Tutor

Dr. Ernesto Nicolás Galindo
Departament de Química Inorgànica i Orgànica

Grau de Química

Treball Final de Grau

Towards the synthesis of a thermolabile linker.
Cap a la síntesi d'un espaiador bifuncional termolàbil.

Eduard Balaguer Garcia

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Veiem les coses, no com són, sinó com som nosaltres.

Immanuel Kant

Al Dr. Ernesto Nicolás i l'Aida López per tot el seu esforç i dedicació en guiar-me al llarg del projecte, malgrat el que ha hagut de suposar reconduir aquest treball enfront la adversa situació actual.

Als meus pares, a la meva germana i a tota la meva família per haver facilitat el camí fins aquí i per l'immens suport moral al llarg de tots aquests anys. Si no fos per ells, no seria qui soc.

A la Sara, els seus ànims al llarg d'aquests últims mesos m'han servit d'incentiu per a seguir treballant com el primer dia. Gracies per escoltar les meves preocupacions i oferir la teva mà sempre que l'he necessitada.

Al Pau per ser el millor company de laboratori que un pot desitjar tenir. I a tota la resta d'amics i amigues que he tingut la sort de conèixer durant aquesta etapa acadèmica.

REPORT

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1. SUMMARY

Thermolabile protecting groups (TPGs) present a significant interest in organic chemistry as the mere deprotection with the increase of the temperature avoids the use of chemicals, complying with the principles of green chemistry. The deprotection is produced generally in mild conditions by an intramolecular cyclization, releasing the compound. This idea of thermal cleavage was taken into consideration by Dr Patrick Gamez's research group to develop versatile nanosystems for the selective delivery of drugs, with the purpose of fighting against non-communicable diseases such as cancer.

2-[*N*-(2-pyridyl)-*N*-[4-(methoxycarbonyl)benzyl]amino]ethanol (**5**) was synthesized to fulfil this function of thermolabile linker. The synthesis was carried out by mainly implying nucleophilic substitution reactions and the final yield was 35%. Consequently, the experimental conditions were analyzed and some modifications were suggested in order to try to improve the performance in the near future. For the same reason, two alternative synthetic paths were proposed involving other nitrogen alkylation reactions, such as the Pd (0) catalyzed Buchwald-Hartwig reaction or the reductive amination.

All compounds obtained in the synthesis have been characterized by ^{1}H-NMR, ^{13}C-NMR and IR spectroscopy.

Keywords: Thermolabile linker, thermolabile protecting group, nanosystem, organic synthesis.

2. RESUM

Els grups protectors termolàbils (TPGs) presenten un interès significatiu en la química orgànica, ja que la mera desprotecció amb l'augment de la temperatura evita l'ús de productes químics, complint així amb els principis de la química verda. La desprotecció es produeix generalment en condicions suaus per una ciclació intramolecular, alliberant el compost. Aquesta idea de ruptura tèrmica ha sigut considerada pel grup de recerca del Dr. Patrick Gamez per desenvolupar nanosistemes versàtils per a l'entrega selectiva de fàrmacs, amb el propòsit de lluitar contra malalties no transmissibles com el càncer.

S'ha sintetitzat el compost 2-[*N*-(2-piridil)-*N*-[4-(metoxicarbonil)benzil]amino]etanol (**5**) per complir amb aquesta funció d'espaiador termolàbil. La síntesi s'ha dut a terme emprant principalment reaccions de substitució nucleòfila i el rendiment total ha sigut del 35%. Per consegüent, s'han analitzat les condicions experimentals a la vegada que s'han suggerit modificacions per tractar de millorar aquest rendiment en un futur pròxim. Per la mateixa raó, s'han proposat dos camins sintètics alternatius implicant altres reaccions d'alquilació de nitrogen, com la reacció Butchwald-Hartwig catalitzada per Pd (0) o l'aminació reductora.

Tots els compostos obtinguts en la síntesi han sigut caracteritzats per ^{1}H-RMN, ^{13}C-RMN i espectroscòpia IR.

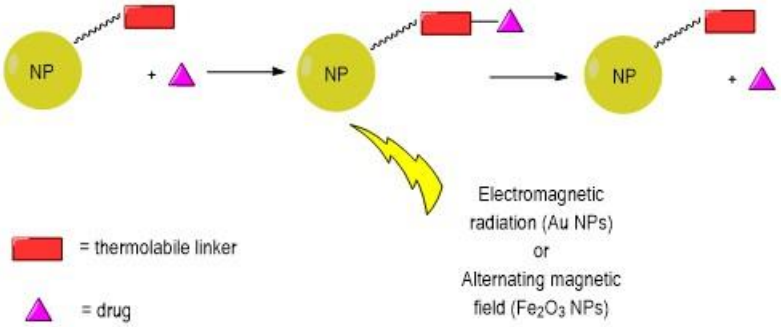
Paraules clau: Espaiador bifuncional termolàbil, grup protector termolàbil, nanosistema, síntesi orgànica.

3. INTRODUCTION

With the ageing of the population, the impact of associated non-communicable diseases (NCDs) will increase dramatically.[1] Hence, it is expected that NCDs (such as cancer, Alzheimer's disease and so on) will represent a major socio-economic challenge for society.

A major problem encountered with most current drugs used clinically is their common lack of selectivity. In order to solve this problem, drug delivery systems, in other words nanocarriers, may provide enhanced efficacy and/or reduced toxicity for therapeutic and diagnostic agents.[2] Various types of nanoparticles (NPs) have been used as drug carriers, especially for oncology applications.[3] Furthermore, thermal properties of metal NPs have received much attention from the scientific community over the last decade, because of the possibility to produce localized heat through the application of an external stimulus.[4] Such heat generation in Au NPs can be achieved under radiofrequency irradiation,[5] as well as in an analogous manner, heat can be produced through the application of an external alternating magnetic field to single-domain nanomagnets, such as Fe_3O_4 NPs.[6]

Consequently, the hyperthermic properties of NPs opens the possibility to develop novel nanosystems. In this context, a bioinorganic research group from the Department of Inorganic and Organic Chemistry of University of Barcelona (with Dr. Patrick Gamez as principal investigator), proposed the development of the nanosystem shown in **Scheme 1**.

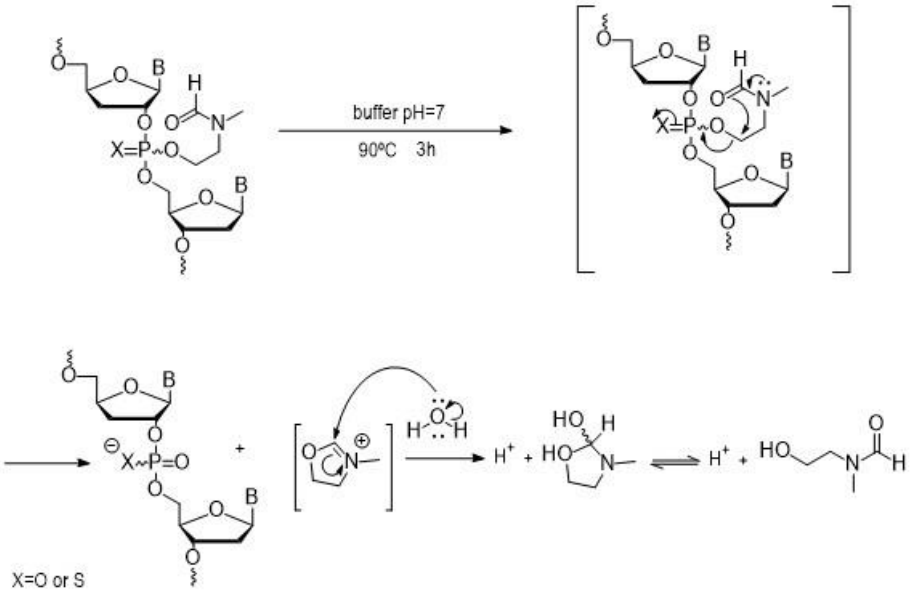


Scheme 1. Schematic representation of the approach to develop thermolabile nanosystems.

These nanosystems are composed by inorganic NPs bearing thermolabile linkers that can be used to covalently bind drugs (*e.g.* a peptide or a metal complex). After being delivered to its target (by using a targeting group linked to the nanoparticle or directing the nanoparticle with a magnet), the drug will be released through the application of electromagnetic radiation (visible, microwave) or alternating magnetic field depending on the nature of the NPs used. The key to the functioning of the nanosystem is to dispose of a linker sensible to the temperature in a way that with a variation of it, a reaction occurs in order to release the drug.

3.1. THERMOLABILE LINKERS

The use of thermolabile protecting groups (TPGs) has received an increased interest in the last decade. It offers profitable advantages such as the capability of thermal removal avoiding dangerous reagents commonly used to cleave protecting groups, complying with the principles of green chemistry.[7] The first application of TPGs was done in context of phosphate/thiophosphate protection in solid-phase oligodeoxyribonucleotide synthesis.[8,9] The thermolytic cleavage of the protecting group involves a well-studied intramolecular cyclization reaction (**Scheme 2**).



Scheme 2. Tentative mechanism of the thermolytic cleavage of 2-(*N*-formyl-*N*-methyl)aminoethyl phosphate/thiophosphate protecting groups.

