Investigating the Mechanism for the Metal-free Dehydrogenative Coupling of Acetone with 1,2,3,4-Tetrahydroisoquinoline

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Abstract

THIQ is a common scaffold in the pharmaceutical industry. Its functionalization usually consists of disparate pathways, often involving transition metal catalysis and numerous synthesis steps. Several studies on variously catalysed functionalisation of THIQ had been proposed in the past, with a particular curiosity towards cross-dehydrogenative coupling reactions. The dehydrogenative coupling reaction of THIQ with acetone, in presence of oxygen and a Brønsted acid, forms the Mannich product (1-(1,2,3,4-tetrahydroisoquinolin-1-yl)propan-2-one) without the need of a metal, photo- or organo-catalyst. This reaction has unknown behaviour and proceeds through the formation of an intermediate containing a peroxy-group. An investigation about the mechanism of the formation of this structure, involving rate, kinetic isotope effect and radical trapping experiments, is here proposed. A route to obtain the pure intermediate and a method to measure the rates and kinetic isotope effect(s) are provided, while radical traps experiments did not produce the expected outcomes, suggesting that more endeavours are needed to completely comprehend this reaction.

Keywords: Metal-free; CDC; THIQ; Acetone

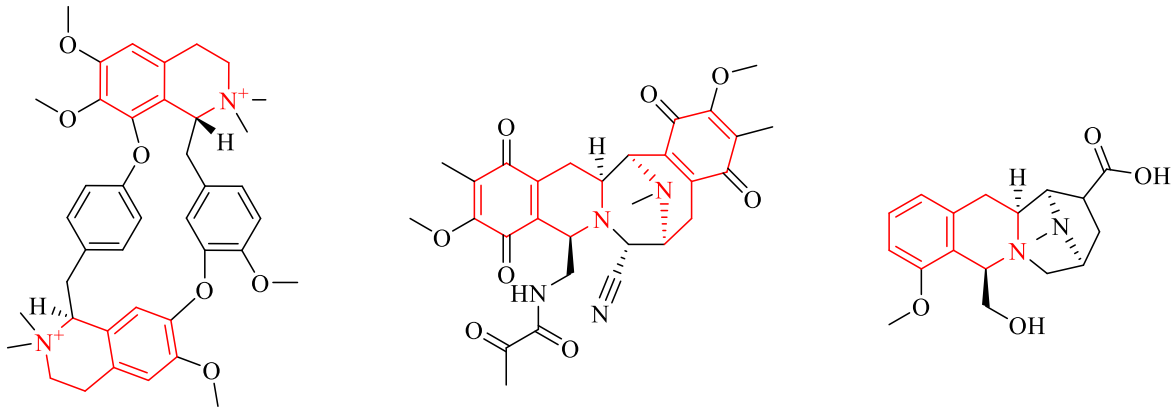
Riassunto

La 1,2,3,4-Tetraidroisochinolina rappresenta una struttura comune nell'industria farmaceutica. La sua funzionalizzazione può avvenire in vari modi, i quali spesso coinvolgono catalizzatori metallici e numerosi step di sintesi. Diversi studi sulla funzionalizzazione della THIQ sono stati proposti in passato, con particolare interesse riguardo alle reazioni di coupling cross-deidrogenativo. La reazione di coupling deidrogenativo della THIQ con acetone, in presenza di ossigeno e un acido di Brønsted, forma il prodotto 1-(1,2,3,4-tetrahydroisoquinolin-1-yl)propan-2-one (Mannich product) senza la necessità di un fotocatalizzatore, di un organocatalizzatore o di un catalizzatore metallico. La reazione procede attraverso la formazione di un intermedio contenente un gruppo perossidico. Viene qui proposto uno studio sul meccanismo della reazione, riguardante la cinetica, l'effetto isotopico cinetico e l'uso di trappole radicaliche. Una via per ottenere l'intermedio puro e un metodo per effettuare esperimenti sulla velocità di reazione sono forniti. Gli esperimenti riguardanti le trappole radicaliche hanno prodotto risultati inattesi, per i quali si necessitano ulteriori investigazioni.

Parole chiave: Metal-free; CDC; THIQ; Acetone

Introduction

1,2,3,4-Tetrahydroisoquinoline (**THIQ**) is a secondary amine of great importance in the pharmaceutical industry.[1] This is because of its large range of bioactive derivatives, which belong to the wide group of bioactive molecules named alkaloids. Thereby, it can be found in different kinds of drugs (Scheme 1), spacing from muscle relaxants to antitumor drugs or antibiotics, such as Metocurine, Saframycin A and Quinocarcinol, respectively. [1–3]



Metocurine
Muscle relaxant

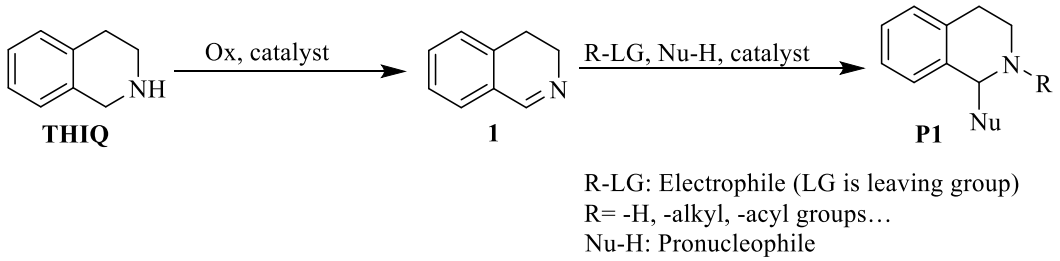
Saframycin A
Antitumor and antibacterical drug

Quinocarcinol
Antibiotic with antibacterical and antitumor activity

Scheme 1 – Examples of alkaloids containing the THIQ scaffold, highlighted in red.

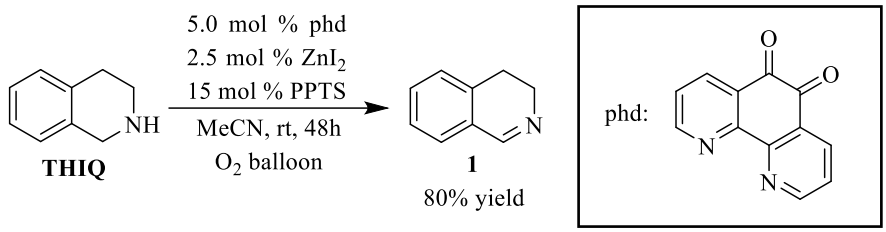
The synthesis of these drugs often involves the functionalization in C1 (benzylic) position, which can be carried out in several ways. On this behalf, much effort has been dedicated to the research of new methods for this kind of functionalization by the scientific community.[4]

Several methods reckon on a pre-functionalization of **THIQ** and, then, its reaction with the desired substrate. Often, the pre-functionalization step is an oxidation that forms either the imine (**1**) or the iminium ion of **THIQ** (whether it is N-substituted or not). These structures then undergo a nucleophilic addition, that is often catalysed, leading to structure **P1** (Scheme 2).[4]



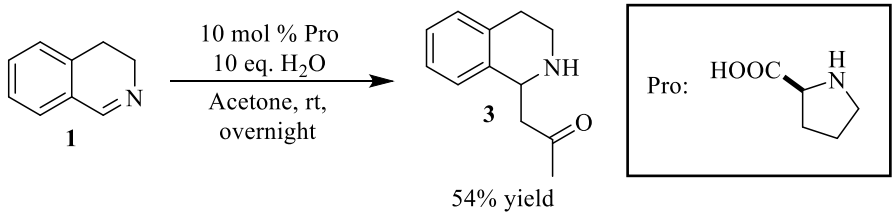
Scheme 2 – General functionalization process workflow.

An example of pre-functionalization can be the catalytic aerobic oxidation proposed by Wendlandt *et al.* (Scheme 3).[5] This makes use of a zinc-centred catalyst in which the ligand is phd, that has the role of oxidising the substrate (**THIQ**). The reduced structure of phd, phd-H_2, is then oxidised back by oxygen.



