Synthesis of Organometallic Compounds with Potential Therapeutic Properties

Síntese de Compostos Organometálicos com Potenciais Propriedades Terapêuticas

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Abstract

The application of inorganic chemistry to medicine is a fast developing field, both from a therapeutic and a diagnosis perspective. In particular, the topic of organometallic pharmaceuticals is of emergent significance, and although efforts have predominantly been directed towards the evaluation of organometallics as anticancer agents, other targets for organometallic therapeutic agents include parasitic, viral and microbial diseases.

One of the strategies regarding the medicinal use of organometallic species involves the incorporation of organometallic moieties into the structures of known active drugs to improve their therapeutic properties. The present work followed this general approach.

One of the objectives of the project was to synthesise new organometallic analogues of the anti-HIV drug, Maraviroc, having the potential to act as antagonists of the CCR5 cell surface receptor, thereby blocking infection. These species were modelled upon the ligand lead-compound used in the screening project that yielded Maraviroc. The target molecules were formulated by replacing a biphenyl group in the lead compound with a 3-ferrocenylpropan-2-one substituent. Moreover, an azabenzimidazole ring, considered to be important for specific binding to the CCR5 receptor, was replaced with other potential pharmacophores, based on imidazole and triazole. The two structural units were joined by diethylamine links. The novel compounds were fully characterized by spectroscopic methods and will soon undergo biological evaluation.

A second objective was to synthesise new analogues of the estrogen receptor antagonist, Tamoxifen, coordinated to an organogermanium moiety (Ge-132). An alkyl ester linker was introduced between the metal and the tamoxifen moiety. These organometallic compounds are expected to combine cytotoxic activity with a stimulation of the immune system while maintaining negligible toxicity. The results of the preliminary attempts towards this goal are reported herein.

Keywords: Bioorganometallics, Tamoxifen, Germanium, Maraviroc, Ferrocene, CCR5

Resumo

A aplicação de química inorgânica à medicina tem contribuído para o rápido crescimento desta área do ponto de vista terapêutico e de diagnóstico. O tópico de compostos farmacêuticos de natureza organometálica tem ganho particular interesse, nomeadamente na avaliação de compostos organometálicos como agentes anti-cancerígenos e outros alvos de agentes terapêuticos, incluindo doenças causadas por microorganismos, incluindo bactérias, vírus e outros parasitas.

Uma das estratégias do uso medicinal de espécies organometálicas envolve a incorporação de fragmentos organometálicos em estruturas de fármacos conhecidos com o objectivo de melhorar as suas propriedades terapêuticas. Esta abordagem foi seguida no presente trabalho.

Um dos objectivos do projecto consistia na síntese de novos análogos organometálicos do fármaco com propriedades anti-HIV, Maraviroc, que desempenha a função de antagonista do receptor presente na superfície celular, CCR5, impedindo a infecção. Estes análogos foram desenhados com a base no modelo do projecto que englobou o rastreio do composto líder do qual resultou o Maraviroc. A formulação da estrutura das moléculas-alvo consistiu na substituição de grupo bifenilo presente no composto líder pelo substituinte, 3-ferrocenylpropan-2-ona. O anel heterocíclico (azabenzimidazole) considerado importante na ligação específica ao receptor CCR5 foi, também, substituído por outros potenciais farmacóforos baseados em anéis de imidazole e triazole. As duas unidades estruturais encontravam-se ligadas por intermédio de dietilamina. Os produtos sintetizados foram totalmente caracterizados por métodos espectroscópicos e em breve estarão sujeitos a testes biológicos.

O segundo objectivo consistiu na síntese de novos análogos do antagonista do receptor de estrógeneo, Tamoxifen, coordenado a um fragmento de Germânio (Ge132). Estas duas unidades foram ligadas por intermédio de um alquil éster. A estratégia de utilização destes compostos organometálicos é combinar o estímulo do sistema imunológico com actividades citotóxicas, enquanto se mantém um reduzido nível da toxicidade para o paciente. Os resultados das tentativas preliminares para atingir o objectivo pretendido encontram-se descritos no presente trabalho.

Palavras-chave: Química Bioorganometálica, Tamoxifen, Germânio, Maraviroc, Ferroceno, CCR5

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List of Abbreviations

Acetone-d_6	Deuterated acetone
AIDS	Acquired immune deficiency syndrome
Ar	Aromatic
t-BUOK	potassium *tert*-butoxide
n-BuLi	*n*-Butyllitium
$CDCl_3$	Deuterated chloroform
COSY	Correlation Spectroscopy
CMV	cytomegalovirus
CCR5	Chemokine (C-C motif) Receptor 5
CXCR4	Chemokine (C-X-C motif) Receptor 4
DBU	Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DMF	Dimethylformamide
DMSO-d_6	Deuterated Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
vDNA	viral Deoxyribonucleic acid
ESI	Electrospray Ionization
EtOAc	Ethyl acetate
Et_2O	Diethyl ether
FDA	Food and Drug Administration
FT/IR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas chromatography-mass spectrometry
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear Single Quantun Coherence
HTLV-III	T-lymphotropic virus type III
IR	InfraRed
LAV	lymphadenopathy-associated virus
K_2CO_3	Potassium carbonate
MAC	*Mycobacterium avium* complex
MeOH	Methanol
Mp	Melting Point
MS	Mass Spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide
NMR	Nuclear Magnetic Resonance

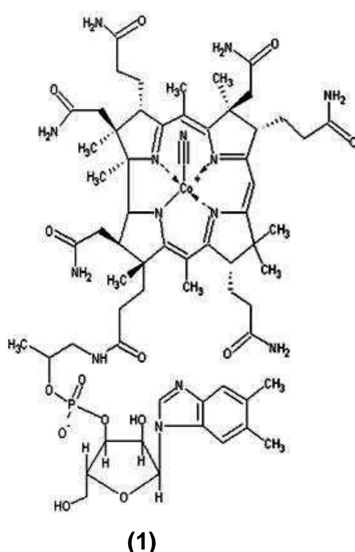
NNRT	Non-nucleotide/nucleoside analogue reverse transcriptase inhibitors
NRTI	Nucleotide/nucleoside analogue reverse transcriptase inhibitors
PCP	*Pneumocystis carinii* pneumonia
PI	Protease inhibitors
PLC	Preparative Layer Chromatography
ppm	parts per million
Py	pyridine
RNA	Ribonucleic acid
m-RNA	Messenger Ribonucleic Acid
r.t.	room temperature
RT	Reverse Transcriptase
SERM	Selective Estrogen Receptor Modulators
S_N2	bimolecular nucleophilic substitution
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMEDA	Tetramethylethylenediamine
U.V.	Ultraviolet

1. Introduction

1.1 - Metals in Medicine

The application of inorganic chemistry to medicine has progressed significantly in recent years, both for therapeutic and diagnostic purposes, although inorganic compounds have been used in medicine for many centuries.[1]

There is evidence that many organic compounds used in medicine do not have a purely organic mode of action. The majority of organic compounds is activated or suffers a biotransformation catalyzed by free metal ions or by metalloenzymes (e.g., vitamin B_{12} in Figure 1). There are also many examples of direct and indirect involvement of metal ions in metabolism.



(1)

Figure 1: Coenzyme Vitamin B_{12} **(1)**. [2]

Inorganic elements play important roles in biological and biomedical processes. Studies have shown that there about 24 elements essential for mammalian life.[1] For instance, both sodium and potassium can balance electrolytes and are responsible for signalling in the nervous system; they are therefore considered two essential elements in humans. Zinc is also known as an essential element. This second most abundant transition metal, following iron, plays catalytic and structural roles in biological systems. It also modulates signalling events. Other inorganic elements, such as nickel, molybdenum, vanadium, selenium and tin have also been shown to be essential for mammalian life. Some of these biologically-essential elements can become toxic at high doses but they are well tolerated at the low doses common in normal living systems.

The design of metal containing bioactive compounds is conducted for specific purposes, by controlling toxicity (side-effects) and targeting to specific tissues and cells where metal ion

activity is expected. On the other hand, metal ions can be also removed by sensitive metal binding molecules (chelating agents).

The uses of organometallic compounds have gained interest for treatment and understanding of diseases which are currently untreatable. However, one fundamental issue of using inorganic elements is the understanding of their mechanism of action based on molecular aspects.

1.2 – History of metallic compounds as medicinal agents

In 1760 Louis Claude Cadet de Gassicourt did some research on inks based on cobalt salts and isolated the first organometallic mixture: a brown highly toxic liquid, cacodyl oxide $[((CH3)_2As)_2O]$, which also contained other cacodly (dimethylarsinyl radical) compounds such as dicacodyl $[(CH_3)_2As)_2]$.[3]

It took nearly 80 years for the explanation of the reaction forming the arsenic compound to be proposed by Bunsen. He also discovered new cacodyl derivatives, cacodylates that were used as tonics and stimulating medicines for conditions related with lowering of haemoglobin levels (tuberculosis, multiple sclerosis, malaria, etc).

Meantime other scientists reported new organometallic complexes. An important milestone was achieved by Paul Ehrlich, who after many years of screening of arsenic complexes obtained the first organometallic drug, Arsphenamine, also known as Salvarsan in 1910.[4] (Figure 2) This drug treated syphilis and trypanosomiasis until the advent of antibiotics in the 1940s. It was the first modern chemotherapeutic agent. This research proved that organometallic compounds, such as organoarsenic complexes, could be less toxic and easier to manipulate than their inorganic counterparts which were famous for toxicity (for example white arsenic, As_2O_3).

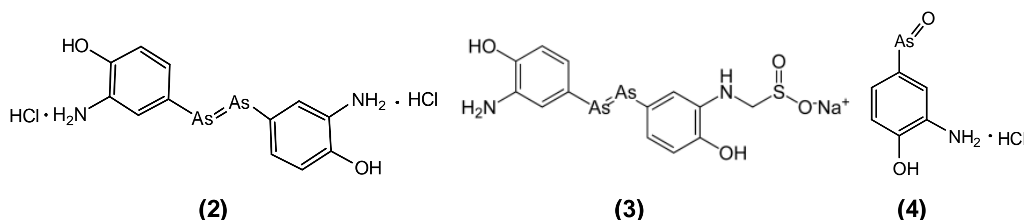


Figure 2: Chemical structure of Salvarsan **(2)**, Neosalvarsan **(3)**, Mapharsen **(4)**.

Nonetheless, although Salvarsan was a major breakthrough, improvements were still needed. Ehrlich came across of poor solubility of Salvarsan in water and its hydrochloride salt was found to be very toxic so the drug was administered in basic solution. The solubility was improved by modifying the amino group and a new derivative of Salvarsan, NeoSalvarsan, was born. Although the problem of solubility was solved, both organoarsenic compounds were easily oxidised in air and had to be stored under nitrogen. Unfortunately, the solution for this problem was only obtained in 1915, after Ehrlich´s death, with an oxidised form of Salvarsan salt, oxophenarsine hydrochloride (Mapharsen) which was also the product obtained in the body

after administration of Salvarsan. As a result the original drug was replaced by Mapharsen because it was stable in air allowing it to become the most used organoarsenic drug in the 1930s. [4]

Since 1930 other metal based drugs have been investigated, such as organometallic mercury derivatives and some very successful anti-cancer drugs: cisplatin and dichlorotitanocene. Other bioorganometallic compounds (Figure 3) have recently started clinical trials such as manganese superoxide dismutase mimics, vanadium insulin mimics, ruthenium nitric oxide scavengers, lanthanide-based photosensitizers.[1]

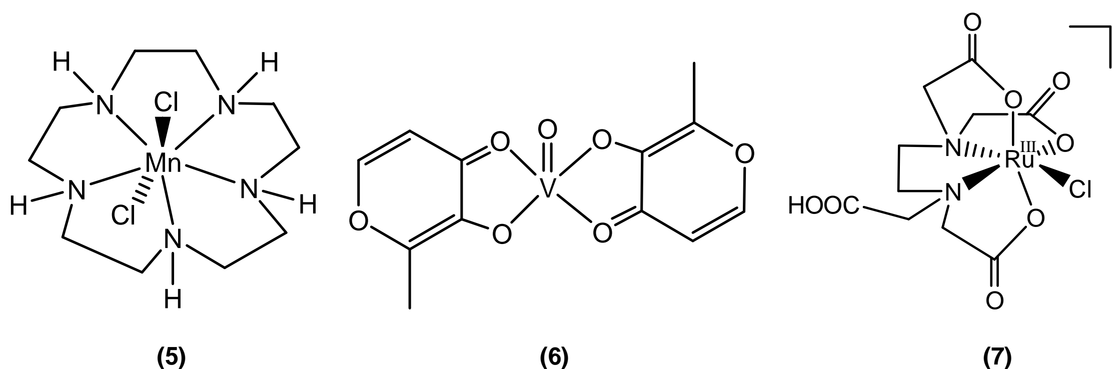


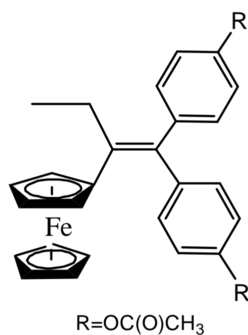
Figure 3: Some examples of: a) Manganese superoxide dismutase mimics **(5)**, b) Vanadium insulin mimics **(6)**, c) Ruthenium nitric oxide scavengers **(7)**.

In 1951 the discovery of the ferrocene sandwich structure allowed to understand the nature of the metal-carbon bond, which proved that the organometallic compounds can form σ, π and δ bonds that explain the formation of metallocenes, metal carbonyls and metal carbenes.[4] The discovery of ferrocene and elucidation of its stability caused by its remarkable structure rendered possible to extend organometallic chemistry into biology and medical applications, which permitted the development of bioorganometallic chemistry.

A pioneering strategy concerning the medicinal use of organometallic species involves the incorporation of organometallic moieties into the structures of known active drugs to improve their therapeutic properties. [4]

One such example is the synthesis of Ferrocenyl-tamoxifene derivative, and their analogues with other metals (Ti, Ru) which have gained biological interest because some of the compounds have achieved better or similar anti-proliferative effect compared with Tamoxifen.(1) [5] Recently, it has been reported that the ferrocenyl diphenol inserted in a Tamoxifen analogue (represented in Figure 4) exhibited strong *in vitro* anti-proliferative effects on both hormone dependent and hormone independent breast cancer cells, while Tamoxifen only acts on hormone-dependent cells.[6] This result is an excellent representative example of the potential of bioorganometallics as effective drugs, provided that no increase in toxicity is associated with their use.

(1) See section 1.4.4 for a more detailed information on Tamoxifen



(8)
Figure 4: An example of Ferrocenyl-Tamoxifene derivative **(8)** with significant anti-proliferative estrogen effects.

1.3 - Anti-HIV treatments

1.3.1 – Acquired immune deficiency syndrome (AIDS)

In broad terms AIDS can be defined as *"a disease formed from the most advanced stages of HIV infection in human beings which severely weakens the body's ability to fight infections and certain cancers"* [7]

1.3.1.1 - Definition of AIDS

Acquired immune deficiency syndrome or acquired immunodeficiency syndrome (AIDS or Aids) is a combination of symptoms and infections resulting from the damage to the human immune system caused by the human immunodeficiency virus (HIV).[8] This virus damages and kills cells of the immune system and progressively destroys the natural resources of defence of the body from infections and certain cancers. HIV is transmitted by direct contact of a mucous membrane, by blood transfusion when the virus is present, for instance between drug addicts or even mother-to-child in partum or during breastfeeding.

When the individual has been infected by the virus for more then a decade severe damages of the immune system by HIV are bound to happen. At this stage the individual is prone to opportunistic disease and at some point the individual may develop AIDS. These diseases are typically caused by organisms which do not induce illness in healthy human beings but are highly effective and dangerous in individuals with compromised immune systems.

The strong connection between HIV and the onset of AIDS is generally recognised although some early controversy disputed this relationship. A person is recognised as carrier of AIDS if the following conditions are obeyed:

- the individual contains HIV antibodies present in his blood (HIV positive);
- the individual has a CD_4^+ cell count lower than 200 cells/mm^3 in the blood or a history of opportunistic disease;

Currently this definition of AIDS, established in 1993 needs to be re-evaluated because AIDS has become a chronic disease which is manageable for many people in certain stages, as long as effective treatment is available. For instance, the majority of HIV-positive people under competent medical care can keep their CD_4^+ count above 200cells/mm^3 and avoid the opportunistic diseases unless they are resistant to the drugs used for the treatment.

1.3.1.2 - The primordial's of Acquired immunodeficiency syndrome (AIDS)

In July 1981 a number of gay men in New York and California developed rare opportunistic infections and cancers which were reported as the "gay cancer". One of the unexpected cancer types was a particulary aggressive form of Karposi´s Sarcoma which was known to affect aged patients and with relatively benign prognosis.[9] At the same time, many healthy young people suffered fevers, symptoms of flu due to lung infection pneumonia called *Pneumocystis carinii*. About a year later this illness was found to be related to blood and highly contagious in certain groups of the population.

In the first year (1982) this disease was called in many different ways such as: "gay compromise syndrome"[10] , GRID (gay-related immune deficiency), AID (acquired immunodeficiency disease), "gay cancer" or "community-acquired immune dysfunction".[11] These designations proved a direct connection between the immune deficiency and the homosexual community. Strangely, this so-called disease started appearing in people who consumed injectible drugs and then it extended to hemophilic patients. These patients had been submitted to blood transfusion which turned out to be responsible for spreading AIDS.

Quickly many cases of AIDS appeared at the same time in different locations from USA and Europe to Africa. Just only in the first year over 1600 cases were diagnosed with close to 700 deaths. More than 25 million people have died of AIDS since 1981. Africa has 11.6 million AIDS orphans. [12]

In the beginning AIDS rates grew exponentially and due to progress in research and a better understanding of this disease the numbers of people infected have started to stabilize (Figure 5).

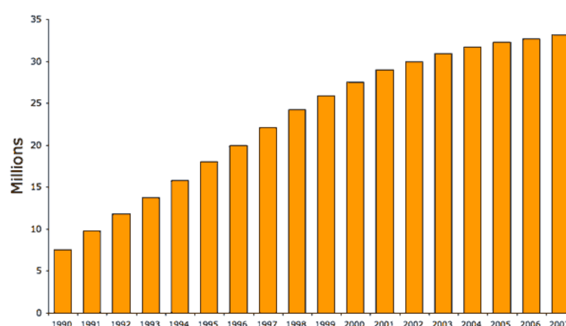


Figure 5: Global number of people (in millions) infected with HIV worldwide.[12]

In the 1980s and early 1990s, HIV/AIDS was a major cause of death among young adults, with the values of mortality rate increasing every year from 1987 to 1994. A significant decrease in number of deaths of people with AIDS was first seen in 1996, the year after the first protease inhibitor (PI) was introduced. In 1997 the number of HIV/AIDS deaths decreased approximately 50%. In the following year (1998) the number of deaths suffered a 20% reduction. However, in 1999 the decline had stopped.[13]

HIV/AIDS deaths decreased from more than 51,000 in 1995 to about 16,000 in 2002 (the latest year for which data are available).[13]

During 2007 more than two and a half million adults and children became infected with HIV. At the end of the year, nearly 33 million people globally were living with HIV/AIDS. Also women accounted for 50% of all adults living with HIV worldwide, and for 59% in sub-Saharan Africa. Young people (under 25 years old) account for half of all new HIV infections worldwide. In developing countries, 9.7 million people were in immediate need of drugs to save their lives from AIDS and unfortunately only 31% received the drugs.[12] Despite recent improvements in access to antiretroviral treatment in 2007 two million deaths from AIDS occurred.[13]

1.3.2 – Human immunodeficiency virus (HIV)

AIDS is caused by a retrovirus (Figure 6), an infectious agent, of two types: type 1 (HIV-1) and type 2 (HIV-2) which is thought to have originated in non-human primates in sub-Saharan Africa and then transferred to humans. HIV-1 is more dangerous and is easily spread, which explains the cause of the majority of HIV infections. HIV-2 is rarely transmitted and it seems to be restricted to West Africa.

