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ENANTIOSELECTIVE ALKYLATION OF SUBSTITUTED OXINDOLES MEDIATED BY ASYMMETRIC ORGANOCATALYSIS

Experimental degree thesis

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Abstract

Numerous chemical sectors, such as pharmaceutical chemistry, are involved in the use of chiral compounds. Indeed, chirality plays a crucial role in drug discovery and development because the biological activity of a chiral compound can be significantly affected by its stereochemistry. Consequently, enantioselective processes are continually developed to increase the synthetic efficiency and to reduce waste. Among them, asymmetric organocatalysis is a recently defined route to the sustainable production of chiral compounds based on the exploitation of small organic molecules. In this work, organocatalytic procedures have been tested to initiate an alkylation reaction, which results in the formation of a quaternary chiral center at C3 of an oxindole scaffold. Molecules bearing the oxindole moiety have been shown to be common in a wide variety of compounds extracted from plant sources. In particular, compounds with an oxindole framework bearing a tetrasubstituted carbon stereocenter at the 3-position are characterized by high biological activity. Some examples are alkaloids, which structure is based on a system of spiro[pyrrolidine-3,3’-oxindole] rings, such as convolutamydines and diazonamides. Therefore, developing an efficient enantioselective route for 3,3’-disubstituted oxindole framework is of high interest especially in the pharmaceutical framework. During this project, conducted at the University of Zaragoza in the Herrera-OrganoCatalisis Asimétrica Group, various oxindole substrates, differing only in the protecting group bonded to the amine of the oxindole scaffold, have been synthesized by adapting synthetic processes used for similar compounds. Subsequently, a screening of different catalysts was conducted to determine the most effective reaction conditions. In particular, different squaramide derivatives have been used as bifunctional catalysts. A screening of diverse benzohydrol alkylating agents was previously carried out by the research group, leading to the identification of Michler's hydrol as the most appropriate reactive species since it offers high yields; thus, it has been chosen as alkylating agent also in this work. Additionally, more oxindole derivatives have been tested as substrates. The catalytic procedure produced similar enantiomeric excesses for all the *N*-substituted substrates under the tested reaction conditions, underscoring the versatility of this methodology.

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1. Introduction

1.1 Asymmetric Organocatalysis

Revealing the potential of catalysts has become a paramount goal in the race towards sustainability, as small chemical agents hold the power to transform the chemical industry, revolutionizing the way reactions are conducted and paving the way for greener and more efficient processes. Indeed, catalysts are chemical species added in low sub-stoichiometric amounts to the reaction environment. Their activity can reduce the activation energy of a reaction intermediate making a reaction accessible at lower temperatures and decreasing reaction times. A significant subcategory of catalysis is asymmetric catalysis, which promotes enantiomerically selective synthesis.

During decades different catalytic strategies have been developed. Initially, they were mainly metal-based catalysts. With List and MacMillan's pioneering research in the early 2000's, the use of fully organic molecules became a concrete possibility to promote organic reactions giving rise to a novel research field: asymmetric organocatalysis. Nowadays, organocatalysis is recognized as the third pillar in asymmetric catalysis, along with bio- and metal- catalysis and covers a wide range of organic processes and methodologies, including diverse activation modes, providing efficient and environmentally friendly access to enantiomerically pure compounds.[1] Asymmetric organocatalysis can be classified as covalent and non-covalent catalysis based on the interaction of the catalyst with the substrate. In covalent organocatalysis, a new covalent bond is formed between the organocatalyst and the substrate. Generally speaking, catalysts used in this category include amine catalysts, *N*-heterocyclic carbenes and other Lewis bases. In non-covalent organocatalysis, the organocatalyst and substrate typically interact through hydrogen bonding in the case of thiourea, squaramide and phosphoric acid catalysts; or through ionic interactions in the case of phase transfer catalysts.[2] This organic chemistry branch is particularly important in the pharmaceutical and agrochemical sectors where the effect and activity of a molecule can be significantly affected by its stereochemistry. Therefore, this interest has prompted intensive research into more efficient asymmetric organocatalytic methodologies. These should involve environmentally friendly and efficient catalysts that work well with easily available starting materials and reagents. They should deliver high yields with limited by-products within short reaction times under mild conditions. In addition, the methods must provide broad substrate scope to allow the construction of diverse molecular structures.[2]

However, despite the significant improvements in homogeneous asymmetric organocatalysis, the catalytic activity and turnover number associated with organocatalysts are still inferior to natural enzymes or metal-based catalysts. There have been several solutions considered in order to overcome these obstacles. Using more acidic organocatalysts is one of the most extended one. Another option is the use of bifunctional organocatalysts: molecules that bear two different reactive functional groups, which can simultaneously interact with different reactants to produce the chiral product. Regardless of the previously mentioned limitations associated to asymmetric organocatalysis, this technology found successful application in scale-up to production of chiral drugs, natural products and enantiomerically pure bioactive compounds. Some examples (Figure 1) are Letermovir (active principle of antiviral drug Prevymis), Censavudine (antiviral under investigation as a treatment for HIV), and Funapide (for postherpetic neuralgia), among many others.[3]

In conclusion, organocatalysis is a useful tool in asymmetric synthesis with considerable potential applications; even if in some cases it can still show lower turnover numbers and catalytic activity compared to other systems, it can be inferred that the relative simplicity of the catalyst structures, as well as the complexity of the target molecules, compensate for such low catalyst proficiency.[3]

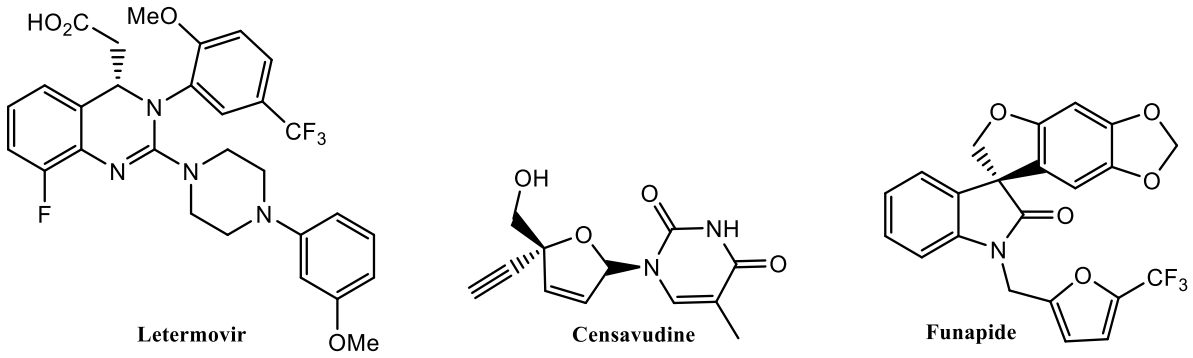


Figure 1. Biologicaly active derivatives produced using organocatalytic reactions: Letermovir,[3] ***Censavudine***[3] ***and Funapide.***[3]

1.1.2 Squaramides

Squaramides are a class of molecules extensively used as organocatalysts. The growing interest in these structures can be attributed to their incorporation of a chiral scaffold with a basic moiety, making them highly effective as bifunctional hydrogen-bonding catalysts (Figure 2).

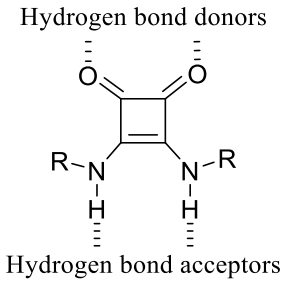


Figure 2. Hydrogen bonding in squaramides.[4]

Most of the squaramide catalysts have a tertiary amine as the basic site, but the squaramide catalysts with a tertiary phosphine as well as secondary and primary amine moieties have also been developed (Figure 3). The additional advantage of these squaramide catalysts lies in their ease of preparation. Moreover, in most cases the catalyst precipitates out of solution, hence no further purification is required.[5]

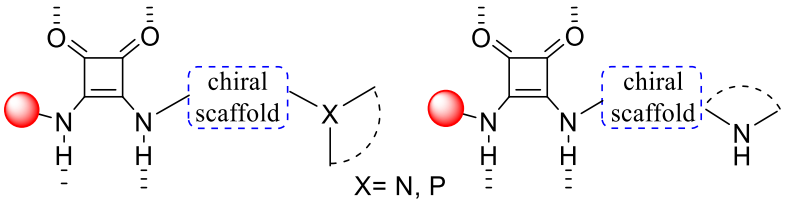


Figure 1. General structures of squaramide organocatalysts.[5]

The squaramide molecule, which is a combination of a rigid planar cyclobutene system, two hydrogen bond donors (NH) and acceptors (C=O), is partially aromatic. Aromaticity in squaramides arises due to the delocalization of the lone electron pair of one of the nitrogen atoms, which is confirmed by Hückel's rule. As mentioned in the previous paragraph they can interact with the reactant forming non-covalent interactions through hydrogen bonds.

It was found that squaramides have a higher acidity compared to other classes of molecules commonly used as catalysts.[6] Indeed, squaramide catalysts have emerged as an effective alternative to the urea/thiourea and guanidine catalysts. Since the potency of these organocatalysts highly depends on the strength of the interactions that they are able to form with the substrates, and since this is linked to the acidity of the aminic hydrogens, a higher acidity will determine an enhanced activity. For instance, the difference in hydrogens' acidity

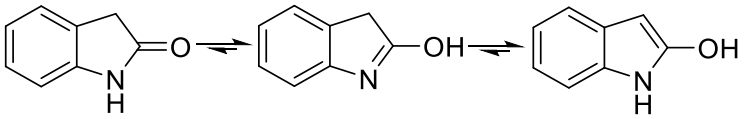
between thioureas and squaramides can be explained by the distance between nitrogen atoms, which in squaramides it is 0.6 Å longer.[6] In the case of a thiourea, the distance between the two N-H groups attached to the same carbon remains fixed at 2.13 Å, whereas in a squaramide this distance is approximately one third (2.73 Å) larger than that in a thiourea. The X-ray crystal structure of a squaramide developed by Rawal's group showed 2.85 Å between the acidic hydrogen atoms, which is very close to the calculated distance.[6] From this, it was concluded that squaramide catalysts can form stronger hydrogen bonds with the reactants of the catalyzed process.

Another important aspect that influences the acidity of the N-H is the delocalization of the double bonds, which becomes important in this context since it weakens the N-H bonds that will have a higher tendency to form hydrogen bonds. In particular, the delocalization can increase during the complex formation with the substrates due to anion–π, lone pair–π, and C–H/π interactions. In both thioureas and squaramides the lone pair on the nitrogen atom is delocalized, thereby restricting the rotation of the C-N bond. However, only in squaramides can further the delocalization occur through the cyclobutenedione system. Therefore, the N-H acidity of a squaramide is higher as compared to that of a urea/thiourea and the delocalization lowers the freedom of rotation around the C_{Ar}–N bond in the squaramide molecule. Hence, the squaramide scaffold is more rigid, which accounts for the limited conformational changes. However, it is important to acknowledge that rotational freedom also depends on the structure of substituents at nitrogen atoms, temperature, solvent, and the concentration of the substance itself. By reducing the conformational freedom of the squaramide-based catalyst, the reactive functional groups responsible for the catalysis become more constrained. As a result, they can interact more selectively with the reacting molecules in a stereoselective manner, enhancing the likelihood of forming the desired enantiomer in a higher yield and with greater enantioselectivity.

In summary, the impact of squaramides on enantioselectivity can be attributed to the formation of a chiral pocket within the solvent environment. This is a result of hydrogen bond formation between the catalyst and the reagents involved.

1.2 Oxindole and Oxindole Derivatives

Along with asymmetric organocatalysis, oxindole derivatives are an emerging area that is gaining consistent importance in medicinal chemistry. Oxindole is a heterocyclic compound that belongs to the indole family. It is characterized by a bicyclic structure consisting of an indole core fused with a lactam ring. The indole core comprises a benzene ring fused to a pyrrole ring, while the lactam ring is a cyclic amide formed by the connection of a carbonyl group (C=O) and a nitrogen atom. The carbon atom within the lactam ring is also connected to the carbon atom of the pyrrole ring in the indole core. Its IUPAC name is 2,3-dihydro-1*H*-indol-2-one, that highlights the existence of tautomeric forms referred to as lactam (1) and lactim (i). A type of tautomerism that indicates the occurrence of a hydrogen's migration between the nitrogen atom and the oxygen atom in such heterocyclic bicyclic rings is known as lactam-lactim interconversion. It is a particular case of tautomerism involving amide-imidol interconversion in which the H of N in amide tautomerizes with the H of the adjacent CH_2 group in the two hydroxyl enol forms (Scheme 1).[7]



Scheme 1. Oxindole tautomerism.

The oxindole molecule exhibits a planar structure due to the fused aromatic rings and the presence of conjugated double bonds, which contribute to its stability and unique electronic properties. The substitution pattern on the benzene ring and the pyrrole nitrogen in oxindole can vary, leading to the generation of diverse derivatives. The term oxindole derivative refers to a wide class of organic compounds and most of them present interesting biological activity that made them a central research topic in medicinal chemistry and drug discovery.[7] For instance, Funapide, cited in the previous paragraph, is an example of oxindole derivative of high pharmaceutical interest. The first known oxindole derivative was naturally extracted in the form of alkaloids from the bark of the Cat claw's plant (Uncaria tomentosa) species, originating in the Amazon region as well as other tropical zones of central and southern parts of South America. Historical sources report that this plant was often present in traditional medicinal practices against infections arthritis and other mild inflammations.

The tunability of its biochemical properties through the addition of different substituent groups at different positions made it a prominent scaffold among the nitrogen and oxygen

containing heterocycles. To date an extensive number of oxindole-based compounds have been synthetized for a wide range of biochemical applications.

This class of compounds can be and are generally obtained starting from isatin. Indeed, the possibility of being used both as an electrophile and nucleophile and the easy availability have made isatin a valuable building block for the organic synthesis of oxindole derivatives. One of the most valued applications of isatins in organic synthesis is undoubtedly due to the highly reactive C-3 carbonyl group that is a prochiral center as well. Indeed, substitution at the C3 position has been found to have a significant impact on the potency and selectivity of oxindole-based compounds.[7] Figure 4 reports a schematic summary of the relationship between activity and substituent positions, highlighting the numerous beneficial functions linked to the presence of an isatins in organic synthesis substituent bonded to that carbon atom. In the following paragraph will be discussed the most significant example of 3,3-substituted oxindole-based drugs.

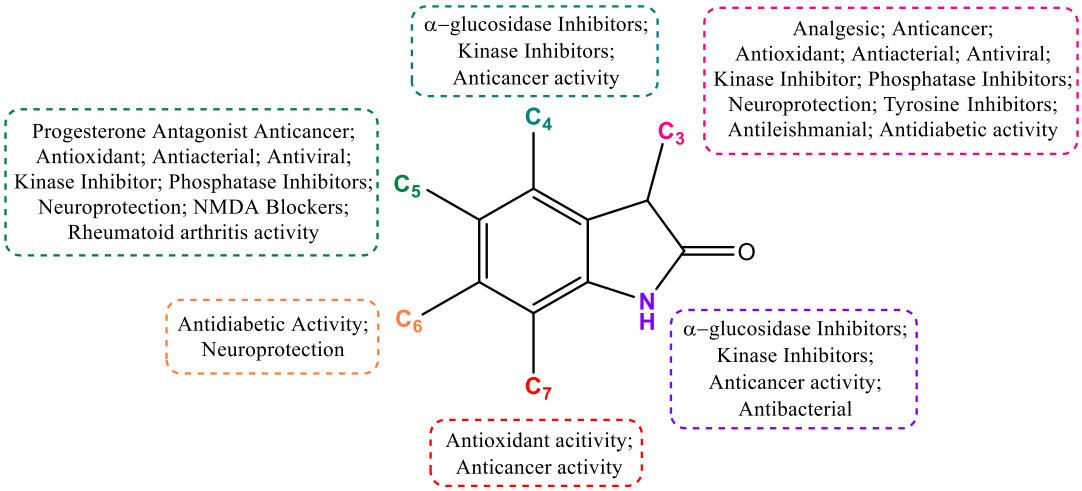


Figure 4. Different positions of oxindole nucleus explored for varied pharmacological activities.

1.2.1 C3 substituted oxindoles with beneficial therapeutic activity.

One of the simplest subclasses of oxindoles bearing substituents in C3 position is 3-alkenyl-oxindoles (Figure 5) and nearly all these compounds can be obtained from natural resources.[7]

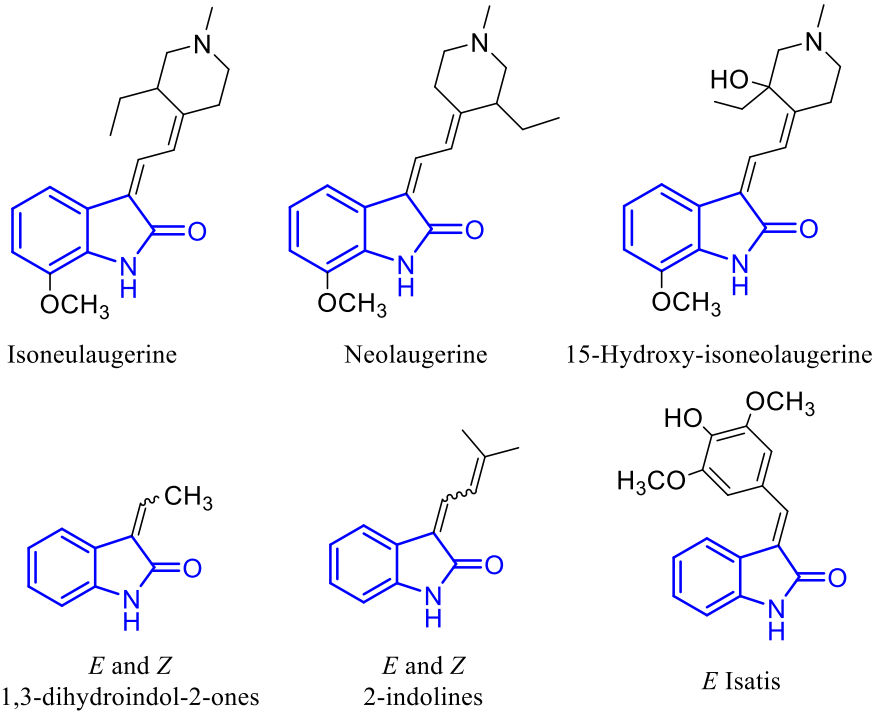


Figure 5. Examples of bioactive oxindole derivatives bearing alkenyl substituent at C3.[7]

They have been extracted for the first time in 1978, from *Cimicifuga dahurica* plant, from the *Cimicifuga* dry rootstock species. These compounds have demonstrated potential in various therapeutic areas, including oncology, neurology, and infectious diseases.

However, a particular interest is growing for oxindole scaffolds bearing a disubstituted quaternary carbon in C3. An example are alkaloids, which structure is based on a system of spiro[pyrrolidine-3,3'-oxindole] rings. Therapeutically, alkaloids are particularly well known as anesthetics, cardioprotective, and anti-inflammatory agents. Due to their proven beneficial bioactivity, there has been a strong focus on investigating simple alkaloids as templated for drug discovery. Alkaloids are abundant in nature and therefore, potentially more easily extracted and readily available for research. Even though most alkaloids present a spirocyclic structure, the non-spirocyclic quaternary stereocenter at the 3 position is also present in nature in multiple molecular structures. A common example is the 3 substituted 3-hydroxy-2-oxindole, obtained from different sources (Figure 6).