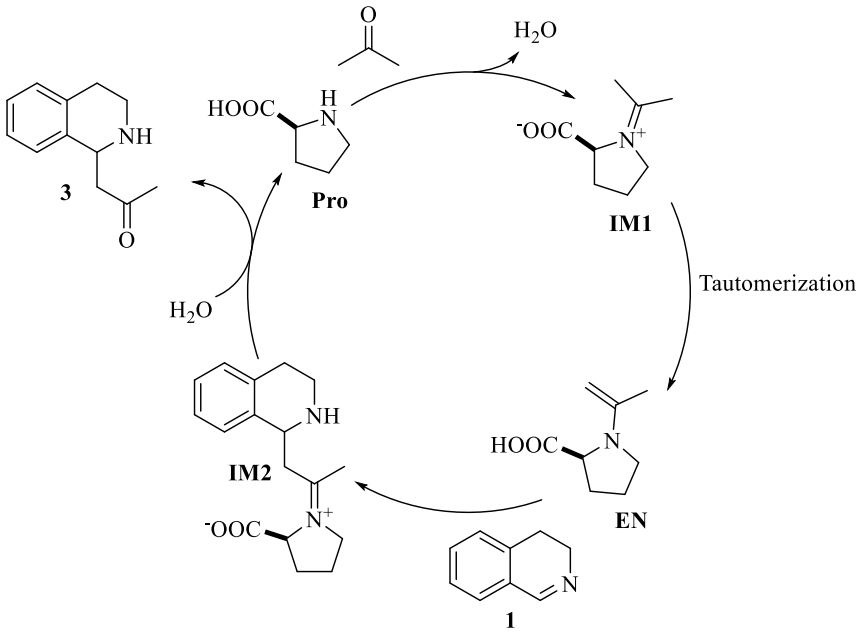
Scheme 3 – Catalytic Aerobic Oxidation of THIQ.

A case of nucleophilic addition in α-position to the nitrogen, after imine (**1**) formation, is the one that has been studied by Monaco *et al.* and reproposed by Veenstra and Harutyunyan, from our research group (Scheme 4).[6,7] In this catalytic reaction, the Mannich product (**3**) is formed by using a bifunctional organocatalyst, L-proline (**Pro)**. An excess of water is used to hydrolyse the iminium ion (**4)** that gets formed when THIQ attacks acetone (Scheme 5).[7]



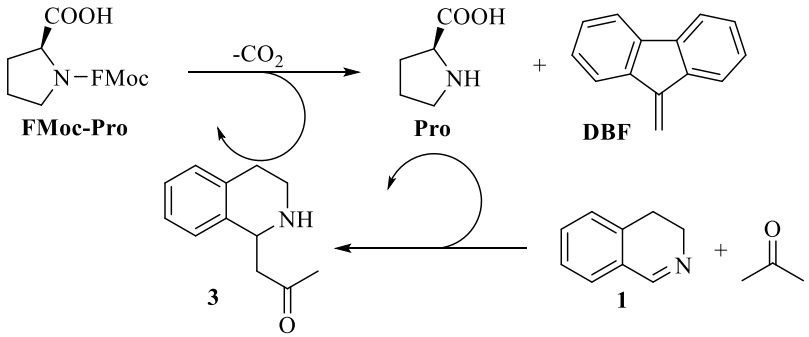
Scheme 4 – Organocatalysed addition of acetone to 3,4-dihydroisoquinoline.

Pro is commonly used in enamine catalysis, which has been largely exploited in reactions such as aldol, Mannich, Michael, Diels-Alder reactions, alkylations and halogenations.[8]
The mechanism of this reaction (Scheme 5) consists mainly in four steps. In the first one, **Pro** attacks acetone, forming the iminium ion (**IM1**). This tautomerizes to the enamine (**EN**), which is now the activated nucleophile. Therefore, this structure can attack the imine (**1**), generating a new iminium ion (**IM2**) that is afterwards hydrolysed by water, eventually giving the Mannich product (**3**).[7,9]



Scheme 5 – Catalytic cycle of L-proline in the Mannich Reaction between acetone and 3,4-dihydroisoquinoline.

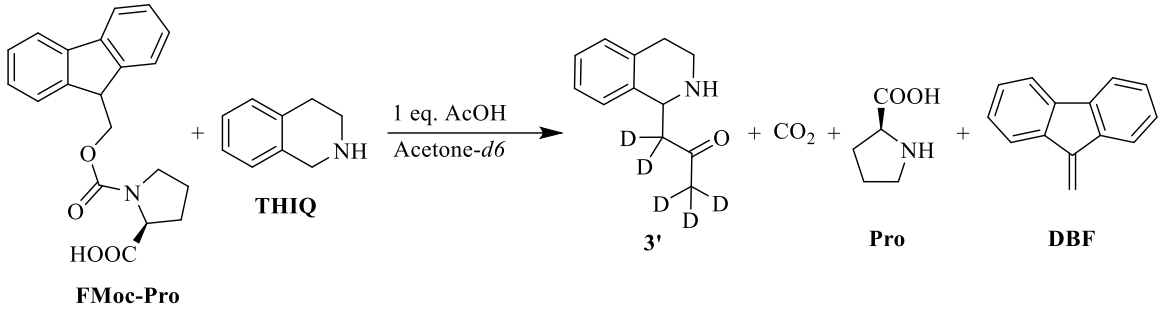
The functionality of the L-proline has been exploited to design a cross-catalytic system (CCS) in which two reaction are carried out and the product of a reaction catalyses the other one (then, the formation of the other product).[7] More in detail, the CCS proposed by Veenstra *et al.* (Scheme 6) make use of protected Pro (**7**), which is catalytically unactive. For its deprotection, a base, such as the Mannich product (**3**), is exploited. Hence, product **3** becomes the catalyst for the deprotection reaction, while the deprotected **Pro** catalyses the Mannich reaction. Thus, the CCS is established.



Scheme 6 – Representation of the cross-catalytic system developed by Veenstra et al.

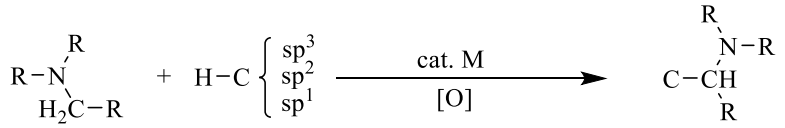
Such systems can be studied as small-scale reproductions of the biological processes. When more cross-catalytic systems are put together, they form an hypercycle (single products catalyse more than one reaction each).[9] This term was first used to describe in some self-replication reactions of biopolymers.[10]

However, another functionalization reaction, initially observed by Veenstra, showed an uncommon behaviour. The Mannich product (**3'**) (Scheme 4) was obtained without pre-fuctionalization of the amine, using acetone-*d*6, one equivalent of acetic acid and **Fmoc-Pro** (Scheme 7).[9]



Scheme 7 – Reaction observed by Veenstra.

The behaviour of this reaction resambles that of a cross-dehydrogenative coupling (CDC). This is a kind of reaction in which a new C-C bond is formed from two different C-H bonds (Scheme 8), under oxidative conditions. These are given by oxidising agents and may differ a lot between one reaction and another. When the oxidant of such a reaction is oxygen, the process is called *aerobic oxidation*.[11] CDC reactions can occur in several fashions, involving various catalysts, and represent viable alternatives to pre-functionalization reaction.[12]



Scheme 8 – General CDC reaction on a secondary amine.