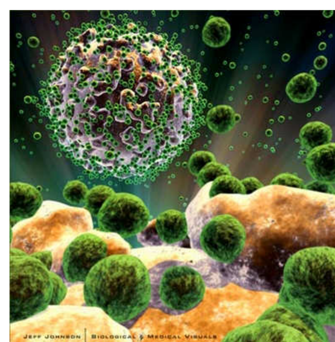


Figure 6: Image of HIV manipulated by software[14]

There are four routes of transmission of HIV which involve contact with fluids and infected tissue [13][15], and can be summarizes as:

- unprotected sexual intercourse;
- contaminated needles;
- breast milk;
- at the birth of a child, through mother-to-child transmission;

An infection of an individual by HIV-1 consists in progressive decrease of the CD_4^+ cell count and an increase in viral load (exact amount of virus present in the body fluid). There are four stages of infection of HIV [11] (Figure 7):

- incubation period – period of two to four weeks with no apparent symptoms.
- acute infection – virulent period of nearly one month where flu like symptoms appear (fever, swollen lymph nodes, sore throat, muscle pain) due to a rapid viral replication which increases the infection. This period is very favourable to transmit the virus to others.
- latency stage – initially the increase of CD_4^+ count shows that the body is creating a strong immune defence which reduces the number of copies of viral RNA. After this phase, the viral load starts progressing gradually, the CD_4^+ count is still acceptable for a healthy human being but at the end it decreases. This stage can extend for decades before it jumps on to the final stage of infection.
- AIDS – when CD_4^+ cell numbers decrease below a critical level, cell-mediated immunity disappears and opportunistic infections appear such as *Pneumocystis carinii* pneumonia (PCP), *Mycobacterium avium* complex (MAC) disease, cytomegalovirus (CMV), toxoplasmosis, and candidiasis. The individual is recognised as carrier of AIDS, which may progress exponentially leading to death in a year's time.

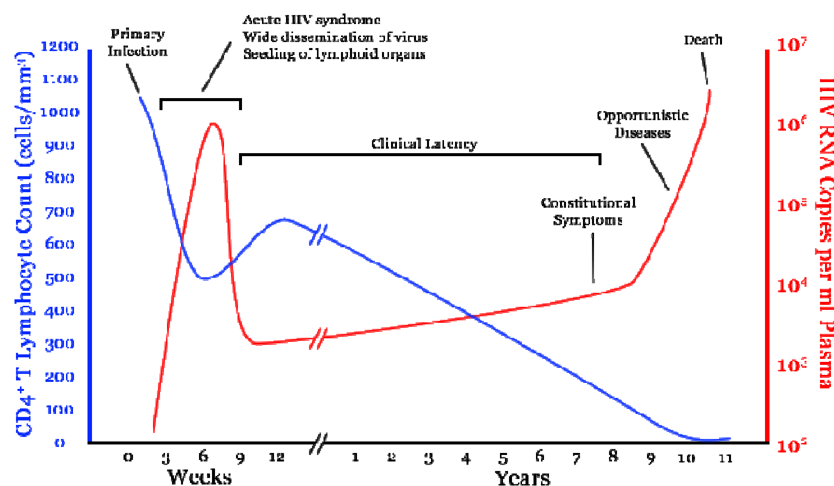


Figure 7: A general graph that relates number of viral RNA copies per mL of plasma (scale and curve in red) with CD_4^+ cell counts per mm^3 (scale and curve in blue) over the average course of untreated HIV infection.[15]

1.3.2.1 – Mode of action

The human immunodeficiency virus was first discovered by a group of researchers at the Pasteur Institute in Paris in 1983 under Dr. Luc Montagnier´s supervision, who was awarded A Nobel Prize in Medicine in 2008 for the discovery of HIV. At this time the virus was named lymphadenopathy-associated virus (LAV). In the following year, Robert Gallo confirmed the discovery of the virus, but changed the name to human T-lymphotropic virus type III (HTLV-III). At present, both scientists are considered responsible for the discovery of the virus and agreed to keep the current name, human immunodeficiency virus (HIV).

After the virus was discovered research quickly developed to discover the mechanism of its action in the body. This mechanism was studied by collaboration of many groups of researchers and clinical specialists worldwide. (Fig. 8)

Like other retrovirus, HIV has also its genetic material (virial RNA) surrounded by a protective cover (capsid). The virial RNA contains information for its own replication and a protein called Reverse Transcriptase (RT) that is crucial for viral replication inside CD_4^+ cells.

HIV contains proteins called antigens that have various functions; for instance the combination of two antigens present on the surface of the virus, gp120 and gp41, link the virus to CD_4^+ cells infecting them. Basically, the white blood cell acts as the host that HIV needs in order to replicate because CD_4^+ cells contain genetic material in the form of DNA that has all the information for a cell to function and survive.

On the surface of T cells there is a protein called CD_4 that has mirror conformation with antigen gp120 present in HIV. The bond between the white blood cell and the HIV only happens if the HIV´s antigen is located in the right place of the receptor site of CD_4, also designated as the docking port for HIV. This connection becomes stronger when linked to a second receptor designated as chemokine (C-C motif) receptor 5 (CCR5) or chemokine (C-X-C motif) receptor 4 (CXCR4). Most HIV strains use CCR5 at time of initial infection.[16] When this connection is established the HIV injects its viral RNA and the reverse transcriptase into the T cell where the capsid is dissolved. The reverse transcriptase uses the material from the cell and copies the viral RNA into a complementary single strand DNA. RT has a high error rate and frequently

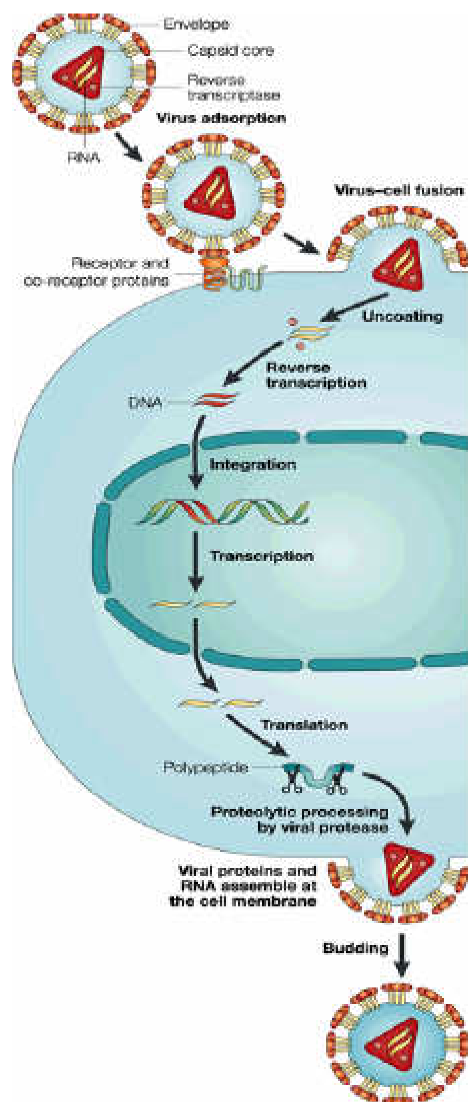


Figure 8: Life Cycle of HIV.[17]

leaves mutations in the copied DNA in resulting variant forms of HIV in the cell. Afterwards, the viral RNA is destroyed and a double chain of DNA is formed using the single chain as a template, resulting into a double-stranded viral DNA intermediate (vDNA). This vDNA is then transported into the cell nucleus and integrates with the cell´s DNA. The integrated viral DNA is known as a provirus. At this stage, if the cell is activated it will start the transcription of this proviral DNA creating several copies of viral m-RNA which contains information to produce the capsid proteins and proteins needed for virial assembly. Also, the form of viral m-RNA spliced by an enzyme called protease contains information to produce other proteins like specific antigens of HIV. All these proteins, RT and viral RNA assemble at the membrane where the lipid membrane involves all this viral components and is destroyed in the process. As a result, the white blood cell is completely destroyed and the immune system starts getting much weaker, which renders the body open to opportunistic infections.

1.3.2.2 – Treatment

Once the virus and it's mechanism of action was discovered scientists have trying to find a cure or vaccine for HIV. Unfortunately, this hasn't been possible although there has been an incredible progress in developing drugs to fight the infection caused by HIV. The studies were performed on the basis of HIV´s life cycle by many researchers. They analysed all the steps involved in the cycle and studied how was it possible to block them in order to avoid replication of HIV or even prevent the infection by blocking its entry into the white blood cell.

Currently, all the anti-HIV drugs produced are classified by their site of action in the life cycle of HIV resulting into five major classes of antiretroviral drugs:

- Nucleotide/nucleoside analogue reverse transcriptase inhibitors (NRTI)

This class belongs to the first type of drugs discovered to treat HIV infection in 1987. These drugs block the function of reverse transcriptase enzyme present in the virus which is crucial for the replication of the viral RNA and prevent the total synthesis of double-stranded viral DNA.

Basically, the NRTI´s are known as competitive inhibitors of RT. These types of drugs are analogues of natural deoxynucleosides for creating the viral DNA and compete with the ones present in the T cell. Yet the major difference between NRTI and natural deoxynucleosides is the missing 3´-hydroxyl group in the former so when a molecule of NRTI is incorporated in the growing viral DNA the following desoxynucleotide will not be linked to it. As a result, the synthesis of viral DNA is stopped and HIV is not multiplied. Some examples of drugs which belong to this class of inhibitors are represented below (Figure 9):

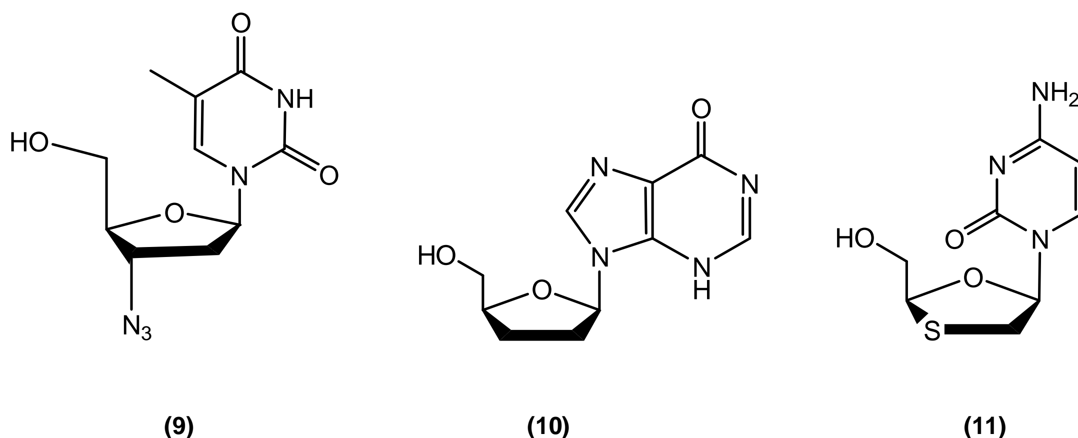


Figure 9: Examples of NRTI´s drugs, first drug approved by the FDA (1987) - Zidovudine **(9)**, second drug approved (1991) – Didanosine **(10)** and lastest drug approved (2004) – Lamivudine **(11)**.

- Non-nucleotide/nucleoside analogue reverse transcriptase inhibitors (NNRTI)

These types of inhibitors are classified as non-competitive inhibitors of RT because they bind to an allosteric site of the reverse transcriptase, restraining its movement and consequently the protein is blocked and no viral DNA is synthesised. Some examples of drugs which belong to this class of inhibitors are represented below (Figure 10):

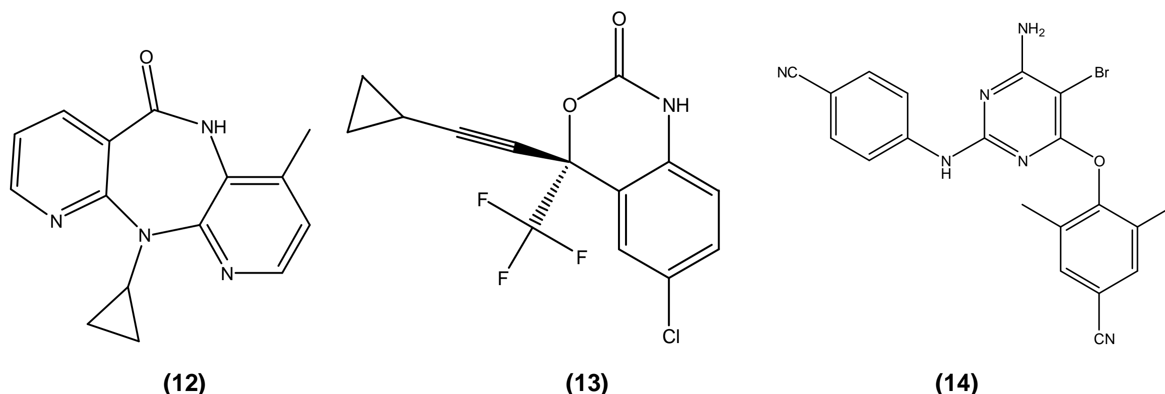
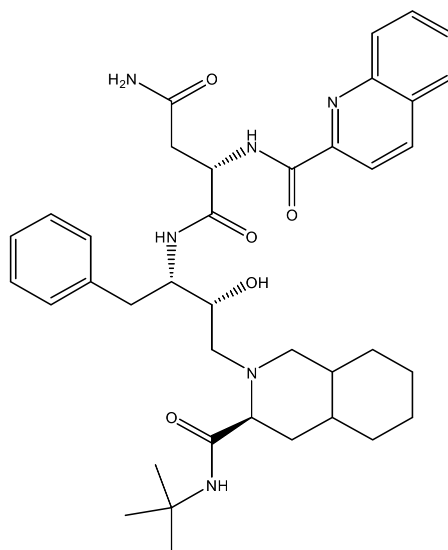


Figure 10: Examples of NNRTI´s drugs, first drug approved by the FDA (1996) – Nevirapine **(12)**, third drug approved (1991) – Efavirenz **(13)** and lastest drug approved (2008) – Etravirine **(14)**.

- Protease inhibitors (PI)

These types of drugs inhibit the function of an enzyme which participates in the splicing of the viral proteins called protease. The first protease inhibitor was approved in 1995 (Figure 11).

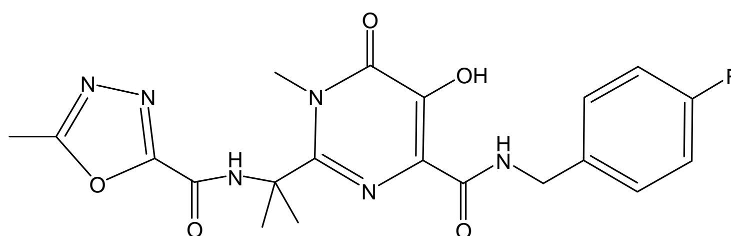


(15)

Figure 11: First protease inhibitor (1995) – Saquinavir **(15)**.

- Integrase inhibitors

This class of drugs inhibits an important enzyme present in the HIV life cycle whose function is to integrate the viral DNA previously formed with the host´s DNA resulting into a provirus. Currently there is only one drug approved named as Raltegravir, introduced in the UK in January 2008 (Figure 12).[18]



(16)

Figure 12: First integrase inhibitor approved in 2007 – Raltegravir **(16).**

- Fusion or entry inhibitors

These types of drugs interfere with the binding, fusion and entry of an HIV virion to a white blood cell. They are also known as fusion inhibitors and currently have gained interest among researchers because these drugs delay the infection from HIV to AIDS. At present there are only two drugs approved Maraviroc (Figure 14) which will be described later and Enfuvirtide.

Presently, the treatment for HIV involves a combination of different classes of drugs currently called as cocktails. This strategy of treatment, designated as highly active antiretroviral therapy (HAART), has benefited many HIV-infected individuals since its introduction in 1996.

The so-called cocktails consist of at least three drugs which correspond to two different antiretroviral classes. The most common combination is two NRTI´s and one PI ou NNRTI. Only when the patients are resistant to this therapy are they introduced to the new classes of drugs (entry and integrase inhibitors).

However, HAART can be less effective in dose to 50% of the patients. This fact is a consequence of intolerance/side effects of the medication, previous exposure to antiretroviral therapy and infection with a certain strain of HIV that is resistant to the drug. Another reason that explains the incomplete efficiency of HAART is poor compliance of the patients in following all the prescriptions with the exact dosage of the drugs to be taken.

Fortunately there are promising new treatments some of them have been already been referred to previously. Newly proposed treatments involve certain enzymes that are able to remove HIV from an infected cell. Basically, the treatment consists in extracting the patient´s stem cells, curing them and then finally, re-injecting them in order to spread the enzyme so that it can find and remove the virus from the body.[19]

1.3.3 – Maraviroc

As referred earlier, presently, there is a new class of antiretroviral drugs that have considered very interesting by many researchers. This new class corresponds to future drugs which act as inhibitors of a certain receptor present in immune cells by blocking the entrance of the virus in the cell.

Maraviroc also known as Selzentry in the USA or Celsentri in the EU is the first in a new class of drugs designed to slow the progression of HIV and received priority review by the FDA.[20]

Maraviroc is a selective, reversible, small molecule CCR5-receptor antagonist. Basically, it prevents the virus from entering healthy cells by blocking a human co-receptor called CCR5 which is located next to CD_4 receptors present on the surface of immune cells. Nearly 50 to 60% of patients who have been treated with other HIV medications have spread CCR5-tropic HIV-1.(2)

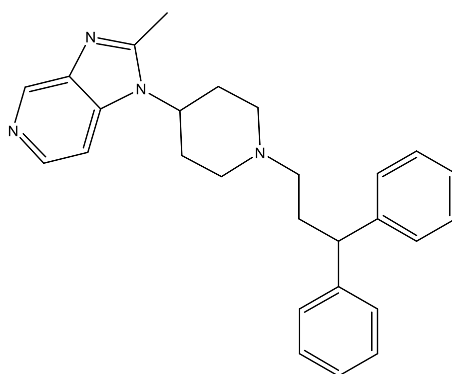
This drug is only used when combined with other antiretroviral drugs for the treatment of adults with CCR5-tropic HIV-1. These patients typically have high levels of HIV in their blood and due to failures of previous antiretroviral treatment regimens.[21]

(2) Identification of the cell surface receptors CCR5 and CXCR4 and the study of their key role in the cell-entry process have permitted a new classification of HIV-1. A virus that exclusively uses CCR5 for cell entry is named as *CCR5-tropic* (formerly *macrophagetropic),* and virus that exclusively uses CXCR4 is designated as *X4-,* or *CXCR4-tropic* (formerly *T-cell-tropic).* A virus that is able to use both receptors with comparable efficiency is termed *R5/X4-,* or *dual-tropic.*

When initiating therapy with Maraviroc certain points have to be considered. Firstly, the patient has to test positive for CCR5-tropic HIV-1 (tropism) because patients with dual or CXCR4-tropic HIV-1 are not taken in account for blocking successfully the entrance of the virus by Maraviroc. Secondly, the patient's treatment history must be very well known in order to avoid undesirable interactions between drugs. Finally, the safety and efficacy of Maraviroc have not been established in treatment of naïve adult patients and young patients (<16 years of age).

1.3.3.1 – Discovery

Maraviroc, formerly designated UK-427857, was formulated in UK labs belonging to the drug company Pfizer. The industry screened a vast compound library using a chemokine radioligand-binding assay to identify a small-molecule CCR5 ligand.[22] From this high-throughput screen an imidazopyridine CCR5 ligand was identified. (Figure 13)



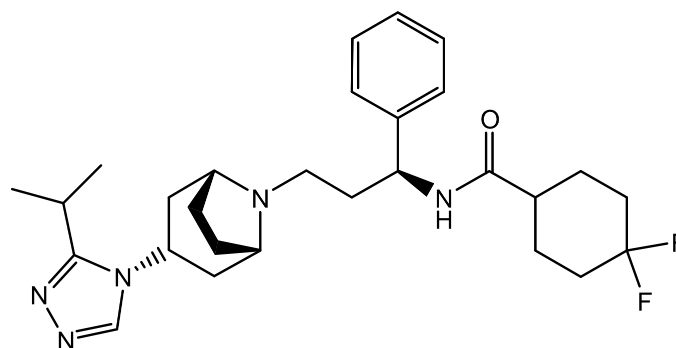
(17)

Figure 13: Imidazopyridine CCR5 ligand **(17)**, UK-107,543

This ligand turned out to be the most potent and was selected as the ligand lead compound. Other two assays (binding and functional experiments) were developed and optimized. One of them measured the binding of the virus to the cell surface receptors and the other modelled the membrane fusion events involved. A programme outlined and synthesized nearly 1000 analogues from which Maraviroc was selected.

On August, 6, 2007 the US Food and Drug Administration approved Maraviroc for treatment of experienced patients and on September, 24 of the same year Pfizer announced that the European Commission approved Maraviroc.(3) (Figure 14)

(3) IUPAC [4,4-difluoro-*N*-{(1*S*)-3-[3-(3-isopropyl- 5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl}cyclohexanecarboxamide].



(18)
Figure 14: Maraviroc **(18)**.

1.3.3.2 – Mechanism of action

The entry of HIV-1 in the immune cells is a complex process with many steps involved which have been described in section 1.3.2.1.

Maraviroc selectively binds to the human chemokine receptor CCR5 present on the cell membrane, preventing the interaction between HIV-1 gp120 and CCR5 necessary for CCR5-tropic HIV-1 to enter cells. CXCR4-tropic and dual-tropic HIV-1 entry is not inhibited by Maraviroc. Basically, this drug blocks the entry of HIV by inducing changes in the conformation of the CCR5receptor.

