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Enantioselective synthesis of Equol with Ir-BARF catalyst and labelling with deuterium

Tesi di laurea sperimentale

CANDIDATO

David Sebastian Casadio

RELATORE

Chiar.mo Prof. Paolo Righi

CORRELATORE

Chiar.ma Prof.ssa Kristiina Wähälä

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Summary

Abstract

In this project we researched and optimized an new synthetic route for R-Equol, a molecule that is attracting increasing interest for the medicine because of its phytoestrogenic properties and the chemoprevention of breast cancer.
To reach this objective we start, from smaller building blocks, with the synthesis of Daidzein followed by a chemoselective borane reduction to obtain an olefin that will be hydrogenated enantioselectively with a commercial Ir-BARF catalyst. The increasing success of these catalysts even with this genre of substrates has already given good results with different catalysts in both e.e. and yield.
For further researches we deuterate the Equol in the aliphatic O-ring and attempt a secondary synthetic route with an hydrogenation using QN-modified Pd.

In questo progetto abbiamo studiato e ottimizzato una nuova via di sintesi per l'R-Equol, una molecola che sta suscitando sempre più interesse nella medicina per le sue proprietà ormonali e per la chemoprevenzione del cancro al seno.
Per raggiungere il nostro obbiettivo siamo partiti da piccoli building blocks per sintetizzare la Daidzeina, processo seguito da una riduzione chemoselettiva con borano per ottenere una olefina che sarà idrogenata enantioselettivamente con Ir-BARF commerciale.
Il crescente successo di questi catalizzatori anche con il nostro tipo di substrati ha già dato buoni risultati in resa ed e.e.
Per ulteriori ricerche abbiamo deuterato l'Equol nell'O-ring alifatico, inoltre abbiamo tentato una seconda via di sintesi con una idrogenazione catalizzata da Pd modificato con chinina.

Abbreviations

Bn	Benzyl
CBS	Corey-Bakshi-Shibata
DCM	Dichloromethane
D_x	Number of deuteriums in the molecule¨
e.e.	Enantiomeric excess
ER	Estrogen receptor
EtOD	D_1-ethanol
ip	Isotopic purity
IR	Infrared
Me	Methyl
MeOD	D_1-methanol
MOM	Methoxymethyl
MS	Mass Spectra
NaHMDS	Sodium bis(trimethylsilyl)amide
NMR	Nuclear Magnetic Resonance
OY	Overall yield
Ph	Phenyl
PTC	Phase transfer catalysis
r.t.	Room temperature
THF	Tetrahydrofuran
TMS	Tetramethyl silane
TON	Turnover number (unit of substrate converted per time)

INTRODUCTION

Chirality

Chirality, a term coined by Lord Kelvin in the end of the 19th century, is a property held by the geometrical figures that are not symmetric so not superimposable in their mirror image.

In everyday life we can find many objects that have this property, both natural and artificial. Our hands are the most immediate example we can see, but even the whole human beings are considered chiral.

Geometrically this feature has no or little consequences, indeed a dice is chiral but this has no consequence in the probabilities, even the simple knot is chiral but not for this reason one will be easier to untie.

In many sciences instead this property is more interesting because it is the source of many other features. In medicine we have dextrocardia that is the congenital defect that goes from having the heart situated in the right side of the body to having a completely mirrored body. In neuropsychology this phenomenon is visible in the control of language functions, which are related almost always only to the left hemisphere. However, the dominance of one hemisphere (which affects handiness) can affect the location of language specific brain areas. For this reason, left-handed people are generally excluded from language related brain studies.

In physics we can see that just the chirality in both massive and massless particles: spins of electrons are the base of VSEPR theory, where the electrons occupy orbitals in pair. This happens because the electron can have two spins: ½ and -½ that generate opposed magnetic moments so 2 electrons can stay in the same orbital because the magnetic moments balance the charge repulsion eliminating the total magnetic moment, a similar phenomenon is present even in light and can be called helicity.

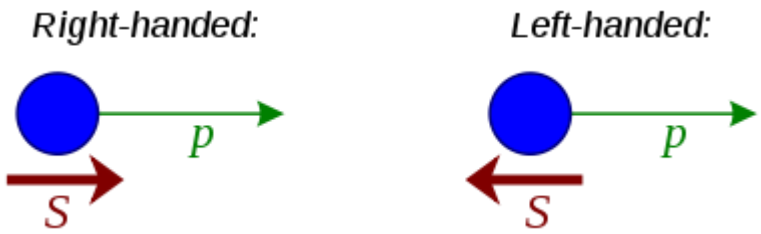


Fig. 1: Helicity in particles spin

In biology we see that evolution has developed a lot of animals and plants that are chiral for example the flatfish that has evolved to swim on one side of the body, the male narwhal has developed the tusk, a protruding tooth on the left side of the upper jaw that looks like a horn and the fiddler crab had developed his claws differently, one for female courtship and one for eating.

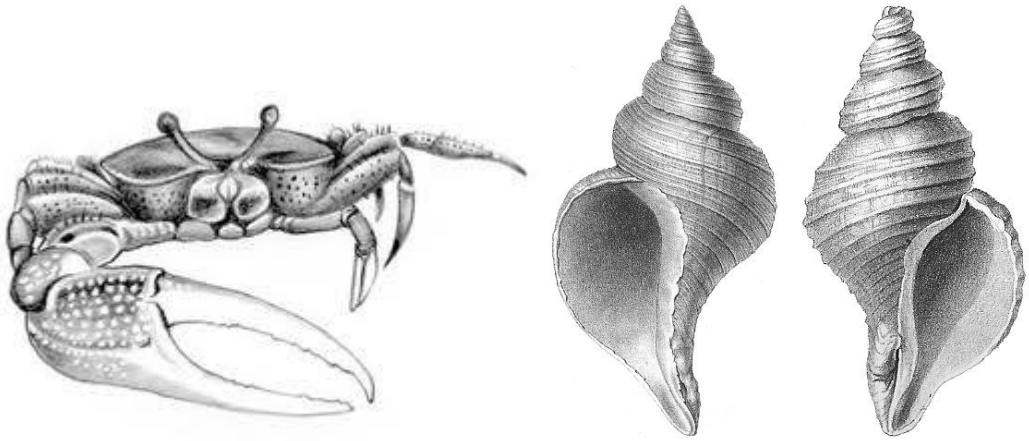


Fig. 2: Chirality in nature

In chemistry the chirality has a fundamental role in the properties of molecules, while two enantiomers have exactly the same group and chemical-physical properties the structures are different and when they interact with other chiral molecules they show different behaviors.
Most of the natural organic molecules present in nature and so even in our body are chiral, amino acids except glycine, for example, are chiral molecules and in nature we can find only one of the possible enantiomers.

For this reason, every time our body interacts with a chiral molecule, the different enantiomers may have different effects in our body. It can be a different taste as R-Asparagine that is sweet while S-Asparagine is bitter, or different smell like R-

Limonene that smells like lemon and S-Limonene that smells like orange. Many times different enantiomers have the same effects just with a different efficiency. Adrenaline, also called R-Epinephrine, is 10 times more efficient than its enantiomer and for this reason is used enantiopure. Sometimes is not needed to have a pure enantiopure molecule, like with Ibuprofen that is administered as a racemic mixture because the body automatically transforms the (R) enantiomer in the most active (S) enantiomer[1].

In other cases, enantiopurity is essential, this is the case of Penicillamine an antirheumatic in the (S)[2] form that is toxic in the (R) form because it blocks vitamin B_6.

Asymmetric Synthesis

So in most cases the purity of an enantiomer is a very important quality for the fine chemistry, for this reason the researchers in the universities and in the big pharmaceutical companies are constantly trying to find ways to obtain their product of interest as enantiopure as possible. There are three main approaches to obtain a single enantiomer: The resolution of a racemic mixture, the use of chiral reagents from the nature and the asymmetric induction.

Racemic Resolution

The chirality has been discovered by Pasteur in 1848 with the crystallization of ammonium tartrate he discovered that the crystals had a particular shape that was not superimposable on its mirror image and he found the presence of both the configuration of these crystals. This was the first resolution of a chiral compound, specifically it was a direct resolution because it happened through a simple crystallization.

The common resolution used nowadays is different from the method utilized by Pasteur, it consist in the simple separation of the two enantiomers induced through the addition of an enantiopure resolving agent, this will produce two diastereoisomers that will behave as different substances and would therefore be separable. It is also possible to separate enantiomer by the use of chiral HPLC or by a reaction (kinetic resolution). With the kinetic resolution we exploit the

different kinetic constant of the same reaction for the two enantiomers, if at the same time we have even a racemization reaction that re-establish the equilibrium of the unreacted reagent we can have a theoretical yield of 100%. Except for this dynamic kinetic resolution method the HPLC and the classical resolution never go beyond 50% of yield, moreover is an expensive method that oblige us to do additional and repetitive steps to add and remove the resolving agent that at least can be recycled. However is the only way we know to obtain compounds whose synthetic route has not been developed yet.

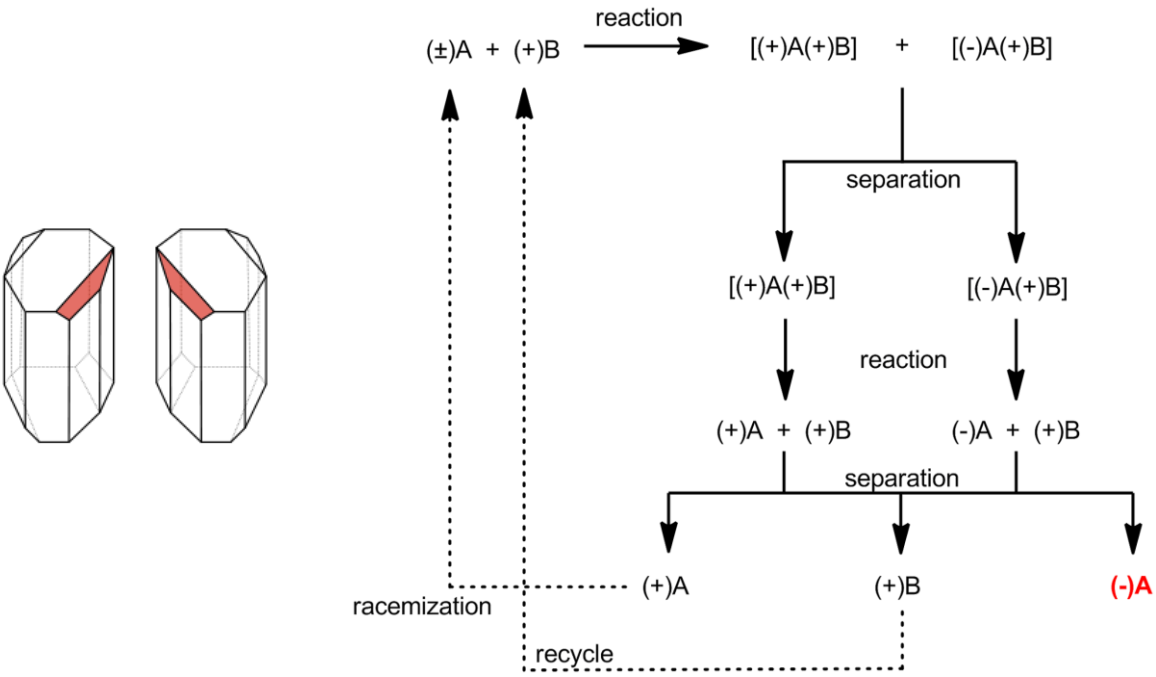


Fig. 3: Enantiomorphic sodium ammonium tartrate crystals observed by Pasteur (left), General resolution procedure with resolving agent (right).

Chiral Pool

The second strategy is the use of natural enantiopure molecules as building blocks for our final product, most of the times these molecules are sugars or amino-acids. This may be an advantage if our final product is similar to the initial substrate but may have big drawbacks if we want to obtain a more complex product. Not always we have chiral molecules close to the product and even if we have them we need molecules with the right chirality, in nature most of the times is present just one of the enantiomers for each optically active molecule. The lack of one of these two needs not always makes the synthesis impossible because we may invert the chirality at a certain point of the synthesis if we need

or we can add different building blocks to the initial substrate to obtain our product, but all these procedures add more unwanted steps in our synthesis that may make our route inconvenient and not efficient.

Enantioselective Synthesis

The enantioselective synthesis is the attempt to copy what before was nature's monopoly of enantiopure molecules' synthesis. Being the most recently discovered strategy and even the most convenient, this method induces the chirality through another chiral substrate, generally we divide further this field in chiral auxiliaries and chiral catalysts.

A chiral auxiliary is an enantiopure molecule that, by being attached near to the prochiral center, will induce the formation of an enantiomer over the other. Introduced by Corey[3] in 1978 this method became famous when Evans[4] started to use oxazolidinones auxiliaries developing a library of available auxiliaries applicable in aldol reactions, alkylations, and Diels-Alder reactions. Although this technique is better than the resolution and most of the times better than using a chiral pool, we still add to our synthesis three additional steps: the covalent attachment of the chiral auxiliary to the substrate, one or more diastereoselective transformations and the detachment of the auxiliary from the compound obtained.

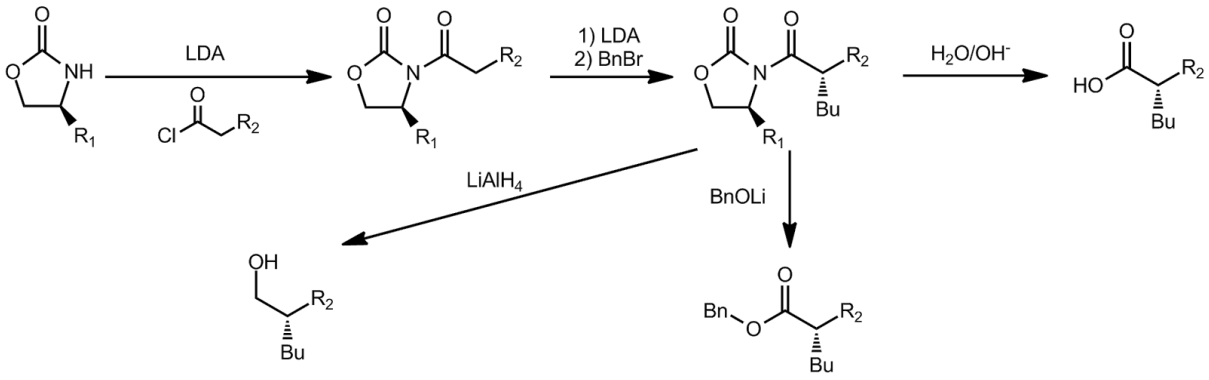


Fig. 4: Example of an enantioselective alkylation with Evans' auxiliary with different detachments[5].

In the asymmetric catalysis instead the chiral source is the enantiopure catalyst, when the substrate interacts with the catalyst the attack from the other reagent will be favored by one side over the other, creating a diastereoselectivity between the two forms of activated substrate. With this method is possible to

give the substrate optical purity without losing time and yield in additional steps by implementing the only chiral inductive step in the normal steps that are needed to obtain the product.

William S. Knowles, Ryōji Noyori and Barry Sharpless where the first pioneer that introduced the asymmetric catalysis. Sharpless developed a set of enantioselective catalyzed oxidations[6] while Knowles and Noyori[7] developed enantioselective catalyzed hydrogenations with chiral catalysts.

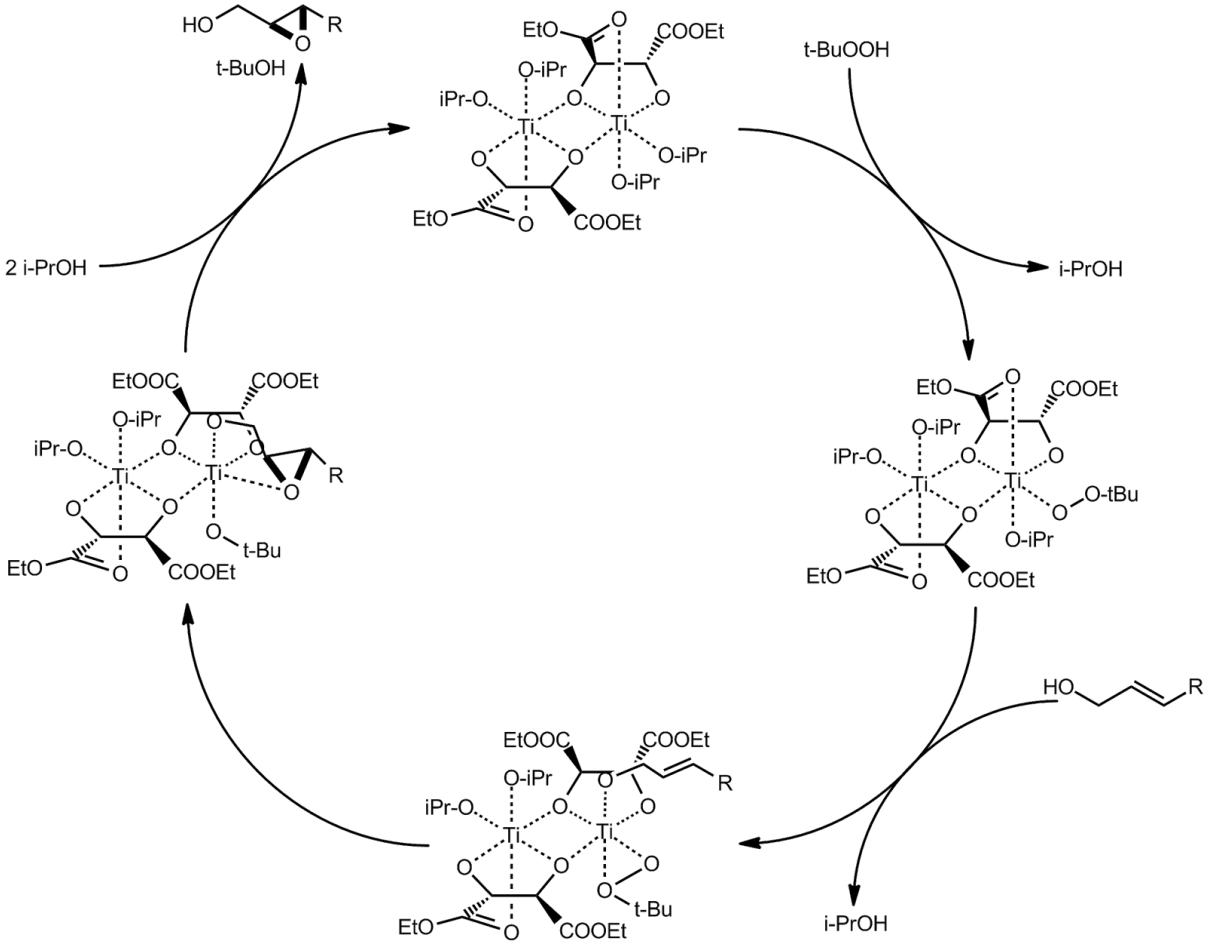


Fig. 5: Sharpless asymmetric epoxidation of allylic alcohols.