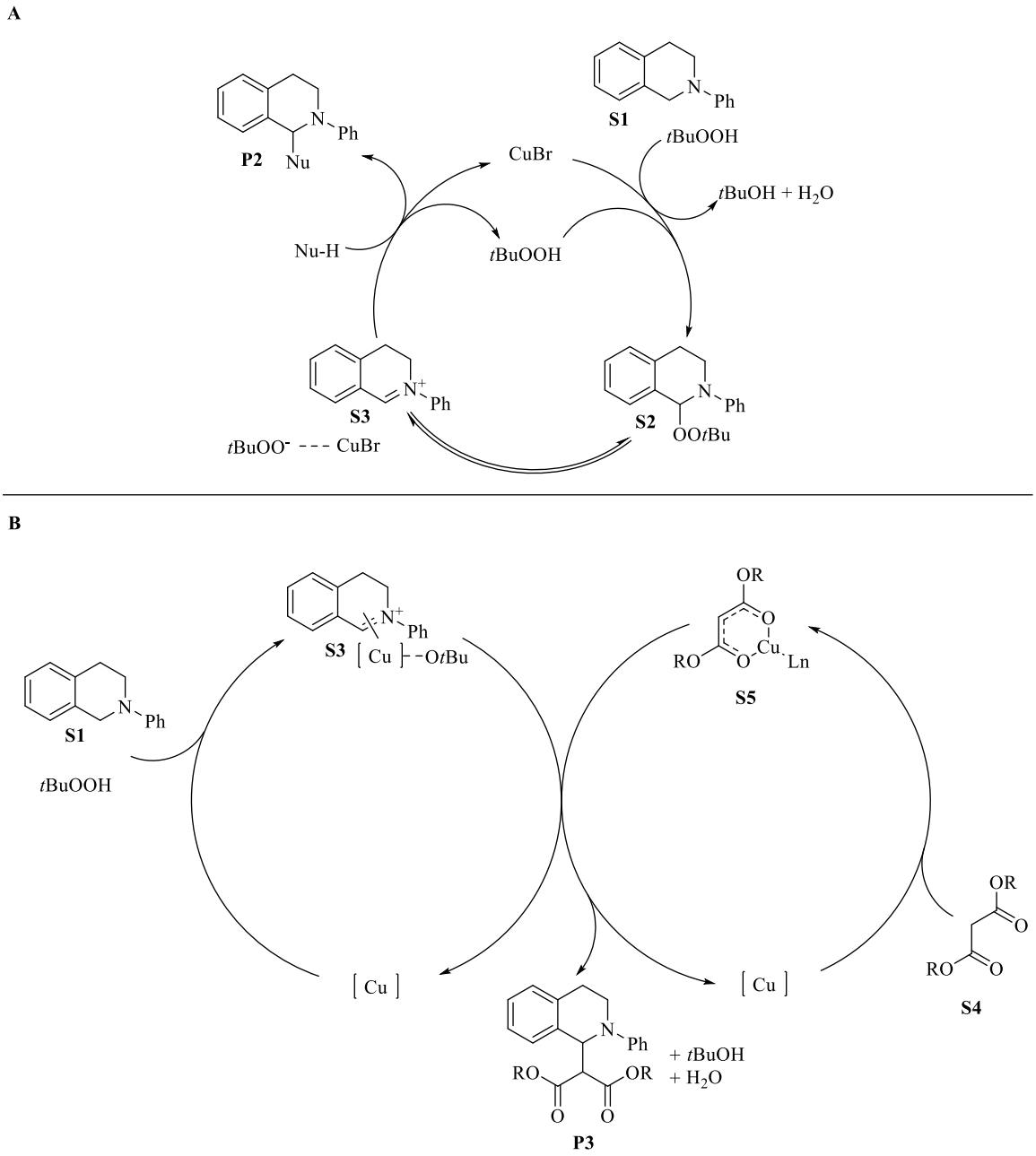
Since they can be catalysed by disparate types of catalysts, a brief introduction to the mechanisms of CDC reactions is given, starting from the metal-catalysed one.

Transition Metal-catalysed CDCs

Tertiary amines can be catalytically oxidised by transition metals, in presence of an oxidant. The reported mechanisms (Scheme 9) exploit Cu(I) as the catalyst and tertbutyl peroxide as the oxidising agent. The usage of this oxidant leads to the formation of the tertbutyl peroxyl-THIQ structure (**S2**), which is supposed to be in acid/base equilibrium with the

iminium ion (**S3**).[13] Thus, this electrophile structure can react with a nucleophile, giving the final product (**P2**).

In some situations, the metal catalyst may have a double function, both oxidizing the substrate and activating the nucleophile: this is the case of CDC reactions between tertiary amines and activate methylene compounds (Scheme 9, B). In the proposed catalytic cycle, the catalyst coordinates to the carboxylic oxygen atoms of malonate, activating the compound for the successive attack.[14]

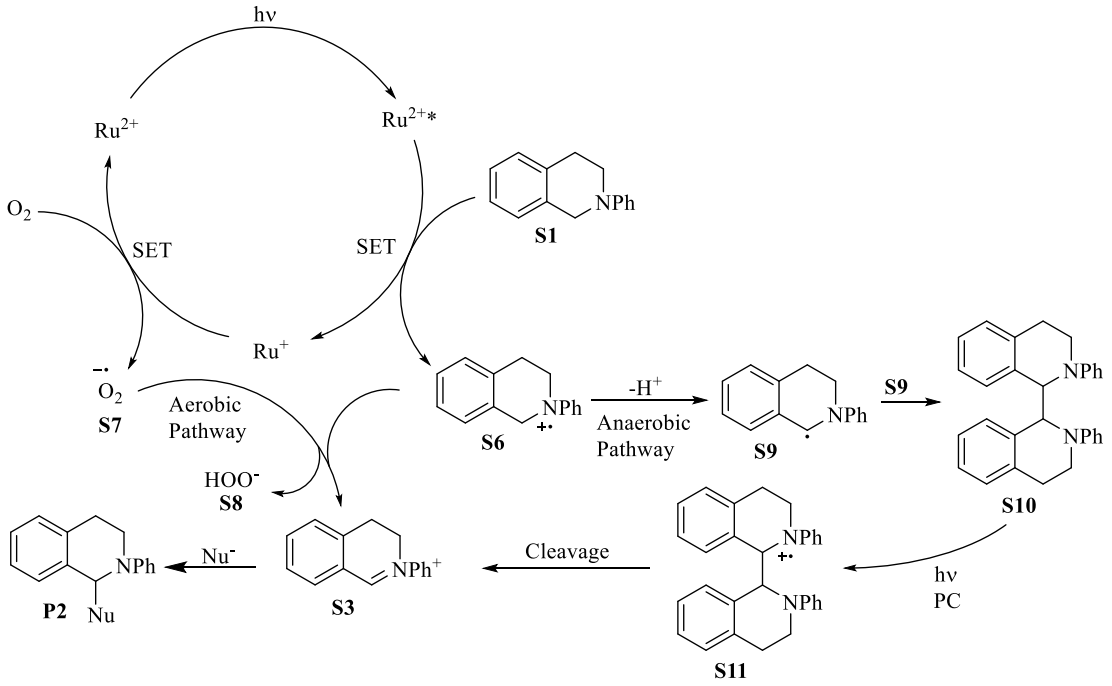


Scheme 9 – A. Cu(I) catalysed CDC reaction between a general nucleophile and a N-substituted THIQ; B. Cu(I) catalysed CDC reaction between an alkyl malonate and a N-substituted THIQ.

Photocatalysed CDCs

In every CDC reaction on N-substituted THIQ (**S1**), the oxidation of the substrate leads to the production of an electrophile compound, the iminium ion (**S3**). Several ways to obtain this structure exist; photocatalysis is one of these and involves, as its fundamental step, a single electron transfer (SET) from the substrate (**S1**) to the photocatalyst. In the reported example (Scheme 10), a Ru(II) complex is exploited.[15] The first step of the catalytic cycle is a light-induced excitation of the catalyst (Ru(II) → Ru(II)*). Then, **S1** undergoes an oxidation step which involves the transfer of one electron (SET) to the ruthenium complex, reducing the metal to Ru(I). This, in its turn, undergoes a SET in which it gets oxidised by oxygen, thereby obtaining again the initial Ru(II) complex. The yielded molecules, in this cycle, are a N-centred radical cation (**S6**) and a superoxide (**S7**). These can react again, giving a dioxidanide (**S8**) and the iminium ion (**S3**), which reacts with the nucleophile, yielding the final product.

