UNIVERSITAT ROVIRA i VIRGILI

Design of Novel Beta-blockers for the Retardation of Breast Cancer Metastasis

Research Project

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Bachelor's Degree in

Chemistry

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Abbreviations

^{13}C NMR	Carbon Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
ADR	Adrenergic Receptor
d	doublet
DCM	Dichloromethane
dd	double of doublets
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DNA	Deoxyribonucleic acid
dt	doublet of triplets
EtOAc	Ethyl acetate
h	Hour(s)
Hz	Hertz
J	Coupling constant
M	Molar
m	multiplet
mCPBA	3-chloropèrbenzoic acid
Min	minute(s)
mL	milliliter(s)
N	Normal
NIS	N-iodosuccinimide
NMR	Nuclear Magnetic Resonance
Pet ether	petroleum ether

pTsOH	p-toluenesulfonic acid
q	quadruplet
s	singlet
t	triplet
TLC	Thin Layer Chromatography
TsCl	Tosyl chloride

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Abstract

The synthesis of new analogues of the beta-blocker propranolol has been explored. According to recent studies, 2-biphenyls rather than naphthalene rings provide compounds with promising β_2-ADR inhibitor properties. The project aimed to produce new derivatives with a methyl group at different positions on the substituted phenyl ring of the biphenyl moiety. Methods for preparing the appropriate iodo-cresols were explored. Two such compounds, derived from the p-cresol and m-cresol, were then converted to the final compounds by Suzuki coupling, reaction with an epoxy tosylate and ring opening with isopropylamine. However, the final products could not be fully purified and only small amounts with a few impurities were obtained. All compounds were characterized by ^{1}H and ^{13}C NMR.

1. Aim

The aim of this project is to synthesize and characterise β_2-adrenergic receptor antagonists with a stronger antimetastatic effect than that of propanolol, that is to synthesize compounds with a similar structure to it.

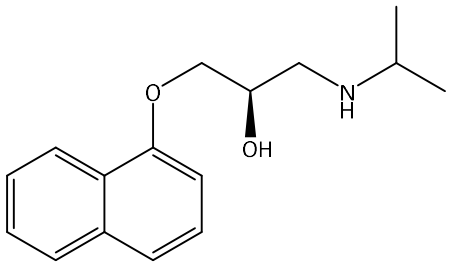


Figure 1-1 Propanolol

2. Introduction

2.1 Cancer

Cancer is one of the leading causes of death, killing millions of people around the world each year. Last year, in 2018, there were 17 million new cases of cancer worldwide and 9.2 million deaths by cancer reported. The four most common cancers occurring worldwide are lung, female breast (there are fewer cases of men breast cancer), bowel and prostate cancer. It is estimated that there will be 27.5 million new cases of cancer worldwide each year by 2040.[1]

Cancer may also be called a tumour and, although this may be correct, not all tumours are carcinogenic, in fact tumours are classified as benign and malignant. A tumour is the result of an uncontrolled proliferation in cells, which means that cells divide at a too high rate. The difference between a malignant and a benign one, is that the benign although growing, does not invade other tissues, whereas, malign ones are capable of invading other tissues and parts of the body (metastasize). When speaking of cancer, we are therefore referring to malignant tumours, which can proliferate and invade other regions of the body.

Cancer is sometimes misconceived as a single disease but is in fact a collection of highly heterogeneous diseases that occurs due to genetic mutations that lead to abnormal cell behavior and metabolism. The accumulation of these mutations result in the progression from normal cells to cancerous providing the last ones with characteristics that normal cells do not

possess such as: uncontrolled cell growth (they can become resistant to growth inhibitors), ability to replicate without limit (their telomeres do not degenerate in each replication due to a telomerase enzyme), evade cell death (apoptosis), and migrate and invade other tissues and organs around the body (metastasize).[2]

There are several ways of classifying a cancer, however the most used worldwide is the classification depending on the tissue of origin, the types of cells from which they are derived. The three main types of cancer are: carcinomas (derived from epithelial cells), lymphomas (derived from blood and immune system cells, lymphocytes) and sarcomas (which arise from mesodermal cells).[3] The most common type of cancer in men is prostate cancer whereas in women is breast cancer.[1]

2.2 Carcinogenesis

The adult human body is composed of approximately 10^{15} cells many of which are required to divide and differentiate in order to repopulate organs that need cell turnover. Some examples are the cells that compose the epithelial layer of the intestine that require a replacement approximately every 10 days or the cells in the basal layer of the skin that differentiate, divide and are finally sloughed. In the human body there are approximately 10^{12} divisions per day and even in organs that show low levels of cell division, such as the liver, a massive proliferation may occur due to a trauma or infection. It is important to remember that chemical damage to DNA itself is not a mutagenic event, it is a compilation of replication and subsequent cell division to convert the chemical damage to an inheritable change in DNA (mutation).[2,13] Therefore, the higher the division rate the higher the possibility of a mutation occurring, in fact, there are millions of mutations in our body daily but normally the cell dies due to the mechanism that regulates cell division (the immune system), and it is only the cells that get to evade this mechanism that have a possibility on becoming carcinogenic.

One of the biggest problems in the treatment of cancer is that divisions take place in the whole body therefore cancer can occur in any of the different kinds of cells within the human body, hence there are hundreds of different types of cancer, each of which are greatly different in morphology, characteristics and response to treatments.[2,3,4]

Spontaneous DNA mutations can occur directly as an error in the replication process or indirectly due to chemical damage leading to an error in the DNA reading and replication by the DNA polymerase. It is a fact that the DNA is constantly suffering chemical damage and that is not

strange because the DNA is a molecule that exists at 37°C in an aqueous environment in the middle of a cell whose existence depends upon making and breaking chemical bonds. Some of this damages are a consequence of spontaneous thermal effects or as consequence of a chemical attack by another molecule.[13]

The violation of just three fundamental processes which regulate cell growth is enough for a cell to go from normal to cancerous. The first rule is that cells should only grow and divide when they receive the appropriate stimulus (cancer cells do not need signals to activate its division). The second rule is that when a cell encounters adverse conditions (provoking stress) it undergoes a pre-programmed self-destruction via processes such as apoptosis instead of allowing DNA replication to occur which could result in DNA damage (cancer cells may avoid this process). Finally, the third rule is that normal cells can only divide a pre-determined number of times because of their telomeres shortening each time they divide until they are gone, in which case the cells can no longer divide. However, cancerous cells can activate an enzyme called telomerase, which keeps the telomeres from shortening, adding on new repeats to the end of each chromosome (on the telomeres), thus allowing infinite self-replication.[2]

The main risk factors that can increase the rate of developing cancer are: lifestyle (smoking, lack of exercise, drinking, drugs), genes and physiology and the surroundings.[3]

2.3 Metastasis

Metastasis is the process by which cancerous cells pertaining to a primary mass, called the primary tumour, migrate and invade other areas of the body creating what are called secondary tumours, also called metastases. This ability of cancerous cells is also one characteristic that distinguishes them from healthy cells.

Once the cancer metastasizes the hope of recovering drastically decays, making it the main cause of cancer related deaths. Tumours that remain localized in a single spot are normally curable as long as they can be removed surgically. In general, metastatic cells tend to be much more aggressive than their non-metastasized counterparts.[2,3]

Currently there is no known method as to how metastasis takes place, but there are two general models accepted based on specific genes that allows the cancerous cell to metastasize. The first is the genetic model which suggests that a metastatic phenotype is determined early when the tumour is formed by a group of genetic mutations. The second is the epigenetic model, which suggests that it is an external stimulus who induces a metastatic phenotype (such as

neurotransmitters and hormones) by enhancing the migratory and invasive capabilities of cancer cells.[2]

The process of metastasis is a highly complex and poorly understood process, sometimes referred to as "metastatic cascade". It is known that this metastatic cascade is a highly effective process and only 1 in 10,000 survive it.[2]

The site of metastasis is normally ruled by the route of dissemination; however, it is thought that there are preferring sites of secondary tumour formation depending on the type of cancer. For example, metastatic breast cancer cells have a need for calcium, therefore they migrate to places rich in it, for example to the bones.[3]

2.4 Breast cancer

Breast cancer is the most common cancer in women worldwide and the leading cause of female death causing millions of deaths per year. There were over 2 million new cases of breast cancer in 2018.[5]

However, as it has been stated before, the main cause of death caused by breast cancer is its metastasis to surrounding tissues, generally to the bones and lung who are rich in calcium.

A characteristic that differentiates breast cancers comes from the expression of hormonal steroid receptors such as: the progesterone receptor (PR), the oestrogen receptor (ER) and the Human Epidermal Growth Factor Receptor 2 (Her2) and basal cytokeratin which are usually the target of cancer therapeutics. However, there is a class of cancer known as basal-type or triple-negative that lack these hormones making it harder to find its correct treatment options.[2]

2.4.1 B_2-adrenergic receptors

A number of laboratories studies have shown that stress hormones play a crucial part in the formation of breast cancer's secondary tumours increasing its migratory and invasive capacity. The stress is mediated through the stimulation of the β_2-adrenergic receptor by the stress hormone norepinephrine. β_2-adrenergic receptors are a subtype of adrenoceptors which are G-protein coupled receptors (GPCRs) who constitute a large protein family of receptors in the cellular membrane that detect molecules outside the cell and activate internal signal transduction pathways (transforming one stimulus into another) and cellular responses.[2,14]

Many breast cancer cell lines have been found to express an elevated concentration of the β_2-adrenergic receptor and, interestingly, the basal-type (that was difficult to treat due to the lack expression of the usual hormone targets) presents the highest concentration among the other classes.[2]

When facing a stress situation, the body's stress system, mediated by the neuroendocrine system, tries to counteract the effects of stressors by activation of adaptive processes while attempting to reach the homeostatic state again. Current studies have shown that chronic stress (stress produced over a prolonged period of time) may play a role in tumour progression and the formation of metastases by means of inducing DNA damage. They have also shown that the positive influence in the metastasis process of stress via the stimulation of the β_2-adrenergic receptor can be blocked by β-adrenoceptor antagonists such as Propanolol. For this reason, the inhibition or retardation of stress induced breast cancer metastasis by blockade of the β_2-adrenergic receptor could be a potential and promising therapeutic option.[2,14] There is some evidence that use of a biphenyl, connected by its 2-position, rather than a naphthalene ring (found in propranolol) provides compounds with promising β_2 -ADR inhibitor properties, so in this project we will aim to make further analogues, concentrating on just adding a methyl group to the substituted phenyl ring.[16]

3. Experimental part

3.1 Synthetic route

As explained before the objective of this project is to synthesis compounds with higher antimetastatic properties than that of Propranolol therefore the objective molecule has to have some highly conjugated system really close to the ether group such as a phenyl substituent as reported by some studies.[16] The starting material proposed to use are the isomers o-cresol, m-cresol and p-cresol.

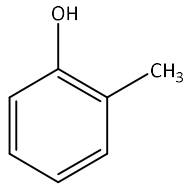


Figure 3-1 o-cresol

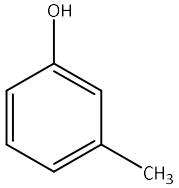


Figure 3-2 m-cresol

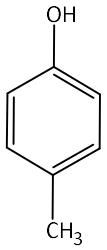
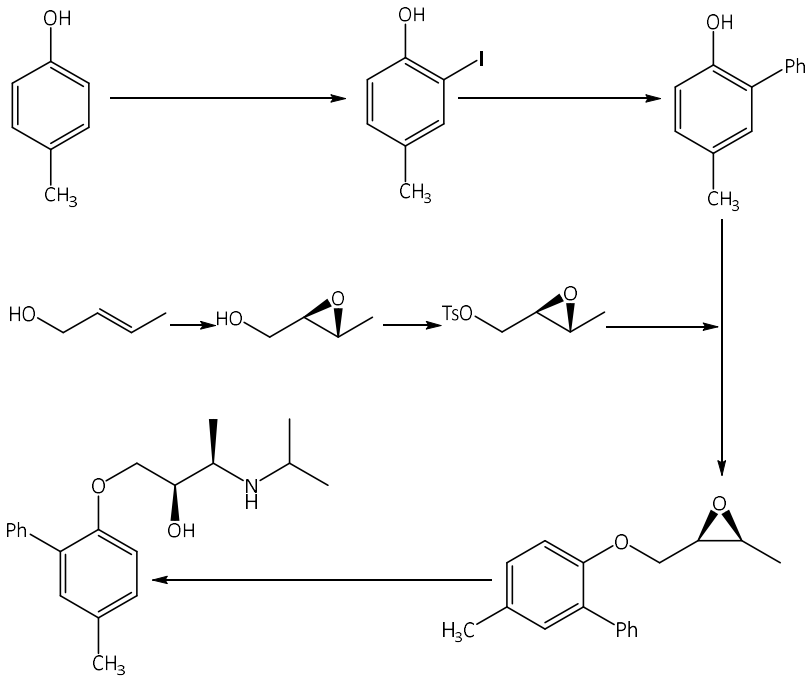


Figure 3-3 p-cresol

Another difference referring the propranolol is that there is a methyl group in the aromatic ring which will also give some steric strain (but a minimal one). One way to attach a phenyl group to an aromatic ring is by a coupling reaction such as a Suzuki reaction, which works well with iodine substrates.