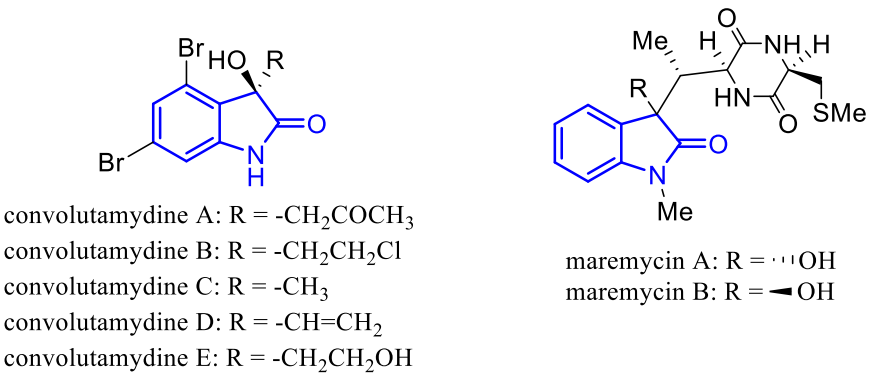


Figure 6. example of non spyrocyclic alkaloids.[8]

Maremycin A and B or Convolutamydines are well-known alkaloids that are found respectively in marine Streptomyces and marine bryozan respectively. In particular, Convolutamydine A has interesting activity in the differentiation of HL-60 human plomyelocytic leukimia cells.[9]

Considering the frequent occurrence in nature, oxindole scaffolds have attracted considering attention also in synthetic chemistry sector. Particularly in pharmaceutical chemistry, the possibility to synthetize novel compounds and test their properties opened new frontiers in drug discovery, leading to numerous successful synthetic drugs with a disubstituted oxindole scaffold at C3 carbon. In Figure 7, a growth hormone secretagogue (SM-130686) and an inductor of systemic hyperplasia in rats (AG041R) are reported.

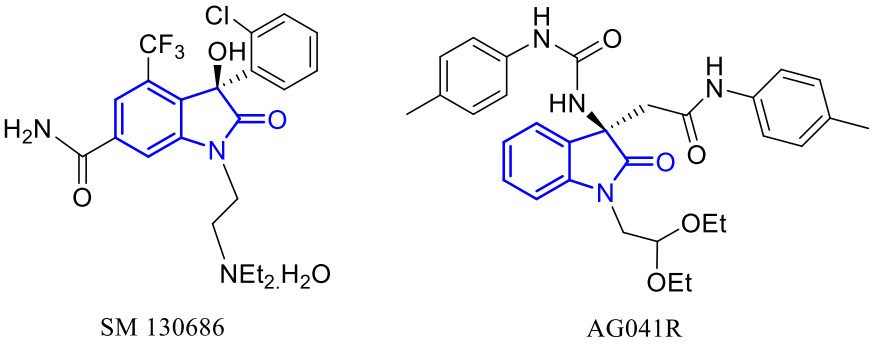


Figure 7. Synthetic drugs with a disubstituted oxindole scaffold at C3 carbon.

Among this class of molecules, several anti-cancerogenic molecules have also been identified as those depicted in Figure 8.[10]

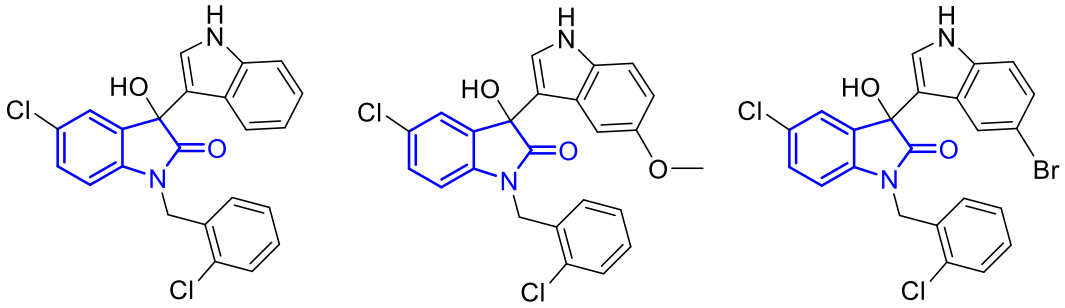


Figure 8. Anticancerogenic with an oxindole scaffold disubstituted at C3 carbon

Oxindole derivatives have also been found to be potent antioxidant, able to reduce oxidative stress that is implicated in the pathogenesis of many diseases such as cancer, cardiovascular diseases or diabetes, among other. The following oxindole derivatives (Figure 9) have shown consistent antioxidant activity against the stable free radical 2,2-diphenil-1-picrylhydrazyl (DPPH) radical.[11]

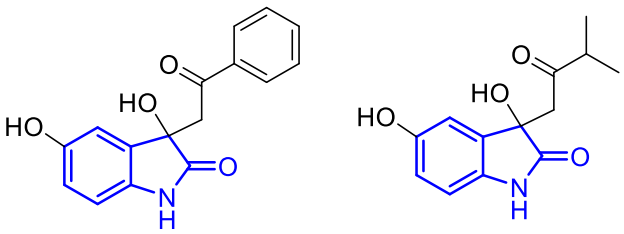


Figure 9. Oxindole derivatives having antioxidant properties.

Moreover, they have also shown considerably lower cytotoxicity in HL60 human cells compared to the BHT antioxidant, which is widely employed as antioxidant food additive.[12] Some oxindole derivatives have also shown contraceptive properties. The molecule represented in Figure 10 has been synthesized by Paira *et. al* and was tested *in vitro* to determine its spermicidal activity. The experiments revealed that the oxindole analogues exerted dose-dependent sperm immobilizing effects.[13] This derivative showed spermicidal potential comparable to the standard spermicide Nonoxynol-9.

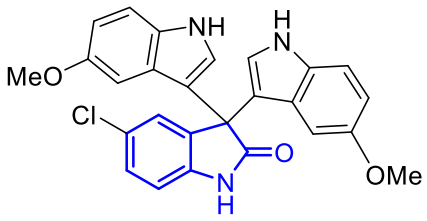


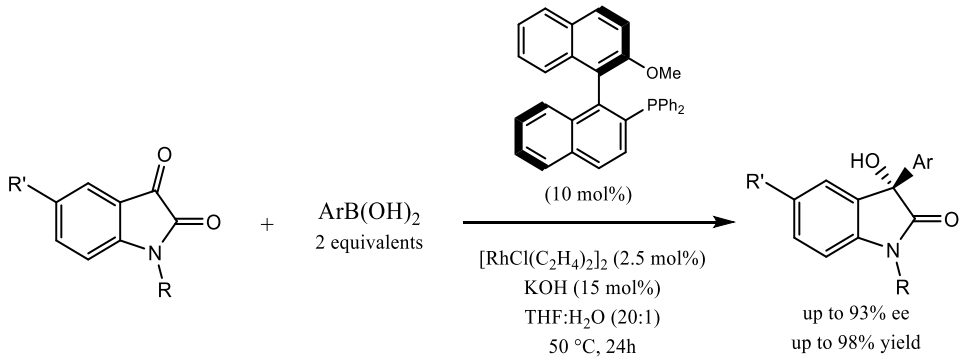
Figure 10. Oxindole derivative with contraceptive properties.

1.2.2 Synthesis of 3,3-disubstituted oxindoles

Considering the wide amount of disubstituted oxindoles that presented beneficial bioactivity, a great interest has grown towards the experimentation of novel synthetic methodologies, in order to provide new type of substitution to the main oxindole core. Finding new or more efficient strategies can promote the advancement of drug design and development, leading to a faster discovery of new therapeutic agents.

Due to the high interest shown in pharmaceutical chemistry for these compounds many synthetic routes have been developed and tested. Among them, nucleophilic addition to isatins has been one of the preferred routes towards oxindole derivatives synthesis. Indeed, isatins are easily accessible and highly available starting materials and they suffer nucleophilic addition at carbonylic C3 or C2 positions. Therefore, this route has constituted and still constitutes one of the most popular options. In the last decades, many methods based both on metal catalysts and organocatalysts have been reported.

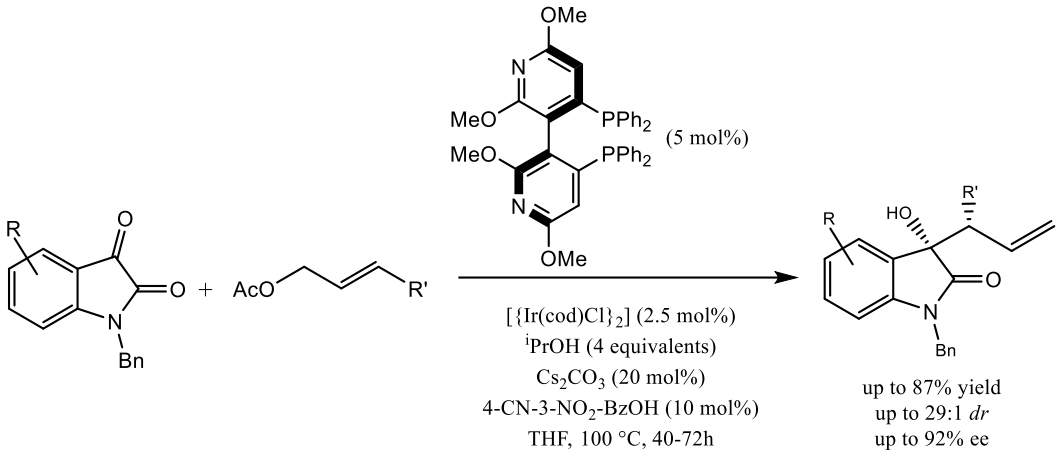
One of the first examples of enantioselective nucleophilic addition to isatin that could be found in literature was obtained through metal-based catalysis by Hayashi's research group (Scheme 2).



Scheme 2. One of the first reported enantioselective nucleophilic addition.[14]

Using a rhodium phosphine complex as catalyst and sub-stoichiometric amount of potassium hydroxide in a mixture 20:1 of THF:H_2O as solvent at 50 °C it was possible to obtain the arylated isatin at C3 with high yields and 93% ee.[14]

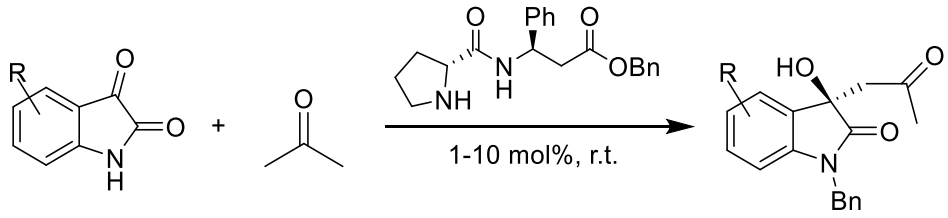
Krische and co-workers proposed another synthetic route based on an iridium complex catalyst (Scheme 3).



Scheme 3. Iridium complex catalyzed enantioselective nucleophilic addition.[15]

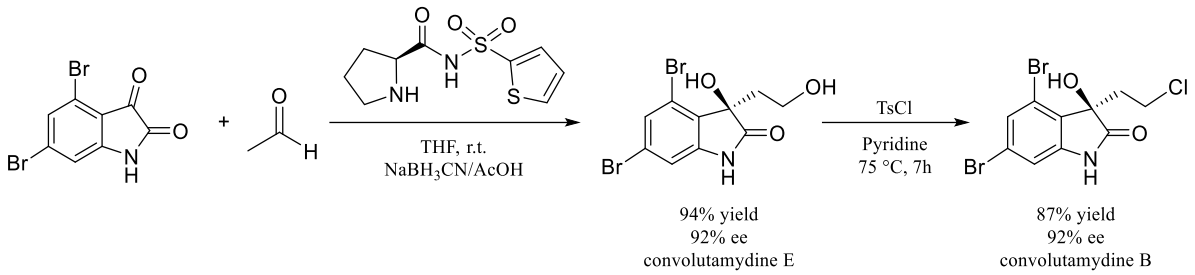
The group was able to obtain enantioselective allylation to the carbonyl group in C3 of the isatin without using allylmetal reagents. They constituted the first examples of enantioselective allylations, crotylations, and prenylations of isatins achieved by isopropanol-mediated transfer hydrogenation. Different nucleophiles were used, and, in all cases, the reported enantiomeric excess always exceed 80% and were able to reach high yields.[15]

Concomitantly, organocatalysis has been investigated as a plausible alternative solution. On a general basis, Brønsted acids have been used to activate the nucleophilic attack. In the last years organocatalyzed processes have been optimized for the synthesis of important bioactive compounds. For example, Tomasini's research group used prolinamide as catalyst to promote enantioselective cross-aldol condensation between acetone and isatin (Scheme 4).[16]



Scheme 4. Example of nucleophilic addition to Isatin C3 through organocatalysis.[16]

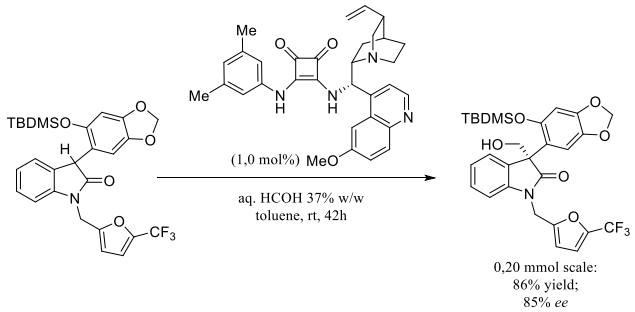
The process consisted of the addition of an acetone at C3 carbon of the isatin with 77% enantiomeric excess.[16] This type of intermediate has been useful for the synthesis of previously reported naturally occurring alkaloids. For instance, Hala and co-workers used a prolinamide modified with a sulfonyl group to catalyze the aldolic reaction between a 4,6-dibromoisatin and different aldehydes (Scheme 5).[8]



Scheme 5. Enantioselective nucleophilic addition of aldehydes to dibromoisatins.

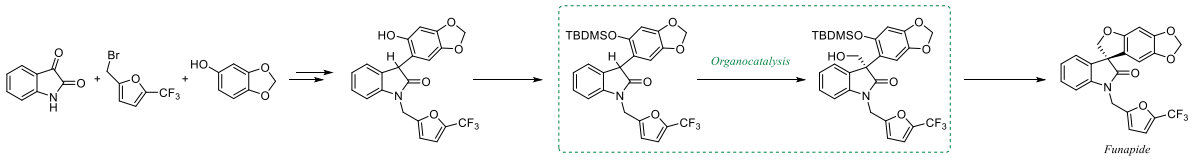
They were able to obtain Convolutamydine E through a two-step synthesis of organocatalyzed aldolic condensation and reduction with a 92% enantiomeric excess. With the addition of a third step, consisting in the reaction with *p*-toluenesulfonyl chloride they managed to get alkaloid Convolutamydine B without any loss of enantiomeric excess.

More recently, Bernardi and Herrera's research groups reported an enantioselective aldol reaction with formaldehyde catalyzed by a squaramide-derived bifunctional organocatalyst. The conditions reported in Scheme 6 allowed to obtain 85% enantiomeric excess and between 84 and 86% yield of the desired product.[17]



Scheme 6. Optimized conditions in the synthesis of Funapide precursor.[17]

The authors successfully reinvestigated the key aldol step of the pilot-plant scale asymmetric synthesis of the drug Funapide (Figure 1) shown in Scheme 7.[17]

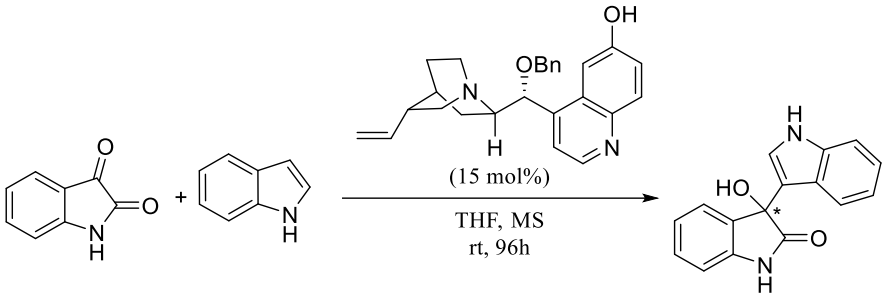


Scheme 7. Reported plant scale synthesis based on an organo-catalytic aldol reaction.[17]

Commonly, this enantioselective step was carried out using thiourea catalyst, characterized by lower selectivity (70.5% ee).

Even though the application of organocatalysts to more complex system is still limited, it is still possible to find promising results in literature. Chimni's research group in 2010 managed

to perform a Friedel–Crafts-type addition of indole to isatin catalyzed by Cinchona alkaloids. The screening of different reaction conditions allowed to define the most effective route as shown in Scheme 8.[18]



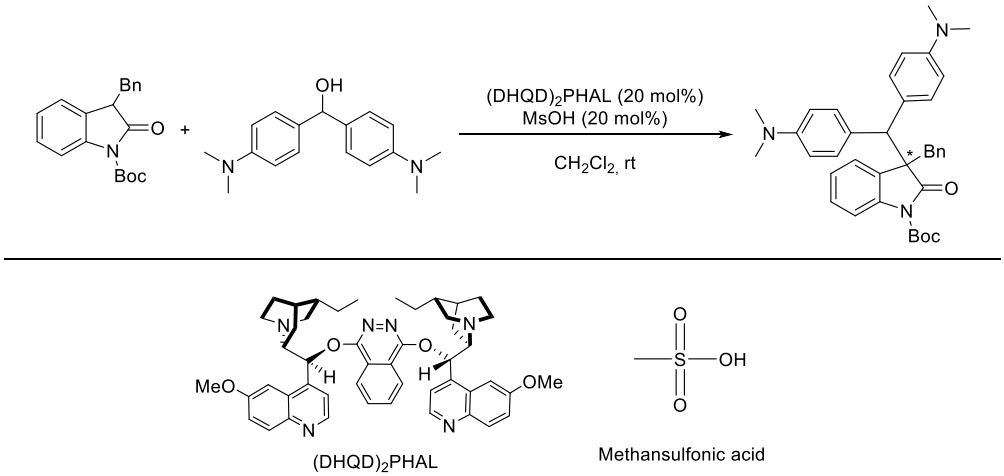
Scheme 8. Organocatalyzed preparation of 3-substituted 3-hydroxyoxindole.[18]

The study showed that the performance of the reaction improved significantly in the presence of 4 Å molecular sieves (MS), while polar protic solvents boosted the reactivity of the reaction but decreased the final enantiomeric excess. The final optimized route gave the desired product in 91% yield and 95% ee.

2. Objectives

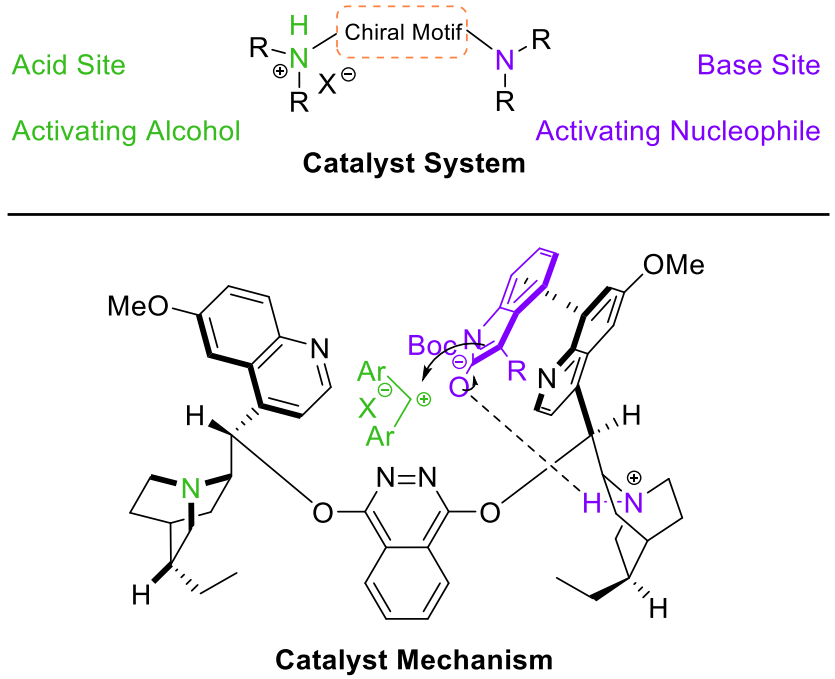
As reported in paragraph 1.2.1, most of the natural occurring oxindole derivatives present chiral stereocenters, and different stereoisomers or even enantiomers can have considerably different biological effects. Therefore, asymmetric catalysis has become a predominant research field.

