the solution is heated and stirred at 40°C. The reaction was monitored by TLC/GC-MS. Once the reaction finished or no evolutions were observed (18-20 hrs), the reaction mixture was quenched with water and diluted with EtOAc. The two phases separated, aqueous layer washed 3 times with EtOAc, the obtained organic phase dried over Na_2SO_4 and solvent evaporated. The obtained crude purified by silica column chromatography (8 Hexane: 2 EtOAc Rf = 0.61). The final product obtained with 75 % yield as a pale yellow oil.

Molecular Formula: $C_{16}H_{24}O_4Si$

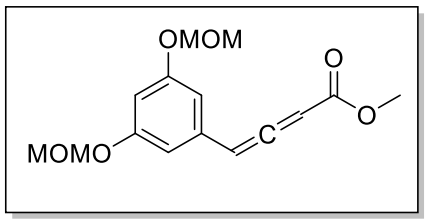
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.72 (m, 2H), 6.61 (m, 1H), 5.15 (s, 4H), 3.59 (s, 2H), 3.47 (s, 6 H), 0.19 (s, 9H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 158.52, 139.05, 109.58, 104.10, 103.43, 94.70, 87.44, 56.26, 26.50, 0.26.

GC-MS (70eV; EI) = 308.1 (M.+), 247, 233, 173, 131, 118, 104, 89, 73, 45.1 (100).

FT-IR (cm^{-1}) = 2958, 2899, 1596, 1465, 1295, 1211, 1140, 1077, 1025, 926, 839.

*METHYL 4-(3,5-BIS(METHOXYMETHOXY)PHENYL)BUTA-2,3-DIENOATE (**62**)*



Same procedure as for the synthesis of (3-(3,5-bis(methoxymethoxy)phenyl)prop-1-yn-1-yl) trimethylsilane **57**, in which methyl propriolate (214 µL, 2.4 mmol, 4.0 eq.) was used instead ethynyl trimethylsilane.

Molecular Formula: $C_{14}H_{19}O_6$

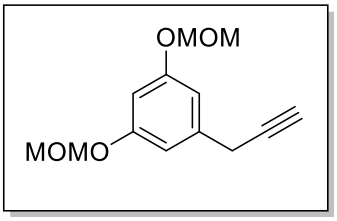
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.66 (t, J = 2.1 Hz, 1H), 6.64 (d, J = 2.2 Hz, 2H), 6.54 (d, J = 6.3 Hz, 1H), 6.02 – 6.00 (m, 1H), 5.14 (s, 2H), 5.14 (s, 2H), 3.75 (s, 3H), 3.46 (s, 6H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 214.98, 165.58, 158.77, 133.34, 109.17, 104.86, 98.86, 94.65, 91.92, 56.31, 52.48.

GC-MS (70eV; EI) = 294 ($M^{\cdot+}$), 263, 235, 217, 203, 191, 175, 131, 115, 45.1 (100).

FT-IR (cm^{-1}) = 2994, 2953, 2901, 2828, 1947, 1721, 1596, 1458, 1434, 1139, 1078, 1014, 925, 844.

*1,3-BIS(METHOXYMETHOXY)-5-(PROP-2-YN-1-YL) BENZENE (**58**)*



To a solution of (3-(3,5-bis(methoxymethoxy)phenyl) prop-1-yn-1-yl) trimethylsilane **57** (200 mg, 0.65 mmol, 1.0 eq) in THF *dry* (7 mL), AcOH (150 µL, 2.6 mmol, 4.0 eq) and TBAF sol 1.0M (2.6 mL, 2.6 mmol, 4.0 eq) was added. The reaction mixture left stirred at r.t for 24 hrs. The reaction was monitored by TLC (8 Hexane : 2 EtOAc Rf = 0.62)/GC-MS. Once the reaction finished (24 hrs), the reaction mixture was quenched with brine and diluted with EtOAc. The two phases separated, aqueous layer washed 3 times with EtOAc, the obtained organic phase dried over Na_2SO_4 and solvent evaporated. The filtrate was concentrated by rotavapor and the crude was purified by column chromatography on silica gel (from 9 Hexane: 1 EtOAc to 8 Hexane: 2 EtOAc), obtaining the final product with a yield of 80% as a very bright yellow oil.

Molecular Formula: $C_{12}H_{17}O_4$

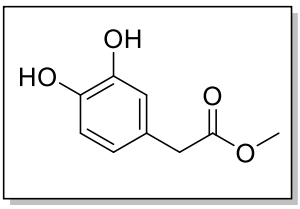
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.71 (m, 2H), 6.63 (m, 1H), 5.15 (s, 4H), 3.55 (m, 2H), 3.47 (s, 6H), 2.19 (m, 1H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 158.59, 138.70, 109.54, 103.38, 94.65, 81.74, 70.94, 56.30, 25.12

GC-MS (70eV; EI) = 236 ($M^{\cdot+}$), 206, 176, 160, 145, 115, 102, 91, 45.1 (100).

FTIR (cm^{-1}) = 3289, 2957, 2900, 2827, 1597, 1459, 1280, 1211, 1138, 1073, 1021, 923, 838, 639.

*METHYL-2-(3,4-DIHYDROXYPHENYL) ACETATE (**78**)*



Sulfuric acid method: In a 200 mL round bottom flask, 3,4-dihydroxy phenyl acetic acid **64** (200 mg, 1.2 mmol, 1.0 eq) dissolved in 6 mL methanol and 70 µL of H_2SO_4 was added dropwise. The solution was refluxed for 5 h. The reaction was monitored by TLC (3 Hex : 7 EtOAc Rf = 0.31)/GCMS. Once the reaction finished, methanol was evaporated and the residue was partitioned between EtOAc and saturated solution of $NaHCO_3$, the obtained organic phase dried

over Na_2SO_4 and solvent evaporated. The final product obtained with a yield of 90 % as colourless oil.

Or

Amberlyst-15 method: In a 25 mL round bottom flask, 3,4-dihydroxyphenyl acetic acid **64** (200 mg, 1.2 mmol, 1.0 eq) is dissolved in 6 mL MeOH dry and 600 mg of Aberlyst-15® (500mg/mmol) were added. The solution was refluxed for 16 h. The reaction was monitored by TLC (4 Hex : 6 EtOAc Rf = 0.48)/GC-MS. Once the reaction finished, the solution is filtered by paper filter and the amberlyst-15® was washed three times with fresh methanol (3x25 mL), the amberlyst is recovered and reactivated, the organic solution is evaporated by rotavapor to give a colourless oil in quantitative way.

Molecular Formula: $C_9H_{10}O_4$

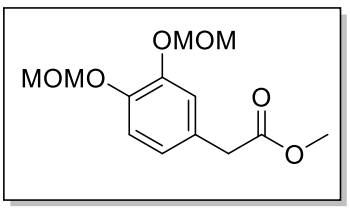
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.73 (m, 1H), 6.61 (m, 2H), 3.67 (s, 3H), 3.48 (s, 2H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 174.23, 144.13, 143.40, 126.14, 121.84, 116.65, 115.75, 52.67, 40.64.

GC-MS (70eV; EI): 168 ($M^{\cdot+}$), 137 (100), 109, 81, 69, 53.1, 39.1, 29.1

FT-IR (cm^{-1}): 3361, 2951, 1705, 1519, 1441, 1279, 1247, 1157, 1113, 1011, 778.

*METHYL 2-(3,4-BIS(METHOXYMETHOXY)PHENYL) ACETATE (**65**)*



To a solution of Methyl-2-(3,4-dihydroxyphenyl) acetate **78** (200 mg, 1.09 mmol, 1.0 eq) dissolved in DCM *dry* (4 mL). DIPEA (N, N-Diisopropylethylamine) (760 µL, 4.36 mmol, 4.0 eq) was added dropwise and stirred 30 minutes at 0° C. To this solution, MOM-Cl (330 µL, 4.36 mmol, 4.0 eq) was added dropwise and left to warm up to room temperature. The reaction was monitored by TLC (6 Hex : 4 EtOAc, Rf = 0.40)/GC-MS. Once the reaction completed, reaction mixture washed with saturated solution of $NaHCO_3$ and the DCM and separated the phases. Aqueous layer was washed 2 times with DCM, the obtained organic phase dried over Na_2SO_4 and the solvent evaporated. The crude obtained is purified by Silica Gel (6 Hex : 4 EtOAc) obtaining the product as a yellow oil with a yield of 80%. If purity

of final product is still not satisfying (mono-protected contamination), it can be purified by a pad of Al_2O_3 basic using DCM as eluent.

Molecular Formula: $C_{13}H_{18}O_6$

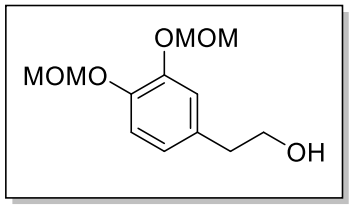
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.73 (m, 1H), 6.61 (m, 2H), 5.19 (s, 2H), 5.17 (s, 2H) 3.64 (s, 3H), 3.52 (s, 2H), 3.47 (s, 3H), 3.46 (s, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 174.23, 144.13, 143.40, 126.14, 121.84, 116.65, 115.75, 95.65, 95.62, 56.38, 56.30, 52.18, 40.64

GC-MS (70eV; EI) = 270 (M.+), 207, 194, 151, 135, 123, 77, 45 (100).

FT-IR (cm^{-1}) = 3361, 2951, 1705, 1519, 1441, 1279, 1247, 1157, 1113, 1011, 778.

*2-(3,4-BIS(METHOXYMETHOXY)PHENYL) ETHANOL (**66**)*



$LiAlH_4$ (74 mg, 1.875 mmol, 2.5 eq) was put in an oven dried 3-neck round bottom flask, suspended in THF dry (1.5 mL) and cooled down to 0°C by an ice water bath. A solution of Methyl 2-(3,4-bis(methoxymethoxy)phenyl) acetate **65** (200 mg, 0.74 mmol, 1.0 eq) in THF dry (0.5 mL) was added dropwise to the first solution. The final mixture was diluted with THF dry (1 mL) and left stirred at r.t. The reaction was monitored by TLC (6 Hex : 4 EtOAc Rf = 0.36)/GC-MS. Once the reaction completed (2 hrs), the reaction mixture was cooled down to 0°C with an ice water bath, quenched with Brine and diluted with EtOAc. The two phases separated, aqueous layer washed 3 times with EtOAc, the obtained organic phase dried over Na_2SO_4 and solvent evaporated. The final product was obtained as a colourless oil with 85 % yield .

Molecular Formula: $C_{13}H_{18}O_5$

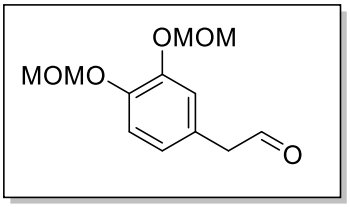
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.91 (m, 2H), 6.67 (m, 1H), 5.04 (m, 4H), 3.61 (t, J= 8Hz, 2H), 3.35 (m, 6H), 2.62 (t, J= 8 Hz, 2H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 147.48, 145.99, 133.31, 123.12, 117.76, 117.21, 95.72, 95.64, 63.77, 56.42, 56.33, 38.85

GC-MS (70eV; EI) = 242 (M.+), 166, 135, 45 (100).

FT-IR (cm^{-1}) = 3398, 2946, 2899, 2827, 1588, 1509, 1429, 1402, 1255, 1148, 1124, 1073, 982, 922, 815.

*2-(3,4-BIS(METHOXYMETHOXY)PHENYL) ACETALDEHYDE (**67**)*



Dess-Martin Method: 200 mg (200 mg, 0.82 mmol, 1.0 eq) of 2-(3,4-bis(methoxymethoxy)phenyl) ethanol **66** were initially charged in 8 mL of dichloromethane and 525mg (1.24 mmol, 1.5 eq) of Dess-Martin reagent were added in portions. The temperature was held at 20° C. The reaction mixture was stirred at r.t for 3 hrs. The reaction was monitored by TLC (6 Hex : 4 EtOAc Rf = 0.47)/GC-MS. Once the reaction completed or no evolutions were detected, $Na_2S_2O_3$ sat. solution was added and aqueous layer is extracted 3 times with fresh DCM. Organic phase is anhydrified over Na_2SO_4 and solvent evaporated by rotavapor. The crude so obtained was purified by column chromatography on silica gel (from 7 Hexane: 3 EtOAc to 5 Hexane: 5 EtOAc), obtaining the final product as colourless oil with a yield of 80%.

Or

DIBAL-H Method: A solution of 2-(3,4-bis(methoxymethoxy)phenyl) acetate **65** (500 mg, 1.85 mmol, 1.0 eq) in Toluene *dry* (7 mL) is cooled down to -78°C, then DIBAL-H (1.0M solution, 2.22 mmol, 1.2 eq.) is added dropwise to first solution and the resultant mixture is warmed up to -60°C and stirred until the starting material is consumed. The reaction is monitored by TLC (6 Hex : 4 EtOAc Rf = 0.47)/GC-MS Once that reaction is finished, the mixture is cooled down to -78°C again and the excess is quenched with fresh methanol (70 mL). The solution is filtered by a pad of celite e washed with Et_2O. The solvent is evaporated, the crude obtained is dissolved in fresh Et_2O and the organic phase is whased with water and brine. The organic layer is anhydrified over Na_2SO_4 and solvent evaporated by rotavapor to give the product as a colourless oil with a yield of 83%.

Molecular Formula: $C_{13}H_{16}O_5$

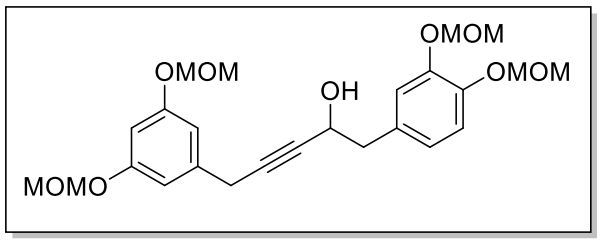
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 9.72 (s, 1H), 7.15 (d, J= 8Hz, 1H), 7.02 (d, J= 2.04 Hz, 1H), 6.88 (dd, J= 8.27 Hz, J= 2.11Hz, 1H), 5.23 (d, J= 1.84 Hz, 4H), 3.62 (d, J= 2.33 Hz, 2H), 3.52 (s, 6H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 199.69, 147.83, 146.78, 126.24, 123.79, 118.16, 117.31, 95.67, 95.64, 56.49, 56.43, 50.22.

GC-MS (70eV; EI) = 240 ($M^{\cdot+}$), 164, 135, 45.1 (100).

FT-IR (cm^{-1}) = 2950, 2827, 1726, 1592, 1512, 1259, 1148, 1069, 986, 918, 819.

*1-(3,4-BIS(METHOXYMETHOXY)PHENYL)-5-(3,5-BIS(METHOXYMETHOXY) PHENYL)PENT-3-YN-2-OL (**68**)*



A solution of compound **58** (210mg, 0.87 mmol, 1.0 eq.) in THF *dry* (0.2M, 4.2 mL) is cooled down to -78°C, then the n-BuLi (1.0 eq, sol. 1.6M, 0.87 mmol, 543 µL) is added very slow and the stirred for 5 minutes at that temperature. After that, a solution of aldehyde **67** (0.3 M in THF dry, 213 mg, 0.87 mmol, 1.0 eq) is added dropwise at the same temperature, then the mixture is left to warm up to r.t and stirred overnight (16 hrs). Reaction is monitored by TLC (6 Hex: 4 EtOAc, Rf = 0.45). When no more evolutions are observed by TLC, DCM is added and organic phase is washed with a saturated aqueous solution of NH_4Cl, the water phase is washed 2 more times with fresh DCM, the organic layer is anhydrified by sodium sulfate anhydrous. The crude obtained is purified by Silica Gel, the product is isolated as a yellow waxy solid with a yield of 71%.

Molecular weight: $C_{32}H_{32}O_9$

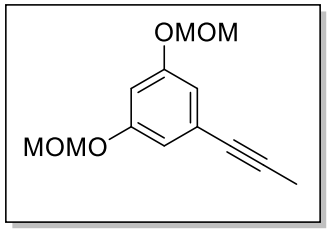
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.10 – 7.06 (m, 2H), 6.87 (dd, J = 8.3, 2.0 Hz, 1H), 6.68 – 6.66 (m, 2H), 6.61 (t, J = 2.2 Hz, 1H), 5.20 (s, 2H), 5.17 (s, 2H), 5.13 (s, 4H), 4.58 (dd, J = 10.6, 4.3 Hz, 1H), 3.56 (d, J = 1.8 Hz, 2H), 3.50 (s, 3H), 3.48 (s, 3H), 3.46 (s, 6H), 3.00 – 2.89 (m, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 158.56, 147.29, 146.39, 139.08, 131.32, 123.99, 118.39, 116.84, 109.56, 103.30, 95.67, 95.64, 94.65, 83.69, 83.33, 63.57, 56.41, 56.36, 56.28, 44.00, 25.36.

ESI-MS (+, m/z) = 499 $[M + Na]^+$

FT-IR (cm^{-1}) = 3468, 2948, 2924, 2827, 1597, 1512, 1459, 1260, 1142, 1073, 1025, 992, 919.

*1,3-BIS(METHOXYMETHOXY)-5-(PROP-1-YN-1-YL)BENZENE (**69**)*



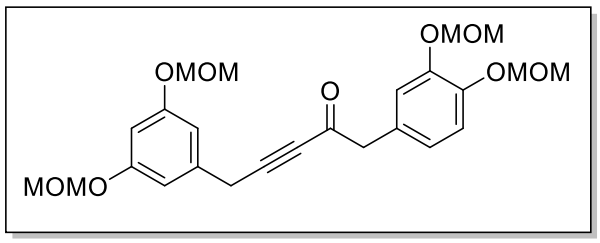
Here the GC-MS, FT-IR and ^{1}H-NMR characterization of isomerized alkyne, isolated from previous reaction, are reported.

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.74 (d, J = 2.3 Hz, 1H), 6.65 (t, J = 2.3 Hz, 2H), 5.12 (s, 4H), 3.45 (s, 6H), 2.01 (s, 3H).

GC-MS $C_{12}H_{17}O_4$ (70eV; EI) = 236 [$M^{\cdot+}$], 204, 191, 176, 160, 132, 102, 76, 45 (100)

FT-IR (cm^{-1}) = 2958, 2917, 2825, 2245, 1585, 1434, 1325, 1211, 1137, 1021, 923, 847, 680.

*1-(3,4-BIS(METHOXYMETHOXY)PHENYL)-5-(3,5-BIS(METHOXYMETHOXY) PHENYL)PENT-3-YN-2-OL (**70**)*



200 mg (0.47 mmol, 1.0 eq) of propargyl alcohol **68** were initially charged in 2 mL of dichloromethane dry and 299mg (0.71 mmol, 1.5 eq) of Dess-Martin reagent were added in portions. The solution is warmed up to r.t. The reaction mixture was stirred at r.t for 7 hrs and monitorated by TLC (8 Hex : 2 EtOAc, Rf = 0.41). Once the reaction was completed or no evolutions were detected, $Na_2S_2O_3$ sat. solution was added and aqueous layer is extracted 3 times with fresh DCM. Organic phase is anhydrified over Na_2SO_4 and solvent evaporated by rotavapor. The crude so obtained was purified by column chromatography on silica gel (from 9 Hexane: 1 EtOAc to 7 Hexane: 3 EtOAc), obtaining the final product with a yield of 50%.

Molecular Weight: $C_{32}H_{30}O_9$

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.11 (d, J = 8.3 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 6.84 (dd, J = 8.4, 2.0 Hz, 1H), 6.63 (d, J = 2.7 Hz, 3H), 5.21 (s, 2H), 5.19 (s, 2H), 5.14 (s, 4H), 3.79 (s, 2H), 3.68 (s, 2H), 3.50 (d, J = 0.4 Hz, 3H), 3.49 (s, 3H), 3.47 (s, 6H).

ESI-MS C (+, m/z) = 497 $[M + Na]^+$

FT-IR (cm^{-1}) = 2952, 2900, 2210, 1674, 1593, 1512, 1260, 1146, 1077, 1021, 988, 923.

1.2i Conclusion

Phenolic compounds are molecules widely distributed in animal and plant reign and they explicate a wide range of biological activities. Due to these bio-characteristics, they can be applied as antiviral agents against novel corona such as virus SARS-CoV-2. In fact, the vaccines, even if they are a potent tools against the spread of the virus of all-over the world and a powerful prevent measure for the human health, new treatment are request for already affected people. Among the possibilities, the small molecules can play an important role to fight COVID-19, since these compounds always have been used to treat several virus infections. Nowadays many scientist are testing and screening chemical libraries with this purpose. Together the already known molecules, the synthesis of new ones is a pathway that can lead to exploit new alternatives to treat several diseases. So, we tried to developed an effective synthetic pathway for a new (poly)phenolic compound that could explicate antiviral action against SARS-CoV-2. We tried to develop two different approaches: one for industrial and more sustainable level, as possible pathway for the synthesis of huge amounts of final product; the second one implicates some lab-optical steps since the use of dangerous reagents and quite harsh conditions leads to the formation of products avoiding additional steps (higher overall yield). Unfortunately, the final target product has not still achieved, but further studies to obtain it are still going on.

Chapter 2

New Sustainable Approaches for the Synthesis of N-heterocycles.

Abstract

Natural and synthetic heterocycles has greatly attracted the interest of scientits because of their widely applications in many fields of science: biological, pharmaceutical, synthetical and industrial applications (plastic, solvents, cosmetics, vulcanization accelerators, etc.)[175] Among them, N-heterocycles have always constituted an important synthetic target due to the pharmaceutical and biological superior properties than non-nitrogen containing cycles[175b] and for their wide distribution in many natural bioactive compounds (such as alkaloids).[176] From a statistical analyses it emerges that 59% of FDA-approved small molecule drugs contain at least one of N-heterocycles moieties in which the six- and five-membered are the size-cycles more diffused, although three-, four-, bicyclic-, macrocyclic N-heterocycle scaffolds are present.[177] Moreover, this last class of compounds have shown useful, not only in medicinal fields, but also in metallurgical applications thanks by their ability to chelate first-row transition metals like Mn(II), Zn(II), Fe(II), Cu(II) and for other industrial applications.[178] Nowadays many scientist are focusing on the development of new sustainable synthetic approaches for this class of compounds[175a] given their potential applicability in many science and everyday life aspects. New approaches consist in employment of UV-light (the most sustainable reactions induces, largely applied in organic synthesis);[179] employment of less toxic, environmentally benign and less expensive Lewis Acid catalysts like Zn, Fe, Cu, Ni cores (minimization of waste, time consuming, improved selectivity, often they allow to build complicated structures under mild conditions);[175a], [180] performing the reaction under microwave irradiations;[181] through multicomponent reactions (which implicate time saving, avoid the separation and purification of intermediates, atom-efficent reactions, type reactions used also for the synthesis of pharmaceuticals, drugs

and compounds by stereoselective routes);[182] employment of less toxic solvents like ionic liquid;[183] just to cite few examples.

This chapter is divided in three sections in which three different N-heterocycle scaffolds are synthesized by new sustainable approaches.

The first section is focused on the synthesis of an aza-macrocycle with the purpose to overcome some problems related to the synthesis and enhanced some aspects making them more suitable for green aspects that rule the scientific world today.

The aim of second section is the synthesis of a six-membered N-heterocycles (1,2-dihydropyridines) through polycondensation of acyclic precursor using a not expensive and low-toxic Lewis acid like $CeCl_3 \cdot 7H_2O$, emphasizing kinetic, thermodynamic aspects of condensation process.

The last section is divided in two more parts in which: the first one focuses on the synthesis of tetrahydro-β-carboline (very important bioactive compound) using not harmful Brønsted acids like graphene oxide and Amberlyst-15® through the Pictect-Spengler reaction; the second part, instead, reports the first results about the development of a new multi-component Pictect-Spengler reaction promoted by Ce(III)-Lewis Acid for the synthesis of N-substituted tetrahydro-β-carboline.

2.1 Improvement of the synthesis of aza-macrocycle: 1,4,7-trimethyl-1,4,7-triazacyclonane

2.1a An overview on aza-macrocycles

The macrocyclic ligands (at least 9-members cycle) form a class of compound that play an important role in coordination chemistry for their ability to complex many metal ions, charged and neutral natural molecules.[184] In particular, the chemistry of aza-macrocycles has under investigation by the scientific community. In fact, inspired by polyazamacrocycles present in nature (like porphyrin and corrin), synthetic chemists has led to the development of numerous organic molecules able to chelate a vast variety of metal ions exactly as natural analogues.[185]

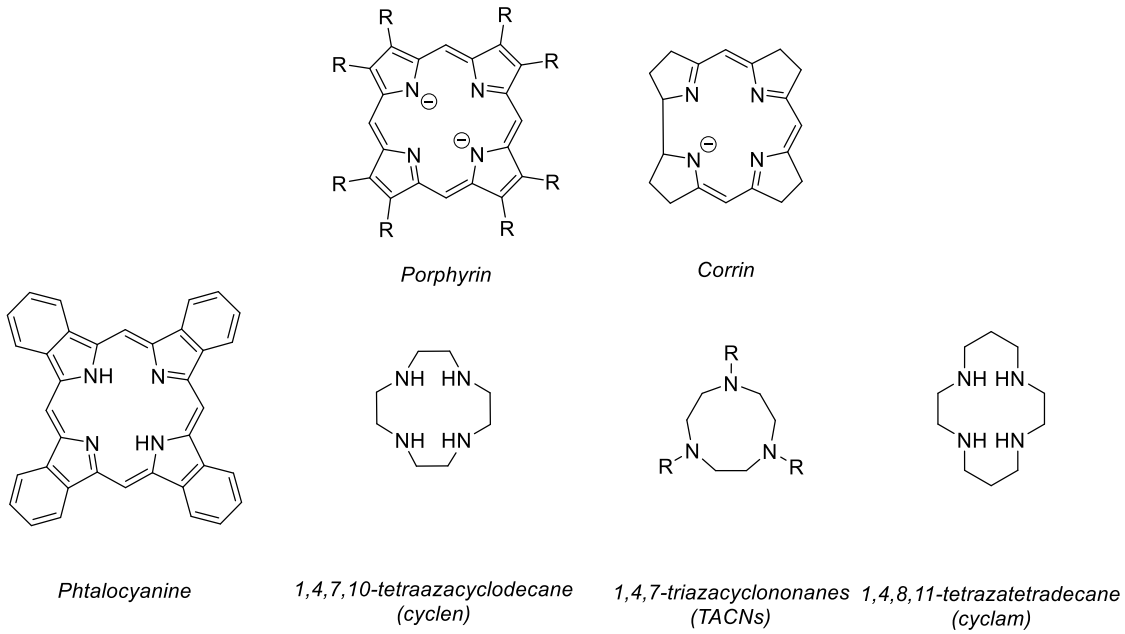


Figure 2.1.1. Structure of natural azamacrocycles (above) and synthetic ones (below)

The presence of nitrogen atoms in the structure of these compounds makes them very incline to associate with cations of transition metals respect to oxygen containing-macrocyclces (strong association with alkali and earth-alkali cations) since the less electronegativity of nitrogen atoms that makes the lone pair more disposable to coordinate metals.[184] These polydentate ligand-metal complexes are more stable than corrispective mono-dentate ones (chelate effect) by thermodynamic and kinetic reasons.

From a thermodynamic point of view, an increment of number of particles in the system means an increment of entropy making the process thermodynamically favored. The second reason for this higher stability is due to a major effective concentration of chelating atoms of ligand around metal core causing a much faster formation of successive M-N bond between ligand and metal centre.[186]

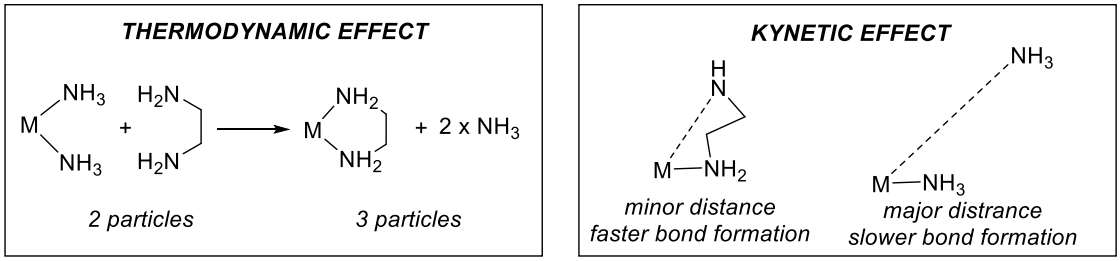


Figure 2.1.2 Two factors of chelate effects

Polycyclic aza-crown ligands form more stable complex respect to the opened-chains analogues, this effect is called "macrocyclic effect": Cabbiness *et al.* discovered, by potentiometry and spectrophotometrically experiments, that Cu(II) complex with meso-1,7-CTH (tet a in *figure 2.1.2*) was at least 10,000 times more stable respect to a Cu(II) complex with a linear poly-amine analogues.[187]

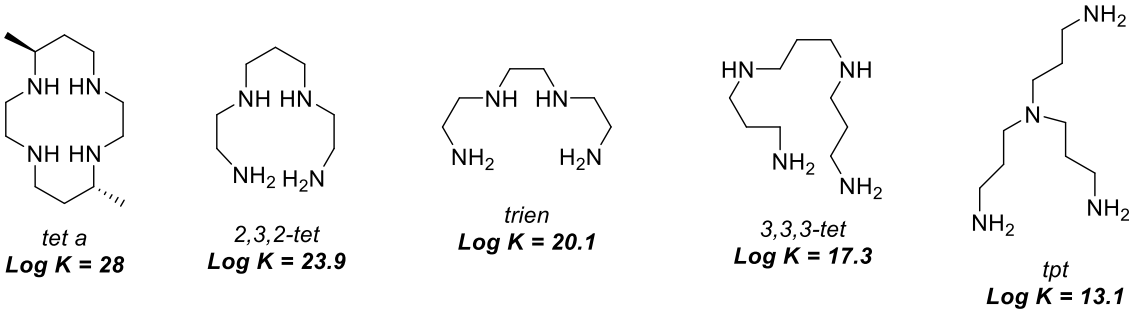
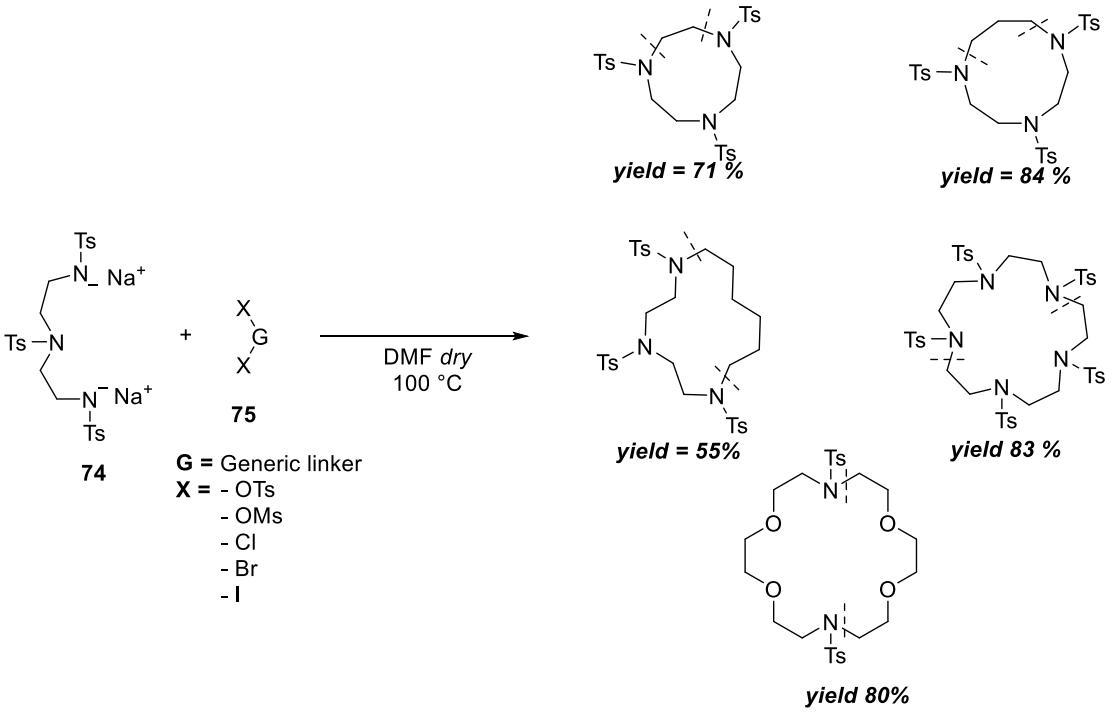


Figure 2.1.3. Aza-ligands studied by Cabbiness et al (nomenclature used by authors) with corrispectives Log K (higher value higher stability of complex)

The stability caused by the macrocyclic effect is explained by the difficult dissociation of interactions between metal and ligand. The dissociation of polydentate ligands proceed through multiple SN_1 replacement steps and this process finishes when the last chelator atom dissociates, but this fact in a macrocycle ligand cannot happen since there is not an end-group.[186]

One of the most famous and versatile methods for the synthesis of this class of compounds was reported by Richman and Atkins in which they prepared different azamacrocycles (from 9 to 21 members) through the reaction between bisodic salt of p-

toulenesuplhonamide **74** and bis-tosylated/mesylated compounds **75** (higher yields when di-halide compounds were employed) in presence of cesium carbonate in DMF *dry* at 100°C. Further remove of Ts-protection gives the free-aza-macrocycle.[188]



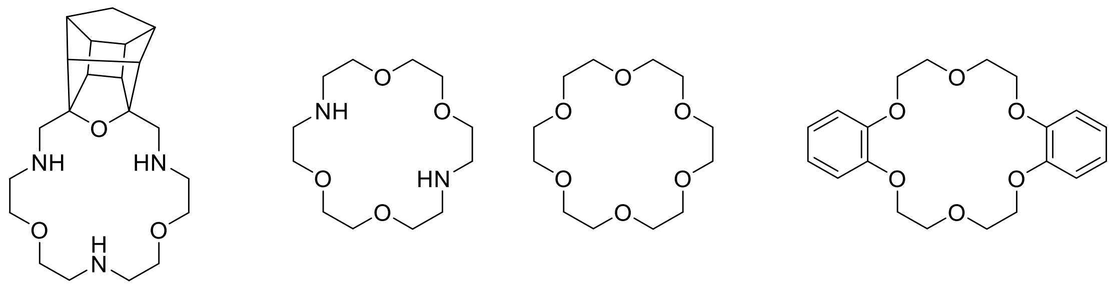
Scheme 2.1.1 Synthesis of different azamacrocycles by Richman-Atkins method, yield reported for G = -OTs

Nowadays several synthetic methods are reported in literature for this class of compounds such as Nuchleophilic Ring Closing,[189] Mitsunobu like Reaction,[190] modification of sulphonamide side chain (allowing to perform the reaction in milder conditions),[191] and many other methods.[192 - 194]

The major feature of these molecules is to form stable complexes with many different divalent and trivalent metal cores like Zn(II), Cu(II), Eu(III), Gd(III), Ca(II), Mg(II), Mn(II), Ni(II);[195] following the Peterson's HSAB theory, coordinational geometrical preference, thermodynamic and kinetic rules.[196]

Importance to notice that the affinity and selectivity toward the metals ions depends on number, arrangement of N-, O-atoms, the size of inner cavity and the substituents on azamacrocycles; Williams *et al.*[197] studied the selectivity of several azamacrocycles toward Ag(I) among different transition and alkali metals in different solutions in which silver ion was one third concentrated respect to others. From ESI-MS studies they observed that in presence of either N- and O-atoms in the macrocycles (like in ligands

76 and **79**), the ligands were selective toward Ag(I) in presence of different alkali ions (Li^+, Na^+, K^+, Rb^+), while ether 18-crown-6 **78** forms only weakly complex with Ag(I) and binds with all alkali metals. On the other hand, in transition metals solutions (Mn(II), Cu(II), Zn(II), Au(III)) the macrocycles **76** and **77** showed higher selectivity for silver ion even if Mn and Zn complexes were weakly detected and ligands **79** (analogue of 18-crown-6) show good selectivity toward Ag(I) respect to its original compound, Au(III) complex was never detected. Moreover, among a second transition metals solution (Fe(III), Ni(II), Pb(II)) the azacycles **76** and **77** showed high selectivity for Ag(I) ion.

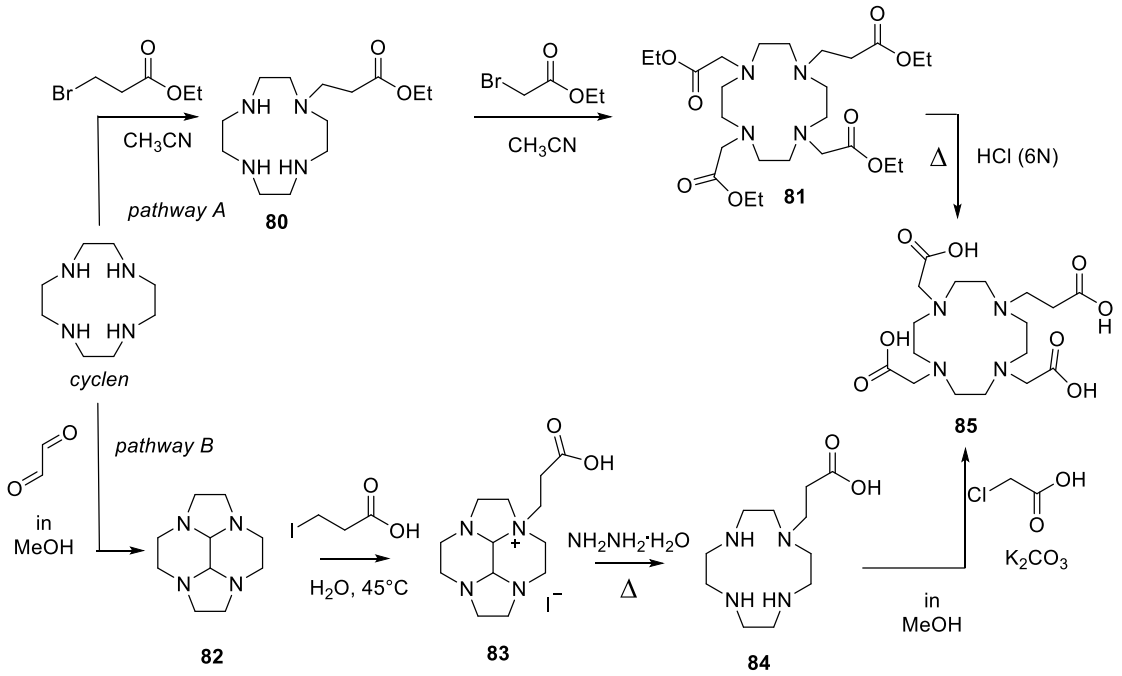


76 **77** **78** **79**

Figure 2.1.4. Structure of some aza-macrocycle studied by Williams et al.

Moreover, azamacrocycle-complexes found different applications in biomedical (i.e. MRI, radiopharmaceuticals, therapeutic applications) and industrial fields, antiviral, as catalyst and recover of metals from (waste) water and others applications.[178a, 186, 195, 197 - 200] For instance, many cyclen-based lanthanides complexes have used as MRI, contrast agents in PET (Positron Emission tomography), SPECT (Single Photon Emission Computed Tomography) due to their low toxicity, higher thermodynamic and kinetic stability.[201] Gadolinium(III) (high paramagnetic metal by its seven unpaired electrons, relatively slow electronic relaxation) complexes with DOTA derivative **86** in which a propionate moiety was presence instead an acetate one enhances the relaxivity properties of DOTA-Gd(III) complex, even if the it results less stable than “original” DOTA complex, it results anyway a good MRI agent and considerably more inert than Gd complex with DTPA (diethylenetriaminepentaacetic acid, polyaza linear compound).[202] This DOTA derivative **86** can be obtained by two possible pathways starting from cyclen, in which the first scope of each method is to attached the propiolate moiety, and then to add the acetate groups to the other nitrogen atoms (*scheme 2.1.2*). The pathway A (overall yield 85%) implicates the mono-proprionation of cyclen (**80**) (present in large excess), and successive acetylation of remaining nitrogen atoms leads to the final product

86, while the pathway B (overall yield 80%) achieves the final ligand through a protection/deprotection strategy of four nitrogen atoms using glyoxaldehyde and formation of iodide salt **83** in the step of mono-proprionation.



Scheme 2.1.2. Synthesis of propiolate-DOTA ligand by Balogh et al.

In addition, NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) and DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) based ligand-^{68}Ga complexes bounded to an oligonucleotide sequence have shown very useful in PET, moreover these studies have explicated that the biodistribution of compound depends by both oligonucleotides sequence and the type of chelator.[203]

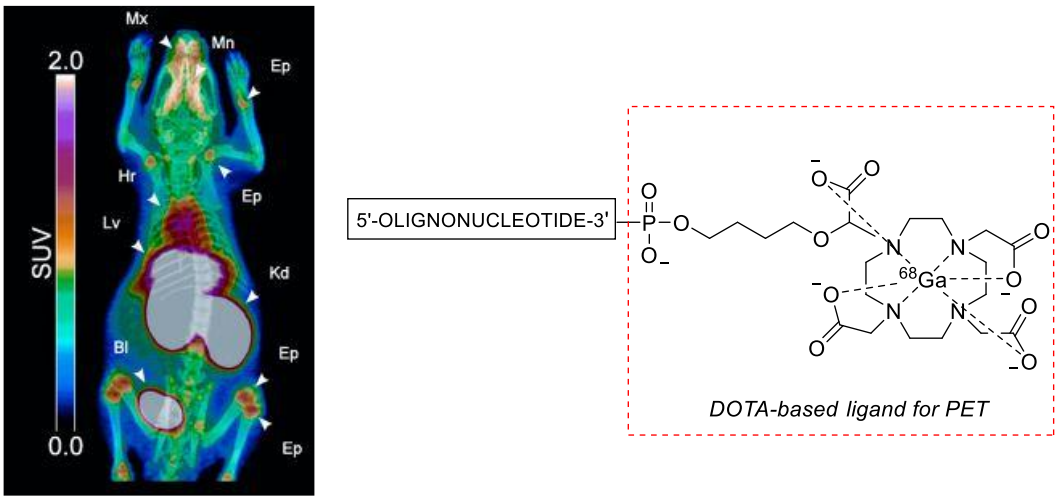


Figure 2.1.5. PET image with DOTA-based ligand-^{68}Ga complex after 60 minutes from injection, image from reference[203], the image shows: accumulates in bone (especially in log bone Ep, in maxilla Mx and mandibular Mn), kidneys (Kd), and liver (Lv). Radioactive urine was observed in the bladder (Bl) and in the heart (Hr)

The employment of 12-crown sized azamacrocycles in which one nitrogen atom belogns to a pyridine moiety has shown very prominent as catalyst for Sonogashira cross-coupling Pd(II)-catalyzed between phenylacetylene and iodobenzene using Et_3N as base and performing the reaction in water. Moreover, the covalently functionalization (π-π stacking interactions) of a nanostructure MWCNT (see *figure 2.1.6.*) leads to the formation of a new green heterogeneous catalysts usable under aerobic conditions. Studies about temperature have shown that these new MWCNT catalysts are very performant at 50°C (conducting the reaction at 30°C it lasts 3 days, at 50°C 2h with a yield of 90% for **87** and 94% for **88** without formation of by-products). The **87** short-MWCNT shown a better recycle of the other one, in fact after four cycles the activity of **87**-MWCNT remains almost similar to the first even if 24h of reaction were necessary to obtain 80% of yield; on the other hand **88**-MWCNT showed a higher degradation just at the second cycle 16h were necessary to obtain an yield of 92%, and at fourth cycle after 24h reaction an yield of 66% was detected.[204]

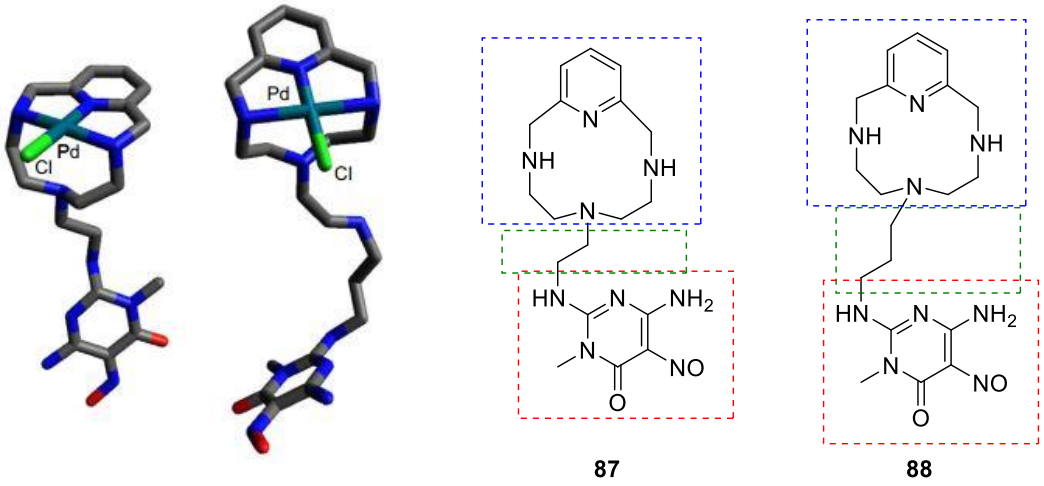
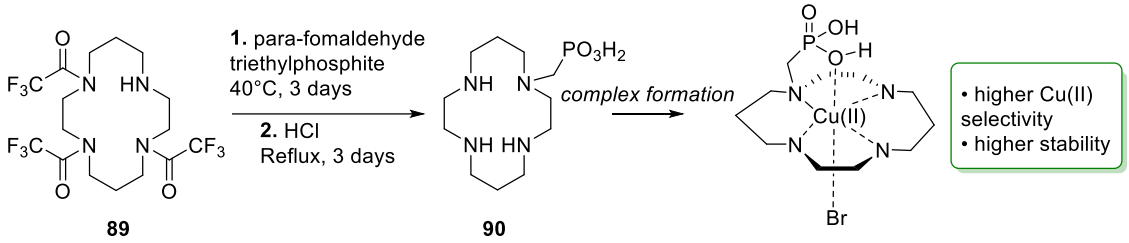


Figure 2.1.6. On the left 3D structure of complexes take from reference[204]; on the right chemical structure of two aza-macrocycles in which the red part hihlights the π-π anchor, the green part highlights the spacer, the blue part highilight the ligand moiety

The chemical modification of aza-macrocycles is an important research area since the modifications improve the stability, activity and the specifity of physiochemical and biological properties of resultant complexes.[201] For example, the modification of cyclam by adding one methyl-phosphonic acid "pendant arm" (obtained after 3 days at 40 °C reaction between dry para-formaldehyde (excess), N,N',N''-tris(trifluoroacetyl)cyclam and triethyl phosphite (large excess) and successive refluxing in HCl, *scheme 2.1.3*) enhances the selectivity toward Cu(II) of original cycle. In fact, cyclam itself coordinates faster Zn(II) under physiological conditions, and an higher selectivity for copper atoms is required for biomedical purpose; since different copper isotopes (^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu) show prominent application in radiopharmaceutical, PET, MRI.[205] Potentiometrically studies have shown a comparable stability between **90**-Cu(II) complex and cyclam-Cu(II), but an higher stability respect other reported pendant-cyclam derivatives. Moreover, the ligand show a very high selectivity for copper among the different bivalent cations like Ca(II), Mg(II), Cd(II), Mn(II), and, more important, it has a 6 magnitude higher selectivity toward Cu(II) than Zn(II). The diffraction study revealed that complex has in a distorted octahedral environment, in which axial bonds are elongated respect to the equatorial ones. The aza-macrocyclic ring coordinates in the basal plane way, and an oxygen atom of the phosphor-moiety is coordinated in one apical position. Finally, rather long coordination

of bromide in opposite position to apical oxygen atom complete the sphere of coordination.[205]



*Scheme 2.1.3. Synthesis of phosphate cyclam derivative **90** and Cu(II)-complex structure*

A huge number of examples can be found in the literature for the applications, synthesis and modifications of this class of compounds; but to avoid going too far and risking boring the reader, only a couple of examples have been reported (those cited previously) to help understand the importance of azamacrocycles. These topics will also be dealt with more specifically for TACN (1,4,7-triazacyclononane) and its derivatives (successive section), topic of this chapter.

2.1b Synthesis and applications of 1,4,7-triazacyclononane and its derivatives

1,4,7-triazacyclononane (TACN) and its derivatives is an important class of aza-macrocycles since they can stabilize high oxidation states of different metal cores like Fe(II), Fe(III), Ru(II), Ru(IV), Cu(II), Zn(II), Co(III) and especially Mn(II) and Mn(IV); in addition, TACNs metal complexes found many applications, molecular imaging, PET, SPECT, catalyses and they are also employed for the preparation of ^{99}Tc radiopharmaceuticals. For this reason this aza-cycles appear in more than 300 patents.[206] An important feature of these molecules is the isoelectronic to Cp (cyclopentadiene) that allow to obtain complexes with a more positive charge.[207]

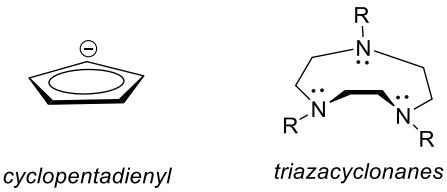
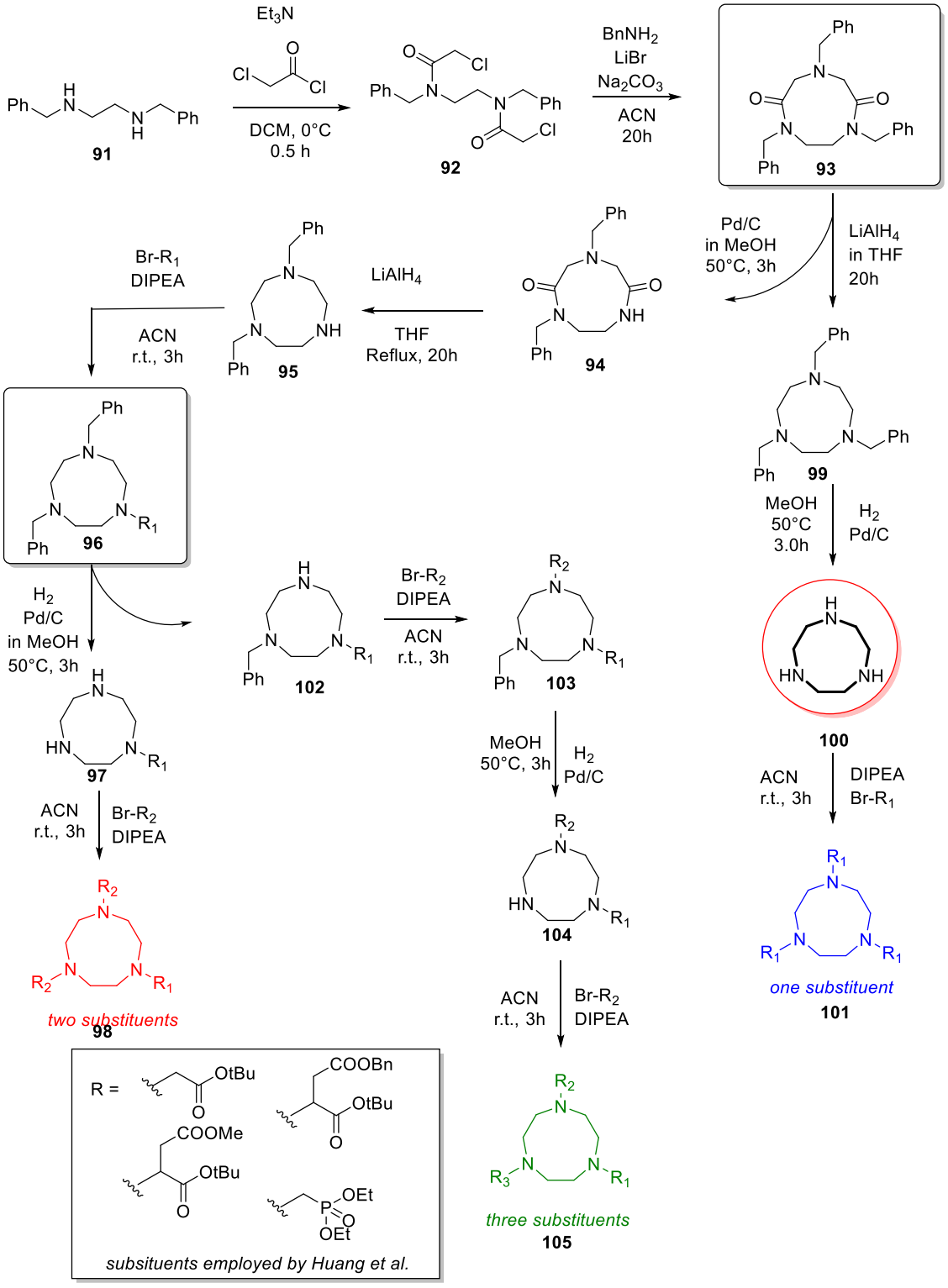


Figure 2.1.7. Isoelectronic structure of cyclopendienyl and TACNs

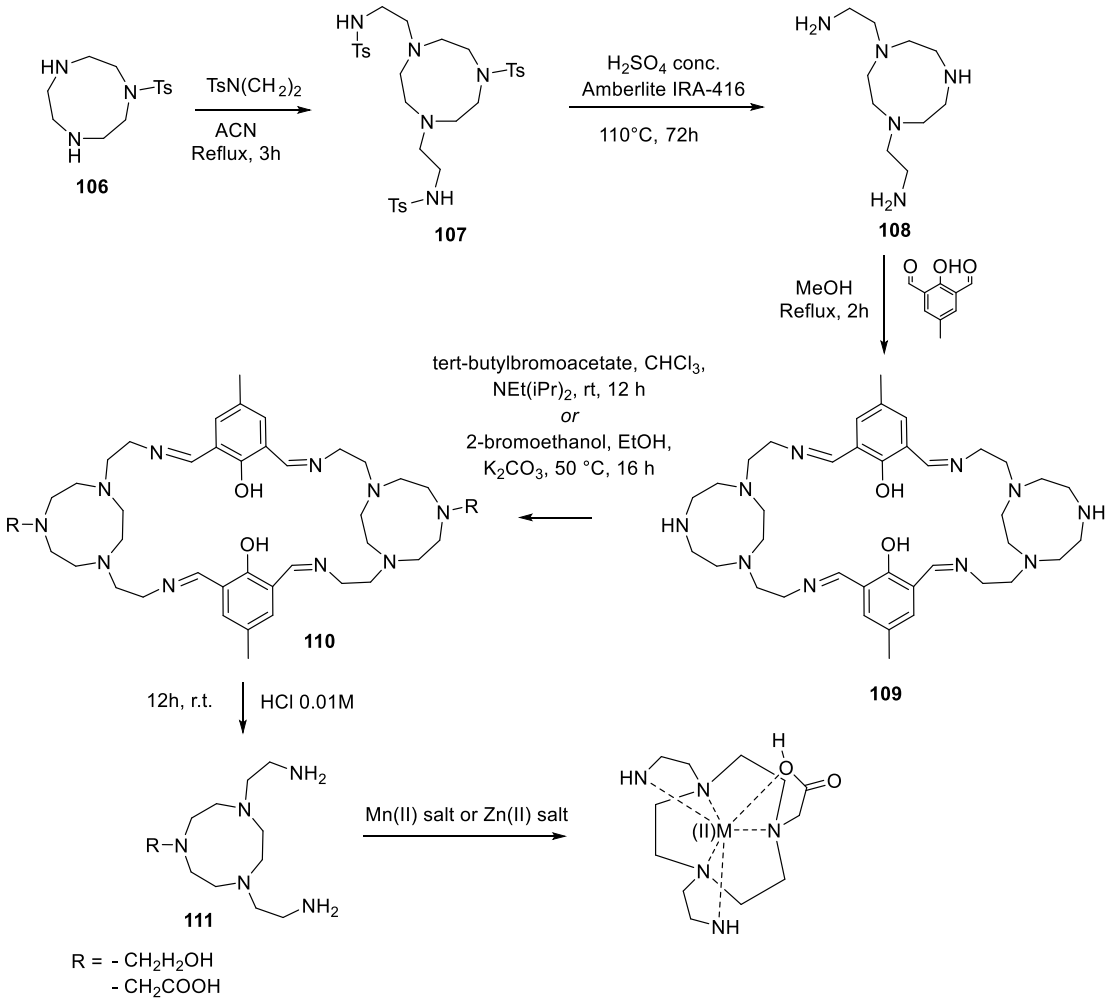
New synthetic strategies and funcionalizations for these compounds are always desirable due to the important features of these ligands and relative complexes. The Richard-Atkins method is one way to obtain this moiety (see *scheme 2.1.1*), it is possible to obtain the triazacyclonanone in deprotected form after the removal of tosylate protecting groups, and going further in other N-functionalizations. A similar work was published by Huang *et al*.[208] in which, starting from N,N-dibenzyldiethylamine **91** they synthesized 1,4,7-tribenzyl-1,4,7-triazonane-2,6-dione **93**, that after a reduction and remove of benzyl groups it is transformed in 1,4,7-triazacyclononane **100** and then going further N-functionalizations. Moreover, the selective mono-deprotection of benzyl group (**94**) open possible pathway in which different N-substituted TACNs can be obtained. In similar way, after the remove of another benzyl from compound **102**, it is possible to synthesize a trisubstituted-TACNs (**105**) in which all three substituents are different one from each other. But this strategy suffer of some drawbacks like long reaction times in some steps and employment of large amount of dangerous reagents like $LiAlH_4$ (*scheme 2.1.4.*).



Scheme 2.1.4. Synthetic scheme for TACN (bold and circle red) and TACNs derivatives by Huang et al.

Functionalization of TACN with pendant arms or synthesis of TACN-pendant arms functionalized is an important research field since these modifications enhance the complexing abilities of aza-macrocycles itself. Starting from N-monotosylated TACN

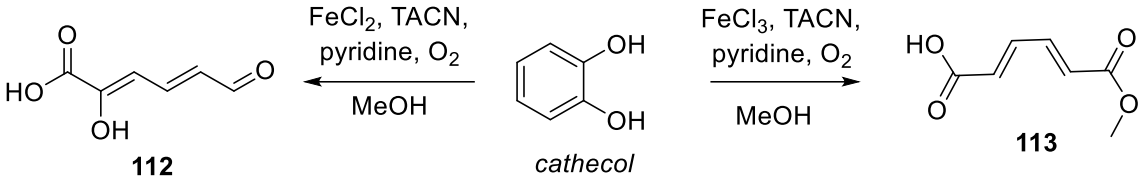
106, it is possible to obtain a TACN derivatives with different pendant arms. The reaction between **106** and ditosylatediamine and a successive deprotection leads to the formation of macrocycle **109** with a free nitrogen atom of TACN moieties that can be functionalized as shown in *scheme 2.1.5*. The resultant ligands **111** can form very stable complexes with Mn(II) and Zn(II).[209a, 209b]



Scheme 2.1.5. Synthesis of TACN pendant arms functionalized

Among the possible applications of TACNs-complexes, biomimics and radiopharmaceutical are ones of the most important due to the high stability of these TACNs-complexes. For example, TACNs coordination environment has widely exploited to coordinate Cu(II) and Cu(I) with the purpose to mimic the active site of some proteins like catechol oxidase, cythocrome c oxidase, nitrite reducetase, galactose oxidase; more over, the linkeage between two TACNs moieties or synthesis of complex-structurated ligands allow to obtained also dinuclear complexes and heteronuclear complexes.[196] TACN itself (**100**) is used as additive in TACN-Fe(II)/Fe(III) systems

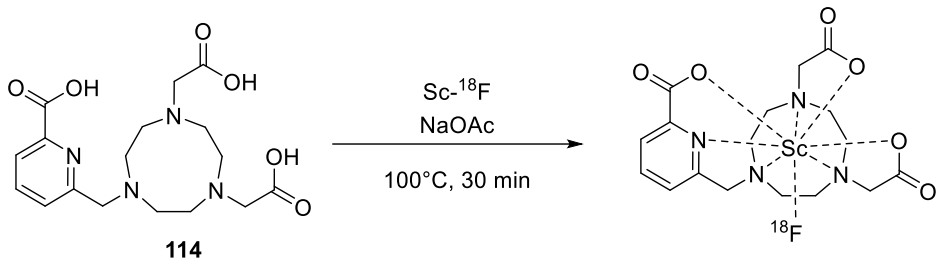
used for the oxidative degradation of catchecol (mimic of dioxygenase family), in fact Lin *et al.*[210] reported that the combination of Fe(II) or Fe(III), TACN and pyridine was able to degrade the catchecol in (2*Z*,4*E*)-2-hydroxy-6-oxohexa-2,4-dienoic acid **112** through Fe(II) reaction and in (2*Z*,4*Z*)-hexa-2,4-dienedioic acid **113** in case of Fe(III) (similar enzyme selectivity). The system with different metal salts (i.e. $MnCl_2$, $CoCl_2$, CuCl) and different bases (i.e. DMAP and DBU) have not shown no activity in the reaction. For what concern the mechanism in Fe(II) model, the reaction, in accordance to author, proceed in similar way to extradiol catechol dioxygenase.



Scheme 2.1.6. Oxidative degradation TACN-catalyzed developed by Lin et al.

Among TACN derivitaves, NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) was intensively studied in complexing metal ions in biological and medicinal field, in fact NOTA-Cu complexes for PET and theraputical are known in literature. Studies about thermodynamic and kinetic stability of NOTA-metal complexes formation have shown that NOTA selectivity bounds with Cu(II) forming more stable and faster complexes respect with Zn(II) and Ni(II) (usual impurities of radiolabelled Copper atoms), the complex formation is not neither affected by the copresence of zinc and nickel atoms. This selectivity is explained by the size of cavity: the relative small size of NOTA cavity does not fit with large divalent cations of lead and cadmium, and weak and long-group decreasing interactions were dectected between NOTA and alkali metals like Ca(II), Mg(II), Sr(II), Li(I), Na(I), K(I) since only one pendant arm is involved in the coordination of metal centre.[211]

Recently, a derivative of NOTA (compound **114**), in which an acetic acid pendant arm is substituted with a picolinic acid one, has shown very ueful in formation of [^{18}F][Sc-F] ternary complex for PET imaging.[212] In fact, due to its high stability (Sc-F energy bound dissociation of 229 kJ/mol), the de-flourination processes do not take place *in vivo* constituting a valid alternative to C-^{18}F compounds and Ga-^{18}F radiofarmaceuticals. This complex can be obtained by the reaction between NOTA derivative **114** and Sc-^{18}F in presence of NaOAC at 100°C only aftr 30 minutes of reaction.



Scheme 2.1.7. Synthesis of [^{18}F][Sc-F] complex for PET

Many others examples of these derivatives are reported and they found wide applications in many fields, especially in radiopharmaceutical field and in the figure below many structure examples are reported.