Additional thermolabile protecting groups were designed taking advantage of this thermocyclation driving force (**Figure 1**), such as 3-(*N-tert*-butylcarboxamido)-1-propyl (**A**),[10] 3-(2-pyridyl)-1-propyl (**B**)[11] and 4-methylthio-1-butyl (**C**).[12]

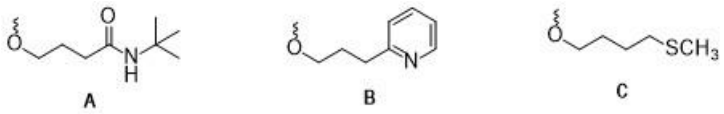
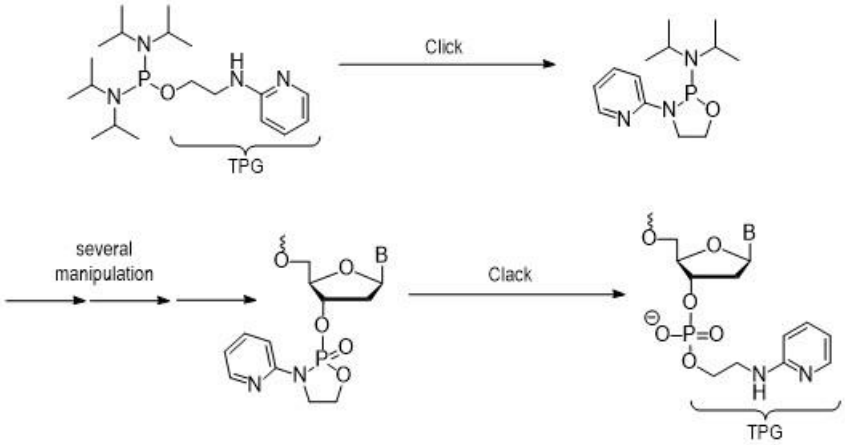


Figure 1

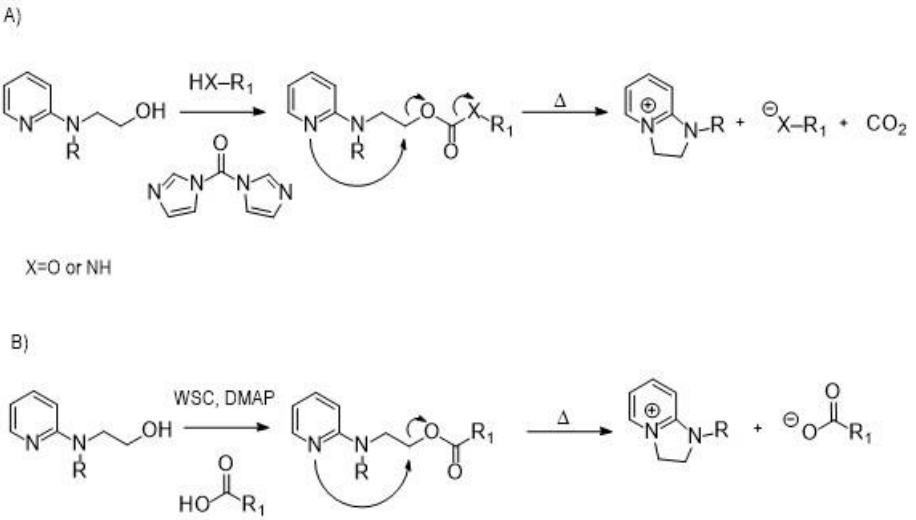
Although in general the release of these TPGs take place at high temperatures, the compounds may not be completely stable at room temperature, and therefore react in a short period of time. For this reason, a new "click-clack" approach was studied in 2009 by Chmielewski (**Scheme 3**).[13] The objective of this method is to increase temporarily the thermostability via an engagement of the phosphate centre in a cyclic structure ("click"). After the desired reactions have been carried out, thermolabile properties are recovered with the opening of the 5-membered ring in mild acidic conditions ("clack").



Scheme 3. Idea of the "Click-Clack" approach using 3-pyridyl-[1,2,3]oxazaphospholidine as the stabilized intermediate.

These groups have shown remarkable efficiency with high yields when protecting phosphate/thiophosphate groups, however do not work correctly for hydroxyl protection due to the great stability of the appropriate carbonate derivatives formed.

Recently, the introduction of nucleophilic 2-aminopyridyl system has permitted to elaborate a new class of thermolabile groups that are useful for the protection of, hydroxyl functional groups,[14] primary amines[15] and carboxylic acids[16] (**Scheme 4**).



Scheme 4. A) Generation of a carbonate/carbamate from the protection of an alcohol/amine, and thermal deprotection; B) analogous protection/deprotection of a carboxylic acid.

The hydroxyl functional group of the pyridine aminoethanol molecule is coupled to the alcohol to be protected via a well-known reaction using 1,1'-carbonyldiimidazole as a coupling agent. This molecule formed can undergo a temperature-induced cyclization (T≈ 90ºC) which ends with a decarboxylation and the release of the alcoholic compound. The same type-reaction occurs with primary amines, forming a carbamate instead. In an analogous manner, a carboxylic acid is protected in this case by esterification with the thermolabile protecting group in presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC), acting as a coupling agent, and *N*,*N*-dimethylaminopyridine (DMAP) which acts as a nucleophilic catalyst. It also can undergo thermocyclization with subsequent release of the carboxylate. However, usually longer reaction times are required.

With the aim of forming the above-mentioned nanosystems, Patrick Gamez research group plans to exploit the benefits of *N*-(2-pyridyl)-2-aminoethanol derivatives, to be employed as the thermolabile linker. The first attempt to fulfil this purpose was by employing ethyl 5-[(2-hydroxyethyl)(pyridin-2-yl)amino]pentanoate (**Figure 2A**), where the drug would be linked to the

hydroxyl group, and the NP to the carboxylate group (once hydrolysed) at the end of the aliphatic moiety. Nonetheless, during its synthesis they encountered numerous chemoselective problems between the hydroxyl and the amino group, as well as very low yields (below 20%).

Later on, in the literature an article was found where a promising thermolabile protecting group was successfully synthesised and characterised by employing 2-(pyridin-2-ylamino)ethanol and a substituted benzylic halide.[17] From this results, it was decided to use 2-[*N*-(2-pyridyl)-*N*-[4-(methoxycarbonyl)benzyl]amino]ethanol (**Figure 2B**) instead, where in the same manner as before, the drug will be covalently loaded to the hydroxyl group and the functionalized NP will be attached to the carboxylate group (once hydrolysed) in *para* position to the benzylic moiety (**Figure 2C**).

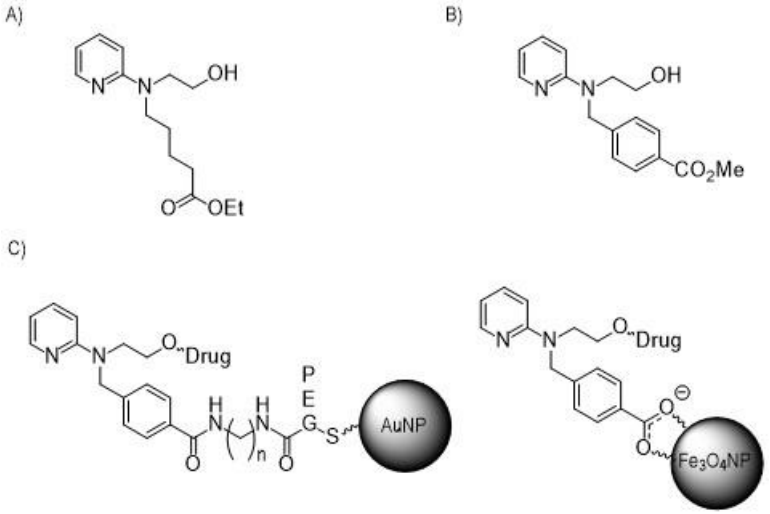


Figure 2. A) ethyl 5-[(2-hydroxyethyl)(pyridin-2-yl)amino]pentanoate; B) 2-[*N*-(2-pyridyl)-*N*-[4-(methoxycarbonyl)benzyl]amino]ethanol; C) structure of nanosystems.

4. OBJECTIVES

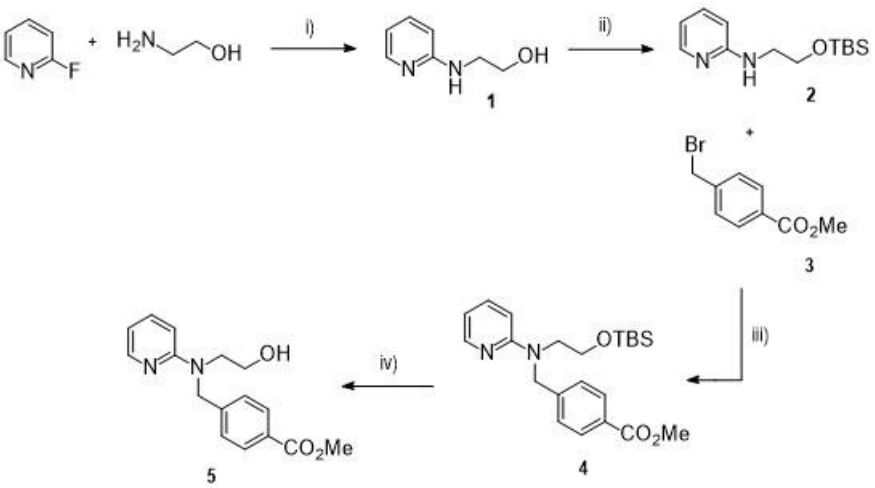
The Final Degree Work has been carried out in the context of a collaboration with the Bioinorganic Chemistry group led by Dr. Patrick Gamez. The main purpose of this work has been to take the first steps towards the synthesis of a thermolabile linker suitable to anchor drugs to carriers designed to drive these drugs to particular therapeutic targets. The specific objectives have been:

- To synthesize 2-[*N*-(2-pyridyl)-*N*-[4-(methoxycarbonyl)benzyl]amino]ethanol.
- To characterize the compounds by IR spectroscopy, ^{1}H-NMR and ^{13}C-NMR.
- To suggest and discuss different synthetic pathways to afford the thermolabile linker.

