Internship report 2nd years :

Research Trainee

Presented by Pauline Ribera

Host Organization : Karolinska Institutet, CBCS Scilifelab

Scilifelab supervisor : Martin Haraldsson

SIGMA Clermont supervisor : Elodie Jagu

From 30/05/2022 to 16/09/2022

Document reference : Ribera_Pauline_InternshipReport.pdf

FICHE D'IDENTIFICATION DU DOCUMENT

Rapport de Stage 2ème Année

TITRE DU DOCUMENT :	Internship report of 2nd years *Internship report 2nd year*
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Date du document	Nbre de pages	Référence du document
15/09/22	47	Ribera_ Pauline_RapportStage2A.pdf

ABSTRACT:

This document presents the internship report of the work I did from May 2022 to September 2022 in Stockholm, as part of the engineering training at Sigma Clermont. I completed this internship as a research intern at SciLifeLab. So the report starts with an introduction to SciLifeLab, followed by a more detailed presentation of the CBCS group I joined for my internship. Then, there is an introduction on my internship topic: molecule synthesis for male contraception. I then continue with a brief presentation of the material and methods I had the opportunity to use. A discussion and a presentation of the results of my experiments followed by the experimental part of them, follows. Finally, I end with a general conclusion of my internship as well as professional and personal.

Keywords: report, internship, SciLifeLab, research, synthesis, inhibitor 1 analogues

RESUME :

Ce document présente le rapport de stage du travail que j'ai effectué de mai 2022 à septembre 2022 à Stockholm, dans le cadre de la formation d'ingénieur à Sigma Clermont. J'ai effectué ce stage en tant que stagiaire en recherche à SciLifeLab. Le rapport commence donc par une présentation de SciLifeLab, suivie d'une présentation plus détaillée du groupe CBCS que j'ai rejoint pour mon stage. Ensuite, il y a une introduction sur mon sujet de stage : synthèses de molécules pour la contraception pour les hommes. Je continue ensuite par une briève présentation du matériel et des méthodes que j'ai eu l'occasion d'utiliser. Une discussion et une présentation des résultats de mes expériences suivie de la partie expérimentale de celles-ci, s'en suit.
Enfin, je termine par une conclusion générale de mon stage ainsi que professionnelle et personnelle.

Mots clés : rapport, stage, SciLifeLab, recherche, synthèse, analogues de l'inhibiteur 1

Visa de l'entreprise :
Ce rapport de stage a été visé par (Nom, Prénom, Qualité) : Martin Haraldsson PhD
Date : 220901
Signature :

Je demande que ce rapport soit confidentiel (non diffusé sur l'intranet de l'école) : €OUI €NON

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ACKNOWLEDGMENTS

First of all, I would like to thank the Swedish National Research Centre Scilifelab and the CBCS group who welcomed me during these 4 months of internship.

Then, I would like to thank my internship tutor **Martin HARALDSSON** who allowed me to complete an internship in full compliance with my expectations.

I would also like to especially thank **Tobias KOOLMEISTER**, who helped me a lot throughout this internship, as well as the DDD group and especially **Remy**, and all the members of Helleday group with who I shared the laboratory. They helped me to resonate with the difficulties I encountered.

I also wanted to thank **Evert** for the time spent explaining to me the biological part of my subject.

All their knowledge and patience allowed me to enrich my chemical knowledge in the field of health. Their friendliness and kindness also allowed me to work in a good atmosphere.

Finally, I would like to thank my school supervisor, **Elodie JAGU**, for being present throughout the internship.

ABREVIATIONS

SciLifeLab: Science Life Laboratory

CBCS: Chemical Biology Consortium Sweden

SAR: Structure-Activity Relationship

RA: Acromosome Reaction

CatSper: Cation channels of Sperm

UV: Ultra-Violet

TLC: Thin Layer Chromatography

Rf: Retention Factor

NMR: Nuclear Magnetic Resonance

LC-MS: Liquid-Chromatography Mass Spectrometry

DMF: Dimethylformamide

DCM: Dichloromethane

EtOAc: ethyl acetate

Iso-Hex: Iso-Hexane

DMSO : Dimethyl Sulfoxyde

HATU : (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium)

RT: Room Temperature

ON: Overnight

Chemical formula :

$CDCl_3$: Deuterated Chloroform

Et_3N : Triethylamine

H_2 Pd/C (X%) : Palladium on Carbon with X percent of Palladium under Hydrogen atmosphere.

I. INTRODUCTION

During my engineering studies, I have to do an internship to validate my second years. This internship lasted 16 weeks and I worked as a research intern in a lab, because I was interested in doing a thesis, so I wanted to know if I liked the field of research.

A. Presentation of Scilifelab

The Science Life Laboratory (SciLifeLab) is a Swedish national research center, especially in molecular biology, with a focus on health and environmental research. SciLifeLab bring together the host universities like Stockholm University, Uppsala University, Royal Institute of Technology (KTH), and Karolinska Institutet (KI). This infrastructure offer access to advanced technologies and services for all sectors, ranging from academic research groups, health care providers, industry, governmental authorities, teachers, and students. Their ambition is to increase quality and international competitiveness of Swedish research through continued efforts on technology-driven infrastructure, as well as a new focus on data-driven life science. SciLifeLab buildings in Stockholm are in Solna.

At SciLifeLab there are different research organizations working in collaboration with each other or with public research organizations, international partners, industry, or healthcare. See **Appendix 1** for the organization chart of SciLifeLab. Chemical Biology and Genome Engineering (CBGE) is a recently established SciLifeLab platform consisting of three units: Chemical Biology Consortium Sweden (CBCS), CRISPR Functional Genomics (CFG), Chemical Proteomics (Chem Prot). In 2012, CBCS became part at SciLifeLab and I did my internship within CBCS group. **[1]**

B. Presentation of CBCS

The Chemical Biology Consortium Sweden (CBCS) was formally established in 2010 through the joint efforts of academic and industrial researchers. CBCS is represented in the 5 bigger Swedish universities : Umeå (UmU), Uppsala (UU), Stockholm, Linköping (LiU), Gothenburg, and Lund. Each university bring their own expertise in new techniques, for example phenotypic profiling using cell painting at UU, screening of ion channels at LiU, biosafety level 3 screening at KI and LiU (and UmU in the future).

This CBCS team is composed of 10 people whose CBCS founding members are: Lars HAMMARSTRÖM, Anna-Lena GUSTAVSSON, Lars JOHANSSON, Mikael ELOFSSON, Per ARTURSSON, Patrik ERNFORS, Annika JENMALM JENSEN, and Martin HARALDSSON. Most of scientific staff members at CBCS have experience from the pharmaceutical industry. Anna-Lena GUSTAVSSON is the platform director of the management group of SciLifeLab Board. Martin HARALDSSON, Tobias KOOLMEISTER, Birger SJÖBERG are chemists at CBCS.

Hanna AXELSSON, Francesco MASSAI, Marianna TAMPERE, Brinton SEASHORE-LUDLOW are biologists at CBCS. The biology team at CBCS is working with assay development and small molecule screening. They are working together with a team of medicinal chemists, on studies of mechanism of action, on the exploration of hit compound and SAR (Structure Activity Relationship).

Flavio BALLANTE, work on cheminformatics. Åsa SLEVIN, Maria ADOLFSSON, and Elisabeth OLSSON take care of the Compound Center. CBCS units comprise compound handling labs (with a high-quality chemical collection around 350 000 compounds), screening labs, and chemistry labs.

This group has provided input to more than 400 research projects during the past 5 years of operation, and the goal now is to have a strong impact in the field of chemical biology and thus, to lead to new biological discoveries, the validation of new drug targets and new concepts for the development of useful therapies and diagnostics. **[2]**

C. Introduction of my subject

An international effort involving labs and organizations in Sweden, UK and Canada has set as a goal to find and develop a new principle for male contraceptives. One of the tracks is targeting a serin hydrolase enzyme ABHD2. There are a few unexplored inhibitors known.

All cells are surrounded by a lipid bilayer. The serine hydrolase ABHD2 is an enzyme that allows the opening of this lipid bilayer. When this enzyme is attached to the lipid bilayer, progesterone, released by the oocyte, can bind to the protein. When progesterone binds, 2-Arachidonoylglycerol (2-AG) is converted into two parts. The glycerol leaves the lipid bilayer (red triangle), and the arachidonic acid (red thread) gets better mobility in the lipid bilayer and approaches the CatSper. After that, there is a release of Ca2+ ions and thus a change in the ionic concentration of cations. These phenomena allow the opening of the sperm cation channels (CatSper) and create sperm hyperactivation. This hyperactivation allows the spermatozoa to enter the zona pellucida, and the acrosomal reaction can take place. **[3]**

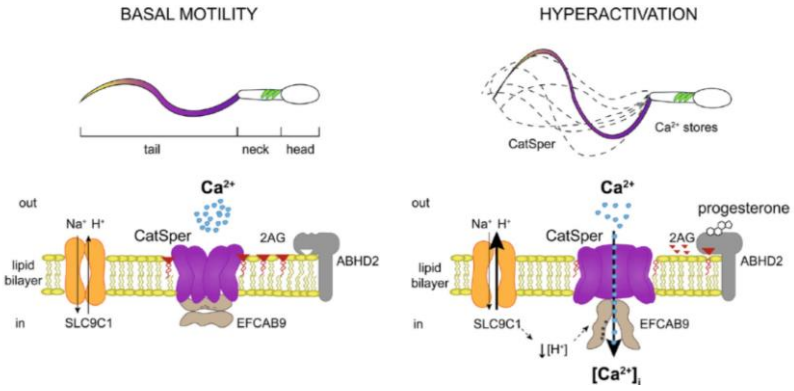


Figure 1 : The mechanism of the sprem hyperactivation.

The acrosome reaction is the reaction that occurs when the sperm comes into contact with the eggshell. The acrosome of the spermatozoa allows attachment between the sperm and the egg and after the fecundation can take place. **[4]**

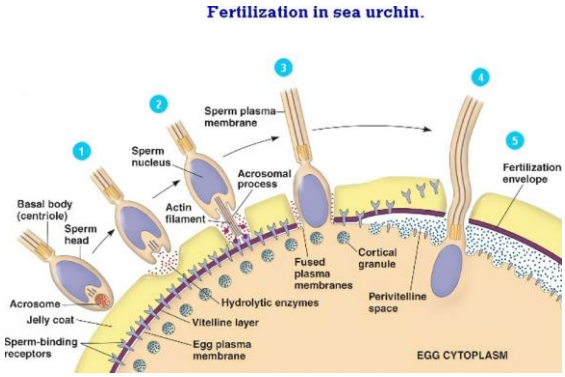
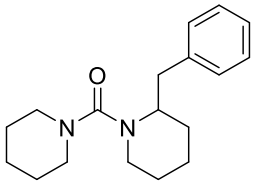


Figure 2 : The acromosome reaction.

Today, Marc P. Baggelaar - *Department of Molecular Physiology,* discovered an inhibitor (inhibitor 1) of the ABHD2. This inhibitor was investigated in *Leiden University*, in native mouse testis proteome, and the progesterone-induced acrosome reaction was reduced in a dose-dependent manner of the inhibitor. Indeed, this molecule allows the regulation of the release Ca2+ ions, and so reduce sperm hyperactivity. So, the ABHD2 have a key role in the acrosome reaction. On this basis, the ABHD2 inhibitor is an excellent starting point for further optimization of ABHD2 inhibitors that can modulate sperm fertility and may lead to novel contraceptives. **[5]**



Inhibitor 1

Today it's complicated to explain how the molecule can interact with the protein. Indeed, no people arrived to have a pure protein, and to know exactly the structure of the protein. For this moment, the molecules are just tested on the cell of insect, because the way to extract a human protein without bacterizes is more difficult.

A protein has 3 different parts: one inside the cell, one inside the lipid bilayer and one outside the cell. The bigger part of the protein is outside the cell, and it's the part that people try to obtain. To have a pure protein, so without bacterizes, the protein must be cut at the outer part of the cell. But this experience was never achieved for the ABHD2. However, a software was created to predict the structure of a protein, so it's possible to see what the ABHD2 looks like and moreover it's possible to see the protein in 3D. This software allows also to see the interactions between the protein and the molecule that was created, thus the things that are supposed about this first inhibitor are that:

- the urea part is inside the protein
- the aromatic is in the hydrophilic part
- there is a dihydrogen bond between the nonbinding doublet and a hydrogen of the protein.

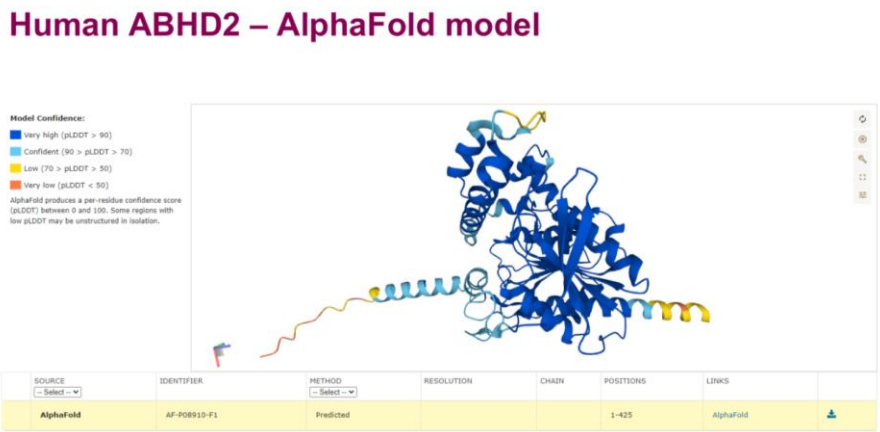


Figure 3 : The model of the ABHD2 protein.

Now, the goal is to create molecules which look like the first inhibitor. So my project aims to work with the inhibitor 1 and try to make new analogues with the goal to get better inhibitory activity and better solubility in water by investigating the structure activity (SAR) of the hit.

A software called DataWarrior, allows to guess different properties of molecules, for example this software predict the solubility in water of a molecule and the log P value which is a measure of lipophilicity or hydrophobicity. So, this software allowed me to start research of the analogues. I calculate on this software the different properties of my target molecule to see if the properties are interesting compared to the first one.

II. MATERIALS AND METHODS

LC-MS :

Agilent Technologies 1260 Infinity II.

Solvent A: TFA 0.1%

Solvent B: CH3CN

Solvent C: NH4HCO3

Method X1097: X-bridge C18, 50x3.0 mm, 3.5u, 10-97% MeCN 1.5min,1ml/min, B: MeCN C: NH4HCO3.

Method A1097: ACE C8, 50x3mm, 3µ, 10-97% MeCN, 1.5min; 1ml/min, A: 0.1% TFA, B: MeCN.

NMR :

Ascend NMR Magnets 400 MHz + sample case by BRUCKER.

Preparative liquid chromatography : (preparative LC)

Liquid Handler 215 + Injection Module 819 + Valvemate + UV/VIS-156 lamp + 2 pumps 306 + Dynamic Mixer 811C + Manometric Module 806 (maximum pressure: 32 MPa) + Prep C18 5µm OBDTM 30x75 mm Column by GILSON.

Eluent used: mixture of aqueous NH4OH (25%) and MeCN.

Flow: 40 mL/min.

UV lamp :

Wavelenght: 365 nm.

Biotage Selekt :

The Biotage Selekt is a high performance automated flash system with two column channels for all flash applications employing columns from 5 g to 1.5 Kg.

Column (Agela Technologies) : C18 spherical 20-35µm 100A 12g. Max pressure : 180psi (12.6Bar).

Gradient of eluent used : From 10% CV to 85% of acetonitrile in water.

Flow : 24mL/min.

III. RESULTS AND DISCUSSIONS

A. Chemistry result

The goal of this internship was to create a maximum of analogues of the inhibitor 1. To start my research, I used the software that I presented before : DataWarrior. With this software, I plotted the value of log S versus log P to get an overview of the different values for all my target molecules. At first, the only objective was to try to create molecules with different solubility values. Later I expanded my research and was able to also add indicators to this graph to observe the difference between molecular weight, the number of donor or acceptor proton and others. This allowed me at first to concentrate on molecules with properties close to the starting molecule, then to widen my field of research and to try to create molecules more varied in terms of properties.

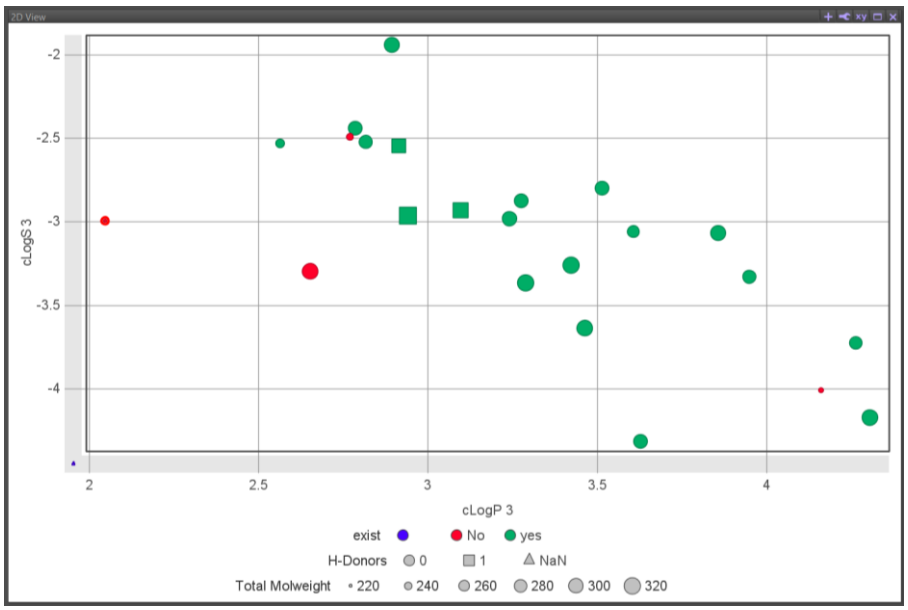


Figure 4 : Graph of Log(S)=f(Log(P)) software simulation.

i. Analogues of (1-benzylpiperidin-2-yl)(piperidin-1-yl)methanone

To start creating different analogues, I did one-step reactions. I started by using reagents that I could find directly in the lab resources, and then I went further.

So first I modified different parts of the molecule as I could with the reagents I had available.

Most of my products, I needed to just purify or extract it to get over 90% of purity each time and I didn't have some difficulties to have a pure product.

Sometimes, to achieve that I used the Preparative liquid chromatography (LC), it is a powerful technique for the isolation or purification of one or more target compounds from a mixture. This machine is useful when on the chromatograph there are two peaks very close to each other, because sometimes it's complicated to separate both of them, with a classic column. Also, this machine is

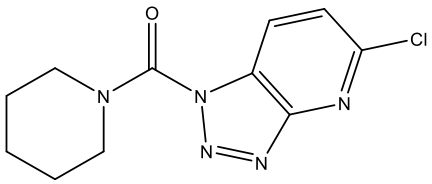
interesting to save time sometimes. However, this preparative LC has an inconvenient. When I used this machine, I get a low yield compared to the classic purification.

Indeed, the reaction to have the *(2-benzylpiperidin-1-yl)(4-methylpiperazin-1-yl)methanone* have a yield of 25%, and for this molecule, I used the preparative LC to purify my product because it was complicated to separate 2 peaks very close to each other. So I used strict condition to have a pure product, so consequently I lost a little of product.

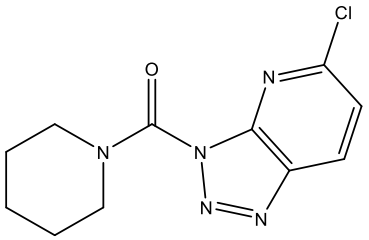
To continue talking about one-step reactions, I encountered problems of selectivity.

Firstly, the formation of the (4-hydroxy-4-phenylpiperidin-1-yl)(piperidin-1-yl)methanone was not very selective because I think there is a competition between the addition on the amine group and the addition on the alcohol group. There was a selectivity for the expected final product, but the yield was less than I tough, I obtained 23% of yield. The selectivity problem can explain this yield. To avoid that, I could have protected the alcohol group and make the reaction and then deprotect it. This method could have increased the yield of this reaction.

