Alma Mater Studiorum – University of Bologna

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Study of the activity and enantioselectivity of alginate-based catalysts in Friedel-Crafts reactions.

Experimental degree thesis

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ABSTRACT:

This thesis is part of a long-term project which aims to demonstrate for the first time that alginate gel beads can be used as chiral heterogeneous catalysts for enantioselective reactions. Alginate barium beads were prepared as previously optimized and applied to the Friedel-Crafts reaction between indoles and nitroalkenes. New substrates were tested, showing that the reaction can accommodate different nitroalkenes and indoles, affording the corresponding products with moderate yields and good enantioselectivities. However, aliphatic nitroalkenes cannot be used as they degrade under the catalytic reaction conditions. Preliminary study on the recyclability of the heterogeneous catalyst indicated a moderate stability of the catalyst, which can be used for few cycles with a slight erosion of enantioinducing power. Some directions for future improvements (storage and work-up solvent, use of ultrasonic bath) have been suggested.

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Summary:

1. Introduction

1.1) Green chemistry

The field of green chemistry has drawn a great deal of interest over the last two decades. This is due to the ability to tackle chemical innovation in order to achieve simultaneously environmental and economic goals. In the previous years, it was pointed out that, the efficiencies of chemical reaction itself and the following separation and purification steps were the main points on which working and implementing necessary improvements. In fact, many highly inefficient atomic processes, non-environmentally friendly compounds, stoichiometric reagents, unrecovered and hardly reused catalysts, and large volumes of volatile organic solvents are routinely employed. One of the major efforts towards reduction of the environmental impact has been directed at reducing waste at the end of the reaction, when the desired organic product needs to be separated from the inorganic reagents, catalysts, and solvents.

Green chemistry is defined as "the design of chemical products and processes to reduce or eliminate the use and consumption of chemicals" in the 1990s by P.T. Anastas and J.C. Warner. **([1])** It is described by careful planning of chemical synthesis and molecular design to lessen significantly negative environmental impacts. Because of its environmental approach and in parallel safety approach as well, it is not surprising that this field has deeply affected many industrial sectors such as cosmetics, aerospace, electronics, agrochemicals, energy, household products, and pharmaceuticals. **([2])**

Taking this into consideration, the search for an approximate optimal synthesis has been accomplished with the application of several technologies including catalysis, as we will find out in this thesis. **([3])**

1.2) Asymmetric catalysis and asymmetric catalysis with biopolymers

Before discussing the concept of catalysis, and specifically asymmetric catalysis, basic notions on stereoselective organic synthesis should be explained. Organic synthesis, aimed at enantiomerically pure compounds, has gained significant importance and relevance in recent decades.

This importance may lie on the fact that, in most cases, molecules in biological systems have different features and properties depending on the enantiomeric form in which they are present, since is now possible controlling the spatial orientation of the newly formed bonds in many reactions.

The control of this absolute stereochemistry is commonly acquired through different strategies such as resolution of racemic mixtures, synthesis from chiral pool or asymmetric synthesis from prochiral compounds. Within the latest, we count on asymmetric catalysis and stoichiometric asymmetric synthesis. The latter is based on stoichiometric amounts of chiral reagents and chiral auxiliaries, that involve diastereoselective reactions. A chiral auxiliary is a specific component that binds to the substrate by covalent bonds, carrying out the reaction, and it is subsequently removed from the product, leaving no trace.

Asymmetric catalysis is traditionally based on enantiopure chiral organic molecules of low molecular weight which include amino acid derivatives, alkaloids, synthetic molecules, and more, acting as enantioselective inducers. These molecules can behave as ligands for all types of metals (organometallic catalysis) or act as catalysts themselves (asymmetric organocatalysis). For several years, research on this subject has been directed towards the use of chiral catalysts that are environmentally friendly and derived from renewable materials. The attention to this issue rose successively with the already explained concept of "green chemistry", and it has been focused on functionalized molecules, already present in nature with their own chirality. These molecules do not require special treatments for disposal, are available in large quantities and do not compete with agricultural production. **([4])**

The first examples of asymmetric catalysis using biopolymers as a source of chirality was revealed in 1956, by Akabori and co-workers. **([5])** Their approach consisted of the ability of a polypeptide, a biopolymer, to induce chirality. The reduction of a Pd (II) salt on the silk fibroin in the formation of a protein bound Pd (0) catalyst, led to hydrogenate some imine derivatives with modest but at the time revolutionary enantioselection. Anyway, because of the not satisfactory outcomes obtained, this approach was put aside for many years, leading to the development of low molecular weight organic molecules uses in the field of asymmetric catalysis.

Likewise, the use of the enzymes encoded by DNA **([6])** in the field of biocatalysis led to the use of this elegant helical and natural structure, the DNA itself, as scaffold for asymmetric catalysis. Today, one of the fields where DNA is widely used is enantioselective nucleic acid catalysis.

Roelfes and Feringa, **([7])** have shown that by considering the incorporation of a metal "cofactor" into the biopolymer structure, the potential of this polynucleotide molecule in asymmetric catalysis was very promising. This incorporation would extend the activity expressed by the biopolymer beyond its natural function. Their study began with a Diels-Alder reaction catalyzed by copper (II) which was brought into contact with the DNA double helix **([8])** via non-chiral ligand, a pyrimidine derivative. By cause of the proximity of the metal catalyst, they were able to obtain high enantiomeric excesses and achieve good regioselectivity and enantioselectivity. They were also able to demonstrate the accelerating effect of DNA on the reaction compared to the copper complex alone **([9])**. The consequent use of the DNA structure in many other types of reactions allowing the formation of C-C bonds, showed that also the Friedel-Crafts alkylation, which not surprisingly is the main reaction of my work, can be performed with this catalyst system **([10])** (Figure 1)

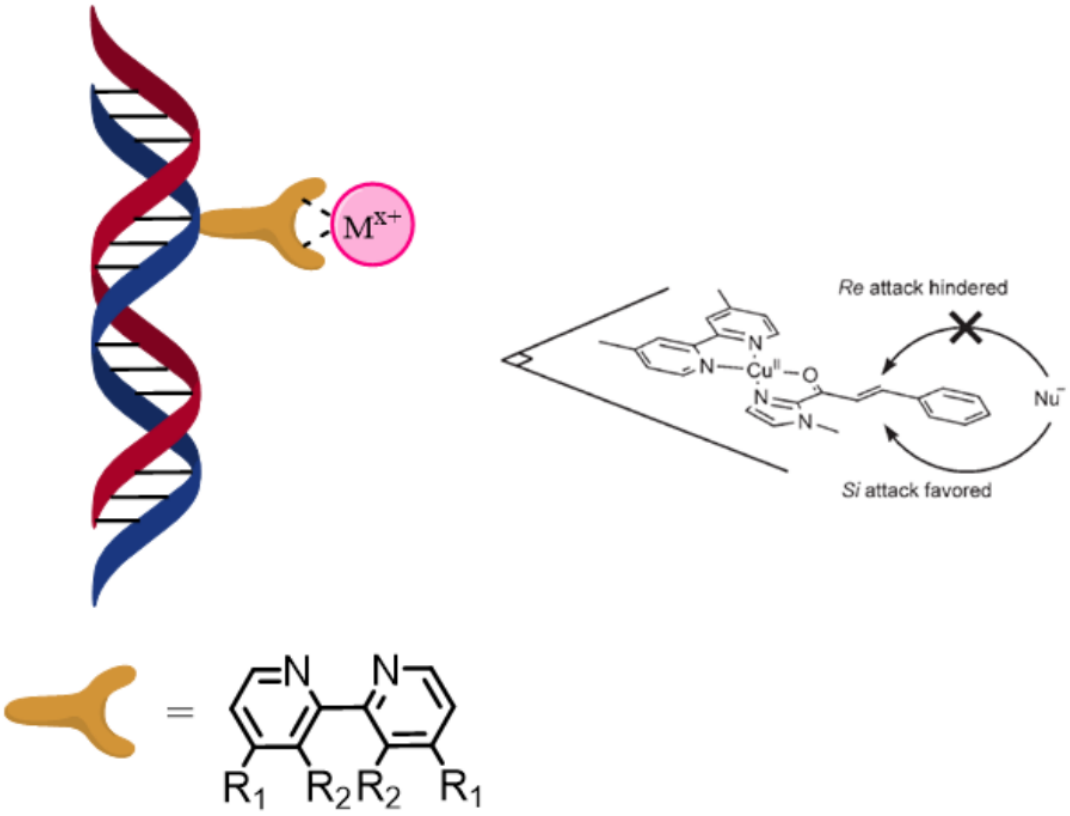


Figure 1: Structure of DNA-based catalyst introduced by Roelfes and Feringa, that catalyzed an asymmetric Friedel-Crafts alkylation of α-β-unsaturated 2-acylimidazoles in water.

1.3) Polysaccharides used in asymmetric catalysis.

Surely, biopolymers that can be attractive for asymmetric catalysis are the polysaccharides. Unfortunately, as far as it is concerned, only a few limited numbers of examples have been reported regarding this topic. Most of them are related exclusively to the use of chitosan **([11]),** a derivative of chitin, a polysaccharide of mainly marine origin and identified by the presence of an amide functionality. It is a D-*N*-acetyl glucosamine polymer. Despite their limited number, these examples still demonstrate how these polysaccharides can have good potential and exploitability, and that the presence of a specific functional group, amine in the case of chitosan, may bring more opportunities than other common polysaccharides including for example cellulose. It is also important to underline how the most common chiral stationary phases applied in enantioselective chromatography are based on polysaccharide materials, mainly amylose,

functionalized and supported on silica. However, this class of biopolymers has received little attention as regards its use for asymmetric induction in catalytic processes.

Polysaccharides obtained from marine sources have some interesting features that can be easily exploited successfully. They can be defined as functionalized molecules, which present in nature their own chirality and that not requiring specific treatments for their disposal. They are available in large quantities and do not compete with agricultural production.

An important class of these molecules is for sure the alginates. These biopolymers possess carboxylic acid groups, which create coordination structures with divalent metals. This peculiarity establishes the possibility of using them in the context of acid catalysis. Another fundamental aspect of these biopolymers is that they can also be used with alkaline earth metals. This is an important feature that should not be underestimated because currently most catalytic systems within organometallic catalysis operate by transition-metals, an active part of the center. The use of alkaline-earth metals, being generally abundant on the earth crust, is accompanied by fewer problems at industrial pollution level, so in line with the general rules of the green chemistry and it has also an advantage from an economic point of view since the price is significantly lower than that of the transition-metals.

Within these premises, the use of alginate-based catalysts will be the main topic of this study and therefore a more detailed description of these compounds is presented in the next paragraph.

1.4) Alginates

Alginates are a family of natural acid polysaccharides found mainly in the intercellular matrix of brown macro-algae as an insoluble mixture of sodium, calcium, magnesium, strontium, and barium salts. **([12])**

From a structural point of view, alginates are binary copolymers composed by residues of mannuronic (**M**) and guluronic (**G**) acid (Figure) linked by 1→4 glycosidic bonds (Figure 2).

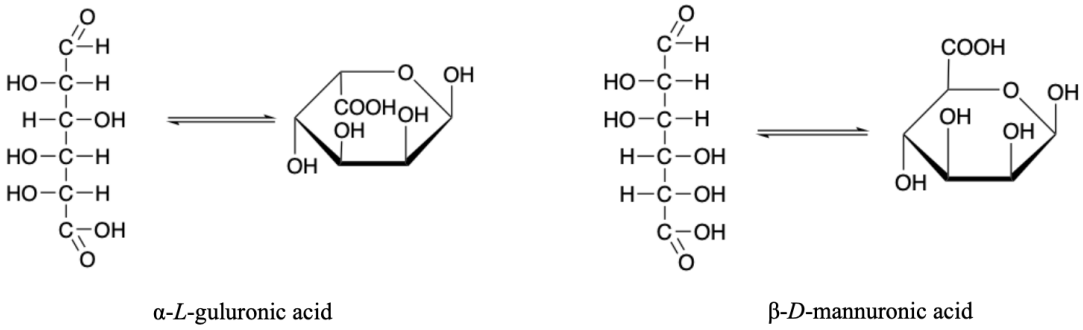


Figure 2 Linear and cyclic structures of α-L-guluronic acid (G) (left) and β-D-mannuronic acid (M) (right).