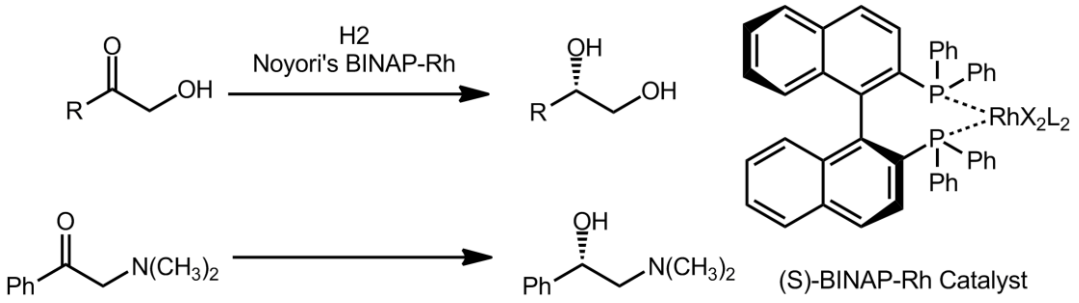


Fig. 6: Noyori's asymmetric hydrogenations (left), Noyori's catalyst (right).

Among the asymmetric catalysts used there are even enzymes, these may be seen as the most specific catalyst found in chemistry but this may be either an advantage or a disadvantage. Because of this specificity due to the shape of the active site we can work only with particular substrates, moreover we have many limits in the reaction conditions, like pH, temperature, solvent and many other parameters that may cause the denaturation of the enzyme, however when we'll have a working reaction we will generally have an excellent e.e.[8]

The research is trying to expand the possible conditions of enzyme catalysts trying to make them more heat resistant and to explore the possibility to use solvents different from water[9].

Even if the research is making giant steps this asymmetric synthesis is still not so used in organic chemistry.

Further developments: Organocatalysis

From when the asymmetric catalysis has been discovered its use had spread in many laboratories all over the world. Initially the catalysts were transition metals complexes with chiral ligands or chiral-at-metal.

This kind of catalyst can be used in low amount due to their high efficiency but they may need sometimes really drastic conditions and sometimes it may be needed to work with an inert atmosphere to avoid the oxidation of the metals, moreover most of these catalysts are extremely expensive.

These conditions and the recent development of "green chemistry" have induced the development of a new field of catalysis by introducing the organocatalysis.

These catalysts that consist in organic molecules have different advantages, generally they are not so toxic and resistant to oxidation so is not absolutely needed to work under inert atmospheres and with water-free solvents, moreover the price is not as high as the metal-complex even if the amount of catalyst needed is higher and the TON generally is lower.

One of the first organocatalytic reaction has been discovered in 1974 by Parrish et al.[10] using L-proline, a natural amino-acid, to catalyze an aldol condensation, but after this reaction there has not been a big development of this field until 2000. In those years the articles about organocatalysis started to increase exponentially especially thanks to the contribution of Barbas, MacMillan and List

that developed further this field discovering new catalysts and mechanisms that represent the “ying and the yang” of asymmetric synthesis because depending by the mechanisms we can attach to our molecule a nucleophile or an electrophile as we can see in the following figures.

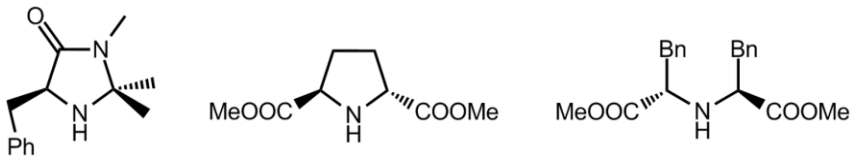


Fig. 7: MacMillan Catalysts[11].

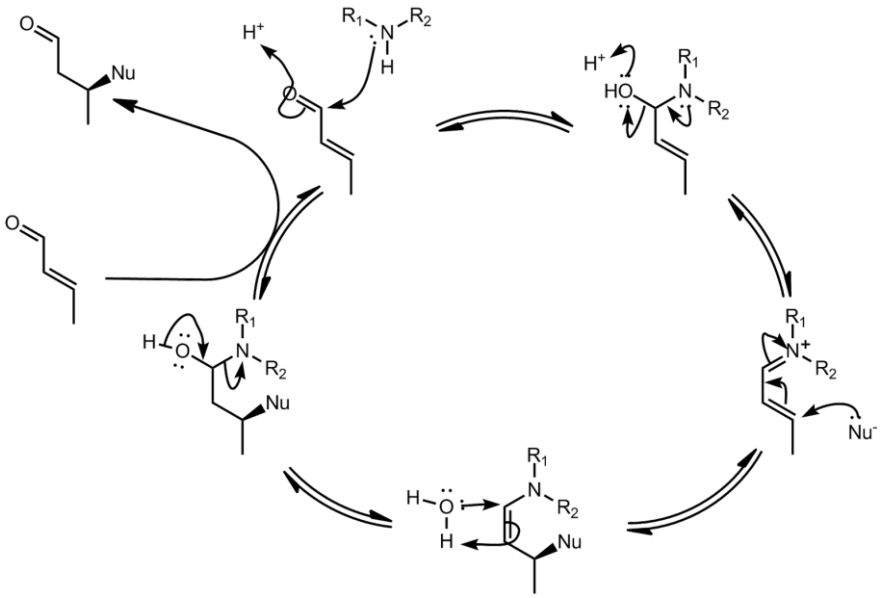


Fig. 8: Micheal addition with MacMillan Catalyst (imine mechanism).

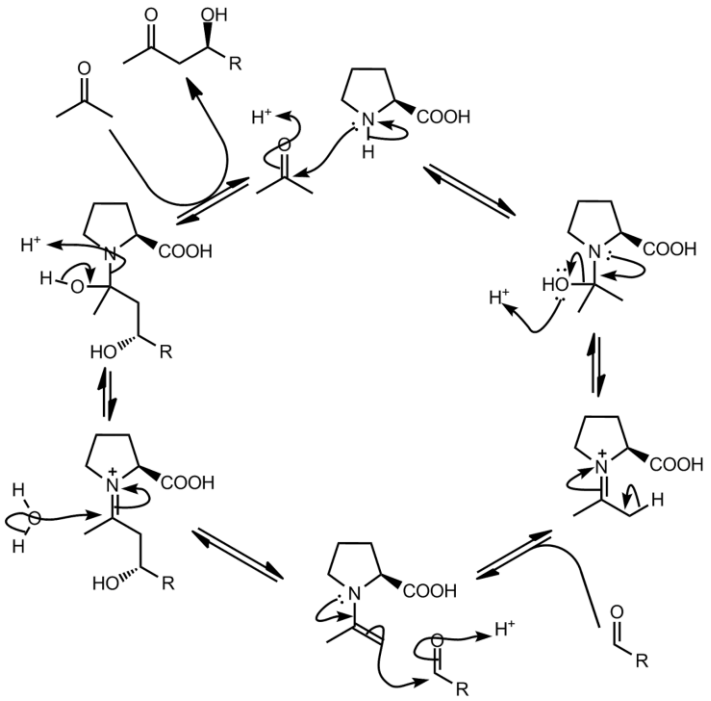


Fig. 9: List & Barbas aldol condensation with L-proline (enamine mechanism)[12].

Another famous catalyst has been developed by Corey, Bakshi and Shibata[13] from L-proline, this is used in reductions with boranes, Diels-Alder and [3+2] cycloaddition. The discovery of this catalyst started with an analysis of the use of a chiral amino alcohol used as auxiliary for the hydroboration this initially led to the discovery of a mechanism that allowed the development of this catalyst.

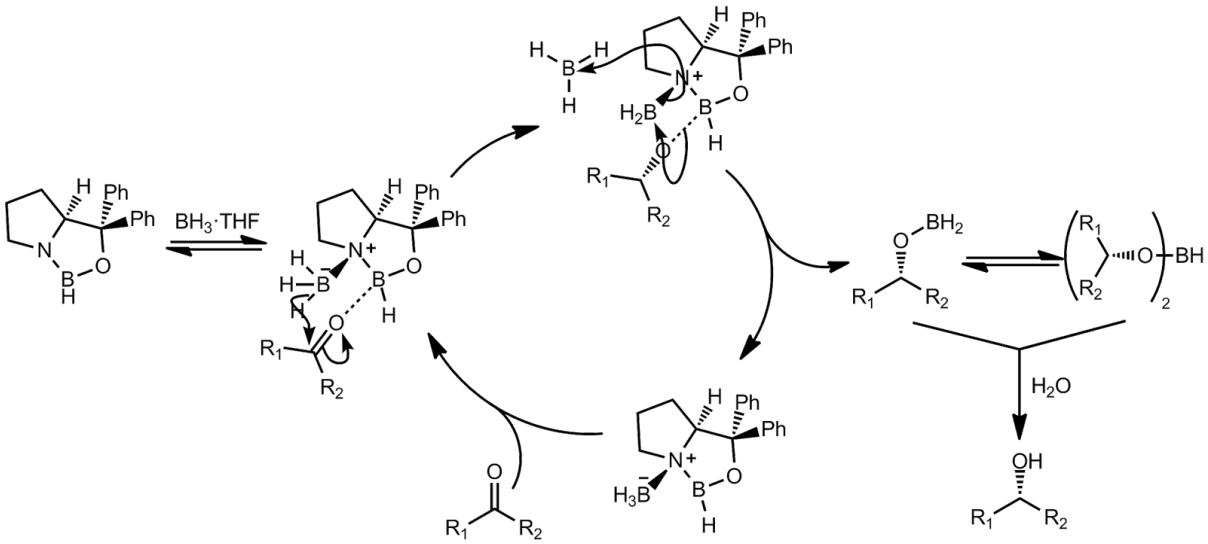
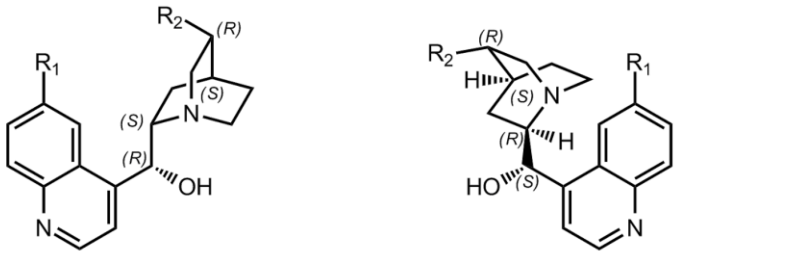


Fig. 10: CBS Catalyst activation and mechanism for hydroboration.

Another big family of organocatalyst are the alkaloids of Cinchona, this plant from South America contains 6 different versions of a singular structure.

These alkaloids in addition to be really cheap have different interesting properties that have attracted the interest of researchers for their use as organocatalysts.



(-)-quinine: R_1= OMe; R_2= vinyl
(-)-cinchonidine: R_1= H; R_2= vinyl
(-)-hydrochinine: R_1= OMe; R_2= ethyl

(+)-quinidine: R_1= OMe; R_2= vinyl
(+)-cinchonine: R_1= H; R_2= vinyl
(+)-hydrochinidine: R_1= OMe; R_2= ethyl

Fig. 11: Alkaloids of cinchona present in nature.

In nature there is not a complete couple of enantiomers of these 6 alkaloids but they have the interesting property to behave as 3 couples of pseudo-enantiomers inducing the opposite chirality to their products.

Moreover these catalyst are bifunctional because of the different groups present on them, the OH group can be a Lewis acid and H-donor while the amino group behaves as a base making these molecule usable as Brønsted basic catalysts.

But the major interest lays in the many possibilities to derivatize these molecules so that they can be used for much more reactions, here we have some examples.

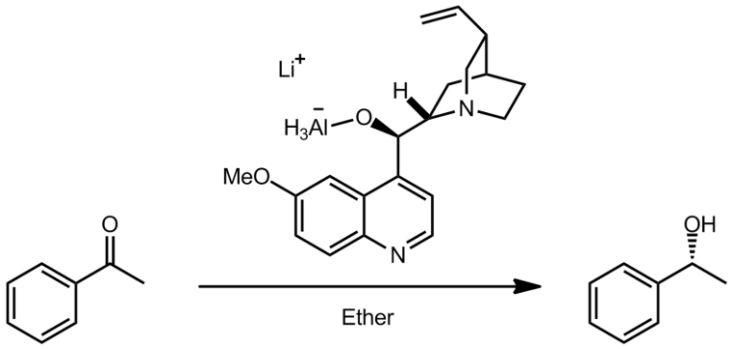


Fig.12: Reduction with QN-AlH_3[14].

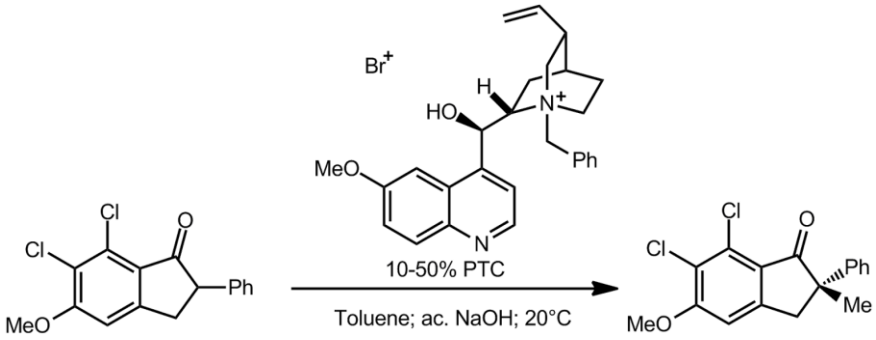


Fig. 13: Alkylation with derivatized QN as PTC[15].

Usually the common hydrogenations are catalyzed by Pd or Pt on charcoal or alumina, but cinchona alkaloids have shown interesting properties even with this reacton, on 1979 Orito et al.[16] have used cinchonidine-modified Pt to hydrogenate methyl pyruvate obtaining an e.e. of 97%.

Further studies have shown that the suitable substrates are those with an electron pair donor in α position of the carbonyl with better e.e. with keto-acids[17], keto-esters[18], keto-acetals[19] and α-methyltrifluoride ketones[20].

Later has been discovered that Pd too has enantioselectivity with the hydrogenations of α,β-insaturated carbonyl to ketones or aldehydes but with a lower efficiency[21].

These modified catalysts are possible because of the interaction of the quinolinic part of the quinine with the metal layer while the quinuclidinic site orientates the substrate, a model has been proposed for the hydrogenation of ethyl piruvate[22,23].

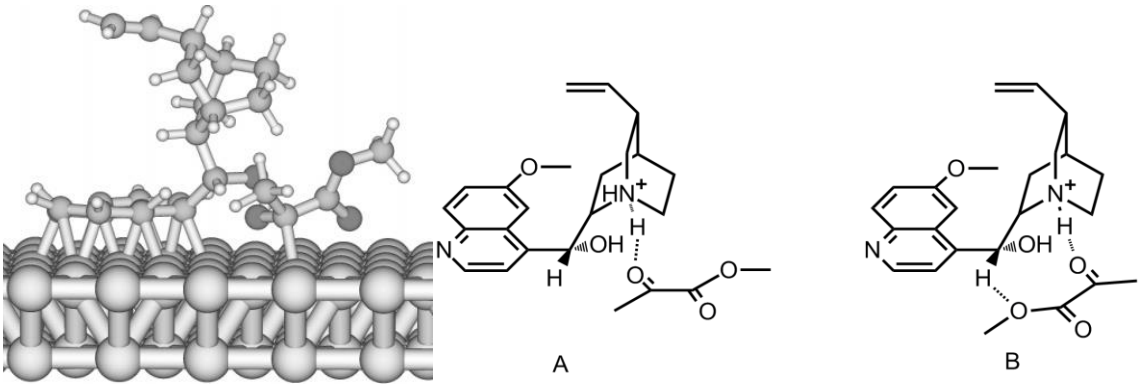


Fig. 14: Model of interaction quinuclidine - Pt - Methyl pyruvate, B is the favourite position.

Asymmetric Hydrogenation with Ir catalysts

Even with the last developments of organocatalysts, the asymmetric hydrogenations is still dominated by metals and the most common catalysts used to induce the chirality are Rh, Ru and Ir based complexes.

Among these metals the Ir had a great development only in the last years, the reason for which it was not so used was due to the excessive stability of the H_2 adduct that fails to dissociate, the solution has been found by Crabtree[24], developing some good Ir-based catalyst that are able to continue the catalytic cycle to the completion. In all the Crabtree catalyst the solvent is fundamental because all the protic solvents or those that could act as ligands were stabilizing the metal hydride. Obviously even the ligands have their importance, the high activity of the best Crabtree catalyst is due probably to its unique structure with a P-donor ligand and a N-donor ligand. Pfaltz[25] decided to add chirality to this catalyst by using optically active phosphino-oxazoline ligands (PHOX), starting with this discovery the development of this new family of ligands. Between the new ligands there are the JM-PHOX developed by Burgess[26] that give good and excellent e.e. with many olefins.

Using a single bidentate ligand instead of two monodentate gives more conformantional rigidity to the complex that has been demonstrated to be an important factor for enantiomeric selectivity in asymmetric synthesis, these catalyst developed by Burgess have a too flexible ethyl ligand between the oxazine and the phosphino, on the base of this assumption Pfaltz[27] develops again the ligands demonstrating that a Me on the oxazoline ring improves the enantioselectivity by increasing the rigidity of the structure.

