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Pharmacomodulation on the piperidinol skeleton: Synthesis of novel PIPD1 derivatives as Mycobacterium abscessus agents

Tesi di laurea sperimentale

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To my Brother, Iheb

Abstract

Mycobacterium abscessus (*M. abscessus*) is a rapidly growing mycobacterium which is able to generate different problems in human infections, such as chronic lung diseases, pulmonary diseases and skin infection. *M. abscessus* is considered as the first emergent opportunistic pathogen and the number of the infections due to this pathogen increases each year. Since the natural multidrug resistance and the absence of specific treatment to fight it, *M. abscessus* has become a serious problem of the public health. In this context, an original approach of a phenotypic screen was performed and allowed to identify a new piperidinol-based molecule, PIPD1, which exhibits a potent activity against *M. abscessus*. The goal of this project was to synthesize and characterize some PIPD1-analogs in order to identify the pharmacophore of PIPD1 and develop a Structure-Activity Relationship (SAR) study.

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Chapter 1

Introduction

The role of microorganisms surrounding us can differ in many aspects: from those important for the environment's decomposition or other digestive processes to those that are cause of the main worldwide diseases. One of the microorganisms on which we focused is *Mycobacterium abscessus*, a *Non-Tuberculous Mycobacterium* of the Family *Mycobacteriaceae*, of the *Bacteria* Kingdom.

As a matter of facts, in the following paragraphs are explained the main features concerning *Mycobacterium*. The genus *Mycobacterium* is best known for the pathogenic species *M. Tuberculosis* which causes the death of million people each year (Hett & Rubin, 2008) but, other species has been found dangerous towards living being, one of them is *M. abscessus*. Hence, after describing the morphology of the *Mycobacteria*, it is presented a possible way to treat them.

1.1. Cellular Structure

Most of the microorganisms are surrounded by a cell membrane which can perform various actions: it can separate the inside of the cell from its external environment; regulate the flow of nutrients and act as a protective barrier (Rogers, 2011). Outside of the cell membrane is present a cell wall, whose common component is peptidoglycan (also known as murein, which contains acylated amino sugars and different amino acids, it is a heteropolymer built out of glycan cross-linked through peptides (Schleifer & Kandler, 1972)). The sugar component consists of *β*-(1,4) linked *N*-acetylglucosamine and *N*-acetylmuramic acid, where the adjacent sugars are linked to each other by peptide bridges that confer rigid stability (Rogers, 2011).

The cell wall presents a different structure depending on whether it is a Gram-positive Bacteria or Gram-negative *Bacteria*. Gram-positive *Bacteria* are *Bacteria* that give positive results in a Gram stain test. They appear blue-coloured after the standard microbiological techniques, due to the presence of peptidoglycan that forms a thick layer of the wall cell trapping in it the blue dye. On the other hand, in Gram-negative *Bacteria*

the peptidoglycan layer is thin, and the blue dye of Gram stain is washed out, therefore they appear light pink (Rogers, 2011).

However, mycobacterial cell wall consists of an inner layer and an outer layer that surround the plasma membrane. The outer compartment consists of lipids and proteins. The lipids are associated with the cell through long- and short-chain fatty acids (Hett & Rubin, 2008).

Among Gram-positive *Bacteria*, *Mycobacterium* is an important genus of bacilli of the Family *Mycobacteriaceae* (Order *Actinomycetales*, Kingdom *Bacteria*). Some of the most important species of which, *M. Tubercolosis* and *M. Leprae*, cause respectively tuberculosis and leprosy in humans (Rogers, 2011).

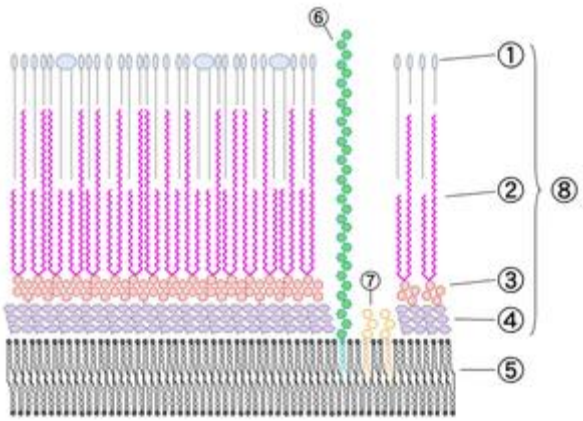


Figure 1: Mycobacterial cell wall (Mycobacterium).

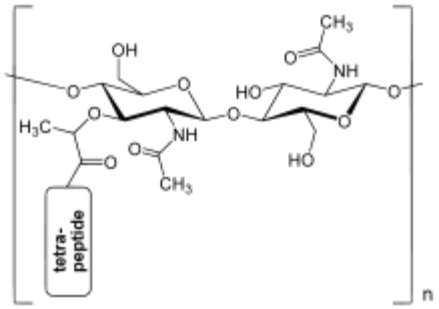


Figure 2: Peptidoglycan structure (Schleifer & Kandler, 1972).

1.2. General characteristics of *Mycobacterium*

Mycobacterium is a genus of obligate aerobic, Gram-positive and acid-fast bacilli. They are non-motile, most of them do not form spores (Ryan & Ray, 2004), unit of asexual reproduction (spore, 2017), and their size varies from 1 to 5 µm (Tortoli & Simonetti,

1990). They can be found in food, soil and water in a free-living form or in diseased tissues of animals.

The acid-fastness property (or acid-alcohol fastness) of *Mycobacteria* is due to the fact that the cell wall lipids make the surface hydrophobic, rendering *Mycobacteria* resistant to staining with basic dyes unless they are applied for long periods of time with detergents or heat. Once stained, *mycobacteria* resist decolourization even when treated with a mixture of acid and alcohol (e.g. 3% hydrochloric acid and 95% ethanol) (Ryan & Ray, 2004).

Mycobacterium wall is distinguished from other many bacteria, as a matter of fact their thick wall consists of: a lipidic layer (phenolic glycolipid), 2-mycolic acid, arabinogalactan, and peptidoglycan (it contains *N*-glycolylmuramic, rather than *N*-acetylmuramic, acid (Ryan & Ray, 2004)) and arabinomannan (Brennan, 2003). This characteristics make mycobacterium harder to defeat. In particular, the outer compartments in slow-growing pathogenic *mycobacteria*, such as *M. Tuberculosis*, the lipoarabinomannan are capped at the terminal residue with mannose residues, whereas the fast-growing *mycobacteria*, such as *M. abscessus*, have phosphoinositol-capped lipoarabinomannan. The outer proteins and lipids are soluble components of the cell wall. Instead, the inner compartments consists of peptidoglycan, arabinogalactan and mycolic acids linked together with covalent bonds, forming a complex insoluble and not easy to break (Hett & Rubin, 2008).

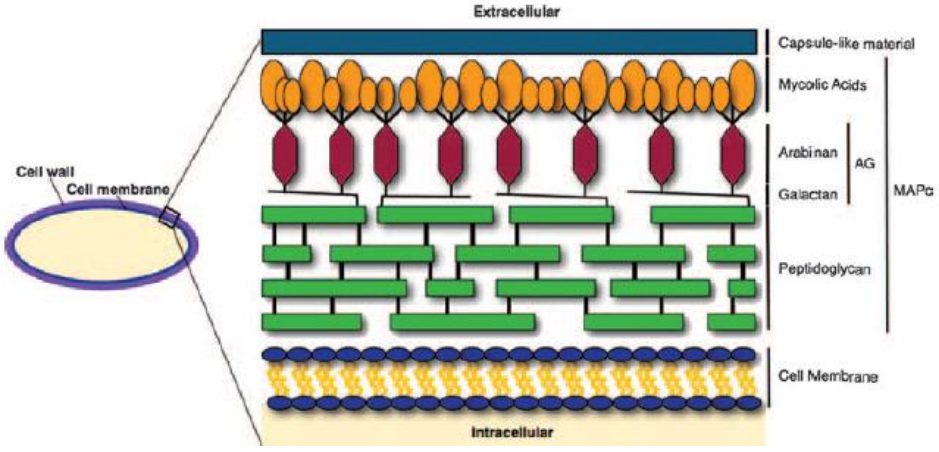


Figure 3: Diagram of the mycobacterial cell wall (Hett & Rubin, 2008).

Mycobacteria can be divided in different species according to their morphology, biochemistry and pathogenic scheme. More than 130 species of them are known. Among

these species, most of them are not pathogenic but some species are responsible of thousands of morbidity and disease in World. In fact, they can colonize various hosts, such as human being, without the host showing any adverse sign, they can also exist in a free living state ((Rogers, 2011) and (Gutierrez MC, 2009)).

However, *mycobacterium* can be subdivided in two main groups for diagnosis and treatment: *M. Tuberculosis complex* and *Non-tuberculosis mycobacterium*. *Mycobacterium tuberculosis complex* are group of *Mycobacterium* species that are capable to cause tuberculosis in hosts. *Non-tuberculosis*, instead, are called opportunists as they infect subjects with predisposing conditions (Cirillo).

In the late 50's, the botanic Runyon introduced a mycobacterium's classification based on their growth's rate, colonies pigmentation and morphology (Koh, Kwon, & Lee, 2002). The *non-tuberculosis mycobacterium* are, thus, divided into four groups: three of them are classified as ***Slowly Growing Mycobacteria*** (they can form colonies in culture in lots of days) and the fourth as ***Rapidly growing Mycobacteria*** (***RGM***, colonies are formed in maximum 7 days).

Examples of *mycobacterium tuberculosis complex* are: *Mycobacterium tuberculosis (*causative agent of tuberculosis*); Mycobacterium africanum* (whose symptoms of infection resemble those of *M. tuberculosis*); *Mycobacterium bovis* (cause tuberculosis in cattle and in humans who drink infected milk); *M. microti* (causes tuberculosis in voles) (Frothingam, Hills, & Wilson, 1994).

Slowly Growing Mycobacteria are: *M. marinum* (typically affects people that work with fish or keep home aquarium and it is the agent of swimming pool Granuloma, that is a skin located infection); *M. kansasii* (can cause serious disease in mammals); *M. scrofulaceum* (cause cervical lymphadenitis in children); *M. leprae* (also known as *Hansen's bacillus spirally*, it is the agent of leprosy); *M. avium* complex etc. (Tortoli & Simonetti, 1990).

On the other hand, examples of *Rapidly Growing Mycobacteria* are: *M. chelonae* (it can cause opportunistic infections of humans); *M. abscessus* (that is a common water contaminant, it can cause chronic lung disease and post-traumatic wound infections); *M. fortuitum* (it causes occasional infections of the lung, bone or soft tissues following trauma (Helou, Viola, Hachem, Han, & Raad, 2013).

1.3- Mycobacterium Abscessus

Among rapidly growing *mycobacterium*, *mycobacterium abscessus* is the most frequently isolated in human diseases.

The first recorded case of *M. abscessus* were identified in 1953, when a 63 year old white woman was admitted to Barnes hospital because she was complaining of pain and limitation of motion of the left knee. This old woman fell down in a farm yard at the age of 14 and since then she started to suffer occasional pains in her knee, but only 48 years later she had a disseminated infection. After suitable analysis (medical and bacteriological) and comparisons with already known microorganisms, Moore and Frerichs stated that probably they were dealing with a new species, which appears after traumatic infection: ***M. abscessus***, indeed, whose name is due to their ability to produce deep abscesses in human tissue (Moore & Frerichs, 1953). *Abscessus* comes from the latin *ab-* (away) and *cedere* (to go), an abscess is named for the notion that humors leave the body through pus (Etymologia: Mycobacterium abscessus subsp. bolletii).

They are long and narrow, short and thick, straight and curved bacilli. Main features to identify these *mycobacteria* are the presence of long chain fatty acids, well known as mycolic acid, and the absence of pigmentation (Brown-Elliott & Richard J. Wallace, 2002). *M. abscessus* complex is prevalent in East Asia. In Taiwan, for example, it comprises 17.2% of clinical *Non-Tuberculosis Mycobacteria* isolates (Meng-Rui Lee, Hung, Yu, Lee, & Hsueh, 2015).

At the beginning, *M. abscessus* were considered by many scientists in the same group of *M. chelonae*, only in 1992 it was considered as an individual species, in fact both these mycobacteria differ by just 4 pair of bases (Brown-Elliott & Richard J. Wallace, 2002).

M. abscessus complex can be divided into 3 different subspecies: *M. abscessus* subsp. *abscessus, M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii*.

M. abscessus subsp. *bolletii* was first isolated in 2006 from a sputum of a patient with chronic pneumonia and then from patients with cystic fibrosis (Choi, Cho, Koh, Chun, Cho, & Shin, 2012).

M. abscessus subsp. *massiliense,* instead, was isolated from a patient with hemoptoic pneumonia (Adékambi, Reynaud-Gaubert, Greub, & Gevaudan, 2004).

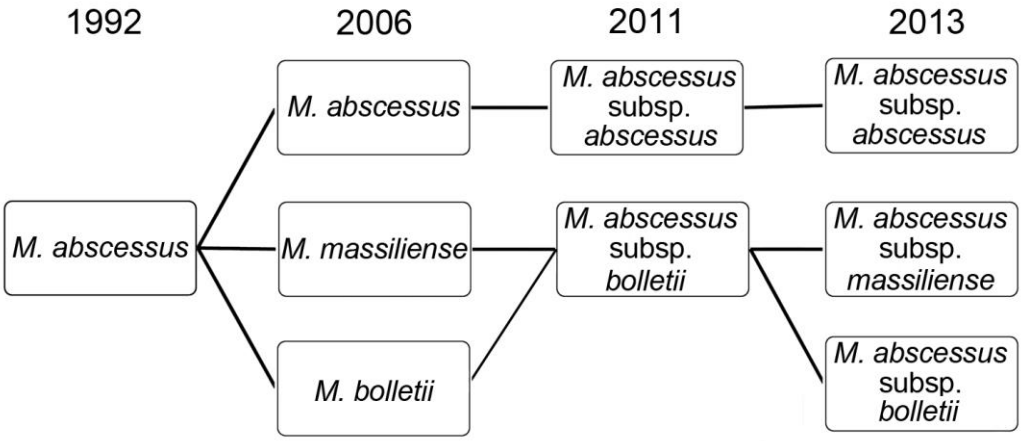


Figure 4: *M. abscessus* classification over the past 20 years (Meng-Rui Lee, Hung, Yu, Lee, & Hsueh, 2015).

However, approximately 80% of chronic pulmonary disease is due to *M. abscessus* (Brown-Elliott & Richard J. Wallace, 2002) especially in vulnerable hosts with lung disease, such as cystic fibrosis and bronchietactasis (Meng-Rui Lee, Hung, Yu, Lee, & Hsueh, 2015). These microorganisms can cause chronic lung infections; localized post-traumatic wound infections; chronic otitis media (Brown-Elliott & Richard J. Wallace, 2002), and rarely also infections to the central nervous system which is manifested by meningitis and cerebral abscesses (Meng-Rui Lee, Hung, Yu, Lee, & Hsueh, 2015). Establishing if *M. abscessus* is the cause of a pulmonary disease is not easy, moreover it is also difficult to establish which subspecies of the group *M. abscessus* is responsible of the disease (Meng-Rui Lee, Hung, Yu, Lee, & Hsueh, 2015) since its isolation from respiratory samples is not diagnostic of pulmonary disease (Meng-Rui Lee, Hung, Yu, Lee, & Hsueh, 2015).

There have been many reports which underline the presence of *M. abscessus* in people with cystic fibrosis and the rising infections by *M. abscessus* in these patients can have multiple factors. Factors that promote transmission can be: contaminated hospital water supplies (KN, JR, & MR, 1996); lack of adherence to maintenance of protocols, poor oversight etc. These conditions could have brought to *mycobacterium* infections and an illustration of this is given from Hawaii in 2012, where it was reported an outbreak of *M. abscessus*. As a matter of fact, of the 19 patient identified with cystic fibrosis in 9 of them there were *M. abscessus* respiratory culture (Johnston, Chisty, Gross, & Park, 2016).

It has been reported, also, that Ireland has the highest incidence of cystic fibrosis in the European Union and therefore the population contains a large number of individuals

susceptibility to *M. abscessus* complex infection (O'Driscoll, Konjek, Heym, Fitzgibbon, Plant, & Chróinín, 2016).

Hence, it was noted a bond between people with cystic fibrosis and *M. abscessus*, since they are more susceptible to develop infections to *M. abscessus* but also to other opportunistic pathogen, due to their deficient immune system. However, this do not exclude the fact that also people without a specific pathology can be exposed to *M. abscessus*.

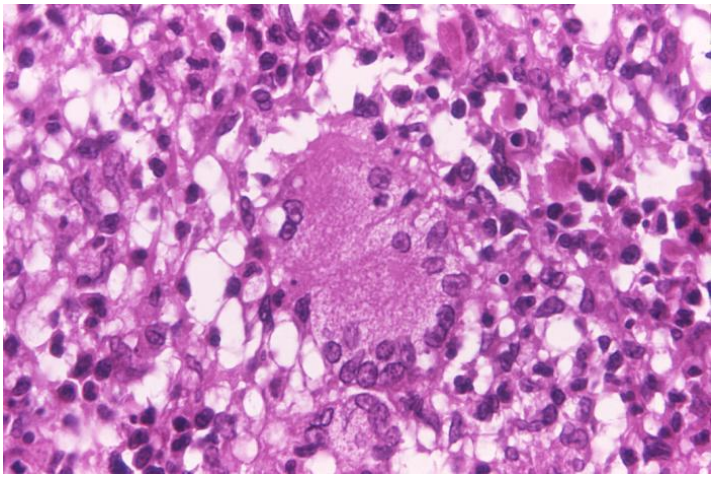


Figure 5: Light photomicrograph of a mycobacterial skin infection (that include those caused by *M. Tuberculosis, M. Leprae* and *M. Abscessus*) (Mycobacterium abscessus in Healthcare Settings).

Treatment of *M. abscessus* is difficult for all the patients since no specific treatments exists nowadays and it is also hard in patients with cystic fibrosis is difficult, because of their constant exposure to antibiotics (Broda, Jebbari, Beaton, Mitchell, & Drobniewski, 2013), moreover *M. abscessus* produces enzymes that degrade or modify antibiotics, which can result in their inactivation. But, drug resistance of *M. abscessus* is showed in both patients with and without cystic fibrosis. For this reason, it is still difficult to find an accurate cure and the right antibiotic to treat these *Mycobacterium*.

1.4- Treatment and Prevention

Infections caused by *M. abscessus* are not easy to treat because of their antimicrobial drug resistance, due to the presence of an impermeable cell wall and antibiotic-modifying enzymes (Harris & Kenna, 2014). They are also resistant to disinfectants and can cause

postsurgical and post-procedural infections (Meng-Rui Lee, Hung, Yu, Lee, & Hsueh, 2015).

Initially, *M. abscessus* were treated with the classical anti-tuberculous drugs, since their sequence similarity in the genome (C, et al., 2016) but it showed its resistance. An example is given when in a young man with cystic fibrosis, in 1999, was suffering of pulmonary infection due to *M. abscessus*. In this patient was administered three different antibiotics but after a month, the patient was still sick, showing the resistance of the *M. abscessus* to the drugs (Sanguinetti, et al., 2001). This resistance to drugs were attributed at first in change in membrane permeability to survive in the environment, change in the target and receptors or in the hydrolytic or drug-modifying enzymes (Ripoll, et al., 2009) but recently was shown that this resistance to amikacin and ciproflaxin, is caused by a point mutation in a region of a determined gene (Lee, et al., 2014).

M. tuberculosis, which is a slow growing organism, is treated with multiple drugs for several months, at least 6 months but sometimes even 12 months. In 2007, however, it was recommended to treat pulmonary *M. abscessus* disease's with a macrolide (a macrocyclic lactone ring to which it is attached one or more deoxy sugars. A macrolide example is Clarithromycin) (Harris & Kenna, 2014).

To treat some *M. abscessus* complex ocular infections, instead, amikacin ($C_{22}H_{43}N_5O_{13}$,) and clarithromycin ($C_{38}H_{69}N_1O_{13}$) can be used.

The influence of antimicrobial to *M. abscessus* were analysed at the UK Health Protection Agency. They were isolated 82 RGM from United Kingdom hospital patients with and without cystic fibrosis disease, over a three year-old period from 2007 to 2009. These isolates were treated with 15 different antimicrobial agents, for example: amikacin, amoxicillin-clavulanic acid ($C_{24}H_{27}KN_4O_{10}S$), cefepime ($C_{19}H_{24}N_6O_5S_2$), cefoxitin ($C_{16}H_{17}N_3O_7S_2$), ceftriaxone ($C_{18}H_{18}N_8O_7S_3$), ciprofloxacin, clarithromycin, trobamycin ($C_{18}H_{37}N_5O_9$). The results showed that isolates were resistant to most of the drugs and that the most active antimicrobial were amikacin and tobramycin (Broda, Jebbari, Beaton, Mitchell, & Drobniewski, 2013).

Other studies, instead, demonstrate that for the treatment of *M. abscessus* infections in cystic fibrosis patients it is recommended a treatment with a macrolide (azithromycin $C_{38}H_{72}N_2O_{12}$), an aminoglycoside (amikacin) and another drug of a different class (Nessar, Cambau, Reyrat, Murray, & Gicquel, 2012). Instead, *M. bolletii* was described as a species resistant to the standard antimicrobial drugs, as clarithromycin (Viana-Niero, et al., 2008).

In conclusion, different methods and drugs are used to treat people with *M. abscessus* strains but it is still uncertain which therapy or drug is the best one.

1.5- Purpose

Main purpose of this study is to synthesize different products for the treatment against *M. abscessus*. *M. abscessus* is a Gram-positive rapidly growing *mycobacterium* (RGM), responsible for many human disease, such as chronic lung disease and skin infections.

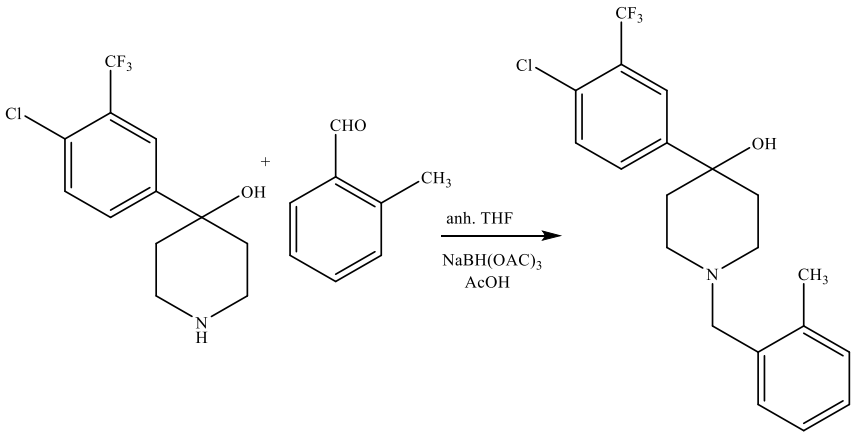
In particular, since the similarity between *M. abscessus* and *M. tuberculosis*, it was decided to start synthesizing those compound which were effective against *M. tuberculosis*. It was demonstrated that the genome of the *M. abscessus* shows similarities with the *M. Tuberculosis,* finding also that *M. abscessus* has homologs of a large number of regulators known to control virulence in *M. tuberculosis* (Ripoll, and al., 2009), that is why it was thought that drugs against *M. tuberculosis* can be used as a starting point to develop drugs against *M. abscessus* (C, and al., 2016).

An example is given by the use of bedaquiline in patients with non-tuberculous mycobacterial disease. Bedaquiline, a drug belonging to the class of diarylquinolines used in patients affected by *M. tuberculosis*, was clinically tested in patients with *M. abscessus* diseases. After 6 months of therapy, some of these patients showed a positive clinical response to this therapy, even if it is not as good as against *M. tuberculosis* (Philley, et al., 2015).

However, having this in mind, it has started to focus on one of the 177 small molecules, able to leas against tuberculosis. These compounds, taken by a GSK collection, have good cell membrane permeability and antitubercular activities (Ballell, Bates, Young, Alvarez-Gomez, and al., 2013).

Among those compounds, only one compound (piperidinol-based molecule) exhibits high activity *in vitro* against *M. abscessus* (C, et al., 2016).

As a matter of fact, recently it was studied the activity of PIPD1,(4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-ol), against *M. abscessus* strains obtained from both cystic fibrosis and non-cystic fibrosis patients. PIPD1 was synthesized, in an inert atmosphere, by reaction of a piperidinol with a substituted toluene under acidic catalysis with an excess of $NaBH(OAc)_3$ in dry THF (C, and al., 2016).



Cytotoxity of the synthesized product was valued in Mycobacterium both *in vitro* et *in vivo* (the drug efficacy was further tested in zebrafish embryos) and it was noted the lowering of multiplication within the cells after 24 and 48 hours of the infection and the decrease of infected cells after the drug treatment.

In conclusion, after subsequent analysis, it was discovered that PIPD1 alters the mycolic acid profile of *mycobacteria* resistant to the major anti-tuberculous drug, inhibiting mycolic acid biosynthesis (C, et al., 2016).

In conclusion, after this work, it was decided to continue synthesizing piperidinol-based molecules with different substituent, in which also different strategy where applied to obtain them with higher yields.

PIPD1 analogs will be evaluate as anti-mycobacterial compounds against different strains of *M. abscessus*.

Chapter 2

Results and Discussions

Different strategies and methods were followed to obtain a piperidinol-based molecule, giving different results, such as yield and products. The various product were identified through an NMR analysis and mass spectrometry. The main characteristic of the different final product was the presence of the piperidinol and the presence of an aromatic group. As a matter of fact, in the NMR spectra the presence of the piperidinol is shown by the peaks between 1.5 and 2.8 ppm; instead, the aromatic groups are shown by the peaks between 7-8 ppm. As it is shown in the figure below, the piperidinol group is identified by the two doublets, a triplet and a triple of doublets, each of which integrates 2 hydrogens:

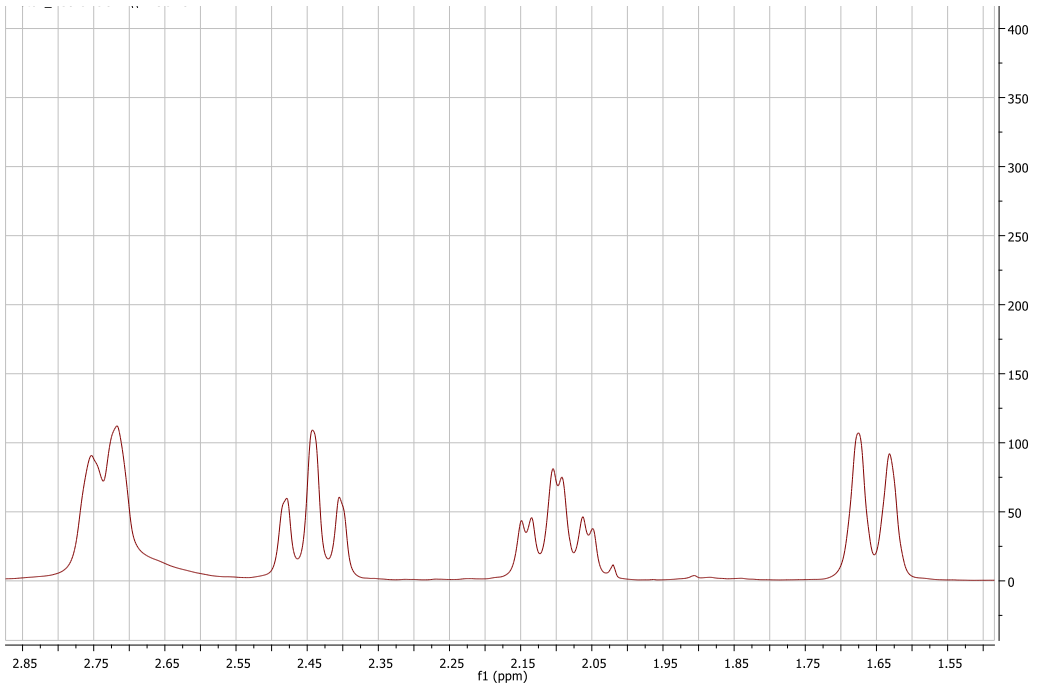
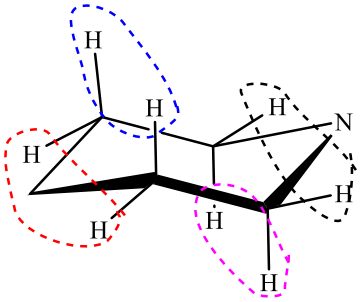


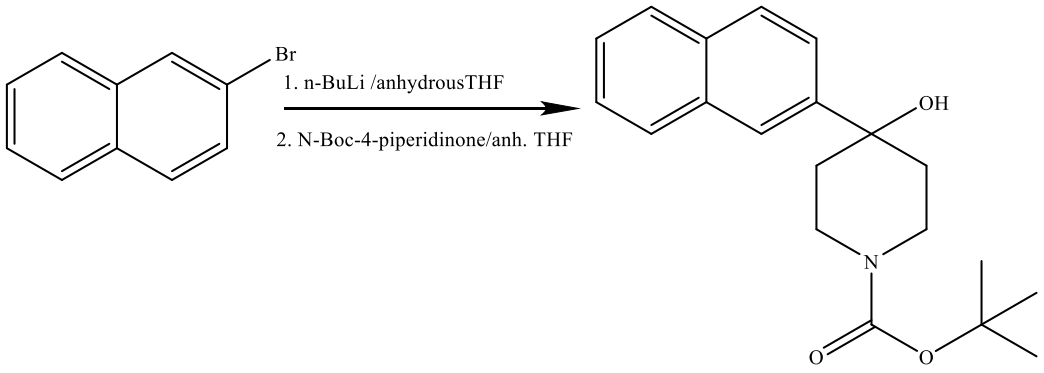
Figure 6: peaks of the piperidinol group

The peaks shown are the result of the chair structure of the piperidinol in which some of the hydrogen cover themselves, as it is possible to see in the molecule below, leading to doublets and triplets.



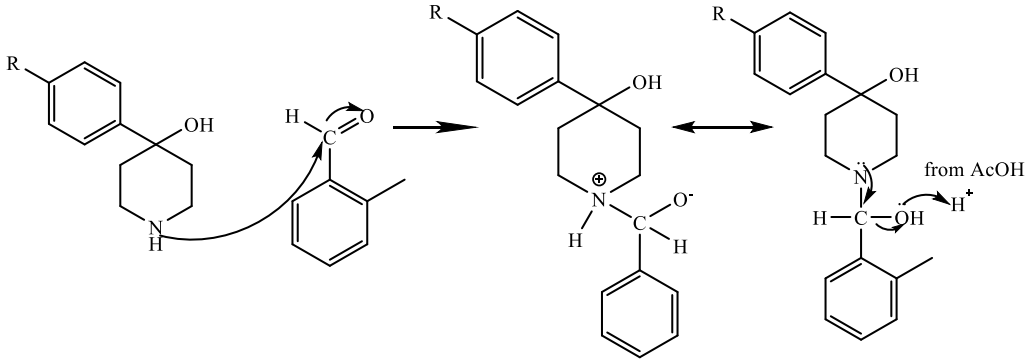
2.1- Method 1

With this method piperidinol-based molecules are obtained following two main reactions.