On the other hand, in order to introduce the ether-linked side chain present in the Propanolol into the final compound, another compound is synthesised starting from crotyl alcohol. The first step is to epoxidase the double bond in the crotyl alcohol. Afterwards, the alcohol group is converted to a good leaving group such as a tosylate group so that in the next step, when the phenol group is introduced the nucleophilic attack takes place at the tosylate rather than at the epoxide. Finally, the epoxide is opened using isopropylamine as a nucleophile obtaining the desired product.

An example of the synthetic route using p-cresol as starting material is shown in Scheme 3.1.



Scheme 3.1 Synthetic route to the propranolol analogue using p-cresol as starting material

3.2 General considerations

This project was carried out in the CELS/NSCR building from the Clifton Campus of the Nottingham Trent University under the supervision of professor John D Wallis. The NMR experiments were performed in the Rosalind Franklin building.

All reagents were bought from Fisher Scientific, Sigma Aldrich, Fluorochem and ACROS organics. Anhydrous ethanol and N,N-dimethylformamide were bought from Sigma Aldrich. Dry DCM was dried using the solvent drying system MB SPS-800.

Reactions were monitored using the thin layer chromatography technique (TLC). The TLC were done in Silica glass plates (2.5 x 7.5 cm) bought from Merk Millipore as "Silica gel 60 F_{254}" and revealed with UV light (in case of compounds with conjugated structures), iodine (crotyl alcohol) and phosphomolibdic acid solution (for the epoxide). Silica gel columns were done with Silica gel 60 angstrom pore size (40-63 Micron) and the columns were packed using the slurry method and loaded using the dry loading method.

NMR spectra were recorded on a ECZ 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) and a ECX 400 (400 MHZ for ^{1}H and 100 MHz for ^{13}C) spectrometer. The ^{1}H and ^{13}C spectra were referenced to the deuterated chloroform signals 7.26 and 77.16 ppm respectively.

3.3 Toxicity and handling of the used compounds

In this section a Table with the more representative reagents used for this project is shown. Information such as purity, toxicity and handling appears in the Table and have been extracted from the SDS of the sources where they were bought from (mainly Sigma Aldrich).

Table 3.3 Purity, toxicity and handling of the reagents used during the project

Compound	*Purity (%)*	*Toxicity*	*Handling*
2-amino-3-methylphenol	96		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Acetic acid glacial	≥99.85		Wear a lab coat, glasses, nitrile gloves and work under the fume hood. Keep away from heat sources.
Acetone			Wear a lab coat, glasses, nitrile gloves and work under the fume hood. Keep away from heat sources.
Acetonitrile			Wear a lab coat, glasses, nitrile gloves and work under the fume hood. Keep away from heat sources.

Ammonium iodide			Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Acetic anhydride			Wear a lab coat, glasses, nitrile gloves and work under the fume hood. Keep away from heat sources..
Crotyl alcohol	Cis and trans mixture 96%		Wear a lab coat, glasses, nitrile gloves and work under the fume hood. Keep away from heat sources.
DCM			Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
DMAP			Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
DMF			Wear a lab coat, glasses, nitrile gloves and work under the fume hood.

Ethyl Acetate			Wear a lab coat, glasses, nitrile gloves and work under the fume hood. Keep away from heat sources.
Hexane			Wear a lab coat, glasses, nitrile gloves and work under the fume hood. Keep away from heat sources.
Hydrogen peroxide	30		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
m-chloroperbenzoic acid	77		Wear a lab coat, glasses, nitrile gloves and work under the fume hood. Use a plastic spoon to manipulate it.
m-cresol	99		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Methanol			Wear a lab coat, glasses, nitrile gloves and work under the fume

			hood. Keep away from heat sources.
NIS			Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
o-cresol	98		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Palladium(II) acetate			Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
p-cresol	≥99		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Phenylboronic acid	≥99		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Potassium carbonate	≥99		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Propan-2-ol	99.5		Wear a lab coat, glasses, nitrile

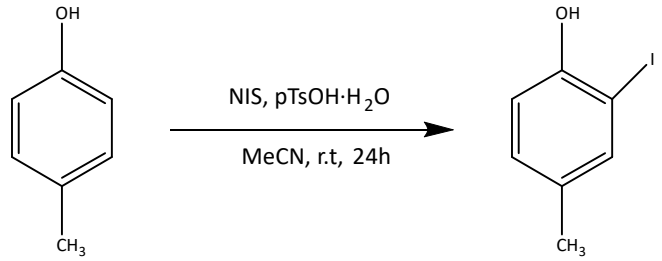
			gloves and work under the fume hood. Keep away from heat sources and from water.
p-toluenesulfonic acid monohydrate	98		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Sodium bicarbonate	99.7	-	Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Sodium carbonate	99.9		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Sodium hydride	60		Wear a lab coat, glasses, nitrile gloves and work under the fume hood. Keep away from heat sources and from water.
Sodium hydroxide (solution)	1N		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.

Sodium nitrite			Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Sodium thiosulfate (solution)	saturated		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Tosyl chloride	99		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Trifluoromehtanesulfonic acid	≥99		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.
Triethylamine	≥99.5		Wear a lab coat, glasses, nitrile gloves and work under the fume hood. Keep away from heat sources and from water.
Triphenylphosphine	99		Wear a lab coat, glasses, nitrile gloves and work under the fume hood.

3.4 Compound's synthesis procedure

3.4.1 From p-cresol

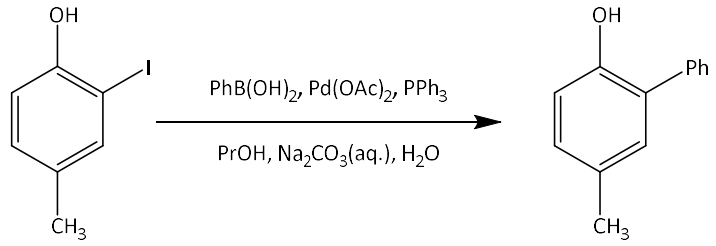
Synthesis of the 2-iodo-4-methylphenol[9]



pTsOH·H2O (17 mmol, 3.233g, 17 mmol) was added to a stirred solution of p-cresol (1.864g, 17 mmol) in acetonitrile (17 ml) at room temperature. After 10 min of stirring at this temperature, NIS (3.824g, 17 mmol) was added in one portion to give an immediate orange solution.

After 24h the solution turned light orange and a TLC using DCM: hexane (1:1) was taken to check the reaction's progress. The reaction was quenched by adding a saturated $Na_2S_2O_3$ solution (10 ml), which changed the colour of the solution to yellow. The mixture was extracted three times with ethyl acetate (3 x 10 ml). The combined organic layer was washed successively with water and brine and dried over magnesium sulphate. Finally the residue was purified by silica gel column chromatography (DCM:hexane= 2:8) to afford the product (3.321 g, 81 %) as a yellow oil. **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.47 (d, J=2.2 Hz, 1H), 7.00 (dd, J=8.2, 2.1 Hz, 1H), 6.86 (d, J=8.2 Hz), 2.23 (s, 3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 152.85, 138.67, 131.99, 130.84, 114.96, 85.3, 20.15.

Synthesis of the 2-phenyl-4-methylphenol[10]



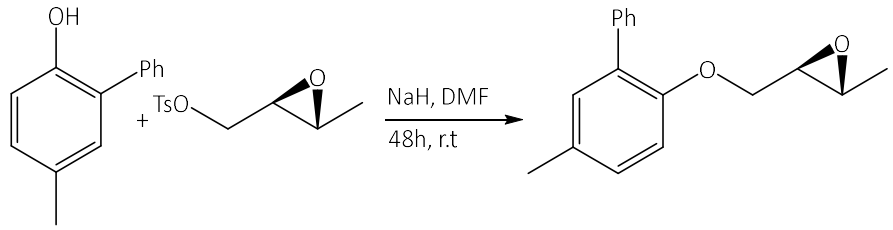
The reaction was carried out under inert conditions to avoid oxygen which could interfere with the Pd reagent.

The 2-iodo-4-methylphenol (4.469 g, 19 mmol), and the phenylboronic acid (2.434 g, 19.997 mmol) were added into a 100 mL two-necked round bottom flask. 2-Propanol (34.2 ml) was added and the system was left degassing under N_2 atmosphere and stirring to form a solution.

After 20 min of degassing, a 2M aqueous solution of Na_2CO_3 (11.4 ml) was added followed by deionized water (6.7 ml) and the mixture was left degassing again for 20 min.

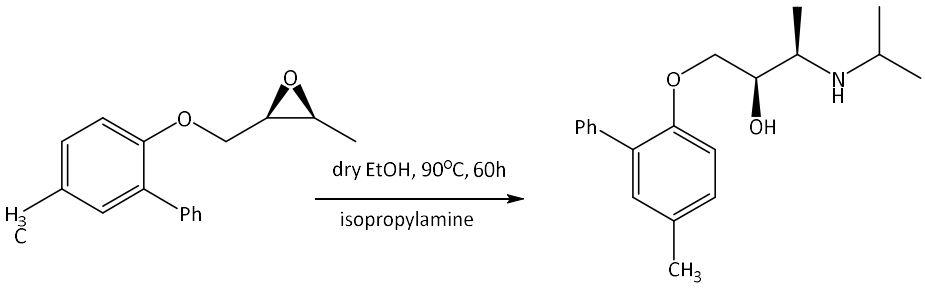
The $Pd(OAc)_2$ (0.015 g, 0.067 mmol), and PPh_3 (0.055 g, 0.2 mmol) were added and an air condenser was connected to the system, degassed for 15 min, and then heated to 95 ^oC for 2h. To quench the reaction, the heat source was removed and deionized water (29 ml) was added. The mixture was left to cool at room temperature for 30 min. The aqueous phase was extracted with ethyl acetate (3 x 30 ml) followed by extraction with $NaHCO_3$ (5%, aq., 20 ml) and brine. Finally, the sample was dried over magnesium sulphate and purified by silica gel column chromatography (DCM : Hexane = 2 : 8) to afford the product (1.957 g, 55%) as a yellowish oil**.** **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.49 (m, 4H), 7.39 (m, 1H), 7.08 (m, 2H), 6.9 (m, 1H), 5.17 (s, 1H), 2.34 (s, 3H); **^{13}C NMR (100MHz, $CDCl_3$) δ (ppm)** 150.31, 137.45, 130.83, 129.17, 129.29, 129.19, 115.78, 20.62.

Synthesis of ±-2-((4'-methyl–biphenyl-2'-yloxy)methyl)-3-methyloxirane[2]



To a stirred yellow solution of 2-phenyl-4-methylpjenol (1.455 g, 7.9 mmol) in dry DMF (60 mL) NaH (507 mg of NaH 60% in oil, 12.6 mmol) was added carefully under N_2 immediately obtaining an orange solution, the system was cautiously heated to allow NaH to dissolve. The formation of bubbles could be seen due to H_2 production, which meant that a gas outlet had to be kept (mantaining the N_2 inlet) until the bubbles stopped. The tosylate (1.818 g, 7.5 mmol) was added and the system was heated to 60 ^oC and stirred for 48h. The reaction was quenched addinng water and diethyl ether (equal volumes, 90 mL). The aqueous phase was extracted with dietly ether (3 x 60 mL). The combined organic layer was washed with a 10% LiCl aquesous solution (3 x 50 mL) in order to take the DMF away. Finally, the sample was dried over magnesium sulfate and purified by silica gel column (DCM : Pet. Et. = 4 : 6, silica pretreated with 1 % Et_3N) to afford a white oil (1.49 g, 74 %). **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.56-7.29 (m, 5H), 7.13 (d, J= 2.17 Hz, 1H), 7.09 (ddd, J=8.32, 2.26, 0.63 Hz, 1H), 6.88 (d, J= 8.23 Hz, 1H), 4.09 (dd, J= 11.01, 3.32 Hz, 1H), 3.95 (dd, J=11.15, 4.97 Hz, 1H), 2.98-2.90 (m, 2H), 2.33 (s, 3H), 1.3 (d, J=5.15 Hz, 3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 153.3, 138.4, 131.7, 131.0, 130.9, 129.5, 128.9, 127.9, 126.8, 113.5, 69.1, 57.2, 52.4, 20.5, 17.3.

Synthesis of the ±-1-([5-methyl-1,1'-biphenyl]-2-yloxy)-3-(isopropylamino)butan-2-ol[2]

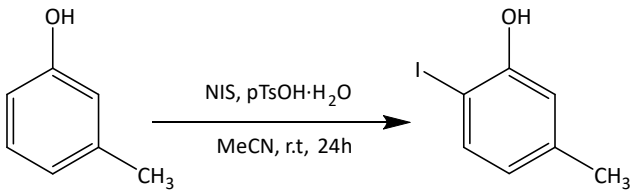


The aromatic epoxide (732 mg, 2.9 mmol) was added to anhydrous EtOH (11 mL) under a N_2 atmosphere and magnetically stirred until fully dissolved. To the stirring solution was then added isopropylamine (0.75 mL, 8.76 mmol) and the solution refluxed for 96 h at 80 °C. After 48h, more isopropylamine was added (0.4 mL, 4.67 mmol) due to a possible evaporation (since it's boiling point is 34 °C and it may have evaporated). Excess isopropylamine was azeotropically removed *in vacuo* with ethanol (6 x 7 mL) and DCM (6 x 7 mL) to afford a dense orange oil crude material (885 mg). The crude was damaged when tried to purify by silica-gel chromatography.