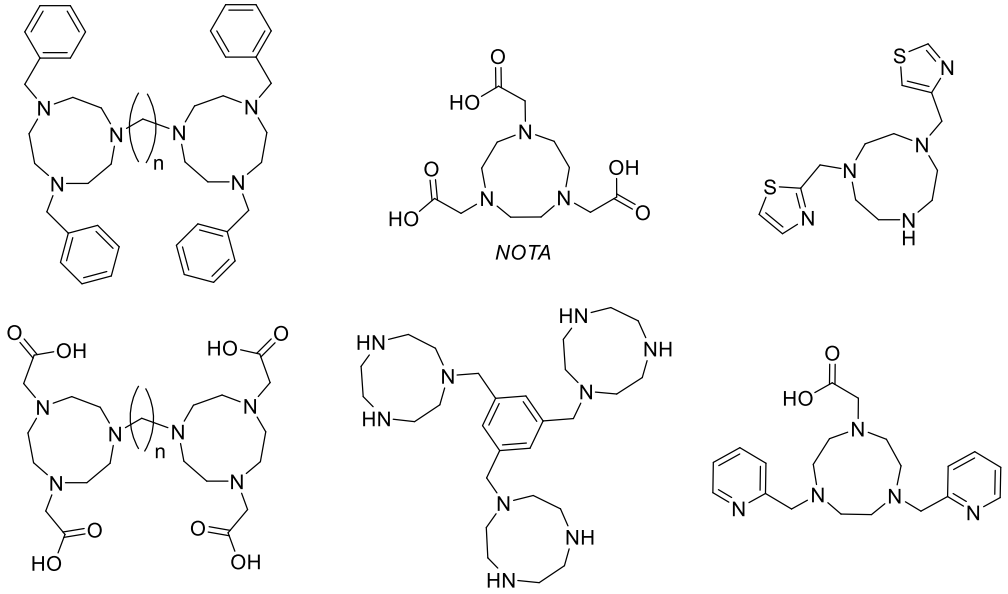


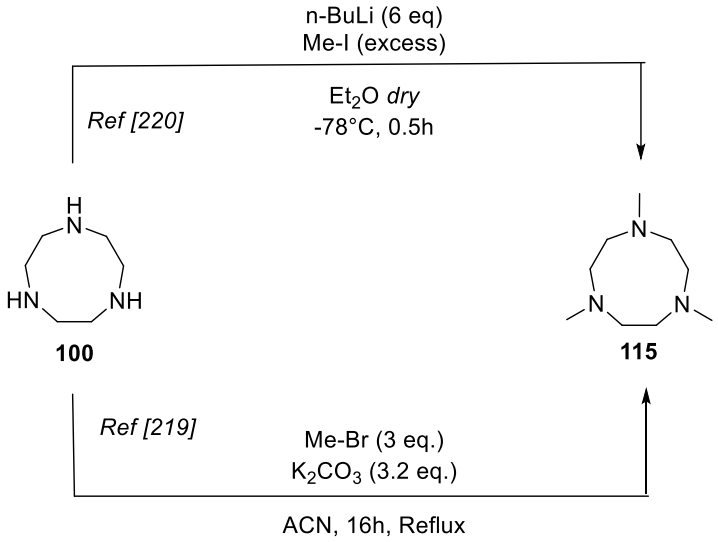
Figure 2.1.8. Structure of somoe TACNs ligands in mimic and radiopharmaceuticals, source refrence[185]

1,4,7-TRIMETHYL-1,4,7-TRIAZACYCLONONANE

Among the possible TACN derivatives, 1,4,7-trimethyl-1,4,7-triazacyclononane (Me_3TACN), focus of this topic, deserves a special mention in this section. Nowadays it is known the complex ability of this ligand for many transition metals, especially for Manganese. These complexes found many applications ranging from organic catalyses, bioinorganics and functional materials.[214] For instance, dinuclear Mn(IV) complex with Me_3TACN, $[Mn_2(Me_3TACN)_2O_3][PF_6]_2$, has shown a very powerfull catalyst for alkanes oxidation in hydrogen peroxide water solution performing the reaction in mild conditions and in presence of a co-catalyst (oxalic acid). This catalyst allow ot overcome some problems related to the employment of metal and metals oxide like cryogenic conditions

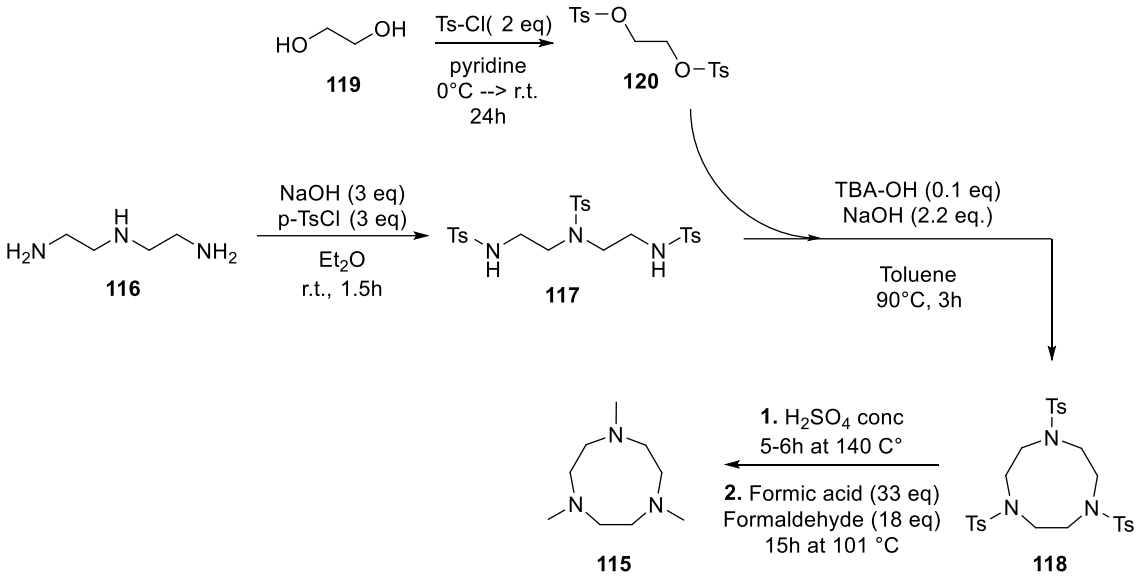
(temperature > 200°C) and selectivity, allowing to oxidise methane to formaldehyde and cyclohexane to corrispective cyclohexanone and cyclohydroperoxyl (ratio 10:1) at 50°C (after 12h for methane and 3h for cyclohexane). On the other hand, the oxidation of linear alkanes (n-heptane and n-hexane) has shown a regioselectivity for $–CH_2-$ of chain (especially for C4 in case of heptane) leaving almost untouched the terminal $–CH_3$ (mixture of carbonyl and alcohol moieties formation was detected).[215] $[Mn(IV)_2(Me_3TACN)_2\text{-}\mu O_3][PF_6]_2$ has shown a green/environmental friendly alternative for Co-based driers used for alkyd emulsion coating. In presence of polyamine (HMTETA, 1,1,4,7,10,10-Hexamethyltriethylenetetramine) this complex has shown higher activity in emulsion of Ethyl Linoleate than to commercial Mn-catalyst (Nuodex Web-Mn) and comparable activity to Co-based commercial catalyst (Nuodex Web-Co). The presence of this polyamine enhances the acitivity of the catalyst, already active by itself, improving the compatibily between complex and ethyl linolate, without detecting any exchange between TACN and HMTETA.[216] Not only Me_3TACN-Mn complexes are known in literature; in fact, Me_3TACN-trinucleare iron complexes, $[(Me_3TACN)_2Fe(II)_2\text{-}\mu Cl_3][Me_3TACNFe(II)Cl_3]$, catalysed efficentely the polymerization of MMA and styrene by ATRP (atom transfer radical polymerization). One important feature of this catalyst is its solubility in polar solvent like methanol, in fact the precipitation of polymer in methanol allow to avoid contamination of polymer by the catalyst and allows also its recovery. In fact, after four recycles, the catalyst still explicates almost the same initial activity (similar conversion and molecular weight of formed PMMA and polystyrene) indicating the robustness and staibility confered by Me_3TACN-ligand to iron (Fe(II) can be easly oxidized to Fe(III) in presence of air oxygen).[217] Just to notice, 1,4,7-trimethyl-1,4,7-triazacyclonane can be used as scaffold for the synthesis of quaternary ammonium compounds (QACs) with antibacterial activity. Studies about antibacterial activity against two gram positive bacterials (*Staphylococcus aureus* MSSA and MRSA) and two gram negatives (*Escherichia coli* and *Pseudomonas aeruginosa*) has shown that limited flexibility (including Me_3TACN) of ammonium counterpart improved the biological activity respect to linear compounds. Mono and triiodide Me_3TACNs salts with 16, 18 and 20 lateral-carbon chains have shown good biological properties against the gram positive ones (MIC values ranging from 1 to 4 μM) and either better properties against negatives than linear tested ammonium salts (TACN MIC values raging from 1 to 16, against 1 to 32).[218]

Apart from the obvious direct methylation of 1,4,7-triazacyclononane **100** using a base and a methyl-halide (see *scheme 2.1.8*) , the most part of synthethic strategies are covered by patents and usually these methods require harsh conditions.[219] The alkylation of TACN (**100**) reported in literature require sometimes the employment of harmfull reagents,[220] and sometimes long reaction time,[221] without considering the dangerous that came from methyl halide (strong alkylating agents of DNA),[222] even if this method allow to obtain different 1,4,7-trisubstituted-1,4,7-TACNs.[221]



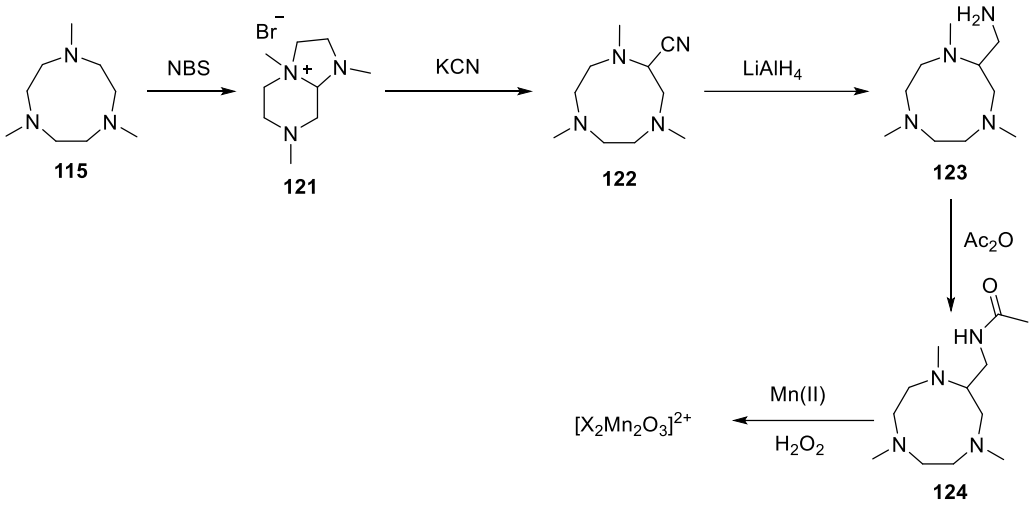
Scheme 2.1.8. Direct alkylations of TACN reported in literature

In addition, it is possible to synthesize this compound starting from a linear polyamine, Schoenfeldt *et al.*[223] synthesized Me_3-TACN starting from diethylenetriamine **116** following a similar Richmann-Atkins pathway. First, they protected the amine with tosyl-group, then after the reaction between protected amine **117** and 1,2-di(p-toluenesulfonyloxy)ethane **120** (previously preparated through the reaction between glycol **119** and p-toluensulfonyl chloride) leads to the 1,4,7-triazacyclononane scaffold **118**. The target cycle **115** is obtained after the reaction of deprotected TACN (deprotection performed in H_2SO_4 conc, 5-6h at 140°C) and formaldehyde after 15hrs of reaction at 101°C.



Scheme 2.1.9. Synthetic strategy for 1,4,7-trimethyl-1,4,7-triazacyclononane developed by Schoenfeldt et al.

Due the important features of Me_3TACN-Mn complexes (i.e. epoxidation of olefin, bleaching activity of hydrogen peroxide), the possible enhancement of resultant complexes has motivated the scientist to explore also the possibility the synthesis of functionalized Me_3TACN. Koek *et al.*[213] published an example of possible mild-condition functionalization of this cycle, they started directly from the Me_3TACN **115**, they performed an oxidation of this cycle using N-bromosuccinimide forming the bromide ammonium salt **121**, further functionalization with KCN and reduction with $LiAlH_4$ allow to obtain the pendant arm $–CH_2NH_2$ (compound **123**), that after protection with acetic anhydride (**124**) and subsequent reaction with Mn(II) leads to the formation of very stable dinuclear trioxo manganese complexes.



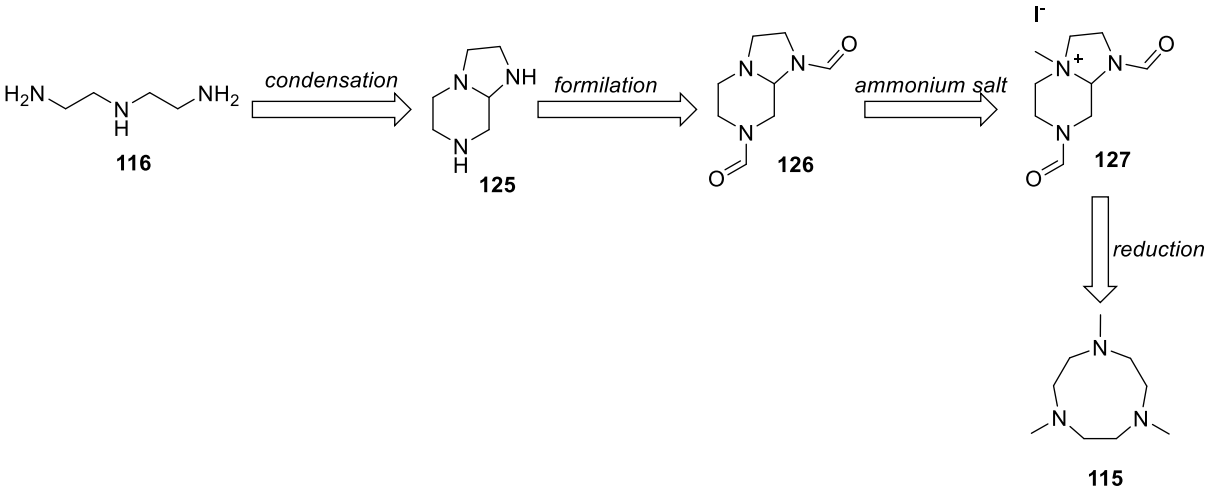
Scheme 2.1.10. Direct functionalization of Me_3TACN

2.1c Results and Discussion

This project was born in collaboration with I.C.A. group of Civitanova Marche (MC), for the studies about dryness effects of 1,4,7-trimethyl-1,4,7-triazacyclononane in painting coating.

BACKGROUND & SYNTHETIC APPROACH

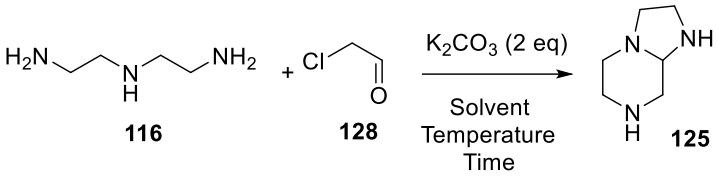
As written in the previous section (2.1b Synthesis and application of 1,4,7-triazacyclononane and its derivatives), there is lack of methods for the synthesis of 1,4,7-trimethyl-1,4,7-triazacyclonane, and they usually require harsh conditions, harmful reagents and sometimes long reaction time. For this reason, we focused our effort to develop a new synthetic strategy for this aza-macrocycle that implicates milder reaction conditions and improve some aspects and overcome some problems met with some methods reported literature; without forgetting the important features of Me_3TACN-complexes can found. Our purpose was to obtain the Me_3TACN through a four steps process starting from an acyclic precursor, diethylenetriamine **116**, that after the condensation with an electrophile leads to the formation of fuse-byclicic intermediates **125**, which further a formilation, formation of ammonium salt **127** and reduction of **127** leads to the final target molecule.



Scheme 2.1.11. Synthetic approach for 1,4,7-trimethyl-1,4,7-triazacyclononane

CURRENT WORK

The first step implicates the condensation between triethylenediamine **116** and chloroacetaldehyde **128** (solution of 50% in water) in presence of a base such as potassium carbonate, and all experiments for the synthesis of pyrazine intermediate **125** are reported in the *table 2.1.1.*



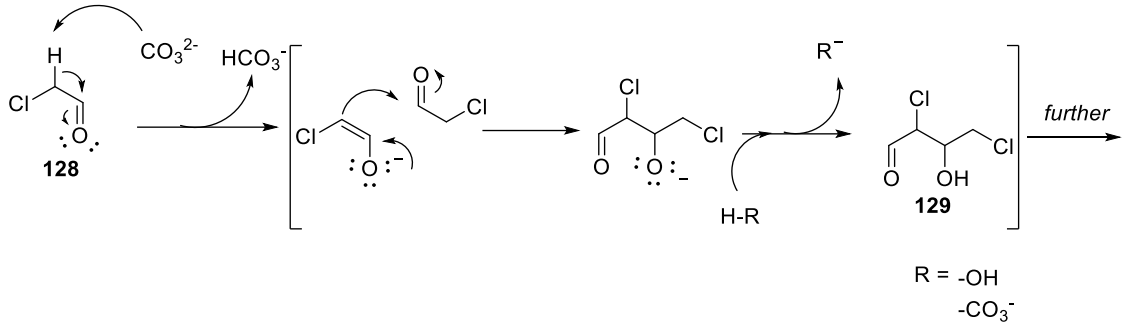
116 128 125

Entry	Chloroacet.	Temp.	Rate Addition	Solvent	Reaction Time	Yield
1	1 eq.	r.t.	Dropwise	CH_3CN	6h	6%
2	1.1 eq.	r.t.	Dropwise	CH_3CN	6h	14%
3	1 eq.	r.t.	Dropwise	CH_3CN:H_2O (10:1)	6h	24%
4	1 eq.	r.t.	Dropwise	CH_3CN:H_2O (12:1)	6h	24%
5	1 eq.	r.t.	Dropwise	THF	6h	7%
6	1 eq.	30 °C	Dropwise	CH_3CN	6h	8%
7	1 eq.	50 M.M.	Dropwise	CH_3CN	0.5h	/
8	1 eq.	0 → 50 °C	Dropwise	CH_3CN	6h	/
9	1 eq.	r.t.	12 mL/h	CH_3CN	16h	45%
10	1 eq.	r.t.	4 mL/h	CH_3CN	24h	66%
11	**1 eq.**	**r.t.**	**2 mL/h**	**CH_3CN**	**24h**	**75%**

Table 2.1.1. Above the scheme of the reaction and under the screening of conditions for the condensation

Applying the conditions reported in literature (Entry 1, *table 2.1.1*)[206] we unfortunately obtained a very low yield of target product, since we observed the formation of autocondensation product of aldehyde (*scheme 2.1.11*) due to the presence of a strong base such as potassium carbonate. Therefore, we tried to modify the parameters of the reaction. First of all we tried to increase the amount of aldehyde (Entry 2, *table 2.1.1*) and a slight and not satisfying increament of yield was detected. Changing the solvent, bad results were obtained again. Performing the reaction in a mixture of acetonitrile/water a modest increase of yield was also detected (Entry 3 and 4, *table 2.1.1*) as probably the solubility of potassium carbonate is increased by the presence of a major amount of H_2O. While performing in other water soluble solvent (THF, Entry 5, *table 2.1.1)* a comparable result with acetonitrile was recorded. Performing the reaction at 30°C, or under microwave irradiation or performing the addition at 0°C and then heat at 50°C any changes in the outcome of the reaction as low yields were still detected or sometimes the product was neither observed (Entry 6, 7 and 8, *table 2.1.1*). In each experiments mentionated above, we always observed the formation of autocondensation product **129** of chloro acetaldehyde. The product was not isolated and characterized (GC-MS

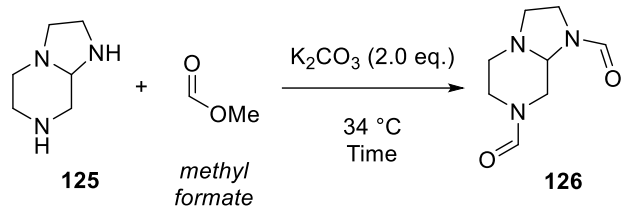
detection), probably it could go to multiple self condensations since the formation of a red and insoluble solid was always observed.



Scheme 2.1.12. Supposed mechanism and structure of the formation of autocondensation product of chloroacetaldehyde

Finally, to overcome the formation of this side product we tried to perform the reaction using an infusion pump as it allow to mantain a costant rate of addition and therefore a constant molar ratio between amine and aldehyde. Adding the solution of aldehyde with a rate of 12 mL/h we suddenly observed an improvment isolating the product with a yield of 45% (Entry 9, *table 2.1.1*). Slowing even more the rate of addition to 4 mL/h and 2 mL/h a 66% and 75% of yield have detected (higher yield respect to literature) after 24h of reaction. Therefore, the control of the addition speed therefore allows to discriminate which type of reaction the aldehyde will follow. The slower addition avoids that aldehyde reacts with the carbonate present in the reaction and forming the autocondensate (thermodynamic process), but it allow to chloroacetaldehyde to react with amine (faster process), present in greater amount (respect to aldehyde) and thus forms the pyrazine target (kinetic process).

Once achivied the intermediate **125** the next step provides the synthesis of di formamide **126** by a formilation process (*table 2.1.2.*).

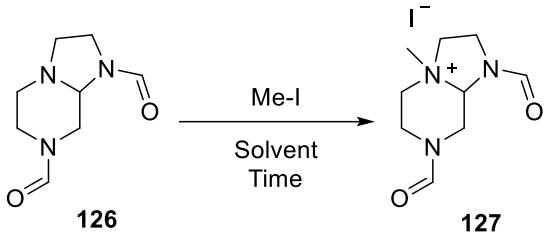


Entry	Methyl Formiate	Temperature	Reaction time	GC Conv.	Yield
1	33 eq.	34 °C	24h	82%	66%
2	**33 eq.**	**34 °C**	**48h**	**93%**	**72%**
3	20 eq.	34 °C	24h	73%	55%
4	10 eq.	40 °C	1h	Complex mixture	/
5	25 eq.	34 °C	24h	95%	58%

Table 2.1.2. Screening conditions for the formilation step

From the *table 2.1.2* it is possible to notice that in major part of experiments the product was achivied with a good yield and good GC conversion. The peculiar of this reaction was that the long time of reaction and a huge employment of methyl formate that acts as solvent of the reaction. The first aspect that can be noticed is that lasting the reaction 48 hrs instead of 24 hrs (respectively Entry 2 and Entry 1, *table 2.1.2*) a better conversion (almost total) and better yield was detected. Trying to perform the reaction under microwave irradiation, with the purpose to speed up the reaction, other secondary process were favoured and formation of complex mixture was detected (Entry 4, *table 2.1.2*). Finally, since the possible large-scale synthesis that this ligand could undergo, we tried to decrease the amount of ethyl formate (reduce amount of waste and reagent) but also in these cases the yield of target product was lower respect to Entry 2.

The ammonium salt formation is the neck-bottle step of this synthetic process. Due to the nature of target TACN derivative **115**, we obviously choose to use methyl iodide as alkyl halide. All the experiment for the synthesis of ammonium salt **127** are reported in *table 2.1.3*.



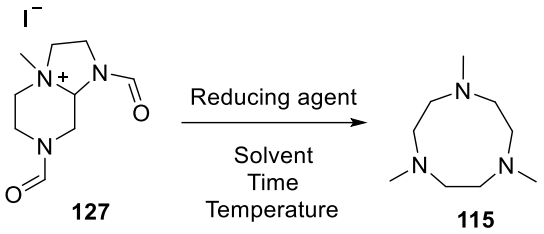
126 127

Entry	Me-I	Solvent	Reaction time	Yield
1	1.02 eq.	CH_3CN	16h	2%
2	1.02 eq.	CH_3CN	24h	52%
3	1.2 eq.	CH_3CN	24h	48%
4	1.2 eq.	CH_3CN	48h	50%
5	**3.0 eq.**	**CH_3CN: MeOH (3:1)**	**24h**	**64%**

*Table 2.1.3. Screeing of conditions for the synthesis of ammonium salt **127***

For this reaction in all experiments the almost same results have recorded. Starting from the employment of little excess of alkyl agent (Entry 1, *table 2.1.3*) a low yield was detected, but leaving the reaction 24 hrs (Entry 2, *table 2.1.3*) an improvement of yield was observed. Increasing a little more the methyl iodide (Entry 3 and 4, *table 2.1.3*) a similar result to Entry 2 was observed. Finally, the employment a larger amount of methyl iodide and performing the reaction in a mixture of acetonitrile/methanol (Entry 5, *table 2.1.3*) a moderate result has obtained this time. The major improvement of this step was the solvent reaction. In fact, one drawback of this reaction is its reproducibility (just confronting the result of Entry 1 with the other ones). In our opinion, the mixture of CH_3CN/MeOH gives to the system a good robustness, since the presence of methanol decreases the solubility of product **127** that precipitates in the reaction and it allows to obtain almost the same results in each experiments. Moreover, the indeherance of tertiary nitrogen atom does not allow the fast trend of the reaction and so good results. Considering also the toxicity of methyl iodide,[222] the employment of higher amount of has not tested.

Finally, the reduction of previously synthesized iodide salts leads to the formation of final Me_3TACN **115**. Different amount and reducing agents were tested and reported in *table 2.1.4.*



Entry	Reducing Agent	Solvent	Temperature	Reaction Time	Yield
1	Red-Al (5.0 eq.)	Toluene	65 °C	5h	5 %
2	$LiAlH_4$ (20 eq.)	THF *dry*	r. t.	24h	Complex Mixture
3	$NaBH_4$ (20 eq.)	THF *dry*	r. t.	24h	Complex Mixture
4	Red-Al (10 eq.)	Toluene	65°C	24h	15%
5	**Red-Al (20 eq.)**	**Toluene**	**65 °C**	**24h**	**77%**
6	**$LiAlH_4$ (5.5 eq.)**	**THF *dry***	**r.t.**	**5h**	**78%**

Table 2.1.4. Screening conditions for the synthesis of 1,4,7-trimethyl-1,4,7-triazacyclononane

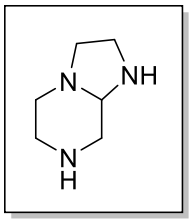
As first reducing agent we tested Red-Al solution but in this case very low yield was observed (Entry 1, *table 2.1.4*). Then, using a very large excess of two different common reducing agents like $NaBH_4$ and $LiAlH_4$ (respectively Entry 2 and Entry 3, *table 2.1.4*) a formation of a complex mixture has observed after 24 hrs. Instead, doubling the amount of Red-Al® a yield of 15% was recorded after 24 hrs reaction (Entry 4, *table 2.1.4*). Anyway, performing the reaction with a higher amount of Red-Al® (Entry 5, *table 2.1.4*) or using 5.5 eq. of lithium aluminium hydride (Entry 6, *table 2.1.4*) a good and similar yields have obtained (77% and 78% respectively). Some considerations have to be done. Even if each two conditions lead to the same result, the $LiAlH_4$ method is preferable to Red-Al® one. First of all, the lithium method is faster and performed in milder conditions (room temperature against 65°C), this aspect means save money, time and energy, and it is very important aspect to consider from an industrial and large scale production point view. Moreover a lower amount of reducing agent is require in case of lithium method that means again save money and also less formation of waste reagent, without considering the major handleness and lower harzardness of $LiAlH_4$ than Red-Al® (highly infiammable). In the end, one aspect to consider is the solvent, the lower boiling point of THF makes this method more suitable during extraction process since the evaporation of it requires less energy.

ALKYD EMULSION

This collaboration between the university and the ICA arises because of the company needs to use a catalyst for alkyd emulsion of resins, since the commercial ones have becomes very expensive as the complexes with Me_3TACN and specific metals found chemotherapic applications. ICA groups tested this ligand in two different resins and the results have already reported in Alessandro Menchi PhD thesis and reported here to show the usefulness of this azamacrocycle. The two resins tested are a long oil alkyd and the second is a urethane alkyd. Once the two products are prepared, they are applied by spreading on a support (glass) and then two catalysing systems are applied over them: the first one si composed of cobalt, zirconium, and sodium salts and it acts as standard, the second one is formed by a combination of manganese salt and the 1,4,7-trimethyl-1,4,7-triazacyclononane. After 16 hours of drying at room temperature, they tested the hardness the paint film to have an objective data on the hardening of catalyst. A precise evaluating the hardness of coating by the two different solutions, they performed Erichsen pen test. This test is used to measure the minimum energy necessary to make the scratch made by a steel tip on the paint film, when the scratch is also seen from the other side of the support, which force is considered the minimum necessary for to do the scratch. Obiousvly, harder paint films requires major intensity of minimum force. Erichsen pen test explicated that the paint coated by standard catalyst system showed a hardness of about 6N (Newton), while the samples coated with the new catalyst system 10N. Therefore the new catalityc system enhanced the drying process since the paints showed a greater hardness and superior quality.

2.1d Experimental Section

*OCTAHYDROIMIDO[1,2-A]PYRAZINE (**125**)*



7.02 mL of Chloroacetaldehyde **128** (50% sol. in water, 55.3 mmol, 1.0 eq.) are dissolved in 40 mL of acetonitrile (1.1 M solution) and it is added dropwise by infusion pump (flow 2.00 mL/h) to a solution of diethyltriamine **116** (5.974 ml, 55.3 mmol, 1.0 eq.) in acetronitrile (80 ml). Then the solution is stirred for 24h at room temperature. The reaction is monitored at GC-MS. When no more evolutions are observed at GC-MS, the mixture reaction is filtered by a pad of Celite and the solvent was evaporated by rotavapor. The final product is a dark yellow oil with a yield of 75% and it is used without further purification.

Molecular Formula: $C_6H_{13}N_3$

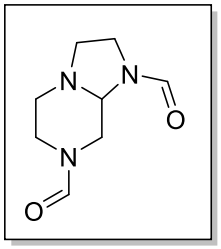
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 2.97 (dd, J = 11.7, 2.8 Hz, 1H), 2.88 – 2.76 (m, 2H), 2.70 – 2.49 (m, 5H), 2.32 (dd, J = 11.7, 8.0 Hz, 1H), 2.25 – 2.11 (m, 2H), 1.97 (s, 2H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 76.61, 52.33, 51.10, 48.31, 45.20, 42.93.

GC-MS (70eV; EI) = 127 ($M^{\cdot+}$), 97, 70 (100), 56, 42.

FT-IR (cm^{-1}) = 3268, 2936, 2882, 2810, 1745, 1656, 1598, 1445, 1311

*HEXAHYDROIMIDAZO[1,2-A]PYRAZINE-1,7-DICARBALDEHYDE (**126**)*



A mixture of 700mg of octahydroimidazo[1,2-a]pyrazine **125** (5.51 mmol, 1.0 eq.) and 1.5g of K_2CO_3 anhydrous (11.02 mmol, 2.0 eq.) are dissolved in 11.20 mL of methyl formiate (181 mmol, 33 eq.), then the reaction is refluxed (34°C) for 48h. The reaction is monitored at GC-MS. When no evolutions are more observed, the reaction mixture is filtered by a pad of celite and the solvent is removed by rotavapor. The final product is a yellowish oil and it is obtained with a yield of 72%. The crude is used without further purifications, but if its purity e is not satisfying, the crude of reaction can be purified by filtration on buffered silica gel (9:1 $CHCl_3$/MeOH, Rf = 0.39). The buffered silica is obtained suspend the silica in dichloromethane, then 1mL of Et_3N is

added to the suspension for each gram of silica, then the suspension is stirred for 16 hrs, the dichloromethane is evaporated under vacuum.

Molecular Formula: $C_8H_{13}N_3O_2$

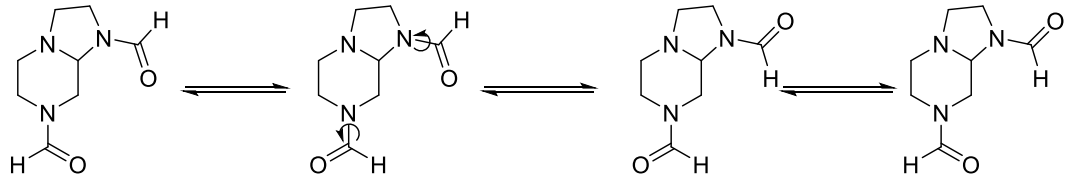
^{1}H-NMR (400 MHz, DMSO-d_6) δ = 8.22, 8.20, 8.18, 8.07, 8.05, 8.02 (s, 1H), 8.01 (s, 1H), 4.77, 4.35, 4.19, 4.09 (dddd, J = 38.9, 34.4, 12.4, 3.3 Hz, 1H), 3.98-2.32 (m, 10H).

^{13}C-NMR (100 MHz, DMSO-d_6) δ = 162.39, 162.34, 162.26, 162.23, 162.10, 159.85, 159.76, 73.00, 72.89, 72.09, 72.03, 49.65, 49.52, 49.38, 49.25, 48.93, 44.50, 44.42, 44.19, 44.07, 42.53, 42.30, 42.10, 41.82, 38.65, 38.44.

GC-MS (70eV; EI) = 183.1 ($M^{\cdot+}$), 154 (100), 125, 98, 72, 56.

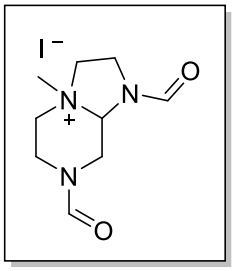
FT-IR (cm^{-1}) = 3519, 2819, 1652, 1432, 1396, 1369, 1275, 1194.

NOTATION: even if the product has a very simple structure, such complicated NMR-spectra are due to the rotational isomer of formamide (*scheme 2.1.10*) that split the signals even if the molecule is pure.[223]



Scheme 2.1.10. Rotational isomers of formamide

*1,7-DIFORMYL-4-METHYLOCTAHYDRO-1H-IMIDAZO[1,2-A]PYRAZIN-4-IUM IODIDE (**127**)*



3.1 g of 1,7-diformyloctahydro-1H-imidazo[1,2-a]pyrazine **126** (16.5 mmol, 1.0 eq.) are dissolved in a solution 3:1 of acetonitrile/methanol (54 mL/18 mL), 3.61 mL of MeI (50.8 mmol, 3.0 eq.) is added dropwise. After one hour formation of a white solid is observed. The reaction mixture is stirred for 24h at r.t. Then the solution is filtered by gouche and a white-like solid is recovered with a yield of 64%.

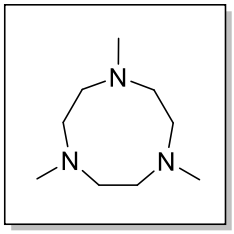
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.42 – 8.06 (m, 2H), 5.15 (dt, J = 7.5, 4.4 Hz, 1H), 4.50-3.35 (m, 10H) 3.31 – 3.21 (s, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 163.30, 162.50, 162.07, 162.04, 161.97, 161.01, 160.78, 78.34, 78.24, 77.04, 75.90, 59.11, 57.98, 57.90, 56.78, 56.59, 55.89, 55.74, 51.49, 50.63, 48.61, 47.15, 37.11, 35.42, 33.60.

FT-IR (cm^{-1}) = 3017, 2985, 2958, 1683, 1656, 1441, 1369, 1306, 1270, 1172.

m.p. (range °C) = 167-169 °C

*1,4,7-TRIMETHYL-1,4,7-TRIAZACYCLONONANE (**115**)*



$LiAlH_4$ method: 642.25 mg of $LiAlH_4$ (16.92 mmol, 5.5 eq.) are suspended in 10 mL of THF *dry* and the solution is cooled down to 0°C with an ice-water bath. Then 1.00g of iodide salt **127** (3.077 mmol, 1.0 eq.) is added slowly to the solution, finished the addition the reaction is left to warm up to r.t and it is stirred for 5h. Then the reaction is diluted in 20 mL of Et_2O with an ice-water bath and then 20 mL of Rochelle Salt are added dropwise for quenching the $LiAlH_4$. Then the reaction is stirred for 1 hour at r.t. The aqueous layer is extracted 2x20mL with Et_2O, the organic layer is anhydrous over Na_2SO_4 and then the solvent is evaporated by rotavapor. The final product is a pale yellow oil and it is obtained with a yield of 78%.

Or

Red-Al® method: 44 mL of 70% toluene solution of Red-Al® (155 mmol, 20.00 eq.) is heated to 65°C under stirring under N_2 atmosphere, followed by – about in 1 hour – adding 2.5 g (7.75 mmol, 1.00 eq.) of the quaternary salt **127**. The reaction mixture is heating up intensively and gas evolution also being detected. After a period of 24 hours has elapsed, no starting compound can be detected anymore. 20 mL of 10% NaOH solution is added for quenching the mixture while applying cooling ice, followed by separating phases. The inorganic phase is extracted with 2x50 mL of toluene, the toluene phases are combined and dried with anhydrous sodium sulphate and the solvent evaporated by rotavapor to give the product as pale yellow oil with a yield of 77%.

Molecular Formula: $C_9H_{18}N_3$

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 2.62 (s, 12H), 2.33 (s, 9H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 57.34, 46.91.

GC-MS (70eV; EI) = 171 ($M^{\cdot+}$), 154, 141, 127, 115, 99, 84, 70, 58 (100).

FT-IR (cm^{-1}) = 3412, 2967, 2868, 2703, 1742, 1450, 1396, 1257.

2.1e Conclusion

The aza-macrocycles constitue a very important class of compounds that has been widely investigated from a synthethical point of view for more than 40 years. This interest by scientific community is due to the important chelating features of these compounds that allow to obtain complexes that can find many applications in different scientific fields, from biomedicals to industrial. Among azamacrocycles, the 1,4,7-triazacyclononane and its derivatives is a special class of compound since these lingads can chelate and stabilaze many different transition metals with different oxidation states. The 1,4,7-trimethyl-1,4,7-triazacyclononane is an important TACN derivative since its complexes are widely used as catalyst in many organic reaction and industrial synthesis, and for these reasons some patents report the synthesis for this special scaffold. The lack of literature synthetic method for this polyamine cycle is an important motivation for developing new synthetic pathways, especially considering the harsh conditions reported in patents. Bearing in mind these aspects, we developed a new synthetic strategy for 1,4,7-trimethyl-1,4,7-triazacyclononane that implicates a four step process for an overall yield of 27% and resulting usefull in drying process made by I.C.A. group. Some important aspects have been improved such as the controlling of addition of aldehyde that avoids the formation of undesidered byproducts, the robusteness of iodide salts step and the new reduction method with $LiAlH_4$ that allow to obtain the final target in mild conditions saving money and energy. We also calculated the cost of our method for the synthesis of 1,4,7-trimethyl-1,4,7-triazacyclononano per 1 gram of final cycle finding a very low cost production for this molecule, respect to other commercial suppliers.

Reagent	**Method cost Red-Al®**	**Method cost $LiAlH_4$**
Diethylentriamine	0,29 €	0,29 €
Chloroacetaldeide	0,25 €	0,25 €
Potassium carbonate	1,76 €	1,76 €
Methyl Formiate	12,90 €	12,90 €
Iodomethane	4,11 €	4,11 €
Acetonitrile	15,56 €	15,56 €
MeOH	2,90 €	2,90 €
Red-Al®	8,50 €	-
Silica Gel	3,13 €	3,13 €
Chloroform	27,40 €	27,40 €
$LiAlH_4$	-	1,26 €
THF	-	3,09 €
TOTAL COST	**76,84 €**	**72,68€**

Table 2.1.5. Analyses cost for 1,4,7-trimethyl-1,4,7-triazacyclononane of our method

2.2 $CeCl_3$-Promoted Condensation of Acyclic Precursors for the Synthesis of 1,2-Dihydropyridines

2.2a 1,2-Dihydropyridines: an important scaffold for the synthesis of bioactive molecules

Among N-heterocycles, dihydropyridines (DHPs) have attracted science community for their important biological properties and for their important role in several areas like medicine, pharmaceutical and drugs/bioactive complex molecules production.[225] Among the five possible isomers, the 1,2-DHPs and 1,4-DHPs attracted most the synthetic chemists for their properties, especially the 1,4-isomer since it is present as scaffold in many drugs like nifedipine and aldomine and in enzyme NADH.[226]

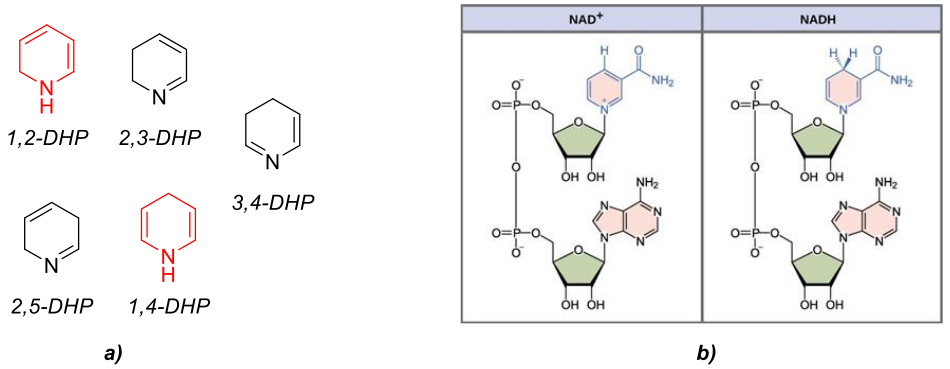
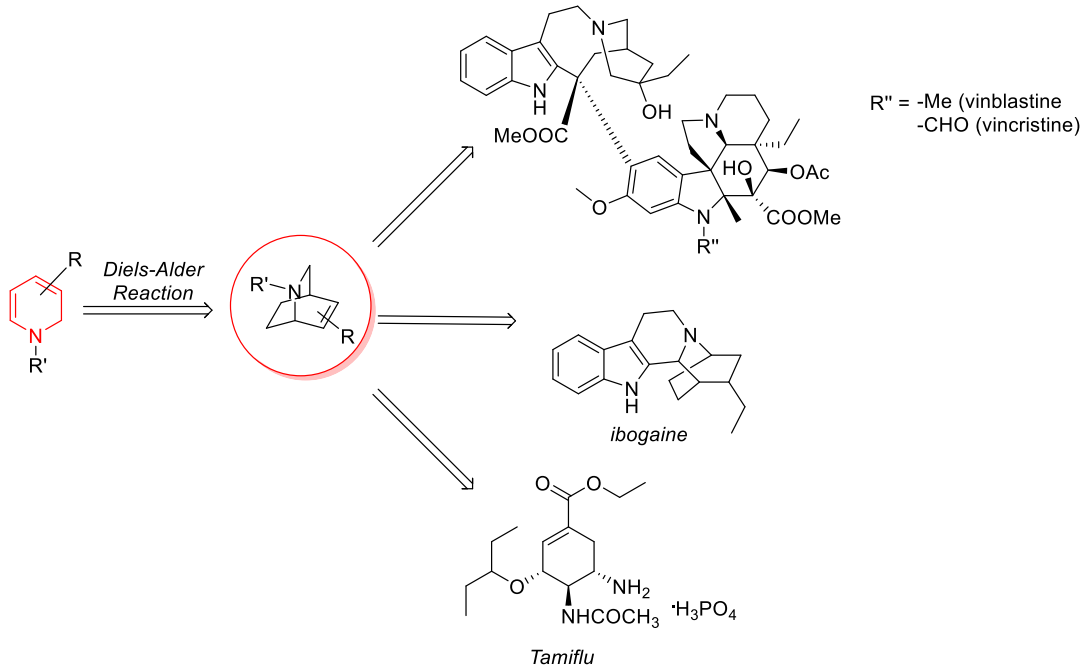


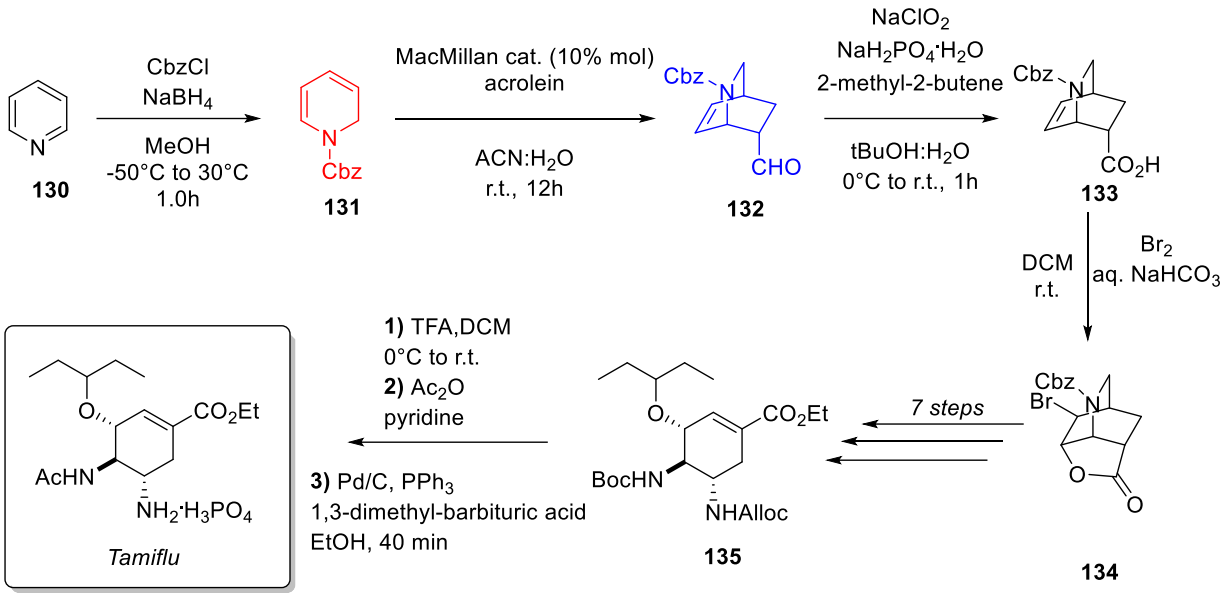
Figure 2.2.1. ***a)*** *structure of five possible isomers of DHPs;* ***b)*** *two oxidation states of NAD^+/NADH*

Although 1,2-DHPs do not find direct biological uses, they have proved very useful for the synthesis of biologically active molecules. It is known in literature the employment of 1,2-DHPs as starting material for the synthesis of isoquinuclidines (2-azabicyclo[2.2.2.]octanes) by Diels-Alder reaction with a dienophile. Isoquinuclidine-scaffold is widely present in many alkaloids (i.e. vinblastine and vincristine), ibogaine (reduces cravings of alcohol and drugs boosting the levels of growth factor) or they can be used as intermediate for the synthesis of bioactive molecules like Tamiflu (phosphate salt of oseltamivir, strong antininfluenza A and B).[227]



Scheme 2.2.1. General scheme for the synthesis of bioactive molecules starting from 1,2-DHPs

Satoh and his collaborators[228] exploited the asymmetrical Diels-Alder reaction between 1,2-dihydropyridine **131**, obtained by the reduction of pyridine (**130**) in presence of benzylchloroformate, acrolein and MacMillan catalyst, as one of key steps of Tamiflu total synthesis, leading the formation of a mixture of isoquinuclidine-aldehydes, included the compound **132**. After a Kraus oxidation of this mixture (compound **133** separated in enantiopure form (e.e. > 99%)) and bromolactonization of **133** leads to the synthesis of lactone **134**. Then, the lactone **134** is transformed in compound **135** after 7 steps (that possesses the oseltamivir core) and the final target Tamiflu is obtained changing the Boc-protection in Acetyl-protection of 4-NH_2, and after the deprotecion and salification of 5-amino group (*scheme 2.2.2.*).

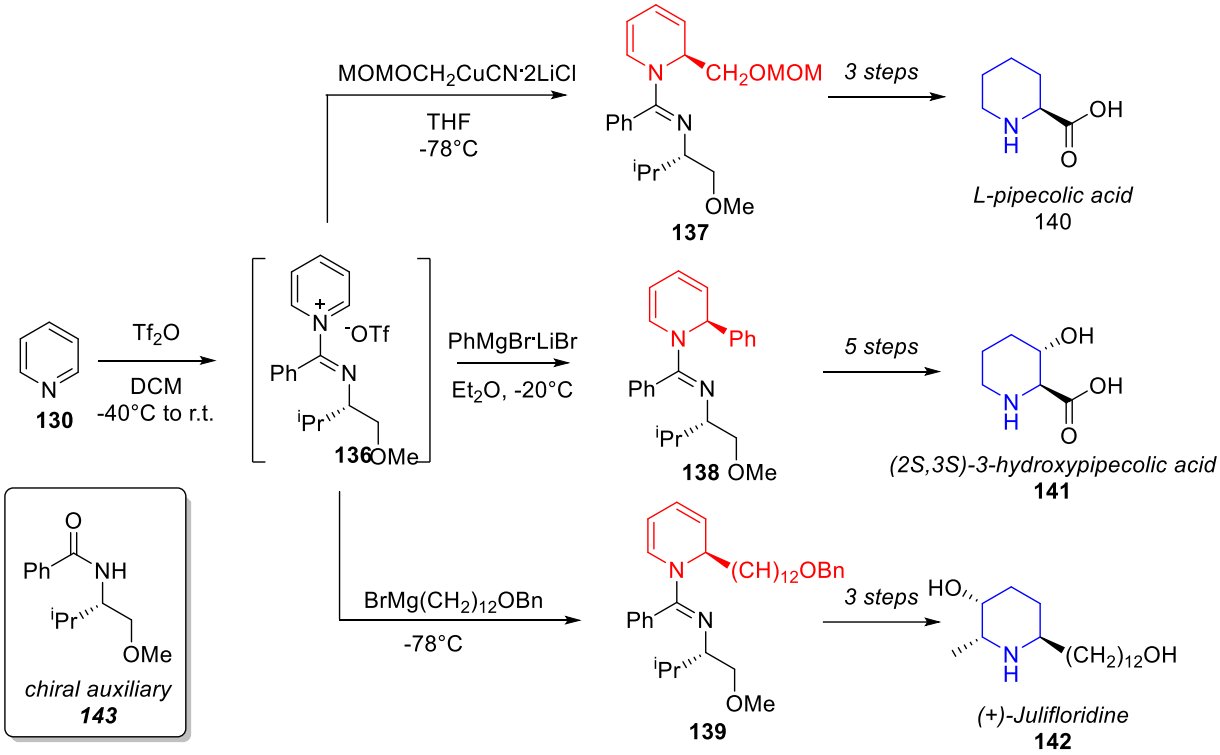


Scheme 2.2.2. Total synthesis of Tamiflu reported by Satoh et al. in which 1,2-dihydropyridine is in red and isoquinuclidine intermediate in blu

Due to the importance of Diels-Alder step in synthesis of Tamiflu of Satoh and relative quite low yield obtained in that step, Nakano *et al.*[227] studied a new oxazolidine organocatalysts for the synthesis of isoquinuclidine **132** (*scheme 2.2.2.*), detecting high enantioselective (e.e.>99%) and high yield for Diels-Alder adduct, studying also different 1,2-DHPs and acrolein derivatives obtaining good results also in those cases.

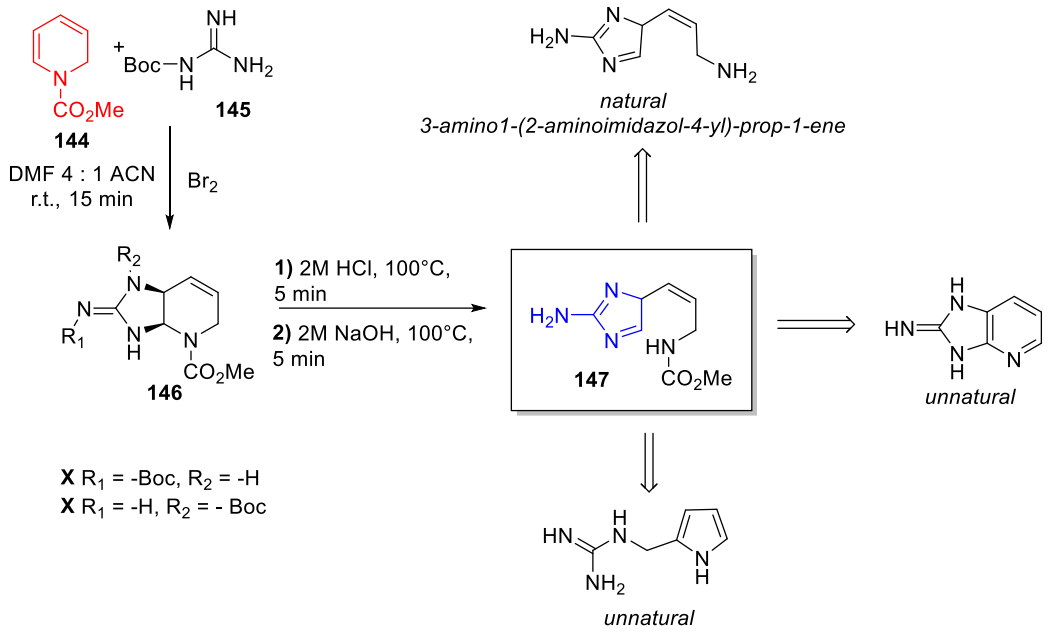
The 1,2-DHPs have shown useful also for the synthesis of natural occurring molecules. For example, Lemire and Charette exploited a 2-substituted-1,2-dihydropyridine for the chiral synthesis of (+)-julifloridine **142**, L-pipecolic acid **140** and its 3-hydroxiderivative **141**.[229] These molecules possess interesting biological properties, in fact, (+)-julifloridine **142** belongs to the family of 2,6-disubstituted piperinidols (alkaloids present in plants *Cassia* and *Proposis*) that displays a wide range of biological activities (i.e. antibacterial, antimycotic, cytoxicity);[230] while L-pipecolic acid **140** is a non-protein amino acid that explicates many roles in microorganisms, plant, humans and it is also consitutent of many natural and synthetic molecules[231] and its 3-hydroxy derivative **141** is a potential antimalarial compound.[229b] Lemir and Charette have obtained chiral 2-substituted-1,2-dihydropyridines starting from the reaction between pyridine **130**, triflic anhydride and chiral auxiliary **143** in dichloromethane, this reaction leads to the formation of chiral enaminium **136,** which can undergo to a enantioselective nucleophilic addition by a Grignard or organocopper compound (*scheme 2.2.3*). Through further transformations, it is possible to obtain the target molecules. Among them, the synthesis of (+)-julifloridine **142** is quite interesting since in the final product is

obtained by a chemo and regioselective reduction of 3,4-double bond in dihydropyridine core (H_2, Pd/C in toluene) with a good yield (69%), leaving inalterate the 5,6-double bond that can be further functionalized to obtain the hydroxyl group in position-5 and methyl in position 6. Successive deprotection of benzyl group gave the final product.[229a]



Scheme 2.2.3. Synthesis of natural occurring molecules exploiting chiral 1,2-DHPs by Lemir and Charette.

Moreover, the 1,2-DHPs have shown usefull for the synthesis of several alkaloids and also other heterocycle and condensated cycles moieties.[232, 233] Abou-Jneid *et al.*[232] have developed a simple and elegant method for the synthesis of 3-amino-1-(2-aminoimidazol-4-yl)-prop-1-ene and some 2-aminoimidazole derivatives starting from N-carboxymethyl-1,2-dihydropyridine **144** (obtained by previous reduction of pyridine in presence of $NaBH_4$ and methyl chloroformate). Starting from the reaction between 1,2-DHP **144** and Boc-guanidine **145**, that leads to byciclic compound **146**, it is possible to obtained the 9-N-carboxymethyl derivative of e 3-amino-1-(2-aminoimidazol-4-yl)-prop1-ene (**147**) after previous remove of Boc-protection by HCl and breaking of byciclic structure by NaOH.



Scheme 2.2.4. Synthesis of natural/unnatural alkaloids by Abou-Jained et al. starting from 1,2-DHP ***144***

The compound **147** can be further transformed in natural 3-amino1-(2-aminoimidazol-4-yl)-prop-1-ene by removing of ester moiety, this last one molecule is an important biogenetic compound for many bioactive alkaloids (i.e. girolline, oroidin, palau'amine, hymenidin, clathrodin); in addition, carboamide **147** can act as precursor foe the synthesis of unnatural compounds.[234] Moreover, this method is quite interesting since it allows the synthesis of 2-aminoimidazole core (in blue in the *scheme 2.2.4.*) in very simple way. Many biological compounds and marine alkaloids like 3-amino1-(2-aminoimidazol-4-yl)-prop-1-ene, girolline, and oroidin possess this type of scaffold.[235] In fact, for example, antitumor properties *in vitro* and *in vivo* of girolline (see *figure 2.2.2.*) are known by scientific community and moreover, this compound has shown prominent antimalarial properties against *P. falciparum in vitro* with a IC_{50} ranging from 77 to 215 nM, showing also good activity *in vivo*.[236] In addition, oroidin and clathrodin (natural analogue of oroidin) were studied as antimicrobials agents, Zidar *et al.* have rescontred that clathorodin possess almost no antibacterial and low antifugial activity, while orodin has shown good antibacterial activity against Gram-positive *S.Aureus* (inhibition growth > 90% at 50µM) and *E.Faecalis* (inhibition growth > 50% at 50µM) but it has not shown acaivity aginst Gram-negative *E.Coli* and fungial *C.Albicans*.[237]

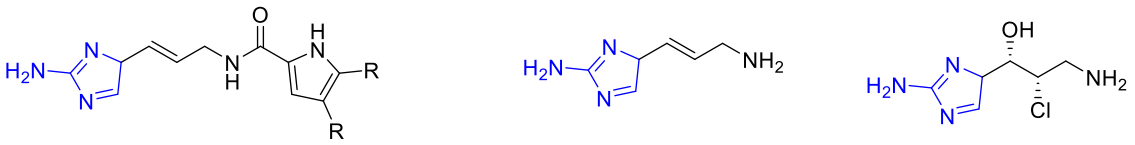


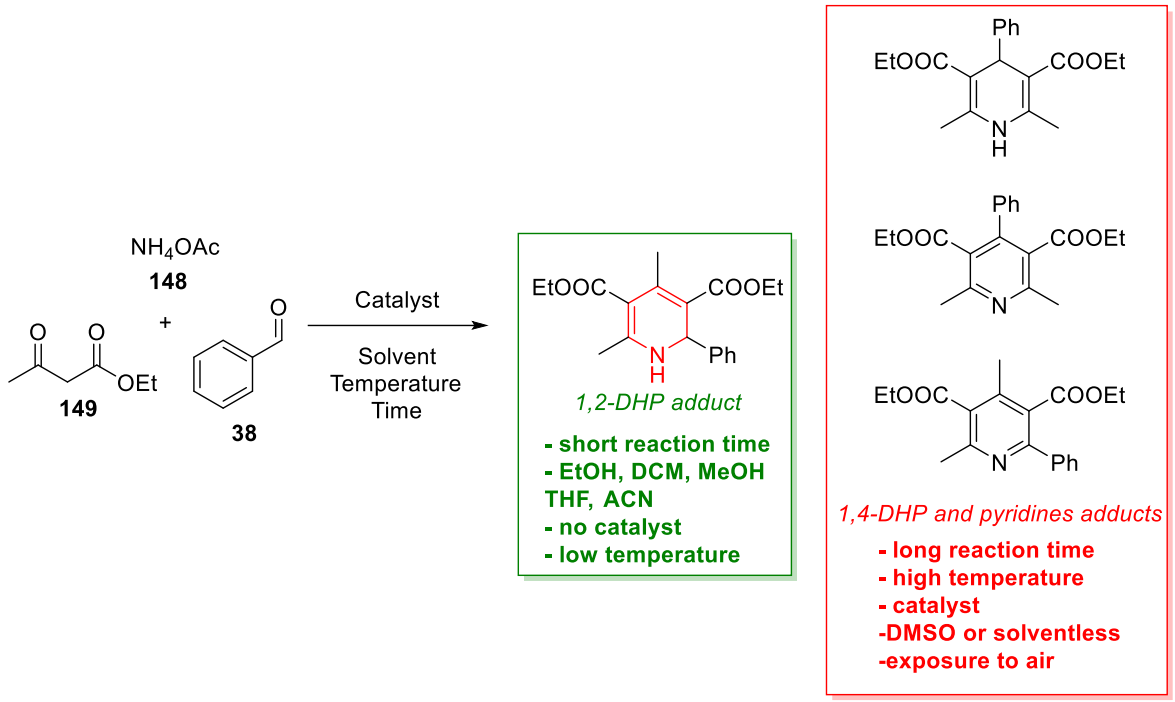
Figure 2.2.2. Some natural alkaloids with 2-aminoimidazole scaffold highlighted in blue

2.2b Synthesis of 1,2-dihydropyridine scaffolds starting from acyclic precursors

Due to the important features covered by 1,2-DHPs in synthetic field of bioactive molecules, they constitute an important target of organic synthesis. The first reported synthesis for dihydropyridine core was reported by Arthur Hantzsch in 1882, but his method provides the formation of symmetrical substituted 1,4-DHP through the reaction between a β-ketoester, an aldehyde (formaldehyde) and ammonia/ammonium acetate as source of nitrogen.[238] However, nowadays rivisations of Hantzsch's method and other methodics are reported in literature apart the direct reduction or nucleophilic addition to pyridine and pyridinum salts,[239] previously cited in the first section of this focus. Moreover, this section is focused on the synthesis of these heterocycles starting from acyclic precursor.

CONDENSATION

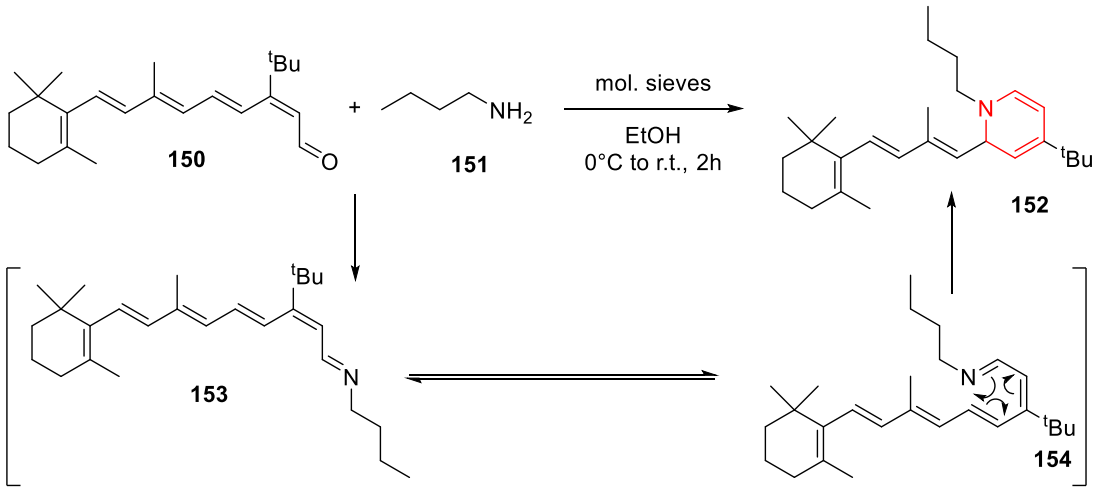
In 2009 Cao and coworkers revisited the Hantzsch's synthesis (benzaldehyde **38**, ammonium acetate **148** as source of nitrogen, 3-keto ethyl butanoate **149**) for dihydropyridine studying discovering that the formation of 1,2-DHP, 1,4-DHP and pyridine adducts was strongly influenced by solvent, temperature, time and presence/absence of the catalyst. Performing the reaction in DMSO or solventless and in presence of catalyst (piperidine, $Yb(PFO)_3$ and $InCl_3 \cdot 4H_2O$) the 1,2-DHP product was not detected, on the other hand its formation is favoured over 1,4-DHPs isomer in solvent like ethanol, dichloromethane, tetrahydrofurane and methanol and performing the reaction at low temperature without lasting the reaction for too much time (oxidation to pyridine). Moreover, these 1,2-DHP adducts are quite unstable since they could oxidize to pyridine-product expose to air for 72h or performing the reaction in AcOH at 20°C.[240]



Scheme 2.2.5. Rivisitation of Hantzsch's Reaction by Shen et al.

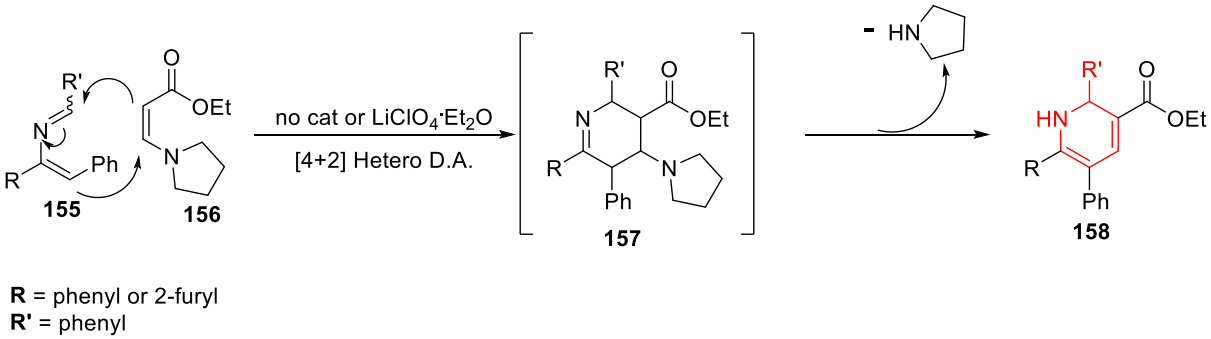
PERICYCLIC REACTION

Pericyclic reaction is one of the most suitable methods for the synthesis of 1,2-dihydropyridines. 1,2-DHPs can be obtained after a 6π-electrocyclization of an azatriene obtained by the previous reaction between a 1,3-dienal compound and a primary amine. Maynard *et al.*[241] reported that after the formation of Schiff base between tert-butyl α,β-cis dienal **150** and butylamine **151** was very fast in mild condition (ethanol at room temperature in absence of any catalyst without observing formation of huge amount of by-products), while the cyclization of triene moiety require harsh conditions (T > 130°C and long reaction time). The determinant step of this reaction is the establishment of equilibrium between the two isomers of imine **153** and **154**, in fact only when the imine assumes this configuration (compound **154**) the 6π-electrocyclization can take place and leading to formation of N-heterocycle core (**152**). Studying in deep several cis-dienals and primary amines, they concluded that the process undergoes to disrotatory cyclization and that electrowithdrawing/donor substituents enhance only in moderate way the reaction, obtaining several 1,2-DHP adducts with a yield ranging 68/83 %.



*Scheme 2.2.6. Synthesis of 1,2-DHP **152** by Maynard et al.*

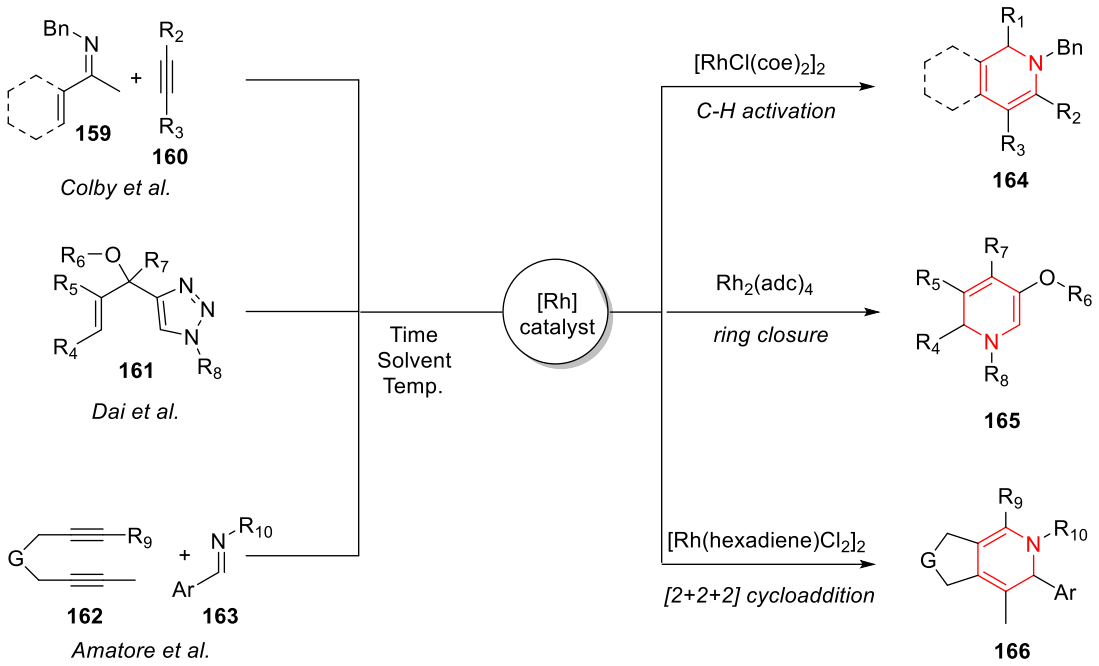
The hetero Diels Alder [4+2] between conjugated imine and a dienophile is another important pathway for the synthesis of 1,2-DHPs, in fact, the reaction between 2-azadienes **155** (phenyl and 2-furyl substituted) and β-enamino ester **156** at elevated temperature (110°C) and after only very long reaction time (> 48h), but in the presence of a catalyst ($LiClO_4 \cdot Et_2O$) the dihydropyridine adducts can be isolated performing the reaction at room tempetaure and with less reaction time.[242]



Scheme 2.2.7. Synthesis of 1,2-DHPs by Palacios et al.

LEWYS ACID CATALYSES

The employment of different catalysts has been also investigated for the synthesis of 1,2-dihydropyridines and nowadays several Lewis Acids are used for this purpose.[243] Rhodium is one of the most employed catalyst for the synthesis of this core by several and different methods allowing to synthesize complicated and multi-substituted 1,2-DHPs (*Scheme 2.2.8.*).



Scheme 2.2.8. Different Rh-catalysed methods for the synthesis of 1,2-DHPs

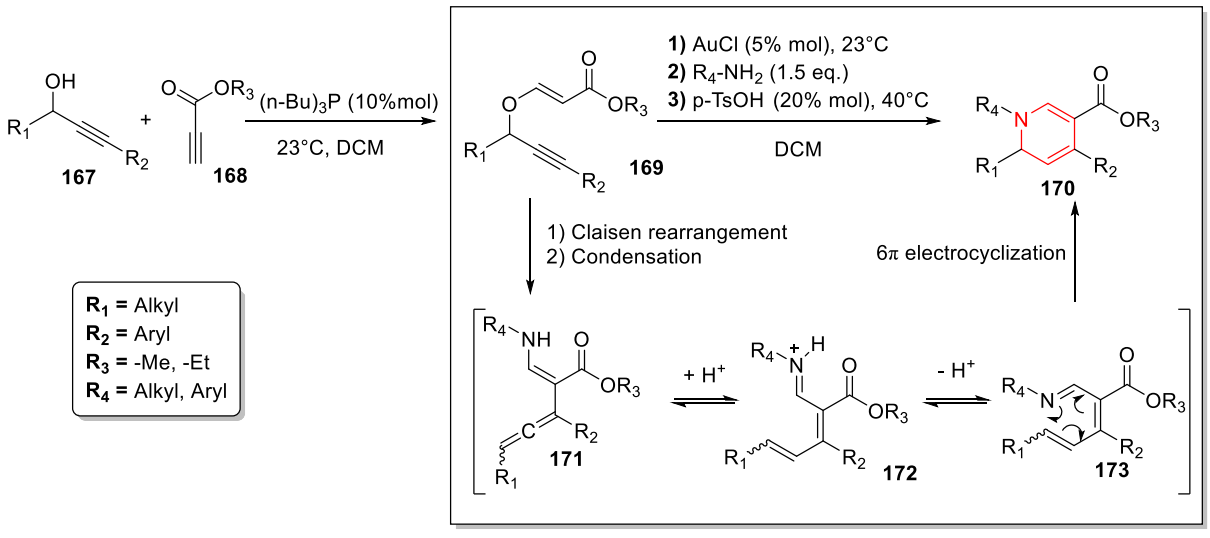
One of the major advantage of Rh-catalysed reaction is the loading of catalyst itself, in fact, Colby *et al.*[244] reported a one pot synthesis in which they synthesized several 1,2-dihydropyridines (**164**) through the reaction between α,β-unsaturated imines (**159**) and alkynes (**160**) in the presence of only 2.5% mol of $[RhCl(coe)_2]_2$ (with coe = cycloctene) and a phosphine as co-catalyst. Performing the reaction at 100°C in toluene many dihydropyridines product **164** (yield ranging from 50% to 99 %) were obtained after short reaction time (2-8h). Only for ester-substituted alkyne 24hrs of reaction were needed to complete the process, but the method was suitable for many α,β,γ-substituted conjugated imines and aryl/alkane/silane substituted alkynes. In accordance to authors, the mechanism proceeds with a complexation of imine by the Rh-catalyst, then after complexation of triple bond in the alkyne by Rh-catalyst, an aza-triene is formed and then a 6π-electrocylization leads to the formation of 1,2-dihydropyridines target.