They are considered as the alter ego of cellulose in algae. However, considering the differences, alginates are formed by gulose and mannose derivatives, instead of glucose and moreover, instead of having only hydroxide groups, alginates are formed by uronic acids, which provides the carboxylic functional groups. The presence of this substituent endows these polymers with special properties regarding their interactions with metal cations: the ability to function as a ligand explains the use of alginates as a carrier for enzyme immobilization **([13])**. In addition to this, the alginates themselves can promote acid catalysis by coordinating with the Lewis acid metals. When metals or metal clusters are incorporated into polymers, specific metal/polymer interactions can lead to exceptional catalytic behavior **([14])**. They are widely used for the entrapment of biologically active materials. In the presence of divalent cations such as calcium, alginates form hydrogels by ionic cross-linking where the gel properties are influenced by the content and length of the G blocks. Alginates are now used in many applications such as biomedical, due to its mild gelling conditions, including immunoselection of cell transplants, slow-release systems, in vitro tissue engineering and 3D bioprinting. **([15]) ([16])**

The structure of the alginates and consequently their characteristics depend on the way in which the monomers are distributed and how they are connected. It comes from the fact that both monomers, guluronate and mannuronate, have two different chair conformations, based on how the carbon atoms are positioned in their respective conformation: the first has a structure named $^{1}C_{4}$, while the second presents a $^{4}C_{1}$ structure (Figure 3).

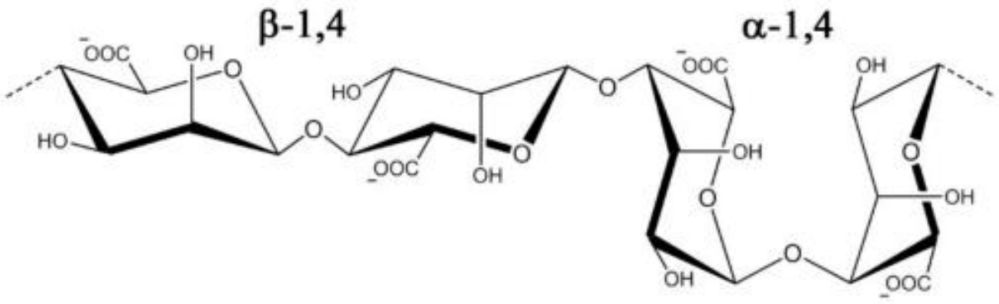


Figure 3 Structure of copolymers 1→4 β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues.

The structure of the alginate is therefore formed by different sequences, each with its own characteristic, depending if they are based on a single type of monomer or the alternation of both **([17])**.

There are three types of sequences: GG, MM and GM or MG (Figure 4).

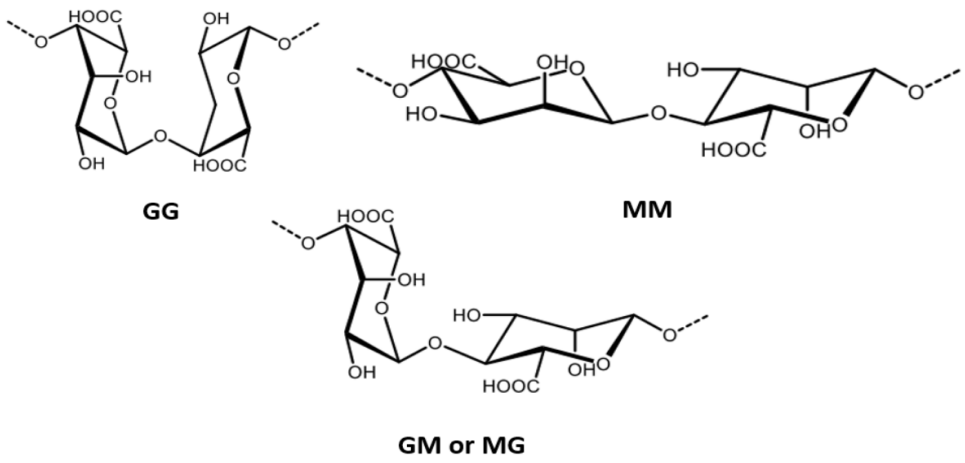


Figure 4: Structure of guluronate (GG), mannuronate (MM) and guluronate- mannuronate (GM or MG) sequences.

This project will use a specific type of alginate, commercialized as Protanal 200S, which is a polymer extracted from a particular alga, *Laminaria digitata*. It has a relatively high guluronate content (G/M approx. 70:30). It has been shown to be able to give better enantioselectivity than those containing a higher percentage of mannuronate. Indeed, having a higher content of guluronic units gives it greater structural rigidity, thus providing better face selectivity, and better management of the directionality of newly formed bonds.

1.5) Alginates gels

Once the corresponding alginate has been attained from the industrial process, one of its applications is in the preparation of *alginate gels*, that can be use in catalysis, as we will observe later.

Alginates tend to form spontaneously stable gels when they are exposed to a dilute aqueous solution of divalent cations or to acidic solution. This capability is due to the predisposition of the carboxyl groups of the guluronate monomer to coordinate with these metals, forming a stable structure, or by lowering the pH, favoring therefore the production of alginic acid gels.

Alginate gels are distinguished according to two categories:

1) the type of counterion of the carboxylic group (ionic alginate gel or alginic acid gel)
2) the type of formulation (hydrogel, solvogel, aerogel, xerogel).

1.5.2) IONIC GEL

One of the alginates' properties is their ability to bind to divalent ions with high selectivity. Indeed, affinity increases following this order: Mn < Zn, Ni, Co < Fe < Ca < Sr < Ba < Cd < Cu < Pb **([18])**. However, it is important to underline that even if the calcium is found down in the order, it is the most used and therefore most studied ion. When these cations establish interactions with guluronic units of the polymer by bonding to the oxygen atoms of the carboxyl groups, also because the

M-block units corresponds to an almost absence of selectivity, a dimerization of two G residues occurs. This promotes the binding of two G chains of opposite sides, producing a diamond-shaped cavity and generating a junction zone known as "egg box" **([19])** (Figure 5).

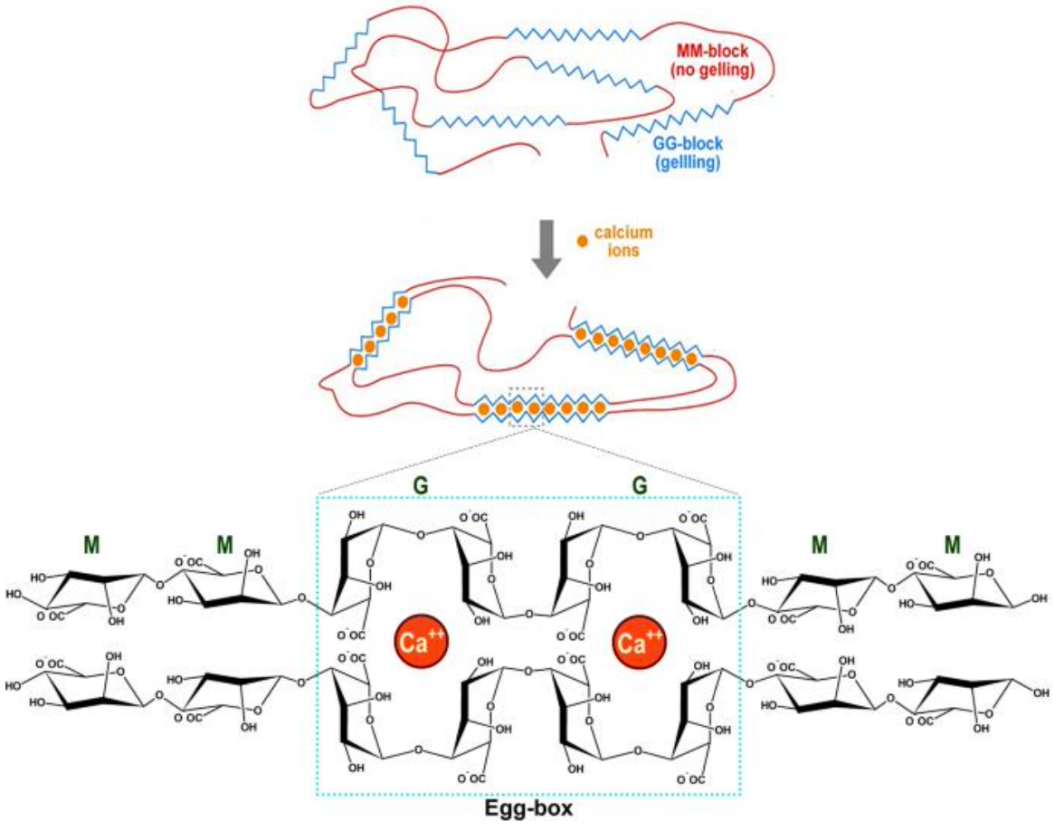


Figure 5: Schematic representation of the egg-box model for calcium alginate gelation.

This stable and three-dimensional structure results in the formation of the gel, in which each cation binds to four monomers of guluronate by multi coordination, exploiting the oxygen atoms of the carboxylic groups. As it was mentioned before, the stability ensured by this structure is another main reason why in this context of catalysis we prefer alginates with a higher percentage of this monomer. Moreover, the length of the **G-blocks** is also a key aspect: between 8-20 of **G** adjacent monomers are necessary to form a stable structure **([20])**

1.5.3) ALGINIC ACID GEL

This gel is formed when an alginate solution is added to another solution with lower pH than the value of acid dissociation constants of the two monomers: guluronate has a p*K*a of 3.65, while mannuronate has a p*K*a of 3.38. This is due to the fact that if the value is lower than the dissociation constants, the acid form of the two monomers will be favored and therefore a cationic exchange will take place between the metal coordinated with the carboxylic groups and the protons present in solution. The formation of the alginic acid gel, however, happens only if its solution is slowly added to the acid one, drop by drop, by formation of the gel stabilize by intermolecular hydrogen bonds, because otherwise a sudden change in pH will provoke the precipitation of insoluble alginic acid. However, the mechanism of formation of the alginic acid gel is not fully comprehend yet. The reason why relies on its relatively limited industrial applications.

Until this point, two types of alginate gels, according to their type of crosslink and medium, have been explained, but if the gels are classified by their formulation the following division must be considered.

1.5.3) HYDROGEL

Hydrogels, in general, are hydrophilic macromolecules capable of absorbing a large amount of water, thus increasing their volume **([21])**. These types of gels can also be obtained from alginates, as was mentioned before. They have a copolymeric network stabilized by hydrogen bond (alginic acid) or cross-linking ions (metal ion alginates). The formation of the gel particles can occur by external (diffusion method) or internal gelation, differing on how the cross-linking ions are introduced into the alginate polymer. However, the method on which we will focus our attention is the diffusion one, which has been used in this work and is detailed in the experimental part.