1.3.3.3 –Efficiency and Toxicity

Some results from the studies of Maraviroc compared with placebo have shown reduction of the concentration of the virus (viral load) when administered with the correct combination of other antiretroviral drugs. Typically, there was a 2-fold decrease in the viral load of patients treated with a combination of Maraviroc and other antiretrovirals compared with patients administered a similar regimen but without Maraviroc.[23] The efficiency was found to be quite similar when the drug was consumed once or twice a day.

As all drugs, Maraviroc has some adverse effects on naïve patients. The most common ones are cough, fever, upper respiratory tract infections, rash, musculoskeletal symptoms, abdominal pain, and dizziness.[20]

At the moment Maraviroc is thought to be non carcinogenic and non genotoxic and does not appear to affect fertility.[23] Patients who previously had liver problems, especially hepatitis B or C are advised not to use Maraviroc because it can severely affect the liver. As like other retroviral drugs, patients consuming this new drug undergo high risk of having new symptoms of infection caused by the recovered immune system and death of bone tissue, osteonecrosis.

The potential hepatotoxicity of Maraviroc is still under scouting because there has been one severe case in a trial reported in a patient with prior liver function abnormalities. Researchers suspect that Maraviroc was not responsible for causing the hepatoxicity but for precaution, Maraviroc´s label contains a warning that indicates a potentially increased risk of hepatoxicity with the use of the drug.[24]

Researchers should be alerted to the long-term safety of blocking CCR5 by Maraviroc because the function of this receptor in a healthy individual is not entirely understood. Another point of concern is the fact that the patients with CCR5 tropic-HIV-1 can induce the entry of the virus by the other receptor, CXCR4, because their CCR5 receptor is being blocked.[25]

1.3.3.4 – Metabolism and Excretion

Across species, Maraviroc is metabolized predominately by the CYP3A isozymes to inactive metobolites via aromatic and aliphatic hydroxylation and *N*-dealkylation. [21] The intact Maraviroc is excreted in the urine and feces. (Figure 15)

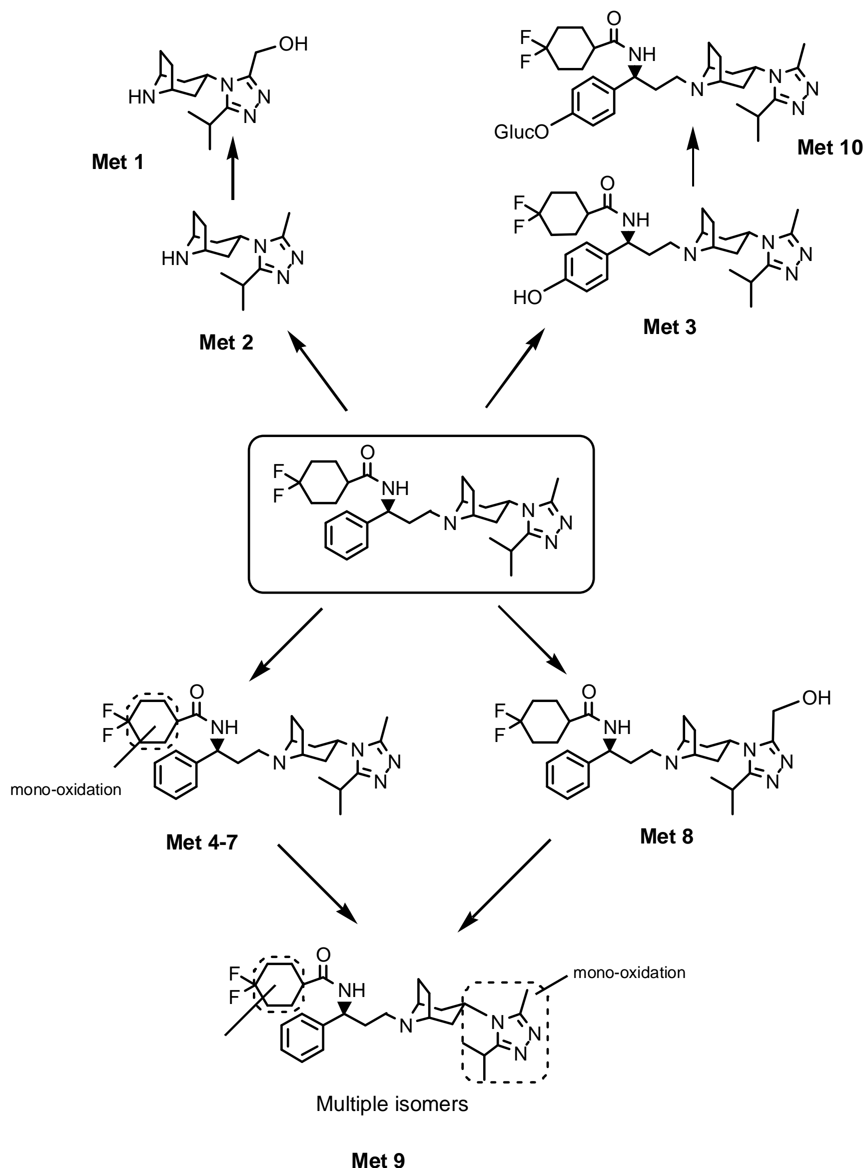


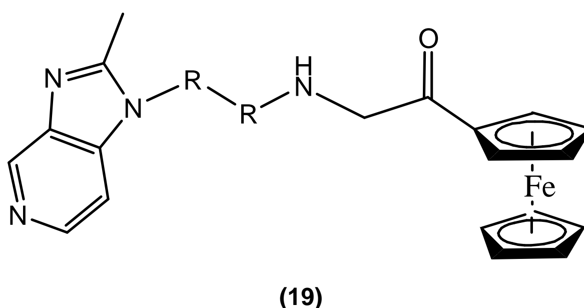
Figure 15: Metabolic pathways of Maraviroc in human, rat, dog, and mouse with percentage of dose in excreta; Maraviroc: human (33%), rat (79%,), dog (45%), mouse (39%), Met **1**: human(2%), dog and rat (<1%) and mouse (1%); Met **2**: human and mouse(7%), dog and rat (5%); Met **3**: human(<1%), dog (4%), rat and mouse (5%); Met **4-7**: human (29%), rat (3%), dog (13%), mouse (9%), Met **8**: human (10%), rat (1%), dog and mouse (8%); Met **9**: human (5%), rat and mouse (<1%), dog (6%); Met **10**: mouse (1%). [26]

Advanced studies have shown that in humans the rate of metabolism of the drug is decreased significantly in the presence of CYP3A4 inhibitors and increased in the presence of CPY3A4 inducers.

1.3.4 – Aim of the project

The aim of the current project is to synthesise novel organometallic analogues of Maraviroc, initially based on the ligand-lead compound discovered by Pfizer which was previously described (Figure 13).

The target molecule was designed by replacing the two phenyl groups of the lead compound (represented in Figure 13) by a ferrocenylethanone substituent, assuming a comparative overall geometry in order to maintain the original interaction of the target with the CCR5 receptor (Figure 16). Another reason for selecting this bioorganometallic compound as target molecule is supported by the biocompatibility of ferrocene derivatives which are found to be stable in aerobic aqueous media, to have synthetic versatility, favourable electrochemistry and low toxicity.[58] As mentioned before Ferrocene derivatives have been widely studied in Medicinal Chemistry and have given promising results in the formulation of potential new drugs.



(19)

Figure 16: Generic structure of the target molecule **(19)**.

Moreover, the initial target molecule maintains the imidazopyridine ring which was found important for affinity to the CCR5 receptor when the high-throughput screening of a chemokine radioligand-binding assay was performed. This ring is located on the opposite side of the ferrocenylethanone substituent; these components are linked by an alkylamino spacer. (Figure 16)

1.4 - Metal based anticancer treatments

1.4.1 – Cancer

In broad terms cancer can be defined as *“a class of diseases in which abnormal cells of the own body divide without control and are able to spread to other parts of the body.”* [27]

1.4.1.1 – Definition of Cancer

Cancer is considered a combination of many diseases associated with a certain type of human cells which are altered genetically. Usually, cells grow and divide to form new cells that the body is demanding and when they grow old they die due to the body’s natural process designated as apoptosis. Sometimes, the body contains genetically altered cells characterised by abnormal proliferation. Basically these cells do not go through the normal process of apoptosis or controlled cell death and as result the extra cells can form a mass of tissue identified as a *tumour*. The uncontrolled growth of the cells is explained by the loss of chemical signals that participate in this process. (Figure 17)

After the proliferation of malignant cells the tumour formed can invade and destroy adjacent tissues and then spread to other remote spots by blood or lymph and establish themselves; this process is designated as metastasis. These processes do not occur in benign tumour cells apart from self limited proliferation of the growth. (Figure 17)

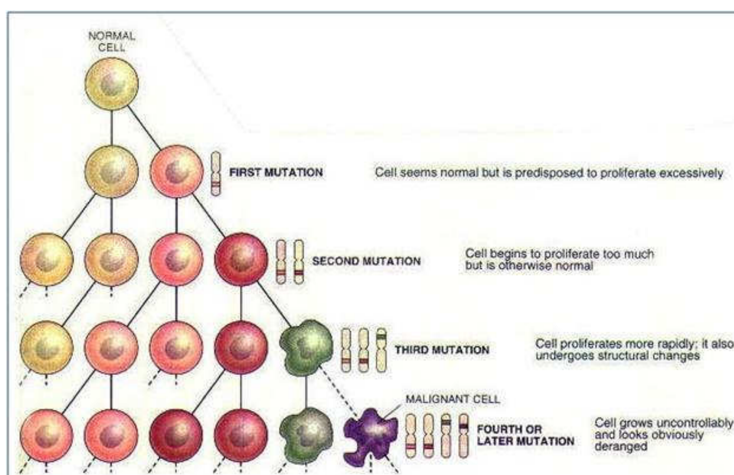


Figure 17: Formation of a malignant cell.[28]

On the whole, cancer incidence rates have increased by one quarter since 1975 but have remained fairly stable over the past decade. Worldwide there were around 11 million new cases of cancer reported in 2002 and a quarter of these were in Europe.[29] Approximately there are 200 types of cancer characterized and classified according to the organ or type of cell in which they originally begin their proliferation. The most common type of cancer is prostate cancer in man and breast cancer in woman.

Cancer depends on many factors, including age, lifestyle and genetic information. It can be caused by certain chemical substances and sources of ionizing radiation that modify and damage the genetic material, DNA, which plays a major role in the growth of cells and metastasis. Other relevant causes of cancer are by viral infections (e.g. HIV), by dysfunctions of the immune system and also by hormones, which will be discussed further on. Rarely, the heredity can be an important factor that explains a certain type of cancers by the presence of specific genes that cause the disease. The following symptoms are common within most cancers: fever, night sweats, weight loss, lack of appetite and fatigue.[29]

1.4.2 – Treatment [31]

The current approaches in the most common types of cancer include surgery (primary treatment), radiation therapy and chemotherapy. These therapies can be used either by their own or in combination with other therapies. For instance, before the primary treatment it is quite common using hormone therapy for prostrate and breast cancer. More recent treatment options include targeted therapies and biologic treatments. In general, the treatment starts with surgery followed by chemotherapy or radiation therapy. The three types of treatment of cancer of any kind are described as follows:

- Surgery consists on the removal of all the cancerous tissue from the body and in some cases it can cure the cancer. This treatment helps to confirm a diagnosis, to identify the stage of the disease, to relieve side effects by obstruction or even to release pain.
- Chemotherapy is a treatment that uses specific drugs to destroy malignant cells by interfering with the proliferation of cancer cells, eventually causing their death. This therapy is used to reduce or eliminate the tumour by destroying and preventing the cells from spreading in the body. However, the treatment has severe side effects because it also damages the healthy cells and as a result the body´s immune system becomes weak and the number of blood cells is reduced.
- Radiation therapy uses high energy X-rays to destroy cancer cells and it only affects one part of the body although this treatment damages both cancer and normal cells of the body. The therapy is commonly used after surgery in order to keep the tumour from returning.
- Hormone Therapy – some types of cancer (e.g., prostate and breast) only grow and develop in the presence of natural hormones. This therapy manages the concentration of the hormones within the body. For example, Tamoxifen (Nolvadex), which has been referred previously, is an anti-estrogen drug used

to treat hormone-responsive breast cancers and will be described in detail ahead.

- Biologic Therapy or Immunotherapy – uses substances which help restore the function of the immune system by stimulating the body's disease-fighting mechanisms or blocking certain proteins that are involved in the growth of cancer cells. Recently, researchers have been developing monoclonal antibodies (e.g. Herceptin) and vaccines.
- Targeted treatments – use drugs that interact with several proteins that contribute to cancer and selectively damage and cause death of only the cancer cells.
- Antisense therapy – uses small strands of DNA that have been modified and which block gene expression by being linked to m-RNA and as result block the production of a single protein.
- Gene therapy – repairs or replaces damaged genetic material or can even add new genetic material.

With the presence of a variety of therapies supported by an intense study of malignant tumours, researchers are still trying to identify a proper target and correspond it to a molecule that can fight cancer efficiently. For example, some researchers are working on anti-angiogenesis drugs, which interrupt a tumour's blood supply and consequently starve the cancer cells and prevent them from growing and spreading.[30] This research is still on experimental basis because one of the problems is the minor differences between the malignant and normal cells of the body. Both have similar metabolic mechanisms and chemical structures, which compromises the potential drug to distinguish a malignant cell from the normal cell of the body; consequently the toxicity caused by the drug is increased.

Bioorganometallic chemistry has played an important role to formulate novel anti-cancer drugs and currently it has gained interest in many other fields of medicine.

In 1960 Rosenberg discovered the anti-cancer activity of *Cis*-platinum also named as Cisplatin. This organometallic compound has achieved remarkable anti-tumoral effects against metastatic testicular and ovarian cancer.[1][31]Also, the discovery of cisplatin has been responsible for promoting research into a new field which combines bioorganometallic chemistry with medicine.

This metal-based compound presents a square-planar geometry in consequence of the four ligands (two chlorines and two amino groups) that are bound to platinum (Figure 18).

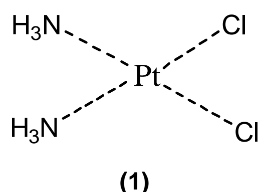


Figure 18: *Cis*-platinum **(1)**.

When cisplatin is inside the cell it undergoes hydrolysis and one of the chlorine ligands is substituted by a molecule of water, producing the reactive charged platinum complex, $[Pt(NH_3)_2ClH_2O]^+$. This complex interacts with N-7 of either a guanine or adenine base present in the DNA. Next, the newly formed platinum complex is again hydrolysed and the remaining chlorine is removed, allowing the metal to bind with a second nucleotide base.[32] Consequently, inter- and intramolecular cisplatin-DNA adducts are formed, which interact with a DNA repair protein promoting the repair mechanism and cell division; however this process turns out to be unsuccessful and it compels the body to initiate apoptosis.[33]

The success of cisplatin has inspired researchers to synthesise and test an extensive variety of novel compounds containing modifications by using different organic substituent and maintaining the geometry of the complex, which has increased the area of organometallic drug development.[34]

Also, researchers have studied other possible metals that might have an antiproliferative effect in some cell lines, like nickel and copper complexes. They seem to be active in inhibiting cell proliferation *in vitro* on human leukemic cell lines (TOM1 and NB4). Vanadium complexes have shown to be cytotoxic towards the hepatoma Morris 5123 tumor cells and chromium complexes have given promising results in combining anti-colorectal cancer properties with insulin resistance.[34]

In this project a germanium moiety was selected for formulating a novel compared with potential anti-cancer properties, by linking to a variety of organic compounds. The choice to use these compounds in the structure will be discussed further on.

1.4.3 – Organogermanium compounds

In 1886, a German chemist named Winkler isolated Germanium from silver ore, argyrodite (silver germanium sulphide, Ag_8GeS_6), and a year later he synthesised the first organogermanium compound – tetraethlygermanium. Unfortunately, only after forty years the investigation of organogermanium compounds was initiated, because before that time derivatives of elements in 14th group, organosilicons and organotins seemed more interesting then organogermanes.

The study of the chemical and biological properties of germanium was only started after the discovery of its semi-conductor properties. It was known that this metal had an important role in the living organisms' processes, after found in Chinese herbs and in various medicinal plants like ginseng, garlic and soy.[35]

Already, in 1970 dietetic supplements of germanium became famous, although its therapeutic properties were not very well understood, which stimulated medical investigations. As a result, a similarity of biological activity between organogermanium and organosilicon derivatives and the fact that both of them are more active than carbon analogs was observed.[36]

The first organogermanium compound with pharmaceutical properties was released under a trade name as Sercion, also known as propagermanium in Japan. This compound has

a broad range of biological activity, characterized by the protection against viruses and by immunostimulation with low levels of toxicity.

Further investigation has resulted into many biologically active organogermanium compounds, although the most promising ones are 2-carboxyethylgermanium sesquioxide, designated as Ge-132, and 2-(3-dimethylaminopropyl)-8,8-diethyl-2aza-8-germaspiro[4,5]decane, subsequently known as spirogermanium.[35] (Figure 19)

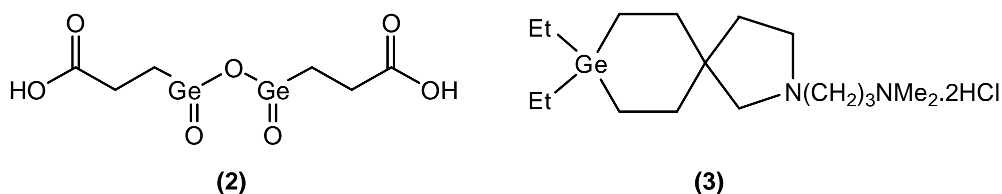


Figure 19: Ge-132 **(2)** and spirogermanium **(3)**.

Both of these compounds have gained interest for increasing an anti-tumour activity effect combined with stimulation of the immune system in low levels of toxicity.

Many studies of Ge-132 have found that this compound decreases the blood arterial pressure and acts as strong analgesic apart from supporting the immune system. More extensive studies have shown, recently, that derivatives of Ge-132 not only are able to break in DNA structures, which compromises the development of cancer, but also can stimulate the production of an interferon and enhance the natural killer cell activity; this is important in both modulating the immune system and inhibiting tumour growth with almost no detectable sign of cytotoxicity.[37][38]

Ge-132 derivatives show inhibiting activity towards various types of cancer cells being more relevant the following ones: EAT, adenocarcinoma LA-795, Lewis lung carcinoma 3LL, melanoma B16 and leukaemia L-1210.[35]

Spirogermanium is an anti-neoplastic agent that has been shown to be useful for the treatment of a variety of solid tumours like metastic prostatic plus ovarian carcinoma, advanced malignant lymphoma and *Plasmodium falciparum* infection.[36]

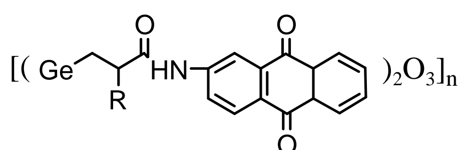
However, the anti-cancer mechanism of organogermanium compounds is not yet completely known. Apparently these compounds are thought to be an important element of chemotherapy which supports treatment of cancer via surgery, as described previously. The crucial roles in chemotherapy are still played by metal-organic preparations, usually platinum and ruthenium complexes. Unfortunately, some of them are not selective and as a result are considerably toxic because undesirable side effects occur during the therapy.

Cytotoxicity of some germanium and silicon derivatives has been accurately tested *in vitro*. Both Ge-132 and Spirogermanium have been taken into clinical trials but only in animals because there has been a strong controversy about the toxicity of these organogermanium compounds in humans. The *in vitro* tests of these compounds evidenced that the risk of using Ge-132 is not primarily the organic compound itself but somewhat the potential for contamination of a substance with the toxic inorganic forms of various germanium salts like the

highly toxic germanium dioxide. This inorganic compound can cause kidney and muscle damages and can also cause irreversible changes in the body leading to death.

The results of an extensive research in the safety of pure preparations of Ge-132 derivatives combined with its successful anti-cancer properties in animal models have encouraged future trials (phase I trials in healthy adults and phase II trials in patients with cancer). Also Ge-132 has showed evidence that it helps the inhibition of osteoporosis and it might be useful, in the future, to treat diabetes and malaria.[35][38]

As an attempt to minimize this risk and also to improve the efficiency of the organogermanium compounds a group of researchers have, recently, linked anthraquinone (or naphthalene) to a Ge-132 derivative. (Figure 20) This compound has shown encouraging results of cytotoxicity against prostrate cancer.[38]



(4)

Figure 20: Anthraquinone linked to a Ge-132 derivative **(4)**.

1.4.4 – Tamoxifen

Some breast cancers are classified as estrogen receptor-positive (also known as hormone sensitive), which means that they have a protein to which estrogen will bind. Tamoxifen is an orally active selective estrogen receptor modulator (SERM) that is used in the treatment of breast cancer. It acts as an antagonist of the estrogen receptor in the breast although it can act as a partial agonist in other tissues. Tamoxifen **(6)** (Fig. 23) is also known as Nolvadex, Istubal, and Valodex.