Secondly, I had another problem of selectivity, I obtained a mixture of two molecules in the reaction to obtain the analogues : (5-chloro-3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)(piperidin-1-yl)methanone. Indeed, the chromatograph shows two peaks glued to each other, so probably there are two molecules, and the NMR analysis confirmed this. I guess I obtained these two molecules :



(5-chloro-1*H*-[1,2,3]triazolo[4,5-*b*]pyridin-1-yl)(piperidin-1-yl)methanone



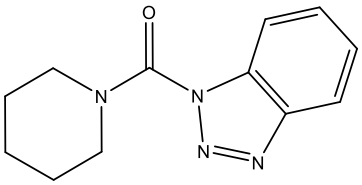
(5-chloro-3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl)(piperidin-1-yl)methanone

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 – 8.23 (m, 2H), 7.55 (dd, *J* = 8.6 Hz, 1H), 7.43 (dd, *J* = 8.9 Hz, 1H), 4.03 – 3.67 (m, 4H), 3.50 – 3.31 (m, 2H), 3.22 – 3.10 (m, 1H), 1.87 – 1.48 (m, 12H), 1.31 – 1.06 (m, 1H).

The peak between 8.38ppm and 7.43ppm correspond to the 2 aromatic protons of my molecule, nevertheless there are 2 other different peaks, therefore 4 different aromatic protons which proves the formation of two isomers. It could have been difficult to avoid this phenomenon and to obtain only one molecule, since before making this reaction I thought that chlorine would have had a selective power on the formation of this or that urea.

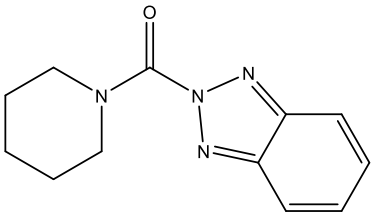
A molecule similar to this one also gave a mixture of two products.

At the beginning, the LC-MS present just one peak, but when I run an NMR, I was surprised by the integration that the different peaks gave me. So, I decided to do a TLC, and I saw two different spots. I tried to separate by a column chromatography these two different spots, and I analyzed both with an NMR. I guess the reaction gave these two molecules :



(1*H*-benzo[*d*][1,2,3]triazol-1-yl)(piperidin-1-yl)methanone

Molecule 1



(2*H*-benzo[*d*][1,2,3]triazol-2-yl)(piperidin-1-yl)methanone

Molecule 2

I supposed that because on the two spectrum that I obtained there are two different types of aromatic peaks. The analysis of the first one molecule (**Appendix 12**), shows that the aromatic proton are not equivalent compared to the other one. On the NMR of the second molecule (**Appendix 13**), there are just 2 peaks for 4 protons, so that means that there is a symmetry in the molecule and that the protons are equivalents. For these different elements I supposed that I created these two molecules.

To finish with the result and discussion of the one-step reactions, the last molecules that I created were complicated to purify because they are not very well visible on the LC-MS because they are not really UV active. When I purify them, sometimes it was complicated to know in what fractions my product was in. So, this fact causes the bad yield that I obtained for these compounds.

After all compound that I created with just one step, I would like to practice new type of chemistry. So, for that, I tried to find other types of analogues, and these new types were created with more than just one step. I had the idea with my colleagues to create an amide and not a urea. To achieve that I thought about this retrosynthetic analysis :

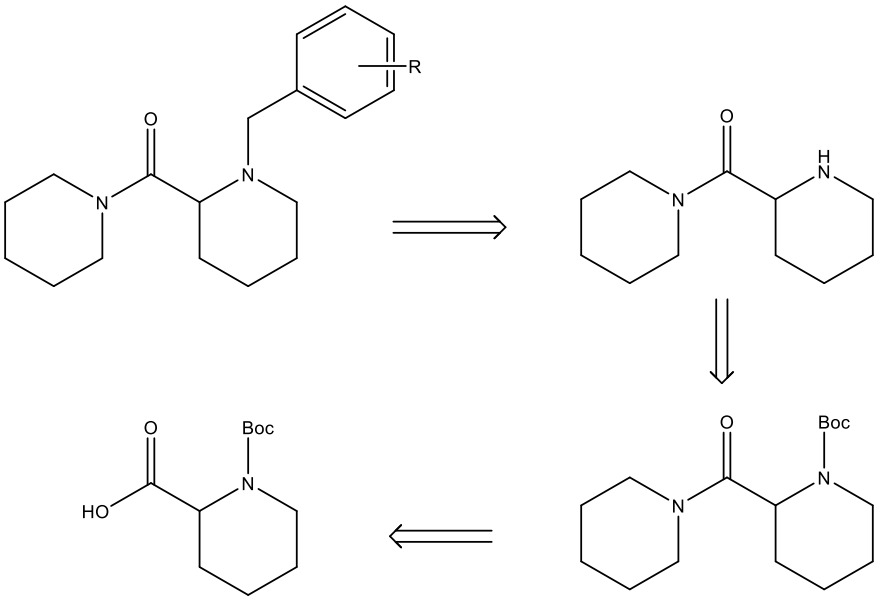


Figure 5 : Retrosynthesis of (1-benzylpiperidin-2-yl)(piperidin-1-yl)methanone analogues.

Compared to the one-step reaction, I obtain a less important yield for the multi-step reaction, it's more around 10-30%. But this comes from the yield of the different step.

The first step of the reaction, with HATU conditions, works well, the yield is 100%, because a there is probably a trace of the decomposition of the HATU as impurity (LC-MS: m/z = 184 [M+H]+).

Regarding the deprotection Boc, I manage to have a yield around 85%. The low yield come from the last step, I obtain just 30% of yield, it's less compared to the literature (70%). Maybe it could be

interesting to change the conditions of the last step, but the other conditions that I read on the literature is more inconvenient.

This type of product took a long time to achieve, so in parallel I continued to create analogues of the first inhibitor but with a new idea of synthesis. I wanted to change the left part of the molecule and not the aromatic part, to have different types of analogues. So, for that, I used the 2-benzylpiperidine as starting material, I transformed it in carbamic chloride, and I changed the other reagent, as below:

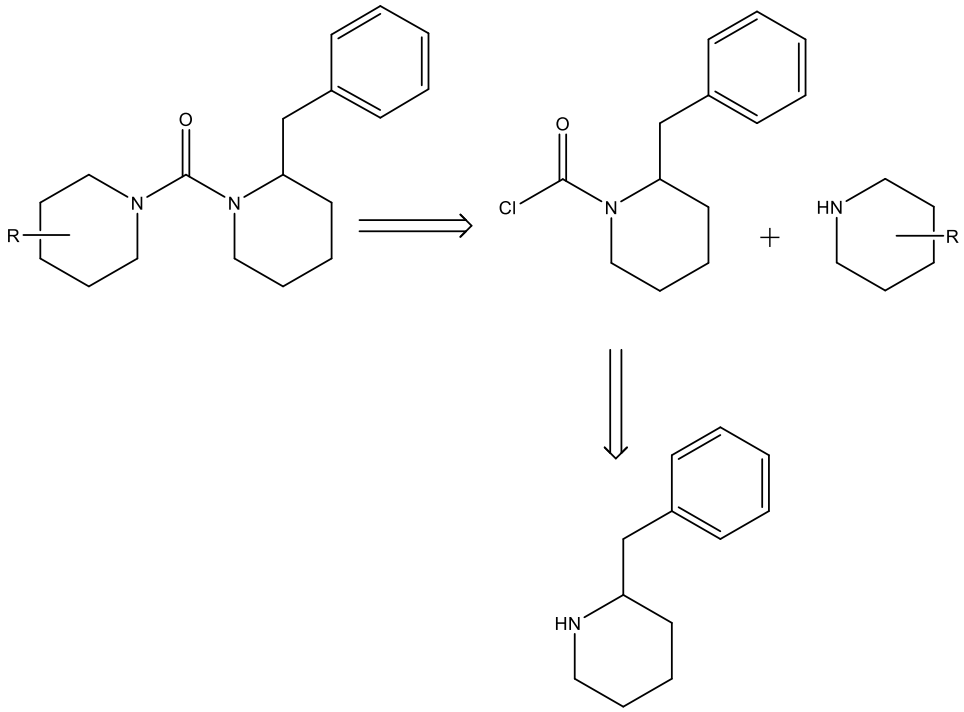


Figure 6 : Retrosynthesis of (2-benzylpiperidin-1-yl)(piperidin-1-yl)methanone analogues.

I tried to find different conditions to follow this retrosynthetic analysis. Finally, this synthesis was complicated to manage, because the first step was difficult to implement. The phosgene reaction is the first step and the yield for this step is just 25%. Indeed, it was difficult to manage this reaction because the difference between the final product and the starting material is not visible on UV, and also on proton NMR. I saw that I created the good product because on the LC-MS I run the starting material alone to have a reference for the retention time, and the peaks for the final product don't have the same retention time, so I continue the reaction without sure analysis. This feature may have increased the risk of poor performance.

So maybe it could be interesting to change the condition of the first step of the reaction to obtain a better yield at the end, with this condition: triphosgene as reagent, in dichloromethane as solvent and an unknown base, at zero degrees, for example.

For the last step for this retrosynthesis, I used the same condition for the one-step reaction, so this yield couldn't increase the risk of a less important yield.

Globally, I didn't have some difficulties to analyze the NMR result, apart the NMR of (2-benzylpiperidin-1-yl)(phenyl)methanone is the only molecule that I didn't have the NMR. This molecule did not give an interpretable NMR in $CDCl_3$ and in DMSO. Moreover, this molecule isn't dilutable in methanol d-4. Actually, I don't find a solution to have a NMR result for this one.

ii. Click chemistry

To continue in the spirit of discovering another chemistry, my tutor proposed me to do click chemistry in order to create the (5-phenyl-1H-1,2,3-triazol-1-yl)(piperidin-1-yl)methanone.

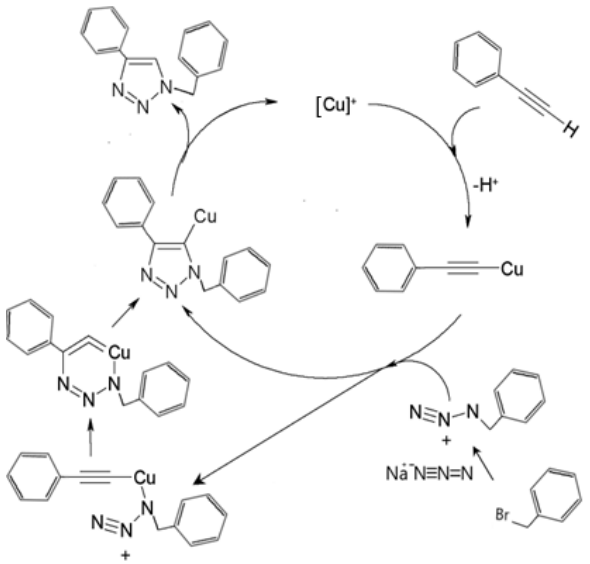


Figure 7 : Synthesis of Click chemistry.

The click chemistry wasn't complicated to achieve, I obtained 58% of yield. After that I would like to remove the benzyl group, but I encountered some difficulties. I tried different conditions. Firstly, I used the high-pressure hydrogen condition: H_2 Pd/C, 5%, in methanol. This reaction never started so I decided to change the experimental condition. So, after that, I tried to disolve an analogue of my compound in DMSO and I added ^{t}BuOK salt, I stirred and I hit until 180°C, but nothing happened on LC-MS analysis. Finally, I would like to try another time the hydrogen deprotection in methanol but in this case, I used H_2 Pd/C 10% and I added a little of acetic acid. This reaction works a little I had 30% of the molecule that is deprotected, however it was not enough, it's maybe 5mg of final product and I need to do the last step. So I tried to use stronger condition with the high pressure to see if it's work better, but nothing more happened. This phenomenon could come from the fact that the palladium is blocked, because the triazole can create 3 different bonds with the palladium and this prevent the reaction to continue.

Finally, I wanted to deprotect all my starting material and I got 50% of deprotected product, so 4.6mg (53% yield) of 5-phenyl-1H-1,2,3-triazole. I purify my final product with the machine : Biotage Selekt, because on my TLC it seems to be complicated to separate the product deprotected and protected properly. I continue the next step and I obtain a mixture of two molecules, but I didn't have the time to purify them and to separate both of them. **Appendix 31**

B. Biology result

Three of them can be tested in biology to see if they have an impact on the protein.

The ABHD2 protein is complicated to purify and to have a structure of this one, she is obtained in Germany but not still in Scilifelab. However, my three first compounds were tested on the ABHD10/11/14B proteins that have a similar structure of the ABHD2 protein.

Unfortunately, no compound significantly stabilized any of the proteins. But this method is not very efficient, normally the activity of the molecule on the protein is shown by a change of colors of the protein. However, this method wasn't possible for the moment. **Appendix 32 / Appendix 33 / Appendix 34**

IV. CONCLUSION

A. Professional conclusion

First of all, the goals of my internship was to create analogues of the first inhibitor, the (2-benzylpiperidin-1-yl)(piperidin-1-yl)methanone. At the end of the internship, I created 23 molecules that are ready to be tested in biology. I created:

-Fifteen ureas with just one-step
-Five ureas with the phosgene reaction for the first step
-Three amides.

And all my molecules, apart 2 of them are a mixture of S and R enantiomers as below :

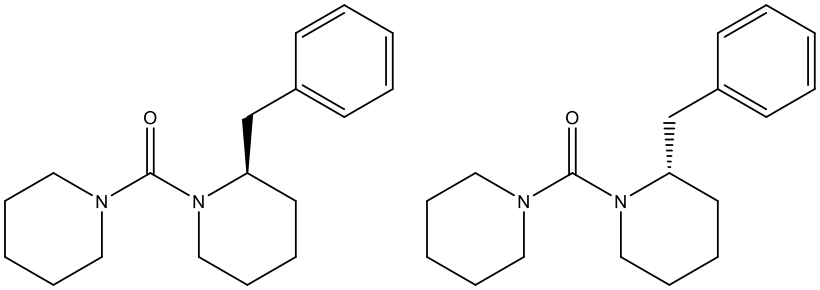


Figure 8 : The two possible enantiomers of inhibitor 1.

So, I made a lot of reaction and purification to achieve that.
In terms of manipulation, considering the number of molecules that are ready to be tested in biology, I mastered well the column chromatography, whereas before I had some difficulties to implement it. Moreover, I know how to use an HPLC and how to analyze its chromatographs.
In terms of knowledge, I know better the properties of solvents, those which can be substituted by others to avoid the use of some. I was able to implement reactions often seen theoretically, but not in practice, which allowed me to understand what could work or not and why, as well as to understand what can happen during a reaction. So, it's interesting to realize that there is a difference between the theory and the practice, and all reaction doesn't work as well as the theory predicts.
Finally, I learned a lot to use software correctly, like MestReNova, ChemLab, DataWarrior, Chemdraw and others, using all the functions that can be useful.

If I had more time at the end of my internship, I think I could have continued with the click chemistry, to look for other conditions and find the right one for deprotection. Also, I could have continued to create different analogues, and come up with new ideas. And maybe I could have had a biological result if my molecules were tested quickly, and that could have helped me focus my attention on one type of compound.

In conclusion, it was interesting to see the connection between biology and chemistry. The products I created must be very pure (over 90% purity) for the bioassay, so purification is a very important part of chemistry research. I also learned that in research you work in small quantities. At first, it was sometimes complicated to work with only a few milligrams for example, but this careful work taught me how to handle correctly. These small amounts come from the fact that in biology, people only need 1mg to test a molecule in a cell.

B. Personal conclusion

Before this internship, I learned to manage on my own, without the help of my parents, because I had to find an apartment on my own, I had to exchange with people who did not speak French for that. I also had to prepare my trip and, had to go alone even though I was with a friend, we had never been on a plane alone or anything like that. So all these events made me learn to be patient (to find my accommodation), to stay calm, to be brave and to take it upon myself.

During this internship I gained confidence, I make my own decisions about the progress of a reaction. I didn't hesitate to try different conditions or to propose my ideas. And the most important thing, is that I improve my English. I choose this destination partly to learn to speak English, and it was good to be here because all of people know to speak English, not just in the lab but everywhere in Sweden. So, this part of this internship was really interesting. The fact that at work all people spoke English was very good, because people trying to speak English when we are with them and that was good to integrate the group.

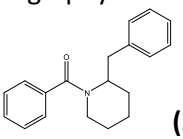
I also discovered the Swedish culture, and it was impressive because it was very different than us. Their daily life is different from ours, their education too. For example, Swedish people eat at 11h and at 17h when they come back at home. So, sometimes we ate out of sync. We known that when we were invited at a gathering to celebrate the summer period. We ate at 18h when all people arrived. Moreover, Swedish people doesn’t have the same education because they are very respectful compared to French people. They are environmentally friendly, the street are very proper, they are respectful of people, of the rules…

Concerning the life at work, I could observe some differences also compared to France. First of all, employees make their own schedules. They don't have to work 8am-6pm, they can choose. My colleagues often arrived around 9am and some come at 7am for example. They can leave when it suits them sometimes. Also, they are allowed to do work at home when they have meetings for example. You just have to warn them and manage their work. The employee-manager relationship is based on a relationship of mutual trust.

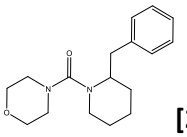
V. EXPERIMENTAL PART

A. One-step reaction

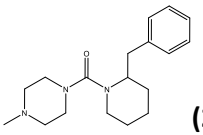
General protocol : To a solution of an unknow amine (one equivalent; 0.3mmol the majority time) in dichloromethane DCM (around 2mL) add (around 80µL) of triethylamine and one equivalent of an unknow acid at room temperate. The solution was stirred a few hours. Sometime, the solution mixture was diluted in water and was extracted with an appropriate solvent. The resulting organic layer was dried and concentrated in vacuo. And sometime, the product was purified by silica column chromatography to obtain the product. **[6]**



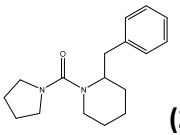
(2-benzylpiperidin-1-yl)(phenyl)methanone The solution was stirred overnight, extracted with HCL (0.1M) and the product was purified by silica column chromatography (80 : 20 of Iso-Hex: EtOAc as eluent) to obtain 8.6mg (12% yield).
^{1}H NMR (400 MHz, $CDCl_3$) X
LC-MS: m/z = 280 [M+H]+



[2-(phenylmethyl)-1-piperidinyl]-4-morpholinylmethanone The mixture was stirred overnight and the resulting was directly purified by a chromatography flash with (7:3 of Iso-Hex: AcEtO) as eluent. The fractions containing the product were combined then concentrated under reduced pressure to yield 50.6 mg (50% yield). **Appendix 3**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.22 (m, 2H), 7.21 – 7.14 (m, 3H), 4.31 – 4.17 (m, 1H), 3.58 – 3.36 (m, 5H), 3.16 – 2.72 (m, 8H), 1.71 – 1.55 (m, 4H), 1.51 – 1.38 (m, 1H).
LC-MS: m/z = 289 [M+H]+

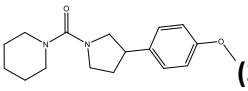


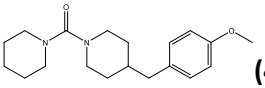
(2-benzylpiperidin-1-yl)(4-methylpiperazin-1-yl)methanone The solution was stirred at room temperature overnight and concentrated under reduced pressure then dissolved in acetonitrile and purified by acidic prep HPLC. The fractions containing the product were combined then concentrated under reduced pressure to yield 22.8mg (25% yield). **Appendix 4**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.20 (m, 2H), 7.19 – 7.13 (m, 3H), 4.24 – 4.16 (m, 1H), 3.48 – 3.39 (m, 1H), 3.14 – 3.02 (m, 3H), 2.99 – 2.90 (m, 3H), 2.84 – 2.77 (m, 1H), 2.32 – 2.15 (m, 7H), 1.77 – 1.54 (m, 5H), 1.50 – 1.36 (m, 1H).
LC-MS: m/z = 302 [M+H]+

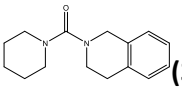


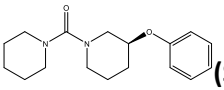
(2-benzylpiperidin-1-yl)(pyrrolidin-1-yl)methanone The reaction was stirred at room temperature overnight. The mixture was purified with 70:30 of Iso-Hex: AcEtO. The fractions containing the product were combined then concentrated under reduced pressure to yield 58.8 mg (72% yield). **Appendix 5**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.23 (m, 2H), 7.24 – 7.16 (m, 3H), 4.27 – 4.19 (m, 1H), 3.57 – 3.50 (m, 1H), 3.32 – 3.23 (m, 2H), 3.16 – 2.87 (m, 5H), 1.81 – 1.42 (m, 10H).