^{1}H NMR (400MHz, $CDCl_3$) δ (ppm) 7.50-7.29 (m, 5H), 7.11-7.08 (m, 2H), 6.89 (d, J= 8.23 Hz, 1H), 4.04-3.94 (m, 2H), 3.75 (q, J= 4.8 Hz, 1H), 2.86-2.73 (m, 2H), 2.32 (s, 3H), 0.94 (dd, J= 7.43, 6.52 Hz, 3H), 0.88 (d, J= 6.17 Hz, 3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 153.6, 138.7, 131.7, 129.6, 129.3, 129, 128.1, 127.0, 112.9, 70.4, 70.3, 51.7, 45.7, 23.7, 23.2, 20.6, 15.7.

3.4.2 From m-cresol

Synthesis of the 2-iodo-4-methylphenol[9]

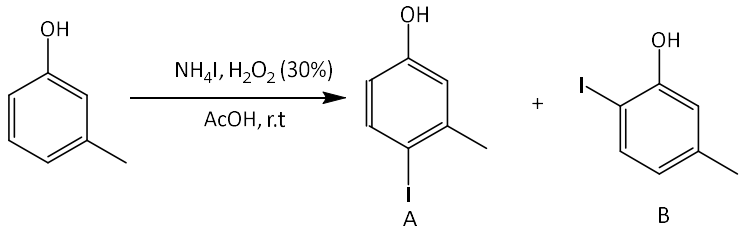


pTsOH·H_2O (0.761 g, 4 mmol) was added to a stirred solution of m-cresol (0.433 g, 4 mmol) in acetonitrile (4 ml), at room temperature. After 10 min of stirring at the same temperature, NIS (0.900 g, 4 mmol) was added in one portion to give an immediate orange solution.

After 24 h the solution had turned light orange and a TLC using DCM : hexane (1:1) was done to check the reaction. The reaction was quenched by adding a saturated $Na_2S_2O_3$ solution (10 ml), which changed the colour of the solution to yellow. The aqueous phase was extracted with ethyl acetate (3 x 10 ml). The combined organic layers were then washed with water (15 ml), followed by brine and lastly dried over magnesium sulphate. Finally the residue was purified by silica gel

column chromatography (DCM:hexane= 2:8) to afford the product (0.80 g, 85%) as white crystals. **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.47 (d, J=2.2 Hz, 1H), 7 (dd, J=8.2, 2.1 Hz, 1H), 6.86 (d, J=8.2 Hz, 1H), 2.23 (s,3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 152.85, 138.67, 131.99, 130.84, 114.96, 85.3, 20.15.

Synthesis of 4-iodo-3-methylphenol and 2-iodo-5-methylphenol[6]

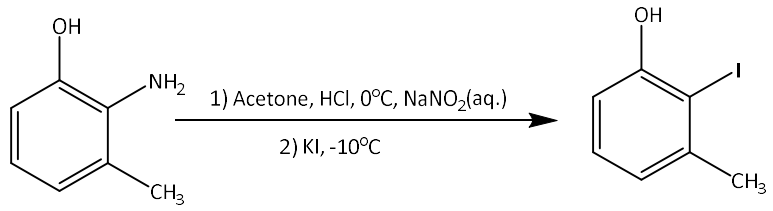


To a stirred solution of m-cresol (453 mg, 4 mmol) and NH_4I (638 mg, 4.4 mmol) in AcOH (8 mL) at room temperature, a 30% solution of H_2O_2 (0.45 mL, 4.4 mmol) was added dropwise. The reaction was quenched after 5 h adding a saturated $Na_2S_2O_3$(aq.) solution (8 mL). The aqueous phase was extracted with ethyl acetate (3 x 15 mL) and the combined organic layers were extracted with brine and finally dried over magnesium sulfate. The residue was purified by silica gel column chromatography (DCM:hexane= 2:8) to afford compound A (319 mg, 18%) and of compound B (418 mg, 23%) as yellow oils.

Compound A: **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.47 (d, J=2.2 Hz, 1H), 7 (dd, J=8.2, 2.1 Hz, 1H), 6.86 (d, J=8.2 Hz, 1H), 2.23 (s,3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 152.85, 138.67, 131.99, 130.84, 114.96, 85.3, 20.15.

Compound B: **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.48 (m, 1H), 6.82 (d, 1H), 6.5 (d, 1H), 2.29 (s,3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 154.5, 140.7, 137.7, 123.6, 115.8, 81.6, 21.

Synthesis of the 2-iodo-3-methylphenol via a diazonium salt[15]



To a stirred solution of 2-amino-3-methylphenol (2 g, 16.24 mmol) in acetone (28 mL) at <0 ^{0}C (using an ice-water-salt bath) HCl (3.4 mL, 40.6 mmol)) was added dropwise carefully without the temperature rising above 0 ^{o}C. Afterwards, a $NaNO_2$ aqueous solution was added (3.3 mL

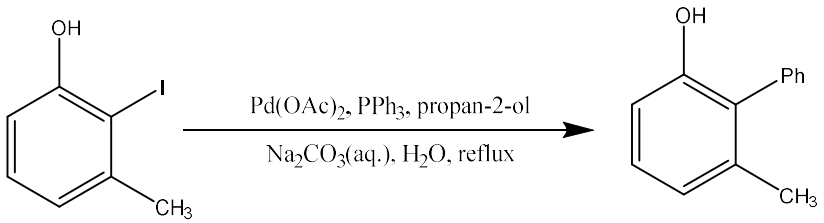
5.4 M, 16.24 mmol) and the reaction was left stirring for 1h at 0 °C. Next, the reaction was cooled to -10 °C (acetone-dry ice bath) and an aqueous solution of KI was added (5.4 mL 6 M, 32.48 mmol).

Since the diazonium salts are explosive due to its chemical and thermal instability, a test was done to check if there was any diazonium salt left. The test consisted in dissolving a minimum fraction of the crude in a 1M NaOH solution in which β-naphthol has been added. If there were any diazonium salt the solution would turn red, due to the formation of an azo compound.

To quench the reaction, the system was left to warm to room temperature and water (

60 ml) was added. The aqueous phase was extracted with ethyl acetate (3 x 30 mL). The organic phase was washed with $Na_2S_2O_3$ (60 mL), followed by distilled water (60 mL) and brine (60 mL). The combined aqueous layers were extracted once with ethyl acetate (30 mL) and the combined organic phases were dried over $MgSO_4$. Finally, the crude material was purified by silica gel chromatography (ethyl acetate : hexane = 0.5 : 9.5) to afford the product as a red oil (2.98 g, 78%). **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.1 (t, J=7.54 Hz, 1H), 6.83-6.78 (m, 2H), 5.5 (s, 1H), 2.43 (s, 3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 155.0, 142.5, 129.3, 122.0, 112.1, 93.3, 28.7.

Synthesis of the 3-methyl-2-phenyl-phenol[10]



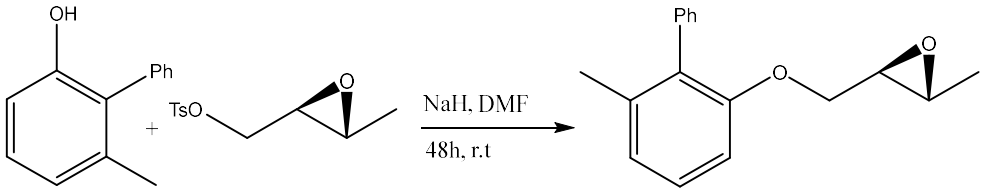
The reaction was carried out under inert conditions to avoid oxygen which could interfere with the Pd reagent.

The 2-iodo-3-methylphenol (2.170 g, 9.2 mmol), and the phenylboronic acid (1.80g, 14.77 mmol) were added into a 100mL two-necked round bottom flask. 2-Propanol (17 ml) was added and the system was left degassing under N_2 atmosphere and stirring to form a solution. After 10 min of degassing, a 2M aqueous solution of Na_2CO_3 (11 ml) was added followed by deionized water (3.2 ml) and the mixture was left degassing again for 20 min.

The $Pd(OAc)_2$ (0.139 g, 0.619 mmol), and PPh_3 (0.488 g, 0.2 mmol) were added and an air condenser was connected to the system, (the mixture left degassing for left 15 min, and heated to 95 °C for 2 h. To quench the reaction, the heat source was removed and deionized water (23 ml) was added. The mixture was left to cool at room temperature for 20 min. The aqueous phase

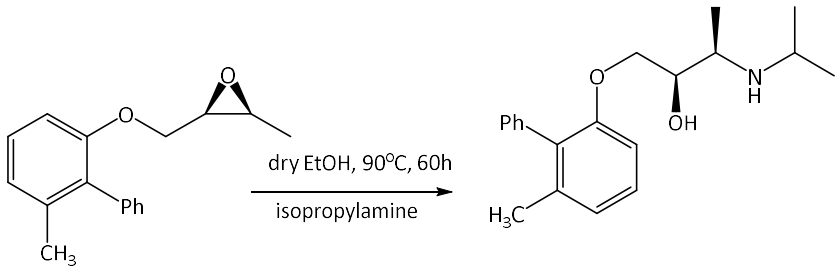
was extracted with ethyl acetate (3 x 90 ml) followed by extraction with $NaHCO_3$ (5%, aq., 150 ml) and brine (150 mL). Finally, the sample was dried over magnesium sulphate and purified by silica gel column chromatography (DCM : Hexane = 1 : 9) to afford the product (1.368 g, 81%) as a red oil. **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.51-7.45 (m, 2H), 7.43-7.39 (m, 1H), 7.30-7.26 (m, 2H), 7.15 (t, J=7.89 Hz, 1H), 6.85 (d, J=1.94 Hz, 1H), 6.84-6.81 (m, 1H), 4.80 (s, 1H),2.06 (s, 3H)**; ^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 152.7, 137.2, 135.2, 130.2, 129.3, 128.5, 128.1, 128, 121.9, 112.6, 20.3.

Synthesis of (±)-2-((3'-methyl–biphenyl-2'-yloxy)methyl)-3-methyloxirane[2]



To a stirred yellow solution of 2-phenyl-3-methylpjenol (1.23 g, 6.68 mmol) in dry DMF(50 mL) NaH (428 mg of NaH 60% in oil, 10.7 mmol) was added carefully under N_2 immediately obtaining an orange solution, the system was cautiously heated to allow NaH to dissolve. The formation of bubbles could be seen due to H_2 production, which meant that a gas outlet gad to be kept (mantaining the N_2 inlet) until the bubbles stopped. Finally, the tosylate (1.538 g, 6.35 mmol) was added and the system was heated to 60 °C and stirred for 48h. The reaction was quenched addinng water and diethyl ether (equal volumes, 70 mL). The aqueous phase was extracted with dietly ether (3 x 50 mL). The combined organic layer was washed with a 10% LiCl aquesous solution (3 x 40 mL) in ordet to take the DMF away. Finally, the sample was was dried over magnesium sulfate and purified by silica gel column (DCM : Pet. Et. = 4 : 6, silica pretreated with 1 % Et_3N) to afford a white oil (1.23 g, 73 %). **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.42-7.19 (m, 6H), 6.91 (d, J= 7.55 Hz, 1H), 6.81 (d, J=8.23 Hz, 1H), 4.04 (dd, J= 11.32, 3.32 Hz, 1H), 3.93-3.89 (m, 1H), 2.84-2.73 (m, 2H), 2.08 (s, 3H), 1.23 (d, J=5.26 Hz, 3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 156.0, 137.9, 137.46, 130.1, 128.0, 126.7, 123.3, 110.7, 68.8, 57.29, 52.35, 20.6, 17.3.

Synthesis of the ±-1-([6-methyl-1,1'-biphenyl]-2-yloxy)-3-(isopropylamino)butan-2-ol[2]

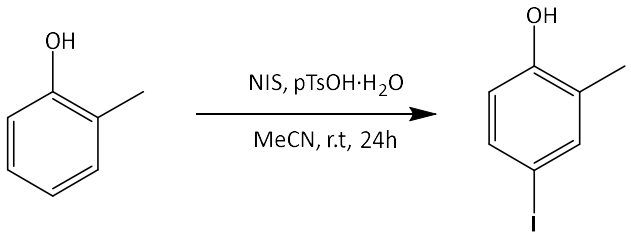


The aromatic epoxide (575 mg, 2.26 mmol) was added to anhydrous EtOH (10mL) under a N_2 atmosphere and magnetically stirred until fully dissolved. To the stirring solution was then added isopropylamine (0.58 mL, 6.78 mmol) and the solution refluxed for 96 h at 80 °C. After 48h, more isopropylamine was added (0.3 mL, 3.51 mmol) due to a possible evaporation (since it's boiling point is 34 °C and it may have evaporated). Excess isopropylamine was azeotropically removed *in vacuo* with ethanol (6 x 7 mL) and DCM (6 x 7 mL) to afford a dense orange oil. The crude was damaged when tried to purify by silica-gel chromatography.