Tamoxifen is used to treat patients with early-stage breast cancer, as well as those with metastatic breast cancer. It helps prevent the original breast cancer from returning and prevent spreading the mallignant cells to the other breast by slowing or even stopping the growth of cancer cells that are present in the body.

1.4.4.1 – Discovery[39]

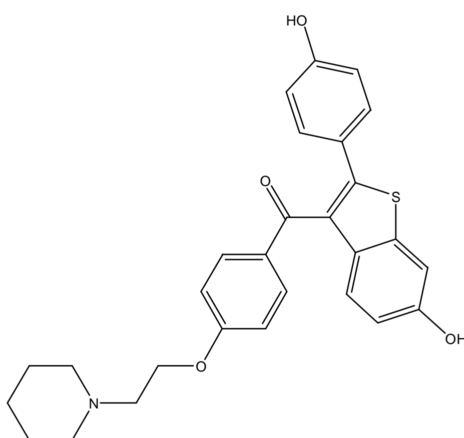
Some researchers were investigating anti-estrogen compounds – a substance that impedes estrogen interacting with specific tissues by preventing it from docking to estrogen receptors present in these tissues. For this research, in 1962 Dora Richardson ended up synthesising Tamoxifen, expected to function as a morning-after contraceptive pill; however, it induced women to become more fertile. This drug received a patent for treatment of fertility and not as contraceptive in the UK in 1962 and in 1980´s in the USA.

Many scientists researched other ways that Tamoxifen could be used in therapy. They discovered that Tamoxifen´s activity appeared to be selective because it only stopped estrogen from functioning in certain tissues such as in the breast. Adding this piece of information and knowing that in breast cancer cases dependent of estrogen (ER+) the hormone promotes the growth of tumour cells suggested that Tamoxifen could actually stop breast cancer growth.

As a result of many clinical trials, firstly Tamoxifen gave notable results in decreasing the growth of cancer cells and consequently the drug was approved for advanced breast cancer therapy, in 1977, by The Food and Drug Administration (FDA). These results led researchers to treat patients after surgery, which provided successful results because this strategy allowed extending the patients survival time. The successes of Tamoxifen in chemotherapy and in women with lymph-node involvement in the cancer permitted the FDA to approve the drug for these patients in the 1980´s.

In short terms, in spite of the potential side effects, such as an increased risk in developing endometrial cancer in women over the age of 50, and a risk of developing blood clots in major veins and lungs Tamoxifen, was found to assume a dual role as a cancer treatment and as a chemopreventive in high-risk women.

Moreover, researchers have already created and tested a second generation SERMS cancer prevention agents, reducing the side effects of tamoxifen; among these raloxifene has gained an outstanding position (Figure 21).



(5)

Figure 21: Raloxifene **(5)**.

1.4.4.2 – Mechanism of action[40]

Currently, the treatment of breast cancer commonly involves a lumpectomy followed by a combination of endocrine therapy, radiotherapy and/or chemotherapy. In some cases the hormonal therapy usually involves tamoxifen, whose hydroxylated metabolite, 4-hydroxytamoxifen (represented in Figure 23), inhibits cancer cell proliferation by competitively binding to the estrogen receptor.

Tamoxifen and some of its metabolites seem to act as estrogen antagonists, because they bind competitively to the estrogen receptors of tumours and other tissue targets. As result a nuclear complex that decreases DNA synthesis is produced. (Figure 22) The mechanism prohibits and suppresses cellular growth and multiplication, which permits accumulation of cells in the G0 and G1 phases. It also shows cytotoxic activity and induces apoptosis independent of estrogen receptor expression. On the other side, tamoxifen is known as an estrogen agonist in the endometrium, bone and lipids.

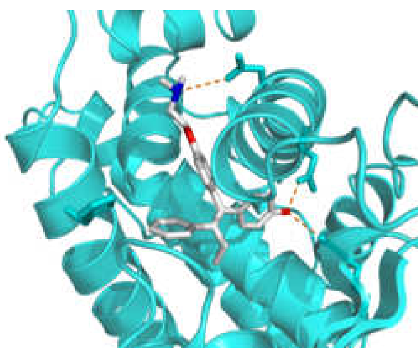


Figure 22: Crystallographic structure of 4-hydroxytamoxifen (white sticks) complexed with the ligand binding domain of the estrogen receptor (cyan cartoon diagram).[40]

1.4.4.3 – Metabolism and Excretion[41]

Tamoxifen is extensively metabolized after oral administration into several Phase **I** by the hepatic cytochrome P450 system, yielding predominantly *N*-desmethyltamoxifen, 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyltamoxifen. (Figure 23)

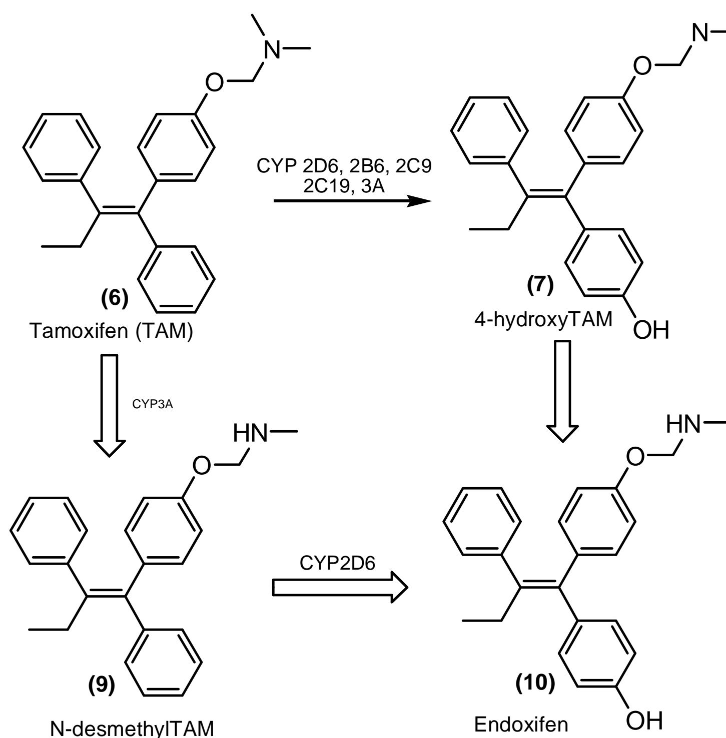


Figure 23: Routes of metabolism of Tamoxifen.[42]

The major metabolite formed is *N*-desmethyltamoxifen, which is very similar to tamoxifen in terms of biological activity and in terms of structure with the exception that the former has a secondary amine. 4-Hydroxytamoxifen and a minor side chain secondary alcohol derivative, α-hydroxytamoxifen, additional metabolites.

The Phase **I** metabolites undergo a conjugation by Phase **II** enzymes resulting mostly in polar conjugates; the parent drug and unconjugated metabolites accounted for less than 30% of the total fecal excretion (primary route of elimination of Tamoxifen). Some studies have shown that 65% of the administered dose is excreted from the body over a period of 2 weeks.[41]

1.4.5 – Aim of the project

The aim of second part of the project was to synthesise novel analogues of a germanium-based Tamoxifen derivative as potential anti-cancer agents.

On one hand the target molecule is formed by a specific organogermanium moiety, which is selected due to its therapeutic properties seen in the past, such as inhibition of growth in certain type of cancers cells in combination with a stimulation of the immune system with no detectable sign of toxicity.

On the other hand the target molecule is formed by an analogue of the estrogen receptor antagonist, Tamoxifen, which acts as the biologically active site (Figure 24). Tamoxifen has given remarkable results in breast cancer on its own and also when associated with metallocene groups, such as ferrocene.[6] A variety of analogues were designed, involving reactions between various combinations of synthetic precursors, such as substituted compounds of 4-hydroxybenzophenone, of propiophenone and of 4-methoxypropiophenone which had also to be synthesised.

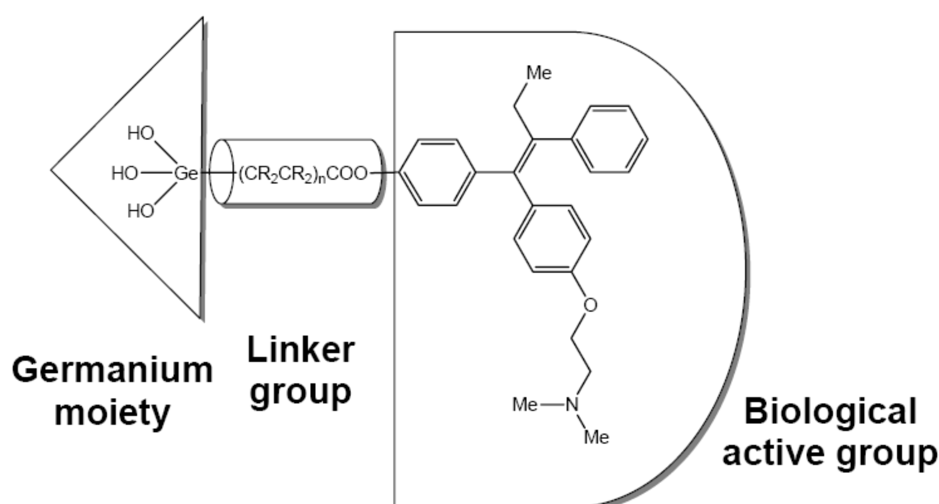


Figure 24: General structure of the second target molecule.

The optimum length of the linker group between the organogermanium moiety and the biological active site had to be considered and investigated because the steric impact of this group is expected to influence the cytotoxic activity associated with DNA. The ability to intercalate in the DNA helix is an important property of these types of components, which may determine their ultimate biological effects.[43] (Figure 24)

Finally, the compounds were proposed to be evaluated *in-vitro* in terms of cytotoxic properties, using an MTT-based assay, and tested against a variety of cancer cell lines.

2. Results and Discussion

2.1 – Organometallic analogues of Maraviroc

2.1.1 – Results and Discussion

The first objective of the project was dedicated to the synthesis of new organometallic analogues of the anti-HIV drug, Maraviroc, which was devised in two main steps. The overall synthetic pathway to achieve the target molecule is described below: (Figure 25)

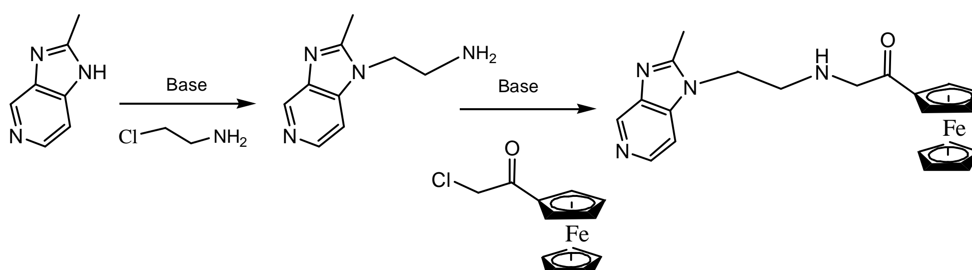


Figure 25: Synthetic pathway to the target molecule.

The first step consisted in *N*-alkylation of the heterocycle by a common alkylating agent, ethanamine-2-chloride hydrochloride. Afterwards, the alkylated product would undergo a reaction with 2-chloro-1-ferrocenylethanone affording the target molecule via S_N2.

By analogy, taking into consideration that the biological activities of various 1*H*-1,2,4–triazole derivatives and their N-bridged heterocyclic analogues, especially the triazole fused with six-membered ring system have diverse applications in the field of medicine, testing of triazole derivatives was also envisioned.[44] Thus, the first reaction carried out consisted in the alkylation of 1*H*-1,2,4-triazole instead of the imidazopyridine (Figure 16) with ethanamine-2-chloride hydrochloride.

Initially, 1*H*-1,2,4-triazole was deprotonated by an excess of a strong base, potassium *tert*-butoxide. (Figure 26) The conjugated base of 1*H*-1,2,4-triazole was expected to attack the carbon of the alkylating agent containing the chlorine atom.

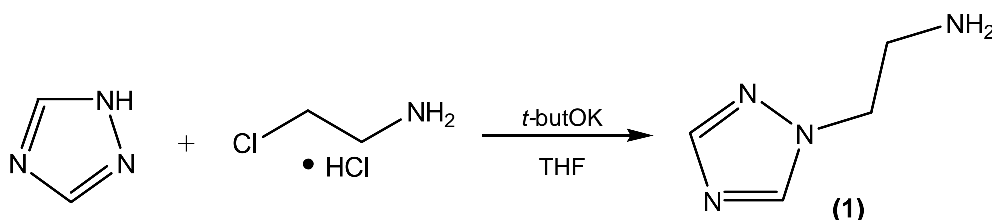


Figure 26: Synthesis of 2-(1H-1,2,4-triazol-1-yl)ethanamine **(1)**.

This reaction was performed twice, in different solvents. In the first attempt, DMF was selected as solvent and the ^{1}H NMR spectrum of the crude mixture demonstrated various peaks assigned to the triazole and the solvent but no evidence of aliphatic protons was seen. In the

second attempt, the solvent was switched to THF while maintaining the relative proportion of the starting materials. Nevertheless, the reaction still did not afford the desired product. The problem maybe related with the amine, which could be participating in an intramolecular reaction affording an aziridine as side product which is represented in Figure 27.[45] In addition, since small molecular weight amines have great affinity for water due to their polarity, the free alkylating agent may have been removed by aqueous washes during the workup; therefore no sign of the starting amine was detected in the ^{1}H NMR spectrum.

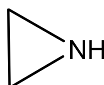


Figure 27: Chemical structure of aziridine.

In order to prevent losing the ethanamine-2-chloride hydrochloride, an alternative for the reaction was investigated and as result two different ways for protecting the alkylating agent were attempted.

The first protection was via formation of an amide by reacting benzoyl chloride with ethanamine-2-chloride hydrochloride as described below: (Figure 28)

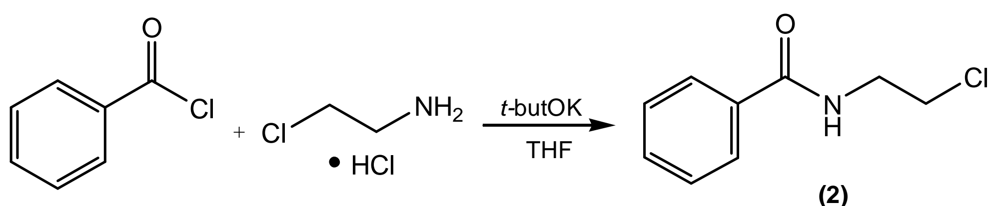


Figure 28: Synthesis of *N*-(2-chloroethyl)benzamide.

Basically, the amine in excess was at first deprotonated by a strong base (tert-butoxide) in THF. Afterwards, the amine was able to attack the carbonyl of benzoyl chloride to form a new N-C bond yielding the *N*-(2-chloroethyl)benzamide **2**. The remaining benzoyl chloride was hydrolysed in the aqueous extraction forming benzoic acid. In basic conditions this acid is deprotonated affording a salt, sodium benzoate, which remains in the aqueous phase. The yield obtained for product **2** was acceptable (~ 51%) given that no column chromatography was performed for the purification step. However, some of the benzoyl chloride, which is known to be very reactive, must have been hydrolysed directly in the reaction mixture, due to the small amount of water present in the THF.

The pure product **2** obtained after the aqueous extraction of the benzoate was identified and characterised mainly by ^{1}H and ^{13}C NMR and mass spectrometry. The structure was confirmed by comparison of the ^{1}H NMR peaks of the product with those of the same compound found in the literature. (Table 1)

Table 1: ^{1}H NMR values of product 2 and from the literature. [46]

	Literature (400 MHz/$CDCl_3$)	Product **2** (400 MHz/$CDCl_3$)
$C_{2,6}$ of C_6H_5	7.79	7.76
C_4 of C_6H_5	7.51	7.51
$C_{3,5}$ of C_6H_5	7.43	7.44
-N*H*	6.84	6.63
-$NHCH_2CH_2Cl$	3.78	3.75
-$NHCH_2CH_2Cl$	3.72	3.70

From the comparison of the values presented in Table 1, where no significant differences were seen, and also because our spectrum was obtained under similar conditions (same solvent) it was possible to conclude that product **2** corresponded to the desired structure. The ^{13}C NMR spectrum was also consistent with the presence of all the expected resonances.

The ESI mass spectrum afforded the mass of the protonated molecule ion for product **2** at m/z 184 [^{35}Cl-MH^+]. Additional fragments corresponding to loss of HCl (m/z 148) and 2-chloroethanamine (m/z 105) further confirmed the structural assignment.

In addition, the melting point (103-105ºC) was entirely consistent with the literature data (103-106ºC).

The next step consisted in deprotonation of 1*H*-1,2,4-triazole in order to react with the amide formed previously (product **2**):

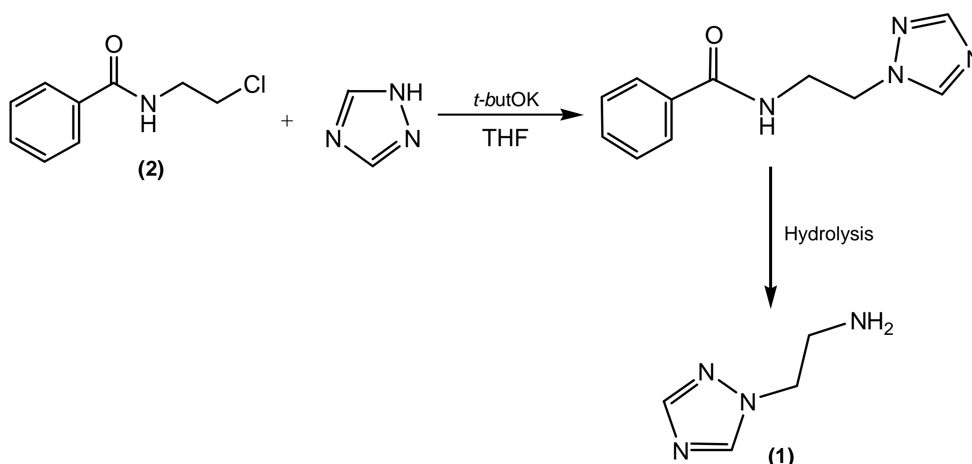


Figure 29: Second attempt of synthesis of product **1**.

Since this reaction was performed in a small scale the purification was achieved through PLC on silica-gel. From the chromatography four fractions were separated and all of them were analysed by ^{1}H NMR. The first fraction with the highest Rf and the last fractions were impurities. The second fraction with the highest Rf was assigned to *N*-(2-chloroethyl)benzamide and the following one seemed to correspond to a side-product. By combination of one-dimensional proton and carbon NMR spectra with the bidimensional NMR spectra (HMBC and HSQC) the

structure of the side product was elucidated. (Table 2) This side product resulted from an intramolecular reaction of the starting material **2**, as described in Figure 30:

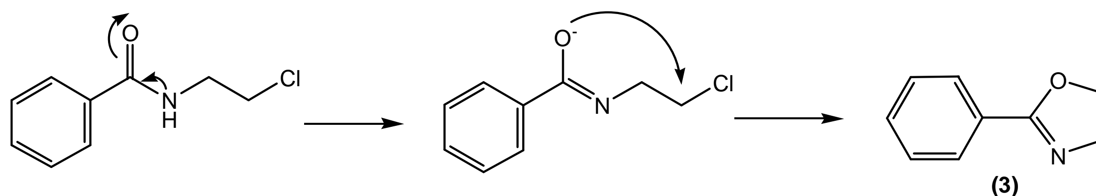


Figure 30: Intramolecular reaction affording product **3**.

The chemical shifts of ^{1}H NMR of products **2** and **3** are compared in Table 2. It was immediately noticed that in product **3** the peak related do –NH did not appear.