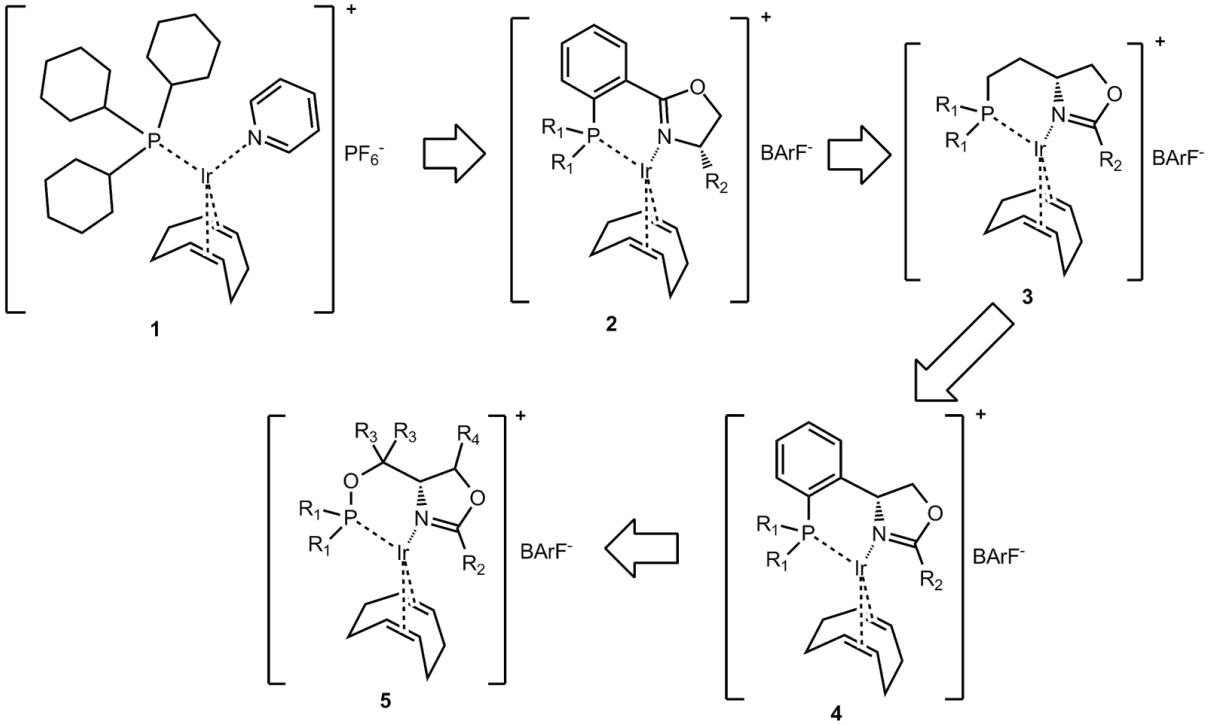


Fig. 15: 1) Crabtree catalyst, 2) Ir-PHOX BARF, 3) Ir-JM-PHOX BARF, 4) Zhang Catalyst, 5) Pflatz Catalyst.

The catalytic cycle for hydrogenation of alkenes with this family of catalysts has been studied through a Density Function Theory analysis (DFT), this method consists in a software modelling of the electronic structures of multiple-body systems like molecules or complexes.

It was already known that the general mechanism was going through an oxidative addition of H_2 and a reductive elimination, but just recent researches have proposed 3 different mechanisms and explored these with the Ir-PHOX catalyst (Fig. 15) and 2 similar substrates (Fig.18).

All the mechanisms start with the reduction of the COD, in the first mechanism (Fig. 16, Mechanism A Ir^{+}/Ir^{3+} cycle) analyzed by Chen[28] we have subsequently the coordination of the Ir^{+} with the alkene and with the H_2 molecule followed by the oxidative addition to form the hydride, then we have migratory insertion and reductive elimination with loss of the product and regeneration of the activated catalyst.

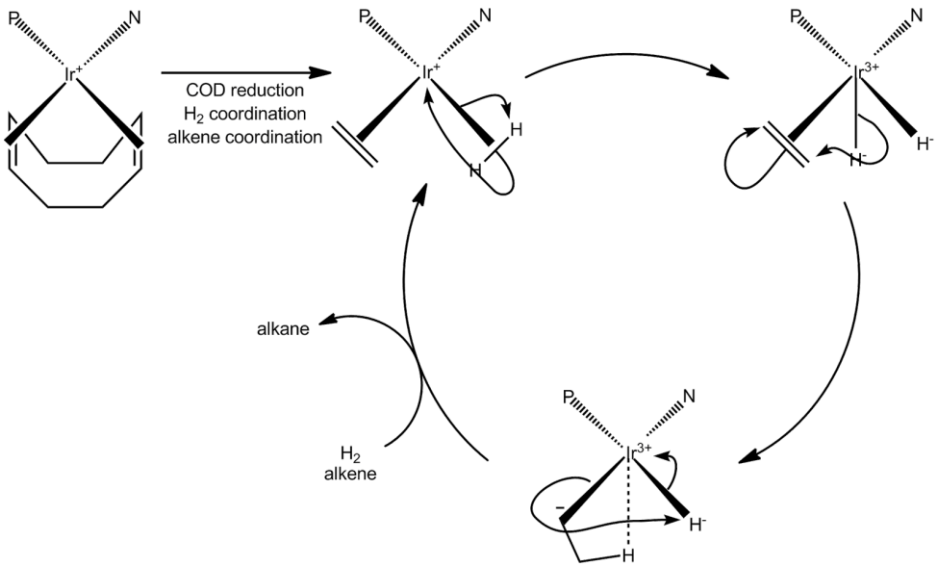


Fig. 16: Mechanism A.

The mechanism B[29] (Fig. 17, Ir^{3+}/Ir^{5+} cycle) in addition to the coordination of the alkene and the H_2 has the oxidative addition of another H_2 in the first step and subsequent migratory insertion of one H from the coordinated H_2 molecule to the alkene in parallel with the oxidative addition of the other to the Ir. Finally the reductive elimination and the product detachment complete the catalytic cycle.

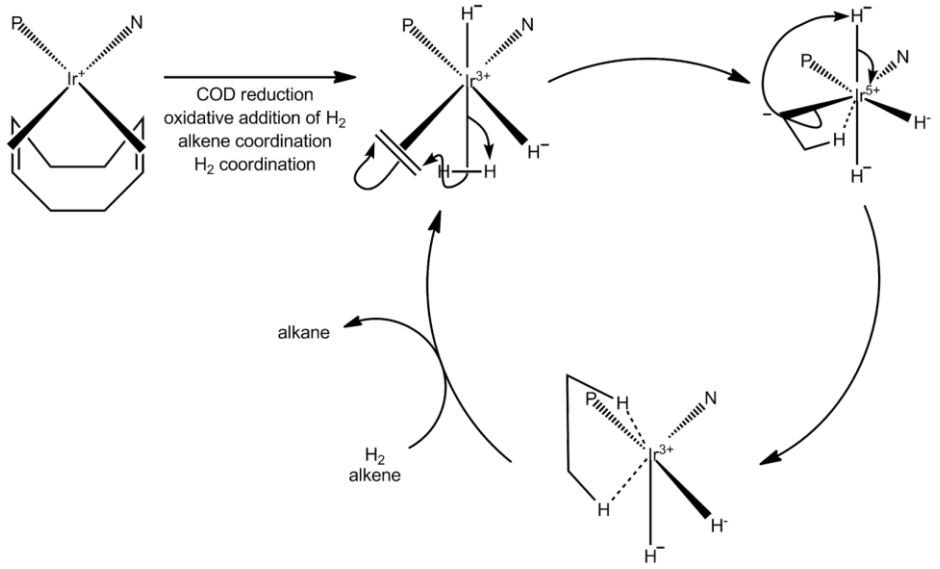


Fig. 17: Mechanism B

The mechanism C (Fig. 18, Ir^{3+}/Ir^{5+} cycle) is slightly similar to B but we have in the beginning a major stabilization due to the coordination of two solvent

molecules (DCM), moreover the second oxidative addition of H_2 happens after the migratory insertion and not in parallel so the first H that attach the alkene is an hydride and is not coming directly from the coordinated H_2 molecule but was one of the hydrides already attached to the Ir.

The most thermodynamically favored mechanism for alkenes hydrogenation is the C, this has been explored further with the prochiral substrate whose hydrogenation has been already tested experimentally with the result of a high e.e. for the R-enantiomer.

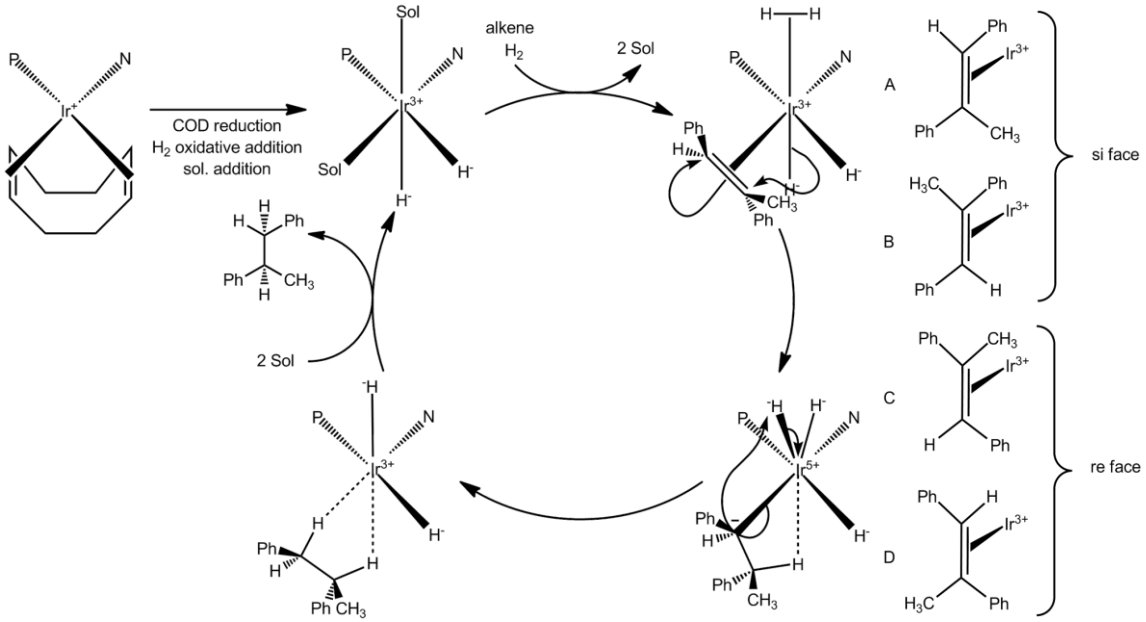


Fig. 18: Mechanism C with prochiral substrate S2 with the favourite structure (left), all the possible positions of the substrate in the complex (right).

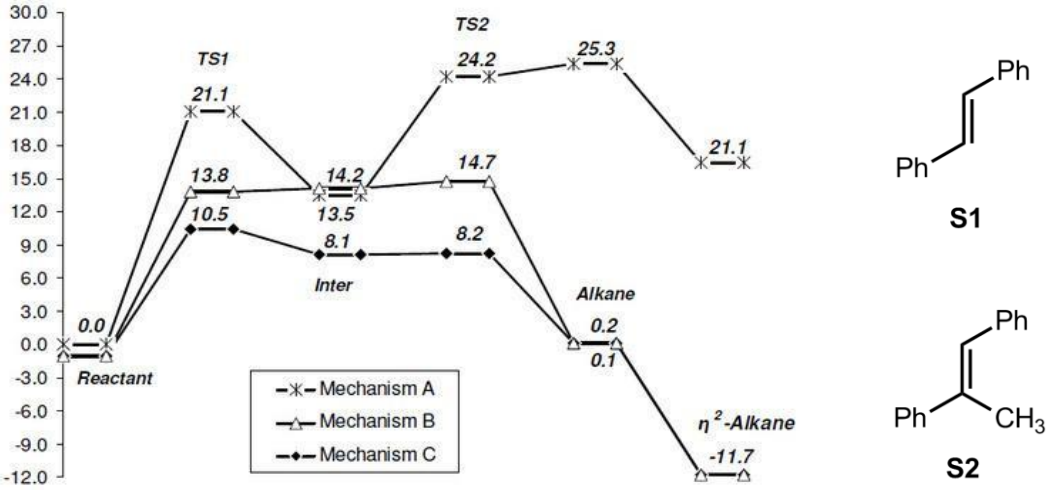


Fig. 19: Energy graph of the 3 possible mechanisms with S1 (left), substrates analyzed (right).

The substrate can interact in 4 different ways, 2 for each face and moreover the hydride formed with the first H_2 oxidative addition can be oriented above or below the basal plane giving the possibility of 2^3 possible combinations. The analysis shows as the most favored the A (Fig. 19) with the hydride below the plane according to the laboratory experiments.

We have to consider that this study has been done with only one particular catalyst and similar substrates, so even mechanisms A and C are not excluded with a catalyst with different ligands and/or substrates.

Equol

Equol is an isoflavandiol phytoestrogen (of the class of isoflavonoids). In nature we can find the (S) enantiomer as a metabolite of Formononetin, Daidzin and Daidzein, three isoflavones that can be found in soy[30].

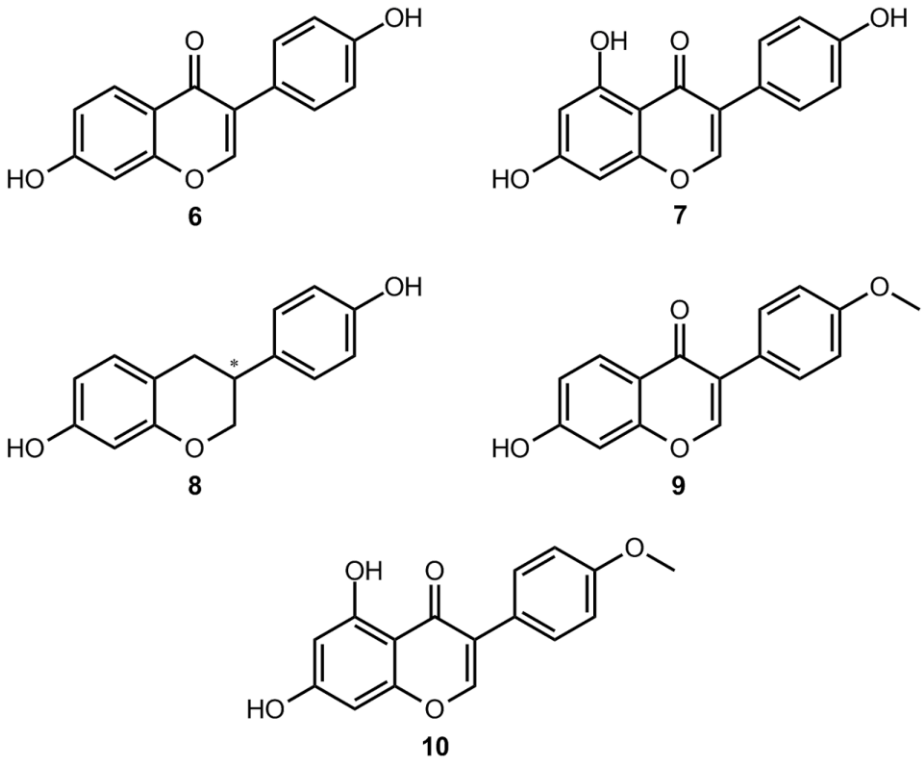


Fig. 20: Isoflavonoids; 6) Daidzein, 7) Genistein, 8) Equol, 9) Formononetin 10) Biochanin A.

This molecule has been isolated for the first time from mare's urine in 1932[31] but successively it has been found even in other animal species including cows, hens, monkeys, chimpanzees, dogs, mice, rats and pigs. In all the species the biosynthesis of Equol comes from a specific microorganism present in the gut. On 1942 a disease (called after this study "Clover disease") in Australia that caused infertility, uterine abnormalities and endometriosis to sheep was caused by the presence of high amounts of Equol in the blood of the animals, this caused by the ingestion of high amounts of the indigen *Trifolium subterranium* clover in which Daidzein and Formononetin are abundant[32]. This study suggested (although didn't prove) that Equol had phytoestrogenic effects. This hypothesis was confirmed only in the mid 60' after the discovery of the first ER[33] whose binding affinity with (±)-Equol was much higher that the binding affinity with daidzein.

From the day of its discovery there has been not so much interest in Equol with the discovery of its presence in human urine and that the presence of Equol in it is depending by the microflora of the body and by the diet this molecule started to attract the first interests. Later came out the so called Equol hypothesis, this hypothesis, proposed by Setchell et al.[34] maintain that the people having the bacterial that convert Daidzein into Equol are more likely to derive benefits from isoflavone and soy exposure than those who don't. This aroused even more interest in Equol as we can see from the graph.

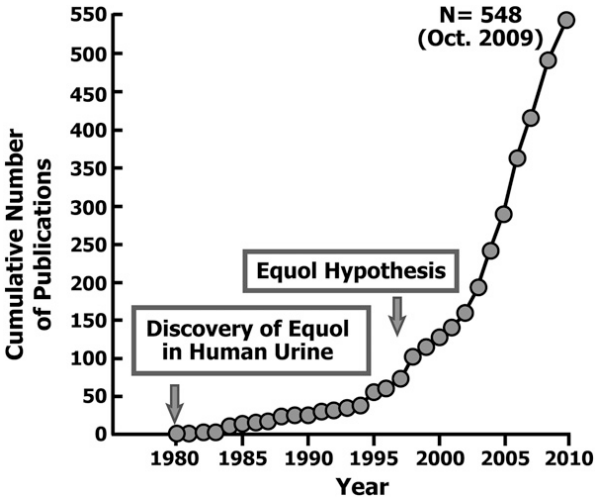


Fig. 21: Chronology of the publications on Equol.

Biological properties of enantiopure Equol

The reason for its affinity with the ER is due to its strikingly similarity with the conformational structure of Estradiol.

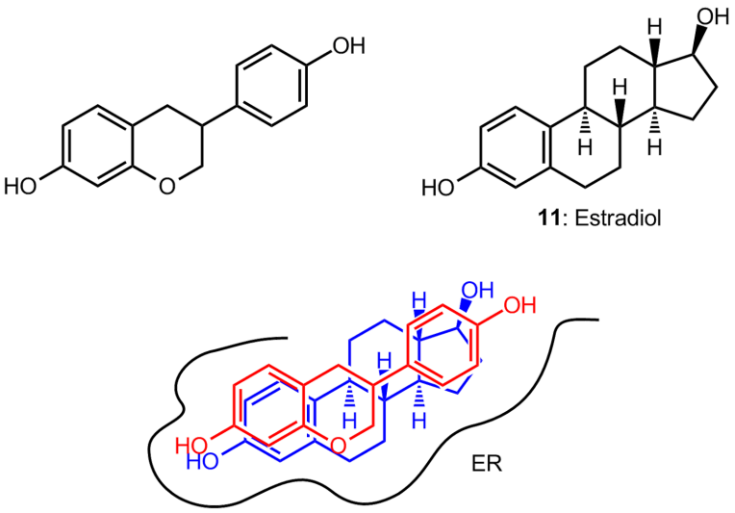


Fig. 22: Comparison of Equol with Estradiol.