5. RESULTS AND DISCUSSIONS

5.1. SYNTHESIS OF 2-[*N*-(2-PYRIDYL)-*N*-[4-(METHOXYCARBONYL)BENZYL]AMINO] ETHANOL

Scheme 5 outlines the first strategy to get the thermolabile linker (**5**).



Scheme 5. Reactions and conditions: (i) Pyridine, microwave heating for 2h at 210ºC; (ii) TBSCl, imidazole, CH_2Cl_2, N_2 atmosphere, microwave heating for 1.5h at 100ºC, then stir at r.t. for 3h; (iii) dry THF, Et_3N, N_2 atmosphere, microwave heating for 6h at 100ºC; (iv) 1% HCl, MeOH, 25ºC, 0.5h.

The first step of the synthesis is based on a S_NAr reaction between 2-fluoropyridyne and 2-aminoethanol to get **1**. At first, pyridine was not added in the mixture and some marks appeared in the glass of the MW vial, due to HF being formed. The reaction has been done successfully before, nevertheless the main objective was to increase the work scale. Previously, DCM was used as the work-up solvent, however multiple extractions were required in order to extract **1** from the aqueous phase quantitatively. For this reason, EtOAc was used instead, hoping that the increased polarity of this solvent would help to reduce the number of extractions. Even though the extractions were reduced to less than half, the 2-aminoethanol was partially extracted as well,

appearing as an impurity in ^{1}H-NMR (**Figure 3**). To avoid this, the organic phase was cleaned repetitively with brine and afterwards, dried with $MgSO_4$. In pursuance of removing the pyridine remaining, it was rotavaporated making and azeotrope with toluene. The product was obtained in a high yield (>90%) and pure as it can be seen in ^{1}H-NMR, ^{13}C-NMR and IR (**Appendix 1**).

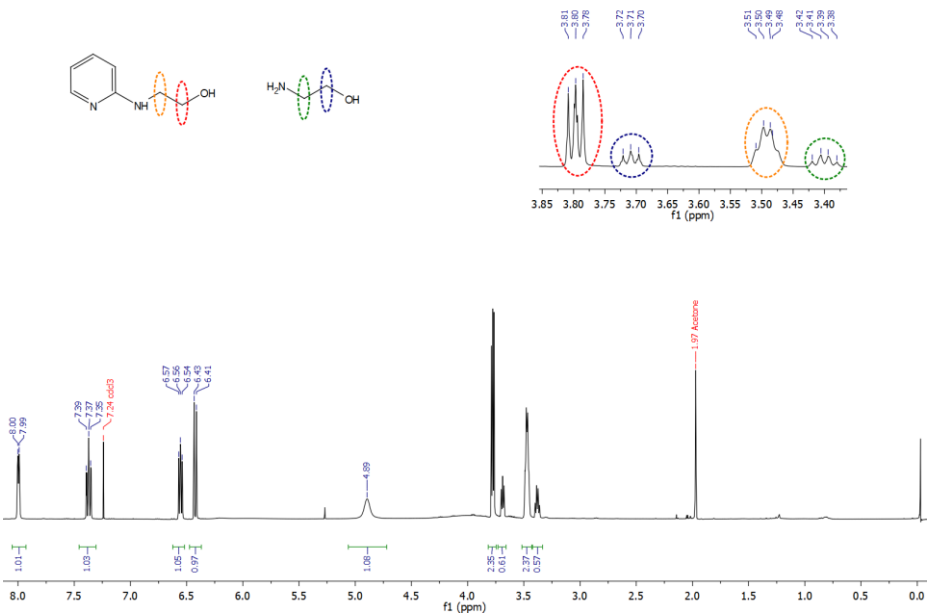


Figure 3. ^{1}H-NMR of **1** contaminated with 2-aminoethanol.

The next objective of the synthesis was to form a tertiary amine via a S_N2 type reaction between **1** and a primary halide. Previously, in the synthesis of the first *N*-(2-pyridyl)-2-aminoethanol thermolabile linker, the research group encountered a chemoselective issue where the hydroxyl moiety competed with the amino group in the reaction, obtaining two main products. This result may be attributed to the fact that the amine is partially disactivated by the pyridine, making it less nucleophile for the S_N2 reaction, and competing with the hydroxyl group. For this reason, it was decided to protect the hydroxyl group with TBSCl.

The reaction was done previously using a ratio of the reagents 1:3 (**1**:TBSCl) but, in order to minimize de reagents used, it was tried a 1:2 ratio (**1**:TBSCl) instead. The material was correctly dried in the oven overnight, and the DCM used as the solvent was distilled (to avoid the hydrolysis of the TBSCl). For the same reason, the reaction was done under inert atmosphere. After 1.5h in

the MW at 100ºC, a TLC with DCM:MeOH (95:5, v/v) of the reaction was done and there was still **1** remaining in the solution, showing that part of the TBSCl was hydrolysed. 1eq of TBSCl was added and after 3h stirring at r.t. another TLC using the same eluent, showed that the conversion of **1** was fully done. The product **2** was isolated in a high yield (88%) and characterized by ^{1}H-NMR, ^{13}C-NMR, and IR (**Appendix 2**).

Continuing with the synthetic plan, the protected aminoalcohol **2** was mixed with the benzyl bromide derivative **3** in more than three times excess. After 6h at 100ºC MW assisted, a TLC with DCM:MeOH (95:5, v/v) was done, appearing a mixture of different products. ^{1}H-NMR and HPLC-MS (**Figure 4**) samples were prepared, indicating that there was nearly no compound **4**. Surprisingly the reagent **3** did not appear in ^{1}H-NMR (even though is in excess) probably because of a side reaction. The reaction was repeated without success, concluding that it was not viable under the conditions described.

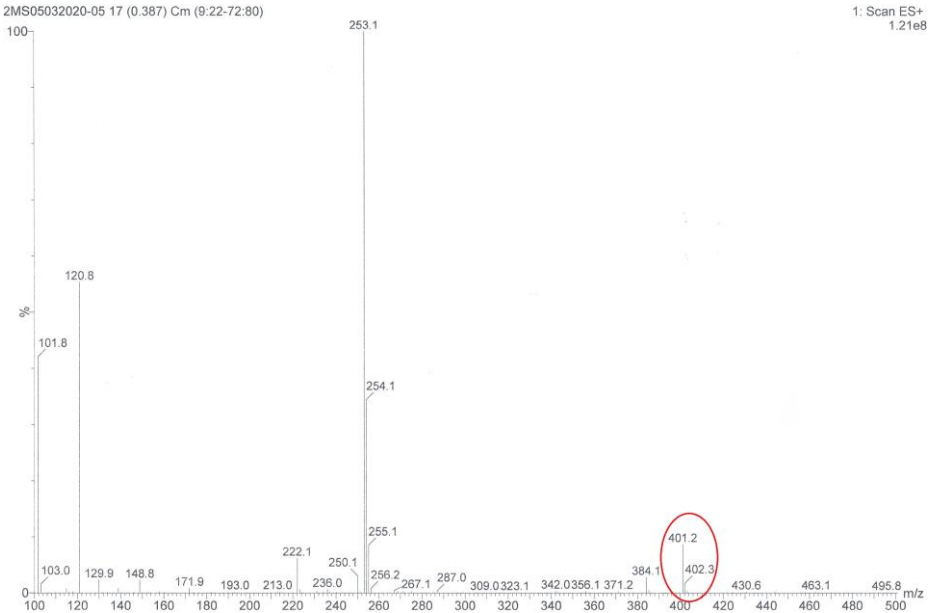
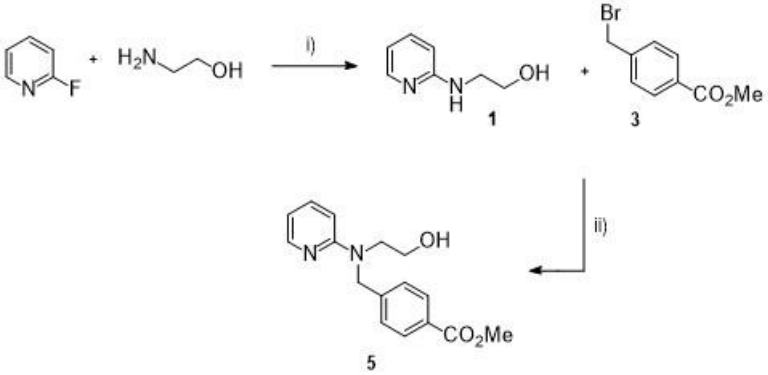


Figure 4. HPLC-MS chromatogram of **4**.

Before trying to improve the reaction conditions to perform this S_N2 type reaction, it was tried the same reaction but with the non-protected compound **1** instead, as it is seen in **Scheme 6**. The main purpose was to confirm if there is actually a competition between these functional groups or

it is not, since the linker is slightly different in terms of structure comparing to the previous one synthesized by the research group.



Scheme 6. Reactions and conditions: (i) Pyridine, microwave heating for 2h at 210ºC; (ii) dry THF, Et_3N, N_2 atmosphere, microwave heating for 6h at 100ºC.