In short, a sodium alginate solution is added dropwise to the corresponding metal chloride solution or acidic solution. The formation of hydrogel spheres is immediately apparent, associated to the rapid jellification of the outer layer of the drop, allowing a rapid shaping and control size of the gel (Figure 6)

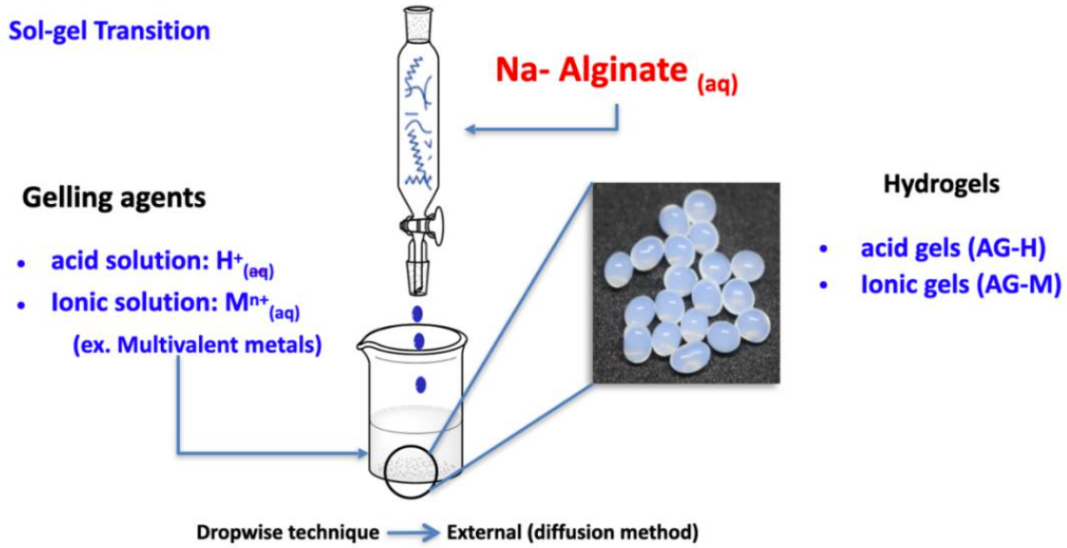


Figure 6: Schematic representation of the first step in the alginate-based hydrogels' synthesis

This diffusion method makes possible to obtain alginate gels thanks to the immediate migration of cations from thier aqueous solution into the alginate solution. The properties and morphology of the final spheres (shape and size) can be influenced by various parameters present during the formation process: for example, a high viscosity of the alginate solution can change the final shape of the sphere: the drop will take longer to descend from the funnel and therefore will have a more elongated than perfectly spherical shape; a high stirring rate may deform the beads, etc.

On the other hand, the internal method consists in mixing the alginate solution with an insoluble source of divalent cations, and a gelation inducer agent. The required low solubility of M^{2+} compound in pure water allows its uniform distribution in alginate solution before gelation occurs. Nevertheless, the simplicity and faster gelling kinetics of diffusion method makes it as one of the most widespread strategies to produce beads at lab scale.

1.5.4) SOLVOGEL

The synthesis of hydrogels is the intermediate step to obtain solvogels, which are the ones used in catalysis. These gels, instead of water, retain organic solvents in

their molecular structure. The alginate spheres used in the laboratory as catalysts were in fact stored as solvogel, specifically in ethanol (also called, in this case, alcogel since the role of the organic solvent is exerted by an alcohol). Ethanol is one of the most used solvents because of the ideal characteristics that the solvent which replaces the water molecules must have: it does not dissolve the structure of the gel, it is miscible with water, it is compatible with the final applications of the solvogel, and it does not promote a dramatic shrinkage of the gel. In fact, the latter is one of the phenomena that required much attention since the release of water involves a reduction in the surface tension of the gel pores, resulting in a reduction in capillary pressure with consequent unfavorable reduction in the volume. **([22])** That's why multistep (soaking with an increase of concentration of the solvent in each step using new- solvent/water mixtures) and low frequency solvent exchange decrease the diffusion rate of water out of the gel, mitigating the shrinkage. Consequently, this protocol is often used to obtain solvogels, instead of soaking the hydrogel directly in the new solvent.

1.5.5) AEROGEL

Aerogels are "dry gels" obtained from alcogels by replacing the organic solvent with a gas, thus generating a light porous structure. These types of gels are principally obtained by extracting alcohol from the spheres using supercritical carbon dioxide (scCO2): this is the reason why they are not obtained from hydrogels or solvogels, since, in general, alcohols are much more soluble in CO2 than water. The use of carbon dioxide allows to preserve the high porosity and the same properties that the starting gel possesses, because the reduced temperature used in this process minimizes the changes that could occur at the molecular level, thus maintaining the conformation and non-covalent interactions between the polymer chains **([23])**. The advantage of using a supercritical fluid is that there are no intermediate situations in which a liquid-vapor transition is present, thus avoiding the collapse of the gel structure **([24])**. A less efficient alternative is to use freeze-drying processes from hydrogels, which, however, generally lead to a slightly shrinkage of the structure.

1.5.6) XEROGEL

The last type are the xerogels. They are always obtained by eliminating the solvent from the alginate structure, but, in this case, by direct evaporation, which leads to a collapse of the porous architecture, thus obtaining a product with a lower volume due to the shrinkage of the material. They are obtained using traditional evaporating drying techniques, resulting in the formation of menisci inside the pores due to the simultaneous presence of a liquid and a gas phase. When the solvent is removed, the surface tension of the liquid contained in the pores creates an intense capillary pressure stress able to collapse them. The use in catalysis of this dried polysaccharide gels suffers from diffusion limitation, due to the low surface area.

1.6) Alginates gels in catalysis

The high surface area, the abundance of functional groups in the polymeric structure of the alginate (5.6 mmol/g of carboxylate groups), their stability in most organic solvents and the porous nature of the gels are some of the characteristics that make these polysaccharide gels as promising compound for catalysis **([25])**.

The three-dimensional structure of the gels allows accessibility of active functions, fundamental for the possible entrapment of catalytic species and interaction with the substrates involved in the reaction. In fact, several studies have been conducted on the use of alginates as supports for organic molecules used in organometallic catalysis (to broaden the field of application of these biopolymers and not limiting their use as supports for enzymes for bio catalysis), alginates as support for metal nanoparticles, heterogeneous Bronsted acid catalysis, also as catalysts creating interactions between the polymer and a metal ion: an example is the Cu^{2+} coordinated alginate, that is a regioselective catalyst in the 1,3-cycloadditions of azides and alkynes **([26])**.

Therefore, given the excellent properties of these eco-friendly polymers obtained from biomass, the use of alginates in catalysis is the subject of ongoing studies

2. Goals

This work is part of a project started some time ago by the research group. The project is based on studying the potential of a biopolymer such as alginates to act as an asymmetric catalyst, with the aim of attaining enantiomerically enriched molecules. Typically, this type of biopolymers is used as a support for catalysts, but their inherent chirality has never been exploited yet.

Indeed, the molecules generally adopted as chirality inducers, excluding enzymatic catalysis, are low molecular weight compounds, ranging from natural derivatives such as alkaloids and amino acids, to semi- and synthetic molecules. To induce enantioselection, these molecules can act as catalysts themselves, as occurring in organocatalysis, or bind to transition and non-transition metals in the field of organometallic catalysis. At this moment, biopolymers have been studied only occasionally as inductors of enantioselection.

The application of biopolymers as chirality inducers may represent not only a scientific challenge but it could also bring significant technical improvement to the syntheses. Thanks to the heterogeneous nature of the gel catalysts, their separation from the reaction environment, and thus their recovery and possible reuse is straightforward. Other interesting features such as complementary selectivity compared to homogeneous systems can be considered too.

Bearing in mind biopolymers coming from marine origin, only chitosan has received some attention as a chirality inducer, demonstrating anyway how these polysaccharides may have promising yet unstudied potential.

My internship work started from the outcomes gained by former colleagues in their projects. These projects have been focused on analyzing the capability of Barium alginate solvogels to operate as catalysts and give enantioselection to different reactions. It was found that barium alginates could indeed induce significant enantioselection in a benchmark reaction: the Friedel-Crafts addition of indoles to nitroalkenes.

Building on these preliminary results, the main goal of my thesis was to study the generality of this reaction, and to preliminarily assess the recyclability of the catalyst. More specifically, the following goals were identified for my internship:

1)Preparation of nitroalkenes

While most nitroalkene substrates were available, some were not. Thus, the preparation of these nitroalkenes was planned via an established approach (Figure 7).

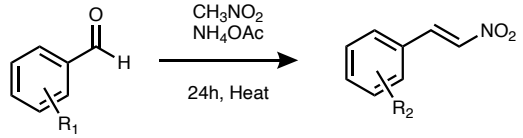


Figure 7: General scheme for nitroalkenes preparation

2)Study of the generality of the nitroalkene partner

Different substituted nitroalkene were tested with a standard nucleophile, the indole, to observe if the catalyst was able to promote the reaction, and to give enantioselection to the adducts (Figure 8).

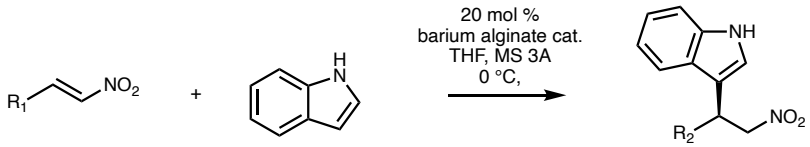


Figure 8: General Friedel-Crafts synthesis using different nitroalkene partner

3)Study of the generality of the indole partner

In the same way, some substituted indoles were tested with the standard Michael acceptor (Figure 9).

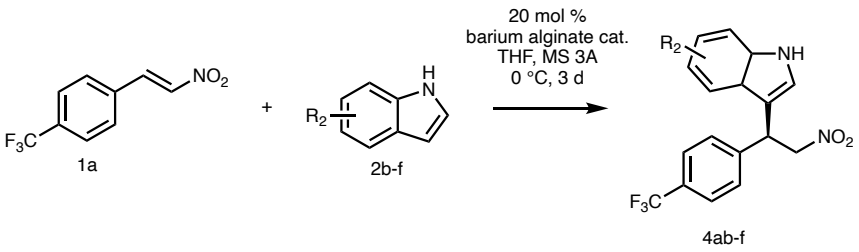


Figure 9: General Friedel-Crafts synthesis using different indole partner

4)Recyclability:

A basic understanding of the preliminary strategies that can be adopted to make efficient the recycling of the catalyst without losing catalytic activity and at the same time indicating which future work have to be pursue, was planned.

3. Results and Discussion

3.1) Preparation of Nitroalkenes

Before studying the main reaction, the substrates **1** were first synthesized through a Henry-type reaction, as shown in Figure 10 and detailed below for substrate **1a**.

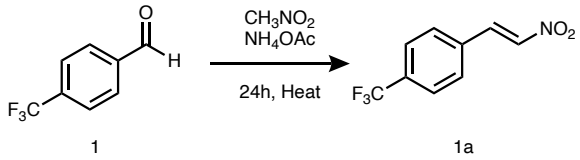


Figure 10: Henry reaction of substrate 1

According to the literature **([27])**, in a 100 ml flask equipped with a magnetic stir and a reflux condenser, nitromethane, 4-(trifluoromethyl) benzaldehyde and ammonium acetate are added. The reaction mixture is heated up to 100°C for at least 6.5 h using a hot plate and an oil bath. The solution at this point of the reaction course appears to be tending to orange-like color. The reaction's control is done with TLC plates using a hexane/ethyl acetate (9:1) mixture and potassium permanganate solution enlightening the products, especially when it is not so clear the dots on the TLC plates. Since we are working with an aldehyde as initial substrate, another stain can be used to visualize the reaction trend, based on 2,4-dinitrophenylhydrazine. The hydrazine is able to react with the aldehyde forming a hydrazone which can be easily visualized. In this way, the operator can easily estimate the course of the reaction and understand if it is gone to total conversion. The following step involves the removing of ammonium acetate, after careful evaporation of excess nitromethane, an explosive compound. A liquid-liquid extraction is performed with a proportion of 1 x 25 ml of water and 3 x 30 ml of dichloromethane (EtOAc would be better from a H&S point of view). The mixture is then cooled to room temperature. The combined organic phases are dried with magnesium sulphate and filtered through a funnel and a folded filter. The resulting

mixture is then carefully dried in a rotavapor to remove dichloromethane. The concentrated product is analyzed by ^{1}H NMR with $CDCl_3$.