Most of the studies have been done with the chemically synthesized racemic compound or with the biosynthesized S-(-) Equol. The main difference between the two enantiomers has been shown only in the last years with comparisons[35] of the binding affinity with ERα and ERβ. This comparison showed that (S)-Equol binds to ERβ more strongly than (R)-Equol, and this last one binds to ERα slightly more strongly than (S)-Equol. These results have increased the interest of the research for the (S)-Equol as a possible pharmaceutical or nutraceutical agent for a number of hormone-dependent disorders, however there was yet the possibility of its carcinogenicity to be checked, especially in the breast cancer because the ER are receptors that are over-expressed in around 70% of breast cancer[36]. Recent studies on different animal models have shown not only that (S)-Equol is not carcinogenic but that (R)-Equol is potently chemopreventive[37].
Other researches have shown that both the enantiomers have a myriad of other biological properties with the potential to be of value in many clinical areas: cardiovascular disease, osteoporosis, and menopausal symptoms, moreover an in-vitro study showed that Equol has the highest antioxidant activity between all the isoflavones. Several studies show Equol to be a vasorelaxant, inducing

endothelial and NO-dependent relaxation[38], suggesting that equol may be helpful in reducing risk of cardiovascular disease.

Synthetic strategies

The studies done on Equol are not complete and most have not been made on human tissue so is still very long the path that researchers have to take for Equol to be a new drug and many are the obstacles that have to be passed. However, there is a growing interest in both the enantiomers of Equol. The research on these two compounds has always been hampered by the lack of an efficient method of synthesis has led many organic chemists in the research of an efficient synthesis of these components to obtain easily both of them in an enantiopure form.

The first studies were based on synthesis of this compound in the racemic form, the most efficient consist in a simple hydrogenation of isoflavones such as Daidzein with 5-10% Pd/C as catalyst (20-30% w/w)[39].

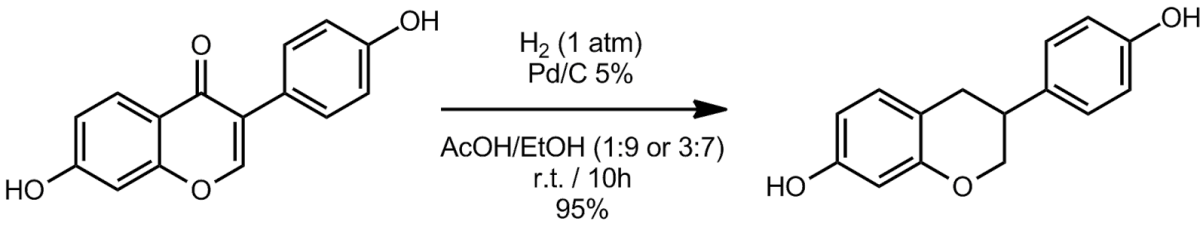


Fig. 23: Catalytic hydrogenation of Daidzein.

Even by having a high yield this method is starting from Daidzein and anyway we obtain only a racemic mixture that we should resolve if we want to study the enantiomeric properties.

The very first attempt of enantioselective synthesis of a derivative of Equol has been done by Heemstra et al.[40] with the help of a chiral auxiliary to obtain the enantioselectivity based on the work of Ferreira et al.[41] that obtained only the dimethylated (S)-Equol.

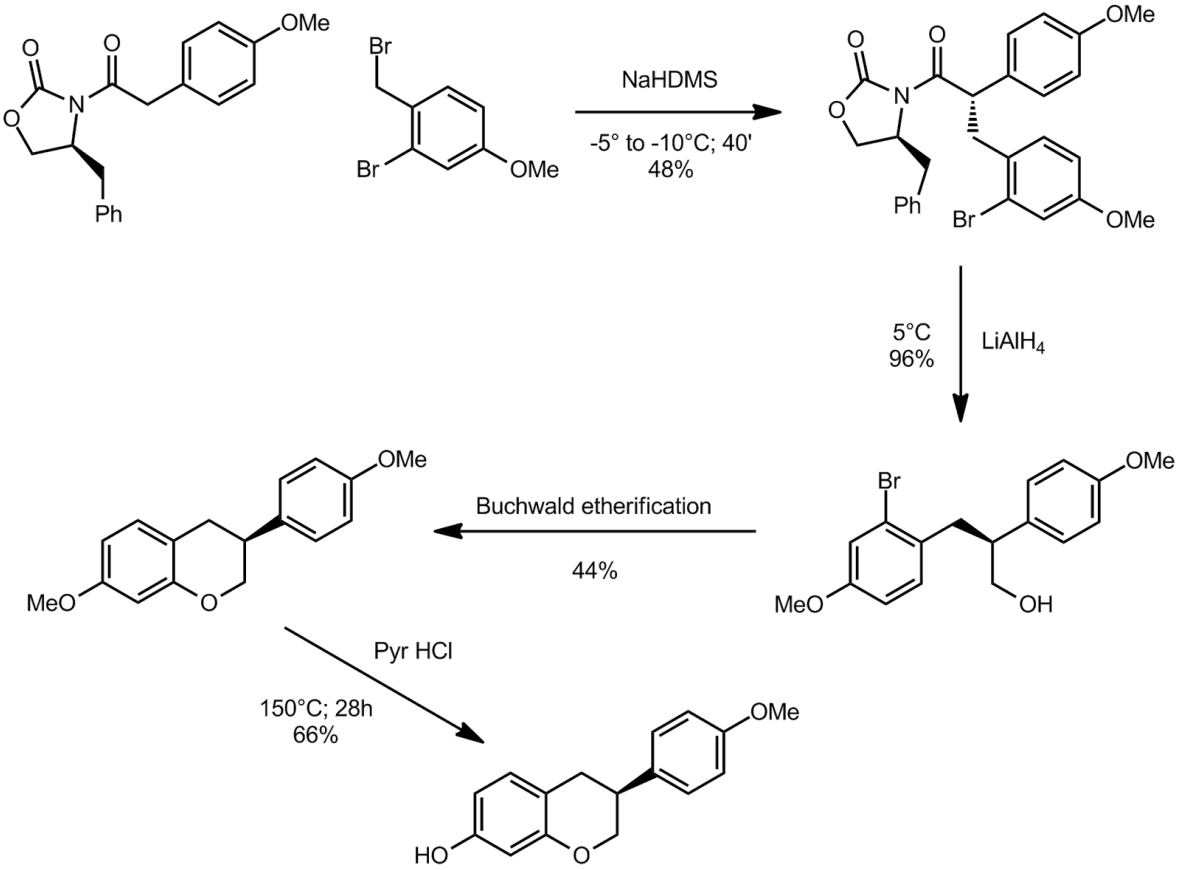


Fig. 24: Heemstra Synthetic route with chiral auxiliaries.

Even if with this first synthesis we obtained a product 99% pure and with an e.e. >99% the overall yield is just 9,8%.

In another catalytic hydrogenation done by Steffan[42] the CBS catalyst has been used to introduce the e.e. in the first step, the process followed by a protection whose sterical hindrance will orientate the successive reduction. In the end the product is obtained after a deprotection step with an OY of 44%.

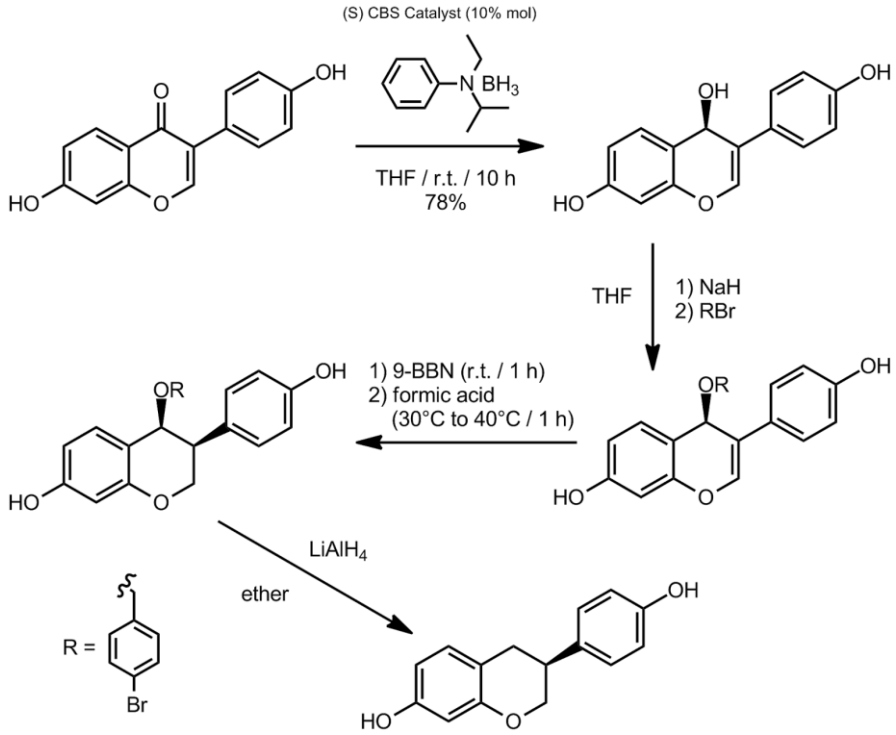
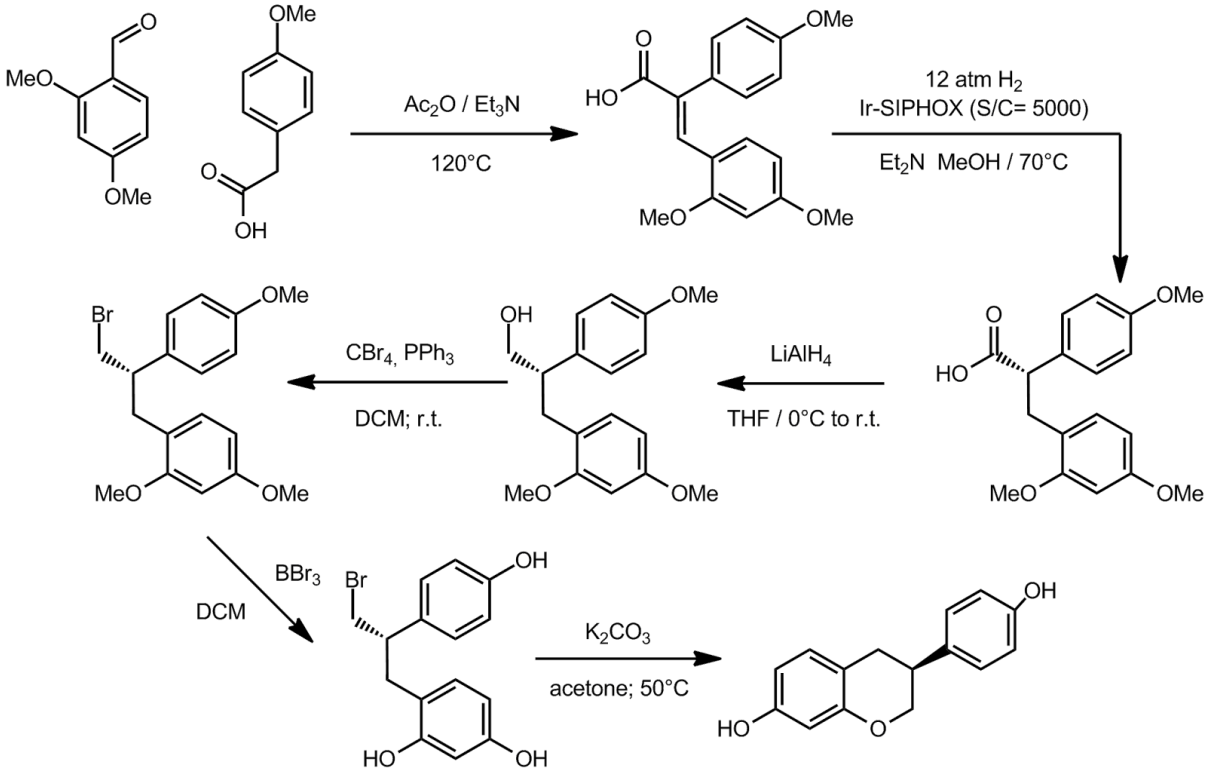


Fig. 25: Steffan synthesis with CBS catalyst.

The last catalytic hydrogenation attempted was done by Zhou et al.[43] with an overall yield of 48,4% this reaction is even the one with the highest OY and an excellent e.e. (98%)



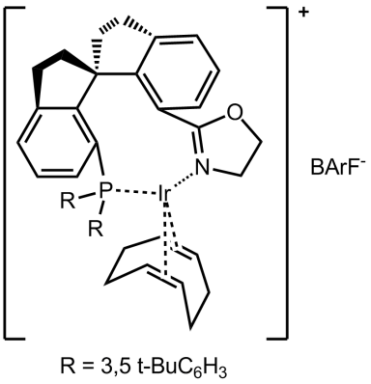


Fig. 26: Zhou's synthesis with Ir catalyst.

There have been even other synthetic strategies like the chiral pool, using a copper-mediated allylic transfer[44] that start from (S)-lactic acid as a source of chirality. (OY: 31,6% non-considering the reagent synt.; e.e.: 91%).

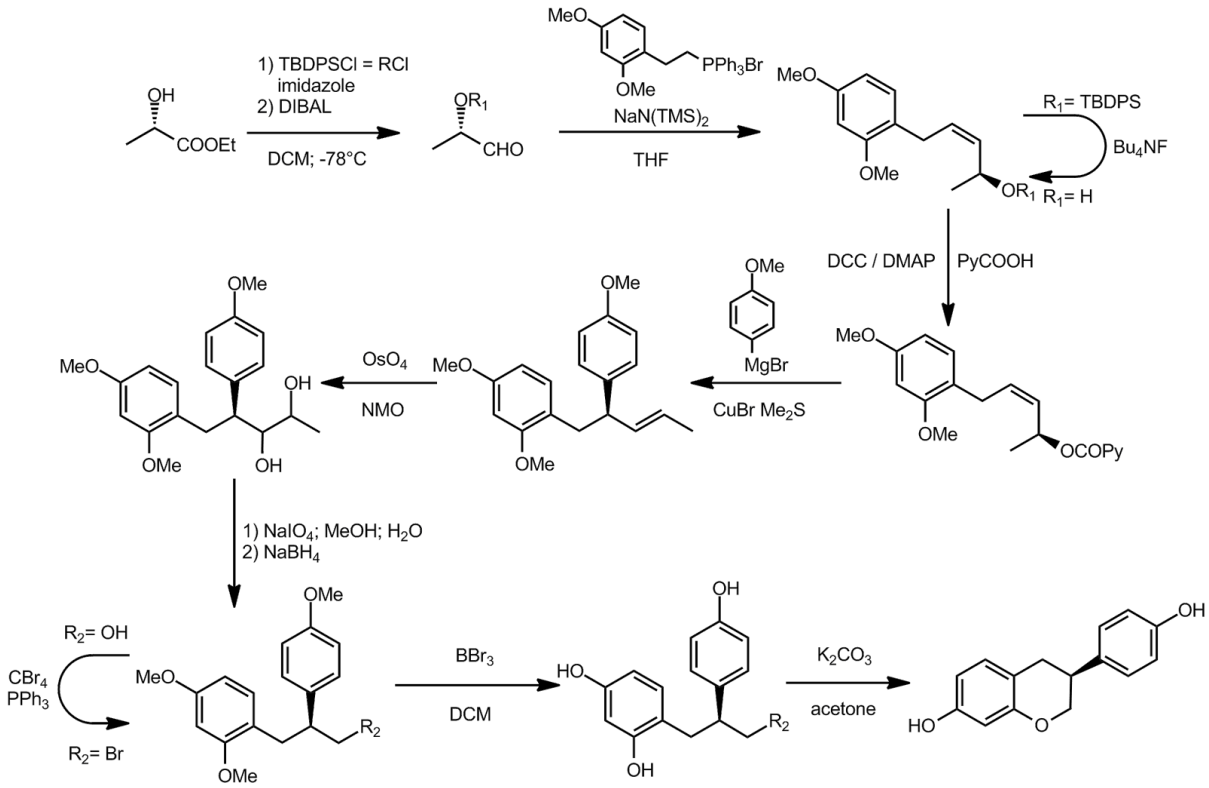


Fig. 27: Kobayashi's synthesis using the chiral pool.

And even cinchona alkaloids have been tested with this synthesis with an enantioselective PTC made from a derivative of cinchonine[45] giving an OY of 31,6%.

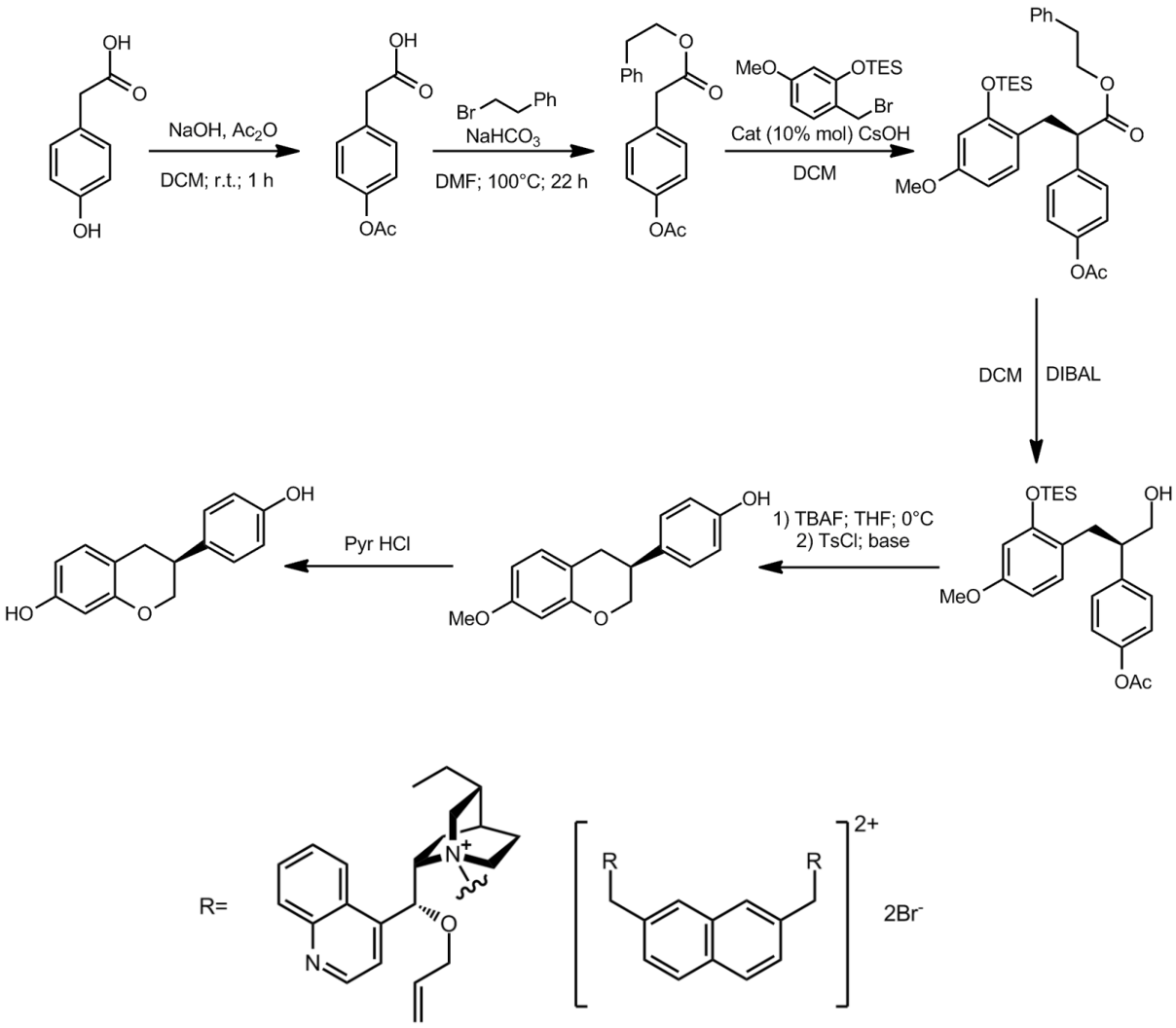


Fig. 28: Equol synthesis with a PTC.