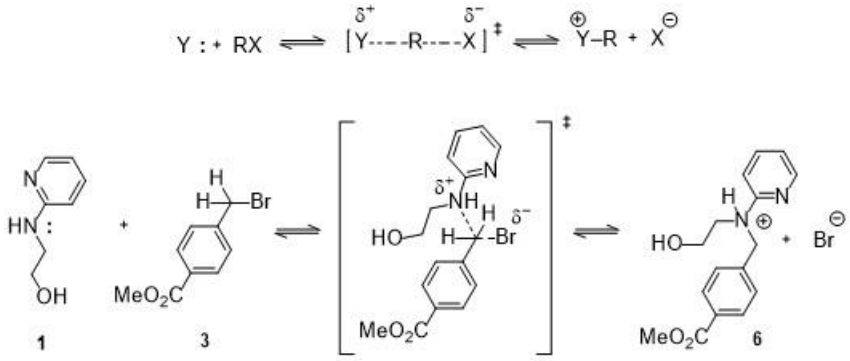
Compound **1** and **3** were mixed in inert atmosphere. After 6h under the suitable reaction conditions, a TLC with EtOAc:Hexane (60:40, v/v) indicated a mixture of compound **1**, **3** and another product stain. The fact that only appeared one product stain in the TLC, indicated either the reaction was chemoselective or the mixture of products does not separate well under the eluent used. A column chromatography with silica gel was done, using EtOAc:Hexane (60:40, v/v) as the eluent. The process was followed by TLC in order to see the relations of the fractions and separate the compounds correctly. The three main fractions were correctly isolated and the ^{1}H-NMR of the product was recorded, confirming that it is the isomer **5**. The yield of the reaction was remarkably low (35%), however it appeared pure in ^{1}H-NMR **(Appendix 3)**. Compound **3** was also collected in order to re-use it in the future. It is noteworthy to mention that the yield of compound **3** recovered after the column chromatography was less than expected.

5.2. SYNTHETIC STUDY

The product **5** was successfully synthesized in two easy steps, using non-expensive commercially available reagents and without protection and deprotection reactions involved. Nonetheless, the S_N2 reaction between the secondary amine **1** and the benzylic halide **3** occurs in low yield, causing an important waste of reagents. Before discarding this synthesis route,

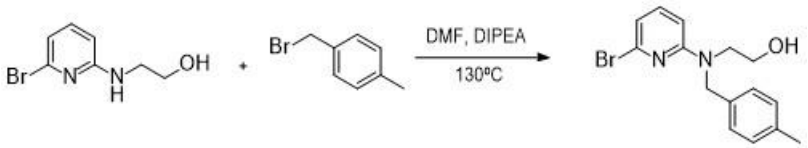
modifications on the conditions of this last reaction could be done with the aim of improving the yield in the near future.

It is well known experimentally that nucleophilic substitution reactions are highly affected by the solvent used. In fact, it is demonstrated that the solvent has the ability to change both, the reaction rate, and the mechanism.[18,19] In our particular case, uncharged reactants transform into charged products via a transition state in which charge is being developed (**Scheme 7**).



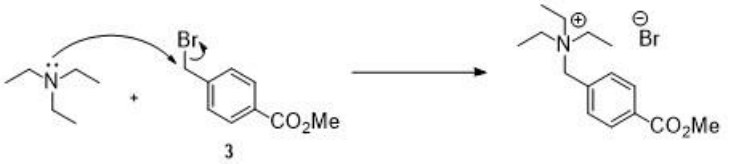
Scheme 7. Course of the S_N2 reaction.

The qualitive Hughes-Ingold solvent theory, predicts that elimination and nucleophilic substitution reactions, in which a charge is being developed in the transition state from neutral (or slightly charged) reactants, will proceed more rapidly if a polar solvent is used. The solvent used in this reaction was anhydrous THF, with a dielectric constant of 7.6 at 298K.[20] Nevertheless, it should be more optimal to use DMF instead, which has a dielectric constant of 36.7 at 298K (being more polar). In fact, DMF has been used as a solvent in similar S_N2 reactions between secondary amines and primary halides. A representative example is shown in **Scheme 8**, where a partially deactivated secondary amine (similar to **1**), reacts with a benzylic halide (similar to **3**). The reaction was described in 2019 by Witkowska obtaining a yield of 68%.[21]



Scheme 8. S_N2 reaction to form 2-[(6-bromopyridin-2-yl)(4-methylbenzyl)amino]ethanol.

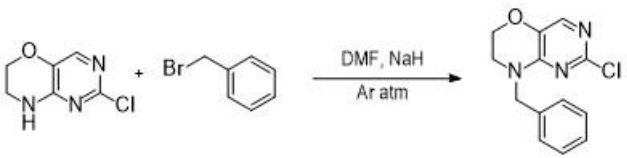
In the reaction done to form **5**, Et_3N is used as a base with the aim of catching the proton of **6**. However, there is a major encounter that is affecting the yield. Et_3N is also a nucleophile and therefore, is able to react with **3** on what is called a Menshutkin reaction, forming a quaternary ammonium salt (**Scheme 9**).



Scheme 9. Mechanism of the Menshutkin reaction.

The Menshutkin reaction is useful and widely employed when synthesizing phase transfer catalysts (PTC),[22] nevertheless for the purpose of the project it is a side reaction. This undesired reaction could explain both, why the excess compound **3** seemed to disappear in ^{1}H-NMR after the reaction between **2** and **3** was completed, and why the amount of **3** recovered in the column chromatography was less than expected. For the sake of avoiding this side reaction, a sterically hindered non-nucleophilic base should be used instead, for instance DIPEA.

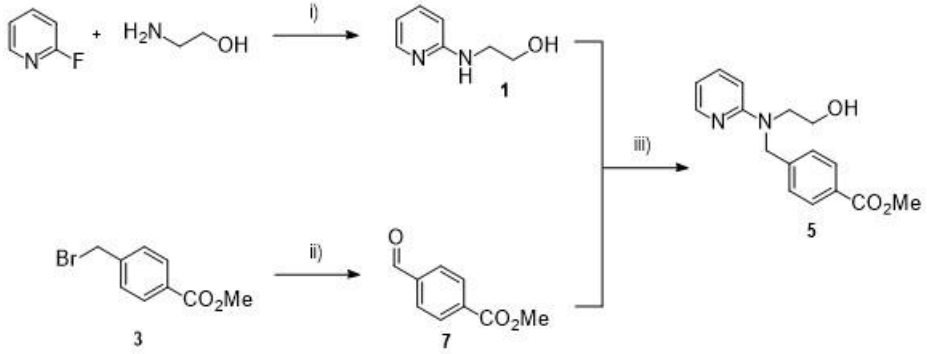
An interesting alternative implies the formation of an amide anion, the conjugate base of **1**, a more nucleophilic compound that can be formed by the addition of NaH. It is crucial to be aware that two equivalents will be needed, since the acidic proton from the hydroxyl group would react as well. In a patent document from 2015,[23] NaH is used to perform the same type of reaction with a remarkable yield of 98% (**Scheme 10**). It is worth to mention that the use of a weak base (such as Et_3N, DIPEA, etc.) is not required, as it does not form the quaternary ammonium cation **6**.



Scheme 10. S_N2 reaction via the formation of the amide anion.

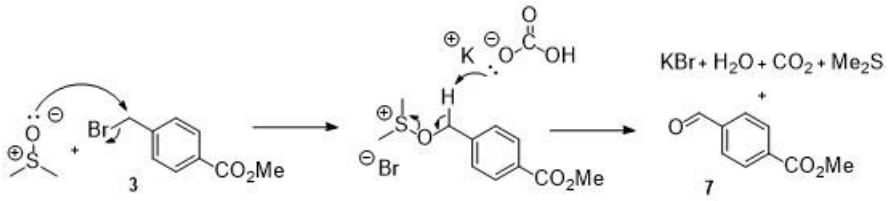
It is clear that the main problem of the current synthetic plan is the S_N2 reaction of the secondary amine **1**. Although this problem can be probably resolved by applying the experimental conditions mentioned, uncertainty exist. For this reason, an alternative synthetic plan is proposed in **Scheme 11**. To design it, the experience from the related reactions already done, the cost as

well as the availability of the reactants involved and the yield of each step are taken into consideration.



Scheme 11. Reactions and conditions: (i) Pyridine, microwave heating for 2h at 210ºC; (ii) DMSO, $KHCO_3$, microwave irradiation (λ =12.2 cm) for 0.05h; (iii) $NaBH(OAc)_3$, Et_3N, AcOH pH=5, DCM, 20ºC for 2h.

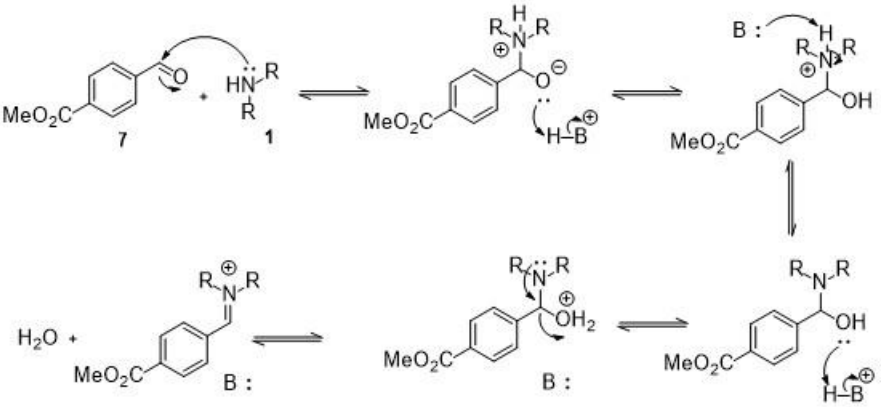
The S_NAr reaction to form **1** works properly with a yield over 90% plus the reagents are already accessible in the laboratory, therefore there is no reason to look for an alternative. On the other hand, the Kornblum oxidation is done in order to obtain the aldehyde **7**. Similar to DMSO-based oxidations, the Kornblum reaction generates an alkoxysulfonium ion, that in presence of a weak base (avoiding Et_3N due to Menshutkin side reaction), decomposes to form the appropriate aldehyde (**Scheme 12**). The same reaction was done in 2008 by George Bratulescu obtaining a high yield of 90%.[24] Both primary halides and dimethyl sulfoxide (DMSO) are strong polar agents that absorb really well microwave radiation. In fact, it was observed that the reaction rate increases remarkably if it is performed under microwave irradiation.[25]



Scheme 12. Hypothetic mechanism of Kornblum oxidation.