The next step regards the purification of the product by column chromatography. The previous steps are not able to give a clean and pure product, so this procedure is mandatory. It is dissolved in toluene, so that the product does not flow with the mixture eluent down the column. It is a fundamental shrewdness to avoid the waste of the product and the repeat of the column's preparation. The mixture used as eluent is petroleum ether/ethyl ether (9:1). It is better to use PE instead of *n*-hexane for economic and safety reasons. The different fractions containing the product, selected by the aid of the TLC analysis, are combined together and the solvents is evaporated using again a rotavapor. The ^{1}H-NMR still shows some impurities in the spectra, therefore a further purification by crystallization from EtOH is performed. A final analysis by ^{1}H and ^{19}F NMR (only for the (*E*)-1-(2-nitrovinyl)-4-(trifluoromethyl) benzene) analysis confirms the purity of the product, which is obtained as yellow needles in 27% yield. The yield is lower than the one reported in the literature (43%). This can be explained either by the fact that there were necessary many steps in the product's purification, that is a chromatography column and additional crystallization, or by the possible low purity of the starting aldehyde used.

Substrate **1b** is prepared in an analogous way (Figure 11), with some modifications as outlined below.

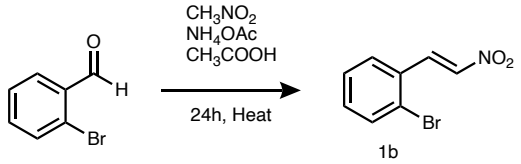


*Figure 11: Synthesis substrate **1b***

This reaction is carried out in the presence of ammonium acetate as catalyst, while reacting 2-bromobenzaldehyde, and nitromethane in excess, but in this case AcOH is also employed, as described in the literature for this specific nitroalkene. The main function of the acetic acid is to promote the dehydration step and lead to the double bond formation. In the procedure, the following step for providing the

product is a liquid-liquid extraction, which it aids to remove the ammonium acetate and so purify the product. In order to avoid the use of this technique and try a much more environmentally friendly method, a precipitation adding just water was proposed. Since no precipitation occurs, this purification procedure is abandoned, and the standard procedure reflecting the one used for **1a**, that is a chromatographic purification (Hex/Et_2O 9:1) followed by a crystallization from EtOH, is used. Such purification works successfully giving the nitroalkene **1b** as yellow needle in a satisfactory 78% yield.

Mechanism of the Henry reaction:

The Henry reaction (Figure 12) starts with the deprotonation of the nitroalkane on the α-carbon position forming an intermediate structure that takes the name of nitronate. Although this structure is nucleophilic both at the deprotonated carbon and at the oxyanions of the nitro group, it is only the carbon which attacks the carbonyl compound. The resulting β-nitro alkoxide is protonated by the conjugate acid of the base that originally deprotonated in the first step the nitroalkane, giving the respective β-nitro alcohol as product. The last step is the dehydration of the β-nitro alcohol in order to give the nitroalkene.

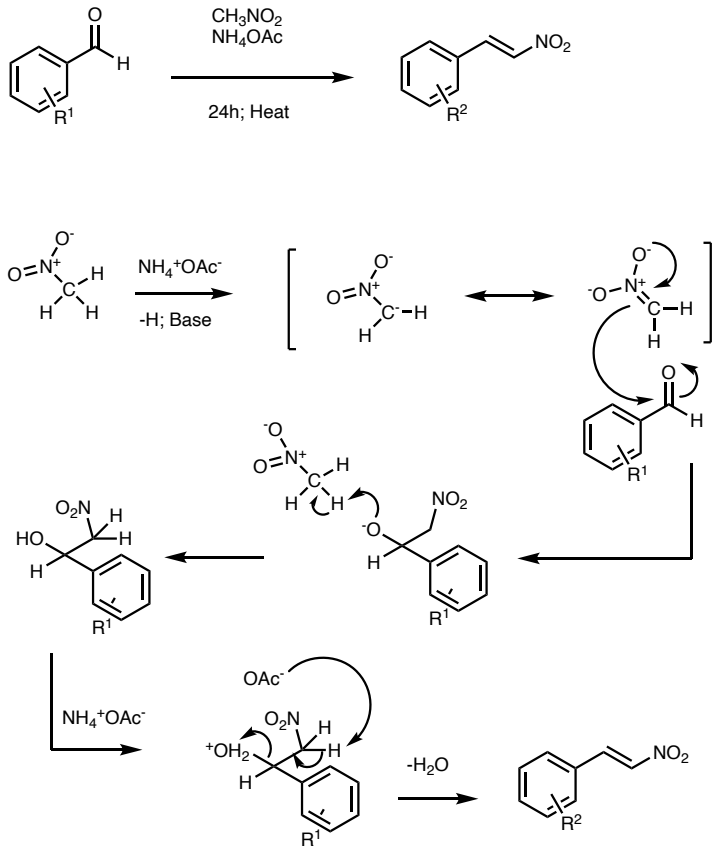


Figure 12: Mechanism of Henry Reaction.

3.2) Optimization of reaction conditions:

The reaction procedure with the standard substrate **1a** and indole **2a** has been already studied in detail and optimized by former colleagues during their work in the laboratory (Figure 13). Anyway, few new upgrades were made during my internship.

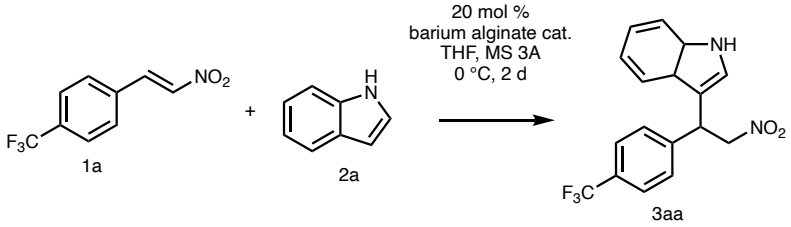


Figure 13: Benchmark Friedel-Crafts addition.

First of all, it worth to explain why substrate **1a** was used for the optimization of this reaction. Substrate **1a** is trifluoromethylated, and the ^{19}F-NMR analysis is a useful tool to follow the course of the reaction. First of all, the ^{19}F-NMR spectra of reaction samples are rather simpler compared to e.g., ^{1}H-NMR analyses, since

the formers contain only two singlets (the starting **1a** and the product **3aa**), making their interpretation easier. Moreover, and more importantly, using ^{19}F-NMR analyses it is possible to analyze reaction samples without removing the solvent, since the solvent is not fluorinated and is not visible by this analysis. This would not be possible using ^{1}H-NMR, wherein the large amount of solvent would saturate the spectrum. It is advisable to avoid solvent evaporation as this operation, besides being time consuming, can influence the result of the analysis, especially at low conversions.

My improvement to the optimized procedure can be summarized in the following paragraphs:

First of all, a new type of test tube was employed. We discovered that the reaction mixture was better accommodated in a tube with a large and deep bottom, wherein all beads can be soaked with the reaction solvent, thus leading to a better reproducibility.

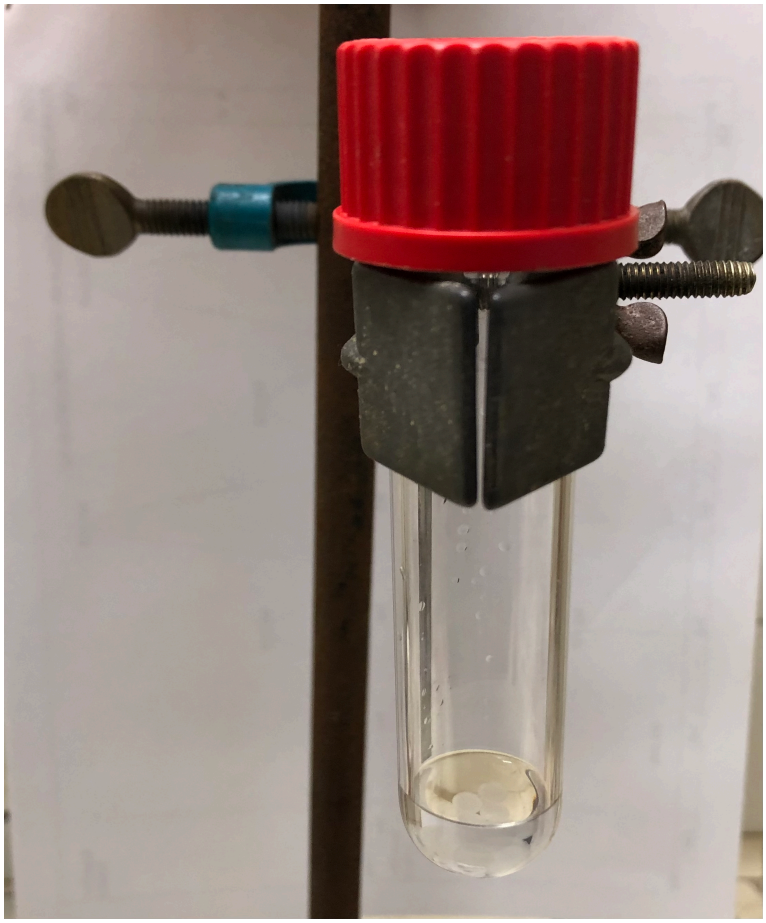


Figure 14: Representation of test tube.

Another upgrade was the optimization of the use of molecular sieves. We know that the presence of water in the reaction mixture can lead to changes in the structure of the alginate beads, or on the coordination spheres of Barium, and thus influence their catalytic activity. We decided therefore to activate new molecular sieves every time we perform a new reaction, instead of re-activating molecular sieves which had already been activated before. The activation process involves heating with a heat gun under vacuum for few minutes, followed by cooling under vacuum.

Besides, another practical improvement was related to the discovery that stirring is not required in these reactions. Therefore, it was possible to simply place the tubes in a Dewar containing ice in a fridge, without using magnetic stirrers or cooling machines. This apparently simple expedient has been decisive since it simplified considerably the procedure and allowed to run reactions over the weekend.

A last detail which was optimized is related to the dimensions of the catalyst beads. In the first stage of the work, the beads were prepared using the spout of a disposable syringe, which gives spheres weighing about 0.5 mg, with 3-4 mm of diameter. We found that using a dropping funnel in the jellification process, spheres weighing around 1 mg and with about 8-10 mm diameter were produced. These beads proved to be more robust, keeping their activity and selectivity for a longer time.

3.3) Study of the generality of the reaction

Before studying the catalytic asymmetric reactions, the racemic reference samples were prepared. The preparation of the racemic samples is necessary in order to devise suitable conditions for the separation of the two enantiomers by chiral stationary phase HPLC, and to know their retention times. As shown in Figure 15 for compound **4ae**, the racemic samples were prepared using an achiral Schreiner's thiourea as catalyst. After chromatographic purification, and a ^{1}H-NMR check of their purity, the samples were analyzed by chiral stationary phase HPLC, changing the stationary phase and the eluent until a satisfactory separation of the two enantiomers was found.

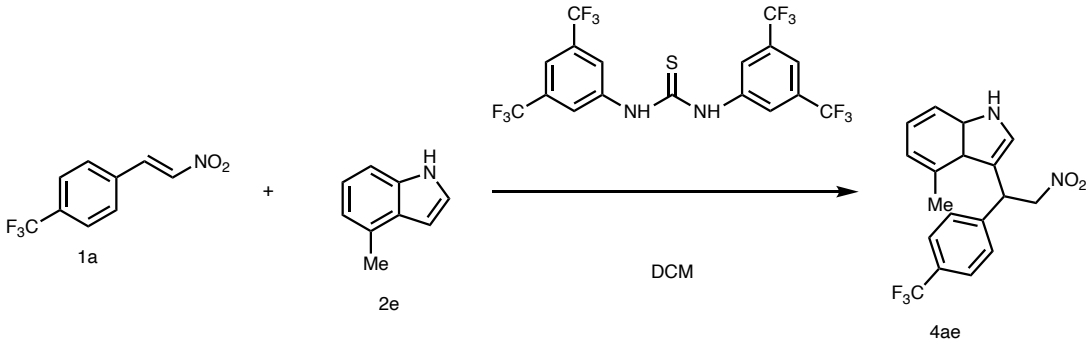


*Figure 15: Racemic reference 4ae → reaction condition: 0.05 mmol of **1a**; 0.075 mmol of **2e**; 100 µL of DCM and 20mol% of Schreiner's Thiourea catalyst.*

For example, compound **4ae** was separated using an analytical AD-H column, eluted with an n-hexane/i-PrOH 90:10 at 0.75 mL/min flux (Figure 16). The enantiomers were detected with a diode array UV detector. Since the two enantiomers present the same molar extinction coefficient, it is not necessary to build calibration curves for these analyses. Indeed, the two peaks integrate equal.