Finally, with an overall yield not reported but e.e. of 85% for Cat. A and 80% for the Cat. B. we have an addition catalyzed by a BINAP atropisomeric catalyst[46].

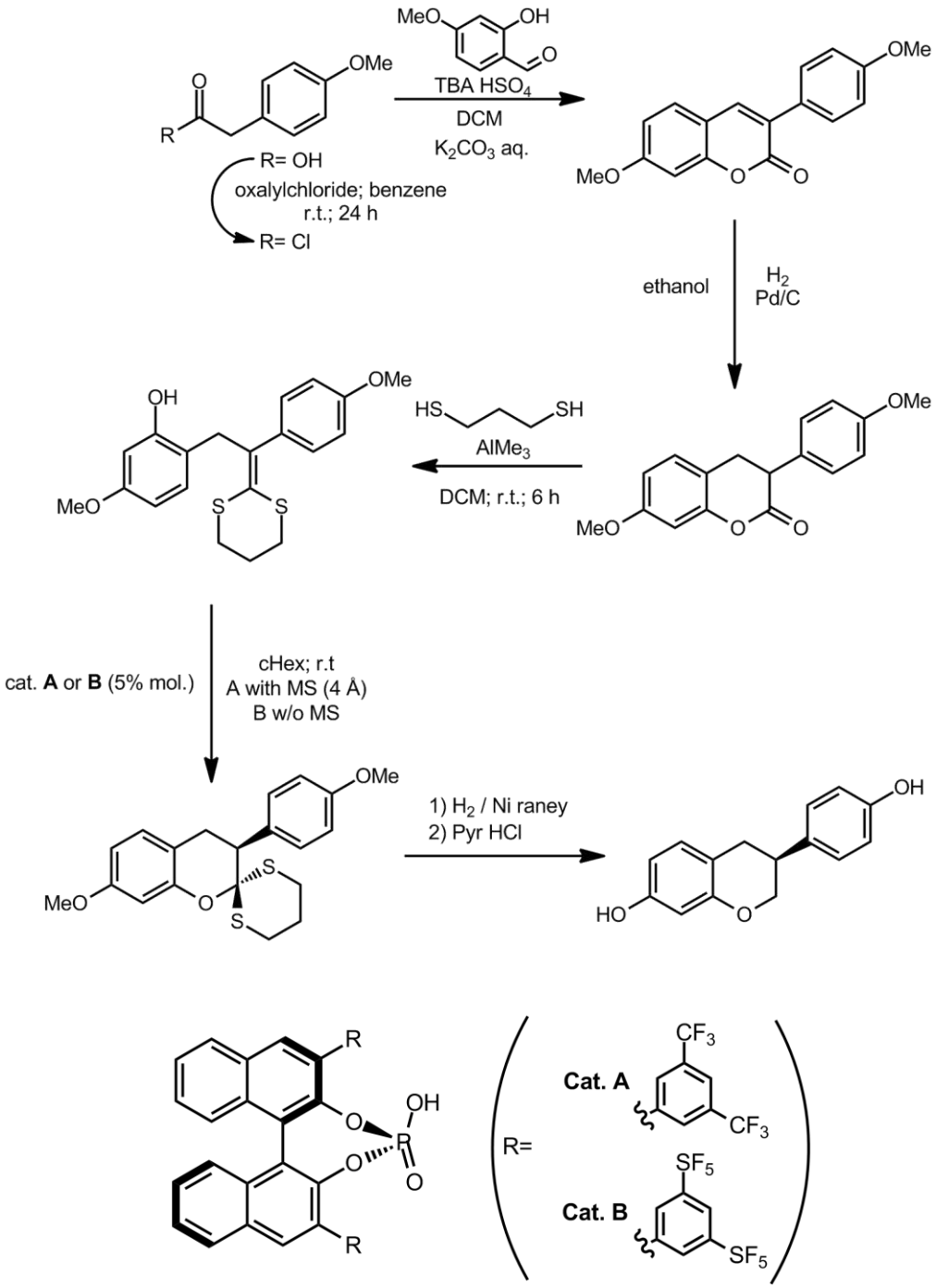


Fig. 29: Equol synthesis (top) with derivatized BINAP (bottom).

PROJECT

Even if the research is starting to explore this field of the synthesis with all the new methods developed for enantioselective synthesis the most common method to produce the enantiomers of equol is still the resolution of the racemate[47].

For this reason the aim of this thesis is to find a synthetic route for this compound in order to give to the medical research a fast and economic method to produce high amounts of both the enantiomers and to open the route of a possible industrial method of synthesis, in case Equol would become a drug.

We want to use a catalyst of the family developed by Pfaltz, specifically with threonine derived phosphinite-oxazoline ligand and BARF counterion. This commercial catalyst will be used to hydrogenate a prochiral double bond situated on the hetero-ring. We choose to use this catalyst because we have seen excellent results in most of the hydrogenation of olefins with a small amount of catalyst.

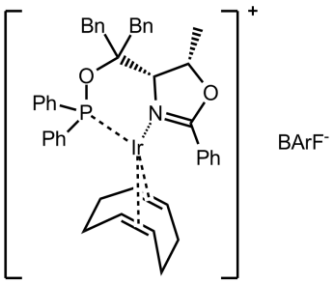


Fig. 30: Ir-BARF catalyst (**A**).

To achieve our hydrogenation we need to have a substrate with a simple insaturation and without alcoholic groups that could coordinate irreversibly with the catalyst and/or destroy the other ligands.

In order to obtain the dehydroequol we decided to reduce the keto group present on Daidzein, the particular combination of groups on the hetero-ring of Daidzein gives us the possibility to use a specific borane reducing agent to obtain straightly our intermediate of interest.

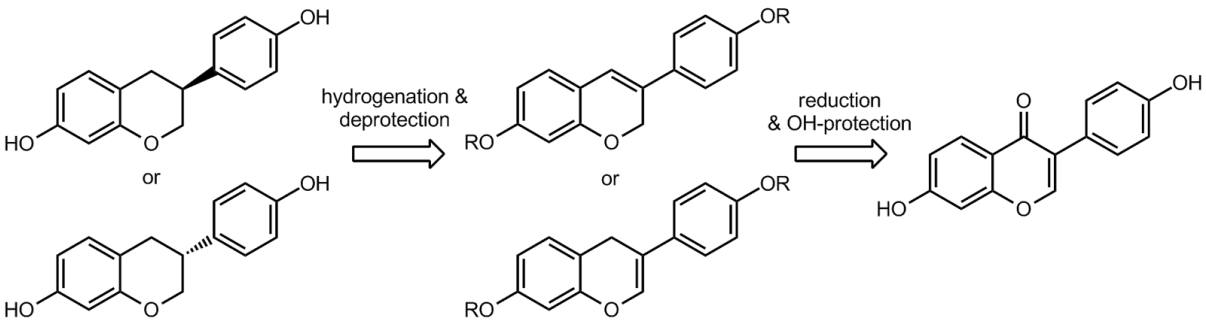


Fig. 31: Project retrosynthesis.

Boranes are between the weakest reducing agent used in organic chemistry, these chemicals have been used for hydroboration and for the partial reduction of carbonyls, having a chemoselectivity for electron rich carbonyl-groups borane are favored the reduction of carboxylic acid over esters and ketones.

Even with amides we have the complete reduction of the carbonyl group, this is due to the higher electron density.

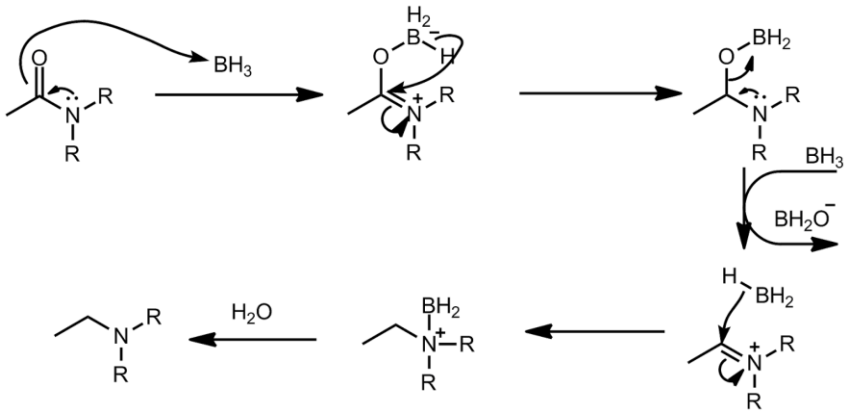


Fig. 32: General reduction with borane of carbonyl with an electron donor in α.

Daidzein has an α,β-unsaturation and an oxygen atom on the hetero-ring we want to reduce, we think that this system can induce an electron pushing that may emulate the mechanism that we have for the amides.

However, there is the presence of the double bond that can be hydroborated while we want to keep it trying to reduce only the carbonyl. For this reason we decided to use 9-BBN. This borane, invented by Knights and studied by him and Brown[48] has proven more reactive and able to be much more regioselective for the less hindered sites.

While many of the synthetic route used in other articles started from Daidzein, we want to develop a complete synthetic route by starting from smaller building blocks to synthesize this product using a procedure developed by our laboratory for general isoflavones.

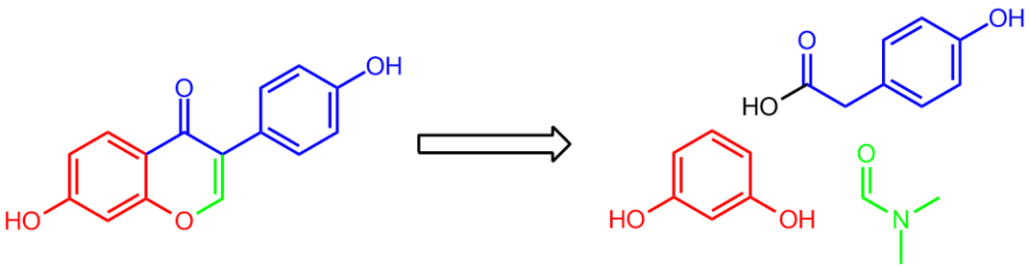


Fig. 33: Retrosynthesis of Daidzein.

During our work we will even produce racemic Equol through a simple Pd-catalyzed hydrogenation of Daidzein that has been proved efficient already and a D-labelling through a D_2 Pd-catalyzed deuteration in order to produce a molecule for further studies in our lab (complete deuteration).

After the completion of this part of the project we tried to develop a shorter and cheaper route to obtain e.e. using Quinine as organocatalyst supported by Pd/Al_2O_3 for the hydrogenation step. This choice has come because of the high price of the Ir-Catalyst and the number of additional steps that are required in order to use such catalyst. Seeing the feasibility of the Daidzein catalytic hydrogenation we wanted to avoid to split the complete reduction of the groups on the heteroring in 2 steps and even to avoid the protection & deprotection but still trying to obtain a good e.e.

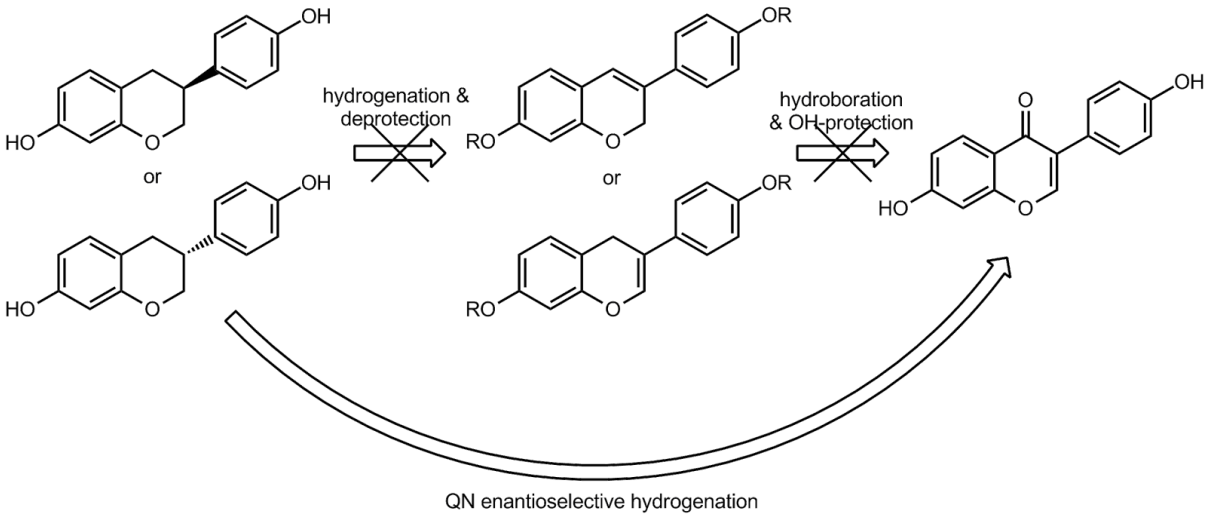


Fig. 34: Second synthetic route analyzed.

RESULTS AND DISCUSSION

Step 1: Synthesis of Deoxybenzoin

For the synthesis of Daidzein (**4**) we used the method developed by Hase[49] through 2 steps one-pot. We start by bonding Resorcinol (**1**) and 4-Hydroxyphenylacetic acid (**2**) with a Friedel-Crafts alkylation using as solvent the BF_3Et_2O obtaining a deoxybenzoin (**3**) (table 1). This reaction is followed by the closure of the central O-ring through a concerted reaction consisting in activated DMF as carbon source acting as an electrophile that will be attached by the deoxybenzoin: first by the aliphatic carbon then by the alcoholic oxygen on the ring (table 2).

These steps have been the most problematic in our synthetic route, I found the experimental data of Hase impossible to reproduce, a problem that has been confirmed by other researchers[50].

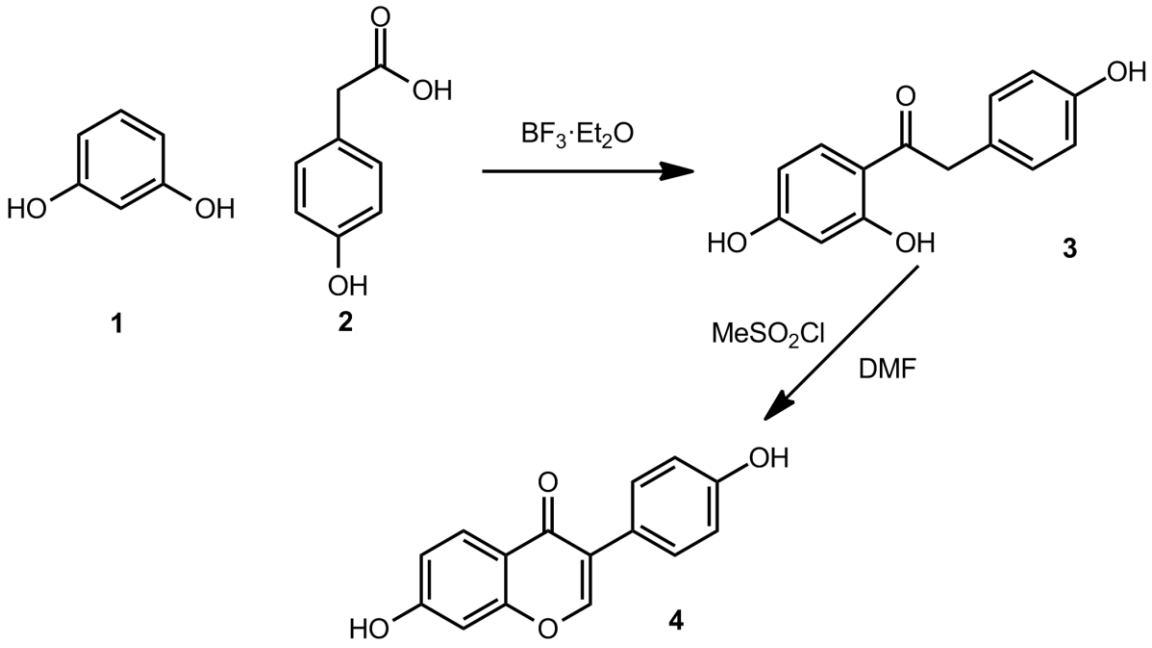
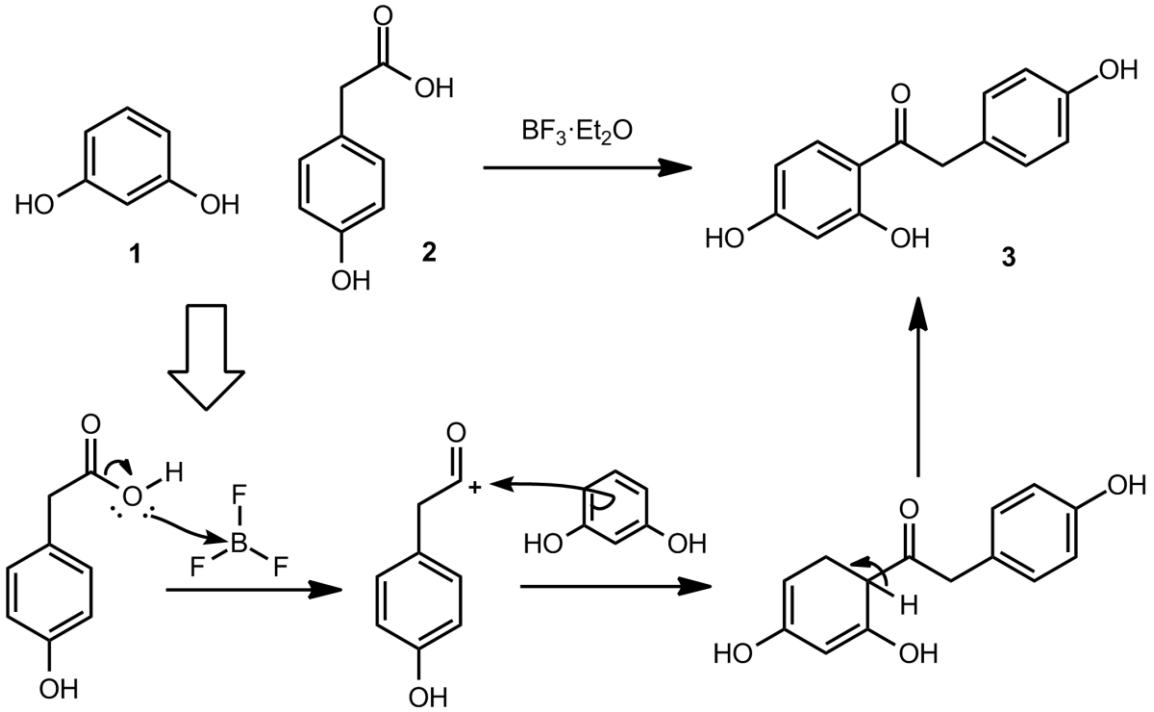


Fig. 35: Steps 1 & 2 one pot.