Table 2: ^{1}H-NMR values of product 2 and 3

	Product **3** (400 MHz/acetone-d_6)	Product **2** (400 MHz/$CDCl_3$)
$C_{2,6}$ of C_6H_5	7.79	7.76
C_4 of C_6H_5	7.55-7.50	7.51
$C_{3,5}$ of C_6H_5	7.50-7.40	7.44
-O$CH_2$$CH_2$N-	4.47	3.75
-O$CH_2$$CH_2$N-	4.04	3.70
-N*H*	-	6.63

In addition, the protons of the –CH_2CH_2– group were deshielded, compared to product **2**. This fact suggested both methylene groups in **3** were under the influence of electron withdrawing effects of the neighbours (oxygen and nitrogen atom). This result was also seen in one dimensional ^{13}C NMR, where the chemical shifts of the carbons assigned to both methylene groups were more deshielded in product **3** than the same carbons present in product **2**. (Figure 31)

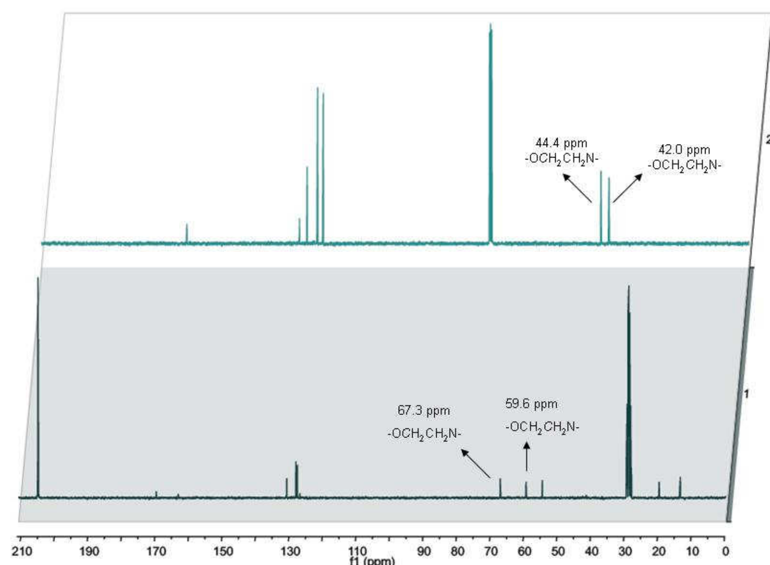


Figure 31: ^{13}C NMR of product 2 (spectrum 2 in $CDCl_3$) and 3 (spectrum 1 in acetone-d_6) both at 300 MHz.

The IR spectrum of **3** had two characteristic bands: a medium intensity, at 1260 cm^{-1}, corresponding to the stretch of a single C-O bond and a medium bond stretch at 1650 cm^{-1}, consistent with the C=N bond.

In order to compete with the intramolecular reaction described previously, several attempts of the same reaction using different bases, sodium hydride, *n*-butyllithium and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), were performed however the product of intramolecular substitution was obtained consistently in all instances.

A second protection of the amino group was attempted by reacting directly a derivative of phthalimide containing the fragment of the alkylating agent with 1*H*-1,2,4-triazole. (Figure 32) The product obtained from this reaction was expected to hydrolise affording product **1**. However, no peaks corresponding to the triazole compound were seen by ^{1}H NMR analysis and as a result no further purification was performed since the desired reaction did not occur.

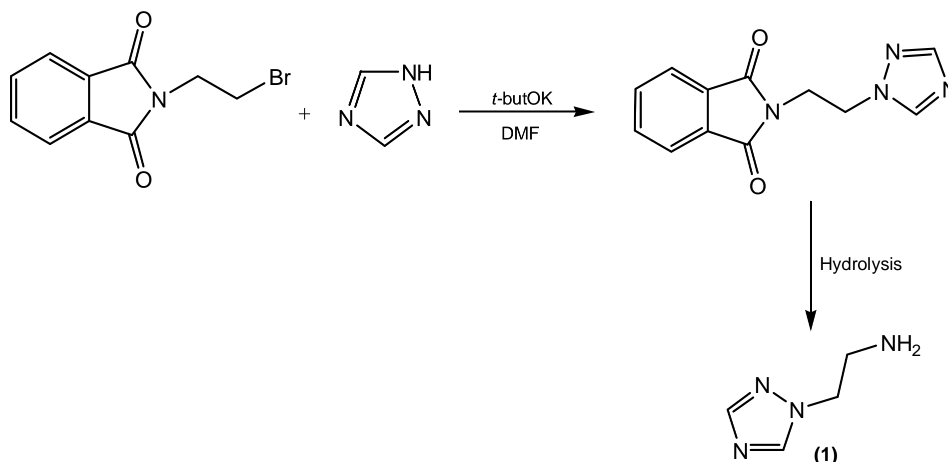


Figure 32: Alternative reaction of protection of the alkylant agent.

In general, the problem observed in every reaction described previously where 1*H*-1,2.4-triazole was a nucleophilic agent and the chlorinated amine an alkylating agent was the role of triazole in the reaction. It appears that the heterocyclic compound preferentially acts as a base rather then a nucleophile. Therefore perhaps different proportions of the starting materials should be experimented in the future. Due to the limited time available for conclusion of this project no further reactions were attempted using this strategy.

The above described results encouraged starting the synthetic pathway (Figure 25) from the opposite side, by introducing first the 2-chloro-1-ferrocenylethanone into the same alkylating agent and at the end inserting the 1*H*-1,2,4-triazole.

2-Chloro-1-ferrocenylethanone had been previously synthesised in the laboratory by Friedel-Crafts acylation of ferrocene. Firstly, ethanamine-2-chloride hydrochloride was deprotonated by a strong base in THF. Afterwards, the amine was expected to attack the primary carbon of the 2-chloro-1-ferrocenylethanone in order to form product **4**. (Figure 33) This reaction was followed by TLC in different systems of solvents but on the silica plate only presence of the 2-chloro-1-ferrocenylethanone was seen. Subsequently, it was thought that the equivalents used for the alkylating agent were not enough so more was added. Since no differences in the TLC were seen and the ^{1}H NMR of the crude product only evidenced presence of the starting material it was concluded that different conditions for the reaction should be studied in the future.

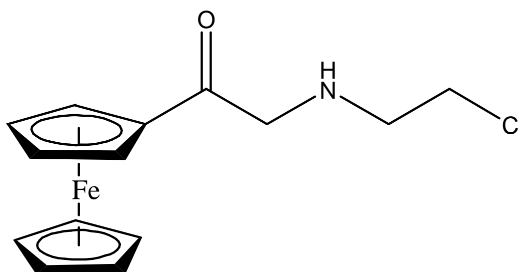


Figure 33: 2-[(2-chloroethyl)amino]-1-ferrocenyletanone **(4)**.

Many alternatives were studied in order to overcome the problem of alkylating the α carbon of 2-chloro-1-ferrocenylethanone with resource to other non-linear amines present in the laboratory such as, piperazine. This group was found attractive because the lead compound (Figure 13) available in the process that yielded Maraviroc, contains a piperdine ring, functioning as a linker; by analogy, a piperazine ring was used in our case to associate 2-chloro-1-ferrocenylethanone with 1*H*-1,2,4-triazole. The general scheme is described below (Figure 34):

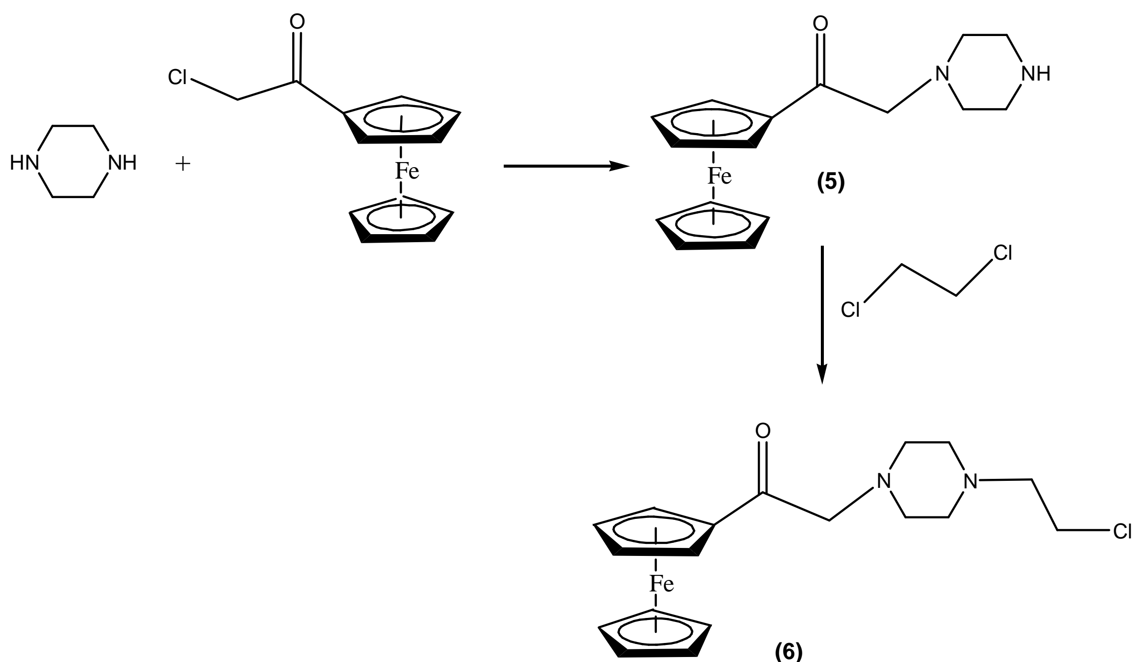


Figure 34: Synthesis of 2-{4-(2-chloroethyl)piperazin-1-yl}-1-ferrocenyletanone **(6)**.

The first step set off successfully affording product **5** with a good yield (~70%) in both attempts performed. For the reaction, piperazine had a dual role: it was the starting material and the base as well, which helped the workup and the purification step.

For characterisation of product **5**, once again, ^{1}H NMR and mass spectrometry were crucial to confirm the structure and purity of the compound. From ^{1}H NMR chemical shifts Table **3**), three main peaks were found important to confirm that reaction between piperazine and 2-chloro-1-ferrocenylethanone had occurred and the desired product had been synthesised

Table 3: ^{1}H NMR values for piperazine in $CDCl_3$ (300 MHz) [46], product **5** and 2-chloro-1-ferrocenylethanone in acetone-d_6 (400 MHz).

Protons	Piperazine	Product **5**	2-chloro-1-ferrocenylethanone
$C_{2,5}$ of -C(O)C_5H_4	-	4.90	4.90
$C_{3,4}$ of C(O)C_5H_4	-	4.55	4.66
-C_5H_5	-	4.23	4.31
-C(O)CH_2-pip.	-	3.54	4.68
-NH	1.66	3.04	-
$C_{2,6}$ of heterocycle	2.840	2.80[a]	-
$C_{3,5}$ of heterocycle		2.50[a]	-

[a] The assignments may be reversed

Firstly, two different peaks for the $–CH_2$ groups of piperazine were observed in the appropriate range, which indicated the methylene groups had different neighbourhood as expected according to the structure of product **5**. Secondly, the $–CH_2$ group originally from 2-chloro-1-ferrocenylethanone was shifted to upfield which is explained by the change in connectivity, from a chlorine to a nitrogen. Finally, the labile proton was shifted downfield, possibly because the pretended conformation of the molecule would place the piperazine ring close to the carbonyl therefore deshielding –NH, compared to the starting material. The very last information taken from the ^{1}H NMR was the integration for –NH proton that accounted for only one proton which also indicated that the piperazine ring was associated to -1-ferrocenylethanone.

In addition, ESI mass spectrometry demonstrated presence of one-of-a-kind peak which corresponded to the protonated molecule at m/z 313. The short melting interval (141-143 ºC), also indicated the purity of product **5**.

The second step consisted in an ordinary substitution reaction between the secondary amine present in the piperazine ring of product **5** and 1,2-dichloroethane. Even using a large excess (up to 17-fold) of the alkylating agent only the starting material was detected on TLC. Yet when the crude product was purified, ^{1}H NMR analysis of the fraction indicated no presence of either product or 1,2-dichloroetane, only product **5** was detected with some impurities. This result could be explained by the high volatility of 1,2-dichloroethane.

Given this result, it was decided to undertake a different approach in the synthetic pathway to achieve the target molecule. Three heterocycles with structural similarities with the target molecule were selected, 1*H*-1,2,4-triazole (**I**), 1H-imidazo[4,5-c]pyridine (**II**) and 2-(1H-indol-3-yl)ethanamine (**III**) and reacted directly with 2-chloro-1-ferrocenylethanone. (Figure 35)

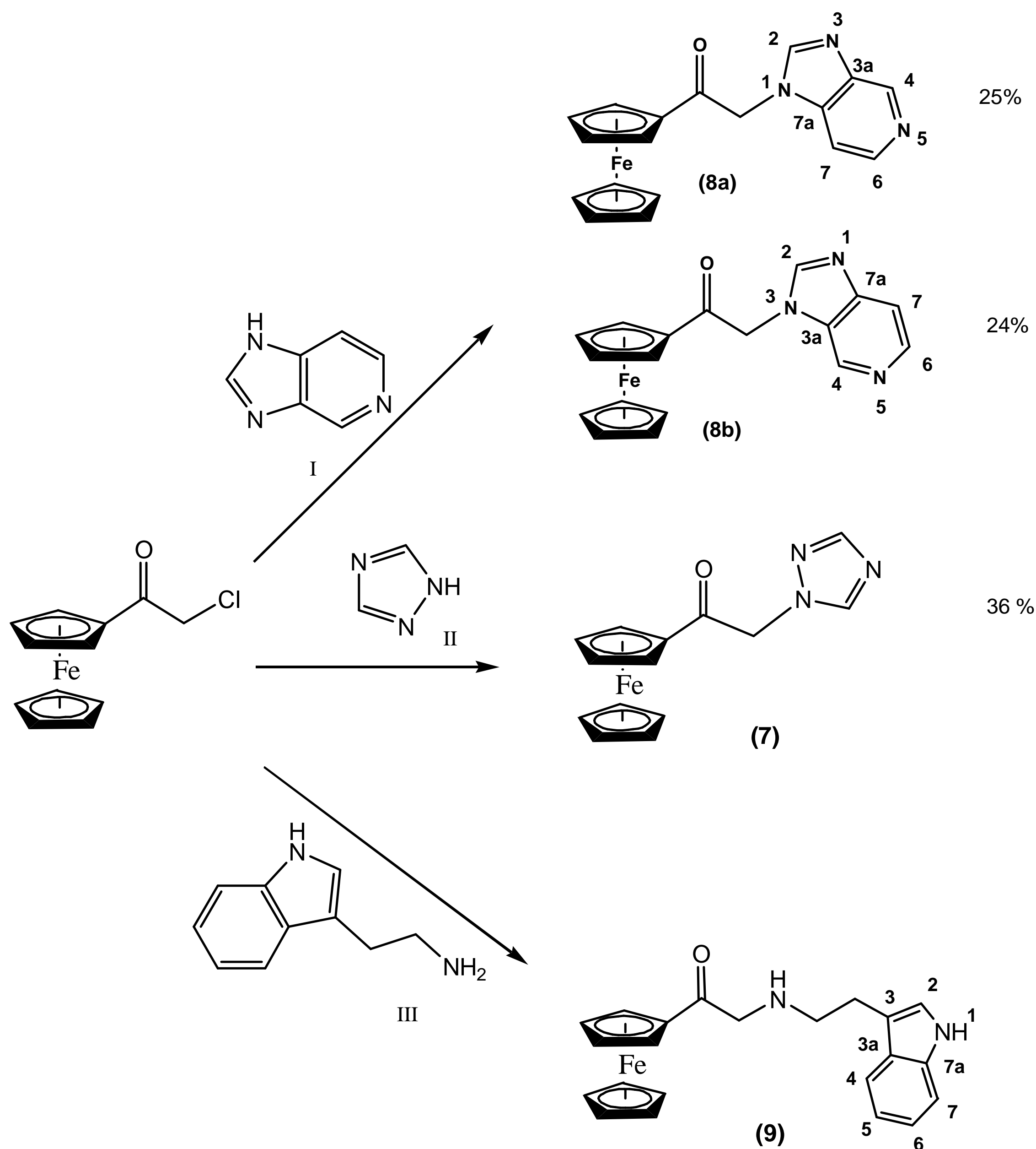


Figure 35: Synthesis of 1-ferrocenyl-2-(1H-1,2,4-triazol-1-yl)ethanone **(7)**, 1-ferrocenyl-{2-[3H-imidazo[4,5-*c*]pyridine]-1-yl}ethanone **(8)** and 1-ferrocenyl-2-[2-(1H-indol-3-yl)ethanamino]ethanone **(9)**.

All the syntheses represented in Figure 35 were undertaken in the same conditions, where THF was used as solvent, with 2 equivalents of the appropriate heterocycle and 1 equivalent of a strong base, potassium *tert*-butoxide. As they were performed in a small scale the purification consisted in preparative layer chromatography on silica-gel. The results obtained are described in detail below.

In the synthesis of compound **7** the relatively low yield (36%) obtained to a certain extent could be caused by having accidentally used half of the equivalents of base, which was not enough to deprotonate all the 1*eH*-1,2,4-triazole present in reaction mixture. This was

confirmed by analysis of the first fraction isolated from chromatography which corresponded to 2-chloro-1-ferrocenylethanone that did not participate in the reaction.

Product **7** was characterised mainly by NMR and mass spectrometry. Based on ^{1}H NMR values (Table 4) combined with the bidimensional spectra and also supported by one dimensional ^{13}C NMR it was concluded that a new compound containing triazole linked to the acetyl ferrocene fragment was synthesised.

Table 4: ^{1}H-NMR values for 1*H*-1,2,4-triazole in D_2O (300 MHz)[46], product **7** and 2-chloro-1-ferrocenylethanone in acetone-d_6 (400 MHz).

Protons	1*H*-1,2,4-triazole	Product **7**	2-chloro-1-ferrocenylethanone
C_5 of heterocycle	8.416	8.42	-
C_3 of heterocycle		7.94	-
$-C(O)CH_2$	-	5.64	4.68
$C_{2,5}$ of $-C(O)C_5H_4$	-	4.97	4.90
$C_{3,4}$ of $C(O)C_5H_4$	-	4.70	4.66
$-C_5H_5$	-	4.39	4.31

From the table above the most noticeable feature corresponds to the 1*H*-1,2,4-triazole protons, which were not equivalent, as expected for the desired product. This immediately indicated that substitution had occurred. Also, the peak for the $–CH_2$ group was shifted downfield, which could be explained by the overall withdrawing effect caused by the 1*H*-1,2,4-triazole fragment. Actually, this shift evidenced the presence of the new bond C-N formed between the starting materials. This was confirmed in the bidimensional spectra, HMBC spectrum, which detects 2- and 3- bond C-H connectivities.

In the ESI mass spectrum, two signals with high intensity were found. One corresponded to the protonated molecule (m/z 296) with 91% intensity. The base peak (m/z 176 MH_2-FeC_5H_5) resulted from loss of FeC_5H_5 fragment.

The melting point for product **7**, 128-130 ºC, had a low interval, consistent with a good purity level.

In the synthesis of compound **8** two isomers were obtained in identical yields. (Figure 35) They were separated by performing two PLC on silica gel.

^{1}H NMR was a key point for the characterisation of the exact structures of each of the isomer which were successfully isolated. (Table 5)

Table 5: ^{1}H NMR values for the two isomers of product **8** (300 MHz) and 2-chloro-1-ferrocenylethanone (400 MHz) in acetone-d_6.

Protons	Product **8a**	Product **8b**	2-chloro-1-ferrocenylethanone
C_4 of heterocycle	8.93	8.98[a]	-
C_6 of heterocycle	8.40	8.38	-
C_2 of heterocycle	8.35	8.26[a]	-
C_7 of heterocycle	7.68	7.54	-
-C(O)CH_2	5.85	5.76	4.68
$C_{2,5}$ of -C(O)C_5H_4	5.05	5.02	4.90
$C_{3,4}$ of C(O)C_5H_4	4.74	4.72	4.66
-C_5H_5	4.02	4.38	4.31

[a] The assignments may be reversed

From the table above minor differences between the ^{1}H NMR were observed apart from two peaks which corresponded to the same protons of the molecule but appeared to have different electronic shield effect. In isomer **8a** (structure represented in Figure 35) the most deshielded peak corresponded to the proton which was positioned at C_4, next to the nitrogen of the heterocycle, and therefore subjected to a substantial electron withdrawing effect. This conclusion resulted from the combination of the bidimensinal NMR (HMBC) and one dimensional carbon and proton NMR spectra which helped assigned all the protons and carbons for each isomer. (e.g. for isomer 8a see Figure 36) The same occurred with isomer **8b** where the most deshielded proton corresponded to the one present in C_4 of the heterocycle. The main difference seen between them was concerning one of the quaternary carbon (C_{7a}) present in heterocycle, which for isomer **8a** appeared more deshielded then the same carbon of isomer **8b**. Also the other quaternary carbon (C_{3a}) appeared more deshielded but this time for isomer **8b** when compared with **8a**.