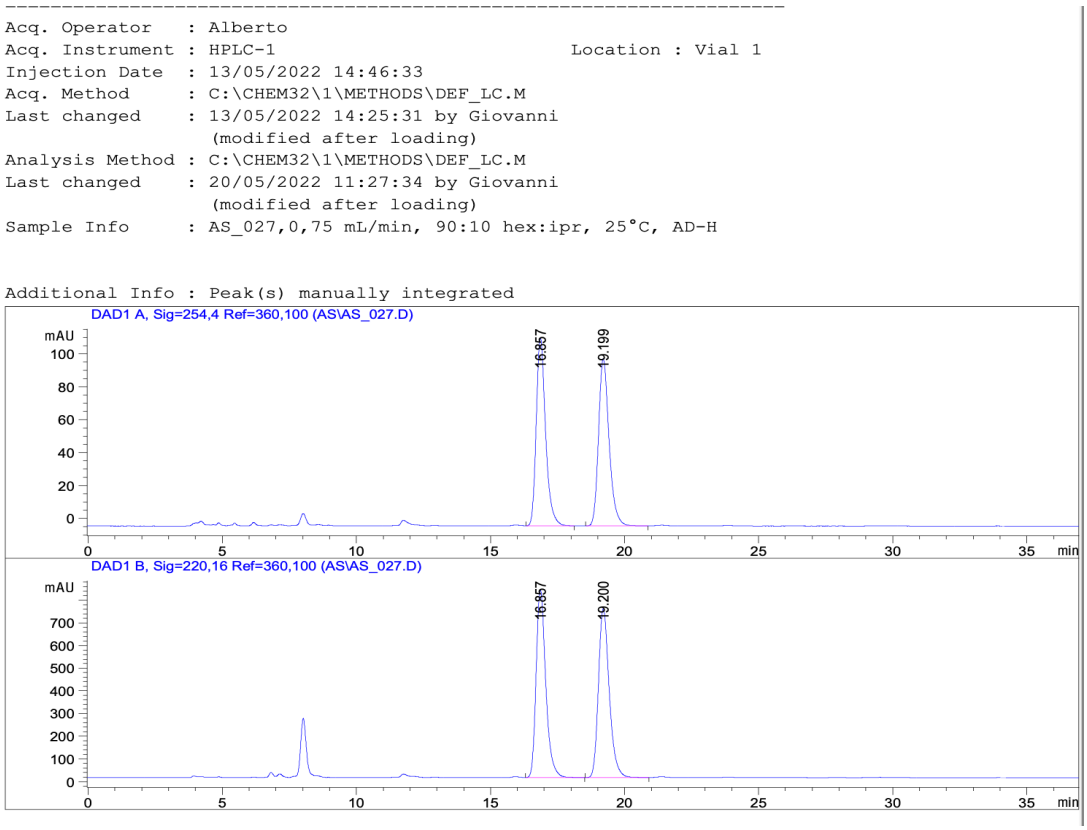


*Figure 16: Racemic sample **4ae** analysis using analytical AD-H column; eluent: n-hexane/i-PrOH 90:10 at 0.75 mL/min flux*

During my internship, after having identified the optimized methodology outlined in the previous subsection, I moved to verify the generality of this procedure by studying the reaction between different nitroalkenes and indoles.

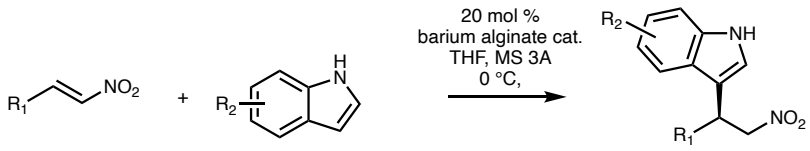


Figure 17: General Friedel-Crafts alkylation synthesis.

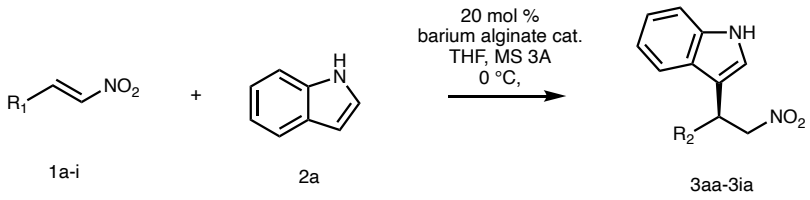
This study complements a previous work wherein the generality of the reaction was inspected with different nitrostyrenes. The data produced from the individual additions were grouped in the two different tables reported in the following sections: the data were obtained on the analysis of the adduct after chromatographic purification on silica gel. The reaction yield was determined gravimetrically, instead the enantioenrichement was assessed by chiral stationary phase HPLC. Racemic reference samples for the HPLC analysis were previously obtained using an achiral catalyst as outlined above.

The products of the syntheses have always been characterized by ^{1}H-NMR spectroscopy, and also by ^{19}F-NMR when fluorinated. Their experimental spectra were compared to the ones already present in literature, or previously recorded. The same was done with HPLC analyses.

A) Variation of the nitroalkene

The structure of the nitroalkene was varied first, by using different nitrostyrenes carrying various substituents at their phenyl ring, some heteroaromatic substrates, and even some aliphatic nitroalkenes. The results are collected in Table 1.

Table 1: Results of Friedel-Crafts additions using different nitroalkenes



Entry	Substrate 1 R1	Contact time	3 - Yield (%)	ee (%)
1	**1a**: *p*-CF_3-C_6H_4	2 days	**3aa**: 80%	85%
2	**1b:** *o-Br*-C_6H_4	2 days	**3ba:** 80%	92%
3	**1c:** o-*Me*-C_6H_4	3 days	**3ca:** 50%	82%
4	**1d:** 2-thiophenyl	3 days	**3da:** 48%	92%
5	**1e:** 2-furyl	3 days	**3ea:** 50%	86%
6	**1f:** 2-pyridyl	3 days	**3fa:** < 40%	31,5%
7	**1g:** *n*-pentyl	4 days	**3ga:** <20%	<10%
8	**1h:** *i*-butyl	4 days	**3ha:** <20%	<10%
9	**1i:** cyclohexyl	4 days	**3ia:** <20%	<10%

The results can be summarized according to the following points:

-First of all, the reaction with ***1a*** with the last adjustments was repeated and the product isolated by chromatography on silica gel. Product ***3aa*** was obtained with a fully satisfactory yield, and with a good 85% enantiomeric excess.

-Then, two substrates carrying an ortho-substituent were tested in the Friedel-Crafts addition: the reaction with the bromo substrate ***1b*** gives the best outcome in terms of both enantioselectivity and yield. Considering the position where the bromide is bound to the benzene in the nitrostirene, the first thought on this specific reaction was that it could have given some trouble, but surprisingly it did not. Possibly, the electron withdrawing properties of the bromide atom could assist the Friedel-Crafts addition, while its “small” dimension didn’t affect the reaction. The second substrate tested was the one labelled ***1c***: it provided a slightly lower result both in terms of yield and enantiomeric excess. The possible reason was attributable to the presence of the methyl functional group in the ortho position on the benzene. The methyl is an electron donor group and can hinder the

nucleophilic addition. In fact, it was necessary to let the reaction go 24h more than the other ones to reach an acceptable conversion. Anyway, both the enantiomeric excess and the yield can be considerable satisfactory and in line with the others.

-Three heteroaromatic substrates (***1d, 1e, 1f***) were later employed in the work. The enantiomeric excesses of both the furyl and thiophenyl derivatives ***3ea*** and ***3da*** were satisfactory but accompanied by moderate yields, whereas pyridine gave unsatisfactory results. A possible explanation for this outcome can be given by the coordination of the pyridine nitrogen to the catalyst, which can justify the low enantiomeric excess. On the other hand, pyridine substrates are known to be challenging, and no enantioselective addition of indole to this substrate has been so far reported.

-Then, a new class of substrate was investigated: aliphatic nitroalkenes (***1g, 1h,*** and ***1i)***. However, none of them led successfully to the production of the desired adducts (**3ga, 3ha, 3ai**). The racemic reference samples were prepared following the usual procedure, using Schreiner's thiourea catalyst. The outcomes were acceptable even if the reaction times were significantly longer. These experiments confirm the lower reactivity of these substrates compared to the nitrostyrene, due to the relatively more electron-rich substituent of the former. Few attempts were thus set in place by modifying the reaction temperature of the barium alginate catalyzed reactions. It is worth reminding that the usual reaction is performed at 0 °C, as it was demonstrated to be the optimal compromise between activity and enantioselectivity. However, a reaction with substrate ***1g*** performed at RT did not give any appreciable improvement, compared to the reaction performed at 0 °C. In fact, the crudes of these reactions presented a different pattern in their ^{1}H-NMR spectra, due to the presence of a side-product. That brings up a new hypothesis about this specific substrate and their difficulties to react and give the desire adduct. Probably substrate ***1g*** reacts with itself producing a new compound. How does it happen? Since the hydrogens in α position with respect to the double bond are acidic, in this specific environment, ***1g*** can be deprotonated, and then act as nucleophile towards another molecule of ***1g***, leading to the formation of another product (Figure 18) which compromise the production of the desired Friedel-Crafts adduct.

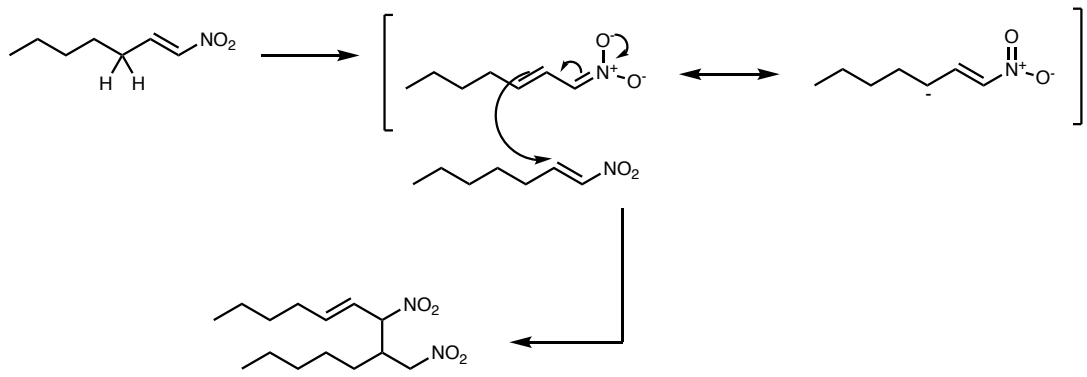


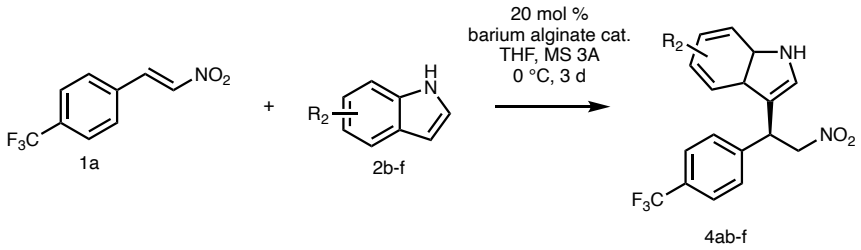
Figure 18: Possible route to the byproduct synthesis

Thus, hypothesizing that the problem was the selectivity of the reaction, rather than the reactivity of the nitroalkene, an experiment was set up at -20 °C (salt-ice bath). However, at this temperature no reactivity was either observed.

B) Variation of the indole

After having analyzed the generality of the reaction with different nitrostyrenes, the following study evaluates different indoles. The results are collected in Table 2.