The problems in these steps lay not only in the reaction but also in the workup process. After trying and failing to reproduce the experiment with the original procedure, we initially increased the reaction times of the first step (Table 2:

entry 3-6) since we were expecting an almost complete reaction and the TLC were still showing reagents present in the reaction mixture (Table 2: entry 1, 2). After entry 6 in table 2 we decided to focus on the first step, deciding to increase the T as suggested by Nair[51] obtaining a slightly better yield in a shorter amount of time optimizing the first step.

Table 1: Screening of the T for the step 1.

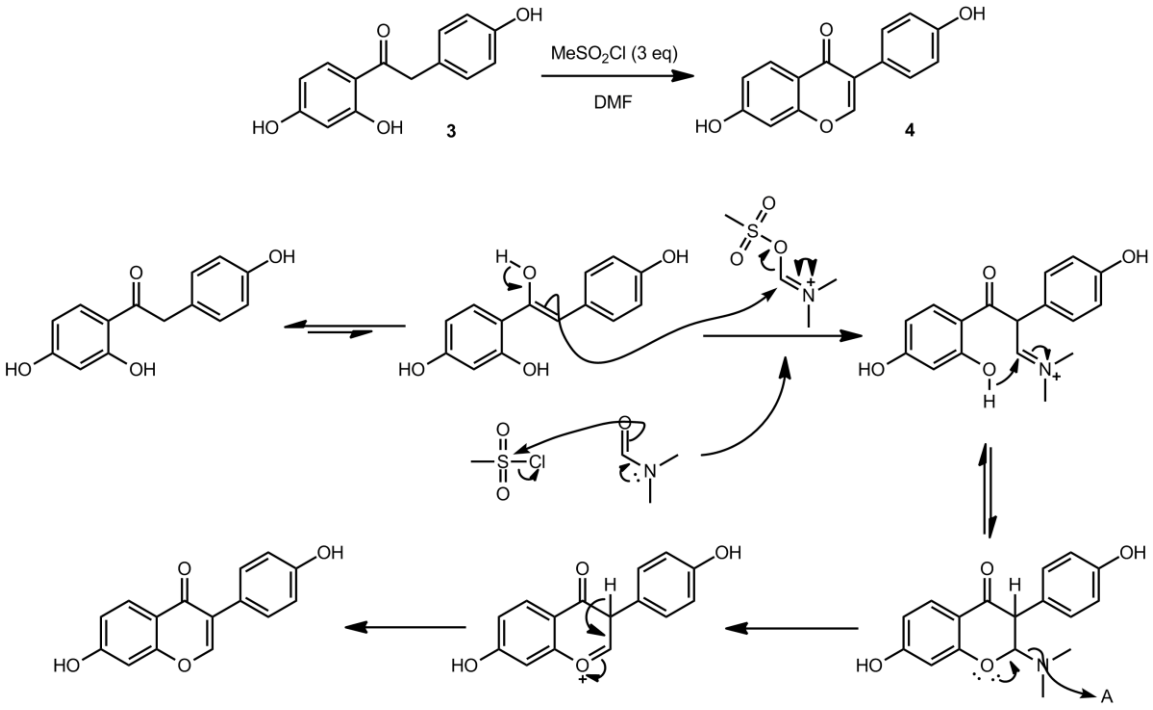


Entry[a]	Time	T	Yield
1	2 h	70°C	74,9%[b]
2	20’	120°C	59,8%[b]
3	16’	120°C	63,2%[b]
4	7’	120°C	79,5%[c]
5	22’	115°C	78,8%[d]
6	17’	115°C	80,1%[c]

a: BF_3Et_2O : **1** : **2** = 20:1:1 (mol.)
b: red-orange colour (impurities)
c: yellow colour
d: orange colour (impurities)

Step 2: O-ring closure

Table 2: Optimization of the synthesis of Daidzein in 2 steps.



	STEP 1		STEP 2		
Entry	**Time**	**T**	**Time**	**T**	**O.Y.**
1	1h	70°C	1h 30’	80°C	13,0%
2	1h	70°C	1h 30’	70°C	14,2%
3	24h	70°C	4h 10’	70°C	28,0%
4	24h	70°C	18h 14’	70°C	38,5%
5	39h 30’	70°C	28h	70°C	41,3%
6	43h	70°C	20h	70°C	18,5%
7	30’	100°C	2h	70°C	24,2%
8	20’	120°C	19h	70°C	38,6%
9	15’	115°C	51h	70°C	73,6%
10	15’	115°C	51h	80°C	68,9%

a: DMF : BF_3Et_2O = 5:4 (mol)

The second step has been more complex to analyze, initially we decided not to increase the reaction time too much in order to avoid what seemed like a general decomposition of the reagents or the product that turned the reaction mixture first red then dark and viscous like tar. However, even in the second step the

intermediate never reacted completely, so we decided to try to keep the reaction until the complete depletion of it. To our great surprise this optimized the reaction up to a yield of 73.6% after a prolongation of the second step from the initial 1h 30' to 50h (Table 2: entry 9).

Even with the incomplete reactions the separation gave us big problems, **4** is really insoluble in most of the common solvents except ethanol[52], methanol (around 0,1 mg/mL) and DMF and DMSO (10 mg/mL and 30 mg/mL). Initially we attempted recrystallization with ethanol and after with methanol as written in the article but this separation is inefficient both for the amount of the product recrystallized and the purity.

We tried a solvent screening in test tube to find good candidates as recrystallization solvent or solvent mixture.

Table 3: Solvent screening for recrystallization.

Solvent	Soluble (r.t.)	Soluble (b.p.)	Recrystallization
Toluene	NO	NO	NO
EtOAc:MeOH (2:1)	NO	YES	NO
EtOAc:MeOH (3:1)	NO	incomplete	NO
EtOAc:MeOH (4:1)	NO	NO	NO
EtOAc:MeOH (5:1)	NO	NO	NO
Acetone	NO	YES	NO
Acetone:MeOH (1:1)	partial	YES	NO
1-Propanol	NO	YES	traces
2-Propanol	NO	YES	traces
1-Butanol	NO	YES	traces
2-Butanol	NO	YES	traces
DMF	YES	YES	NO
EtOAc	NO	NO	NO

THF	NO	YES	NO
Benzene	NO	NO	NO
THF:Acetone (1:1)	incomplete	YES	traces
THF:Acetone (1:2)	incomplete	YES	traces
THF:Acetone (2:3)	incomplete	YES	NO
H_2O	NO	incomplete	traces
MeOH:Toluene	NO	YES	NO
1-Propan1ol : ciclohexene	NO	YES	YES
Propan1ol : ciclohexene	NO	YES	YES

a: Test with approx 10mg of **4** in 3mL of solvent, results after 1 week.

The few good candidates proved themselves inefficient too, we decided so to pass to another purification method, for a short time we considered a chromatographic column and we did also a screening for the solvent mixtures but its low solubility in solvents and the extreme complexity of the only suitable solvent mixture make us drop this separation method. Finally, we decided to simply clean the product from its impurities with cold methanol, the choice of this solvent was due by the fact that even the solubility properties of the impurities are similar to **4**.

After optimizing the synthesis of **4** and obtaining good amount of it we planned the following steps of our synthesis. Our plan was to obtain only a prochiral unsaturation on the O-ring that would allow us to use our Ir-BARF catalyst to do an asymmetric hydrogenation.

To use the Ir-BARF catalyst we need to protect the alcoholic groups because they would destroy it.

We decided to use BnCl for the protection and 9-BBN to eliminate the keto group. Initially we planned to try both the possible paths to obtain the protected

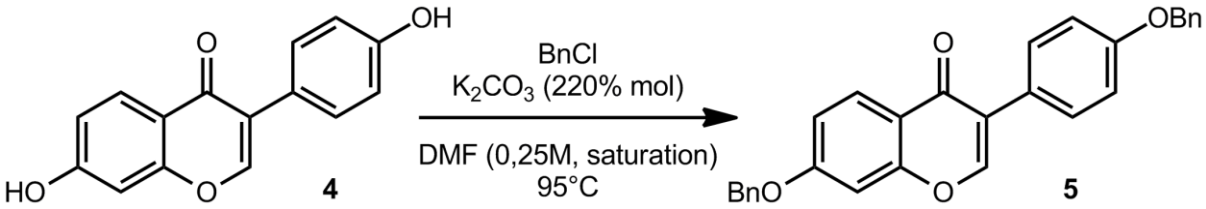
Dehydroequol (**6**): using as first reaction the protection and using as first reaction the reduction.

Step 3: Protection

Our first attempt was using the protection first, thinking we would have less troubles with the 9-BBN used in the reduction. This first attempt was successful and gave an excellent yield. Moreover, the isolation of the DiBnDaidzein (**5**) is really simple with recrystallization.

The simplicity of the separation and purification suggested us to try it with using directly the crude **4** from the previous steps with a bigger excess of BnCl. The first attempt gave a really low yield (Table 4: entry 2) and the HPLC revealed the presence of many products, while second attempt with a higher excess of BnCl has given again an excellent yield (Table 4: entry 3).

Table 4: Protection step with BnCl



Entry	Time	BnCl : 4 (mol)	Reagent 4	Yield
1	3h	2,08	Pure (>99%)	91,5%
2	1h 33’	2,22	Crude (73,6%)	10,3%[a]
3	1h 42’	2,97	Crude (73,6%)	95,6%

a: crude product yield.

This has solved the problem we had with the separation of **4** from its impurities, seen this results we decide to attempt, without so much hope, a three-step one-pot reaction trying to obtain **5** from the initial reagents in one pot. We obtained no results from this so we have not tried this strategy anymore being already satisfied with the results obtained so far.

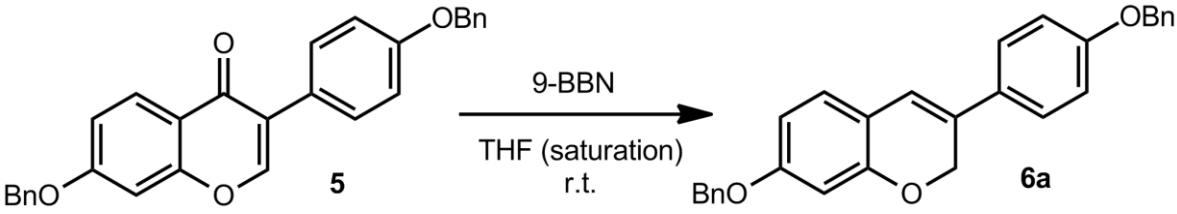
At this point we decided to not try the inverse order of reactions because we would lose the separation advantage given by the dibenzylation.

Step 4: Reduction with 9-BBN

We proceeded so with the reduction reaction, trying initially with a big excess of reducing agent and obtaining a good yield (Table 5: entry 1), we tried then to see the results by using a lower excess of 9-BBN obtaining poor results (Table 5: entry 2). The biggest amount of yield was around 60% when we increased more the 9-BBN (Table 5: entry 3) revealing that a big excess is positive for the yield but not convenient. Leaving the reaction for more time seems to eliminate most of the product and to produce impurities (Table 5: entry 6-7).

We didn't try to use different solvents because protic solvent would have interfered in the reaction by doing unwanted acylation or by destroying the 9-BBN.

Table 5: Step 4, Reduction with 9-BBN.



Entry	Time	9-BBN (eq)	Yield
1	22h 18'	8,5	59,8%
2	22h 16'	5,0	4,5%
3	22h 00'	10,1	59,9%
4	14h 57'	8,7	52,7%
5	15h 58'	8,7	57,9%
6	62h 23'	10,5	0%[a]
7	41h 37'	9,1	0%[a]

a: too many impurities, traces of product inseparable

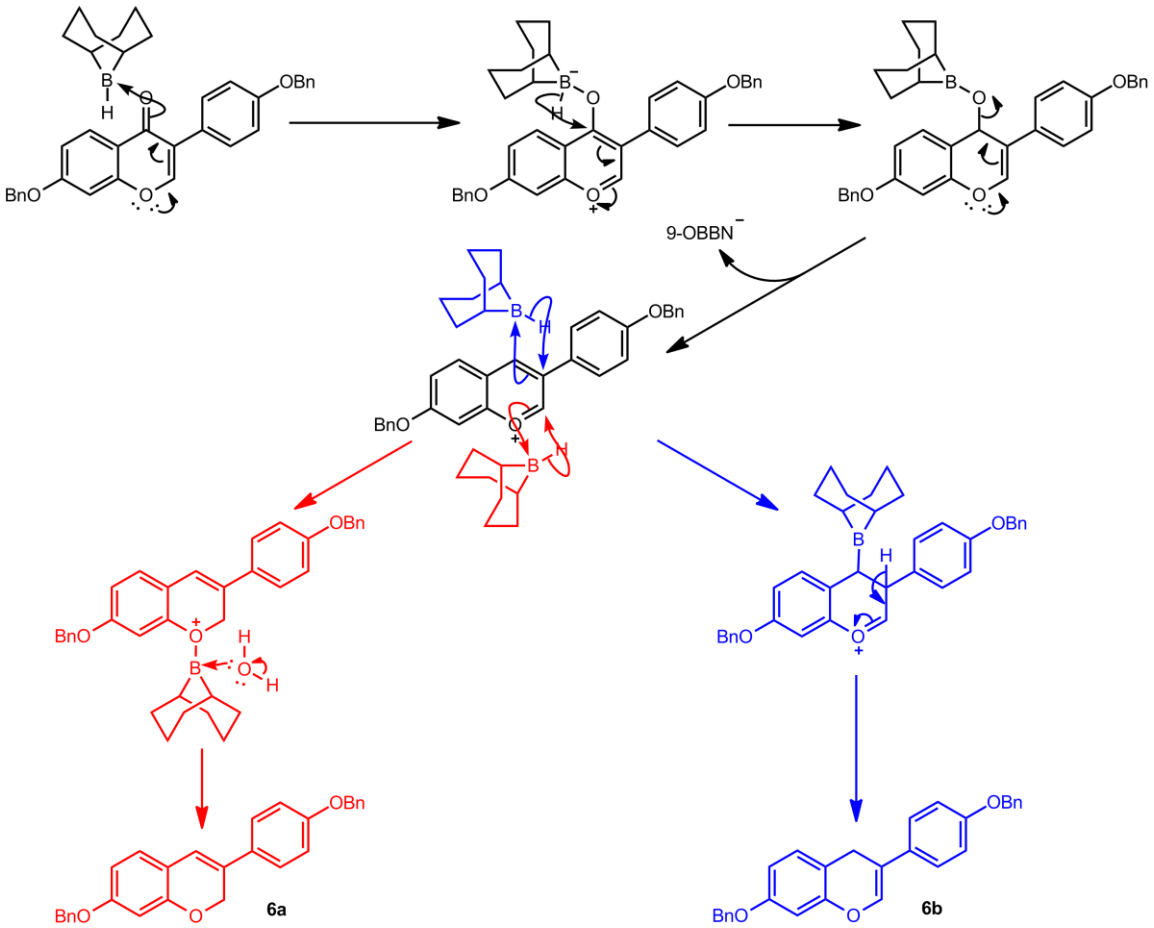


Fig. 36: 9-BBN reduction mechanisms and possible products.

For this reaction we had hypothesized two mechanisms and two possible products, initially we have in both the reduction of the carbonyl with attach of it to the borane, the mesomeric effect of this special substrate and the sterical hindrance favors the initial attack and permit the elimination of 9-OBBN obtaining an intermediate stabilized probably by the temporary aromaticity. Subsequently we need have a second reduction, in this case we have two possible targets the C=C (**blue path**) or the $C=O^+$ (**red path**). The steric hindrance seems to play a role even here, moreover the $C=O^+$ is more polar so the red mechanism seems favored over the blue. The NMR, confronted to the NMR databases, confirm our hypothesis showing **6a** as the major product and just traces of **6b**.

Step 5: Reduction with Ir-BARF

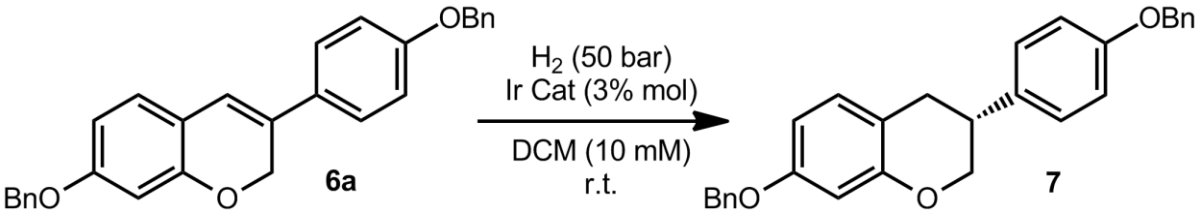
Once we have eliminated the keto group and we have left on the O-ring only the unsaturation we started to try the hydrogenation with our Ir-BARF. We used as

model the procedure of Pfaltz[27] and as catalyst we used the commercial one showed in project chapter (**A**).

The reaction time in the article of Pfaltz for the hydrogenation of aryl-olefins is just 2 h with 50 bar of H_2 and the solvent used is DCM, even in this experiment we didn't checked the effect of different solvents because most of the others would permanently deactivate or destroy the catalyst[23].

The initial result had a really poor yield and increasing the time didn't improve the reaction in a significant way (Table 6: entries 1-4). This has been solved by drying completely the solvent; initially the solvent contained around 3 ppm of water. Since the catalyst with the $BARF^-$ counter-ion was resistant to small traces of water[53] and rigorous exclusion of water was not necessary we decided to not dry it completely. After the first attempts, showing almost no reactivity we decided to dry the solvent through distillation under CaH_2 and storage under molecular sieves 3Å[54]. This allowed the reaction to happen with an excellent yield of (R)-DiBnEquol (**7**) showing that even small traces of water are enough to deactivate completely all this catalyst.