Due to the importance biological features of N-heterocyles, in 2017 Dai *et al.*[245] developed a Rh(II)-catalysed method in which the 1,2-dihydropyridine (**165**) was obtained starting from a triazole derivatives (**161**) in which after the formation of α-iminodiazo compound and the migration of ester moiety ($-OR_6$ = ester, in *scheme 2.2.8.*), leads to the formation of an aza-triene and then, the heterocyclic product is formed after a 6π-electrocylization. The most important aspect of this method is the presence of Rh(II) catalyst (in this method $Rh_2(adc)_4$) since without it, the process does not take place and 1,2-DHP adduct is not isolated (key step

for the substitution of diaza-moiety and migration of ester moiety). In addition, the method requires mild conditions (50°C, toluene and short time reaction) and it is suitable for different triazole substrates (R_8 = different sulfonate derivatives), ester moieties and R_4 substitutents. (see *Scheme 2.2.8.*, yield ranging from 37% to 54%).

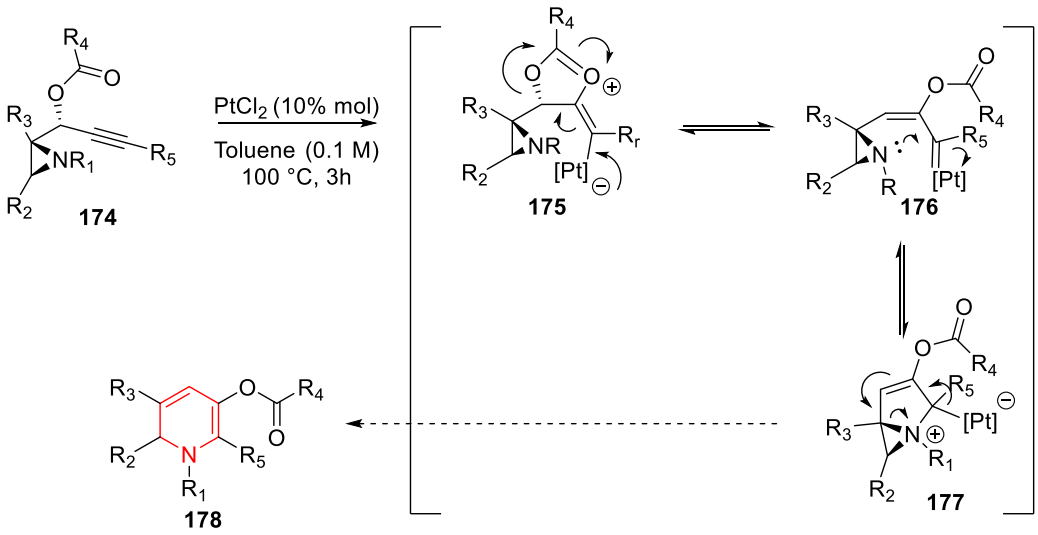
The [2+2+2] Rh-catalysed cycloaddition has shown a powerful way for the synthesis of condensated cycle in which one cycle was constituted by a 1,2-DHP moiety. Amatore *et al.*[246] published a paper, in 2013, in which they synthesized several fused-1,2-DHPs **166** between particular diynes **162** and sulfonimines **163**, performing the reaction in mild conditions (r.t., DCE as solvent, 3hrs) obtaining good yields and good enantioselectivity. The catalyst-system is constituted by $[Rh(hexadiene)_2Cl]_2$, a silver salt ($AgSbF_6$) and a phosphate ligand ((R)-Tol-Binap) allow to obtain an azatriene after a β^H-elimination of a 7-cycle formed by sequential Rh-pyrrole (formed in the first step of mechanism) and complexation of sulfonimine by Rhodium metal centre. The method shows wide scope with many different substrates.

In 2011, Harshneck *et al.*[247] reported an elegant one-pot method for the synthesis of 1,2-dihydropyridines (**170**) (*scheme 2.2.9.*) starting from propargyl vinyl ethers (obtained from the reaction between propargyl alcohol and propargyl ester), which, after a Au(I)-catalysed Claisen-rearrangement and a successive condensation with a primary amine leads to the formation of conjugated enamine **171** (1 hour for rearrangement and 30 minutes for condensation at r.t.), that after a protonation by p-TSA leads to the formation of imonium-intermediate **172** and the final product is obtained after a rearrangement and a 6π-electrocylization (15 hrs at 40°C). The conditions has shown wide suitability for many different ether and primary amine derivatives (yields ranging from 55 to 89%).



Scheme 2.2.9. One-Pot Au(I)-catalysed for the synthesis of 1,2-DHPs developed by Harshneck et al.

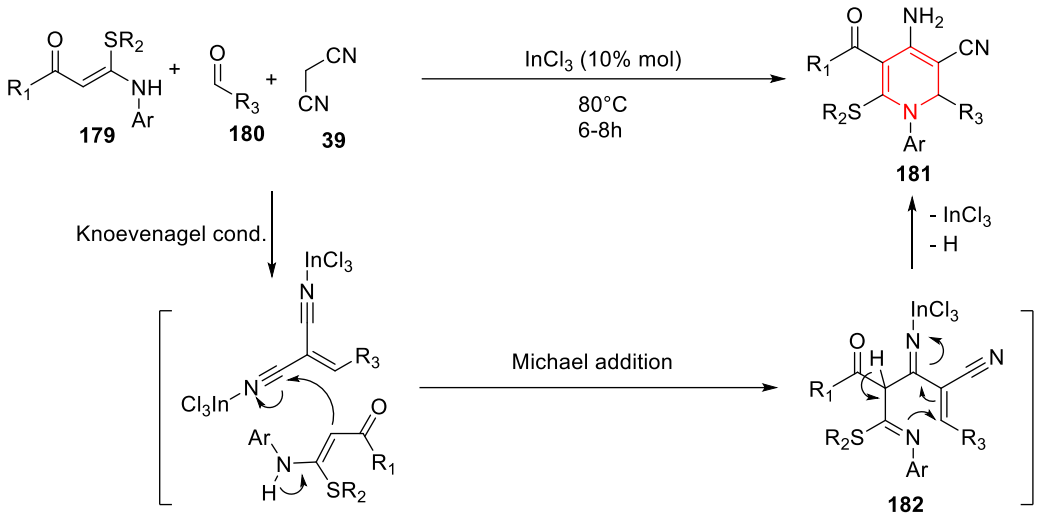
Pt(II) catalysed-cycloisomerization of aziridinyl propargylic esters **174** has shown a powerfull pathway for the synthesis of 1,2-dihydropyridine products (**178**), also in enantioselective way performing the reaction in toluene at 100°C. In this method the target heterocycles **178** is obtained through a 5-exo-dig of ester moiety **174** that leads to the formation of zwitterionic compound **175**, the subsequent rearrangement could afford the metallocarbenoid **176**. The nucleophilic attack of nitrogen to metallocarbenoid functionality allows the formation of byciclic intermediate **177**, which, after the rearrangement, it is transformed to the final product **178** (*scheme 2.2.10.*) in moderate up to good yield with different aziridinyl compounds.[248]



Scheme 2.2.10.Pt(II)-catalysed cycloisomerization reported by Motamed et al.

MUTLTICOMPONENT REACTION

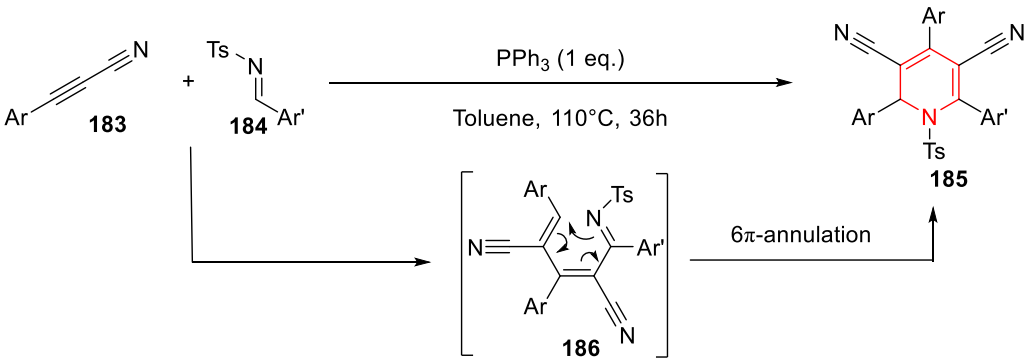
$InCl_3$ has shown as powerful catalyst for the synthesis of 1,2-DHPs through a Multi Component Reaction (minimization time, waste, energy, cost). Common Lewis Acids like $FeCl_3$, $AlCl_3$, $InBr_3$, $Sc(OTf)_3$ and $Y(OTf)_3$ catalysed in minor efficient way the reaction between aldehydes **180**, malonitriles **39** and α-oxoketene-N,S-arylaminoacetals **181** in a solventless process performed at 80°C, in which the final product is obtained after a first Knoevenagel condensation between aldehyde and malonitrile, then a Michael addition, assisted by $InCl_3$, provides the intermediate **182**, that undergoes to an intramolecular cyclization leading to the formation of final dihydropyridine target **181** (*scheme 2.2.11.*). Performing the reaction in solvent media the process stop at Knoevenagel step and it does not go further.[249]



Scheme 2.2.11. MCR for the synthesis of 1,2-DHPs-Indium catalysed with mechanism in accordance to authors.

PHOSPHINE CATALYSED ANNULATION

Nucleophile tertiary phosphine have shown a useful promoter for several annulation reactions for alkynes or allenes to obtain many different multisubstituted cyclic products, for this reason Zhang *et al.*[250] have exploited the possibility to used triphenylphosphine as promoter for the synthesis of 1,2-dihydropyridines thorugh a [2+2+2] annulation between N-tosylimines **184** and cyanoacetylynes **183**. In their work, Zhang and his coworkers have tested many different tertiary amines, but triphenylphosphine has shown the most suitable for the process, since electrodonating/withdrawing substituents on phenyl ring or alkyl side chain of phosphines have not promoted as satisfying as Ph_3P. Otherwise, performing the reaction in toluene at 110°C in presence of 1.0 eq. of phosphine, they were able to isolate several 1,2-DHPs fully substituted as the method is suitable for many substrates. In accordance to authors, there is not clear evidence for the real pathway of the process, but they assumed that the intermediate **186** is formed after a complicated cascade process (that provides a nucleophilic attack of triphenylphosphine to cyanoacetylene and a subsequent attack by imine, and two consecutive proton trasnfers ([1,2]-H-transfer and [1,6]-H transfer and an other successive attack by triphenylphosphine), then, the 6π-annulation of **186** to the formation of the target heterocycle **185**.



Scheme 2.2.12. Annulation of Phosphine-Catalysed-Annulation reported by Zhang et al. with proposed intermediate

2.2c $CeCl_3$ in organic synthesis

Catalysis has always played a very important role in the field of organic synthesis. In this sense, Lewis Acids have always received great attention for their ability to accelerate the reaction and reduce the formation of secondary products. Among them, the lanthanides have long considered as the "forgotten elements" of periodic table, but they gained grothwing interest during the last decades and nowadays they found several applications and they represent as ideal promoters for many organic transformations. Cerium is one of the most abundant Lanthanides and Ce(III) and Ce(IV) are the most stable oxidation states.[251]

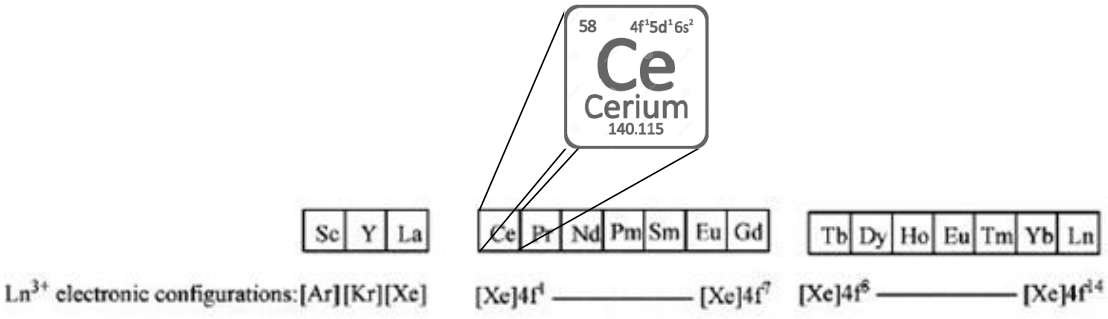


Figure 2.2.3. Cerium Element in Lanthanides Period.

Even if the Lanthanides are so called "rare earths", Cerium is more abundant than more "famous" metals like cobalt, zinc and tin. More over, $CeCl_3 \cdot 7H_2O$ is the most adbundant of Ce(III) salts and it represents a very "friendly" alternative due to the low cost, moisture/air/water tolerant, easy handling, recoverability from water, and very low toxicity (toxicity similar to sodium chloride). $CeCl_3$ is considered a "hard Lewis Acid" by Pearson's HSAB and it shows strong affinity toward "hard base" like oxygen and nitrogen. Even if Ce(III)

is considered as an hard Lewis acid, $CeCl_3$ is considered as a mild lewis acid because of its ratio between charge density and atomic radius.[252]

Salt	LD_{50} (mg/Kg)
$AlCl_3$	3450
$PtCl_2$	3423
NaCl	3000
$CeCl_3 \cdot 7H_2O$	2800
$InCl_3$	>2000
$RhCl_3$	1302
$FeCl_3$	1300
$ZnCl_2$	1100
$SnCl_2 \cdot 2H_2O$	700
$CuCl_2$	584
$PtCl_4$	276

Table 2.2.1. Relative toxicity of some common chloride salts (Safe Data Sheet of Merck-Sigma Adrich, data relative to female mice).

Despite its mildness, $CeCl_3 \cdot 7H_2O$ found, nowadays, many applications in several aspect of organic synthesis and it is used as catalyst/promoter for many reactions. Among the possible applications, it can be used to build new carbon-carbon and carbon-heteroatom bond, it can mediate the reduction of carbonyl and phospho moiety thanks to its "Lewis hardness", mediate addition to carbon-carbon/carbon-oxygen/carbon-nitrogen multiple bond, usefull to form organocerium compounds.[251] To avoid boring the reader too much, here only few examples are reported just to help to understand the usefulness of this "friendly" salt.

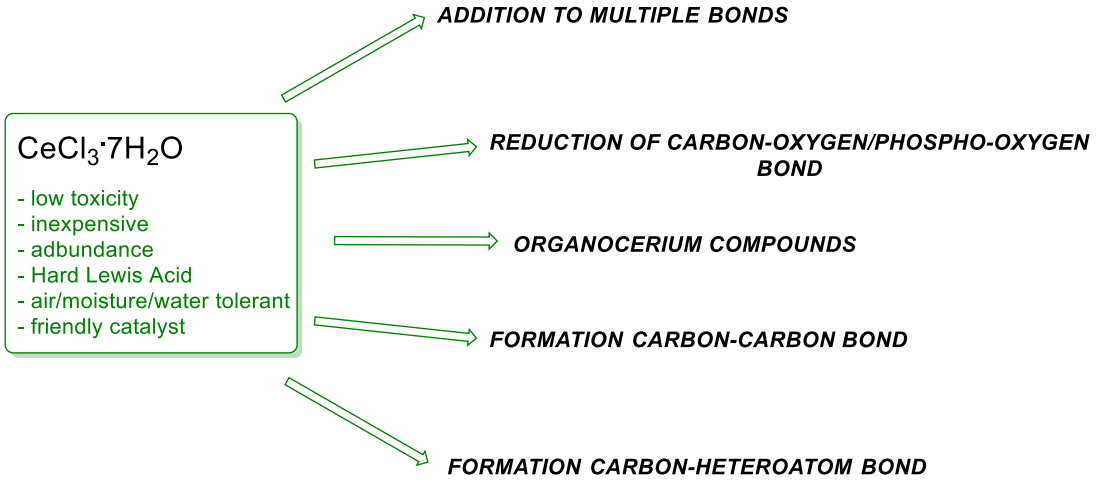
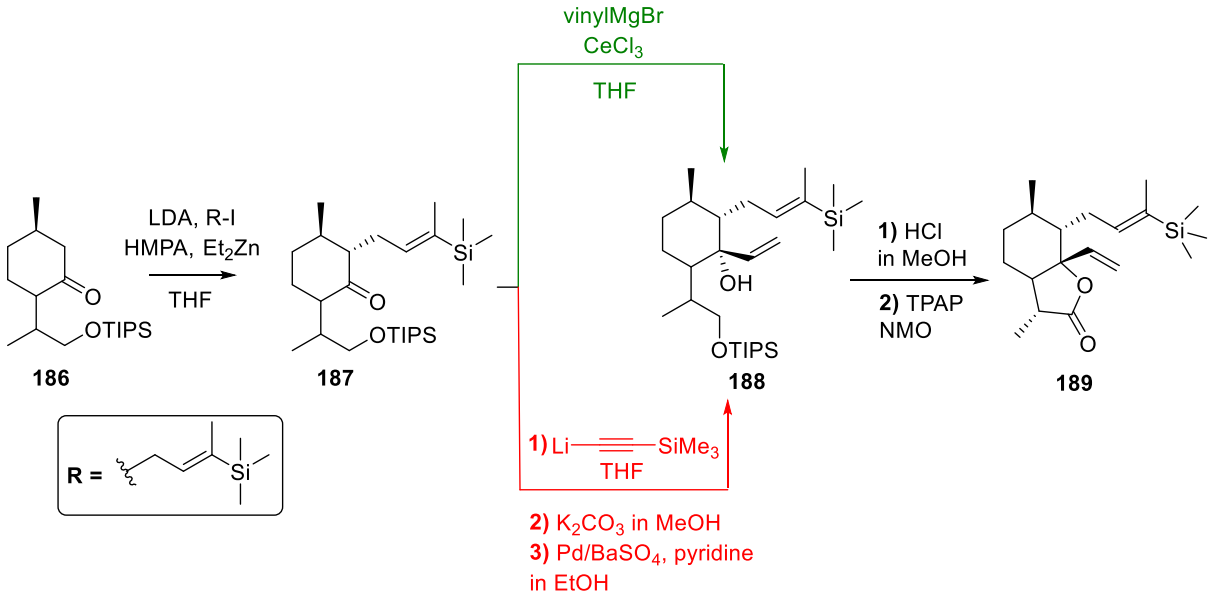


Figure 2.2.4. "Friendly aspects" of $CeCl_3 \cdot 7H_2O$ and some of its applications in organic synthesis

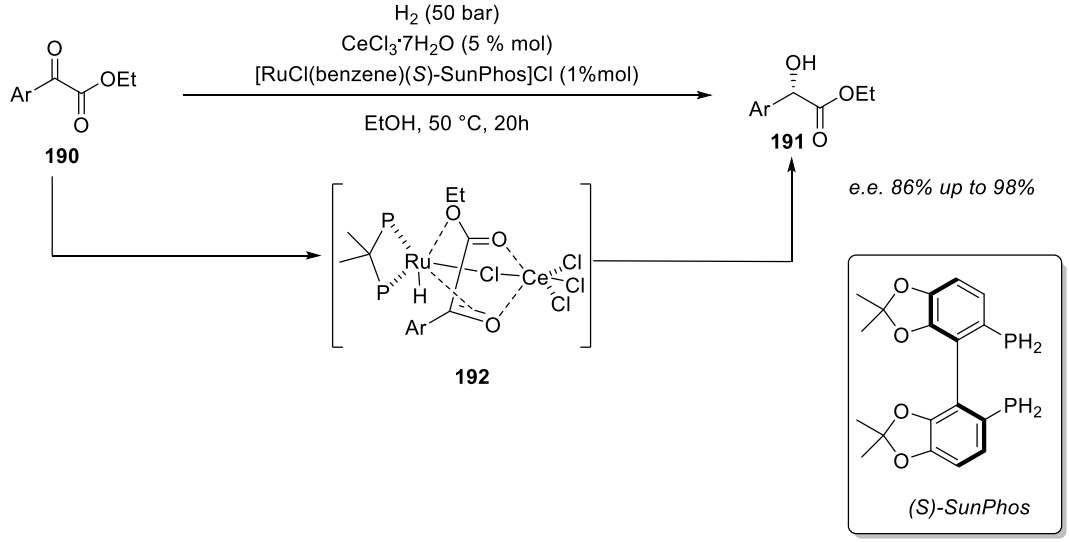
The building up of new carbon-carbon bonds has always been one of most important target of organic synthesis, and the addition of organo-metal compounds to carbonyl moieties is one of viable pathways for this scope. Considering these aspects, the employment of organocerium addition to carbon-oxygen double bond proceeds more smoothly respect to its corrispective organolithium or organomagnesium.[251] In this sense, the organocerium formed from vinyilmagnesium bromide and $CeCl_3$ *dry* (each two freshly prepared) allows to overcome some problems related to the addition of simple Grignard for the synthesis of (+)-dihydro-*epi*-deoxyarteannuin B **189**. This last one is a natural compound extracted from *Artemisia annua* and it has been investigated as starting material for the synthesis of Artemisin (potent anti malaria). Based on Noyori's zincate alkylation of **186** for the synthesis of intermediate **187,** Dudley *et al.*[253] utilized the organocerium to overcome the steric congestion of intermediate and avoid a tedious three steps pathway (which includes an addition of organolithium, deprotection and reduction) increasing the overall yield from **187** to **188** up to 91% from 85%.



*Scheme 2.2.13. Usefulness of Organocerium in synthesis of (+)-dihydro-epi-deoxyarteannuin B **189.***

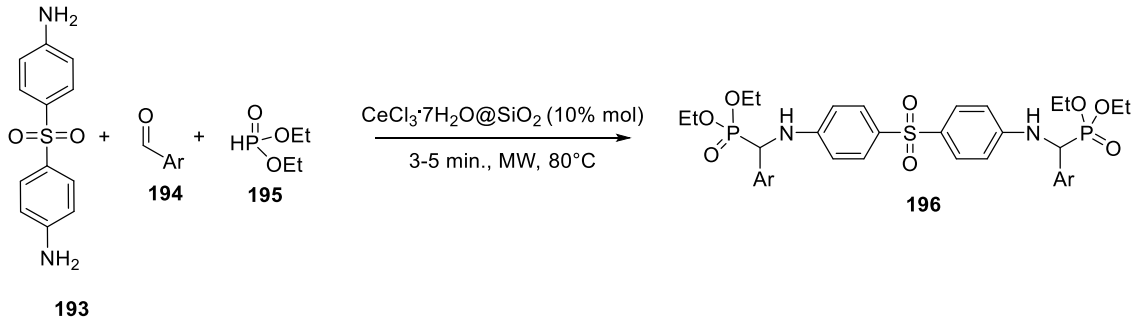
Development of enantioselective reduction is an important pathway for the synthesis of α-hydroxy acids and derivatives since this moieties are widely distributed in various biological compounds and they can be used in many stereoselective reactions.[254] The synergistic system $CeCl_3 \cdot 7H_2O$/[RuCl(benzene)(*S*)-SunPhos]Cl (with (*S*)-SunPhos as chiral auxilary) has shown an efficient catalyst for the enantioselective reduction of α-hydroxy-α-arylacetates **190**. The addition of Ce(III) salt not only enhances the enantioselectivity of the process (e.e. from 86% up to ~98% with several substrates) but it also stabilyzes the [Ru] catalyst. In accordance to authors, the cerium salts coordinate the carbonyl moieties (both the carbonyl of the ketone and

the ester, intermediate **192**) by a σ-coordination, making the carbon more susceptable to nuchleophilic attack by the hydride. Moreover this coordination by Ce(III) alterates the geometry coordination of the [Ru] salt from a σ-type to π-type leading to a very rigid transition state enhancing the enantioselective hydrogen transfer.[255]



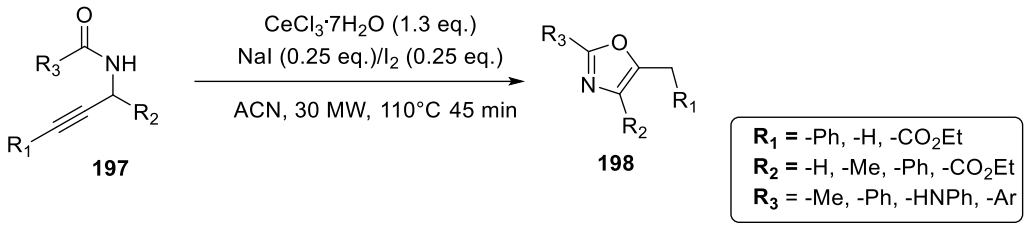
Scheme 2.2.14. Enantioselective reduction promoted by$CeCl_3{\cdot}7H_2O$/[Ru] and reported by Meng et al.

Supported Lewis acids constitutes a class of catalysts/promoters that represent an important friendly alternative to classical system catalyses. Supporting the $CeCl_3{\cdot}7H_2O$ on SiO_2 gel (it is possible to support it on Al_2O_3)[256] has provided as an efficient heterogenous catalyst for a-diaminophosphonate derivatives **196** (potent antifungial compounds) by one pot reaction between amine **193**, aldehydes **194** and diethylphosphate **195** under microwave irradiation. Moreover, the catalyst can be recovered and recyclated. From the studies of Divenevi and his coworkers it emerges that the SiO_2 supported-$CeCl_3{\cdot}7H_2O$ has been the most suitable catalyst in neat and both in batch (7 hrs reaction) and microwave conditions (5 minutes reaction), the system has shown more activity than common Lewis Acid like $FeCl_3$, $AlCl_3$, $ZnCl_2$ and more efficient respect to not-supported Ce(III) salt in ethanol media.[257]



*Scheme 2.2.15. One pot synthesis of a-diaminophosphonate derivatives **196** $CeCl_3·7H_2O@SiO_2$ reported by Dinenevi et al.*

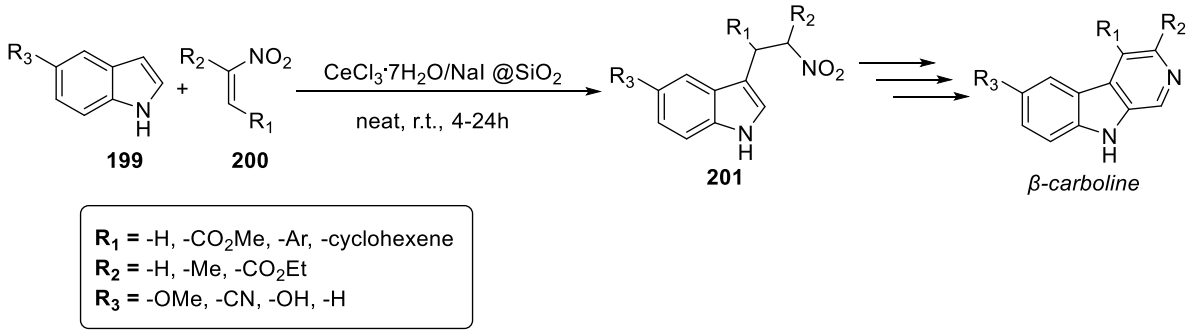
Other Ce(III) halides salt like $CeBr_3$ or CeI_3 show a lower activity respect to the chloride salt, but the combination of a iodide source like NaI ($CeCl_3·7H_2O/MI_n$ systems have shown almost same activity) enhances the electrophilicity and the activity of Cerium(III) by breaks out of dimer of crystalline structure of $CeCl_3·7H_2O$, but X-ray studies demonstrated that there are not direct interactions between iodide and cerium core.[251] This system was also exploited in prof. Enrico Marcantoni's laboratory, in fact, the combination of $CeCl_3·7H_2O/NaI/I_2$ has demonstrated a powerful catalityc system for the synthesis of heterocycle, in this case oxazoles **198** strarting from different propargylamides **197** (*Scheme 2.2.16*), under microwave irradiations with high yields and low reaction time. Moreover, this method allows to overcome some problems related to employment of expensive like Pd(0) and Au(III) and to enlarge the substrates scope and also enhancing the yield respect to aluminium catalyses. The presence of Cerium salt is essential since performing the reaction without it a very poor yield is obtained, in the same way, the presence of iodine is crucial since enhances the yield up to 95%.[258]



Scheme 2.2.16. Microwaved-Ce(III)/NaI/I_2 assisted synthesis of oxazoles reported by Marcantoni et al.

$CeCl_3·7H_2O$/NaI system supported on SiO_2 has proven to be an efficient and friendly catalyst for Michael addition for the funcionalization of heterocycles like indoles **199**. The reaction between indoles **199** and michael acceptors, like α,β-substituted nitroalkenes **200**, in presence of $CeCl_3·7H_2O$/NaI system supported on SiO_2, has provided the synthesis of many 2-substituted indole derivatives in neat condition at room temperature. The most important aspect of this

method is the potential synthesis of tryptamine precursor in friendly and easy way, which, after further transformation in tryptamine derivatives and leading to the possibility to synthesize different β-carbolines that are bioactive-molecules wide diffused in nature.[259]



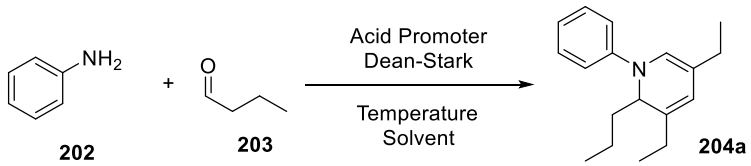
Scheme 2.2.17. Synthesis of 2-substituted indoles Ce(III)/NaI supported on SiO_2 reported by Marcantoni et al.

2.2d Results and Discussion

As highlighted above, the methods shown in literature for the synthesis of 1,2-dihydropyridines by cyclization reaction of acyclic precursors require sometimes expensive and water sensible catalysts like rhodium, gold and long time reaction. For these reasons, we focused our effort to synthesize the 2,3,5-trisubstituted 1,2-dihydropyridines by the polycondensation between primary amines and alkyl aldehydes.

PREVIOUS WORK

Co-workers of prof. Enrico Marcatoni have already faced with this type of reaction and here the most significant and useful results for this work are reported. At the beginning aniline **202** and butyraldehyde **203** were used as pilot agents and performing the reaction in solvent like toluene or hexane. These preliminary studies were focused to figure out and confirm the final structure of the product of this process.



202 **203** **204a**

ENTRY[a]	Aniline	Butyraldehyde	Acid Promoter	Solvent	GC-Conv.
1	10 mmol	50 mmol	/	Toluene (0.3 M)	2.4%
2	10 mmol	50 mmol	pTSA·H_2O (7.5 mmol)	Toluene (0.3 M)	< 1%
3	10 mmol	100 mmol	/	Toluene (0.3 M)	5.3%
4	10 mmol	50 mmol	AcOH 37% (10 mmol)	Toluene (1.0 M)	49.8 %
5	10 mmol	50 mmol	AcOH 37% (10 mmol)	Hexane (1.0 M)	28.4%
6	**10 mmol**	**50 mmol**	**AcOH 37% (10 mmol)**	**Toluene (1.0 M)**	**61.8%**

Table 2.2.2. Condensation performed in Dean Stark apparatus. [a]Reactions last 24h

The *table 2.2.2.* shows the importance of acid catalyses since performing the reaction without it the product is formed in very low conversion (Entry 1 and 3, *table 2.2.2.*). Moreover also the type of the solvent and the concentration are impornt for the good outcome of the reaction, the best result has obtained performing the reaction in toluene (1.0 M) in presence of stochoimetric amount of acetic acid with an excess of aldehyde (Entry 6, *table 2.2.2.*). Also supported acids like Hydrotalcite, Montmorillonite, HSZ-30, Al_2O_3, Al_2OSO_3H have been tested, but in these cases bad result and conversion lower than 10% were observed (results not reported in the table).

From the GC-MS analyses it emerges the presence of two signals with 255 m/z, molecular weight of 1,2-dihydropyridine **204a**, possible related to three structural isomers (*figure 2.2.3.*).

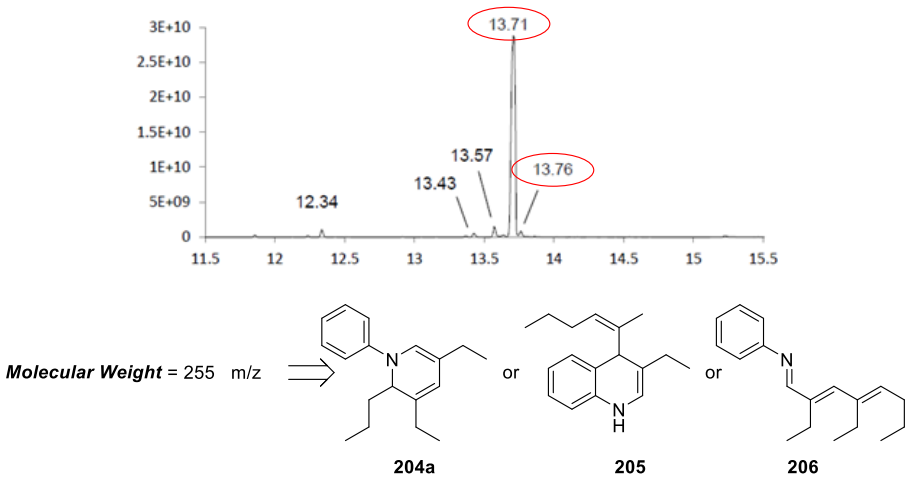


Figure 2.2.5. Cromathogram of crude reaction

From the systematic studied of fragment patterns (*figure 2.2.4.*) it suddenly emerges that the two peaks are related two structurally different compounds since these two signals posses two very different base peaks and fragmentation signals.

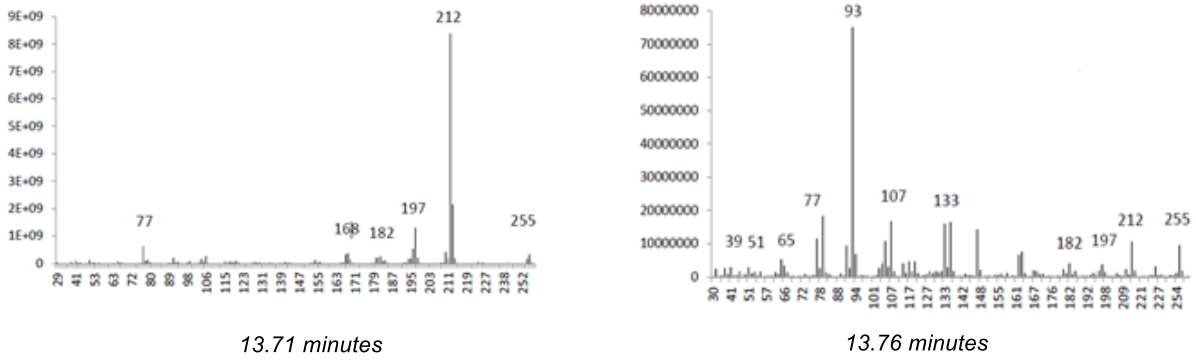


Figure 2.2.6. Fragmantation patterns of peak at 13.71 minutes and 13.76 minutes.

The so high abundance of 93 m/z peak in signal at 13.76 minutes it can be explained by formation of azepinium ion, analogue of trypolinium ion, and it can be formed only from linear azatriene **206**, since the nitrogen atom "is free" to rearrange, while in other cyclic isomers this process cannot takes place. On the other hand, the base peak 212 m/z it can be explained only by the formation of a very stable ion. This ion can be formed in a stable way in all three isomers, since each three molecules can loose a propyl and high conjugated cation can be formed from each molecule.

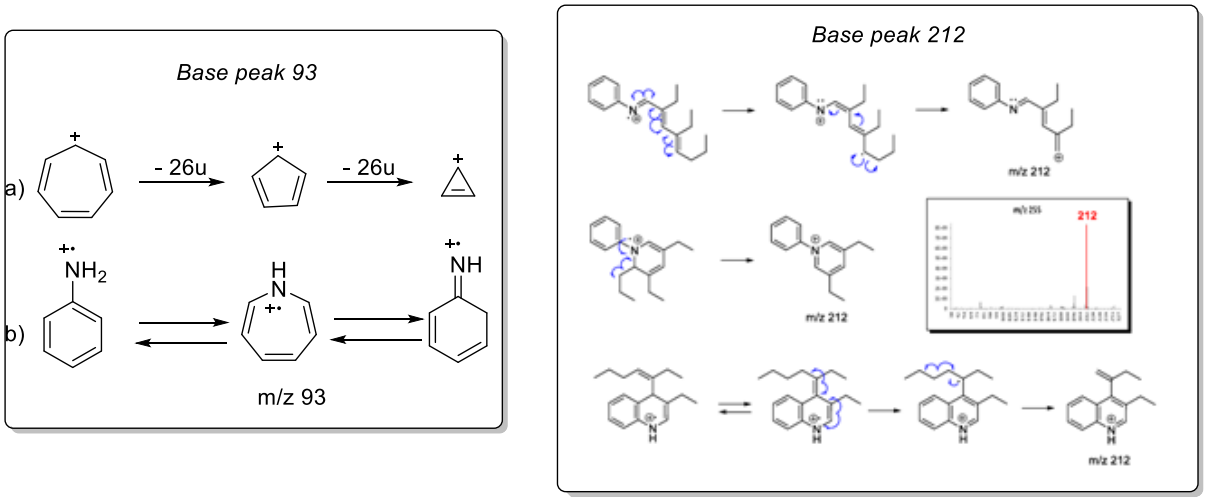
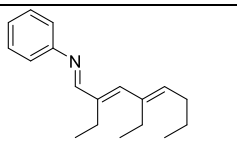


Figure 2.2.7. Explanation of two base peaks 93 m/z (left) and 212 m/z (right)

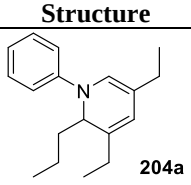
The final assignment of two peaks was done by deeper analyses due to the different possibile structure that come out from these two base peaks. The presence of many fragments of peaks at 13.76 minutes suggest a possible linearity of the related compound, more over the presence of azepinium, phenilium ions and fragment related to the looseness of different $–CH_3$ and propryl ion can confirm the linear and branched structure like the compound **206**.

m/z	Ion	Lose	Structure
255	M^+	1 e^-	
212	M-43	-Pr	
197	M-43-15	-Pr, -Me	
182	M-43-15-15	-Pr, -Me, -Me	
93	Azepinium	-	
77	Phenylium	-	

206

Table 2.2.3. Fragment analyses of peak at 13.76 minutes

The assignment of structure/fragmentation pattern of the peak at 13.71 minutes is not possible only from the explanation of 212 m/z nature, but the presence of phenylium is the crucial fragment as it can only come out from dihydropyridine adduct, since the phenyl moiety is fused with an other cyclic core.

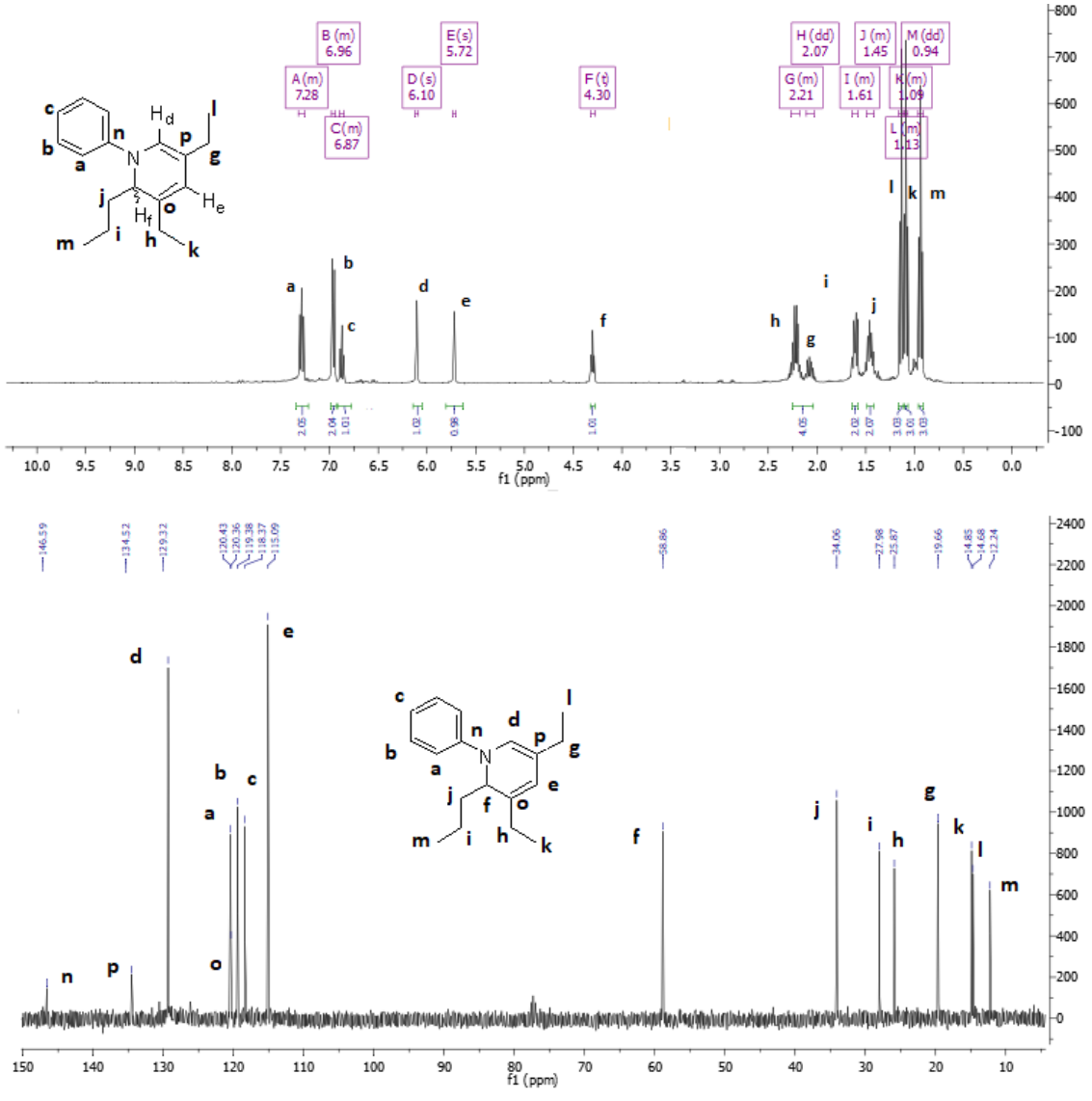
m/z	Ion	Lose	Structure
255	M^+	1 e^-	
212	M-43	-Pr	
197	M-43-15	-Pr, -Me	
182	M-43-15-15	-Pr, -Me, -Me	
168	M-43-29-15	-Pr, -Et, - Me	
77	Phenylium	-	

204a

Table 2.2.4. Fragment analyses of peak at 13.76 minutes

The final structure of the product was also confirmed by NMR-analyses (^1H, ^{13}C and DEPT). From proton spectrum of peak at 13.76 minutes it is possible to exclude the linear compound

206 since no imine proton is present in the spectrum. Moreover, the 1-substitution pattern of aromatic protons can confirm the possible structure of 1,2-dihydropyridine adduct. An other important signals are the two singlets at 6.10 ppm and 5.72 ppm since these signals can only come-out from the proton in structure of 1,2-DHP **204a**. Moreover, from DEPT spectrum can be noticed that the carbon at 58.86 ppm is a –CH (usually chemical shift of carbon near to heteroatom like nitrogen) presents only in **204a**. So, thanks to the NMR analyses we can confirm the real structure of 1,2-dihydropyridine product that come out from the polycondensation: N-phenyl-3,5-diethyl-2-propyl-1,2-dihydropyridine.



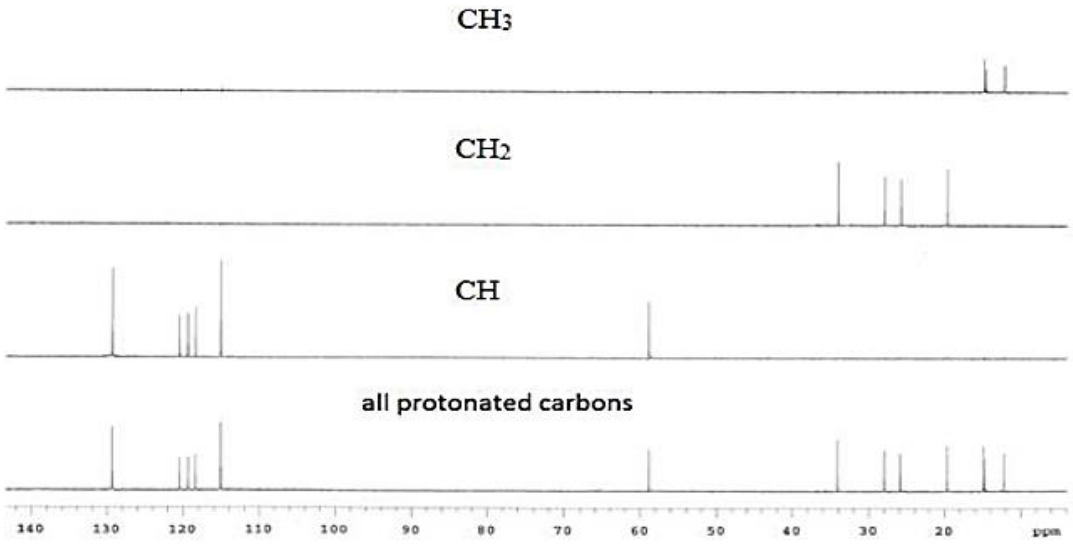
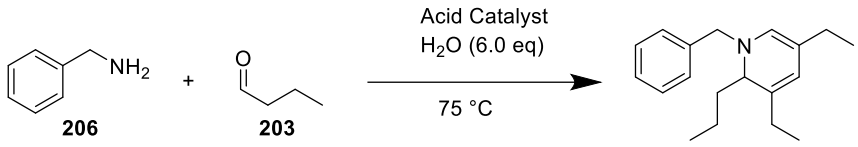


*Figure 2.2.8. [1]H-NMR, [13]C-NMR and DEPT of 1,2-dihydropyridine **205a**.*

CURRENT WORK

Once determinated the 1,2-dihydropyridine structure obtained from this process, we want to restyle and develop a more environmental friendly method for the polycondensation process between primary amines and aldehydes involving $CeCl_3 \cdot 7H_2O$ as acid catalyst. For these studies we have used butyraldehyde as pilot aldehyde and benzylamine **206** as pilot amine since it is less toxic than aniline (relative high toxicity to human liver).[260] At the beginning we investigated different acids, Brønsted and Lewis ones (reported in the *table 2.2.4.*), starting from a solventless protocol in which the acid was present in catalytic amount (2% mol), 6 equivalents of H_2O and 4 equivalents of aldehyde **203**.

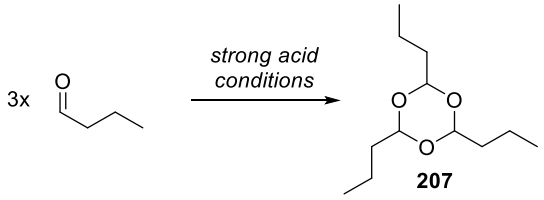


206 1.0 eq + **203** 4.0 eq → **204b**

ENTRY	Acid Catalyst (2% mol)	Time	GC Conversion
1	Acetic Acid 37%	1.5 h	66.89 %
2	**$CeCl_3 \cdot 7H_2O$**	**0.5 h**	**80.93 %**
3	$FeCl_3$	1.0 h	70.65 %
4	$Ce(OTf)_3$	4.0 h	10.78 %
5	$AlCl_3$	6.0 h	48.37 %
6	$Ce(OAc)_3$	3.5 h	64.12 %
7	$Cu(OTf)_2$	4.0 h	20.87 %
8	$CeCl_3 \cdot 7H_2O$/NaI	1.0 h	52.21 %
9	CuI	1.0 h	42.43 %
10	I_2	1.0 h	57.84 %

Table 2.2.5. Screening of Acid Catalysts

Among the tested acids, the $CeCl_3 \cdot 7H_2O$ and $FeCl_3$ have shown as the best promoters for the reaction, in which a conversion of 80.93% and 70.65% were obtained after 0.5 h and 1.0 h of reaction respectively (Entry 2 and 3, *table 2.2.5.*). The counter ion plays an important role in the good coming out of process. In fact, performing the reaction in presence of different cerium and copper salts very different results have obtained. A very low conversion of the 1,2-DHP product was obtained in presence of cerium (Entry 4, *table 2.2.5.*) and copper triflate (Entry 7, *table 2.2.5.*), this fact can be explained by the formation in situ of triflic acid, due to the presence of water,[261a], [261b] that favourites the formation of cyclic trioxane **207**, a typical subproduct of linear and alkylic aldehyde in too strong acid conditions.[262]



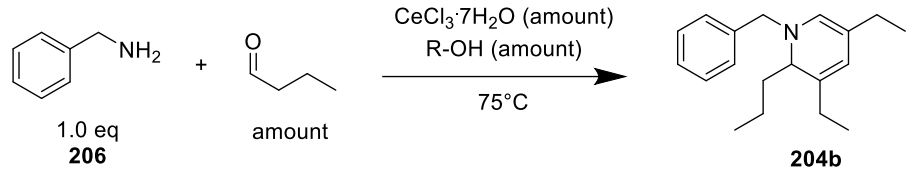
207

Scheme 2.2.18. Formation of trioxane in strong acid conditions

Since the presence of an iodide source enhances the Ce(III) chloride activity by breaking the crystalline of dimer of cerium salt, we performed a reaction in which $CeCl_3 \cdot 7H_2O$/NaI were presence in equal amount, but also in this case a lower conversion was detected at GC even if with a longer time reaction (1.0 h). Final, using other lewis acids such as $AlCl_3$, CuI, I_2 and $Ce(OAc)_3$ lower conversion were obtained, especially in presence of Al(III) chloride (Entry 5,

table 2.2.5.) the reaction was very slow since the maximum conversion was obtained only after 6 hrs. On the other hand $Ce(OAc)_3$ and I_2 are good alternative to Acetic acid as similar conversion were obtained in each three cases (Entry 6, 10 and 1 respectively, *table 2.2.5.*). Other Brønsted acids have been not tested since too strong acid could lead to the formation of trioxane of butyraldehyde (*scheme 2.2.18*). In each experiment, an increase of product conversion was observed until to reach a plateau and a slow degradation and formation of other condensation product was observed.

The $CeCl_3 \cdot 7H_2O$ has shown the best catalyst for the process. So starting from this point we performed the optimization of reaction conditions. All experiments were reported in the table below. For each experiment were reported the maximum conversion and the time at which it was reached.



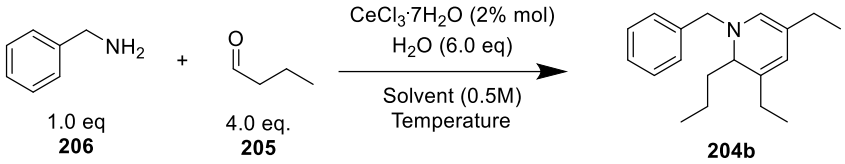
ENTRY	Butyrald.	Acid Cat.	R-OH	Time	GC Conv.
1	**4.0 eq**	**$CeCl_3 \cdot 7H_2O$ (2% mol)**	**H_2O (6 eq.)**	**0.5 h**	**80.93 %**
2	4.0 eq	$CeCl_3 \cdot 7H_2O$ (5% mol)	H_2O (6 eq.)	3.0 h	43.45 %
3	4.0 eq	$CeCl_3 \cdot 7H_2O$ (10% mol)	H_2O (6 eq.)	2.0 h	54.55 %
4	4.0 eq		H_2O (6 eq.)	1.5 h	49.19 %
5	3.0 eq	$CeCl_3 \cdot 7H_2O$ (2% mol)	H_2O (6 eq.)	0.5 h	66.76 %
6	4.0 eq	$CeCl_3$ *dry* (2% mol)	H_2O (6 eq.)	2.0 h	65.65 %
7	4.0 eq	$CeCl_3 \cdot 7H_2O$ (2% mol)		1.0 h	66.24 %
8[a]	4.0 eq	$CeCl_3 \cdot 7H_2O$ (2% mol)	H_2O (6 eq.)	1.0 h	56.92 %
9	4.0 eq			1.5 h	58.74 %
10[b]	4.0 eq	$CeCl_3 \cdot 7H_2O$ (2% mol)	H_2O (6 eq.)	0.5 h	5.71 %
11	4.0 eq	$CeCl_3 \cdot 7H_2O$ (2% mol)	MeOH (6 eq.)	3.0 h	56.55 %

Table 2.2.6. Optimization of reaction conditions. [a]Reaction performed at 50C°; [b]Reaction performed with hydrochloride benzylamine

First of all we have tried to employ different amounts of Cerium salt, but increasing the amount has corrisponded to a decrease in conversion and the maximum of conversion was detected after longer reaction time. Entry 4 of *table 2.2.6.* confirms that the presence of the catalyst was necessary for speeding up the reaction since the maximum was reached after 1.5 h of reaction, even if a good but lower detection was observed (at least ~49%). Moreover, also the little excess of aldehyde is necessary for the good trend of process, since, for the Le-Chatelier principle, the process is less favoured with a stoichiometric amount of aldehyde (Entry 5, *table 2.2.6.*) as all the intermediate species are in equilibrium. Performing the reaction at 50 °C makes reaction

slower and less effective (Entry 8, *table 2.2.6.*). Thinking that the crystal water of Ce(III) could be a problem, we have also tested $CeCl_3$ *dry* obtaining in this case also a good conversion (about 66.76%) but the reaction also is slower. Also the role of water is a complicated question since its presence could decrease the conversion and favoures the back formation of starting material, but it is reported that water could promoted also the intramolecular Diels-Alder,[263] in fact, performing the reaction without H_2O a lower GC-conversion. The direct reaction between amine and butyraldehyde leads to a good conversion of the product after 1.5h reaction (Entry 9, *table 2.2.6.*) it confirms another time the importance co-presence of catalyst and water. Finally, we tested hydrochloride salt of benzylamine to avoid the formation of side product due to basic nature of amines, but substantially not formation of the product was detected. Finally, using methanol instead water a decrease of the speed and conversion of the reaction were detected (Entry 11, *table 2.2.6.*).

Then we have studied how the solvent influence the process, testing some quite different solvents and controlling after 30 minutes of reaction the conversion of the product. Each experiment were conducted with the best conditions of Entry 1 of *table 2.2.6* with a concentration of 0.5 M for the reaction conducted in solvent.

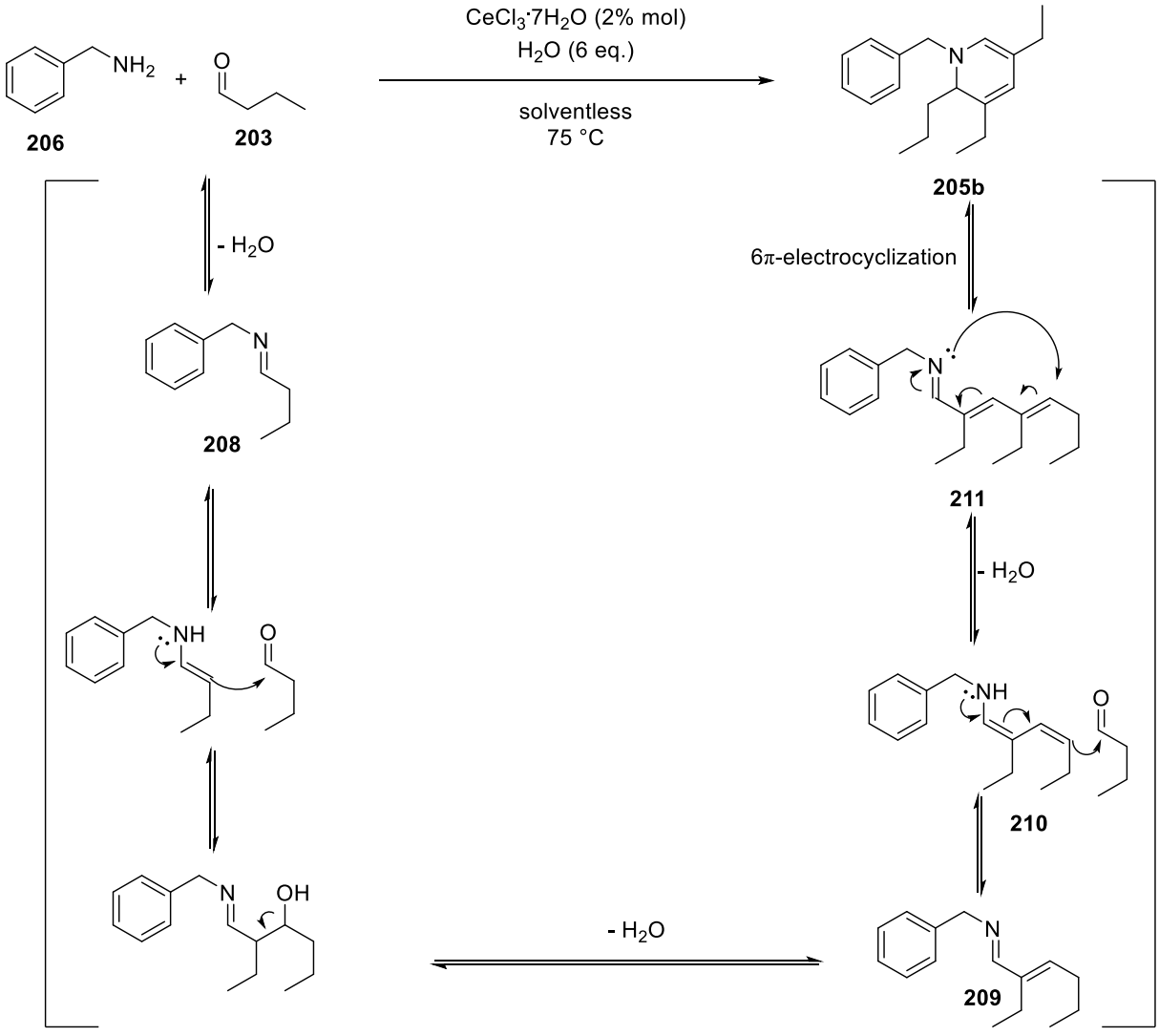


ENTRY	Solvent	Temperature	Time	GC-Conversion
1	**-**	**75 °C**	**0.5 h**	**80.93 %**
2	DCM	Reflux	0.5 h	60.89 %
3	ACN	75 °C	0.5 h	49.74 %
4	Toluene	75 °C	0.5 h	66.44 %
5	THF *dry*	Reflux	0.5 h	16.35 %
6	H_2O	75 °C	0.5 h	26.99 %
7	EtOH	75 °C	0.5 h	32.71 %

Table 2.2.7. Screening of solvent

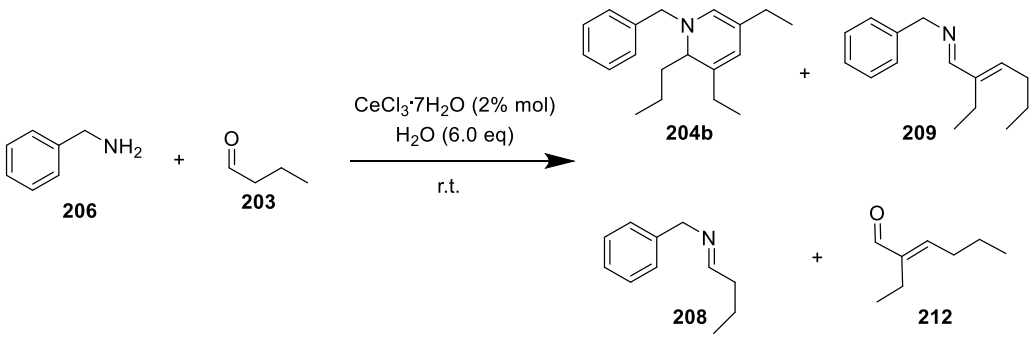
The best reaction conditions remains the solventless conditions, but very interesting results were obtained. From the table it is possible to notice that with apolar solvents and water-immisciable like dichloromethane and toluene a good conversion were obtained even if slightly worse than neat protocol. On the other hand, quite interesting results were observed ìn the case of polar solvent. Expect for acetonitrile, as a quite good conversion was detected, performing the reaction in THF, H_2O and EtOH poor results were observed. The 27% of conversion of

reaction performed in water, which is a higher conversion as compared to THF, it could be due to another confirm of importance of water in the whole process and how it is influenced by it, and it also confirm the water-tollerance of process and catalyst. Performing the reaction in alcoholic solvent the conversion was still low. An interesting result was the reaction performed in THF, this very low conversion could be to the fact of coordination by oxygen atom of tetrahydrofuran to Cerium core, lowering its activity.[264a, 264b] In our opinion, the whole process proceed by multiple step of condensation favorites by the equilibrium between imine/enamine. After the first condensation, the enamine, that as nuchleophilic character, attacks another aldehyde molecule forming a dicondensated, or azadiene **209**, then a successive attack to a third molecule of aldehyde leads to the formation of azatriene **210** and final product was formed after an intramolecular 6π-electrocyclization.



Scheme 2.2.19. Possible mechanism for formation of product.

To finally prove the possible multi-addition process, we have conducted an experiment in which we have started with a benzylamine/butyraldehyde 1:1, then after one huor the mixture was analyzed by GC-MS to figure out the species present in the system, then, an other equivalent of aldehyde was added to the mixture and the solution is stirred for one hour more. Here are reported the most principal peaks written in the table below. The experiment was conducted at room temperature to avoid degradations of compounds present in the mixture.

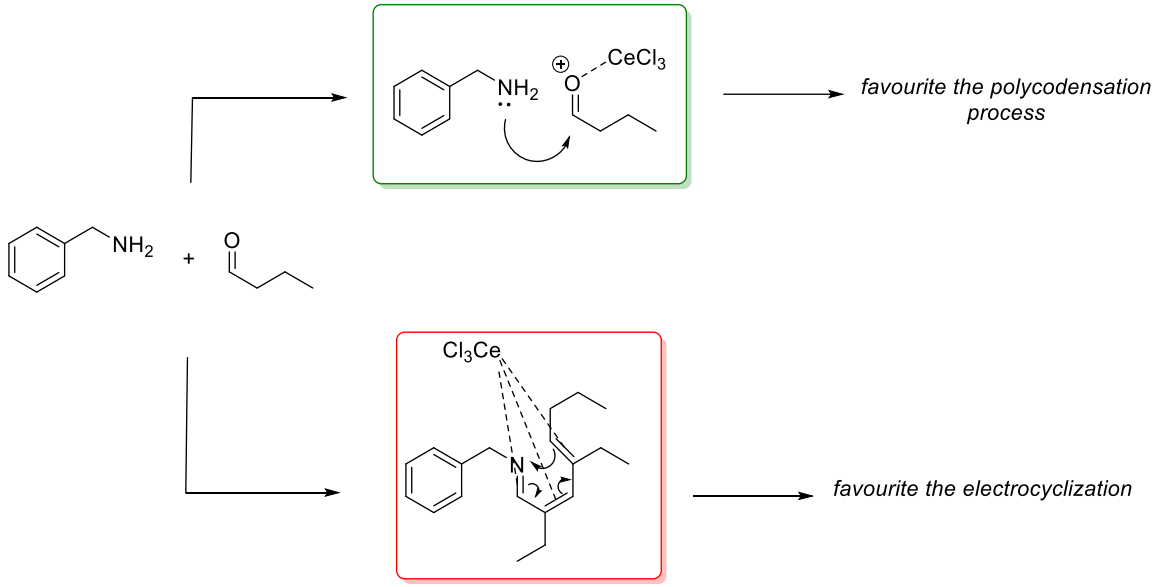


ENTRY	204	Time	207	209	210	205b	213
1.0h react.	1.0 eq	1.0 h	10.50 %	71.83 %	11.04 %	3.82%	1.43 %
2.0h react	2.0 eq	2.0 h	2.03 %	20.08 %	26.35 %	40.58%	3.27 %
3.0h react	3.0 eq	3.0 h	-	7.07 %	11.14 %	45.84%	2.84 %
4.0h react	4.0 eq	4.0 h	-	2.80 %	4.76 %	56.12 %	3.30 %
5.0h react	5.0 eq	5.0 h	-	-	2.32 %	49.73 %	2.69 %

Table 2.2.8.Analyses of multiple additions

For the Le-Chatelier principle, after each addition, the formation of the next condensated intermediate gradually be favored: after the first addition the majority species should be the imine **208**, after the second, the azadiene **209** and so on. In fact, after one hour of reaction the major intermediate present in the reaction was the imine, but the presence of benzylamine was still detected, and the presence of di-condesate **209** and little percentage of target product was already detected (~4%). After the second addition of aldehyde, an important decrese of imine was observed and the product become unexpctedly the major component of the mixture. This could mean that the azadiene **209** is not so stable, so it undergoes to the third addition of aldehyde forming the linear precursor of 1,2-DHP **210** that cyclizes, through a 6π-electrocyclization, forming the N-heterocycle product **204b**. Further additions of aldehydes, third and fourth, consume the imine and condensate product leading to the formation of 1,2-DHP which reach "its maximum" after the fourth addition (same equivalent of best reaction condition). Finally, after the fifth addition, the conversion of target product decrease as other multiple condensation compounds are formed, four and five and so on addition products, were favored and detected at GC-MS. Moreover, the linear-three condensation product was not

observed or observed in very little conversion, this means that once formed the intermediate **210**, it suddenly undergo to cyclication forming the very more stable cyclic product. The role of the cerium is not so clear, it can act by two ways: it can favourite the condensation process coordinating the aldehyde, increasing the electrophilicity of carbonyl, or it can favor the electrocyclization, but to the best of our knowledge no works are reported in which a Ce(III) salt favourites the 6π-electrocyclization. It could also favor both processes.