However, photochemically catalysed CDC can be more complex than this. Several mechanisms may occur, even producing the same product. An example of this is reported by Bartling *et al.* in their mechanistic investigation: **S1**, after the SET, can undergo deprotonation (**S9**) and dimerization (**S10**).[15] A SET may newly occur between the dimer and the photocatalyst, leading to structure **S11**, which rearranges into the iminium ion (**S3**) *via* homolytic C-C cleavage.[15]



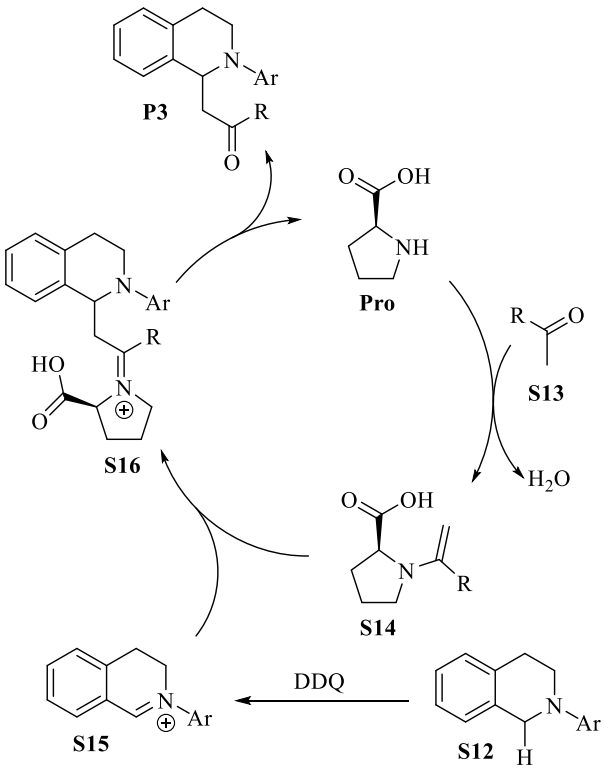
Scheme 10 – Mechanism of the Ru(II) catalysed CDC reaction between THIQ and a generic nucleophile.

Organocatalysed CDCs

Metal-containing catalysts are useful and efficient, but their environmental impact and toxicity constitute a problem for large-scale productions. These problems are lessened in organocatalysis, in which no transition metals are exploited. For this reason, organocatalysts are generally more attractive for green chemistry.

A case in which organocatalysis was used is represented by Scheme 5, with **Pro**. That is a kind of covalent catalysis, enamine catalysis, which reckons on the nucleophile activation of a carbonyl compound through the formation of an enamine. This allows the formation of the new C-C bond.[16,17]

The same mechanism and, also, the same catalyst can be exploited in CDC reactions (Scheme 11). The catalytic cycle of **Pro** is exactly the same, the difference resides in the substate: N-Aryl THIQ (**S12**) is the starting material, which is directly oxidised by a stoichiometric oxidant (for instance, DDQ), yielding the iminium ion (**S15**). Hence, this undergoes the nucleophilic attack of the enamine (**S14**), which gets formed by the condensation between **Pro** and the ketone. After the C-C bond formation, the resulting iminium ion is cleaved by the hydration of the double bond, giving the α-substituted structure (**P3**) and **Pro**.[16]



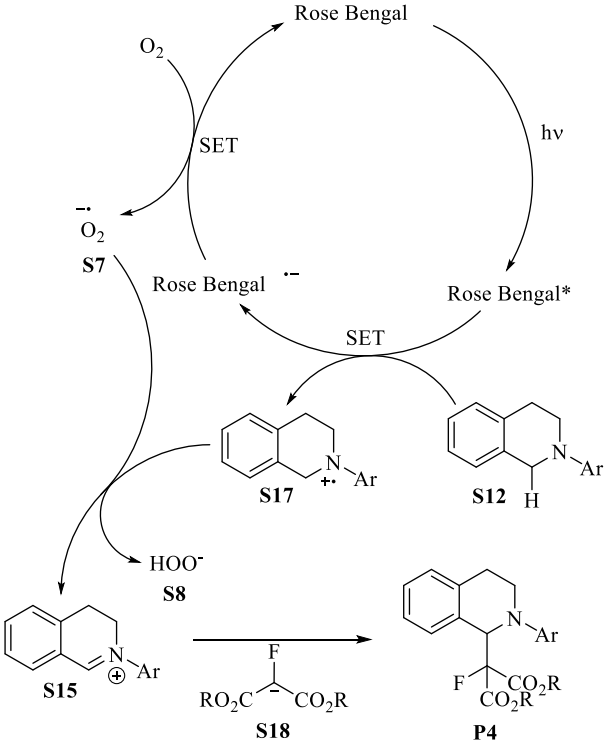
Scheme 11 – Catalytic cycle of an Organocatalysed CDC reaction which exploits L-proline.

The main difference between this and the previously seen types of catalysis stands in the oxidation step. In the photo- and transition metal-catalysis the formation of the iminium ion is guided by the catalyst and the addition of the nucleophile is sometimes assisted by the metal catalyst.

In the case of enamine catalysis, the situation is reversed: the nucleophile gest activated by the organic catalyst while the electrophile is stoichiometrically generated using a single electron oxidant (i.e., the oxidation occurs *via* multiple transfers of single electrons; e.g., DDQ, DEAD).

Photo-organocatalysed CDCs

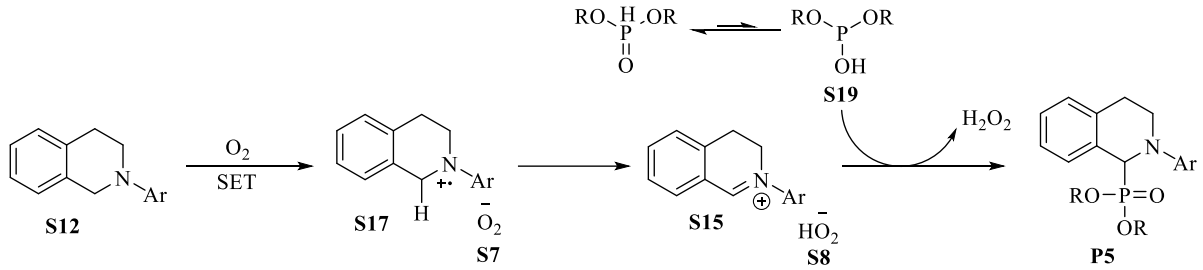
Returning to the photocatalysed CDC reactions, there are photocatalysts that don't involve any transition metal centre, which can be referred to as photo-organocatalysts. These attained great interest from the scientific community because of their low environmental impact, toxicity and cost.[18] Similarly to photocatalysed CDCs, the mechanism of these reactions involves a SET between the excited catalyst and the substrate. Thus, an oxidant, for instance O_2 (Scheme 12), restores the catalyst, which can again undergo photo-excitation.[19] The obtained radical cation (**S17**) reacts with a superoxide (**S7**), yielding dioxidanide (**S8**) and the iminium ion (**S15**), which reacts with the enol form of the fluorinated malonate (**S18**) to give the final product (**P4**).



Scheme 12 – Mechanism of a Photo-Organocatalysed CDC reaction involving Rose Bengal as the catalyst.

Catalyst-free CDCs

Many tertiary amines can be directly oxidised by a stoichiometric oxidant, leading to the formation of the iminium ion, which can be attacked by the nucleophile.
For example, in the reaction observed by Dhineshkumar *et al.* (Scheme 13) the substrate is a N-aryl THIQ (**S12**) and the oxidant is oxygen.[20] The fundamental step in the proposed mechanism resides in a SET between the substrate and oxygen. This, similarly to the cases of photocatalysis and photo-organocatalysis, leads to the production of a superoxide (**S7**) and a N-centred radical cation (**S17**). These react together to give the iminium ion (**S15**), which then undergoes a nucleophilic attack by the tautomerized phosphite (**S19**), giving the final product (**P5**) and liberating hydrogen peroxide.