Table 2: reaction with different indoles



Entry	Substrates 2 R2	Contact time	4-Yield (%)	ee (%)
1	**2b:** 5-MeO	2 days	**4ab:** 61,5%	83%
2	**2c:** 5-Cl	3 days	**4ac:** 45%	85%
3	**2d:** 5-Me	3 days	**4ad:** 50%	89%
4	**2e:** 4-Me	3 days	**4ae:** 55%	85%
5	**2f:** 6-Cl	3 days	/	/

- Firstly, it was investigated variations at a specific position in the indole, in particular position 5. Substrates ***2b, 2c*** and ***2d*** were thus assessed. 5-Methoxyindole gave a reaction with a good yield and a typical value of enantiomeric excess. The reaction promoting the adduct ***4ab*** was carried out following the common procedure even if a change in the indole equivalents was considered. Usually, in the reaction the proportion stands for 1 equivalent of nitrostirene and 2.5 equivalent of indole. In this specific reaction, however, the amount of 5-methoxyindole ***2b*** could be reduced to 1.5 equivalents since it is more reactive than the simple indole ***2a*** due to the activation of the ring by the methoxy group. Moreover, it is important to note that the mixture eluent used for the chromatographic purification is more polar than usual, due to the presence of this functional group in the structure. The substrate ***2c*** has provided instead the adduct ***4ac*** with a relatively good yield and an enantiomeric excess value in line with expectations. This substrate presents a chlorine atom, which acts as electron withdrawn group and therefore can influence the reaction course. For this reason, expecting a lower reactivity, the reaction was set up at RT, but it gave anyway an unsatisfactory enantiomeric excess (75%). Thus, reactions were performed at 0 °C, one using the typical conditions and the other one with a slightly higher catalyst loading (30 mol%). Both reactions gave improved selectivity (83 and 85% ee, respectively), and the experiment using higher loading gave slightly higher yield (45% vs 41%). Finally, the methyl substituted substrate ***2d*** reacted with substrate ***1a*** affording adduct ***4ad*** with values of enantiomeric excess and yield in line with the expectations. Even in this case, considering the presence of the electron donating methyl group in the structure, a variation was attempted, using a minor quantity of indole. However, this reaction was found to be less clean compared to the one performed under standard conditions.

-The substrate ***2e*** reacting with ***1a*** gave an adduct ***4ae*** with an acceptable enantiomeric excess and a moderate yield. Both the racemic reference and the catalytic adduct were produced following the usual procedure and without any relevant issue.

-The reaction involving the substrate ***2f*** did not give any product. Firstly, it was formulated the racemic reference sample using the Schreiner's thiourea catalyst, but even in this case, the product was not able to be formed. The catalytic reaction did follow the racemate outcome, indeed the TLC analysis as the ^{1}H-NMR and ^{19}F-NMR spectra, showed the presence of a significant amount of nitrostyrene even after few days. It has been thought that the electron withdrawing chlorine atom at the para position to the nucleophilic carbon impedes the nucleophilic addition.

Mechanism of the Friedel-Crafts reactions:

The reaction under study is a nucleophilic addition of indoles to nitroalkenes promoted by the Ba-alginate, which catalyzes the reaction by acting as a Lewis acid. The doublet on nitrogen is delocalized on the double bond, making it electron-rich and favoring the nucleophilic attack of C3 to the double bond of the nitrostyrene, which acts as electrophile, further activated by the Lewis acid. After the addition, the aromatic indole nucleus is restored thanks to a proton shift (Figure 19).

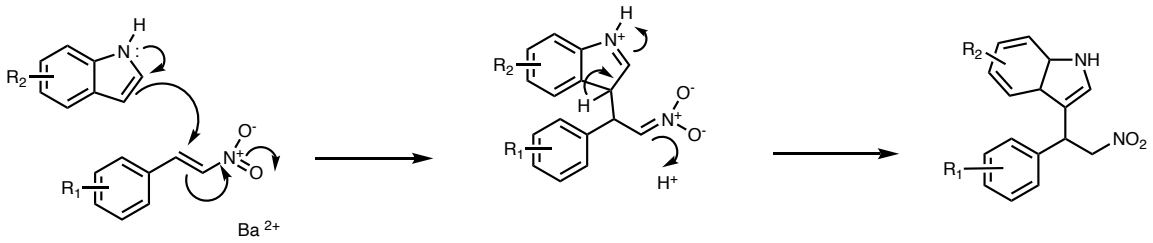


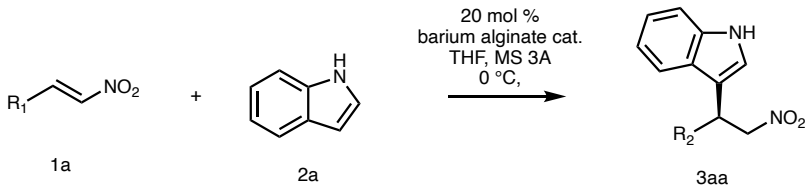
Figure 19: Mechanism of the Friedel-Crafts reaction

3.4) Recyclability:

In the last part of the project, the focus was directed toward a preliminary study of the recyclability of the catalyst. The main function of a catalyst is to lower the activation energy of the reaction and promote the formation of the desired product, but a further and fundamental feature is its ability to be recycled. One of the advantages of using heterogeneous catalysts is the easiness of their separation from the reaction mixtures, thus making their recovery straightforward. However, to be recycled, the catalyst must not degrade during the reaction. The study therefore was towards the analysis of the catalytic ability of the Ba-alginates based

catalyst to operate in few ensuing reactions and moreover to understand more about the factors affecting its loss of activity and selectivity.

Table 3: Recyclability study of Ba^{2+} alginate catalyst in Friedel-Crafts addition.



Cycle	Reaction time (h)	Storage beads solvent	Reaction Solvent	Conversion (%)	Enantiomeric excess
1st	48	EtOH	THF	100	89,5
2nd	~48	THF	THF	100	83,5
3rd	~60	THF	THF	100	80,1
4th	~65	THF	THF	100	64,5
5th	<48	EtOH	THF	100	75

In the table 3 are reported few interesting data from which is possible to make some considerations. Foremost, considering just the first three cycles, it is found that the reaction time increases after every single cycle, indicating probably a slow degradation of the catalytic function of the Ba^{2+} alginate solvogel, while the enantiomeric excess decreases, but still remains above 80%, which may indicate that the catalyst shows anyway an encouraging stability. However, the 4th cycle displays a completely change of value. The enantiomeric excess' value plummeted about 20 points to 65%. The explanation may be due to a modification in the catalyst structure during the recycling of the beads, confirming that the degradation of the structure can be a relevant issue. It was moreover noticed that

in order to achieve 100% conversion, the reaction time has constantly increased as the cycles progressed, indicating a less catalytic activity. This last cycle outcome made us reconsider few situations about the reaction condition, including the conservation of the catalyst after each cycle. From previous studies, it was demonstrated that the perfect solvent for the reaction was the anhydrous THF, even if it is a flammable solvent and not an environment-friendly, whereas the optimal solvent for storage the heterogenous catalyst was the EtOH. Taking in consideration this information, a new reaction cycle was proposed. The beads from the last cycle were recovered, washed five times with 2 ml EtOH, and finally stored overnight. The solvent was then swapped to anhydrous THF according to the general procedure, and the reaction carried out. The result after HPLC analysis was really promising and encouraging. It was indeed witnessed an improvement of 10% of the enantiomeric excess, comparing the 4th with 5th cycle in Table 3 and at the same time, an important decrease of the reaction time from 65 to less than 48 hours. This outcome is very promising for the future of the project. It is assumed that storing the spheres after the reaction in ethanol will first of all allow them to regain their initial shape and interactions and secondly provide a more efficient removal of organic/inorganic impurities which might poison the catalyst surface.

An interesting study has emerged from the analysis of the wash solvent residue which showed a yellowish in color. In fact, after cleaning the beads with ethanol, the wash solvent dyed. This might suggest that there was product residue in/on the beads that had dissolved in the ethanol during washing. ^{1}H and ^{19}F NMR analyses were therefore run to investigate the origin of the color of the solution. Unfortunately, although specific peaks were detected, these had a very low intensity, which did not allow a valid explanation for the color of the solution. Thus, the color could be due to an impurity or a by-product of the reaction. As has been observed, washing, and storing the spheres in ethanol improved the catalyst performances.

3.5) Sustainability aspects:

Considering the master's degree and all the connected applications in the sustainable and green chemistry, I decide to explore the feasibility and sustainability of the work I have being doing for the last few months. Although the catalyst is defined as "green", because of its origin, other forms of this work are inevitable less environmentally friendly.

In this paragraph the sustainability of this project will be deeply assessed under different points:

Firstly, the focus was set on the heterogenous catalyst itself. It is classified as a biopolymer since it is extracted from the family of brown algae, a third-generation biomass, which production does not compete with agriculture and therefore is considered non-food competitive. This characteristic puts it directly in the field of green chemistry. For what concern the metal, even if barium chloride is toxic by ingestion, it is an abundant alkaline earth metals on earth **([28])**, which guarantees its unlimited use for this purpose and not only. Moreover, it is much better than most transition metals, that instead, being overused, are on the verge of extinction **([29])**. Acknowledging the preliminary test on the recyclability, it can be stated that the heterogeneous catalyst is recyclable.

By analyzing the solvents adopted in the project, starting from the preparation of the catalyst to the reaction itself, one can attest to the almost total eco-friendliness of the step. The catalyst's development requires only water and ethanol as solvents. If the water can be easily labelled as the greenest possible solvents, the ethanol can be as well grouped in this list if it is produced from a fermentation process. Its impact on the environment is from low to neutral. If instead the contribute of anhydrous THF is examined, the process is less green. THF is a solvent that is not very good for the environment and is very flammable. However, some solutions can be made so that the process is more sustainable: it is possible indeed to recycle the THF on a larger scale by distilling it in order to eliminate impurities and reduce waste. Anyway, it must be considered the amount of energy spent during the distillation process.

The process of preparation and use of this catalyst can be generally classified as environmentally sustainable.

However, the weak point of this project, from a sustainable point of view, is the reaction itself. Although the catalyst is "green", the substances used in the reaction are not all so green. Making in fact some evaluation on the substrates employed in the reaction, the synthesis of the nitroalkene is not so environmentally friendly since it requires nitromethane, which is a toxic and dangerous compound. Moreover, in the liquid-liquid extraction step, the general procedure proposes the use of DCM, which is classified as “solvents to be limited” in the ICH solvent classes. For this reaction’s step, it can be swapped with EtOAc. Considering the reaction, the work up step uses again anhydrous THF. The purification of the product, which is achieved by chromatographic column, uses a lot of solvent, here petroleum ether and ethyl acetate. Anyway, considering we have operated at laboratory scale, it has been worked with small number of substrates. Petroleum ether is also a very flammable solvent and dangerous to health. Surely, it will be necessary studying the optimization of the purification method in order to improve both the safety and the sustainability of the process. It must be stressed that crystallization could not be used as a purification at this stage, since it would alter the enantiomeric excess of the product thus impeding the measurement of the enantio-inducing properties of the catalyst. In fact, the goal of this project is not to provide a sustainable approach to these compounds, but rather a conceptual advance in asymmetric synthesis, that is, that alginates can induce enantioselectivity and can be used as heterogeneous catalysts.

Finally, analyzing other important features, long process reaction times were highlighted as a non-negligible contributor to increased energy costs in the case of industrial scaling. Another related aspect that can lead to difficulties on a large scale is temperature control. Indeed, the main reaction of the compound studied is set up at 0°C. On a laboratory scale it is easy to control and cool down a mixture but as the scale increases, the energy required to reach the low temperatures rises as well, albeit 0°C can be considered as a mild reaction temperature.

4. Conclusions and Outlook

In conclusion, we can confirm that alginates can be used as heterogeneous chiral catalysts for a Friedel-Crafts addition. The protocol used for catalyst preparation is robust, and we were able to optimize further the protocol of the catalytic reaction to make it more effective, by resorting to new reaction set up. We were also able to gather more data on the proficiency of this catalyst on more substrates, such as substituted nitroalkenes and indoles. It has also been noticed that aliphatic nitroalkenes, and an electron poor indole, were unable to react. Anyway, as said, by following the methodology it is possible to promote the formation of different adducts with good yields and enantioselection.