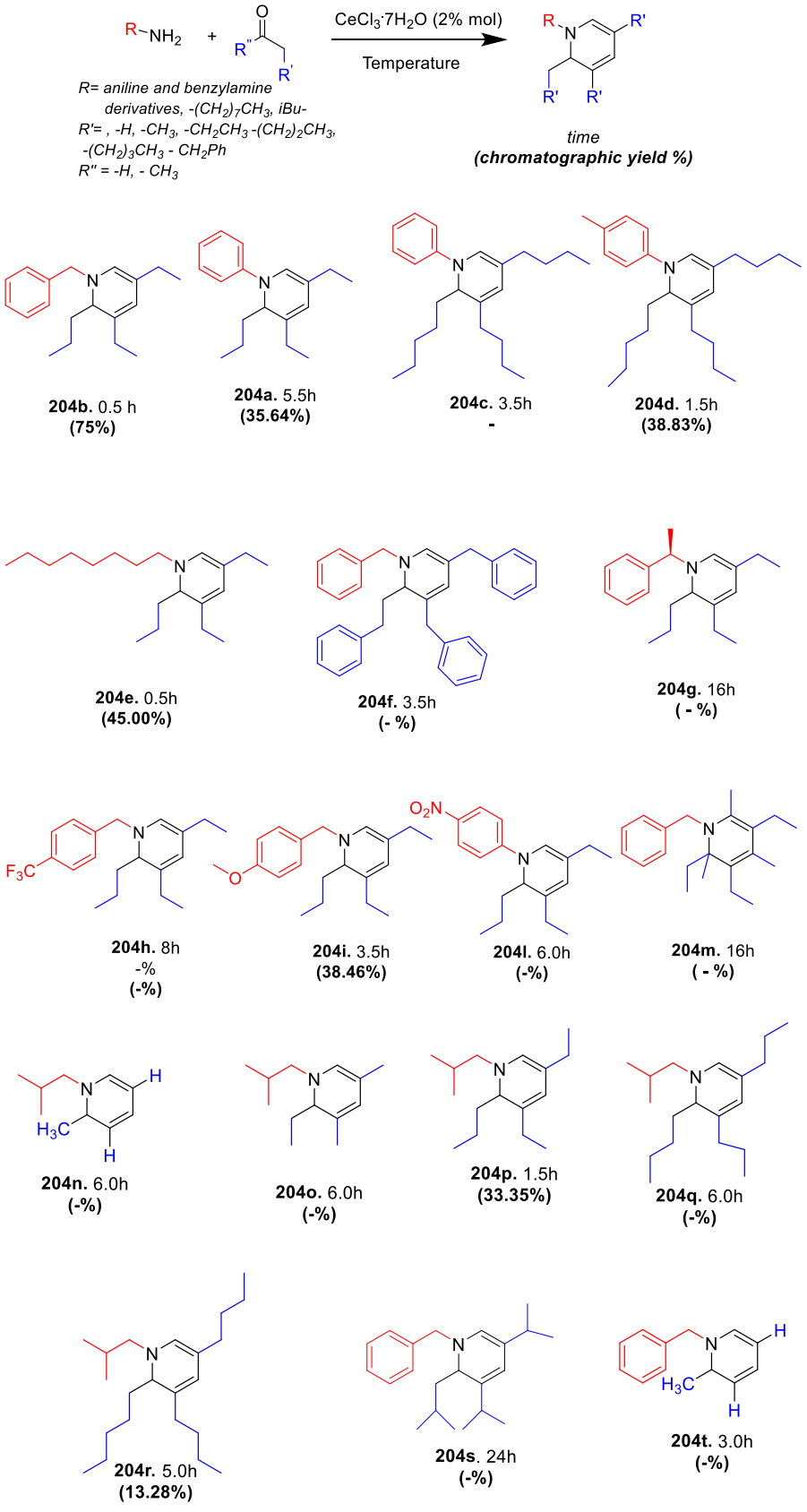


Scheme 2.2.20. Possible roles of Ce(III) Chloride in poolycondensation process

After that we have figured out the best conditions for the process and mechanism, we have tried to expand the scope of the reaction. We have tested several different aldehydes and primary amines performing the reaction in solventless condition, at 75°C (otherwise, the reaction was performed at boiling point of reagent), in presence of catalytic amount of $CeCl_3 \cdot 7H_2O$ and 6 eq. of water (*Scheme 2.2.20.*). For each example was reported the time at which the reaction was stopped, the percentage of the product detected in mixture at that time and the cromathographic yield. It can be suddenly observed that changing the substrates the reactivity changes considerably. Performing the reaction using aniline instead benzylamine the conversion, speed, and yield of the reaction decrease and the product **204a** was isolated only after 5.5 hrs of reaction and with an yield of 35%, probably this relative low yield could be explained by the less disposale of lone pair of aniline-nitrogen that are delocalizated in aromatic ring. Moreover, using a bulky aldehyde, like hydrocynammaldehyde, the product **204f** was only observed at GC-MS with a very low conversion. As predictable, the EDG- and EWG- substitutents greatly influence the reactivity and the trend of the process. Aliphatic and EDG-substituted

benzylamine/aniline have shown the most suitable for the reaction, in fact, when octylamine, p-methoxybenzylamine and p-toluidine were involved, the corrispective 1,2-DHPs were detected with a good conversion at GC-MS and isolated with a good cromathographic yield, respectively 45% for **204e**, 39% both for **204i** and **204d**. On the other hand, performing the reaction with EWG-substituted primary amine, the 1,2-DHP-products **204h** (p-trifluoromethylbenzylamine) and **204i** (p-nitroaniline) were not observed or detected with a very low conversion, probably due to less available of lone pair due to the electronwithdrawing nature of substitutents that make less available the electrons. In case of p-nitroaniline the process stop to azadiene, probably due to strong electron withdrawing behaviour of nitro groups prevents the third attack to another aldehyde stopping the process. The length of aldehyde it seems also influence the process and purification, confronting the GC-conversion and yield between **204a** and **204c**, performing the reaction with hexanal the product was detected with a lower conversion and lower cromathographic yield. This influence of lenghtness and bracheness of aldehyde was also confirmed by the reaction between isoproprylamine and from acetaldehyde to hexanal (**204n-r**). As other primary amines, the 1,2-DHPs between isoproprylamine and butanal/hexanal was observed and isolated observing a lower yield with the reaction performed with hexanal (33% of yield with butanal meanwhile 13% with hexanal). Meanwhile the cyclic product **204o** and **204q** were not observed in a clear way, since a complex mixture of compound was observed at GC-MS. Moreover, performing the reaction in presence of acetaldehyde, the product was not observed neither at GC-MS, both involving isobutylamine and benzylamine. This fact could be explained by the constrain of azadiene (double addition of aldehyde to amine) product obtained from primary amines and acetaldehyde that could not undergo to the third condensation. We tried also the exploit the stereoselectivity of the process since the presence of a stereocentre in the final product, for this reason, we tried to employ a chiral amine, α-(*R*)-methylbenzylamine (**204g**), but unfortunately the product was observed in very low conversion and it was not possible to determinate the diastomeric ratio of final two 1,2-DHPs.

Employing a ketone, rather an aldehyde, **204m**, the reaction between benzylamine and butanone leads to no results and only the imine was observed. The purification of these products has showed a very difficult task, since in some cases, even if a good conversion of the product was detected at GC-MS, sometimes it was no possible to recover almost the product present in the crude of reaction.



Scheme 2.2.21. Scope of the reaction.

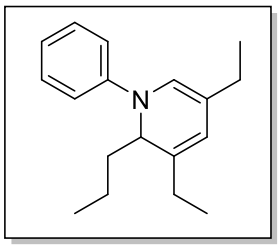
Different purification methods were tested, such as filtration through basic alumina, neutral alumina, silica, buffered silica (the SiO_2 was buffered by Et_3N) and Phase Inverse Chromathography. In most cases, only traces of product were collected after the purification expect in two cases: basic alumina and buffered SiO_2. This fact could be explained by basic nature of 1,2-dihydropyridines, typical of amine, in fact, in the –OH present in neutral alumina and SiO_2 could react with the nitrogen present in the scaffold of molecule forming a salt and block the product inside the cromathographic column, while in basic alumina and buffered SiO_2 this aspect is almost suppressed. It was no possible to recover only little traces of the product by phase Inverse cromathography.

2.2d Experimental Section

*GENERAL METHOD FOR THE SYNTHESIS OF 2,3,5-TRISUBSTITUTED-1,2-DIHYDROPYRIDINES (**204a - 204t**)*

1 equivalent of amine (1 mmol) is added dropwise to a solution of aldehyde (4.0 eq., 4 mmol), $CeCl_3 \cdot 7H_2O$ (2% mol, 0.02 mmol) and H_2O (6 eq., 6 mmol) at 0°C, present in a two necked round flask. Then, after the addition the reaction is heated (75°C or to boiling point of reagents if they are lower the 75°C) until no more starting material or evolutions are observed at TLC (95 Hexane : 5 EtOAc) or GC-MS. Then, the reaction is cooled down and it is portioned in dichloromethane (20 mL) and a saturated solution of $NaHCO_3$ (20 mL) and the organic phase is extracted. The aqueous layer is extracted two more times with dichloromethane (2x20 mL). The organic phase is anhydrified with Na_2SO_4 and the solvent was evaporated by rotavapor. The crude is purified by buffer Silica Gel (The Silica is buffered by a 1% v/v of Et_3N in hexane).

*N-PHENYL-3,5-DIETHYL-2-PROPYL-1,2-DIHYDROPYRIDINE (**204a**)*



Following the general procedure, the reaction was carried out using aniline and butanal as reagents, pure product **204a** was isolated after 5.5 h by Flash Cromatography on buffered Silica Gel (from 100 Hexane to 90:10 Hexane/EtOAc, Rf = 0.46 in 95:5 Hex/EtOAc) with a yield of 35.64% as an orange-yellow oil.

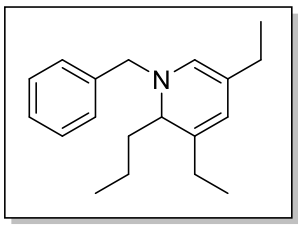
Molecular Formula: $C_{18}H_{25}N$

^{1}H-NMR ($CDCl_3$, 400 MHz) δ = 7.32 – 7.26 (m, 2H), 6.99 – 6.96 (m, 2H), 6.88 (dd, J = 7.8, 6.8 Hz, 1H), 6.12 (s, 1H), 5.73 (s, 1H), 4.31 (t, J = 6.2 Hz, 1H), 2.23 (ddd, J = 6.8, 6.1, 4.2 Hz, 2H), 2.09 (dt, J = 23.9, 8.3 Hz, 2H), 1.62 (ddd, J = 9.2, 4.6, 3.1 Hz, 2H), 1.50 – 1.44 (m, 2H), 1.14 (dd, J = 8.1, 6.8 Hz, 3H), 1.12 – 1.08 (m, 3H), 0.94 (q, J = 2.0 Hz, 3H)

^{13}C-NMR ($CDCl_3$, 100 MHz) δ = 146.62, 134.49, 129.31, 120.45, 120.37, 119.40, 115.11, 58.89, 34.08, 31.88, 27.98, 25.88, 19.67, 14.81, 14.65, 12.21

GC-MS (EI, 70eV) = 255 [M+], 212 (100), 197, 182, 168, 106, 91, 77.

*N-BENZYL-3,5-DIETHYL-2-PROPYL-1,2-DIHYDROPYRIDINE (**204b**)*



Following the general procedure, the reaction was carried out using benzylamine and butanl as reagents, pure product **204b** was isolated after 0.5 h of reaction by Flash Chromatography on buffered Silica Gel (from 100 Hexane to 90:10 Hexane/EtOAc, Rf = 0.49 in 95:5 Hex/EtOAc) with 75% yield as an orange-yellow oil.

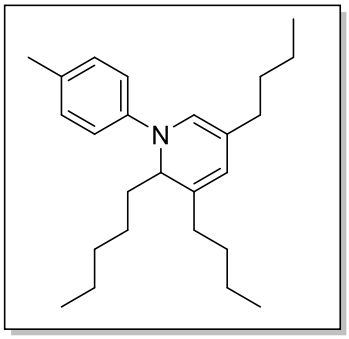
Molecular Formula: $C_{19}H_{27}N$

^{1}H-NMR ($CDCl_3$, 400 MHz) δ = 7.33 – 7.21 (m, 5H), 5.72 (s, 1H), 5.62 (d, J = 1.2 Hz, 1H), 4.21 (d, J = 4.7 Hz, 2H), 3.62 (t, J = 5.1 Hz, 1H), 2.06 – 1.88 (m, 4H), 1.50 – 1.32 (m, 4H), 1.04 – 0.98 (m, 3H), 0.98 – 0.94 (m, 3H), 0.90 – 0.87 (m, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ = 140.16, 129.25, 128.65, 128.58, 127.50, 127.11, 119.55, 111.70, 59.40, 58.15, 34.08, 27.67, 25.64, 18.99, 15.12, 14.75, 12.50.

GC-MS (EI, 70 eV) = 269 [M+], 226 (100), 134, 91.

*N-(P-TOLUIDINE)-3,5-DIBUTYL-2-PENTYL-1,2-DIHYDROPYRIDINE (**204d**)*



Following the general procedure, the reaction was carried out using p-methylaniline and hexanal as reagents, pure product **204d** was isolated after 1.5h by Flash Chromatography on buffered Silica Gel (from 100 Hexane to 90:10 Hexane/EtOAc, Rf = 0.50 in 95:5 Hex/EtOAc) in 38.83% yield as a red oil.

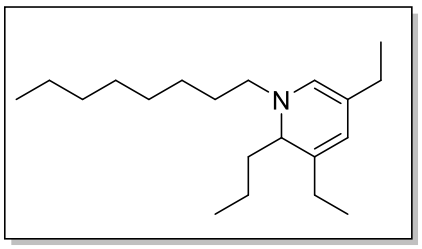
Molecular Formula: $C_{25}H_{36}N$

^{1}H-NMR ($CDCl_3$, 400 MHz) δ = 7.08 (d, J = 8.2 Hz, 2H), 6.89 – 6.79 (m, 2H), 6.04 (s, 1H), 5.68 (s, 1H), 4.24 (t, J = 6.3 Hz, 1H), 2.30 (s, 3H), 2.10 (dddd, J = 22.7, 15.4, 9.9, 6.7 Hz, 4H), 1.60 – 1.28 (m, 16H), 0.95 (t, J = 7.2 Hz, 3H), 0.92 – 0.83 (m, 6H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ = 144.47, 132.35, 129.82, 128.70, 121.46, 119.58, 118.17, 115.32, 59.04, 34.99, 32.59, 32.52, 32.44, 31.72, 30.32, 26.03, 22.87, 22.79, 22.49, 20.65, 14.32, 14.26, 14.22.

GC-MS (EI, 70 eV) = 353 [M+], 310, 282 (100), 252, 226, 197, 91.

*N-OCTYL-3,5-DIETHYL-2-PROPYL-1,2-DIHYDROPYRIDINE (**204e**)*



Following the general procedure, the reaction was carried out using octylamine and butanl as reagents, pure product **204e** was isolated after 0.5 h by Flash Chromatography on buffered Silica Gel (from 100 Hexane to 90:10 Hexane/EtOAc, Rf = 0.45 in 95:5 Hex/EtOAc) in 45% yield as an orange-yellow oil.

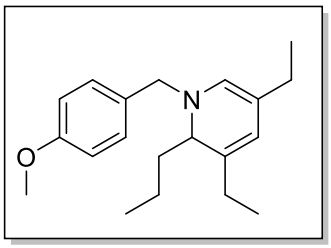
Molecular Formula: $C_{20}H_{37}N$

^{1}H-NMR ($CDCl_3$, 400 MHz) δ = 5.62 (s, 1H), 5.56 (d, J = 1.3 Hz, 1H), 3.61 (t, J = 5.0 Hz, 1H), 2.93 (ddd, J = 13.6, 7.4, 6.0 Hz, 2H), 2.15 – 1.91 (m, 4H), 1.48 – 1.24 (m, 16H), 1.05 (t, J = 7.4 Hz, 3H), 0.97 (t, J = 7.4 Hz, 3H), 0.87 (t, J = 7.1 Hz, 6H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ = 128.73, 128.45, 119.34, 110.83, 60.11, 54.31, 34.01, 32.04, 30.18, 29.65, 29.50, 27.74, 27.07, 25.62, 22.88, 19.03, 15.14, 14.77, 14.32, 12.56

CG-MS (EI, 70 eV) = 291 [M+], 262, 248 (100), 176, 162, 149, 136, 121.

*N-(P-METHOXYBENZYL)-3,5-DIETHYL-2-PROPYL-1,2-DIHYDROPYRIDINE (**204i**)*



Following the general procedure, the reaction was carried out using p-methoxybenzylamine and butanal as reagents, pure product **204i** was isolated after 3.5 h by Flash Chromatography on buffered Silica Gel (from 100 Hexane to 90:10 Hexane/EtOAc, Rf = 0.43 in 95:5 Hex/EtOAc) in 38.46% yield as an orange-yellow oil.

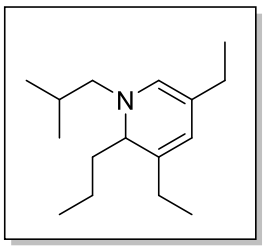
Molecular Formula: $C_{20}H_{26}NO$

^{1}H-NMR ($CDCl_3$, 400 MHz) δ = 7.29 – 7.13 (m, 2H), 6.94 – 6.82 (m, 2H), 5.76 (s, 1H), 5.65 (d, J = 1.1 Hz, 1H), 4.19 (t, J = 10.5 Hz, 2H), 3.81 (s, 3H), 3.66 (t, J = 5.3 Hz, 1H), 2.13 – 1.90 (m, 4H), 1.57 – 1.36 (m, 4H), 1.03 (dt, J = 14.7, 7.4 Hz, 6H), 0.93 (t, J = 7.0 Hz, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ = 158.94, 132.03, 129.16, 128.79, 128.74, 119.61, 113.98, 111.65, 59.25, 57.54, 55.41, 34.04, 27.73, 25.73, 19.02, 15.18, 14.81, 12.57.

GC-MS (EI, 70 eV) = 299 [M+], 256 49, 134, 121 (100), 91, 77.

*N-ISOPROPYL-3,5-DIETHYL-2-PROPYL-1,2-DIHYDROPYRIDINE (**204p**)*



Following the genarl procedure, the reaction was carried out using isobutylamine and butanal as reagents, pure product **204p** was isolated after 1.5h by Flash Chromatography on buffered Silica Gel (from 100 Hexane to 90:10 Hexane/EtOAc, Rf = 0.38 in 95:5 Hex/EtOAc) in 33.35% yield as a yellow oil.

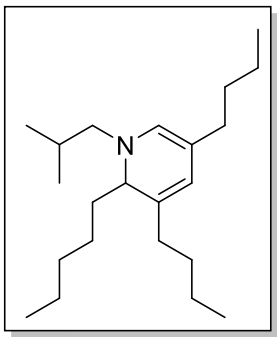
Molecular Formula: $C_{16}H_{26}N$

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 5.59 (s, 1H), 5.57 (d, J = 1.3 Hz, 1H), 3.60 – 3.53 (m, 1H), 2.78 (d, J = 7.2 Hz, 2H), 2.02 (dddt, J = 23.4, 14.4, 13.5, 7.1 Hz, 4H), 1.75 (dt, J = 13.7, 7.0 Hz, 1H), 1.51 – 1.23 (m, 4H), 1.06 (t, J = 7.4 Hz, 3H), 0.98 (t, J = 7.4 Hz, 3H), 0.91 – 0.79 (m, 9H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 128.81, 128.26, 119.44, 110.32, 62.83, 60.65, 34.07, 29.58, 27.84, 25.63, 20.37, 20.21, 19.21, 15.28, 14.74, 12.76.

GC-MS (EI, 70eV) = 235 [M+], 192 (100), 178, 149, 136, 121, 106, 91, 77.

*N-ISOPROPYL-3,5-DIBUTYL-2-PENTYL-1,2-DIHYDROPYRIDINE (**204r**)*



Following the general procedure, the reaction was carried out using isobutylamine and hexanal as reagents, pure product **204r**, was isolated after 5.0h by FC on buffered Silica Gel (from 100 Hexane to 90:10 Hexane/EtOAc, Rf = 0.41 in 95:5 Hex/EtOAc) in 13.28% yield as yellow oil.

Molecular Formula: $C_{22}H_{38}N$

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 5.57 (s, 1H), 5.53 (d, J = 1.2 Hz, 1H), 3.53 (ddd, J = 6.9, 4.4, 1.4 Hz, 1H), 2.82 – 2.70 (m, 2H), 2.03 – 1.85 (m, 4H), 1.72 (dd, J = 13.6, 6.9 Hz, 1H), 1.52 – 1.18 (m, 20H), 0.92 – 0.85 (m, 12H), 0.82 (t, J = 6.0 Hz, 3H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ = 129.64, 126.46, 120.81, 108.60, 62.82, 60.59, 34.84, 33.11, 32.56, 32.34, 31.67, 30.60, 29.53, 25.71, 22.97, 22.76, 22.33, 20.34, 20.20, 14.32, 14.27, 14.25.

GC-MS (EI, 70eV) = 319 [M+], 248 (100), 218, 192, 176, 149, 107, 79, 57, 41

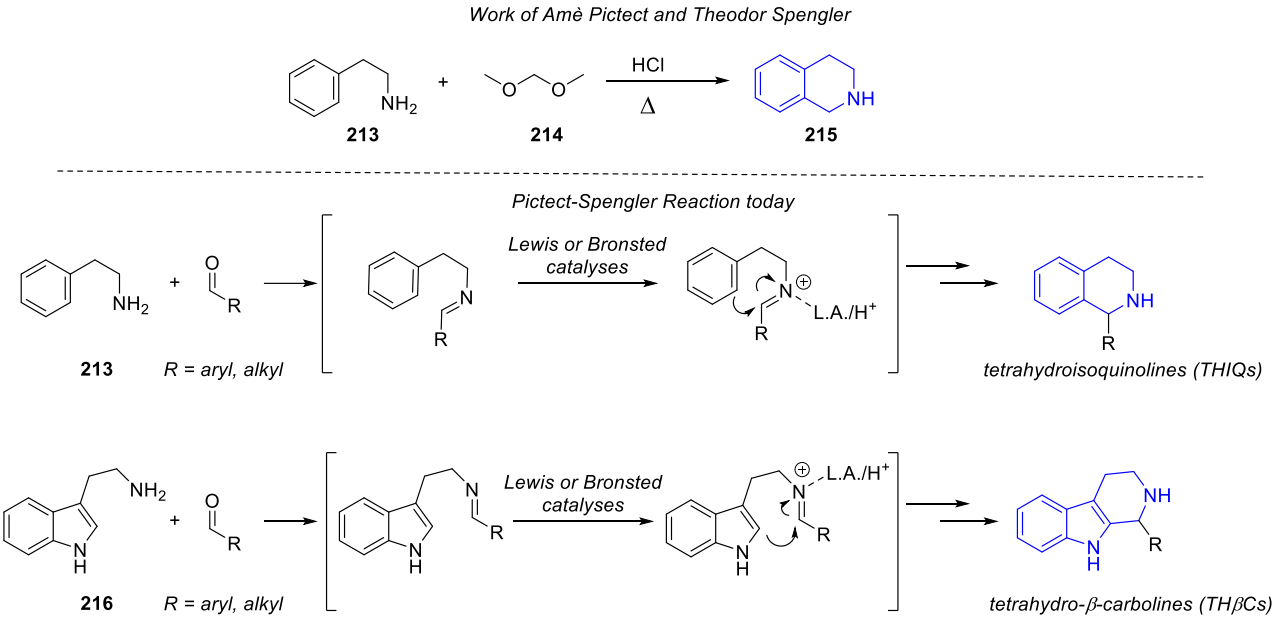
2.2e Conclusion

Although 1,2-dihydropyridines have received less attention than their 1,4-DHP isomers during recent years, 1,2-DHPs have received more attention by the scientific world, and they have found important synthetic applications for biologically active compounds such as Tamiflu, alkaloids and other natural chiral molecules. The most important synthetic application of 1,2-DHPs is to act as diene in pericyclic reactions together with a dienophile, a reaction that can allow to obtain molecules even with complex structures. Nowadays many synthetic processes are reported in the literature such as the reduction of pyridinium salts, or even reactions between acyclic precursors. The latter turns out to be a very effective way and for the synthesis of many types of heterocycles. Furthermore, many Lewis Acids are used for the synthesis of 1,2-DHPs, but unfortunately they are either expensive, sensitive to water and humidity, or the reactions require long reaction time. Bearing in mind all these aspects, in our laboratory we have tried to develop a method for the synthesis of 2,3,5-trisubstituted-1,2-dihydropyridines starting from acyclic precursors such as primary amines and aldehydes, using $CeCl_3 \cdot 7H_2O$ as a "friendly, green and water-tolerant" catalyst. During the study of this polycondensation process it was possible to establish that the Ce(III) salt was the most effective among different and well-known Lewis acids, obtaining the product with only after half an hour of reaction and with good yields. From the GC-MS studies it was also possible to establish that at ratio 1:2 between amine and aldehyde the formation of the 1,2-DHP target is favored over other intermediates such as imine and azadiene, indicating an high stability of cyclic product respect to the other compounds. Moreover, the solventless reaction has shown the most suitable conditions since performing the reaction in common solvents the conversion of 1,2-DHPs was lower caused by, sometime, the possible de-activation of Ce(III) by solvent itself. The major drawbacks of this process is the limitation of substrates that can be employed. In fact 1,2-DHPs were isolated only using butanal and hexanal as aldehyde and with benzylamine, aniline, alkyl-amine and EDG-substituted benzylamine/aniline, on the other hand the EWG-substituted amine were not suitable for the process. Moreover, the common filtration on silica is a problem due to the basic nature of these compounds that can be overcome by using buffered silica or basic alumina. The 1,2-dihydropyridines synthesized by us can find industrial applications as accelerators in crosslinking of polymeric products. These additives are usefull when a crossiling agent must be used at the lowest possible temperature to prevent decomposition of the polymer. They may also enhance tensile properties and improve age resistance of final product.

2.3 Brønsted and Lewis Promotion of Pictect-Spengler Reaction under Microwave Irradiation

2.3a Pictect-Spengler Reaction

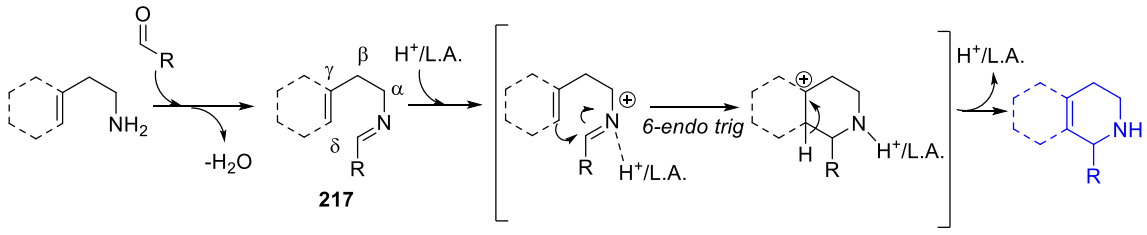
The two chemists, Amè Pictect and Theodor Spengler, published a paper in 1911 in which, under acidic conditions, they were able to synthesize the tetrahydroisoquinoline skeleton **215** through the reaction between phenylethylamine **213** and dimethoxymethane **214**.[265] Nowadays this reaction is known as Pictect-Spengler Reaction (PSR) or Pictect-Spengler Cyclization. In more general application, the PRS can be useful for the synthesis of tetrahydroisoquinoline and tetrahydro-β-carboline scaffolds starting from β-arylethylamine, like tryptamines or phenylethylamines, and carbonyl compounds, like aldehydes and ketones, undergoing Lewis/Brønsted catalyses.[266]



Scheme 2.3.1. "Original" PSR (above) and "Current" PSR (below)

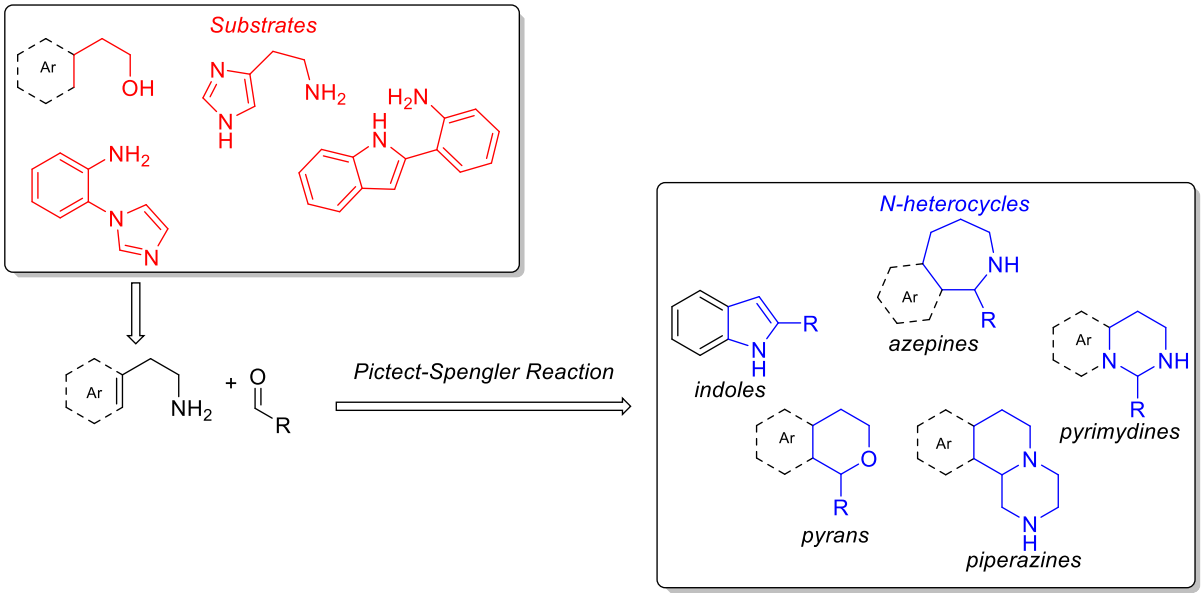
Isoquinolines and β-carbolines are important natural compounds that belongs to the class of widely known "Alkaloids", which can explicate a large, potent and important biological activities[267] (which will be discussed later). Beyond the investigations of the Lewis/Brønsted catalyses of PSR by scientists, it is known that many enzymes (called PSases, such as norcoclaurine synthase (NCS) and strictosidine synthase (STR))

performed the PSR to obtain the alkaloids and other bioactive compounds. These enzymes catalyse the reaction with a high stereoselectivity (preference for (*S*)-enantiomer both natural and unnatural substrates), leading to the synthesis of many bioactive compounds and making them very attractive for biocatalyses applications for the production of numerous alkaloids in laboratory.[268] Both enzymatic and Brønsted/Lewis catalysed Pictect-Spengler Reaction proceed by two steps-mechanism in which, in the first step the imine **217** is formed by the condensation of imine and aldehyde (or ketone), then, consequently, the activation of imine in acidic environment leads to the formation of active-specie iminium which undergoes to a 6-endo trig cyclization obtaining finally a new heterocyclic compound by the formation of a new C-C bond, further deprotonation restores the previous aromacity of aryl moiety (*scheme 2.3.2.*).[269]



Scheme 2.3.2. Mechanism of Pictect-Spengler Reaction

Although the "classic" Pictect-Spengler is limited to tryptamines and phenylethylamines, interesting works are reported in the literature in which, leaving the α-δ layout of the primary amine and aryl carbon intacts (see *scheme 2.3.2.*), it is possible to synthesize a plethora of N-heterocyclic compounds through a modified Pictect-Spengler Reaction (see *scheme 2.3.3.*).[269, 270] Further modification of PSR by using β-arylethylalcohols as substrates, and carbonyl compounds (aldehydes and ketones) it is possible to obtained pyran derivatives fused with aromatic rings by an oxo-version of Pictect-Spengler Reaction.[271] Even if the so interesting and wide scope of PSR can find nowadays, here we will limit to discuss the synthesis of tetrahydro-β-carboline and tetrahydroisoquinoline by Brønsted and Lewis catalyses, object of this chapter.

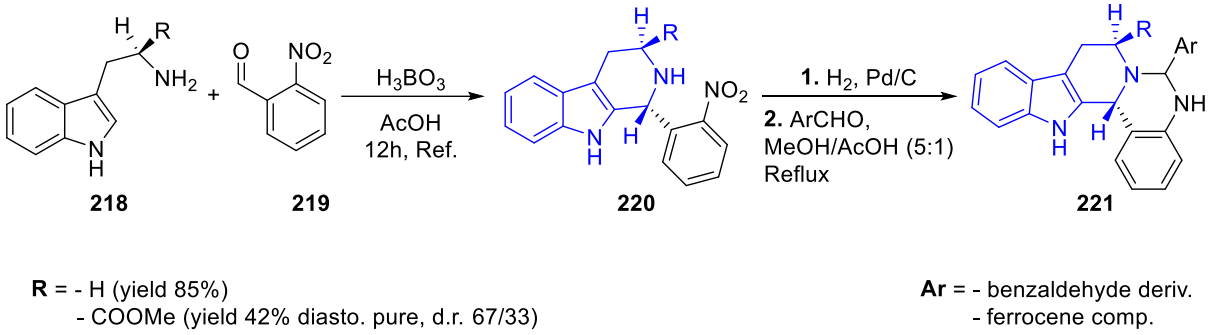


Scheme 2.3.3. Enlargement of Pictect-Spengler substrates and heterocycles.

BRØNSTED CATALYSES

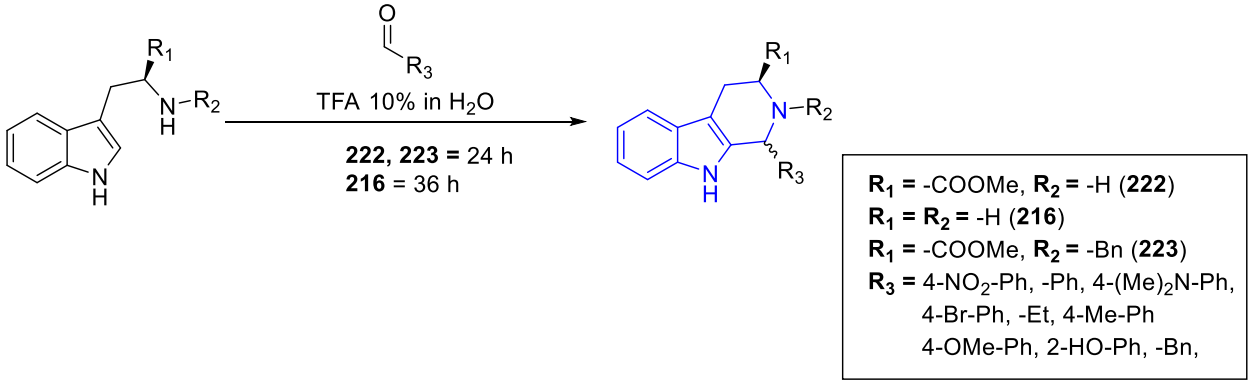
The first PSR provides the formation of tetrahydroisoquinoline by employing a common Brønsted acid, hydrogen chloride (see *Scheme 2.3.1.*). Nowadays many papers report the Brønsted catalyses of this reaction employing common mineral and organic acids like HCl,[272] Acetic Acid,[273] Trifluoroacetic acid,[274] Sulfuric acid,[275] Boric acid;[276] requiring sometime long reaction time or/and harsh conditions, despite the fact that the very large substrates scope of this type of catalyses.

Just to cite some few examples, Fodor *et al.*[276] demonstrated the usefulness of PSR, catalysed by a system composed by H_3BO_3/AcOH, through which they synthesized the tetrahydro-β-caroboline **220** (THβC), obtained through the reaction between tryptamine/methyl ester of L-tryptophane and 2-nitrobenzaldehyde after 12h of reaction at reflux. Then subsequent reduction of nitrogroup present in THβC and successive reaction with other benzaldehyde-derivatives leads to the formation of 6-aryl-5,6,8,9,14,14b-hexahydroindolo-[20,30:3,4]pyrido-[1,2-c]quinazolines **221**, a novel class of N-condensated heterocycles that showed potent and prominent antiproliferative/cytotoxic activities against human cancer cell lines PANC-1, COLO-205, A2058 and EBC-1.



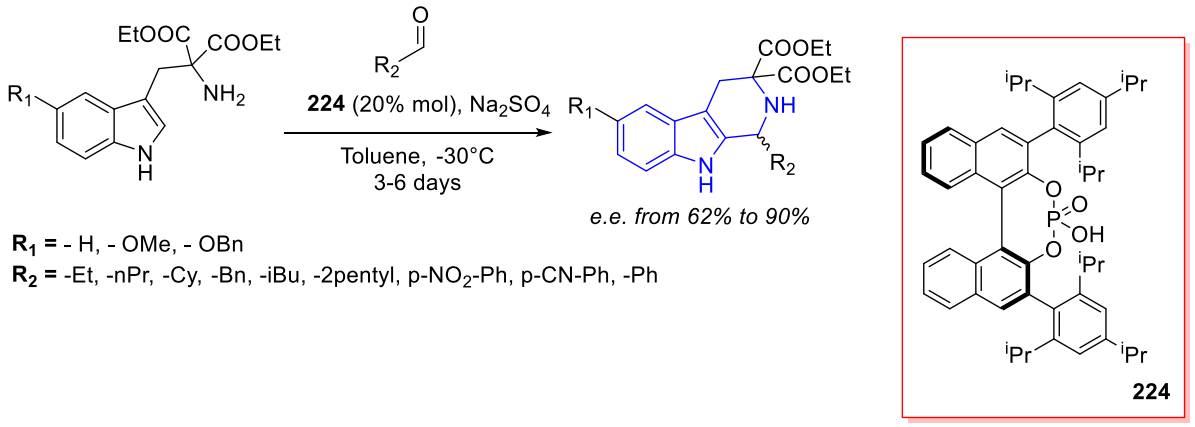
Scheme 2.3.4. Exploiting of PSR for the synthesis of new anticancer N-heterocycles by Fodor et al.

Even if many solvent media have tested for this type of the reaction, Saha and his coworker have investigated the possibility to perform the PSR in water media (green choice, especially for industrial scale synthesis).[277] In this work, the reaction proceeds smoothly with different benzaldehydes derivatives, methyl ester of L-triptophane (Trp-OMe, **222**) and is N-benzylated derivative (**223**) (24 hrs of reaction) and tryptamine **216** (36h), in presence of 10% of TFA in water at room temperature (yields ranging from 45% to 83%). Performing the reaction in presence of less concentrated TFA solutions (5% and 2%), Acetic Acid (AcOH), p-toluenesulphonic acid (p-TsOH) and $Yb(OTf)_3$, common Brønsted/Lewis catalysts of this reaction, the product was obtained with a lower yield (23% in case of AcOH) or the product was not detetced (p-TsOH and Yb(III) triflate). Deeper investigations have showed that the unique electronic properties of water favor the trend of the reaction respect to other common solvents like dichloromethane (DCM) or toluene, in fact, the reactions between tryptamine/Trp-OMe and Salycilaldehyde/4-dimethylamminobenzaldehyde leads to an higher product performed in water respect to DCM/toluene-solvent, this fact can be exaplined, in accordance to authors, by the promotion of endo-cyclization in water. The protocol has showed a good diastereoselectivity for the cis-products, especially for 2-hydroxy substituted benzaldeyde (d.r. 90/10).



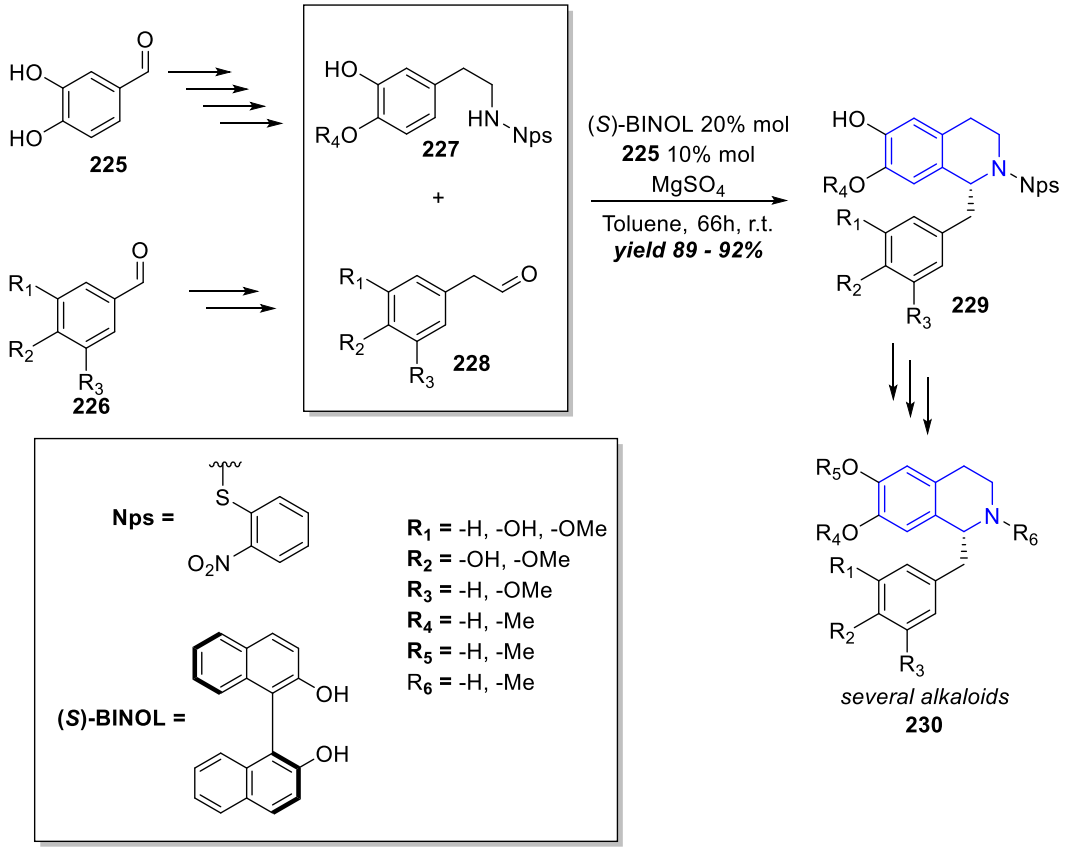
Scheme 2.3.5. PSR protocol in water developed by Saha et al.

Entatioselectivity of reaction has always been a very important research topic of organic synthesis. The scientists are trying to mimics the enatioselective "power" of enzymes and to develop even more efficient and mild protocols for the synthesis of enantiopure molecules, since enantiopure compounds explicate important and sometime different biological actions. Chiral phosphoric acid **224** has shown very useful chiral Brønsted acid for Pictect-Spengler Reaction among others different substituted-phosphoric acids (e.e. 66%, highest respect to others, performing the reaction in toluene at room temperature). Testing different substrates, the enantioselectivity was improved lowering the temperature of reaction (-30°C) and lasting the reaction for 3-6 days in presence of Na_2SO_4.[278]



Scheme 2.3.6. Enantioselective synthesis of chiral THβC by Seayad et al.

The same catalyst **224** was exploited by Ruiz-Olalla *et al*.[279] for the enantioselective synthesis of tetrahydroisoquinolines **229** starting from secondary phenylethylamine **227** (obtained from 3,4-dihydroxybenzaldehyde **225** after several steps) and phenylacetaldehyde derivatives **228** (1.05 equivalents). The concomitant use of phosphoric acid **224** and the diol (*S*)-Binol resulted in THIQ (tetrahydroisoquinoline) **229** after 66 hrs of reaction in toluene at room temperature with an e.e. about ~90%. This protocol provides a very useful pathway for the synthesis of many alkaloids since the tolerance for different dopamine derivatives and aldehydes. The removal of Nps protecting group allow to obtain several N-substituted THIQs **230**.

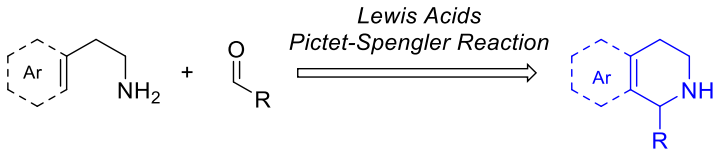


Scheme 2.3.7. Enantioselective synthesis of THIQ by PSR Phosphoric acid catalysed reported by Ruiz-Olalla et al.

LEWIS CATALYSES

The employment of Lewis acids as catalyst for different reactions has always been one of most vast area of organic research, and the Pictect-Spengler Reaction makes no expetions. Many Lewis acids have been tested for this reaction showing different activity depends by the metal core and counter ions (*table 2.3.1.*).

Both Imine (In, Bi, Sc, etc.) and Aldehyde (Al, Ti, Sn, etc) selective Lewis Acids (Kobayashi Classification[281]) should be prominent and active catalysts for the PSR.[280] Srinivasan *et al.* have tested the efficiency of several Lewis Acids performing the Pictet-Spengler Reaction using as pilot reagents tryptamine/methyl ester of L-tryptophane, a little excess of benzaldehyde in dichloromethane undermicrowave irradiation (100°C, 30 minutes of reaction).

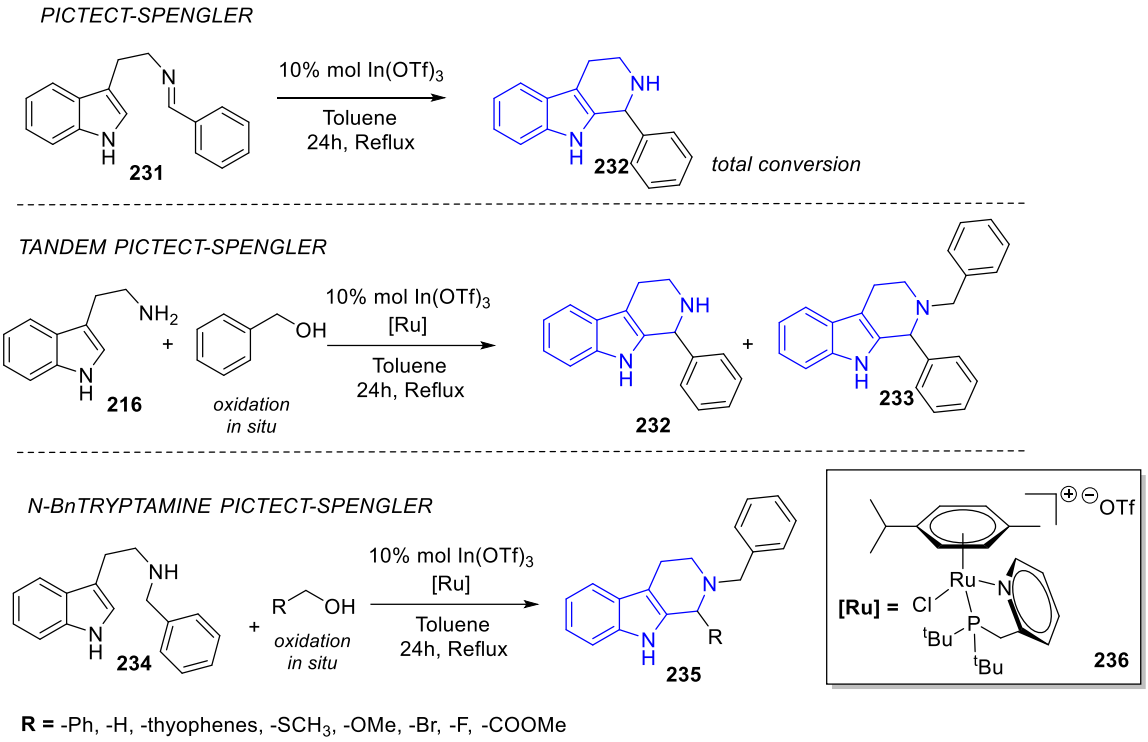


HIGH ACTIVITY[a]	MODERATE ACTIVITY	POOR ACTIVITY
$In(OTf)_3$, $Sm(OTf)_3$, $YbCl_3$, $Sc(OTf)_3$, YCl_3, $Ce(OTf)_3$, $Y(OTf)_3$, [bmim]Cl–$AlCl_3$	$InCl_3$, $Zn(OTf)_2$, $AlCl_3$, $TiCl_4$, $SiCl_4$, $Hg(OAc)_2$, $Cu(OTf)_2$, $Ho(OTf)_3$, $Tb(OTf)_3$, $Gd(OTf)_3$, $Nd(OTf)_3$, $Dy(OTf)_3$, $Eu(OTf)_3$, $Pr(OTf)_3$, [bmim][BF_4]	$Ba(OTf)_2$, AgOTf, CuOTf, $Sn(OTf)_2$, $La(OTf)_3$, $CoCl_2$, $BiCl_3$

Table 2.3.1. Activity of different Lewis Acids for Pictect-Spengler Reaction. Table reported by Srinivasan et al.

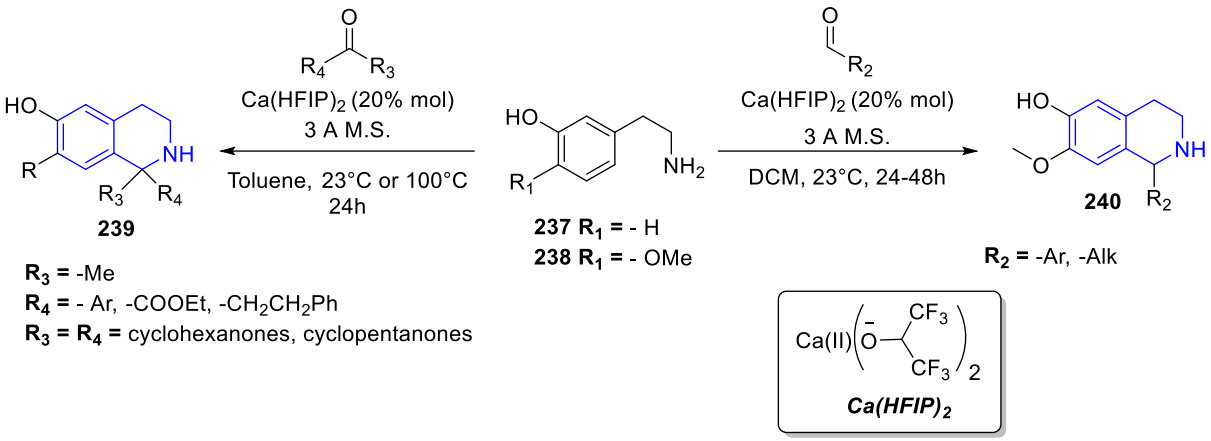
From their studies it has emerged that the triflate salts of Sc(III) (10% mol) and Yb(III) (9% mmol) were the most active catalyst for this reaction, leading respectively to a 86% and 85% of yield of THβC. Good results have been obtained by other triflate salts of Ce(III), In(III) and Sm(III). On the other hand, the chloride salts of this metal atoms have shown lower activity even with higher loading of catalyst like $AlCl_3$ (45% mol, yield 70%) and $TiCl_4$ (40% mol, yield 65%).[279]

Further, $In(OTf)_3$ has shown as good co-catalyst in a tandem process in presence of [Ru] catalyst **236**, used as hydrogen borrowing. In the protocol developed by Nalikezhathu *et al.* they observed, at the beginning, that the In(III) triflate totally converted the imine **231** to Pictect-Spengler **232** product after 24h of toluene reflux. Then, the one-pot protocol with $In(OTf)_3$/[Ru] has shown very powerfull system for the synthesis of N-benzyl substituted THβC **233**, only in presence of a large excess of benzylalcohol (10 equivalents, formation of corrispective aldehyde in situ). Performing the reaction in presence of 1.5 or 2.5 equivalents of alcohol a mixture of THβC **232** and N-benzyl-THβC **233** has obtained. Finally, the co-catalytic system has shown large susbtrates scope for the secondary tryptamine **234** obtaining different N-benzyl-THβCs **235** using several and different functionalyzed alcohols.[282]



Scheme 2.3.8. Tandem protocol for Pictect-Spengler Reaction by Nalikezhathu et al.

Among several lewis acids, the 1,1,1,3,3,3-hexafluoroisopropoxide Ca(II) salt has shown a powerful Lewis Acid for the synthesis of several tetrahydroisoquinolines by Pictet-Spengler Reaction. Catalytic amount of Ca(II) catalyst allowed to obtain the THIQs **240** through the reaction between 4-OMe substituted dopamine **238** and different aldehydes performing the reaction in DCM and room temperature after 24/48 h of reaction. Further enlargement of scope, this protocol has shown useful for the synthesis of 1,1-disubstituted THIQs **239** by the reaction between dopamine derivative **237**/3'-hydroxyphenyl ethylamine **238** and ketones, but harder conditions are required (toluene, 100°C, at least 24h reaction, 3 Å M.S.). Despite the harsh conditions, the method has shown suitable also for the synthesis of spyrocycles when cyclohexanone or cyclepentanone or their derivatives were involved (*scheme 2.3.9*).[282]

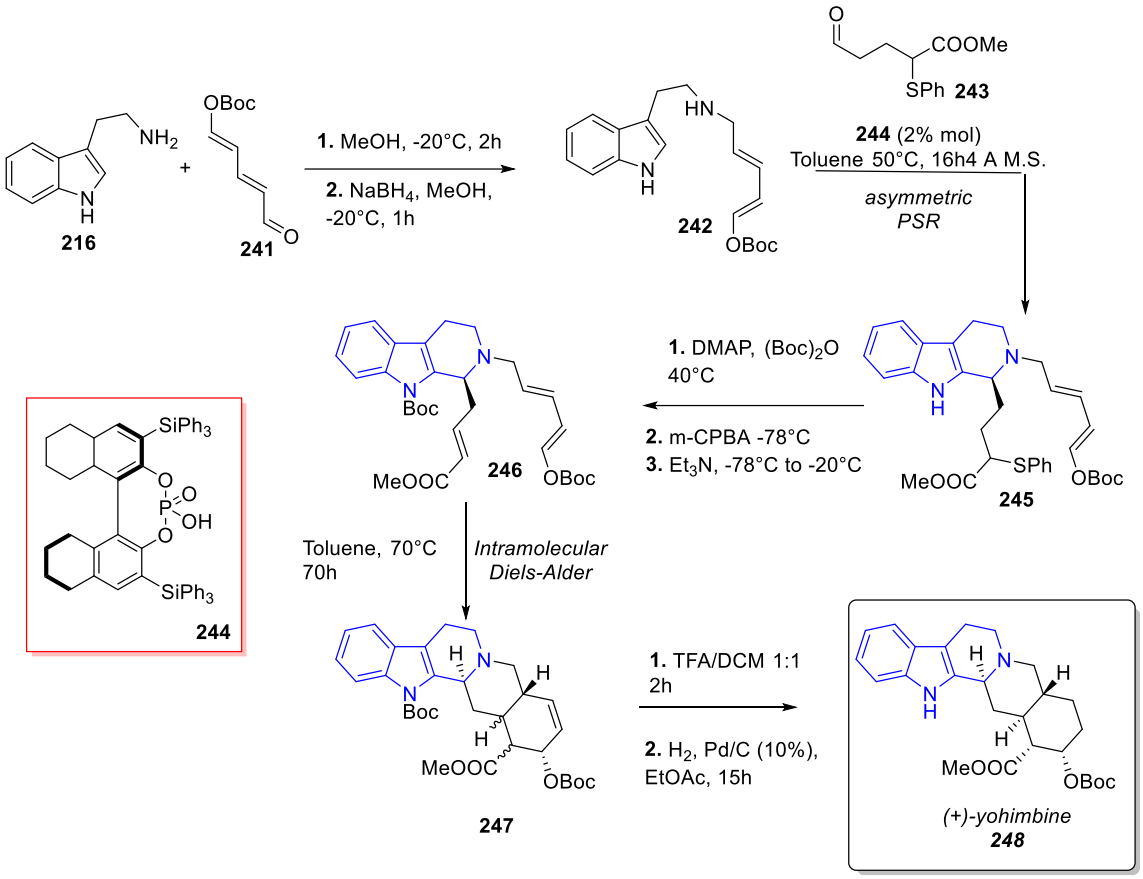


Scheme 2.3.9. Calcium catalyzed Pictet-Spengler Reaction developed by Eiden et al.

PICTET-SPENGLER IN TOTAL SYNTHESIS OF NATURAL PRODUCTS

The β-carboline and isoquinoline cores are widely present in many bioactive alkaloids present in nature and extracted from many plants and organism, so the Pictet-Spengler Reaction has demonstrated, over the years, a very powerful instrument and a crucial step for the total synthesis of many natural products with important biological activities.[267]

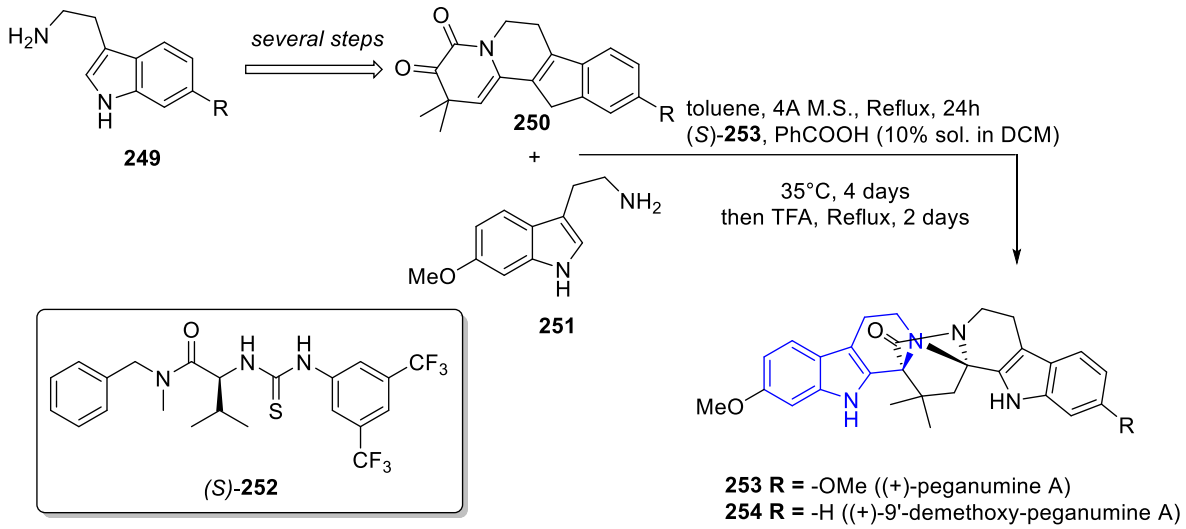
In 2011, for example, Herlè *et al.*[284] synthesized (+)-Yohimbine **248** exploiting an asymmetric Pictect-Spengler Reaction using a chiral phosphoric acid. Yohimbine (17a-hydroxyyohimban-16a-carboxylic acid methylester, YOH) is an indole alkaloid extracted from *Rauwolfia* root and it is the major component of alkaloids extracted from *Pausinystalia yohimbe* tree, nowadays it can find many clinical and biological applications.[285] Starting from tryptamine **216**, Herlè and coworkers have synthesized the secondary amine **242** by the reaction of **216** and Boc-glutaconaldehyde **241**, the subsequent PSR with **242** and aldehyde **243** (reaction performed in toluene, 50°C, 16h in presence of molecular sieves) gave the THβC **245** as a mixture of diasteroisomers. The successive two steps, that provides the Boc-protection of N-indole and remotion of –SPh, lead to the formation of intermediate **246** with e.r. of 92:8. Then the intramolecular Diels-Alder allow to obtain the yohimbine precursor **247** with 6:1 *endo/exo* products. The removal of Boc-protection and reduction of double bond gaves finally the (+)-yohimbine **248** with an e.r. > 99:1.



Scheme 2.3.10. Total synthesis of (+)-yohimbine reported by Tam et al.

The Pictet-Spengler Reaction has shown a powerful tool for the synthesis of octacylic architecture core of (+)-peganumine. Wang and his coworkers has extracted this alkaloid from *Peganum harmala*, this unique structure has shown good citotoxicity properties against cancer cell lines HL-60 (IC_{50} = 5.8 µM), MCF-7 (IC_{50} = 38.5 µM), PC-3 (IC_{50} = 40.2 µM) and HepG52 (IC_{50} = 55.4 µM).[286]

Further, the enatioselective synthesis for (+)-peganumine A **253** and its demethoxy derivative **254** was achivied by Piemontesi *et al.*[287] who synthesis these alkaloids by a enantioselective Pictet-Spengler Reaction performing the reaction in presence of benzoic acid and thiourea derivative **252** (10% solution in DCM for both acid and chiral auxiliary) in reflux of toluene, obtaining the (+)-peganumine A (69% of yield and e.r. 96:4) and (+)-9'-demethoxy-peganumine A (67% of yield and e.r. 96:4) after 6 days of reaction between **250** and 7-methoxy tryptamine **251** (see *Scheme 2.3.11.*).



Scheme 2.3.11. Total synthesis of (+)-peganumine A and its 9'-demethoxy derivatives by Piemontesi et al.

2.3b Biological Activities of Tetrahydro-β-carbolines and tetrahydroisoquinolines.

The β-Carboline is a special class of alkaloids that covers an important role in medicinal field. They posses a pyrido[3,4-b]indole ring structure and they can be divided, in accordance to the unsaturation of pyrido core, in tetrahydro-β-carboline (THβC), dihydro-β-carboline (DHβC) and full aromatizated β-carboline (βC).[288] The first β-carboline alkaloid have extracted from *Peganum harmala* in 1847, a common herbal drugs used in the Middel east and North Africa.[289] Nowadays, several sources of this class of compounds are known (i.e. fruits, tomato, beer, wine, chocolate, cocoa, et al.), and they are very attractive for their important biological properties like: antifungial, antiviral, antitumor, anti-leishmanial, antioxidant and many others.[290] In the same way, the tetrahydroisoquinolines compose a very important bioactive class of N-heterocycle alkaloids, in fact, they can be extracted from several plants and they can exert numerous biological properties too (i.e. anti-inflammatory, antiviral, antitumor, neuroprotective, antioxidant and others).[291] Here below some important properties are just cited in deeper way.

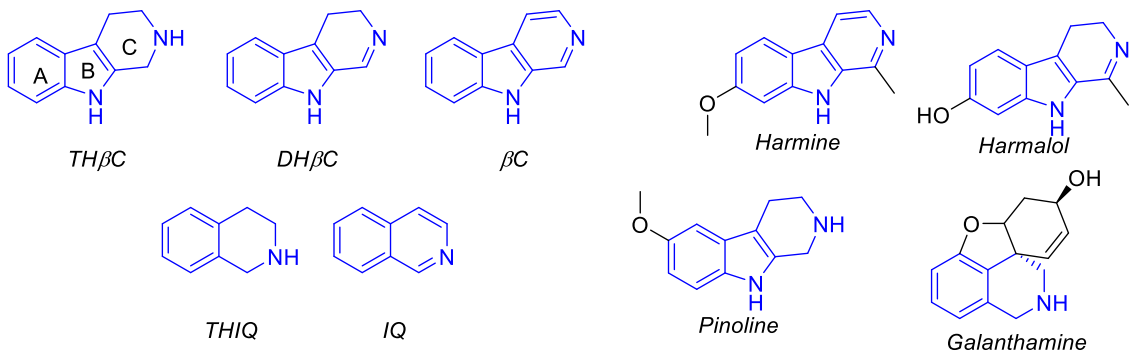
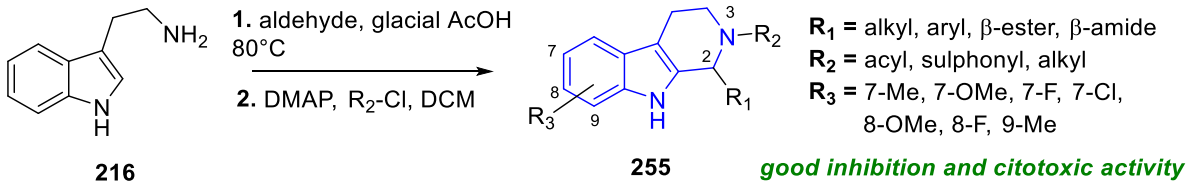


Figure 2.3.1. β-carboline and isoquinoline core of many natural compounds

ANTIVIRAL

Antiviral activity is one of most important bioactivity explicated by tetrahydro-β-carboline and tetrahydroisoquinolines. N-substituted tetrahydro-β-carbolines, obtained through two-step process in which first the THβC core **255** (see *Figure 2.3.2*), synthesized by the reaction between tryptamine **216** and p-tolualdehyde in presence of glacial acetic acid and the N-functionalization was performed in presence of DMAP and chloride compound (like acyl, aryl etc.), have shown a powerful agent against human papilloma virus. SAR (structure activity relantionship) has shown the influence of 2-/3-/7,8,9- substitutions are well tolerated and all carboline derivatives have shown good inhibition activity, sometimes very good, but only in some cases they possess good citytoxicity activity too. Amide and carbamide derivatives (3-substitution) possess high inhibition activity (IC_{50} ranging from 0.9 to 23 µM) but relative high cytotoxicity (CC_{50} from 31 over 100 µM). 3-Benzyl substitution has shown good inhibition and cytotoxicity activity. On the other hand, aldehydes (2-substituion) greatly influence the activity, in fact, for example, the THβC synthesized with isobutylaldehyde and 3-phenylpropyl chloride shown an IC_{50} about 0.33 µM and a CC_{50} about 22 µM, meanwhile using methyl glyxylate the THβC shown an IC_{50} about 20 µM and a CC_{50} over 100 µM. All indole ring substitutions (7/8/9-substituion) shown high inhibition activity (IC_{50} ranging from 0.0062 to 5.8 µM) but a low citotoxicity CC_{50} >100 µM.[292]



Scheme 2.3.12. Synthesis and SAR of anti-papilloma virus THβCs studied by Miller et al.

The tetrahydroisoquinoline alkaloids corydine and norisoboldine, hydroalcoholic extraction (70% EtOH) from *Croton echinocarpus*, has shown potential anti-HIV activity. Further partitionation of ethanolic crude between methanol and hexane were evaluated. The studies conducted on transciptase enzyme of HIV-1 (enzyme that covers an important role in replication of virus) have demonstrated that the hexane-portion did not explicate any activity, meanwhile the methanol phase was more active either of ethanolic crude (32% inhibition at concentration 100 µg/mL). Moreover norisolboldine is more potent against RT-HIV 1 than corydine, in fact it explicates an inhibition of 40% at 100 µg/mL while corydine at 450 µg/mL.[293] Moreover, piperizine-β-carbolines obtained starting from tryptophane have shown prominent anti HIV activity. The electronwithdrawing and electron-donating substituents attached on aryl ring linked to piperizine influence the activity of N-heterocycles. The 2-substitution and 4-susbtition on phenyl ring have shown the most influential positions, in fact 3-substitution and pyridine moiety enhanced the cytotoxicity without increasing the anti HIV acitivity. The (4-(4-fluorophenyl)piperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone **257** has shown the most potent anti-HIV β-carboline among the studied compounds.[294]

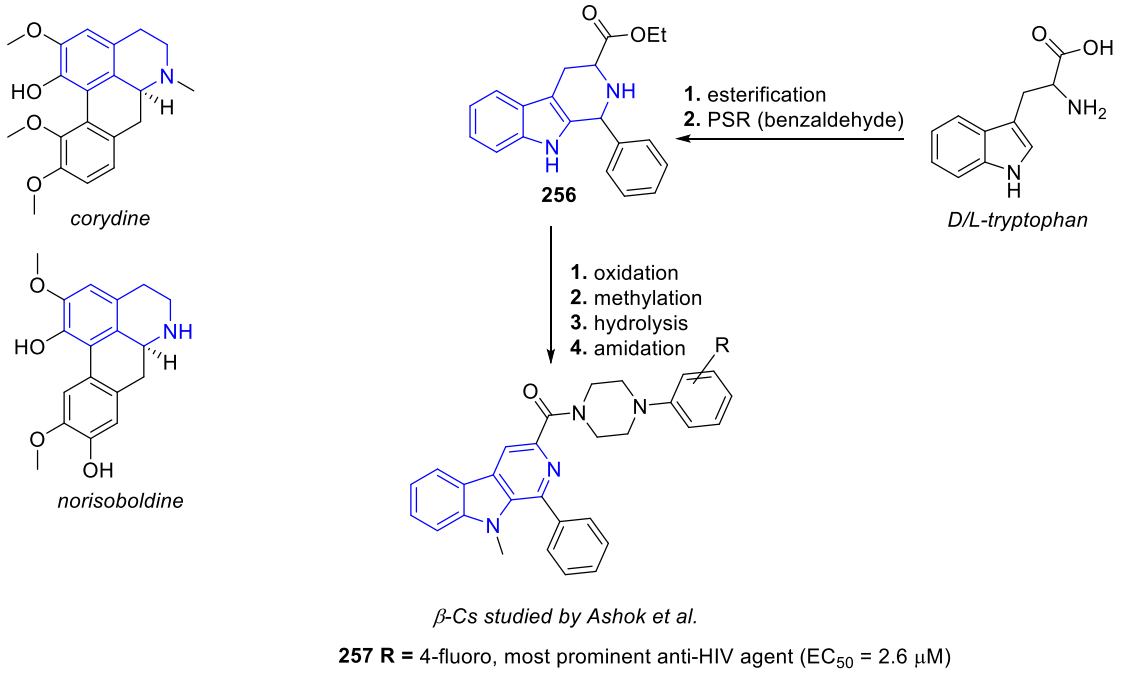


Figure 2.3.2. THIQs and βCs with anti-HIV properties

ANTICANCER

Cancer is the principal cause of deaths around of the world exceeded only by cardiovascular deaseas,[295] for this reason the development and study of natural or new chemotherapy is

always desiderable. Among alkaloids, β-carbolines and isoquinolines have shown potential anticancer agents. The study about anticancer activity of 22 alkaloids extracted from *Amaryllidaceae* (plants distributed around tropical and subtropical areas) against 17 cancer cell lines has shown that haemanthamine, lycorine and haemanthidine exerted the highest antiproliferative activity (see *figure 2.3.3.*). Haemanthamine, lycorine and haemanthidine decreased the proliferation below of 25% respectively of 12, 10 and 8 cell lines. Moreover, lycorine has shown the most potent cytotoxicity action with an IC_{50} about 0.7-1.4 μM, meanwhile the haemanthamine and haemanthidine are slightly less effective (IC_{50} respectively ranging from 0.3 – 9.8 μM and 1.3 – 9.7 μM). Unfortunately, even the promising anticancer activity *in vitro* of haemanthamine, its efficancy *in vivo* is not so marked due to the very short half-life of alkaloid inside the body (about 70 minutes).[296]

It is already known the anticancer activity against cell lines UMUC3, PACA2, MDA231, and FDIGROV of imidazolinium salts[297] and the combination of these salts and tetrahydro-β-carboline core gave birth to a new potential class of organic molecules **258** that explicate good anticancer activity.[298] These N-substituted tetrahydro-β-carboline–imidazole derivatives **258**, obtained by synthetic pathway, have demonstrate good cytotoxic activity against HL-60, SMMC-7721, A-549, MCF-7 snd SW480 cell lines with IC_{50} ranging from 2.27 μM over 40 μM. SAR data have explicated that the type of substituent of imidazolium salt is the most activity-influencer since the 2-ethyl-imidazole, benzimidazole, 5,6-dimethyl-benzimidazole and a 3-naphthylmethyl or 1-(naphthalen-2-yl)ethan-1-one at position-3 are the most active compounds.