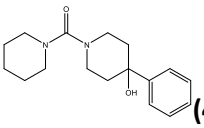
LC-MS: m/z = 273 [M+H]+

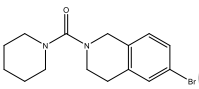
 **(3-(4-methoxyphenyl)pyrrolidin-1-yl)(piperidin-1-yl)methanone** This solution was stirred at room temperature overnight. The crude mixture was purified by silica gel chromatography with a gradient from pure Iso-Hex to 60:40 of Iso-Hex: EtOAc. The final product was obtained as a light yellow viscous liquid in 39% yield (33.8mg) with a purity of 98%. **Appendix 6**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 – 7.15 (m, 2H), 6.88 – 6.84 (m, 2H), 3.79 (s, 3H), 3.72 – 3.45 (m, 4H), 3.37 (t, *J* = 9.8 Hz, 1H), 3.29 – 3.19 (m, 5H), 2.24 – 2.15 (m, 1H), 1.99 – 1.87 (m, 1H), 1.63 – 1.48 (m, 7H).
LC-MS: m/z = 289 [M+H]+

 **(4-(4-methoxyphenyl)piperidin-1-yl)(piperidin-1-yl)methanone** This solution was stirred at room temperature 1H, and not purified. The product obtained is pure at 98%, to yield 67.8mg (71% yield). **Appendix 7**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 – 7.01 (m, 2H), 6.84 – 6.79 (m, 2H), 3.77 (s, 3H), 3.66 – 3.54 (m, 4H), 3.17 – 3.13 (m, 4H), 2.71 – 2.61 (m, 2H), 2.47 (d, *J* = 6.8 Hz, 2H), 1.64 – 1.50 (m, 7H), 1.24 – 1.12 (m, 2H).
LC-MS: m/z = 317 [M+H]+

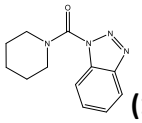
 **(3,4-dihydroisoquinolin-2(1H)-yl)(piperidin-1-yl)methanone** The reaction was stirred at room temperature. The mixture was purified with 3:1 of Iso-Hex: EtOAc. The fractions containing the product were combined then concentrated under reduced pressure to yield 99.6mg (100% yield). **Appendix 8**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 – 7.08 (m, 4H), 4.44 (s, 1H), 3.51 (t, *J* = 5.9 Hz, 2H), 3.29 – 3.22 (m, 4H), 2.93 (t, *J* = 5.8 Hz, 2H), 1.68 – 1.54 (m, 6H).
LC-MS: m/z = 245 [M+H]+

 **(S)-(3-phenoxypiperidin-1-yl)(piperidin-1-yl)methanone** In the solution added water and EtOAc to wash and extract it, to obtain 100% of purity, and 76.0mg (88% yield). **Appendix 9**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.23 (m, 2H), 6.98 – 6.90 (m, 3H), 4.34 (tt, *J* = 8.3, 3.8 Hz, 1H), 3.83 – 3.72 (m, 1H), 3.42 – 3.34 (m, 1H), 3.23 – 3.12 (m, 4H), 3.05 – 2.97 (m, 1H), 2.96 – 2.88 (m, 1H), 2.13 – 2.03 (m, 1H), 1.88 – 1.78 (m, 1H), 1.74 – 1.37 (m, 8H).
LC-MS: m/z = 289 [M+H]+

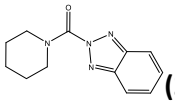
 **(4-hydroxy-4-phenylpiperidin-1-yl)(piperidin-1-yl)methanone** Add water and EtOAc to the reaction mixture to obtain 20.1mg pure at 100% (23.2% yield). **Appendix 10**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.40 (m, 2H), 7.31 – 7.25 (m, 2H), 7.22 – 7.16 (m, 1H), 3.55 – 3.48 (m, 2H), 3.28 – 2.94 (m, 6H), 2.04 – 1.92 (m, 4H), 1.71 – 1.14 (m, 7H).
LC-MS: m/z = 289 [M+H]+

 **(6-bromo-3,4-dihydroisoquinolin-2(1H)-yl)(piperidin-1-yl)methanone** The solution was stirred at room temperature during 4 hours. The crude mixture was purified by silica gel chromatography with an eluent 95:5 of Iso-Hex : EtOAc. The final product was obtained in 36% yield (35.1mg) with a purity of 100%. **Appendix 11**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 – 7.18 (m, *J* = 5.7, 3.8 Hz, 15H), 6.92 – 6.86 (m, 7H), 4.28 (s, 14H), 3.39 (t, *J* = 5.8 Hz, 14H), 3.20 – 3.08 (m, *J* = 19.8, 5.2 Hz, 30H), 2.81 (t, *J* = 5.7 Hz, 14H), 1.60 – 1.43 (m, 47H).
LC-MS: m/z = 324 [M+H]+

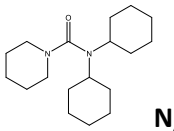

(1H-benzo[d][1,2,3]triazol-1-yl)(piperidin-1-yl)methanone (molecule 1)

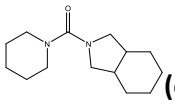
And

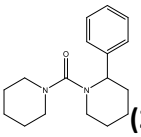

(2H-benzo[d][1,2,3]triazol-2-yl)(piperidin-1-yl)methanone (molecule 2)

The solution of this two molecules was stirred at room temperate during the week-end, diluted in DCM and extracted with HCL (0.5M) then purified with an eluent 9:1 of Iso-Hex : EtOAc.
The white solid that I obtained weight 31.8mg (46% yield) of the **molecule 1** with a purity of 100%. **Appendix 12**
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, 1H), 7.88 (dd, *J* = 8.3, 0.8 Hz, 1H), 7.51 (ddd, *J* = 7.1, 5.5, 1.0 Hz, 1H), 7.36 (ddd, *J* = 8.1, 7.1, 1.0 Hz, 1H), 3.80 – 3.65 (m, *J* = 5.2 Hz, 4H), 1.80 – 1.60 (m, *J* = 27.6 Hz, 6H).
LC-MS: m/z = 231 [M+H]+

Also, I obtained 7.7mg (11% yield) of the **molecule 2** with a purity of 97.5%. **Appendix 13**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.82 (m, 2H), 7.42 – 7.35 (m, 2H), 3.83 – 3.68 (m, 2H), 3.43 – 3.28 (m, 2H), 1.81 – 1.56 (m, 6H).
LC-MS: m/z = 231 [M+H]+

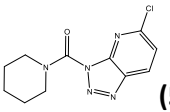

N,N-dicyclohexylpiperidine-1-carboxamide The solution was stirred at room temperate during the week-end, diluted in DCM and extracted with HCL (0.5M) and then I purified it with 9:1 of iso-hex : EtOAc as eluent to afford 1.9mg. **Appendix 14**
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06 – 2.93 (m, 6H), 1.87 – 1.77 (m, 3H), 1.76 – 1.68 (m, 4H), 1.62 – 1.54 (m, 5H), 1.49 – 1.45 (m, 6H), 1.27 – 0.99 (m, 7H), 0.83 – 0.75 (m, 1H).
LC-MS: m/z = 293 [M+H]+


(octahydro-2H-isoindol-2-yl)(piperidin-1-yl)methanone The solution was stirred at room temperate during the week-end, and for the purification I used a gradient from 9:1, to 7:3 of iso-hex : EtOAc. I had just 0.6mg. **Appendix 15 / Appendix 16 / Appendix 17**
^{13}C NMR (101 MHz, $CDCl_3$) δ 52.2, 47.2, 37.0, 25.9, 25.7, 24.8, 22.8.
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.28 (dd, *J* = 10.2, 6.8 Hz, 2H), 3.18 (dd, *J* = 10.2, 5.6 Hz, 2H), 3.14 – 3.08 (m, 4H), 2.13 – 2.01 (m, 2H), 1.56 – 1.44 (m, *J* = 20.4, 11.8 Hz, 8H), 1.40 – 1.24 (m, 4H), 0.85 – 0.73 (m, 2H).
LC-MS: m/z = 237 [M+H]+

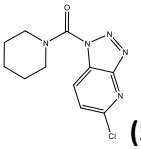
 **(2-phenylpiperidin-1-yl)(piperidin-1-yl)methanone** The solution was stirred during 4days, and was purified thanks to the preparative LC with a gradient of the solvent B from 54% to 84%, to obtain 27.8mg (34%). **Appendix 20**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.18 (m, 4H), 7.16 – 7.10 (m, 1H), 4.75 (t, *J* = 4.8 Hz, 1H), 3.34 – 3.26 (m, 1H), 3.24 – 3.13 (m, 4H), 2.98 – 2.89 (m, *J* = 12.9, 9.7, 2.9 Hz, 1H), 2.07 – 1.97 (m, *J* = 15.3, 5.0 Hz, 1H), 1.90 – 1.76 (m, *J* = 11.3, 9.9, 6.0 Hz, 1H), 1.61 – 1.40 (m, 10H).

LC-MS: m/z = 273 [M+H]+

 **(5-chloro-3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)(piperidin-1-yl)methanone**

and

 **(5-chloro-1H-[1,2,3]triazolo[4,5-b]pyridin-1-yl)(piperidin-1-yl)methanone**

This mixture of two compounds was stirred overnight, and purified with 7:3 of iso-Hex : EtOAc as eluent, to obtain 52mg as global mass. **Appendix 18 / Appendix 19**

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 – 8.23 (m, 2H), 7.55 (dd, *J* = 8.6 Hz, 1H), 7.43 (dd, *J* = 8.9 Hz, 1H), 4.03 – 3.67 (m, 4H), 3.50 – 3.31 (m, 2H), 3.22 – 3.10 (m, 1H), 1.87 – 1.48 (m, 12H), 1.31 – 1.06 (m, 1H).

LC-MS: m/z = 266 [M+H]+

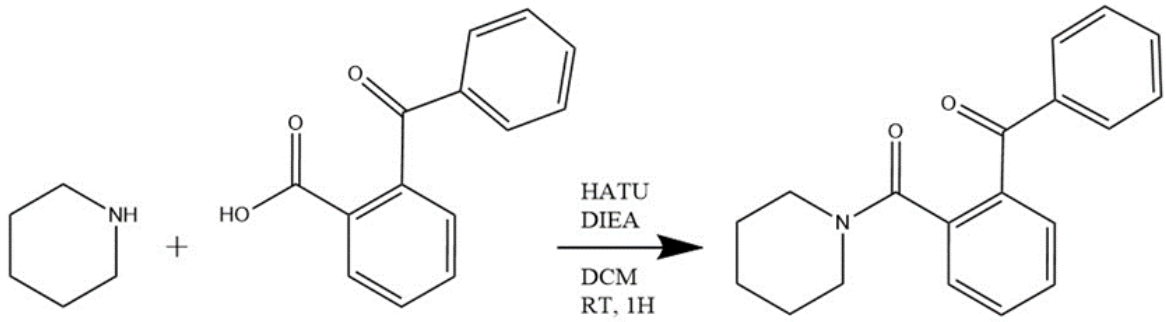


Figure 9 : Synthesis with use of HATU.

Protocol: To a solution of 2-Benzoylbenzoic acid (0,086mmol ; 19,8mg ; 15µL) in dichloromethan DCM (around 380µL) add (0,18mmol ; 66,5mg) of HATU and (0,1mmol ; 8,5mg ; 9,9µL) of piperidine at room temperate. The solution was stirred overnight. The solution mixture was disolved in water and was extracted with EtOAc. The resulting organic layer was dried and concentrated in vacuo. The product was purified by silica column chromatography (70 : 30 of Iso-Hex: EtOAc as eluent) to obtain 6.8mg (27% yield) of (2-Benzoyl-phenyl)-piperidin-1-yl-methanone. [7] **Appendix 21**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.77 (m, 2H), 7.60 – 7.49 (m, 3H), 7.48 – 7.37 (m, 4H), 3.60 – 3.45 (m, 2H), 3.34 – 3.21 (m, *J* = 4.9 Hz, 2H), 1.68 – 1.61 (m, *J* = 7.4 Hz, 3H), 1.60 – 1.52 (m, 3H).

LC-MS: m/z = 294 [M+H]+

B. Multi-step reaction

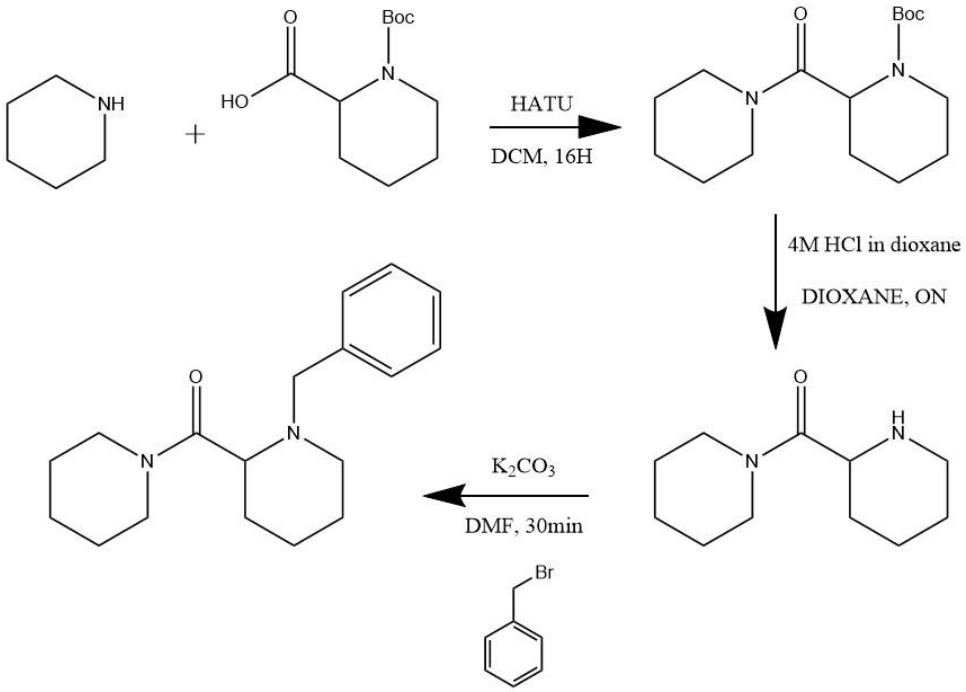
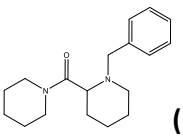
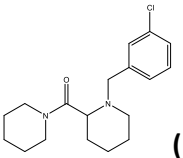


Figure 10 : Synthesis of (1-benzylpiperidin-2-yl)(piperidin-1-yl)methanone analogues.

Protocol: To a solution of 1-(1,1-Dimethylethyl) 1,2-piperidinedicarboxylate (68.8 mg ; 0.3 mmol ; 59.3µL) in dichloromethan DCM (around 3mL) add (228 mg ; 0.6 mmol) of HATU and an excess of piperidine (0.9mmol ; 89.2µL) at room temperate. The solution was stirred overnight. The solution mixture was disolved in DCM and was extracted with HCl (0.5M in water). The resulting organic layer was dried with $MgSO_4$ and concentrated in vacuo.
The solution was disolved in 5mL of dioxane. Add 2mL of HCl in dioxane (4M) and stir the solution during overnight. The amine was deprotected.
To a solution of piperidin-1-yl(piperidin-2-yl)methanone (0.3mmol ; 59.1mg) and K_2CO_3 (124.2mg) in DMF (around 2.5mL) is stirred at 0°C. Then an analogue of Bromobenzyl (0.3mmol) is added to the reaction mixture dropwise and stirred 30min. The reaction mixture is washed with water and extracted with EtOAc. The organic layer is dried, filtered and concentrated under reduced pressure. The crude product is purified by flash silica gel chromatography. **[7] [9] [10] Appendix 2**

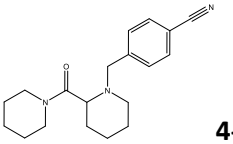


(1-benzylpiperidin-2-yl)(piperidin-1-yl)methanone The eluent chosen for the purification of this product was : 100% of EtOAc to afford (19.6mg ; 23% yield). **Appendix 22**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.23 (m, 5H), 4.01 – 2.79 (m, 8H), 2.10 – 1.23 (m, 13H).
LC-MS: m/z = 287 [M+H]+



(1-(3-chlorobenzyl)piperidin-2-yl)(piperidin-1-yl)methanone The eluent used for this one is : Iso-Hex: EtOAc (3 : 1) to obtain 27.8mg (29%). **Appendix 23**
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.17 (m, 4H), 3.90 – 3.46 (m, 5H), 3.36 – 3.20 (m, *J* = 18.2, 11.6 Hz, 2H), 3.05 – 2.92 (m, *J* = 6.1 Hz, 3H), 2.10 – 1.50 (m, 12H), 1.41 – 1.13 (m, 1H).

LC-MS: m/z = 321 [M+H]+



4-((2-(piperidine-1-carbonyl)piperidin-1-yl)methyl)benzonitrile Firstly, the eluent for this purification was Iso-Hex: EtOAc (6 : 4) to have 20mg and 80% of purity, the second purification was made with the preparative LC. I have chosen a gradient from 43% of B to 73% and finally to obtain 14.8mg (16%). **Appendix 24**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.56 (m, 2H), 7.48 – 7.43 (m, *J* = 8.1 Hz, 2H), 3.92 – 3.55 (m, 4H), 3.54 – 3.44 (m, *J* = 8.5, 5.5 Hz, 1H), 3.33 – 3.23 (m, 2H), 2.97 – 2.87 (m, *J* = 11.3 Hz, 1H), 2.08 – 1.22 (m, 15H).

LC-MS: m/z = 312 [M+H]+

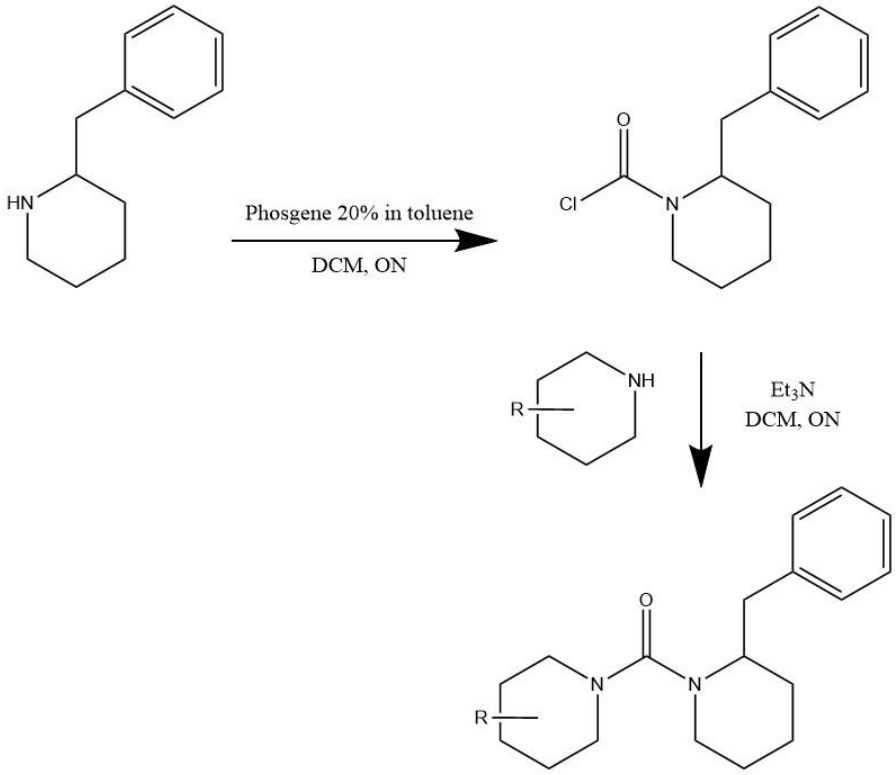
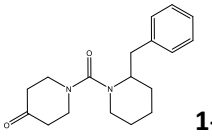


Figure 11 : Synthesis of (2-benzylpiperidin-1-yl)(piperidin-1-yl)methanone analogues.