The last reaction consists of a reductive amination, in which the carbonyl group of **7** is converted into the amine **5** via the formation of an imine intermediate. Even though secondary amines tend to form enamines instead, the aldehyde **7** does not have any hydrogen bonded to

the C in alpha that allows it. The imine is then reduced *in situ* by the action of a reducing agent. It is essential that the reducing agent only reacts with the imine intermediate and does not reduce the aldehyde or the methyl ester. With this objective it is used $NaBH(OAc)_3$ alternatively to the typical and more powerful reductants $NaBH_4$/$LiAlH_4$. The formation of the imine is an equilibrium reaction shown in **Scheme 13**, which can be shifted towards the imine formation by the removal of the formed water.

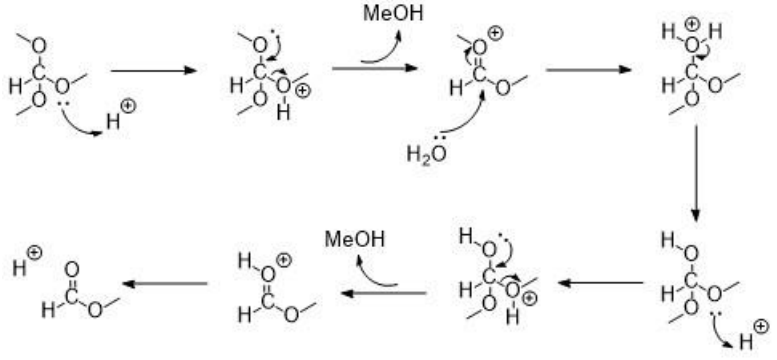


Scheme 13. Hypothetic mechanism of the imine formation.

It exists several ways to remove the water from the reaction, implying both physical and chemical techniques. One of the most widely employed, due to its simplicity, is the addition of molecular sieve with a pore diameter of four Angstroms. A molecular sieve is a type of material which presents high porosity, employed to absorb small molecules (such as H_2O, SO_2, C_2H_4, etc.) inside it. The pores present a uniform size, being the diameter what determines the functioning of the particular molecular sieve. In particular, four Angstroms molecular sieve is considered to be the universal drying agent in polar and non-polar media.[26] Moreover molecular sieves have proven to be more efficient comparing to other traditional techniques, which often operate with aggressive dessicants.[27]

An additional wide employed technique involves the addition of trimethyl orthoformate (TMOF), the simplest orthoester, which reacts with water forming methanol and methyl formate

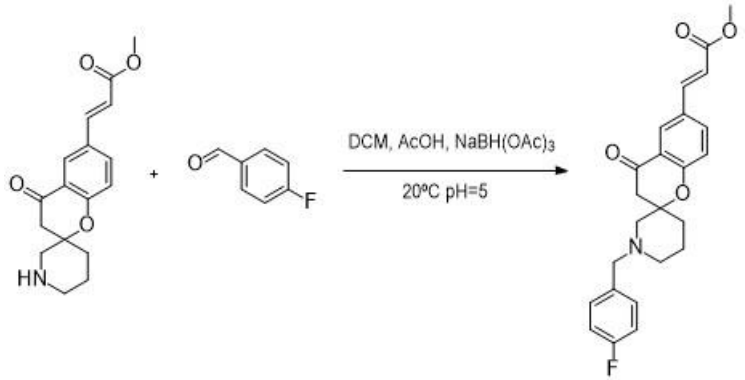
(**Scheme 14**). The formation of methanol does not suppose a problem, since a possible transesterification reaction of compound **7** results in the same methyl ester.



Scheme 14. Mechanism of the TMOF action.

In 2017, Michael O. Frederick performed a reductive amination reaction working with similar reagents compared to **1** and **7**.[28] With the main objective of shifting the reaction towards the imine formation, they employed various water scavengers such as molecular sieves, but no positive results were obtained until trimethyl orthoformate was used. Not only was the reaction rate accelerated, but the conversion was complete. In fact, TMOF is considered an excellent dehydrating reagent for solid phase and solution imine formation.[29]

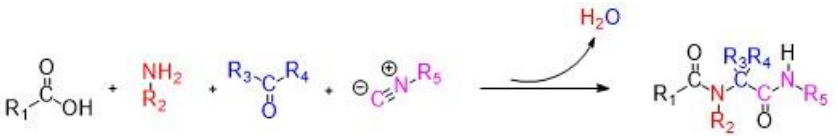
The reductive amination to form **5** also avoids possible chemoselective issues that can be produced, since the reaction only occurs between the aldehyde and the amine, the hydroxyl group of **1** does not intervene at all. In 2016, Florian Thaler carried out a similar reductive amination, shown in **Scheme 15**, obtaining the desired product with a 90% yield.[30]



Scheme 15. Synthesis of a spirochromane, acting as a potent HDAC inhibitor.

The synthesis towards the compound **5** can be seen as a consecutive *N*-alkylation reaction starting from the 2-aminoethanol. Generally, the formation of tertiary amines starting from primary amines has been widely done by the reactions of the latter with electrophiles. Nonetheless, alkylated amines usually are more nucleophile and have the tendency to undergo further alkylation. Hence, the reaction between primary amines and electrophiles such as alkyl halides, frequently results in a mixture of polyalkylation products. Consequently, tertiary amines bearing three different substituents have regularly been synthesized by the progressive nitrogen alkylation with some experimental manipulations in order to ensure the unique formation of secondary amines in the first steps, minimizing posterior alkylations.[31]

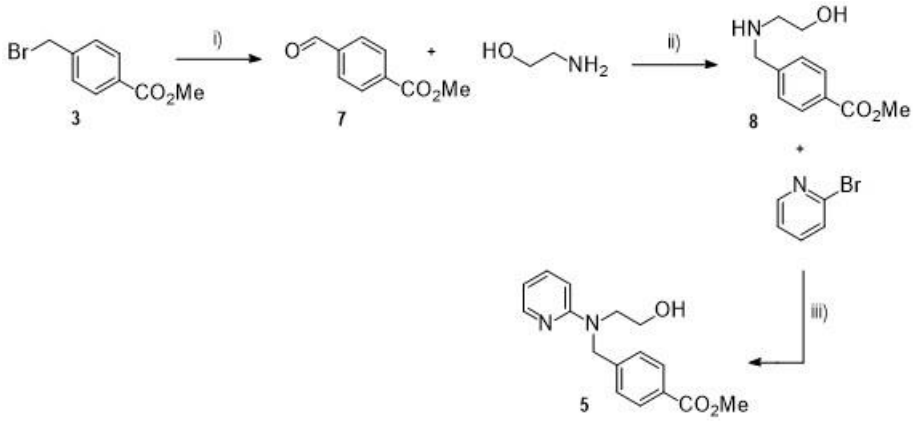
The formation of tertiary amines from primary amines by the concurrent formation of different C-N bonds at the same time, has not been reported yet. However, a four-component reaction (known as the Ugi reaction) involving a primary amine, a carboxylic acid, an isocyanide and a ketone/aldehyde to form a bis-amide (**Scheme 16**), has been actively investigated.



Scheme 16. Multi-component Ugi reaction.

The Ugi reaction is an uncatalyzed reaction giving only water as a by-product. Thus, the high atom economy as well as general high chemical yields, makes it a reaction that arouses great interest.[32] The main drawback that makes it unviable to employ it for the synthesis of **5**, is the lack of chemical diversity of the products.

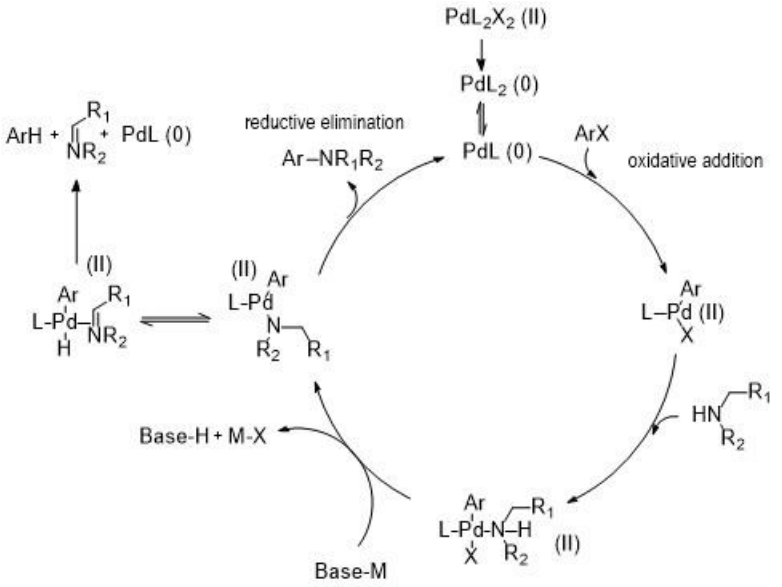
The Buchwald-Hartwig amination, is another widely used chemical reaction to form C-N bonds between amines and aryl halides. This Pd-catalysed reaction presents an excellent group tolerance and has replaced, to a certain extent, other methods such as S_NAr and the Ullmann-type reaction.[33] Benefiting from the aryl halide used in the first steps of the synthesis, an alternative synthesis plan taking this coupling reaction into consideration is shown in **Scheme 17**.



Scheme 17. Reactions and conditions: (i) DMSO, $KHCO_3$, microwave irradiation (λ =12.2 cm) for 0.05h; (ii) $NaBH(OAc)_3$, Et_3N, AcOH pH=5, DCM, 20ºC for 2h; (iii) $[Pd_2(dba)_3]$, Cs_2CO_3, RuPhos, N_2 atmosphere, TAA, 80ºC overnight.