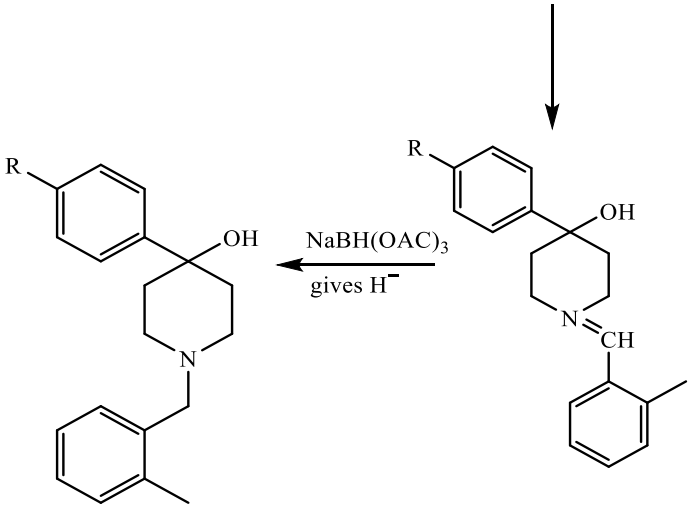


First an halogen-lithium exchange: n-butyllithium reacts with the organic bromide, in an exchange reaction to form the corresponding organolithium derivative. Reaction of BuLi in THF is conducted at low temperature (-78 °C). After this step, a nucleophilic addition of the organolithium derivative with the ketone was realised, in this case the *N*-Boc-4-piperidinone allowed to obtain a tertiary alcohol. Since the presence of the protecting group, Boc group (tert-butyloxycarbonyl group), a deprotection step is required. To the protected amine is added slowly a solution of 2 mL of HCl (37%) in 3 mL of MeOH. The reaction mixture is stirred at room temperature during two hours to give the relative amine. Nevertheless, the desired product is obtained in small quantity due to the presence of a tertiary alcohol, which can react with a E1 mechanism with HCl.

But, despite that, the desired product was obtained and thus the next step was performed. Finally, to the piperidinol based molecule is added methylbenzaldehyde, according to the following scheme:

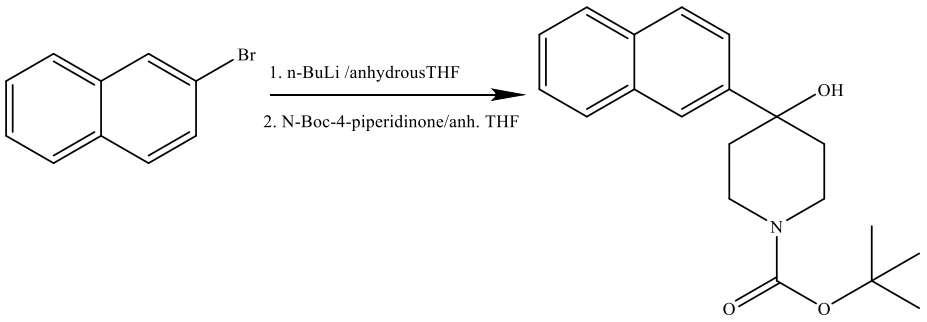


A reaction of reductive amination produced the piperidinol moiety.

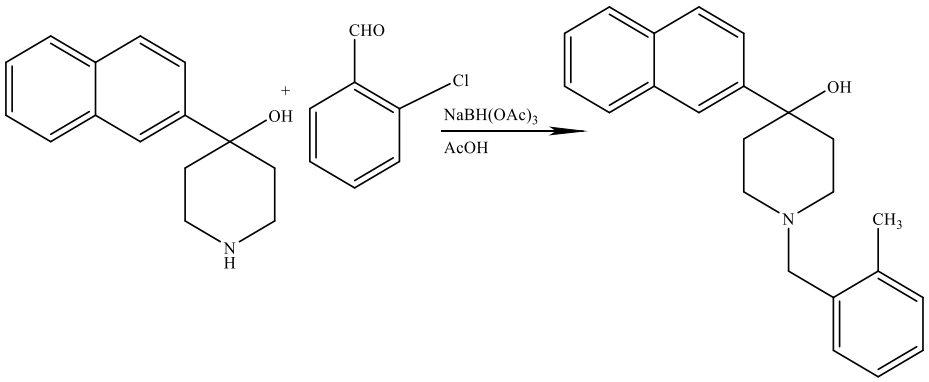


The same reaction was realised with other bromine derivatives that lead to bad results, as a matter of fact, except for the first reaction, which gave a 21% of yield, they were not obtained the other desired bromine derivative products.

However, another reaction was realised with 2-Bromonaphthalene with the difference of using 2-chlorobenzaldehyde in the last step:



After a deprotection step with HCl/MeOH, the obtained product undergoes a nucleophilic addition with 2-methylbenzaldehyde, in the presence of $NaBH(OAc)_3$ and AcOH.



The same reaction was repeated but, in the last step of nucleophilic reaction, instead of 2-methylbenzaldehyde it was used 2-chlorobenzaldehyde. Though, the reaction was conducted in the same way, the desired product was not obtained. As it is possible to see in the following proton and carbon NMR spectra, in which the final product lacks of the peaks typical of the piperidinol group:

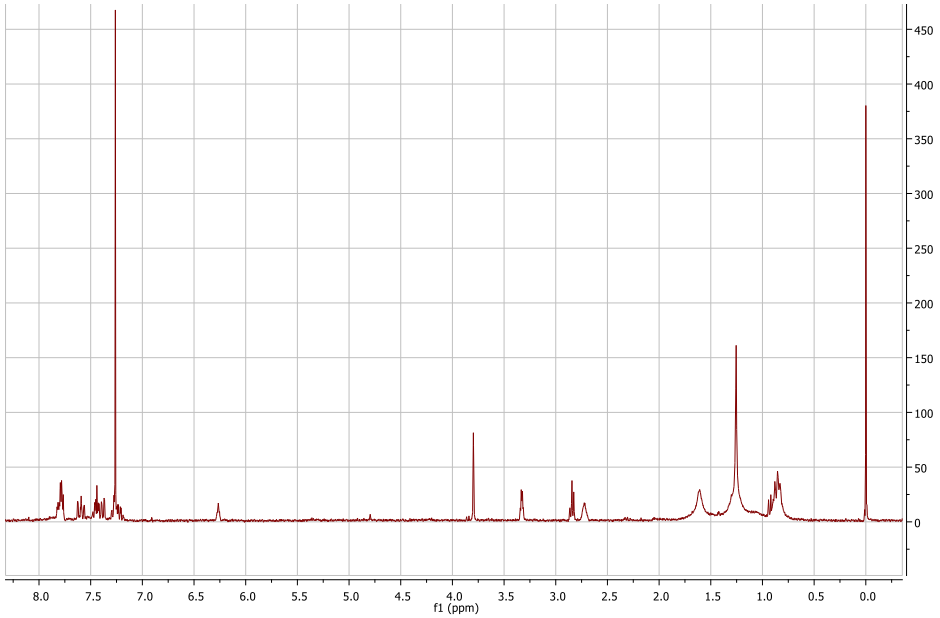


Figure 7: example of a product's proton NMR spectra that lacks of the peaks of the piperidinol group

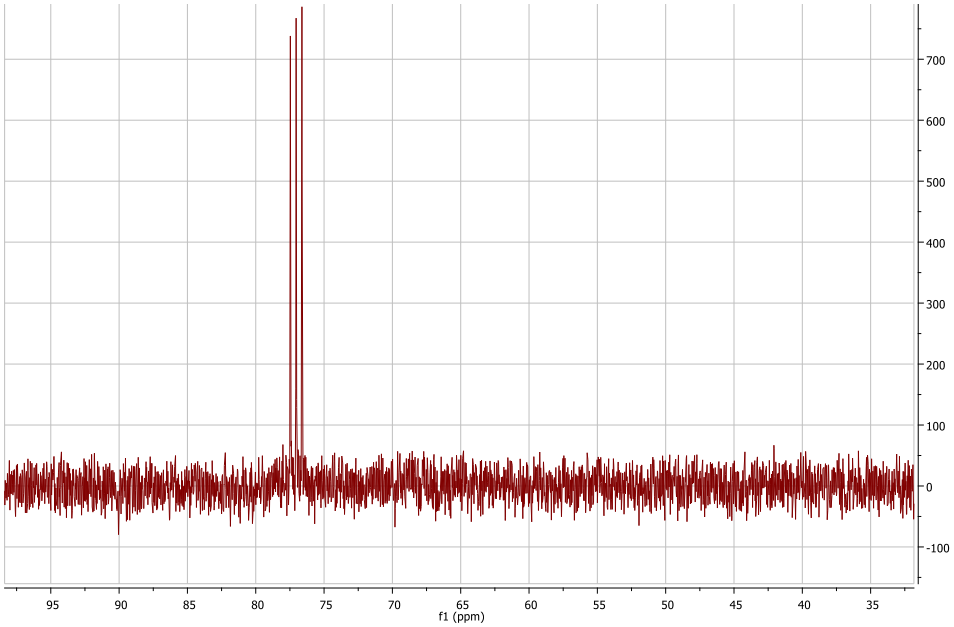


Figure 8: example of a product's carbon-13 NMR spectra that lacks of the peaks of the piperidinol group

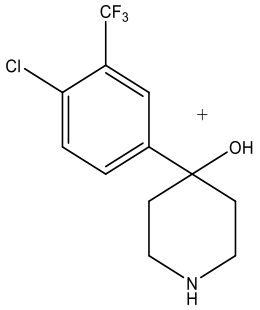
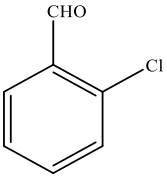
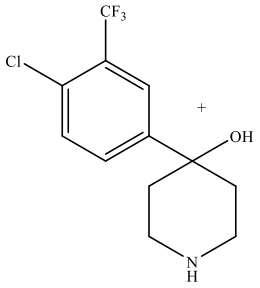
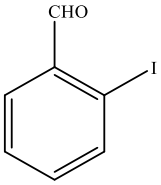
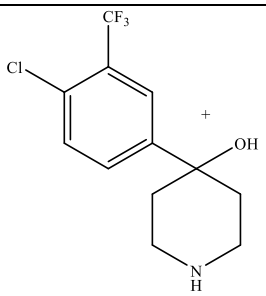
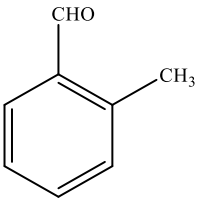
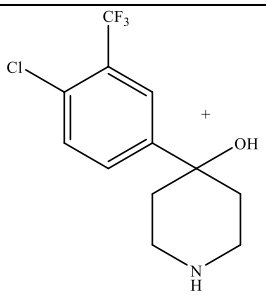
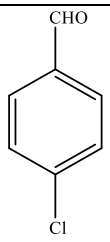
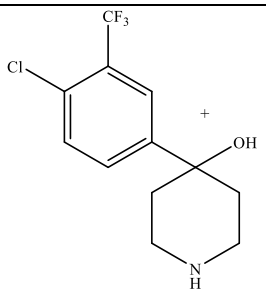
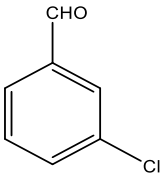
Finally, other reactions were conducted using different reagents such as: 1-bromo-4-(tert-butyl)benzene and 1-bromo-3,5-bis-(trifluoromethyl)benzene, and in the last step reactions were conducted with 2-methylbenzaldehyde. But, the desired products were not obtained.

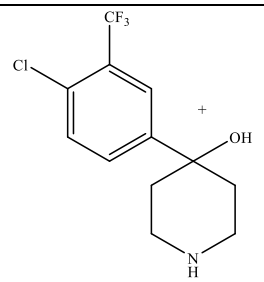
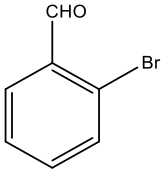
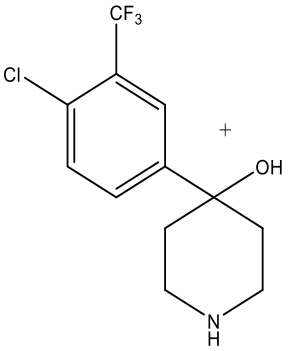
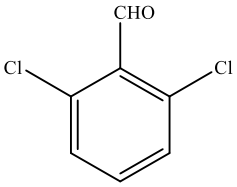
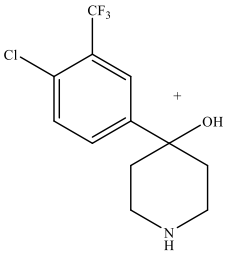
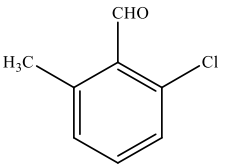
For this reasons, it was decided to follow a second strategy in order to obtain the products and to improve the final yield.

2.2- Method 2

The second method used to obtain the piperidinol based molecule, removed the first two steps typical of *method 1*. In fact, it was decided to use the commercial reagent 4-(4-chloro-3(trifluoromethyl)phenyl)piperidin-4-ol, removing the steps regarding the halogen-lithium exchange followed by the nucleophilic addition with *N*-Boc-4-piperidinone and the deprotection step. In this way, changing the starting material was possible to ease the process development.

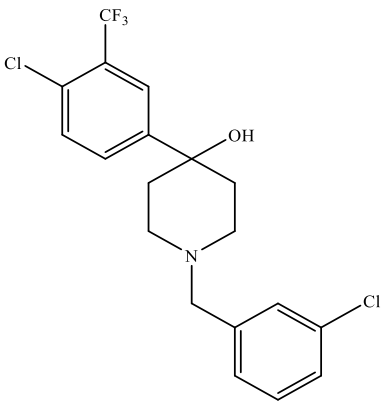
However, to the commercial amine is added an halogen benzaldehyde, in presence of $NaBH(OAc)_3$ and AcOH, using an ether solvent (anhydrous THF). The various halogen benzaldehyde reagents which react with 4-(4-chloro-3(trifluoromethyl)phenyl)piperidin-4-ol are shown in the following table, with the obtained yield.

Reagent A	Reagent B	Yield
		63%
		38%
		54%
		80%
		98%

		68%
		37%
		17%

The various reactions follow a nucleophilic addition of the amine group to the carbonyl group, C=O. The intermediate produced is protonated with AcOH, leading to the formation of an immonium ion, which in presence of $NaBH(OAc)_3$ is reduced to the relative amine, with a variable yield from 17% to 98%.

The NMR spectra of the different products vary by the presence of the halogens in the different position of the aromatic group. An example of the proton NMR spectrum is given:



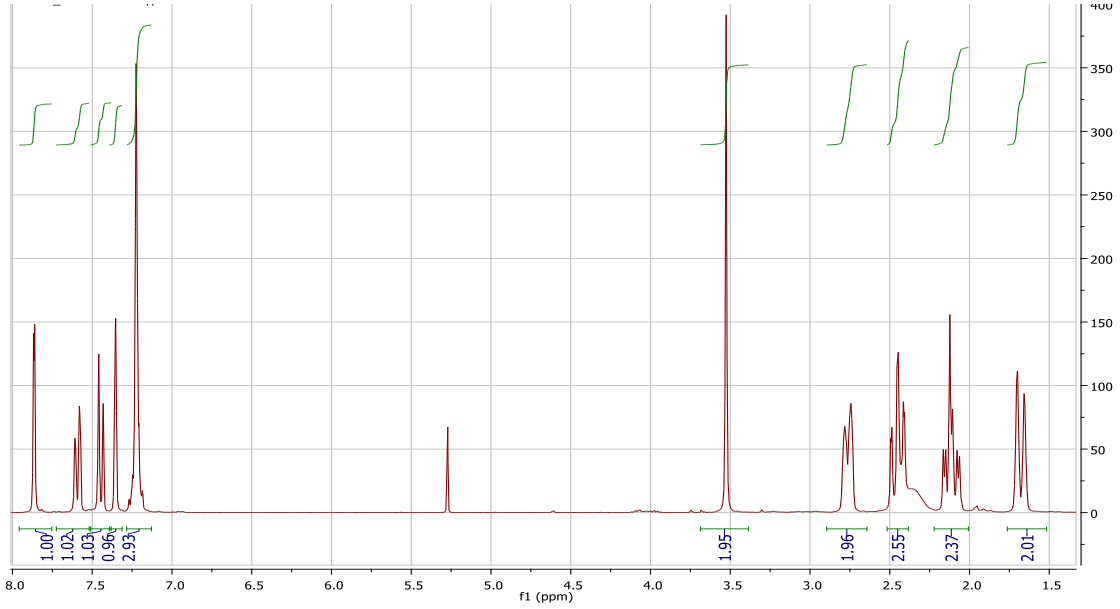
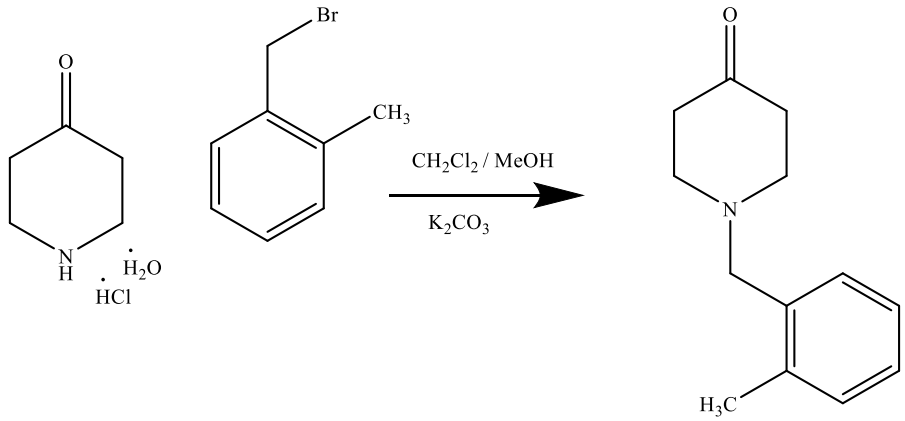


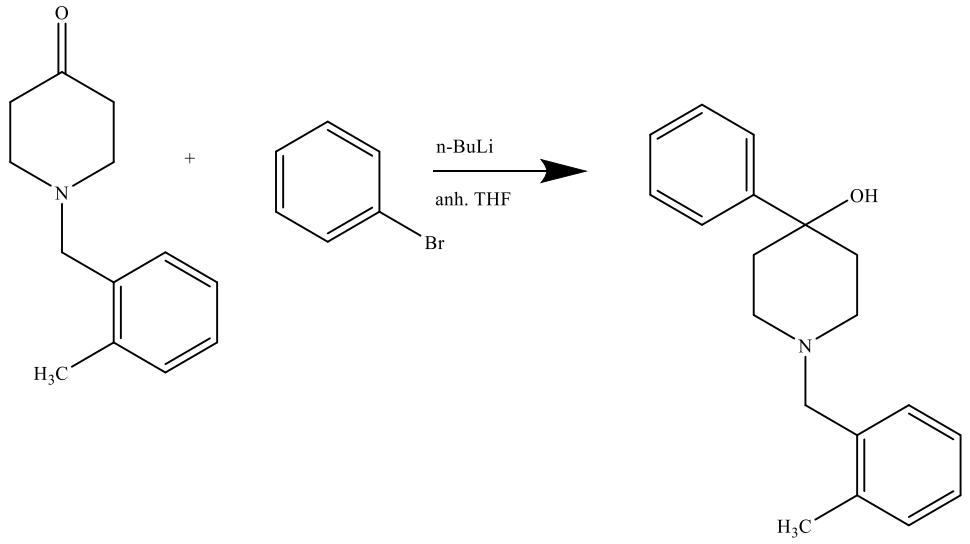
Figure 9: proton NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(3-chlorobenzyl)piperidin-4-ol

2.3- Method 3

Another synthetic path was outlined in the figure below

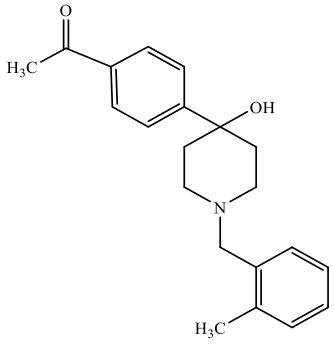


This reaction begins with the alkylation of the commercial 4-piperidinone monohydrate hydrochloride with α-bromo-o-xylene, in presence of K_2CO_3 as a base and dichloromethane in methanol as solvents. The reaction is conducted at room temperature, overnight. Then, a purification step by flash silica gel chromatography eluting with CH_2Cl_2/MeOH is carried out. Once the product, 1-(2-methylbenzyl)piperidin-4-one, is obtained a second step is carried out. As it is possible to see in the following scheme, for example:



Under N_2 atmosphere, the halide compound reacts with n-BuLi at -78 °C, to form the corresponding organolithium derivative. At this point, the organolithium derivative reacts with the piperidinone, previously produced, in a nucleophilic addition in order to obtain the relative tertiary alcohol, as a final product.

However, the reaction that brought to the production of the methylbenzyl-piperidinone was repeated in variable amounts. As a matter of fact, while the first reaction used 1.0 g of 4-piperidinone monohydrate hydrochloride (giving a 40% yield) then, it was decided to use 4.0 g of 4-piperidinone monohydrate hydrochloride, giving a 50% yield. The reactions, were carried out with different kind of bromide compound, some of which were already used in the previous methods, in order to compare with the different strategies. From the various halide compound, a different range of yield were obtained. Except for the reaction with bromobenzene that gave a 9% of yield, reactions with 1-bromo-3,5-bis(trifluoromethyl)benzene, 2-chloro-5-bromobenzotrifluoride and 2-naphthalene gave yield from 27% to 46%. Nevertheless, reactions with 4-bromo-chlorobenzene and 4-bromoacetophenone, after purification, did not led to the desired product and an example of which it is shown in the following NMR spectrum:



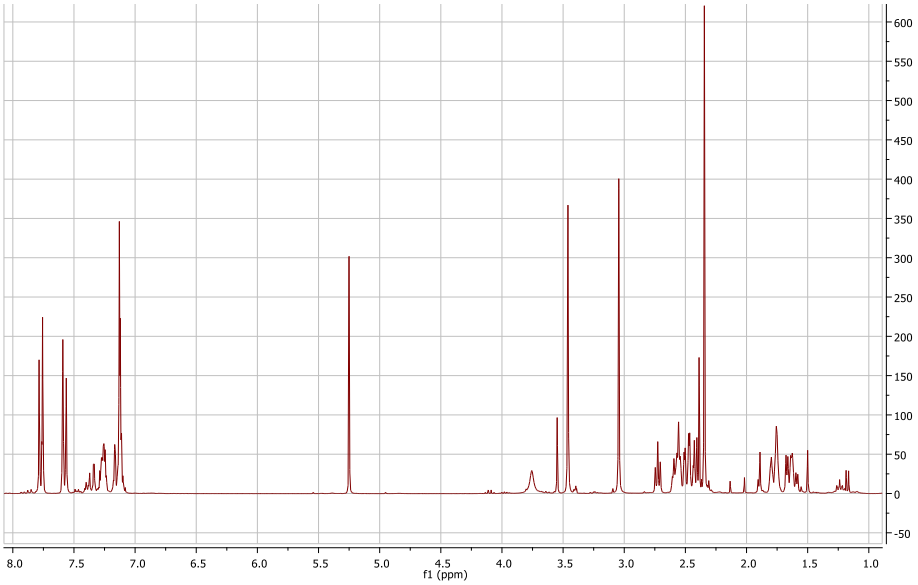


Figure 10: proton NMR spectrum of 1-(4-(4-hydroxy-1-(2-methylbenzyl)piperidin-4-yl)phenyl)ethan-1-one

Chapter 3

Experimental part

The solvents and reagents were purchased from commercial suppliers (Sigma Aldrich and TCI). Dry THF was obtained from a solvent purification system. Column chromatography was performed using an Interchim Machine, Puriflash 430, and carried out on an Interchim's flash column(30 μm).

Nuclear magnetic resonance (^{1}H, ^{13}C) spectra were recorded at room temperature on a Brüker AC 300 spectrometer. TMS was used as an internal standard and $CDCl_3$ as the solvent. ^{1}H-NMR analysis were obtained at 300 MHz (s: singlet, d: doublet, t: triplet, td: triplet of doublets, dd: double doublet, m: multiplet), and ^{13}C NMR analyses were obtained at 75.4 MHz. The chemical shifts (δ) are given in parts per million relative to TMS (δ = 0.00).

Mass spectra were recorded on a Maldi/Tof machine.

Reaction were monitored by TLC using coated silica gel plates and detection by UV lamp at 245 nm and either vanillin in H_2SO_4 with heat as developing agents.

NMR spectra are in the APPENDIX.

3.1- METHOD 1

3.1.1- Protocol A:

Under N_2, a bromide compound (2.5 eq) is added in a round bottom flask, properly closed. It is added 5 mL of anhydrous THF and a magnetic stir bar. At this point, the system is cooled to -78°C by using acetone and dry ice. It is treated with n-BuLi (2.5 M, 2.5 eq) and it is stirred for one hour. In another round bottom flask, under N_2, N-Boc-4-piperidinone (1eq) is dissolved with 5 mL of anhydrous THF. After one hour, this solution is added to the mixture of bromide and n-BuLi. Stir for two hours (making sure that the temperature is still -78°C). The reaction is quenched with saturated aqueous NH_4Cl (~30 mL) and then, a liquid-liquid extraction is carried out with ethyl acetate (2x). At the organic phase is dried by Na_2SO_4 before filtration. Finally the organic layer is

concentrated under reduce pressure. The residue is purified on column chromatography with petroleum ether/ethyl acetate or petroleum ether/dichloromethane as eluent conditions.

3.1.2- Protocol B

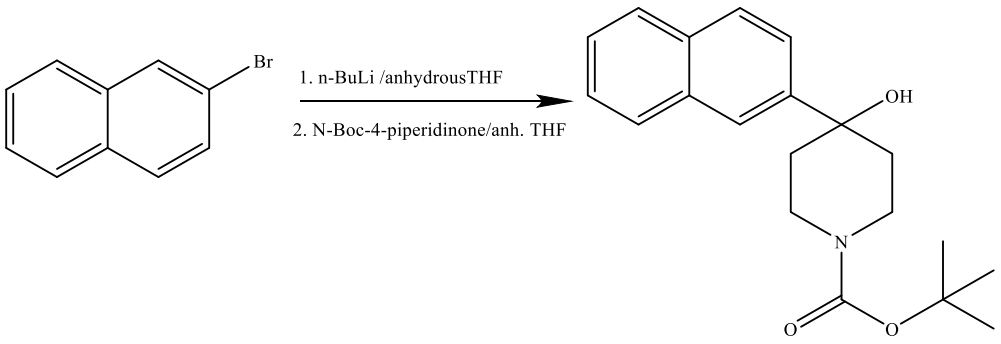
2 mL of HCl (37%) is dissolved in 3 mL of MeOH. This MeOH acidic solution is added to the protected piperidine derivate product obtained from **Protocol A**. The mixture is stirred for two hours at room temperature. The advancement of the reaction is checked by TLC analysis each hour and it is compared to the starting material. When the reaction is finished, the mixture is cooled to 0°C, using ice. A solution of NaOH (4 g of NaOH in 25 mL of distilled water) is added slowly. An extraction with ethyl acetate is carried out (3x). After that, the organic layer is washed by brine (3x). Finally, the organic phase, collected in a conical flask, is dried by Na_2SO_4 and it is filtered in a round bottom flask.

3.1.3- Protocol C:

Under N_2, 2 mL of anhydrous THF is added to $NaBH(OAc)_3$ (4 eq). The mixture is stirred. Meanwhile, to the round flask containing the deprotected product is added the aldehyde (1 eq) and 3 mL of anhydrous THF. When the product is well dissolved, it is transferred to the round bottom flask containing $NaBH(OAc)_3$. In conclusion, AcOH is added and the mixture is stirred for 24 hours.

After 24 hours, the mixture is quenched in ethyl acetate (100 mL) and the organic layer is washed first by an aqueous saturated solution of $NaHCO_3$ (2x) then, with brine (an aqueous saturated solution of sodium chloride). The solution is dried by Na_2SO_4, filtered and finally concentrated.

Reaction 1



The product was synthesized following **Protocol A**: 2-Bromonaphthalene (1.413 g; 6.80 mmol); *N*-Boc-4-piperidinone (0.541g; 2.7 mmol); n-BuLi (2.6 mL, 6.5 mmol).

Flash column chromatography of the crude product: 25 g silica gel (30 μm), petroleum ether 100% for 20' then, petroleum ether/ ethyl acetate 70/30 and finally petroleum ether/ethyl acetate 50/50 for 30'. TLC analysis performed with petroleum ether/ ethyl acetate 50/50.

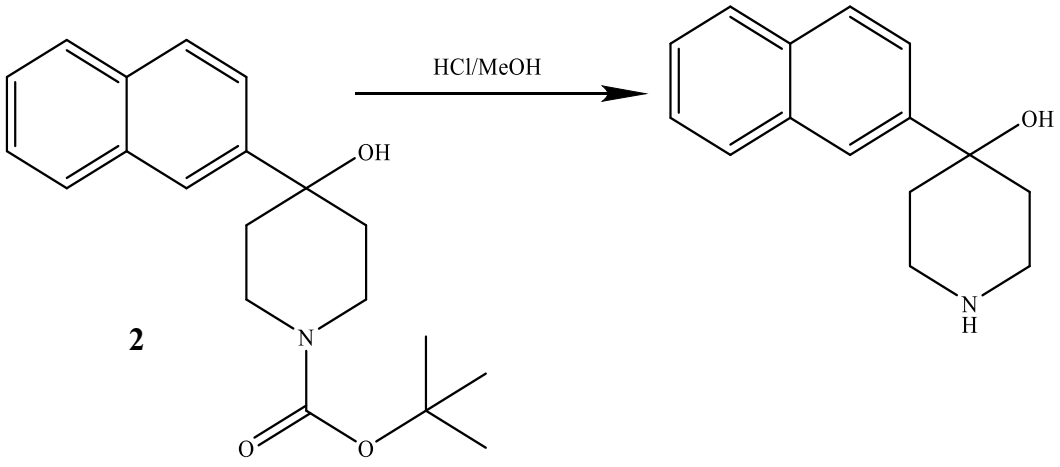
A yellow product is obtained. Yield 5.4% (0.1192 g, $3.6x10^{-4}$mol).

^{1}H NMR (*$CDCl_3$, 300 MHz*): δ 1.50 ppm (s, 9H, 3xCH_3); 1.71-1.75 ppm (d, 2H, 1xCH_2); 1.96-2.01-2.05 ppm (t, 2H, 1xCH_2); 3.21 ppm- 3.24 ppm- 3.27 ppm (t, 2H,1xCH_2); 3.95 ppm- 4.00 ppm (d, 2H, 1xCH_2); 7.41 ppm- 7.43 ppm- 7.44 ppm (t, 2H, 2xCH_{arom}); 7.51 ppm- 7.55 ppm (d, 2H, 2xCH_{arom}); 7.76 ppm- 7.79 ppm (d, 2H, 2xCH_{arom}).