Protocol : A solution of phosgene in toluene (300µL, 20%) was added at rt to a solution of 2-benzylpiperidine (52.5mg ; 0.3mmol) in DCM (40mL). After the night the solution was disolved in DCM, washed with H_2O and the organic layer was dried over $MgSO_4$. The solvent was evaporated under reduced pressure.

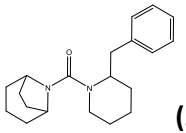
Then I follow the first protocol that I described. The solution was stirred overnight, then my product was diluted in DCM and extracted with HCL (0.5M) all the time.**[6][11]**



1-(2-benzylpiperidine-1-carbonyl)piperidin-4-one The product was purified by silica column chromatography (1 : 1 of Iso-Hex: EtOAc as eluent) to obtain 20.4mg (23% yield). **Appendix 25**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.18 (m, 5H), 4.44 – 4.34 (m, 1H), 3.59 – 3.51 (m, *J* = 14.5 Hz, 1H), 3.38 – 3.29 (m, 2H), 3.25 – 3.05 (m, 4H), 2.86 – 2.77 (m, *J* = 13.7, 6.6 Hz, 1H), 2.39 – 2.28 (m, *J* = 9.1, 6.7, 3.4 Hz, 2H), 2.15 – 2.04 (m, 2H), 1.87 – 1.65 (m, 5H), 1.58 – 1.16 (m, 3H), 0.96 – 0.79 (m, 1H).

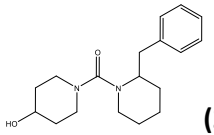
LC-MS: m/z = 301 [M+H]+



(2-benzylpiperidin-1-yl)(8-azabicyclo[3.2.1]octan-8-yl)methanone I purified this product with the preparative LC with a gradient of solvent B from 65% to 100%. At the end, I had 11.2mg (14%) of the pure product. **Appendix 26**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.17 (m, 5H), 4.40 – 4.31 (m, 1H), 4.00 – 3.94 (m, *J* = 7.0 Hz, 1H), 3.75 – 3.65 (m, 2H), 3.16 – 3.07 (m, *J* = 13.1, 2.7 Hz, 1H), 2.98 (dd, *J* = 13.4, 7.3 Hz, 1H), 2.87 (dd, *J* = 13.4, 8.3 Hz, 1H), 1.90 – 1.36 (m, 17H).

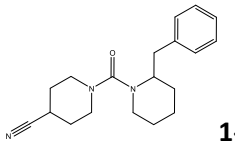
LC-MS: m/z = 313 [M+H]+



(2-benzylpiperidin-1-yl)(4-hydroxypiperidin-1-yl)methanone The solution was stirred overnight, purified by silica column chromatography with a gradient from 1 : 1 of Iso-Hex: EtOAc as eluent to 8:2 of the same eluent. Finally, I obtained 5.1mg (5% yield). **Appendix 27**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 – 7.08 (m, *J* = 1.4 Hz, 5H), 4.15 – 4.07 (m, *J* = 4.1 Hz, 1H), 3.71 – 3.64 (m, 1H), 3.42 – 3.36 (m, *J* = 13.2 Hz, 1H), 3.31 – 3.16 (m, *J* = 27.5, 13.2 Hz, 1H), 3.08 – 2.99 (m, *J* = 11.7 Hz, 1H), 2.91 (dd, *J* = 13.5, 7.7 Hz, 1H), 2.79 – 2.68 (m, *J* = 13.3, 5.3 Hz, 1H), 2.64 – 2.54 (m, *J* = 16.7, 6.5 Hz, 1H), 1.75 – 1.13 (m, 12H), 0.89 – 0.77 (m, *J* = 15.5, 10.7, 7.6 Hz, 1H).

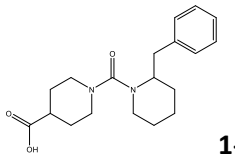
LC-MS: m/z = 303 [M+H]+



1-(2-benzylpiperidine-1-carbonyl)piperidine-4-carbonitrile The purification of this product was made with 8 : 2 Iso-Hex: EtOAc as eluent to afford 12.5mg (16% yield). **Appendix 28**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 – 7.17 (m, 2H), 7.14 – 7.09 (m, *J* = 7.1, 5.2 Hz, 3H), 4.19 – 4.02 (m, *J* = 7.7 Hz, 1H), 3.40 – 3.32 (m, *J* = 12.1 Hz, 1H), 3.18 – 2.80 (m, 4H), 2.75 – 2.64 (m, 2H), 2.59 (q, 1H), 1.77 – 0.73 (m, 11H).

LC-MS: m/z = 312 [M+H]+



1-(2-benzylpiperidine-1-carbonyl)piperidine-4-carboxylic acid The solution was stirred overnight, was purified by the preparative LC, with a gradient of the solvent B from 9% to 39%. I had 1.2mg of final product. **Appendix 29**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.29 (m, *J* = 7.1 Hz, 1H), 7.28 – 7.25 (m, 1H), 7.24 – 7.15 (m, 2H), 4.27 – 4.11 (m, 1H), 3.54 – 3.29 (m, 2H), 3.11 (t, *J* = 12.1 Hz, 1H), 3.00 (dd, *J* = 13.4, 7.7 Hz, 1H), 2.84 (dd, *J* = 13.5, 7.8 Hz, 1H), 2.78 – 2.65 (m, 1H), 2.62 – 2.51 (m, 1H), 1.89 – 1.20 (m, 13H).

LC-MS: m/z = 312 [M+H]+

Protocol of click chemistry : Add in a screw-top reaction tube equipped with a magnetic stirrer, the benzylbromide (0.5 mmol; 85,5mg; 49,1µL), the phenylacetylene (0.5 mmol; 51,1mg; 54,9µL), and the sodium ascorbate (spatula tip, 15 mg, 0.05 mmol). To this, 2 mL *t*-BuOH/water** (1:1) solution is added as solvent. Lastly, add 1 M aqueous sodium azide solution (550 µl, 0.55 mmol).

The reaction mixture is stirred for 5 minutes and then 1 M (aq) copper (II) sulfate pentahydrate (50 µL, 0.05 mmol) is added and the reaction mixture is stirred at 60 °C for 2 hours. After 2 hours, the reaction mixture is cooled to room temperature with continued stirring overnight.

Add 1 mL 10% aqueous ammonia solution followed by ca 5 mL water to complete precipitation of the product. After stirring an additional 5 minutes, collect the solid precipitates by filtration in a Hirsch funnel. Wash the collected solid product by rinsing the solid on the filter paper with some water (ca 5 -10 mL) and methanol (1-2 mL) through the filter paper. **[8] [12]**

I obtained 68.5mg of 1-benzyl-4-phenyl-1H-1,2,3-triazole so 58% of yield. **Appendix 30**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.80 (m, 2H), 7.68 (s, 1H), 7.46 – 7.32 (m, 7H), 5.61 (s, 2H).

To deprotect the benzyl group I used H_2 Pd/C 10%, as conditions, during 4days and I purified it with the Biotage Selekt machine with a gradient from 10% to 85% of acetonitrile in water to obtain 4.6mg (53% yield) of 5-phenyl-1H-1,2,3-triazole.

The 5-phenyl-1H-1,2,3-triazole was dissolved in DCM (around 1mL). Add in this solution 5µL of Et_3N and (3.7µL; 1eq) of piperidine-1-carbonyl chloride, to obtain (5-phenyl-1H-1,2,3-triazol-1-yl)(piperidin-1-yl)methanone.

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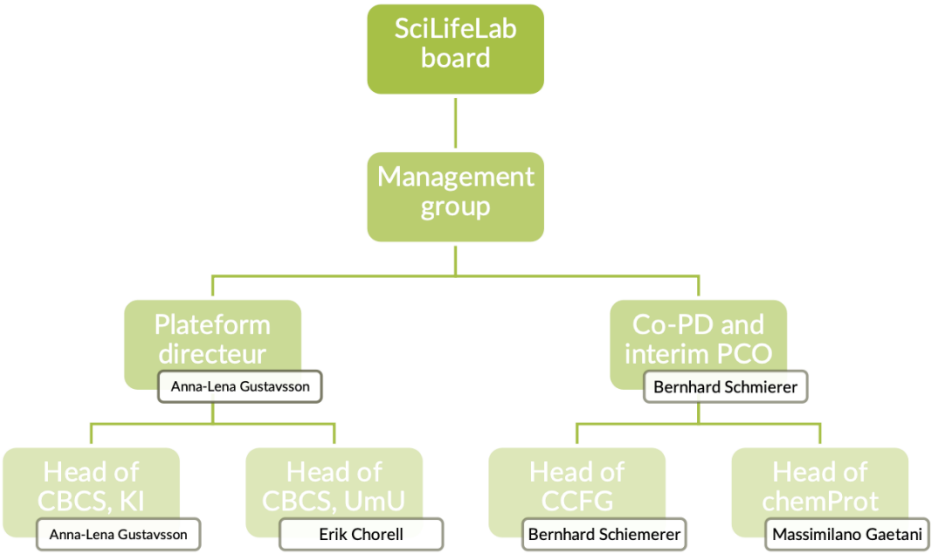
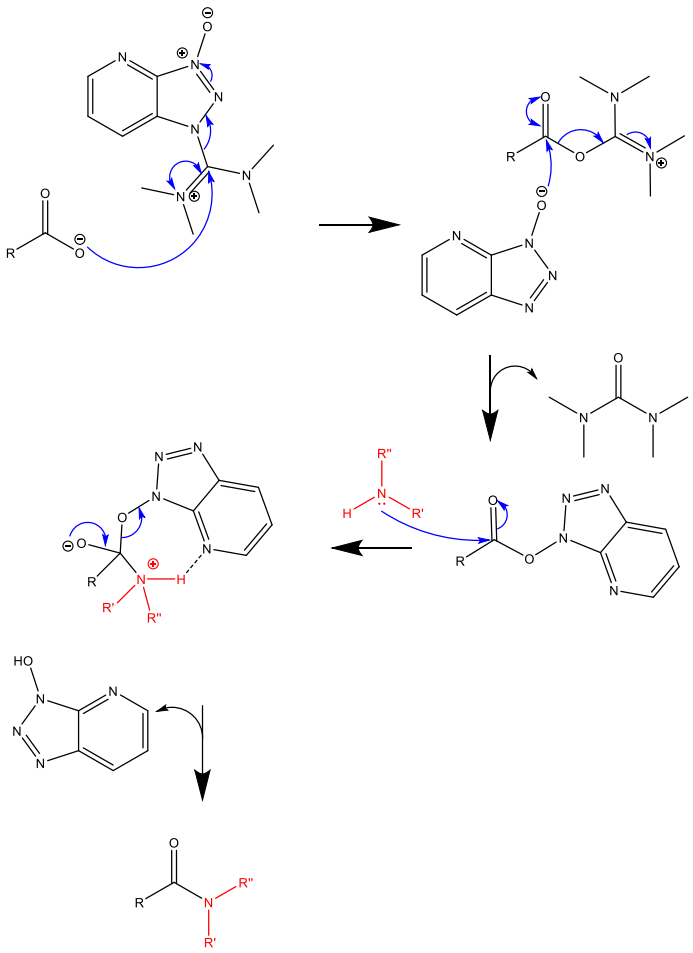
APPENDIX



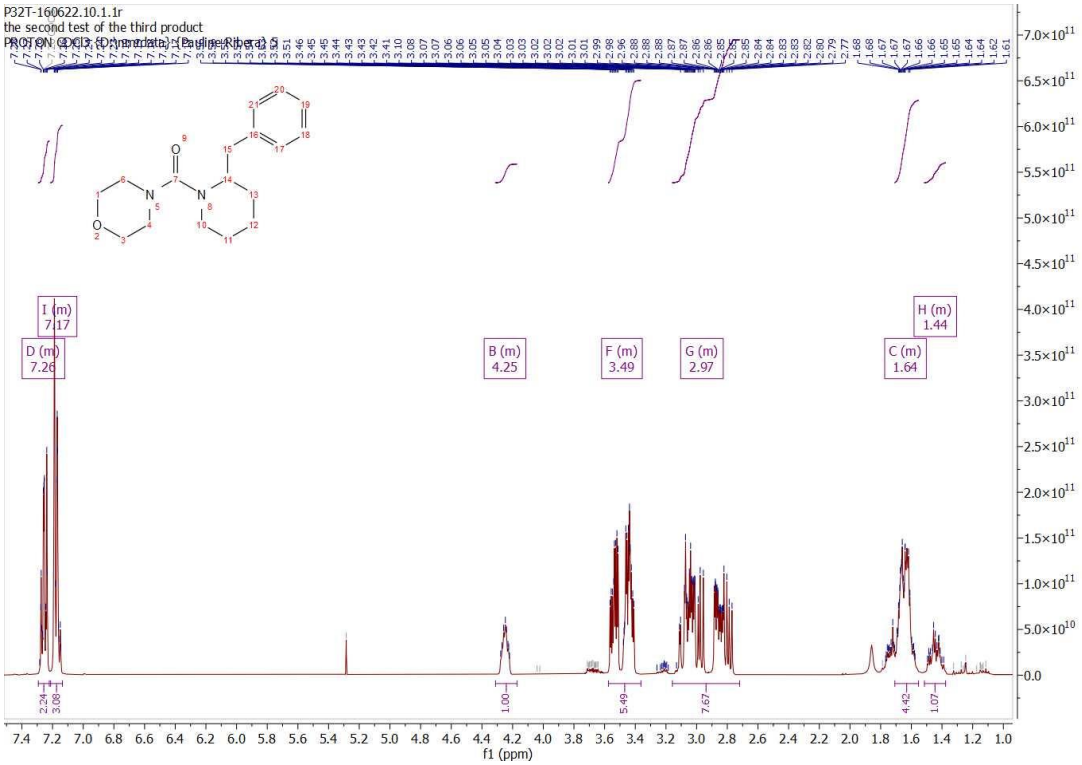
Appendix 1 : Infrastructure organization



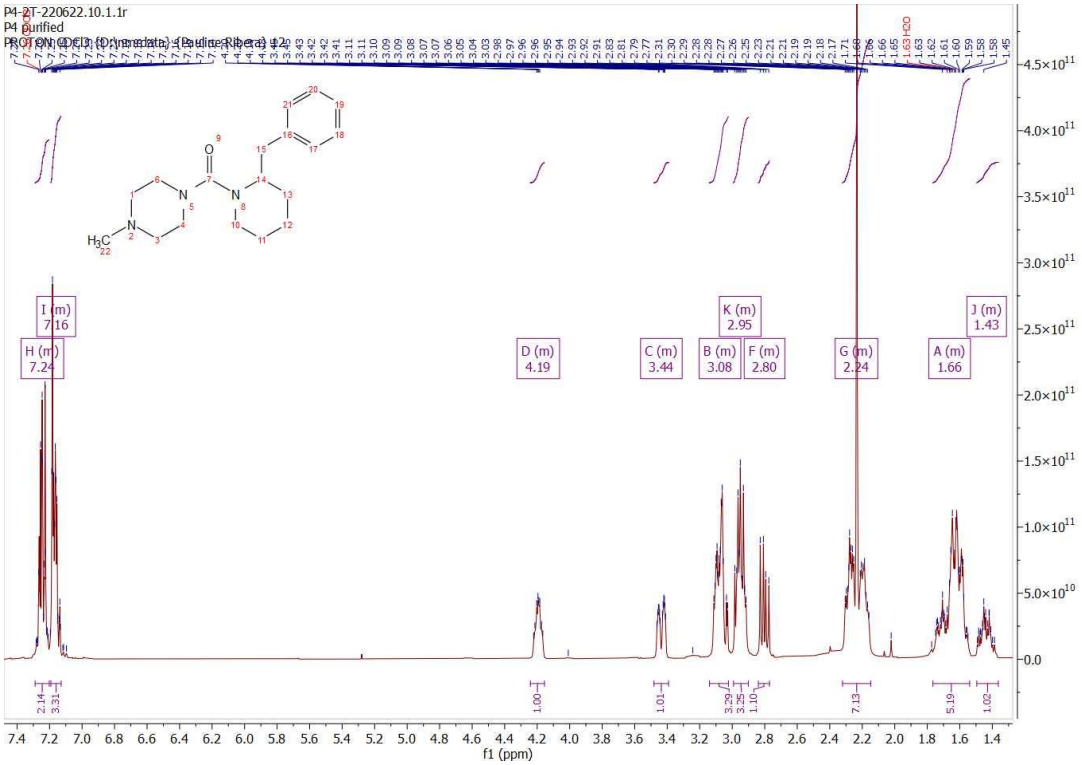
Appendix 2 : Mechanism of N-acylation using HATU

A/ ANALYSIS RESULT

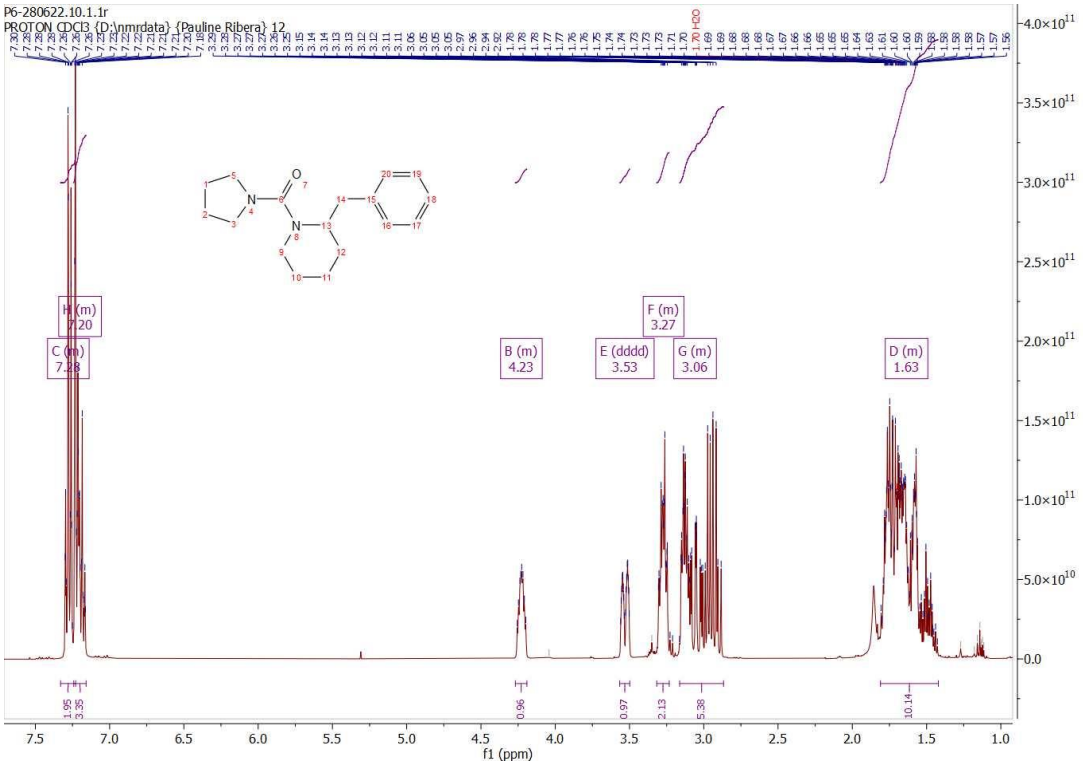
i. Analysis of one-step reaction



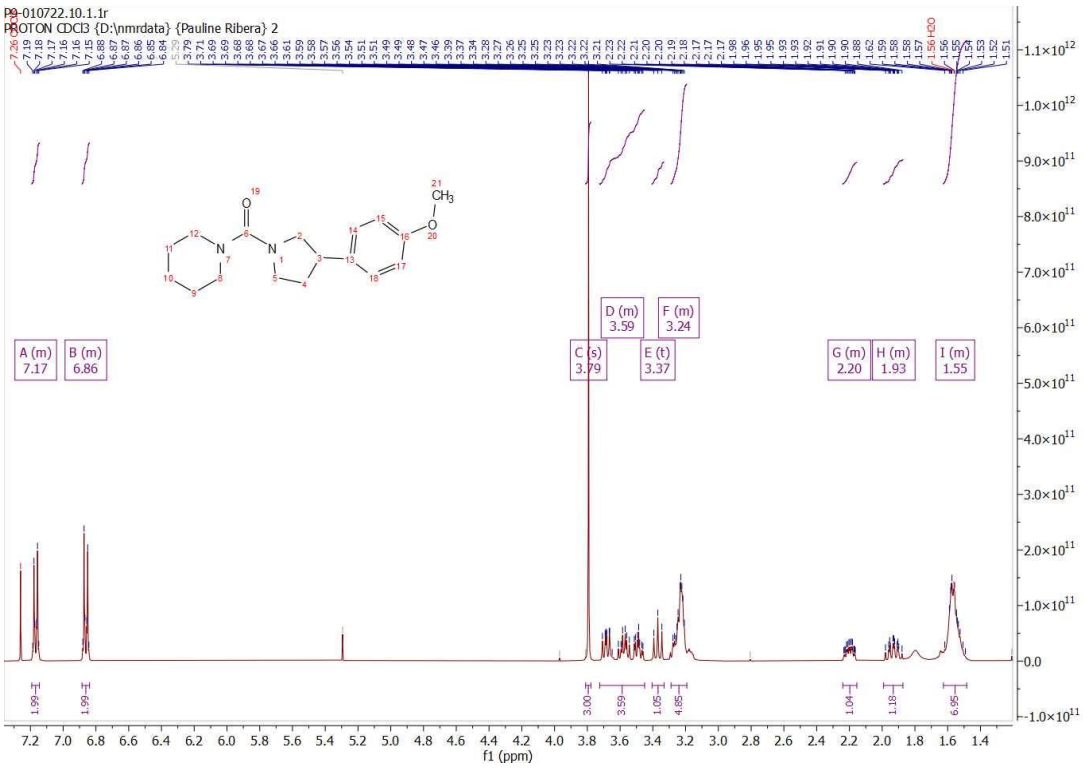
Appendix 3 : NMR of [2-(phenylmethyl)-1-piperidinyl]-4-morpholinylmethanone