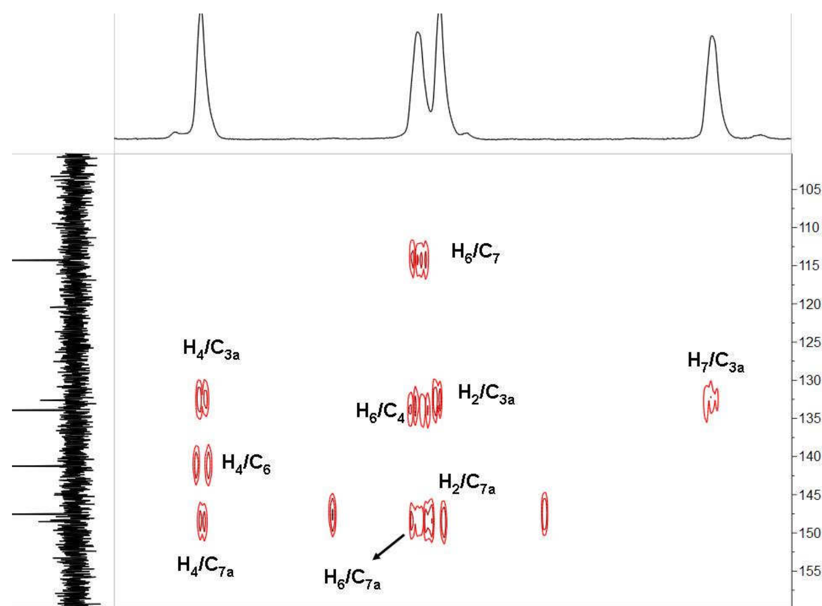


Figure 36: 2- and 3- C-H correlations (HMBC) obtained for product **8a**.

The structure of both isomers was essentially derived from analysis of the NMR spectra. ESI mass spectrometry gave results consistent with the assigned structures. Isomer **8a** gave a unique peak at m/z 346 which corresponded to the protonated molecule with 100% of intensity. This result indicated that isomer **8a** is very stable upon protonation. For isomer **8b**, the mass spectrum consisted of two peaks, one with 80% intensity peak at m/z 346 which corresponded to the pronated molecule and the base peak at m/z 226 (MH_2-FeC_5H_5).

With these results it was possible to conclude that both isomers were formed with an approximately 1:1 ratio. Isomer **8a** was synthesised and isolated with a significant purity level. Isomer **8b** was more difficult to crystallize but the spectroscopic data are entirely consistent with the assigned structure. Analysis of the crystal structure form of both isomers by X-ray diffraction is expected to further confirm these conclusions.

The ^{1}H NMR values of the two starting materials (one taken from the literature) that were used for the synthesis of product **9** are in Table 6. This comparison with the data obtained for **9** helped to characterise the structure of the product.

Table 6: ^{1}H-NMR values for 2-(1*H*-indol-3-yl)ethanamine in DMSO-d_6(400 MHz) [46] , product **9** and 2-chloro-1-ferrocenylethanone in acetone-d_6 (400 MHz).

Protons	2-(1*H*-indol-3-yl)ethanamine	Product **9**	2-chloro-1-ferrocenylethanone
-N*H*	10.8	10.13	
C_4 of heterocycle	7.349	7.64	-
C_7 of heterocycle	7.519	7.42	-
C_6 of heterocycle	7.061	7.13	-
C_2 of heterocycle	7.128	7.23	-
C_5 of heterocycle	6.970	7.05	-
-C(O)CH_2	-	1.98	4.68
$C_{2,5}$ of -C(O)C_5H_4	-	4,84	4.90
$C_{3,4}$ of C(O)C_5H_4	-	4.55	4.66
-C_5H_5	-	4.23	4.31
-CH_2N*H*CH_2CH_2-	2.0	3.93	-
-NHC$H_2$$CH_2$-	2.766	3.00	-
-NHCH_2CH_2-	2.828	2.84	-

From the above table, the peaks concerning 2-(1*H*-indol-3-yl)ethanamine were fairly similar and the small differences between the heterocycle in product **9** and the starting material were mostly noticed in the vicinity of the new substituintes. Two main shifts of the ^{1}H NMR values were observed and distinguished from the starting materials, which helped elucidate the structure of the product. One of them concerned the protons of the –CH_2 group originally from the metallocene compound, which were shifted substantially upfield, presumably reflecting proximity to the shielding zone of the heterocyclic ring. Also, the –NH group of the alkyl was

shifted, which suggested that the molecule had arranged in a certain conformation that promoted the carbonyl to form a hydrogen bond with the same proton. The assignments for all protons and carbons (cf. Experimental Section) were achieved by combination of one dimensional ^{1}H NMR and ^{13}C NMR and two-dimensional (HMBC and HSQC spectra represented in Figure 37).

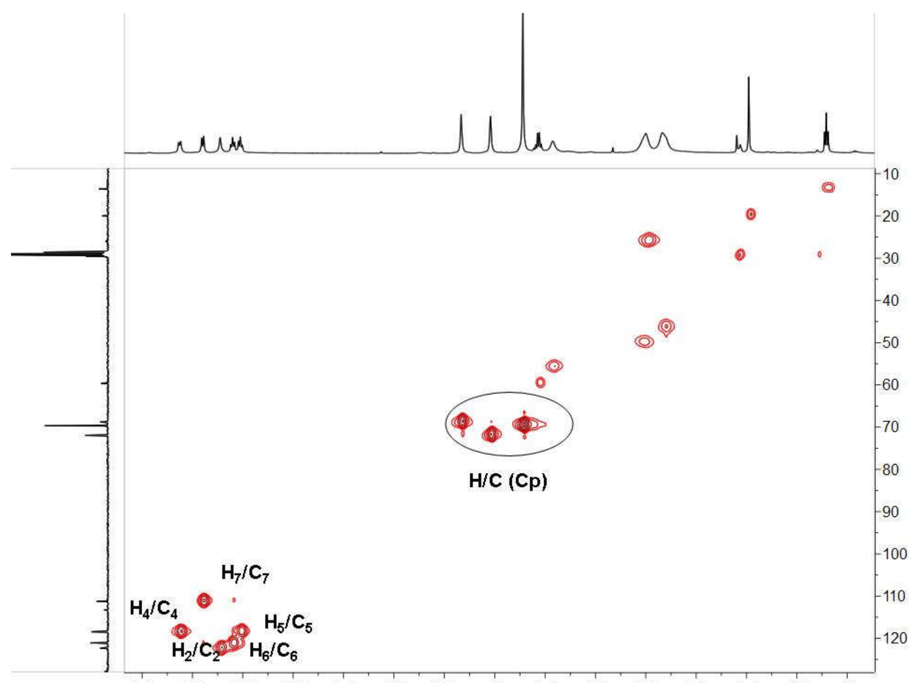


Figure 37: HSQC spectrum obtained for 1-ferrocenyl-2-[(1H-indol-3-yl)ethanmino]etanone **(9)**

The product was found very unstable at room temperature. The overall product was decomposed and as a result mass spectrometry was very unlikely to perform as well as the measurement of the melting point.

2.1.2 – Conclusions and Outlook

As an overall analysis of the results obtained in the first part of this project, it was possible to conclude that direct alkylation of 1*H*-1,2,4-triazole needs further investigation since in our reactions the heterocycle did not act as a nucleophilic agent with the exception of the synthesis of compound **7**, possibly due to poor selection of the alkylating agent. In fact, the use of the primary amine, ethanamine-2-chloride hydrochloride, as an alkylating agent was found problematic due to the intramolecular reaction described previously, which was difficult to compete with (represented in Figure 30). On the other hand, alkylation of the α carbon of 2-chloro-1-ferrocenylethanone with secondary amines (piperazine, 1*H*-1,2,4-triazole and 1*H*-imidazo[4,5-*c*]pyridine) yielded, successfully, stable products whereas with the primary amine present in the heterocycle (2-(1*H*-indol-3-yl)ethanamine) it afforded an unstable product. This suggested that, in the future, for this type of reactions the use of secondary amines could be advantageous. A very last conclusion taken from the results obtained was the fact that substitution reactions in heterocyclic rings are capable of affording different substituted products (isomers), as seen for compound **8**, for which the corresponding isomers could be separated.

The small library of products synthesised are fairly far from Maraviroc regarding structural similarities, although products **7-9** were found important. All of them had in common a heterocyclic ring on one side of the product, linked with a ferrocenyl substituent (on the other side), which was in agreement with the proposed structure of the target molecule represented in Figure 25 (section 2.1.1). These products are planned to undergo evaluation of pharmacological relevant properties (eg, partion coefficients, redox potentials) and *in vitro* assessment of CCR5 binding affinity, which are important indicators for therapeutic potential. Also, more research will be carried out to investigate the effect of changing the linker length/type for the same compounds and/or their derivatives.

2.2 – Organometallic analogues of Tamoxifen

2.2.1 – Results and Discussion

For the second part of the project, the synthesis of germanium-based tamoxifen derivatives, there were three main synthetic steps involved to obtain the target molecule described previously in the introduction: Generation of precursor molecules, coupling of precursors to tamoxifen-like compounds, and coupling of the germanium moiety with the biologically active part.

The first step was focused essentially on generating precursors required to form analogues of Tamoxifen. The precursors were mainly obtained by substitution of the OH group of [(4-hydroxyphenyl)phenyl]methanone or 1-(4-hydroxyphenyl)propan-1-one with alkoxy substituents using appropriate alkyl chloride reactants and resulting into four different types of starting materials for the second step [**1-4**].

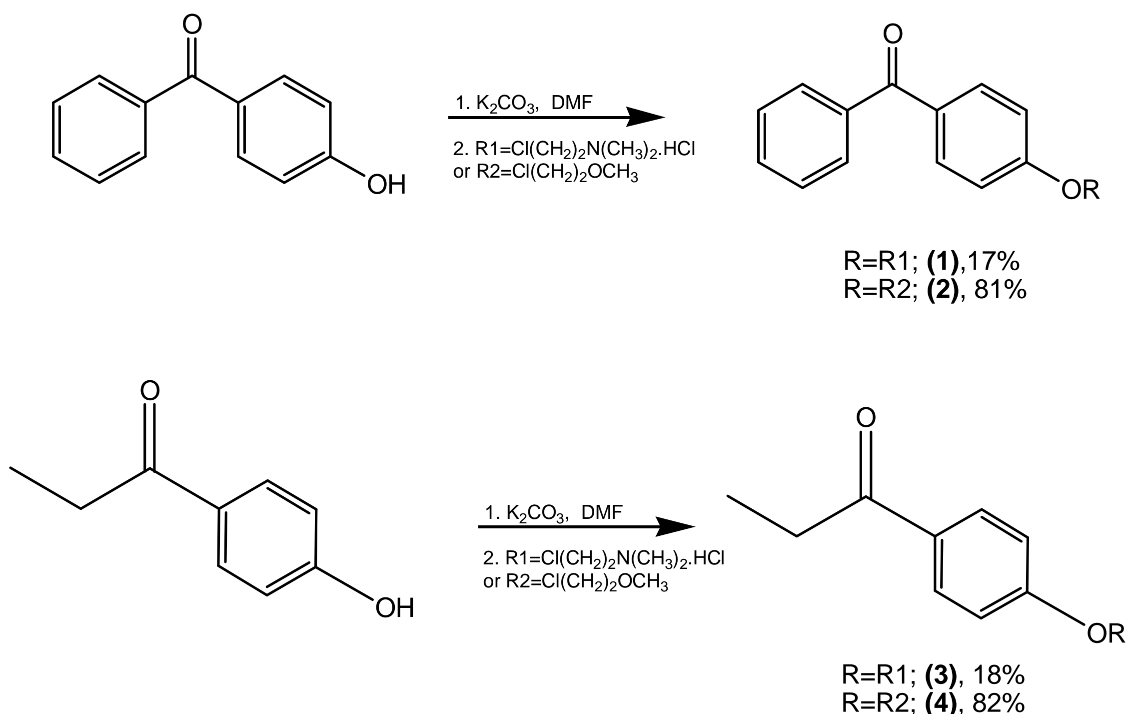


Figure 38: Reaction for the synthesis of precursors **(1)-(4**)

Both reactions described in Figure 38 start with an acid-base reaction, where an OH group of the [(4-hydroxyphenyl)phenyl]methanone (or 1-(4-hydroxyphenyl)propan-1-one) is deprotonated by a weak base in an aprotic solvent (DMF). The resulting 4-benzoylphenolate (or 4-propionylphenolate) attacks the carbon of 2-(dimethylamino)ethylchloride hydrochloride (or 2-chloroethyl methyl ether), via S_N2, driving out the chlorine from the alkylating agent to afford compounds **1-4**. The synthesis was performed according to an experimental procedure described by Meegan[47] although instead of acetone DMF was used and the equivalents of the

starting materials adopted (1:3,5:3; ketone /base/alkylating agent) were from Brummer[48] only for compounds **2**, **3** and **4**.

In the synthesis of compound **1** the ratio of reactants used was 1:3:2.3 (4-hydroxybenzphenone/K_2CO_3/$Cl(CH_2)_2N(CH_3)_2{\cdot}HCl$) which explains the presence of 4-hydroxybenzophenone in the first fraction of column chromatography and could be responsible for the low yield (17%). Furthermore, the published conditions for the column separation (1:1 methanol/DCM) had to be altered as they were not efficient in initial TLC tests. The isolated product was identified mainly by ^1H and ^{13}C NMR. The peaks with the appropriate integration obtained in ^1H NMR, especially the ones concerning the methyl groups of the amine (2.30 ppm) and the two –CH_2 groups with same coupling constant (4.09 ppm, 2.71 ppm; J=5.0Hz) combined with the location of the aromatics peaks compared to 4-hydroxybenzophenone supported the expected structure. Also, the presence of the carbon of the carbonyl detected in ^{13}C NMR (~195.4ppm) and confirmed by IR (~1645 cm^{-1}) helped characterise the desired product.

The values of ^1H NMR shifts for compound **1** were compared with the ones obtained in the literature for the same compound and in the same solvent (Table 7). From this comparison the structures of both of them were confirmed and some slight differences between the products obtained with the literature were found, which could be due to the dissimilar properties of the NMR apparatus used.

Table 7: ^1H-MR values of compound 1 and from the literature in $CDCl_3$

Protons	Compound **1**/ppm (250 MHz)	Literature/ppm (400 MHz)
$-OCH_2CH_2N(CH_3)_2$	4.09, t, *J*= 5.0 Hz	4.15, t, *J*= 5.6 Hz
$-CH_2N(CH_3)_2$	2.71, t, *J*= 5.0 Hz	2.74, t, *J*= 5.6 Hz
$-N(CH_3)_2$	2.30, s	2.32, s

In the synthesis of compound **2** the ratio of reactants was altered to 1:3.5:3 (4-hydroxybenzphenone/K_2CO_3/$Cl(CH_2)_2OCH_3$) according to the literature[48]. This ratio turned out to be appropriate for the reaction and no further purification of the crude product was necessary, apart from the aqueous extraction. This suggests that nearly all starting materials were consumed in the reaction or separated, and consequently a good yield of the product (~81%) was obtained. Again, NMR analysis was crucial for the characterisation of the product, where the carbon of the carbonyl (~196 ppm) and the aromatic protons appeared in a different range from the starting material. The thin white crystals isolated from the aqueous layer were adequate to confirm and to recognize the three-dimensional structure of the product by X-ray analysis (

Figure 39). The structure and the correspondent data are presented below (Table 8):

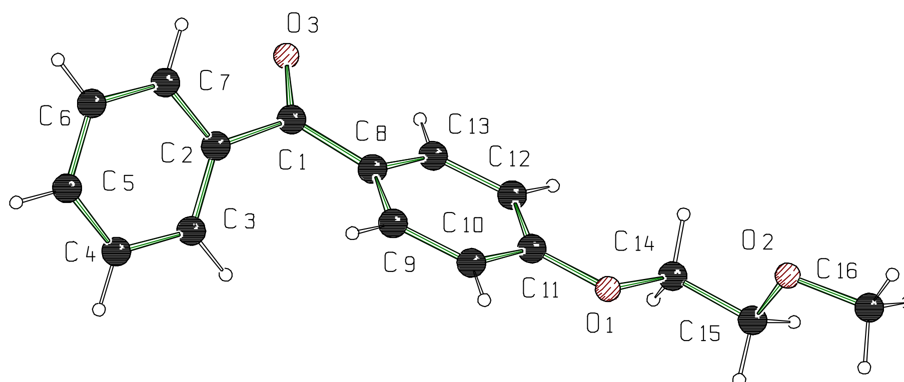


Figure 39: Three-dimensional structure of [4-(2-methoxyethoxy)phenyl]phenylmethanone **2**.

Table 8: Crystal data and structure refinement for **<**Compound **2>**.

Identification code	**<Compound 2>**	
Empirical formula	$C_{16}H_{16}O_3$	
Formula weight	256.29 g·mol^{-1}	
Temperature	1732 K	
Wavelength	0.71073 Å	
Unit cell dimensions	a = 4.6190(5) Å	α= 90°.
	b = 10.1441(11) Å	β= 90°.
	c = 28.1623 Å	γ= 90°.
Volume	1319.52 Å^3	
Unit per cell	Z = 4	
Density (calculated)	1.290 g·cm^{-1}	
Absorption coefficient	0.088 mm^{-1}	
F(000)	544	
Crystal size	0.50 x 0.20 x 0.20 mm^3	
Theta range for data collection	2.13 to 27.00°.	
Index ranges	-5<=h<=5	
	-12<=k<=12	
	-35<=l<=35	
Reflections collected	32408	
Independent reflections	2867 [R_{int} = 0.0393]	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2867 / 0 / 173	
Goodness-of-fit on F^2	1.041	
Final R indices [I>2sigma(I)]	*R1* = 0.0340, w*R2* = 0.0893	
R indices (all data)	*R1* = 0.0350, w*R2* = 0.0902	
Absolute structure parameter	1.9(10)	
Largest diff. peak and hole	0.199 and -0.177 e.Å^{-3}	

Again, an alkylation with the amine 2-(dimethylamino)ethylchloride hydrochloride was applied in the synthesis of compound **3**, by the same procedure used previously. The yield was fairly low and therefore a second attempt was performed by using a different aprotic solvent in order to improve the yield. Unfortunately, this attempt was unsuccessful, because it was not possible to establish an efficient system for separation by column chromatography and also distillation did not afford a pure product. An ultimate resource for purifying the crude product was to form an amine salt of the product followed by an extraction in basic conditions in order to pull out the product from the aqueous phase. This strategy was also found unsuccessful and consequently compound **3** was synthesised by using DMF as solvent. The compound was characterised by NMR where a notable deshielding of peaks in the area of the aromatic protons originally from 1-(4-hydroxyphenyl)propan-1-one was seen. The protons of the ethyl substituent group were less affected when compared to the starting material, as the new substituent of the product was too far away to affect the electronic environment of those protons. These results were also found for compound **4** and in both cases the same protons were crucial to confirm the structure of the new desired compounds (Table 9).

Table 9: ^{1}H-NMR of 4-hydroxypropiophenone and compounds **3** and **4**

	4-Hydroxypropiophenone (300 MHz, CDCl3+DMSO-d_6)	Compound **3** (250 MHz, $CDCl_3$)	Compound **4** (250 MHz, $CDCl_3$)
C-3,5 of $OC_6\mathit{H}_4$	7.75	7.96	7.97
C-2,6 of $OC_6\mathit{H}_4$	6.75	6.97	6.99
$-OC\mathit{H}_2CH_2OCH_3$	-	-	4.20
$-OC\mathit{H}_2CH_2-$	-	4.15	-
$-C\mathit{H}_2OCH_3$	-	-	3.80
$-OC\mathit{H}_3$	-	-	3.46
$-C(O)C\mathit{H}_2CH_3$	2.90	3.00	3.00
$-C\mathit{H}_2N(CH_3)_2$	-	2.78	-
$-N(C\mathit{H}_3)_2$	-	2.35	-
$-C(O)CH_2C\mathit{H}_3$	1.25	1.24	1.24

The second step of the synthetic pathway was dedicated to synthesis of the biological active part of our target molecule – the coupling of the previously synthesized precursors. This step contains the coupling of two carbonyl compounds, one containing two aromatic rings (4-hydroxybenzophenone or compounds **1** or **2**) and the other just one (1-(4-hydroxyphenyl)propan-1-one, 1-(4-methoxyphenyl)propan-1-one, compounds **3** or **4**) resulting into a Tamoxifen-like compound (e.g. Figure 40).

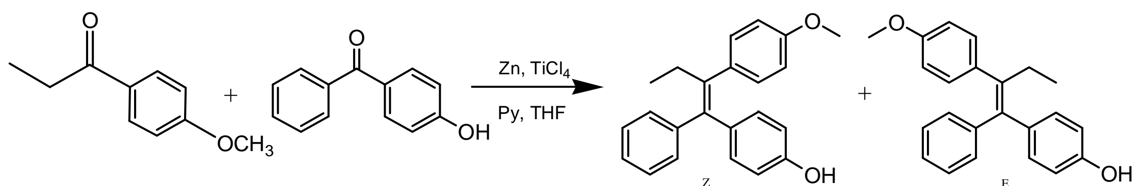
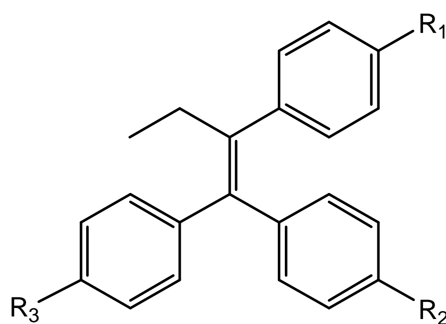


Figure 40: Synthesis of product **6**.