The two first reactions of the synthesis have already been discussed previously, with the only difference that the reductive amination employs 2-aminoethanol instead of **1**. Eventually, the secondary amine **8** would be coupled to the 2-bromopyridine by the Buchwald-Hartwig reaction. For this reaction the only halogens suitable are chlorine, bromine and iodine, hence 2-fluoropyridine cannot be used. The conditions required for the success of the Buchwald-Hartwig reaction are substrate dependant, which implies considering both steric and electronic properties of reagents.

The general mechanism as well as the function of each reactant is exposed in **Scheme 18**. Firstly, Pd (II) is reduced to Pd (0) by amines that contain α-H or by the ligand. Next, a ligand dissociates from Pd, and it is formed a Pd (II) complex by the oxidative addition of 2-bromopyridine. Then, **8** attack Pd substituting the bromine with help of a base. Lastly, a reductive elimination gives the product **5**, regenerating the catalyst. An undesired side reaction would be the formation of an imine due to a β-elimination of the amine.[34] It is crucial for the reduction of Pd (II) in the first steps the presence of amines that contain α-H, otherwise an excess of ligand should be added to the reaction. Alternatively, Pd (0) complex can be used instead of PdL_2X_2.



Scheme 18. Catalytic cycle of the Buchwald-Hartwig reaction.

The key part fort the successful outcome of this reaction is the use of a suitable base and ligand. Buchwald in 2011 proposed a general ligand design strategy shown in **Figure 5**.[35] Depending on the type of nucleophile, the ligand may vary to ensure high efficiency.

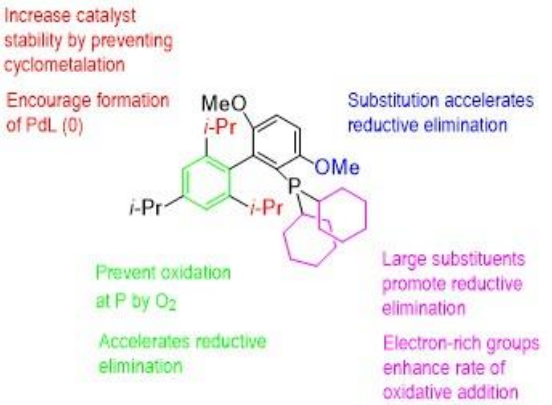


Figure 5. Structural features of dialkylbiaryl phosphine ligands.

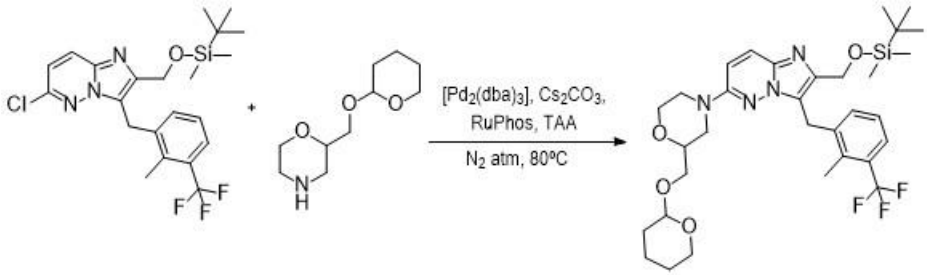
A specific ligand called RuPhos has demonstrated high performances for secondary amines.[36] Nevertheless, it should be noted that if there is any primary amine in the media

selectivity is not assured. Referring to the type of base used, Buchwald gave useful information (resumed in **Table 1**) in "*use guide*". [35]

Base	Advantages	Disadvantages
NaOtBu	Higher reaction rates	Can react with some electrophilic groups
LHMDS	Useful at T↓ and preferred in presence of protic groups	At T↑ can undergo side reactions
Cs_2CO_3	Usually the most efficient weak base, also compatible with most of the functional groups	Expensive plus it aggregates in large scales
K_3PO_4 and K_3CO_4	Cheap and optimal, especially when working with aryl sulfonates instead of aryl halides	Usually requires an excess and higher reaction times

Table 1. Base comparison.

Concerning the election of the base, strong bases as for example alkoxides (NaOtBu) are not compatible for the purpose of our reaction, since the molecule **8** presents an ester group which can react. Weak inorganic bases such as Cs_2CO_3, K_3CO_4 and K_3PO_4 would be more suitable to our purpose, since it provides more general conditions for reactants containing electrophilic groups. It was observed that grinding the weak base before using it can increase the yield of the reaction.[37] A reaction that illustrates the huge potential of the Buchwald-Hartwig reaction is shown in **Scheme 19**. The indicated reaction appears in a 2016 patent,[38] and despite the apparent complexity of the reactants the reaction is performed with >85% yield.



Scheme 19. Synthesis of an imidazopyridazine derivative acting as PI3Kβ inhibitor.

6. EXPERIMENTAL SECTION

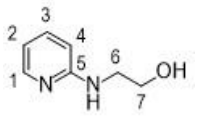
6.1. MATERIALS AND METHODS

Microwaves were irradiated by a Biotage Initiator. Infrared spectra were recorded on a Nicolet 6700 FT-IR spectrophotometer using ATR sampling technique and the value of each band is given in cm^{-1}. ^1H NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ at room temperature with Mercury 400 spectrometer. Chemical shifts are given in δ (ppm) relative to TMS and coupling constants in Hz. Multiplicity of signals are indicated as: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet) and br (broad signal). Column chromatography was performed manually employing silica gel (40-63 μm) as the stationary phase.

6.2. PREPARATION OF THE COMPOUNDS

6.2.1. Preparation of 2-(pyridin-2-ylamino)ethanol (1)

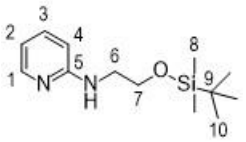
To a 20 mL MW vial, 2-aminoethanol (0.2 mol, 12 mL) and 2-fluoropyridine (0.023 mol, 2 mL) were dissolved in excess of pyridine (6 mL). The mixture was stirred under microwave irradiation for 1.5h at 210ºC and then, let it cool down until room temperature. Saturated $NaHCO_3$ solution (25 mL) was added to the mixture and the two phases were separated. The aqueous layer was washed with EtOAc (5 x 20 mL). All organic layers were combined and washed several times with brine (5 x 20 mL). Then it was dried with $MgSO_4$ and concentrated under reduce pressure. The pyridine remaining was removed under reduced pressure by making an azeotrope with toluene. Compound **1** was obtained as a pale yellow solid (2.9 g), with 90% yield.



Pale yellow solid. IR (ATR): 3284, 3143, 2945, 2852, 1615, 1455, 1376, 1339, 1052, 978 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 7.99 (d, $J_{H\text{-}H}$= 3.5 Hz, 1H, H^1), 7.36 (t, $J_{H\text{-}H}$= 7.8 Hz, 1H, H^3), 6.54 (t, $J_{H\text{-}H}$= 5.9 Hz, 1H, H^2), 6.41 (d, $J_{H\text{-}H}$ = 8.4 Hz, 1H, H^4), 4.95 (br, 2H, O-H + N-H), 3.79-3.74 (m, 2H, H^7), 3.45 (t, $J_{H\text{-}H}$ = 5.2 Hz, 2H, H^6). ^{13}C NMR ($CDCl_3$, 101 MHz): δ 159.01, 147.36, 137.81, 113.18, 108.53, 63.30, 45.47.

6.2.2 Preparation of *N*-[2-[*tert*-butyl(dimethyl)silyl]oxyethyl]pyridin-2-amine (2)

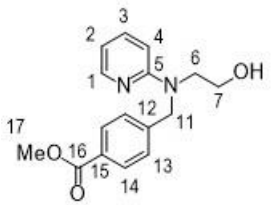
To a 20 mL MW vial, compound **1** (0.7 mmol, 0.10 g) was dissolved in dry DCM (15 mL) with TBSCl (1.4 mmol, 0.22 g) and imidazole (1.4 mmol, 0.10 g) under N_2 atmosphere. Additional TBSCl (0.7 mmol, 0.11g) was added after 1.5h under microwave irradiation at 100ºC. The mixture is then stirred at room temperature for 3h and quenched with water (10 mL). The two layers were separated, washing the aqueous layer with DCM (2 x 5 mL). Organic layers were combined, washed with distilled water (5 x 20 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure giving **2** as a yellow oil (0.17 g) with 88% yield.



Yellow oil. IR (ATR): 2923, 2852, 1597, 1459, 1250, 1103, 1076, 827, 765 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.06 (d, $J_{H\text{-}H}$ = 4.2 Hz, 1H, H^1), 7.38 (t, $J_{H\text{-}H}$ = 6.9 Hz, 1H, H^3), 6.54 (t, $J_{H\text{-}H}$ = 6.0 Hz, 1H, H^2), 6.39 (d, $J_{H\text{-}H}$ = 8.4 Hz, 1H, H^4), 4.79 (s, 1H, N-H), 3.79 (t, $J_{H\text{-}H}$ = 5.3 Hz, 2H, H^7), 3.40 (q, $J_{H\text{-}H}$ = 5.5 Hz, 2H, H^6), 0.88 (s, 9H, H^{10}), 0.04 (s, 6H, H^8). ^{13}C NMR ($CDCl_3$, 101 MHz): δ 158.99, 148.19, 137.50, 112.98, 107.47, 62.11, 44.35, 26.10, 18.51, -5.16.

6.2.3 Preparation of 2-[*N*-(2-pyridyl)-*N*-[4-(methoxycarbonyl)benzyl]amino]ethanol (5)

To a 5 mL MW vial, compound **1** (0.7 mmol, 0.10 g) and **3** (1.4 mmol, 0.33 g) were dissolved in dry THF (4 mL) with triethylamine (0.5 mL) under N_2 atmosphere. The mixture was stirred under microwave irradiation for 6h at 100ºC and then, let it cool down until room temperature. The solvents were evaporated under reduced pressure obtaining **5** as well as other undesired compounds. The product was purified by column chromatography employing EtOAc:Hexane (60:40, v/v) as the eluent. Pure fractions were combined and rotaevaporated to dryness giving **5** as a brown-yellow oil (0.07g) with 35% yield.