For this reason, this research project has the objective of applying asymmetric organocatalysis to form a quaternary chiral stereocenter at the C3 of an oxindole scaffold. One of the most versatile and used strategy entails the direct functionalization of already substituted oxindoles. The starting point of the project was the article published by Zhang's research group in 2013 that proposed an asymmetric α-alkylation of functionalized oxindoles using Michler's hydrol as alkylating agent.[19] The oxindole substrate presented a Boc protecting group bonded to the nitrogen and a benzyl group at C3. The research group tested various reaction conditions entailing different catalyst, co-catalysts and solvents. At first, Cinchona alkaloids were considered as organocatalyst, but it only brought to racemic mixtures. Thus, the focus shifted to dimeric Cinchona alkaloids, also considering their proven tendency to form stronger interactions with substrates. Even if the enantioselectivity remained low, the obtained results were better than the ones achieved in previous reaction conditions. Therefore, considering the promising results, the group focused on dimeric Cinchona alkaloids derivatives as organocatalysts. However, more tests were carried out to improve both the yields and enantiomeric excess. The reaction condition that provided the best results (yield: 86%; ee: 76%) used the homodimeric catalyst $(DHQD)_2PHAL$, showed in Scheme 9, and methane sulfonic acid as co-catalyst in dichloromethane. However, it is important to notice that the nature of the employed co-catalyst had more effect on the reactivity rather than on the enantioselectivity.[19]



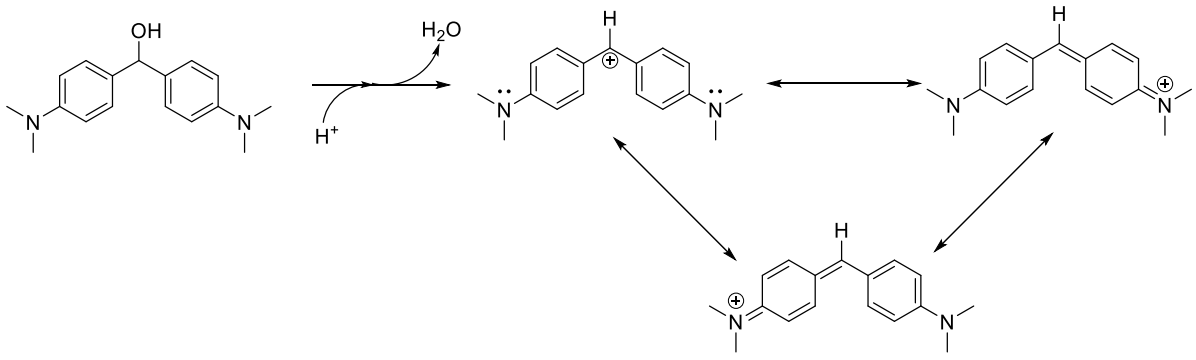
Scheme 9. Organocatalyzed alkylation of oxindole derivative.[19]

To explain the catalytic activity of the bischincona alkaloid, a S_N1-type mechanism (Scheme 10) was proposed, which involves the formation of a carbocation before the nucleophilic attack.



Scheme 10. Proposed S_N1 mechanism for the biscinchona alkaloid catalyzed alkylation.[19]

The catalyst system of the dimeric Cinchona alkaloid with two tertiary amine moieties and an additional acid (1:1) simultaneously activates both oxindoles and alcohol. Highly efficient stereocontrol was attributed to the ability of the dimeric Cinchona alkaloid catalyst to form a chiral pocket, which can firmly fix both the formed carbocation and enolated 2-oxindole.[19] Indeed, the choice of Michler's hydrol as alkylating agent is also related to the S_N1-type mechanism since the resulting carbocation is highly resonance-stabilized and can easily undergo a nucleophilic attack (Scheme 11). Moreover, water is the only co-product resulting from their activation resulting in high atom efficiency.



Scheme 11. Resonance Isomers of Michler's hydrol carbocation.

Taking into consideration the preliminary results of this study, the intention was to test similar organocatalytic procedures using different bifunctional organocatalysts and oxindole based substrates to see if similar performances could be achieved. More specifically, the starting materials to prepare the substrates considered are the ones shown in Figure 11.

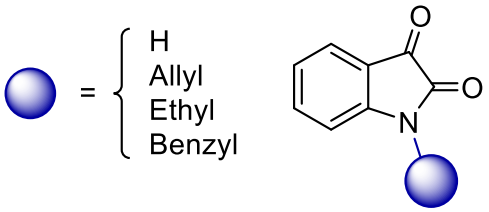


Figure 11. Oxindoles for the preparation of the substrates for the organocatalyzed alkylation in C3.

The choice for this class of molecules was driven primarily for the extensive interest for indole derivates and the easily accessible addition of indole scaffold in C3 position. Previous research carried out by the research group executed a screening in different conditions of different bifunctional organocatalysts, bearing two different functional groups in their structure, and homodimeric catalysts, characterized by the presence of two identical functionalities. Three different alkylating agents (Figure 12) were tested.

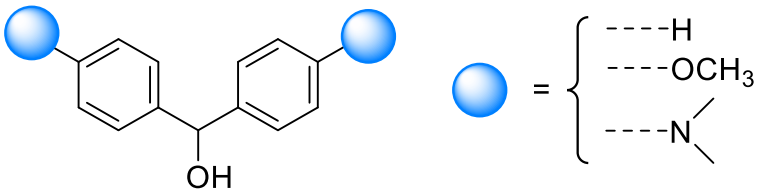
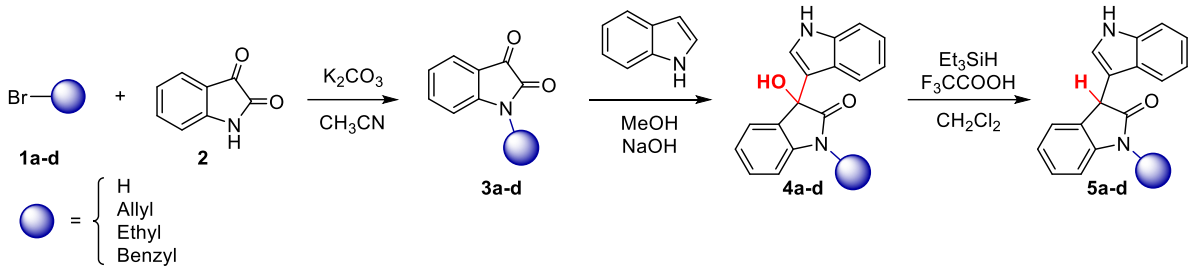


Figure 12. Tested alkylating agents.

However, the experimental evidence confirmed that Michler's hydrol was the one able to provide better performances. While, regarding catalysts, it was observed that homodimeric catalysts required an acid co-catalyst to achieve comparable enantioselectivity (ee) and conversion values to a bifunctional catalyst. On the contrary, bifunctional catalysts exhibited better results (both in terms of conversion and ee) in the absence of an acid. In some cases, it was recorded a negative impact of acid co-catalysts when using bifunctional catalysts.
Therefore, starting from the previously reported results, the objectives of this project were the following:

- Synthesis of four substrates on a laboratory scale, by adapting synthetic procedures already present in literature. The final route was based on three steps: isatin protection, attack of indole moiety to the C3 of the *N*-substituted isatin, reductive dihydroxylation (Scheme 12).



Scheme 12. Laboratory scale synthesis for the chosen substrates

- Catalysts screening using the benzylated oxindole derivative at standard conditions to define the most enantioselective one among a series of squaramide derivatives.

- Determination of the conditions to isolate the alkylation products and define the feasibility of the separation of the two enantiomers through HPLC on chiral stationary phase.

- Application of the asymmetric catalytic procedure to the other three synthesized substrates to check the versatility of this methodology.

3. Results and Discussions

3.1 Substrate synthesis

The substrate on which the screening of the catalysts was conducted is the 1-benzyl-3-(1*H*-indol-3-yl) indolin-2-one, the same used during the previous experimental tests.

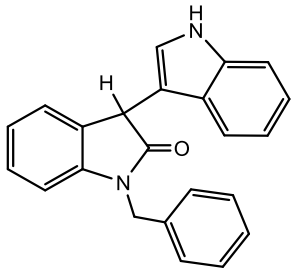


Figure 13. 1-benzyl-3-(1H-indol-3-yl) indolin-2-one.

As anticipated in chapter 2, this molecule has been prepared on laboratory scale following a 3-step synthesis using isatin as starting reactant. The first step[17] consisted in the installation of a benzyl protecting group to the amine of the isatin. This step is useful to remove the polar NH group that could give problems during product purification and further synthetic steps. On the other hand, adding substituents to the oxindole nitrogen can affect the acidity of the C3 hydrogen (Figure 14).[20]

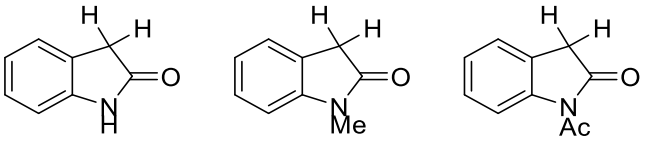
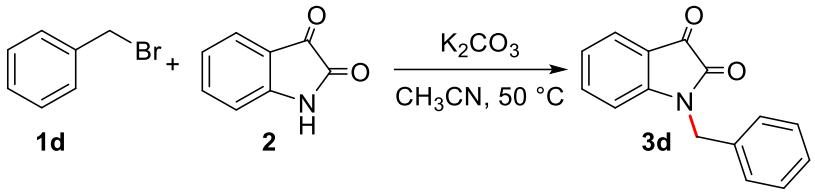


Figure 14. Oxindole C3 hydrogen acidity depending on nitrogen's substituent.[20]

The presence of an electron withdrawing group bonded to the nitrogen increases the acidity (decrease the pK_a) of the C3 hydrogen and thus his tendency to react with a base. This inclination could be ascribed to the resonance-driven stabilization of the conjugate base when an electron-withdrawing group is present. On the other hand, an electron donor causes the increase in the pK_a, that corresponds to a lower acidity and thus, lower reactivity. Increasing the acidity of the protons in C3 position would favor the formation of the nucleophilic intermediate through deprotonation. This would enhance the reactivity of the substrate towards a nucleophilic substitution, and thus benefit the performance of the overall reaction. However, during this project only electron-donor substituents have been considered as protecting groups for the amine, even though it was focused on the catalysis of a nucleophilic alkylation. Indeed, despite decreasing the reactivity of the substrates, these groups are extremely common in already identified bioactive compounds with therapeutic activity (see

chapter 1.2.1). Similarly, the indole moiety has been preferred mainly for the ease of synthesis and the elevated interest of indole-based bioactive compounds.[21,22]

The protection was obtained by making react one equivalent of isatin with one equivalent of benzyl bromide in CH_3CN in the presence of 3 equivalents of K_2CO_3 (Scheme 13).



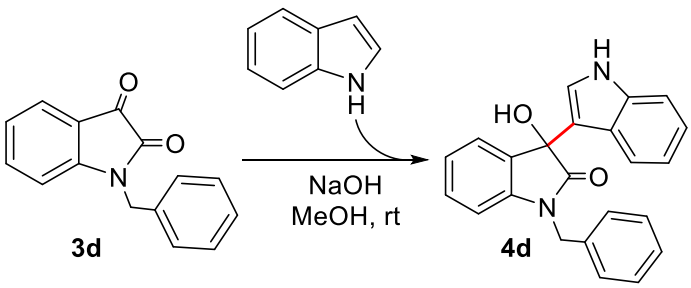
Scheme 13. Isatin protection.

The reaction mixture was then heated to 50 °C in a reaction bath with a magnetic stirrer and left for approximately 24 hours. The advancement of the reaction was checked through TLC. The reaction product was then isolated following the two steps: liquid-liquid extraction and the resulting organic phase was dried with $MgSO_4$.

Liquid-liquid extraction was carried out using CH_2Cl_2 phase and an aqueous phase to extract all the potassium carbonate. The aqueous phase was then extracted with two 15 ml aliquots of dichloromethane.

Subsequently, any remaining trace of water was removed from the organic phase by gravity filtration with $MgSO_4$. After the two purification steps the procedure consists of the crystallization of the product in ethanol. However, on some occasion the resulting liquid phase was not pure enough to allow crystallization. Therefore, in those cases, to isolate the product a column chromatography was employed using an eluent mixture of 8:2 hexane: ethyl acetate. The obtained product was then characterized through ^1H NMR. This step was associated with an average yield of 73%. Similar, procedure was followed for the different protection groups (see experimental section 5.5).

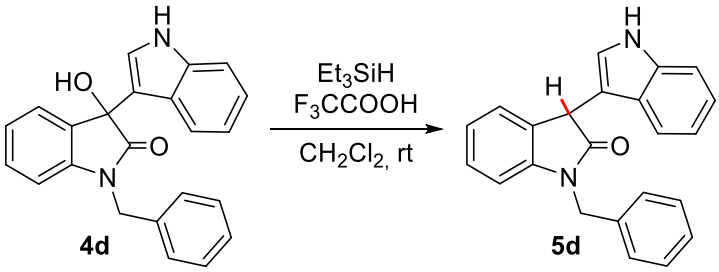
In the next synthetic step, indole was added by making react at room temperature in methanol 1 equivalent of the protected isatin (2) with 1.1 equivalent of indole, with 0.4 equivalent of NaOH (Scheme 14).[23]



Scheme 14. Indole addition to isatin derivative 3d.

The reaction mixture was stirred using a magnetic stirrer and the advancement of the reaction was checked through TLC. After approximately 6h the solvent was evaporated in the rotavapor and then, the product was separated through a column chromatography using an eluent mixture of 6:4 hexane: ethyl acetate.

The last synthetic step was carried out at room temperature. Using 1 equivalent of the reactant **4d** was dissolved in CH_2Cl_2.[17] Then, 3 equivalents of triethyl silane and 5 equivalents of trifluoroacetic acids were added to the mixture (Scheme 15).

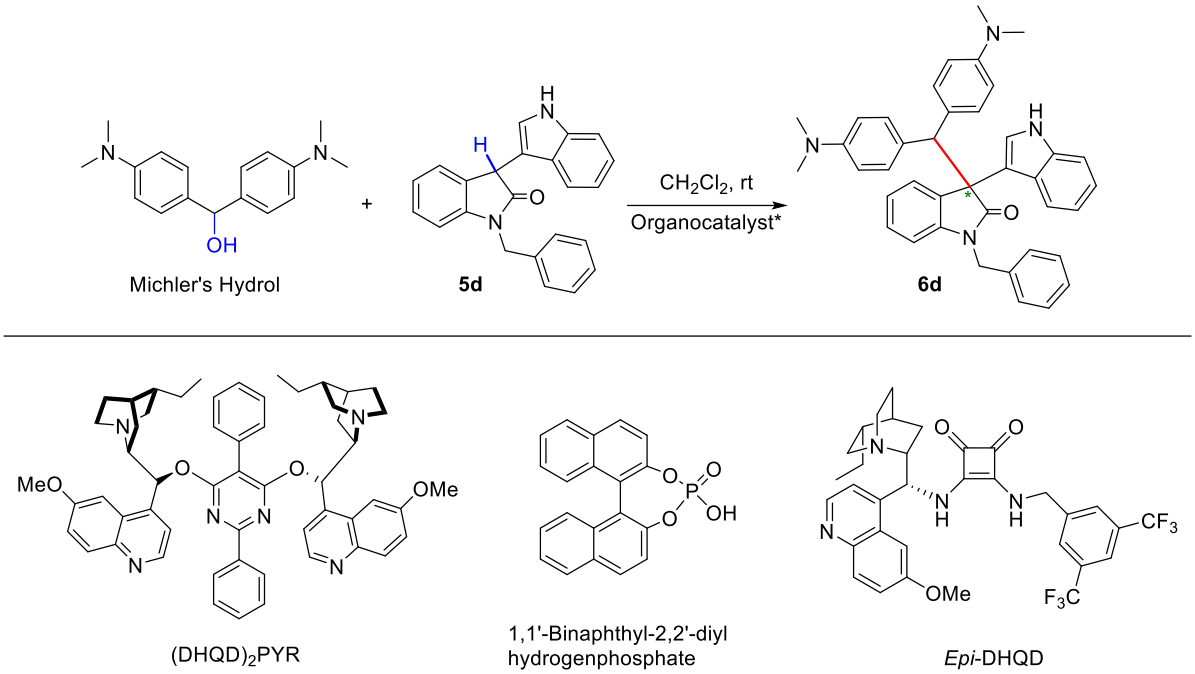


Scheme 15. Synthesis of substrate 5d.

After approximately 2h it could be observed the competition of the reaction through TLC. The pH of reaction mixture was then increased by adding with a pasteaur pipette an alkaline solution 1M of NaOH until it reached pH=5. The pH level was controlled using the pH test paper. Then, a liquid-liquid extraction was carried out between the reaction mixture and an aqueous phase. The aqueous phase was washed with 2 x 15 ml aliquots of dichloromethane. To remove the water traces from the organic solvent, gravity filtration was performed using $MgSO_4$. Finally, the product was isolated through column chromatography using eluent mixture of 5:5 hexane: ethyl acetate. On average, this step gave a yield of 77%.

3.2 Reproduction of previous studies' results

Before starting with the catalyst screening, the first objective has been to reproduce the best results obtained from the previous research project.[24] The reaction showed in Scheme 16 has been carried out using the dimeric and bifunctional catalyst that exhibited the highest enantiomeric excess, and two attempts were made to replicate the previously achieved results reported in Table 1.



Scheme 16. Model reaction studied.

Table 1. Results obtained in previous experiments.

Catalyst (20%)	Co-catalyst (20%)	Time (h)	Conversion (%)	Ee (%)
$(DHQD)_2PYR$	BINPA	48	57	28
Epi-DHQD	None	48	60	41

For the CP-13 (Table 2), the same exact conditions have been reproduced: in 250 µl of CH_2Cl_2 1 equivalent of Michler's hydrol (0.050 mmol) was made react with 1.1 equivalents of reactant **5d** (0.055 mmol). Both the catalyst $(DHQD)_2PYR$ and the co-catalyst BINPA were 20 mol% with respect to the limiting reactant (0.2 equivalents, 0.010 mmol). Both the catalyst and co-catalysts are commercially available compounds and we had them in the laboratory.

On the other hand, the CP-12 was carried out using half of the mmol used in the original study because of the little quantities available of the catalyst. Thus, CP-12 was carried out always in 250 µl of CH_2Cl_2, but with 0.025 mmol of Michler's hydrol (1 equivalent) and 0.0275 mmol of reactant **5d** (1.1 equivalents) and the catalyst (20 mol%) with respect to the limiting reactant (0.2 equivalents, 0.005 mmol). No co-catalyst was necessary in this case. The reactions were kept under stirring conditions and at room temperatures.

As shown in Table 2, the preliminary experiments brought to slightly higher enantiomeric excesses compared to the previous study. It is important to mention that during this project, the yield has been considered instead of the conversion, making the comparison in this sense

less effective. However, the yield for the dimeric Cinchona catalyzed reaction turned out to be significantly lower because not all the alcohol reacted and the separation through column chromatography was not efficient. On the other hand, in CP-12 catalyzed by the squaramide, the Michler's hydrol completely reacted after 48h. Indeed, the obtained yield for the CP-12 is significantly higher.

Table 2. Results of the preliminary experiments.

Reference	Catalyst (20%)	Co-catalyst (20%)	Time (h)	Yield (%)	Ee (%)
CP-12	*Epi*-DHQD	None	48	84	52
CP-13	$(DHQD)_2PYR$	BINPA	48	32	40

At this point, it was decided to focus for the rest of the project on squaramide catalysts. This was mainly driven not only by the better results obtained, but also by the fact that such results could be achieved without the use of additional co-catalyst, making the process simpler.