Shancaraiah *et al.*[298] have designed a series of tetrahydro-β-carboline-hydantoin hybrids structurally similar to hexahydro-imidazo[1,5-b]-β-carboline-1,3-dione (HR22C16) **259** (compounds **260a/260b),** already known for its inhibition of Eg5 (kinase-type enzyme that plays a crucial role in the development and function of the mitotic spindle and controls mitosis through bipolar spindle formation and chromosome separation). These new heterocycles were obtained starting from L-tryptophane, which after a PSR with benzaldehyde derivatives catalyzed by H_2SO_4 (0.1N conc and reflux), and successive reaction with isocyanates and inversion of configurations. The *in vitro* cytotoxicity against A549 (lung adenocarcinoma), ME-180 (cervical carcinoma), HeLa (cervical carcinoma), MCF-7 (breast adenocarcinoma), and PC-3 (prostate cancer) were evaluated and these compounds explicated highes cytotoxicity against PC-3 cell lines (p-Cl-phenyl derivatives is the most active), even if high anticancer activity was recordered against A549 and MCF-7 too. Moreover, all tested compounds have expressed an IC_{50} lower than 100 μM against HeLa cell lines following, in general, the five

Lipinski's principles. The position and type of substituent attached to hydantoin moiety greatly influence togheter to the configuration of stereogenic centre, the anticancer activity of substrates.

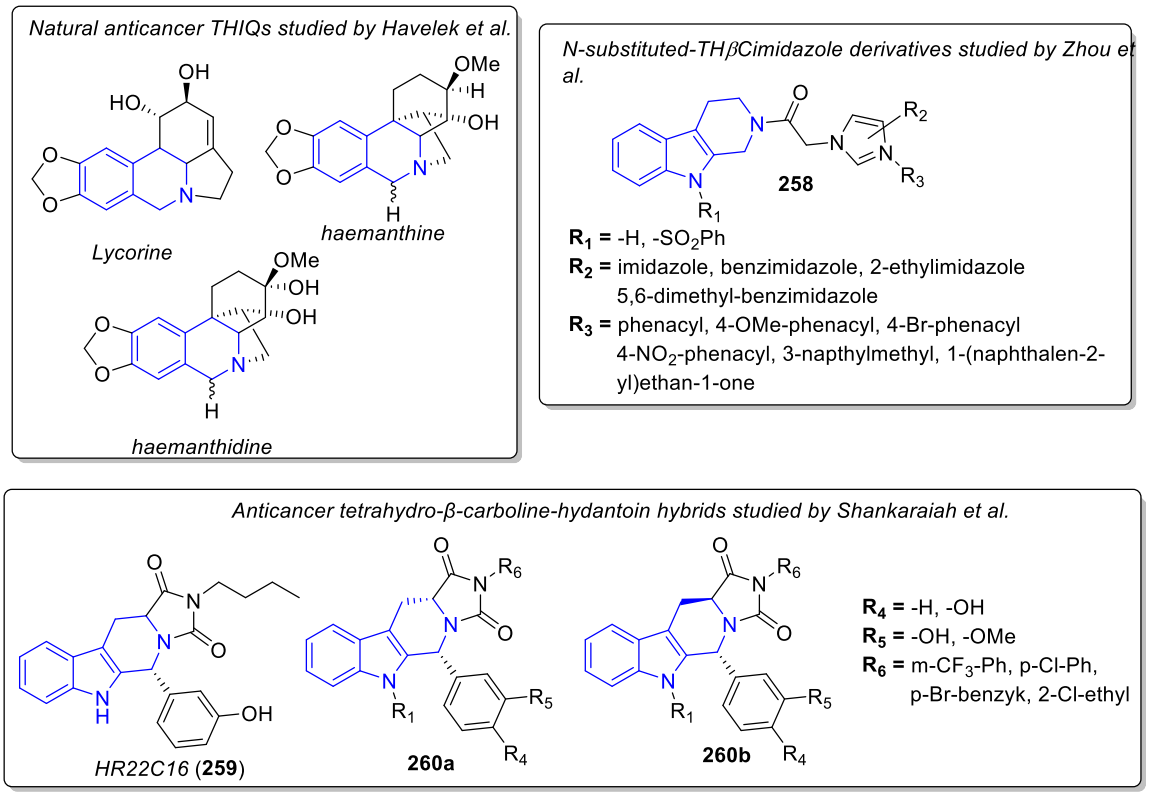
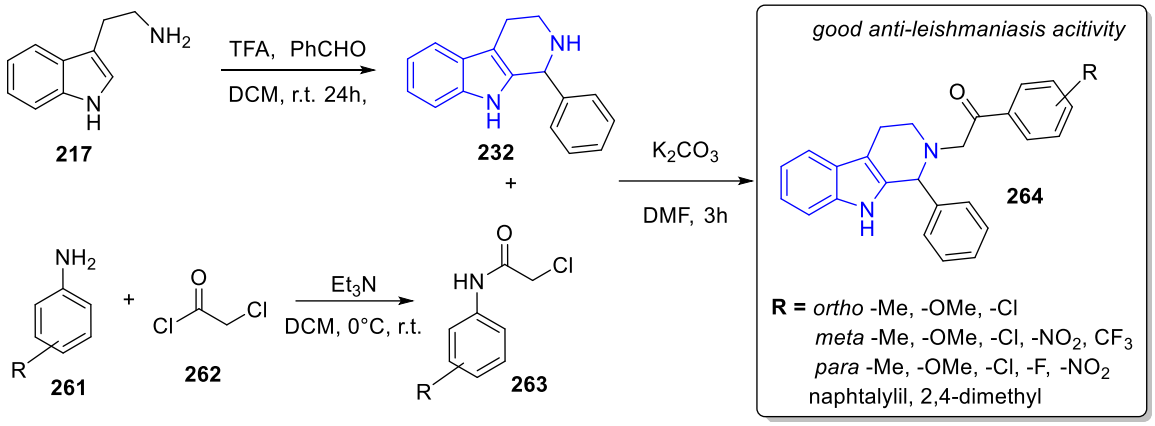


Figure 2.3.3. THIQs and THβCs with anticancer activities.

OTHER BIOACTIVITIES

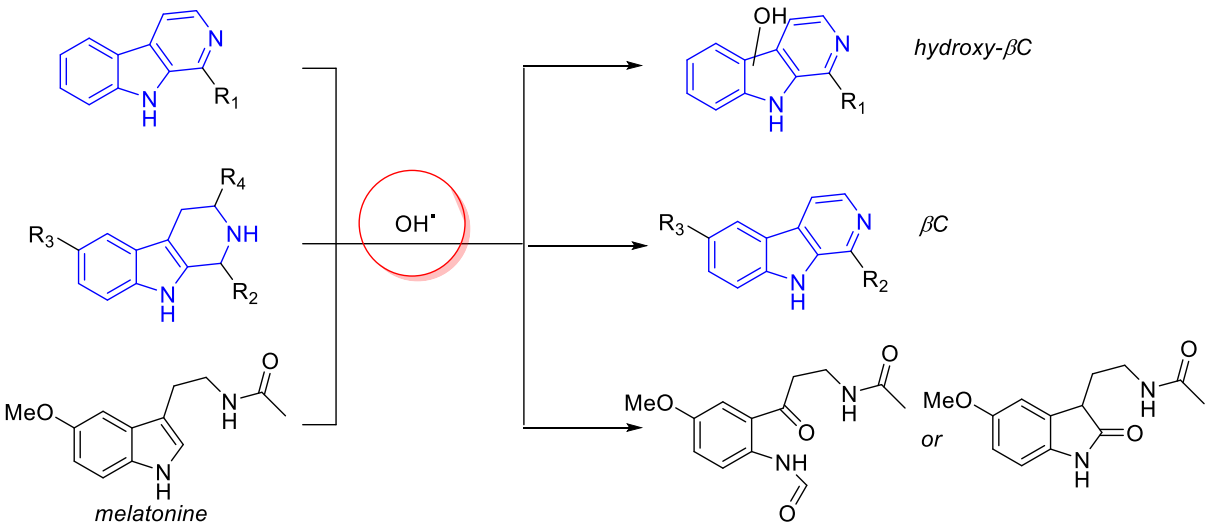
These alkaloids, together with the antiviral and anticancer activity, can explicate several others biological properties. For example, 3-substituted tetrahydro-β-carbolines have shown good anti-leishmaniasis agents (anti-parasitic activity). Ashok *et al.* synthesized and studied several THβCs (**264**) obtained by a convergent strategy in which the THβC core **233** was synthesized by tryptamine **217** and benzaldehyde through a TFA catalyzed PSR, and the substitution was performed in presence of K_2CO_3 in DMF using an alkyl chloride **263** obtained by the reaction between aniline derivatives **261** and chloroacetylchloride **262** (see *figure 2.3.3.*). All synthesized derivatives have shown potent inhibition activity against promastigotes of *L. infantum* (IC_{50} ranging from 1.99 to 20.69 µM, lower activity respect to anti-leishmaniasis drug amphotericin B that has a IC_{50} 0.77 ± 0.15 µM) and amastigote of *L. infantum* (IC_{50} ranging from 0.67 to 6.46 µM). The position of substitutents in amide-phenyl ring influences the activity of final products. From SAR analyses, it emerged that the type of substitutent greatly influences when it is in *ortho* position, while *meta* substitution does not influence so much the activity. On

the other hand, nitro group in *para* position leads to the formation of a potent anti-leishmainiasis β-carboline.[300]



Scheme 2.3.13. Synthesis and SAR of anti-leishmaniasis THβCs studied by Ashok et al.

Herraiz and Galisteo evaluated the antioxidant acitivity of different THβCs as hydroxyl radical scavenger generated in situ by Fenton Reaction.[301] They noticed that norharman, harman, THCβ, MeTHβC, pinoline and melatonin inhibited the OH· in dose dependent way, showing also good scavenger activity in presence of pro-oxidant like ascorbic acid or 6-hydroxydopamine. On the other hand, the acid derivatives **270** and **271** have shown low or no anti-oxidant acitivity. Depending on the dregradation of the substrate (benzoate, 2-deoxyribose, 2'-deoxyguanosine) these alkaloids explicated a IC_{50} about 20~40 μM in case of benzoate, except for **270** and **271** that shown no activity; meanwhile higher carbolines concentrations were required in other two cases of deoxyribose and 2'-deoxyguanosine degradation (about 180~600 μM, slightly higher in presence of prooxidant). The defence mechanism is different for each type of substrate. In case of full aromatized β-carbolines, the formation of hydroxyl-product was detected at HPLC-ESI MS; tetrahydro-β-carbolines are oxided to β-carbolines; melatonine, instead, is transformed to oxindole or amide after cleavage of indole ring.



R_1 = -H (norharman **265**)
R_1 = -Me (harman **266**)
R_2 = R_3 = R_4 = -H (THβC **267**)
R_2 = -Me, **R_3 = R_4 =** -H (MeTHβC **268**)
R_2 = -H, **R_3 =** -OMe, **R_4 =** -H (pinoline **269**)
R_2 = **R_3 =** -H, **R_4 =** -COOH (TCA **270**)
R_2 = -H, **R_3 =** -Me, **R_4 =** -COOH (MTCA **271**)

Scheme 2.3.14. Antioxidant action of melatonine, β-carbolines and tetrahydro-β-carbolines

A study conducted in 2010 has revealed that a series of mono ammonium β-carbolines/THβCs and bivalent ammonium β-carbolines/THβCs (linked by an alkyl spacer) explicate moderate or strong inhibition activity of acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and NMDA receptor blockers, proving as potential drugs for Alzheimer's disease treatment. Disfunction of AChE and BChE leads to lose of memory and causes cognition disturbances. In the same way, an extraconcentration of (*S*)-glutamate (mediator normal physiological excitatory synaptic transmission) in extracellular enviroment influences the NMDA activity, that allows the entrance of Ca-ions in neuronal cells forming free radicals that cause brain damages (excitoxicity). This mechanism is probably the cause of many other neurodegenerative disorder like Morbus Parkinson, Huntigton disorder, epilepsy, etc. All tested N-heterocycles shown an IC_{50} of nM order for AChE and BChE inhibition, for what concern the NMDA inhibition, it requires an higher concentration of μM order; the bivalent ammonium β-carbolines/THβCs shown the most potent inhibition action.[302]

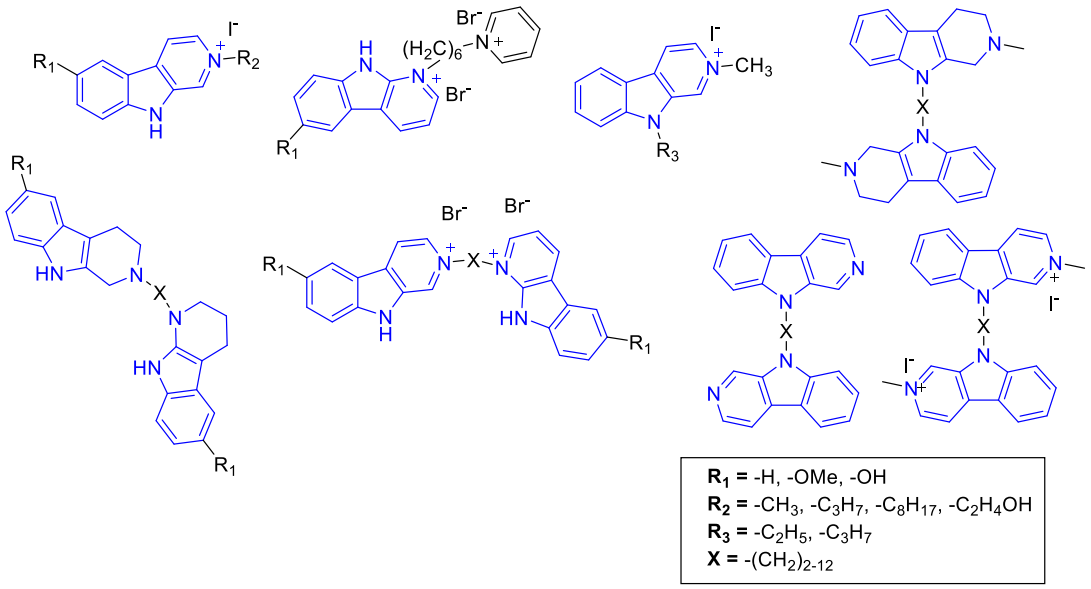


Figure 2.3.4. ammonium βCs and THβCs studied for AD treatment by Rook et al.

Finally, Song *et al.*[303] studied natural and new synthesized βCs, DHβCs and THβCs against Tobacco Mosaic Virus (TMV) and 14 types of fungi. From their studies, natural harmane, harmalan, tetrahydroharmane, tetrahydroharmine and new derivative **272** exhibited higher inhibition than Ribavirin (common commercial anti-TMV). DHβCs and THβCs showed higher activity respect to the full aromatic compounds; in fact, harmalan, tetrahydroharmane, and tetrahydroharmine and **272** explicated an inhibition respectively of 58.6%, 63.7%, 58.2%, 46.5% at 500 µg/mL (at the same concentration Ribavirin explicated 40% of inhibition) and also good curative and protection activity (*in vivo* analyses). On the other hand, full aromatized compound explicated better antifungi activity than to partial/total saturated carbolines, the synthesized compounds **273**, **274** and **275** shown the best antifungi activity against all 14 types, even higher to the natual carbolines.

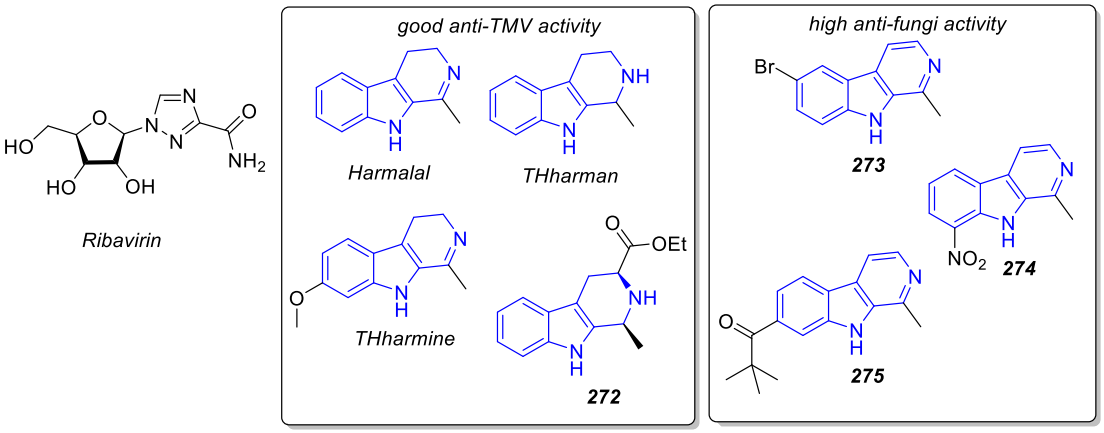


Figure 2.3.5. βCs, DHβCs, THβCs with anti-TMV and anti-fungi activity.

2.3c Microwave irradtion in organic synthesis

The first MAOSs (Microwave Assisted Organic Synthesis) were reported in 1986 in which, Giguere[304] and Geyde[305] performed a series of organic synthesis, in closed vessel, under microwave irradiation generated from commercial microwave oven. They suddenely observed the incredible power toward speed-increament of microwave irradiations. This increbile rate enhancement is due to transfer of energy from microwave irradiation to the system and this "propensity to microwave heating" depends by two main factors: dielectric loss factor (e", it represents the ability of a substance to transform this energy into heat) and the dielectric constant of media (e', it represents the ability of a substance to absorb microwaves). The whole phenomen is described by dissipation factor (tan d = e"/e') and a high dissipation factore means high susceptibility to microwave. Moreover, microwave region radiations enables to heat entire sample without any major temperature gradient. This heating mechanism depends, not only by dielectric properties of a given material, but it is influenced also by specific heat capacity, the emissivity, the geometry, the volume (or mass) and by the strength of the applied field. These factors are characteristic of microwave-radio wavelength radiations since other forms of electromagnetic radiation have a too small penetration depth and/or limited thermal conductivity (like infrared irradiation).[306]

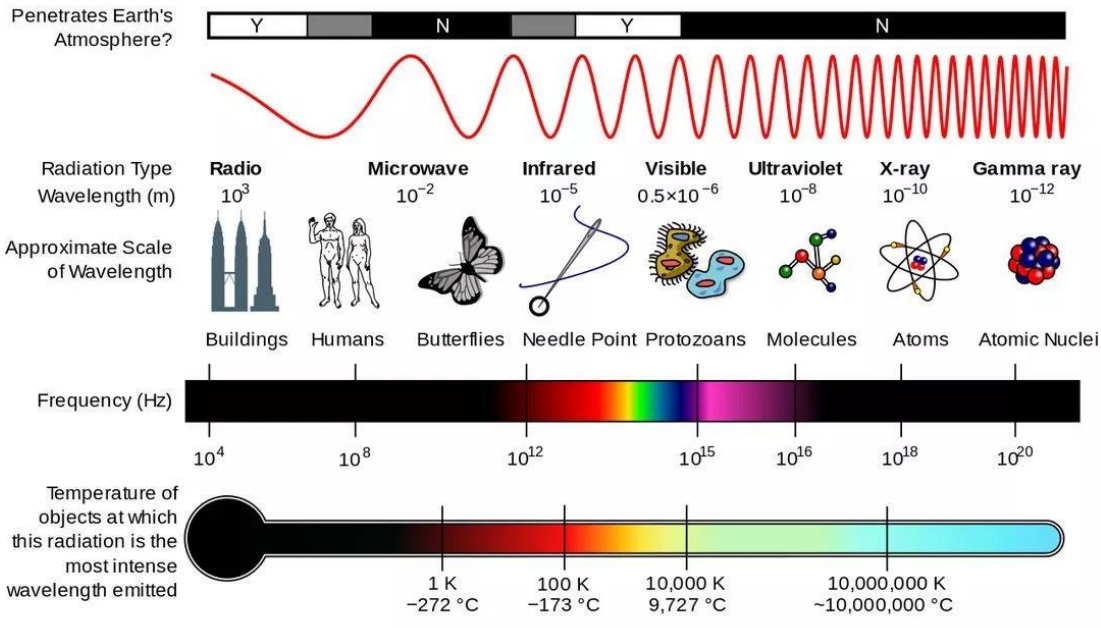


Figure 2.3.6. Electromagnetic Spectrum

Profoundly and different interactions are involved in microwave heating and conventional. In fact, microwave heating possesses an highly selectivity toward different materials dependent by the properties of different materials, as the transformation of energy into heat takes place at

molecular level by interaction between electromagnetic radiation and molecules; meanwhile the conventional heating is strongly affected by efficiency of heat distibution inside the sample.[307]

Microwave Heating	Conventional Heating
Energetic Coupling	Conduction/convection
Couplig at molecular level	Superficial heating
Rapid	Slow
Volumetric	Superficial
Selective	Non selective
Depend on material properties	Less dependet

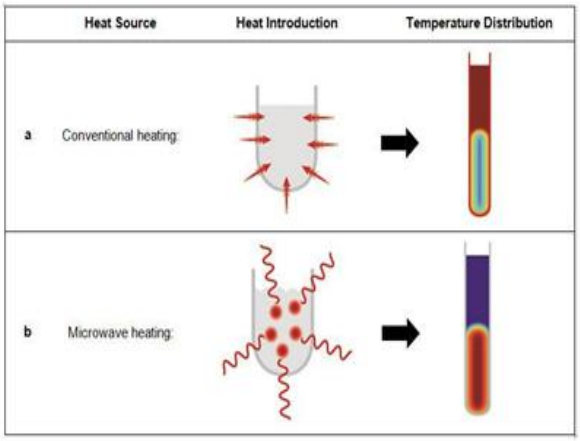


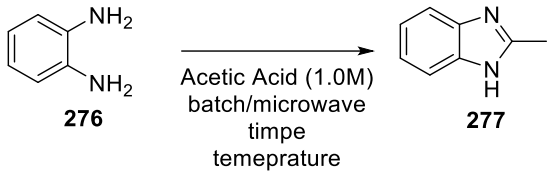
Figure 2.3.7. On the right Table 2.3.2. in which features of microwave/conventional heating are reported; on the left the temperature distribution in microwave/conventional heating (image took from reference[308]

The reactions performed under microwave radiations present some and important advantages like faster reaction time, higher returns, higher levels of purity of the products, best reproducibility, better control of the reaction and milder reaction condtions. Not all these improvements caused by microwave radiation can be explained by only one motivation, but by a series of factors called "microwave effect":[308 - 310]

- Thermal effect; it depends by the dielectric characteristic of different materials and it describes the ability of given material to convert energy into heat;[306]
- Overheating; ability to perform the reaction at higher temperature than boiling point of solvents;
- Hot Spots; they are caused by inhomogeneity of applied field and they are spots with a higher temperature respect to the whole system (not rappresentative);
- Selectivity toward solvents, catalysts, reagents, products;
- Non-thermal effects (also called microwave effects); they arise from the interaction of radiation and material and they are under controversial debates;
- No wall effects; elimination of invert gradient temperature due to conventional heating.

Nowadays the microwave irradiations are applied in many organic synthesis. Just to cite few examples, Damm *et al.* studied the synthesis of benzyimidazole **277** (heterocyclic core widely present in many bioactive compounds) starting from 1.0 M acetic acid solution of

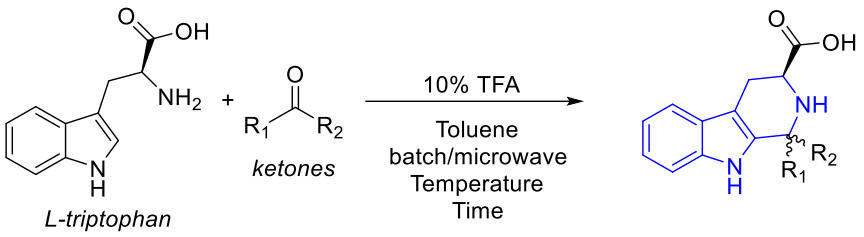
o-phenylendiamine **276** comparing the kinetic of conventional batch conditions and microwave conditions (using 400 W Initiator Eight 2.5 EXP platform (Biotage AB) as instrument). Performing the reaction at different temperature, starting from 25°C, they observed that the full conversion of starting material was achievied after 9 weeks. Increasing the temperature, the total conversion of starting material (SM) is achievied only after 5 hrs reaction (100°C), but performing the reaction at 130°C under microwave irradiation the SM was consumed after only 1 hr and no SM was detected after 1s of reaction at 270°C reaction.[311]



Temperature	Batch/microwave	Pressure	Time
25 °C	Batch	-	9 weeks
60 °C	Batch	-	3 days
100 °C	Batch	-	5 hrs
130 °C	Microwave	2 bar	1hrs
160°C	Microwave	4 bar	10 min
200 °C	Microwave	9 bar	3 min
270 °C	Microwave	29 bar	1 s

Table 2.3.3. Kinetic influence of microwave irradiation on the synthesis of benzymidazole reported by Damm et al.

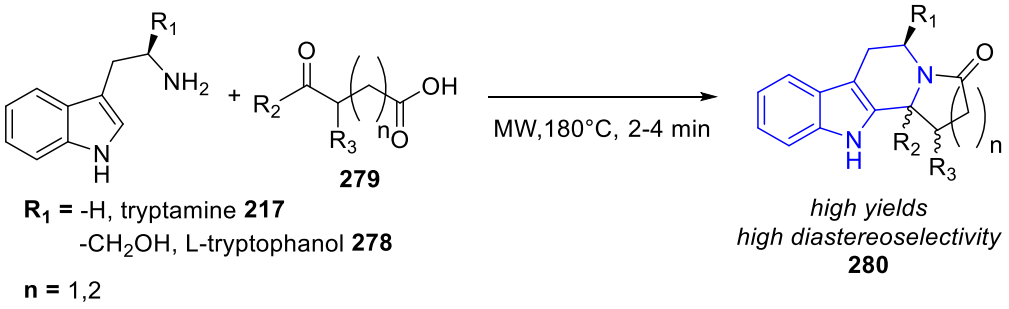
The Pictet-Spengler reaction between L-triptophan and ketones can be smoothly and cleanly enhanced by microwave irradiation. In fact, Kuo *et al.*[312] compared the time, diastereoselectivity and yield of batch/microwave reactions performed in presence of different ketone substrates at room temperature or 60°C. They observed that the room temperature and 60°C batch reactions, even if leads to high yields, their kinetic were tremendously slow, but performing the reaction under microwave heating, they were able to obtain the products with comparable yields in shorter time. Reactions that require days/several hours, under microwave irradiation, could be perfomed only in few hours obtaining good results. Performing the reaction at 100°C other side-reaction were favoured and leads to degradation of substrates lowering the overall yield of reaction.



	Room Temperature			Batch 60°C			Microwave 60°C		
Ketones	**Time**	**Yield**	**d.r.**	**Time**	**Yield**	**d.r.**	**Time**	**Yield**	**d.r.**
acetophenone	49d	90%	20:80	95h	96%	25:75	40h	67%	23:77
2-butanone	18h	74%	51:49	3.5h	99%	55:45	20 min	96%	50:50
3-methyl-2-butanone	11.5d	87%	68:32	62h	86%	60:40	15h	76%	65:45
3-pentenone	5.5d	96%	-	52h	99%	-	3.3h	91%	-
cyclohexanone	6h	68%	-	75 min	99%	-	10 min	99%	-
cyclopentenone	2.5d	95%	-	55 min	99%	-	20 min	99%	-

Table 2.3.4. Influence of microwave irradiation on Pictet-Spngler Reaction between L-tryptophan and ketones reported by Kuo et al.

More recently, Jida *et al.* developed a protocol in which they synthesized several 4-cycles fused compounds **280 i**n which a THβC moiety was present. These N-heterocycles **280** were obtained in solvent and catalyst-free conditions under microwave irradiation starting from tryptamine **217**/L-tryptophanol **278** and ketocarboxylic acids **279**. With respect to batch conditions that require 48h of reflux in toluene with a Dean-Stark apparatus, using "microwave pathway" it is possible to obtain the corrispective substrates in only 2-4 minutes at 180 °C with high yield and high d.e. (<99%) when tryptophanol is involved. In accordance to authors, the whole process proceed by two steps in which the first step is a Meyers' reaction and the second one a PSR.[313]



Scheme 2.3.15. Pictet-Spengler Reaction protocol developed by Jida et al.

2.3d Graphene Oxide in Organic Synthesis

During the last years, Graphene Oxide (GO) and Carbonaceous materials have shown promising as carbocatalysts for developing new green and suistainable pathways.[314] The intrinsic Brønsted acidity of GO is due to the presence of several alcohol, ketone, epoxide and carboxylic moieties attached to 2D layers of graphene and nowadays it is applied as catalyst for several organic reactions (i.e. Fischer reaction, Kabachnik-Fields, transamidation, etc.).[314] One of the most advantages of Graphene Oxide is its dispersionability in solvent media and for this aspect it can find several applications in different fields (i.e. electronic devices, energy storage devices, biosensors, biomedical application, coating technology) in addition to organic synthesis. These heterogeneous catalyst possess some green properties like high stability, safety, insolubility and recyclability that make it a very versatile catalyst.[316] Graphene oxide can be generally prepared by the oxidation of graphite using strong oxidant agents like $KMnO_4$, $NaNO_3$, $KClO_3$ and H_2SO_4, then, the successive exfoliation gives the GO-sheets.[317]

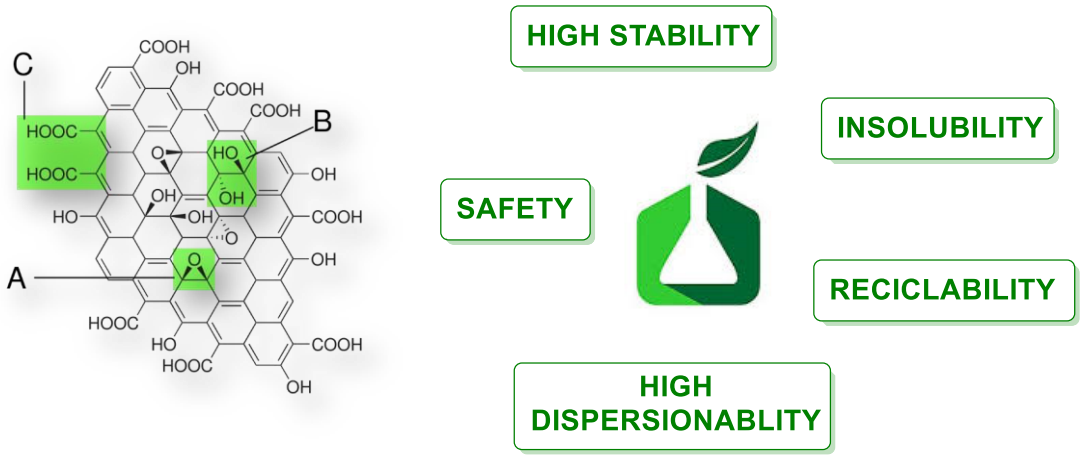
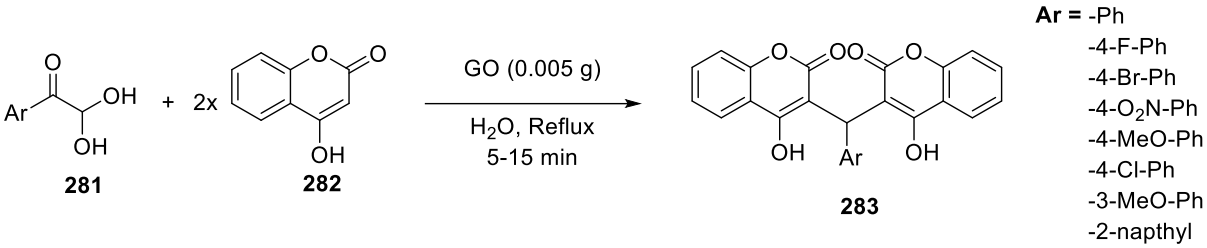


Figure 2.3.8. On the left the structure of GO and on the right the green properties of GO

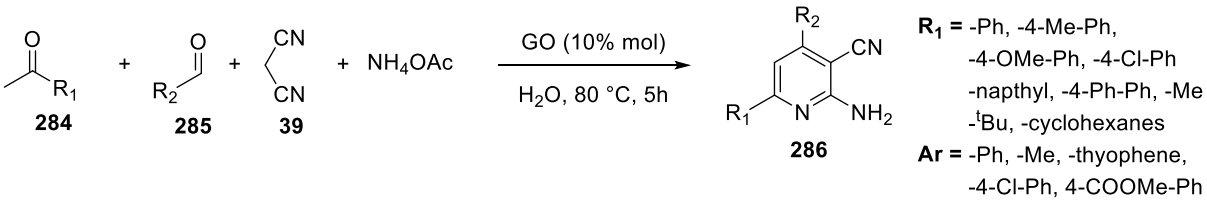
Graphene Oxide has shown very usefull for the synthesis of compounds **283** through the condensation between glyoxal derivatives **281** and 4-hydroxycoumarins **282**; performing the reaction in H_2O-reflux and in presence of catalytic amount of GO-nano sheets leads to the formation of product in only 10 minutes. Nano-particles of Fe_3O_4, ZnO and TiO_2 were less effective than GO, and performing the reaction in other polar solvent like MeOH, EtOH and THF similar but lower results were obtained, meanwhile DCM has shown the worse solvent in which perfom the reaction (50% yield against 83% in H_2O). Moreover, the method shown good scope of reaction with different substrates and graphite and partially oxidized GO possess lower

activity, and NaOH-deactivated graphene oxide shown no activity after 30 minutes of reaction, confirming the importance of acidic moieties present in GO-nanosheets.[314]



*Scheme 2.3.16. Synthesis of dicoumarol **283** by Khodabakhshi et al.*

GO has shown as an efficient catalyst for the synthesis of 2-amino-3-cyanopyridines through one-pot pathway (Hantzsch like process) in very mild conditions conserving its catalytic activity for consecutive 5 runs (passing from an yield of 90% to 86% after fifth run). In the protocol developed by Khalili, the pyridine derivatives **286** were achievied performing the reaction in water at 80 °C using malonitrile **39**, acetonephenone substrates **284** and other aldehydes **285**, ammonium acetate (as nitrogen source) in presence of 10% mol of GO in an open flask after 5h of reaction. The presence of catalyst is foundamental since perfoming reaction in catalyst-free condition the product was isolated with a yield < 10% after 24h reaction. The Graphene Oxide has shown more efficient respect other heterogenous catalyst like graphite, reduced-GO, Silica, H-β zeolite or metal oxide like TiO_2. Different pyridines were isolated using various ketones and aromatic aldehydes with yields ranging from 75 to 90%.[318]



Scheme 2.3.17. GO-catalysed synthesis of 2-amino-3-cyano pyridines by Khalili

2.3e Amberlyst-15® in organic synthesis

Amberlyst-15® (Amb-15®) is a commercial proton exchange resin of polystyrene in which acid sulfonic groups are attached to phenyl ring of styrene moiety; it is considered as a strong acid and it can be easly removed from by reaction mixture and it can be reactivated and reused reseveral times.[319] This resin has attracted the scientific field for its unique and green properties like environmental compatibility, reusability, non-corrosivness, chemical and physical stability, non-toxicity.[320]

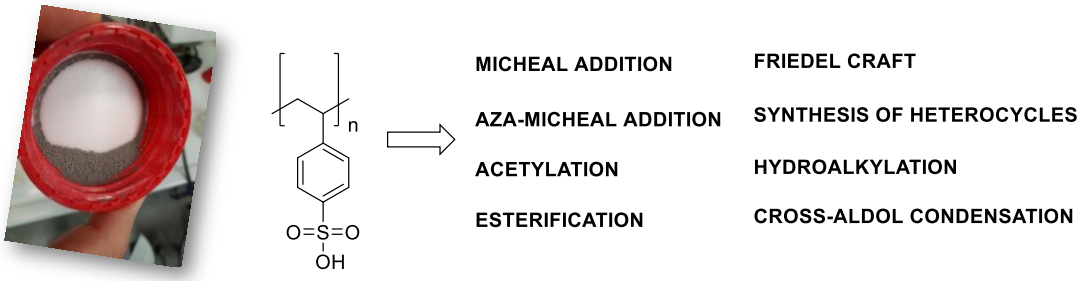
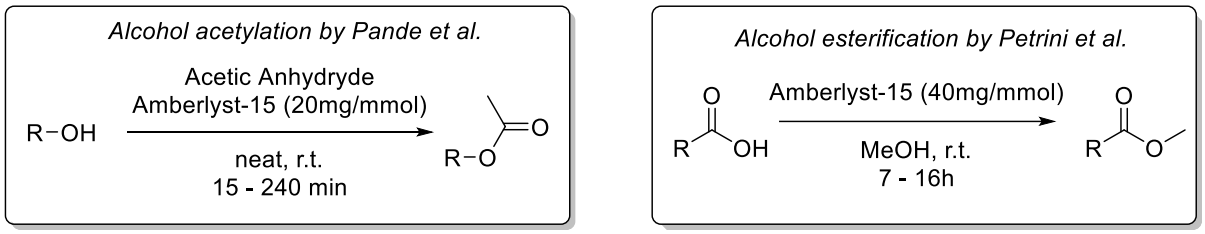


Figure 2.3.11. Structure and image of Amberlyst-15® and some reaction catalysed by Amb-15®

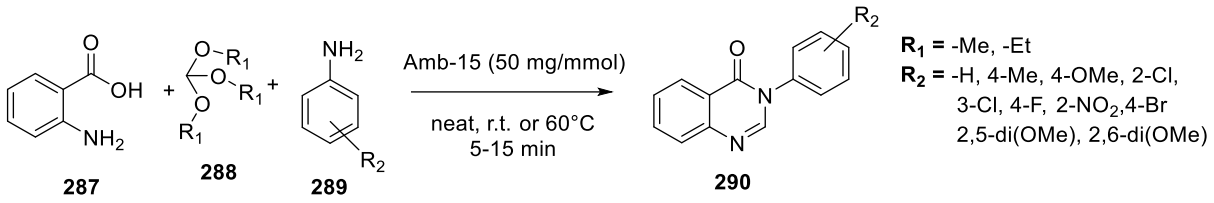
Due to these unique properties, amberlyst-15® has found several application in many different reactions (i.e. Micheal/aza-Micheal addition, Friedel Craft reaction, metal-free hydroarylation, synthesis of heterocycles, etc.).[319] Just to cite few reaction, acetylation[320] and esterification[164] of alcohol performed in presence of amberlyst-15® can be performed in mild and selective conditions.



Scheme 2.3.18. Alcohol acetylation reported by Pande et al.; alcohol esterification reported by Petrini et al.

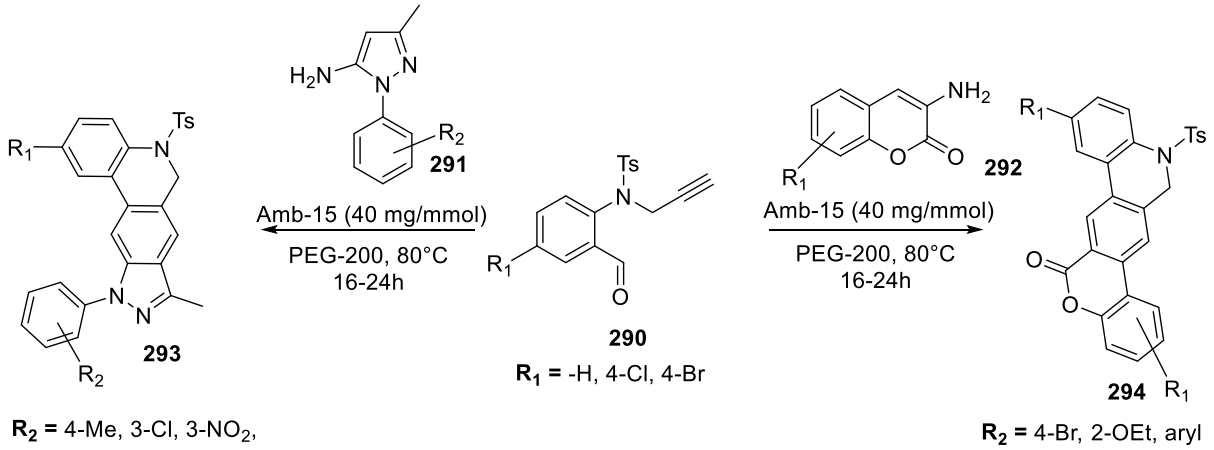
More recently, the esterification of free fatty acids (FFAs), using ethanol as alcohol counter-part, performed in presence of Amb-15®, has shown a green pathway for the synthesis of biodiesel performing the reaction at 60 °C with a FFA/EtOH molar ratio 1:1 with a yield of 53% after 6h of reaction.[319]

Amb-15® has shown a powerful catalyst for the one-pot synthesis of 4(3H)-quinazolines **290** (broad spectrum of biological properties) starting from anthranilic acid **287**, orthoformates **288** and substituted anilines **289**. The method provides a series of quinazolines in solvent free-conditions obtained at room temperature after few minutes reaction with high yield, except when nitroaniline and 2,5-dimethoxyaniline are used (the reaction was performed at 60°C). Moreover, the method provides a very broad reaction aims and it smoothly works with several anilines.[322]



Scheme 2.3.19. Synthesis of quinazolines by Das et al.

Muthukrishnan *et al.*[323] exploited in 2019 the employment of Amberlyst-15® as catalyst for the synthesis of complex heterocycles in which a [1,6]-naphthyridine (compounds **293**/**294**) moiety was present using 2-(N-propargylamino)-arylaldehydes **290** and 3-aminocoumarins **292**/3-methyl-1-aryl-1H-pyrazol-5-amines **291** as starting material by a Povarov-type reaction. The ion resin was more efficient than a common Lewis Acid (CuCl) in a green solvent like PEG-200 at 80°C (67% after 24 hrs against 80% of yield after 16 hrs of reaction), but performing the reaction at higher temperature (100°C) or in other solvents only worse results were detected. Testing N-propargylamines and coumarins it was possible to isolate many different products after 16hrs-24hrs of reaction with good yields (ranging from 67% to 81%) in case of coumarines. For what concern pyrazol amines, the products were obtained with yield ranging from 60 to 81% but with slower reaction (24 hrs – 30 hrs of reaction).



Scheme 2.3.20. Synthesis of [1,6]-naphthyridines by Muthukrishnan et al.

2.3f Results and Discussion

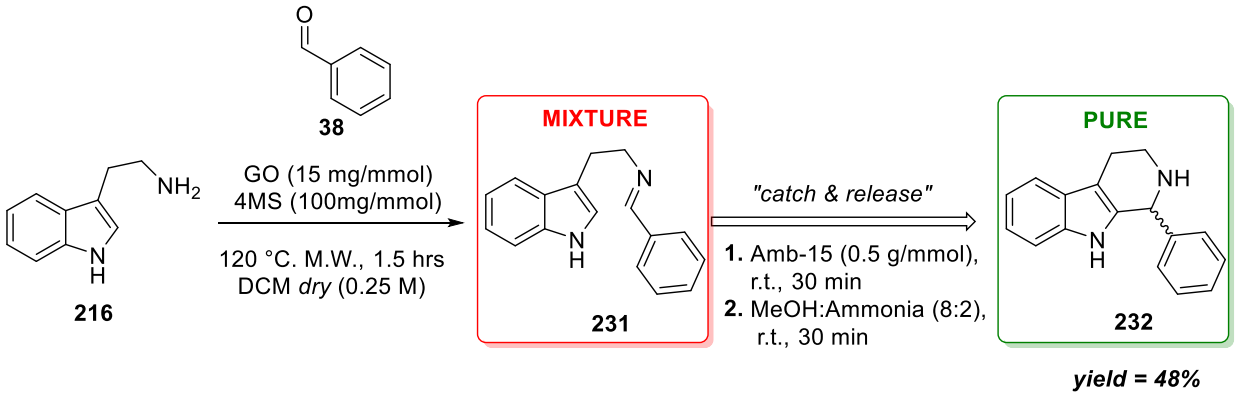
BACKGROUND OF THE PROJECT

Due to the biological importance of tetrahydro-β-carbolines, tetrahydroisoquinolines and N-substituted tetrahydro-β-carbolines we tried to develop new synthetic pathways for the synthesis of these aza-heterocycles exploting the Brønsted and Lewis promotion of Pictet-Spengler Reaction. In fact, this chapter is divided in other two sections:

- Brønsted catalyses; we investigated the possible employment of Graphene Oxide as green and suistainable Brønsted acid for PSR performing the reaction under microwave irradiation, exploiting Amberlyst-15® as purification tool. The focus of this part is to replace the common Brønsted and harmful acids like TFA, H_2SO_4, HCl and so on with the purpose to speed up the reaction by using microwave.
- Lewis catalyses; we investigated the possible employment of $CeCl_3 \cdot 7H_2O$ as green metal catalyses in PRS, studying in deep a one-pot pathway for the synthesis of N-substituted THβCs under microwave irradiation. In fact, these heterocycles are usually synthesied by two step process in which: first, THβC is synthesized and then substitution is performed, the one-pot protocol has the scope to avoid the formation of much waste and saving time for the synthesis of these molecules.

BRØNSTED CATALYSES

Since mineral acids are often used as catalysts in PRS, we wanted to investigate Graphene Oxide as new Brønsted acid for this reaction. Our investigations started on two step process using tryptamine and benzaldehyde as model system: in the first step we performed the reaction between tryptamine **216** and a stoichiometric amount of benzaldehyde **38** in DCM *dry* (0.25M) in presence of catalytic amount of Graphene Oxide (15 mg/mmol) at 120 °C under microwave irradiation. The second step provides the final THβC **232** after an extraction process called "catch & release" in which the acid functionalities of Amb-15 react with basic ones of our target product forming an ammonium salt, then, washing the resin with a basic solution (8:2 of MeOH:33% v/v ammonia in H_2O) the N-heterocycle is released in the solution. This solution is diluted with DCM and portionated with a sat. $NaHCO_3$ solution. Extraction of the aqueous phase provided our target product. After 1.5 hrs in a microwave reactor, the tryptamine **216** is totally consumed and after the "catch & release" process the final product was obtained with a yield of 48%.



Scheme 2.3.21. First approach for 2-steps protocol for GO-PSR

Studying in deep the process by GC-MS and NMR analysis we discovered that the first reaction step stops after the formation of the imine and the final heterocycle is obtained only after the reaction with Amberlyst-15®.

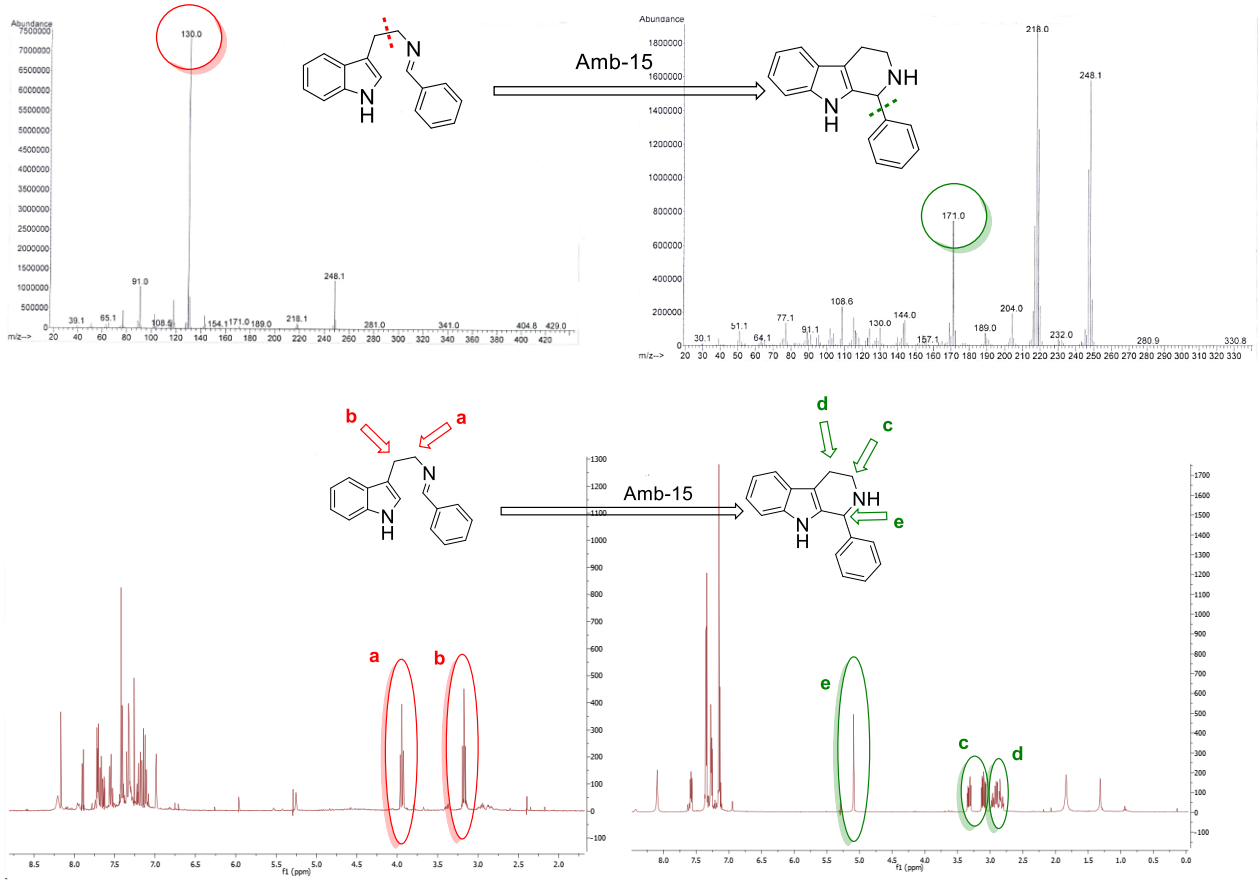
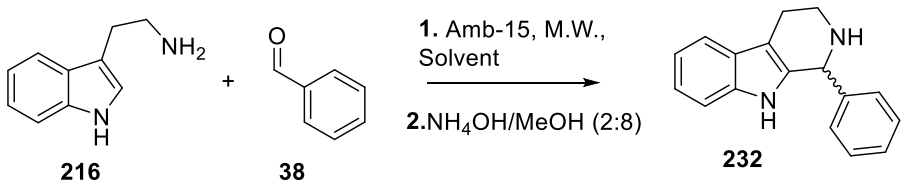


Figure 2.3.9. GC-MS and NMR analyses of 2-steps protocol

By GC-MS it is possible to notice the difference between the two spectra, the imine spectrum shows a 130 m/z as base peak that can be referred only to the imine due to the high stability of formed fragment. On the other hand, the product presents three typical peaks in its spectrum: 248 m/z (molecular weight), 218 m/z (loss of ethane) and 171 m/z (loss of phenyl ring derived from benzaldehyde). Moreover, the imine presents two triplets at around 3.87 ppm (proton **a**) and 3.18 ppm (protons **b**); meanwhile the corrispective $–CH_2$ signals in THβC split in a more complex pattern (caused by the coupling with a vicinal stereocenter). Formation of the product is underlined also by the characteristic singlet of –CH (proton **e**) present at 5.09 ppm.

Due to these results, we tried the direct employment of ionic resin as catalyst of PSR. After performing the reaction at room temperature in DCM (5.0 hrs) and isolating the product with a "catch & release" process, compound **232** was isolated with a yield of 37% recovering most of the starting material (entry 1, *table 2.3.5*). But encouraged by this result, we tried to optimize the reaction conditions performing the step of PSR both in batch and in microwave reactor.



216 **38** **232**

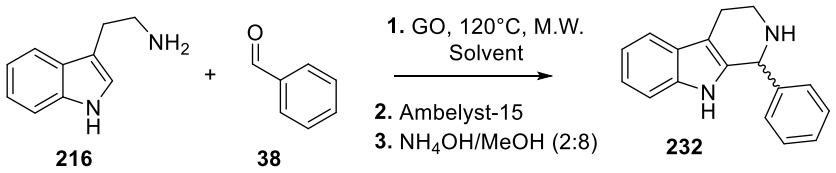
ENTRY	216	38	Solvent	Amb-15	Conditions	Yield (%)
1	1.0 eq.	1.2 eq.	DCM (0.25M)	0.2 g/mmol	r.t., 5.0h	37%
2	1.0 eq.	1.2 eq.	ACN (0.25M)	0.2 g/mmol	120°C, M.W., 0.5h	33%
3	1.0 eq.	1.2 eq.	ACN (0.25M)	0.5 g/mmol	120°C, M.W., 0.5h	38%
4	1.0 eq.	1.2 eq.	ACN (0.25M)	1.0 g/mmol	120°C, M.W., 0.5h	41%
5	1.0 eq.	1.2 eq.	DCM (0.25M)	1.0 g/mmol	120°C, M.W., 0.5h	28%
6	1.0 eq.	1.2 eq.	EtOH abs. (0.25M)	1.0 g/mmol	120°C, M.W., 0.5h	/
7	1.0 eq.	1.2 eq.	ACN (0.25M)	1.0 g/mmol	60°C, M.W., 0.5h	48%
8	**1.0 eq.**	**1.2 eq.**	**ACN (0.25M)**	**1.0 g/mmol**	**Reflux, 5.0h**	**61%**

Table 2.3.5. Optimization for Amb-15 protocol.

We suddenly passed from batch conditions to microwave ones with the purpose to speeding up the reaction between tryptamine **216** and benzaldehyde **38** since the acidic moieties of Amb-15 could react with the starting material forming the corrispective ammonium salt and lowering the yield of the process. But unfortunately no increments were detected. In fact perfoming the reaction in different solvent the product was isolated with almost the same yield of entry 1, without detecting the product when the reaction is performed in ethanol. Increasing the the loading of the resin the yield passed from 33% to 41% performing the reaction in acetonitrile (entry 2, entry 3 and entry 4, *table 2.3.5*). Performing the reaction at lower temperature (M.W.

irradiation at 60°C, entry 7 *table 2.3.5*) the product was isolated with a yield of 48%, refluxing of acetonitrile has shown the most suitable conditions since the product was isolated with a yield of 61%. The low yield of Amb-15® under microwave irradiation can be explained by several factor: first; the acidic moieties present in the resin that traps the tryptamine, forming the ammonium salt, lowering of overall yield. Second, the NMR of crude obtained present a complex NMR spectra, even if with good GC-MS conversion (>95%). This complexity could be due to the degradation of the resin when it is irradiated by microwave.

Given these poor results, we have decided to deeply investigate the 2-step one process proprosed as first approach and the optimization is reported in *table 2.3.5.*



216 **38** **232**

ENTRY	216	38	M.W. Time	G.O.	Solvent	Yield
1[a]	1 eq.	1 eq.	1.5 hrs	15 mg/mmol	DCM (0.25M)	48%
2	1 eq.	1 eq.	1.5 hrs	15 mg/mmol	DCM (0.25M)	50%
3	1 eq.	1.2 eq.	1.5 hrs	15 mg/mmol	DCM (0.25M)	53%
4	1 eq.	1.2 eq.	1.0 h	15 mg/mmol	EtOH abs (0.25M)	69%
5	1 eq.	1.2 eq.	1.5 hrs	/	EtOH abs (0.25M)	51%
6	**1 eq.**	**1.2 eq.**	**1.0 h**	**15 mg/mmol**	**ACN (0.25M)**	**76%**
7	1 eq.	1.2 eq.	1.0 h	15 mg/mmol	EtOAc (0.25M)	73%
8	1 eq.	1 eq.	1.0 h	15 mg/mmol	ACN (0.25M)	70%
9[a]	1 eq.	1.2 eq.	1.0 h	15 mg/mmol	ACN (0.25M)	71%
10	1 eq.	1.2 eq.	1.0 h	7.5 mg/mmol	ACN (0.25M)	72%
11	1 eq.	1.2 eq.	1.0 h	30 mg/mmol	ACN (0.25M)	75%

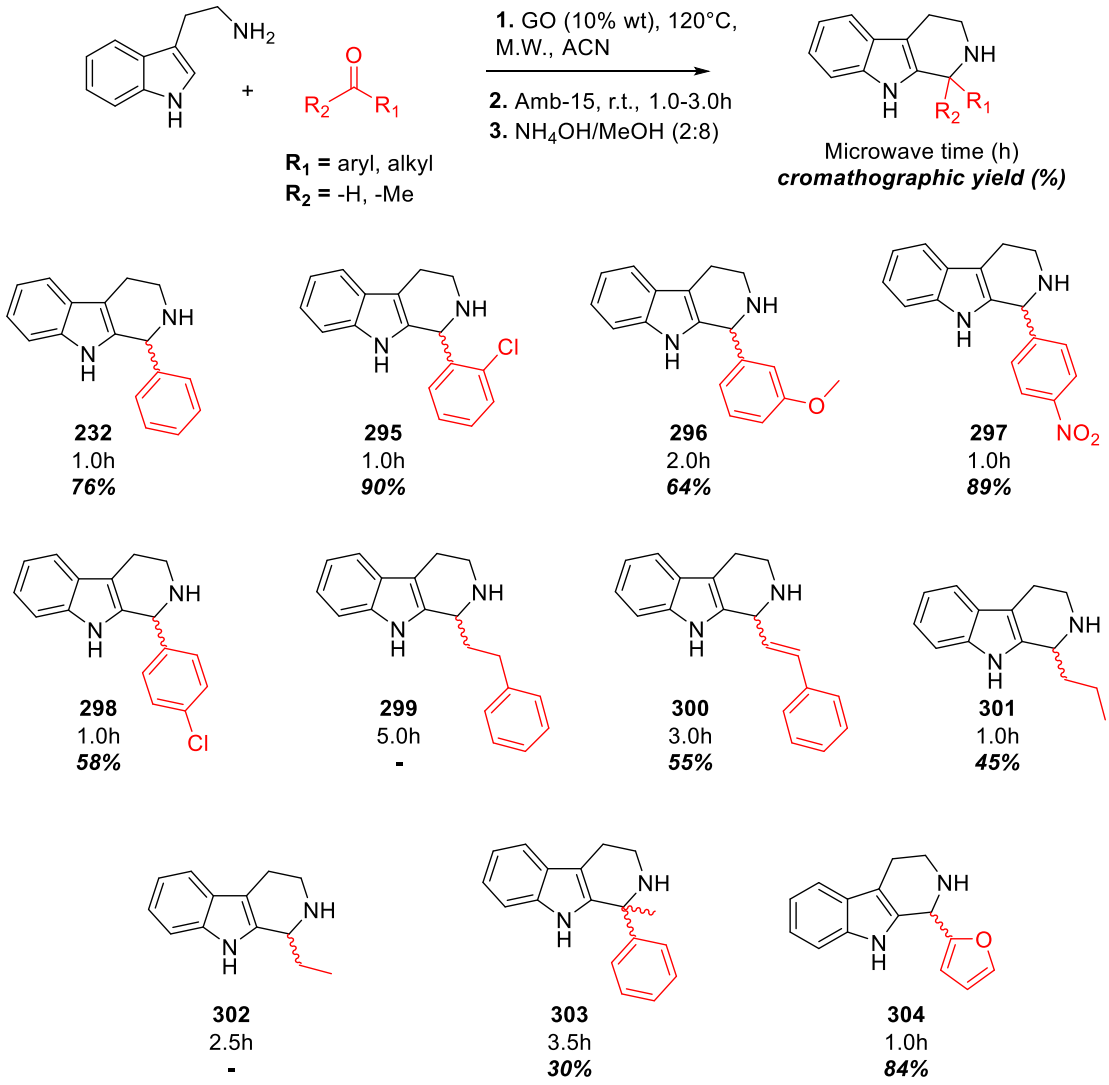
Table 2.3.6. Screening of first step of 2-step one flask protocol; [a]the reaction is performed in presence of molecular sieves (100mg/mmol).

In general, the reaction procced smoothly in all tested conditions and it is quite influenced by the solvent: performing the reaction in other polar solvent like EtOH (entry 4, *table 2.3.6*), ACN (Entry 6, *table 2.3.*6) and EtOAc (entry 7, *table 2.3.6*) the product is isolated with higher yield with a yield og 69%, 76% and 73%. Entry 5 confirms the importance of GO as catalyses to favour the formation of the imine increasing the final yield of the product, and speeding up also the formation of imine itself. The sligh excess is necessary since to increase the final yield of the product, even if with stoichiometric amount aldehyde the THβC is isolated with similar yields, even if quite slighter low. The amount of Graphene oxide does not influce so much the process since with half and double amount, the product was isolated with similar yield. The

formation of the imine proceeds smoothly also performing the reaction in water (result not reported in table), using an excess of aldehyde (1.2 eq.), the extraction with DCM of water solution gives a crude in which the benzaldehyde is not present, but the imine is present with a conversion of ~62% and the SM about ~30%. The amount of ion resin was not subjected to investigation since the higher amount should only speed up the "catch" process.

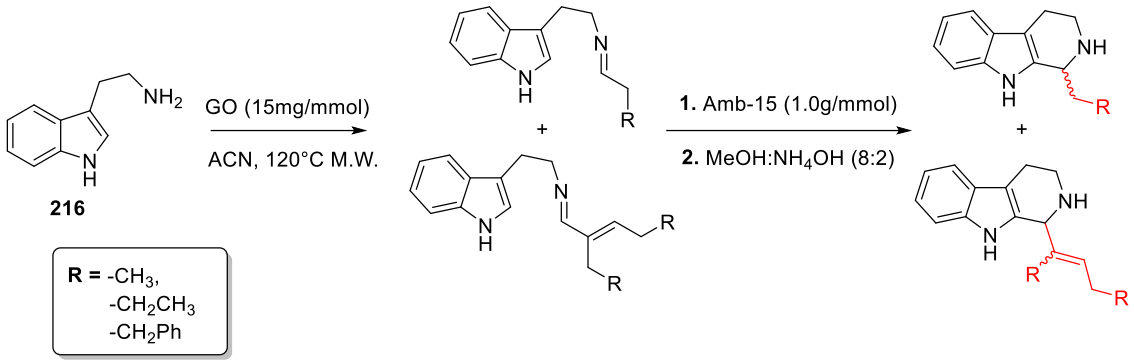
Once obtained the best reaction conditions, we performed, first a screening of aldehyde, second a screening of tryptamine derivatives.

Using different substituted benzaldehydes the relative tetrahydro-β-carbolines were separated with good yield ranging from 58% to 90% of yield. Moreover, the EDG-substituents like methoxy group (**296**) probably de-activates the relative imine increasing the electrondensity near the imine-carbon disfavoring the ring closure. On the other hand, when EWG-substituted benzaldehyde is involved in the reaction, the relative products were isoltated with high yield respect to non-substituted benzaldehyde. This fact can confirm that contrary behavior of 3-methoxybenzaldehyde. In fact, 4-nitro (**297**), 4-Cl (**298**) and 2-Cl (**295**) benzaldehyde allows to obtain the relative THβCs with a yield of 89%, 58% and 90%. These yields confirming that the nature and the position of substituent greatly influence the process. In fact nitro group is more electron withdrawing than chloride, so when these substituents are present in 4-position, the nitro decrease the electron density favoring the process, meanwhile the -Cl does not. Instead, in 2-position the chlorine decrease the electron density in imine-carbon favouring the 6-endo trig ring closure.



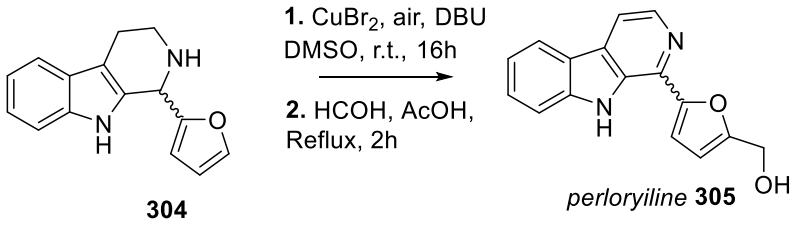
Scheme 2.3.22. Screening of aldehyde.

On the other hand, when an aliphatic aldehyde like hydrocynammaldehyde, butanal and propanal are involved, the corrispective heterocycles were isolated with a low yield or not isolated. This fact is explained by the formation of self condensation product under microwave irradiation. In fact, the radiation incredibly increases the reaction rate of these side-products formation, if at room temperature or 2-8 °C (fridge storage) the reaction takes place after days and days, under microwave irradiations the process takes palce after a few hours. From GC-MS analyses it was possibile to observe the imine between self condensating aldehydes and tryptamine and even the relative THβC products after work-up with Amberlyst-15®. The only product was isolated when butanal (**301**) was involved.