Scheme 13 – Mechanism of a Catalyst-free CDC reaction between THIQ and a phosphite.

Nevertheless, there is a lack of proof concerning how the SET between **S12** and oxygen would (spontaneously) happen.

Similarities in CDC reactions

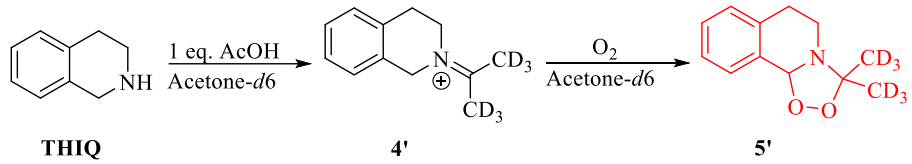
To summarise, a few common points of the previously analysed mechanisms are deepened. The first one regards the electrophile of these reactions. In all the reported CDCs, the final step, that leads to the formation of the product, is the nucleophilic attack.
This is allowed if the structure that reacts with the nucleophile is an electrophile. Hence, the reaction shall involve the iminium form (activated electrophile) of the substrate.

Another factor that can be discussed is the N-substitution on the amine, since free amines are never involved in the reactions, because they may act as nucleophiles. Thereby, a common procedure to obtain C-functionalised free amines starts with a prior protection step of the amine group. After the CDC reaction has occurred, the deprotection step gives back the substituted free amine. These steps lower the Atom Economy (AE) and the

Environmental Factor (EF) of the whole processes and, hence, the green-chemistry aspects related to them.

Nevertheless, the reaction explored by Veenstra (Scheme 7) allows the α-functionalization to occur, while using a free amine as the substrate. It doesn't require any prior protection at the nitrogen and it yields the Mannich product in one step without the need of a metal- or photocatalyst. Some experiments have been carried out and it was observed that the organocatalyst (**Pro**) is not required for the reaction to occur.

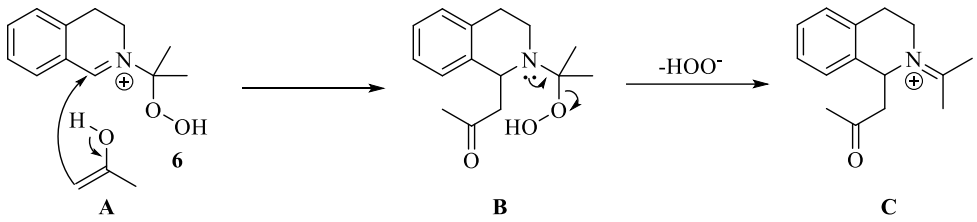
The reaction has been investigated, providing initial insights of the mechanism (Scheme 14).[9] It starts with the formation of the iminium ion (**4'**), given by the condensation between **THIQ** and acetone. Then, the obtained structure reacts with oxygen, yielding the intermediate (**5'**).



Scheme 14 – Intermediate formation reaction. The intermediate is highlighted in red.

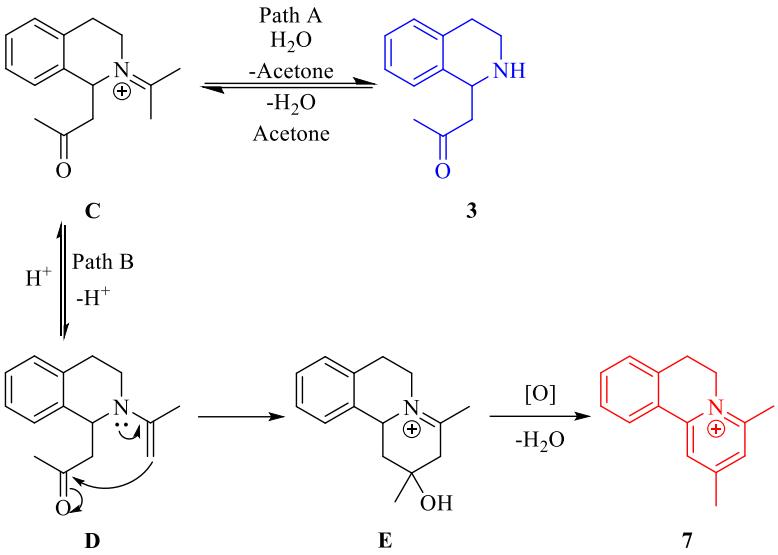
5 may get protonated, leading to **6**. The reaction, from this point on is completely unknown; therefore, the reported mechanisms are simple hypotheses.

Structure **6** can react with the enol form of the acetone, 2-propenol (**A**), with loss of dioxidanide and consequent formation of another iminium ion (**C**) (Scheme 15).[9]



Scheme 15 – Proposed mechanism for the addition of acetone in α-position.

C can either be hydrated, giving the Mannich product (**3**), or dehydrated and oxidised, yielding a pyridinium product, 2,4-Dimethyl-6,7-dihydropyrido[2,1-a]isoquinolin-5-ium (**7**).[9]



Scheme 16 – Possible pathways for the formation of the Mannich (blue) and pyridinium (red) products.

Nonetheless, this last part goes beyond the actual aim of this research project.

Previously, the reaction has been investigated mostly in the part that leads to the intermediate **5** transformation into manifold products. Now, we are focusing on the part concerning the production of the intermediate itself.

Results

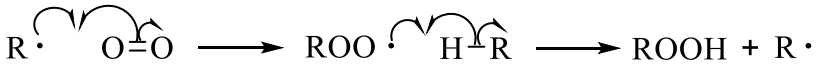
Several experiments have been carried out to explain the formation of the intermediate, validating or not the hypothesised mechanisms. The main hypothesis is that the reaction occurs *via* radical pathway. This links all the proposed mechanisms and initiation steps.

Radical Mechanisms – Autoxidation and SET

An autoxidation process is defined as the oxidation reaction of an organic molecule carried out by oxygen, at or near room temperature. It represents a common way to obtain many commercial chemicals, such as acetone, cyclohexanone or phenol, but also an important path for the degradation of (bio-)molecules.[21,22]

The main pathway of autoxidation reactions consists of a radical chain of peroxyl radicals (Scheme 17). The initial alkyl radical is generated by abstraction of a hydrogen atom, which carried out by a radical species (e.g., given by a radical initiator). Then, the alkyl radical reacts with molecular oxygen, forming a peroxyl radical. This one, in turn, can react with a hydrocarbon chain, leading to the production of an alkyl hydroperoxide and, newly, an alkyl radical.[21,22]

Other reactions may occur, for instance the homolytic cleavage of the hydroperoxide, which is considered to be the dominant chain-initiation reaction in absence of a catalyst (both RO· and HO· are very reactive towards hydrocarbon substrates).[22]