^{13}C NMR (*$CDCl_3$, 75 MHz*): 28.38 ppm (CH_3); 37.94 (CH_2); 41.04 ppm (CH_2); 71.27 ppm (C^{IV}); 79.57ppm (C^{IV}); 122.96 ppm- 123.38 ppm- 125.92 ppm- 126.15 ppm- 127.49 ppm- 128.09 ppm- 128.19 ppm (CH_{arom}); 132.52 ppm- 133.20 ppm- 145.60 ppm- 154.95ppm (C^{IV}).

m/z calculated for $C_{20}H_{25}NO_3$ was 327.42 g/mol.

Deprotection

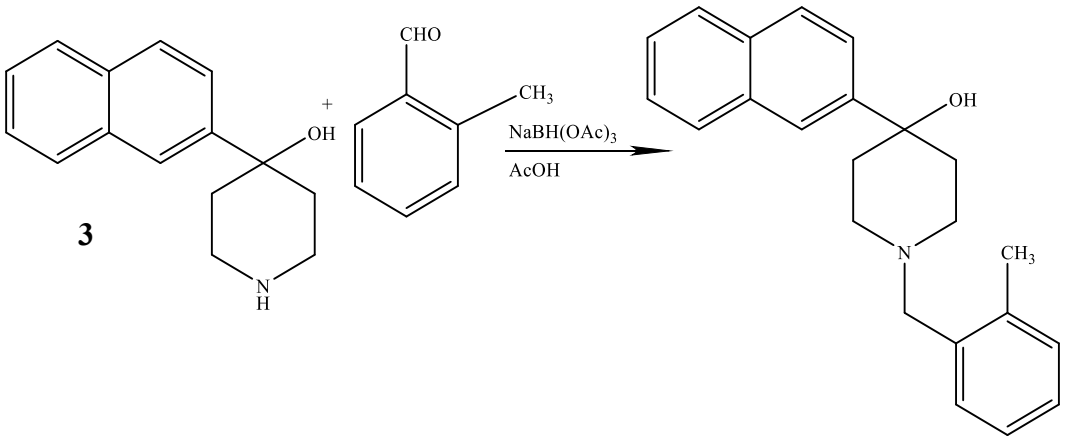


2

This product was obtained following **Protocol B**: **2** (0.1192g, 0.36 mmol). Yield 47% (0.039g; $1.7x10^{-4}$mol).

^{1}H-NMR (CDCl3, 300MHz), C-Apt NMR and a **HSQC** analysis were done. The spectrum showed different and several peaks due to the presence of the solvents and co-products but, since the presence of the peaks typical of the piperidine, it was decided to continue the reaction.

Nucleophilic addition



3

The product was obtained following **Protocol C**: 3 (0.0386 g; 0.17 mmol); 2-methylbenzaldehyde (0.0312g; 0.26 mmol); $NaBH(OAc)_3$ (0.146 g; 0.68 mmol). Flash column chromatography of the crude product: 25 g silica gel (30 μm), CH_2Cl_2 100% for 20' then, CH_2Cl_2/ MeOH (95/5).

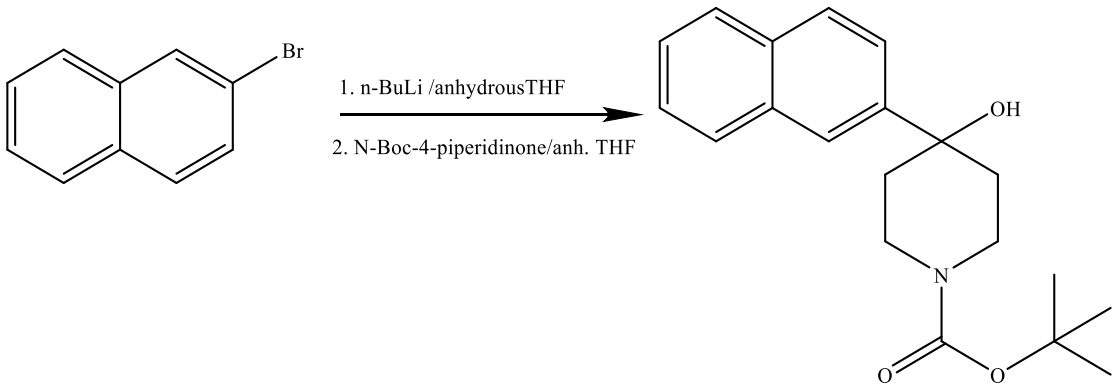
A solid yellow-orange product is obtained. Yield: 21.5% (12.1 mg, $3.65x10^{-5}$mol).

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***): δ 1.50 ppm (s, OH); 1.79-1.83 ppm (d, 2H, $2xCH_2$); 2.21- 2.31 ppm (td, 2H, $2xCH_2$); 2.52- 2.60 ppm (t, 2H, $2xCH_2$); 2.81- 2.85 ppm (d, 2H, $1xCH_2$); 2.41 ppm (s, 3H, $1xCH_3$)- 3.57 ppm (s, 2H, $1CH_2$)- 7.17-7.18 ppm (d, 3H, $3xCH_{arom}$); 7.34 ppm (t, 1H, $1xCH_{arom}$); 7.48-7.50 ppm (m, 2H, $2xCH_{arom}$); 7.62-7.65 ppm (dd, 1H, $1xCH_{arom}$); 7.80-7.82-7.85 ppm (m, 3H, $3xCH_{arom}$); 7.95 ppm (s, 1H, $1xCH_{arom}$).

^{13}CNMR(***$CDCl_3$, 75 MHz***): 19.40 ppm (CH_3); 38.57 ppm (CH_2); 49.62 ppm (CH_2); 60.85 ppm (CH_2); 71.64 ppm (C^{IV}); 122.96 ppm (CH_{arom}); 123.50 ppm (CH_{arom}); 125.58 –127.04 –130.30 ppm (CH_{arom}); 125.87-126.13 ppm (CH_{arom}); 127.50-128.05-128.22 ppm (CH_{arom}); 129.84 ppm (CH_{arom}); 123.84-132.46-133.20-137.53 ppm (C^{IV}).

m/z calculated for $C_{23}H_{25}NO$ was 331.46 g/mol.

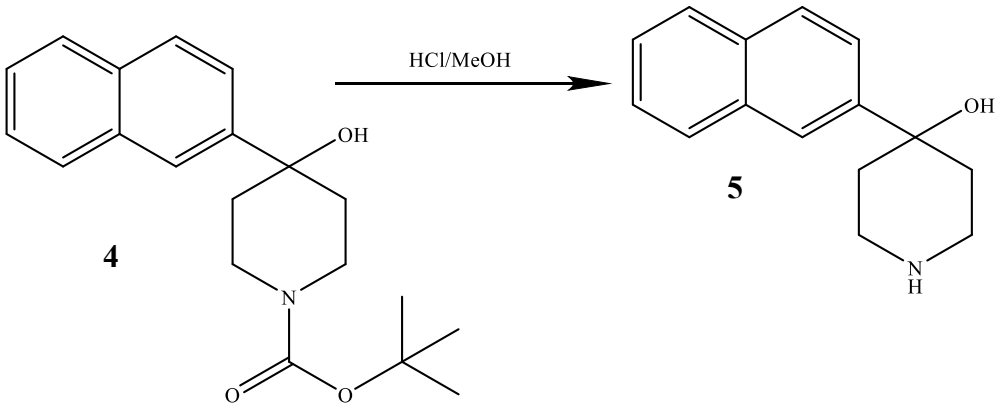
Reaction 2



Product obtained following **Protocol A:** 2-bromonaphthalene (1,395g; 6,700 mmol); n-BuLi (2,5 M; 2,6 mL; 6.700 mmol); anhydrous THF (10 mL; 0.1233 mol); N-Boc-4-piperidinone (0.515g; 2,6 mmol). The product was purified by flash column chromatography (silica gel 30 μm, 25g) eluting with petroleum ether.

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***): peaks didn't identify the structure of the final product, but however, it was decided to keep going with the following reaction.

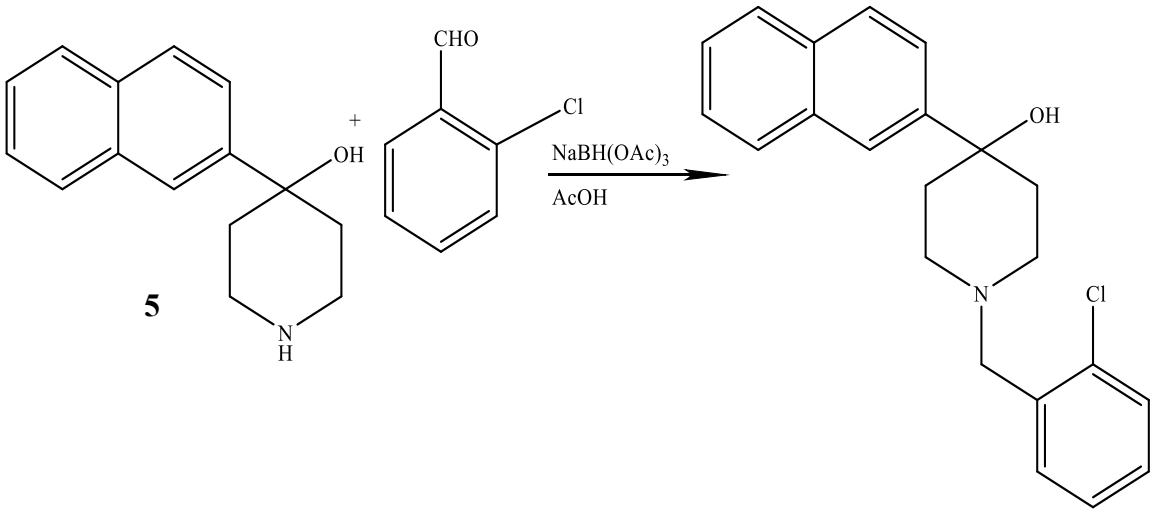
Deprotection step



Product obtained following **protocol B**: **4** (0. 2187g, 0.67mmol).

Yield 65% (98.3 mg; 0.43 mmol).

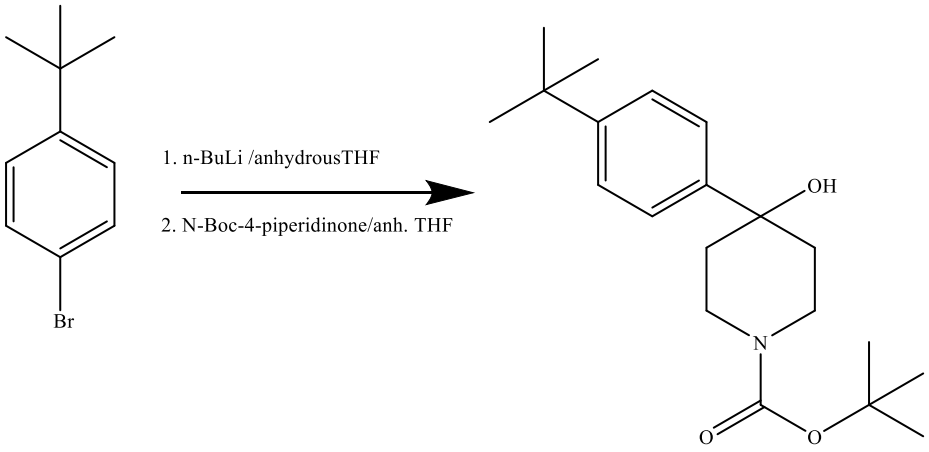
^{1}H-NMR (CDCl3, 300MHz), C-Apt NMR and a **HSQC** analysis were done. Peaks didn't identify the structure of the product, but it was decided to follow the next step.



Product obtained following **Protocol C: 5** (98.3 mg; 0.433 mmol); aldehyde (76,4 mg; 0.544 mmol); $NaBH(OAc)_3$ (0.367g;1.73 mmol); AcOH (0.025 mL, 0,437 mmol); anhydrous THF (5mL). Purify the product by silica gel flash chromatography (silica gel 12 g, 30 μm) eluting with CH_2Cl_2/MeOH (95/5) for other 20 minutes. Purification monitored by TLC 95/5 CH_2Cl_2/MeOH.

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***) and **^{13}C-NMR Analysis ($CDCl_3$, 75 MHz)**:peaks didn't identify the structure of the final product.

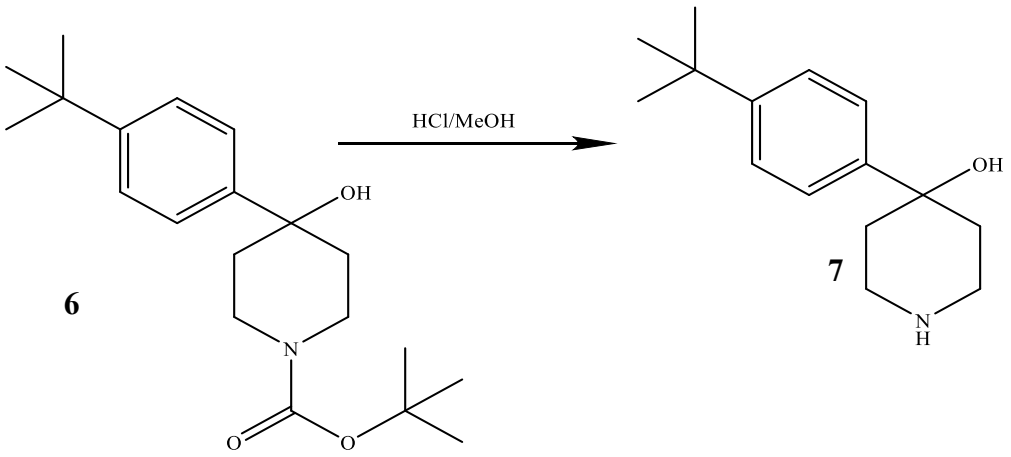
Reaction 3



Product obtained following **protocol A**: 1-bromo-4-tert-butylbenzene (1,1 mL; 6.3 mmol); n-BuLi (2.5 M; 2.6 mL, 6.6mmol); *N*-Boc-4-piperidinone (0.547 g; 2.7 mmol); anhydrous THF (10 mL). Purify the product by silica gel flash chromatography (silica gel 25 g, 30 μm) eluting with CH_2Cl_2 (100%) for 20 min then CH_2Cl_2/MeOH (95/5) for other 20 minutes. Purification, finally, monitored by TLC.

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***): peaks didn't identify the structure of the final product, peaks identifying the piperidine are absent.

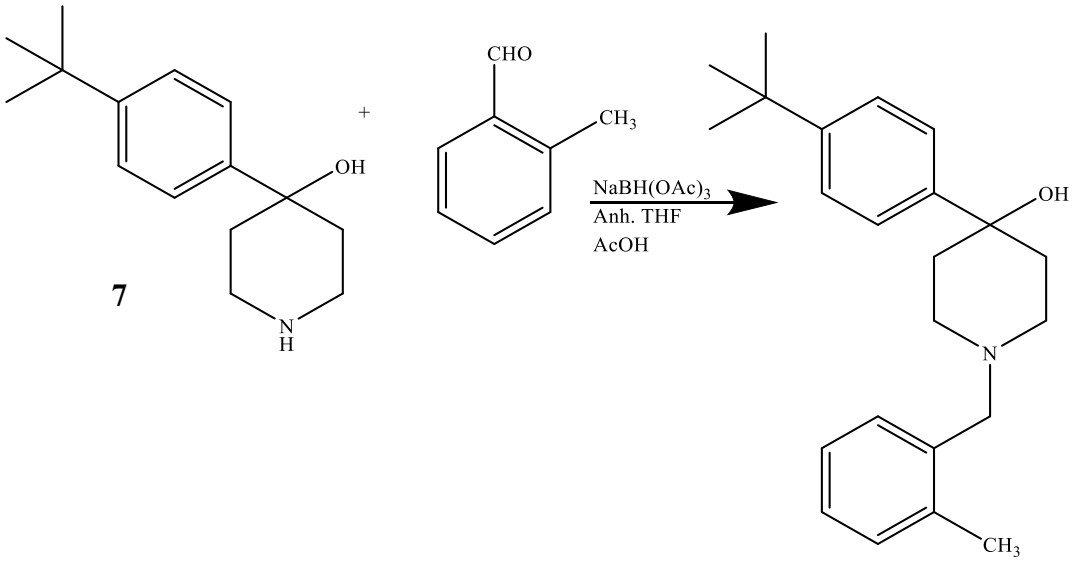
Deprotection



Product obtained following **protocol B: 6** (0.4428g, 1.35 mmol).

A white/colourless product is obtained. Yield 80% (0.252g; 1.1 mmol).

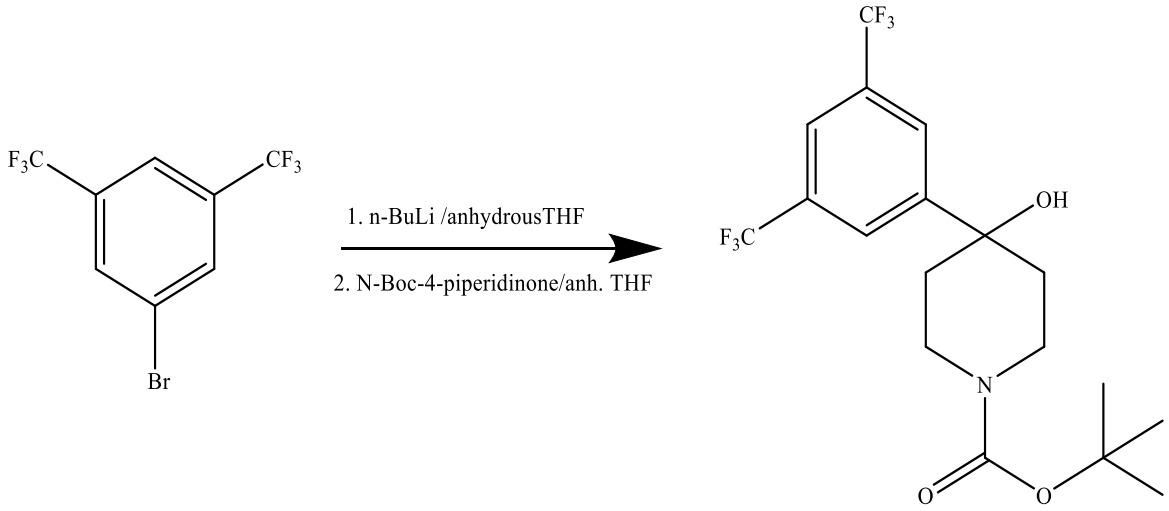
^{1}H-NMR (CDCl3, 300MHz), C-Apt NMR and a **HSQC** analysiswere done. Peaks didn't identify the structure of the desired product.



Product obtained following **Protocol C: 7** (0.252g, 1.1 mmol); 2-methylbenzaldehyde (0.142g, 1.2mmol)-$NaBH(OAc)_3$ (0.935g, 4.4 mmol); Acetic acid (0.063 mL, 1.1 mmol). Purify the product by silica gel flash chromatography (silica gel 12 g, 30 μm) eluting with CH_2Cl_2/MeOH (95/5). Purification monitored by TLC 95/5 CH_2Cl_2/MeOH.

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***) and **^{13}C-NMR Analysis**(***$CDCl_3$, 75 MHz***):peaks didn't identify the structure of the final product.

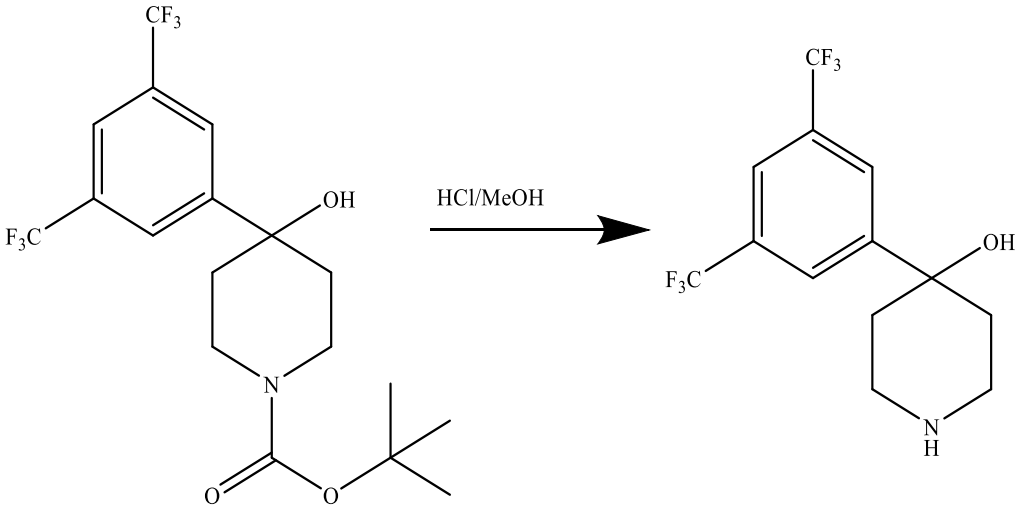
Reaction 4



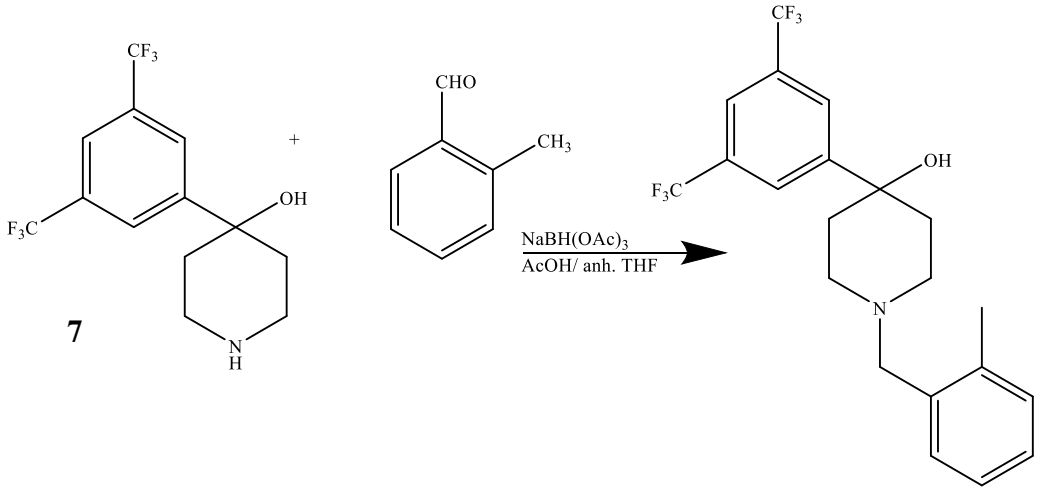
Product obtained following **protocol A**: 1,3-bis(trifluoromethyl)-5-bromobenzene (1,1 mL; 6.4mmol); n-BuLi (2.5 M; 2.6 mL, 6.5 mmol); *N*-Boc-4-piperidinone (0.528 g; 2.7 mmol); anhydrous THF (10 mL).Purify the product by silica gel flash chromatography (silica gel 25 g, 30 μm) eluting with CH_2Cl_2 (100%). Purification monitored by TLC petroleum ether.

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***): peaks didn't identify the structure of the final product, peaks identifying the piperidine are absent.

Deprotection



Product obtained following **protocol B:**



Product obtained following **Protocol C: 7** (4.8 mg, 0.0153 mmol); 2-methylbenzaldehyde (2.3 mg, 0.0666 mmol); $NaBH(OAc)_3$ (0.0135g, 00637 mmol); Acetic acid (0.004g; 1.05 g/mol, 0.0637 mmol).

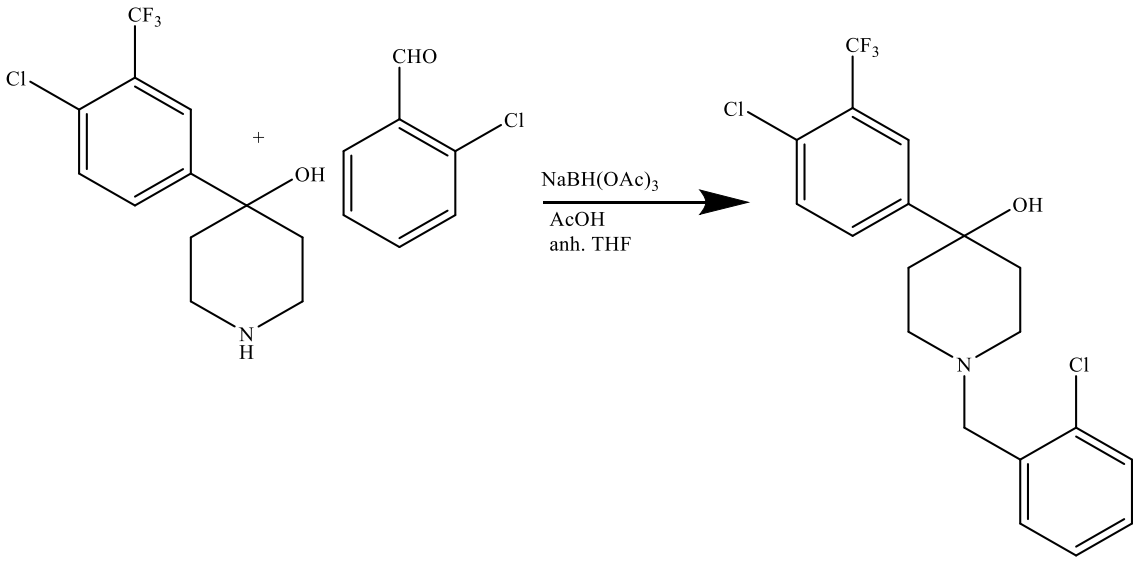
A black product is obtained. It was decided not to keep going with this reaction.

3.2- METHOD 2

3.2.1- Protocol D:

Under nitrogen atmosphere, add $NaBH(OAc)_3$ (4 eq) and 10 mL of Anhydrous THF in a round bottom flask properly closed, with a magnetic stir bar. Stir this solution. Meanwhile, in another round bottom flask, under N_2, add 4-(4-chloro-(trifluoromethyl)phenyl)piperidin-4-ol (1 eq), a benzyl derivated (1 eq) and 10 mL of anhydrous THF. When this mixture is well dissolved, transfer it in the round bottom flask containing $NaBH(OAc)_3$. Finally Acetic acid (1 eq) was added and the reaction mixture was stirred for 24 hours at room temperature. The day after, the reaction mixture was dried under pressure and the organic layer residue diluted with ethyl acetate. The organic layer was washed with $NaHCO_3$ (twice), brine (once) then, dried with Na_2SO_4 and concentrated under pressure after filtration. Purification of the residue by flash silica gel chromatography (25 g, 30 µm) eluting with CH_2Cl_2/ MeOH 95/5. Monitor the reaction by TLC (CH_2CL_2/MeOH 5%).

Reaction 5



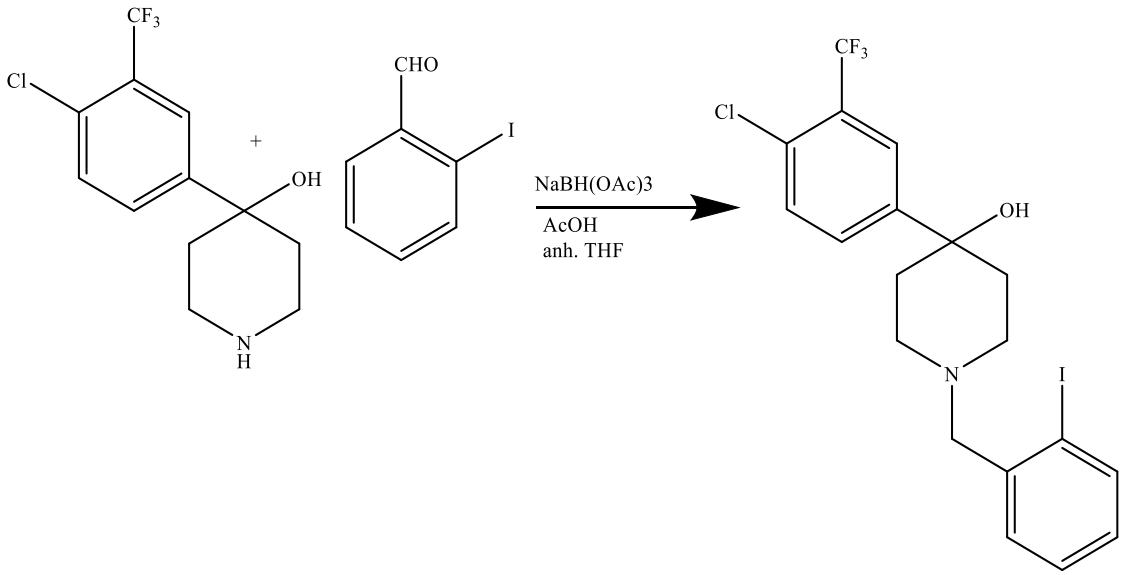
Product obtained following Protocol D: 4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol (0.501 g, 1.8 mmol); 2-chlorobenzaldehyde (0.183g, 1.3 mmol); $NaBH(OAc)_3$ (1.50 g, 7.1 mmol); AcOH (0.1 mL, 1.8 mmol). After purification, an orange oil was obtained with 63 % yield (0.333g, 0.8 mmol).

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 1.67 ppm (d, 2H, 1xCH_2)- 2.12 ppm (m, 2H, 1xCH_2)- 2.53 ppm (m, 2H, 1xCH_2)- 2.81 ppm (d, 2H, 1xCH_2)- 3.69 ppm (s, 2H, 1xCH_2)- 7.22 ppm (m, 2H, 2xCH_{arom})- 7.34 ppm (d, 1H, 1xCH_{arom})- 7.48 ppm (m, 2H, 2xCH_{arom})- 7.60 ppm (d, 1H, 1xCH_{arom})- 7.86 ppm (s, 1H, 1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***): 38.61 ppm (CH_2)- 49.33 ppm (CH_2)- 59.43 ppm (CH_2)- 71.15 ppm (C^{IV})- 121.28 ppm (C^{IV})- 124.31 ppm (CH_{arom})- 124.91 ppm (C^{IV})- 126.75 ppm (CH_{arom})- 128.00 ppm (C^{IV})- 128.34 ppm (CH_{arom})- 129.44 ppm (CH_{arom})- 129.61 ppm (CH_{arom})- 130.74 ppm (C^{IV})- 130.85 ppm (CH_{arom})- 131.41 ppm (CH_{arom})- 136.01 ppm (C^{IV})- 147.92 ppm (C^{IV}).

m/z calculated 404.25 g/mol, found 404.15 g/mol.