3.3 Catalysts Screening

After determining the class of catalysts, the project proceeded with the testing of the activities of the squaramide derivatives reported in Figure 15 as organocatalysts for the same model alkylation reaction shown in Scheme 16.

All reactions were carried out under the same conditions. In 250 µl of CH_2Cl_2, 1 equivalent of Michler's hydrol (0.025 mmol) was made react with 1.1 equivalents of reactant **5d** (0.0275 mmol) and the catalyst (20 mol%) with respect to the limiting reactant (0.2 equivalent, 0.005 mmol). The reactions were kept under stirring conditions and at room temperatures. After 48 hours, the advancement of the reaction was assessed using TLC. Depending on the remaining amount of alkylating agent in the reaction mixture, the reaction was allowed to proceed for a maximum of 96 hours.

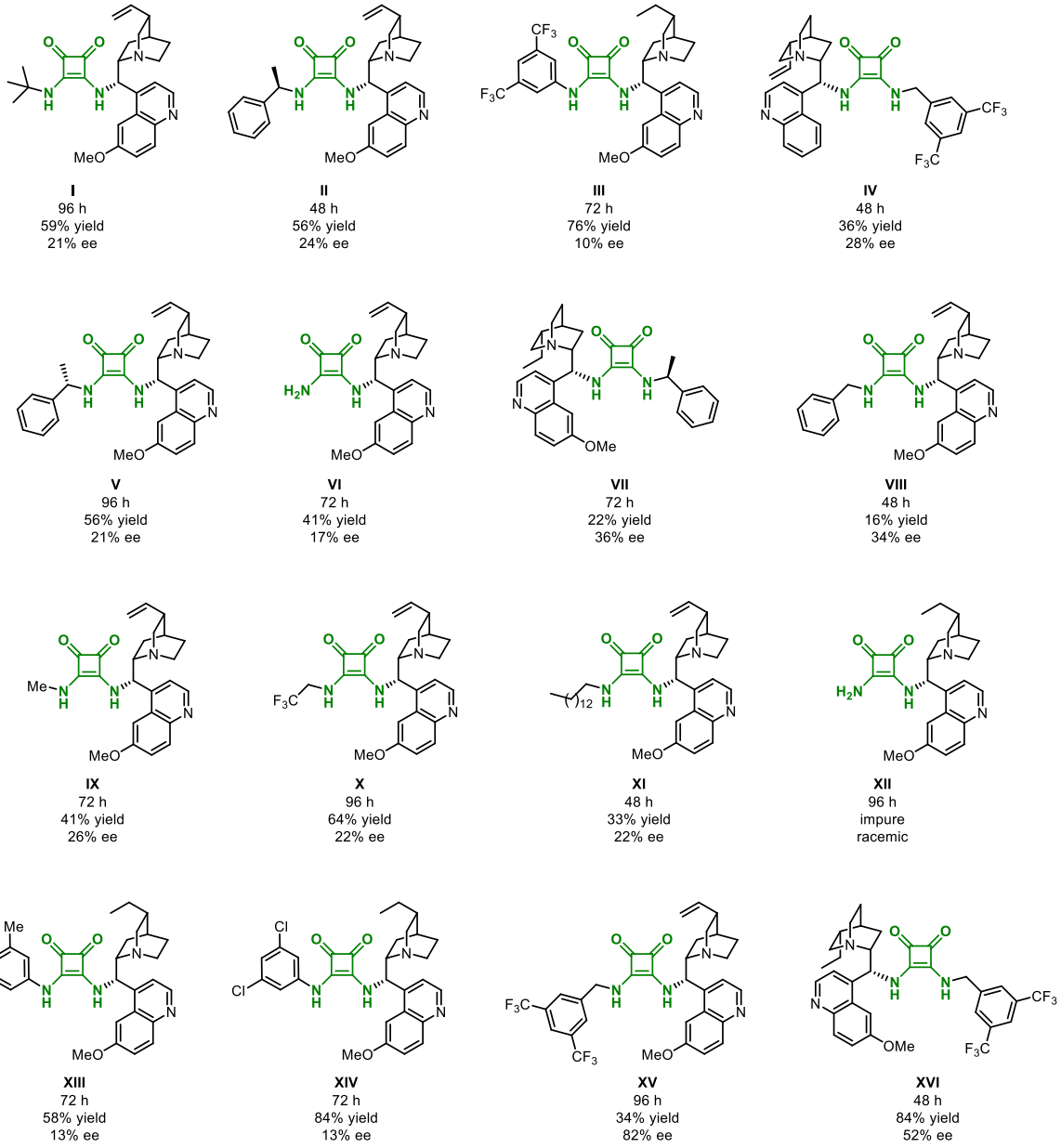


Figure 15. Squaramide derivatives tested as organocatalysts.

The reaction was stopped by adding the reaction mixture in a silica column and the reaction product **6d** was isolated. The enantiomeric excess was obtained using an HPLC equipped with chiral stationary phase columns. During the first experiments, the eluent mixture for the column chromatography consisted of 3:7 hexane: ethyl acetate. However, this mixture did not allow the complete and correct separation in chromatographic conditions of the product from the alkylating agent, which significantly affected the yield.

TLCs were conducted to determine whether alternative solvent ratios could improve the separation. However, none of the tested options was able to provide sufficient separation.

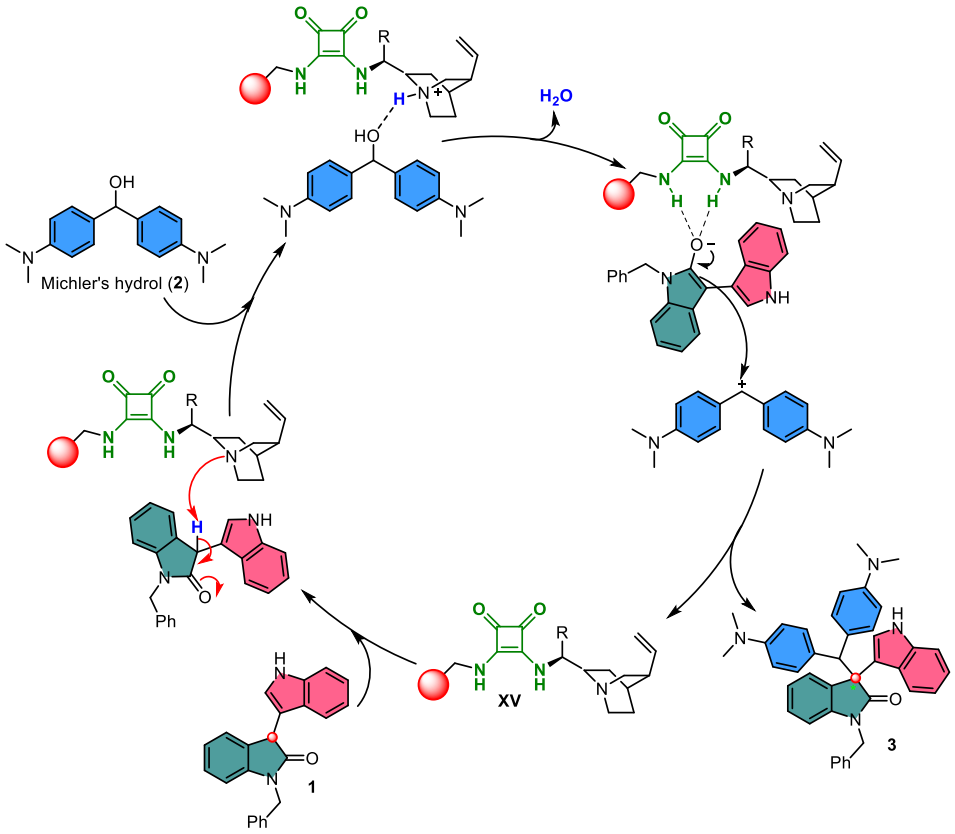
Therefore, other solvent pairings have been taken into consideration. Noticeable separation was finally observed using a mixture of 6:1 toluene: ethyl acetate. With the change in the eluent mixture a significant increase in the average reaction yields was recorded. Indeed, the average yield passed from 40% to 60%, and the final products were obtained clean. The results of the screening are reported in Table 3.

Table 3. Results of the catalysts screening

Reference	Catalyst	Yield (%)	Ee%	t(h)	Eluent mixture
CP12	*Epi*-DHQD **XVI**	84	52	48h	Hex:EtOAc (3:7)
CP19	*Epi*-CN **IV**	36	28	48h	Hex:EtOAc (3:7)
CP23	*Epi*-QN **VIII**	16	34	48h	Hex:EtOAc (3:7)
CP24	*Epi*-QN **II**	56	24	48h	Hex:EtOAc (3:7)
CP25	*Epi*-QN **XI**	33	22	48h	Hex:EtOAc (3:7)
CP31	*Epi*-QN **XV**	36	82	96h	Hex:EtOAc (3:7)
CP32	*Epi*-DHQN **XII**	impure	racemic	96h	Tol:EtOAc (6:1)
CP33	*Epi*-QN **I**	59	21	96h	Tol:EtOAc (6:1)
CP34	*Epi*-QN **VI**	41	17	72h	Tol:EtOAc (6:1)
CP35	*Epi*-DHQD **VII**	22	36	72h	Ex:EtOAc (3:7)
CP36	*Epi*-QN **V**	56	21	96h	Tol:EtOAc (6:1)
CP41	*Epi*-QN **X**	64	22	96h	Tol:EtOAc (6:1)
CP42	*Epi*-QN **IX**	41	26	72h	Tol:EtOAc (6:1)
CP43	*Epi*-DHQN **III**	76	10	72h	Tol:EtOAc (6:1)
CP44	*Epi*-DHQN **XIII**	58	13	72h	Tol:EtOAc (6:1)
CP45	*Epi*-DHQN **XIV**	84	13	72h	Tol:EtOAc (6:1)

QN: Quinine, CN: Cinchonine, DHQD: Dihydroquinidine, DHQN: Dihydroquinne

The obtained results could be partially explained using the concepts already discussed in paragraph 1.1.2. It has been reported that the enantioselectivity promoted by a squaramide-based organocatalyst is heavily linked to its tendency to create a chiral pocket where the reaction will take place. Indeed, the hypothesized reaction mechanism for the generic bifunctional catalyst under consideration in this project is derived from its ability to perform two distinct activities, drawing parallels with the mechanism proposed by Zhang and co-workers, as depicted in Scheme 17.



Scheme 17. Proposed reaction mechanism for the organocatalyzed alkylation.

The tertiary amine fragment, which possess an electron pair not shared with conjugated double bonds and thus available, acts as a Brønsted base, deprotonating substrate **5d**, leading to the formation of an ammonium ion and generating a nucleophilic enolate. The positively charged ammonium ion will then interact with the hydroxyl group of the alkylating agent, causing the further protonation of the oxygen atom and the departure of a water molecule, leading to the formation of the electrophile. On the other hand, the two N-H groups of the squaramide stabilize the negative charge on the oxygen of the enolate activating the C3 as nucleophilic center. The shape of the catalyst, together with the presence of interactions between it and the nucleophile, contribute to the formation of a chiral pocket, which is responsible for the enantioselective induction in the reaction.

According to this, the nature of the squaramide amine plays an important role in affecting the enantioselectivity of the process.

Indeed, despite high ee have not been achieved with any of the tested catalysts, it is visible from the acquired data that the catalysts having amines functionalized with groups with little steric hindrance, or even non substituted, provided extremely low enantiomeric excess (Figure 16).

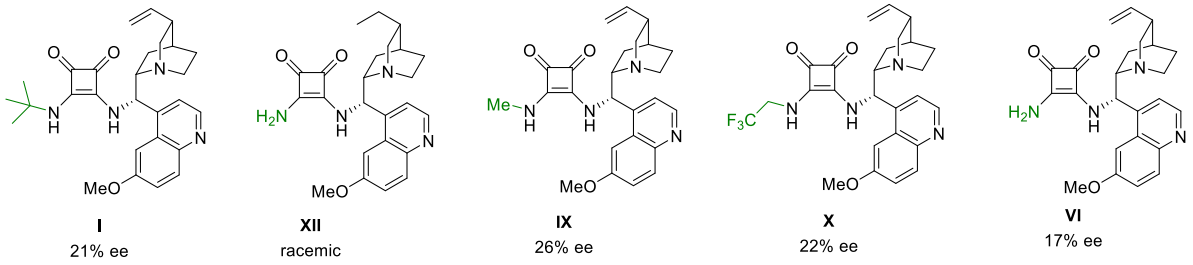


Figure 16. Tested squaramides bearing non bulky substituents to one of the amines.

This tendency could be attributed to the fact that low-profile substituent bonded to the nitrogen atoms are not sufficient to form selective chiral pocket. Even bulky substituents like phenyl rings or substituted phenyl rings were not able to provide significative selectivity when directly attached to squaramide amide (Figure 17), although in this case the aryl group could influence the electronics of the catalyst.

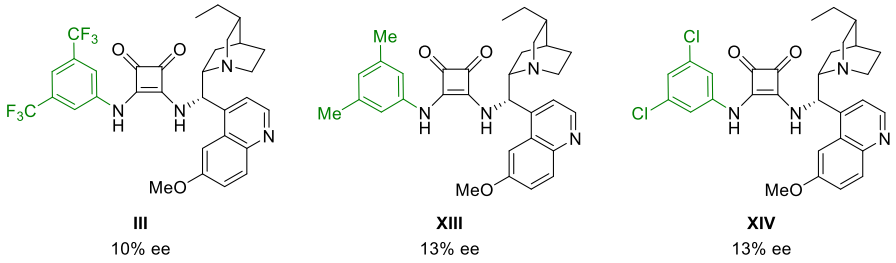


Figure 17.

However, adding a single carbon atom to the substituent group can increase the enantiomeric excess by at least 10% (Figure 18).

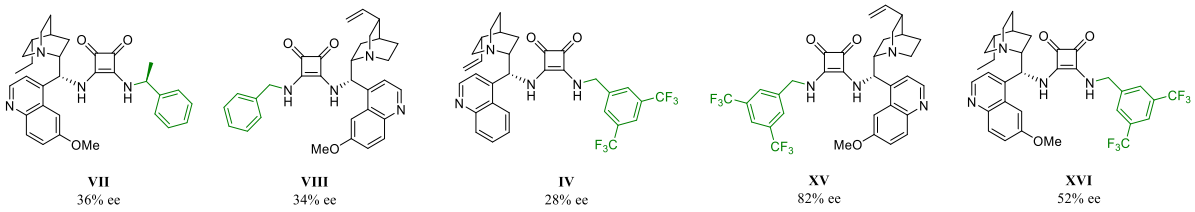
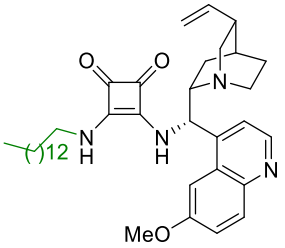


Figure 18.

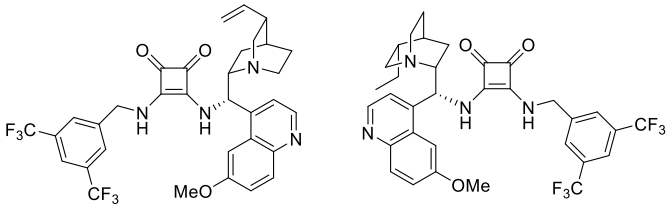
Plausibly, the increased conformational freedom provided by the additional C-C bond allowed a better catalyst-substrate interaction and the formation of a more effective chiral pocket.[19,25] A final valuable result was obtained by considering a 14-carbon hydrocarbon chain. Even though the length of the chain constitutes a significantly bulky substituent, the resulting enantiomeric excess was comparable to that of squaramides with no or low-profile substituents (Figure 19).



XI
22% ee

Figure 19.

This could be explained by considering the mobility of the chain: even though long, it is still characterized by high mobility, preventing the formation of an effective chiral pocket.[4,19] However, the efficiency of a catalyst's enantiomeric selectivity cannot be predicted solely by the nature of the substituents. Even squaramides with similar substituents performed differently in terms of enantiomeric excess, suggesting that other factors, such as the substrate's affinity for the substituents, can also affect the catalyst's performance. From the screening the two best performing catalyst appeared to be the following:



XV
82% ee

XVI
52% ee

Figure 20.

After the catalyst screening, the best performing squaramide (*Epi*-QN **XV**) has been considered to perform the solvent screening in order to define the best reaction condition for the alkylation reaction.

3.4 Solvent screening

Since the amount of original catalyst was not sufficient for the screening, more squaramide **XV** has been synthesized in laboratory. For the solvent screening, the same reaction conditions have been maintained while changing only the employed solvent. The results are reported in Table 4, together with the dielectric constants of the respective solvents.

Table 4. Results of the solvent screening.

Reaction	Catalyst	Yield (%)	Ee%	t(h)	Solvent	Dielectric constant (ε)[1]
CP62	Epi-QN **XV**	89	37	73h	CH_2Cl_2	8.93
CP63	Epi-QN **XV**	80	10	92h	$CHCl_3$	4.81
CP64	Epi-QN **XV**	40	Rac.	92h	Toluene	2.38
CP65	Epi-QN **XV**	93	40	92h	CH_3CN	36.64

1 The values are reported from CRC (87th edition), or Vogel's Practical Organic Chemistry (5th ed.).

Unfortunately, during this step, the high enantiomeric excess that was obtained during the catalyst screening was not reproduced. Therefore, further trials have been made to understand why the results differ from the first registered enantiomeric excess.

However, interesting considerations can still be made considering the results from the solvent screening. The primary distinction that stands out is the notably poorer catalyst performance in toluene. Reaction CP-64 did not produce enantioselectivity or a satisfactory overall yield, unlike other tested conditions. This discrepancy could be attributed to the low polarity of toluene. Both substrates, particularly Michler's hydrol, and the squaramide catalyst exhibit high polarity. During the reaction setup, it was apparent that the reactants and catalyst did not fully dissolve due to this polarity mismatch.[25]

The catalytic procedure in acetonitrile and dichloromethane yielded the product with comparable enantiomeric excesses (ee), although the ee was slightly higher in acetonitrile. In chloroform, the yield was comparable to dichloromethane and acetonitrile, but the ee was significantly lower. The dielectric constants of the solvents, which determine their polarity, showed a trend of increasing ee with increasing polarity. Hypothetically, this could be explained by considering that a solvent with high polarity or that can form a hydrogen bond with the substrate may favor the formation of a transition state with a more polar face, which would lead to the preferential formation of one enantiomer of the product. However, it was observed that the increase was not linear. The increase in ee between dichloromethane and chloroform was more than double the increase between acetonitrile and dichloromethane, even though the dielectric constants of the former two solvents differ by only 4 points, while the dielectric constants of dichloromethane and acetonitrile differ by 28 units. In other words, it was found that the polarity of the solvent might have a significant impact on the enantioselectivity of the reaction, but it is not the only factor since once reached a maximum

level, even with high increase in polarity the enantiomeric excess tends to a plateau, as shown in Figure 21.

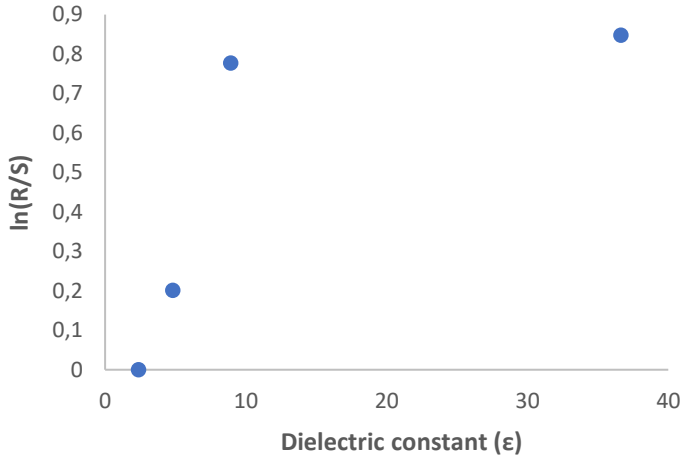


Figure 21. Plotted enantioselectivity against polarity of the solvents.