^{1}H NMR (400MHz, $CDCl_3$) δ (ppm) 7.42-7.38 (m, 2H), 7.33-7.29 (m, 1H), 7.24-7.2 (m, 3H), 6.91 (d, J= 7.55 Hz, 1H), 6.84 (d, J= 8.12 Hz, 1Hz), 3.93 (dd, J=5.09, 1.03 Hz, 2H), 3.61 (q, J=4.92 Hz, 1H), 2.72-2.66 (m, 2H), 2.08 (s, 3H), 0.91-0.88 (m, 9H).

3.4.3 From o-cresol

Synthesis of the 4-iodo-2-methylphenol[9]

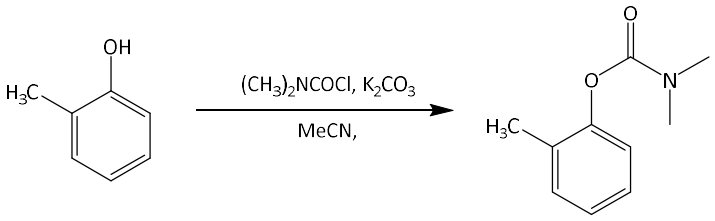


To a stirred solution of o-cresol (150 mg, 1.4 mmol) in acetonitrile (1.4 mL), pTsOH·H2O (264 mg, 1.4 mmol) was added at room temperature. After 10 min of stirring at the same temperature, NIS (313 mg, 1.4 mmol) was added in one portion to immediately obtain an orange solution.

After 19 h the solution had turned light orange and a TLC using DCM : hexane (1 : 1) was done to check the reaction. The reaction was quenched adding a saturated $Na_2S_2O_3$ solution (5 mL, which changed the colour of the solution to yellow). Extractions were done to the aqueous

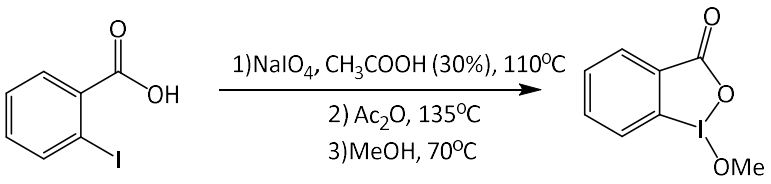
phase using ethyl acetate (3 x 10 mL). The combined organic layers were washed with water, followed by brine and lastly dried over magnesium sulphate. The residue was purified by silica gel column chromatography (DCM : hexane = 2 : 8) to afford the product as a yellow oil (300 mg, 91%). **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.40 (s, 1H), 7.30 (dd, J=8.5, 2.2 Hz, 1H), 6.54 (d, J=8.5 Hz, 1H), 5.12 (s, 1H), 2.18 (s, 3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)**153.97, 139.5, 135.8, 126.9, 117.2, 82.5, 15.6.

Synthesis of O-2-methylphenyl-N,N-dimethylcarbamate[8]



o-Cresol (3.244 g, 30 mmol), Me_2NCOCl (4.838 g, 45 mmol) and K_2CO_3 (6.221 g, 45 mmol) were dissolved in acetonitrile (62 ml), and the solution was heated reflux (90 °C). After 3.5 h the reaction was quenched by turning off the heat source, leaving it to cool to room temperature before removing the acetonitrile on the rotary evaporator. The sample was dissolved in water and extracted with DCM (3 x 40 ml). Afterwards, in order to destroy the remaining Me_2NCOCl, the combined organic fractions were extracted with a 1M NaOH aqueous solution (2 x 100 ml). Finally, extractions with water and then brine were done and it was dried over magnesium sulphate to afford the product (4.892 g, 91%) as a yellow liquid**. ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 7.18 (m, 2H), 7.08 (m, 1H), 7.05 (m, 1H), 3.12 (s, 3H), 3.01 (s, 3H), 2.21 (s, 3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 154.85, 150.1, 131, 130.4, 126.9, 125.5, 122.3, 36.8, 36.5, 16.2.

Synthesis of 1-methoxy-1,2-benziodoxol-3-(1H)-one (I-OMe)[8]

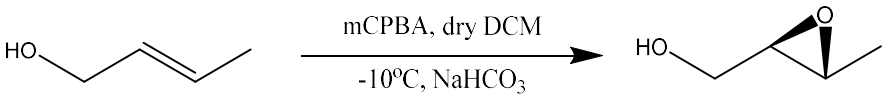


2-Iodobenzoic acid (0.744 g, 3 mmol) and $NaIO_4$ (0.674 g, 3.15 mmol) were added together with an aqueous solution of 30% acetic acid (5 mL) in a 25 mL round bottom flask. The reaction was heated up to 110 °C and stirred. After 4 h the heat source was turned off and the system cooled by adding ice-water so that the product precipitated. After 10 min the product was washed in

ice-water and dried in vacuum. The product was treated with Ac_2O (5 ml) and the system taken to 135 °C. The solution became orange when it got to 135°C and all solid was dissolved. After 15 min the heating was stopped and the system left to cool to room temperature, and the flask put into the freezer at -20 °C until it crystallised. Filtration, washing with water and drying in vacuum gave the product as a white solid. This material was dissolved in MeOH (5 ml) and put to reflux (70 °C) until it dissolved after around 10 min. When the reaction was finished it was cooled to room temperature followed by crystallisation at -20 °C, filtration, washing with the minimal amount of MeOH and drying under vacuum to afford the product (355mg, 40%) as yellow crystals. **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 8.24 (d, J=7.7 Hz, 1H), 7.88 (t, J=7.7 Hz, 1H), 7.64-7.76 (m, 2H), 4.26 (s,3H).

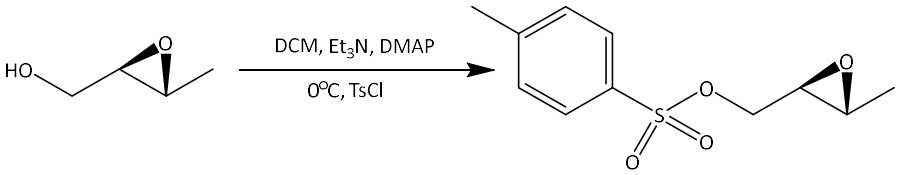
3.4.4 From crotyl alcohol

Synthesis of the ((±)-3-methyloxiran-2-yl)methanol[2]



Crotyl alcohol, as a mixture of *cis* and *trans* 96%, (8 g, 110 mmol) and $NaHCO_3$ (11.1 g, 132 mmol) were dissolved in dry DCM (160 mL) and the system was cooled to -10 °C. mCPBA (37.4 g of commercial 77% mCPBA, 167 mmol) was then added cautiously over a 20 min period to the stirring solution (using a plastic spoon, because the mCPBA is a sensitive peracid and strong oxidant so metal spatulas should not be used). Once the addition of the reagents was complete, a drying tube (filled with $CaCl_2$) was fitted to the flask and the mixture was allowed reach room temperature and was left overnight. Once complete a white solid had precipitated and was filtered and washed in a Buchner funnel using DCM as washing solvent. Afterwards the solution was filtered again, because some mCPBA went through the Buchner. The solvent of the liquid filtrate was evaporated in the rotary evaporator to obtain a yellow liquid (8.126 g, 83%). No further purification was needed for the next step but the presence of the mCPBA had to be checked (no significant amount should be present) with a starch iodine paper which goes blue in the presence of an oxidizing agent. **^{1}H NMR (400MHz, $CDCl_3$) δ (ppm)** 3.91 (dd, J=12.46, 2.63 Hz, 1H), 3.62 (dd, J=12.58, 4.35 Hz, 1H), 3.04 (m, 1H), 2.89 (m, 1H), 1.34 (d, J=5.26 Hz, 3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 61.8, 59.8, 52.3, 17.1.

Synthesis ((±)-3-methyloxiran-2-yl)methyl 4'-methylbenzenesulfonate[2]



To a solution of (±)-3-methyloxiran-2-yl)methanol, (4.0 g, 45 mmol) in dry DCM (160 mL) at -10 °C triethylamine (9.5 mL, 68.0 mmol) and DMAP (0.49 g, 4 mmol) were added. Toluenesulfonyl chloride (8.58 g, 45 mmol) was dissolved in dry DCM (20 mL) in a separate flask and added dropwise to the reaction mixture, which was then left overnight in an ice-salt-water bath for 18h. Upon completion, the solution was washed with 10% tartaric acid solution (100 mL), saturated sodium hydrogen carbonate solution (150 mL) and saturated brine solution (100 mL). The solvent was removed in the rotary evaporator to afford a brown oil which was purified *via* flash chromatography (EtOAc : pet ether = 2 : 8) to afford the product as a pale yellow crystalline solid (4.789 g, 44%). **^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm)** 7.8 (d, J=8 Hz, 2H), 7.36 (d, J=8 Hz, 2H), 4.18 (dd, J=11.32, 3.89 Hz, 1H), 3.98 (dd, J=11.32, 5.72, 1H), 2.92 (ddd, J=5.83, 3.89, 2.17 Hz, 1H), 2.87 (qd, J= 5.18, 2.06 Hz, 1H), 2.46 (s, 3H), 1.32 (d, J=5.26 Hz, 3H); **^{13}C NMR (100MHZ, $CDCl_3$) δ (ppm)** 145.2, 132.7, 130.0, 128.0, 70.2, 55.5, 52.8, 21.8, 17.1.

4. Results and conclusion

4.1 Iodinated compounds

The first approach in the synthetic route was to directly iodinate the cresol isomers using NIS as the iodine source.[9] The reaction was good for the *para*-cresol which gave a unique desired product, easily separated and purified by flash column. However, the reaction of NIS with the *ortho* and *meta* cresols did not work too well, the reaction did not give the desired products, and the crude was too complex to purify so a new approach was needed.

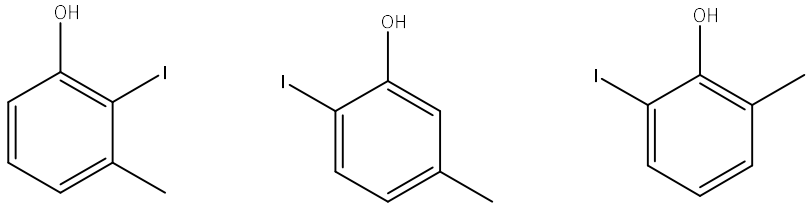


Figure 4.4-1.1 Desired iodinated compounds for the m- and o- cresol

A new procedure consisting in using NH_4I as iodinating source and hydrogen peroxide (30%) was tested since it seemed to be an environmental and economic friendly approach.[6] Although this new approach worked because it gave the desired products, the yield of the reaction was below 20% and the purification and separation of the products from the by-products was too complicated. The main by product in this procedure was always the double iodination product and even if the reaction conditions were changed it always formed in the beginning of the reaction.

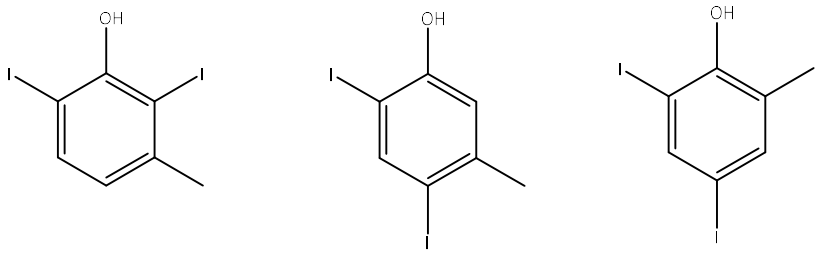


Figure 4.1.4-2 Main by products in the reaction using NH_4I and H_2O_2 for the m- and o- cresol

Then it was thought that maybe reducing the reactivity of the aromatic benzene ring the reaction could work. Therefore, the *o*-cresol was reacted with dimethylcarbamyl chloride to form the corresponding carbamate which would make the aromatic ring less reactive, and would introduce a protecting group which is easily taken off. In the same article in which we found a good procedure to synthesize the carbamate they reported the use of a I(III) compound they

synthesized from 2-iodobenzoic acid and palladium acetate to iodinate the carbamate. Yet when we tested this in the lab the reaction it did not work.[6] Treating the carbamate with NIS or NH_4I did not give the desired compounds either, rather the results were similar to those obtained with the unprotected cresol.

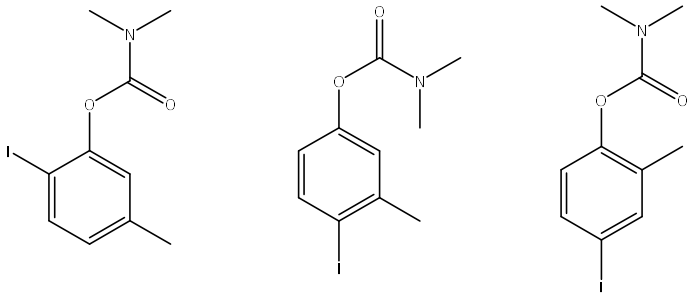


Figure 4.1.4-3 Main products obtained via carbamate procedure .