Reaction 6



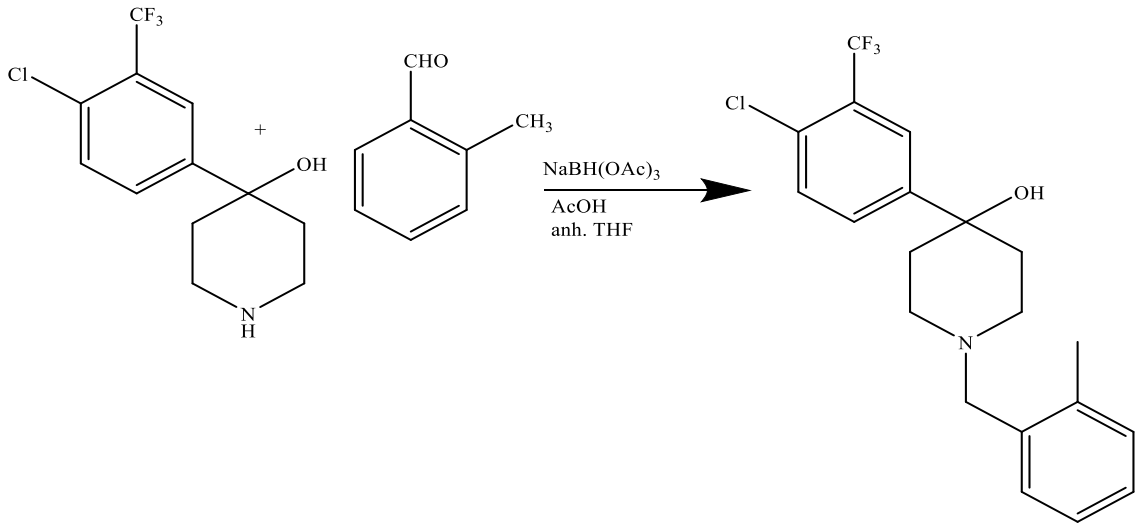
Product obtained following Protocol D: 4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol (0.524g, 1.9 mmol); 2- iodobenzaldehyde (0.421g, 1.8 mmol); NaBH(OAc)3 (1.50 g, 7.1 mmol); AcOH (0.1 mL, 1.8 mmol). After purification, yellow solid was obtained with 38 % yield (0.338g, 0.7 mmol).

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 1.66 ppm (d,2H, 1xCH_2)- 2.12 ppm (t, 2H, 1xCH_2)- 2.58 ppm (t, 2H, 1xCH_2)- 2.79 ppm (d, 2H, 1xCH_2)- 3.59 ppm (s, 2H, 1xCH_2)- 6.94 ppm (t, 1H, 1xCH_{arom})- 7.31 ppm (t, 1H, 1xCH_{arom})- 7.42 ppm (d, 2H, 2xCH_{arom})- 7.58 ppm (d, 1H, 1xCH_{arom})- 7.84 ppm (t, 2H, 2xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***): 38.50 ppm (CH_2)- 49.14 ppm (CH_2)- 66.56 ppm (CH_2)- 71.10 ppm (C^{IV})- 100.81 ppm (C^{IV})- 121.24 ppm (C^{IV})- 124.20 ppm (CH_{arom})- 124.86 ppm (C^{IV})- 128.11 ppm (CH_{arom})- 128.84 ppm (CH_{arom})- 129.44 ppm (CH_{arom})- 130.36 ppm (CH_{arom})- 130.64 ppm (C^{IV})- 131.34 ppm (CH_{arom})- 139.60 ppm (CH_{arom})- 140.63 ppm (C^{IV})- 147.95 ppm (C^{IV}).

m/z calculated 495.71 g/mol, found 496.09 g/mol.

Reaction 7



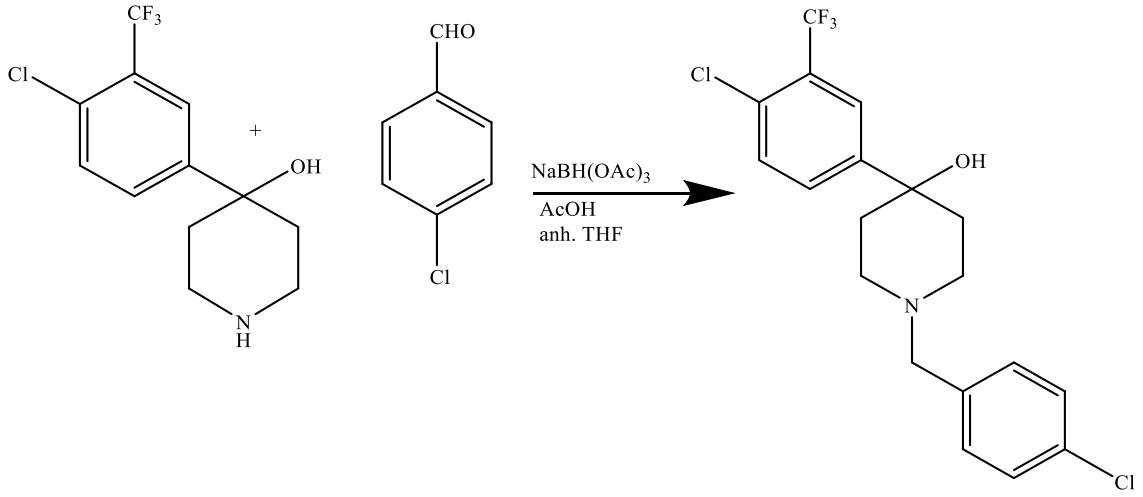
Product obtained following Protocol D: 4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol (0.501g, 1.8 mmol); 2- methylbenzaldehyde (0.421g, 1.8 mmol); $NaBH(OAc)_3$ (1.53 g, 7.2 mmol); AcOH (0.1 mL, 1.8 mmol). After purification, a brownish-yellow oil was obtained with 54 % yield (0.370g, 0.96 mmol).

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 1.65 ppm (d, 2H, 1xCH_2)- 2.11 ppm (m, 2H, 1xCH_2)- 2.38(s, 3H, 1xCH3)- 2.42 ppm (t, 2H, 1xCH_2)- 2.76 ppm (d, 2H, 1xCH_2)- 3.51 ppm (s, 2H, 1xCH_2)- 7.16 ppm (m, 3H, 3xCH_{arom})- 7.28 ppm (m, 1H, 1xCH_{arom})- 7.42 ppm (d, 1H, 1xCH_{arom})- 7.57 ppm (d, 1H, 1xCH_{arom})- 7.85 ppm (s, 1H, 1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***): 19.34 ppm (CH_3)- 38.67 ppm (CH_2)- 49.38 ppm (CH_2)- 60.88 ppm (CH_2)- 71.28 ppm (C^{IV})- 121.29 ppm (C^{IV})- 124.24 ppm (CH_{arom})- 124.91 ppm (C^{IV})- 125.64 ppm (CH_{arom})- 127.17 ppm (CHarom)- 127.94 ppm, 128.35ppm (C^{IV})- 129.46 ppm (CH_{arom})- 129.86 ppm (CH_{arom})- 130.41 ppm (CH_{arom})- 130.68 ppm (C_{IV})- 131.36 (CH_{arom})- 136.61 ppm (C^{IV})- 137.53 ppm(C^{IV})- 148.03 ppm (C^{IV}).

m/z calculated 383.84 g/mol, found 383.21 g/mol.

Reaction 8



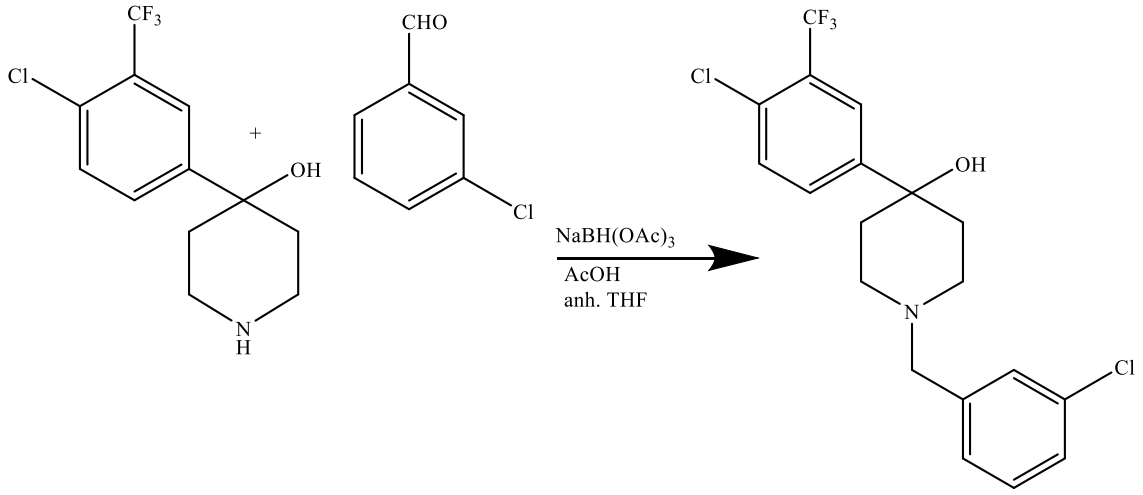
Product obtained following Protocol D: 4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol (0.505g, 1.8 mmol); 4- chlorobenzaldehyde (0.183g, 1.3 mmol); $NaBH(OAc)_3$ (1.51 g, 7.1 mmol); AcOH (0.1 mL, 1.8 mmol). After purification, a white solid was obtained with 80 % yield (0.422 g, 1.04 mmol).

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 1.70 ppm (d, 2H, 1xCH_2)- 2.10 ppm (td, 2H, 1xCH_2)- 2.45 ppm (t, 2H, 1xCH_2)- 2.75 ppm (d, 2H, 1xCH_2)- 3.53 ppm (s, 2H, 1xCH_2)- 7.28 ppm (s, 4H,4xCH_{arom})- 7.46 ppm (d, 1H, 1xCH_{arom})- 7.58 ppm (d, 1H, 1xCH_{arom})- 7.86 ppm (d, 1H, 1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***): 38.38 ppm (CH_2)- 49.07 ppm (CH_2)- 62.27 ppm (CH_2)- 71.04 ppm (C^{IV})- 121.13 ppm (C^{IV})- 124.13 ppm (CH_{arom})- 124.80 ppm (C^{IV})- 128.45 ppm (2xCH_{arom})- 129.31 ppm (CH_{arom})- 130.49 ppm (2xCH_{arom})- 130.72 ppm (C^{IV})- 131.35 (CH_{arom})- 132.90 ppm (C^{IV})- 136.59 ppm(C^{IV})- 147.85 ppm (C^{IV}).

m/z calculated 404.25 g/mol, found 404.17 g/mol.

Reaction 9



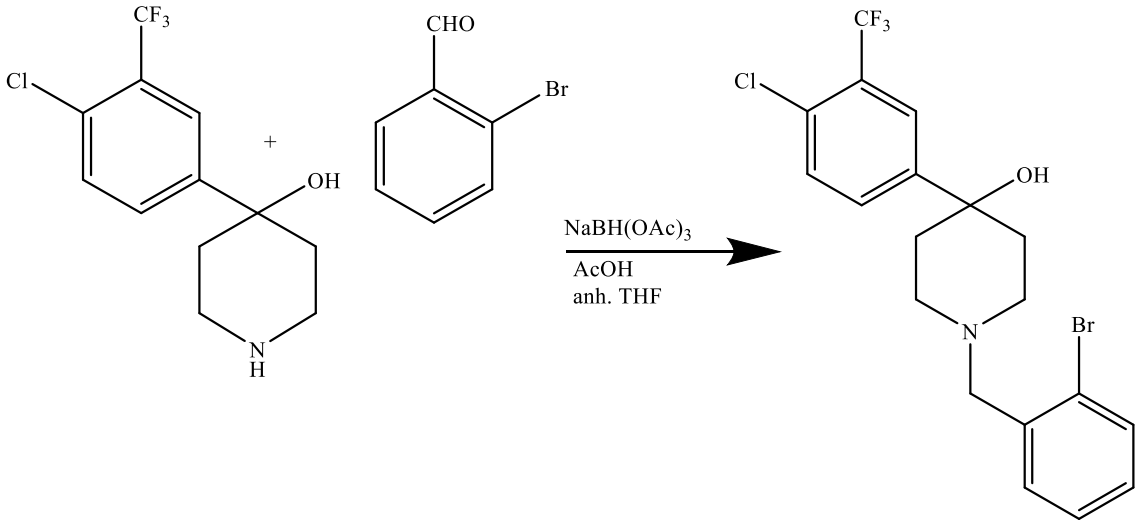
Product obtained following Protocol D: 4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol (0.502 g, 1.8 mmol); 3- chlorobenzaldehyde (0.147 mL, 1.3 mmol); $NaBH(OAc)_3$ (1.51 g, 7.1 mmol); AcOH (0.1 mL, 1.8 mmol). After purification, a brownish-yellow oil was obtained with 98.5 % yield (0.519g, 1.28 mmol).

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 1.77 ppm (d, 2H, 1xCH_2)- 2.20 ppm (td, 2H, 1xCH_2)- 2.53 ppm (dd, 2H, 1xCH_2)- 2.82 ppm (d, 2H, 1xCH_2)- 3.60 ppm (s, 2H, 1xCH_2)- 7.30 ppm (m, 3H,3xCH_{arom})- 7.43 ppm (s, 1H, 1xCH_{arom})- 7.53 ppm (d, 1H, 1xCH_{arom})- 7.65 ppm (d, 1H, 1xCH_{arom})7.94 ppm (d, 1H, 1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***): 38.40 ppm (CH_2)- 49.15 ppm (CH_2)- 62.47 ppm (CH_2)- 70.79 ppm (C^{IV})- 121.20 ppm (C^{IV})- 124.21 ppm (CH_{arom})- 124.82 ppm (C^{IV})- 127.35 ppm (2xCH_{arom})- 129.09 ppm (CH_{arom})- 129.34 ppm (CH_{arom})-129.56 ppm (CH_{arom})- 130.70 ppm (C^{IV})- 131.34 (CH_{arom})- 134.23 ppm (C^{IV})- 140.41 ppm(C^{IV})- 147.82 ppm (C^{IV}).

m/z calculated 404.25 g/mol, found 404.18 g/mol.

Reaction 10



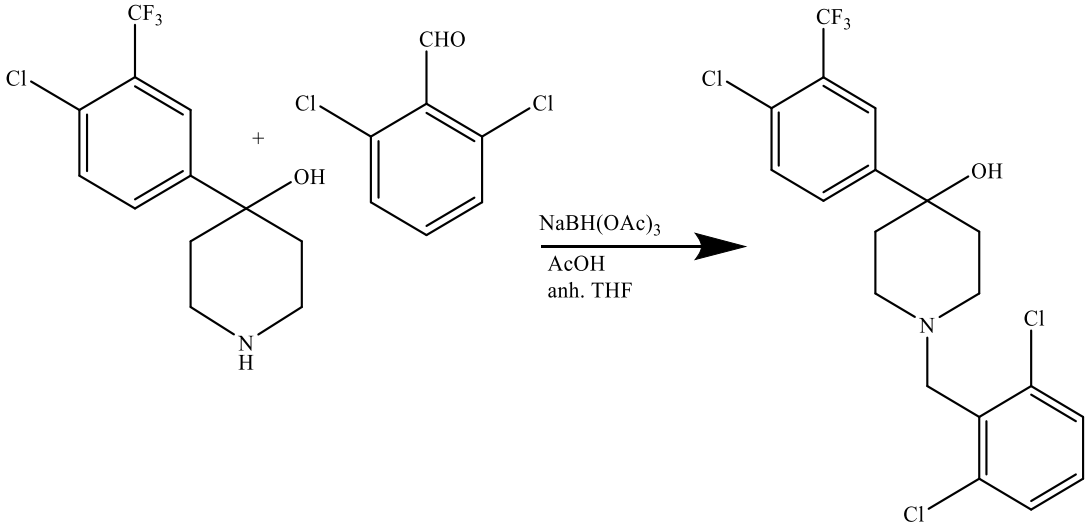
Product obtained following Protocol D: 4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol (0.502 g, 1.8 mmol); 2- bromobenzaldehyde (0.152 mL, 1.3 mmol); $NaBH(OAc)_3$ (1.50 g, 7.1 mmol); AcOH (0.1 mL, 1.8 mmol). After purification, a brownish-yellow oil was obtained with 67.9 % yield (0.396g, 0.88 mmol).

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 1.67 ppm (d, 2H, 1xCH_2)- 2.12 ppm (td, 2H, 1xCH_2)- 2.57 ppm (t, 2H, 1xCH_2)- 2.80 ppm (d, 2H, 1xCH_2)- 3.66 ppm (s, 2H, 1xCH_2)- 7.10 ppm (t, 1H,1xCH_{arom})-7.28 ppm (t, 1H, 1xCH_{arom})- 7.52 ppm (m, 3H, 3xCH_{arom})- 7.59 ppm (d, 1H, 1xCH_{arom})- 7.87 ppm (s, 1H, 1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***): 38.54 ppm (CH_2)- 49.23 ppm (CH_2)- 61.88 ppm (CH_2)- 71.11 ppm (C^{IV})- 121.19 ppm (C^{IV})- 124.22 ppm (CH_{arom})- 124.78 ppm (C^{IV})- 127.29 ppm (CH_{arom})- 127.94ppm (C^{IV})- 128.53ppm (CH_{arom})-129.39 ppm (CH_{arom})- 130.69 ppm (C^{IV})- 130.77 ppm(CH_{arom})- 131.34 ppm (CH_{arom})-132.84 (CH_{arom})- 137.64 ppm(C^{IV})- 147.76 ppm (C^{IV}).

m/z calculated 448.71 g/mol, found 450.13 g/mol.

Reaction 11



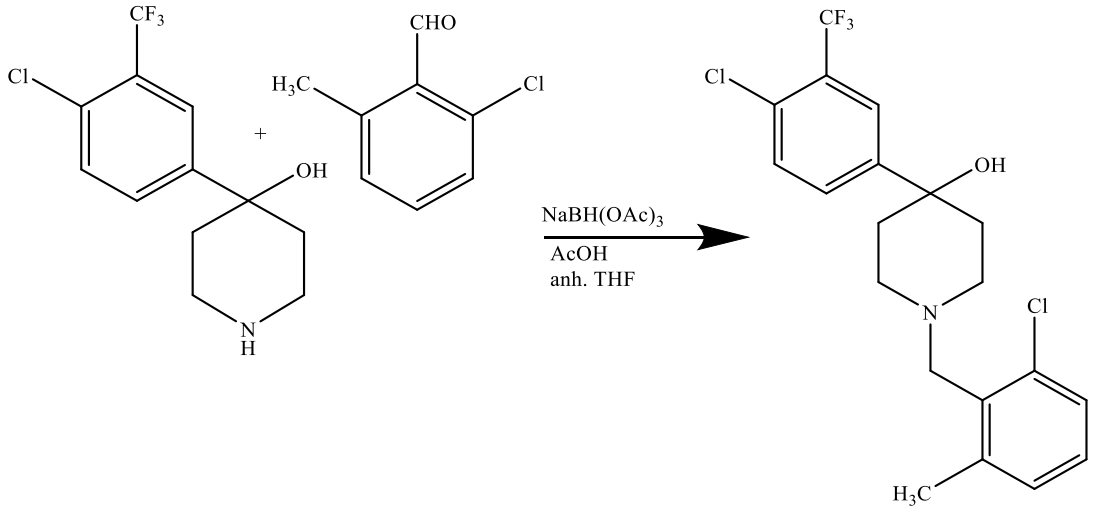
Product obtained following Protocol D: 4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol (0.507 g, 1.8 mmol); 2.6-di chlorobenzaldehyde (0.230 mL, 1.3 mmol); $NaBH(OAc)_3$ (1.50 g, 7.1 mmol); AcOH (0.1 mL, 1.8 mmol). After purification, a yellow solid was obtained with 37 % yield (0.210 g, 0.48 mmol).

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 1.70 ppm (d, 2H, 1xCH_2)- 2.06 ppm (td, 2H, 1xCH_2)- 2.68 ppm (t, 2H, 1xCH_2)- 2.85 ppm (d, 2H, 1xCH_2)- 3.82 ppm (s, 2H, 1xCH_2)- 7.15 ppm (m, 1H,1xCH_{arom})-7.30 ppm (d, 2H, 2xCH_{arom})- 7.43 ppm (d, 1H, 1xCH_{arom})- 7.58 ppm (dd, 1H, 1xCH_{arom})- 7.84 ppm (d, 1H, 1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***): 38.47 ppm (CH_2)- 49.12 ppm (CH_2)- 56.57 ppm (CH_2)- 71.10 ppm (C^{IV})- 121.18 ppm (C^{IV})- 124.14 ppm (CH_{arom})- 124.77 ppm (C^{IV})- 128.29 ppm (C^{IV})- 128.43 ppm (2xCH_{arom})- 128.88ppm (CH_{arom})-129.37 ppm (CH_{arom})- 130.64 ppm (C^{IV})- 131.27 ppm(CH_{arom})- 134.48 ppm (C^{IV})-137.04 (C^{IV})- 147.78 ppm (C^{IV}).

m/z calculated 438.70 g/mol, found 440.11 g/mol

Reaction 12



Product obtained following **protocol D:** 4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol (0.545 g, 1.9 mmol); 2-chloro-6-methylbenzaldehyde (0.280g; 1.8 mmol); $NaBH(OAc)_3$ (1.50g, 7.1 mmol)- AcOH (0.1 mL, 1.8 mmol). After purification an oily, dark-yellow product is obtained.

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***): peaks didn't identify the structure of the final product.

The same reaction was repeated in the same conditions.

Product obtained following **protocol D:** 4-(4-chloro-3-(trifluoromethyl)phenyl)piperidin-4-ol (0.504 g, 1.8 mmol); 2-chloro-6-methylbenzaldehyde (0.288g; 1.9 mmol); $NaBH(OAc)_3$ (1.50g, 7.1 mmol)- AcOH (0.1 mL, 1.8 mmol). After purification an oily, dark-yellow product is obtained with 17 % yield (0.130; 0.31 mmol).

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 1.70 ppm (d, 2H, 1xCH_2)- 2.03 ppm (td, 2H, 1xCH_2)- 2.48 ppm (s, 3H, 1xCH_3)-2.61 ppm (t, 2H, 1xCH_2)- 2.75 ppm (d, 2H, 1xCH_2)- 3.72 ppm (s, 2H, 1xCH_2)- 7.10 ppm (m, 2H, 2xCH_{arom})-7.21 ppm (m, 1H, 1xCH_{arom})- 7.44 ppm (d, 1H, 1xCH_{arom})- 7.60 ppm (dd, 1H, 1xCH_{arom})- 7.83 ppm (d, 1H, 1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***):19.97 ppm (CH_3)- 38.69 ppm (CH_2)- 48.95 ppm (CH_2)- 56.30 ppm (CH_2)- 71.22 ppm (C^{IV})- 121.17 ppm (C^{IV})- 124.19 ppm (CH_{arom})- 124.79 ppm (C^{IV})- 127.08 ppm (CH_{arom})- 128.09 ppm (CH_{arom})- 129.07 ppm (CH_{arom})-129.35 ppm (CH_{arom})- 131.28 ppm (CH_{arom})- 134.32 ppm(C^{IV})- 135.70 ppm (C^{IV})-140.99 (C^{IV})- 147.78 ppm (C^{IV}).

m/z calculated 418.28 g/mol.

3.3- METHOD 3

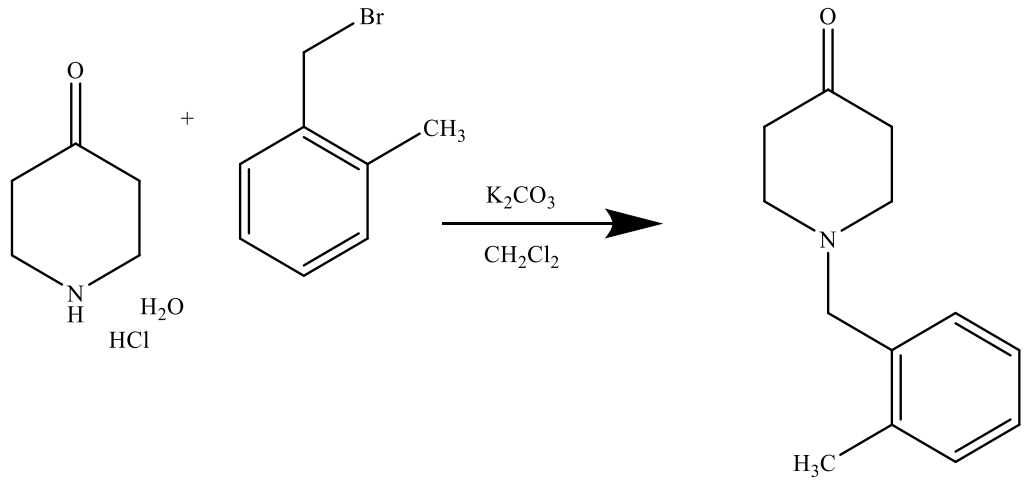
3.3.1- Protocol E:

In a round bottom flask, 4-piperidinone monohydrate hydrochloride (1 eq)was added in CH_2Cl_2 and MeOH (15 eq). Then add K_2CO_3 (2.3 eq). Stir for few minutes, then add α-bromo-o-xylene (1 eq). Stir overnight at room temperature. A purification is, finally, performed: flash silica gel column chromatography eluting with CH_2Cl_2/MeOH 95/5.

3.3.2- Protocol F:

Under N_2, a solution of a bromide compound (2.5 eq) with 5 mL of anhydrous THF was cooled to -78 °C. n-BuLi solution in THF (2.5 M, 2.5 eq) was added and the reaction mixture was stirred for one hour. A solution of the piperidinone compound (prepared with protocol E, 1 eq) with 5 mL of anhydrous THF it is added to the solution with the bromide compound, still at -78 °C. Stir for two hours. Quench by adding a saturated aqueous solution of NH_4Cl, at room temperature. Then, add distilled water to dissolve the product, proceed with an extraction with ethyl acetate (3 times). The organic layer is dried by Na_2SO_4, then after filtration, it was concentrated under reduce pressure. Finally, carry out a flash silica gel column chromatography (CH_2Cl_2/MeOH 5%).

Reaction 13

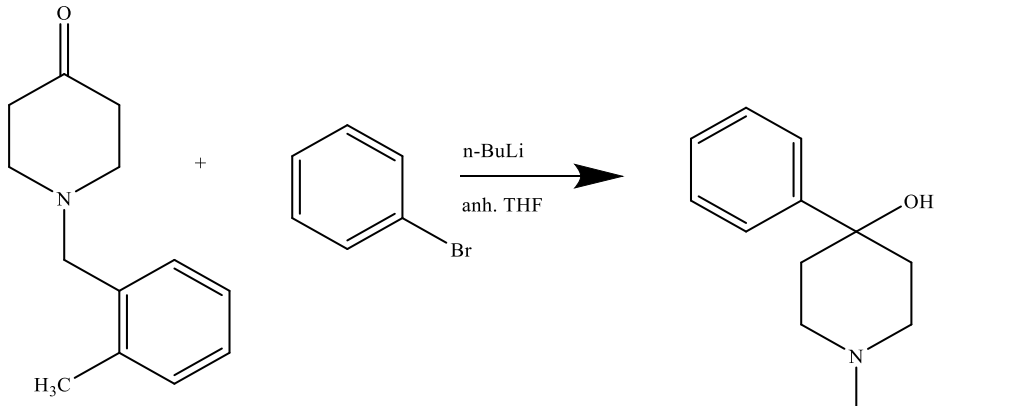


Product obtained by following **Protocol E**: 4-piperidinone monohydrate hydrochloride (1.00 g; 6.55 mmol); CH_2Cl_2 (120 mL); K_2CO_3(2.01g, 14.5 mmol); α- Bromo - o-xylene (0.86 mL, 6.4 mmol). After purification a light yellow oil is obtained with a 40% yield.

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 2.33 ppm (m, 7H, 2xCH_2- 1xCH_3)- 2.67 ppm (t, 4H, 2xCH_2)- 3.50 ppm (s, 2H, 1xCH_2)-7.12 ppm (m, 3H, 3xCH_{arom})- 7.23 ppm (m, 1H, 1xCH_{arom}).

Since the presence of the desired product was confirmed by the proton NMR analysis, the Carbon NMR analysis was not performed.

Reaction 14

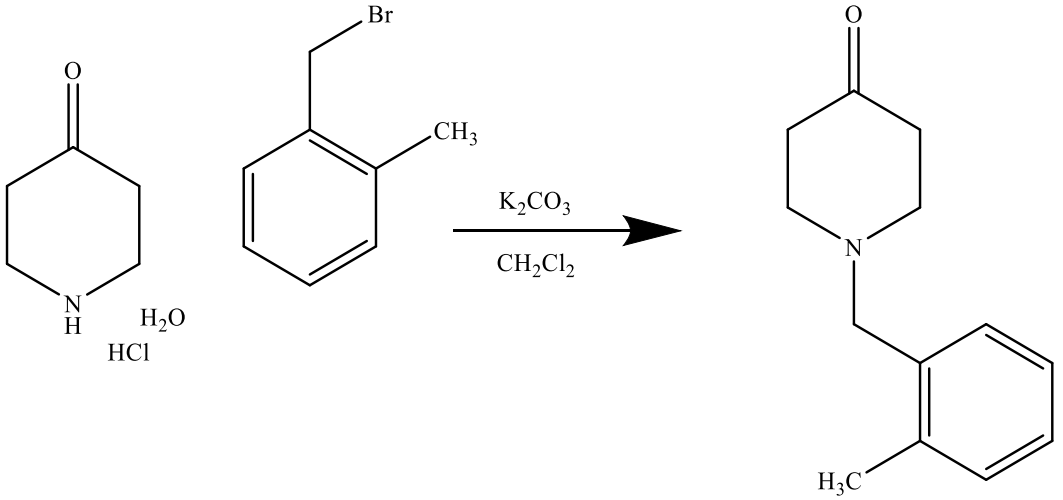


Obtained from reaction 13

Product obtained following protocol F: bromobenzene (0.26 mL; 2.5 mmol); 1-(2-methylbenzyl)piperidin-4-one (0.2 g, 0.98 mmol); n-BuLi (1 mL, 2.5 mmol). A colourless product is obtained with a 9% yield.

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 1.71 ppm (d, 2H, 1xCH_2)- 2.11 ppm (td, 2H, 1xCH_2)- 2.37 ppm (s, 3H, 1xCH_3)-2.47 ppm (t, 2H, 1xCH_2)- 2.71 ppm (d, 2H, 1xCH_2)- 3.50 ppm (s, 2H, 1xCH_2)- 7.14 ppm (s, 3H, 3xCH_{arom})-7.22 ppm (m, 1H, 1xCH_{arom})- 7.31 ppm (m, 3H, 3xCH_{arom})- 7.46 ppm (d, 2H, 2xCH_{arom}).