After determining that acetonitrile appeared to be the best solvent, other catalysis have been performed to understand why the initial enantiomeric excess of 82% obtained during the preliminary screening could not be reproduced. The first suspect fell over the newly synthetized catalysts, hypothesizing that maybe something negatively affected the synthesis or that the resulting product was not pure. Therefore, in the following step the same reaction have been executed using in one case the old catalyst and in the second case the new one, while keeping the same conditions.

3.5 Repetition of previous reactions

As previously mentioned, during this phase the main goal was to repeat experiments in order to understand the lack of reproducibility. Considering the slightly better performance obtained in acetonitrile, this solvent has been chosen for the comparison.

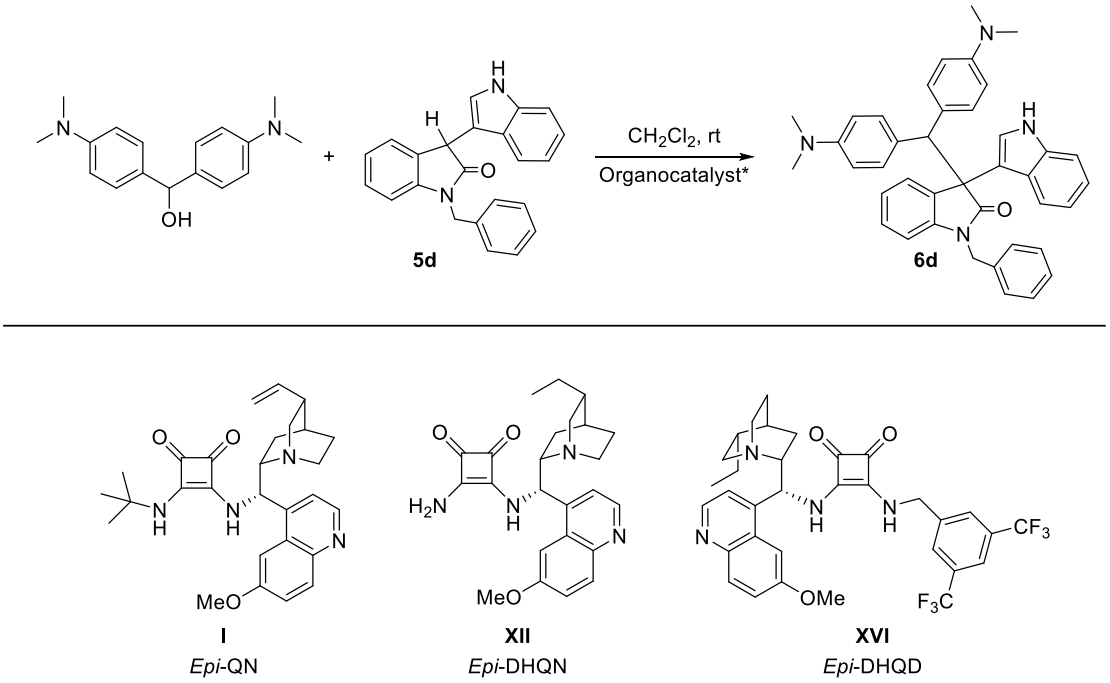
The results to compare the performances of the two catalysts are reported in Table 5. The results obtained for the newly synthesized squaramide confirmed the enantioselectivity obtained during the solvent screening discussed in paragraph 3.4, supporting its reproducibility. However, the old catalyst used during the catalyst screening, discussed in paragraph 3.3, produced an enantioselectivity significantly lower compared to the one previously registered. The explanation for this difference can be linked to the uneven stirring of the solution, as hypnotized further in the document in paragraph 3.6. This supposition finds further confirmation when taking into consideration the results obtained during the solvent

screening at low temperature reported in Table 7. Regardless, this experimental evidence indicated that there has been no mistake in the synthesis of the newly produced catalyst.

Table 5. Comparison between original catalyst and newly synthesized.

Reaction	Catalyst	Yield (%)	Ee%	t(h)	Solvent
CP71	Epi-QN **XV** (old)	79	28	92h	CH_3CN
CP72	Epi-QN **XV** (new)	81	41	92h	CH_3CN

It was suspected that during the screening, there might have been a mix-up with the squaramides, leading to the use of different catalysts on the same day as the *Epi*-QN **XV** test. As a result, three additional tests were conducted using as catalysts the squaramides that were tested on the same day as *Epi*-QN **XV**: *Epi*-DHQN **XXII** and *Epi*-QN **I**. Additionally, another trial with the initial catalyst *Epi*-DHQD **XVI** was made (Scheme 18). The results are shown in Table 6.



Scheme 18.

Table 6. Reproduced tests.

Reaction	Catalyst	Yield (%)	Ee%	t(h)	Solvent
CP73	*Epi*-DHQN **XII**	63	racemic	96h	CH_2Cl_2
CP74	*Epi*-QN **I**	85	22	96h	CH_2Cl_2
CP75	*Epi*-DHQD **XVI**	99	40	96h	CH_2Cl_2

The obtained enantiomeric excesses from the repetition of the catalytic reactions performed on the same day of the *Epi*-QN **XV** squaramide are perfectly compatible with the ones registered during the catalyst screening shown in Table 3. Therefore, these results proved that the catalysts have not been exchanged and some other phenomenon must have caused the significant divergence of the ee.

3.6 Screening at lower temperature and different solvent quantities

Subsequently, further experiments have been executed to generate more insight over the effect that lower temperatures and increasing volumes of solvents could have over the enantioselectivity of the reaction. Four vials were prepared using the same ratio and amount of reactants, with DCM and CH_3CN as solvents. The only difference between the vials was the volume of solvent used. The temperature was kept constant at 7 °C. To prevent the reaction from starting at room temperature, the alkylating agent was added after the test tubes had been cooled down in the fridge. The results are reported in Table 7.

Table 7. Volume and solvent screening at low temperatures.

Reaction	Catalyst	Yield (%)	Ee%	t(h)	Solvent	µL	Temperature
CP76	Epi-QN **XV**	84	39	72h	CH_2Cl_2	250	7 °C
CP77	Epi-QN **XV**	73	51	96h	CH_2Cl_2	500	7 °C
CP84	Epi-QN **XV**	64	40	96h	CH_2Cl_2	1000	7 °C
CP85	Epi-QN **XV**	53	38	96h	CH_2Cl_2	1500	7 °C
CP78	Epi-QN **XV**	80	28	168h	CH_3CN	250	7°C
CP88	Epi-QN **XV**	86	14	96h	CH_3CN	500	7 °C

The effect caused by the modified reaction temperature can be difficult to predict. Indeed, at higher temperatures more energy enters the system, and the reaction intermediates will be more accessible. However, for the same reason the molecules' freedom of mobility is enhanced, and since it has already been stated that in this case an effective enantioselectivity is achieved through the formation of a chiral pocket, this higher mobility could negatively

affect the registered ee%. However, since the enantiomeric excess obtained for CP76 is similar to the one obtained in CP62 (Table 4), and since they differ only for the temperature at which the reaction has been performed, it can be assessed that a decrease in temperature did not affect significantly the enantioselectivity of the catalytic system in DCM. On the other hand, a significant increase of more than 10% in the ee has been registered when doubling the volume of the solvent. This means that the volume of the reaction might have a more relevant effect over the performance of the catalyst compared to the temperature.

Considering this outcome, other reactions with increasing volumes of dichloromethane have been carried out. However, the enantioselectivity didn't further increase. Reaction CP-84 was performed in 1 ml and produced an enantioselectivity like the one obtained in 250 µl, with a 40% ee. An additional volume increase to 1.5 ml in CP-85 caused a slight decrease in enantioselectivity, with a 38% ee. Furthermore, there has been a noteworthy drop in the reaction yield, amounting to a 20% reduction compared to the previously recorded average. Given that the isolation conditions have already been fine-tuned as part of the project, this decline could potentially be attributed to the increased dilution of the reaction. Indeed, a slight decrease in the reactivity of the system has also been registered with a yield decrease in CP-84.

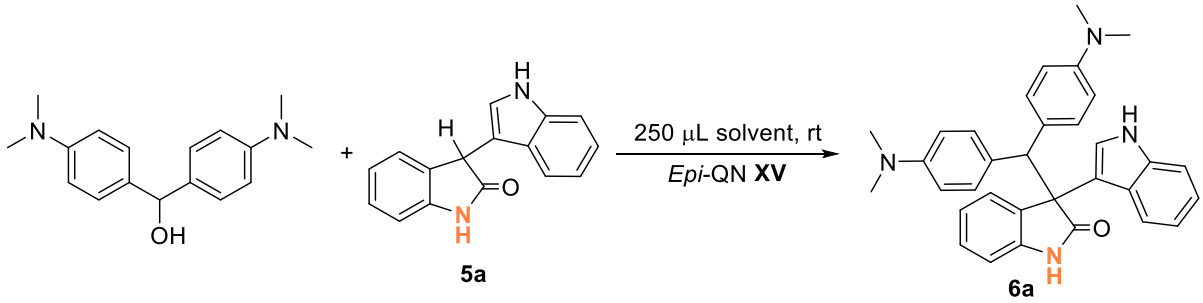
An unexpected 14% enantiomeric excess has been registered in reaction CP-88. The reaction was carried out in acetonitrile in 500 µl. Instead of observing an enhanced enantioselectivity, the enantiomeric excess in these conditions appeared to be significantly lower. However, it should be specified that the chromatogram resulting from the analysis of the CP-88 after isolation was not clean, meaning that some mistakes must have been done during the isolation of the final product.

As final observations, the enantiomeric excess (ee%) obtained in CP-78 was significantly lower than the results of CP-65 (Table 4) and CP-72 (Table 5), which were carried out under the same conditions except for the temperature. A similar decrease in ee% was observed for CP-71 (Table 5), which used the original batch of *Epi*-QN **XV** in acetonitrile and was also carried out at room temperature. Indeed, also in this case, the ee% also dropped to 28%. Since the decrease in temperature did not have a significant impact on enantioselectivity, this discrepancy could be attributed to discontinuous and inefficient stirring. In fact, it was observed that the magnetic stirring bars inside the tubes did not move at all in both CP-71 and CP-78, preventing the reactions from being carried out under continuous stirred conditions. The identical ee% of these two reactions suggests that discontinuous stirring may have played a significant role in decreasing the enantioselectivity of the system.

3.7 Screening with different substrates

Concomitantly to the solvent volume screening at low temperatures, the alkylation reaction was tested with the other synthesized oxindole derivatives as substrates. Each reaction was performed at room temperature under continuous stirring in 250 µL of solvent. Every reaction was performed in both dichloromethane and acetonitrile. After 96h the reaction were stopped and the product isolated through column chromatography. To define the enantiomeric excess the purified product was subjected to chiral stationary phase HPLC. In order to define the right conditions, a test reaction using 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate as non-chiral catalyst was performed for each substrate. However, the obtained product turned out to be extremely impure in all three cases and has not been used as reference.

The reaction for the non-protected oxindole derivative is shown in Scheme 19 and the relating results reported in Table 8.



Scheme 19. Organocatalytic alkylation of the non-protected substrate.

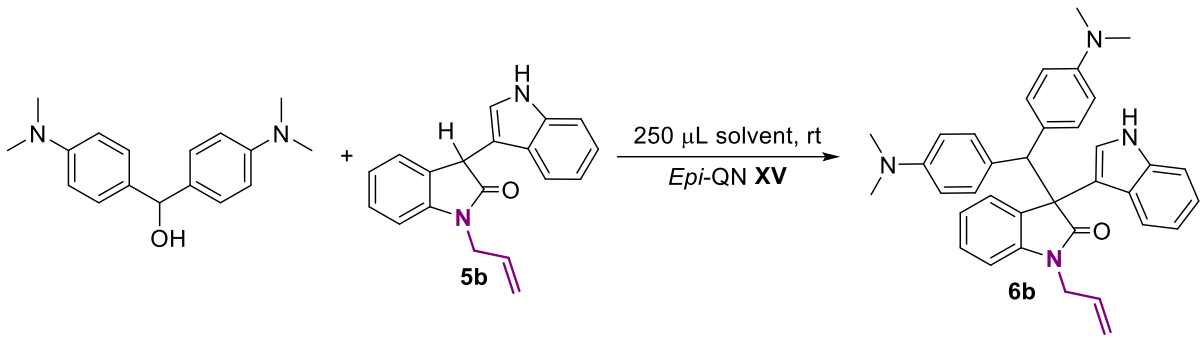
Table 8. Results of the non N-substituted substrate.

Reaction	Catalyst	Yield (%)	ee%	t(h)	Solvent	µL
CP80	*Epi*-QN **XV**	43	14	72	CH_2Cl_2	250
CP82	*Epi*-QN **XV**	49	racemic	168	CH_3CN	250

The successful isolation of the final product was accomplished using column chromatography, with a carefully selected eluent mixture of toluene and ethyl acetate in a 5:2 ratio. Interestingly, the experimental results exhibited a noteworthy decrease in selectivity, marking a significant departure from the expected outcome. Specifically, when the reaction was conducted in dichloromethane, an enantiomeric excess of merely 14% was recorded. The situation was even more severe in acetonitrile, where virtually no enantioselectivity could be obtained. A possible cause for these results could be the presence of two -NH functional groups within the molecular structure under study, in contrast to the presence of just one. As a result, the

molecule polarity is enhanced, which could potentially influence its interactions with both the catalyst and the solvent environment. Indeed, the strength of intramolecular interaction, especially hydrogen bonds, can control the population of different conformers for a molecule.[26] Notably, the hydrogen atom positioned at the 1 position of the oxindole scaffold serves as a hydrogen-bond acceptor, opening possibilities for novel interactions with both the organocatalyst and the surrounding solvent molecules. The interactions that the oxindole scaffold can form are strongly influenced by the presence of the NH group in the heterocycle.[27] The alteration in hydrogen bonding interactions might have disrupted the previously well-established stereochemical control of the reaction by modifying the interaction pattern between the organocatalyst and the substrate. Consequently, the perturbation of the transition state geometry could potentially account for the significant decline in the observed enantioselectivity. This hypothesis might find further confirmation looking at the results obtained for the *N*-allyl and *N*-ethyl oxindole derivatives.

The *N*-allyl protected oxindole derivative **5b** represented another substrate subjected to testing. The reaction is shown in Scheme 20 and the obtained results are reported in Table 9.



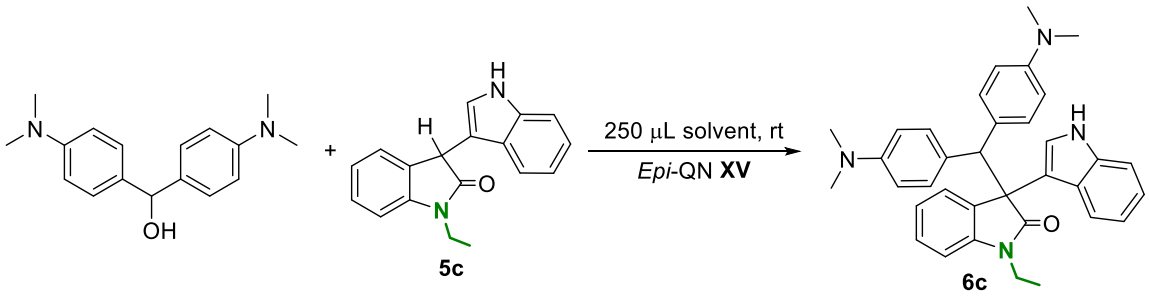
Scheme 20. Organocatalytic alkylation of the N-allyl substrate.

Table 9. Results of the N-allyl substrate.

Reaction	Catalyst	Yield (%)	ee%	t(h)	Solvent	µL
CP86	*Epi*-QN **XV**	62	40	96h	CH_2Cl_2	250
CP89	*Epi*-QN **XV**	66	33	96h	CH_3CN	250

The final alkylated product has been isolated using toluene: ethyl acetate 6:1 eluent mixture. With this substrate, the enantioselectivity of the reactions appeared to be consistent with the one registered for the other protected substrates, even though a slight decrease was observed in acetonitrile, while the highest selectivity was registered in dichloromethane.

The reaction scheme for the *N*-ethyl alkylation product is reported in Scheme 21, while the obtained results are shown in Table 10.



Scheme 21. Organocatalytic alkylation to the N-ethyl substrate.

Table 10. Results of the N-ethyl substrate.

Reaction	Catalyst	Yield (%)	ee%	t(h)	Solvent	µL
CP81	*Epi*-QN **XV**	30	N/D	92h	CH_2Cl_2	250
CP83	*Epi*-QN **XV**	38	40	168h	CH_3CN	250

In both cases the alkylation product was isolated through column chromatography using as liquid phase a toluene: ethyl acetate mixture in 6:1 ratio. The product obtained by the CP81 reaction wasn't pure and thus it was impossible to determine a reliable enantiomeric excess through HPLC. While the CP83 was characterized by an enantiomeric excess equal to the one registered for the *N*-benzyl substrate. On the other hand, the final yields are significantly lower if compared to the ones registered for the initial substrate. This could be attributed to the use of a non-optimal eluent mixture during the isolation phase that impeded the complete separation of the final product from the alkylating agent.

In conclusion, the investigation into the enantioselectivity of the *Epi*-QN **XV** organocatalyst using different oxindoles yielded insightful results. The catalyst displayed consistent enantioselectivity levels similar to those of the *N*-benzyl substrate when applied to other protected substrates. This observation suggests a degree of substrate tolerance within the scope of this study, indicating the potential versatility of the catalyst in accommodating various substrate modifications. Notably, the performance of the non-protected substrate stood out with a pronounced reduction in enantioselectivity. This underlines the importance of specific substrate features and their interaction with the catalyst in determining the outcome of asymmetric reactions. The contrasting enantioselectivity patterns observed in different

solvents for different substrates emphasize the sensitivity of these reactions to the surrounding environment. However, the incomplete data collection (such as the missing ee for CP-81) impede a thorough model delineation. Therefore, further studies are necessary to develop rigorous purification and analysis procedures to draw more reliable conclusions about enantiomeric excess.

4. Conclusions and Perspectives

In conclusion, the results obtained from this research project offer valuable insights into several key aspects of the catalytic processes exploiting squaramides and involving protected oxindole derivatives. The following conclusions have been drawn from this study:

The methodologies present in the existing literature can be successfully adopted to produce a diverse array of protected oxindole derivatives on a laboratory scale.

Even though the initial high enantiomeric excess was not reproduced, interesting conclusions can be drawn from the catalysts screening. The utilization of bifunctional squaramides as catalysts for the alkylation reaction, as illustrated in Scheme 16, facilitates the synthesis of the target product **6** with a moderate degree of enantiomeric excess. Through systematic catalyst screening, it was possible to discern the squaramide variants that exhibit optimal enantioselectivity.

Notably, advancements have been made in refining the isolation conditions for the alkylation product. This is pivotal in ensuring the purity and quality of the synthesized compounds, contributing to the reproducibility of the results.

The solvent's polarity, as employed in the reaction, emerged as an important factor impacting the enantioselectivity of the system. This underlines the importance of considering the solvent's nature in designing and optimizing enantioselective reactions.

Intriguingly, variations in temperature didn’t affect significantly neither the reaction yield nor the enantiomeric excess. While stirring conditions appeared to have a more significant impact.

The organocatalyst *epi*-QN **XV** showcased comparable performance levels of enantioselectivity when considering the range of substituted substrates. However, a remarkable reduction was evident when dealing with the non-substituted oxindole derivative.