Brown-yellow oil. IR (ATR): 3341, 2914, 1713, 1593, 1486, 1428, 1272, 1103, 769 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.09 (d, $J_{H\text{-}H}$ = 4.7 Hz, 1H, H^1), 7.98 (d, $J_{H\text{-}H}$ = 8.2 Hz, 2H, H^{14}), 7.37 (t, $J_{H\text{-}H}$ = 6.9, 1H, H^3), 7.27 (d, $J_{H\text{-}H}$ = 8.1 Hz, 2H, H^{13}), 6.60 (t, $J_{H\text{-}H}$ = 6.0 Hz, 1H, H^2), 6.39 (d, $J_{H\text{-}H}$ = 8.6 Hz, 1H, H^4), 4.70 (s, 2H, H^{11}), 4.10 (q, $J_{H\text{-}H}$ = 7.1 Hz, 1H, O-H), 3.89 (s, 3H, H^{17}), 3.88-3.84 (m, 2H, H^7), 3.82-3.79 (m, 2H, H^6). ^{13}C NMR ($CDCl_3$, 101 MHz): δ 167.02, 159.06, 147.35, 143.41, 138.26, 130.36, 129.48, 126.55, 113.30, 107.15, 63.34, 53.92, 53.24, 52.32.

7. CONCLUSIONS

In this work, 2-[*N*-(2-pyridyl)-*N*-[4-(methoxycarbonyl)benzyl]amino]ethanol was synthesized and purified in a 35% yield. In an attempt to improve the performance of the last reaction, some modifications on the experimental conditions were suggest. Firstly, to substitute Et_3N for a more hindered base (such as DIPEA) with the objective of avoiding the Menshutkin reaction. Secondly, to use DMF as the solvent as a replacement of THF, stabilizing the transition state. Lastly, to employ the amide anion instead of the amine **1** as the nucleophile, via the addition of NaH.

It is widely known that S_N2 reactions implying amines are not usually the most effective method, since it can derive to some issues such as undesired polyalkylations. Therefore, two alternative synthetic plans were proposed after reviewing the literature, taking advantage of the chemoselective reductive amination and the Pd (0) catalysed Buchwald-Hartwig reaction.

All the intermediates as well as the thermolabile linker **5** were characterized by ^{1}H-NMR, ^{13}C-NMR and IR spectroscopy.

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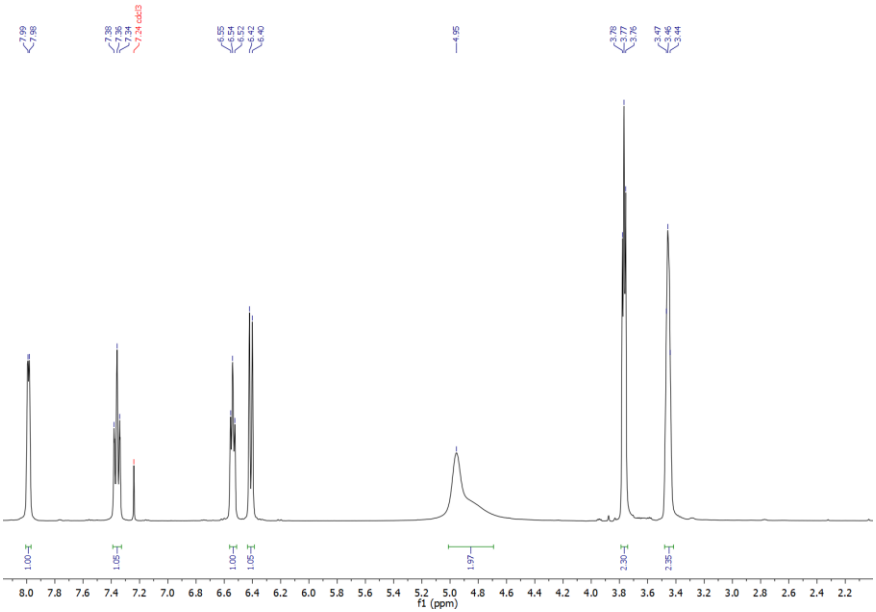
9. ACRONYMS

NCDs	Non-communicable diseases
NPs	Nanoparticles
TPGs	Thermolabile protecting groups
WSC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
DMAP	*N*,*N*-dimethylaminopyridine
DCM	Dichloromethane
MW	Microwave
S_N2	Bimolecular nucleophilic substitution
S_NAr	Aromatic nucleophilic substitution
EtOAc	Ethyl acetate
TBSCl	*tert*-Butyldimethylsilyl chloride
TLC	Thin-layer chromatography
r.t.	Room temperature
THF	Tetrahydrofuran
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
LHMDS	Lithium bis(trimethylsilyl)amide
Dba	Dibenzylideneacetone
TAA	*tert*-amyl alcohol
TMS	Tetramethylsilane
ATR	Attenuated total reflection