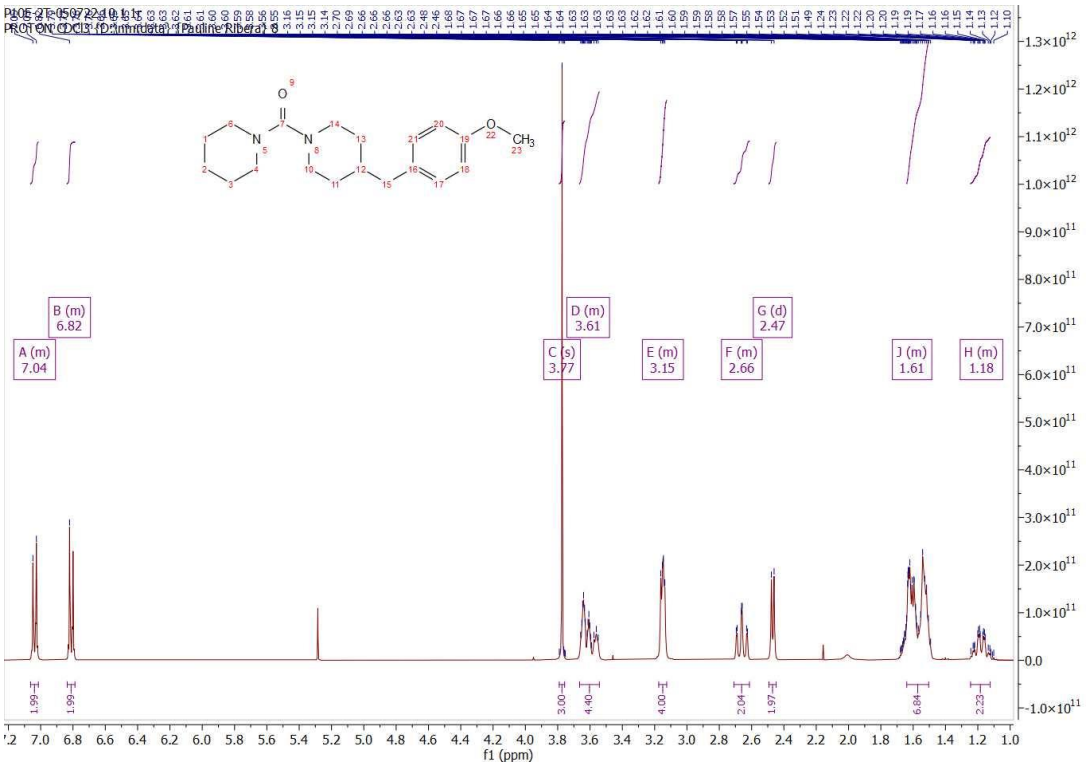
Appendix 4 : NMR of (2-benzylpiperidin-1-yl)(4-methylpiperazin-1-yl)methanone



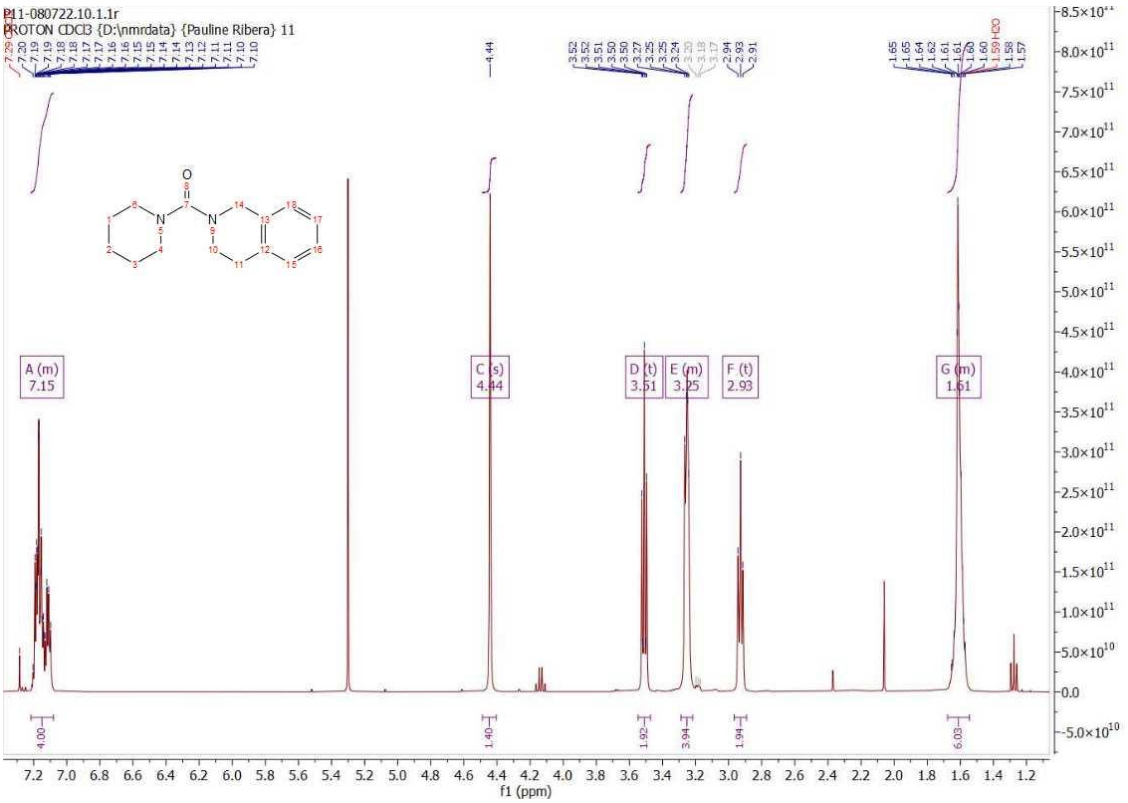
Appendix 5 : NMR of (2-benzylpiperidin-1-yl)(pyrrolidin-1-yl)methanone



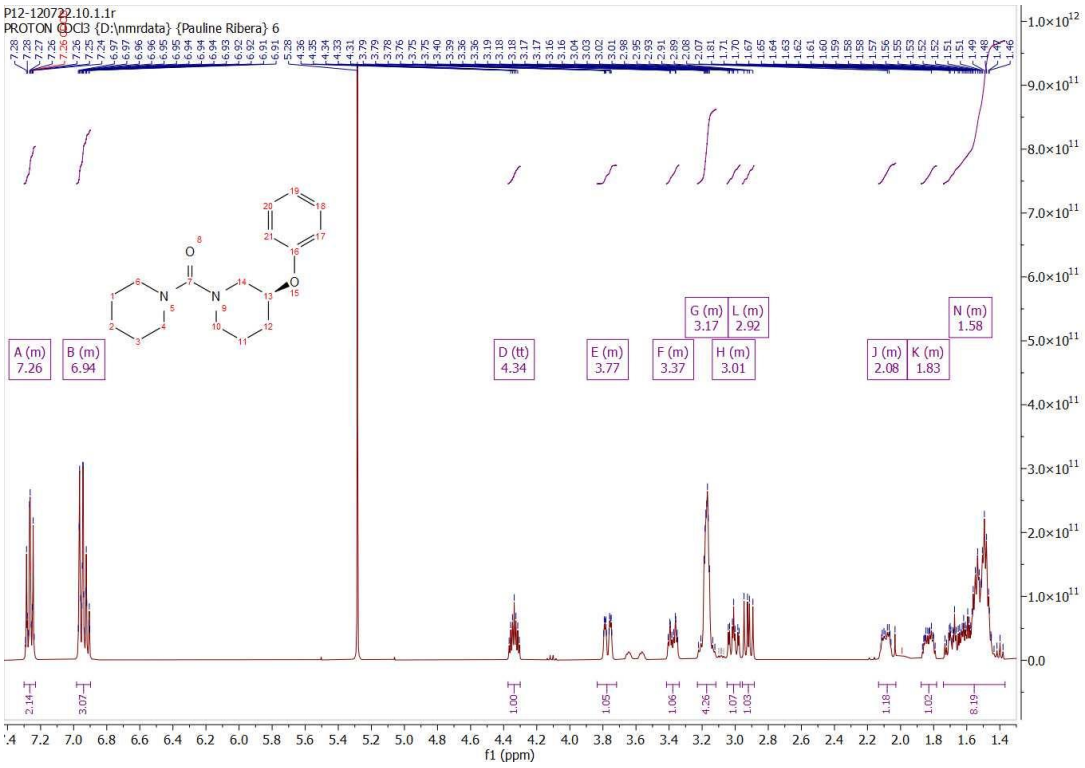
Appendix 6 : NMR of (3-(4-methoxyphenyl)pyrrolidin-1-yl)(piperidin-1-yl)methanone



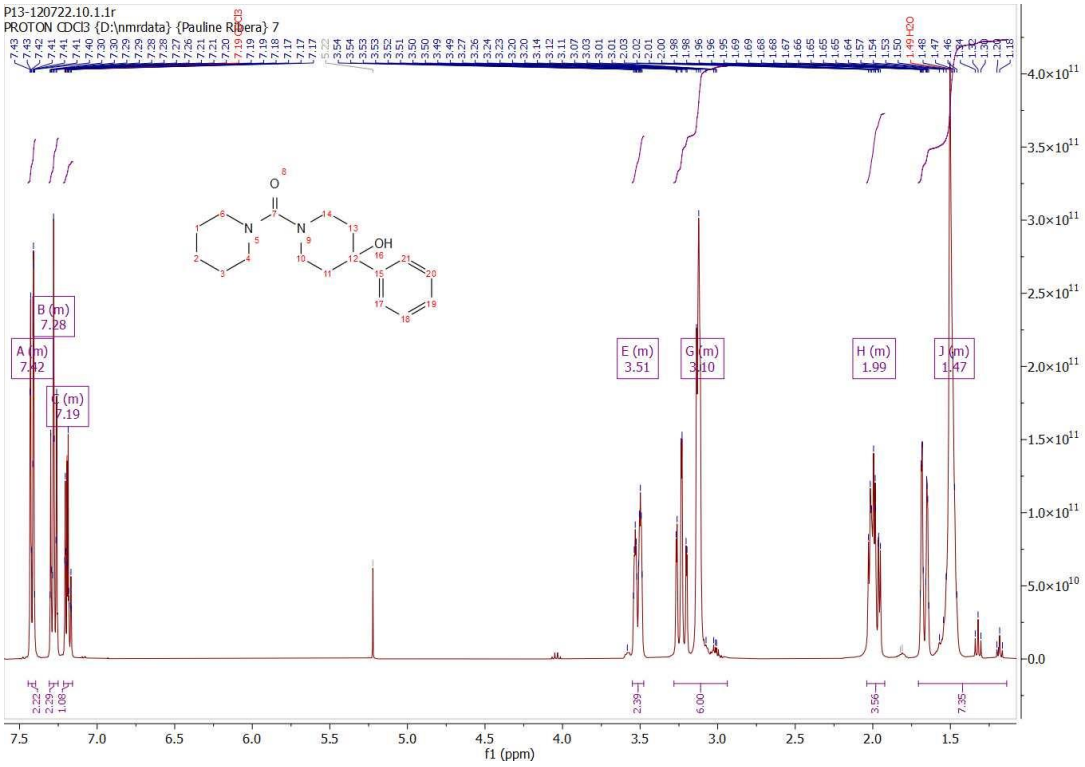
Appendix 7 : NMR of (4-(4-methoxybenzyl)piperidin-1-yl)(piperidin-1-yl)methanone



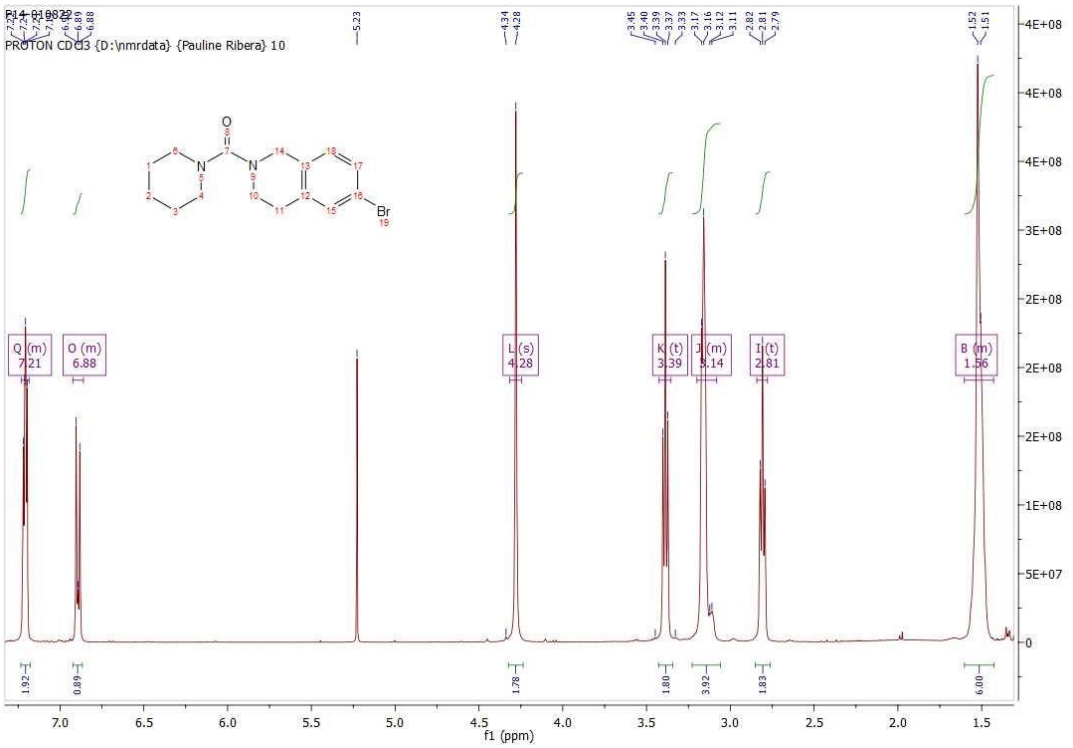
Appendix 8 : NMR of (3,4-dihydroisoquinolin-2(1H)-yl)(piperidin-1-yl)methanone



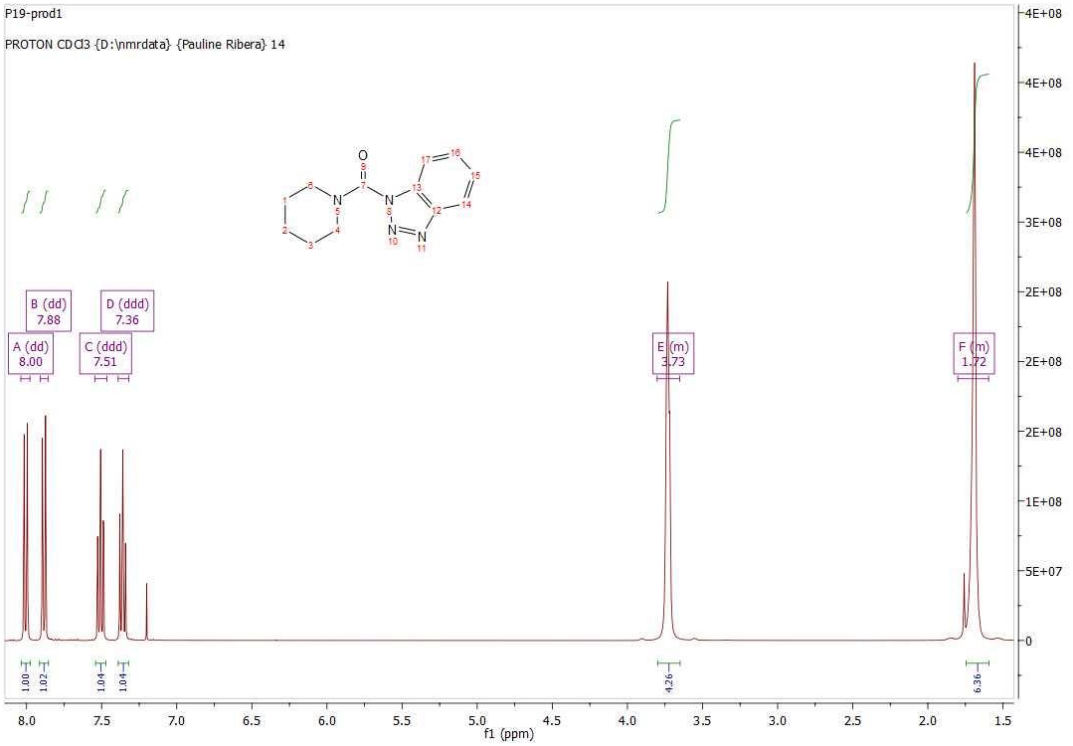
Appendix 9 : NMR of (S)-(3-phenoxypiperidin-1-yl)(piperidin-1-yl)methanone



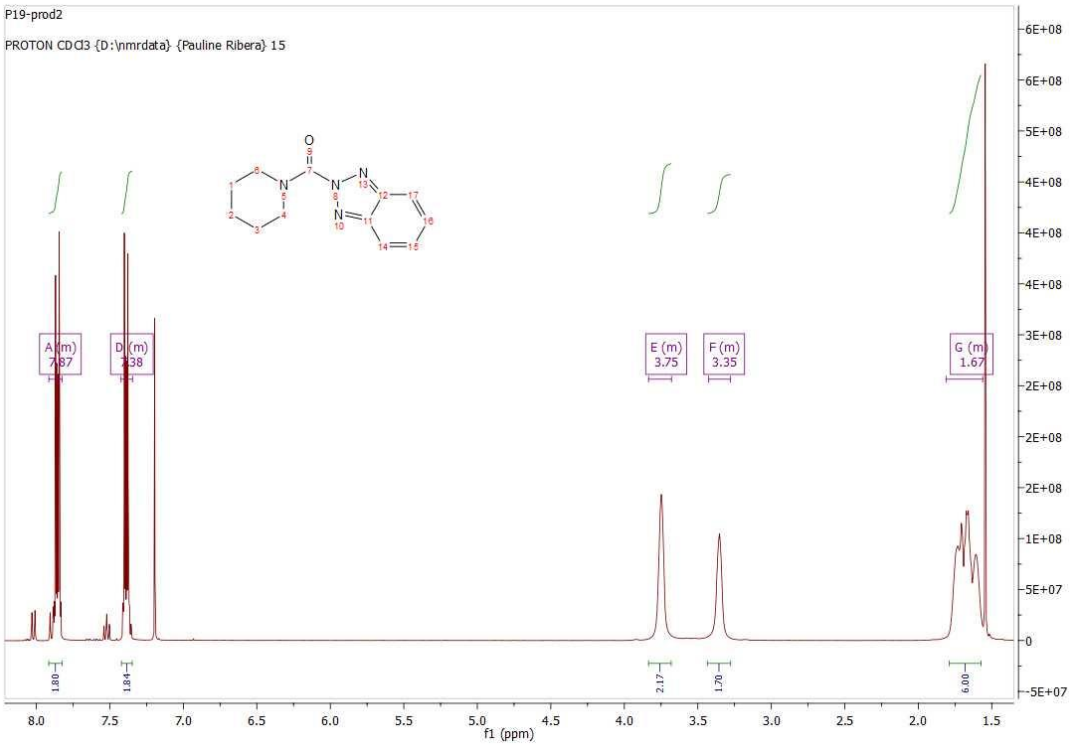
Appendix 10 : NMR of (4-hydroxy-4-phenylpiperidin-1-yl)(piperidin-1-yl)methanone