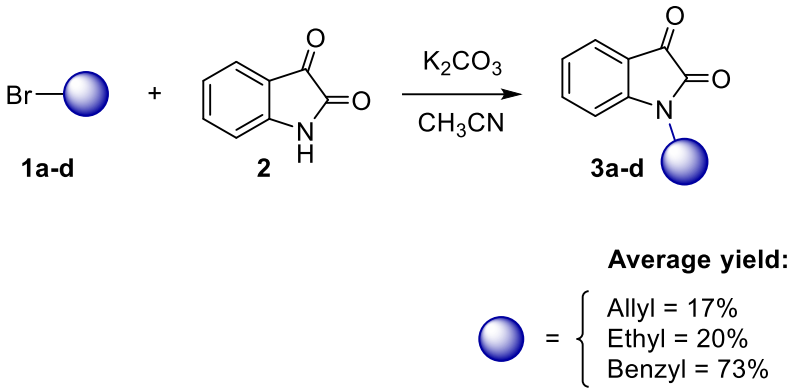
Expanding upon these findings, could represent interesting basis for further research. A compelling prospect would involve an expanded screening taking into consideration a broader spectrum of substrates and conditions. This endeavor would facilitate a more holistic understanding of the organocatalyst's applicability and its interaction preferences across varied substrates. Moreover, the elucidation of the underlying factors contributing to the diminished enantioselectivity observed with the non-substituted oxindole derivative requires further in-depth investigation.

5. Experimental part

5.1 Materials and Methods

Commercial or laboratory-available reagents and solvents were used. To verify the purity of the products obtained and the course of the reactions, ^{1}H NMR spectra were taken using a Varian Mercury 400 spectrometer. The enantiomeric excess was obtained by using a chiral stationary phase HPLC with a DIOD-ARRAY UV detector. In order to obtain a reference for the two enantiomers produced by the catalytic reactions, the racemate was first obtained using (-)-1,1'-binaphthyl-2,2'-diyl hydrogenphosphate as a catalyst in order to obtain the corresponding retention times.

5.2 Synthesis of *N*-substituted isatins



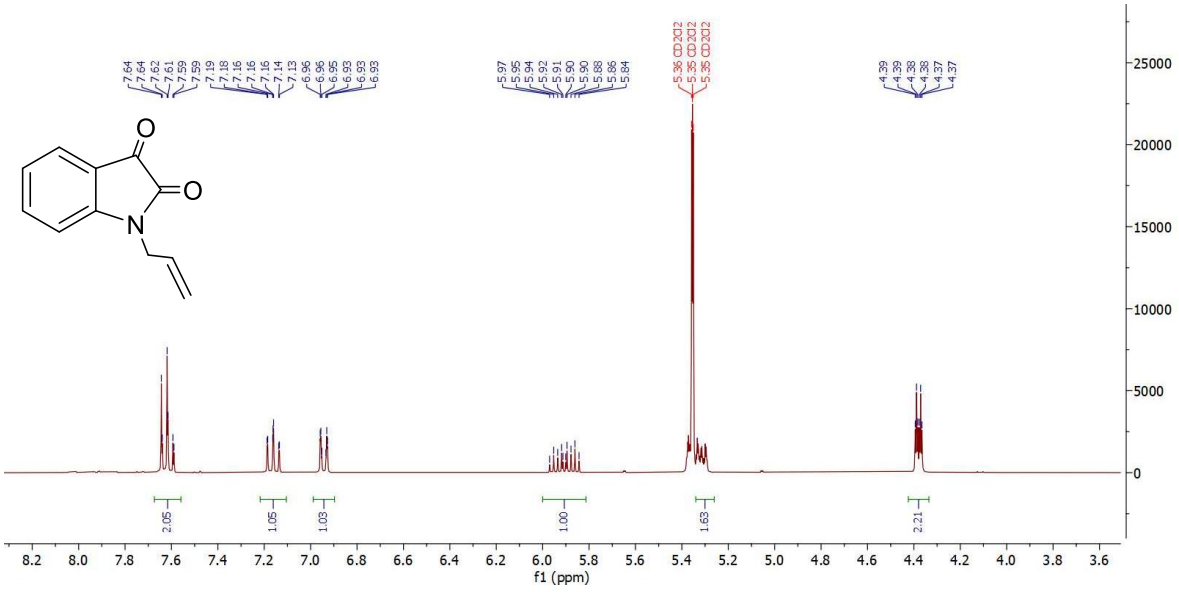
Scheme 22. Addition of protecting group to isatin amine.

To add the protecting group to the N of the isatin scaffold the reaction showed in Scheme 22 has been carried out.[17] 0.444 g of isatin (3 mmol, red-orange crystalline solid) were inserted in a 50 ml reaction flask together with 3 equivalents of potassium carbonate (1.24 g, white powder). Then 10 ml of acetonitrile were versed. After inserting the magnetic stirrer, 1 equivalent of organic bromide (benzyl bromide 360 µL; ethyl bromide 315 µL; allyl bromide 260 µl) were added with a micropipette. Right after the addition of the last reactant the flask is immersed in a silicon bath and heated at 50 °C. The reaction course was checked through TLCs. The reaction time could vary between the 24 and 168 hours, according to the type of protecting group. Indeed, the time to reach completion of the benzylation reaction were lower compared to the ethylation and allylation ones. While the alkylation reaction never reached complete conversion, some isatin always remained even after 48h. To stop the reaction, the solvent was evaporated using rotavapor, and the solid precipitate was resuspended in 25 ml of DCM. The organic phase was washed with 25 ml approximately of water through liquid-liquid extraction. Then the aqueous phase was further extracted with two 25 ml phases of DCM. The remaining traces of water were eliminated from the DCM organic phases through gravity

filtration using $MgSO_4$. After this phase, if the obtained mixture presented a black color, the solution was subjected to column chromatography to isolate the desired product. The eluent phase consisted of 7:3 hexane: ethyl acetate. On the other hand, if the color was bright orange and the solution is limpid, the solvent was evaporated to reduce the volume of at least 2/3 of the initial one and then left to crystallize. In average it took between the 3 and 5 days to obtain bright orange crystals presenting high purity verified through ^{1}H NMR experiment. However, crystallization has been observed only for the benzylation reactions, while the other two protecting group addition all required column chromatography.

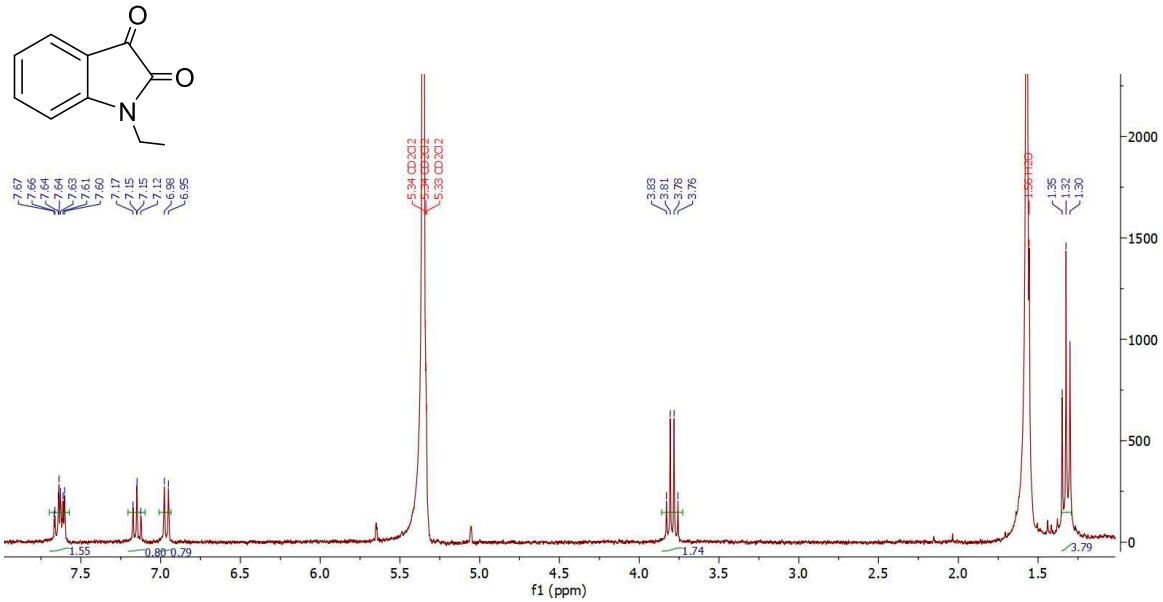
5.2.1 3b: *N*-allyl isatine

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.62 (td, *J* = 7.6, 1.3 Hz, 2H), 7.16 (td, *J* = 7.6, 0.8 Hz, 1H), 6.94 (dt, *J* = 7.4, 0.9 Hz, 1H), 5.91 (ddt, *J* = 17.2, 10.4, 5.3 Hz, 1H), 5.41 – 5.26 (m, 2H), 4.38 (dt, *J* = 5.3, 1.7 Hz, 2H).



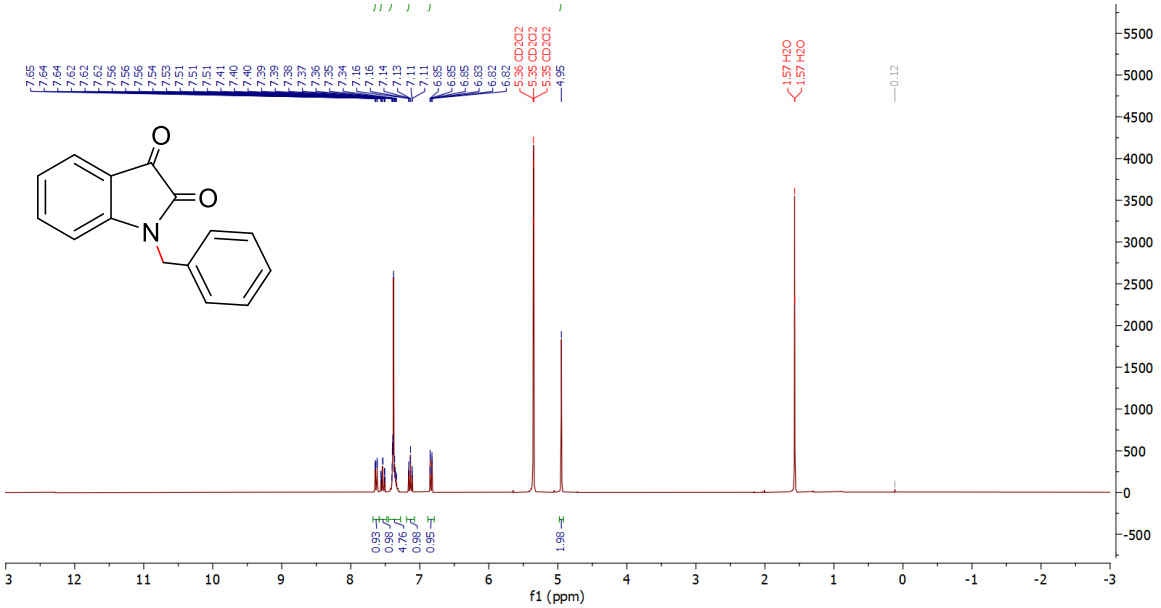
5.2.2 3c: *N*-ethyl isatine

^{1}H NMR (300 MHz, CD_2Cl_2) δ = δ 7.70 – 7.57 (m, 2H), 7.20 – 7.10 (m, 1H), 6.96 (d, *J* = 7.8 Hz, 1H), 3.79 (q, *J* = 7.2 Hz, 2H), 1.32 (t, *J* = 7.3 Hz, 3H).

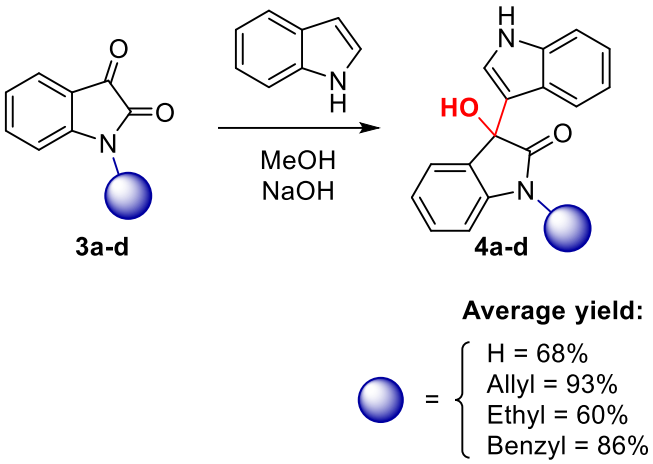


5.2.3 3d: *N*-benzyl isatine

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.63 (ddd, *J* = 7.5, 1.4, 0.7 Hz, 1H), 7.54 (td, *J* = 7.8, 1.3 Hz, 1H), 7.45 – 7.28 (m, 5H), 7.14 (td, *J* = 7.6, 0.9 Hz, 1H), 6.84 (dt, *J* = 8.0, 0.8 Hz, 1H), 4.95 (s, 2H).



5.3 Addition of indole molecule at C3



Scheme 23. Indole addition at C3.

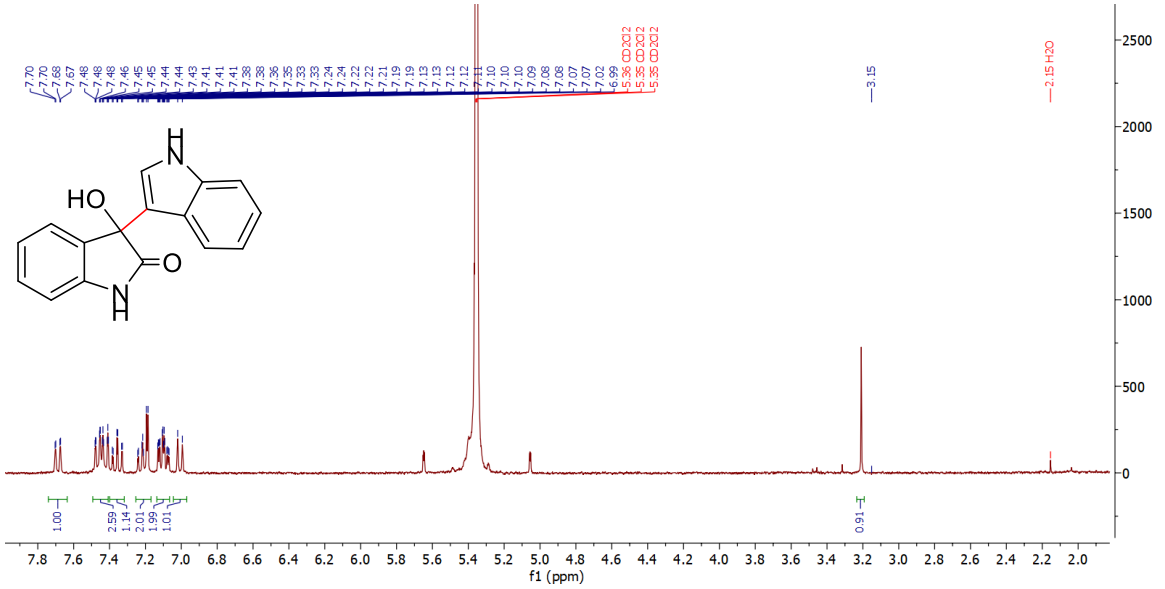
To add the indole moiety to the C3 of the protected isatins previously obtained, procedure reported in Scheme 23 was followed.[23] In a 50 mL flask 2 mmol of molecule **3a-d** were added with 1.1 equivalents of indole (0.25773 g). Next, 10 mL of ethanol were versed and 0.4 equivalents of NaOH are added. If the crystals of reactant number one did not dissolve completely, the flask was slightly immersed in an ultrasound bath to favor the dissolution. The reaction was kept under stirred conditions at room temperatures. The reaction was controlled through TLCs.

After approximately 6 hours the complete disappearance of molecules **3a-d** was observed, meaning that complete conversion was achieved. To separate the product from the excess of indole, and other eventual impurities, a chromatographic column was executed. The used eluent mixture was 6:4 hexane: ethyl acetate.

Then, the fraction containing the product were collected and the solvent evaporated. The resulting products **4a-d** consisted in a rose-orange powder.

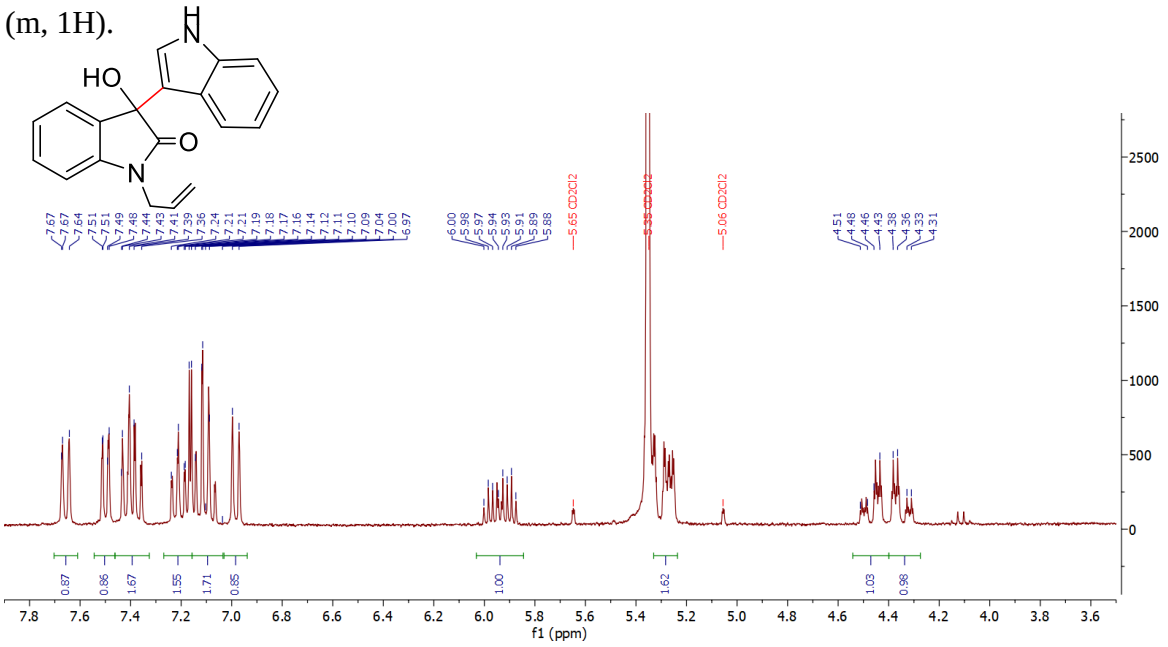
5.3.1 4a: Indole addition at C3 non protected isatin

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.69 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.49 – 7.41 (m, 2H), 7.36 (td, *J* = 7.7, 1.3 Hz, 1H), 7.25 – 7.17 (m, 2H), 7.10 (tdd, *J* = 7.1, 2.6, 1.1 Hz, 2H), 7.01 (dd, *J* = 7.8, 0.9 Hz, 1H).