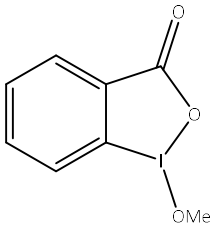
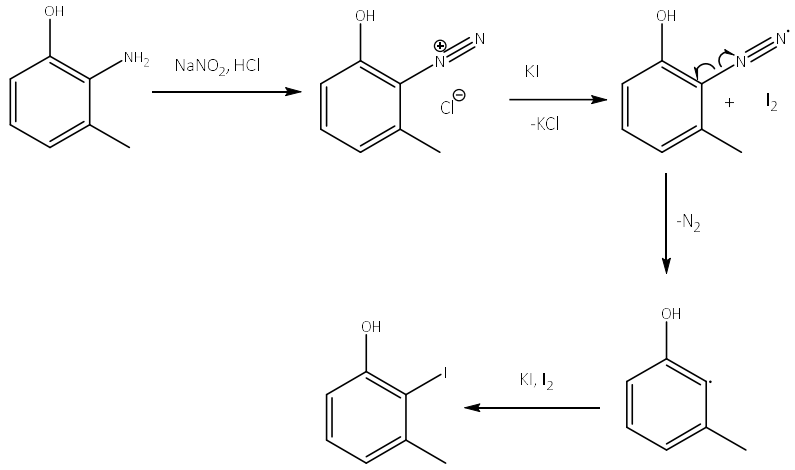


Figure 4.1.4-4 I(III) reagent synthesized from the 2-iodobenzoic acid

A new approach was then proposed, changing groups already in the desired position of the aromatic ring for iodide. Derivatives were found with an amino group in the desired position for the iodine, hence a Sandmeyer-like reaction was proposed using KI as iodinating source. When using KI, it is not necessary to use the typical Cu(I) salt used in the Sandmeyer that forms the radical in the diazonium salt by oxidizing to Cu(II) because the iodide itself is easily and reversibly oxidizable hence the iodide claims the function of the copper salt in this reaction. A proposal for the mechanism of the iodination via diazonium salt can be found in Scheme 4.1.1.



Scheme 4.1.2 Mechanism for the iodination via diazonium salt.

This path proved to successfully and easily achieve the desired products but due to a lack of time only the 2-iodo-3-methylphenol was synthesized in this way with a 78 % yield.

4.1.1 Characterization of the iodinated compounds

To characterize the iodinated compounds, that is to know whether or not the product obtained is the expected compound, the ^{1}H and ^{13}C NMR techniques were mainly used. Furthermore, in order to identify the molecule, parameters such as multiplicity, number of protons integrated, number of signals and chemical shifts were analysed and compared with the expected theoretical ones.

For example, in the case of the 2-iodo-4-methylphenol in Figure 4.1.1.1, as expected, 4 signals were observed, one that integrates 3 protons in the aliphatic region and corresponding to the methyl group, another corresponding to the O-H group and 3 signals in the aromatic region. The main difference from the p-cresol would be in the aromatic region. Since p-cresol is symmetric, the aromatic region should only show 2 signals integrating two protons each and with a doublet multiplicity. However, the NMR of the product had three signals, two doublets and a double doublet showing that the aromatic ring has a new substituent.

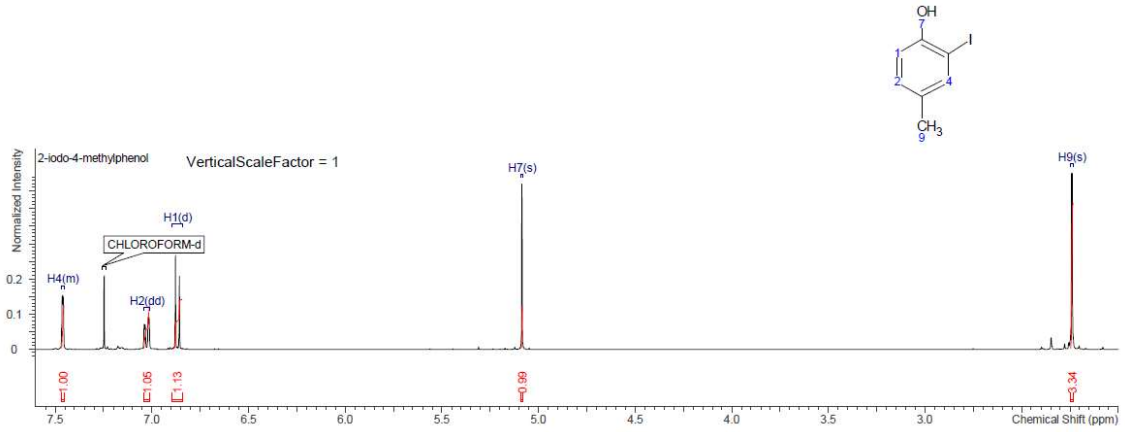


Figure 4.1.1.1 ^{1}H NMR spectrum for the 2-iodo-4-methylphenol

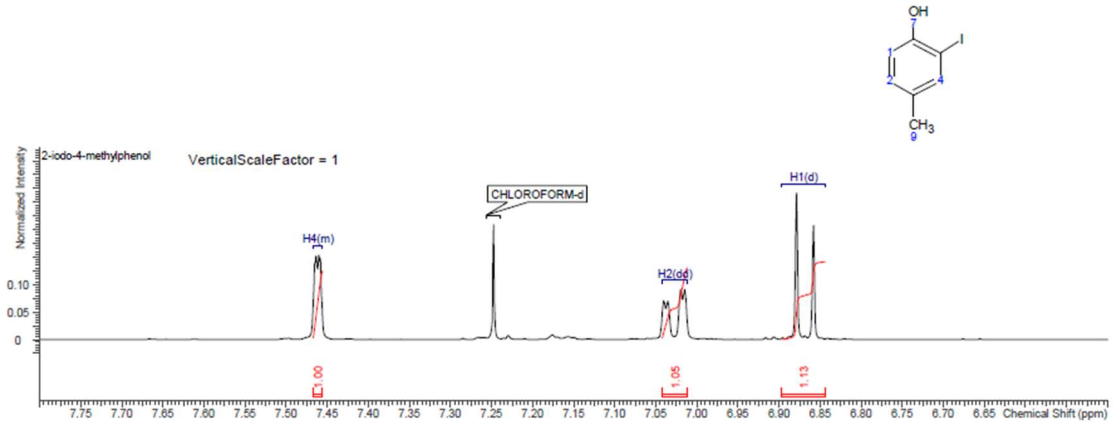


Figure 4.1.1.2 Aromatic region of NMR spectrum of the 2-iodo-4-methylphenol

In order to confirm that the crude was the desired product ^{13}C NMR was required. Iodobenzene compounds present a characteristic peak in the aromatic region of the spectrum around 90ppm for the carbon bounded to iodine (Figure 4.1.1.3). Furthermore, it is known that signals from C-H have a higher intensity than C-X, which was also used to assign each signal to their corresponding carbons in the molecule.

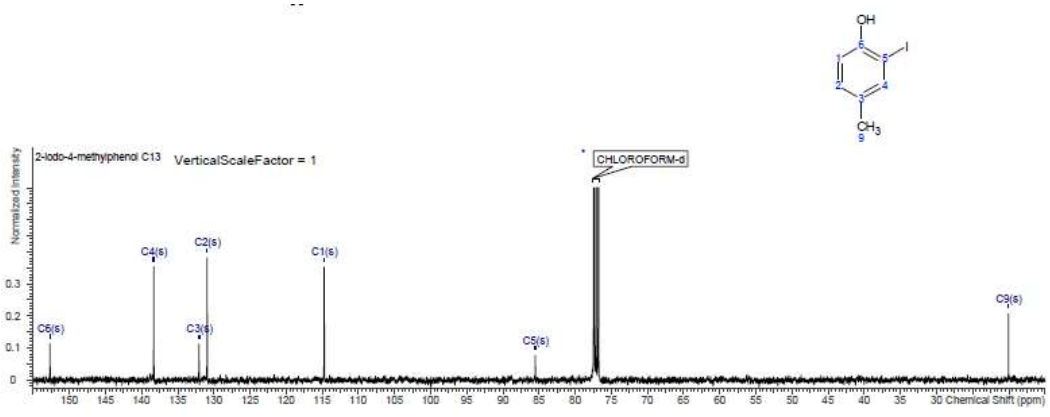
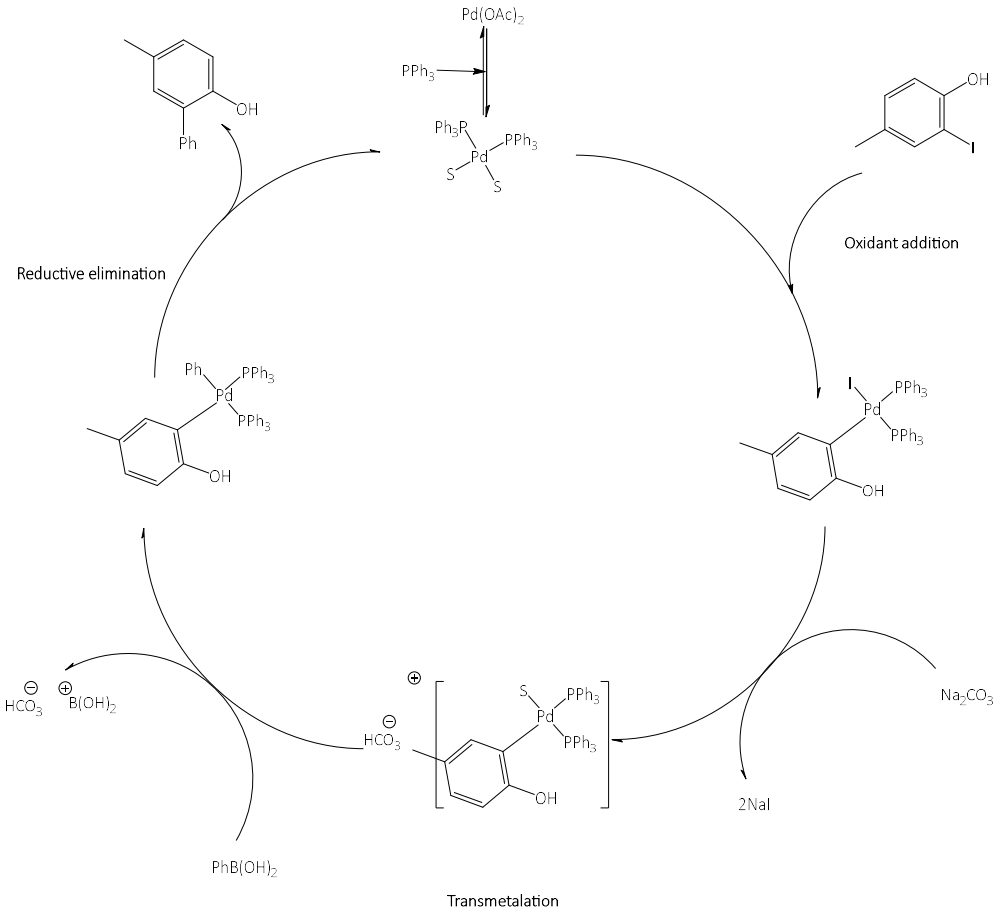


Figure 4.1.1.3 ^{13}C NMR spectrum of 2-iodo-4-methylphenol

4.2 Phenyl compounds

The next step in the synthetic route was the substitution of the iodo group for a phenyl group. A well-known reaction and with good results is the Suzuki Coupling reaction. After testing with different conditions it was found that the method using palladium acetate, triphenylphosphine, phenylboronic acid and propan-2-ol as solvent was the one that gave the best results. The reaction had to be carried out under nitrogen atmosphere because the $Pd(OAc)_2$ is sensitive to air especially when heated to 90ºC, the reaction temperature conditions.

This reaction was carried out with the 2-iodo-4-methylphenol and the 2-iodo-3-methylphenol in order to obtain their corresponding biphenyls with 55 and 81 % yield respectively. In Scheme 4.1.2 an example of a possible mechanism for the Suzuki reaction using the 2-iodo-4-methylphenol as starting material is shown.



Scheme 4.2.1 Possible Suzuki reaction mechanism

4.2.1 Characterization of the phenyl compounds

In this case, to confirm the structure ^{13}C NMR spectra were used (Figures 4.2.1.1 and 4.2.1.2), which showed the disappearance of the C-I peak (C_5) and the appearance of more aromatic signals.