Table 6: Step 5, asymmetric hydrogenation



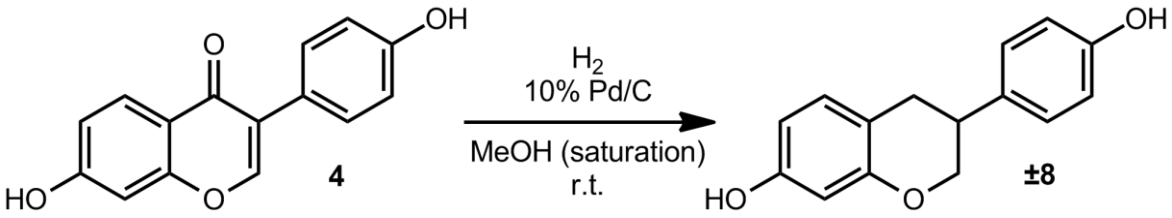
Entry	Time	Yield
1	10 h	0%
2	66 h	5,5%
3	90 h	11,5%
4	149 h	12,0%
5	10 h	62,4%
6	22 h	97,0%

After optimizing the reaction we produced racemic Equol (**±8**) with a simple hydrogenation of **4** to test the chiral column and the eluent. Then we deprotected the -OH of **7** through a hydrogenation with $Pd/BaSO_4$ and analyzed the e.e.

through an HPLC with inverse phase, comparing it with **±8** synthesized with a Pd/C catalyzed hydrogenation.

Hydrogenation of Daidzein to (±)-Equol with Pd/C

Table 7: Hydrogenation of Daidzein.



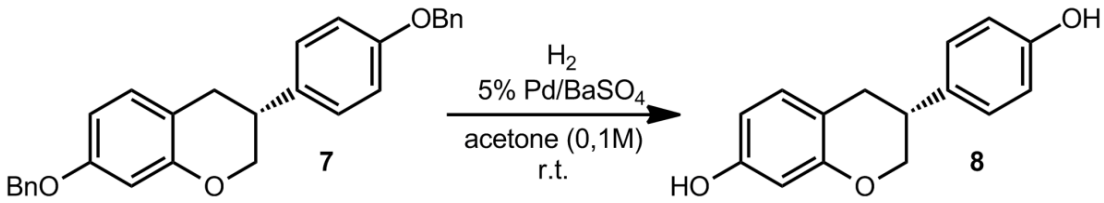
Entry	Time	Cat. (% m/m)	Yield
1	27h 10’	200%	74,3%
2	18h 21’	20%	87,1%
3	24h	20%[a]	0%

a: using $Pd/BaSO_4$

Initially we were using a procedure that was using a huge amount of catalyst[55] the high amount of catalyst was justified in the article by the low reactivity of **4**, however for us even with 1/10 of the amount suggested the reaction seems to work perfectly and less product is kept by the charcoal.

Step 6: Hydrogenation to Equol with Pd/C

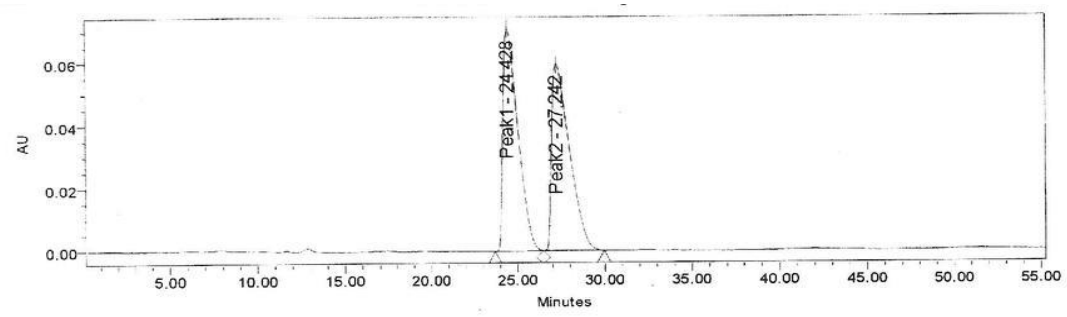
Table 8: Deprotection of 7



Entry	Time	Cat. (% m/m)	Yield
1	3 h	10%	95,1%

The deprotection of **7** has been made with $Pd/BaSO_4$, a poisoned catalyst, to avoid every minimal unwanted hydrogenation.

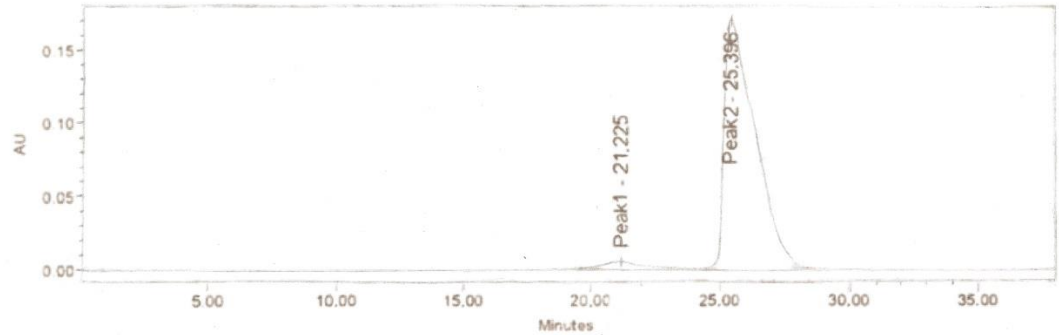
The HPLC revealed that we obtained an e.e. of 97%.



Peak Results

	Name	RT	Area	Height	% Height	% Area	Start Time	End Time
1	Peak1	24.428	4249534	71296	54.33	49.95	23.697	26.463
2	Peak2	27.242	4258285	59920	45.67	50.05	26.463	29.963

Fig. 37: HPLC graph of racemic Equol.



Peak Results

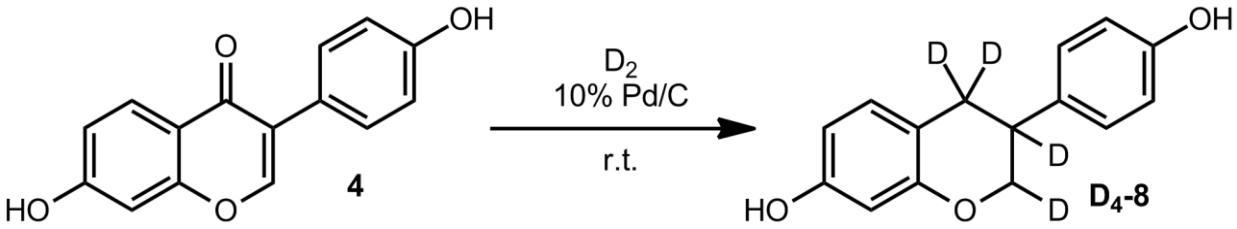
	Name	RT	Area	Height	% Height	% Area	Start Time	End Time
1	Peak1	21.225	216032	3898	2.23	1.51	20.495	24.295
2	Peak2	25.396	14051801	171007	97.77	98.49	24.295	26.878

Fig. 38: HPLC of our final product.

Deuteration to D_4-Equol with Pd/C

After having improved the hydrogenation of **4** we used the same procedure to obtain labelled Equol using D_2. This product will be used by a colleague for further deuterations of the aromatic rings.

Table 9: Deuteration of Equol.



Entry	Time	Solv. (saturation)	Cat:4 (% m/m)	Yield	Isotopic purity
1	24h 36’	EtOH	20%	68,61%	0%
2	48h 49’	EtOD	10%	90,70%	>99%

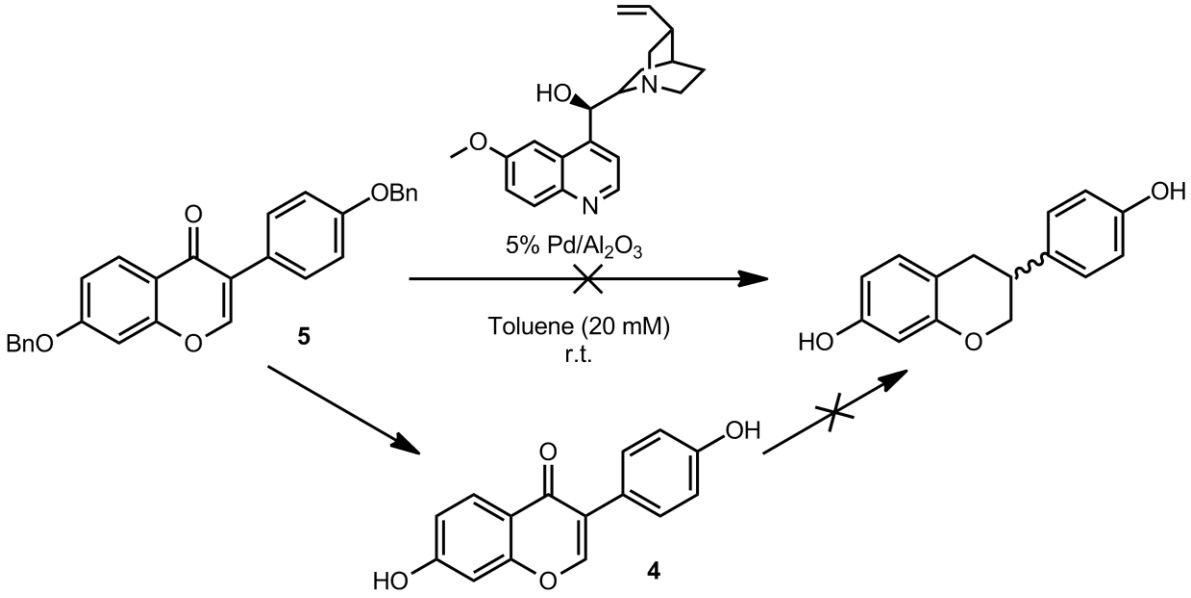
Completed this part of the project we attempted and hydrogenation of **4** with Pd/Al_2O_3 and quinine as modifier.

Hydrogenation with QN-modified Pd

It is known that the modifier poisons the catalyst itself so we use as reagent the DiBn-Daidzein to check if the catalyst is able to deprotect the alcohol or if it is totally deactivated.

We used a small amount of reagent in a big amount of catalyst, in entry 1 we decided to add more catalyst because after 24h there were no trace of activity, the rate QN : Pd was already lower compared to the amounts used in other studies[21,c], but even decreasing the amount of QN (entry 2) the reaction never goes to completion being able only to deprotect the OH but not to hydrogenate the carbonyl and the double bond. This may be due to the big sterical hindrance that impede **4** to attack to a catalyst modified with QN.

Table 10: Hydrogenation with QN-modified Pd



Entry	Time	Cat : 5 (m/m)	QN : Pd (m:m)	Yield
1	51h	8:5[a]	5:6[b]	0%
2	66h	3:1	2:3	0%

a: initial rate = 6:5 after 24h more catalyst has been added to reach the rate in the table

b: initial rate = 3:2 after 24h more catalyst has been added to reach the rate in the table

CONCLUSION AND FUTURE DEVELOPMENTS

In this thesis we have achieved the objective to experiment and develop a new path for the synthesis of Equol with an overall yield of 38% and a e.e. of 97%.

Deciding to not start from Daidzein but to synthesize it in the beginning of our path has decreased the expenses but has revealed to be one of the weakest point of our path, the decision to not analyze singularly the second step was due to the will to improve it in the one-pot procedure estimating its yield through the yield of the first step.

The decision to reduce the keto group with the 9-BBN has revealed an interesting mechanism of the hydroboration further studies could be done in this argument through the deuteration of 9-BBN with D_2 according to Nelson[56] to have a further confirmation and a new labelling of equol.

The necessity of a high excess of this reagent for this reaction is probably linked to the last step of the mechanism where we have the attachment of 9-BBN to the $C{=}O^+$, we suppose this because from the TLC seems that the first attachment happens in the first 2 hours of the reaction showing a new fluorescent spot that never disappears, we attempted to isolate what we were thinking was the intermediate but without success.

For the key step of our path, the hydrogenation through Ir catalyst, we used a commercial catalyst that had previously showed really good results in the hydrogenations of aryl substituted olefins and even with our intermediate we obtained an excellent result. This could be further improved by testing different Ir catalyst considering the possible mechanisms that have been proposed for this catalyst.

The necessity for a protection and deprotection increased the length of our synthesis, for this reason we decided to try the hydrogenation with QN-modified Pd on alumina, previous hydrogenations with Pd/C have reduced completely both the carbonyl and the unsaturation making us hope to obtain the same result with an e.e., even obtaining not a complete reduction but only a reduction of the unsaturation would anyway have shortened the synthetic route.

We used only QN as modifier because due to the extreme similarity of all the alkaloids of cinchona we wanted just to explore the potential of this route,

obtaining just Daidzein and just minimal traces of other products suggested us to stop developing this route.

The substrates previously used for this asymmetric hydrogenation were smaller and able to coordinate with both the alcoholic group and the aminic nitrogen on the QN, our molecule, being flat and having the OH groups in the extremities has not only problems in the occupation of the active site under the quinuclidine but in case it would, probably the difference of energy between re and si face would be almost none.

The possibility to obtain better result in the synthesis of Equol with this strategy could be tested using a smaller substrate.

We have seen that good result have been obtained in the synthesis of O-DMA through the same process of friedel-craft acylation used by us in the very first step[47].

So it may be a good idea to explore the asymmetric hydrogenation of 2-(4-hydroxyphenyl)-propionic acid and, in case of success, try to develop a new synthesis starting from here.

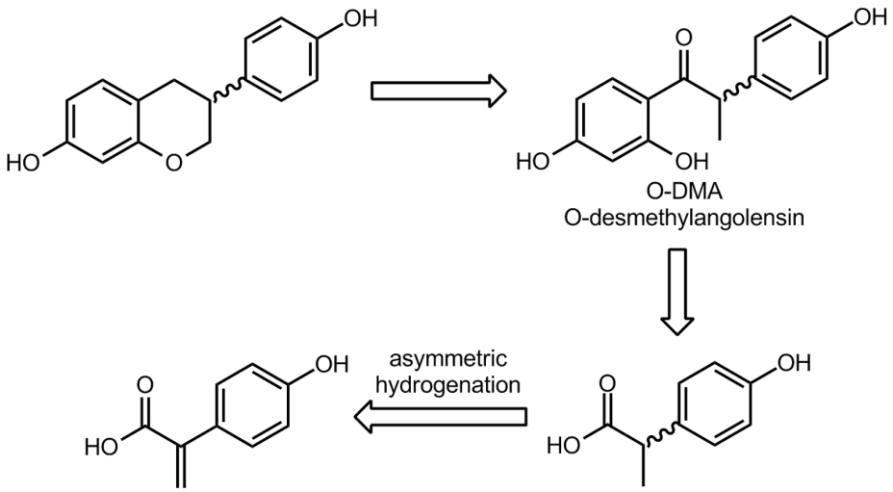


Fig. 39: possible new route.

EXPERIMENTAL SECTION

Reagents

From Sigma-Aldrich:
Resorcinol (ReagentPlus)
$BF_3 \cdot Et_2O$ (≧47% BF_3)
9-BBN (0,5M in THF)
Pd/C (10% m/m)
$Pd/BaSO_4$ (10% m/m)
Pd/Al_2O_3 (5% m/m)
d_1 EtOD (>99,9%)
DCM
DMF

From KeboLab:
4-hydroxyphenyl acetic acid

From Merck:
$MeSO_2Cl$
BnCl

From Fisher Chemicals:
K_2CO_3 (dry)

From Altia Oyj.:
EtOH (96%)

The water content of every solvent for the step 1-2 one pot, 3 and 5 has been checked with a Karl-Fischer titration.

Instruments

All the products have been analyzed with H^1-NMR and C^{13}-NMR spectroscopy with a Varian Mercury 300 MHz at room temperature. The reference is TMS in all the samples in $CDCl_3$ and the solvent in all the samples in DMSO.
To indicate the multiplicity we used the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad signal.
The reactions have been followed through TLC and the purifications for the analysis have been made through column (except for the Step 1-2 one pot and Step 3 in which recrystallization is used) with silica.
The solvents have been used without further purification if not stated otherwise, the amount of water in solvent has been determined though a Karl-Fisher titration in a Mettler Toledo DL32 coulumeter titrator.

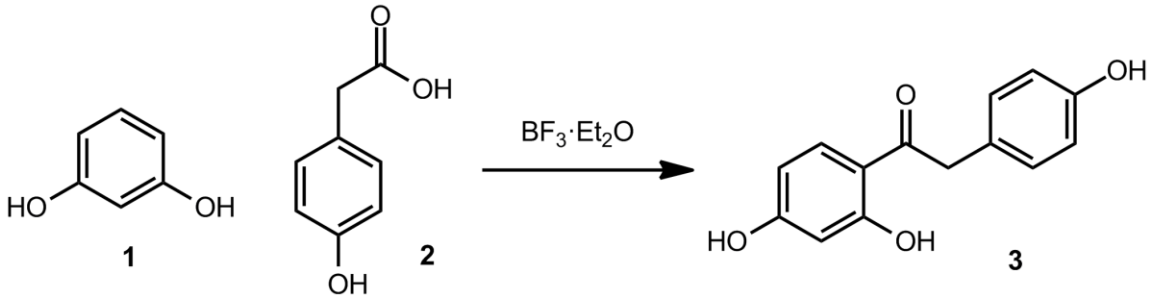
For additional analysis to confirm our product and to check the isotopic purity of the deuterated ones a MS has been taken of all of them in a EI-MS Jeol JMS-700.

The enantiomeric excess has been analyzed with a Polarimeter Jasco DIP-1000 and determined with a column Cyclobond I RSP 2000; 250 x 10mm; Bonded phase: (R,S)-Hydroxypropyl modified beta-cyclodextrin. Eluent used ACN : aq. HCOOH 1% 35:65, 3,5 mL/min.

Optimized procedures

STEP 1 (Table 1)

Synthesis of 1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)ethanone (3)



A mixture of **1** (1,1011 g, 1 mol) and **2** (1,5216 g, 1 mol) are inserted in a dry, 2-neck round bottom flask and dried overnight under vacuum, the flask has been refilled with Ar from a balloon attached to the flask and evacuated 3 times leaving Ar in the end. $BF_3 \cdot Et_2O$ (25mL, 20 mol eq) is added dropwise and the reaction mixture is left under magnetic stirring for 15' at 115°C. The reaction was monitored with TLC in DCM:EtOAc (7:2).