Reaction 15

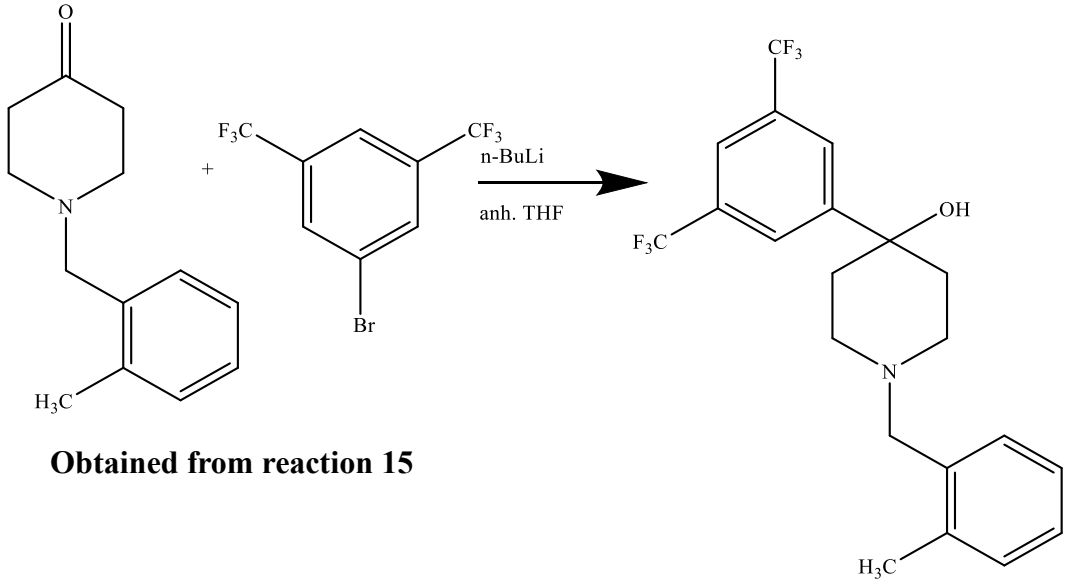


Product obtained following protocol E: 4-piperidinone monohydrate hydrochloride (4.00 g, 0.0261 mol), α-bromo-o-xylene (3.4 mL, 0.0254 mol), CH_2Cl_2 (480 mL, 7.49 mol); MeOH (16 mL, 0.395 mol); K_2CO_3 (8.00 g, 0.0579 mol). A yellow oil is obtained with a 50% yield.

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 2.39 ppm (s, 3H, 1xCH_3)- 2.41 ppm (d, 4H, 2xCH_2)- 2.72 ppm (m, 4H,2xCH_2)- 3.55 ppm (s, 2H, 1xCH_2)- 7.16 ppm (m, 3H, 3xCH_{arom})-7.26 ppm (m, 1H, 1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***):19.26 ppm (CH_3)- 41.40 ppm (CH_2)- 53.06 ppm (CH_2)- 59.89 ppm (CH_2)- 125.70 ppm (CH_{arom})- 127.37 ppm (CH_{arom})- 129.71 ppm (CH_{arom})- 130.47 ppm (CH_{arom})- 136.16 ppm(C^{IV})- 137.50 ppm (C^{IV})- 209.69 ppm (C^{IV}).

Reaction 16



Obtained from reaction 15

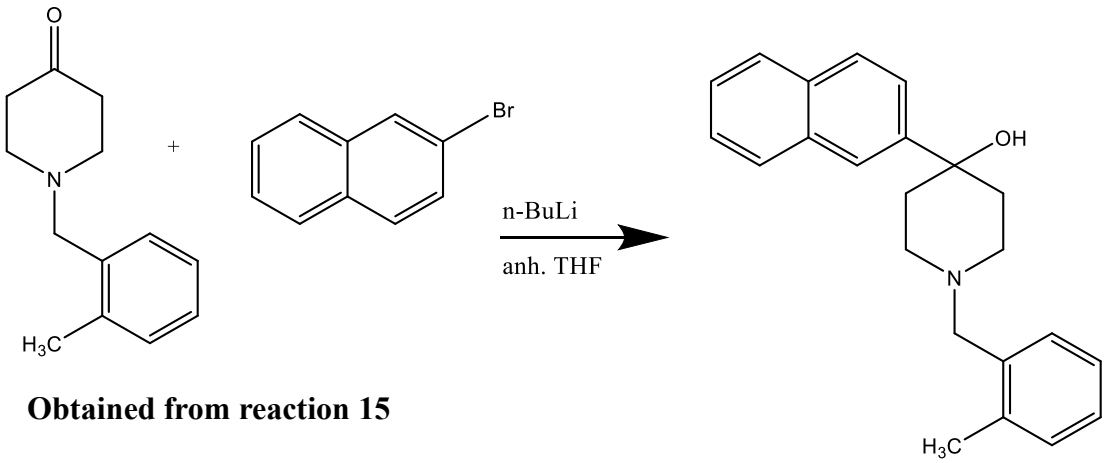
Product obtained following **protocol E:** 1-(2-methylbenzyl)piperidin-4-one (0.511 g, 2.5 mmol); 1-Bromo-3,5-bis(trifluoromethyl)benzene (1.08 mL; 6.3 mmol); n-BuLi (2.5 mL, 6.3 mmol); anhydrous THF (10 mL). After purification an oily, brown product is obtained with 26.8 % (0.280; 0.67 mmol).

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***): 1.79 ppm (10-13, d, 2H, 1xCH_2)- 2.27 ppm (10-13, t, 2H, 1xCH_2)- 2.51 ppm (21, s, 3H, 1xCH_3)- 2.61 ppm (11-12, t, 2H, 1xCH_2)- 2.93 ppm (11-12, d, 2H, 1xCH_2)- 3.66 ppm (14, s, 2H, 1xCH_2)- 7.27 ppm (18-19-20, s, 3H, 3xCH_{arom})- 7.40 ppm(17, s, 1H, 1xCH_{arom})- 7.91 ppm (6, s, 1H, 1xCH_{arom})- 8.19 ppm (4-2, d, 2H,1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***): 19.26 ppm (21, CH_3)- 38.60 ppm (10-13, CH_2)- 49.25 ppm (11-12, CH_2) - 60.73 ppm (14, CH_2)- 71.54 ppm (C^{IV})– 120.92 (6, CH_{arom}) -121.77 ppm (3, CH_{arom}) – 125.28 ppm (4-2, CH_{arom}) - 125.67 ppm (18-19-20, CH_{arom})- 127.27 ppm (18-19-20, CH_{arom})- 129.91 ppm (17, CH_{arom})- 130.45 ppm (18-19-20, CH_{arom})- 131.00 (C^{IV})- 131.44 ppm (C^{IV})- 131.88 ppm (C^{IV})- 132.32 ppm (C^{IV})- 136.24 ppm (C^{IV})- 137.52 ppm (C^{IV})- 151.47 (C^{IV}).

m/z calculated 417.40 g/mol, found 418.14 g/mol.

Reaction 17



Obtained from reaction 15

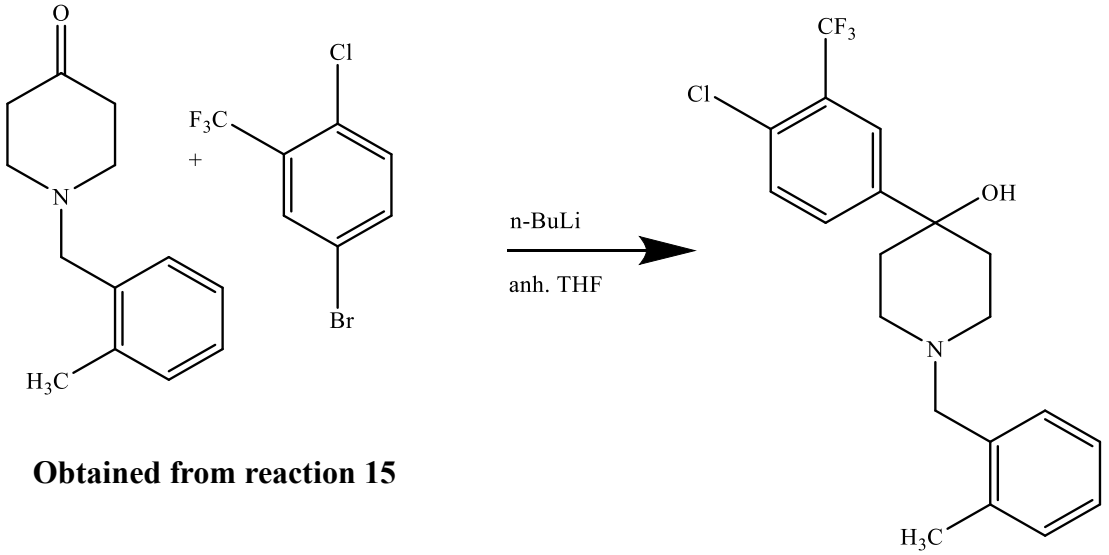
Product obtained following **protocol E:** 1-(2-methylbenzyl)piperidin-4-one (0.373 g, 1.8 mmol);2-naphthalene (0.949 g; 4.6 mmol); n-BuLi (1.8 mL, 4.6 mmol); anhydrous THF (10 mL). After purification an solid, white product is obtained with 46 % (0.279; 0.84 mmol).

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***): 1.74 ppm (12-15, dd, 2H, 1xCH_2) – 2.21 ppm (12-15, dt, 2H, 1xCH_2)- 2.38 ppm (24, s, 3H, 1xCH_3)- 2.50 ppm (14-13, dt, 2H, 1xCH_2)- 2.74 ppm (14-13, d, 2H, 1xCH_2)- 3.51 ppm (17, s, 2H, 1xCH_2) – 7.15 ppm (21-22-23, d, 3H, 3xCH_{arom})- 7.30 ppm (20, m, 1H, 1xCH_{arom})- 7.43 ppm (2-1, m, 2H, 2xCH_{arom})- 7.58 ppm (3, dd, 1H 1xCH_{arom})- 7.76 ppm (6-9-10, m, 3H, 3xCH_{arom})- 7.91 ppm (7, d, 1H, 1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***): 19.33 ppm (24, CH_3)- 38.63 ppm (12-15, CH_2)- 49.68 ppm (14-13, CH_2) - 60.96 ppm (17, CH_2)-71.64 ppm (C^{IV}) – 123.04 (7, CH_{arom}) -123.60 ppm (3, CH_{arom}) - 125.61 ppm (21 or 22 or 23, CH_{arom}) - 125.89 ppm (1 or 2, CH_{arom})- 126.14 ppm (1 or 2, CH_{arom})- 127.05 ppm (21 or 22 or 23, CH_{arom})- 127.55 ppm (6 or 9 or 10, CH_{arom})- 128.06 (6 or 9 or 10, CH_{arom})- 128.28 ppm (6 or 9 or 10, CH_{arom})- 129.87 ppm (20, CH_{arom})- 130.34 ppm (21 or 22 or 23, CH_{arom})- 132.54 ppm (C^{IV})- 133.29 ppm (C^{IV})- 136.89ppm (C^{IV})- 137.54 ppm (C^{IV})- 145.92 ppm (C^{IV}).

m/z calculated 331.46 g/mol, found 332.26 g/mol.

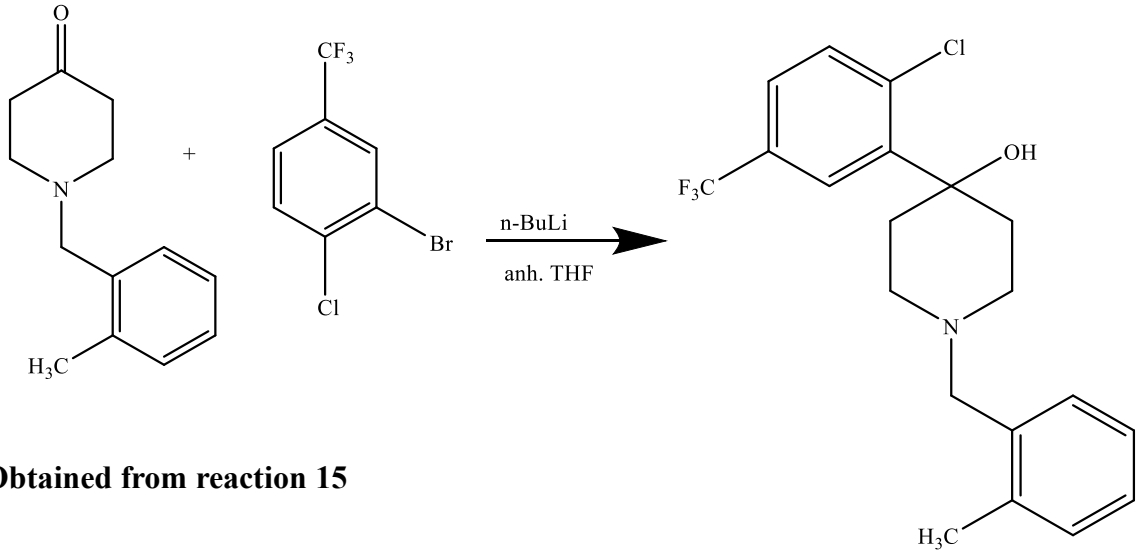
Reaction 18



Obtained from reaction 15

Product obtained following **protocol E:** 1-(2-methylbenzyl)piperidin-4-one (0.498 g, 2.5 mmol); 2-chloro-5-Bromobenzotrifluoride(0.91 mL; 6.1 mmol); n-BuLi (2.4 mL, 6.0 mmol); anhydrous THF (10 mL). After purification an oily, brown product is obtained. **^{1}H NMR Analysis** (***$CDCl_3$, 300 MHz***): peaks didn't identify completely the structure of the final product.

Reaction 19



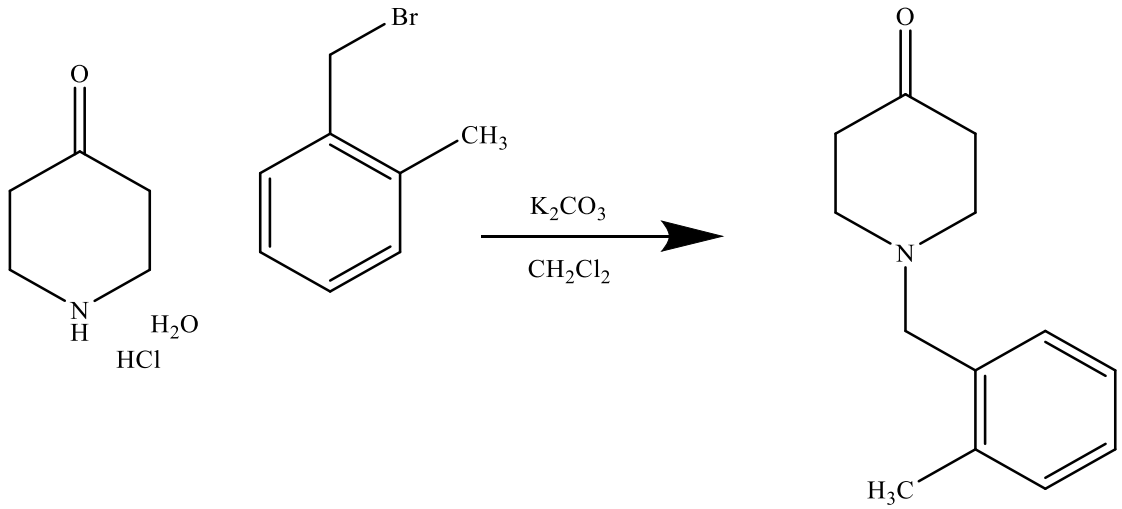
Obtained from reaction 15

Product obtained following **protocol E:** 1-(2-methylbenzyl)piperidin-4-one (0.517 g, 2.5 mmol); 4-chloro-3-Bromobenzotrifluoride(0.96 mL; 6.4 mmol); n-BuLi (2.6 mL, 6.5

mmol); anhydrous THF (10 mL). After purification an oily, dark-yellow product is obtained

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***): peaks didn't identify the structure of the final product.

Reaction 20

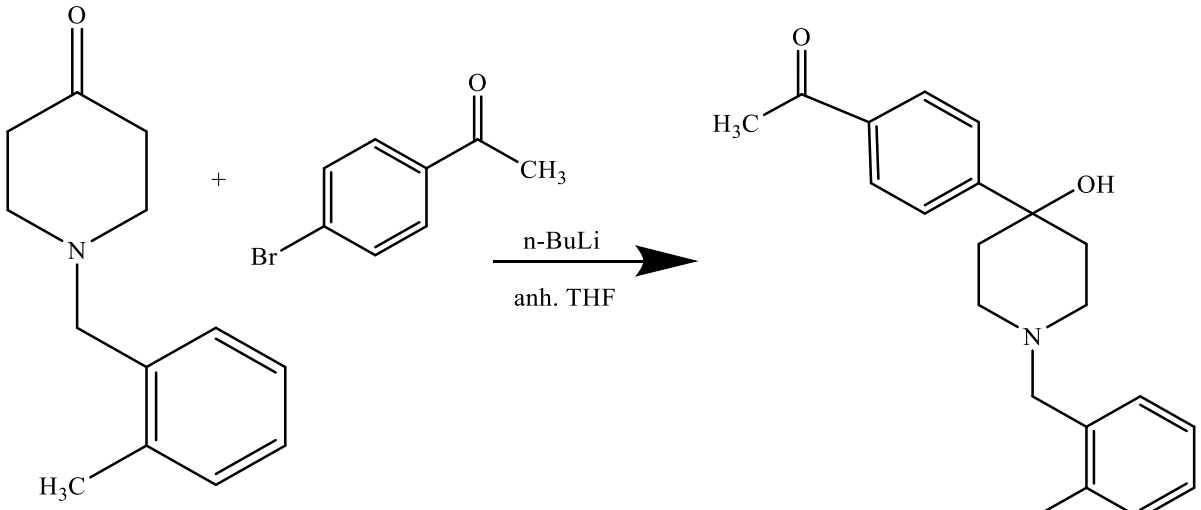


Product obtained following **protocol E**: 4-piperidinone monohydrate hydrochloride (4.00 g, 0.0261 mol), α-bromo-o-xylene (3.4 mL, 0.0254 mol), CH_2Cl_2 (480 mL, 7.49 mol); MeOH (16 mL, 0.395 mol); K_2CO_3 (8.00 g, 0.0579mol). A yellow oil is obtained with a yield of 46%.

^{1}H-NMR (***$CDCl_3$, 300 MHz***): 2.40 ppm (m, 7H, 1xCH_3, 2xCH_2)- 2.70 ppm (m, 4H,2xCH_2)- 3.52 ppm (s, 2H, 1xCH_2)- 7.15 ppm (m, 3H, 3xCH_{arom})-7.27 ppm (m, 1H, 1xCH_{arom}).

^{13}C-NMR (***$CDCl_3$, 75 MHz***):18.96 ppm (CH_3)- 41.40 ppm (CH_2)- 53.07 ppm (CH_2)- 59.89 ppm (CH_2)- 125.61 ppm (CH_{arom})- 127.37 ppm (CH_{arom})- 129.59 ppm (CH_{arom})- 130.47 ppm (CH_{arom})- 136.15 ppm(C^{IV})- 137.22 ppm (C^{IV})- 209.23 ppm (C^{IV}).

Reaction 21

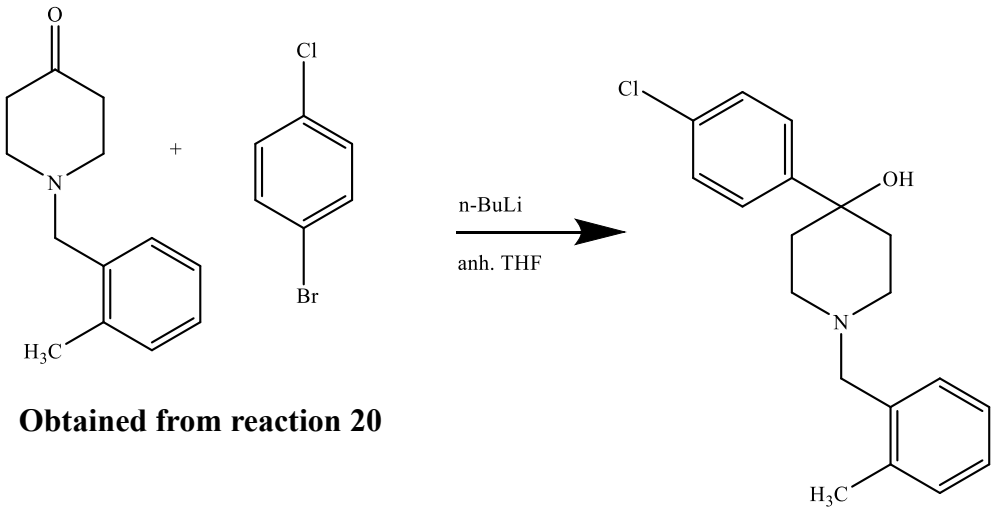


Obtained from reaction 20

Product obtained following **protocol E:** 1-(2-methylbenzyl)piperidin-4-one (0.272 g, 1.3 mmol); 4-bromo-acetophenone (0.667 g; 3.4 mmol); n-BuLi (1.34 mL, 3.4 mmol); anhydrous THF (10 mL). After purification an oily, orange product is obtained.

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***): peaks didn't identify completely the structure of the final product, due to the presence of others peaks.

Reaction 22



Obtained from reaction 20

Product obtained following **protocol E:** 1-(2-methylbenzyl)piperidin-4-one (0.295 g, 1.5 mmol); 4-bromo-chlorobenzene (0.696 g; 3.6 mmol); n-BuLi (1.45 mL, 3.6 mmol); anhydrous THF (10 mL). After purification a colorless product is obtained.

^{1}H NMR Analysis (***$CDCl_3$, 300 MHz***): peaks didn't identify the structure of the final product.

Chapter 4

CONCLUSIONS

An ongoing theme, nowadays, is how to improve our health and in particular how to resolve those problems that are harmful to us. For example, considering microorganisms, some of them can be really harmful to us. For this reason, the main point in this study is about synthesising different piperidinol based molecule that are effective against *M. abscessus*.

M. abscessus, first isolated from an old woman which complained of constant pain to her left knee, is a rapidly growing mycobacterium (RGM) and it is responsible of different diseases, such as: chronic lung disease, post traumatic wound infections, skin infection etc. It was also observed that these mycobacterium are present in patients with cystic fibrosis, slowing their healing process. Even if it was noted a bond between people with cystic fibrosis and *M. Abscessus* this have not excluded the fact that everyone can be exposed to M. *abscessus*.

M. abscessus are microorganisms difficult to treat, since their intrinsic resistance to the common antibiotic due to their cell envelope. Nevertheless, some recent studies observed that aromatic-piperidinol based molecule are effective against *M. tuberculosis*, and since some similarities with *M. abscessus* it was decided to synthesize different aromatic-piperidinol based molecule to test in *M. abscessus*.

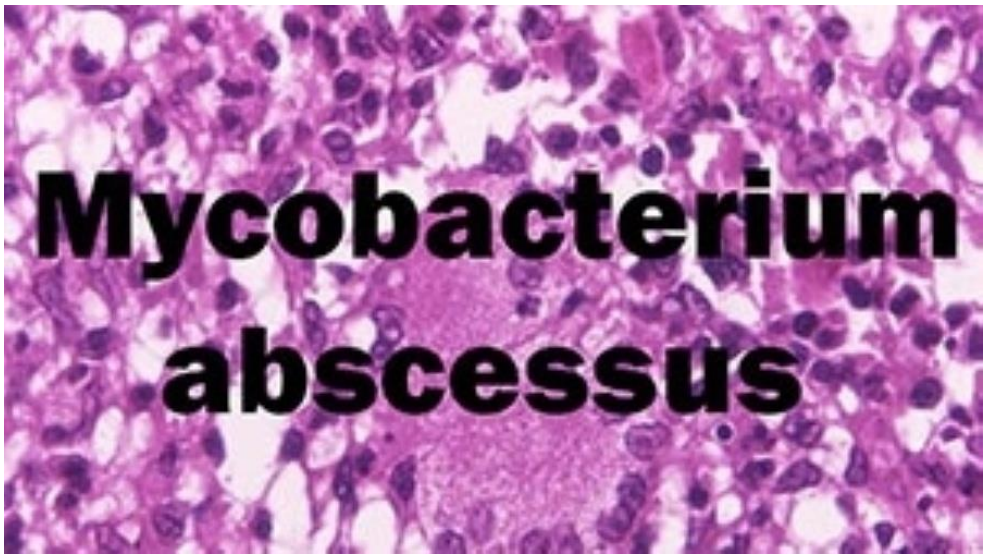


Figure 11: *Mycobacterium abscessus* (http://keywordsuggest.org/gallery/695141.html)

Hence, three different strategy of synthesis were applied:

1. A three steps reaction. Through this method were not easy to obtain the desired products, as a matter of fact only the first reaction with 2-bromonaphthalene gave the desired product, even if in low yield, it was about 20%.Instead for the other reactions tried none of them were obtained.

   The reasons for these problems could be different. The fact that is a three step reaction maybe this has lead to lose some of the main reagent/products, lowering the yield. Moreover, the first reaction used a concentrated reaction of n-BuLi in an exchange reaction to form the corresponding organolithium derivate. The concentration over time tends to decrease since the instability of n-BuLi to air, that is why the reaction might have not reacted completely. Another reasons might be the fact that some of the product could have been lost in the purification column.

2. A one step reaction in which most of the desired product were obtained with a variable yields, from 17% to 98%. Also with this method the main problem that brought to a low yield could be due to the fact that some of the product has been lost in the purification phase.

3. A two step reaction in which, at first, is synthesised 1-(2-methylbenzyl)piperidin-4-one and then the product reacts with different halide compound, leading to a variable yields. Also in this case the presence of n-BuLi, as I said instable to air, and the purification phase could have brought to lose the final product, lowering the yield.

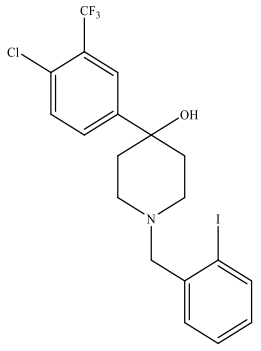
Therefore, in this work it was decided to synthesis piperidinol-based molecule but, during the course of the project some difficulties had arisen: low yield, presence of instable compound. To resolve the main problem, the low yield, they were realised different route, decreasing the number of steps needed to obtain the final product and using more stable reagents.

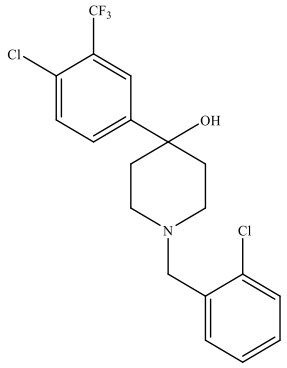
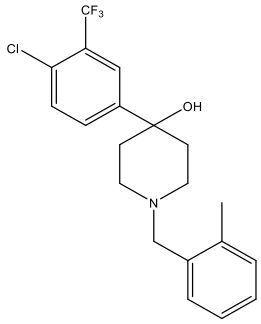
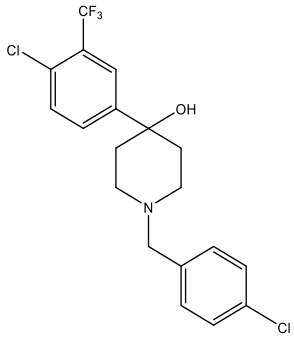
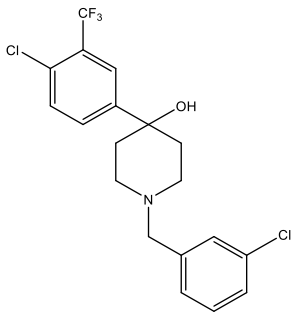
However, only some of these strategies reactions brought to the desired product that is why it was decided to continue on finding a clean route that brings to a higher amounts. Among these methods, maybe the second one was the better one, since it improved the yields, it brought to a cleaner route and thus it minimized the waste produced: just one step against a two or three-step reaction. As a matter of fact, considering the first method, the presence of the protecting group meant the adding of other steps (deprotection), that could increase general costs.

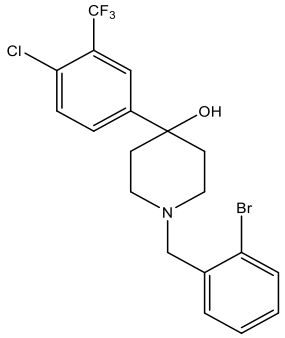
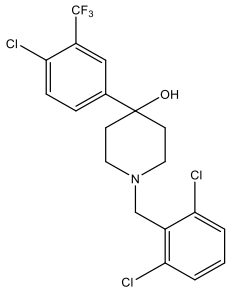
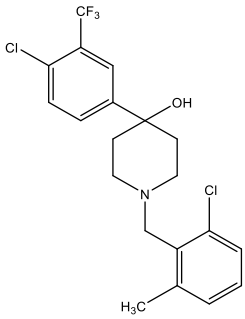
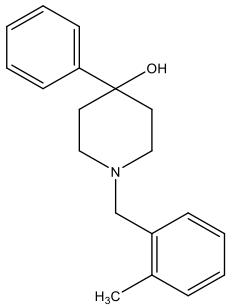
However, the synthesised products were sent to biologist in order to test if based-on-them drugs are useful against *Mycobacterium abscessus*. In particular some word are first explained:

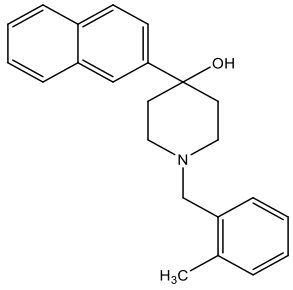
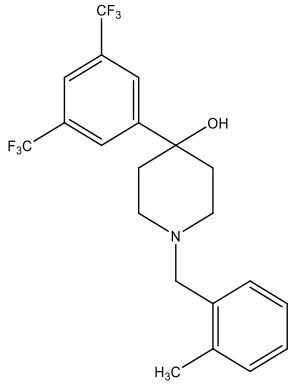
- Cytotoxity is a biological, chemical or physical effect that brings to the toxicity of the cell, compromising cell membrane integrity. When survivals of *Mybobacterium* are below 50%, compounds are considered cytotoxic.
- MIC, minimum inhibitory concentration, is the lowest concentration of a chemical that prevents the growth of a bacterium and it is measured in µg/ml after highlighting the interested compounds in blue.

It is shown below a table in which it is illustrated the percentage of survival of *Mycobacterium abscessus* and the minimum inhibitory concentration for some different compounds.

DRUGS	MIC µg/ml	Survival (%)	
		50 x MIC	100 x MIC
	0.25	93.74	72.35

DRUGS	MIC µg/ml	Survival (%)	
		50 x MIC	100 x MIC
	0.125	83.57	62.17
	0.0625	80.00	68.00
	0.25	45.30	43.39
	4	46.09	36.87

DRUGS	MIC µg/ml	Survival (%)	
		50 x MIC	100 x MIC
	0.125	90.35	88.00
	0.5	69.13	35.22
	0.125	50.52	31.57
	4	25.74	25.04

DRUGS	MIC µg/ml	Survival (%)	
		50 x MIC	100 x MIC
	4	50.87	38.35
	4	45.91	35.30

As it is possible to see from the previous table, some of the synthesized products are effective against *M. abscessus*, since their survival to the applied drug is less than 50%. In particular, it is possible to note that the molecule that gives the lowest percentage of survival is 1-(2-methylbenzyl)-4-phenylpiperidin-4-ol as a matter of fact only 25% of the microorganisms treated with this molecule survived.

In conclusion, we can say that for the future other strategies could be realized, such as finding new starting materials or routes that if improve the yields and decrease the waste could be realized in larger scale. Thanks to this training I learned a lot about organic synthesis and how to collaborate with other people, that's why in the future I would like to work with capable people and in a well-organised structure as the one that I worked in.