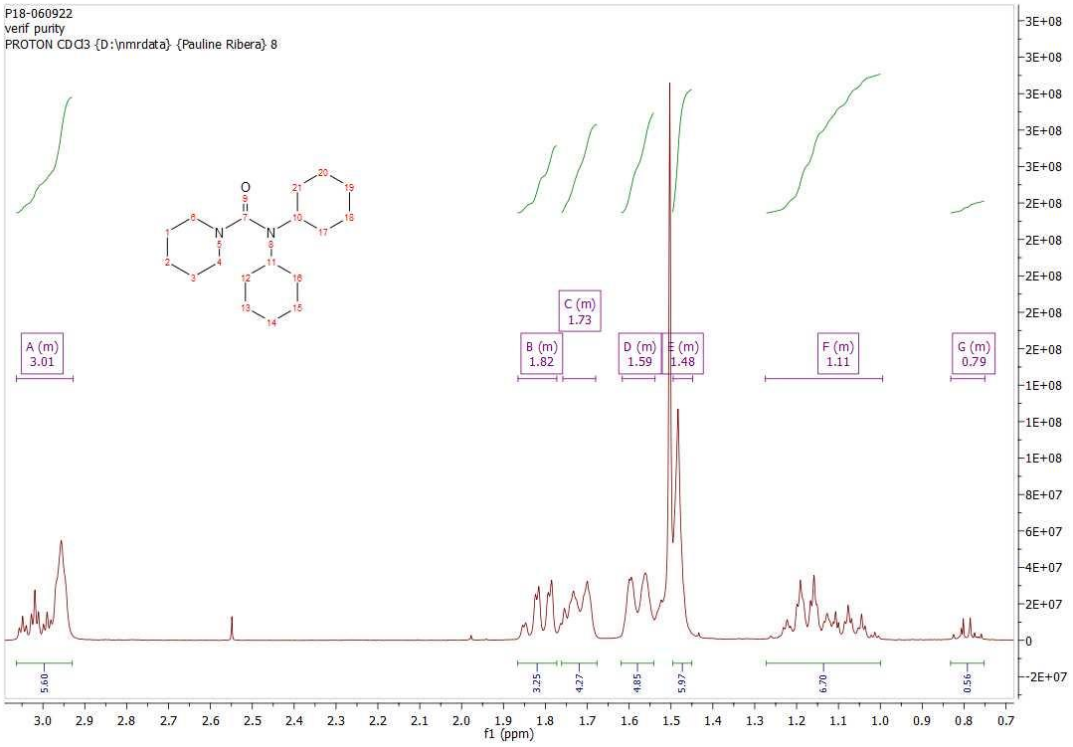
Appendix 11 : NMR of (6-bromo-3,4-dihydroisoquinolin-2(1H)-yl)(piperidin-1-yl)methanone



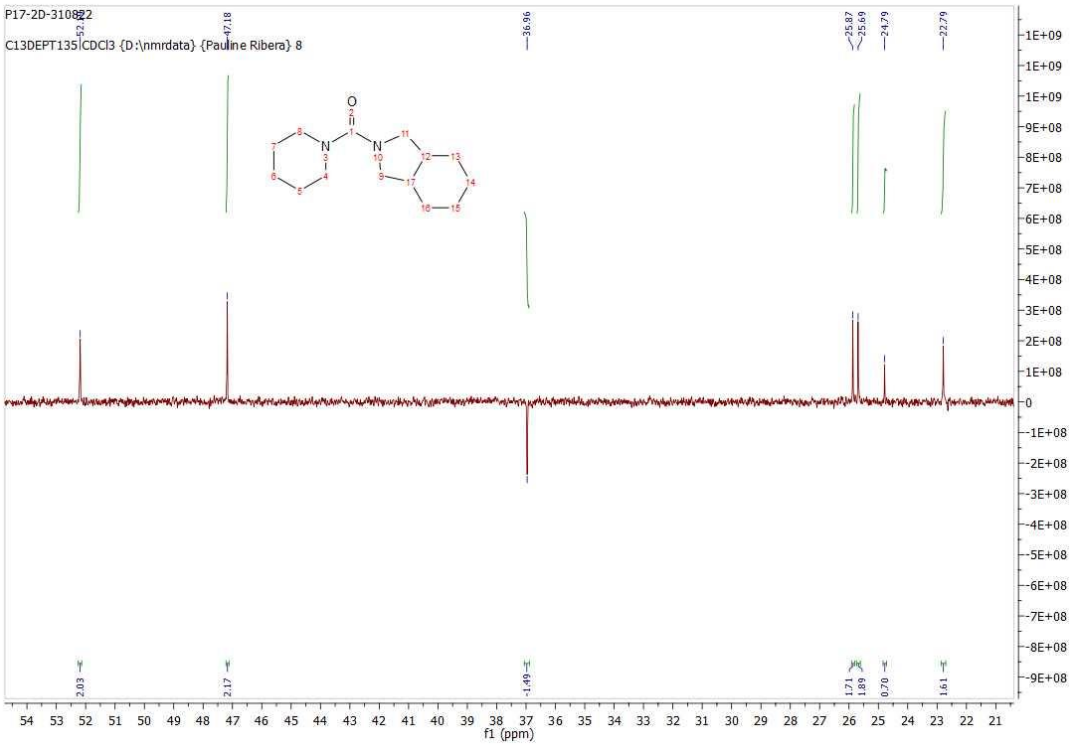
Appendix 12 : NMR of (1H-benzo[d][1,2,3]triazol-1-yl)(piperidin-1-yl)methanone (molecule 1)



Appendix 13 : NMR of (2H-benzo[d][1,2,3]triazol-2-yl)(piperidin-1-yl)methanone (molecule 2)



Appendix 14 : NMR of N,N-dicyclohexylpiperidine-1-carboxamide



Appendix 15 : NMR of N,N-dicyclohexylpiperidine-1-carboxamide (Carbone 13)

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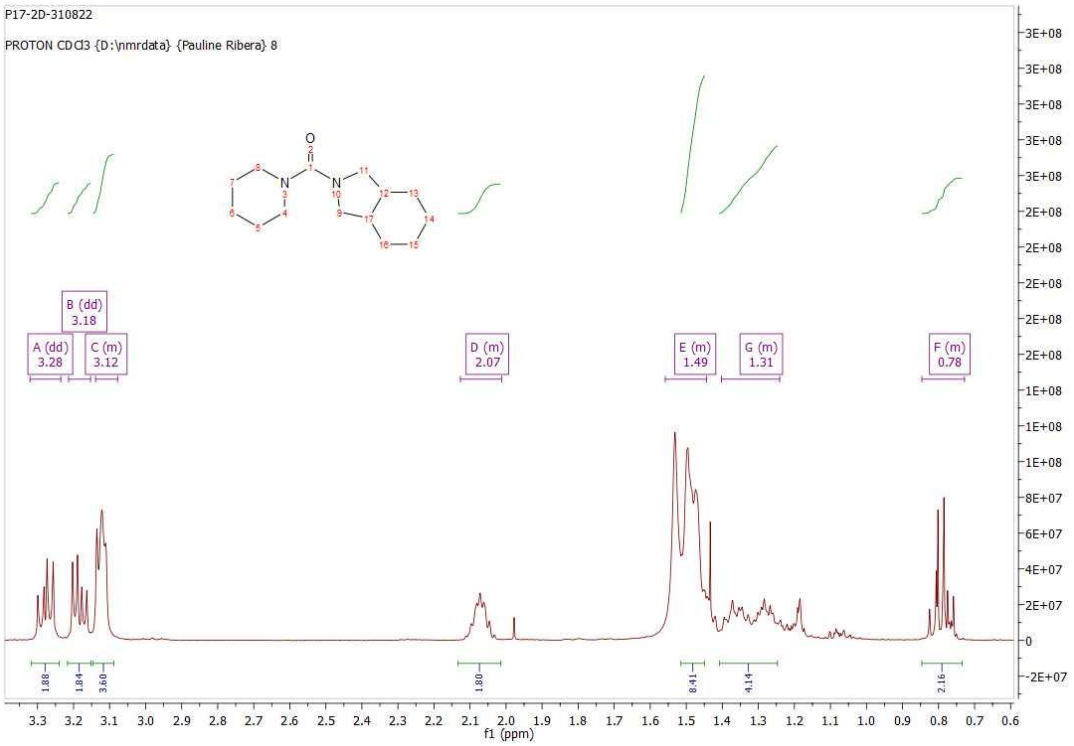
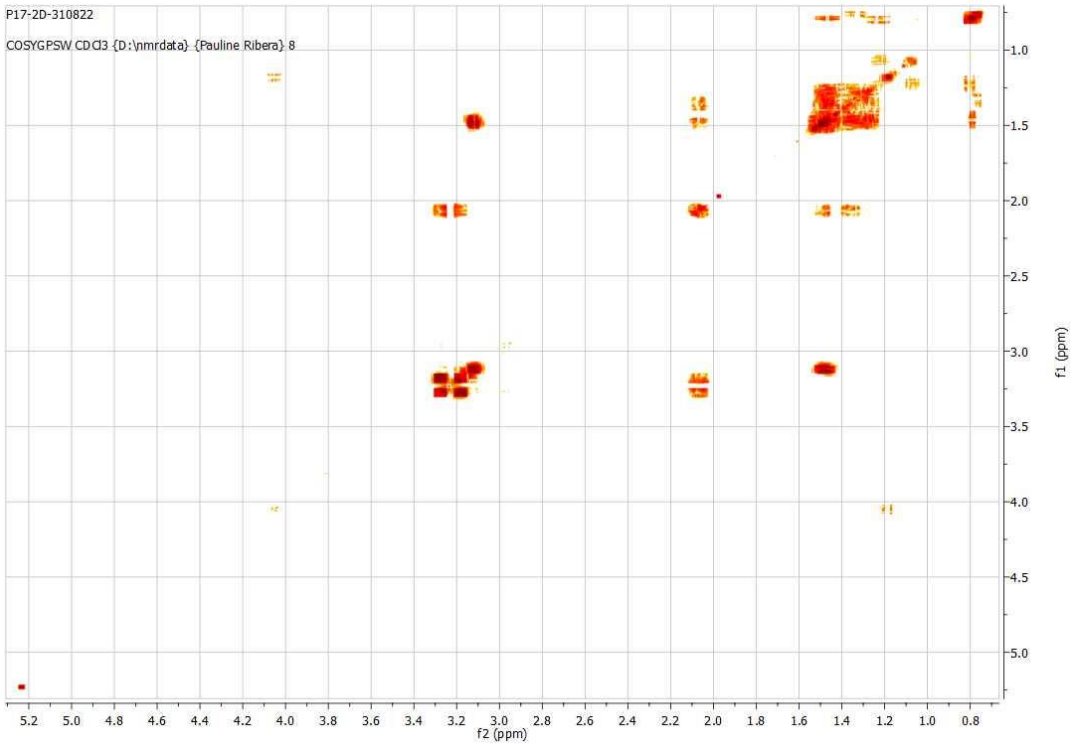
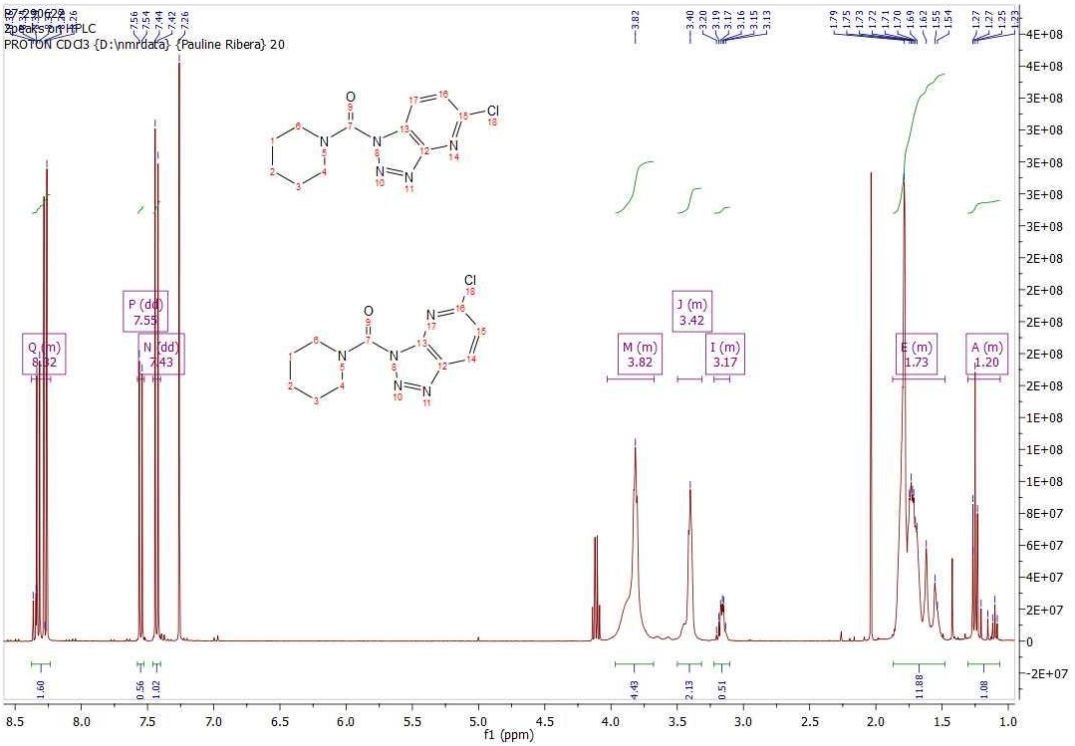


Appendix 16 : NMR of N,N-dicyclohexylpiperidine-1-carboxamide (proton 1)



Appendix 17 : COSY of N,N-dicyclohexylpiperidine-1-carboxamide



Appendix 18 : NMR of the mixture of (5-chloro-3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)(piperidin-1-yl)methanone and (5-chloro-1H-[1,2,3]triazolo[4,5-b]pyridin-1-yl)(piperidin-1-yl)methanone