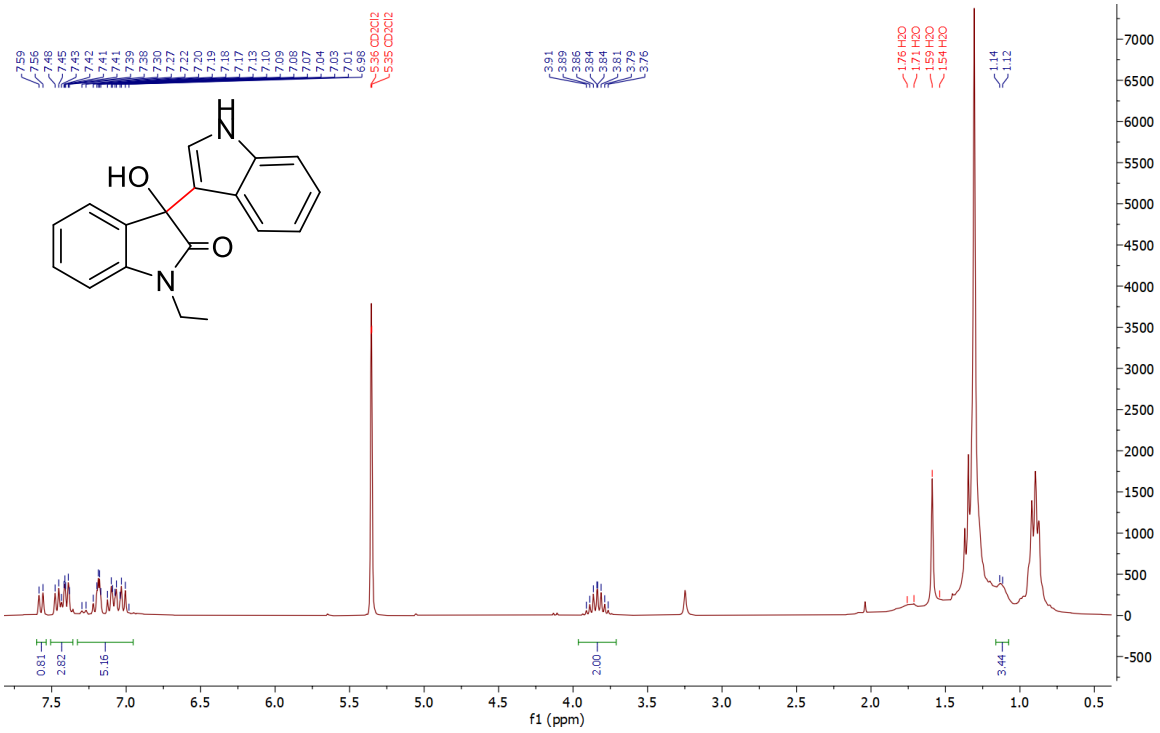
5.3.2 4b: Indole addition at C3 N-allyl isatin

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.70 – 7.61 (m, 1H), 7.54 – 7.46 (m, 1H), 7.46 – 7.33 (m, 2H), 7.27 – 7.16 (m, 2H), 7.16 – 7.03 (m, 2H), 6.98 (dd, *J* = 7.9, 0.9 Hz, 1H), 5.94 (ddt, *J* = 17.3, 10.4, 5.3 Hz, 1H), 5.33 – 5.24 (m, 2H), 4.47 (ddt, *J* = 16.4, 5.3, 1.7 Hz, 1H), 4.40 – 4.27 (m, 1H).



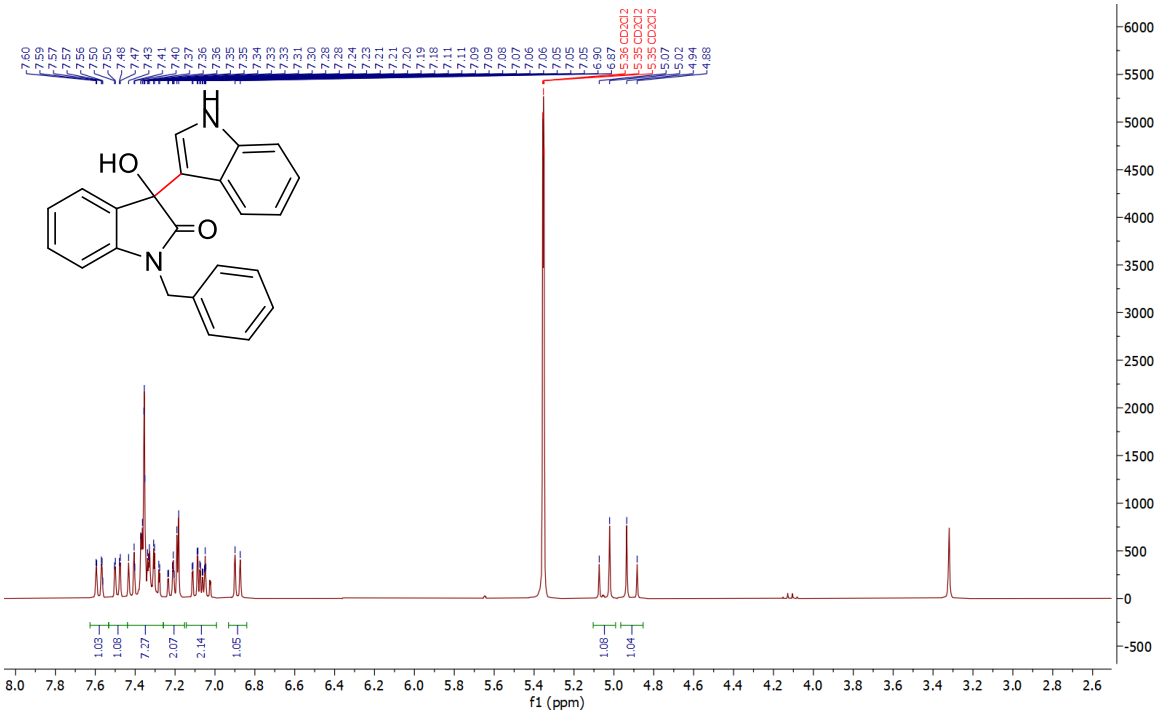
5.3.3 4c: Indole addition at C3 N-ethyl isatin

^{1}H-NMR (300 MHz, CD_2Cl_2) δ = 7.57 (d, *J* = 8.1 Hz, 1H), 7.51 – 7.36 (m, 3H), 7.33 – 6.95 (m, 5H), 3.96 – 3.71 (m, 2H), 1.12 (d, *J* = 7.9 Hz, 3H).

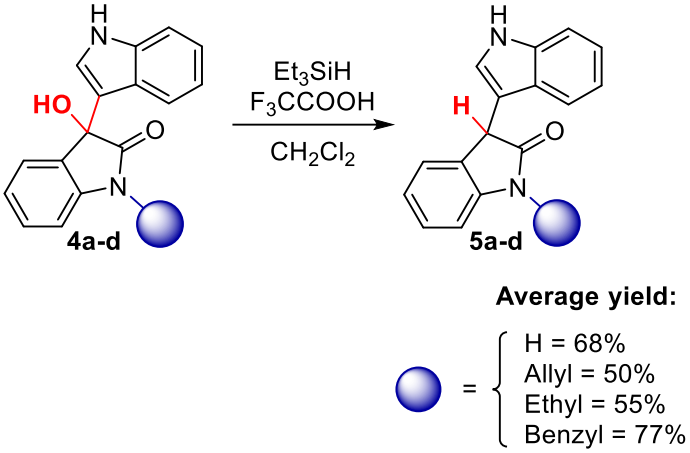


5.3.4 4d: Indole addition at C3 *N*-benzyl isatin

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 8.35 (s, 1H), 7.58 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.49 (dd, *J* = 7.4, 1.3 Hz, 1H), 7.44 – 7.26 (m, 7H), 7.26 – 7.15 (m, 2H), 7.14 – 6.99 (m, 2H), 6.89 (dt, *J* = 7.8, 0.8 Hz, 1H), 5.05 (d, *J* = 15.4 Hz, 1H), 4.91 (d, *J* = 15.7 Hz, 1H).



5.4 Reductive dehydration in C3



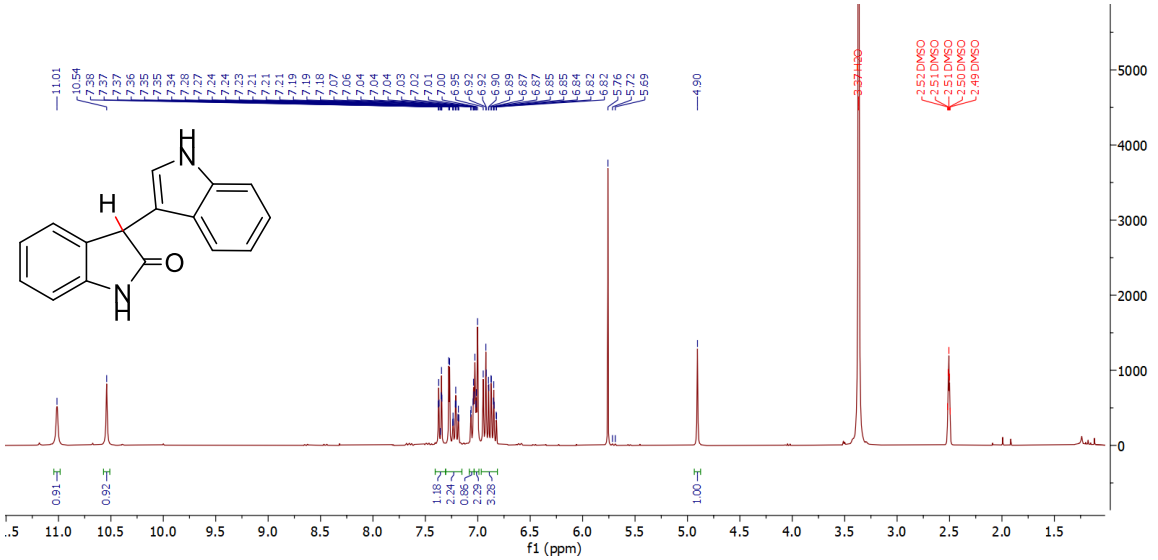
Scheme 24. Reductive dehydration at C3.

To remove the hydroxyl group in C3 1 mmol of the reactant **4a-d** was added in a 50 ml reaction flask with 20 ml of dicholoromethane. Subsequently, 3 equivalents of triethylsilane (440 µl) and 5 equivalents of trifluoroacetic acid (385 µl) were added using a micropipette. The reaction was kept under stirring conditions with a magnetic stirrer at room temperature. The reaction changes color with time passing from pitch black, to bright red, to orange to yellow. The reaction was followed through TLC and complete conversion was observed after approximately two hours, qualitatively when the solution assumed a light-yellow color. To stop the reaction mixture acidity was brought to pH = 5 by adding 1M NaOH aqueous solution (pH=14) with a Pasteur pipette. The pH of the solution during the procedure was checked using the pH indicator paper. Subsequently the aqueous and organic phases have been separated trough liquid-liquid extraction. The aqueous phase was further washed with 3 x 25 ml volumes of DCM. The organic phases were collected and dried using $MgSO_4$ on paper filter (gravity filtration).

The final product was separated through column chromatography using the eluent mixture 5:5 hexane: ethyl acetate. The collected phases were evaporated with the rotavapor and the product isolated. The products resulted in a white/slightly orange powder. The purity was checked through ^1H NMR experiments.

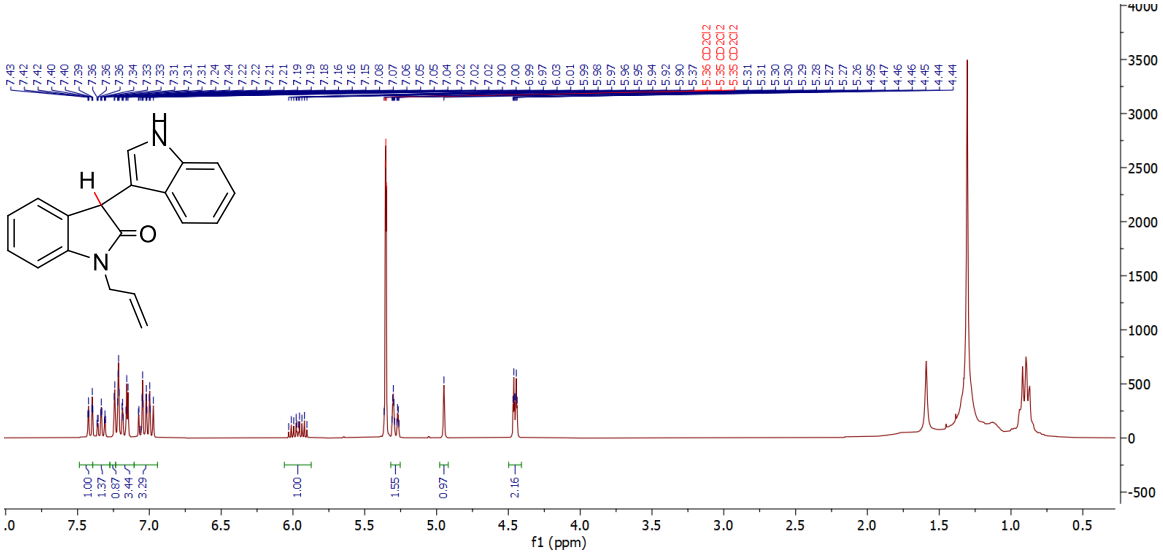
5.4.1 5a: Reductive dehydration of non-protected oxindole derivative

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 11.01 (s, 1H), 10.54 (s, 1H), 7.36 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.31 – 7.15 (m, 2H), 7.08 – 7.03 (m, 1H), 7.03 – 6.99 (m, 2H), 6.97 – 6.81 (m, 3H), 4.90 (s, 1H).



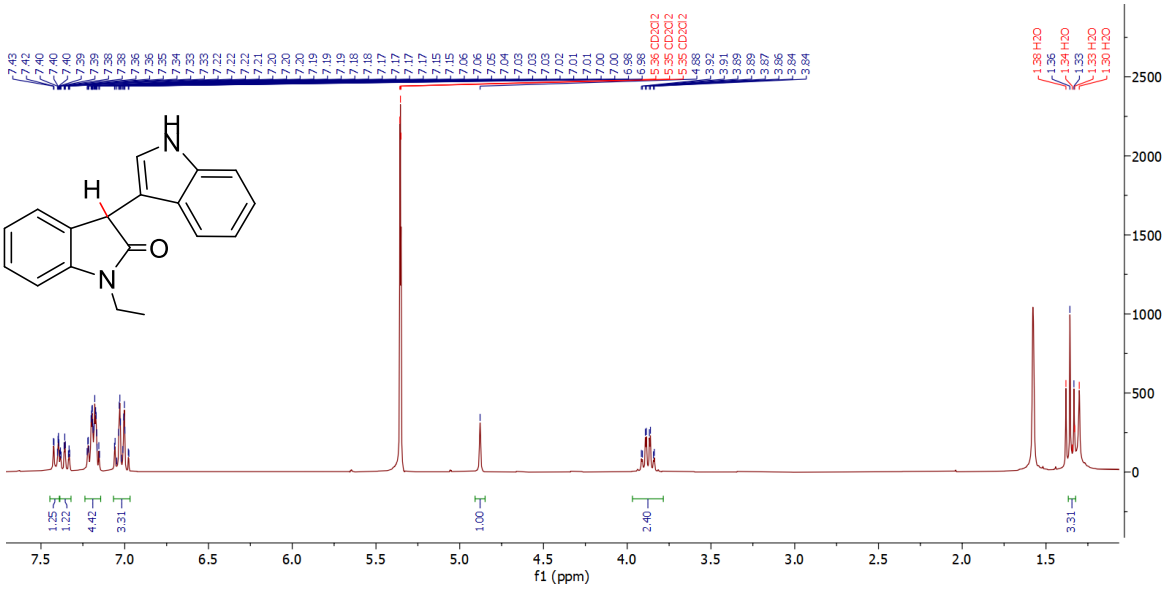
5.4.2 5b: Reductive dehydration of *N*-allyl oxindole derivative

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.49 – 7.39 (m, 1H), 7.39 – 7.28 (m, 1H), 7.24 (d, *J* = 1.2 Hz, 1H), 7.24 – 7.11 (m, 3H), 7.11 – 6.94 (m, 3H), 5.97 (ddt, *J* = 17.2, 10.4, 5.3 Hz, 1H), 5.28 (dq, *J* = 9.0, 1.4 Hz, 2H), 4.95 (s, 1H), 4.45 (dt, *J* = 5.3, 1.7 Hz, 2H).



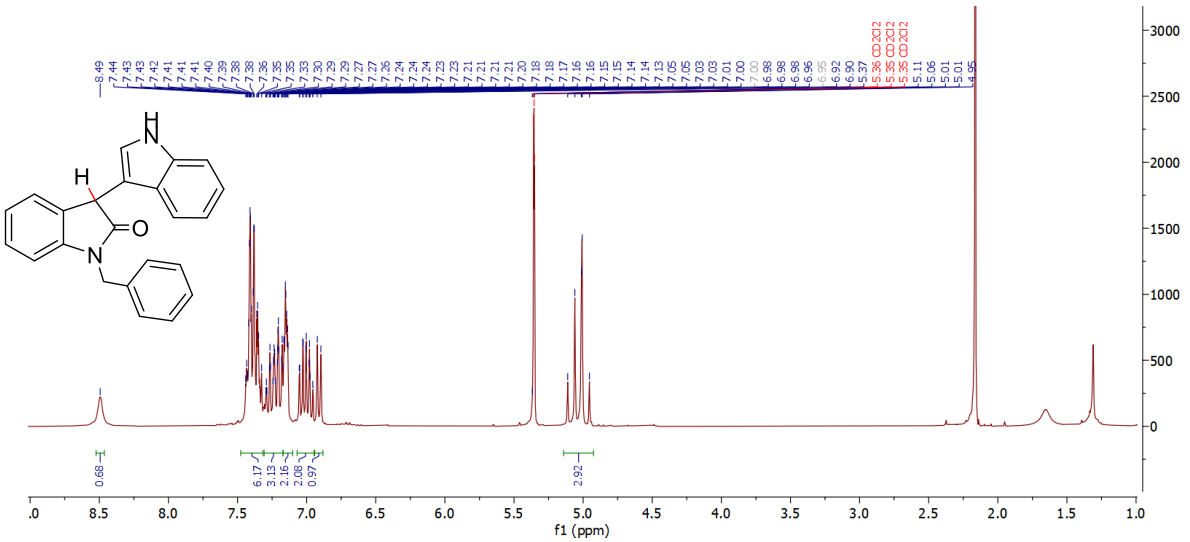
5.4.3 5c: Reductive dehydration of *N*-ethyl oxindole derivative

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.45 – 7.39 (m, 1H), 7.39 – 7.32 (m, 1H), 7.24 – 7.14 (m, 4H), 7.02 (dddd, *J* = 9.2, 7.9, 7.3, 1.0 Hz, 3H), 4.88 (s, 1H), 3.88 (qd, *J* = 7.1, 1.8 Hz, 2H), 1.34 (d, *J* = 7.2 Hz, 3H).

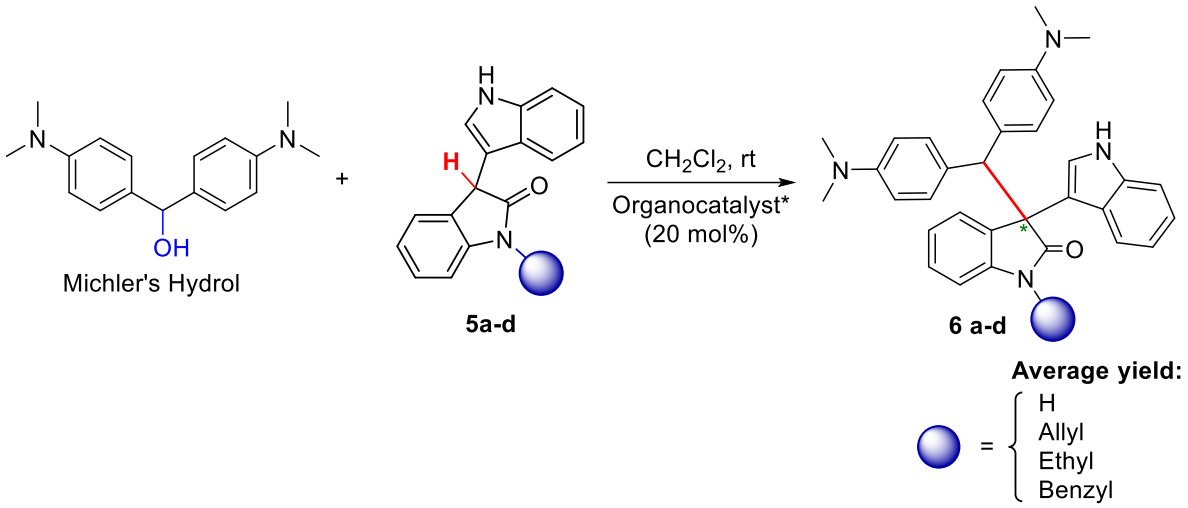


5.4.4 5d: Reductive dehydration of N-benzyl oxindole derivative

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 8.49 (s, 1H), 7.54 – 7.31 (m, 6H), 7.31 – 7.11 (m, 5H), 7.08 – 6.94 (m, 2H), 6.91 (d, *J* = 7.8 Hz, 1H), 5.17 – 4.89 (m, 3H).