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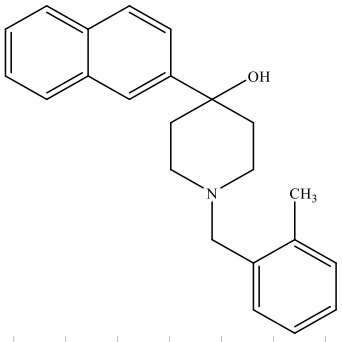
Appendix

Reaction 1



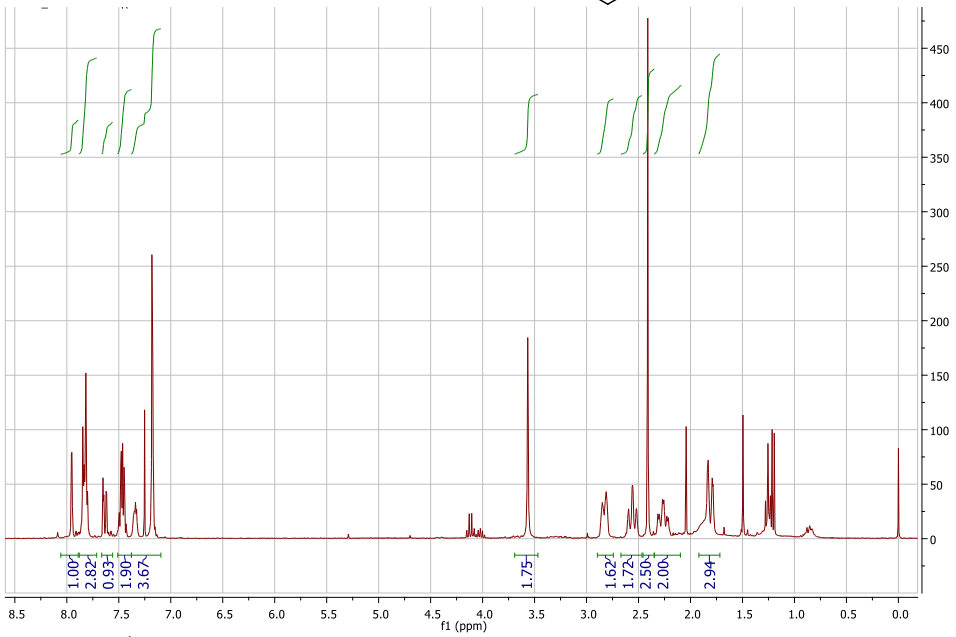


Figure 12: ^{1}H-NMR spectrum of 1-(2-methylbenzyl)-4-(naphthalen-2-yl)piperidin-4-ol

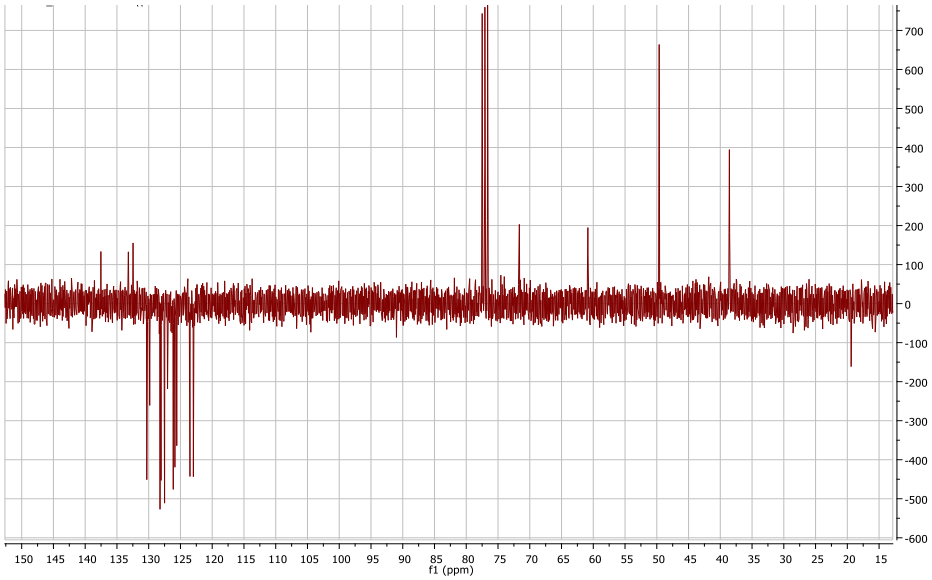
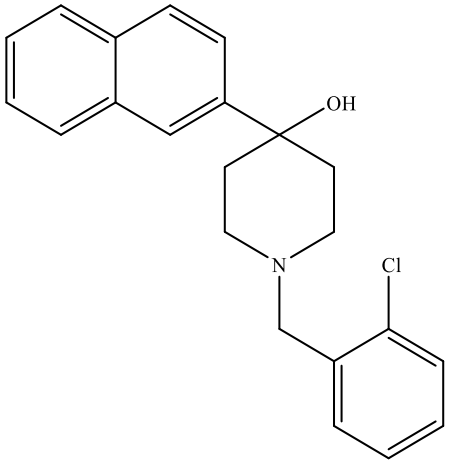


Figure 13: ^{13}C-NMR spectrum of 1-(2-methylbenzyl)-4-(naphthalen-2-yl)piperidin-4-ol

Reaction 2



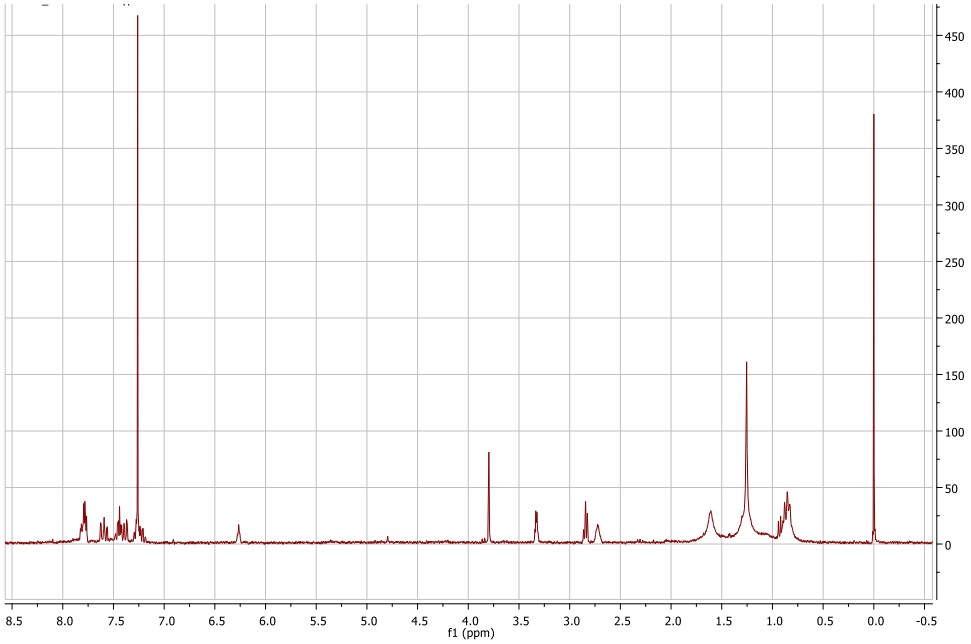
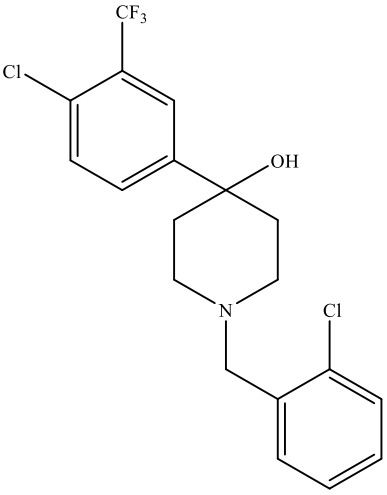


Figure 14: ^{1}H-NMR spectrum of 1-(2-chlorobenzyl)-4-(naphthalen-2-yl)piperidin-4-ol

Reaction 5



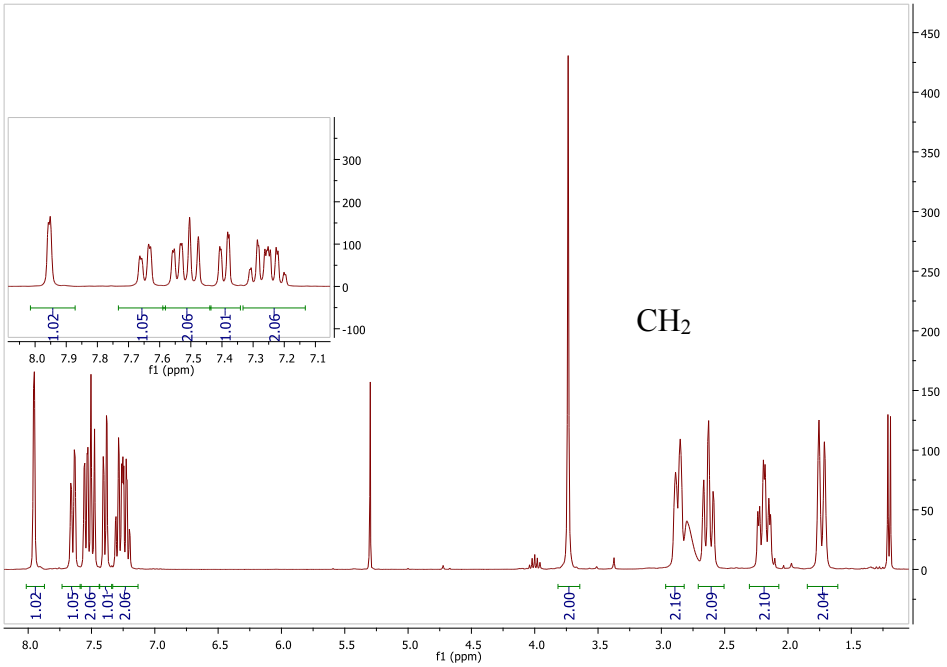


Figure 15: ^{1}H-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-chlorobenzyl)piperidin-4-ol

Reaction 5

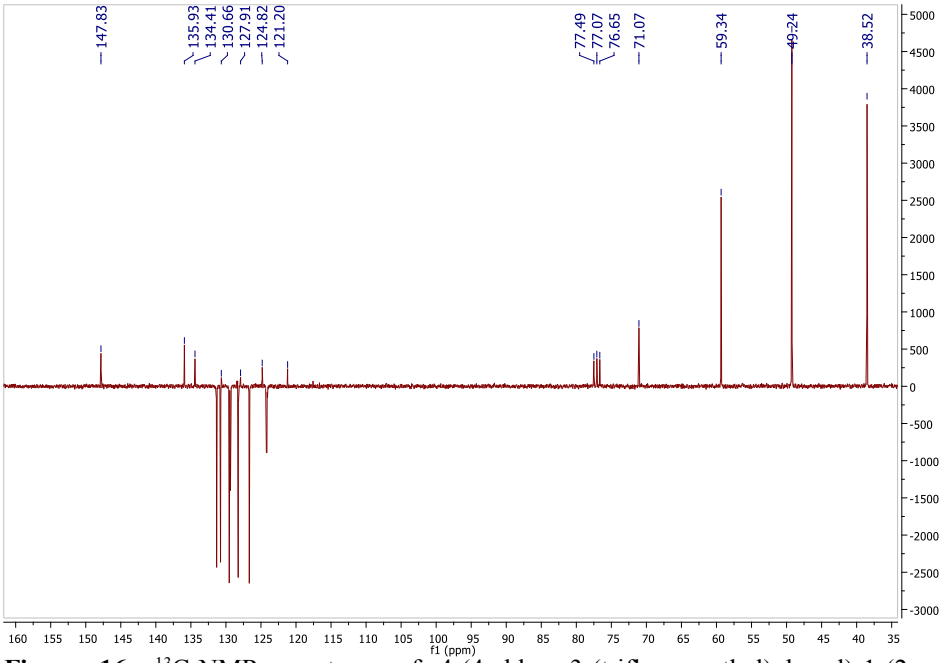


Figure 16: ^{13}C-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-chlorobenzyl)piperidin-4-ol

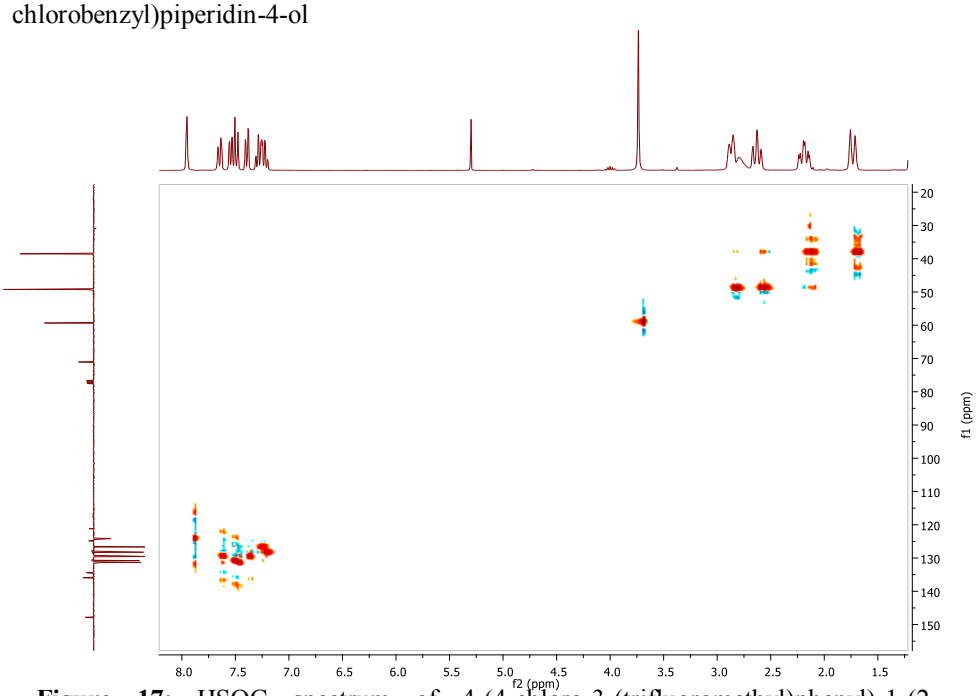
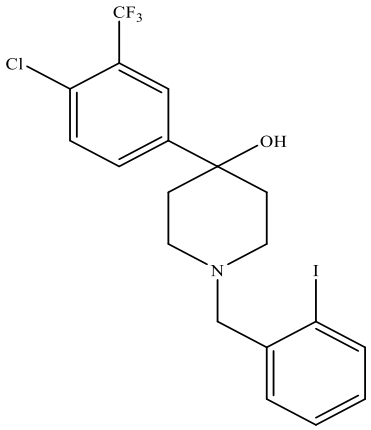


Figure 17: HSQC spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-chlorobenzyl)piperidin-4-ol

Reaction 6



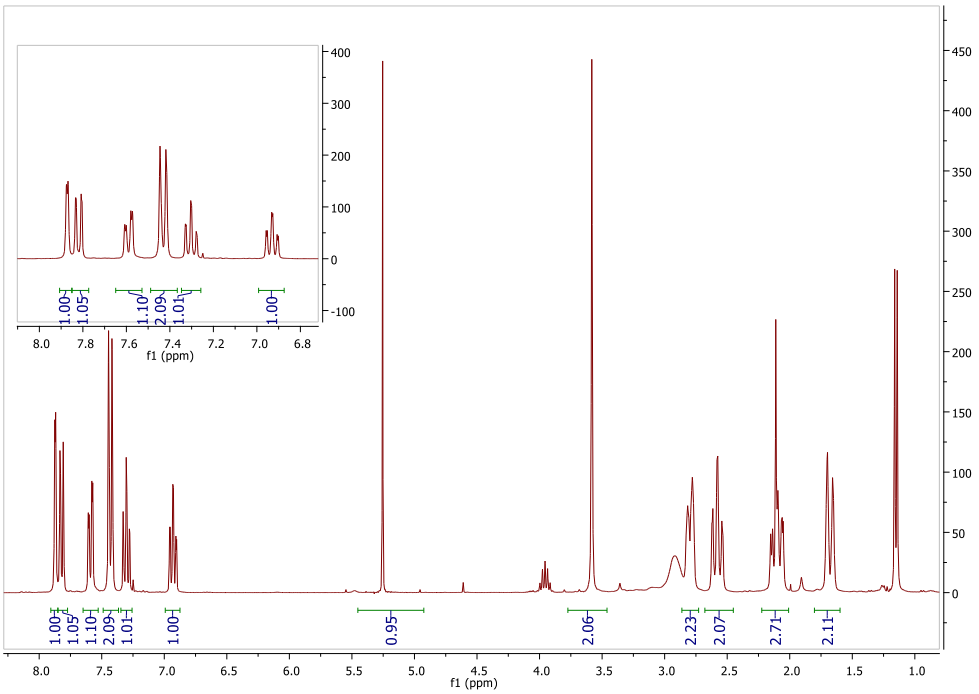


Figure 18: ^{1}H-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-iodobenzyl)piperidin-4-ol

Reaction 6

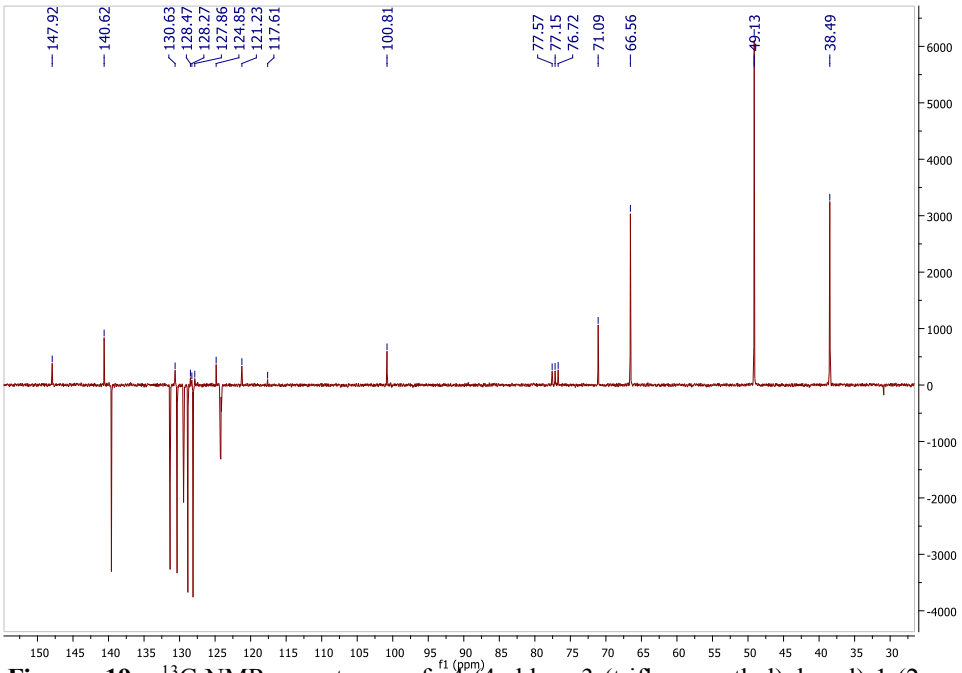


Figure 19: ^{13}C-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-iodobenzyl)piperidin-4-ol

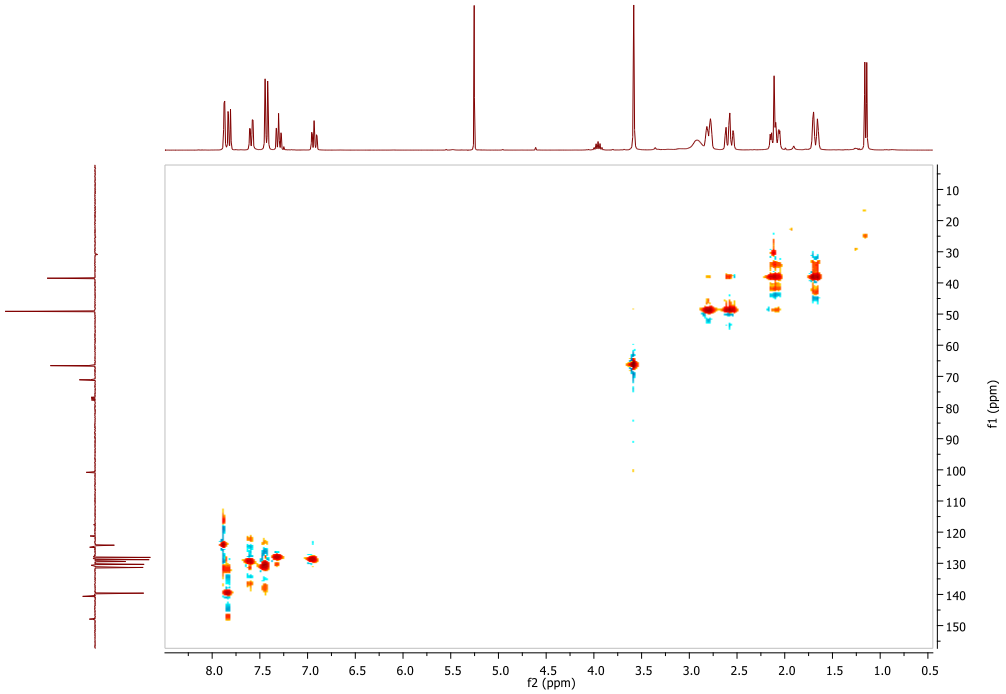
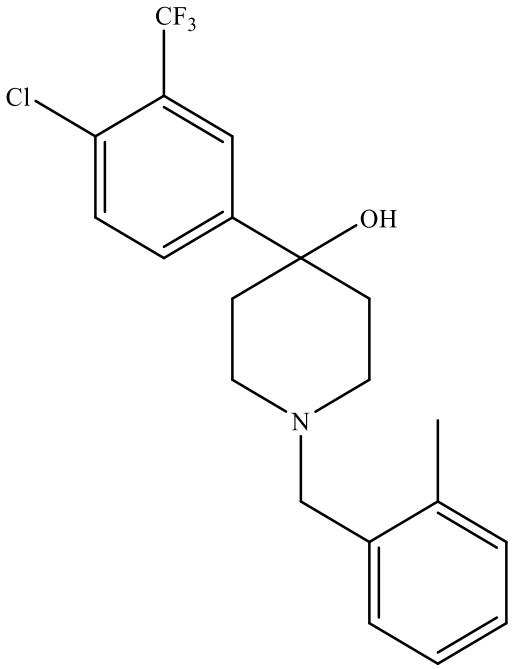


Figure 20: HSQC spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-iodobenzyl)piperidin-4-ol

Reaction 7



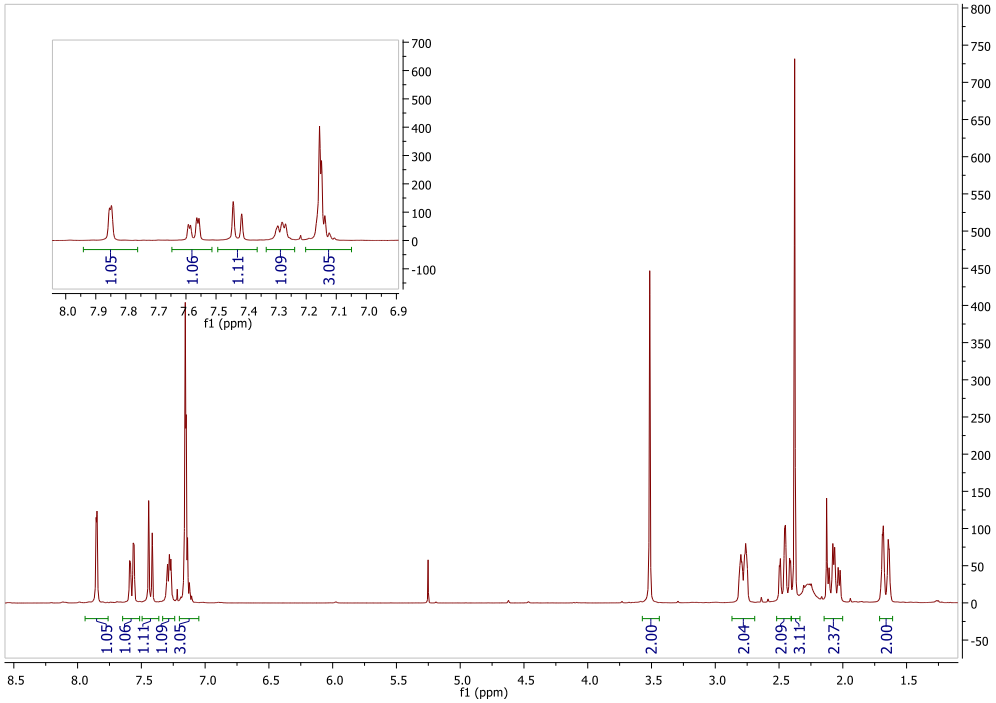


Figure 21: [1]H-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-ol

Reaction 7

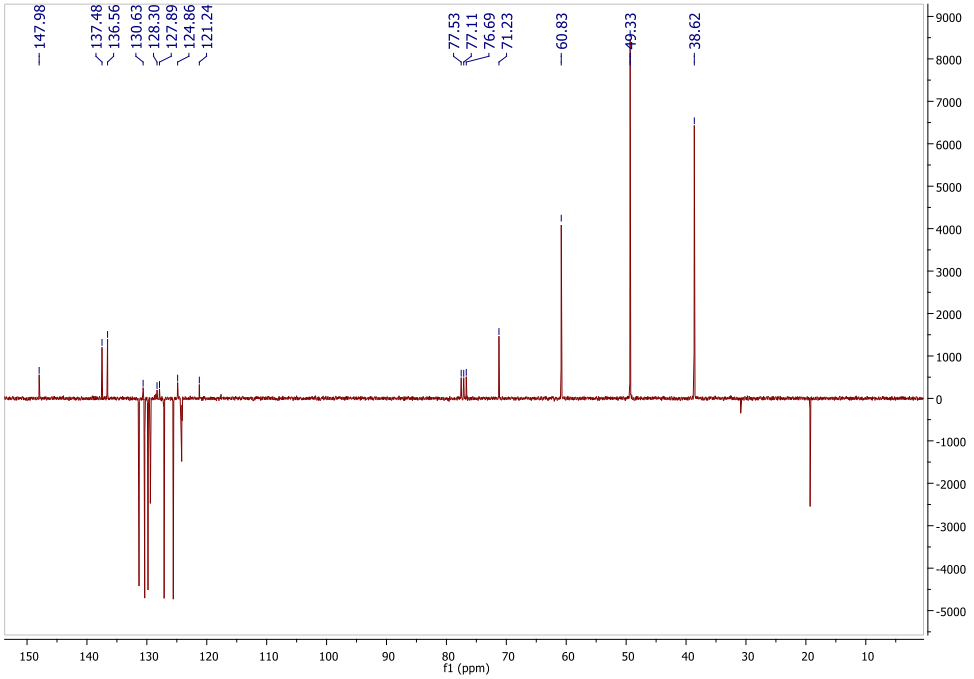


Figure 22: ^{13}C-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-ol

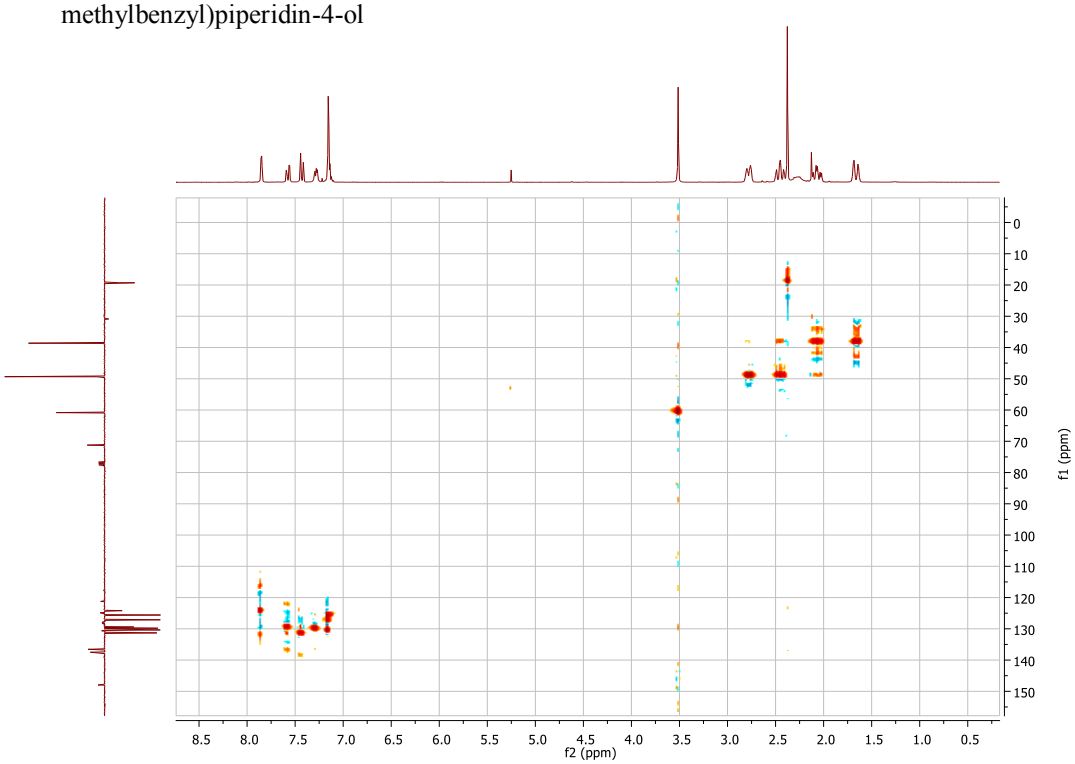
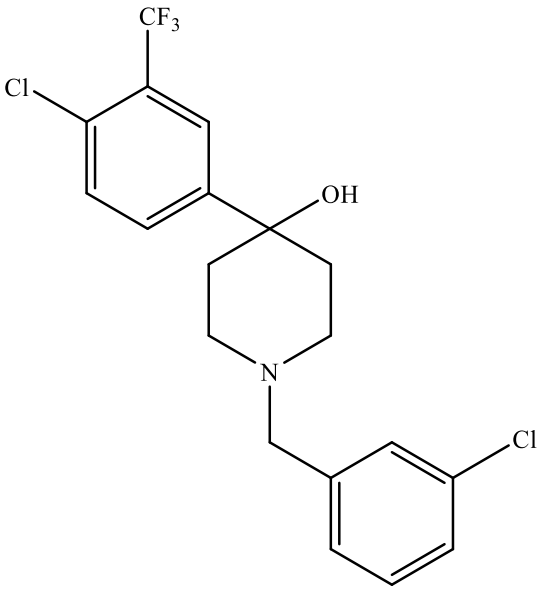