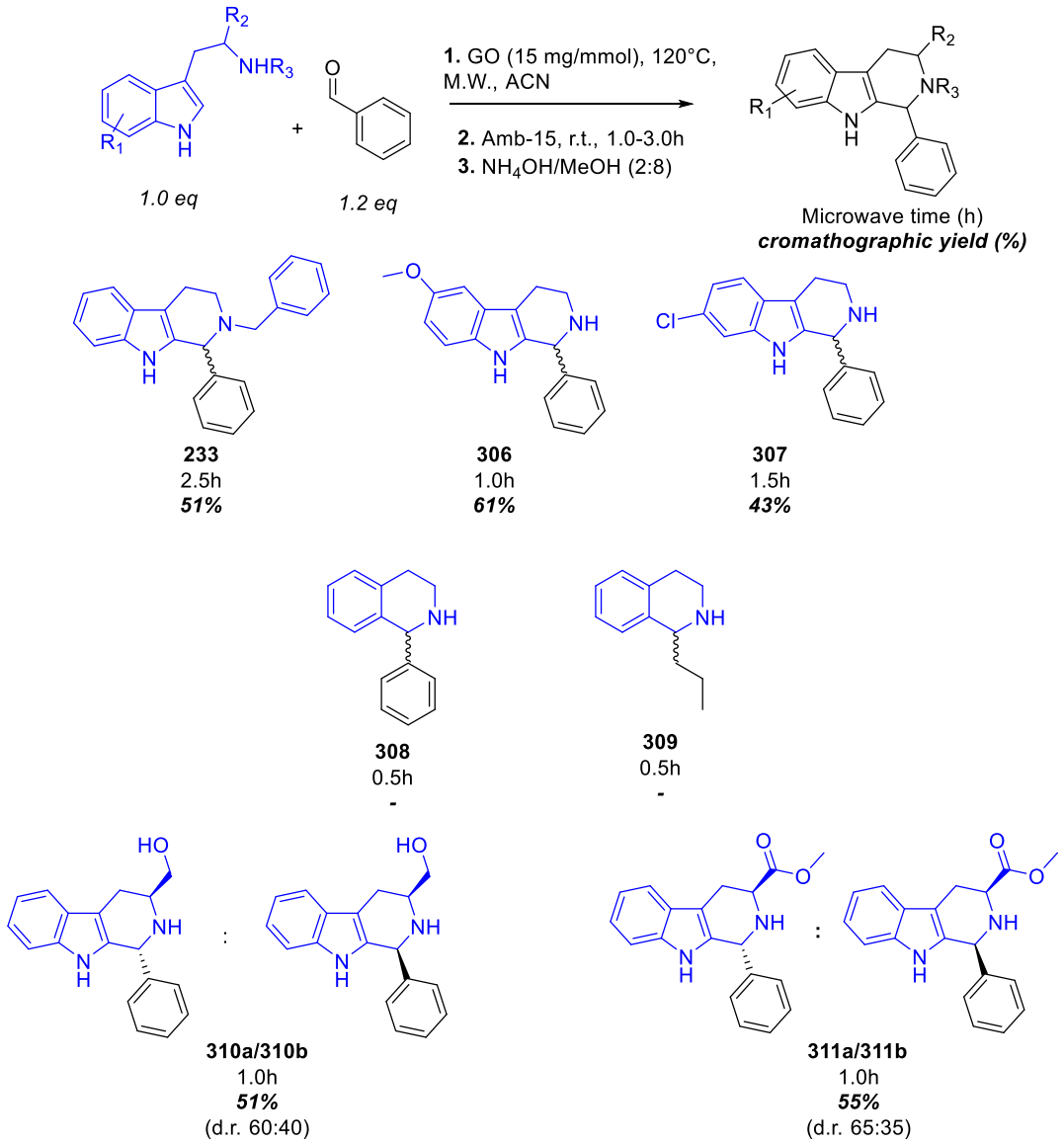
Scheme 2.3.23. Formation of self condensated side product of alkyl-aldehydes

It is known in literature that the ketones are less reactive in PSR protocols, but in this 2-step one flask protocol it was possible, even if with quite low yield (30%) the relative THβC adduct **303** deriving from acetophenone and tryptamine, the maximum to imine conversion was reached after 3.5h. Very interesting is the THβC **304** obtained thourgh the Pictet-Spengler Reaction between tryptamine and furfural. This carboline is a precursor of perloryline **305**, that is a β-carboline that can counteract against the stomach tumor cells and it seem also an activator of TRPV1 (transient receptor potential vanilloid 1) that is a protein that regulates the perception of pain in human.[324] In literature it is reported the synthesis of this precursors using trifluoroacetic acid as Brønsted acid after 2h of reaction at room temperature, but due to high harmful of this acid, this method could preferable to the green properties of GO and Amberlyst-15®; then once obtained the THβC, the carboline is achievied after an oxidation performed in presence of a Cu(II) salt and a functionalization of furfural moiety.[325]



Scheme 2.3.24. Synthesis of perloryline reported by Zheng et al.

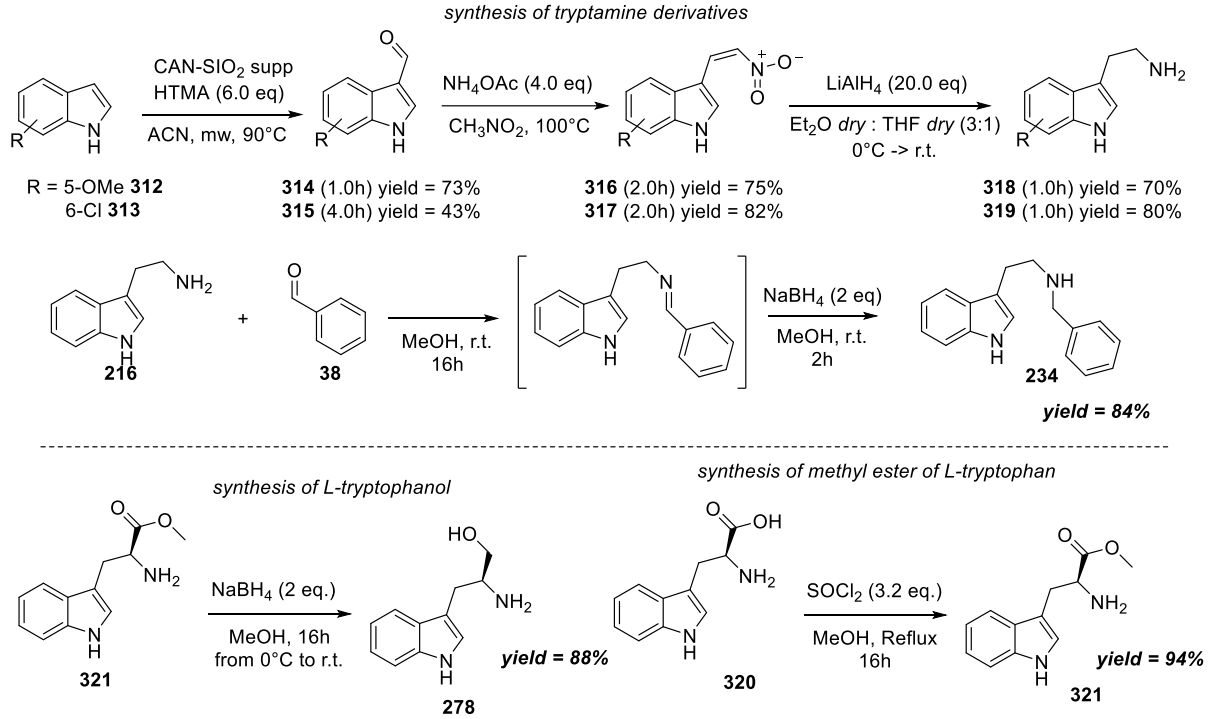
Here below the screening of β-ethyl aryl amines is reported using again benzaldehyde as pilot aldehyde. The reaction proceed smoothly also with different amine-substrates.



Scheme 2.3.25. Screening of different β-arylethylamines

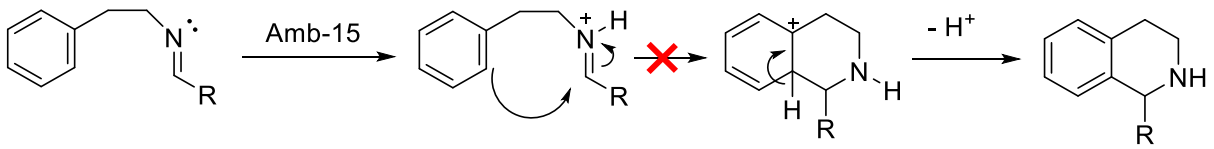
In the case of secondary amine (N-benzylated **234**) the formation of the product was suddenly detected in the step of Graphene oxide, since the reaction of the secondary amine and aldehyde leads to the formation of immonium intermediate that is already active toward the ring closure, and the step of Amberlyst-15® acts as purification tool extracting the product from the reaction mixture. The β-carbolines derived from 6-methoxytryptamine **318** and 7-chlorotryptamine **319** (obtained by 3-step process reported in *scheme 2.3.26*) were isolated with good yields, in addition, the 7-Cl-tryptamine is less reactive probably due to electron-withdrawing nature of chloride, but even the methoxy group negatively influences the trend of PSR since the adduct **306** was isolated with a lower yield respect to non-substituted tryptamine. Two L-tryptophan derivatives were evaluated: the methyl ester **321** (esterification performed with $SOCl_2$ at reflux for 16 hrs in MeOH, yield = 92%) and the L-tryptophanol **278** (obtained by reduction of methyl

ester **321** with $NaBH_4$ (16h, from 0°C to r.t., yield 88%). In each case the total conversion of starting material to imine was observed after only one hour of reaction in the microwave reactor, the successive cyclization with amberlyst-15® gives the 2 diasteroisomers with similar yields (51% for **310a/310b**, and 55% for **311a/311b**) and similar d.r. (60:40 for **310a/310b**, and 63:35 for **311a/311b**) meaning that the two different moieties do not influence so much the trend of the reaction.



Scheme 2.3.26. Synthesis of tryptamine derivatives

Finally we tested also phenylethylamine with two different aldehydes: benzaldehyde and butanal. But unfortunately, in each reaction the products were not achieved. In fact, the reaction stop to imine, even the work up with Amb-15® does not active the imine and the ring closure does not take place, probably because this reaction conditions are to mild to break the aromacity of phenyl moiety and obtain the cyclic product.



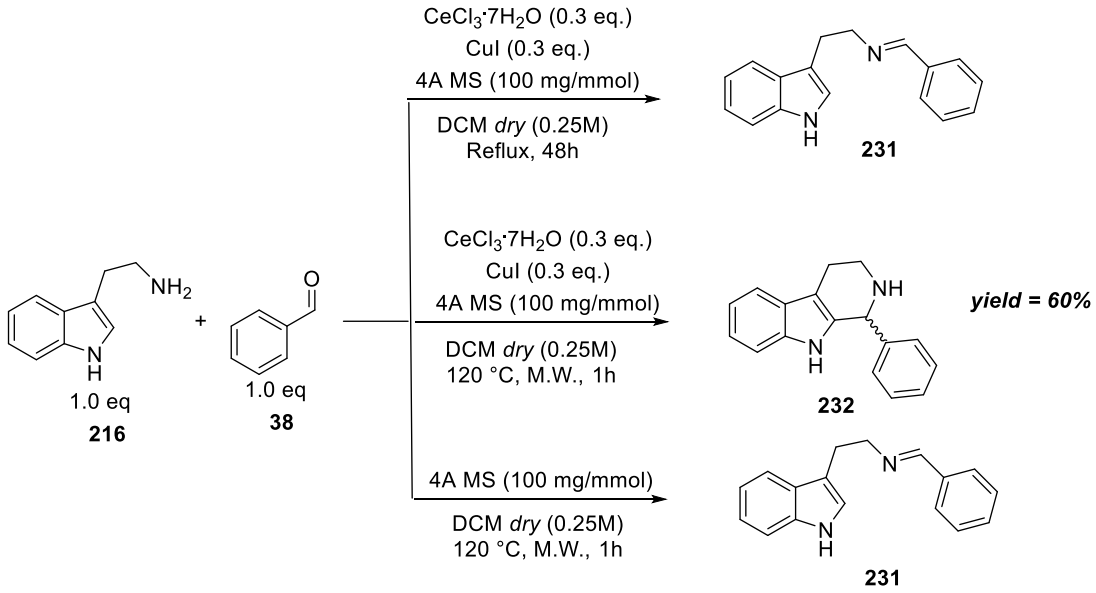
Scheme 2.3.27. Limitation of the scope of Amberlyst-15® in PSR

As expected, the resin used for a second run did not work and the product was recovered only in a small portion, but as reported in literature (cited above), the resin can be reactivated and

resused for other runs. The Graphene oxide was not subjected to reuse studies since it is reported (cited above) the possible employment for different runs.

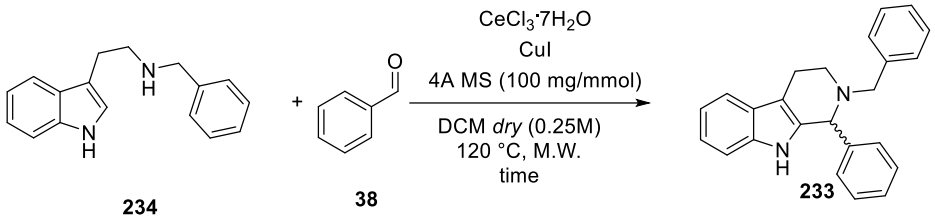
LEWIS CATALYSES

Our investigation about Ce(III) chloride as environmental metal catalyst for one-pot PSR reaction started from the screening of reaction conditions in three different experiments reported in the scheme below. In these synthetic protocol the product was not observed in the reflux of DCM, even after 48h reaction and the reaction stop at imine stage. But switching from batch to microwave irradiation the product was isolated with a relative low yield, and perfoming the reaction under microwave irradiation without catalytic system the process stop at imine (*scheme 2.3.28*).



Scheme 2.3.28. First approaches for one-pot PSR Ce(III) catalysed

As expected, using phenylethylamine as amine, the reaction stop at imine stage even under microwave irradiation (scheme not reported). Further, we decided to used a secondary tryptamine (N-benzylated **234**, see *scheme 2.3.25*) since its predictable more reactivity toward the Pictet-Spengler Reaction, observing the formation of tetrahydro-β-carboline **233** after 1h of the reaction with a yield of 48%. Encouraged by this result, we performed a little screening of reaction conditions to figure out the best conditions for the synthesis of this N-substituted heterocycle.



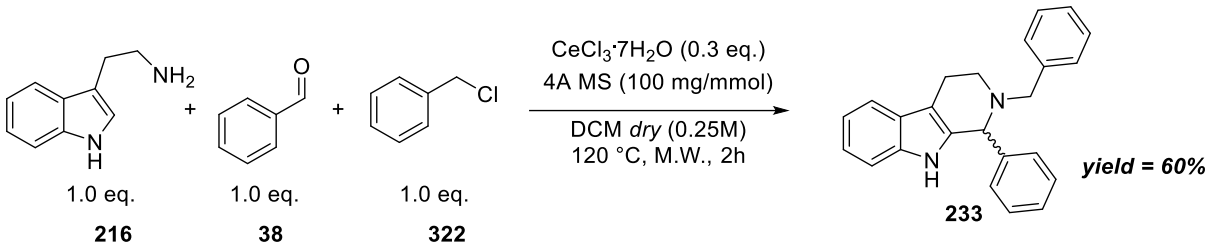
234 38 233

ENTRY	234	38	$CeCl_3 \cdot 7H_2O$	CuI	Time	GC conv.	Yield
1	1.0 eq	1.0 eq	0.3 eq.	0.3 eq.	1.0h	60 %	48%
2	1.0 eq	1.0 eq	0.3 eq.	0.3 eq.	1.5 h	90 %	71 %
3	**1.0 eq**	**1.0 eq**	**0.3 eq.**	**0.3 eq.**	**2.0 h**	**95%**	**79 %**
4	1.0 eq	1.0 eq	0.5 eq.	0.5 eq.	2.0 h	90 %	70 %
5	1.0 eq	1.0 eq	0.3 eq.	-	2.0 h	92 %	75 %
6	1.0 eq	1.0 eq	-	0.3 eq.	2.0 h	84 %	63 %
7	1.0 eq	1.0 eq	-	-	2.0 h	80 %	60 %

*Table 2.3.7. Screening of the reaction conditions for the synthesis of N-benzylated THβC **233** starting from secondary tryptamine*

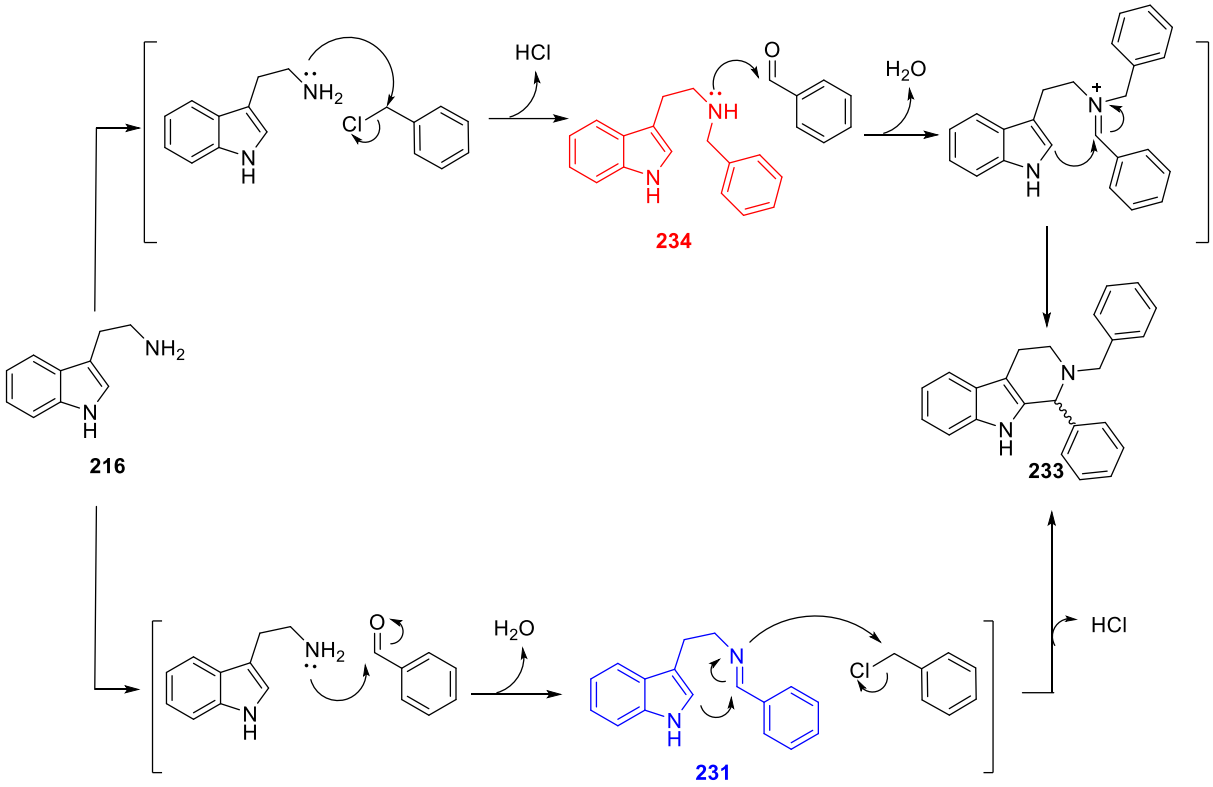
As reported in the table 2.3.7, the product was obtained in each experimented conditions. At first glance, increasing the reaction time goes along with an increment of target product, which is isolated with the highest yield after 2h of reaction (entry 3, *table 2.3.7.*). Involving a higher amount of each two metal catalysts (entry 4, *table 2.3.7.*) the product was formed with less conversion (90%) and isolated with a lower yield (70%). Perfoming the reaction withouth any catalysts the reaction proceeds anyway and the product was isolated with a yield of 60%. Moreover, from the table, it is possible to notice that Ce(III) chloride is a more active catalyst than Cu(I) for this reaction, in fact, performing the reaction with only $CeCl_3 \cdot 7H_2O$ (entry 5, *table 2.3.7.*) the GC-converion and yield of the β-carboline were comparable to the co-catalytic system, meanwhile when CuI (entry 6, *table 2.3.7.*) is used as metal catalyst the reaction goes on by an inefficient way. The higher yield in the Ce(III)/Cu(I) could be explained by the enhancement of Cerium activity by the presence of a iodide source, since the iodide breaks the Cerium dimer increasing its activity.

Questioning about the possibility to perform the reaction through a one-pot process, we conducted an experiment in which we employed 1.0 eq. of tryptamine, benzaldehyde and benzylchloride in DCM *dry* (0.25 M) under microwave irradiation at 120 °C in presence of molecular sievies; and the product was isolated with a 60% of yield.



Scheme 2.3.29. First One-Pot approach for the synthesis of N-benzylated THβC

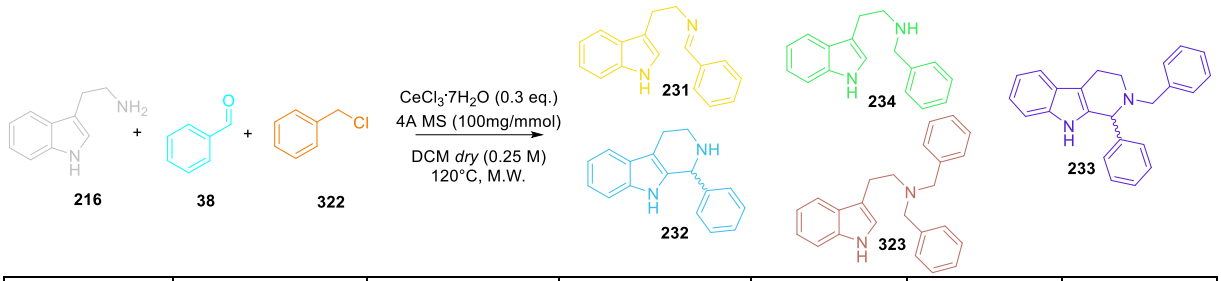
Even if the the product **233** is formed with a good yield (higher yield and less reaction time respect to Graphene Oxide 2-step one flask, see *scheme 2.3.25.*) by easy way, the answer to the question "by which way is it formed?" is quite more difficult. The product can be formed by two ways (see *scheme 2.3.30*): from a simple GC-MS kinetic analyses it appears that one path is under kinetic control and the other pathway under thermodynamic control.



Scheme 2.3.30. Two possible mechanism of one-pot protocol for PSR

The substantial difference between these two paths lies in the fact that tryptamine would attacks the benzylchloride by SN_2 mechanism forming the secondary amine and then, this intermediate would attack the benzaldehyde forming the immonium ion which would suddenly undergo to ring closure by a PSR reaction forming the THβC **233**. Likewise the imine **231** was formed

firstly, then a consecutive PSR and a nucleophilic attack to benzylchloride (**322**) would take place leading to the formation of target product **233**.



Compound	1 min	5 min	15 min	30 min	60 min	120 min
38	22,65%	21,48%	13,44%	13,48%	11,77%	4,80%
322	52,62%	49,89%	35,31%	34,49%	31,96%	14,30%
216	12,17%	1,08%	1,20%	1,51%	1,80%	1,26%
231	12,56%	27,44%	47,10%	41,64%	33,47%	13,56%
232	-	-	-	-	-	-
234	-	-	-	-	-	-
233	0,00%	0,11%	2,84%	8,62%	26,80%	63,91%
323	0,00%	0,00%	0,11%	0,26%	0,20%	2,17%

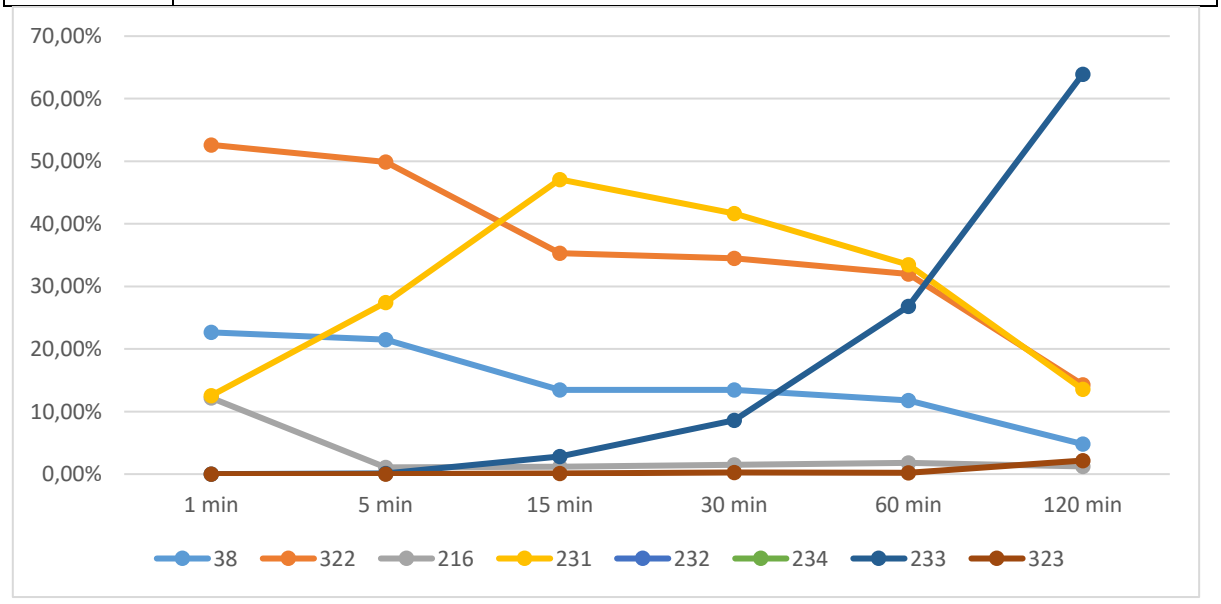
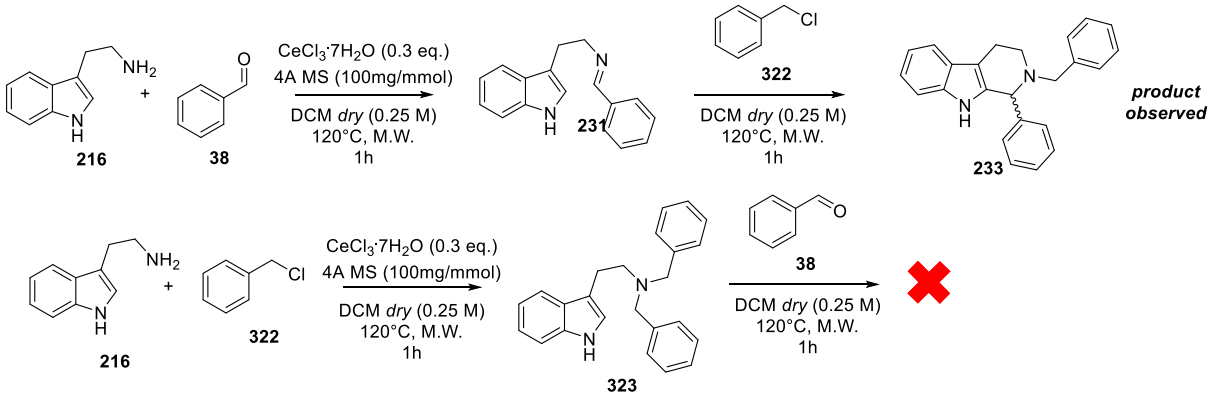


Table 2.3.8. Above GC-percentage conversion of the mixture, below the resultanting graphic of kinetic of one-pot PSR protocol

The data confirm that the process follow the 2-steps pathways. In fact, the secondary amine **234** was never detected and the product of double addition **323** is observed in low percentage only after 2 hrs of reaction. But, by deeply exams, it is clear that at the beginning of the reaction the benzylchloride is the major component in the reaction mixture and the second is the

benzaldehyde **38** and the third the imine **231**, moreover the tryptamine **216** is almost totally consumed only after 5 minutes. After 15 minutes the imine **231** is the major component of the mixture a small formation of the N-substituted product was detected. From this point the imine **231**, aldehyde **38**, benzylchloride **322** are consumed and the percentage conversion of the target product increasing until it reaches the maximum after 2h of reaction. For all these reasons, we can think that the three-steps path can operate under kinetic control, while followed by the reaction under thermodynamic control. This mechanism could be explain by two other experiments in which 2 "two step one flask" pathway were tested:

- In the first experiment: first, the imine is synthesized after one hour of the reaction between stoichiometric amount of tryptamine and benzaldehyde in presence of $CeCl_3 \cdot 7H_2O$, then benzylchoride (1 eq.) is added to the mixture and it is stirred for one hour more
- In the second experiment, first the tryptamine is left to react with stoichiometric amount of benzylchloride with the purpose to obtain the secondary amine, then benzylaldehyde (1 eq.) is added and the reaction is left in the microwave reactore for one hour more.

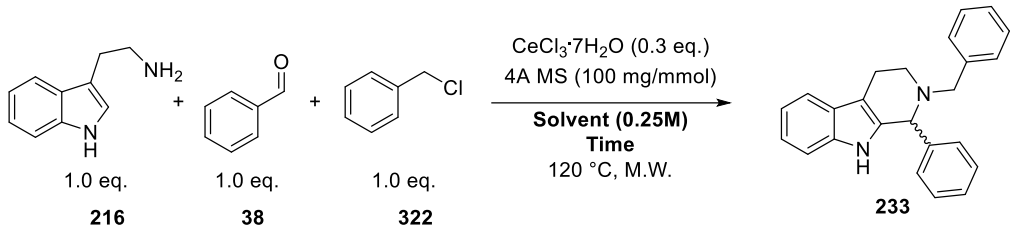


Scheme 2.3.31. The 2 "two step-one flask" experiments

The first experiment could be a confirm of our theory in which the process undergoes to the thermodynamic pathway, in which the tryptamine is totally converted to imine after one hour of the reaction; then, after one hour of reaction from the benzylchloride addition the product was detetcted in good percentage of conversion, even if the imine was still detected. On the other hand, in the second two step-one flask experiment was detected the formation of only tertiary tryptamine due to the second addition of molecule of benzylchloride. This result is not surprising at all, in fact, the nucloephilicity of amines makes benzylchloride high susceptible to multiple attacks by tryptamine itself, and bearing in mind this concept, it is not possible to exclude the formation of ammonium salt as side reaction in presence of too much chloride. So,

in the one-pot protocol, the formation of imine prevails over the nucleophilic attack to benzyl chloride, then, undergoing to contemporary ring closure and attack to chloride. To increase the efficiency of the reaction towards PSR product it is necessary to favor the formation of the imine with respect to amine **234**, and for this Ce(III) salt is optimal. Its oxyphilic character allows to bind to carbonyl oxygen making the aldehyde even more electrophilic and favor the formation of the imine. Better PSR results were obtained when Cu(I) is added to $CeCl_3$ for the azaphylic character of copper.[326]

Once that we clarified the kinetic of the the process, we tried to optimize the reaction conditions with the purpose to find some confirms to our hypothesis and enhance the final yield of target heterocycle. First, we evaluated different solvents in the reaction and all the experiments were reported in the table below.



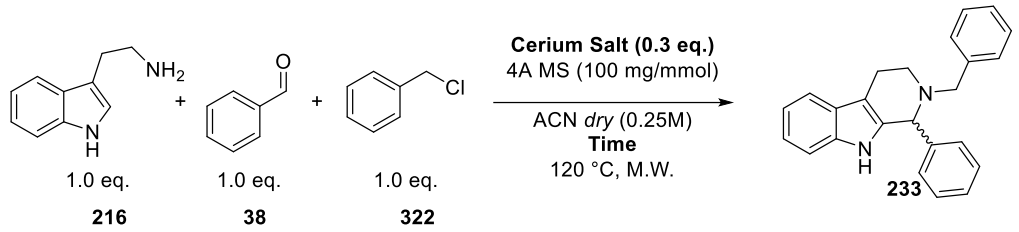
ENTRY	Solvent	Time	Yield
1	**DCM *dry***	**2h**	**60%**
2	**ACN *dry***	**2h**	**60%**
3	EtOH abs	2h	52%
4	THF *dry*	2h	16%
5	Toluene *dry*	2h	trace
6	ACN:EtOH (1:1)	3.5h	41%
7	neat	0.5h	36%

Table 2.3.9. Screening of solvent for one-pot PSR protocol

From the table it is clear that the solvent has a profound influence in the trend of the process. Perfoming the reaction in polar aprotic solvent like dichloromethane and acetonitrile the best results were obtained with an equal yield of about 60% (entry 1 and entry 2, *table 2.3.9.*). Meanwhile, the reaction performed in a polar protic solvent like ethanol the product was isolated with a slight lower yield (52%, entry 3, *table 2.3.9.*). The so low yield detected in THF could be explained by the lowering of Ce(III) activity by THF-coordination[264a, 264b] (see *table 2.2.9.*), in fact, the imine is the major component of reaction mixture. Instead, when the reaction is performed in apolar solvent like toluene the reaction stop at imine stage even if only traces were detetcted at GC-MS after 2 hrs of reaction. Finally, performing the reaction in a mixture of ACN:EtOH abs (1:1) the reaction becomes slow and less effective since the THβC **233** was isolated with a yield of 41%. The neat conditions were ineffective and even if the tryptamine

216 was totally consumed, the product **233** was recovered with a yield of 36% and a series of side-products were detected at GC-MS. Finally we choose acetonitrile as solvent since it is quite less toxic than dichloromethane.

After find the most suitable solvent, we switched our investigation on different Cerium salts and other catalysts of which the screening it is reported below.



1.0 eq. **216** 1.0 eq. **38** 1.0 eq. **322**

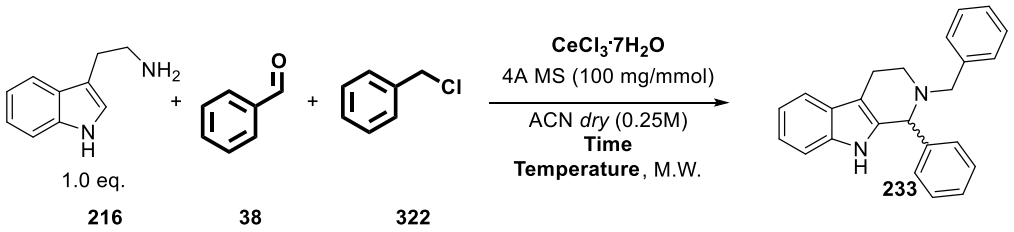
ENTRY	Promoter	Time	Yield
Entry 1	**$CeCl_3 \cdot 7H_2O$ (0.3 eq.)**	**2h**	**60%**
Entry 2	$Ce(OTf)_3$ (0.3 eq.)	2h	52%
Entry 3	$Ce(OAc)_3$ (0.3 eq.)	2h	17%
Entry 4	$CeCl_3 \cdot 7H_2O$/NaI (1:1) (0.3 eq./0.3 eq.)	2h	43%
Entry 5	$CeCl_3 \cdot 7H_2O$/NaI (1:1) (0.3 eq./0.3 eq.)	1h	57%
Entry 6	$CeCl_3$ *dry* (0.3 eq.)	2h	39%
Entry 7	NaI (0.3 eq.)	2h	62%
Entry 8	-	2h	36%
Entry 9	$CeCl_3 \cdot 7H_2O@Al_2O_3$ acid (0.3 eq.)	1h	28%
Entry 10	Graphene Oxide (15mg/mmol)	2h	50%

Table 2.3.10. Screening of solvent for one-pot PSR protocol

Among the tested catalyst the $CeCl_3 \cdot 7H_2O$ has shown the best catalyst. Using other two different Ce(III) salts like $Ce(OTf)_3$ (entry 2, *table 2.3.10.*) and $Ce(OAc)_3$ (entry 3, *table 2.3.10.*) worse results were detected. In case of Ce(III) triflate a good conversion of the product was observed but it was isolated with a 52% of yield, meanwhile the basicity of acetate could favor the formation of other drawbacks (like formation of ammonium salt) by depronating the tryptamine and increasing its nucleophilicity toward multiple attacks and lowering the yield of compound **233**. Entry 4 and entry 5 are tentative to enhance the activity of $CeCl_3 \cdot 7H_2O$ by the presence of iodide source like NaI. Actually, the reaction provides a higher yield when it proceeds 1 h instead of 2, probably, leaving react too much this system the formation of side-products is favored, leading to a yield of 43% (entry 4, *table 2.3.10.*) instead 57% (entry 5, *table 2.3.10.*). Removing the crystalline water of Ce(III) chloride, the product was isolated with a 39% of yield detetcting a huge amount of benzylchlorde in the reaction mixture. The 62% of yield obtained with only NaI (entry 7, *table 2.3.10.*) can be explained by the probable halogen substitution which could favor the whole process, in fact also a 5% of conversion of tertiary tryptamine **216** was detected (usually it was around 1% or lower as conversion). The presence of the catalyst is

necessary since performing the reaction without (entry 8, *table 2.3.10.*) the heterocycle **233** is obtained with a lower yield, moreover, in this reaction, a slight higher formation of tertiary amine **323** was observed, thus it means again the Ce(III) probably favourite the formaton of imine by coordination favouring the attack to benzaldehyde over benzylchloride avoiding the formation of kinetic byproducts. Finally, even using supported Ce(III) chloride on acid Al_2O_3 and a green Brønsted acid like Graphene oxide the reaction procceds in less effective way. The low result in supported cerium salt could be explained by the instrisic acidity of alumina, in fact it can react with tryptamine, catching it and forming an ammonium salt, making impossible the formation of target product; meanwhile the GO allow to obtained the **233** with a yield of 50%, still lower to entry 1. We finally choose $CeCl_3{\cdot}7H_2O$ since the results with it and NaI are comparable between them and probably the 62% of NaI is probably due to a side reaction of halo exchange.

Finally, when we found the best solvent and the best catalyst, we investigated and tried to optimize the final yield changing the amount of different component of this protocol.



216 **38** **322**

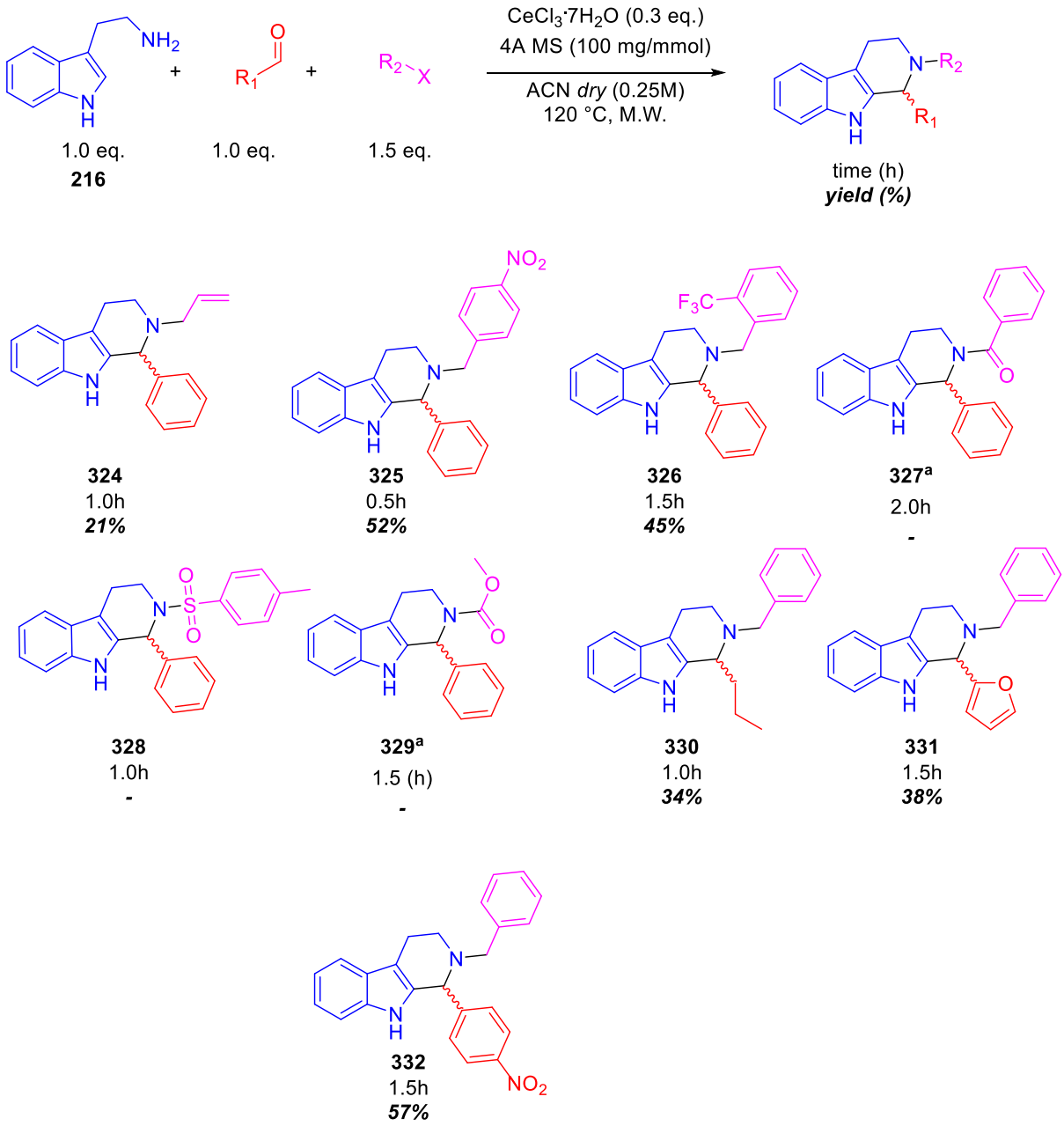
ENTRY	38	322	$CeCl_3{\cdot}7H_2O$	Temperature	Time	Yield
1	1.0 eq	1.0 eq.	0.3 eq.	120 °C	2h	60%
2	1.0 eq	1.0 eq.	0.3 eq.	180 °C	0.5h	Complex mixture
3	1.0 eq	1.0 eq.	0.3 eq.	90 °C	2h	traces
4	1.0 eq	1.0 eq.	0.1 eq.	120 °C	2h	63%
5	1.0 eq	1.0 eq.	1.0 eq.	120 °C	10 min	Complex mixture
6[a]	1.0 eq	1.0 eq.	0.3 eq.	120 °C	2h	43%
7	1.5 eq	1.0 eq.	0.3 eq.	120 °C	2h	34%
8	**1.0 eq**	**1.5 eq.**	**0.3 eq.**	**120 °C**	**2h**	**67%**
9	1.0 eq	2.5 eq.	0.1 eq.	120 °C	2h	17%
10	1.0 eq	1.5 eq.	0.1 eq.	120 °C	0.5h	18%
11	1.5 eq	1.5 eq.	0.3 eq.	120 °C	1h	47%
12	1.0 eq	1.5 eq.	0.3 eq.	Reflux	24	traces

Table 2.3.11. Final screening for one-pot PSR protocol; [a]the reaction was performed in presenc of 1 eq of 2,6-di-terbutyl4-methyl-pyridine.

Performing the reaction at higher temperature (180 °C, entry 2, *table 2.3.11.*) or at lower temperature (90 °C, entry 3, *table 2.3.11.*) two different results were observed: at higher temperature just only after 0.5h of reaction we observed the formation of a complex mixture of products in which the partially and total oxidize (dihydro-β-carboline and β-carboline) were detected as major components of the mixture and product was neither detected. On the other hand, at lower temperature become very slow and after 2h a huge amount of imine was still detected with trace of products and tertiary tryptamine. Involving less amount of Ce-catalyst the product were isolated with a slight higher yield but comparable to entry 1 (*table 2.3.11.*). Instead, involving a stoichiometric amount of catalyst favours too much the side-reactions and a complex mixture is formed only after 10 mintues. Since HCl is released during the reaction and favours the formation of side products (i.e. ammonium salts formation), we performed the reaction in presence of a common proton-sponge like 2,6-di-terbutyl-4-methyl-pyridine, but unlikely the expected result, we observed a decrease of the yield (43%) of cycle **233** This demonstrates that the realesed HCl not only has an undesirable effect, but it could act as co-catalyst favourating the PSR stage and so the attack to benzylchloride. Combining different amount of benzalhyde, benzylchloride and catalyst very interesting results were observed. When an excess of aldehyde was employed (entry 7, *table 2.3.11.*) the formation of target product **233** is suppressed isolating it with a lower yield (34%) favouring the formation of non-susbtituted THβC **232** and its partial and total oxidized products. On the other hand, involving an excess of of benzylchloride **322** (entry 8, *table 2.3.9.*) the product was isolated with a yield of 67% confirming that the process proceed through an SN_2 pathway (higher concentration of chloride, higher reaction rate) and that the attack of imine **231** to chloride is the bottleneck step of reaction. So, we tried to use an even greater amount of benzylchloride, but as said before, many side reactions could take place in the mixture (included the multiple attack of tryptamine **216** toward benzylchloride), in fact, this low yield of this experiment (17% entry 9, *table 2.3.11*) can be explained by the formation of a huge amount of ammonium salt and tertiary amine **323**. Instead, performing the reaction with less amount of $CeCl_3 \cdot 7H_2O$ and an excess of benzylchloride (entry 10, *table 2.3.11.*) a yield of 18% was obtained after 30 minutes of reaction. Using a contemporary excess of aldehyde **38** and benzylchloride **322** (entry 11, *table 2.3.11.*) with the purpose to enhance both formation of the imine and the SN_2 attack, we unfortunately isolated the product with a yield of 47% after one hour of reaction, because of the formation of non-substituted-THβC, non-substituted-DHβC and non-substituted-βC were favoured over the formation of the product in this conditions. The microwave irradiation play

an important role for the reaction since performing the reaction in batch the process stop at imine **231** and trace of products were detetcted after 24 hrs.

Once assumed that the entry 8 of *table 2.3.11.*, we tried to enlarge the aim of substrates of the protocol, this screening is still carrying out in our laboratory but here the preliminary results, expecially for what concern the employment of different halogen source are reported.



Scheme 2.3.32. Preliminary Screening of different halogen components and aldehyde; [a]reaction performed also in stoichiometric amount of halogen compound.

As expected, the trend of the reaction is tremendously influenced by the substrates. The N-substituted-THβCs **324**, **325**, **326** were respectively obtained using allyl-, p-nitrobenzyl- and 2-

trifluoromethylbenzyl- bromide. In each experiment a not trascurable formation of non-substituted tetrahydro-β-carboline were observed (probably due to the formation of HBr in the reaction mixture), and the product **324** was isolated with a yield of 21%, meanwhile EWG substituted benzylbromide worked quite well and the relative product were isolated with a corrispective yield of 52% for **325** and 45% for **326**. This last relative low yield could be explained by the higher susceptibility of trifluoromethylbenzylbromide toward nucleophilick attack, in fact the tertiary tryptamine (double attack to bromide) was detetcted with a higher GC-conversion respect to the target product **326** (high influene of electronwithdrawing nature of $–CF_3$ near to benzyl-carbon). Using Ts-Cl, the product **328** was detected in traces at ESI-MS. The acyl chloride (benzylchloride and methylchloroformate) deserved a deeper investigation. The product **327** was only detected in trace by ESI-MS, but we tried to perform the reaction with stoichiometric amount of benzoyl chloride we observed by GC-MS a huge formation of amide derivative and traces of target product, this fact can be explained by the higher electrophility of carbonyl compound of acyl moiety. Thus the tryptamine **216** is more inclined to attack the acyl chloride instead of the benzaldehyde and this behaviour is greatly enhanced when benzoyl chloride is present in excess. Similar results were observed when methyl chloroformate was involved, in fact, the product **329** was not detected, while it is present in stochiometric amount the relative amide and N-substituted THβC **329** were observed at GC-MS (higher conversion of carboammide). Finally, when different aldehydes were involved, the reaction proceeds less smoothly respect to benzaldehyde, but the product **331** (furfural) and **330** (butanal) were isolated with a yield 42% and 34% after 1.5h and 1.0h of reaction. The THβC **332** was isolated with good yield when p-nitrobenzaldehyde was involved.

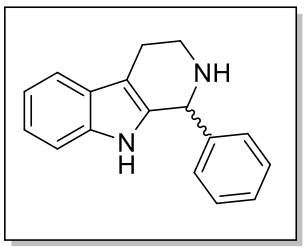
Studies to improve this synthetic methodology of PSR products, particularly with regard to develop an equally efficient and sustainable method are currently underway in prof. Marcantoni's laboratory.

2.3g Experimental Section

*GENERAL METHOD FOR THE SYNTHESIS OF TETRAHYDRO-β-CARBOLINES by GRAPHENE OXIDE/AMBERLYST-15 (**232, 233, 295-304, 306-311**):*

β-Arylethylamine (1.0 eq.) is dissolved in ACN *dry* forming a solution of 0.25M in a microwave vial, aldehyde (1.2 eq.) is added dropwise and GO (15mg/mmol) is added one pot and the vial is put in the microwave reactor at 120 °C. The reaction is monitored by TLC (95 $CHCl_3$: 5 MeOH) and GC-MS. Once that the starting material is consumed and imine is formed (or no more evolution were observed) the reaction mixture is filtered by a pad of Celite and washed with fresh solvent, then 1.0g/mmol of Amb-15® is added to the solution and stirred for 2.0h (monitored by TLC). After that the Amb-15® is filtered by gooch and the resin is put in a round bottom flask and it is washed by organic basic solution (2 NH_4OH 33% : 8 MeOH) and it is stirred for 30 minutes. Then the Amberlyst-15 is filtered and the solution is portioned by DCM (15mL) and sat. $NaHCO_3$ (15 mL), and the aqueous layer is washed 2 time with fresh DCM (2x15 mL). The organic phase is dried over anhydrous sodium sulphate and the solvent is evaporated by rotavapor to give the product. If necessary, the product can be purified by a pad of Silica Gel (gradient from 100 $CHCl_3$ to 90 $CHCl_3$: 10 MeOH).

*1-PHENYL-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**232**)*



Following the general procedure, the product **232** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.45, 95 $CHCl_3$: 5 MeOH) with an yield of 76%, after 1.0 h of reaction at microwave, using tryptamine **216** and benzaldehyde **38** as substrates.

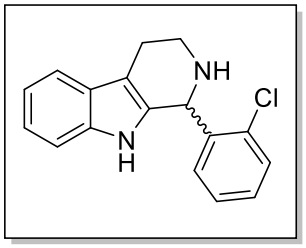
Molecular Formula: $C_{17}H_{16}N_2$

GC-MS (EI, 70 eV) = 248 ($M^{+\cdot}$), 218 (100), 204, 171, 144, 109, 77. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ = 7.96 (s, 1H), 7.56 (dd, J = 7.6, 4.2 Hz, 1H), 7.37 – 7.32 (m, 3H), 7.29 – 7.24 (m, 2H), 7.17 – 7.10 (m, 4H), 5.09 (d, J = 1.6 Hz, 1H), 3.32 (ddd, J = 12.5, 5.2, 3.9 Hz, 1H), 3.14 – 3.06 (m, 1H), 2.97 – 2.79 (m, 2H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 142.08, 136.13, 134.71, 129.04, 128.81, 128.42, 127.57, 121.90, 119.55, 118.44, 111.12, 110.37, 58.27, 42.99, 22.76.

FT-IR (cm^{-1}) = 3402, 3056, 2917, 2844, 1453, 1298, 1140, 906, 730, 700.

*1-(o-CHLOROPHENYL)-2,3,4,9-TETRAHYDRO- PYRIDO[3,4-b]INDOLE (**295**)*



Following the general procedure, the product **295** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.42, 95 $CHCl_3$: 5 MeOH) with an yield of 90%, after 1.0 h of reaction at microwave, using tryptamine **216** and o-chloro-benzaldehyde as substrates.

Molecular Formula: $C_{17}H_{15}N_2Cl$

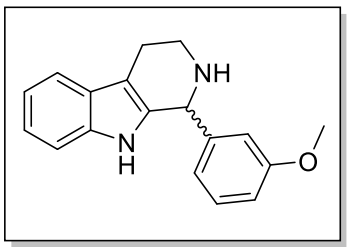
GC-MS (EI, 70 eV) = 282 ($M^{+\cdot}$), 253, 218 (100), 191, 171, 144, 108, 75, 51.

^{1}H-NMR (400 MHz, $CDCl_3$) δ= 7.79 (s, 1H), 7.58 – 7.53 (m, 1H), 7.44 (dd, J = 8.0, 1.2 Hz, 1H), 7.32 – 7.02 (m, 6H), 5.68 (s, 1H), 3.61 (t, J = 6.7 Hz, 1H), 3.29 – 3.17 (m, 1H), 3.17 – 3.07 (m, 1H), 2.87 (tdd, J = 9.8, 8.7, 7.2 Hz, 2H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ= 139.09, 136.02, 133.96, 132.98, 130.36, 129.98, 129.35, 127.26, 127.12, 121.95, 119.52, 118.34, 111.00, 53.67, 41.54, 29.82, 22.42 ppm.

FT-IR (cm^{-1}) = 3398, 2922, 2841, 1468, 1446, 1051, 1037, 741.

*1-(m-METHOXYPHENYL)-2,3,4,9-TETRAHYDRO- PYRIDO[3,4-b]INDOLE (**296**)*



Following the general procedure, the product **296** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.41, 95 $CHCl_3$: 5 MeOH) with an yield of 64%, after 2.0 h of reaction at microwave, using tryptamine **216** and m-methoxy-benzaldehyde as substrates.

Molecular Formula: $C_{18}H_{18}N_2O$

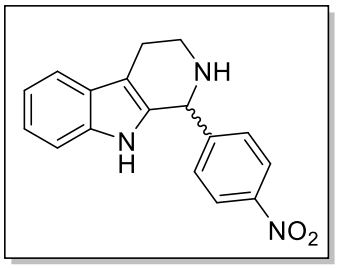
GC-MS (EI, 70 eV) = 278 ($M^{+\cdot}$), 249, 218, 191, 171, 144, 115, 96, 77.

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.53 (d, J = 7.3 Hz, 1H), 7.29 – 7.21 (m, 3H), 7.14 (ddd, J = 9.0, 7.1, 1.3 Hz, 2H), 6.89 (dd, J = 13.2, 4.8 Hz, 3H), 5.25 (s, 1H), 3.76 (s, 3H), 3.37 (dt, J = 12.3, 4.7 Hz, 1H), 3.18 – 3.09 (m, 1H), 2.97 (dd, J = 14.8, 7.4 Hz, 1H), 2.85 (d, J = 15.1 Hz, 1H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 160.25, 141.66, 136.20, 133.02, 130.13, 127.34, 122.25, 121.26, 119.78, 118.56, 114.63, 114.27, 111.18, 110.12, 58.02, 55.60, 42.60, 21.83**.**

FT-IR (cm^{-1}) = 3398, 2837, 1603, 1859, 1491, 1450, 1261, 1041, 741

*1-(p-NITROPHENYL)-2,3,4,9-TETRAHYDRO- PYRIDO[3,4-b]INDOLE (**297**)*



Following the general procedure, the product **297** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.41, 95 $CHCl_3$: 5 MeOH) with an yield of 89%, after 1.0 h of reaction at microwave, using tryptamine **216** and p-nitro-benzaldehyde as substrates.

Molecular Formula: $C_{17}H_{15}N_3O_2$

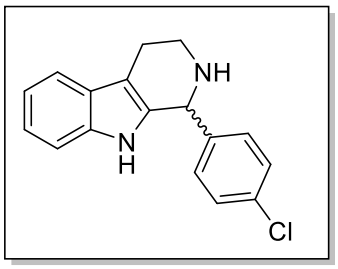
GC-MS (EI, 70 eV)= 293.1($M^{+\cdot}$), 130 (100), 103, 77.

^{1}H-NMR (500 MHz, DMSO-d_6) δ = 10.53 (s, 1H, -NH indole), 8.24 – 8.20 (m, 2H), 7.58 (dd, J = 6.4, 4.6 Hz, 2H), 7.44 (d, J = 7.7 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 7.05 – 6.96 (m, 2H), 5.24 (s, 1H), 2.99 (ddd, J = 12.4, 8.7, 5.5 Hz, 2H), 2.76 – 2.66 (m, 2H).

^{13}C-NMR (125 MHz, DMSO-d_6) δ = 151.51, 147.18, 136.51, 134.63, 130.18, 127.25, 123.73, 121.28, 118.84, 118.17, 111.57, 109.13, 56.24, 41.45, 22.54**.**

FT-IR (cm^{-1}) = 3407, 3232, 2850, 1598, 1517, 1450, 1347, 1298, 1091, 1010, 857, 736.

*1-(p-CHLOROPHENYL)-2,3,4,9-TETRAHYDRO- PYRIDO[3,4-b]INDOLE (**298**)*



Following the general procedure, the product **298** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.47, 95 $CHCl_3$: 5 MeOH) with an yield of 58%, after 1.0h of reaction at microwave, using tryptamine **216** and p-chloro-benzaldehyde as substrates.

Molecular Formula: $C_{17}H_{15}N_2Cl$

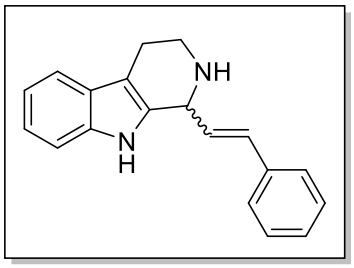
GC-MS (EI, 70 eV) = 282 ($M^{+\cdot}$), 253, 218 (100), 189, 171, 144, 108, 75, 51**.**

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.55 (dd, J = 6.7, 2.2 Hz, 1H), 7.32 – 7.29 (m, 2H), 7.24 – 7.20 (m, 3H), 7.16 – 7.11 (m, 2H), 5.13 (s, 1H), 3.35 – 3.29 (m, 1H), 3.16 – 3.09 (m, 1H), 2.96 – 2.88 (m, 1H), 2.82 (dtd, J = 8.9, 4.8, 1.7 Hz, 1H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 140.52, 136.10, 134.21, 134.03, 130.11, 129.18, 127.51, 122.14, 119.74, 118.53, 111.10, 110.64, 57.57, 42.87, 22.66.

FT-IR (cm^{-1}) = 3394, 3053, 2927, 2850, 1486, 1450, 1086, 1015, 821, 741.

*1-(STYRYL)-2,3,4,9-TETRAHYDRO- PYRIDO[3,4-b]INDOLE (**300**)*



Following the general procedure, the product **300** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.54, 95 $CHCl_3$: 5 MeOH) with an yield of 55%, after 3.0h of reaction at microwave, using tryptamine **216** and hydrocynnamaldehyde as substrates.

Molecular Formula: $C_{19}H_{18}N_2$

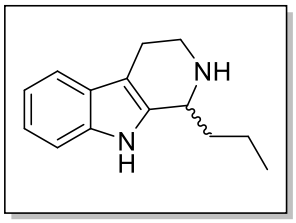
GC-MS (EI, 70 eV) = 274 ($M^{+\cdot}$), 244, 197, 171, 154, 128, 91, 65.

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.92 (s, 1H, -NH indole), 7.53 (d, J = 7.5 Hz, 1H), 7.43 – 7.38 (m, 1H), 7.30 (dddd, J = 8.6, 7.7, 2.9, 1.5 Hz, 5H), 7.18 – 7.09 (m, 2H), 6.68 (d, J = 15.8 Hz, 1H), 6.35 (dd, J = 15.8, 8.1 Hz, 1H), 4.76 (d, J = 8.0 Hz, 1H), 3.38 (dt, J = 12.3, 4.9 Hz, 1H), 3.13 (ddd, J = 12.7, 8.1, 5.0 Hz, 1H), 2.87 – 2.77 (m, 2H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ =136.35, 135.89, 133.81, 133.16, 129.51, 128.82, 128.17, 127.69, 126.70, 121.83, 119.50, 118.37, 110.96, 109.29, 56.08, 46.09, 42.42, 22.48, 10.41.

FT-IR (cm^{-1}) = 3398, 3295, 3052, 2922, 1719, 1612, 1446, 1302, 974, 741, 691.

*1-(PROPYL)-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**301**)*



Following the general procedure, the product **301** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.40, 95 $CHCl_3$: 5 MeOH) with an yield of 45%, after 1.0 h of reaction at microwave, using tryptamine **216** and butanal as substrates.

Molecular Fomrula: $C_{14}H_{18}N_2$

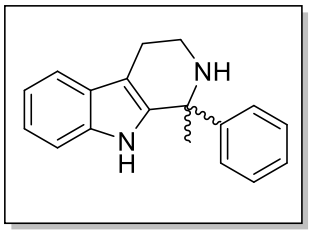
GC-MS (EI, 70 eV) = 214 ($M^{+\cdot}$), 184, 171 (100), 154, 143, 130, 115, 85.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H, -NH indole), 7.49 (d, J = 7.5 Hz, 1H), 7.31 (d, J = 7.9 Hz, 1H), 7.18 – 7.09 (m, 2H), 4.13 – 4.03 (m, 1H), 3.37 (dt, J = 12.8, 4.6 Hz, 1H), 3.08 – 3.00 (m, 1H), 2.75 (tdd, J = 8.3, 5.5, 3.0 Hz, 2H), 1.89 – 1.80 (m, 1H), 1.72 – 1.63 (m, 1H), 1.53 (dddd, J = 21.5, 17.8, 10.2, 5.1 Hz, 2H), 1.00 (t, J = 7.3 Hz, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 136.56, 135.84, 127.77, 121.68, 119.56, 118.26, 110.92, 109.12, 52.68, 42.84, 37.46, 22.93, 19.38, 14.50.

FT-IR (cm^{-1}) = 2956, 2925, 2849, 1452, 1319, 1297, 1154, 1122, 1006, 734.

*1-METHYL-1-PHENYL-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**303**)*



Following the general procedure, the product **303** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.51, 95 $CHCl_3$: 5 MeOH) with an yield of 30%, after 3.5 hrs of reaction at microwave, using tryptamine **216** and phenylacetone as substrates.

Molecular Formula: $C_{18}H_{18}N_2$

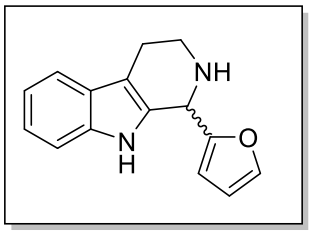
GC-MS (EI, 70 eV) = 262 ($M^{+\cdot}$), 247 (100), 232, 217, 185, 144, 115, 77.

^{1}H-NMR (400 MHz, $CDCl_3$)= δ 7.77 (s, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.36 – 7.06 (m, 11H), 3.16 – 3.08 (m, 1H), 2.95 – 2.83 (m, 3H), 2.83 – 2.69 (m, 2H), 1.82 (s, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) =δ 146.34, 138.31, 135.84, 128.38, 127.49, 127.35, 126.95, 121.96, 119.60, 118.55, 110.98, 109.84, 56.98, 39.85, 28.43, 22.89.

FT-IR (cm^{-1}) = 3398, 3208, 3052, 2927, 1450, 1298, 911, 736, 696.

*1-(FURAN-2-YL)-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**304**)*



Following the general procedure, the product **304** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.48, 95 $CHCl_3$: 5 MeOH) with an yield of 84%, after 1.0 h of reaction at microwave, using tryptamine **216** and furfural as substrates.

Molecular Formula: $C_{15}H_{14}N_2O$

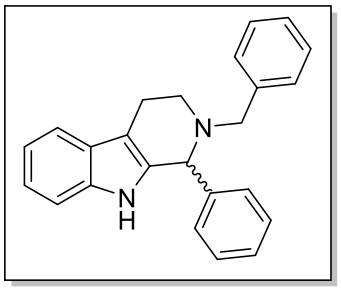
GC-MS (EI, 70 eV) = 238 ($M^{+\cdot}$), 209, 180, 167, 152, 130, 115, 90, 77.

^{1}H-NMR (500 MHz, $CDCl_3$) δ = 8.14 (s, 1H), 7.56 (d, J = 7.7 Hz, 1H), 7.45 (dd, J = 1.8, 0.8 Hz, 1H), 7.28 (dd, J = 5.7, 2.2 Hz, 2H), 7.16 (dtd, J = 14.5, 7.0, 1.0 Hz, 2H), 6.37 (dd, J = 3.2, 1.9 Hz, 1H), 6.21 (dd, J = 3.2, 0.7 Hz, 1H), 3.32 (dt, J = 11.6, 5.0 Hz, 1H), 3.20 – 3.14 (m, 1H), 2.89 – 2.80 (m, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ = 154.52, 142.48, 135.64, 131.79, 127.28, 121.93, 119.32, 118.41, 111.08, 110.31, 109.85, 107.71, 50.71, 41.43, 22.45, 22.80.

FT-IR (cm^{-1}) = 3403.4, 3058.4, 2928.5, 2843.3, 1455.2, 1297, 1142.7, 1012.9, 907.33, 598.87, 436.53.

*N-BENZYL-1-PHENYL-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**233**)*



Following the general procedure, the product **233** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.48, 95 $CHCl_3$: 5 MeOH) with an yield of 51%, after 2.5 hrs of reaction at microwave, using N-benzyl tryptamine **234** and benzaldehyde **38** as substrates.

Molecular Formula: $C_{24}H_{22}N_2$

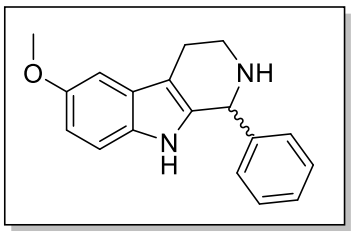
GC-MS (EI, 70 eV) = 338 ($M^{+\cdot}$), 261, 219 (100), 189, 169, 155, 91.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.60 (dd, J = 7.8, 4.0 Hz, 2H), 7.51 (dd, J = 8.1, 1.3 Hz, 3H), 7.47 – 7.37 (m, 11H), 7.35 – 7.32 (m, 1H), 7.29 (s, 1H, -NH indole), 7.29 (s, 1H), 4.70 (s, 1H), 3.97 (d, J = 13.5 Hz, 1H), 3.44 (d, J = 13.6 Hz, 1H), 3.33 – 3.26 (m, 1H), 3.03 – 2.95 (m, 2H), 2.91 – 2.83 (m, 2H), 2.73 (ddd, J = 11.8, 9.4, 4.3 Hz, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ = 141.75, 139.85, 136.57, 135.10, 129.34, 129.30, 129.05, 129.03, 128.89, 128.55, 128.38, 127.49, 127.24, 121.79, 119.65, 118.61, 111.12, 109.21, 64.79, 58.59, 48.56, 21.44.

FT-IR (cm^{-1}) = 3052, 2927, 2850, 1450, 1298, 736, 696.

*6-METHOXY-1-PHENYL-1,2,3,4-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**306**)*



Following the general procedure, the product **306** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.42, 95 $CHCl_3$: 5 MeOH) with an yield of 61%, after 1.0 h of reaction at microwave, using 6-methoxy tryptamine **318** and benaldehyde **38** as substrates.

Molecular Formula: $C_{18}H_{18}N_2O$

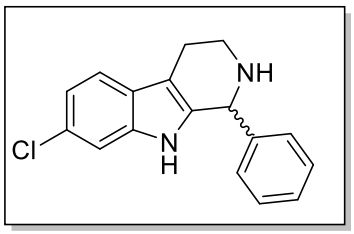
GC-MS (EI, 70 eV) = 278 ($M^{+\cdot}$, 100), 248, 218, 201, 178, 158, 130, 102, 77.

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.64 (s, 1H), 7.36 – 7.26 (m, 5H), 7.08 – 7.05 (m, 1H), 7.00 (d, J = 2.4 Hz, 1H), 6.79 (dd, J = 8.7, 2.5 Hz, 1H), 5.11 (s, 1H), 3.87 (d, J = 3.5 Hz, 3H), 3.34 (ddd, J = 12.5, 5.2, 3.9 Hz, 1H), 3.14 – 3.07 (m, 1H), 2.89 (dddd, J = 14.2, 8.8, 5.3, 1.9 Hz, 1H), 2.78 (ddd, J = 7.0, 6.2, 3.5 Hz, 1H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 154.22, 142.02, 135.64, 131.18, 129.03, 128.73, 128.40, 127.95, 111.75, 111.69, 110.21, 100.68, 58.40, 58.36, 43.05, 22.80.

FT-IR (cm^{-1}) = 3399.3, 2936.6, 2831.1, 1585.1, 1479.6, 1451.2, 1288.8, 1215.8, 1029, 728.75, 700.34.

*7-CHLORO-1-PHENYL-1,2,3,4-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**307**)*



Following the general procedure, the product **307** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.45, 95 $CHCl_3$: 5 MeOH) with an yield of 43%, after 1.0 h of reaction at microwave, using 7-chlorotryptamine **319** and benzaldehyde **38** as substrates.