5.5 Alkylation reactions



Scheme 25. Organocatalysed alkylation reaction at C3

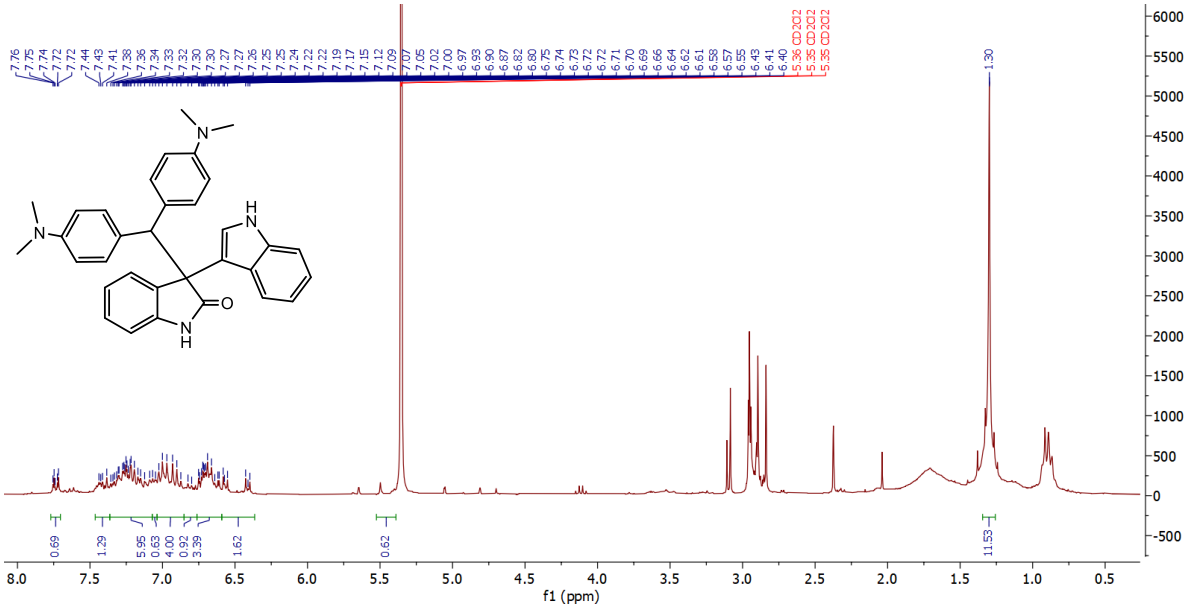
The alkylation reaction (Scheme 25) was performed by adding the substrates **5a-d** and the alkylating reagent in a molar ratio of 1.1:1 (equivalent to 0.0275 and 0.025 mmol, respectively) to a vial equipped with a magnetic stir bar. Additionally, 0.0050 mmol of catalyst (20% molar ratio relative to the limiting reagent) were added, which varied for each catalysis experiment. The entire mixture was dissolved in 250 µL of solvent. The vial was then closed and sealed with Parafilm to prevent solvent evaporation.

After a variable period (from 48 to 168 hours), the reaction was stopped by doing a chromatographic column on silica stationary phase. Initially used eluent mixture consisted in hexane: ethyl acetate in 4:6 ratio, however the separation between the alkylating agent and the product was not good enough to obtain a good yield. Therefore, it was changed half-way with a mixture of toluene: ethyl acetate in 6:1 ratio. The isolated product was then analyzed using ^{1}H NMR to confirm the collected material's identity as the desired target compound and assess its purity.

Successively, HPLC analysis with a chiral stationary phase was conducted to separate the two enantiomers of product **6a-d** and evaluate their enantiomeric excess.

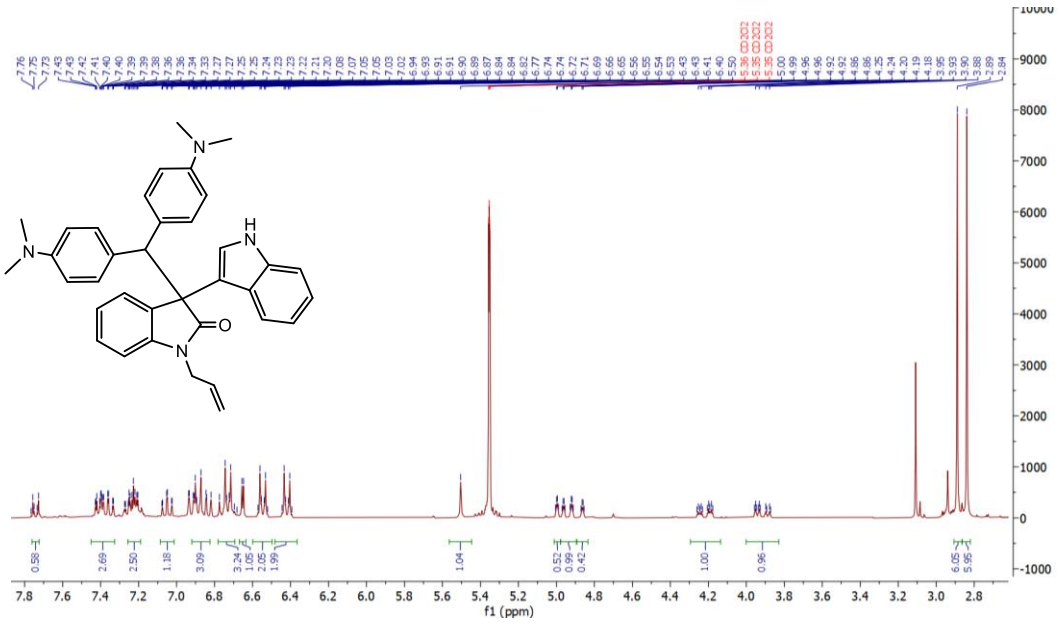
5.5.1 6a: Alkylation reaction with non-protected substrate

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.74 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.46 – 7.36 (m, 1H), 7.36 – 7.10 (m, 5H), 7.10 – 7.04 (m, 1H), 7.04 – 6.95 (m, 2H), 6.94 – 6.86 (m, 1H), 6.81 (d, *J* = 7.7 Hz, 1H), 6.76 – 6.54 (m, 4H), 6.41 (d, *J* = 8.9 Hz, 1H), 5.45 (d, *J* = 29.6 Hz, 1H), 1.30 (s, 12H).



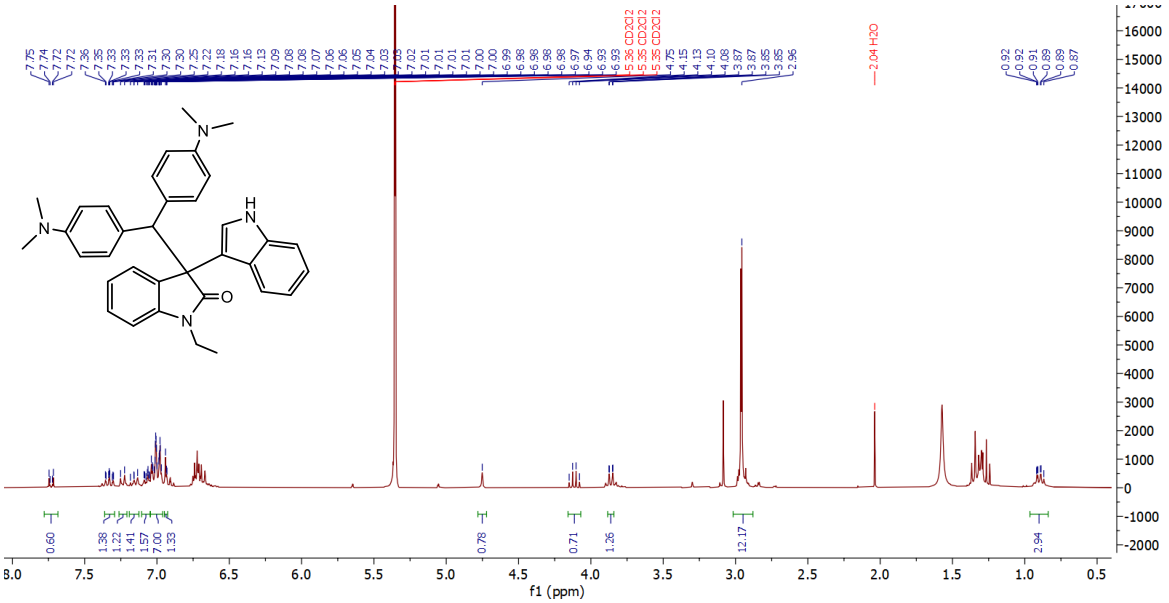
5.5.2 6b: Alkylated reaction with *N*-allylated substrate

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.80 – 7.67 (m, 1H), 7.45 – 7.33 (m, 3H), 7.23 (ddd, *J* = 7.0, 4.7, 1.7 Hz, 2H), 7.05 (td, *J* = 7.6, 1.1 Hz, 1H), 6.92 – 6.83 (m, 3H), 6.73 (d, *J* = 8.9 Hz, 3H), 6.65 (d, *J* = 2.6 Hz, 1H), 6.55 (d, *J* = 8.8 Hz, 2H), 6.42 (d, *J* = 8.9 Hz, 2H), 5.50 (s, 1H), 5.00 (q, *J* = 1.5 Hz, 1H), 4.94 (dq, *J* = 13.0, 1.6 Hz, 1H), 4.86 (q, *J* = 1.6 Hz, 1H)., 4.29 – 4.15 (m, 1H), 3.99 – 3.84 (m, 1H), 2.89 (s, 6H), 2.84 (s, 6H).



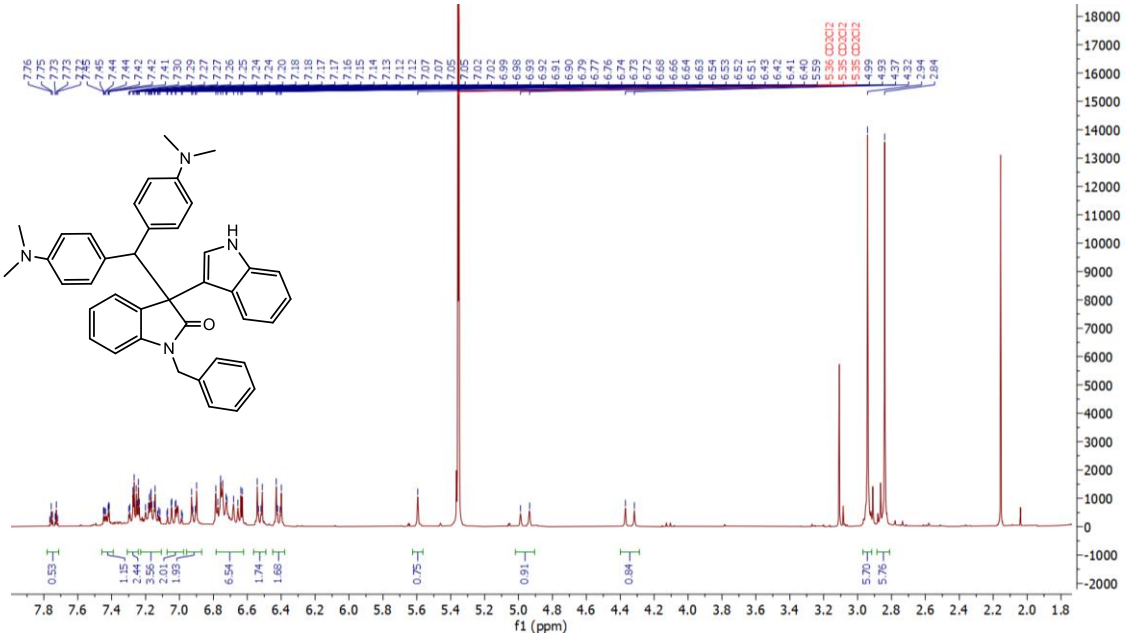
5.5.3 6c: Alkylation reaction with *N*-ethylated substrate

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.75 – 7.71 (m, 1H), 7.36 – 7.29 (m, 1H), 7.24 (d, *J* = 8.2 Hz, 1H), 7.19 – 7.12 (m, 1H), 7.11 – 7.05 (m, 2H), 7.05 – 6.96 (m, 7H), 6.94 (d, *J* = 3.0 Hz, 1H), 4.75 (s, 1H), 4.11 (q, *J* = 7.1 Hz, 1H), 3.86 (dd, *J* = 7.2, 0.9 Hz, 1H), 3.02 – 2.88 (m, 12H), 0.96 – 0.84 (m, 3H).



5.5.4 6d: Alkylation reaction with N-benzylated substrate

^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.77 – 7.71 (m, 1H), 7.46 – 7.39 (m, 1H), 7.31 – 7.24 (m, 2H), 7.23 – 7.11 (m, 4H), 7.07 – 6.98 (m, 2H), 6.96 – 6.87 (m, 2H), 6.78 – 6.62 (m, 6H), 6.56 – 6.49 (m, 2H), 6.45 – 6.38 (m, 2H), 5.56 (s, 1H), 4.96 (d, *J* = 15.8 Hz, 1H), 4.34 (d, *J* = 15.8 Hz, 1H), 2.94 (s, 6H), 2.84 (s, 6H).



References

1. Herrera, R. P, Ardevines, S., Marqués-López, E. Horizons in Asymmetric Organocatalysis: En Route to the Sustainability and New Applications. *Catalysts* **12**, (2022).

2. Han, B. *et al.* Asymmetric organocatalysis: An enabling technology for medicinal chemistry. *Chemical Society Reviews* vol. 50 1522–1586 Preprint at https://doi.org/10.1039/d0cs00196a (2021).

3. Carlone, A. & Bernardi, L. Enantioselective organocatalytic approaches to active pharmaceutical ingredients - Selected industrial examples. *Physical Sciences Reviews* vol. 4 Preprint at https://doi.org/10.1515/psr-2018-0097 (2019).

4. Alemán, J., Parra, A., Jiang, H. & Jørgensen, K. A. Squaramides: Bridging from molecular recognition to bifunctional organocatalysis. *Chemistry - A European Journal* vol. 17 6890–6899 Preprint at https://doi.org/10.1002/chem.201003694 (2011).

5. Chauhan, P., Mahajan, S., Kaya, U., Hack, D. & Enders, D. Bifunctional amine-squaramides: Powerful hydrogen-bonding organocatalysts for asymmetric domino/cascade reactions. *Advanced Synthesis and Catalysis* vol. 357 253–281 Preprint at https://doi.org/10.1002/adsc.201401003 (2015).

6. Popova, E. A. *et al.* Squaramide-Based Catalysts in Organic Synthesis (A Review). *Russian Journal of General Chemistry* vol. 92 287–347 Preprint at https://doi.org/10.1134/S107036322203001X (2022).

7. Khetmalis, Y. M., Shivani, M., Murugesan, S. & Chandra Sekhar, K. V. G. Oxindole and its derivatives: A review on recent progress in biological activities. *Biomedicine and Pharmacotherapy* vol. 141 Preprint at https://doi.org/10.1016/j.biopha.2021.111842 (2021).

8. Ortega Martínez, A. *Synthesis of 3,3-disubstituted2-oxindoles by deacylative alkylation and photocataytic alkylation of olefins by zinc-sulfinates*. http://iso.ua.es;

9. Zhang, H. *et al. Isolation and Structure of Convolutamydines B ~ D from Marine Bryozoan Amathia convoluta*. *Tetrahedron* vol. 51 (1995).

10. Cane, A., Tournaire, M. C., Barritault, D. & Crumeyrolle-Arias, M. The endogenous oxindoles 5-hydroxyoxindole and isatin are antiproliferative and proapoptotic. *Biochem Biophys Res Commun* **276**, 379–384 (2000).

11. Midoh, N. *et al.* Antioxidative activities of oxindole-3-acetic acid derivatives from supersweet corn powder. *Biosci Biotechnol Biochem* **74**, 1794–1801 (2010).

12. Annual Report of the EFSA Journal 2012. *EFSA Supporting Publications* **10**, (2013).

13. Paira, P. *et al.* Efficient synthesis of 3,3-diheteroaromatic oxindole analogues and their in vitro evaluation for spermicidal potential. *Bioorg Med Chem Lett* **19**, 4786–4789 (2009).

14. Shintani, R., Inoue, M. & Hayashi, T. Rhodium-catalyzed asymmetric addition of aryl- and alkenylboronic acids to isatins. *Angewandte Chemie - International Edition* **45**, 3353–3356 (2006).

15. Itoh, J., Han, S. B. & Krische, M. J. Enantioselective allylation, crotylation, and reverse prenylation of substituted isatins: Iridium-catalyzed C - C bond-forming transfer hydrogenation. *Angewandte Chemie - International Edition* **48**, 6313–6316 (2009).

16. Luppi, G. *et al.* Dipeptide-catalyzed asymmetric aldol condensation of acetone with (N-alkylated) isatins. *Journal of Organic Chemistry* **70**, 7418–7421 (2005).

17. Sonsona, I. G. *et al.* Investigation of Squaramide Catalysts in the Aldol Reaction En Route to Funapide. *European J Org Chem* **2022**, (2022).

18. Chauhan, P. & Chimni, S. S. Asymmetric addition of indoles to isatins catalysed by bifunctional modified cinchona alkaloid catalysts. *Chemistry - A European Journal* **16**, 7709–7713 (2010).

19. Zhang, T. *et al.* Asymmetric direct α-alkylation of 2-oxindoles with michler's hydrol catalyzed by bis-cinchona alkaloid–Brønsted acid via an SN1-type pathway. *Chemical Communications* **49**, 1636–1638 (2013).

20. Heterocyclic Aromatic Anions with in+ 2 «-Electrons. doi:10.0.

21. Mahmoud, E., Hayallah, A. M., Kovacic, S., Abdelhamid, D. & Abdel-Aziz, M. Recent progress in biologically active indole hybrids: a mini review. *Pharmacological Reports* vol. 74 570–582 Preprint at https://doi.org/10.1007/s43440-022-00370-3 (2022).

22. Singh, T. P. & Singh, O. M. Recent Progress in Biological Activities of Indole and Indole Alkaloids. *Mini-Reviews in Medicinal Chemistry* **18**, (2017).

23. Babu, K. N., Roy, A., Singh, M. & Bisai, A. Thiourea-Catalyzed Enantioselective Malonate Addition onto 3-Sulfonyl-3'-indolyl-2-oxindoles: Formal Total Syntheses of (-)-Chimonanthine, (-)-Folicanthine, and (+)-Calycanthine. *Org Lett* **20**, 6327–6331 (2018).

24. Vecchi M., RELATORE Luca Bernardi & CORRELATORE Raquel Herrera. Alchilazione enantioselettiva di ossindoli sostituiti mediata da organocatalizzatori.

25. Susam, D. & Tanyeli, C. Enantioselective aza-Henry reaction of: t -Boc protected imines and nitroalkanes with bifunctional squaramide organocatalysts. *New Journal of Chemistry* **41**, 3555–3561 (2017).

26. Arya, N., Mishra, S. K. & Suryaprakash, N. Intramolecular hydrogen bond directed distribution of conformational populations in the derivatives of: N '-benzylidenebenzohydrazide. *New Journal of Chemistry* **43**, 13134–13142 (2019).

27. Czeleń, P. Investigation of the inhibition potential of new oxindole derivatives and assessment of their usefulness for targeted therapy. *Symmetry (Basel)* **11**, (2019).