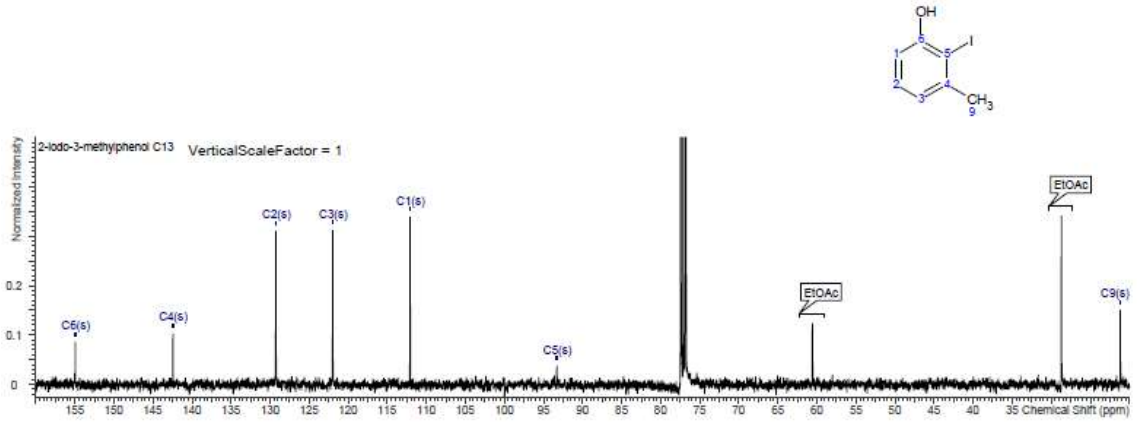


Figure 4.2.1.1 ^{13}C NMR spectrum of the 2-iodo-3-methylphenol

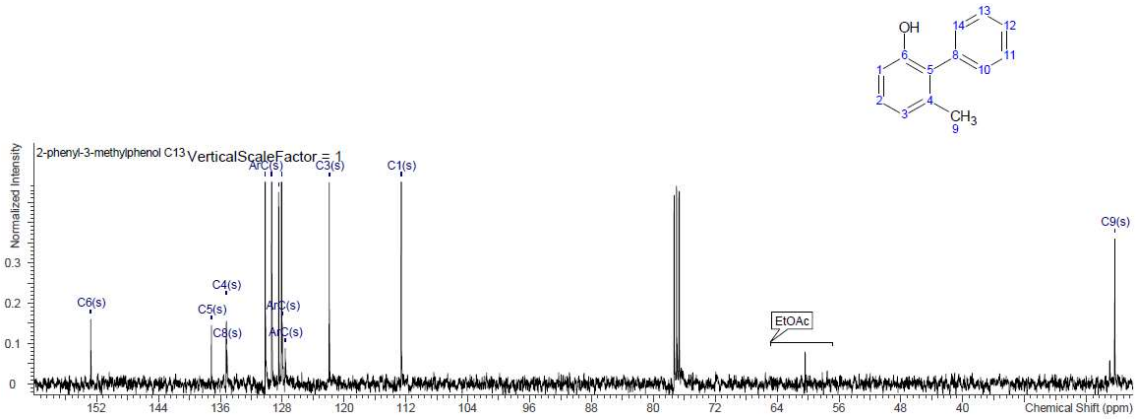


Figure 4.2.1.2 ^{13}C NMR spectrum of 2-phenyl-3-methylphenol

In the case of the 2-phenyl-3-methylphenol (Figure 4.2.1.4), the ^{1}H NMR spectrum also showed the same multiplicity in the already existent aromatic signals (from the starting material, Figure 4.2.1.3) and there were three additional signals integrating all together 5 protons corresponding to the new phenyl substituent. Furthermore, the phenyl substituent deshields the methyl group by 0.3 ppm due to the apparent field generated by the aromatic system.

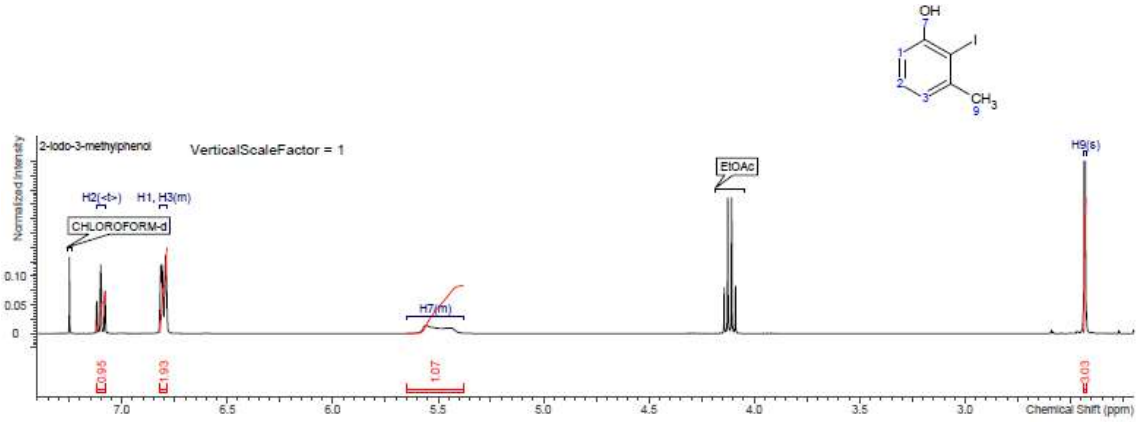


Figure 4.2.1.3 ^{1}H NMR spectrum of 2-iodo-3-methylphenol

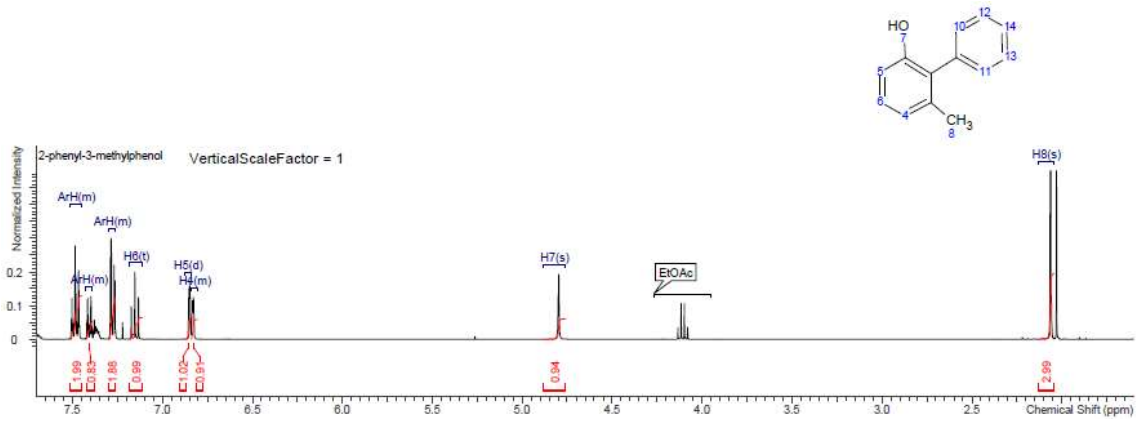


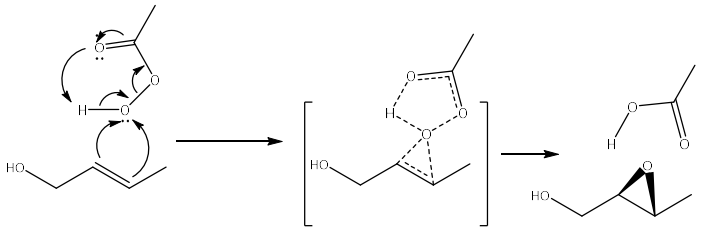
Figure 4.2.1.4 ^{1}H NMR spectrum of 2-phenyl-3-methylphenol

4.3 Crotyl alcohol derivatives

Once the two 2-phenylphenols substrates were prepared the ether part of the final product was synthesized.

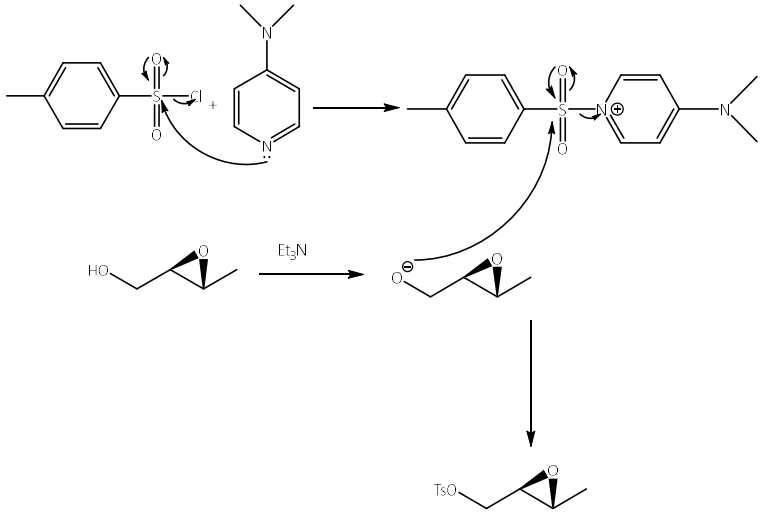
A widely used method to introduce substituents in positions 1,3 in an alkylated chain, one of them being an alcohol with a specific stereoselectivity, is by an epoxide ring opening.

The epoxide is a sensitive and unstable group so preparing it had to be the last step in the synthetic route. Starting from crotyl alcohol, the first step was to epoxidize the double bond using mCPBA, which is a peroxycarboxylic acid often used as oxidizing agent, affording the epoxide as a racemic mixture with 2R,3S and 2S, 3R stereochemistry. If we wanted to obtain the enantiomers, Sharpless's asymmetric epoxidation method which gives the epoxides with ee > 90% could be used.[18]



Scheme 4.3.1 Crotyl alcohol epoxidation mechanism.

Afterwards, a labile leaving group is introduced in place of the alcohol so that in the next step the nucleophilic substitution is done at the alcohol centre rather than at the epoxide. A good leaving group to make from an alcohol is a tosylate due to the anion's stability. The introduction of the tosyl group causes a formal change in the stereochemical descriptor of the stereocentre at C_2 from 2S,3R to 2S,3S.



Scheme 4.3.2 Tosylation mechanism of the (±) 2-methanol-3-methyloxirane

As observed in scheme 4.3.2, DMAP's role is to act as a catalyst to an acyl transfer reaction. DMAP acted as a nucleophile in a nucleophilic acyl substitution in which DMAP took chlorine's place in the tosyl chloride making it even more electrophile so that the alkoxyde can attack the sulphur centre afterwards and obtain the desired tosylate product with a 44 % yield.

4.3.1 Characterization of the epoxide

In the ^{1}H NMR spectrum the resulting epoxide from crotyl alcohol reaction with mCPBA presented no alkene peaks (Figure 4.3.1.1) and showed two multiplets at around 2.7 – 3.1 ppm. Additionally, two doublet of doublets have appeared in the region of 3.5 - 4 ppm integrating one

proton for each peak and corresponding to the diastereotopic protons of the CH_2 neighbouring the hydroxyl group and the epoxide. Some impurities were present in the spectrum due to the presence of some possible residual mCPBA or its reduced form, the *m*-chlorobenzoic acid, but there was no need of further purification and the next reaction could be carried out from the obtained crude material with 83 % yield from crotyl alcohol.

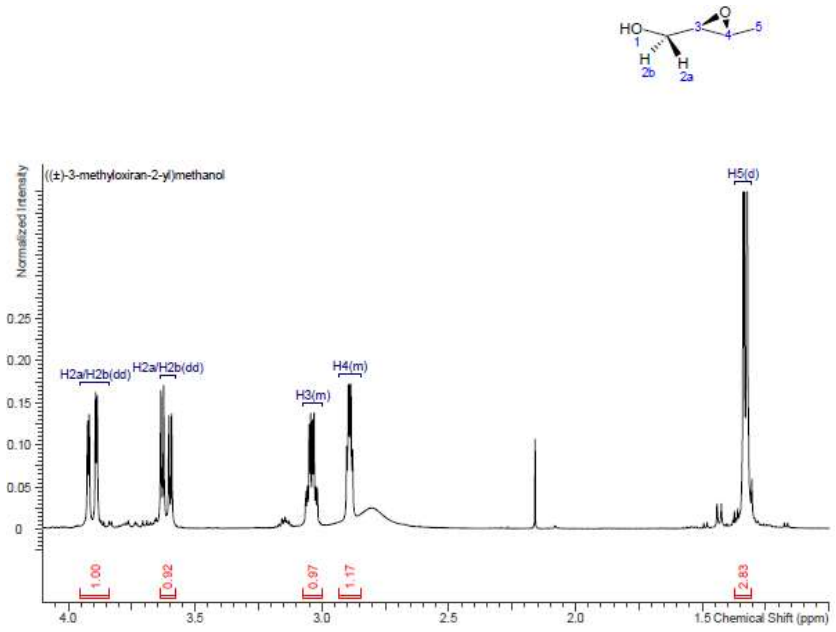


Figure 4.3.1.1 ^1H spectrum of the ((±)-3-methyloxiran-2-yl)methanol

When the tosyl group is introduced in the molecule, aromatic protons appeared in the spectrum (Figure 4.3.1.2): two aromatic peaks due to the symmetry of the tosyl group and in the aliphatic region an additional peak at 2.46 ppm corresponding to the methyl group in the sulfonate. Moreover, the tosylate group deshields the two diastereotopic protons by *ca.* 0.3 ppm compared to its hydroxyl counterpart. The coupling constant for these protons also varied from 12.6 to 11.3 Hz. The protons of the epoxide ring also suffered an upfield shift which was more notable in the proton neighbouring the tosyl group (H_3) because of its proximity (from 3.04 ppm to 2.92 ppm) and an almost insignificant change can be seen in the other proton (H_4).