Molecular Formula: $C_{17}H_{15}N_2Cl$

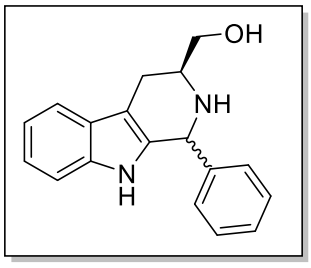
GC-MS (EI, 70 eV) = 282 ($M^{+\cdot}$), 253, 217, 200, 178, 143, 108, 77, 51

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.34 (dt, J = 4.8, 2.5 Hz, 4H), 7.30 – 7.24 (m, 3H), 7.16 (d, J = 1.8 Hz, 1H), 7.08 (dd, J = 8.4, 1.8 Hz, 1H), 5.11 (t, J = 1.9 Hz, 1H), 3.39 – 3.31 (m, 1H), 3.16 – 3.06 (m, 1H), 2.94 – 2.84 (m, 1H), 2.82 – 2.73 (m, 1H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 141.66, 136.41, 135.41, 129.13, 128.67, 128.56, 127.63, 126.23, 120.24, 119.25, 111.06, 110.51, 58.25, 58.22, 42.91, 22.60

FT-IR (cm^{-1}) = 3147.5, 2921.2, 2844.4, 1620.2, 1454.5, 1301, 1058.6, 905.05, 800, 593.94.

*(1RS, 3S)-3-HYDROXYMETHYL-PHENYL-1,2,3,4-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**310a/310b**)*



Following the general procedure, the product **310** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.36, 95 $CHCl_3$: 5 MeOH) with an yield of 51%, after 1.0 h of reaction at microwave, using L-tryptophanol **278** and benzaldehyde **38** as substrates.

Molecular Formula: $C_{18}H_{18}N_2O_2$

GC-MS (EI, 70 eV) = 278.0 ($M^{+\cdot}$), 245 (100), 218, 144, 115, 77.

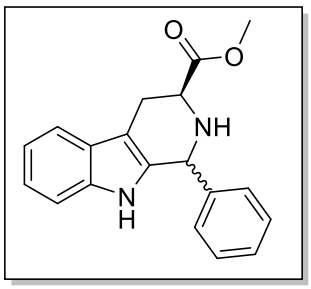
^{1}H-NMR (400 MHz, $CDCl_3$, as mixture of diastereoisomers) δ = 7.53 – 7.49 (m, 1H), 7.38 – 7.31 (m, 4H), 7.21 – 7.09 (m, 4H), 5.15 (t, J = 2.2 Hz, 1H), 3.84 (dd, J = 10.8, 3.7 Hz, 1H), 3.59

(dd, J = 10.6, 8.0 Hz, 1H), 3.33 – 3.26 (m, 1H), 2.79 (tdd, J = 7.8, 5.0, 3.0 Hz, 2H), 2.63 – 2.55 (m, 1H).

^{13}C-NMR (100 MHz, $CDCl_3$, as mixture of diastereoisomers) δ 141.42, 136.30, 135.32, 129.21, 128.79, 128.70, 127.47, 122.16, 122.03, 119.70, 118.40, 111.14, 109.84, 66.38, 59.01, 58.98, 56.65, 46.19, 24.87.

FT-IR (cm-1) = 3276, 3058, 2922, 1538, 1454, 1320, 1308, 1019, 909, 731, 699.

*(3S)-METHYL-(1RS)-PHENYL-1,2,3,4-TETRAHYDRO-9H-PYRIDO-[3,4-b]-INDOLE-3-CARBOXYLATE (**311a/311b**)*



Following the general procedure, the product **311** was isolated as a dark-yellow oil by SiO_2 chromathography (Rf = 0.41, 95 $CHCl_3$: 5 MeOH) with an yield of 55%, after 1.0 h of reaction at microwave, using methyl ester of L-tryptophan **320** and benzaldehyde **38** as substrates.

Molecular Formula: $C_{18}H_{18}N_2O_2$

GC-MS (EI, 70 eV) = 306 ($M^{+\cdot}$), 275, 247, 218 (100), 189, 169, 144, 115, 77.

^{1}H-NMR (400 MHz, $CDCl_3$ as mixture of diastereoisomers) δ = 7.63 (s, 1H, -NH indole), 7.57 – 7.53 (m, 2H), 7.47 (s, 1H, -NH indole), 7.40 – 7.10 (m, 16H), 5.43 (s, 1H), 5.25 (s, 1H), 3.98 (dd, J = 11.0, 4.1 Hz, 3H), 3.81 (s, 3H), 3.71 (s, 3H), 3.33 – 3.21 (m, 2H), 3.15 (dd, J = 14.8, 6.2 Hz, 1H), 3.06 – 2.99 (m, 1H).

^{13}C-NMR (100 MHz, $CDCl_3$ as mixture of diastereoisomer) δ 174.12, 173.40, 143.70, 141.77, 140.83, 136.94, 136.41, 136.37, 134.81, 133.13, 129.97, 129.21, 129.00, 128.88, 128.72, 128.46, 127.30, 127.14, 122.24, 122.20, 119.87, 119.77, 118.49, 118.44, 111.18, 109.12, 108.60, 58.90, 57.10, 55.19, 52.72, 52.53, 52.44, 25.91, 24.75.

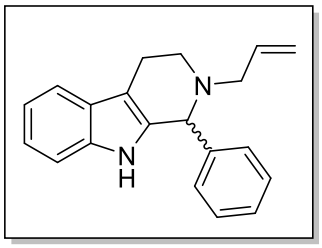
FT-IR (cm^{-1}) = 3393, 2925, 2853,, 1733, 1457, 1270, 1216, 1176, 734, 703**.**

*GENERAL METHOD FOR THE SYNTHESIS OF N-SUBSTITUTED TETRAHYDRO-β-CARBOLINES by ONE-POT CERIUM PROTOCOL (**324-332**):*

Tryptamine **216** (100 mg, 0.625 mmol, 1.0 eq.) is dissolved in ACN *dry* forming a solution of 0.25M in a microwave vial, aldehyde (0.625 mmol, 1.0 eq.) is added dropwise, then $CeCl_3 \cdot 7H_2O$ (69 mg, 0.19 mmol, 0.3 eq.) is added one pot and then

halogen-compound (0.92 mmol, 1.5 eq.) is added dropwise and molecular sieves (62 mg, 100 mg/mmol) are added, then the vial is put in the microwave reactor at 120 °C. The reaction is monitored by TLC (8 Hex : 2 EtOAc) and GC-MS. Once that the starting material is consumed or no more evolution were observed, the solution is diluted by DCM (15 mL) and sat. $NaHCO_3$ (15 mL), and the aqueous layer is washed 2 time with fresh DCM (2x15 mL). The organic phase is dried over anhydrous sodium sulphate and the solvent is evaporated by rotavapor to give the product. If necessary, the product can be purified by a pad of Silica Gel (gradient from 95 Hex : 5 EtAOAc to 70 Hex : 30 EtOAc).

*N-ALLYL-1-PHENYL-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**324**)*



Following the general procedure, the product **324** was isolated as a bright yellow oil by SiO_2 chromathography (Rf = 0.54, 80 Hex : 20 EtOAc) with an yield of 21%, after 1.0 h of reaction at microwave, using tryptamine **216**, benzaldehyde **38** and allyl bromide as substrates.

Molecular Formula: $C_{20}H_{20}N_2$

GC-MS (EI, 70 eV) = 288($M^{+\cdot}$), 245, 218 (100), 189, 169, 144, 115, 77.

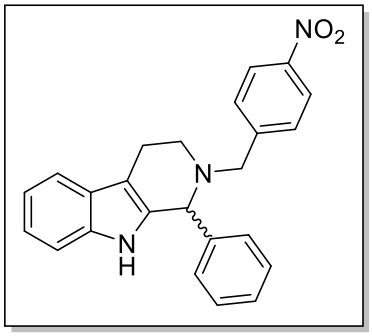
^{1}H-NMR (500 MHz, $CDCl_3$) δ = 7.60 – 7.56 (m, 1H), 7.43 – 7.36 (m, 4H), 7.32 – 7.28 (m, 1H), 7.21 (dd, J = 6.0, 3.1 Hz, 1H), 7.17 – 7.12 (m, 2H), 5.96 (dddd, J = 11.9, 7.1, 5.5, 2.6 Hz, 1H), 5.29 – 5.20 (m, 2H), 4.69 (d, J = 1.1 Hz, 1H), 3.37 (ddd, J = 9.2, 6.0, 3.7 Hz, 2H), 3.07 – 2.98 (m, 2H), 2.90 (ddd, J = 8.1, 5.6, 4.1 Hz, 1H), 2.83 – 2.74 (m, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) δ = 141.08, 136.34, 135.61, 134.72, 129.11, 128.71, 128.09, 127.23, 121.54, 119.38, 118.31, 117.70, 110.80, 109.06, 63.89, 57.10, 48.22, 21.08.

FT-IR (cm^{-1}) = 3412, 3061, 2919, 2809, 1456, 1452, 1303, 1267, 928, 739, 699.

m.p. (range °C) = 109-110 °C

*N-(4'-NITROBENZYL)-1-PHENYL-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**325**)*



Following the general procedure, the product **325** was isolated as a yellow-orange solid by SiO_2 chromathography (Rf = 0.63, 80 Hex : 20 EtOAc) with an yield of 52%, after 0.5 h of reaction at microwave, using tryptamine **216**, benzaldehyde **38** and p-nitrobenzyl bromide as substrates.

Molecular Formula: $C_{24}H_{21}N_3O_2$

ESI (+) = 384 $[M+H]^+$.

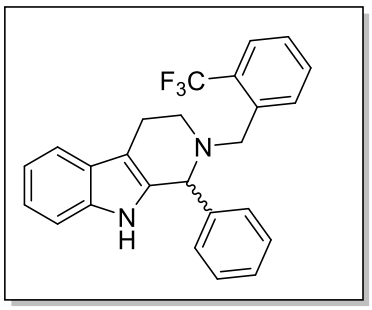
^{1}H-NMR (500 MHz, $CDCl_3$) δ = 8.25 – 8.19 (m, 2H), 7.58 (dd, J = 11.7, 5.8 Hz, 3H), 7.50 (dd, J = 5.2, 3.1 Hz, 2H), 7.47 – 7.38 (m, 3H), 7.34 (s, 1H, -NH indole), 7.24 – 7.15 (m, 3H), 4.72 (s, 1H), 3.99 (d, J = 14.5 Hz, 1H), 3.53 (d, J = 14.5 Hz, 1H), 3.19 (ddd, J = 11.5, 5.0, 3.5 Hz, 1H), 3.06 – 2.98 (m, 1H), 2.87 (dd, J = 12.4, 2.9 Hz, 1H), 2.79 – 2.71 (m, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) δ = 147.81, 147.15, 140.93, 136.41, 134.50, 129.10, 129.05, 128.97, 128.47, 127.13, 123.58, 121.76, 119.55, 118.37, 110.93, 108.84, 65.02, 57.78, 48.89, 21.37.

FT-IR (cm^{-1}) = 3406, 3056, 3033, 2947, 2908, 2839, 1601, 1512, 1341, 1105, 852, 735, 697.

m.p. (range °C) = 98-100 °C

*N-(2'-TRIFLUOROMETHYL BENZYL)-1-PHENYL-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**326**)*



Following the general procedure, the product **326** was isolated as a bright grey solid by SiO_2 chromathography (Rf = 0.63, 80 Hex : 20 EtOAc) with an yield of 52%, after 0.5 h of reaction at microwave, using tryptamine **216**, benzaldehyde **38** and 2-trifluoromethylbenzyl bromide as substrates.

Molecular Formula: $C_{25}H_{21}N_2F_3$

GC-MS (EI, 70 eV) = 406 ($M^{+\cdot}$), 245, 218 (100), 189, 159, 143, 109, 77.

^{1}H-NMR (500 MHz, $CDCl_3$) δ = 8.07 (dd, J = 7.7, 4.0 Hz, 1H), 7.73 – 7.68 (m, 1H), 7.63 (ddd, J = 12.6, 7.2, 3.2 Hz, 2H), 7.58 – 7.54 (m, 2H), 7.48 – 7.38 (m, 4H), 7.35 (s, 1H, -NH indole), 7.25 – 7.19 (m, 3H), 4.77 (d, J = 1.4 Hz, 1H), 3.99 (dd, J = 15.1, 3.9 Hz, 1H), 3.88 – 3.82 (m,

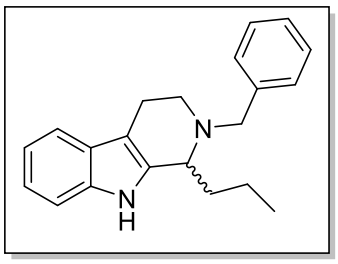
1H), 3.28 – 3.21 (m, 1H), 3.07 (ddtd, J = 15.2, 9.8, 5.0, 2.2 Hz, 1H), 2.89 (ddd, J = 15.3, 2.7, 1.2 Hz, 1H), 2.78 (tt, J = 11.7, 3.8 Hz, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) δ = 141.24, 139.21, 136.47, 135.06, 132.00, 129.86, 129.13, 128.90, 128.32, 127.28, 126.64, 125.52, 125.48, 121.65, 119.49, 118.39, 110.94, 108.99, 65.55, 53.86, 49.17, 21.54.

FT-IR (cm^{-1}) =3413, 3063, 3031, 2908, 2844, 2803, 1606, 1453, 1156, 1112, 1035, 907, 731.

m.p. (range °C) = 120-122°C

*N-BENZYL-1-PROPYL-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**330**)*



Following the general procedure, the product **330** was isolated as a bright yellow oil by SiO_2 chromathography (Rf = 0.54, 80 Hex : 20 EtOAc) with an yield of 34%, after 1.0 h of reaction at microwave, using tryptamine **216**, butanal and benzyl chloride **322** as substrates.

Molecular Formula: $C_{21}H_{22}N_2$

GC-MS (EI, 70 eV) = 304 ($M^{+\cdot}$), 261 (100), 234 (100), 169, 91, 65.

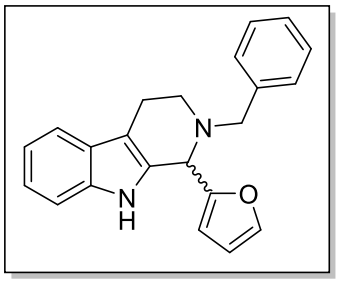
^{1}H-NMR (500 MHz, $CDCl_3$) δ = 7.67 (s, 1H, -NH indole), 7.56 (d, J = 7.7 Hz, 1H), 7.43 (d, J = 7.1 Hz, 1H), 7.40 – 7.28 (m, 5H), 7.22 – 7.15 (m, 2H), 3.80 (dt, J = 26.9, 13.4 Hz, 1H), 3.71 – 3.66 (m, 1H), 3.29 (ddd, J = 14.6, 9.7, 5.9 Hz, 1H), 3.05 – 2.92 (m, 1H), 2.67 – 2.58 (m, 1H), 1.87 – 1.71 (m, 1H), 1.60 – 1.43 (m, 1H), 0.91 (t, J = 7.4 Hz, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) δ 135.84, 128.98, 128.84, 128.22, 128.20, 127.43, 126.96, 121.38, 119.33, 118.08, 110.67, 107.84, 57.32, 56.46, 44.73, 36.93, 19.48, 17.94, 14.16.

FT-IR (cm^{-1}) = 3410, 3057, 3029, 2962, 2930, 2871, 1671, 1453, 1303, 1148, 1002, 736, 701.

m.p. (range °C) = 115-117 °C

*N-BENZYL-1-(FURAN-2-YL)-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**331**)*



Following the general procedure, the product **331** was isolated as a bright yellow oil by SiO_2 chromathography (Rf = 0.54, 80 Hex : 20 EtOAc) with an yield of 38%, after 1.5 h of reaction at microwave, using tryptamine **216**, furfural and benzylchloride **322** as substrates.

Molecular Formula: $C_{22}H_{19}N_2O$

GC-MS (EI, 70 eV) = 328 ($M^{+\cdot}$), 237, 209 (100), 180, 144, 91.

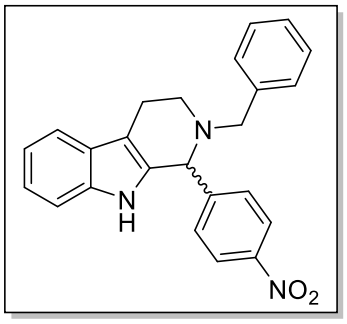
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.62 (s, 1H), 7.53 (d, J = 7.2 Hz, 1H), 7.45 – 7.41 (m, 3H), 7.36 – 7.25 (m, 5H), 7.13 (dtd, J = 18.6, 7.2, 1.2 Hz, 3H), 6.36 (dd, J = 3.2, 1.8 Hz, 1H), 6.24 (d, J = 3.2 Hz, 1H), 4.95 (s, 1H), 3.86 (d, J = 13.7 Hz, 1H), 3.70 (d, J = 13.7 Hz, 1H), 3.27 – 3.19 (m, 1H), 2.92 – 2.79 (m, 4H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 154.10, 142.81, 139.27, 136.35, 131.97, 129.04, 128.53, 128.42, 127.35, 127.27, 122.06, 119.61, 118.62, 111.09, 110.47, 109.60, 109.21, 58.30, 56.31, 47.02, 20.35

FT-IR (cm^{-1}) = 3043, 3058, 3030, 2847, 1495, 1451, 1301, 1138, 1008, 736, 696.

m.p. (range °C) = 132 – 135 °C

*N-BENZYL-1-(4'-NITROPHENYL)-2,3,4,9-TETRAHYDRO-PYRIDO[3,4-b]INDOLE (**332**)*



Following the general procedure, the product **332** was isolated as a bright yellow oil by SiO_2 chromathography (Rf = 0.61, 80 Hex : 20 EtOAc) with an yield of 57%, after 1.5 h of reaction at microwave, using tryptamine **216**, p-nitrobenzaldehyde and benzyl chloride **322** as substrates.

Molecular Formula: $C_{24}H_{22}N_3O_2$

ESI-MS (+) = 384 $[M+H]^+$

^{1}H-NMR (500 MHz, $CDCl_3$) δ = 8.21 – 8.17 (m, 2H), 7.62 – 7.57 (m, 3H), 7.43 (s, 1H, -NH indole), 7.39 – 7.30 (m, 5H), 7.28 – 7.25 (m, 1H), 7.21 – 7.14 (m, 2H), 4.80 (s, 1H), 3.84 (d, J = 13.5 Hz, 1H), 3.53 (d, J = 13.5 Hz, 1H), 3.25 – 3.20 (m, 1H), 2.95 – 2.89 (m, 2H), 2.77 (ddd, J = 12.5, 8.0, 4.7 Hz, 1H)

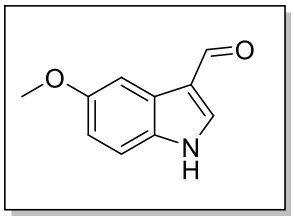
^{13}C-NMR (125 MHz, $CDCl_3$) δ = 149.55, 147.59, 138.85, 136.47, 132.72, 129.77, 128.71, 128.46, 127.32, 126.98, 123.89, 122.09, 119.70, 118.54, 111.01, 109.70, 63.06, 58.40, 47.64, 20.63.

FT-IR (cm^{-1}) = 3436, 3392, 3061, 3028, 2892, 2799, 1598, 1512, 1450, 1350, 1304, 831, 819, 743, 694, 427

m.p. (range °C) = 168 – 170 °C

Here below the synthetic methods and characterizations of intermediates for the synthesis of 6-methoxy and 7-chloro tryptamine and other tryptamine derivatives are reported.

*5-METHOXY-3-CARBOXALDEHYDE INDOLE (**314**)*



In a microwave vial 400mg of 5-methoxy indole **312** (2.72 mmol, 1.0 eq.), 1.35g of CAN@SiO_2 (149 mg of CAN, 0.1 eq.) and 1.29g of HTMA (6.8 mmol, 2.5 eq.) and then they are dissolvend in 13.6 mL of Acetonitrile and put in a microwave reactor at 90°C. The reaction is monitored by TLC (95 $CHCl_3$: 5 MeOH, Rf = 0.46) and GC-MS. Once that starting material is consumed, the mixture is transferred in a round bottom flask, the solvent is removed by rotavapor and the crude so obtained is purified by a Pad of SiO_2 (gradient from 100 $CHCl_3$ to 90 $CHCl_3$:10MeOH) to give the product as a bright gray solid with a yield of 77%.

Molecular Forumla: $C_9H_8NO_2$

GC-MS (EI, 70 eV) = 175 [$M^{+\cdot}$, 100], 160, 132, 103, 77, 51.

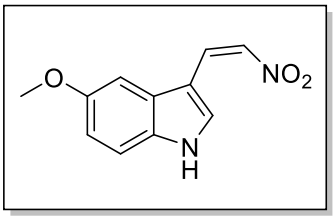
^{1}H-NMR (400 MHz, DMSO-d_6) δ = 9.87 (s, 1H), 8.18 (d, J = 3.2 Hz, 1H), 7.56 (d, J = 2.5 Hz, 1H), 7.38 (d, J = 8.8 Hz, 1H), 6.86 (dd, J = 8.8, 2.6 Hz, 1H), 3.76 (s, 3H).

^{13}C-NMR (100 MHz, DMSO-d_6) δ = 186.18, 156.83, 132.52, 126.45, 117.96, 113.93, 114.02, 103.41, 56.23

Spectroscopy data are in accordance with literature.[327]

Method for synthesis of supported CAN: 1.0g of Cerium Ammonium Nitrate (CAN) is dissolved in 2 mL of H_2O, this solution is added dropwise to SiO_2 (9.0 g) under magnetic stirring and te mixture is stirred for one hour. Then the solvent is evaporated under reduced pressure at 60°C for 4 h.

*5-METHOXY-3-(2-NITROVINYL) INDOLE (**316**)*



In a round bottom flask of 25 mL, 210 mg of 5-methoxy-3-carboxaldehyde indole **314** (1.2 mmol, 1.0 eq.) are dissolvend in 7.5 mL of nitromethane, then 369 mg of NH_4OAc (4.8 mmol, 4 eq.) are added and the reaction is refluxed (102 °C) until the starting material is consumed. The reaction is monitored by TLC (95 ($CHCl_3$: 5 MeOH, Rf = 0.54) and GC-MS. Once that starting material is consumed, the solvent is evaporated by rotavapor and the crude so obtained is purified by purified by a pad of SiO_2 gel (isocratic 100 $CHCl_3$) to give the product as an orange solid with an yield of 75%.

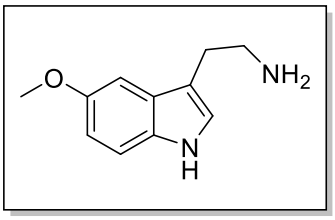
Molecular Formula: $C_{10}H_9N_2O_4$

GC-MS (EI, 70 eV) = 218 [$M^{+\cdot}$, 100], 201, 175, 156, 132, 115, 92, 75

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.78 (1H), 7.25 (m, 1H), 7.20 (m, 1H), 7.06 – 7.02 (m, 2H), 6.82 (d, J = 3.8 Hz, 1H), 3.81 (s, 3H)

FT-IR (cm.1): 3228, 2909, 1612, 1544 (-nitro), 1455, 1374, 1289.

*5-METHOXY TRYPTAMINE (**318**)*



In a round bottom flask of 50 mL 364 mg of $LiAlH_4$ (9.57 mmol, 20 eq.) is suspendend in a mixture 1:2 of THF *dry*/Et_2O *dry* (1.5 mL/3 mL), 106 mg of 5-methoxy-3-(2-nitro vinyl) indole **316** (0.48 mmol, 1.0 eq.) are dissolved in 1.0 mL of THF *dry* and added dropwise to the first solution that was cooled at 0°C with an ice water bath. The reaction is monitorated by TLC (90 $CHCl_3$:10 MeOH, Rf = 0.31) and GC-MS. Once that starting material is consumed, the solution is diluted with Et_2O (20 mL) and quenched with NaOH 1N (20 mL), the soda solution is added until formation of bubbles is observed. Once that $LiAlH_4$ is totally quenched, other 10 mL of water are added and the organic phase is separated and the aqueous layer is extracted 2 more times (2x30 mL) with fresh Et_2O. The organic phase is anhydrified with anhydrous sodium sulfate and the solvent is evaporated by rotavapor to grive the product as a grey solid with a yield of 70%. The product so obtained is used without further purifications.

Molecular Formula: $C_{10}H_{13}N_2O$

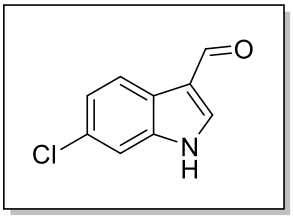
GC-MS (EI, 70 eV) = 190 [$M^{+\cdot}$], 160 (100), 145. 130, 117, 102, 89.

^{1}H-NMR ($CDCl_3$, 400 Hz) = 8.23 (s, 1H), 7.09 (d, J = 2.4 Hz, 1H), 7.04 (s, 1H), 6.90 (dd, J = 8.7 Hz, 1H), 3.91 (s, 3H), 3.08 (t, J = 6.8 Hz, 2H), 2.92 (t, J = 6.8 Hz, 2H).

^{13}C-NMR (100 MHz, DMSO-d_6) δ = 153.18, 131.34, 131.23, 128.67, 124.03, 112.93, 112.55, 111.67, 100.23

Spectroscopy data are in accordance with literature.[328]

*6-CHLORO-3-CARBOXALDEHYDE INDOLE (**315**)*



In a microwave vial 300 mg of 6-chloro indole **313** (1.97 mmol, 1.0 eq.), 981 mg of CAN@SiO_2 (108 mg of CAN, 0.1 eq.) and 937 mg of HTMA (4.9 mmol, 2.5 eq.) and then they are dissolvend in 10.2 mL of Acetonitrile and put in a microwave reactor at 90°C. The reaction is monitorated by TLC (95 $CHCl_3$: 5 MeOH, Rf = 0.51) and GC-MS. Once that starting material is consumed, the mixture is transfered in a round bottom flask, the solvent is evaporated by rotavapor and the crude so obtained is purified by a Pad of SiO_2 (gradient from 100 $CHCl_3$ to 95 $CHCl_3$: 5 MeOH) to give the product as a white solid with a yield of 47%.

Molecular Formula: C_9H_5NOCl

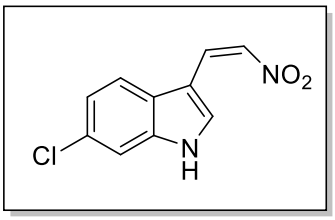
GC-MS (EI, 70 eV) = 178 [$M^{+\cdot}$, 100], 150, 123, 89, 75, 63.

^{1}H-NMR (400 MHz, DMSO-d_6) δ = 9.90 (s, 1H, -NH indole), 8.31 (s, 1H), 8.06 – 8.03 (m, 1H), 7.55 (d, J = 1.9 Hz, 1H), 7.24 – 7.21 (m, 1H).

^{13}C-NMR (100 MHz, DMSO-d6) δ = 185.76, 140.02, 137.78, 128.33, 123.23, 122.67, 122.63, 118.38, 111.91

Spectroscopy data are in accordance with literature.[329]

*6-CHLORO-3-(2-NITRO VINYL) INDOLE (**317**)*



In a round bottom flask of 25 mL, 260mg of 6-chloro-3-carboxaldehyde indole **315** (1.48 mmol, 1.0 eq.) are dissolvend in 9.2 mL of nitromethane, then 456 mg of NH_4OAc (5.94 mmol, 4 eq.) are added and the reaction is refluxed (102 °C) until the starting material is consumed. The reaction is monitorated by TLC (95 ($CHCl_3$: 5 MeOH, Rf = 0.57) and GC-MS. Once that starting material is consumed, the solvent is evaporated by rotavapor and the crude so obtained is purified by purified by a pad of SiO_2 gel (gradient 100 $CHCl_3$ to 95 $CHCl_3$: 5 MeOH) to give the product as an orange solid with an yield of 82%.

Molecular Formula: $C_{11}H_6N_2O_2Cl$

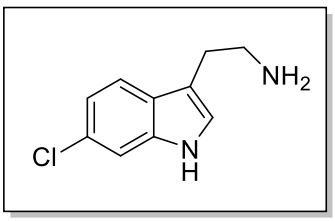
GC-MS (EI, 70 eV) = 222 [$M^{+\cdot}$, 100], 192, 175, 141, 113, 85, 63, 30.

^{1}H-NMR (400 MHz, DMSO-d_6) δ = 11.93 (1H, s), 8.42 (d, J = 12.45 Hz, 1H), 8.12 (d, J = 13.2 Hz, 1H), 8.14 (1H, s), 8.04 (d, J = 8.9 Hz, 1H), 7.61 (d, J = 2.1 Hz, 1H), 7.20 (dd, J = 1.8, 8.4 Hz, 1H)

^{13}C-NMR (100 MHz, DMSO-d_6) δ = 138.85, 137.27, 134.36, 132.42, 128.24, 123.82, 122.91, 121.23, 112.14, 108.76.

Spectroscopy data are in accordance with literature.[329]

*6-CHLORO TRYPTAMINE (**319**)*



In a round bottom flask of 250 mL 800 mg of $LiAlH_4$ (21.05 mmol, 20 eq.) is suspendend in a mixture 1:2 of THF *dry*/Et_2O *dry* (7 mL/14 mL), 160 mg of 5-methoxy-3-(2-nitro vinyl) indole **317** (1.05 mmol, 1.0 eq.) are dissolved in 1.0 mL of THF *dry* and added dropwise to the first solution that was cooled at 0°C with an ice water bath. The reaction is monitorated by TLC (90 $CHCl_3$:10 MeOH, Rf = 0.36) and GC-MS. Once that starting material is consumed, the solution is diluted with Et_2O (30 mL) and quenched with NaOH 1N (40 mL), the soda solution is added until formation of bubbles is observed. Once that $LiAlH_4$ is totally quenched, other 20 mL of water are added and the organic phase is separated and the aqueous layer is extracted 2 more times (2x30 mL) with fresh Et_2O. The organic phase is anhydrified with anhydrous sodium sulfate and the solvent is removed by

rotavapor to give the product as a grey solid with a yield of 80%. The product so obtained is used without further purifications.

Molecular Formula: $C_9H_{10}N_2Cl$

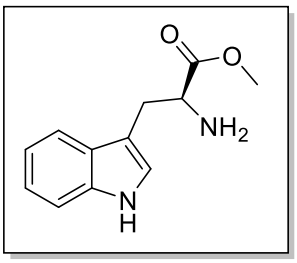
GC-MS (EI, 70 eV) = 194 [$M^{+\cdot}$, 100], 164 (100), 128, 101,75.

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.23 (s, 1H, -NH indole), 7.54 (d, J = 8.7 Hz, 1H), 7.32 (d, J = 1.7 Hz, 1H), 7.13 (dd, J = 8.1, 1.9 Hz, 1H), 7.04 (s, 1H), 3.12 (td, J = 6.9, 1.4 Hz, 2H), 2.88 (t, J = 6.3 Hz, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ = 137.43, 128.83, 126.31, 123.56, 120.75, 120.14, 114.22, 111.72, 42.27, 29.83.

Spectroscopy data are in accordance with literature.[329]

*L-TRYPTOPHAN METHYL ESTER (**321**)*



200 mg of L-tryptophan **320** (0.98 mmol, 20 eq.) are dissolved in 1 mL of MeOH inside a round bottom flask of 25 mL, then 225 µL of $SOCl_2$ (3.10 mmol, 3.2 eq.) are added dropwise to the solution and refluxed overnight. The reaction is monitored by TLC (95 $CHCl_3$: 5 MeOH, Rf = 0.42) and GC-MS. Once that the starting material is consumed, the reaction is portionated by $CHCl_3$ (10 mL) and sol. sat. of $NaHCO_3$ (10 mL), the organic phase is separated and the aqueous layer is washed two more times with fresh $CHCl_3$ (2x10 mL). The organic phase is anhydrified with anhydrous sodium sulfate and the solvent is removed by rotavapor to give the product and white-yellow solid with a yield of 94%.

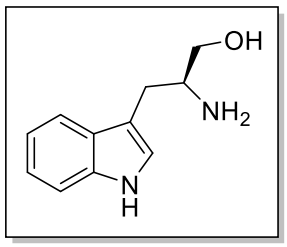
Molecular Formula: $C_{11}H_{13}N_2O_2$

GC-MS (EI, 70 eV) = 218 [$M^{+\cdot}$], 159, 130 (100), 103, 77, 51.

^{1}H-NMR (400 MHz, DMSO-d_6) δ = 10.85 (s, 1H), 7.48 (s, 1H), 7.32 (d, J = 7.0 Hz, 1H), 7.10 (s, 1H), 7.04 (s, 1H), 6.96 (s, 1H), 3.62 (s, 1H), 3.54 (s, 3H), 2.97 (d, J = 18.9 Hz, 2H).

^{13}C-NMR (101 MHz, DMSO-d_6) δ = 176.29, 136.77, 128.05, 124.32, 121.54, 118.97, 118.91, 112.04, 110.54, 55.81, 52.03, 31.38.

*L-TRYPTOPHANOL (**278**)*



200mg of L-tryptophan methyl ester **321** (0.917 mmol, 20 eq.) are dissolved in 2 mL of MeOH *dry* inside a r.b.f. of 25 mL and it is cooled down to 0°C with an ice/water bath, then 317 mg of $NaBH_4$ (9.17 mmol, 10 eq.) are added to the solution and stirred overnight. The reaction is monitorated by TLC (90 $CHCl_3$: 10 MeOH, Rf = 0.37) and GC-MS. Once that the starting material is consumed, the reaction is portionated by EtOAc (10 mL) and H_2O (10 mL), the organic phase is separated and the aqueous layer is washed two more times with fresh EtOAc (2x10 mL). The organic phase is anhydrified with anhydrous sodium sulfate and the solvent is evaporated by rotavapor to give the product and white-yellow solid with a yield of 88%.

Molecular Formula: $C_{11}H_{14}N_2O$

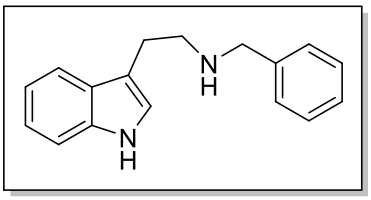
GC-MS $C_{11}H_{14}N_2O$ (EI, 70 eV) = 204 [$M^{+\cdot}$], 159, 130 (100), 103, 77, 60, 42.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H, -NH indole), 7.62 – 7.59 (m, 1H), 7.39 – 7.33 (m, 1H), 7.20 (ddd, J = 8.2, 6.1, 1.2 Hz, 1H), 7.14 – 7.10 (m, 1H), 7.03 (d, J = 2.3 Hz, 1H), 3.70 (dd, J = 10.6, 3.9 Hz, 1H), 3.44 (dd, J = 10.6, 7.2 Hz, 1H), 3.29 – 3.23 (m, 1H), 2.93 (ddd, J = 14.3, 5.1, 0.9 Hz, 1H), 2.70 (dd, J = 14.3, 8.5 Hz, 1H).

^{13}C-NMR (101 MHz, $CDCl_3$) δ = 136.18, 127.34, 122.59, 121.97, 119.38, 1198.93, 112.63, 111.04, 66.74, 52.74, 30.23.

Spectroscopy data are in accordance with literature.[330]

*N-BENZYL TRYPTAMINE (**234**)*



To a solution of tryptamine **216** (500 mg, 3.12 mmol, 1 eq.) in methanol (13 ml) is added benzaldehyde **38** (350 ml, 3.43 mmol, 1.1 eq.). The mixture is left under stirring at room temperature overnight. After that, solid $NaBH_4$ (177 mg, 4.68 mmol, 1.5 eq.) is added to reduce the imine to secondary amine. The solution is left under stirring at room temperature for 2 hours and then 6 mL saturated $NaHCO_3$ solution was added, concentrated, dissolved in dichloromethane, and washed with brine. Organic layer was extracted, dried over anhydrous sodium sulfate and concentrated in vacuo. The mixture was purified by flash column chromatography (1:3 hexanes: ethyl acetate) to yield 670 mg of the pure N-benzyltryptamine **234** (84%) as pale-yellow oil.

Molecular Formula: $C_{17}H_{17}N_2$

GC-MS (EI, 70 eV) = 250[$M^{+\cdot}$], 131, 114, 91(100), 65, 39.

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.17 (bs, 1H), 7.63 (d, J = 8.0, 1H), 7.37 – 7.28 (m, 5H), 7.25 – 7.18 (m, 2H), 7.13 (t, J = 8.0, 1H), 7.01 (d, J = 2.3 Hz, 1H), 3.84 (s, 2H), 3.02 (s, 4H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 140.4, 136.5, 128.5, 128.2, 127.6, 127.0, 122.1, 122.1, 119.4, 119.0, 114.1, 54.0, 49.5, 25.9.

Spectroscopy data are in accordance with literature.[331]

2.3h Conclusion

Tetrahydro-β-carbolines and tetrahydroisoquinolines constitue a very important subclass of alkaloids due to their wide and important biological applications. Apart the extraction from natural sources like plants, the Pictet-Spengler reaction is the most powerful way to synthesize this particular molecules through the formation of a new C-C bond. From the first paper about this reaction (1911), it was subjected to many studies by the synthetic scientific community and, how many other reactions, it can undergo to Brønsted and/or Lewis catalyses. In the recent year, carbocatalyses has shown as prominent catalyst for organic reaction due to its green properties, for this reason we tried to study the possible employment of this acid for PSR. From this point, we developed a two step one flask type protocol for the synthesis of THβCs exploiting the Graphene Oxide as Brønsted acid, microwave irradiation (green synthetic tool) for the synthesis imine-intermediate and Amerblyst-15® (green ion resin) for the synthesis of target cycle. This protocol has shown useful and it allows to obtain several and different heterocycles after few hours of reaction (respect the usual 24h) avoiding toxic and mineral acids like TFA, sulphuric acid and so on. Moreover, even if it was not investigated, the possible reuse and reactivation of resin (reported in literature) make this protocol green and respectful of green principles that rule the world today. For what concern the Lewis catalyses, the Ce(III) chloride has demonstrated an useful Lewis Acid for one pot-PSR for the synthesis of N-substituted tetrahydro-β-carbolines avoiding long reaction time, toxic solvent and formation of much amount of waste. GC-MS analyses has clarified the possible mechanism which proceeds by fist the formation of imine, second the contemporary ring-closing and nuchleophilic attack to halo-compound. Even if the studies about this reaction and the enlargement of substrate aim has been carrying on, good preliminary results were obtained for the synthesis of these important heterocycles employing a green catalyst as $CeCl_3 \cdot 7H_2O$.

Bibliography

1) Stockwell, B.R. Exploring biology with small organic molecules. *Nature* **2004**, *432*, 846–874.
2) Galloway, W. R.; Bender, A.; Welch, M.; Spring, D. R. The discovery of antibacterial agents using diversity-oriented synthesis. *Chemm. Comm*. **2009**, 2446-2462
3) Min, J.; Kwon, O.K.; Cui, C.; Park, J.H.; Wu, Y.; Park, S.Y.; Li, Y.; Brabec, C:J. High performance all-small-molecule solar cells: engineering the nanomorphology via processing additives. *J. Mater. Chem. A.* **2016**, *4*, 14234-14240.
4) McPherson, A.; Nguyen, C.; Cudney, R.; Larson, S.B. The Role of Small Molecule Additives and Chemical Modification in Protein Crystallization. *Cryst. Growth Des*. **2011**, *11*, 1469–1474.
5) He, Z.; Zhang, Z.; Bi, S. Small-molecule additives for organic thin film transistors. *J. Mater Sci: Mater Electron* **2019**, *30*, 20899–20913.
6) Chan, J.; Dodani, S.C.; Chang, C.J. Phosphine-Catalyzed Enantioselective Synthesis of Oxygen Heterocycles. *Nat. Chem.* **2012**, *4*, 973–984.
7) Hoelder, S.; Clarke, P.A.; Workman, P. Discovery of small molecule cancer drugs: Successes, challenges and opportunities. *Mol. Onc.* **2012**, *6*, 155-176.
8) Adams, J.L.; Smothers, J.; Srinivasan, R.; Hoos, A. Big opportunities for small molecules in immuno-oncology. *Nat. Rev. Drug Discov.* **2015**, *14*, 603–622.
9) Galloway, W. R.J.D.; Spring, D.R. The discovery of antibacterial agents using diversity-oriented synthesis. *Expert. Opin. Drug Dis.* **2009**, *4*, 467-472.
10) Burke, M.D.; Berger, E.M.; Schreiber, S.L. A Synthesis Strategy Yielding Skeletally Diverse Small Molecules Combinatorially. *J. Am. Chem. Soc.* **2004**, *126*, 14095-14104.
11) Varma, V.S. Greener and Sustainable Trends in Synthesis of Organics and Nanomaterials. *ACS Sustainable Chem. Eng.* **2016**, *4*, 5866–5878.
12) Cioc, R.C.; Ruijter, E.; Orru, R.V.A. Multicomponent Reactions: Advanced Tools for Sustainable Organic Synthesis. *Green Chem.* **2014**, *16*, 1-18.
13) Pathania, S.; Rawal, R.K. Green synthetic strategies toward thiazoles: a sustainable approach. *Chem. Heteroc. Comp.* **2020**, *56*, 445–454.

14) Bribi, N. Pharmacological activity of Alkaloids: A Review. *Asian Journal of Botany* **2018**, *1*, 1-6.

15) Kaur, S.; Kumari,P.; Singh,G.; Bhatti,R.; Singh, P. Design and Synthesis of Aza-/Oxa Heterocycle-Based Conjugates as Novel Anti-Inflammatory Agents Targeting Cyclooxygenase-2. *ACS Omega* **2018**, *3*, 5825−5845.

16) Ricciarelli, R.; Zingg, J.M.; Azz, A. The 80th Anniversary of Vitamin E: Beyond Its Antioxidant Properties. *Biol. Chem.* **2002**, *38*3, 457 – 465.

17) Galankis, C.M. *Polyphenols: Properties, Recovry and Applications* 1st Ed.; eBook ISBN 9780128135730; Woodhead Publishing; AT; Vienna; 2018.

18) Jangale, A.D.; Dalal, D.S. Green synthetic approaches for biologically relevant organic compounds. *Synthethic Comm*. **2017**, *47*, 2139 – 2173.

19) Paz,V.; Zalewska, K.; Santos, M.M.; Branco, L.C. *Synthetic Approaches to Nonaromatic Nitrogen Heterocycles*; Editor: Phillips, A.M.M.M.F.; Online ISBN:9781119708841; John Wiley & Sons Ltd.; PT, Lisbon; 2020, pp. 729 – 758.

20) Palucki, M.; Wolfe, J.P.; Buchwald, S.L. Synthesis of Oxygen Heterocycles via a Palladium-Catalyzed C-O Bond-Forming Reaction. *J. Am. Chem. Soc*. **1996**, *118*, 10333-10334.

21) Flores-Conde, M.I.;de la Cruz, F.N.; López, J.; Jiménez-Halla, J.O.C.; Peña-Cabrera, E.; Flores-Álamo, M.; Delgado, F.; Vázquez, M.A. Unexpected reactivity of pyridinium salts toward alkynyl Fischer complexes to produce oxo-heterocycles. *Appl. Organometal. Chem.* **2017**; *32*, e4202.

22) Xie, Y.; Floreancig; P.E. Stereoselective Synthesis of Oxo-Heterocycles via Rhenium Catalysis. *Angew. Chem. Int. Ed.* **2014**, *53*, 4926–4929.

23) Chung, Y.K.; Fu, G.C. Phosphine-Catalyzed Enantioselective Synthesis of Oxygen Heterocycles. *Angew. Chem. Int. Ed.* **2009**, *121*, 2259 –2261.

24) Ahmad, T.; Ullah, N. The oxa-Michael reaction in the synthesis of 5- and 6-membered oxygen-containing heterocycles. *Org. Chem. Front.* **2021**, *8*, 1329-1344.

25) Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouységu, L. Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. *Angew. Chem. Int. Ed*. **2011**, *50*, 586–621.

26) Obika, S.; Nambu, D.; Hari, Y.; Morio, K.I.; In, Y.; Ishida, T.; Iminashi, T. Stability and structural features of the duplexes containing nucleoside analogues with a fixed N-type conformation, 2'-O,4'- C-methyleneribonucleosides. *Tetrahedron Lett.* 1997, *38*, 8735-8738.

27) Koshkin, A.A.; Singh, S.K.; Nielsen, P.; Rajwanshi V.K.; Kumar, R.; Meldgaard, M.; Olsen, C.E.; Wengel, J. LNA (Locked Nucleic Acids): Synthesis of the Adenine, Cytosine, Guanine, 5-Methylcytosine, Thymine and Uracil Bicyclonucleoside Monomers, Oligomerisation, and Unprecedented Nucleic Acid Recognition. *Tetrahedron* **1998**, *54*, 3607 – 3630.

28) Mirsky, A. E. The Discovery of DNA. *Sci. Am.* **1968**, *218*, 78-90.

29) Jones, M.E. Albrecht Kossel, A Biographical Sketch. *Yale J. Bio. Med.* **1953**, *26*, 80-97.

30) Olby, R. DNA before Watson-Crick. *Nature* **1974**, *248*, 782 - 785.

31) Watson, J. D.; Crick, F.H.C. Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. *Nature* **1953**, *171*, 737 - 738.

32) Zamecnik, P.C.; Stephenson, M.L. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. *Proc. Natl. Acad. Sci.* **1978**, *7*5, 285-288.

33) Jespen, J.S.; Sørensen, M.D.; Wengel, J. Locked Nucleic Acid: A Potent Nucleic Acid Analog in Therapeutics and Biotechnology. *Oligonucleotides* **2004**, *14*, 130-146.

34) Dwaine, A.B.; Corey, D.R. Locked nucleic acid (LNA): fine-tuning the recognition of DNA and RNA. *Chem. Biol.* **2001**, *8*, 1-7.

35) Eckstein, F. Nucleoside phosphorothioates. *J. Am. Chem. Soc.* **1966**, *88*, 4292–4294.

36) Eckstein, F. Phosphorothioates, Essential Components of Therapeutic Oligonucleotides. *Nucleic Acid Ther.* **2014**, *24*, 374 – 387.

37) Miller, P. S.; Agris, C. H.; Murakami, A.; Reddy, P. M.; Spitz, S. A.; Ts'o, P. O. P. Preparation of oligodeoxyribonucleoside methylphosphonates on a polystyrene support. *Nucleic Acids Res.* **198**3, *11*, 6225.

38) McCuen, H. B.; Noé, M. S.; Sierzchala, A. B.; Higson, A. P.; Caruthers, M. H. Synthesis of Mixed Sequence Borane Phosphonate DNA. *J. Am. Chem. Soc.* **2006**, *128*, 8138 -8139.

39) Chaturvedi, S.; Horn, T.; Letsinger, R. L. Stabilization of Triple-Stranded Oligonucleotide Complexes: Use of Probes Containing Alternating Phosphodiester and Stereo-Uniform Cationic Phosphoramidate Linkages. *Nucleic Acids Res.* **1996**, *24*, 2318 – 2323.

40) Gupta, A.; Mishra, A.; Puri, N. Peptide Nucleic Acids: Advanced Tools for Biomedical Applications. *J. Biotech.* **2017**, *259*, 148 – 159.

41) a) Turner, B.J.; Cheah, I.K.; Macfarlane, K.J.; Lopes, E.C.; Petratos, S.; Langofrd, S.J.; Cheema, S.S. Antisense peptide nucleic acid-mediated knockdown of the p75 neurotrophin receptor delays motor neuron disease in mutant SOD1 transgenic mice. *J. Neurochem* **2003**, *87*, 752–763. b) Torres, A.G.; Threlfall, R. N.; Gait, M. J. Potent and sustained cellular inhibitionof miR-122 by lysine-derivatized peptide nucleic acids (PNA) and phosphorothioate locked nucleic acid (LNA)/2'-O-methyl (OMe) mixmer anti-miRs in the absence of transfection agents. *Artif. DNA PNA XNA* **2011**, *2*, 71–78.

42) Zhao, X.L.; Chen, B.L.; Han, J.C.; Wei, L.; Pan, X.B. Delivery of cell-penetrating peptide-peptide nucleic acid conjugates by assembly on an oligonucleotide scaffold *Sci. Rep-UK* **2015**, *5*, 17640.

43) Froheler, B.C.; Wadwani, S.; Therhorst, T.J.; Gerrard, S.J. Oligodeoxynucleotides Containing C-5 Propyne Analogs of 2'-Deoxyuridine and 2'-Deoxycytidine. *Tetrahedron Lett.* **1992**, *33*, 5307 – 5310.

44) Balow, G.; Mohan, V.; Lesnik, E.A.; Johnston, J.F.; Monia, B.P.; Acevedo, O.L. Biophysical and antisense properties of oligodeoxynucleotides containing 7-propynyl-, 7-iodo and7-cyano-7-deaza-2-amino-24-deoxyadenosines. *Nucleic acid Research* **1998**, *26*, 3350-3357.

45) Buhr, C.A.; Wagner, R.W.; Grant, D.; Froheler, B.C. Oligodeoxynucleotides containing C-7 propyne analogs of 7-deaza-24-deoxyguanosine and 7-deaza-24-deoxyadenosine. *Nucleic acid research* **199**6, *24*, 2974 – 2980.

46) Sagi, J., Szakonyl, M., Vovlickova, M. and Kypr, J. Unusual Contribution of 2-Aminoadenine to the Thermostability of DNA. *J. Biomol. Struct. Dyn*. **1996**, *13*, 1035 -1037.

47) Prakash, T.P. An Overview of Sugar-Modified Oligonucleotides for Antisense Therapeutics. *Chem. Biodivers.* **2011,** *8*, 1616 – 1641.

48) Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E. Synthesis and hybridization studies on two complementary nona(2'-O-methyl)ribonucleotides. *Nucleic Acids Res.* **1987**, *15*, 6131– 6148.

49) Kawasaki, A.M.; Casper, M.D.; Freier, S.M.; Lesnik, E.A.; Zounes, M.C.; Cummins, L.L.; Gonzalez, C.; Cook, P.D. Uniformly Modified 2'-Deoxy-2'-fluoro Phosphorothioate Oligonucleotides as Nuclease-Resistant Antisense Compounds

with High Affinity and Specificity for RNA Targets. *J. Med. Chem.* **1993**, *36*, 831–841.

50) Lesnik, E.A.; Guinosso, C.J.; Kawasaki, A.M.; Sasmor, H.; Zounes, M.; Cummins, L.L.; Ecker, D.J.; Cook, P.D.; Freier, S.M. Oligodeoxynucleotides Containing 2'-OModified Adenosine: Synthesis and Effects on Stability of DNA:RNA Duplexes. *Biochemistry-US* **1993**, *32*, 7832–7838.

51) Wahlested, C.; Salmi, P.; Good, L.; Kela, J.; Johnsson, T.; Hökfelt, T.H.; Broberger, C.; Porreca, F.; Lai, J.; Ren, K.; Ossipov, M.; Koshkin, A.; Jakobsen, N.; Skouvi, J.; Oerum, H.; Jacobsen, M.H.; Wengel, J. Potent and nontoxic antisense oligonucleotides containing locked nucleic acids. *PNAS* **2000**, 97, 5633 – 5368.

52) a) Braasch, D.A.; Corey, D.R. Locked nucleic acid (LNA): fine-tuning the recognition of DNA and RNA. *Chem. Biol.* **2001**, *8*, 1–7. b) Singh, S.K.; Nielsen, P.; Koshkin, A.A.;Wengel, J. LNA (locked nucleic acids): synthesis and high-affinity nucleic acid recognition. *Chem. Commun.* **1998**, 455–456.

53) Bondensgaard, K.; Petersen, M.; Singh, S.K.; Rajwanshi, V.K.; Kumar, R.; Wengel, J.; Jacobsen, J.P. Structural Studies of LNA:RNA Duplexes by NMR: Conformations and Implications for RNase H Activity. *Chem. Eur. J.* **2000**, *6*, 2687–2695.

54) Koshkin, A.A.; Nielsen, P.; Meldgaard, M.; Rajwanshi, V.K.; Singh, S.K.; Wengel, J. LNA (Locked Nucleic Acid): An RNA Mimic Forming Exceedingly Stable LNA:LNA Duplexes. *J. Am. Chem. Soc*. **1998**, *120*, 13252–13253.

55) Levin, A.A. A review of the issues in the pharmacokinetics and toxicology of phosphorothioate antisense oligonucleotides. *Biochim. Biophys. Acta* **1999**, *1489*, 69–84.

56) Arzumanov, A.; Walsh, A.P.; Rajwanshi,W.K.; Kumar, R.; Wengel,J.; Gait, M.J. Inhibition of HIV-1 Tat-Dependent Trans Activation by Steric Block Chimeric 2'-O-Methyl/LNA Oligoribonucleotides. *Biochemistry* **2001**, *40*, 14645 – 14654.

57) Fluiter, K.; ten Asbroek, A.L.M.A.; de Wissel, M.B.; Jakobs, M.E.; Wissenbach, M.; Olsson, H.; Olsen, O.; Oerum, H.; Baas, F. In vivo tumor growth inhibition and biodistribution studies of locked nucleic acid (LNA) antisense oligonucleotides. *Nucleic Acids Res*. **200**3, *31*, 953-962.

58) Wengel, J. Synthesis of 3'-C- and 4'-C-Branched Oligodeoxynucleotides and the Development of Locked Nucleic Acid (LNA). *Acc. Chem. Res.* **1999**, *32*, 301-310.

59) Vester, B.; Wengel, J. LNA (Locked Nucleic Acid): High-Affinity Targeting of Complementary RNA and DNA. *Biochemistry* **2004**, *43*, 13233 – 13241.

60) Braasch, D.A.; Liu, Y.; Corey, D.R. Antisense inhibition of gene expression in cells by oligonucleotides incorporating locked nucleic acids: effect of mRNA target sequence and chimera design. *Nucleic Acids Res.* **2002**, *30*, 5160–5167.

61) Sakari, K.; Verster, B.; Wengel, J. Locked nucleic acid (LNA): High affinity targeting of RNA for diagnostics and therapeutics. *Drug Discov. Today: Technol.* **2005**, *2*, 287-290.

62) Arzumanov, A.; Stetsenko, D.A.; Malakhov, A.D.; Reichelt, S.; Sørensen, M.D.; Babu,B.R.; Wengel, J.; Gait, M.J. A Structure-Activity Study of the Inhibition of HIV-1 Tat-Dependent Trans-Activation by Mixmer 2'-O-Methyl Oligoribonucleotides Containing Locked Nucleic Acid (LNA), a-L-LNA, or 2'-Thio-LNA Residues. *Oligonucleotides* **2003**, *13*, 435–453.

63) Laxton, C.; Brady, K.; Moschos, S.; Turnpenny, P.; Rawal, J.; Pryde, D.C.; Sidders, B.; Corbau, R.; Pickford, c.; Murray E.J. Selection, Optimization, and Pharmacokinetic Properties of a Novel, Potent Antiviral Locked Nucleic Acid-Based Antisense Oligomer Targeting Hepatitis C Virus Internal Ribosome Entry Site. *Antimicrob. Agents Chemiother.* **2011**, *55*, 3105–3114.

64) Kloosterman, W.P.; Wienholds, E.; de Bruijn, E.; Kauppinen, S.; Plasterk, R.H.A. In situ detection of miRNAs in animal embryosusing LNA-modified oligonucleotide probes. *Nat. Methods* **2006**, *3*, 27 – 29.

65) Elmén, J.; Lindow, M.; Schütz, S.; Lawrence, M.; Petri, A.; Obad, S.; Lindholm, M.; Hedtjärn, M.; Hansen, H.F.; Berger, U.; Gullans, S.; Kearney, P.; Sarnow, P.; Straarup, E.M., Kauppinen, S. LNA-mediated microRNA silencing in non-human primate. *Nature* **2005**, *452*, 896-900.

66) Eyring, E. J.; Ofegand, J. *Biochemistry* **1967**, *6*, 2500-2510.

67) Maguire, A.R.; Meng, W.D.; Roberts, S.M.; Willets, A.J. Synthetic Approaches towards Nucleocidin and Selected Analogues; anti- HIV Activity in 4'- Fluorinated Nucleoside Derivatives. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1795-1806.

68) Agrawal, V.K.; Bansal, A.; Kumar, R.; Kumawat, B.L.; Mahajan, P. Potassium permanganate toxicity: A rare case with difficult airway management and hepatic damage *Indian J. Crit. Care Med.* **2014**, *18*, 819–821.

69) a) Schaffrat,C.; Cobb, S.L; O'Hagan, D. Cell-Free Biosynthesis of Fluoroacetate and 4-Fluorothreonine in Streptomyces cattleya. *Angew. Chemie Int. Ed.* **2002**, *41*,

3913 – 3915. b) Wei, W.,; Shi, W.K.; Wang, P.F.;Zeng, X.T.; Li, P.; Zhang, J.R.; Li, Q.;Tang, Z.P.; Peng, J.; Wu, L.Z.; Xie, M.Q.; Liu, C.; Li, X.H.; Wang, Y.C.; Xiao, Z.P.; Zhu, H.L. Adenosine analogues as inhibitors of tyrosyl-tRNA synthetase: design, synthesis and antibacterial evaluation. *Bioino. Medicin. Chem*. **2015**, *23*, 6602 – 6611. c) Müller, H.; Gabrielli, V.; Agoglitta, O.; Holl, R. Chiral pool synthesis and biological evaluation of C-furanosidic and acyclic LpxC inhibitors. *Eur. J. Med. Chem.* **2016**, *110*, 340-375.

70) Shahidi, F.; Yeo, J.D. Bioactivities of Phenolics by Focusing on Suppression of Chronic Diseases: A Review. *Int. J. Mol. Sci.* **2018**, *19*, 1573 – 1589.

71) Quideau,S.; Deffieux, D.; Douat-Casassus, C.; Pouységu, L. Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. *Angew. Chem. Int. Ed.* **2011**, *50*, 586 – 621.

72) Swain, T.; Bate-Smith in Comparative Biochemistry, Vol. 3 (Eds.: H. S. Mason, A. M. Florkin), Academic Press, New York, 1962, pp. 755 – 809.

73) Haslam, E.; Cai, Y. Plant polyphenols (vegetable tannins): gallic acid metabolism. *Nat. Prod. Rep.* **1994**, *11*, 41 – 66.

74) Brydson, J.A. in Plastic Materials 7th ed.; ISBN 0 7506 4132 0; Butterworth-Heinemann, UK, Oxford, 1999, Vol. 1, p. 635 – 667.

75) Garcìa, D.E.; Glasser, W.G.; Pizzi, A.; Laborie, M.P. Polyphenolic resins prepared with maritime pine bark tannin andbulky-aldehydes. *Ind. Crop. Prod.* **2014**, *62*, 84 – 93.

76) McNaught, A.D.; Wilkinson, A. *Compendium of Chemical Terminolgy*, 2nd ed., Blackwell Science, UK, Oxford, 1997, p. 299.

77) Tsao, R. Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients* **2010**, *2*, 1231 – 1246.

78) Gonthier, M.P.; Remesy, C.; Scalbert, A.; Cheynier, V.; Souquet, J.M.; Poutanen, K.; Aura, A.M. Microbial metabolism of caffeic acid and its esters chlorogenic and caftaric acids by human faecal microbiota in vitro. *Biomed. Pharmacother.* **2006**, *60*, 536-540.

79) Ozcan, T.; Akpinar-Bayizit, A.; Yilmaz-Ersan, L.; Delikanli, B. Phenolics in Human Health. *Int. J. Chem. Eng. App.* **2014**, *5*, 393 - 396.

80) De la Rosa, L.A.; Moreno-Escamilla, J.O.; Rodrigo-García, J.; Alvarez-Parrilla, E. *Postharvest Physiology and Biochemistry of Fruits and Vegetables*; Ed. Yahia,

E.M.; Carillo-López, ISBN (online): 978-0-12-813279-1, Woodhead Publishing, Chennai, IN, 2018, pp. 253 - 271.

81) Balasundram, N.; Sundram, K.; Samman, S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.* **2006**, *99*, 191 - 203.

82) Kawabata, J.; Mishima, M.; Kurihara, H.; Mizuntani, J.; Kobophenol, B. A tetrastilbene from Carex pumila. *Phytochemistry* **1991**, *2*, 645–6470.

83) Sabnis, R.W. Patents on Natural Products for Diagnosis/Preventing/Treating Alzheimer's Disease. In *Studies in Natural Products Chemistry*, Aha-Mr-Rahman, Ed.; Elsevier Science Publisher: Amsterdam, 2019, Vol. 61, pp 85 - 141, 85 -141.

84) Cassidy, A.; Hanley, B.; Lamuela-Raventos, R.M. Isoflavones, lignans and stilbenes – origins, metabolism and potential importance to human health. *J. Sci. Food Agr*. **2000**, *80*, 1044 - 1062.

85) Worachartcheewana, A.; Nantasenamata, C.; Isarankura-Na-Ayudhya, C.;Prachayasittikulc, S.; Prachayasittikul, V. Predicting the free radical scavenging activity of curcumin derivatives. *Chemometr. Intell. Lab.* **2011**, *109*, 207-216.

86) Lacy, A.; O'Kennedy, R. Studies on Coumarins and Coumarin-Related Compounds to Determine their Therapeutic Role in the Treatment of Cancer. *Curr. Pharm. Des*. **2004**, *10*, 3797–3811.

87) Milner, J.A. *In Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals;* Goldberg, I., Ed.; Chapman & Hall: New York, NY, USA, 1994; pp. 39-70.

88) a) Storz, G.; AImlayt, J. Oxidative stress. *Curr. Opin. Microbiol.* **1999**, *2*, 188-194. b) Neudèrffer, A.; Desvergne, J.P.; Bonnefont-Rousselot, D.; Legrand, A.; Fleury, M.B.; Largeron, M. Protective Effects of 4-Hydroxycinnamic Ethyl Ester Derivatives and Related Dehydrodimers against Oxidation of LDL: Radical Scavengers or Metal Chelators. *J. Agric. Food Chem.* **2006**, *54*, 1898 – 1905.

89) Belščak-Cvitanović, A.; Durgo. K.; Huđek, A.; Bačun-Družina, V.; Komes, D. in *Polyphenols: Properties, Recovery, and Applications*, Ed. Galanakis, C.M.; Woodhead publishing, AT, Vienna, 2018, pp. 3-44.

90) Fang, J.G.; Zhou, B. Structure−Activity Relationship and Mechanism of the Tocopherol-Regenerating Activity of Resveratrol and Its Analogues. *J. Agric. Food Chem.* **2008**, *56*, 11458 – 11463.

91) Leopoldini, M.; Marino, T.; Russo, N.; Toscano, M. Antioxidant Properties of Phenolic Compounds: H-Atom versus Electron Transfer Mechanism. *J. Phys. Chem. A* **2004**, *108*, 4916 – 4922.

92) Wright, J.S.; Johnson, E.R.; DiLabio, G.A. Predicting the Activity of Phenolic Antioxidants: Theoretical Method, Analysis of Substituent Effects, and Application to Major Families of Antioxidants. *J. Am. Chem. Soc.* **2001**, *123*, 1173 – 1183.

93) Dangles, O. The physico-chemical properties of polyphenols. How do they relate to their roles in plants, foods and human health. *Agro Food Ind. Hi Tec.* **2006**, *17*, 64–67.

94) Perron, N.R.; Brumaghim, J.L. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochem. Biophys.* **2009**, *53*, 75–100.

95) Van Acker, S.A.B.E.; Van Den Berg; D.J.; Tromp, M.N.J.L.; Griffioen, D.H.; Van Bennekom, W.P.; Van Der Vijgh, W.J.F.; Bast, A. Structural aspects of antioxidant activity of flavonoids. *Free Radical Bio. Med.* **1996**, *20*, 331 - 342.

96) Pearson, R.G. Hard and Soft Acids and Bases. *J. Am. Chem. Soc.* **1963**, *85*, 3533 – 3539.

97) Perron, N.R.; García, C.R.; Pinzón, J.R.; Chaur, M.N.; Brumaghim, J.L. Antioxidant and prooxidant effects of polyphenol compounds on copper-mediated DNA damage. *J. Inorg. Biochem.* **2011**, *105*, 745 -7 53.

98) Wang, Q.; Ping, Y.; Qian, Dai, F.; Lu, D.L.; Yan, W.J.; Chen, Y.; Zhou, B. Ortho-dihydroxychalcones as cupric ion-dependent prooxidants: Activity and mechanisms. *Food Chem.* **2013**, *141*, 1259 - 1266.

99) Kamboj, A.; Saluja, A.K.; Kumar, M.; Atri, P. Antiviral activity of plant polyphenols *J. Pharm. Res.* **2012**, *5*, 2402-2412.

100) Kumar, N.; Goel, N. Phenolic acids: natural versatile molecules with promising therapeutic applications. *Biotechnol. Reports* **2019**, *24*, e00370.

101) Rashed, K.; Zhang, X.Y.; Luo, M.T.; Zheng, M.T. *Phytopharmacology* **2012**, *3*, 199 - 207.

102) Montenegro-Landívar, M.F.; Tapia-Quirós, P.; Vecino, X.; Reig, M.; Valderrama, C.; Granados, M.; Cortina, J.L.; Sauri, J. Polyphenols and their potential role to fight viral diseases: An overview. *Sci. Total Environ.* **2021**, *801*, 149719.

103) Smith, J.J., Kremer, M.J., Luo, M., Vriend, G., Arnold, E., Kamer, G., Rossman, M.G., Mckinlay, M.A., Diana, G.D., Otto, M.J. The Site of Attachment in Human Rhinovirus 14 for Antiviral Agents That Inhibit Uncoating. *Science* **1986**, *233*, 1286 -1293.

104) Selway JT, Antiviral activity of flavones and flavans, In *Plant flavonoids in biology and medicine: biochemical, pharmacological, and structure–activityrelationships*, Alan R. Liss, Inc.; Ed. Cody, V., Middleton E., Harborne J.B.; New York, NY, US:. 1986, pp. 521 – 536.

105) a) Roberts, N.A.; Martin, J.A.; Kinchington, D.; Broadhurst, A.V.; Duncan, B.; Galpin, S.A. Rational design of peptide based HIV Protease inhibitors. *Science* **1990**, *248*, 358-361. b) Ubillas. R.; Jolad, S.D.; Bruening, R.C.; Bernard, D.; Safrin, S.; Orlando, R.; Nakanishi, K. SP-303 an Antiviral Oligometric Proanthocyanidin from the Latex of Croton lechleri (Sangre de Drago). *Phytomed.* **1994**, *1*, 77-106.

106) Liu, A.L.; Wang, H.D.; Lee, S.M.Y.; Wang, Y.T.; Du, G.H. Structure–activity relationship of flavonoids as influenza virus neuraminidase inhibitors and their in vitro anti-viral activities. *Bioorg. Med. Chem*. **2008**, *16*, 7141–7147.

107) Bellavite, P.; Donzelli, A. Hesperidin and SARS-CoV-2: New Light on the Healthy Function of Citrus Fruits. *Antioxidants* **2020**, *9*, 742 – 760.

108) Rockx, B.; Feldmann, F.; Brining, D.; Gardner, D.; LaCasse, R.; Kercher, L.; Long, D.; Rosenke, R.; Virtaneva, K.; Sturdevant, D.E.; Porcella, S.F.; Mattoon, J.; Parnell, M.; Baric, R.S.; Feldmann, H. Comparative Pathogenesis of Three Human and Zoonotic SARS-CoV Strains in Cynomolgus Macaques. *PLOS One* **2011**, *6*, e18558.

109) Rockx, B.; Baas, T.; Zornetzer, G.A.; Haagmans, B.; Sheahan, T.; Frieman, M.; Dyer, M.D.; Teal, T.H.; Proll, S.; van den Brand, J.; Baric, R.; Katze, M.G. Early Upregulation of Acute Respiratory Distress Syndrome-Associated Cytokines Promotes Lethal Disease in an Aged-Mouse Model of Severe Acute Respiratory Syndrome Coronavirus Infection. *J. Virol.* **2009**, 83, 7062-7074.

110) van den Brand, J.M.A.; Haagmans, B.L.; van Riel, D.; Osterhaus, A.D.M.E.; Kuiken, T. The Pathology and Pathogenesis of Experimental Severe Acute Respiratory Syndrome and Influenza in Animal Models. *J. Comp. Pathol*. **2014**, *151*, 83–112.