The product is extracted with DCM and purified (if needed) with a column using DCM:EtOAc (7:5) as eluent.

Yield: 80,1%

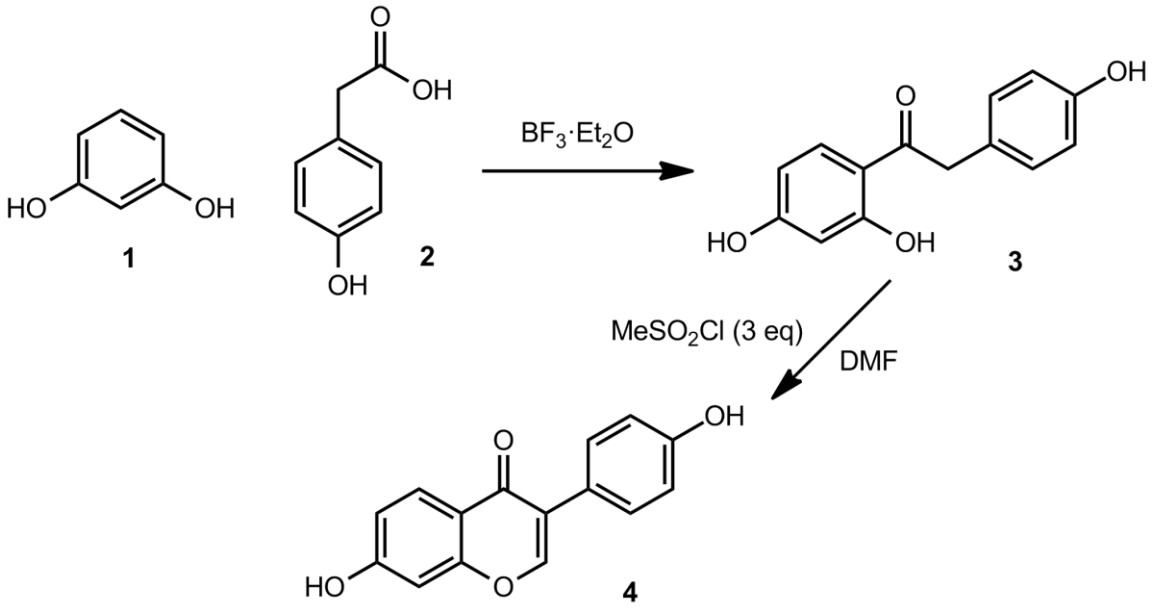
^{1}H-NMR: 3,37 (dd, 1H J_1= 2,4 Hz J_2= 9,0 Hz), 4,12 (s, 2H), 6,24 (d, 1H J= 2,4 Hz), 6,75-6,60 (m, 2H), 7,12-7,00 (m, 2H), 7,92 (d, 1H, J= 8,7 Hz), 9,26 (s, 1H), 10,63 (s, 1H), 12,59 (s, 1H)

^{13}C-NMR: 43,26; 102,45; 108,20; 112,05; 115,20; 125,16; 130,33; 133,56; 164,70; 164,87; 202,67.

MS-EI: 244 (24%), 137 (100%), 122(10%), 107(18%), 97(11%), 81(16%), 69(17%), 58(82%)

STEP 2 (Table 2)

Synthesis of Daidzein (4)



A mixture of **1** (1,1011 g, 1 mol) and **2** (1,5216 g, 1 mol) are inserted in a dry, 2-neck round bottom flask and dried overnight under vacuum, the flask has been refilled with Ar from a balloon attached to the flask and evacuated 3 times leaving Ar in the end. $BF_3 \cdot Et_2O$ is added dropwise and the reaction mixture is left under magnetic stirring for 15' at 115°C. The reaction was monitored with TLC in DCM:EtOAc (7:2).

When the reaction is completed the reaction is cooled down to 0°C and dry DMF (15 mL) is added dropwise successively $MeSO_2Cl$ (2,4 mL) is mixed with DMF (4 mL), cooled to 0°C and added dropwise to the reaction mixture. The temperature is increased to 80°C for the first 24h then decreased to 70°C for the final 24h. The reaction is monitored with TLC in DCM:EtOAc (7:2).

The reaction is quenched in a sol. of NaOAc, the reaction flask is cleaned with the minimum amount of methanol.

The quenched reaction is kept under vacuum to eliminate most of the methanol and is left to rest for 1 week.

Successively the reaction mixture is filtered and 4 is cleaned until the desired purity with methanol or ethanol (the time intervals between adding the solvent and filtering has to be at least of 1 day).

Yield: 73,6%

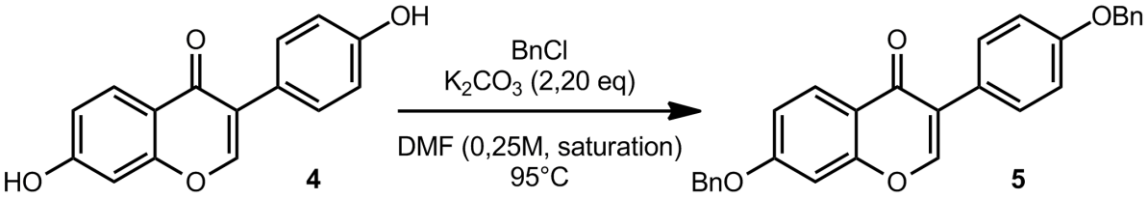
^{1}H-NMR: 6,69-6,88 (m, 3H), 6,94 (d, 1H, J=2,4 Hz), 7,38 (m, 2H, J=8 Hz), 7,96 (d, 1H, J=8,7 Hz) 8,27 (s, 1H), 9,51 (s, 1H), 10,75 (s, 1H).

^{13}C-NMR: 102,08; 114,93; 115,10; 116,63; 122,53; 123,48; 127,27; 130,05; 152,77; 157,15; 157,40; 162,47; 174,46.

MS-EI: 254(78%), 137(39%), 118(21%)

STEP 3 (Table 3)

Synthesis of 7-(benzyloxi)-3-(4'-(benzyloxi)phenyl)4H-chromen-4-one (5)



The compound **4** (1g, 4,2 mol) and K_2CO_3 (1,42 g, 2,4 eq) are inserted in a dry, 2-neck round bottom flask and dried overnight under vacuum, the flask has been refilled with Ar from a balloon attached to the flask and evacuated 3 times leaving Ar in the end. Successively DMF (20 mL) is added through a septum and the reaction mixture is stirred magnetically until complete dissolution.

BnCl (1mL, 2,05 eq with pure 4, 1,5 mL, 3 eq with crude 4) is added dropwise and the reaction mixture is left stirring for 2h at 95°C monitoring the reaction with TLC in DCM:EtOAc (7:2).

The reaction is quenched in distilled water, cooled in ice and filtered. If some daidzein is present or if the daidzein used is the crude product of the previous steps further purification in a flash column with DCM is needed. If the Daidzein used was not purified the purification is done through recrystallization in DCM.

Yield: 95,6%

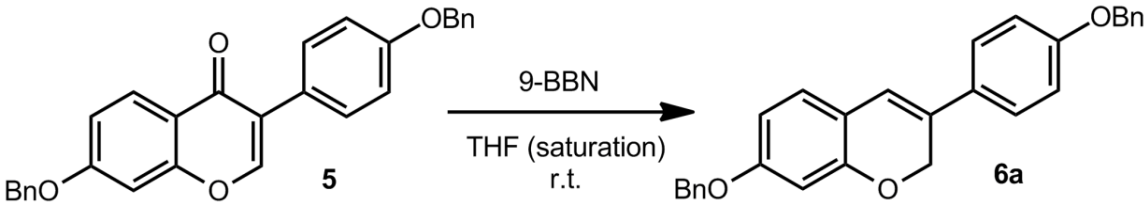
^{1}H-NMR: 5,03 (s, 2H), 5,10 (s, 2H), 6,85 (d, 1H, J=2,4 Hz), 6,90-7,15 (m, 3H), 7,23-7,46 (m, 12H), 7,83 (s, 1H), 8,15 (d, 1H, J= 8,7 Hz)

^{13}C-NMR: 70,20; 70,69; 101,39; 115,05; 115,15; 118,77; 123,63; 125,01; 127,58; 127,65; 128,02; 128,10; 128,55; 128,74; 128,91; 130,29; 135,88; 137,09.

MS-EI: 434(55%), 343(29%), 91(100%), 77(18%)

STEP 4 (Table 4)

Synthesis of 7-(benzyloxi)-3-(4'-(benzyloxi)phenyl)2H-chromene (6a)



The compound **5** (0,5 g 1,17 mol) is inserted in a dry, 2-neck round bottom flask and dried overnight under vacuum, the flask has been refilled with Ar from a balloon attached to the flask and evacuated 3 times leaving Ar in the end. Successively THF (approx 78 mL) is added while stirring until all the compound is dissolved and 9-BBN (8,5 eq) is added dropwise. The reaction mixture is left under stirring for 22 h and is monitored with TLC in DCM. When the reaction is complete it is quenched in water and filtered. If further purification is needed is possible to purify in a flash column with DCM.

Yield: 59,9%

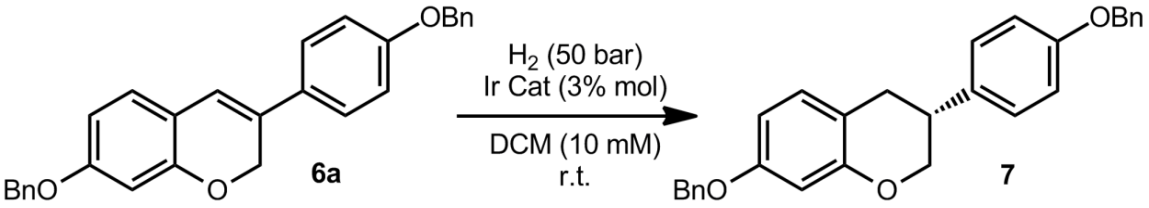
^{1}H-NMR: 5,05 (s, 2H), 5,06-5,12 (m, 2H), 5,09 (s, 2H), 6,50-6,57 (m, 3H), 6,68 (s, 1H), 6,69-7,05 (m, 2H), 7,26-7,49 (m, 12H)

^{13}C-NMR: 31,08; 67,44; 70,26; 102,55; 108,38; 115,13; 115,24; 116,81; 118,32; 125,64; 125,93; 127,55; 127,61; 128,12; 128,62; 128,73; 128,76; 129,94; 137,00.

MS-EI: 420(55%), 329(64%), 106(55%), 105(54%), 91(100%), 77(48%)

STEP 5 (Table 5)

Synthesis of (R)-7-(benzyloxi)-3-(4'-(benzyloxi)phenyl)chroman (7)



The compound **6a** (0,080 g, $1{,}91*10^{-4}$ mol) is inserted in a dry test tube for autoclave and dried overnight under vacuum, the flask has been refilled with Ar from a balloon attached to the flask and evacuated 3 times leaving Ar in the end. keeping everything under Ar atm the catalyst **A** (9 mg, 0,03 eq) then dry DCM (9 mL) are added and the test tube inserted in the autoclave and this one is closed with a manometer.

Successively 50 bar of H_2 are added to the manometer and evacuated slowly, after repeating this procedure three times the reaction is left under slow stirring until the completion of the reaction (22h). The H_2 left is evacuated slowly and the reaction mixture is plugged in a column with DCM.

Yield: 97%

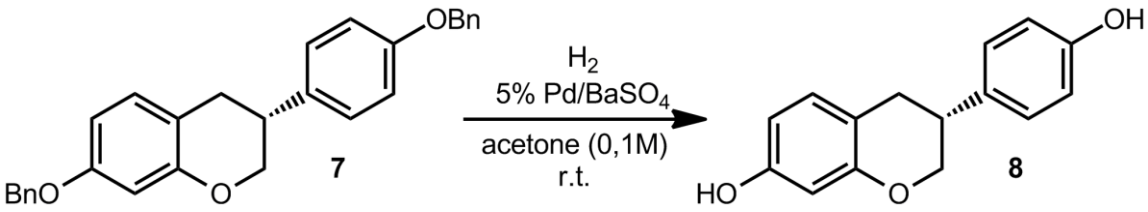
^{1}H-NMR: 2,93 (dd, 2H, J_1= 1,2 Hz, J_2= 9 Hz), 3,17 (m, 1H), 3,96 (t, 1H, J= 10,5 Hz), 4,29 (ddd, 1H, J_1= 1,5 Hz, J_2= 3,3 Hz, J_3= 10,5 Hz), 5,02 (s, 2H), 5,05 (s, 2H), 6,50 (d, 1H, J= 2,7 Hz), 6,54 (dd, 1H, J_1= 2,7 Hz, J_2= 8,1 Hz), 6,96 (m, 3H), 7,14 (m, 2H), 7,26-7,48 (m, 10H)

^{13}C-NMR: 32,03; 38,04; 70,23; 71,25; 102,63; 108,25; 114,64; 115,28; 127,61; 127,69; 128,04; 128,12; 128,49; 128,71; 128,75; 130,33; 133,86; 137,16; 137,27; 155,14; 158,01; 158,49.

MS-EI: 422(73%), 210 (42%), 106(49%), 105(46%), 91(100%), 77(44%)

STEP 6 (Table 6)

Synthesis of (R)-Equol (8)



Compound **7** (0,2 g, 4,73*10^{-4} mol) and 10% $Pd/BaSO_4$ (20 mg, 20% m/m) are inserted in a flask, dissolved in acetone (5 mL) and the flash is evacuated and refilled with H_2 3 times.

The reaction is left under magnetic stirring at r.t. and is monitored by TLC in DCM:EtOAc 7:5.

When the reaction is complete the reaction mixture is plugged in a filter with celite and the product is separated in a column with DCM:EtOAc 7:5.

Yield: 95,1%

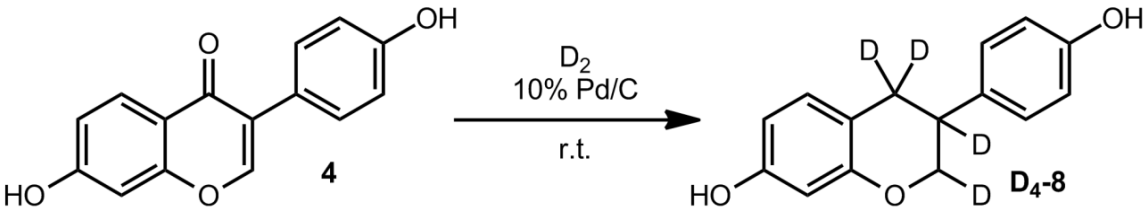
^{1}H-NMR: 2,71-2,90 (m, 2H), 2,94-3,08 (m, 1H), 3,89 (t, 1H J= 10,2 Hz), 4,14 (ddd, 1H J_1= 1,8 Hz, J_2= 3,6 Hz , J_3= 10,5 Hz), 6,18 (d, 1H, J= 2,4 Hz), 6,27 (d, 1H, J= 8,1 Hz), 6,71 (m, 2H), 6,85 (d, 1H, J= 8,1 Hz), 7,10 (m, 2H), 9,13 (s, 1H), 9,25 (s, 1H)

^{13}C-NMR: 31,30; 31,16; 48,59; 70,25; 102,47; 112,55; 115,23; 128,29; 130,04; 131,64.

MS-EI: 242(73%), 135(17%), 123(43%), 120(53%), 107(14%), 58(14%)

$[\alpha]_D^{25}$: +18,5° (Lit.[57] : +23°)

Synthesis of D_4-Equol



Compound **4** (1 g, 3,93*10^{-3} mol) and 10% Pd/C (50 mg, 20% m/m) are inserted in a flask, dissolved in MeOD (25 mL) and the flash is evacuated and refilled with D_2 3 times.

The reaction is left under magnetic stirring at r.t. and is monitored by TLC in DCM:EtOAc 7:2.

When the reaction is complete the reaction mixture is plugged in a filter with celite and the product is separated in a column with DCM:EtOAc 7:2.

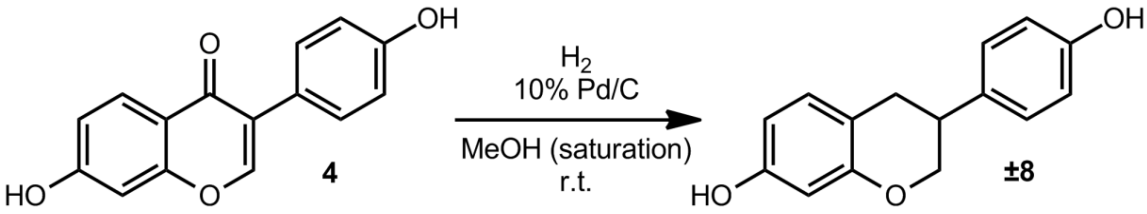
Yield: 90,7% ; I.P.= >99%

^{1}H-NMR: 3,87 (s, 1H), 4,11 (s, small signal), 6,18 (d, 1H, J= 2,4 Hz), 6,27 (d, 1H, J= 8,1 Hz), 6,71 (m, 2H), 6,85 (d, 1H, J= 8,1 Hz), 7,10 (m, 2H), 9,13 (s, 1H), 9,25 (s, 1H)

^{13}C-NMR: 31,30; 31,16; 48,59; 70,25; 102,47; 112,55; 115,23; 128,29; 130,04; 131,64.

MS-EI: 246(12%), 125(13%), 122(17%), 58(42%)

Synthesis of (±) Equol



Compound **4** (1 g, 3,93*10^{-3} mol) and 10% Pd/C (50 mg, 20% m/m) are inserted in a flask, dissolved in MeOH (25 mL) and the flash is evacuated and refilled with H_2 3 times.

The reaction is left under magnetic stirring at r.t. and is monitored by TLC in DCM:EtOAc 7:2.

When the reaction is complete the reaction mixture is plugged in a filter with celite and the product is separated in a column with DCM:EtOAc 7:2.

Yield: 87,1%

^{1}H-NMR: 2,71-2,90 (m, 2H), 2,94-3,08 (m, 1H), 3,89 (t, 1H J= 10,2 Hz), 4,14 (ddd, 1H J_1= 1,8 Hz, J_2= 3,6 Hz , J_3= 10,5 Hz), 6,18 (d, 1H, J= 2,4 Hz), 6,27 (d, 1H, J= 8,1 Hz), 6,71 (m, 2H), 6,85 (d, 1H, J= 8,1 Hz), 7,10 (m, 2H), 9,13 (s, 1H), 9,25 (s, 1H)

^{13}C-NMR: 31,30; 31,16; 48,59; 70,25; 102,47; 112,55; 115,23; 128,29; 130,04; 131,64.

MS-EI: 242(73%), 135(17%), 123(43%), 120(53%), 107(14%), 58(14%)

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