Certainly, we can assert that the catalyst is recyclable after few cycles, even if it is necessary to store it, after every reaction, in ethanol, in order to preserve it better and avoid drastic drops in enantio-inducing power.

Keeping in consideration the recyclability and acknowledging that most of the substrates have been tested, for the continuation of the project it would be appropriate to focus both on the structure of the catalyst, with chemical and physical studies, and at the same time gathering more evidence on recyclability.

In this context, it could be interesting to work on the possibility to leverage an ultrasonic bath. A first attempt was carried out, giving a satisfactory enantiomeric excess value and moderate yield. The ultrasonic bath was tried out both in the solvent swap step and in the work up phase. In the first case, it was performed to understand if the waves produced by the equipment could disrupt the catalyst structure, whereas in the second case, it was accomplished to extract a major amount of product entrapped in the catalyst. Considering the promising outcomes, a few studies may be pursuit to improve the recyclability results.

5. Experimental Section

5.1 Materials and Methods

Reagents and solvents in commercial use or available in the laboratory were used. To verify the purity of the products obtained and the progress of the reactions, ^{1}H NMR and ^{19}F NMR spectra were performed using Varian Mercury 400 and 600 spectrometers. The enantiomeric excess of the products (*ee*), on the other hand, was determined by chiral stationary phase HPLC (Daicel Chiralpak AD-H or Chiralcel OD-H columns), using an UV detector operating at 254 nm. In order to obtain a reference for the two enantiomers produced by the catalytic reactions, the racemes of the two enantiomers were first obtained using the achiral N, N'-di[3,5-di(trifluoromethyl)phenyl]-thiourea catalyst. In this way, the relative retention times were discovered.

5.2 Synthesis of Nitroalkenes

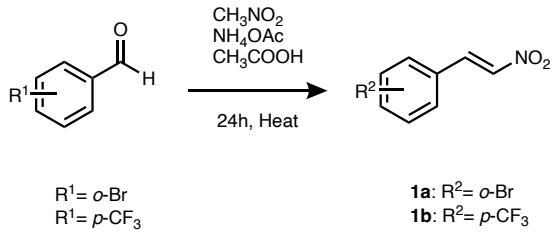


Figure 20: General Henry-reaction synthesis

The following procedure was adopted to synthesize substrates **1a** and **1b**. This procedure had also been used by my previous colleagues as a method to synthesize all the non-commercial substrates used in the studies.

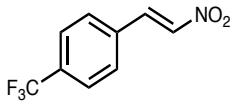
According to the procedure **([30])**, 0.3 mol of nitromethane, 10 mmol of functionalized aldehyde and 2.5 mmol of ammonium acetate (in order to generate a buffered environment) are placed in a 100-mL flask equipped with a magnetic stirring bar and a reflux condenser; the system is placed in an oil bath at 100°C for at least 6.5 h. The progress of the reaction is monitored by TLC analysis using hexane/ethyl ether in a 90:10 ratio as the eluent mixture and a potassium permanganate solution to visualize the formation of the product **1a** or **1b**.

After confirming the formation of nitroalkene **1a** or **1b**, the mixture is cooled to room temperature, after which the ammonium nitrate in the mixture is separated by liquid-liquid extraction with water and DCM. Alternatively, an eco-friendlier solvent such as EtOAc can be applied. The organic phase obtained from the extraction is anhydrified with anhydrous MgSO4 and subsequently filtered. The outcome of this separation is dried by rotavapor evaporation.

The product is then purified by chromatographic column (LC) using the same eluent mixture used in the TLC analysis (hexane/ethyl ether in a 90:10 ratio). The fractions containing the product are pooled in a flask and once again the solvent is evaporated using a rotavapor. In both cases, the acquired compound is further purified by crystallization with ethanol since the product obtained after column chromatography is not as pure as it should. Finally, the obtained product is analyzed by ^{1}H NMR to confirm the purity.

1a: (*E*)-1-(2-nitrovinyl) -4-(trifluoromethyl)benzene:

Following the general procedure, using 1.4 mL of 4-(trifluoromethyl) benzaldehyde, and reacting it with 16.20 mL of nitromethane and 193 mg of ammonium acetate, it yields product **1a** as a brown-ish-yellow-ish solution. The purified product appears as a solid in the form of very pale-yellow chips.

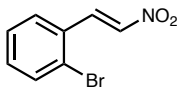


^{1}H-NMR ($CDCl_3$; 400MHz): δ = 8.02 (d, J = 13.8 Hz, 1H), 7.72 (d, J = 8.5 Hz, 2H), 7.67 (d, J = 8.8 Hz, 2H), 7.61 (d, J = 13.8 Hz, 1H).

^{19}F-NMR ($CDCl_3$; 400MHz): δ = -63.19 (s, 3F)

1b: (*E*)-1-bromo-2-(2-nitrovinyl) benzene:

Following the general procedure, but using 1,16ml of 2-Bromobenzaldehyde (10 mmol), and reacting it with 3,5 mL of nitromethane (65,5 mmol) and 77 mg of ammonium acetate (1,00 mmol), it yields product **1b** as a brown-ish solution. The purified product appears as a solid in the form of very yellow crystals.



^{1}H-NMR ($CDCl_3$; 400MHz): δ 8.41 (d, J = 13.7 Hz, 1H), 7.65 – 7.55 (m, 2H), 7.50 (d, J = 8.0 Hz, 1H), 7.43 (m, 1H), 7.34 (m, 1H).

5.3 Preparation of the alginate barium spheres

The procedure **([31]) ([32])** for producing the alginate hydrogel beads (AG-M), specifically of Ba^{2+} consist of preparing 2% w/V solution of sodium alginate, by adding 1 g of 200S Protonal alginate to 50 mL of deionized water in an Erlenmeyer flask and stirring it until a clear and viscous solution is obtained.

This mixture is then added dropwise (using a separating or dropping funnel) to a Beaker containing 100 mL of a 0,1 M solution of barium chloride, under magnetic stirring and at ambient temperature and pressure.

The formation of hydrogel beads is instantaneous and clearly visible. The resulting beads are stirred slowly overnight to allow their maturation. Then, they are filtered and washed carefully with water, 5 times for 10 minutes each.

These hydrogels are then transformed in their corresponding solvogel (SG). They are dehydrated by immersion in a series of Ethanol/H_2O baths, with increasing solvent content (10, 30, 50, 70, 90 and 100%) for 15 minutes each, and three last baths of pure ethanol. To ensure total conversion from hydrogel to solvogel, the beads in ethanol are placed in contact with activated 3Å molecular sieves for 3 days. Molecular sieves were previously activated by heating with a vacuum heat gun, then placed under an inert atmosphere of N2 and cooled to room temperature. After that, the sieves are removed, and the beads stored as solvogel in EtOH.

The last task of the procedure is to evaluate and determine the weight of the single beads, in order to calculate the amount of barium present in each bead, and therefore how many beads have to be used in the catalytic reactions to reach a certain loading. To do that, 10 solvogel beads are collected and dried to xerogel using the vacuum pump, until constant weight.

5.4 General procedure for catalytic reactions

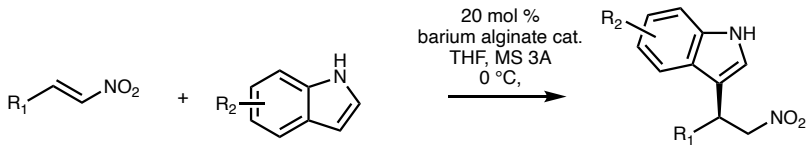


Figure 21: General Friedel-Crafts alkylation synthesis

In a specific test tube, 0.13 mmol of nitroalkene, 0.325 mmol of indole, 0.48 mL of anhydrous THF, previously distilled over sodium, as solvent and five activated molecular sieves of 3Å pore, are set up. After cooling the system to 0°C by means of an ice bath, 8 barium alginate spheres are added, corresponding to 20 mol% barium relative to the substrate nitroalkene. The alginate spheres, stored as ethanol alcogels, were previously conditioned before use by five washes with few mL of anhydrous THF, lasting at least ten minutes each. The reaction has to run at 0°C for 48h, trying to keep the temperature as constant as possible by the aid of transportable cryogenic tank, known as Dewar, thoroughly placed in the fridge, while the progress is checked by TLC analysis. After confirming the actual presence of the product and satisfactory conversion of the substrate, the solution is removed from the test tube and filtered through a silica plug. EtOAc is then added to the tube and left in contact with the beads for few minutes, in order to extract all product, and filtered through the same plug. This step is repeated several times. The solution obtained from filtration over the plug is subsequently evaporated and purified on a chromatographic column. TLC analysis is used to identify the product-containing fractions, while the same eluent mixture used in the column is applied to develop the plate. Usually, a potassium permangate solution is used to highlight the product. The product-containing fractions are pooled in a flask and then dried through rotavapor and subsequently characterized by NMR spectroscopy, using $CDCl_3$ as the solvent, and by chiral stationary phase HPLC to determine the enantiomeric excess. After all these steps, the product is stored, preferably in fridge, in a labelled vial.

5.4.1 3-(2-nitro-1-(4-(trifluoromethyl)phenyl)ethyl)-3a,7a-dihydro-1*H*-indole

Following the general procedure, using 28,20 mg substrate **1a** and reacting it with 38,1 mg indole (**2a**), product **3aa** is obtained with complete conversion after 48 h

of reaction. Following chromatography column purification, using as an eluent a mixture of hexane/ethyl acetate 75:25, product **3aa** is obtained as a solid with a yield of 80%.

The product was injected into HPLC (AD-H, 0.75 mL/min, 90:10=hexane:isopropanol, λ=254 nm, tmin=22.07 min and tmaj=27.39 min), so that the enantiomeric excess' value can be determined. (85%)

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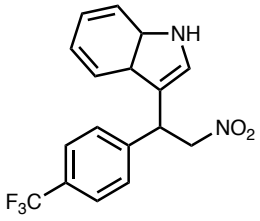


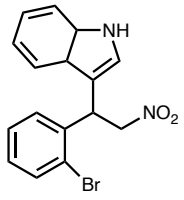
^{1}H-NMR ($CDCl_3$; 400MHz): δ = 8.16 (br s, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.1 Hz, 1H), 7.37 (d, J = 8.1 Hz, 1H), 7.22 (t, J = 7.9 Hz, 1H), 7.09 (t, J = 7.9 Hz, 1H), 7.03 (d, J = 2.7 Hz, 1H), 5.26 (t, J = 8.3 Hz, 1H), 5.08 (dd, J = 7.6 Hz, J = 12.9 Hz, 1H), 4.96 (dd, J = 8.7 Hz, J = 12.9 Hz, 1H).

^{19}F-NMR ($CDCl_3$; 400MHz): δ = -62.61.

5.4.2 3-(1-(2-bromophenyl)-2-nitroethyl)-3a,7a-dihydro-1*H*-indole

Following the general procedure, using 36,48 mg substrate **1b** and reacting it with 46,8 mg indole (**2a**), product **3ba** is obtained with complete conversion after 48 h of reaction. Following chromatography column purification, using as an eluent, a mixture of hexane/ethyl acetate 75:25, product **3ba** is obtained as a solid with a yield of 78%.

The product was injected into HPLC (OD-H, 0.75 mL/min, 70:30=hexane:isopropanol, λ=254 nm, tmin=21.72 min and tmaj=36.98 min), so that the enantiomeric excess' value can be determined. (85%).

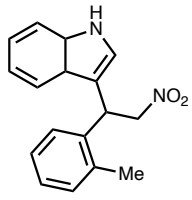


^{1}H-NMR ($CDCl_3$; 400MHz): δ = 8.20 (br s, 2H), 7.63 (dd, J = 7.9, 0.9 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 0.9 Hz, 1H), 7.26 (d, J = 0.3 Hz, 0H), 7.24 – 7.03 (m, 9H), 5.76 – 5.66 (m, 1H), 5.04 – 4.90 (m, 3H).