Data File C:\MassHunter\Walkup\DataFiles\Cleopatra\CBCS\22-06\P7P-290622_0720.D
Sample Name: P7P-290622

```
    ======================================================================
    Acq. Operator   : Administrator
    Sample Operator : Pauline Ribera
    Acq. Instrument : CLEOPATRA                        Location :   D2F-A8
    Injection Date  : 6/29/2022 4:03:08 PM                Inj :   1
                                                    Inj Volume : Inj prog
    Method          : D:\ChemStation\1\Methods\X1097-3.M
    Last changed    : 6/29/2022 4:01:23 PM by Administrator
                      (modified after loading)
    Method Info     : X-bridge C18, 50x3.0 mm, 3.5u, 10-97% MeCN 3min,1ml/min, B: MeCN C:
                      NH4HC03

    Sample Info     : Walkup method: 'X1097-3'
                      Target:
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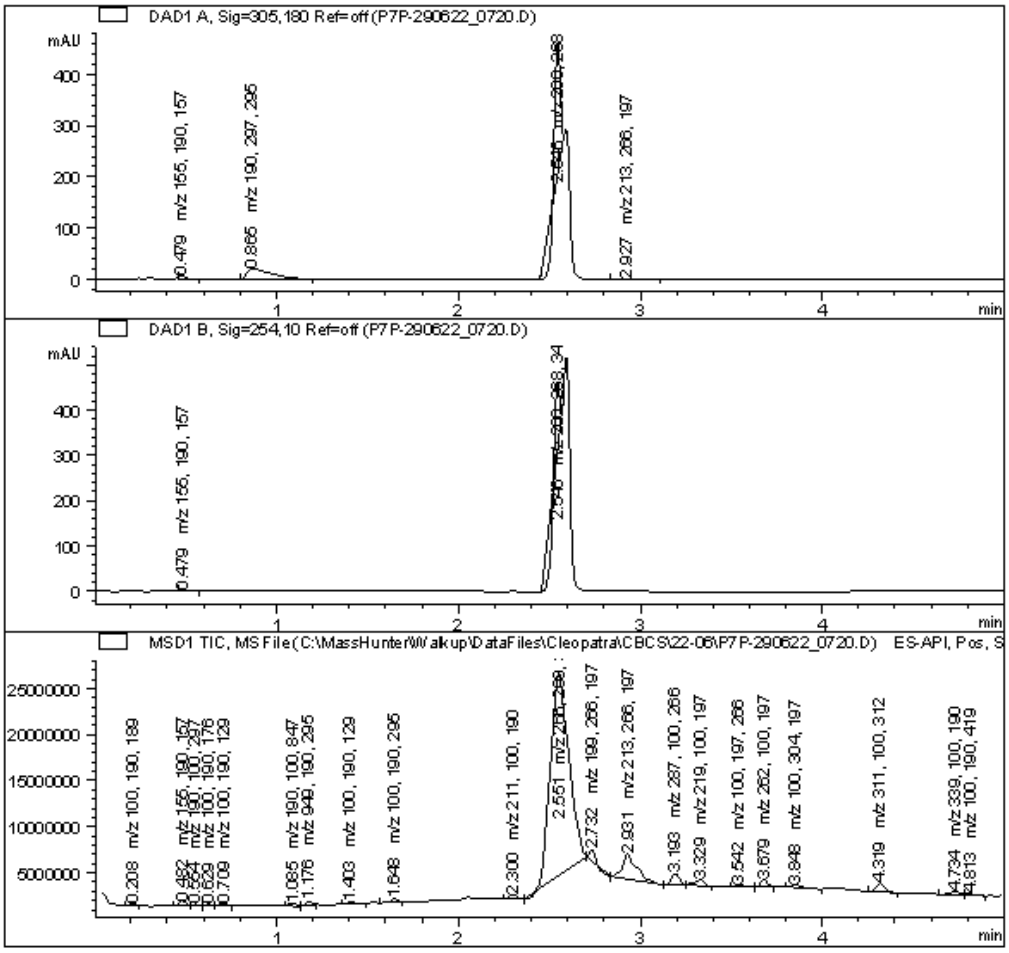
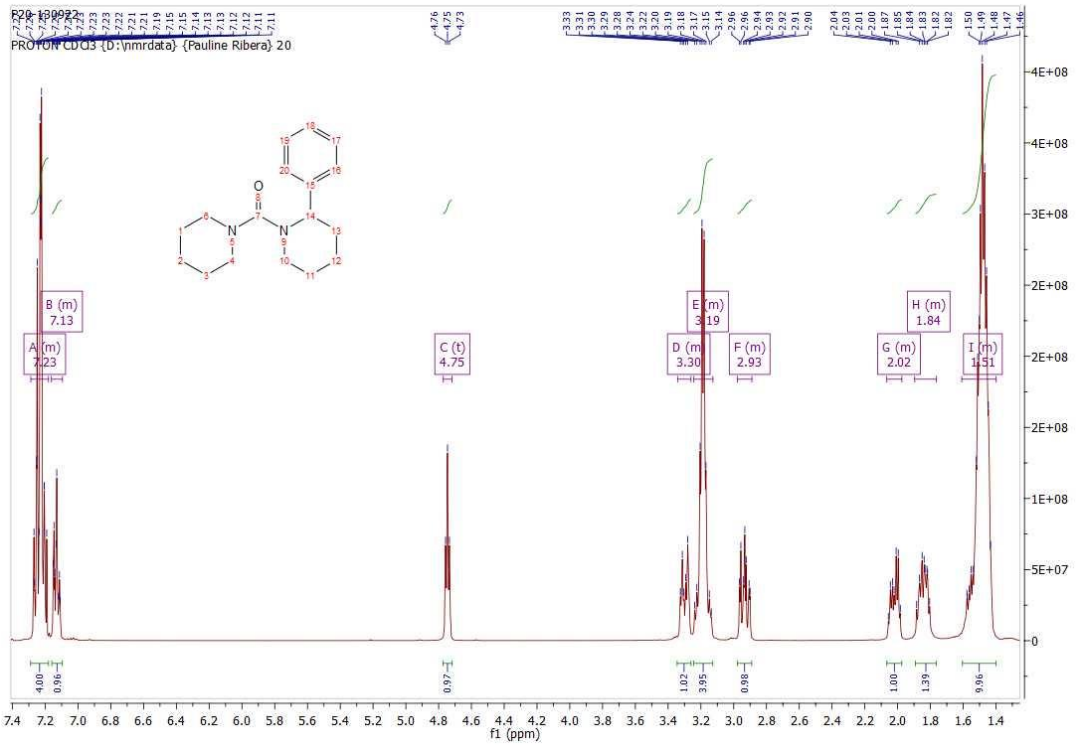
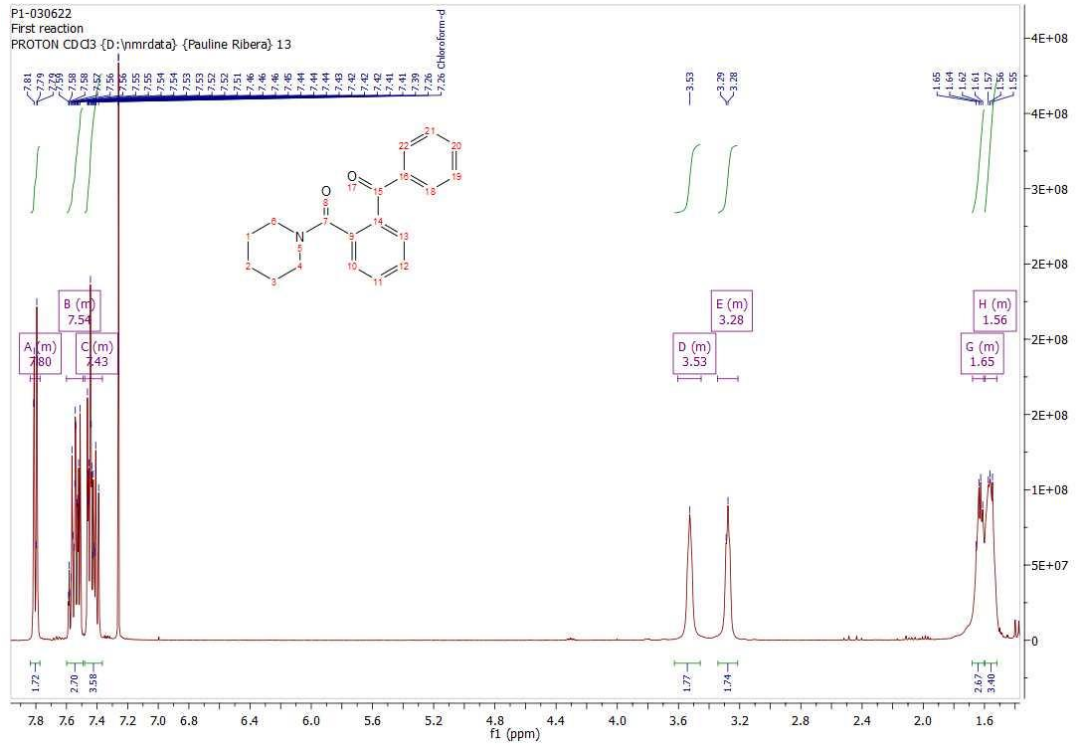


Appendix 19 : LC-MS of the mixture of (5-chloro-3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)(piperidin-1-yl)methanone and (5-chloro-1H-[1,2,3]triazolo[4,5-b]pyridin-1-yl)(piperidin-1-yl)methanone

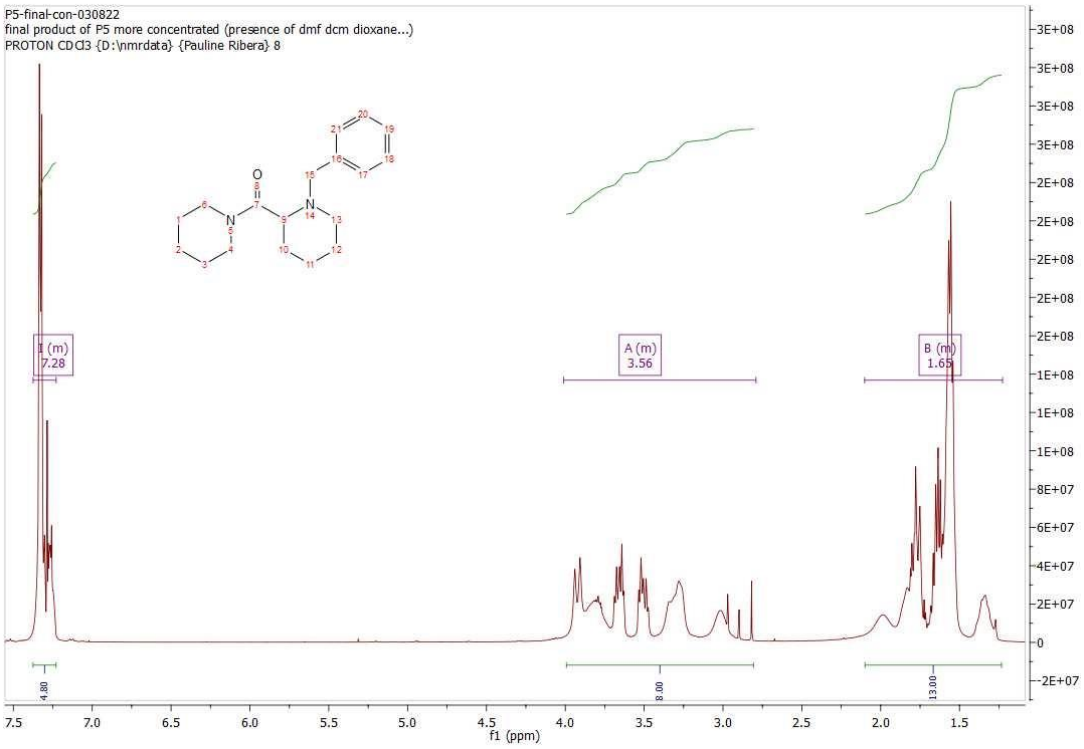


Appendix 20 : NMR of (2-phenylpiperidin-1-yl)(piperidin-1-yl)methanone

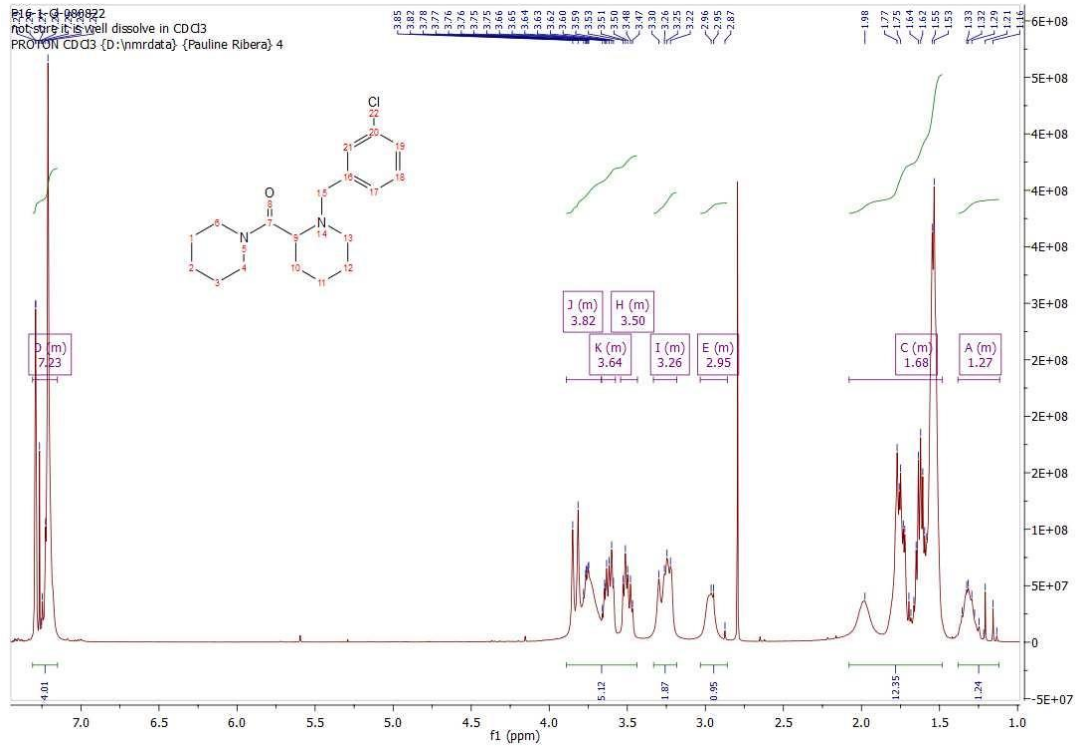


Appendix 21 : NMR of (2-benzoylphenyl)(piperidin-1-yl)methanone

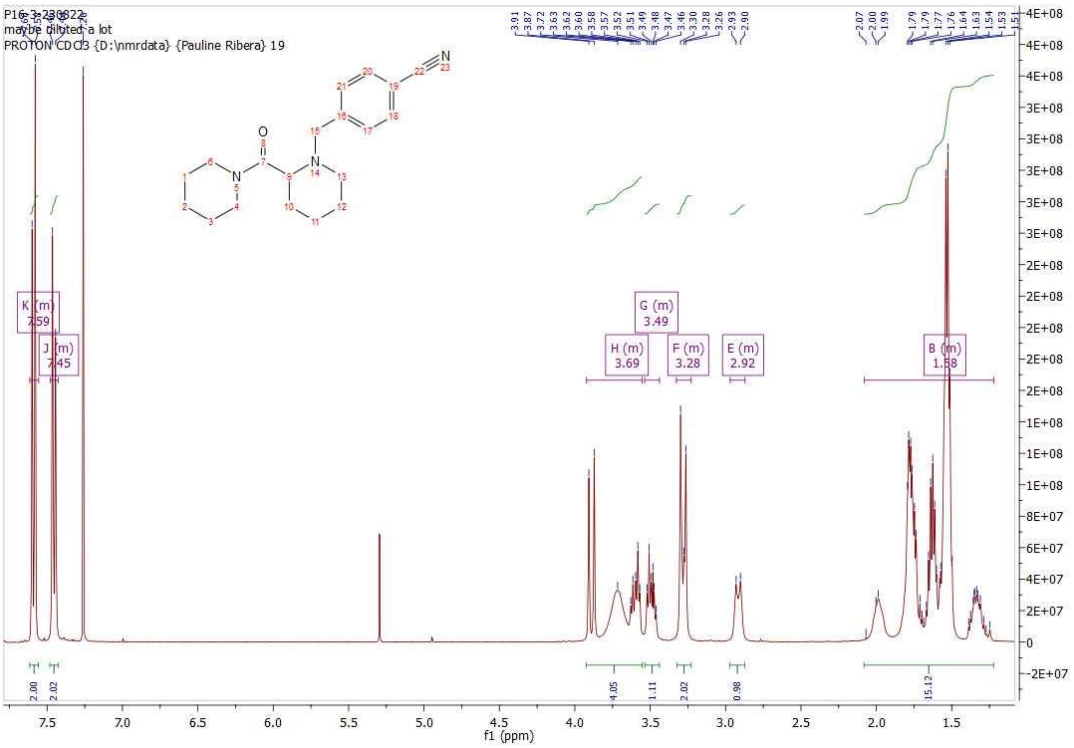
ii. Analysis of multi-step reaction



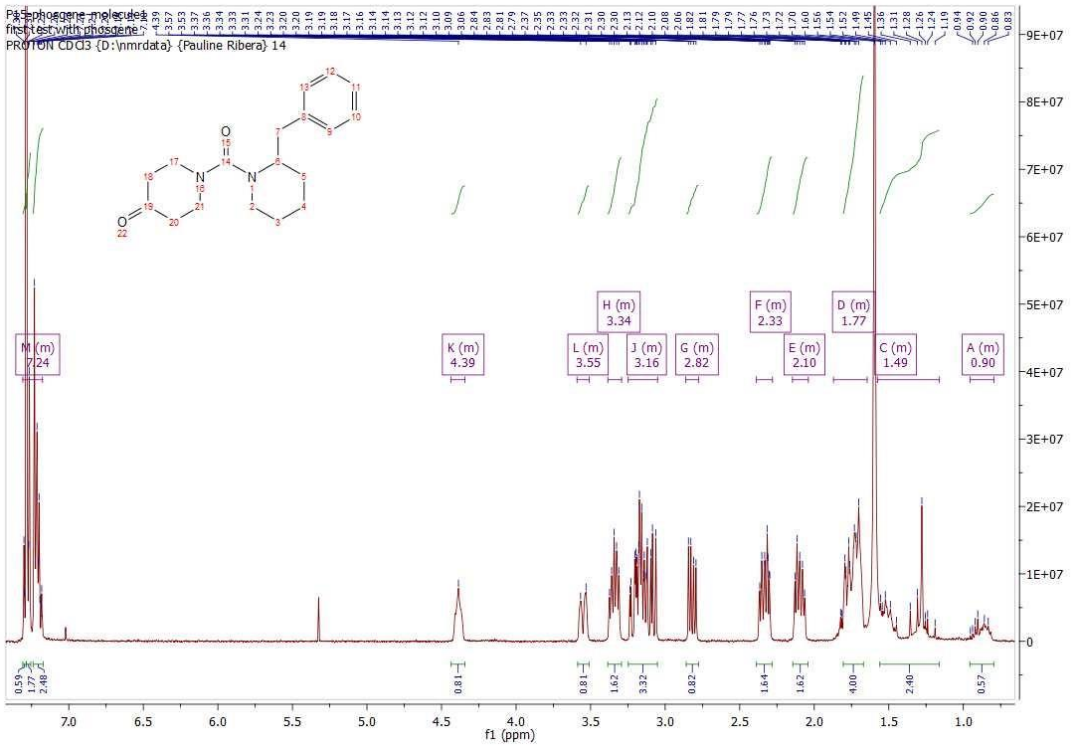
Appendix 22 : NMR of (1-benzylpiperidin-2-yl)(piperidin-1-yl)methanone



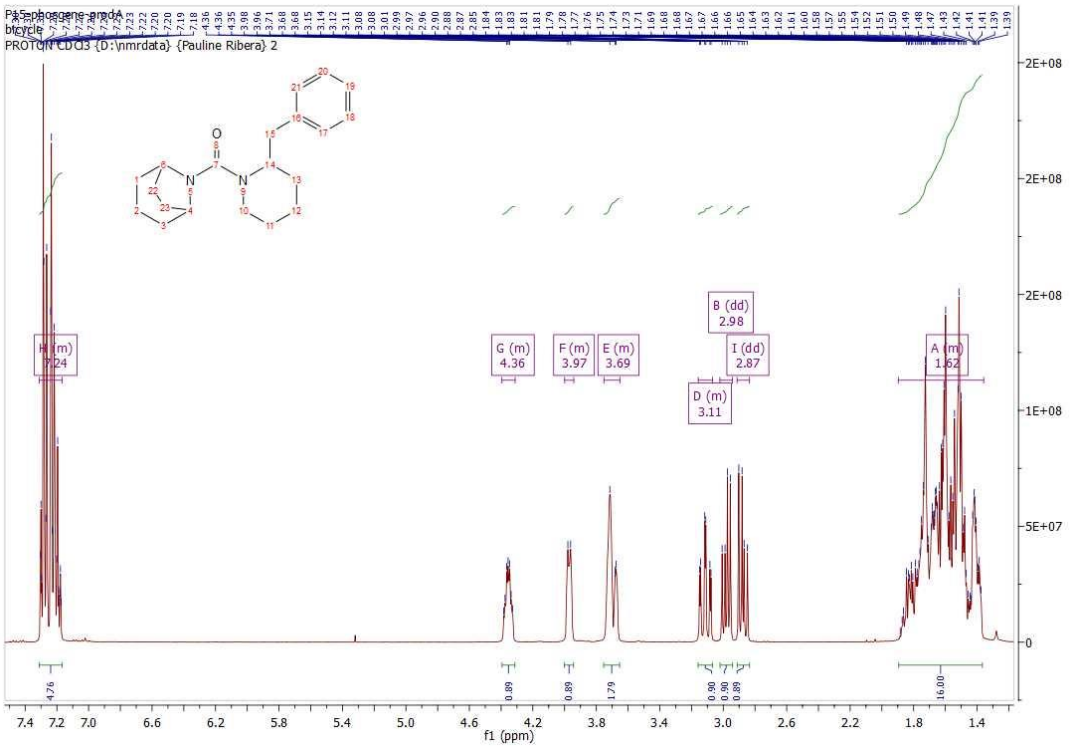
Appendix 23 : NMR of (1-(3-chlorobenzyl)piperidin-2-yl)(piperidin-1-yl)methanone



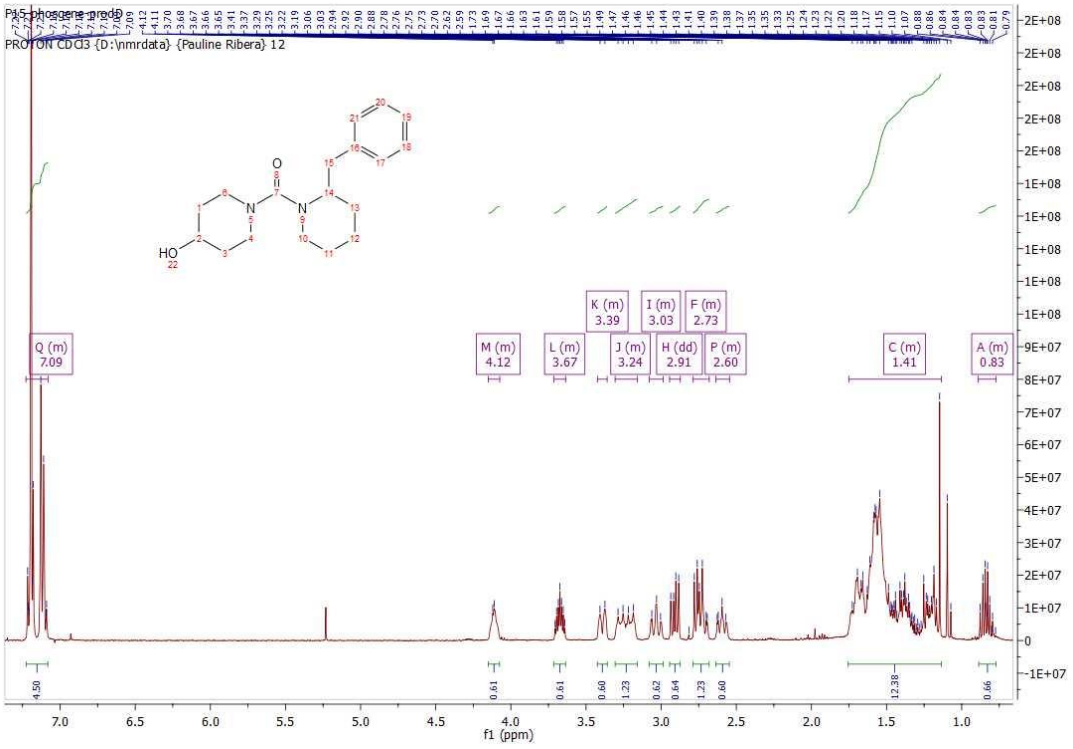
Appendix 24 : NMR of 4-((2-(piperidine-1-carbonyl)piperidin-1-yl)methyl)benzonitrile



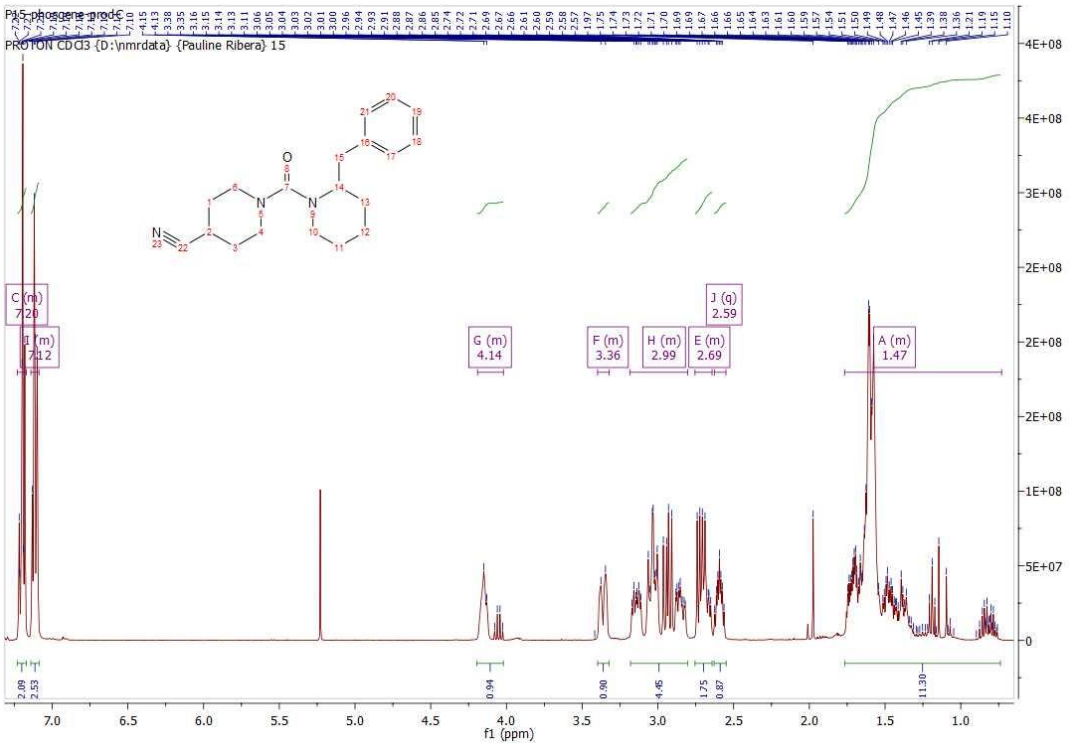
Appendix 25 :NMR of 1-(2-benzylpiperidine-1-carbonyl)piperidin-4-one



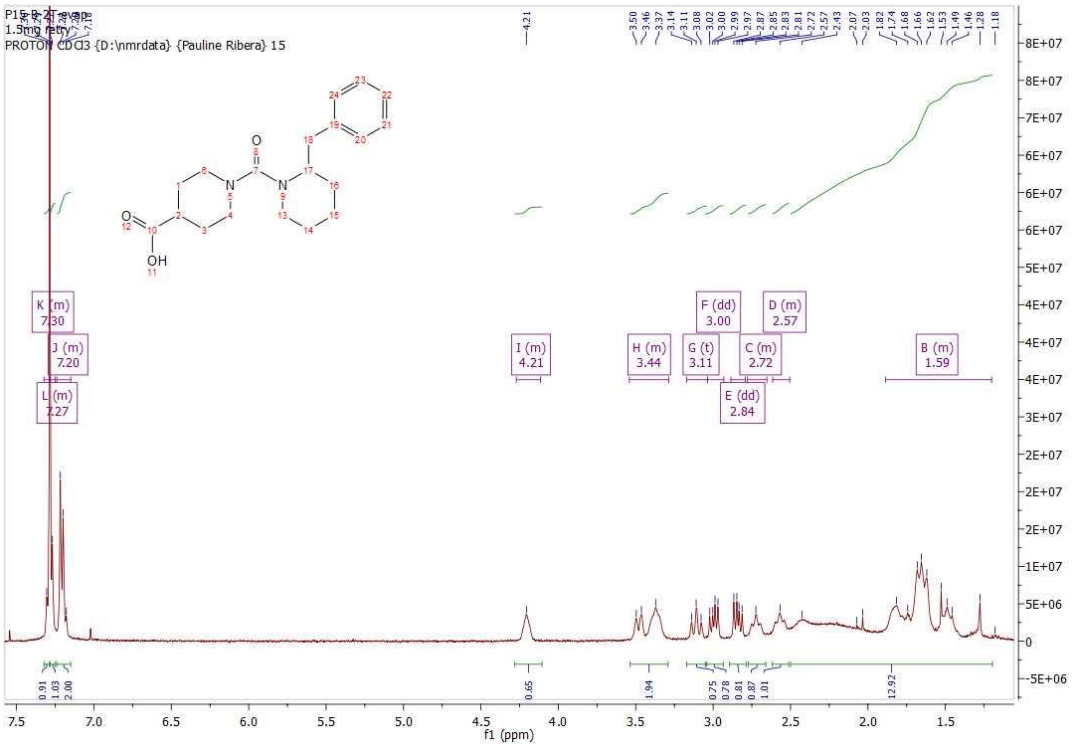
Appendix 26 : NMR of (2-benzylpiperidin-1-yl)(8-azabicyclo[3.2.1]octan-8-yl)methanone



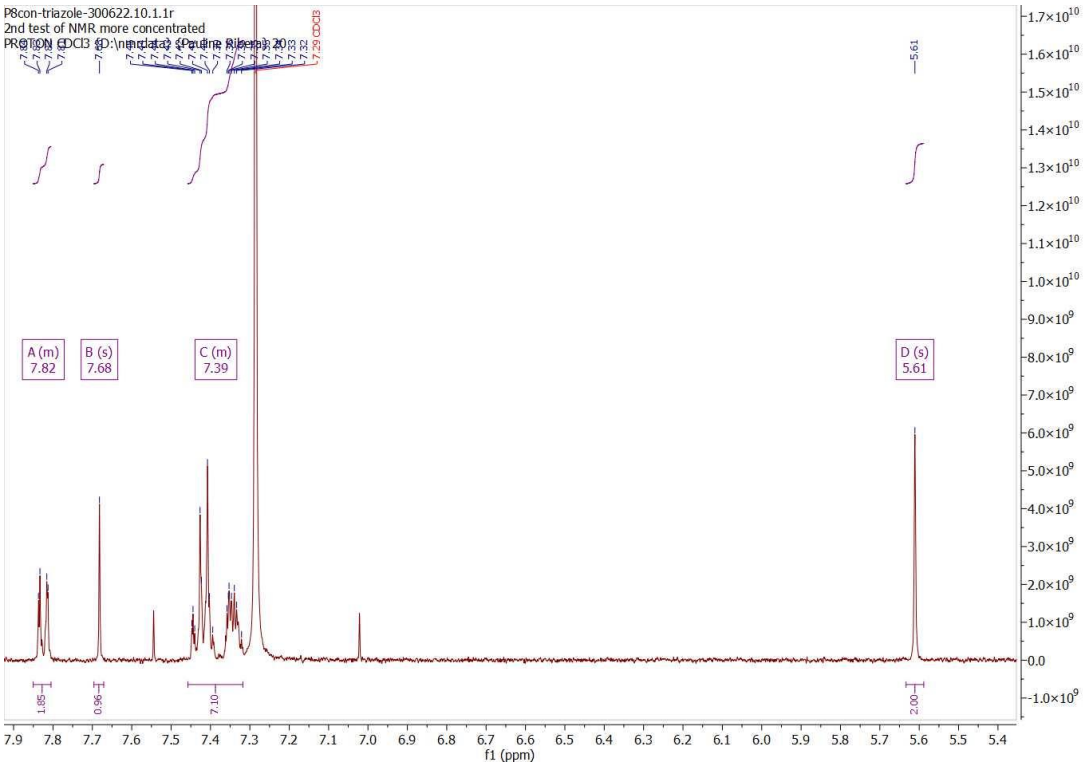
Appendix 27 : NMR of (2-benzylpiperidin-1-yl)(4-hydroxypiperidin-1-yl)methanone



Appendix 28 : NMR of 1-(2-benzylpiperidine-1-carbonyl)piperidine-4-carbonitrile



Appendix 29 : NMR of 1-(2-benzylpiperidine-1-carbonyl)piperidine-4-carboxylic acid



Appendix 30 : NMR of 1-benzyl-4-phenyl-1H-1,2,3-triazole (click chemistry)

Data File C:\MassHunter\Walkup\DataFiles\Cleopatra\CBCS\22-09\urea-click-lastLCMS_2877.D
Sample Name: urea-click-lastLCMS