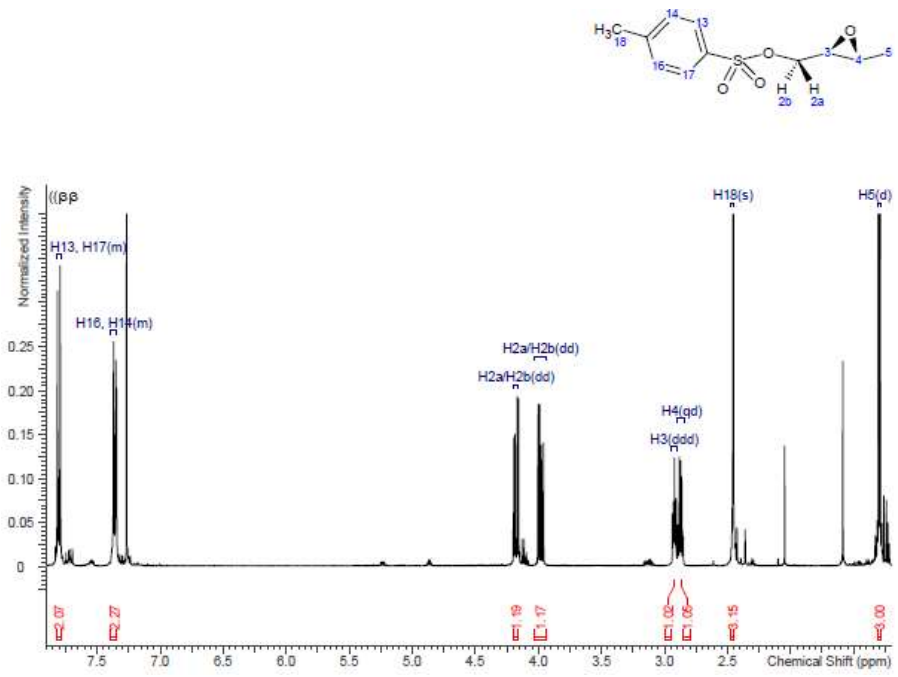
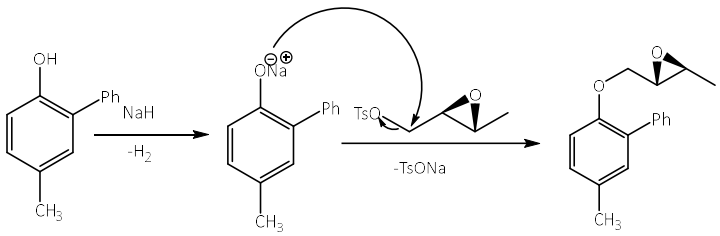


Figure 4.3.1.2 Aliphatic region of the ^{1}H NMR spectrum of ((±)-3-methyloxiran-2-yl)methyl 4'-methylbenzenesulfonate

4.4 Final compounds

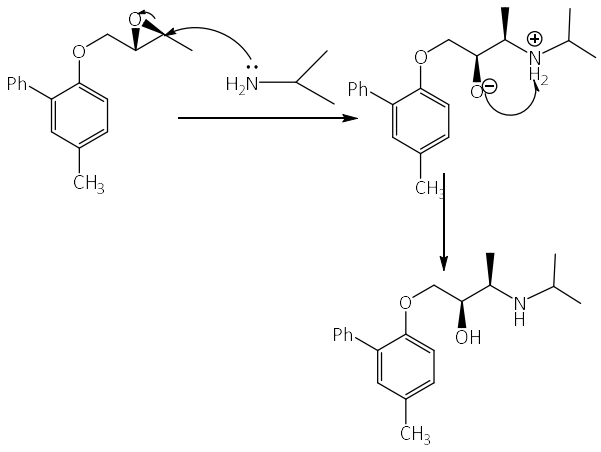
Once the tosyl group is introduced, the next step in the synthetic route is to substitute the tosylate with the phenol in a nucleophilic substitution reaction. In order to do so, the nucleophilic properties of the phenol had to be increased by converting it into an phenoxide. However, a non-nucleophilic base, such as NaH, had to be used so that it does not attack the epoxide or tosyl group. Sodium hydride had to be carefully manipulated. In fact, it was stored as a 60% mixture in oil and had to be previously washed with cyclohexane and carefully added to the reaction system which had been previously purged with nitrogen. No water should enter into the system because sodium hydride reacts rapidly and violently with water, so everything that got into contact with the sodium hydride while manipulating it had to be washed with isopropanol and then water afterwards. (±)-2-((4'-methyl–biphenyl-2'-yloxy)methyl)-3-methyloxirane and (±)-2-((3'-methyl–biphenyl-2'-yloxy)methyl)-3-methyloxirane were obtained with 74 and 73 % yields respectively.



Scheme 4.4.1 Nucleophilic substitution mechanism

The final step of the synthesis is to open the epoxide ring with isopropylamine. When opening an epoxide, the regioselectivity of the ring opening is governed by the substitution of the epoxide ring, the strength of the nucleophile and whether the reaction is carried out in acidic or basic conditions. In general, in acidic conditions, in which the epoxide protonates, nucleophiles attack the epoxide at the more substituted end, due to the formation of a stablished semi-carbocation intermediate via a SN_1 like mechanism. However, in basic conditions there is no semi-carbocation, positive charge, created as an intermediate. Since the epoxide oxygen is a poor leaving group (it is an alkoxide), it will only react with strong nucleophiles. In this case, in basic conditions, only the strain of the epoxide ring makes it a reactive electrophile and the steric hindrance becomes the main factor in the control of the regioselectivity making it so that nucleophiles will preferably react at the less sterically hindered end of the epoxide ring.

The epoxides synthesized in this project are unsymmetrical and contain the phenol ring which introduce some steric hindrance to the nucleophile. The isopropylamine is a primary amine, a relatively strong and non-sterically bulky nucleophile, therefore the attack should take place at the less hindered end of the epoxide.



Scheme 4.4.2 Epoxide ring opening mechanism using isopropylamine as nucleophile

The reaction was carried out under nitrogen because the presence of water had to be prevented (since it could act as a nucleophile and open the epoxide ring). The system had to be put to reflux for three days in ethanol and using a water condenser (air condensers proved to not be effective enough to condense isopropylamine that has a low boiling point). Upon completion, in order to remove excess isopropylamine from the reaction mixture, azeotropical distilations *in vacuo* were performed using EtOH and DCM.

The desired products could be obtained as crude solids which by NMR seemed to be quite pure but showed some impurities when it was run by silica TLC plate. When tried to purify by column chromatography none of the fractions fitted with the desired product but a very slow moving spot whose NMR suggested it was the desired compound but with some impurities.

4.4.1 Characterization of the final products

Both epoxy products are really similar and only differ in the aromatic region so similar comments are relevant to the other compound.

To ascertain that the nucleophilic substitution with the phenoxide had taken place in the tosyl group rather than the epoxide, both ^{13}C and ^{1}H NMR spectra were recorded. The integration of the peaks in the ^{1}H NMR spectrum fitted with the expected number of protons from the product and with their respective integration in the molecule.

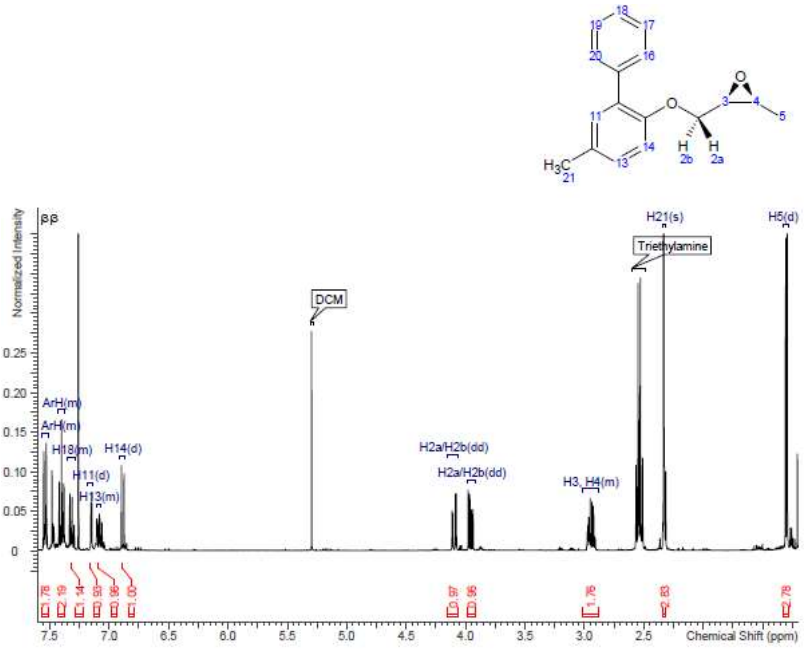


Figure 4.4.1.1 ^{1}H spectrum of (±)2-((4'-methyl–biphenyl-2'-yloxy)methyl)-3-methyloxirane

In the ^{13}C NMR spectrum (Figure 4.4.1.2) the aliphatic and aromatic region of the parts from each starting material (crotyl alcohol derivative and biphenyl-ol) could be observed. In the aliphatic region 5 carbon peaks can be observed, four from the crotyl alcohol's part of the molecule and one from the methyl group in the aromatic region (*ca* 21 ppm). As for the aromatic peaks, some of the peaks overlapped which is why there are not 12 aromatic peaks.

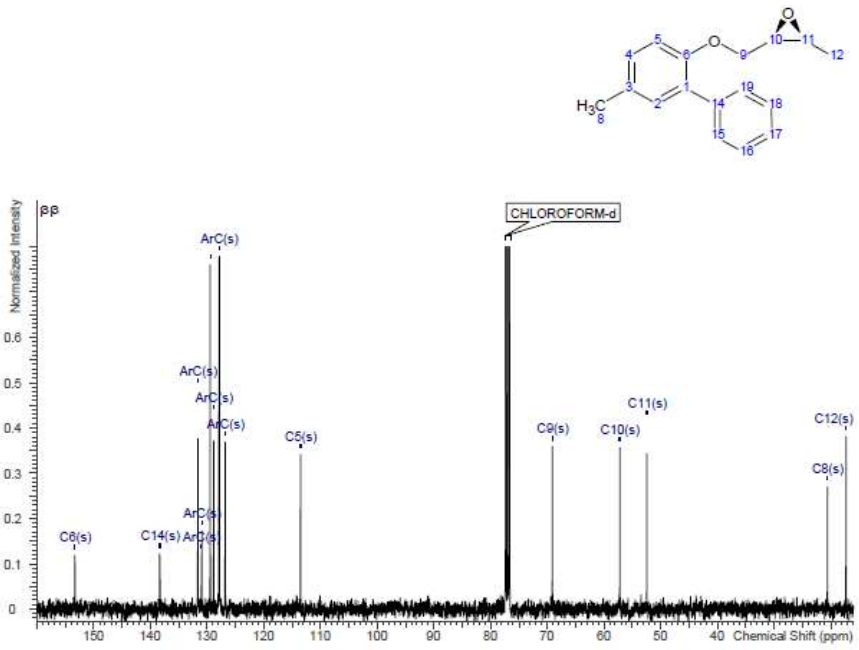


Figure 4.4.1.2 ^{13}C spectrum of (±)2-((4'-methyl–biphenyl-2'-yloxy)methyl)-3-methyloxirane

Finally, the final product could not be fully purified, after trying to purify it only a small amount could be obtained which contained some impurities. However, the crude's NMR was clear enough to identify the presence of the final product and the disappearance of the starting material (the epoxide). The presence of the final product was confirmed by the notable change in the signal of the proton from the methyl group neighbouring the epoxide in the starting material (H_5, Figure 4.4.1.1) which is deshielded by the nitrogen of the isopropylamine moiety by *ca.* 0.5ppm (Figure 4.4.1.3) and for the appearance of new peaks integrating 6 H as a dd although in reality they are two overlapping doublets.

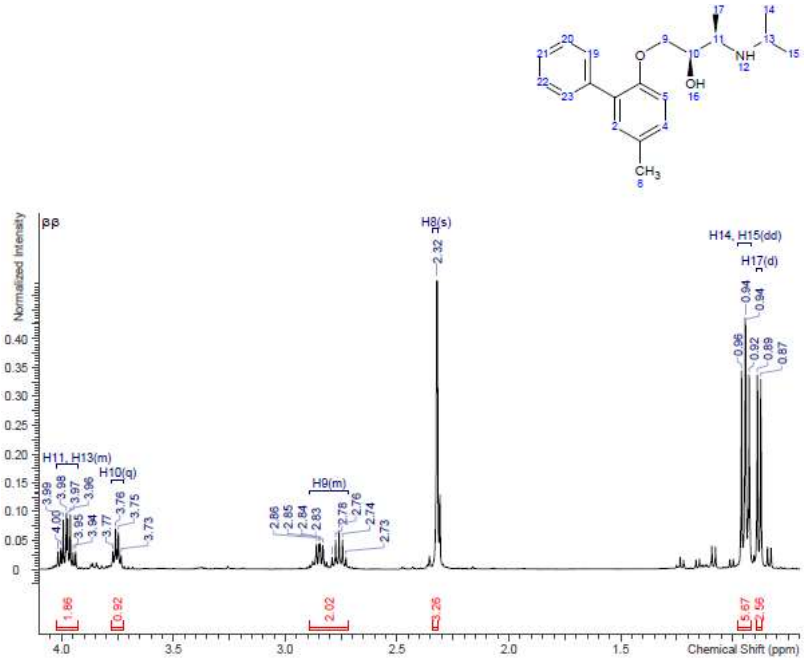


Figure 4.4.1.3 ^{1}H NMR spectrum of crude (±)-1-([5-methyl-1,1'-biphenyl]-2-yloxy)-3-(isopropylamino)butan-2-ol

The ^{13}C NMR spectrum also confirmed the appearance of 3 new signals in the aliphatic region of the spectrum (Figure 4.4.1.4) from the isopropylamine and changes in the already existent aliphatic peaks.