5.4.3 3-(2-nitro-1-(*o*-tolyl)ethyl)-3a,7a-dihydro-1*H*-indole

Following the general procedure, using 21,20 mg substrate **1c** and reacting it with 38,1 mg indole (**2a**), product **3ca** is obtained with complete conversion after 48 h of reaction. Following chromatography column purification, using as an eluent, a mixture of hexane/ethyl acetate 85:15, product **3ca** is obtained as a solid with a yield of 50%.

The product was injected into HPLC (OD-H, 0.75 mL/min, 70:30=hexane:isopropanol, λ=254 nm, tmin=25.08 min and tmaj=29.25 min), so that the enantiomeric excess' value can be determined. (82%).

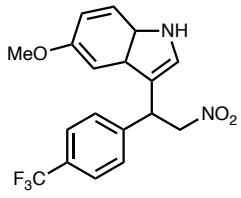


^{1}H-NMR ($CDCl_3$; 400MHz): δ = 8.26 (br s, 1H), 7.47 (dd, J = 8.0, 1.0 Hz, 1H), 7.35 (dt, J = 8.2, 0.9 Hz, 1H), 7.29 – 7.23 (m, 1H), 7.24 – 7.14 (m, 4H), 7.13 – 7.04 (m, 1H), 6.89 (dd, J = 2.5, 0.9 Hz, 1H), 5.50 – 5.36 (m, 1H), 5.03 (dd, J = 12.9, 7.6 Hz, 1H), 4.91 (dd, J = 12.8, 8.3 Hz, 1H), 2.17 (s, 3H).

5.4.4 5-methoxy-3-(2-nitro-1-(4-(trifluoromethyl)phenyl)ethyl)-3a,7a-dihydro-1*H*-indole

Following the general procedure, using 28,20 mg substrate **1a** and reacting it with 28,7 mg (0,195 mmol) indole (**2b**), product **4ab** is obtained with complete conversion after 48 h of reaction. Following chromatography column purification, using as an eluent, a mixture of hexane/ethyl acetate 70:30, product **4ab** is obtained as a solid with a yield of 61,5%.

The product was injected into HPLC (AD-H, 0.75 mL/min, 80:20=hexane:isopropanol, λ=254 nm, tmin=10.90 min and tmaj=14.45 min), so that the enantiomeric excess' value can be determined.(82,5%).



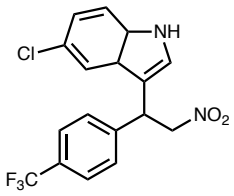
^{1}H-NMR ($CDCl_3$; 400MHz): δ = 8.07 (br s, 1H), 7.59 (d, J=8.51 Hz, 2H), 7.47 (d, J=8.41 Hz, 2H), 7.26 (d, J=8.82 Hz, 1H), 7.00 (d, J=2.81 Hz, 1H), 6.88 (dd, J=8.81, 2.42 Hz, 1H), 6.80 (d, J=2.42 Hz, 1H), 5.21 (t, J=7.83 Hz, 1H), 5.07 (dd, J=12.7,7.2 Hz, 1H), 4.95 (dd, J=12.7, 8.7 Hz, 1H), 3.78 (s, 3H).

^{19}F NMR **($CDCl_3$; 376MHz)** δ = -62.65.

5.4.5 5-chloro-3-(2-nitro-1-(4-(trifluoromethyl)phenyl)ethyl)-3a,7a-dihydro-1*H*-indole

Following the general procedure, using 28,20 mg substrate **1a** and reacting it with 49,3 mg indole (**2c**), product **4ac** is obtained with complete conversion after 72 h of reaction. Following chromatography column purification, using as an eluent, a mixture of hexane/ethyl acetate 75:25, product **4ac** is obtained as a solid with a yield of 45%.

The product was injected into HPLC (AD-H, 0.75 mL/min, 90:10=hexane:isopropanol, λ=254 nm, tmin=17.51 min and tmaj=22.17 min), so that the enantiomeric excess' value gets out (82,5%).



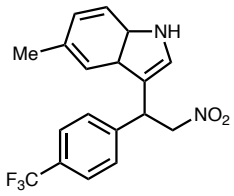
^{1}H-NMR ($CDCl_3$; 400MHz): δ = 8.20 (br s, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.49 – 7.41 (m, 1H), 7.36 (d, J = 2.0 Hz, 1H), 7.32 – 7.27 (m, 1H), 7.17 (dd, J = 8.7, 2.0 Hz, 1H), 7.09 (dd, J = 2.6, 0.9 Hz, 1H), 5.19 (t, J = 8.0 Hz, 1H), 5.04 (dd, J = 12.7, 7.7 Hz, 1H), 4.94 (dd, J = 12.7, 8.3 Hz, 1H).

^{19}F NMR**, ($CDCl_3$; 376MHz):** δ = -62.65.

5.4.6 5-methyl-3-(2-nitro-1-(4-(trifluoromethyl)phenyl)ethyl)-3a,7a-dihydro-1*H*-indole

Following the general procedure, using 28,20 mg substrate **1a** and reacting it with. 42,6 mg indole (**2d**), product **4ad** is obtained with complete conversion after 72 h of reaction. Following chromatography column purification, using as an eluent, a mixture of hexane/ethyl acetate 75:25, product **4ad** is obtained as a solid with a yield of 50%.

The product was injected into HPLC (AD-H, 0.75 mL/min, 90:10=hexane:isopropanol, λ=254 nm, tmin=15.67 min and tmaj=21.05 min), so that the enantiomeric excess' value gets out (89%).



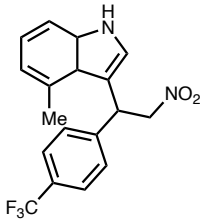
^{1}H-NMR ($CDCl_3$; 400MHz): δ = 8.35 (br s, 1H), 7.61 – 7.54 (m, 1H), 7.48 – 7.42 (m, 2H), 7.23 (d, J = 0.7 Hz, 1H), 7.20 – 7.18 (m, 1H), 7.02 (ddt, J = 8.3, 1.7, 0.5 Hz, 1H), 6.95 (dd, J = 2.5, 0.9 Hz, 1H), 5.21 (dd, J = 8.8, 7.2 Hz, 1H), 5.05 (dd, J = 12.7, 7.1 Hz, 1H), 4.94 (dd, J = 12.8, 8.9 Hz, 1H), 2.39 (s, 1H).

^{19}F NMR **($CDCl_3$; 376MHz):** δ = -62.62.

5.4.7 4-methyl-3-(2-nitro-1-(4-(trifluoromethyl)phenyl)ethyl)-3a,7a-dihydro-1*H*-indole

Following the general procedure, using 28,20 mg substrate **1a** and reacting it with 42,61 mg indole (**2e**), product **4ae** is obtained with complete conversion after 72 h of reaction. Following chromatography column purification, using as an eluent, a mixture of hexane/ethyl acetate 80:20, product **4ae** is obtained as a solid with a yield of 55%.

The product was injected into HPLC (AD-H, 0.75 mL/min, 90:10=hexane:isopropanol, λ=254 nm, tmin=17.03 min and tmaj=19.36 min), so that the enantiomeric excess' value gets out (85%).



^{1}H-NMR ($CDCl_3$; 400MHz): δ = 8.20 (s, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.45 – 7.35 (m, 2H), 7.25 – 7.19 (m, 1H), 7.15 – 7.04 (m, 2H), 6.86 – 6.79 (m, 1H), 5.62 (t, J = 8.0 Hz, 1H), 5.00 (dd, J = 12.8, 8.1 Hz, 1H), 4.87 (dd, J = 12.8, 8.0 Hz, 1H), 2.55 (s, 3H)

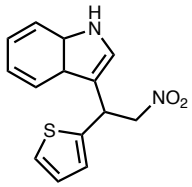
^{19}F NMR ($CDCl_3$; 376MHz) δ = -62.60.

5.4.8 3-(2-nitro-1-(thiophen-2-yl)ethyl)-3a,7a-dihydro-1*H*-indole

Following the general procedure, using 20,15 mg substrate **1d** and reacting it with 38,05 mg indole (**2a**), product **3da** is obtained with complete conversion after 72 h of reaction. Following chromatography column purification, using as an eluent, a

mixture of hexane/ethyl acetate 80:20, product **3da** is obtained as a solid with a yield of 48%.

The product was injected into HPLC (OD-H, 1,00 mL/min, 80:20=hexane:isopropanol, λ=254 nm, tmin=37.47 min and tmaj=41.33 min), so that the enantiomeric excess' value gets out (92%).

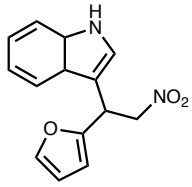


^{1}H-NMR ($CDCl_3$; 400MHz): *δ*=8.12 (br s, 1H), 7.52 (d, *J*=8.0 Hz, 1H), 7.37 (d, *J*=8.1 Hz, 1H), 7.25–7.19 (m, 2H), 7.11 (t, *J*=7.4 Hz, 2H), 6.99–6.93 (m, 2H), 5.45 (t, *J*=7.9 Hz, 1H), 5.06–4.95 (m, 2H)

5.4.9 3-(1-(furan-2-yl)-2-nitroethyl)-3a,7a-dihydro-1*H*-indole

Following the general procedure, using 22,40 mg substrate **1e** and reacting it with 38,05 mg indole (**2a**), product **3ea** is obtained with complete conversion after 72 h of reaction. Following chromatography column purification, using as an eluent, a mixture of hexane/ethyl acetate 80:20, product **3ea** is obtained as a solid with a yield of 50%.

The product was injected into HPLC (OD-H, 0,75 mL/min, 80:20=hexane:isopropanol, λ=254 nm, tmin=32.31 min and tmaj=47.52 min), so that the enantiomeric excess' value gets out (83,5%).

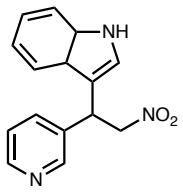


^{1}H-NMR ($CDCl_3$; 400MHz): δ = 8.13 (s, 1H), 7.66 – 7.51 (m, 1H), 7.41 – 7.31 (m, 2H), 7.24 (dd, J = 7.1, 1.2 Hz, 1H), 7.17 – 7.09 (m, 2H), 6.32 (dd, J = 3.2, 1.9 Hz, 1H), 6.17 (d, J = 3.3 Hz, 1H), 5.26 (dd, J = 8.2, 7.3 Hz, 1H), 5.06 (dd, J = 12.5, 8.1 Hz, 1H), 4.93 (dd, J = 12.5, 7.4 Hz, 1H).

5.4.10 3-(2-nitro-1-(pyridin-3-yl)ethyl)-3a,7a-dihydro-1*H*-indole

Following the general procedure, using 21,00 mg substrate **1f** and reacting it with 24,6 mg (1,5 eq) indole (**2a**), product **3fa** is obtained with complete conversion after 72 h of reaction. Following chromatography column purification, using as an eluent, a mixture of hexane/ethyl acetate 80:20, product **3fa** is obtained as a solid with a yield of 40%.

The product was injected into HPLC (AD-H, 0,75 mL/min, 80:20=hexane:isopropanol, λ=254 nm, tmin=18.48 min and tmaj=19,79 min), so that the enantiomeric excess' value gets out (31,4%).



^{1}H-NMR ($CDCl_3$; 400MHz): δ = 8.67 (dt, J = 2.4, 0.7 Hz, 1H), 8.53 (dd, J = 4.8, 1.6 Hz, 1H), 8.40 (s, 1H), 7.78 – 7.55 (m, 1H), 7.50 – 7.33 (m, 2H), 7.31 – 7.17 (m, 2H), 7.10 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 7.04 (dd, J = 2.5, 0.9 Hz, 1H), 5.22 (dd, J = 8.7, 7.2 Hz, 1H), 5.10 (dd, J = 12.7, 7.2 Hz, 1H), 4.97 (dd, J = 12.7, 8.7 Hz, 1H).

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