Scheme 17 – General peroxyl radical chain in autoxidation reactions

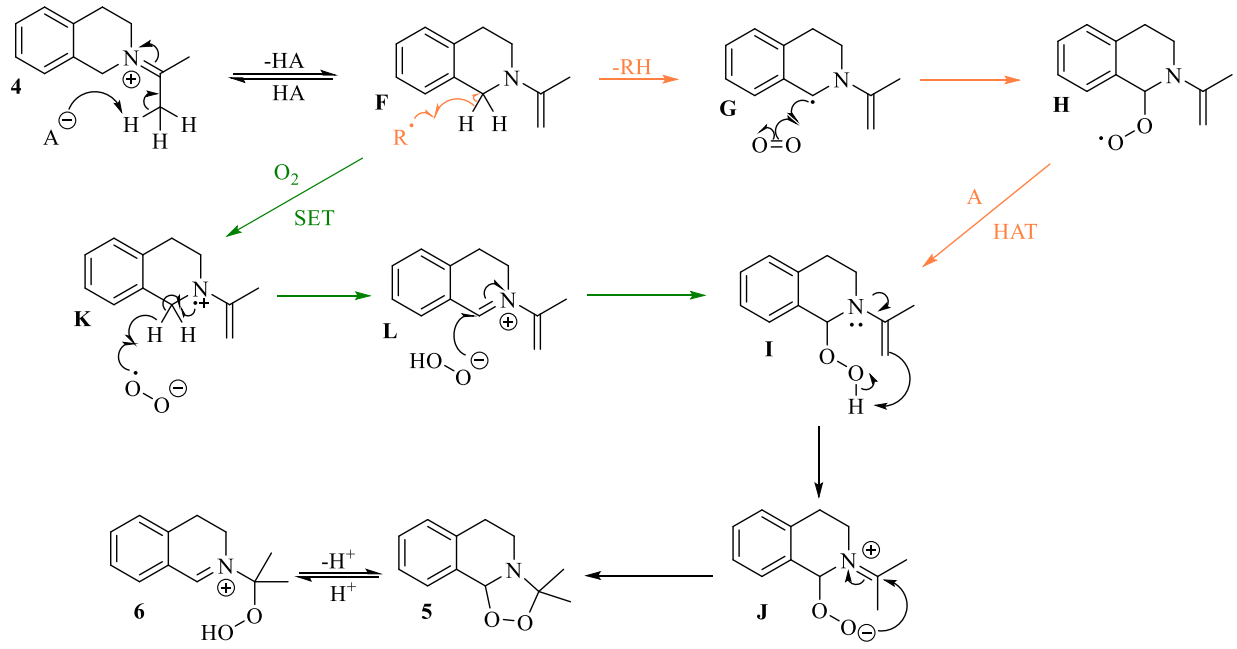
This may fit the reaction under study, since a peroxy-group can be observed both in the deprotonated and protonated intermediate.

Autoxidation Mechanism

A secondary amine and a ketone are involved in acidic conditions; therefore, the enamine (**F**) formation can occur. This structure may undergo a hydrogen atom abstraction at benzylic position. The resulting radical species (**G**) would be able to react with oxygen, resulting in a THIQ-peroxyl radical (**H**), which may abstract a hydrogen atom from some other enamine molecule. The resulting structure (**I**) undergoes a tautomerization and, eventually, the intermediate gets formed (Scheme 18).

Single Electron Transfer (SET)

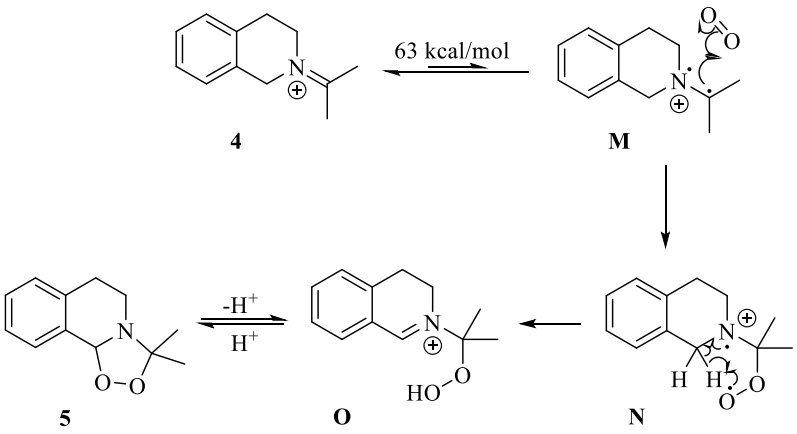
In the introduction, an example of catalyst free CDC has been depicted (Scheme 13). The fundamental step of that reaction is a Single Electron Transfer between the tertiary amine (N-substituted THIQ) and oxygen.[20] The same might happen in our reaction (Scheme 18), involving the enamine (**F**), because of its lone pair of electrons (on the nitrogen atom) which can be involved in the SET. After the electron transfer, the produced radical N-centred radical cation (**K**) and superoxide react together, giving the iminium ion **L** and, after its reaction with dioxidanide, structure **I**. This last structure, just as in the autoxidation hypothesis, rearranges to the intermediate structure (**5**).



Scheme 18 – Hypothesised mechanisms for the intermediate formation. The Autoxidation mechanism is highlighted in orange, while in green is the mechanism involving SET.

Iminium ion – A possible initiator?

When a secondary or primary amine reacts with a ketone, the first stable intermediate that is reached is the iminium ion. This structure might give a transition from the singlet to the triplet state of the electrons in the iminium π-system (ΔG= 63 kcal/mol, computationally calculated by DFT), leading to the formation of a biradical structure (**M**) which can react with triplet oxygen and, hence, produce the intermediate (**5**) *via* intramolecular (**N**) hydrogen abstraction and deprotonation (Scheme 19).[9]



Scheme 19 – Hypothesised mechanism involving the formation of a biradical structure of the iminium ion.

This reaction might act as the initiator of a proper autoxidation process, even though it is difficult to determine if the peroxyl radical (**N**) reacts with more ease in an intra- or intermolecular fashion and the energy barrier that leads to the biradical structure is large enough to consider this mechanism as minimised at room temperature.

All these mechanisms, or steps, might occur simultaneously and influence each other. Thereby, we cannot automatically exclude one or another: the aim of the mechanistic research is to find the one which is most probable.

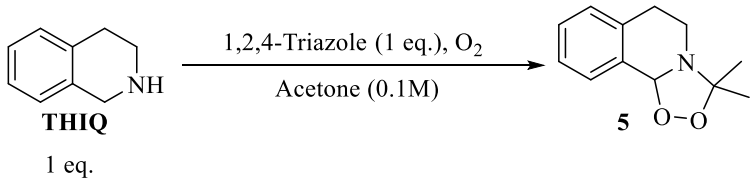
In order to test these possible mechanisms, the experiments focused mainly on four aspects:

1- Reaction rate;
2- Kinetic Isotope Effect;
3- Intermediate isolation;
4- Radical Trapping.

Rate Experiments

Initially, the reaction has been carried out at the same conditions reported in Scheme 14. The outcome showed the necessity of an optimization. This because the intermediate has been pushed towards the pyridinium product (**7**), while the reaction was too slow for an accurate measurement of the rate. The formation of **7** is undesired because it does not allow to study the kinetics for the formation of the intermediate. Therefore, we firstly tried to avoid the intermediate over-reaction.

We tested 1,2,4-triazole as an alternative to acetic acid. The lower strength of this acid ($pKa_{triazole}$ = 10.3; pKa_{AcOH} = 4.756) allowed the observation of a larger amount of intermediate, without further conversion (Scheme 20). This is probably due to the slower rate of the acetone enolization and/or formation of the protonated structure **6**.



Scheme 20 – Intermediate formation reaction carried out with 1 equivalent of 1,2,4-triazole. O_2 pressure was 300mbar.

Nevertheless, the reaction is too slow, since full conversion has been reached in more than 48h.

In order to enhance the velocity at which the starting material is converted into the intermediate, several changes were actuated (Table 1). The reaction has revealed a good behaviour at 40°C, while at 60°C a significant amount of cross-aldol products could be observed.

Furthermore, the presence of molecular sieves improved the rate, probably because of their ability to segregate the water from the reaction mixture (which favours the formation of the iminium ion **4**).

Entry	Condition variation	Outcome
1	1 eq. 1,2,4-triazole	Slow reaction, no pyridinium formed
2	$MgSO_4$	No significant change
3	MS	Full conversion in 48h
4	40°C	Full conversion in 24h
5	60°C	Faster reaction, large amount of aldol products
7	2 eq. triazole, 40°C	Full conversion in 16h

Table 1 – Conditions optimization experiments. Positive results are highlighted in green. The variation relates to the first entry.