Figure 23: HSQC spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-ol

Reaction 9



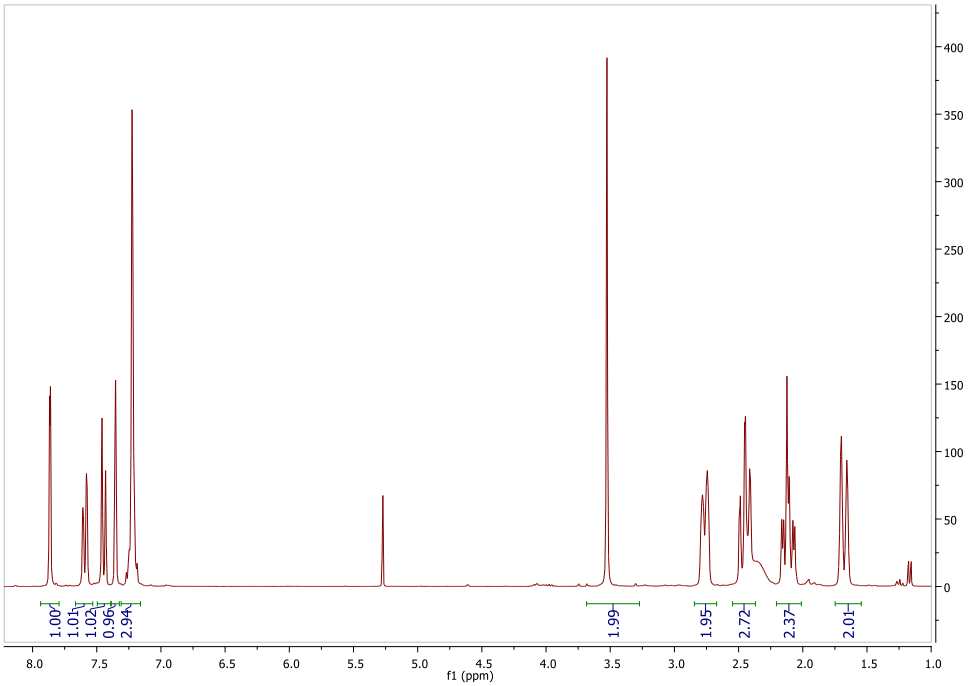


Figure 24: [1]H-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(3-chlorobenzyl)piperidin-4-ol

Reaction 9

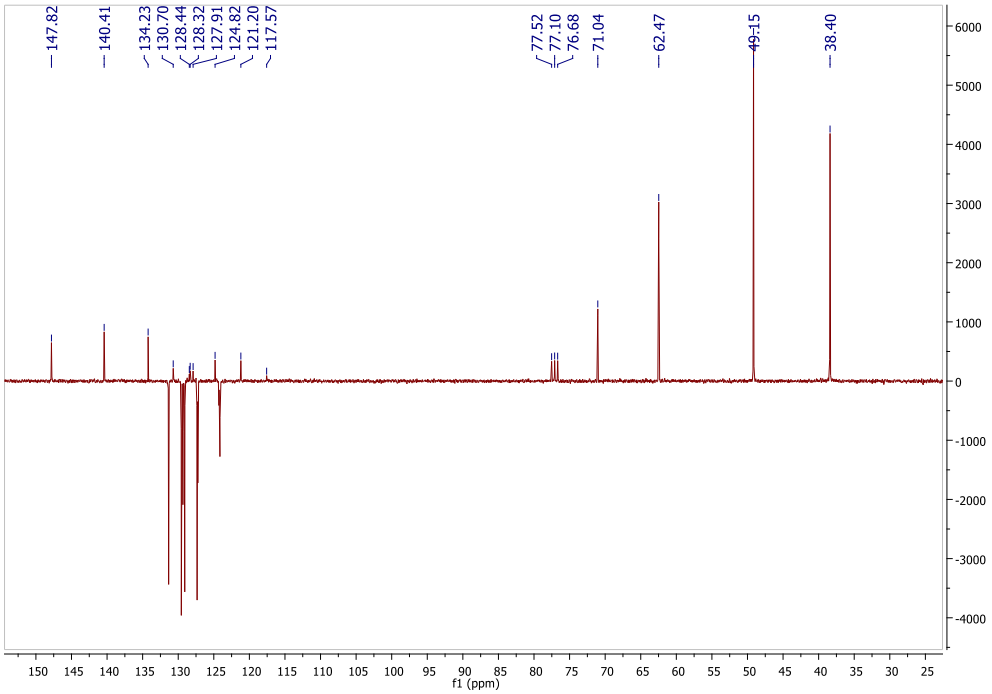


Figure 25: ^{13}C-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(3-chlorobenzyl)piperidin-4-ol

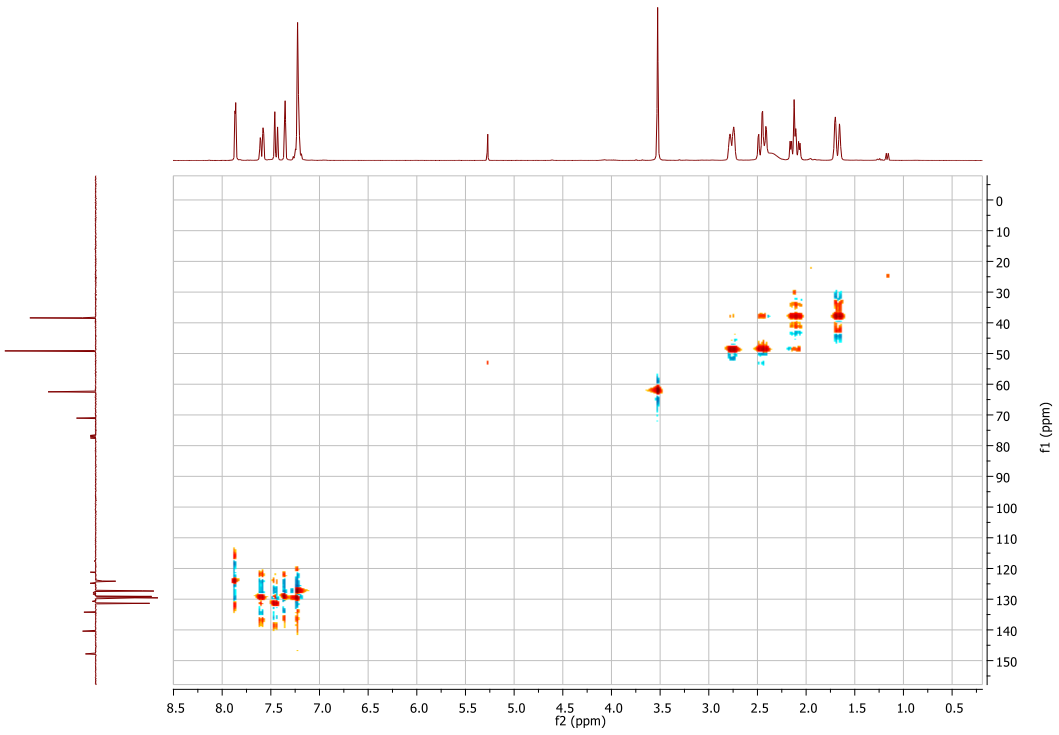
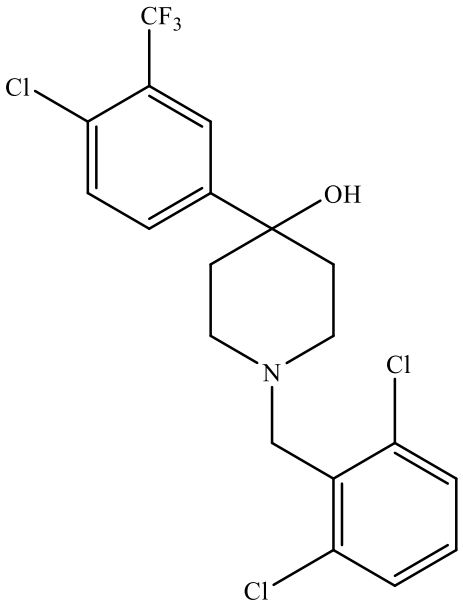


Figure 26: HSQC spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(3-chlorobenzyl)piperidin-4-ol

Reaction 11



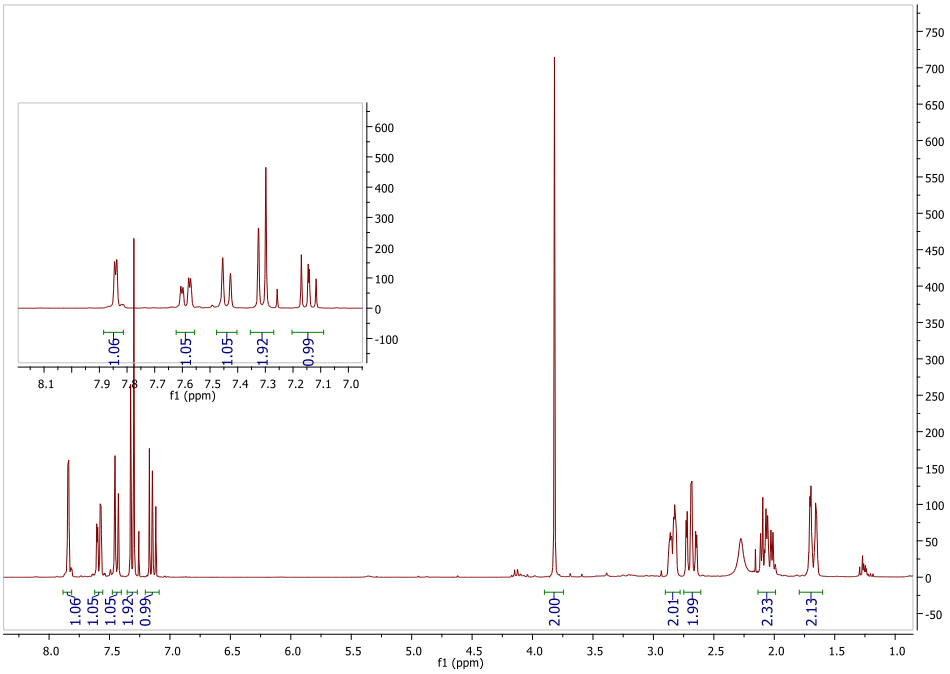


Figure 27: [1]H-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2,6-dichlorobenzyl)piperidin-4-ol

Reaction 11

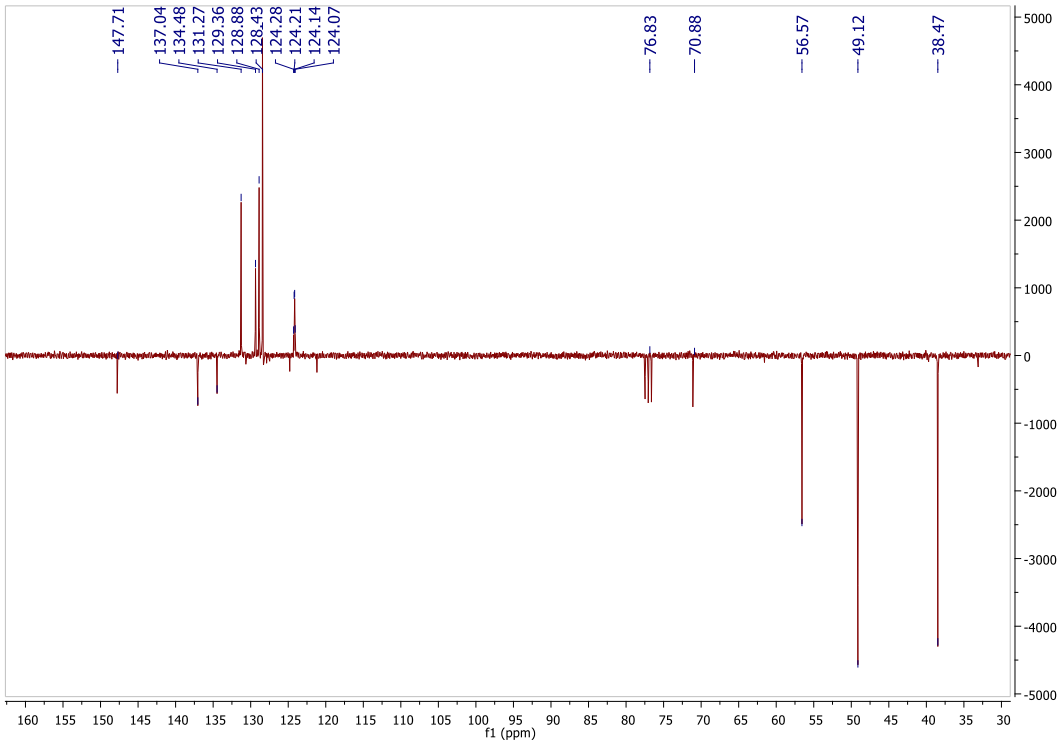


Figure 28: ^{13}C-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2,6-dichlorobenzyl)piperidin-4-ol

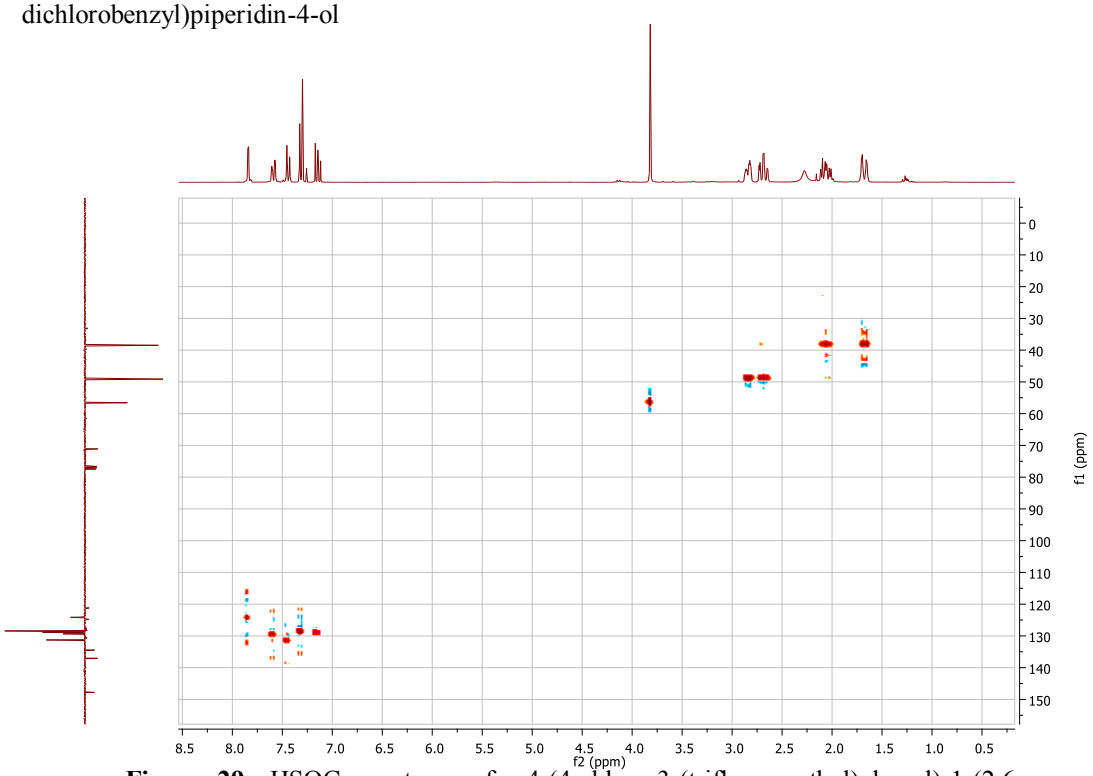
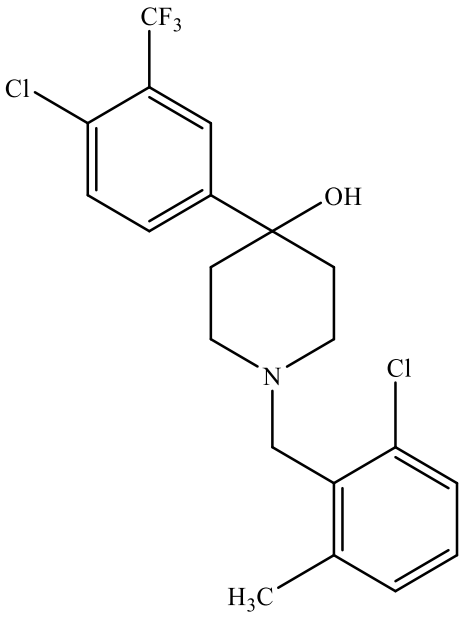


Figure 29: HSQC spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2,6-dichlorobenzyl)piperidin-4-ol

Reaction 12



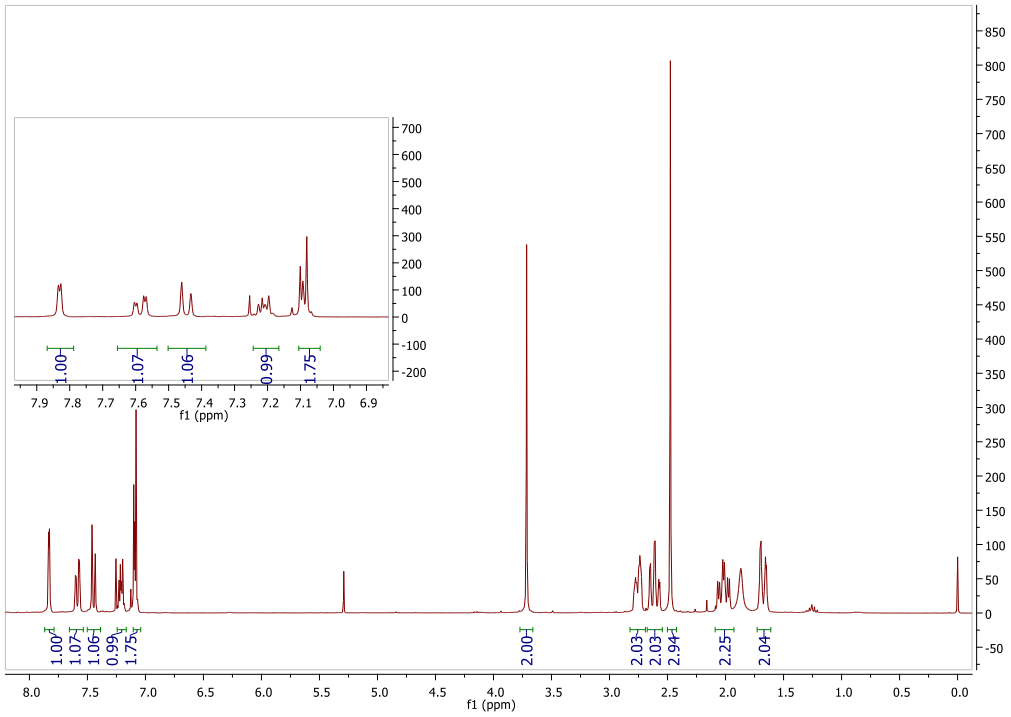


Figure 30: ^{1}H- NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-chloro-6-methylbenzyl)piperidin-4-ol

Reaction 12

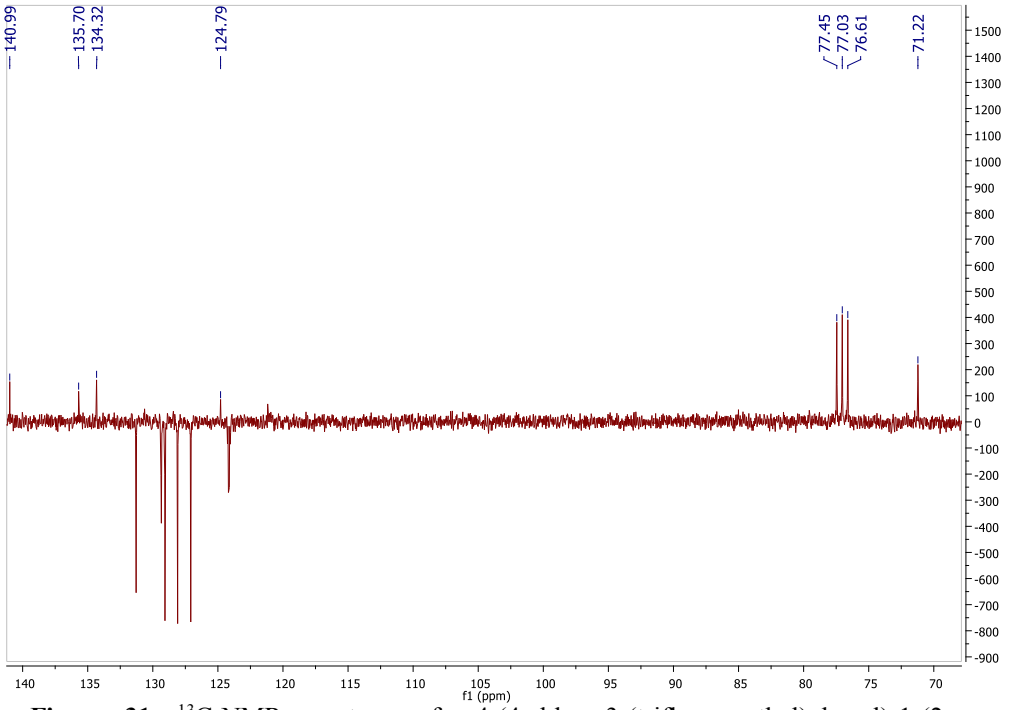


Figure 31: ^{13}C-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-chloro-6-methylbenzyl)piperidin-4-ol

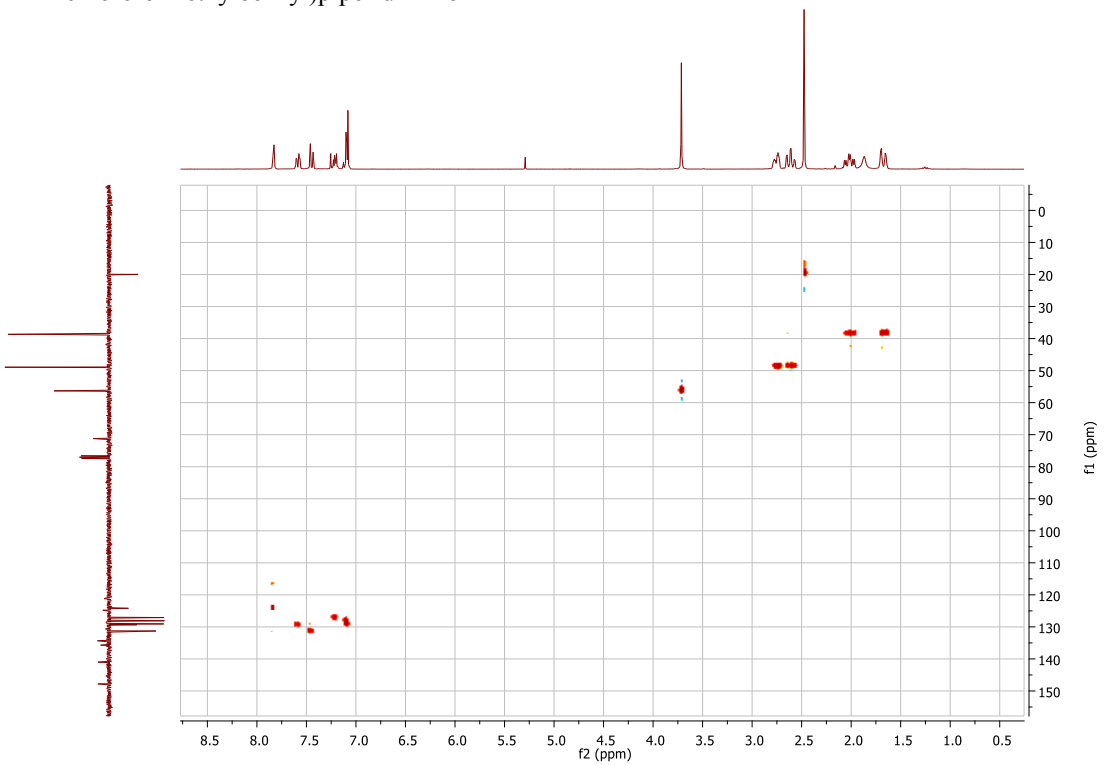
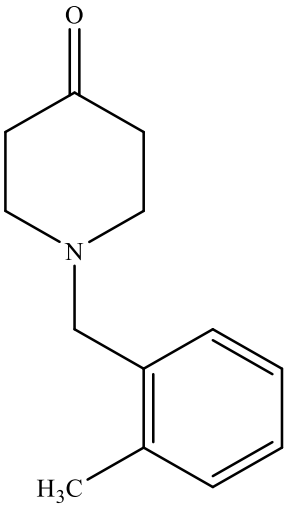


Figure 32: HSQC spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-chloro-6-methylbenzyl)piperidin-4-ol

Reaction 13



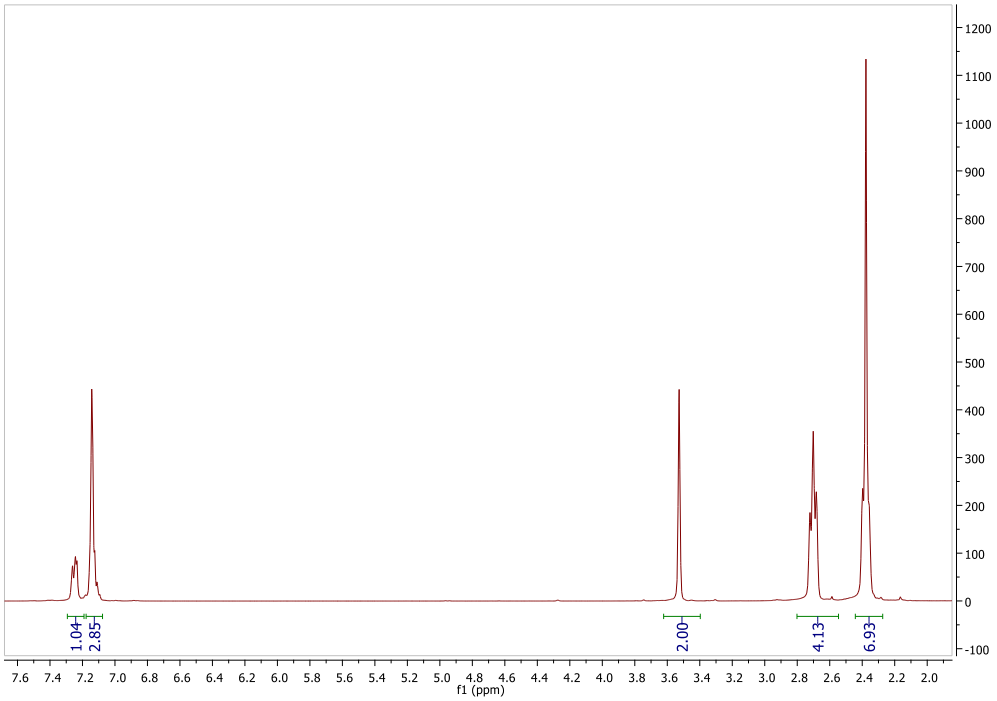


Figure 33: ^{1}H-NMR spectrum of 1-(2-methylbenzyl)piperidin-4-one

Reaction 13

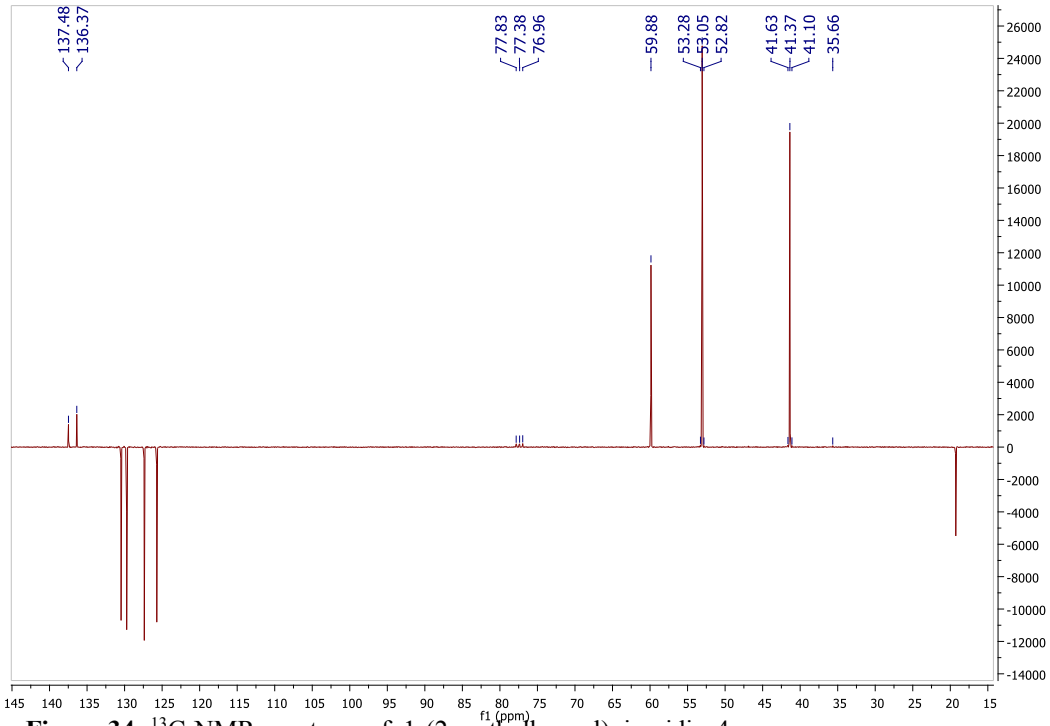


Figure 34: ^{13}C-NMR spectrum of 1-(2-methylbenzyl)piperidin-4-one

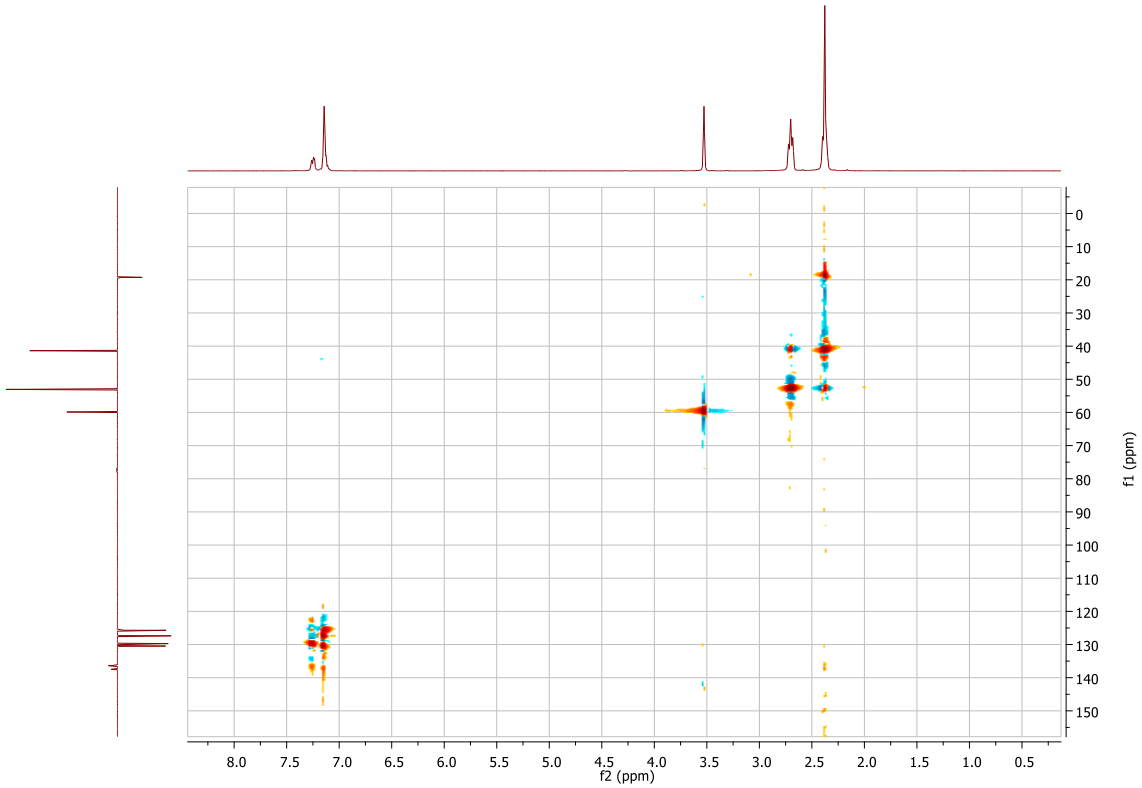
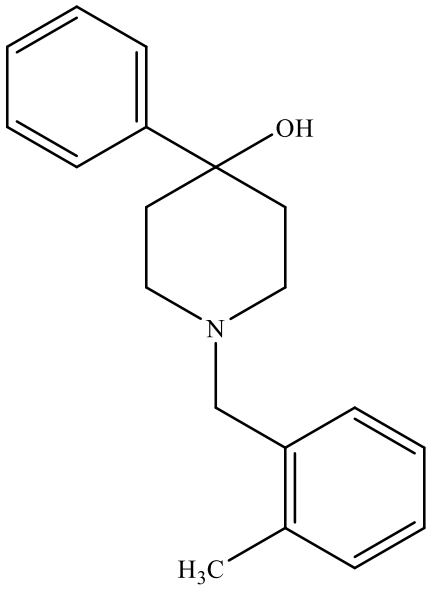