The new signals in both NMR spectra were then compared with pure isopropylamine and the solvents used (ethanol, methanol, DCM and ethyl acetate) to eliminate the possibility of any error but none fitted with the experimental ones obtained confirming with the crude NMR that the desired product has formed and that there is no starting material left.

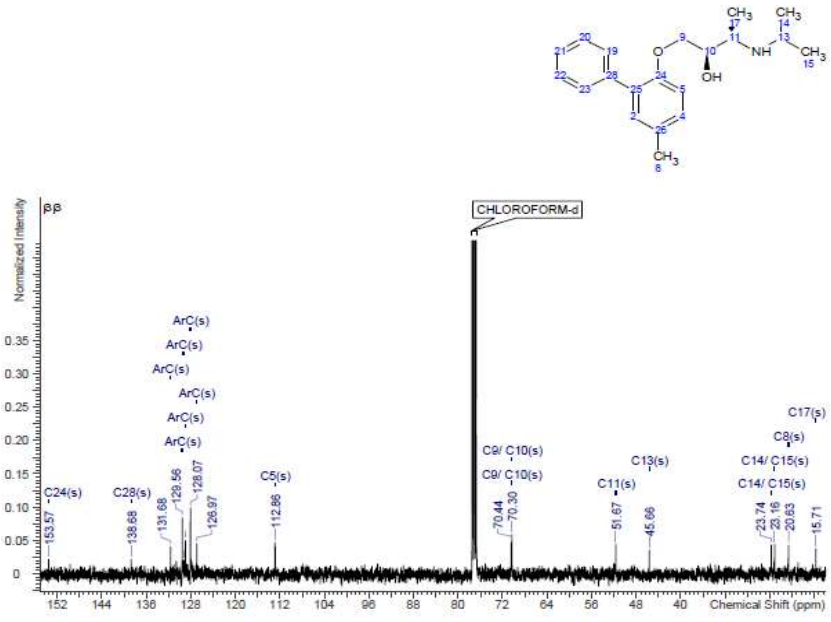


Figure 4.4.1.4 ^{13}C NMR spectrum of crude (±)-1-([5-methyl-1,1'-biphenyl]-2-yloxy)-3-(isopropylamino)butan-2-ol

As for the other final product, the (±)-1-([6-methyl-1,1'-biphenyl]-2-yloxy)-3-isopropylamino)butan-2-ol, its NMR looked very promising, with only a few impurities, but when trying to further purify the same problem arose. (See Appendix for the NMR spectra)

5. Conclusions

The most relevant conclusions that can be extracted from this research project are summarised as follows:

- The inhibition or retardation of stress induced breast cancer metastasis by blockade of the β_2-adrenergic receptor using biphenyl-ether derivatives could be a promising therapeutic option in the future.
- The hardest part of these syntheses was to find a good route to pure iodo compounds for subsequent Suzuki reactions.

  Iodination methods using NIS as iodinating source proved to be poorly selective and only a viable option in the case of the p-cresol. The Sandmeyer like reaction proved to be the most efficient method to synthesize the desired product when there was a possibility of multiple iodinated by products.
- Two new biphenyl-ols could be synthesized and converted to their respective desired amino alcohols in two steps, but some further work is needed to find a better purification method for the final products. Once purified to the highest standard, the final products will be sent for biological testing.
- The products obtained are racemic but the enantiomers could be made using Sharpless' asymmetric epoxidation methodology which gives the epoxides with ee > 90%. Since receptors in the body are also chiral the two enantiomers might have different biological properties.

6. Acknowledgments

I would like to thank my supervisor in the Nottingham Trent University professor John Wallis for all his support and guidance during the project. I would also like to acknowledge the NTU, the URV and the Erasmus program for granting me this opportunity. Finally, I would also like to thank my lab partners and the staff who helped me in everything they could.

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Appendix

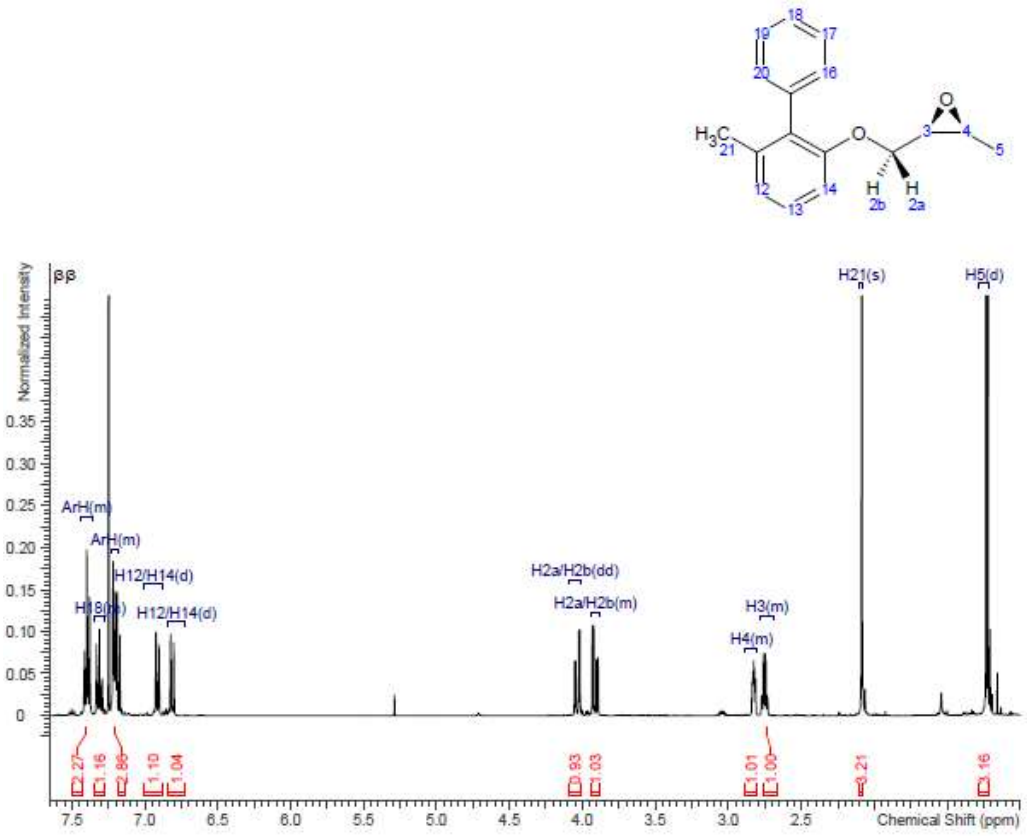

Figure 0-1 ^{1}H NMR spectrum of (±)-2-((3'-methyl–biphenyl-2'-yloxy)methyl)-3-methyloxirane

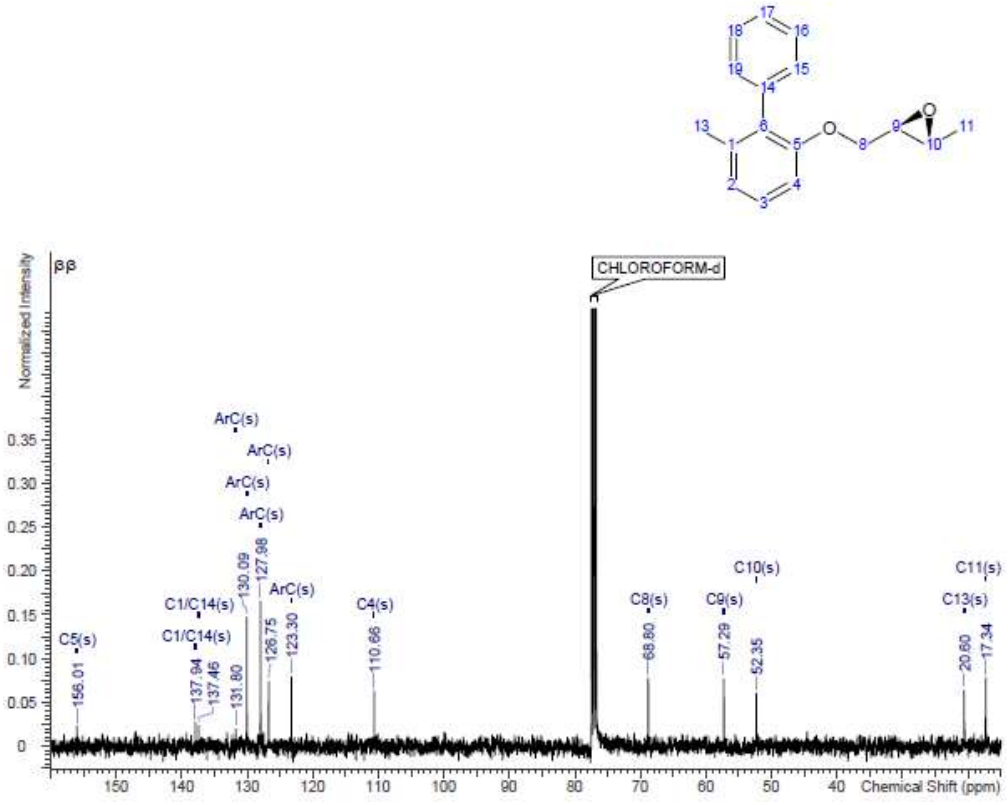

Figure 0-2 ^{13}C NMR spectrum of (±)-2-((3'-methyl–biphenyl-2'-yloxy)methyl)-3-methyloxirane

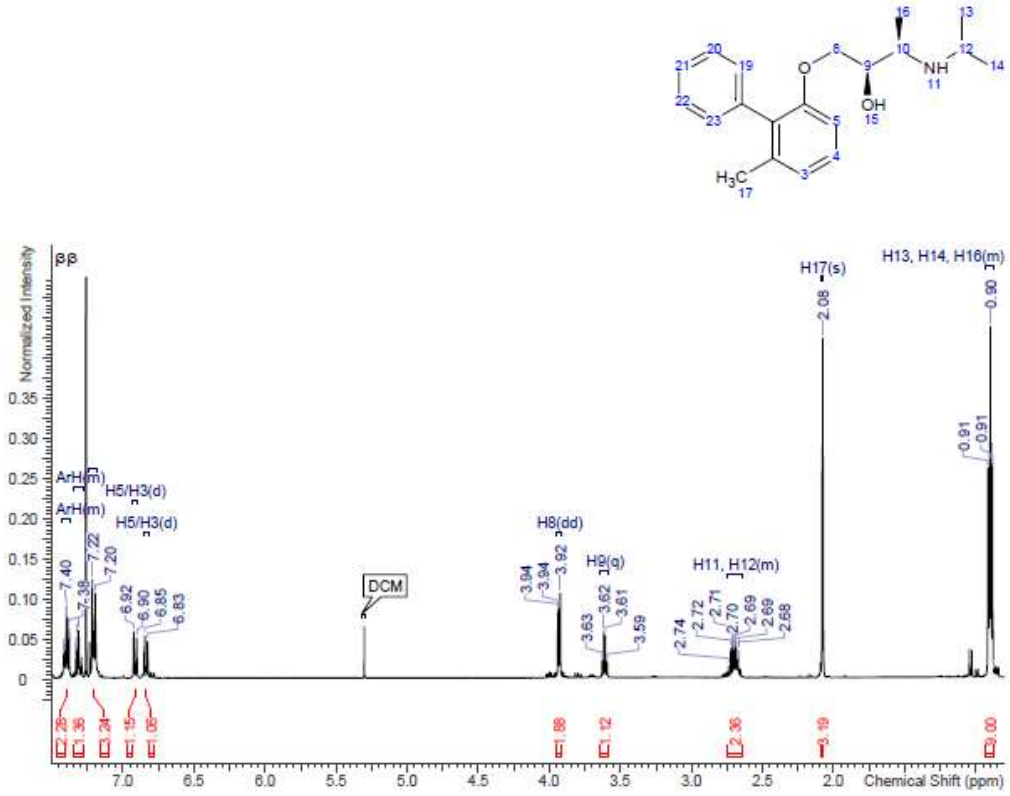

Figure 0-3 ^{1}H NMR spectrum of crude ±-1-([6-methyl-1,1'-biphenyl]-2-yloxy)-3-(isopropylamino)butan-2-ol