All of the analogues of this drug were synthesised with the purpose of maintaining a certain structure composed of a double bond between the carbons originally from the two carbonyl compounds with four substituents of which one is an ethyl group and the others are three aromatic rings. These three rings contain one OH group free to react with the germanium moiety (third step) and a substituent composed by the alkoxy group inserted previously. In some cases the compound also had another substituent ($-OCH_3$) originally from the starting carbonyl compounds. (Figure 41)



$R_1=OCH_3$; $R_2=OH$; $R_3=H$; **(6)**; 77%
$R_1=OH$; $R_2=OCH_2CH_2OCH_3$; $R_3= H$; **(8)**
$R_1=OCH_3$; $R_2=R_3=OH$; **(9)**; 79%
$R_1=OCH_3$; $R_2=R_3=OCH_2CH_2OCH_3$; **(11)**; 80%
$R_1=OCH_2CH_2OCH_3$; $R_2=OH$; $R_3=H$; **(12)**; 65%
$R_1=OCH_2CH_2OCH_3$; $R_2=OC(O)CH_2CH_2GeCl_3$; $R_3=H$; **(15)**; 57%

Figure 41: General structure for analogues of Tamoxifen.

As result of several searches into the synthesis of olefins published in the past it was concluded that the reaction to form tetra-substituted double bonds is well accomplished by McMurry coupling, although this reaction is bound to be less successful for unsymmetrical olefins.[49] At first this reaction consists in forming "*in situ*" a catalyst surface composed by Ti(0) which was previously introduced as titanium tetrachloride, Ti(IV), and then reduced to Ti(0) by zinc. McMurry reaction engages two important stages: the first stage refers to a pinacol radical-radical coupling where the small Ti(0) particles present in the highly active-surface donate an electron to the ketone which generates a ketyl that endures dimerization affording a 1,2-diol intermediate.[50] The pinacol product can be isolated from the reaction when the temperature is lowered than the room temperature which assures that the pinacol reaction is indeed observed. (Figure 42)

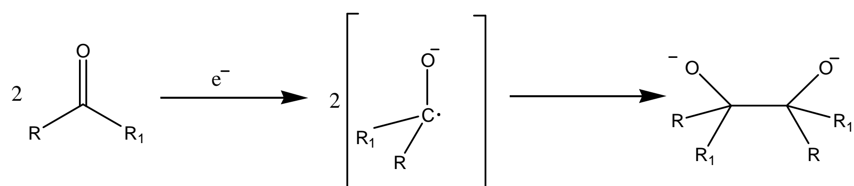


Figure 42: Formation of the pinacol product

The second stage corresponds to a deoxygenation step; although the mechanism of this reaction is not yet understood, three possible pathways have been proposed (represented in Figure 43).[50] From the three mechanisms evidences have shown that both OH groups of the diol coordinate to the surface of a heterogeneous titanium particle, Ti(0). Afterwards, the two carbon-oxygen bonds are cleaved generating the double bond of the desired olefin. (Figure 43)

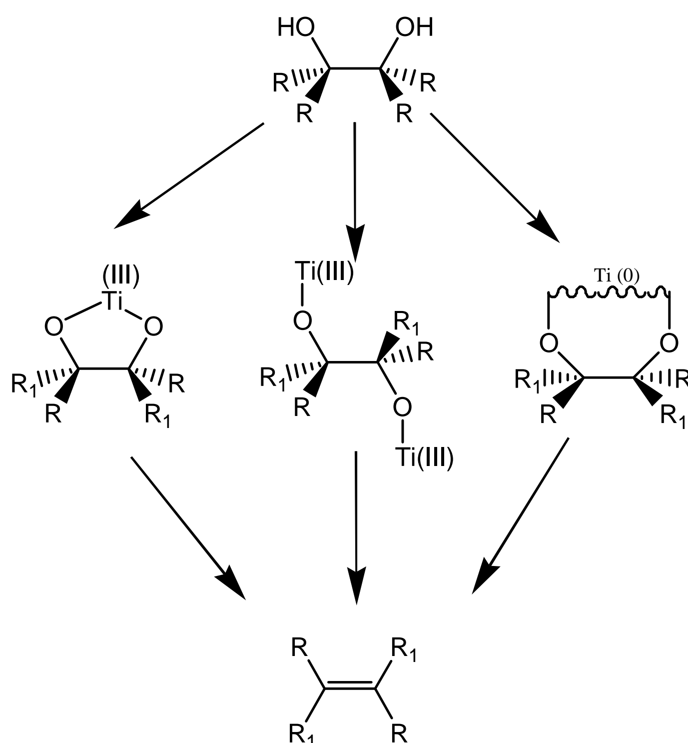


Figure 43: Three possible approaches for the mechanism of deoxygenation in the second stage of the McMurry coupling reaction.

Many new combinations of cross-coupling between compounds **1** to **4** were carried out, also taking into account the structure needed at the end. Moreover, taking into consideration the past researches the differences of the yield obtained for compounds **6**, **8** and **12** (for structure of the compounds see Figure 41), where all of them were synthesised using the same equivalents of the starting materials and the experimental procedure described by Duan[51], were predominantly caused by difference in the affinity of reactants to the titanium surface. Basically, the substituents present in the precursors synthesised before and also in the product formed, namely the hydroxyl, methoxy and dimethylaminoethoxy groups, have strong affinity to the

titanium surface. Consequently, both the product and/or starting material were trapped in the catalyst and all the workup that followed the reaction hardly got hold of all of these compounds. This piece of evidence was found in the synthesis of compound **6** where the purification step was problematic because many attempts of column chromatography resulted in various fractions that seemed pure by TLC but impure by NMR. When a sample of one of the fractions was submitted to GC-MS the nature of the impurity suggested that a side product was also present (homo-coupling of the aryl compound). To solve this problem various protecting groups of –OH and $–N(CH_3)_2$ were considered before performing the McMurry reaction. Previous research suggested that this strategy might not improve the yield but it could affect the product selectivity.[52][53] In fact, the groups to be protected were found to have strong affinity to the titanium surface, which could favour a certain reaction, improving the selectivity of the cross coupling in a slower rate. As a result it was decided not to protect the groups although it was used unsuccessfully for purification of the crude of compound **8**. [51][52] [54]

In order to improve the reaction time, and relying on literature models, the syntheses of compounds **6**, **8** and **12** were used in specific conditions where the selectivity of the starting material and respective product were decreased allowing a considerable rate of the reaction. These specific conditions comprised the use of anhydrous pyridine after the titanium surface was assembled which influenced the interaction and affinity between the Ti(0) particles and the substituents present in the reactants and/or product. For the workup process that was followed as suggested by the literature[51], 10% potassium carbonate solution was used with the purpose of scrapping from the catalyst all the remaining reactants and product. This procedure was suitable for the synthesis of compound **12**, which also explains the yield obtained for the same (65%). This compound was characterized by NMR combined with some information given by IR. The following table contains the NMR peaks of the two starting materials and the coupling product (compound **12**) between them, which was synthesized in success and has not been described previously in literature (Table 10).

Table 10: ^{1}H-NMR values of 1-[4-(2-Methoxy-ethoxy)-phenyl]-ethanone **(4)**,(E)(Z)-4-{2-[4-(2-methoxyethoxy)phenyl]-1-phenylbut-1-enyl}phenol **(12)** and 4-Hydroxybenzophenone.

Protons (ppm)	Compound **4**	Compound **12**	4-Hydroxybenzophenone
C-2,6 of C_6H_5	-	7.35-6.40	7.70
C-3,5 of OC_6H_4	-		7.53
C-4 of C_6H_5	-		7.45
C-3,5 of C_6H_5	-		7.36
C-3,5 of $H_2COC_6H_4$	7.97		-
C-2,6 of $H_2COC_6H_4$	6.99		-
C-2,6 of OC_6H_4	-		6.83
-O*H*	-	5.19, 4.89	5.0
$-OCH_2CH_2$	4.20	4.10-4.00	-
$-CH_2OCH_3$	3.80	3.75-3.70	-
$-OCH_3$	3.46	3.52, 3.51	-
$-C(O)CH_2CH_3$	3.00	2.50-2.40	-
$-C(O)CH_2CH_3$	1.24	0.95-0.90	-

The NMR values concerning the protons of the ethyl group originally from compound **4** have shifted slightly and the existence of two peaks related to hydroxyl groups indicated the presence of two isomers of compound **12** and confirmed the desired structure (Figure 44). Also, in ^{13}C-NMR no peak regarding to carbonyl was seen, which pointed out that no starting material was present in the pure product isolated and the reaction indeed had been successful.

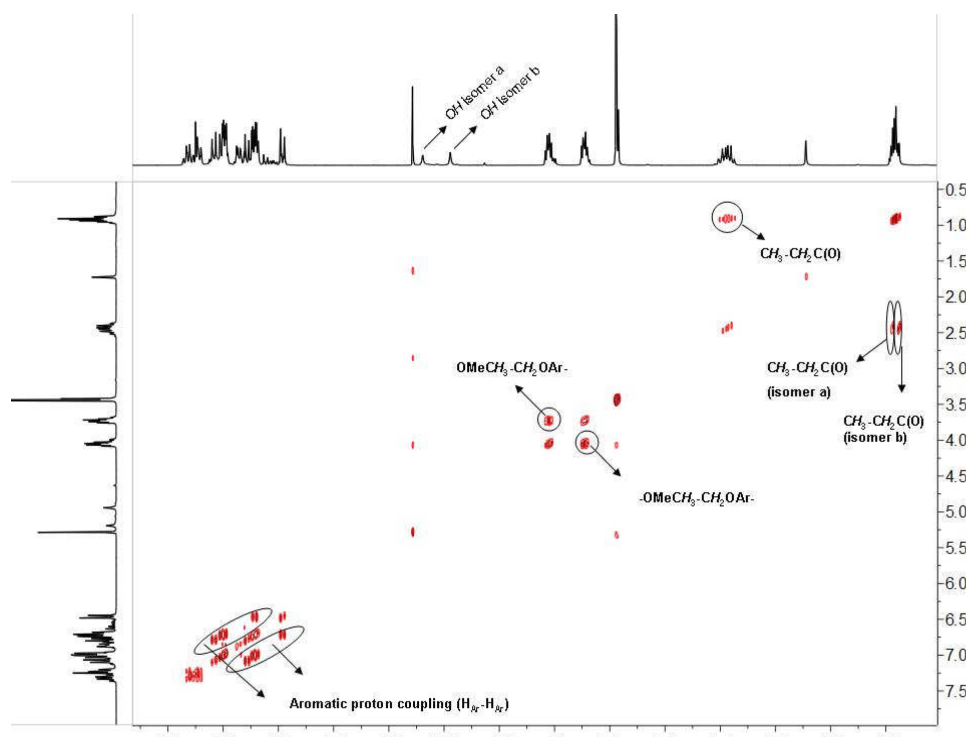


Figure 44: COSY spectrum of product **12.**

However, surprisingly, the synthesis of product **6** (Figure 41) was found problematic due to the reasons discussed above. Therefore more research was carried out and a different experimental procedure found for literature models was seen in the article by D. Yu and B. Forman[53] and applied in a second attempt for synthesis of product **6**. The major differences between the two experimental procedures were that the first one used pyridine to activate the catalyst and the latter one did not use a base, but on the other side the reaction between the catalyst and both ketones was left in the dark in a significant lower reaction time, when compared to the original procedure described for compounds **8** and **12**. This fact suggested that perhaps 1-(4-methoxyphenyl)propan-1-one (starting material for compound **6**) did not have great affinity to the titanium surface as other starting materials used in other McMurry reactions and also that the darkness might have helped to keep the catalyst activated for both steps of the reaction. Again, NMR was important to characterise a new product, compound **6,** and the values of ^{1}H NMR are listed below (Table 11):

Table 11: ^{1}H-NMR values of 1-(4-methoxyphenyl)propan-1-one, (E)(Z)-4-[2-(4-methoxyphenyl)-1-phenylbut-1-enyl]phenol **(6)** and 4-Hydroxybenzophenone

Protons (ppm)	1-(4-methoxyphenyl)propan-1-one	Compound **6**	4-Hydroxybenzophenone
C-2,6 of C_6H_5	-	7.35-6.45	7.70
C-3,5 of $OC_6\mathit{H}_4$	-		7.53
C-4 of C_6H_5	-		7.45
C-3,5 of C_6H_5	-		7.36
C-3,5 of $H_3COC_6\mathit{H}_4$	7.78		-
C-2,6 of $H_3COC_6\mathit{H}_4$	6.85		-
C-2,6 of $OC_6\mathit{H}_4$	-		6.83
-O*H*	-	4.86, 4.64	5.0
-OCH_3	3.73	3.76, 3.75	-
-C(O)CH_2CH$_3$	2.59	2.50-2.40	-
-C(O)CH$_2$CH_3	1.18	1.00-0.90	-

The aromatic protons of compound **6** (structure represented in Figure 41) indicated in the table were apparently shifted although the peaks were not very clear because the product isolated corresponded to the 1:1 mixture of isomers (E) and (Z). This piece of information was also confirmed by the incidence of two peaks referring to the hydroxyl and methoxy substituents present in the product. The OH group of the compound were shifted upfield compared to 4-hydroxbenzophenone because the latter undergoes a withdrawing effect from the carbonyl and the former had an opposite effect due to the double bond and the presence of a donating substituent (-$H_3COC_6H_4$).

In the synthesis of compound **8** (Figure 41), again the purification step was problematic because it seemed that the reaction resulted into many side products which did not help to

isolate the product from the impurities. Also, the presence of two isomers present did not help to identify the product from the impurities. Nevertheless, after establishing an appropriate eluenting system a column chromatography was performed, but it did not seem reproducible as expected by TLC because there was a solvent effect with the compounds present in the crude, which modified its affinity for silica. As a result it was decided to purify a small sample in a preparative plate, but unfortunately the new product isolated was only enough for NMR and so its respective yield was not possible to calculate. Once again, the NMR was important to characterise compound **8** and the values of ^{1}H NMR are listed below (Table 12):

Table 12: ^{1}H-NMR values of 1-(4-hydroxyphenyl)propan-1-one, (E)(Z)-4-{1-[4-(2-methoxyethoxy)phenyl]-1-phenylbut-1-en-2-yl}phenol **(8)** and [4-(2-methoxyethoxy)phenyl]phenylmethanone **(2)**

Protons (ppm)	1-(4-hydroxyphenyl)propan-1-one	Compound **8**	Compound **2**
C-2,6 of C_6H_5	-	7.30-7.10	7.84-7.81
C-3,5 of OC_6H_4	-	7.05-7.00	7.01-6.98
C-4 of C_6H_5	-	7.30-7.10	7.60-7-50
C-3,5 of C_6H_5	-	7.30-7.10	7.50-7-40
C-3,5 of OC_6H_5	7.72	6.55-6-50	-
C-2,6 of OC_6H_5	6.81	6.90-6.80	-
C-2,6 of OC_6H_4	-	6.70-6.65	7.77-7.74
$-OCH_2CH_2$		4.17-4.09	4.20-4.10
$-OH$	5.0	5.20, 4.95	-
$-CH_2OCH_3$		3.84-3.78	3.75-3.70
$-OCH_3$	-	3.37	3.40
$-C(O)CH_2CH_3$	2.59	2.40, 2.35	-
$-C(O)CH_2CH_3$	1.18	1.30, 0.90	-

All the aromatic protons were shifted compared to the starting materials as it was expected, because the double bond formed extended the conjugate system allowing the protons to be more shielded. Again, the existence of two peaks for the hydroxyl groups indicates the presence of two isomers and in this case the protons of the ethyl group of each isomer were possible to discriminate and appeared in a different range then the starting material.

An alternative for the second step of the synthetic route towards the target molecule consisted in initially performing a McMurry coupling followed by an alkylation of just one of the hydroxyl groups, in an attempt to improve the yields and solving the problems of purification mentioned previously (Figure 45).

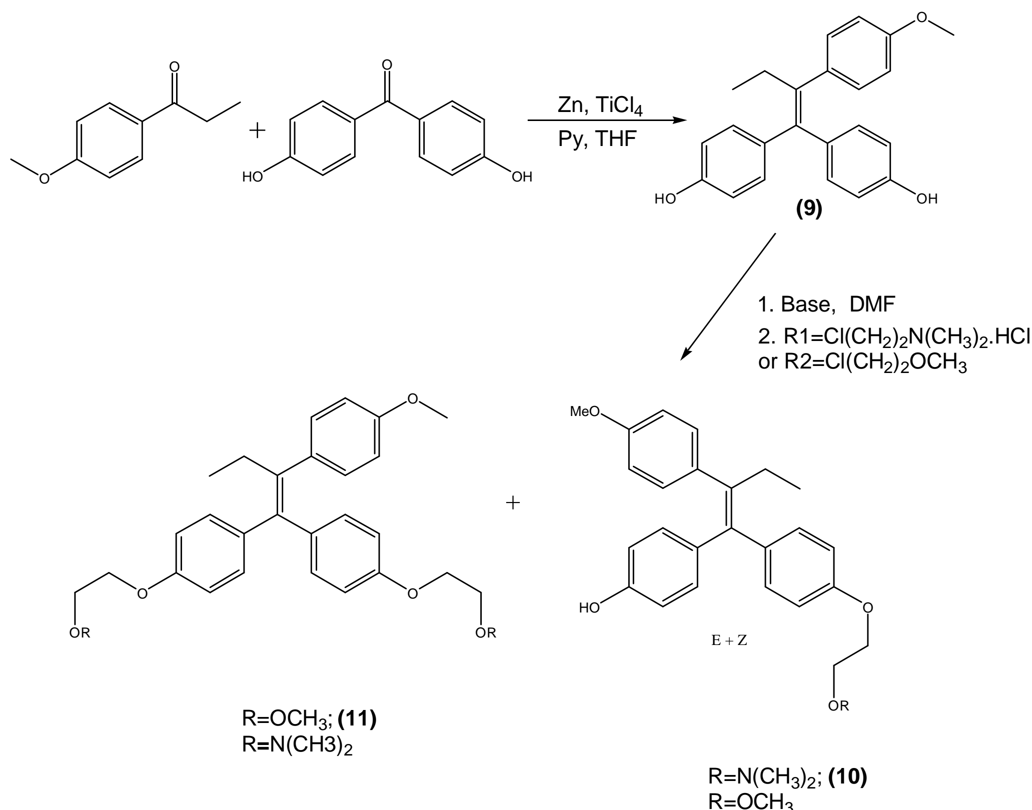


Figure 45: Scheme of an alternative for the second step of the synthetic route to achieve the target-molecule

In the first stage, a McMurry reaction was carried out according to the experimental procedure used for compound **6** (without pyridine)[53] affording a red compound **9** in a substantial yield (79%) (Figure 45). This piece of evidence suggested that none of the starting materials had great affinity for the titanium surface, which consequently did not interfere in the reaction time. This result had also been observed in the synthesis of compound **6**, where the affinity of the starting material, 1-(4-methoxyphenyl)propan-1-one was found to be in the same range. Compound **9** was essentially characterised by NMR and compared with the literature values for compound, 4-(1-(4-hydroxyphenyl)-2-phenylbut-1-enyl)phenol, (Table 13). The comparison of ^{1}H NMR values presented in the table below showed minor differences for relevant peaks and the only exceptions highlighted were the protons of C-3,5 of $H_3COC_6\mathit{H}_4$ or of C-3,4,5 of C_6H_5, that were actually caused by the methoxy group that was present merely in compound **9**.

Table 13: ^{1}H-NMR shifts of compound **9** obtained and of 4-(1-(4-hydroxyphenyl)-2-phenylbut-1-enyl)phenol taken from the literature [53] in $CDCl_3$

Protons	Compound **9**/ppm (250 MHz)	Literature/ppm (500 MHz)
C-3,5 of OC_6H_4	7.10-7.00	7.17-7.10
C-3,5 of $H_3COC_6H_4$ or of C-3,4,5 of C_6H_5	6.83	
C-2,6 of $H_3COC_6H_4$ or of C-2,6 of C_6H_5	6.50-6.45	6.49
C-2,6 of OC_6H_4	6.80-6.75	6.86, 6.75
-O*H*	5.20	4.65
-O*H*	4.95	4.43
-OC*H*$_3$	3.76	-
-C(O)C*H*$_2$CH$_3$	2.49	2.49
-C(O)CH$_2$C*H*$_3$	0.95	0.92

The second stage was problematic in the alternative route because the exact equivalents of alkylating reagent and base used for the same reaction in the literature[55] turned out to not to work for the reaction with 2-(dimethylamino)ethylchloride hydrochloride. This alkylation was not successful because on one hand the equivalents were not appropriate (3.7 equivalents of alkylating agent) and on the other hand reactions with β-halo amines are bound to be difficult because they can form side-products easily, e.g. aziridines, which probably happened in this reaction too. As a result of some research, the number of equivalents (1.2 equivalents) necessary for monoalkylation given in a paper [54] and using 2-chloroethyl methyl ether as alkylating agent for the reaction was used. The reaction was followed by TLC, which showed that no reaction occurred and so more alkylating reagent was added in excess, which led to a dialkylated product (compound **11**) and, unfortunately, no monoalkylated product was isolated (Fig 45). The excess of alkylating reagent and the short time (~ 2h) before adding the third portion could have prevented forming the monoalkylated product initially desired and also explained the substantial yield obtained for compound **11** isolated from column chromatography (80%).