The reaction reaches full conversion of the starting material in 24h at 40°C with 1 equivalent of 1,2,4-triazole. It becomes even faster when the amount of acid is doubled. An experiment involving 2 equivalents of 1,2,4-triazole and molecular sieves at 40 °C revealed full conversion in less than 16 h. Thereby, the experiments that followed were performed under these conditions.

Some rate experiments were attempted. Initially, it was expected to directly observe the intermediate in GC-FID. Nevertheless, a large quantity of imine **1**, together with the intermediate (**5**), was observed. We deepened this phenomenon by comparing the GC-FID data to the ^{1}H-NMR data resulting from the same reaction mixture. The comparison revealed that the imine **1** was produced due to the high temperatures reached in the GC. This led to the major problem: it was not possible to precisely determine the total concentration of intermediate, because of the partial conversion of the intermediate (**5**) during the analysis of the sample.

Thereby, we tried to analyse the samples with HPLC and UPLC-MS, but the same phenomenon was observed. Thus, another approach was attempted: to convert the intermediate into a more stable structure, which can be easily analysed with GC or UPLC. It was expected that the intermediate (5) would be unstable towards basic conditions. This was supported by the results acquired for the intermediate isolation experiments. Various experiments were tried out, using different bases and conditions (Table 2).

Among the experimented conditions, the best results were achieved by sodium hydroxide in water (entry 3), with the total conversion of the intermediate.

Entry	Base	Eq. or conc.	Solvent	Instrument	Outcome
1	Et_3N	100	MeOH	GC-FID	Low conversion
2	LDA	200	MeOH	GC-FID	Low conversion
3	NaOH	0.1M	H_2O	UPLC-ESI	Full conversion

Table 2 – Quenching of the intermediate at various conditions. In green are the positive outcomes.

After a few ^{1}H-NMR analyses (Figure 1), it was found out that the quenching fully converts the intermediate (**5**) to the imine **1**. In both spectra, the peaks of the intermediate (3.76 and 6.14 ppm) are visible, they are shifted because of the of the strong change in acidity (addition of NaOH). The blue spectrum was taken in neutral D_2O; a characteristic peak of the intermediate (**5**) is visible (benzylic proton). This is a sharp peak situated between 6 and 6.25 ppm. When sodium hydroxide was added to the solution (red graph), this peak could not be observed anymore, while another peak at 8.29 ppm appeared. This is a peak relative to the imine (**1**).

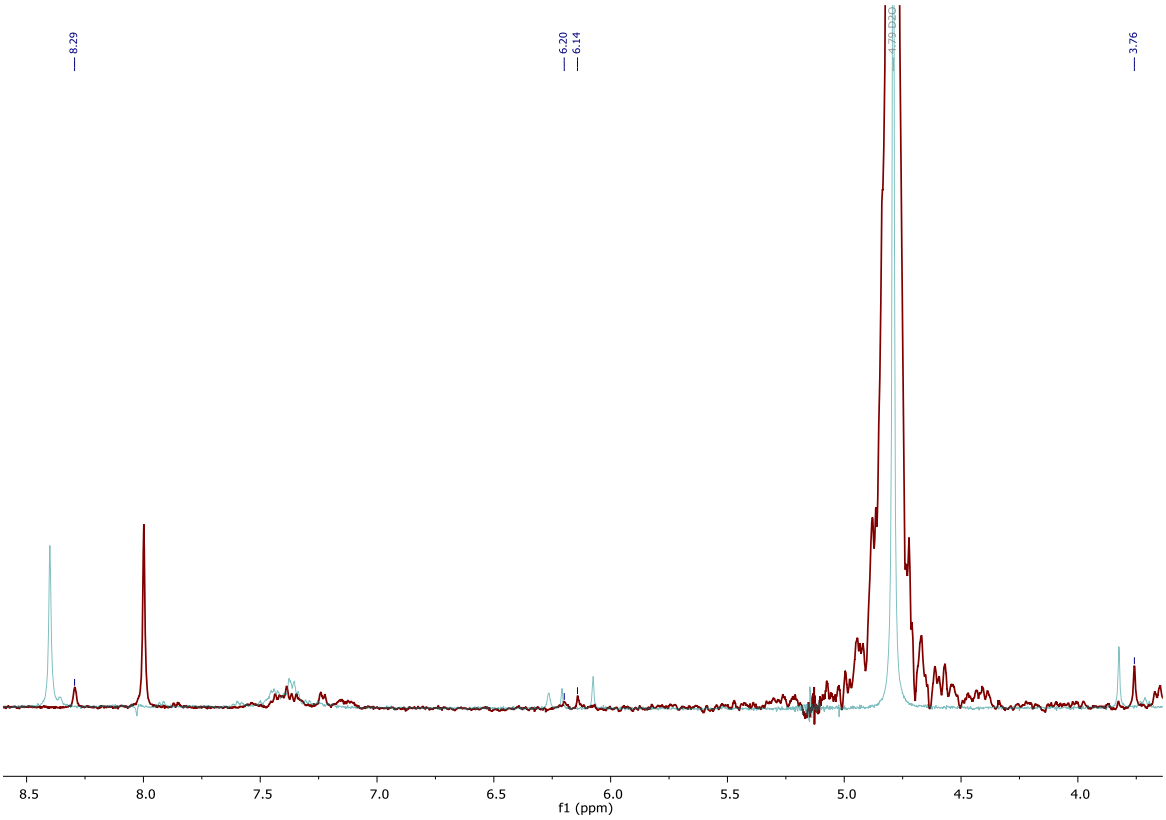
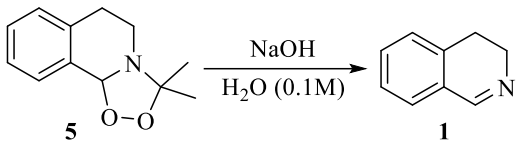


Figure 1 – ^{1}H-NMR spectra of the reaction mixture before and after addition of NaOH. In both cases, the solvent is D_2O and the utilised instrument is a 400MHz NMR spectrometer.

The trapping reaction can be then schematised as in Scheme 21.



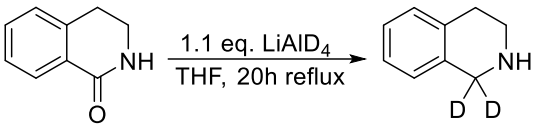
Scheme 21 –Intermediate trapping reaction.

Kinetic Isotope Effect (KIE)

The possibility of measuring the reaction rates can be exploited to perform other rate-related experiments, such as those for the kinetic isotope effect (KIE).

In order to get more information about the rate determining step, measuring the KIE is of crucial importance. According to the proposed mechanisms, the benzylic hydrogen is removed during the reaction. For this reason, a primary KIE is expected. This would be observed whenever the C-H bond breaking event occurs along the highest peak on the reaction coordinate diagram.

To test this out, the deuterated structure of THIQ (**9**) was synthesised (Scheme 22) *via* reduction of 3,4-Dihydroisoquinolin-1(2H)-one (8), using $LiAlD_4$.



8 **9**

1 eq. 84.8% yield

Scheme 22 – Synthesis of THIQ-d2.

The reaction gave a good yield. Nevertheless, due to lack of time, it was not possible to perform any KIE experiment.

Intermediate isolation

Having the pure intermediate can be useful research-wise because it can be used to confirm its structure and prove its involvement in the reaction. Since it was possible to selectively synthesise the intermediate (**5**), the intermediate isolation could be attempted. The correct way for the purification of **5** had to be found. This may involve several purification steps and different means (Table 3). The main aim of all these experiments is to achieve the complete removal of the acid from the solution.

The extraction with saturated $NaHCO_3$ solution did not allow the purification (entry 1). Furthermore, the reaction at 40 °C generated some impurities, such as aldol and pyridinium (7) products. The subsequent experiments were performed at room temperature.