111) Kumar, P.; Khanna, M.M Srivastava, Kumar Tyagi, Y.; Raj, H. G.; Ravi, K. Effect of Quercetin supplementation on lung antioxidants after experimental influenza virus infection. *Exp. Lung Res.* **2005**, *31*, 449–459.

112) Wu, J. Tackle the free radicals damage in COVID-19. *Nato Sci. S. A. Lif. Sci.* **2020**, *102*, 39 – 41.

113) a) Park, H.-K.; Kang, S.W.; Park, M.S. Hesperidin Ameliorates Hepatic Ischemia-Reperfusion Injury in Sprague-Dawley Rats. *Transplant. Proc.* **2019**, *51*, 2828 – 2832. b) Wu, C.; Liu, Y.; Yang, Y.; Zhang, P.; Zhong, W.; Wang, Y.; Wang, Q.; Xu, Y.; Li, M.; Li, X.; Zheng, M.; Chen, L.; Li, H. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharm. Sin. B* **2020**, *10*, 766–788.

114) Chen, Y.W.; Yiu, C.B.; Wong, K.Y. Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease (3CL pro) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates. *F1000Research* **2020**, 9:129.

115) a) Du, K.; McGill, M.R.; Xie, Y.; Bajt, M.L.; Jaeschke, H. Resveratrol prevents protein nitration and release of endonucleases from mitochondria during acetaminophen hepatotoxicity. *Food Chem. Toxicol.* **2015**, *81*, 62–70. b) La Vecchia, C.; Chatenoud, L.; Franceschi, S.; Soler, M.; Parazzini, F.; Negri, E. Vegetables and fruit and human cancer: Update of an Italian study. *Int. J. Cancer* **1999**, *82*, 151 - 152. c) Bahadoran, Z.; Tohidi, M.; Nazeri, P.; Mehran, M.; Azizi, F.; Mirmiran, P. Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial. *Int. J. Food Sci. Nut.* **2012**, *63*, 767 – 771. d) Daglia, M. Polyphenols as antimicrobial agents. *Curr. Opin. Biotech.* **2012**, *23*, 174 – 181.

116) Srinivasa, U.S.; Tana, B.W.K.; Vellayappan, B.A.; Jeyasekharana, A.D. ROS and the DNA damage response in cancer. *Redox Biol*. **2019**, *25*, 101084.

117) Iacopetta, D.; Grande, F.; Caruso, A.; Mordocco, R.A.; Plutino, M.R.; Scrivano, L.; Ceramella, J.; Muià, N.; Saturnino, C.; Puoci, F.; Rosano, C.; Sinicropi, M.S. New insights for the use of quercetin analogs in cancer treatment. *Future Med. Chem.* **2017**, *9*, 2011 – 2028.

118) Perrotta, C.; Buonanno, F.; Zecchini, S.; Giavazzi, A.; Proietti Serafini, F.; Catalani, E.; Guerra, L.; Belardinelli, M.C.; Picchietti, S.; Fausto, A.M.; Giorgi, S.; Marcantoni, E.; Clementi, E.; Ortenzi, C.; Cervia, D. Climacostol reduces tumour

progression in a mouse model of melanoma via the p53-dependent intrinsic apoptotic programme. *Sci Rep.* **2016**, 6:27281.

119) Fahrioglu, U.; Dodurga, Y.; Elmas, L.; Seçme, M. Ferulic acid decreases cell viability and colony formation while inhibiting migration of MIA PaCa-2 human pancreatic cancer cells in vitro. *Gene* **2016**, *576*, 476 – 482.

120) Fang, H.Y.; Wang, H.W.; Chang, K.F.; Hu, H.T.; Hwang, L.J.; Fu, T.F.; Lin, Y.C.; Chang, W.C.; Chiu, T.P.; Wen, Z.H.; Chien-Chi, Y.F.; Chen, C.B.H. Feruloyl-l-arabinose attenuates migration, invasion and production of reactive oxygen species in H1299 lung cancer cells. *Food Chem. Toxicol*. **2013**, *58*, 459 - 466.

121) Vaquero, R.M.J.; Manca de Nadra, M. C. Growth Parameter and Viability Modifications of Escherichia coli by Phenolic Compounds and Argentine Wine Extracts. *Appl. Biochem. Biotechnol.* **2008**, *151*, 342 – 352.

122) Vaquero, R.M.J.; Fernàndez, A.P.A.; de Nadra, M.M.C.; de Saad, S.A.M. Phenolic Compound Combinations on Escherichia coli Viability in a Meat System. *J. Agric Food Chem* **2010**, *58*, 6048 – 6052.

123) Pragasam, S.J.; Venkatesan, V.; Rasool, M. Immunomodulatory and Anti-inflammatory Effect of p-Coumaric Acid, a Common Dietary Polyphenol on Experimental Inflammation in Rats. *Inflammation* **2013**, *36*, 169–176.

124) Chao, C.Y.; Mong, M.C.; Chan, K.C.; Yin, M.C. Anti-glycative and anti-inflammatory effects of caffeic acid and ellagic acid in kidney of diabetic mice. *Mol. Nutr. Food Res.* **2010**, *54*, 388–395.

125) Menon, V.P.; Sudheer, A.R. chapter Antioxidant and Anti-Inflammatory Properties of Curcumin; In *The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease*, Springer; Boston, 2007, Vol. 595, pp 105 – 125.

126) Małgorzata, K.; Jadwiga, J.L. Polyphenols in Parkinson's Disease: A Systematic Review of In Vivo Studies. *Nutrients* **2018**, *10*, 642.

127) Choi, D.Y.; Lee, Y.J.; Hong, J.T.; Lee, H.J. Antioxidant properties of natural polyphenols and their therapeutic potentials for Alzheimer's disease. Brain Res. Bull. **2012**, 87, 144 – 153.

128) Floris, B.; Galloni, P.; Conte, V.; Sabuzi, F. Tailored Functionalization of Natural Phenols to Improve Biological Activity. *Biomolecules* **2021**, *11*, 1325.

129) Hirpara, K.V.; Aggarwal, P.; Mukherjee, A.J.; Joshi, N.; Burman, A.C. *Anti-Cancer Agent. Me.* **2009**, *9*, 138-161.

130) Yuan, J.; Wong, I.L.K.; Jiang, T.; Wang, S.W.; Liu, T.; Wen, B.J.; Chow, L.M.C.; Wan Sheng, B. Synthesis of methylated quercetin derivatives and their reversal activities on P-gp- and BCRP-mediated multidrug resistance tumour cells. *Eur. J. Med. Chem*. **201**2, *54*, 413 – 422.

131) Shi, Z.H.; Li, N.G.; Tang, Y.P.; Shi, Q.P.; Tang, H.; Li, W.; Zhang, X.; Fu, H.-A.; Duan, J.A. Biological evaluation and SAR analysis of O-methylated analogs of quercetin as inhibitors of cancer cell proliferation. *Drug Dev. Res*. **2014**, *75*, 455 – 462.

132) Walle, T. Methylation of Dietary Flavones Increases Their Metabolic Stability and Chemopreventive Effects. *Int. J. Mol. Sci.* **2009**, *10*, 5002–5019.

133) Juvale, K.; Stefan, K.; Wiese, M. Synthesis and biological evaluation of flavones and benzoflavones as inhibitors of BCRP/ABCG2. *Eur. J. Med. Chem.* **2013**, *67*, 115 - 126.

134) Massi, A.; Bortolini, O.; Ragno, D.; Bernardi, T.; Sacchetti, G.; Tacchini, M.; De Risi, C. Research Progress in the Modification of Quercetin Leading to Anticancer Agents. *Molecules* **2017**, *22*, 1270.

135) Martins, I.L.; Charneira, C.; Gandin, V.; Ferreira Da Silva, J.L.; Justino, G.C.; Telo, J.P.; Vieira, A.J.S.C.; Marzano, C.; Antunes, A.M.M. Selenium-Containing Chrysin and Quercetin Derivatives: Attractive Scaffolds for Cancer Therapy. *J. Med. Chem*. **2015**, *58*, 4250 – 4265.

136) Mangiavacchi, F.; Botwina, P.; Menichetti, E.; Bagnoli, L.; Rosati, O.; Marini, F.; Fonseca, S.F.; Abenante, L; Alves, D.; Dabrowska, A.; Kula-Pacurar, A.; Ortega-Alarcon, D.; Jimenez-Alesanco, A.; Ceballos-Laita, L; Vega, S.; Rizzuti, B.; Abian, O.; Lenardão, E.J.; Velazquez-Campoy, A.; Pyrc, K.; Sancineto, L.; Santi, C. Seleno-Functionalization of Quercetin Improves the Non-Covalent Inhibition of Mpro and Its Antiviral Activity in Cells against SARS-CoV-2. *Int. J. Mol. Sci.* **2021**, *22*, 7048.

137) Cho, S.Y.; Kim, M.K.; Mok, H.; Choo, H.; Chong, Y. Separation of Quercetin's Biological Activity from Its Oxidative Property through Bioisosteric Replacement of the Catecholic Hydroxyl Groups with Fluorine Atoms. *J. Agric. Food Chem.* **2012**, *60*, 6499 − 6506.

138) Beevers, C.S.; Huang, S. Pharmacological and clinical properties of curcumin. *Botanics: Targets and Therapy* **2011**, *1*, 5–18.

139) Oglah, M.K.; Mustafa, Y.F. Synthesis, Antioxidant, and Preliminary Antitumor Activities of New curcumin Analogues. *Journal of Global Pharma Technology* **2020**, *12* , 854 - 862.

140) Bairwa, K.; Grover, J.; Kania, M.; Jachak, S.M. Recent developments in chemistry and biology of curcumin analogues. *RSC Adv.* **2014**, *4*, 13946 – 13978.

141) Liang, G.; Shao, L.; Wang, Y.; Zhao, C.; Chu, Y.; Xiao, J.; Zhao, Y.; Li, X.; Yang, S. Exploration and synthesis of curcumin analogues with improved structural stability both in vitro and in vivo as cytotoxic agents. *Bioorg. Med. Chem*. **2009**, *17*, 2623 – 2631.

142) Zohreh, T.; Dangolani, S.K.; Yousefi, R.; Panahi. F.; Shahsavani, M.B.; Khalafi-Nezhad, A. Synthesis of new curcumin derivatives as influential antidiabetic α-glucosidase and α-amylase inhibitors with anti-oxidant activity. *Carbohyd. Res.* **2020**, *494*, 108069.

143) Quassinti, L.; Ortenzi, F.; Marcantoni, E.; Ricciutelli, M.; Lupidi, G.; Ortenzi, C.; Buonanno, F.; Bramucci, M. DNA binding and oxidative DNA damage induced by climacostol–copper(II) complexes: Implications for anticancer properties. *Chem.-Biol. Interact.* **2013**, *206*, 109–116.

144) Petrelli, D.; Buonanno, F.; Vitali, L.A.; Ortenzi, C. Antimicrobial activity of the protozoan toxin climacostol and its derivatives. *Biologia* **2012**, *67*, 525 – 529.

145) Buonanno, F:; Ortenzi, C. The protozoan toxin climacostol and its derivatives: Cytotoxicity studies on 10 species of free-living ciliates. *Biologia* **2010**, *65*, 675–680.

146) Buonanno, F.; Quassinti, L.; Bramucci, M.; Amantini, C.; Lucciarini, R.; Santoni, G.; Ilo, H.; Ortenzi, C: The protozoan toxin climacostol inhibits growth and induces apoptosis of human tumor cell lines *Chem. Biol. Interact.* **2008**, *176*, 151–164.

147) Fiorini, D.; Giuli, S.; Marcantoni, E.; Quassinti, L.; Bramucci, M.; Amantini, C.; Santoni, G.; Buonanno, F.; Ortenzi, C. A Straightforward Diastereoselective Synthesis and Evaluation of Climacostol, A Natural Product with Anticancer Activities. *Synthesis* **2010**, *9*, 1550 - 1556.

148) Buonanno, F.; Catalani, E.; Cervia, D.; Proietti Serafini, F.; Picchietti, S.; Fausto, A.M.; Giorgi, S.; Lupidi, G.; Rossi, F.V.; Marcantoni, E.; Petrelli, D.; Ortenzi, C. Bioactivity and Structural Properties of Novel Synthetic Analogues of the Protozoan Toxin Climacostol. *Toxins* **2019**, *11*, 42.

149) Akthar, J. COVID19 inhibitors: A prospective therapeutics. *Bioorg. Chem.* **2020**, *101*, 104027.

150) Yang, P.; Wang, X. COVID-19: a new challenge for human beings. *Cell. Mol. Immunol.* **2020**, *17*, 555 - 557.

151) Kitajima, M.; Ahmed, W.; Bibby, K.; Carducci, A.; Gerba, C.P.; Hamilton. K.A.; Haramoto, E.; Rose, J.B. SARS-CoV-2 in wastewater: State of the knowledge and research needs *Sci. Total. Environ,*. **2020**; *739*, 139076.

152) Boni, M.F.; Lemey, P.; Jiang, X.; Tsan-Yuk Lam, T.; Perry, B.; Castoe, T.; Rambaut, A.; Robertson, D.L. volutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19 pandemic. *Nat. Microbiol*. **2020**, *5*, 1408 - 1417.

153) Kargesa, J.; Cohen, S.M. Metal Complexes as Antiviral Agents for SARS-CoV-2. *Chem Bio Chem* **2021**, *22*, 2600 – 2607.

154) Colalto, C. Volatile molecules for COVID-19: A possible pharmacological strategy? *Drug Develop. Res*. **2020**, *81*, 950 - 968.

155) Gurunathan, S.; Qasim, M.; Choi, Y.; Do, J.T.; Park, C.; Hong, K.; Kim, J.H.; Song, H. Antiviral Potential of Nanoparticles—Can Nanoparticles Fight Against Coronaviruses? *Nanomaterials-Basel* **2020**, *10*, 1645.

156) Liu, W.; Morse, J.S.; Lalonde, T.; Shiqing Xu, S. Learning from the Past: Possible Urgent Prevention and Treatment Options for Severe Acute Respiratory Infections Caused by 2019-nCoV. *Chem. Bio. Chem.* **2020**, *21*, 730 – 738.

157) Zhu, W.; Xu, M.; Chen, C.Z.; Guo, H.; Shen, M.; Hu, X.; Shinn, P.; Klumpp-Thomas, C.; Michael, S.G.; Zheng, W. Identification of SARS-CoV-2 3CL Protease Inhibitors by a Quantitative High-Throughput Screening. *ACS Pharmacol. Transl. Sci*. **2020**, *3*, 1008 − 1016.

158) Abian, O.; Ortega-Alarcon, D.; Jimenez-Alesanco, A.; Ceballos-Laita, L.; Vega, S.; Reyburn, H.T.; Rizzuti, B.; Velazquez-Campoy, A. Structural stability of SARS-CoV-2 3CLpro and identification of quercetin as an inhibitor by experimental screening. *Int. J. Biol. Macrol.* **2020**, *164*, 1693-1703.

159) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P.J.J.A.D.D.R. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliver. Rev.* **1997**, *23*, 3-25.

160) Stefani, M.; Rigacci, S. Beneficial properties of natural phenols: Highlight on protection against pathological conditions associated with amyloid aggregation. *Biofactors* **2014**, *40*, 482-493.

161) Catalani, E.; Buonanno, F.; Lupidi, G. Bongiorni, S.; Belardi, R.; Zecchini, S.; Giovarelli, M.; Coazzoli, M.; De Palma, C.; Perrotta, C.; Clementi, E.; Prantera, G.; Marcantoni, E.; Ortenzi, Fausto, A.M.; Picchietti, S.; Cervia, D. The Natural Compound Climacostol as a Prodrug Strategy Based on pH Activation for Efficient Delivery of Cytotoxic Small Agents. *Front. Chem.* **2019**, *7*, 463.

162) Andrus, M.B.; Liu, J. Synthesis of polyhydroxylated ester analogs of the stilbene resveratrol using decarbonylative Heck couplings. *Tetrahderon Lett.* **2006**, *47*, 5811 - 5814.

163) Wong, Y.; Osmond, G.; Brewer, K.I.; Tyler, D.S.; Andrus, M.B. Synthesis of 4'-ester analogs of resveratrol and their evaluation in malignant melanoma and pancreatic cell lines. *Bioorgan. Medic. Chem. Lett.* **2010**, *20*, 1198 – 1201.

164) Petrini, M.; Ballini, R.; Marcantoni, E.; Rossini, G. Amberlyst 15: A Practical, Mild and Selective Catalyst for Methyl Esterification of Carboxylic Acids. *Synth Comm*. **1988**, *18*, 847-853.

165) Lamba, R.; Kumar, S.; Sarkar, S. Esterification of decanoic acid with methanol using Amberlyst 15: Reaction kinetics. *Chem. Eng. Commun*. **2018**, *205*, 281-294.

166) Davies, K.A.; Abel, R.C.; Wulff, J.E. Operationally Simple Copper-Promoted Coupling of Terminal Alkynes with Benzyl Halides. *J. Org. Chem.* **2009**, *74*, 3997 – 4000.

167) Princivala, J.L.; Ferreira, J.G. $CeCl_3$-mediated addition of acetylenic bis-lithium salts to aldehydes and ketones: An efficient route to bis-substituted alkyne diols. *Tetrahedron Lett.* **2017**, *58*, 3525 - 3528.

168) Jeanbourquin, L.N.; Scopelliti, R.; Tirani, F.F.; Severin, K. Synthesis and Reactivity of 1-Allenyltriazenes. *Org. Lett.* **2017**, *19*, 2070 – 2073.

169) a) Pasto, D.J.; Chou, S.K.; Waterhouse, A.; Shults, R.H.; Hennion, G.F. Transition Metal Catalysis in Aliene Formation from Grignard Reagents and Propargyl Chlorides. *J. Org. Chem.* **1978**, *3*, 1385 – 1387. b) Zhao, Y.; Song, Q. Copper-catalyzed tandem A3-coupling–isomerization–hydrolysis reactions of aldehydes and terminal alkynes leading to chalcones. *Org. Chem. Front.* **2016**, *3*, 294 - 297. c) Otsuka, M.; Tsuchida, N.; Ikeda, Y.; Lambert, N.; Nakamura, R.; Mutoh, Y.; Ishii,

Y.;Takano, K. Theoretical Study on Internal Alkyne/Vinylidene Isomerization in Group 8 Transition-Metal Complexes. *Organometallics* **2015**, *34*, 3934 – 3943.

170) Kawaguchi, Y.; Yasuda, S:; Kaneko, A.; Oura, Y.; Mukai, K. Rhodium(I)-Catalyzed Cycloisomerization of Benzylallene-Alkynes through C-H Activation. *Angew. Chem. Int. Ed.* **2014**, *53*, 7608 – 7612.

171) Boyer, J.; Allenbach, Y.; Ariza, X.; Garcia, J., Georges, Y.; Vicente, M. New Stereoselective Approach to 1,2,3-Triols: Application to a Straight forward Access to Polyoxamic Acid Array. *Syn. Lett*. **2006**, *12*, 1895 – 1898.

172) Mukai, C.; Inagaki, F.; Yoshida, T.; Yoshitani, K.; Hara, Y.; Kitagaki. S. Rh(I)-Catalyzed Pauson−Khand Reaction and Cycloisomerization of Allenynes: Selective Preparation of Monocyclic, Bicyclo[m.3.0], and Bicyclo[5.2.0] Ring Systems. *J. Org. Chem.* **2005**, *70*, 7159 – 7171.

173) Zhou, J.C.; Zhao, L.; Li, Y.; Fu, D.Q.; Li, Z.C.; Huang, W.C. Alkynylation of aldehydes mediated by zinc and allyl bromide: a practical synthesis of propargylic alcohols. *Res. Chem. Intermediat.* **2017**, *43*, 4283 - 4294

174) a) Sharma, S.; Das, Y.; Das, P. Hydrogenation of nitroarenes to anilines in a flow reactor using polystyrene supported rhodium in a catalyst-cartridge (Cart-Rh@PS). *New J. Chem.* **2019**, *43*, 1764 - 1769. b) Tukacs, J.M.; Jones, R.V.; Darvas, F.; Dibó, G.; Lezsák, G.; Mika, L.T. Synthesis of γ-valerolactone using a continuous-flow reactor. *RSC Adv.* **2013**, *3*, 16283 – 16287.

175) a) Kaur, N.J. Metal catalysts: applications in higher-membered N-heterocycles synthesis. *Iran Chem. Soc*. **2015**, *12*, 9 – 45. b) Wang, Y.; Zhang, W.X.; Xi, Z. Carbodiimide-based synthesis of N-heterocycles: moving from two classical reactive sites to chemical bond breaking/forming reaction. *Chem. Soc. Rev.* **2020**, *49*, 5810 – 5849. c) Liu, J.; Jiang, J.; Zheng, L.; Liu, Z.Q. Recent Advances in the Synthesis of Nitrogen Heterocycles Using Arenediazonium Salts as Nitrogen Sources. *Adv. Synth. Cat.* **2020**, *362*, 4876 – 4895.

176) Prasat, K.; Mahidol, C.; Ruchirawat, S. Alkaloids as Important Scaffolds in Therapeutic Drugs for the Treatments of Cancer, Tuberculosis, and Smoking Cessation. *Curr. Top. Med. Chem.* **2014**, *14*, 239 - 252.

177) Vitaku, E.; Smith, D.T.; Njardarson, J.T. Analysis of the Structural Diversity, Substitution Patterns, and Frequency of Nitrogen Heterocycles among U.S. FDA Approved Pharmaceuticals. *J. Med. Chem.* **2014**, *57*, 10257 − 10274.

178) a) Fenton, D.E. Metal ion recognition: The story of an oxa-aza macrocycle. *Pure App. Chern.* **1993**, *65*, 1493 – 1498. b) Bencini, A.; Bianchi, A.; Bazzicalupi, C.; Ciampolini, M.; Fusi, V.; Micheloni, M.; Nardi, N.; Paoli, P.; Valtancoli, B. Proton inclusion properties of a new azamacrocycle. Synthesis, characterization and crystal structure of $[H_3L][Cl]_3 \cdot 2H_2O$ (L = 4,10-dimethyl-1,4,7,10-tetraazabicyclo [5.5.2] tetradecane). *Supramol. Chem.* **1994**, *3*, 141 - 146.

179) Srivastava, A.; Singh, P.K.; Ali, A.; Singh, P.P.; Srivastava, V. Recent applications of Rose Bengal catalysis in N-heterocycles: a short review. *RSC Adv.* **2020**, *10*, 39495 – 39508.

180) Krishan, K.K.; Ujwaldev, S.M.; Saranya, S.; Anilkumar, G.; Beller, M. Recent Advances and Perspectives in the Synthesis of Heterocycles via Zinc Catalysis. *Adv. Synth. Cat.* **2019**, *361*, 382 – 404.

181) Kaur, N. Review of Microwave-Assisted Synthesis of Benzo-Fused Six-Membered N,N-Heterocycles. *Synth. Commun.* **2015**, *45*, 300 - 330.

182) Bhaskaruni, S.V.H.S.; Maddila, S.; Gangu, K.K.; Jonnalagadda, S.B. A review on multi-component green synthesis of N-containing heterocycles using mixed oxides as heterogeneous catalysts. *Arab. J. Chem.* **2020**, *13*, 1142 – 1178.

183) Cao, D.; Zhang, Y.; Liu, C.; Wang, B.; Sun, Y.; Abdukadera, A.; Hu, H.; Liu, Q. Ionic Liquid Promoted Diazenylation of N-Heterocyclic Compounds with Aryltriazenes under Mild Conditions. *Org. Lett.* **2016**, *18*, 2000 – 2003.

184) Bradshaw, J.S.; Krakowiak, K.E.; Izzat, R.M.; Aza-Crown Macrocycles, In *Chemistry of Heterocyclic compounds*; ISBN-10: 0471524859, John Wiley & Sons Inc., New York, US, 2009, Vol. 51, pp. 1 – 30.

185) Timmons, J.C.; Hubin, T.J. Preparations and applications of synthetic linked azamacrocycle ligands and complexes. *Coordin. Chem. Rev.* **2010**, *254*, 1661 – 1685.

186) Hubin, T.J. Synthesis and coordination chemistry of topologically constrained azamacrocycles. *Coordin. Chem. Rev.* **2003**, *241*, 27 - 46.

187) Cabbiness, D.K.; Margerum, D.W. Macrocyclic Effect on the Stability of Copper(II) Tetramine Complexes. *J. Am. Chem. Soc.* **1969**, *91*, 6540.

188) Richman, J. E.; Atkins, T.J. Nitrogen Analogs of Crown Ethers. *J. Am. Chem. Soc.* **1974**, *96*, 2268.

189) Vriesema, B.K.; Buter, J.; Kellogg, R.M. Synthesis of Aza Macrocycles by Nucleophilic Ring Closure with Cesium Tosylamides. *J. Org. Chem.* **1984**, *49*, 110 - 113.

190) Hovinen, J.; Sillanpää, R. Synthesis of azamacrocycles via a Mitsunobu reaction. *Tetrahedron Lett*. **2005**, *46*, 4387 – 4389.

191) Hoye, R.C.; Richman, J.E.; Dantas, G.A.; Lightbourne, M.F.; Shinneman, L.S. Synthesis of Polyazamacrocyclic Compounds via Modified Richman−Atkins Cyclization of β-Trimethylsilylethanesulfonamides. *J. Org. Chem.* **2001**, *66*, 2722 - 2725.

192) Shutalev, A.D.; Fesenko, A.A.; Yankov, A.N.; Tafeenko, V.A.; Chernyshev, V.V. Membered cyclic bis-semicarbazones: Stereoselective synthesis and structural features. *J. Mol. Struct*. **2017**, *1150*, 349 - 357.

193) Menon, S.C.; Panda, A.; Singh, H.B.; Patel, R.P.; Kulshreshtha, S.K.; Darby, W.L.; Butcher, R.J. Tellurium azamacrocycles: synthesis, characterization and coordination studies. *J. Organomet. Chem*. **2004**, *689*, 1452 – 1463.

194) Rathjens, A.; Thiem, J. Synthesis of carbohydrate-based azamacrocycles by Richman–Atkins cyclisation. *C. R. Chim.* **2011**, *14*, 286 - 300.

195) Mewis, R.E.; Archibald, S.J. Biomedical applications of macrocyclic ligand complexes. *Coordin. Chem. Rev.* **2010**, *254*, 1686 – 1712.

196) Joshi, T.; Kubeil, M.; Nsubuga, A.; Singh, G.; Gasser, G.; Stephan, H. Harnessing the Coordination Chemistry of 1,4,7-Triazacyclononane for Biomimicry and Radiopharmaceutical Applications. *Chem. Plus. Chem.* **2018**, *83*, 554 - 564.

197) Williams, S.M.; Brodbelt, J.S.; Huang, Z.; Lai, H.; Marchand, A.P. Complexation of silver and co-recovered metals with novel aza-crown ether macrocycles by electrospray ionization mass spectrometry. *Analyst* **2003**, *128*, 1352 – 1359.

198) Masllorens, J.; Roglans, A.; Anticò, E.; Fontas, C. New applications of azamacrocyclic ligands in ion recognition, transport and preconcentration of palladium. *Anal. Chim. Acta* **2006**, *560*, 77 - 83.

199) Honzíček, J. Curing of Air-Drying Paints: A Critical Review. *Ind. Eng. Chem. Res*. **2019**, *58*, 12485–12505.

200) Liang, X.; Sadler, P.J. Cyclam complexes and their applications in medicine. *Chem. Soc. Rev.* **2004**, *33*, 246.

201) Cakic, N.; Gündüz, S.; Rengarasu, R.; Angelovski, G. Synthetic strategies for preparation of cyclen-based MRI contrast agents. *Tetrahedron Lett.* **2015**, *56*, 759–765.

202) Balogh, E.; Tripier, R.; Fouskova, P.; Reviriego, F.; Handel, H.; Toth, E. Monopropionate analogues of $DOTA^{4-}$ and $DTPA^{5-}$: kinetics of formation and dissociation of their lanthanide(III) complexes. *Dalton Trans*. **2007**, 3572 – 3581.

203) Kiviniemi, A., Mäkelä, J., Mäkilä, J., Saanijoki, T., Liljenbäck, H., Poijärvi, Virta, P. Solid-Supported NOTA and DOTA Chelators Useful for the Synthesis of 3′-Radiometalated Oligonucleotides. *Bioconjugate Chem.* **2012**, *23*, 1981 – 1988.

204) Savastano, M.; Arranz-Mascarós, P.; Bazzicalupi, C.; Clares, M.P.; Godino-Salido, M.L.; Gutiérrez-Valero, M.D.; Inclán, M.; Bianchi, A.; García-España, E.; López-Garzón, R. Construction of green nanostructured heterogeneous catalysts via non-covalent surface decoration of multi-walled carbon nanotubes with Pd(II) complexes of azamacrocycles. *J. Cat.* **2017**, *353*, 239 –2 49.

205) Fuzerova, S.; Kotek, J.; Cisarova, I.; Hermann, P.; Binnemans, K.; Lukes, I. Cyclam (1,4,8,11-tetraazacyclotetradecane) with one methylphosphonate pendant arm: a new ligand for selective copper(II) binding. *Dalton Trans.* **2005**, 2908 – 2915.

206) Désogère, P.; Rousselin, Y.; Poty, S.; Bernhard, C.; Goze, C.; Boschetti, F.; Denat, F. Efficient Synthesis of 1,4,7-Triazacyclononane and 1,4,7-TriazacyclononaneBased Bifunctional Chelators for Bioconjugation. *Eur. J. Org. Chem*. **2014**, 7831 – 7838.

207) Hajela, S.; Schaefer, W.P.; Bercaw, J.E. Highly electron deficient group 3 organometallic complexes based on the 1,4,7-trimethyl-1,4,7-triazacyclononane ligand system. *J. Organomet. Chem.* **1997**, *532*, 45 - 53.

208) Huang, Y.; Liu, Y.; Wu, R.; Wu, Z. An Efficient Synthesis of N,N,N-Substituted 1,4,7-Triazacyclononane. *Eur. J. Org. Chem.* **2018**, 1546 - 1551.

209) a) Tei, L.; Blake, A.J.; Wilson, C.; Schröde, M. Synthesis of asymmetric derivatives of 1,4,7-triazacyclononane and trigonal prismatic Mn(II) complexes. *J. Chem. Soc., Dalton Trans.* **2002**, 1247–124. b) Blake, A.J.; Tei, L.; Wilson, C.; Schroèder, M. A zinc±lithium complex of 4,7-bis-(2-aminoethyl)-1,4,7-triazacyclo-nonane-1-acetate. *Acta Cryst.* **2003**, C59, m43 - m45.

210) Lin, G.; Reid, G.; Bugg, T.D.H. Extradiol Oxidative Cleavage of Catechols by Ferrous and Ferric Complexes of 1,4,7-Triazacyclononane: Insight into the

Mechanism of the Extradiol Catechol Dioxygenases. *J. Am. Chem. Soc.* **2001**, *123*, 5030 – 5039.

211) Kubíček, V.; Böhmová, Z.; Ševčíková, R.; Vaněk, J.; Lubal, P.; Poláková, Z.; Michalicová, R.; Kotek, J.; Hermann, P. NOTA Complexes with Copper(II) and Divalent Metal Ions: Kinetic and Thermodynamic Studies. *Inorg. Chem*. **2018**, *57*, 3061 – 3072.

212) Whetter, J.N.; Vaughn, B.A.; Koller, A.J.; Boros, E. An Unusual Pair: Facile Formation and In Vivo Validation of Robust Sc-^{18}F ternary Complexes for Molecular Imaging. *Angew. Chem. Int. Ed.* **2021**, e20211423.

213) Koek, J.H.; Kohlen, E.W.J.M. Direct ring functionalisation of 1,4,7-trimethyl-1,4,7-triazacyclononane and its application in the preparation of functional $[L_2Mn_2O_3]$-type complexes. *Tetrahderon Lett.* **2006**, *47*, 3673 – 3675.

214) Qian, B.F.; Wang, J.L.; Jia, A.Q.; Shi, H.T.; Zhang, Q.F. Syntheses, reactivity, structures and photocatalytic properties of mononuclear ruthenium(II) complexes supported by 1,4,7-trimethyl-1,4,7-triazacyclononane (Me3tacn) ligands. *Inorg. Chim. Acta* **2022**, *516*, e120128.

215) Shul'pin, G.B.; Nizova, G.V.; Kozlov, Y.N.; Arutyunov, V.S.; dos Santos, A.C.M.; Ferreira, A.C.T.; Mandelli, D. Oxidations by the system "hydrogen peroxide–$[Mn_2L_2O_3][PF_6]_2$ (L = 1,4,7-trimethyl-1,4,7-triazacyclononane)–oxalic acid". Part 6. Oxidation of methane and other alkanes and olefins in water. *J. Organometal. Chem*. **2005**, *690*, 4498 – 4504.

216) Oymana, Z.O.; Minga, W., Micciché, F.; Oostveenb, E.; van Haverenb, J.; van der Lindea, R. A promising environmentally-friendly manganese-based catalyst for alkyd emulsion coatings. *Polymer* **2004**, *45*, 7431 – 7436.

217) Niibayashi, S.; Hayakawa, H.; Jina, R.H.; Nagashima, H. Reusable and environmentally friendly ionic trinuclear iron complex catalyst for atom transfer radical polymerization. *Chem. Commun*. **2007**, 1855 – 1857.

218) Mitchell, M.A.; Iannetta, A.A.; Jennings, M.C.; Fletcher, M.H.; Wuest, W.M.; Minbiole, K.P.C. Scaffold-Hopping of Multicationic Amphiphiles Yields Three New Classes of Antimicrobials. *Chem Bio Chem* **2015**, *16*, 2299 – 2303.

219) a) Denat, F.; Desogere, P.; Bernhard, C.; Rousselin, Y.; Boschetti, F. Synthesis of [1,2-A]pyrazine-4-ium salts for the synthesis of 1,4,7-triazacyclononane (TACN) and N- and/or C- functionalized derivatives thereof **US Patent 2014/0142298 A1** May 22, 2014. b)Kertèsz, M.; Répàasi, J.; Szabò, A.; Tasi, G. *Process for producing*

1,4,7-triazacyclononane derivatives and novel intermediates **WO Patent 2017/055885 A1** April, 06 2017. c)Hideo, N.; Shota, N.; Ren-Hua, J.; Hitoshi, H. Method of Producong Polymer Using Iron Complex as Catalyst **US Patent 2009118448 (A1)** 07 May**, 2009.**

220) Geraldes, C.F.G.C.; Sherry, A.D.; Marques, M.P.M.; Alpoim, M.C.; Cortes, S. Protonation scheme for some triaza macrocycles studied by potentiometry and NMR spectroscopy. *J. Chem. Soc., Perkin Trans. 2* **1991**, 137 - 146.

221) Nakanishi, S.; Kawamura, M.; Kai, H.; Jin, R.H.; Sunada, Y.; Nagashima, H. Well-Defined Iron Complexes as Efficient Catalysts for "Green" Atom-Transfer Radical Polymerization of Styrene, Methyl Methacrylate, and Butyl Acrylate with Low Catalyst Loadings and Catalyst Recycling. *Chem. Eur. J.* **2014**, *20*, 5802 – 5814.

222) Gansewendt, B.; Xu, D.; Foest, U.; Hallier, E.; Bolt, H.E.; Peter, H. DNA binding of methyl iodide in male and female F344 rats. *Carcinogenis* **1991**, *12*, 463 – 467.

223) Schoenfeldt, N.J.; Korinda, A.W.; Notestein, J.M. A heterogeneous, selective oxidation catalyst based on Mn triazacyclononane grafted under reaction conditions. *Chem. Comm*. **2010**, *46*, 1640 – 1642.

224) Lopes, A.B.; Miguez, E.; Kümmerle, A.E.; Rumjanek, V.M.; Manssour Fraga, C.A.; J. Barreiro, E.J. Characterization of Amide Bond Conformers for a Novel Heterocyclic Template of N-acylhydrazone Derivatives. *Molecules* **2013**, *18*, 11683-11704.

225) Vichislo, N.V. α,β-Unsaturated Aldehydes as C-Building Blocks in the Synthesis of Pyridines, 1,4-Dihydropyridines and 1,2-Dihydropyridines. *Asian J. Org. Chem*. **2019**, *8*, 1207 – 1226.

226) Sharma, V.K.; Singh, S.K. Synthesis, utility and medicinal importance of 1,2-& 1,4-dihydropyridines. *RSC Adv*. **2017**, *7*, 2682.

227) Nakano, H.; Osone, K.; Takeshita, M.; Kwon, E.; Seki, C.; Matsuyama, H.; Takanoa, N.; Koharia, Y. A novel chiral oxazolidine organocatalyst for the synthesis of an oseltamivir intermediate using a highly enantioselective Diels–Alder reaction of 1,2-dihydropyridine. *Chem. Commun.* **2010**, *46*, 4827 – 4829.

228) Satoh, N.; Akiba, T.; Yokoshima, S.; Fukuyama, T. A Practical Synthesis of (−)-Oseltamivir. *Angew. Chem. Int. Ed.* **2007**, *46*, 5734 – 5736.

229) a) Lemire, A.; Charette, A.B. Stereoselective Synthesis of 2,6-Disubstituted 3-Piperidinols: Application to the Expedient Synthesis of (+)-Julifloridine. *Org. Lett.* **2005**, *7*, 2747-2750. b) Lemire, A.; Charette, A.B. Stereoselective Syntheses of l-

Pipecolic Acid and (2*S*,3*S*)-3-Hydroxypipecolic Acid from a Chiral N-Imino-2-phenyl-1,2-dihydropyridine Intermediate. *J. Org. Chem.* **2010**, *75*, 2077 – 2080.

230) Zhai, H.; Parvez, M.; Back, T.G. A Highly Stereoselective Synthesis of (-)-(ent)-Julifloridine from the Cyclization of an Alanine-Derived Chloroamine with an Acetylenic Sulfone. *J. Org. Chem*. **2007**, *72*, 3853 - 3858.

231) Vranova, V.; Lojkova, L.; Rejsek, K.; Formanek, P. Significance of the Natural Occurrence of L- Versus D-Pipecolic Acid: A Review. *Chirality* **2013**, *25*, 823 - 831.

232) Abou-Jneid, R.; Ghoulami, S.; Martin, M.T.; Dau, E.T.H.; Travert, N.; Al-Mourabit, A. Biogenetically Inspired Synthesis of Marine C6N4 2-Aminoimidazole Alkaloids: Ab Initio Calculations, Tautomerism, and Reactivity. *Org. Lett.* **2004**, *6*, 3933 - 3936.

233) Silva, E.M.P.; Rocha, D.H.A.; Silva, A.M.S. Diels–Alder Reactions of 1,2-Dihydropyridines: An Efficient Tool for the Synthesis of Isoquinuclidines. *Synthesis* **2018**, *50*, 1773 - 1782.

234) Mourabit, A.A.; Potier, P. Sponge's Molecular Diversity Through the Ambivalent Reactivity of 2-Aminoimidazole: A Universal Chemical Pathway to the Oroidin-Based Pyrrole-Imidazole Alkaloids and Their Palau'amine Congeners. *Eur. J. Org. Chem.* **2001**, 237 – 243.

235) Picon, S.; Zaparucha, A.; Al-Mourabit, A. N-Pyridinium imidates as new sources of 2-aminoimidazole and imidazoline derivatives. *Tetrahedron Lett.* **2009**, *50*, 6826 – 6829.

236) Benoit-Vical, F.; Saléry, M.; Soh, P.N.; Ahond, A.; Poupat, C. Girolline: A Potential Lead Structure For Antiplasmodial Drug Research. *Planta Med*. **2009**, *74*, 438 – 444.

237) Zidar, N.; Montalvão, S.; Hodnik, Z.; Nawrot, D.A.; Žula, A.; Ilaš, J.; Kikelj, D.; Tammela, P.; Mašič, L.P. Antimicrobial Activity of the Marine Alkaloids, Clathrodin and Oroidin, and Their Synthetic Analogues. *Mar. Drugs* **2014**, *12*, 940 - 963.

238) Hantzsch, A. Uelm die Synthese pyridinartiger Verbindungen aus Acotessigiither und Aldehydainmcniak. *Justus Liebigs Ann. Chem.* **1882**, *215*, 1.

239) Silva, M.P.; Varandas, P.A.A.M.; Silva, A.M.S. Developments in the Synthesis of 1,2-Dihydropyridines. *Synthesis* **2013**, *45*, 3053 – 3089.

240) Shen, L.; Cao, S.; Wu, J.; Zhang, J.; Li, H.; Liu, N.; Qian, X. A revisit to the Hantzsch reaction: Unexpected products beyond 1,4-dihydropyridines. *Green Chem*. **2009**, *11*, 1414 – 1420.

241) Maynard, D.F.; Okamura, W.H. 6π-Electrocyclization of 1-Azatrienes to 1,2-Dihydropyridines. *J. Org. Chem*. **1995**, *60*, 1763 – 1770.

242) Palacios, F.; Alonso, C.; Rubiales, G.; Ezpeleta, J.M. Cycloaddition Reactions of Neutral 2-Azadienes with Enamines − Regiospecific Synthesis of Highly Substituted Dihydropyridines and Pyridines. *Eur. J. Org. Chem.* **2001**, 2115 - 2122.

243) a) Ichikawa, E.; Suzuki, M.; Yabu, K.; Albert, M.; Kanai, M.; Shibasaki, M. New Entries in Lewis Acid−Lewis Base Bifunctional Asymmetric Catalyst: Catalytic Enantioselective Reissert Reaction of Pyridine Derivatives. *J. Am. Chem. Soc*. **2004**, *126*, 11808 - 11809. b) Kobayashi, T.; Hatano, S.; Tsuchikawa, H.; Katsumura, S. A novel strategy for the synthesis of 2-arylpyridines using one-pot 6π-azaelectrocyclization. *Tetrahedron Lett.* **2008**, *49*, 4349 - 4351. c) Yamakawa, T.; Yoshikai, N. Annulation of α,β-Unsaturated Imines and Alkynes via Cobalt-Catalyzed Olefinic C–H Activation. *Org. Lett.* **2013**, *15*, 196 - 199. d) Binder, J. T.; Kirsch, S. F. Synthesis of Highly Substituted Pyrroles via a Multimetal-Catalyzed Rearrangement−Condensation−Cyclization Domino Approach. *Org. Lett*. **2006**, *8*, 2151 - 2153.

244) Colby, D.A.; Bergman, R.G.; Ellman, J.A. Synthesis of Dihydropyridines and Pyridines from Imines and Alkynes via C−H Activation. *J. Am. Chem. Soc*. **2008**, *130*, 3645 - 3651.

245) Dai, H.; Yu, S.; Cheng, W.; Xu, Z.F.; Li, C.Y. Rhodium-catalyzed synthesis of 1,2-dihydropyridine by a tandem reaction of 4-(1-acetoxyallyl)-1-sulfonyl-1,2,3-triazole. *Chem. Comm.* **2017**, *53*, 6417 - 6420.

246) Amatore, M.; Lebœuf, D.; Malacria, M.; Gandon, V.; Aubert, C. Highly Enantioselective Rhodium-Catalyzed [2+2+2] Cycloaddition of Diynes to Sulfonimines. *J. Am. Chem. Soc.* **2013**, *135*, 4576 – 4579.

247) Harschneck, T.; Kirsch, S.F. One-Pot Synthesis of 1,2-Dihydropyridines: Expanding the Diverse Reactivity of Propargyl Vinyl Ethers. *J. Org. Chem*. **2011**, *76*, 2145 – 2156.

248) Motamed, M.; Bunnelle, E.M.; Singaram, S.W.; Sarpong, R. Pt(II)-Catalyzed Synthesis of 1,2-Dihydropyridines from Aziridinyl Propargylic Esters. *Org. Lett.* **2007**, *9*, 2167 – 2170.

249) Koley, S.; Chowdhury, S.; Chanda, T.; B.; Ramulu, J.; Samai, S.; Motisa, L.; Singh, M.S. Lewis acid mediated three-component one-flask regioselective synthesis of densely functionalized 4-amino-1,2-dihydropyridines via cascade Knoevenagel/Michael/cyclization sequence. *Tetrahedron* **2015**, *71*, 301 – 307.

250) Zhang, J.; Zhang, Q.; Ji, X.; Meng, L.G. Highly Regioselective Phosphine-Promoted [2+2+2] Annulations of Cyanoacetylenes and N-Tosylimines to 1,2-Dihydropyridine-3,5-dicarbonitrile Derivatives. *Synt. Lett.* **2019**, *30*, 1095 - 1099.

251) Bartoli, G.; Marcantoni, E.; Marcolini, M.; Sambri, L. Applications of $CeCl_3$ as an Environmental Friendly Promoter in Organic Chemistry. *Chem. Rev.* **2010**, *110*, 6104 – 6143.

252) Bartoli, G.; Marcantoni, E.; Sambria, L. The $CeCl_3 \cdot nH_2O$/NaI System in Organic Synthesis: An Efficient Water Tolerant Lewis Acid Promoter. *Synlett.* **2003**, *14*, 2101 – 2116.

253) Dudley, G.B., Engel, D.A.; Ghiviriga, I.; Lam, H.; Poon, K.C.P.; Singletary, J.A. Synthesis of (+)-Dihydro-epi-deoxyarteannuin B. *Org. Lett.* **2007**, *9*, 2839 – 2842.

254) Oijima, I.; *Catalysis Asymmetric Synthesis*, 2nd ed.; Ed.; Oijima, I.; Wiley-VCH: New York, US, 2000.

255) Meng, Q.; Sun, Y.; Ratovelomanana-Vidal, V.; Genêt, J.P.; Zhang, Z. $CeCl_3 \cdot 7H_2O$: An Effective Additive in Ru-Catalyzed Enantioselective Hydrogenation of Aromatic α-Ketoesters. *J. Org. Chem.* **2008**, *73*, 3842 – 3847.

256) Ahmed, N.; van Lier, J. E. Alumina supported-$CeCl_3 \cdot 7H_2O$–NaI: an efficient catalyst for the cyclization of 2′-aminochalcones to the corresponding 2-aryl-2,3-dihydroquinolin-4(1H)-ones under solvent free conditions. *Tetrahedron Lett.* **2007**, *48*, 13 – 15.

257) Devineni, S.R.; Doddaga, S.; Donka, R.; Chamarthi, N.R. $CeCl_3 \cdot 7H_2O$-SiO_2: Catalyst promoted microwave assisted neat synthesis, antifungal and antioxidant activities of α-diaminophosphonates. *Chinese Chem. Lett.* **2013**, *24*, 759 – 763.

258) Bartoli, G.; Cimarelli, C.; Cipolletti, R.; Diomedi, S.; Giovannini, R.; Mari, M.; Marsili, L.; Marcantoni, E. Microwave-Assisted Cerium(III)-Promoted Cyclization of Propargyl Amides to Polysubstituted Oxazole Derivatives. *Eur. J. Org. Chem.* **2012**, *2012*, 630 – 636.

259) Bartoli, G.; Bosco, M.; Giuli, S.; Giuliani, A.; Lucarelli, L.; Marcantoni, E.; Sambri, L.; Torregiani, E. Efficient Preparation of 2-Indolyl-1-nitroalkane Derivatives Employing Nitroalkenes as Versatile Michael Acceptors: New Practical Linear

Approach to Alkyl 9H-β-Carboline-4-carboxylate. *J. Org. Chem.* **2005**, *70*, 1941 - 1944.

260) Khan, M.F.; Wu, X.; Kaphalia, B.S.; Boor, P.J.; Ansari, G.A. Acute hematopoietic toxicity of aniline in rats. *Toxicol. Lett.* **1997**, *92*, 31 – 37.

261) a) Yashiro, T.; Khrickeldorf, H.R.; Schwartz, G. Polymerization of Cyclosiloxanes by Means of Triflic Acid and Metal Triflates. *Macromol. Chem. Phys.* **2010**, *211*, 1311 - 1321. b) Dalpozzo, R.; De Nino, A.; Maiuolo, L.; Procopio, A.; Tagarelli, A.; Sindona, G.; Bartoli, G. Simple and Efficient Chemoselective Mild Deprotection of Acetals and Ketals Using Cerium(III) Triflate. *J. Org Chem*. **2002**, *67*, 9093 – 9095.

262) Sato, S.; Furuta, H.; Sodesawa, T.; Nozaki, F. Cyclotrimerization of aliphatic aldehydes catalysed by Keggin-type heteropoly acids and concomitant phase separation. *J. Chem. Soc., Perkin Trans. 2* **1993**, *2*, 385 – 390.

263) Soto-Delgado, J.; Aizman, A.; Contreras, R.; Domingo, L.R. On the Catalytic Effect of Water in the Intramolecular Diels–Alder Reaction of Quinone Systems: A Theoretical Study. *Molecules* **2012**, *17*, 13688 – 13703.

264) Krygowski, T.M.;Fawcett, W.R. Complementary Lewis Acid-Base Description of Solvent Effects. I. Ion-Ion and Ion-Dipole Interactions. *J. Am. Chem. Soc.* **1975**, *97*, 2143 – 2148. b) Maria, P.C.; Gal, J.F. A Lewis Basicity Scale for Nonprotogenlc Solvents: Enthalpies of Complex Formation with Boron Trifluoride In Dichloromethane. *J. Phys. Chem.* **1985**, *89*, 1296 – 1304.

265) Pictet, A.; Spengler, T. Über die bildung von isochinolin-derivaten durch einwirkung von methylal auf phenyl-äthylamin, phenyl-alanin und tyrosin. *Ber. Dtsch. Chem. Ges*. **1911**, *44*, 2030 – 2036.

266) Zhao, J.; Méndez-Sánchez, D.; Ward, J.M.; Hailes, H.C. Biomimetic Phosphate-Catalyzed Pictet–Spengler Reaction for the Synthesis of 1,1′-Disubstituted and Spiro-Tetrahydroisoquinoline Alkaloids. *J. Org. Chem.* **2019**, *84*, 7702 – 7710.

267) Heravi, M.M.; Zadsirjan, V.; Malmir, M. Application of the Asymmetric Pictet–Spengler Reaction in the Total Synthesis of Natural Products and Relevant Biologically Active Compounds. *Molecules* **2018**, *23*, 943 – 991.

268) Sheng, X.; Himo, F. Enzymatic Pictet–Spengler Reaction: Computational Study of the Mechanism and Enantioselectivity of Norcoclaurine Synthase. *J. Am. Chem. Soc*. **2019**, *141*, 11230 – 11238.

269) Kundu, B.; Agarwal, P.K.; Sharma, S.K.; Sawant, D.; Mandadapu, A.K.; Saifuddin, M.; Gupta, S. Pictet-Spengler Reaction Revisited: Engineering of Tetherd Biheterocycles into Annulated Polyheterocycles. *Curr. Org. Synth.* **2012**, *9*, 357 - 376.

270) Parisa, G. Chapter 3 - The Pictet–Spengler Reaction: A Powerful Strategy for the Synthesis of Heterocycles, In *Advanced Heterocycles Chemistry* 1st ed.; ISBN: 978-0-12-817149-3; Ed., Scriven, E.F.V.; Ramsden, C.A.; Elsevier Inc., Oxford, UK; **2019**, Vol. 127, pp. 153 – 226.

271) Larghi, E.L.; Kaufman, T.S. Synthesis of Oxacycles Employing the Oxa-Pictet–Spengler Reaction: Recent Developments and New Prospects. *Eur. J. Org. Chem.* **2011**, 5195 – 5231.

272) Fang, H.W.; Liao, Y.R.; Hwang, T.L.; Shieh, P.C.; Lee, K.H.; Hung, H.Y.; Wu, T.H. Total synthesis of cordatanine, structural reassignment of drymaritin, and anti-inflammatory activity of synthetic precursors. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3822 – 3824.

273) Huang, Y.Q.; Song, H.J.; Liu, Y.X.; Wang, Q.M. Dehydrogenation of N-Heterocycles by Superoxide Ion Generated through Single-Electron Transfer. *Chem-Eur. J.* **2018**, *24*, 2065 – 2069.

274) Liao, Q.; Li, Q.; Zhao, Y.; Jiang, P.; Yan, Y.; Sun, H.; Liu, W.; Feng, F.; Qu, W. Design, synthesis and biological evaluation of novel carboline-cinnamic acid hybrids as multifunctional agents for treatment of Alzheimer's disease. *Bioinorg. Chem.* **2020**, *99*, 103844.

275) Maresh, J.J.; Giddings, L.A.; Friedrich, A.; Loris, E.A.; Panjikar, S.; Trout, B.L.; Stöckigt, J.; Peters, B.; O'Connor, S.E. Strictosidine Synthase: Mechanism of a Pictet−Spengler Catalyzing Enzyme. *J. Am. Chem. Soc.* **2008**, *130*, 710 – 723.

276) Fodor, K.J.; Hutai, D.; Jernei, T.; Takács, A.; Szász, Z.; Sulyok-Eiler, M.; Harmat, V.; Szabó, R.O.; Schlosser, G.; Hudecz, F.; Kohidai, L.; Csámpai, A. Novel Polycondensed Partly Saturated β-Carbolines Including Ferrocene Derivatives: Synthesis, DFT-Supported Structural Analysis, Mechanism of Some Diastereoselective Transformations and a Preliminary Study of their In Vitro Antiproliferative Effects. *Molecules* **2020**, *25*, 1599.

277) Saha, B.; Sharma, S.; Sawant, D.; Kundu, B. Water as an efficient medium for the synthesis of tetrahydro-β-carbolines via Pictet–Spengler reactions. *Tetrahedron Lett.* **2007**, *48*, 1379 – 1383.

278) Seayad, J.; Seayad, A.M.; List, B. Catalytic Asymmetric Pictet−Spengler Reaction. *J. Am. Chem. Soc.* **2006**, *128*, 1086 - 1087.

279) Ruiz-Olalla, A.; Würdemann, M.A.; Wanner, M.J.; Ingemann, S.; van Maarseveen, J.H.; Hiemstra, H. Organocatalytic Enantioselective Pictet–Spengler Approach to Biologically Relevant 1-Benzyl-1,2,3,4-Tetrahydroisoquinoline Alkaloids. *J. Org. Chem.* **2015**, *80*, 5125 – 5132.

280) Srinivasan, N.; Ganesan, A. Highly efficient Lewis acid-catalysed Pictet–Spengler reactions discovered by parallel screening. *Chem. Commun.* **2003**, 916–917.

281) Kobayashi, S.; Busujima, T.; Nagayama, S. A Novel Classification of Lewis Acids on the Basis of Acidity and Selectivity. *Chem. Eur. J.* **2000**, *6*, 3491.

282) Nalikezhathu, A.; Cherepakhin, V.; Williams, T.J. Ruthenium Catalyzed Tandem Pictet–Spengler Reaction. *Org. Lett.* **2020**, *22*, 4979–4984.

283) Eynden, M.J.V.; Kunchithapatham, K.; Stambuli, J.P. Calcium-Promoted Pictet-Spengler Reactions of Ketones and Aldehydes. *J. Org. Chem.* **2010**, *75*, 8542–8549.

284) Herlé, B.; Wanner, M.J.; van Maarseveen, J.H.; Hiemstra, H. Total Synthesis of (+)-Yohimbine via an Enantioselective Organocatalytic Pictet–Spengler Reaction. *J. Org. Chem.* 2011, *76*, 8907 − 8912.

285) Tam, S.W.; Worcel, M.; Wyllie, M. Yohimbine: a clinical review. *Pharmacol. Therapeut.* **2001**, *91*, 215 – 243.

286) Wang, K.B.; Di, Y.T:; Bao, Y.; Yuan, C.M.; Chen, G.; Li, D.H.; Bai, J.; He, H.P.; Hao, X.J.; Pei, Y.H.; Jing Y.K.; Li, Z.L.; Hua, H.M. Peganumine A, a β-Carboline Dimer with a New Octacyclic Scaffold from Peganum harmala. *Org. Lett.* **2014**, *16*, 4028 – 4031.

287) Cyril Piemontesi, Qian Wang, and Jieping Zhu Enantioselective Synthesis of (+)-Peganumine A. *J. Am. Chem. Soc.* **2016**, *138*, 11148 – 11151.

288) Cao, R.; Peng, W.; Wang, Z.; Xu, A. β-Carboline Alkaloids: Biochemical and Pharmacological Functions. *Curr. Med. Chem.* **2007**, *14*, 479 - 500.

289) Mahmoudian, M.; Jalilpour, H.; Salehian, P. Toxicity of Peganum harmala: Review and a Case Report. *Iranian J. Pharmacol. Ther*. **2002**, *1*, 1.

290) Maity, P.; Adhikari, D.; Jana, A.K. An overview on synthetic entries to tetrahydro-β-carbolines. *Tetrahedron* **2019**, *75*, 965 – 1028.

291) Shang, X.F.; Yang, C.J.; Morris-Natschke, S.L.; Li, J.C.; Yin, X.D.; Liu, Y.Q.; Guo, X.; Peng, J.W.; Goto, M.; Zhang, J.Y:; Lee, K.H. Biologically active isoquinoline alkaloids covering 2014–2018. *Med. Res. Rev.* **2020**, *40*, 2212 - 2289.

292) Miller, J.F.; Turner, E.M.; Sherrill, R.G.; Gudmundsson, K.; Spaltenstein, A.; Sethna, P.; Brown, K.W.; Harvey, R.; Romines, K.R.; Golden, P. Substituted tetrahydro-β-carbolines as potential agents for the treatment of human papillomavirus infection. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 256 – 259.

293) Ravanelli, N.;Santos, K.P.; Motta, L.B.; Lago, J.H.G.; Furlan, C.M. Alkaloids from Croton echinocarpus Baill.: Anti-HIV potential. *S. Afr. J. Bot.* **2016**, *102*, 153 - 156.

294) Ashok, P.; Chander, S.; Balzarini, J.; Pannecouque, C.; Murugesan, S. Design, synthesis of new β-carboline derivatives and their selective anti-HIV-2 activity. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1232 - 1235.

295) Varmus, H. The New Era in Cancer Research. *Science* **2016**, *312*, 1162 – 1165.

296) Havelek, R.; Muthna, D.; Tomsik, P.; Kralovec, K.; Seifrtova, M.; Cahlikova, L.;Hostalkova, H.; Safratova M.; Perwein, M.; Cermakova, E:; Rezacov, M. Anticancer potential of Amaryllidaceae alkaloids evaluated by screening with a panel of human cells, real-time cellular analysis and Ehrlich tumor-bearing mice. *Chem-Biol Interact.* **2017**, *275*, 121 - 132.

297) Cui, B.; Zheng, B.L.; He, K.; Zheng, Q.Y. Imidazole Alkaloids from Lepidium meyenii. *J. Nat. Prod.* **2003**, *66*, 1101 – 1103.

298) Liu, LX.; Wang, X.Q.; Zhou, B.; Yang, L.Y.; Li, Y.; Zhang, H.B.;Yang, X.D. Synthesis and antitumor activity of novel N-substituted carbazole imidazolium salt derivatives. *Sci. Rep.* **2015**, *5*, 13101.

299) Shankaraiah, N.; Nekkanti, S.; Chudasama, K.J.; Senwar, K.R.; Sharma, P.; Jeengar, M.K.; Naidu, V.G.M.; Srinivasulu, V.; Srinivasulu, G.; Kamal, A. Design, synthesis and anticancer evaluation of tetrahydro-β-carboline-hydantoin hybrids. *Bioorg. Med. Chem. Lett.* **2014,** *24*, 5413 – 5417.

300) Ashok, P.; Chander, S.; Tejería, A.; García-Calvo, L.; Balana-Fouce, R. Murugesan, S. Synthesis and anti-leishmanial evaluation of 1-phenyl-2,3,4,9-tetrahydro-1H-β-carboline derivatives against Leishmania infantum. *Eur. J. Med. Chem.* **2016**, *123*, 814 – 821.

301) Herraiz, T.; Galisteo, J. Hydroxyl radical reactions and the radical scavenging activity of β-carboline alkaloids. *Food Chemistry* **2015**, *172*, 640 – 649.

302) Rook, Y.; Schmidtke, K.U.; Gaube, F.; Schepmann, D.; Wunsch, D:; Heilmann, J.; Lehmann, J.; Winckler, T. Bivalent β-Carbolines as Potential Multitarget Anti-Alzheimer Agents. *J. Med. Chem.* **2010**, *53*, 3611 – 3617.

303) Song, H.; Liu, Y.; Liu, Y.; Wang, L.; Wang, Q. Synthesis and Antiviral and Fungicidal Activity Evaluation of β-Carboline, Dihydro-β-carboline, Tetrahydro-β-carboline Alkaloids, and Their Derivatives. *J. Agric. Food Chem.* **2014**, *62*, 1010 − 1018.

304) Giguere, R.J.; Bray, T.L.; Duncan, S.M. Application of commercial microwave ovens to organic synthesis. *Tetrahedron Lett.* **1986**, *27*, 4945 – 4948.

305) Gedye, R.; Smith, F.; Westaway, K.; Ali, H.; Baldisera, L.; Laberge, L.; Rousell, J. The rapid synthesis of organic compounds in microwave ovens. *Tetrahedron Lett.* **1986**, *27*, 279 – 282.

306) De La Hoz, A.; Díaz-Ortiz A.; Moreno, A. Selectivity in Organic Synthesis Under Microwave Irradiation. *Curr. Org. Chem.* **2004**, *8*, 903 - 918.

307) Díaz-Ortiz, A.; Prieto, P.; de la Hoz, A. A Critical Overview on the Effect of Microwave Irradiation in Organic Synthesis. *Chem. Rec.* **2018**, *19*, 85 – 97.

308) de la Hoz, A.; Díaz-Ortiz, A.; Moreno, A. Microwaves in organic synthesis. Thermal and non-thermal microwave effects. Microwaves in organic synthesis. Thermal and non-thermal microwave effects. *Chem. Soc. Rev.* **2005**, *34*, 164 – 178.

309) Lindstrom, P.; Tierney, J.; Wathey, B.; Westman, J. Microwave assisted organic synthesis-a review. *Tetrahedron* **2001**, *57*, 9225.

310) Kappe, C.O. Controlled Microwave Heating in Modern Organic Synthesis. *Angew. Chem. Int. Ed.* **2004**, *43*, 6250 – 6284.

311) Damm, M.; Glasnov, T.N.; Kappe, C.O. Translating High-Temperature Microwave Chemistry to Scalable Continuous Flow Processes. *Org. Process Res. Dev*. **2010**, *14*, 215 – 224.

312) Kuo, F.; Tseng, M.C.; Yen, Y.H.; Chu, Y.H. Microwave accelerated Pictet–Spengler reactions of tryptophan with ketones directed toward the preparation of 1,1-disubstituted indole alkaloids. *Tetrahedron* **2004**, *60*, 12075 – 12084.

313) Jida, M.; Soueidan, O.M.; Deprez, B.; Laconde, G.; Deprez-Poulain, R. Racemic and diastereoselective construction of indole alkaloids under solvent- and catalyst-free microwave-assisted Pictet–Spengler condensation. *Green Chem.* **2012**, *14*, 909 - 911.

314) Lombardi, L.; Bandini, M. Graphene Oxide as a Mediator in Organic Synthesis: a Mechanistic Focus. *Angew. Chem. Int. Ed.* **2020**, *59*, 20767 - 20778

315) Ebajo, V.D. Jr., Santos, C.R.L.; Alea, G.V.; Lin, Y.A.; Chen, C.H. Regenerable Acidity of Graphene Oxide in Promoting Multicomponent Organic Synthesis. *Sci Rep*. **2019**, *9*, 15579.

316) Khodabakhshi, S.; Karami, B.; Eskandari, K.; Hoseinia, J.; Rashidi, A. Graphene oxide nanosheets promoted regioselective and green synthesis of new dicoumarols. *RSC Adv.* **2014**, *4*, 17891 – 17895.

317) Mohammadi, O.; Golestanzadeh, M.; Abdouss, M. Recent advances in organic reactions catalyzed by graphene oxide and sulfonated graphene as heterogeneous nanocatalysts: a review. *New J. Chem.* **2017**, *41*, 11471 - 11497.

318) Khalili, D. Graphene oxide: a reusable and metal-free carbocatalyst for the one-pot synthesis of 2-amino-3-cyanopyridines in water. *Tetrahedron Lett.* **2016**, *57*, 1721 - 1723.

319) Pal, R.; Sarkar, T.; Khasnobis, S. Amberlyst-15 in organic synthesis. *Arkivoc* **2012**, 570 - 609.

320) Pande, M.A.; Samant, S.D. Amberlyst-15 catalyzed acetylation of phenols and alcohols under solvent free conditions. *Recyclable Catalysis* **2012**, *1*, 6 - 9.

321) Hykkerud, A.; Marchetti, J.M. Esterification of oleic acid with ethanol in the presence of Amberlyst-15. *Biomass Bioenerg.* **2016**, *95*, 340 - 343.

322) Das, B.; Banerjee, J. Silica-supported Sodium Hydrogen Sulfate and Amberlyst-15 : Two Efficient Heterogeneous Catalysts for Single-step Synthesis of 4(3H)-Quinazolinones from Anthranilic Acid, Ortho Esters, and Amines under Solvent Free Conditions. *Chem. Lett*. **2004**, *33*, 960 - 961.

323) Muthukrishnan, I.; Vachan, B.S.; Karuppasamy, M.; Eniyaval, A.; Maheswari, C.U.; Nagarajan, S.; Menéndez, J.C.; Sridharan, V. Heterogeneous Amberlyst-15-catalyzed synthesis of complex hybrid heterocycles containing [1,6]-naphthyridine under metal-free green conditions. *Org. Biomol. Chem.* **2019**, *17*, 6872 - 6879.

324) Nagai C.; Noda, K.; Kirihara, A.; Tomita, Y.; Murata, M. A Low-Molecular Weight Maillard Pigment from Beer was Identified as Perlolyrine, a Maillard Reaction Product from Tryptophan. *Food Sc. Tech. Res.* **2019**, *25*, 81 - 88.

325) Zheng, B.; Trieu, T.H.; Meng, T.Z.; Lu, X.; Dong, J.; Zhang, Q.; Shi, X.X. Cu-catalyzed mild and efficient oxidation of THβCs using air: application in practical total syntheses of perlolyrine and flazin. *RSC Adv.* **2018**, *8*, 6834 – 6839.

326) Cimarelli, C.; Di Nicola, M.; Diomedi, S.; Giovannini, R.; Hamprecht, D.; Properzi, R.; Sorana, F.; Marcantoni, E. An efficient one-pot two catalyst system in the construction of 2-substituted benzimidazoles: synthesis of benzimidazo[1,2-c]quinazolines. *Org. Biomol. Chem.* **2015**, *13*, 11687 – 11695.

327) Tongkhan, S.; Radchatawedchakoon, W. Kruanetr, S.; Sakee, U. *Tetrahderon Lett.* **2014**, *55*, 3909-3912.

328) Ling, F.; Cheng, D.; Liu, T.; Liu, L.; Li, Y.; Li, J.; Zhong,W. Recyclable and reusable n-Bu_4NBF_4/PEG-400/H_2O system for electrochemical C-3 formylation of indoles with Me_3N as a carbonyl source. *Green Chem.* **2021**,*23*, 4107 – 4113.

329) Hara, T.; Durell, S.R.; Myers, M.C.; Appella, D.H. Probing the Structural Requirements of Peptoids That Inhibit HDM2−p53 Interactions. *J. Am. Chem. Soc.* **2006**, *128*, 1995–2004.

330) Marson, C.M.; Matthews, C.J.;Yiannaki, E.; Atkinson, S.J.; Soden, P.E.; Shukla, L; Lamadema, N.; Thomas, N.S.B. Discovery of Potent, Isoform-Selective Inhibitors of Histone Deacetylase Containing Chiral Heterocyclic Capping Groups and a N-(2-Aminophenyl)benzamide Binding Unit. *J. Med. Chem.* **2013**, *56*, 6156–6174.

331) Ying, J.; Gao, Q.; Wu, X.F. Site-Selective Carbonylative Synthesis of Structurally Diverse Lactams from Heterocyclic Amines with TFBen as the CO Source *J. Org. Chem.* **2019**, *84*, 14297–14305.