Figure 35: HSQC spectrum of 1-(2-methylbenzyl)piperidin-4-one

Reaction 14



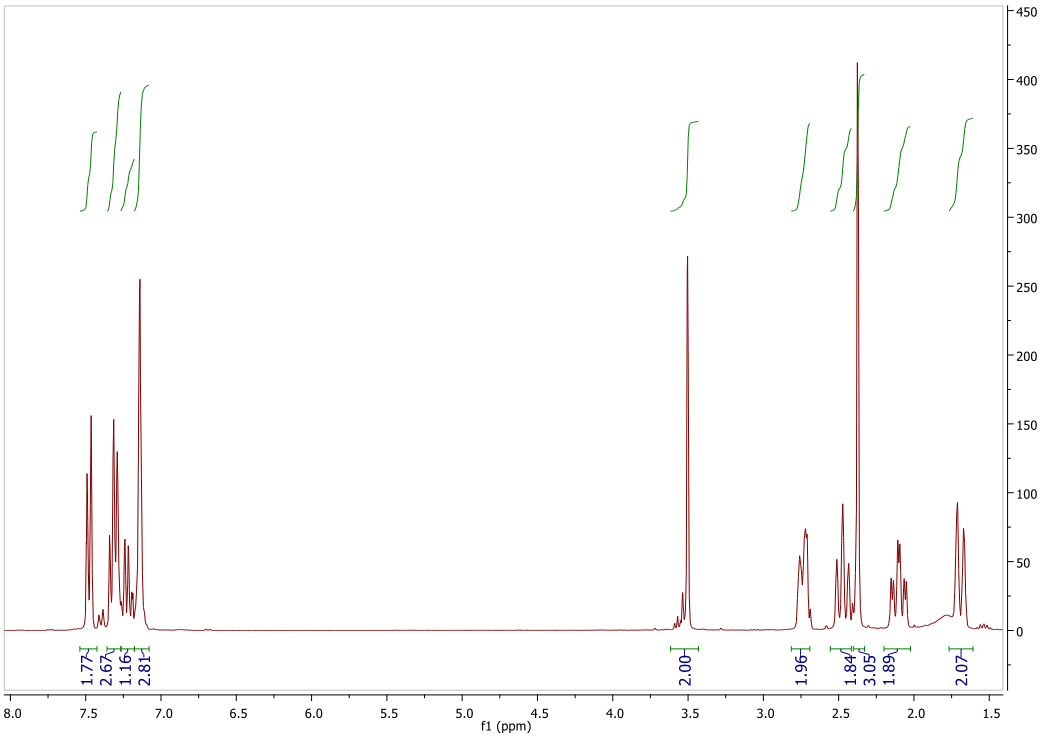
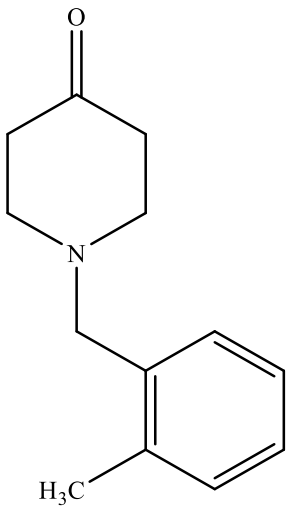


Figure 36: ^{1}H-NMR spectrum of 1-(2-methylbenzyl)-4-phenylpiperidin-4-ol

Reaction 15



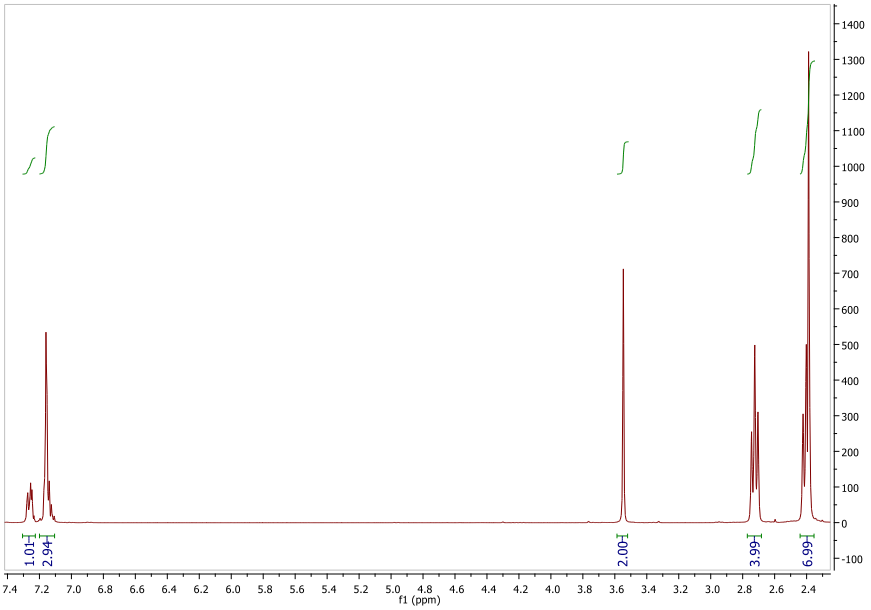


Figure 37: ^{1}H-NMR spectrum of 1-(2-methylbenzyl)piperidin-4-one

Reaction 15

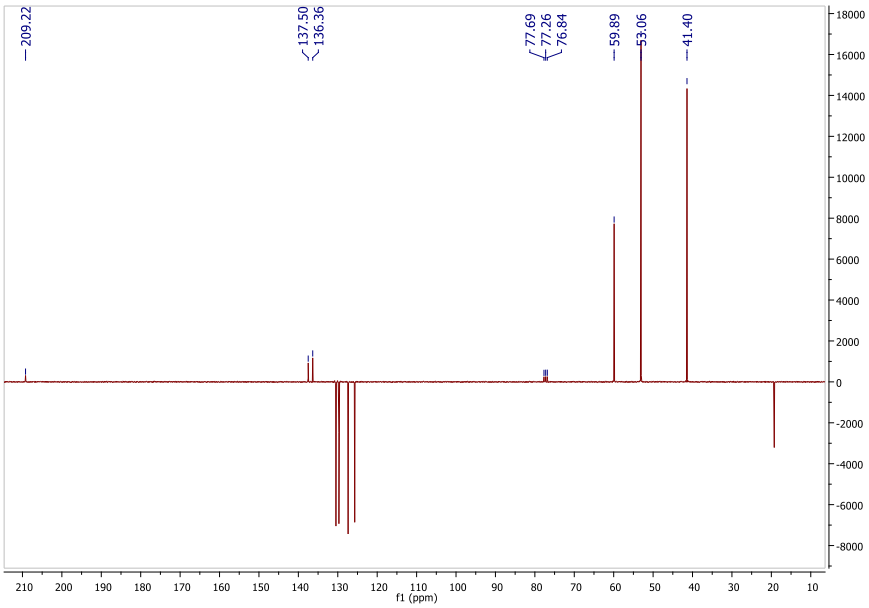


Figure 38: ^{13}C-NMR spectrum of 1-(2-methylbenzyl)piperidin-4-one

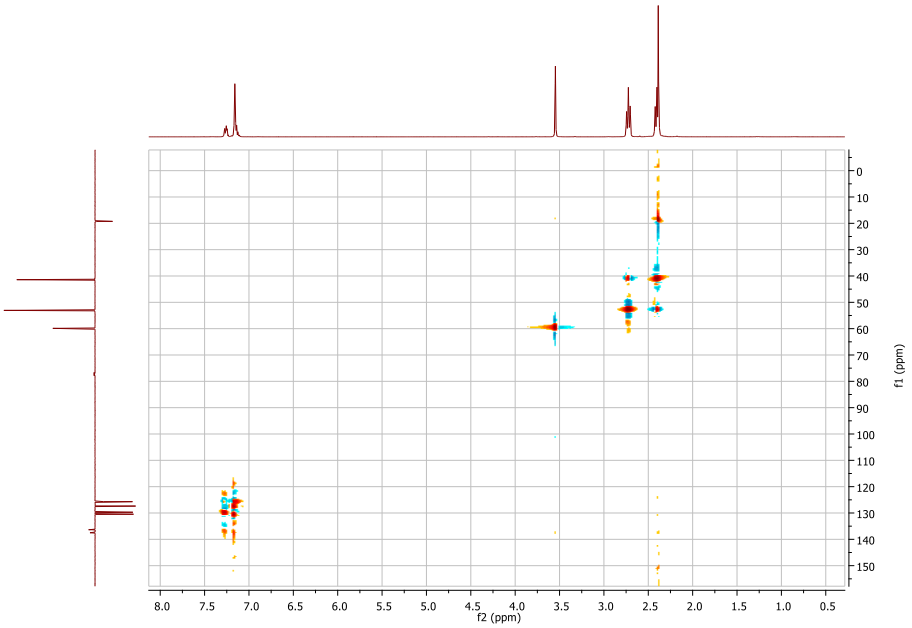
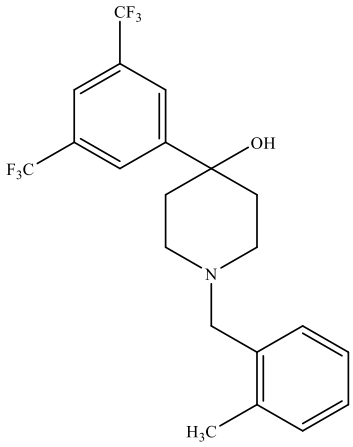


Figure 39: HSQC spectrum of 1-(2-methylbenzyl)piperidin-4-one

Reaction 16



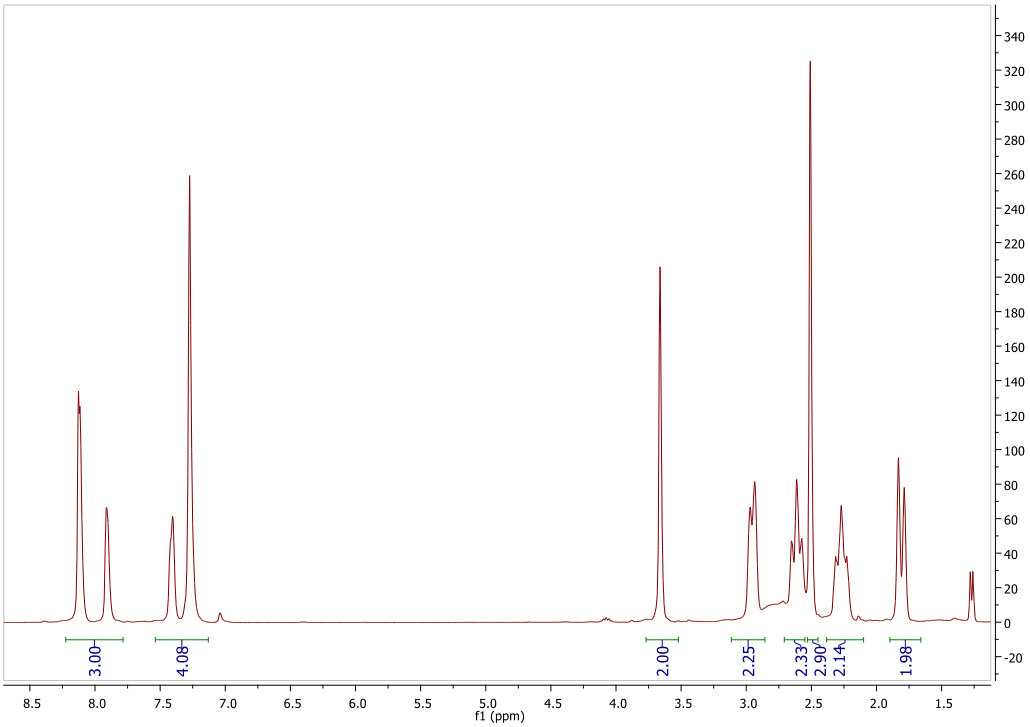


Figure 40: ^{1}H-NMR spectrum of 4-(3,5-bis(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-ol

Reaction 16

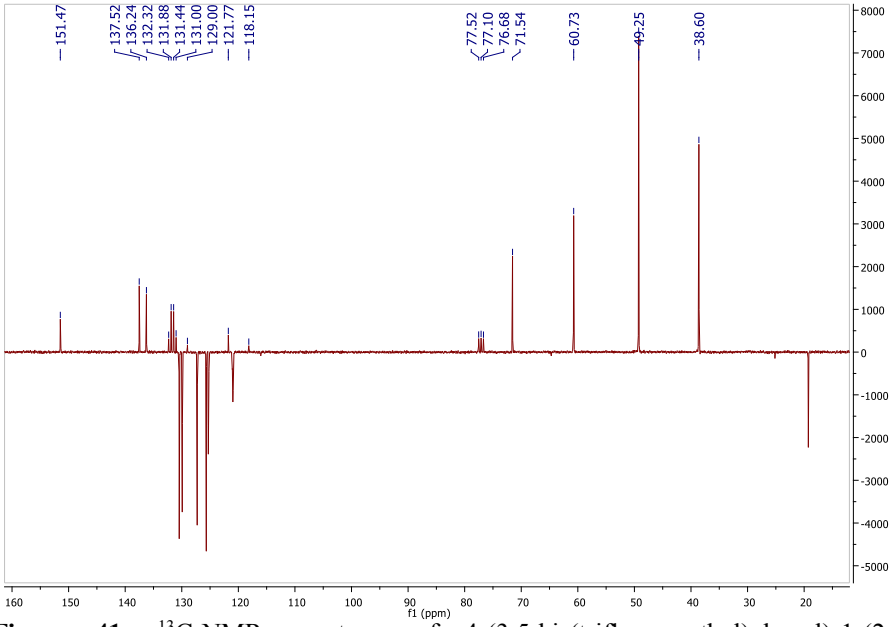


Figure 41: ^{13}C-NMR spectrum of 4-(3,5-bis(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-ol

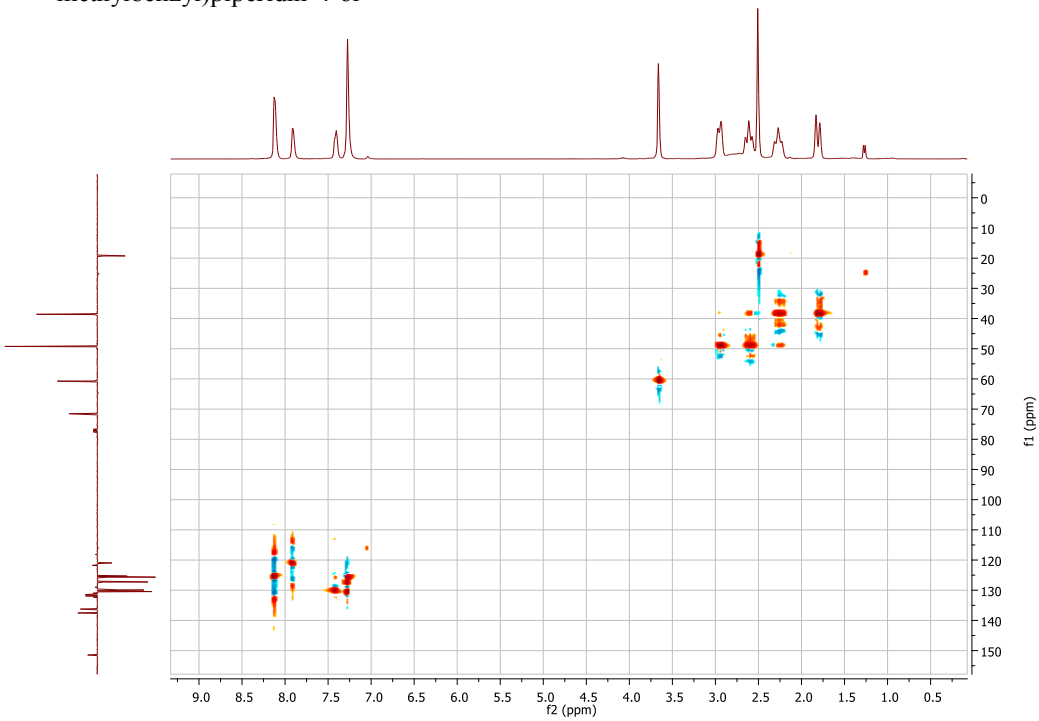
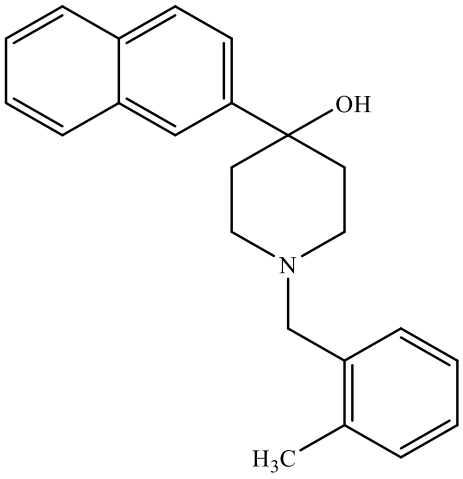


Figure 42: HSQC spectrum of 4-(3,5-bis(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-ol

Reaction 17



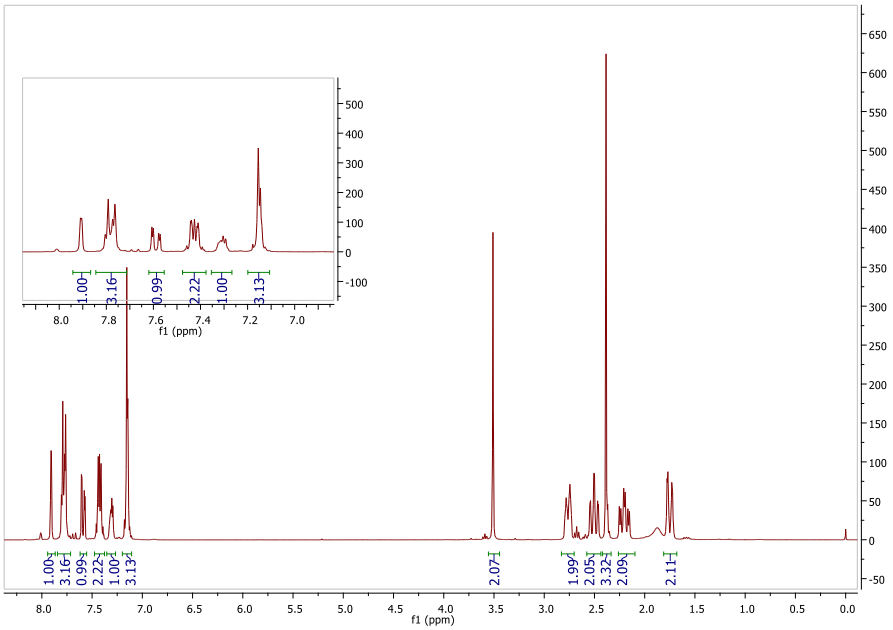


Figure 43: [1]H-NMR spectrum of 1-(2-methylbenzyl)-4-(naphthalen-2-yl)piperidin-4-ol

Reaction 17

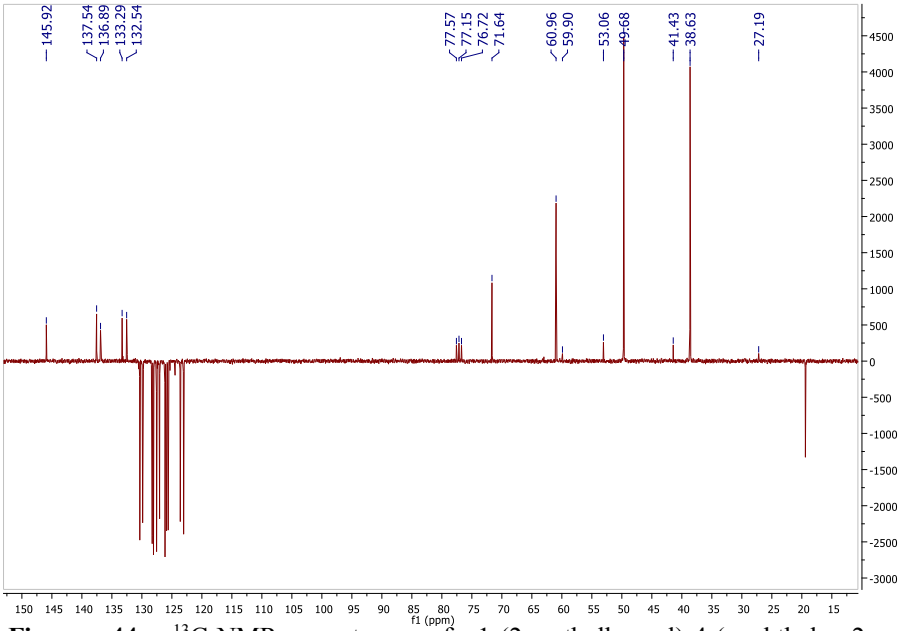


Figure 44: ^{13}C-NMR spectrum of 1-(2-methylbenzyl)-4-(naphthalen-2-yl)piperidin-4-ol

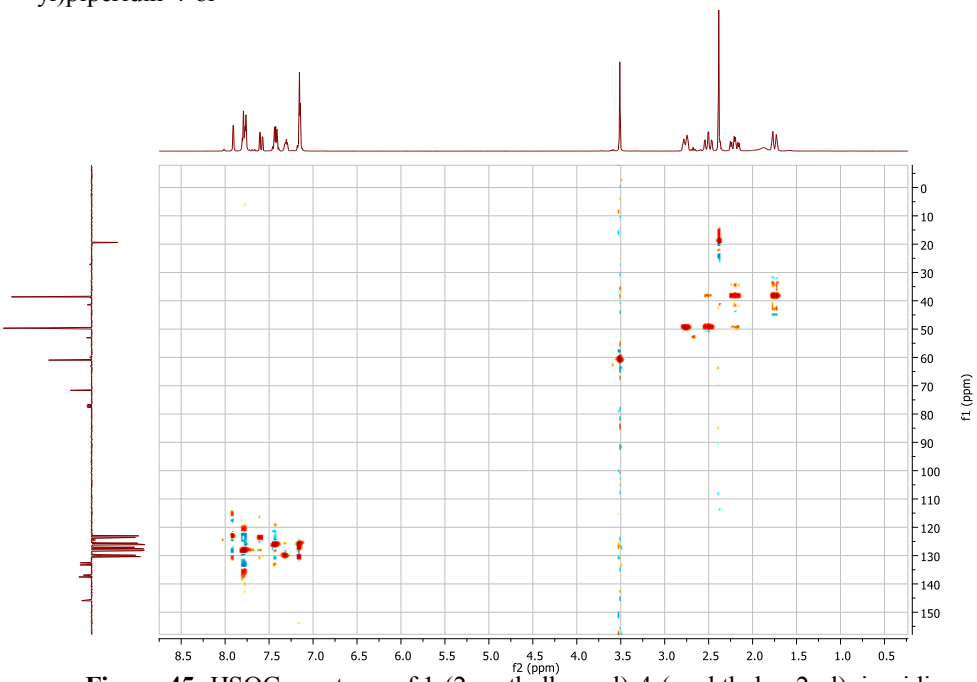
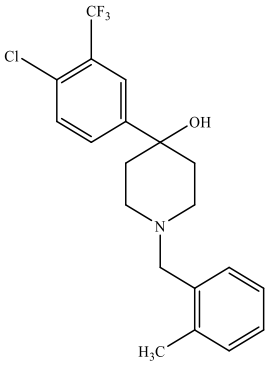


Figure 45: HSQC spectrum of 1-(2-methylbenzyl)-4-(naphthalen-2-yl)piperidin-4-ol

Reaction 18



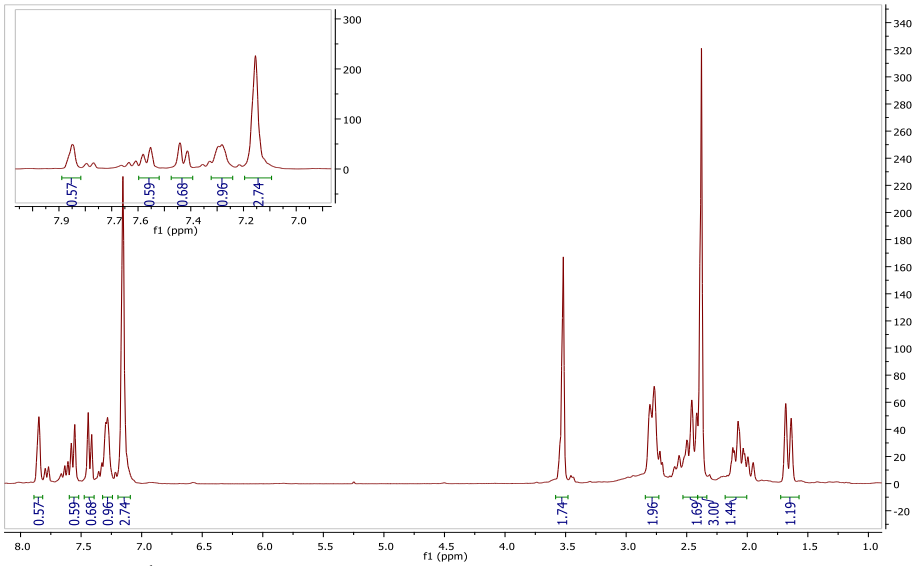


Figure 46: ^{1}H-NMR spectrum of 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-ol

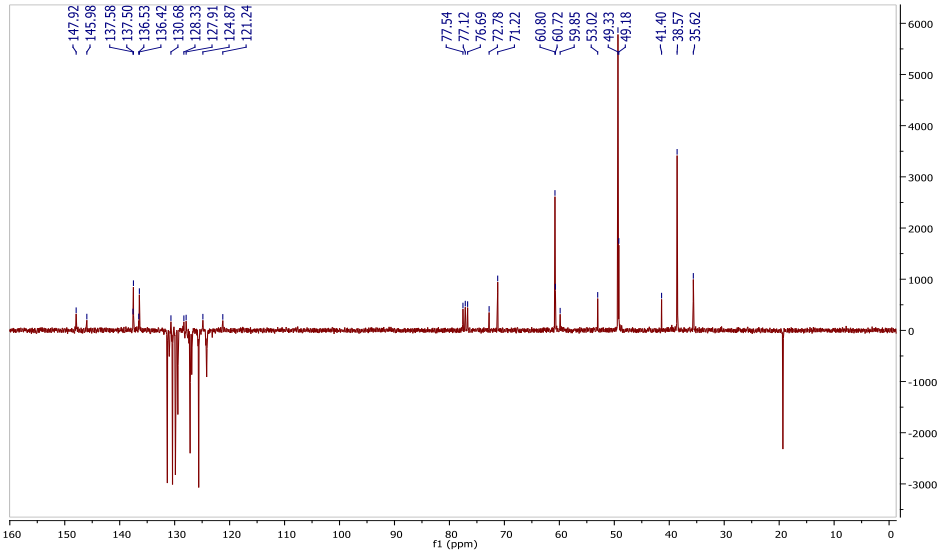
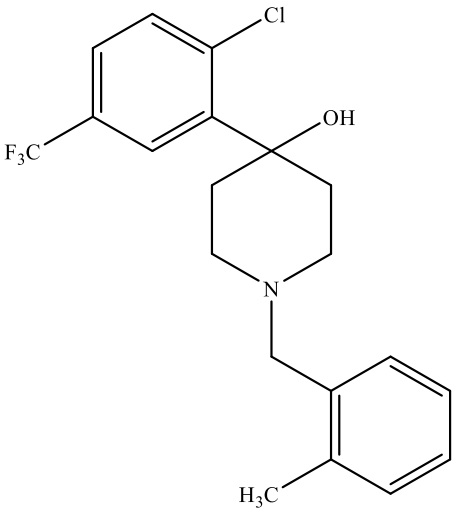


Figure 47: ^{13}C-NMR spectrum 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-ol

Reaction 19



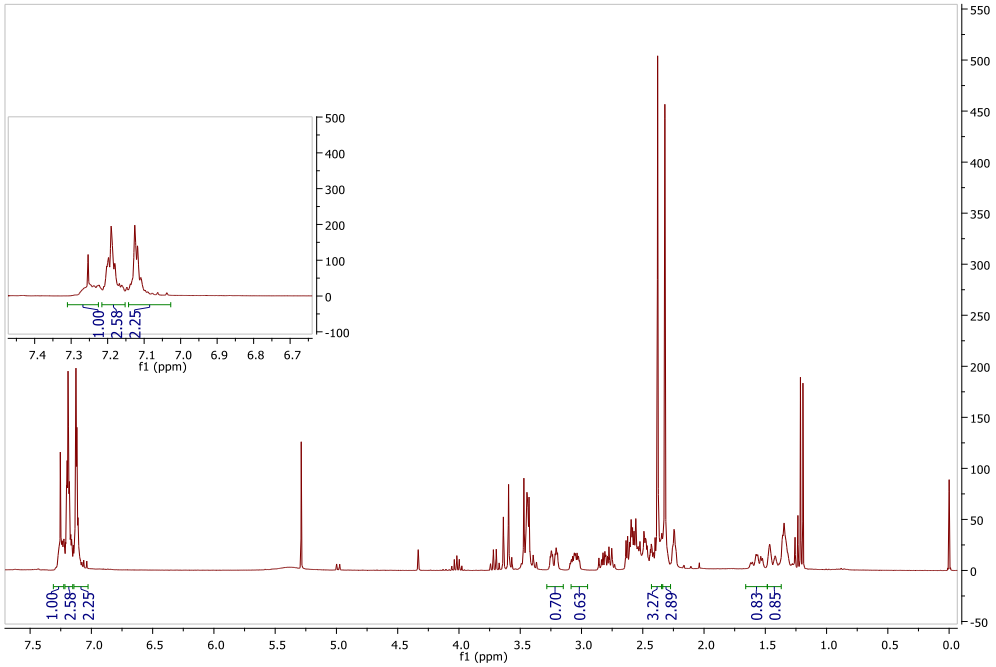


Figure 48: ^{1}H-NMR spectrum of 4-(2-chloro-5-(trifluoromethyl)phenyl)-1-(2-methylbenzyl)piperidin-4-ol