Other ways to separate the acid from the mixture were tested out. Filtration over small amounts of neutral aluminium oxide (AlO_x) and silica (SiO_2) were conducted separately, using acetone as the solvent. The filtration over SiO_2 converted the intermediate (**5**) into other species, whereas that over AlO_x revealed the preservation of the structure. However, a large amount of aldol products deriving from acetone was detected by ^{1}H-NMR.

In order to prevent these aldol reactions, a change in solvent was attempted and ethyl ether was chosen. However, after filtration, the intermediate (**5**) could not be observed. This was probably due to the long waiting time for the solvent evaporation and the multiple changes in conditions (solvation, temperature and pressure mainly).

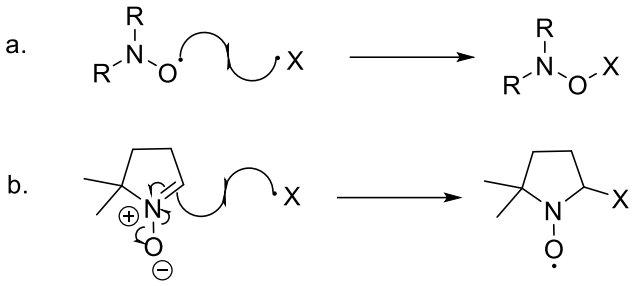
Entry	Reaction			Purification step 1	Purification step 2	Outcome
	Eq. Triazole	T (°C)	Time (h)			
1	2	40	24	Extraction	Evaporation (rotavapor)	No product obtained
2	2	rt	48	Filtration over neutral AlO_x or SiO_2		AlO_x preserves the intermediate structure; aldol reactions occur
3	2	rt	36	Evaporation (airflow)	Filtration over AlO_x	No product obtained

Table 3 – Experiments for the intermediate purification.

Since acetone was an excellent eluent for the separation of the acid from the mixture, it was decided to return to use it. The reaction mixture has been partially evaporated to concentrate the solution and limit the quantity of aldol products in the final product. Meanwhile, a small AlO_x plug has been set up using Et_2O for its initial elution, in order to prevent aldol reactions. Then, the reaction mixture was quickly eluted, without flushing with acetone. After solvent evaporation with air-flux and 5 minutes on rotatory evaporator (rt, 400 mbar), the obtained yellowish oil was analysed by NMR. The results showed a high purity of intermediate (**5**) (Supporting Information).

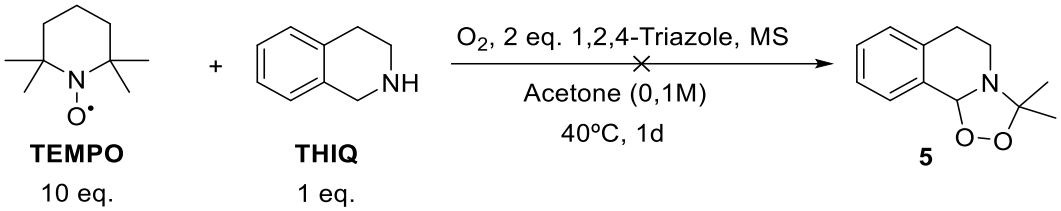
Radical trapping

The hypothesised mechanisms involve some radical species. These are predictably very reactive (short-lived). The commonly available radical traps react with the free radicals *via* two mechanisms, depending on the kind of trap (Scheme 23). The first, named “Cross-coupling mechanism” (Scheme 23a) involves a stable radical (generally an aminoxyl radical) which traps the free radical species by coupling of the two spare electrons. The other common mechanism is the “Spin trapping” one (Scheme 23b). It often involves nitrone groups, which can be depicted as a zwitterion with a (dative) double bond between the (positively-charged) nitrogen and a neighbouring carbon atom. This bond can undergo a radical addition, which causes the formation of an aminoxyl radical (stable).[23]



Scheme 23 – a. Cross-coupling trapping; b. Spin trapping.

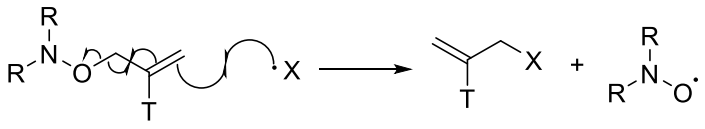
A reaction in which we put **TEMPO** in excess (to exploit the cross-coupling mechanism) was attempted. This led to a quite interesting, but not yet fully comprehended phenomenon (Scheme 24). The intermediate (**5**) formation did not occur and the starting material was unaltered. However, no trapped radicals were detected.



Scheme 24 – Reaction for free radical intermediates trapping, exploiting TEMPO. O_2 pressure was 300mbar.

The spin traps are applicable to the most common short-lived radicals in both liquid and gas phases.[23] Nevertheless, in some situations this trap type revealed false positives and, most important, poor sensitivity. For these reasons, we decided to use the recently developed traps, proposed by Williams *et al*.[23] These involve a different trapping mechanism (

Scheme *25*). They comprise three main parts: a stable aminoxyl radical living group, a tuning group and a terminal double bond. The unsaturation is the functionality which is directly affected by the presence of free radicals, which bind to it, leading to a radical substitution mechanism (S_H2'). The extent to which the trap is reactive towards unstable free radicals is regulated by the tuning group (T). But this is not its sole function; it may also tune the solubility of the molecule or the MS-ionization efficiency, as an example of physical property.[23]

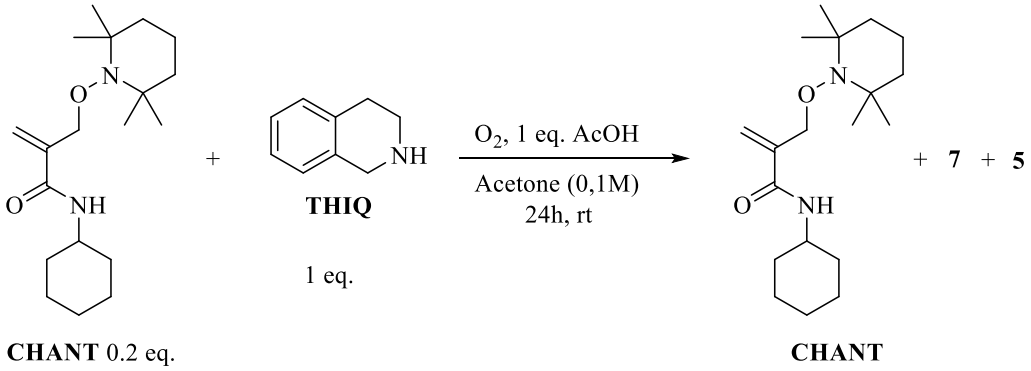


Scheme 25 – S_H2' trapping mechanism (homolytic substitution reaction).

Both of the proposed[23] synthesis for the traps were tried out. We were able to synthesise and purify the radical trap CHANT, while DEADANT was synthesised, but not purified (Scheme 26).

Scheme 26 – Radical traps CHANT and DEADANT.

The radical trap **CHANT** was tried out. 0,2 equivalents of the trap were put in the reaction mixture, containing acetic acid instead of 1,2,4-triazole.



Scheme 27 – Reaction for radical intermediates trapping. O_2 pressure was 300mbar.

After 24 hours, no **TEMPO** could be detected in the reaction mixture. Furthermore, the radical trap was still intact, without substitution or side-products.

Another experiment was attempted, this time with a higher concentration of the trap (1 equivalent) and 2 equivalents of 1,2,4-Triazole. However, no reaction could be observed even that time.

Conclusions

To conclude, the intermediate synthesis was enhanced, reducing the reaction time from more than 48 h to less than 16 h and a way to convert the intermediate into a stable structure and, hence, study the kinetics of the reaction was found. The synthesis of THIQ-*d*2 (**9**) was performed with a high yield. However, it was not possible to carry out any KIE experiment. A method for the intermediate purification was achieved.

TEMPO did not let the reaction to occur and the radical trap CHANT was successfully synthesised, but no radicals were trapped in both cases.

Further efforts are needed to comprehend the behaviour of this reaction. Nevertheless, a few steps forward, under the experimental point of view, have been done.

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