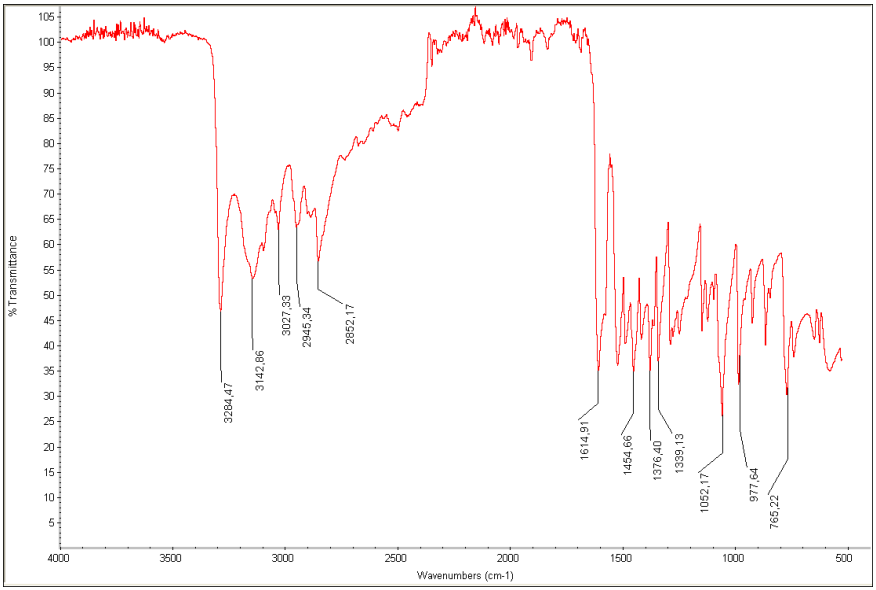
APPENDICES

APPENDIX 1: SPECTRA DATA OF 1

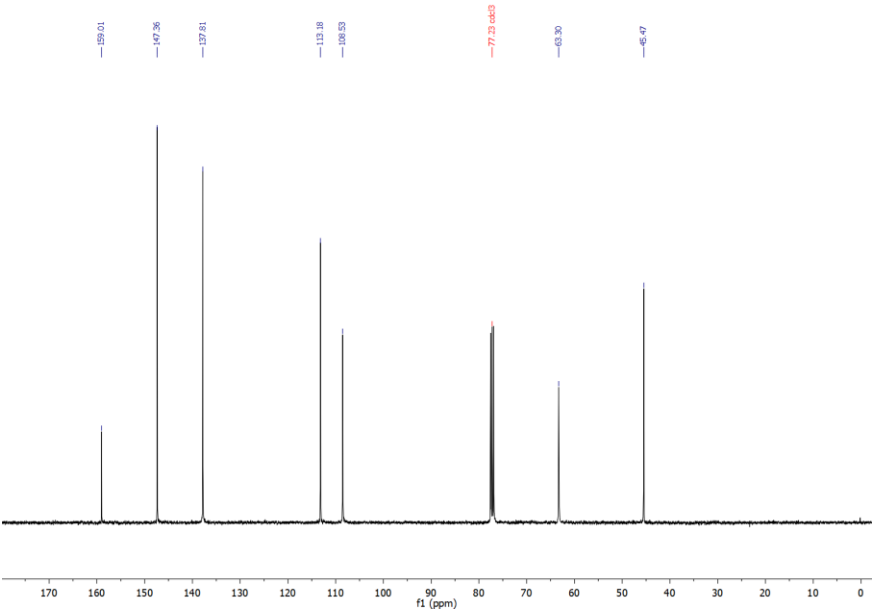
^{1}H-NMR



IR

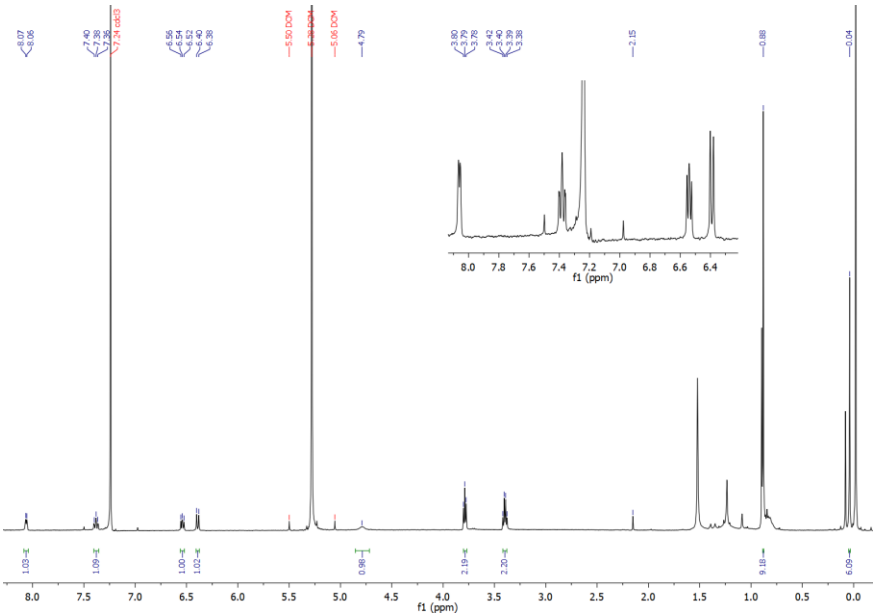


^{13}C-NMR

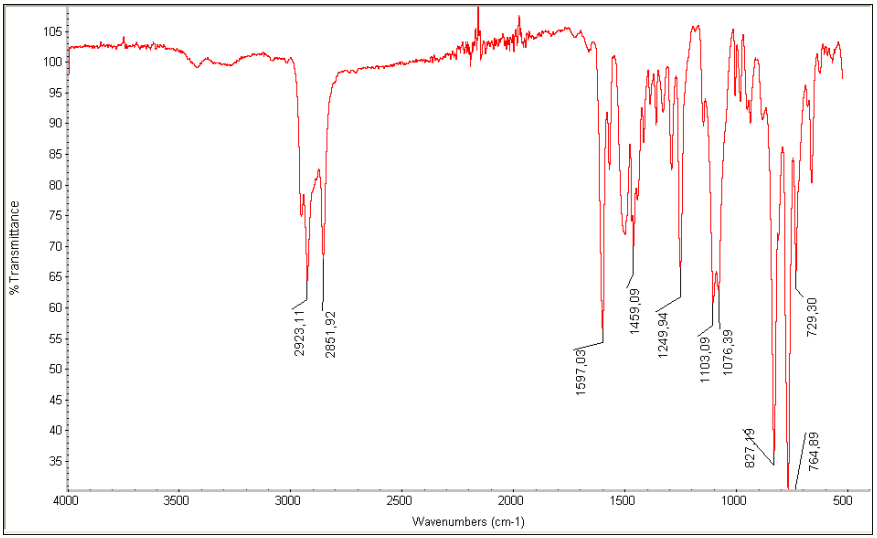

159.01
147.36
137.81
113.18
108.53
77.23 cdcl3
63.30
45.47
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

APPENDIX 2: SPECTRA DATA OF 2

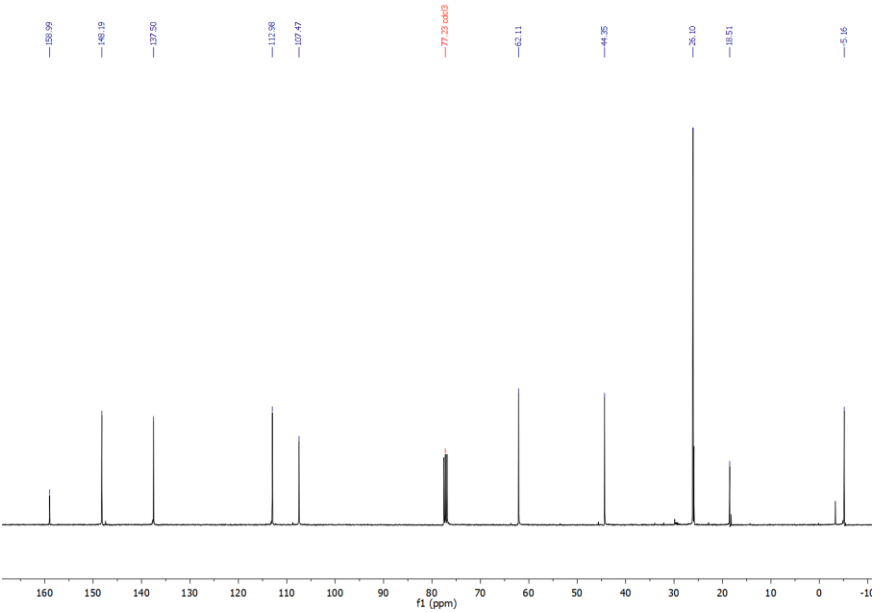
^{1}H-NMR



IR

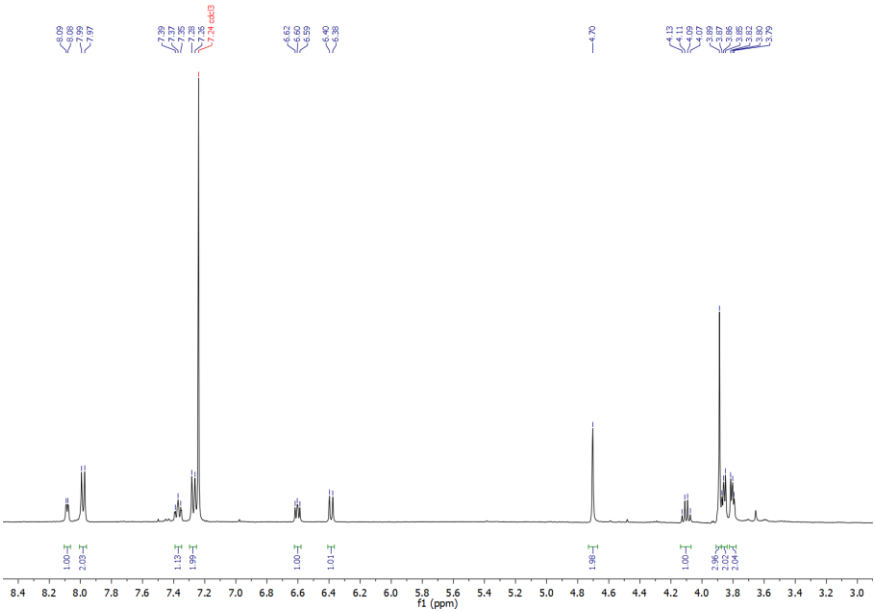


^{13}C-NMR

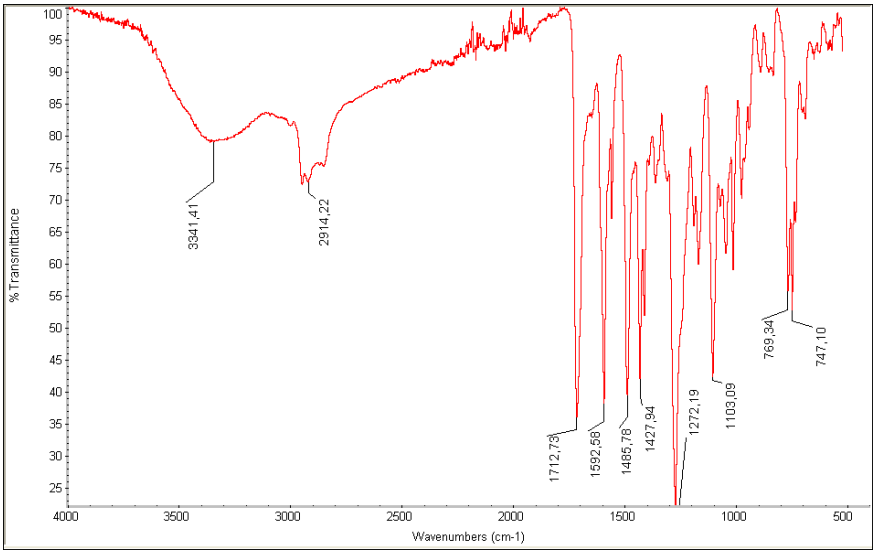

158.99
148.19
137.50
112.98
107.47
77.23 cdcl3
62.11
44.35
26.10
18.51
5.16
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

APPENDIX 3: SPECTRA DATA OF 5

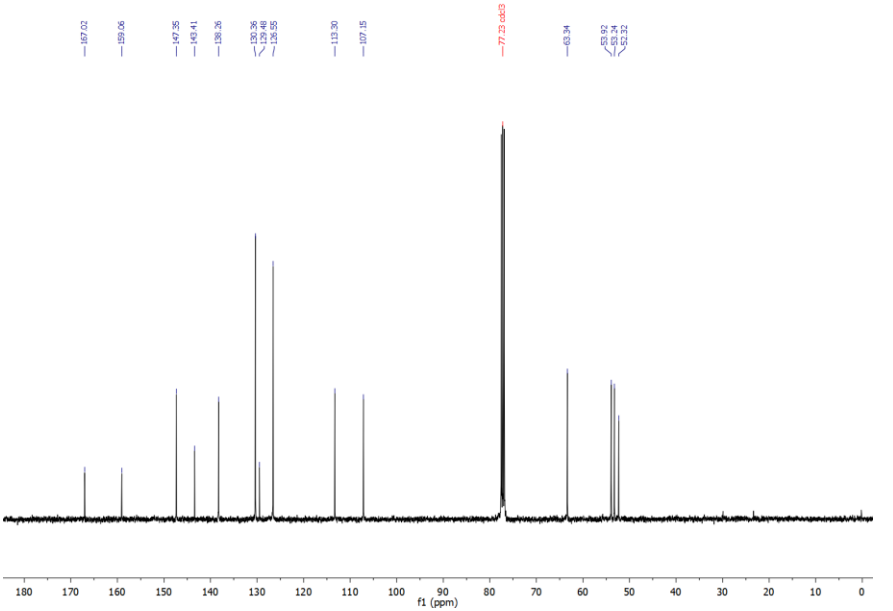
^{1}H-NMR



IR



^{13}C-NMR


167.02
159.06
147.35
143.41
138.26
130.36
129.48
126.55
113.30
107.15
77.53 cdcl3
63.34
53.92
53.24
53.22
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)