```
    ======================================================================
    Acq. Operator   : Administrator
    Sample Operator : Pauline Ribera
    Acq. Instrument : CLEOPATRA                        Location :   D1F-B9
    Injection Date  : 9/15/2022 2:17:45 PM                Inj :   1
                                                     Inj Volume : Inj prog
    Method          : D:\ChemStation\1\Methods\X1097-3.M
    Last changed    : 9/15/2022 2:15:59 PM by Administrator
                      (modified after loading)
    Method Info     : X-bridge C18, 50x3.0 mm, 3.5u, 10-97% MeCN 3min,1ml/min, B: MeCN C:
                      NH4HCO3

    Sample Info     : Walkup method: 'X1097-3'
                      Target:
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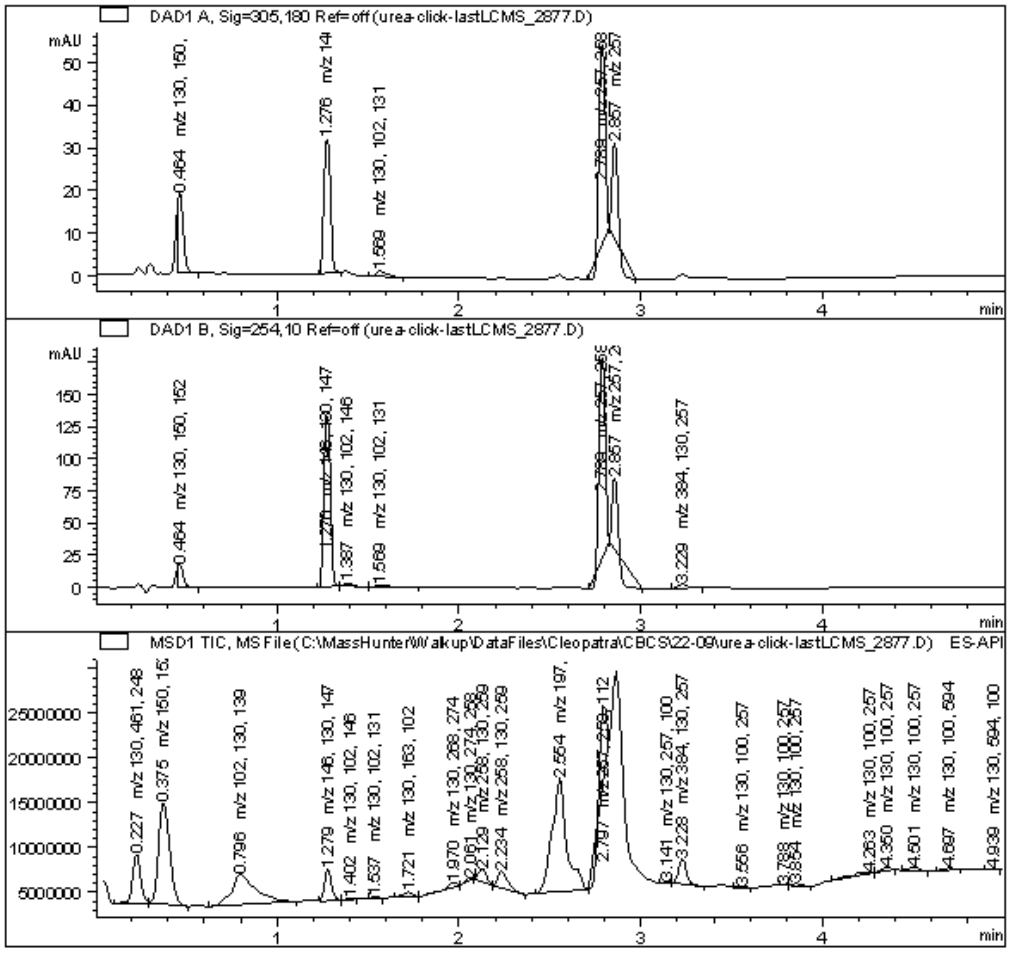


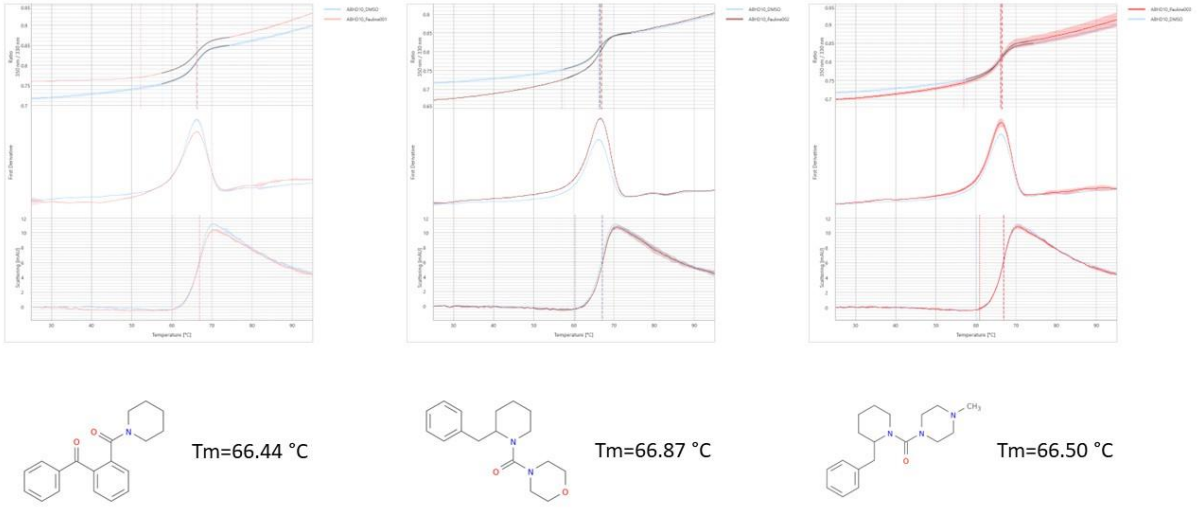
Appendix 31 : LC-MS of the formation of (5-phenyl-1H-1,2,3-triazol-1-yl)(piperidin-1-yl)methanone (click chemistry)

B/ BIOLOGY RESULT

ABHD10 (4 µM) + Compound (100 µM)

T_m(control)=66.39 °C

Control with 1% DMSO; Samples with compound added up to 1% DMSO; recorded in duplicates

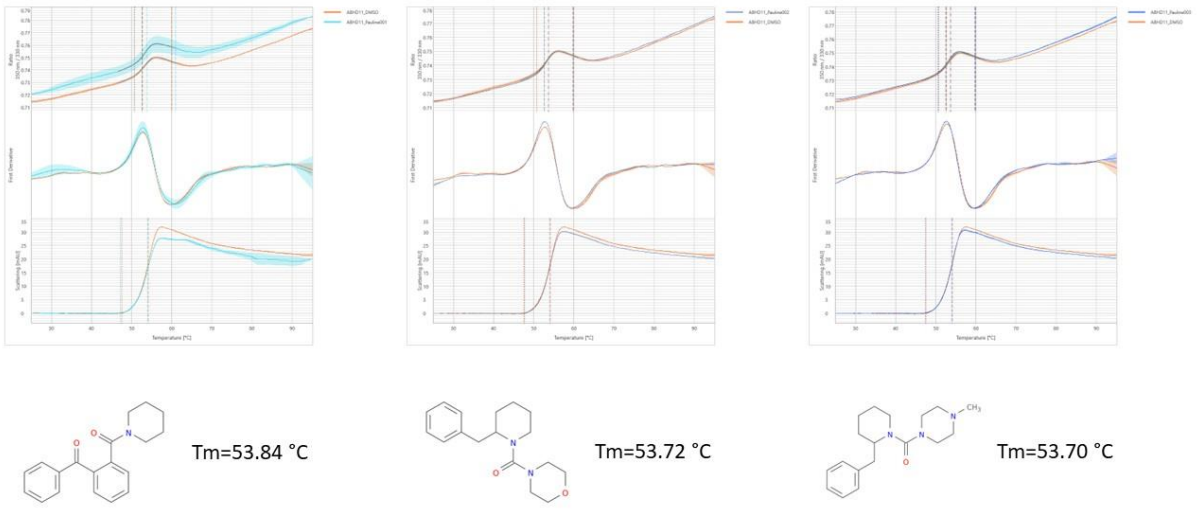


Appendix 32 : Test on ABHD10 of (2-Benzoyl-phenyl)-piperidin-1-yl-methanone, [2-(phenylmethyl)-1-piperidinyl]-4-morpholinylmethanone, (2-benzylpiperidin-1-yl)(4-methylpiperazin-1-yl)methanone

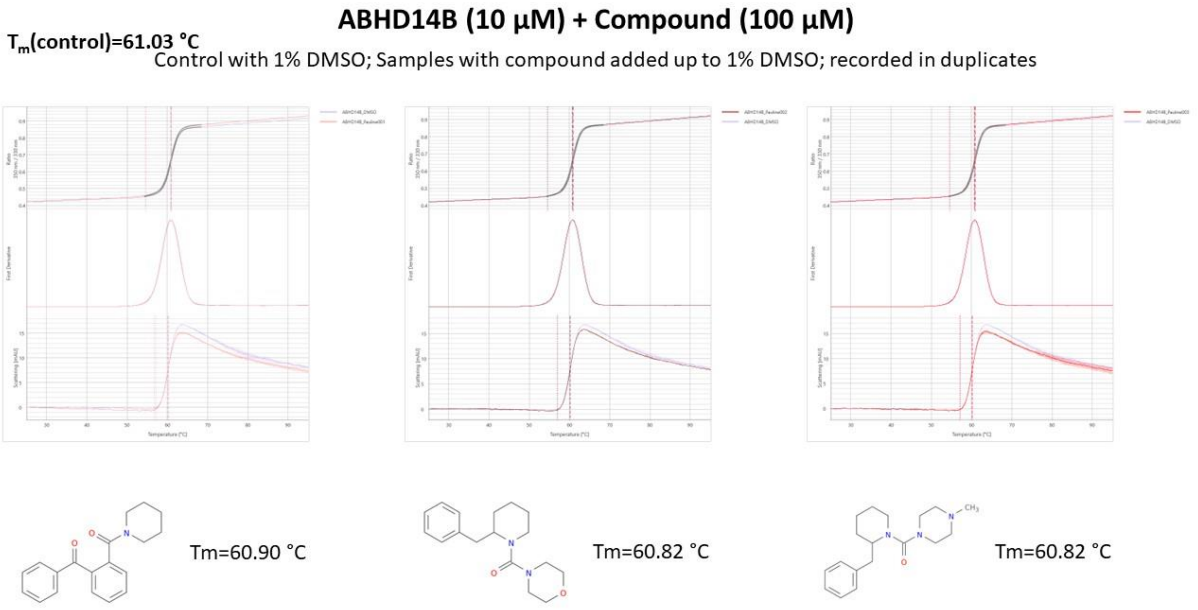
ABHD11 (10 µM) + Compound (100 µM)

T_m(control)=53.81 °C

Control with 1% DMSO; Samples with compound added up to 1% DMSO; recorded in duplicates



Appendix 33 : Test on ABHD11 of (2-Benzoyl-phenyl)-piperidin-1-yl-methanone, [2-(phenylmethyl)-1-piperidinyl]-4-morpholinylmethanone, (2-benzylpiperidin-1-yl)(4-methylpiperazin-1-yl)methanone



Appendix 34 : Test on ABHD14B of (2-Benzoyl-phenyl)-piperidin-1-yl-methanone, [2-(phenylmethyl)-1-piperidinyl]-4-morpholinylmethanone, (2-benzylpiperidin-1-yl)(4-methylpiperazin-1-yl)methanone