The structure of compound **11** was characterised mostly by comparing the proportion of the integration of the peaks corresponding to $–CH_2$ present in the ethyl group with the $–CH_2$ of the alkoxy substituent. The peak corresponding to the methylene protons of the ethyl group integrated for two protons whereas the peak concerning the same group present in the substituent integrated for four protons. Therefore, the ratio 1:2 of the peaks mentioned, previously, immediately indicated that two molecules of alkylating reagent were present in the product. Also, a slight shift of the protons of $C_{3,5}$ of $H_3COC_6H_4$ was seen when compared with compound **9**, which also elucidated that a reaction had occurred and confirmed the structure of the product.

All the analogues obtained (Compound **6**, **8**, **9**, **11** and **12**) were only characterised by NMR, IR and when possible, comparison to literature values because mass spectra was not available. However the characterization appears unambiguous and they should give the expected molecular ions, when submitted to mass spectrometry in the near future. Since all products from the McMurry reactions were obtained as 1:1 mixtures of E/Z isomers, no melting points were determined.

As the McMurry reaction was problematic, especially for the purification of the crude product, a third and final approach to the second step of the synthetic route for the target-molecule was considered. After some research a paper regarding a Tamoxifen and Toremifene alternative synthesis was found.[55] The key step of the synthesis was a condensation of a fully functionalised benzophenone compound, such as compound **5,** with an anion of propylbenzene generated by a super-base (LICKOR) yielding a carbinol which underwent dehydration resulting in Tamoxifen or Toremifene. (Figure 46)

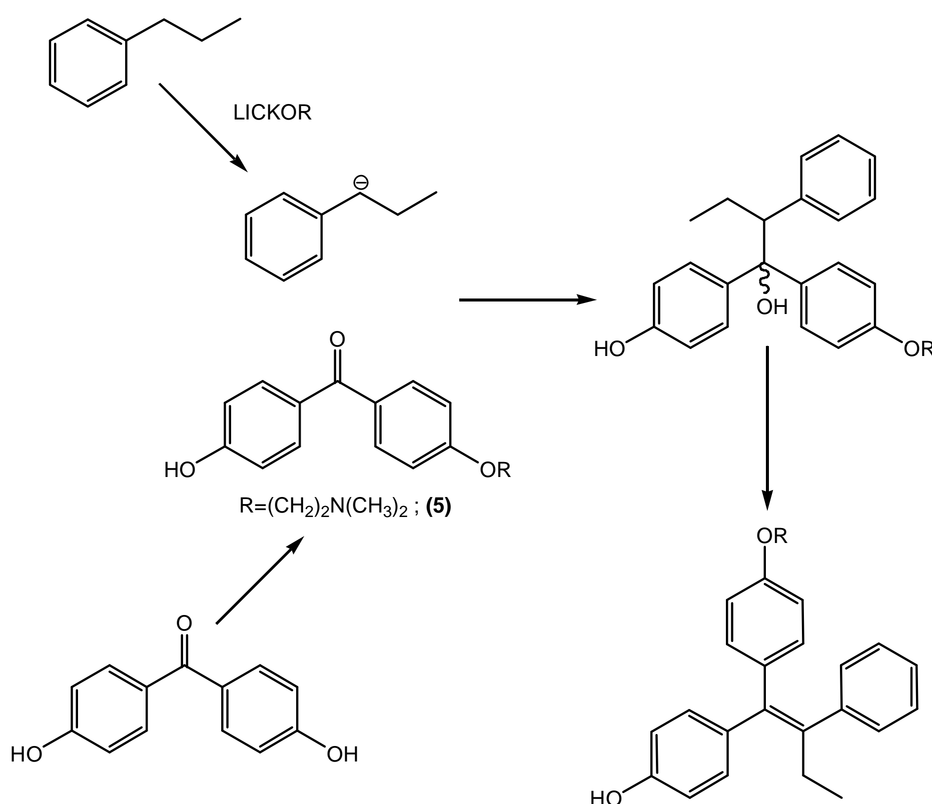


Figure 46: Third approach of synthesis of an analogue of Tamoxifen

This strategy was also applied in this project by firstly alkylating one of the hydroxyl groups of 4,4-dihydroxybenzophenone. The alkylation was undertaken in two different conditions. The first one used a strong base, sodium hydride, and although the base seemed fairly altered the equivalents used for the reaction (2 equivalents) explained the small yield obtained for compound **5** (6%). Another factor which contributed to the low yield was the short

time of reaction (~ 1h). Even tough TLC was done to follow the reaction, it was inconclusive because many impurities appeared in the TLC plate which encouraged to working up the reaction instead of using longer reactions times.

In order to improve the yield, the alkylation was repeated by increasing the equivalents of a different base chosen (3 equivalents), caesium carbonate, and all the experimental procedure was modified accordingly, on basis of an article written by Sylvain Gauthier. [54] As a result the yield obtained for compound **5** increased significantly, 44%, coinciding with the yield obtained in the literature. Improvements could include the change of column conditions to a different system of eluents or the change from a silica based column to alumina, as the formed amine seems to have a high affinity to silica. Also, the dialkylated product could form, although the yield is fairly low which is consistent in not obtaining the by-product of the reaction.

Once again, the characterisation of compound **5** (Figure 46) was compared to the same compound synthesised by Sylvain Gauthier (Table 14) and consequently the structure was confirmed by the similarities in the literature between the peaks of compound **5** and those reported.

Table 14: ^{1}H-NMR values of 4-(2-(dimethylamino)ethoxy)phenyl)(4-hydroxyphenyl)methanone **(5)** and from the literature.[54]

	Compound **5** (250 MHz, CD_3OD)	Literature (300 MHz, CD_3OD)
C-2,6 of $OC_6\mathit{H}_4$	7.78	7.72
C-2,6 of $OC_6\mathit{H}_5$	7.71	7.65
C-3,5 of $OC_6\mathit{H}_4$	7.11	7.00
C-3,5 of $OC_6\mathit{H}_5$	6.91	6.85
$-OC\mathit{H}_2CH_2$	4.25	4.18
$-C\mathit{H}_2N(CH_3)_2$	2.87	2.81
$-N(C\mathit{H}_3)_2$	2.30	2.36

The second starting material for the reaction described in Figure 46 consisted in forming propylbenzene anion “in situ” by using super-basic reagents. These reagents were partially composed by binary combinations, BuLi-TMEDA, which is known to metallate alkyl-substituted benzenes at the α-carbon centres and/or the aromatic ring. [55] Also a third base was used to form the anion as it is known that a 1:1 combination of alkyllithium (“LiC”) with bulky potassium alcoholates (“KOR”) allows smooth metallation of low acidity hydrocarbons. Basically, LICKOR promotes a hydrogen/metal exchange with a high regioselectivity of the proton abstracted. [56]In the reaction this step was performed successfully for the two attempts of synthesis of compound **14** (represented in Figure 47). The red reaction mixture was seen immediately, as expected, which proved the presence of the anion formed.

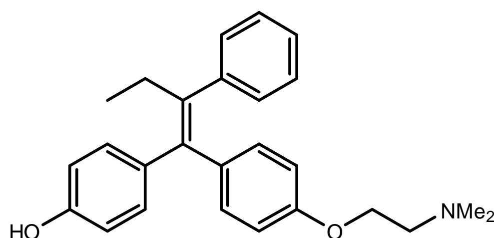


Figure 47: Structure of compound **14**

The addition of a solution of compound **5** to the mixture containing the anion was problematic because, although the first reaction was an acid-base reaction where the anion (base) abstracted the proton of the hydroxyl group of the ketone the main reaction (condensation of the anion with compound **5**) seemed unlikely to have happened as minor changes of colour of the reaction mixture was seen. The NMR of the crude product, including the bi-dimensional spectra (COSY and HSQC) and IR indicated the respective peaks of compound **5** and of propylbenzene appeared in the same range as expected and no new peaks were observed; this led us to conclude that no reaction had occurred. This result was explained by the presence of the respective phenolate of compound **5**, which may have prevented the benzylic anion from attacking the carbonyl, due to repulsive interactions of the negative charges present in both of reactants.

Finally, the last step of the synthetic pathway was unfortunately less explored due to time restrictions. Nevertheless, it was possible to investigate the reaction of one of the newly synthesized tamoxifen analogues with 3-(trichlorogermyl)propanoyl chloride. The analogue chosen for this reaction was compound **12**. This reaction was taken relying on the supporting information section in a paper [43], where a similar reaction between a quinoline and the same germanyl compound used to form compound **15** was described. Firstly, compound **12** was deprotonated by triethlyamine and then the respective phenolate formed attacked the carbonyl group of the germanyl compound which lost the chlorine substituent (Figure 48).

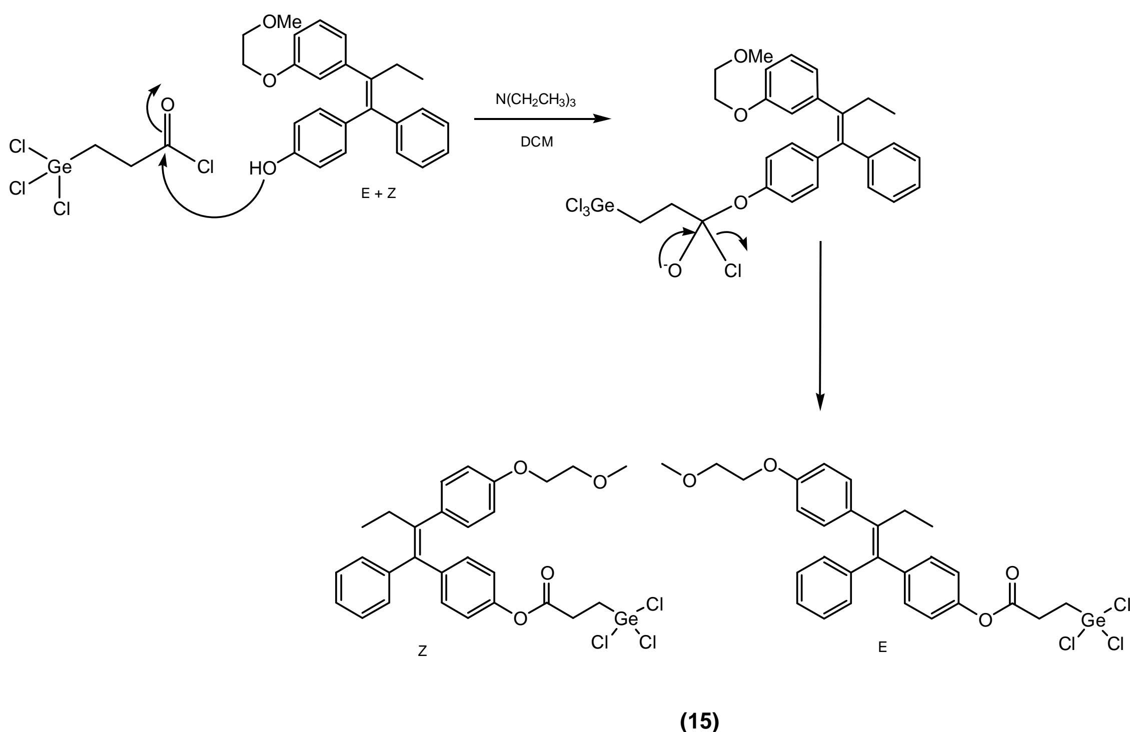


Figure 48: Synthesis of compound **15** (isomers E and Z).

Workup and purification were undertaken according to the literature mentioned previously, which afforded the respective germanium sesquioxide hydrochloride salt of compound **12** (first target molecule **15**). The structure of the second target molecule was formed by hydrolysis of compound **15** which undergoes a intra-molecular reaction affording a dimer of the same compound (Figure **49**). Some modifications had to be made in the experimental procedure as the salt did not precipitate in THF. The filtrate was concentrated, which unfortunately contributed to partially decomposition of the product due to the temperature used in the rotary evaporator. However, the product was characterised by NMR, which showed that the peaks referring to each proton were situated in the same range as seen for the two starting materials. This result was not very surprising, since the presence of germanium was relatively remote from the aromatic location. Also, the IR was crucial to confirm the new desired structure of one of the target molecules, compound **15**, because the C-Ge (medium peak at 586 cm^{-1}) bond appeared fairly close to the wave numbers achieved by the product seen in the literature regarding to a quinoline (medium peak at 576 cm^{-1}).[43] At the end, the compound was submitted to mass spectrometry and three relevant fragments were seen. The base peak, appearing at m/z 397 was assigned to the fragment arising from compound **15** by loss of methanol and $Ge(OH)_3$, [M-MeOH-$Ge(OH)_3]^+$. A small peak observed at m/z 577, corresponded to sodiated **17** molecules. Another important peak appeared at m/z 537, which corresponded loss of water from the protonated **17** molecule. From analysis of the mass spectrum, it can be concluded that the Ge moiety was effectively incorporated although it appears to have been very prone to hydrolysis. This result is not surprising, in view of the known properties of 3-(trichlorogermyl)propanoyl derivatives.

In the same paper [43] an experimental procedure was described for the product with the quinoline and the germanyl moiety containing the three hydroxyl groups instead of the chlorines. Following this procedure, basically, compound **15** was hydrolysed (affording **17**) and tried to precipitate in cold acetone. This did not happen because the starting material was found to be impure at the time of the experiment. However, after analysis of mass spectrum of **15** it was concluded that the second target molecule (compound **17**) was also present.

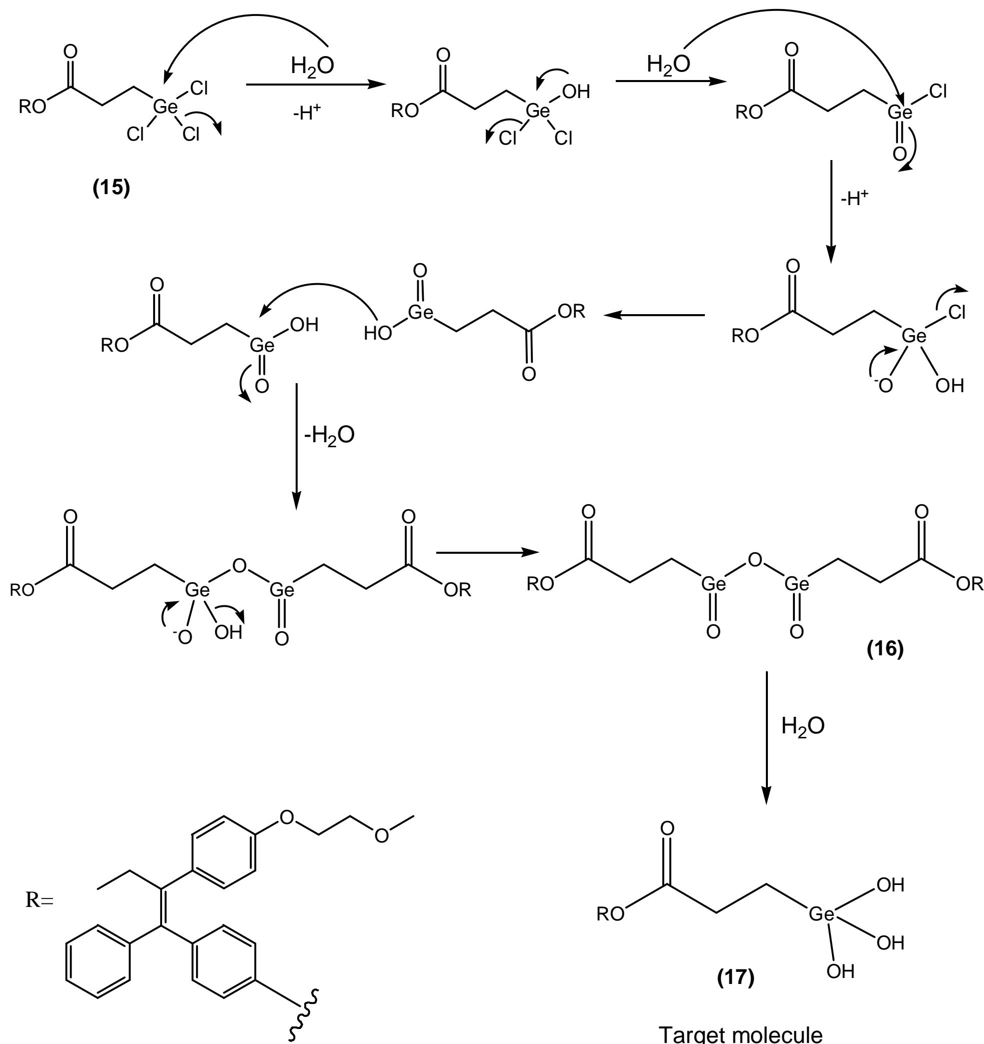


Figure 49: Mechanism of the reaction to form the target molecule

As an alternative, some patents were found referring to the same reaction (quinoline + 3-(trichlorogermyl)propanoyl chloride), one of them using pyridine instead of triethylamine to deprotonate the quinoline and the other did not use any base in the first step. Unfortunately, this strategy was not further explored due to time restrictions.[57]

2.2.2 – Conclusions and Outlook

The second part of this project was an exploratory exercise, firstly, for the synthesis of analogues of Tamoxifen and secondly for the coordination of the same to a germanium moiety.

The alkylation reactions on phenolic oxygens, where a β-halo amine [2-(dimethylamino)ethylchloride hydrochloride] was participating, were found problematic compared to the same reactions using 2-chloroethyl methyl ether. Also, the presence of alkoxy amino substituents was found to compromise the efficiency of McMurry coupling.

The second stage of the synthetic pathway, which involves the coupling of the precursors synthesised in the first step, via McMurry reactions afforded various products (**6**, **8**, **9**, **11** and **12**). The limiting step found in this stage was the purification of the crude product which is worth optimising in the future. For instance by using HPLC or even column chromatography on Alumina, which could afford an efficient separation not only of the isomers formed but also the impurities present.

The coordination of the biologically active compound (only attempted for compound **12**) to the germanium moiety (final products **15** and **17**) was also problematic, due to the poor stability of the metal fragment and to limitations in the methods used for purification for this type of compounds. Also not much literature was available for this specific reaction. Nonetheless, evidence for incorporation of the germanium moiety was obtained by ESI mass spectrometry. In the future, more investigation regarding the coupling of the organogermanium compound with all the analogues of Tamoxifen obtained is envisioned. Once the pure compounds are formed they will be assessed for cytotoxicity using MTT based assays; their potential as anti-cancer agents is planned to be tested for a number of different cancer cell lines.

3. Experimental Section

3.1 - General

^{1}H NMR spectra were recorded on either a Bruker AMX 400 / Bruker Avance II 400 NMR (400 MHz), or a Bruker DPX 250 (250 MHz) / Bruker Avance II 300 (300 MHz) spectrometer in deuterated chloroform (CDCl3) or methanol (MeOH-d_4) and referenced to the residual solvent peaks or to tetramethylsilane as an internal standard. Signal positions were recorded as chemical shifts with the abbreviations s, bs, d, dd, ddd, t, q and m denoting singlet, broad singlet, doublet, double doublet, double double doublet, triplet, quartet and multiplet. ^{13}C NMR spectra were recorded on the same spectrometers listed above at either 60 or 100 MHz. All NMR chemical shifts are quoted in parts per million (ppm). Coupling constants are recorded in Hertz to the nearest 0.5 Hz.

Mass spectra (MS) were recorded on a Varian 500-MS LC Ion Trap mass spectrometer operated in the electrospray ionization (ESI) mode. The spray was set ± 5 kV and the capillary voltage was set at 10 V. Fragment percent intensities and fragment assignments are indicated in parentheses and square brackets, respectively.

Melting points were recorded using an Electrothermal digital melting point apparatus or a Leica Galen III hot stage apparatus and are uncorrected.

Infrared spectra were recorded as thin films between sodium chloride plates or potassium bromide discs on a Perkin-Elmer 1720 –X-FT-IR spectrometer or on a Jasco FT/IR 4100. All absorption values are quoted in wave numbers (cm^{-1}). Major features of each spectrum are reported with the abbreviations s, m, w denoting strong, medium and weak peaks.

Column chromatography was performed on silica gel supplied by Merck, particle size: 40-63 µm using head pressure by means of hand bellows. Thin layer chromatography analysis was carried out on aluminium plates pre-coated with Merck 0.2mm $60F_{254}$ silica gel. Spots were visualised either by UV fluorescence at a wavelength of 254 nm, by staining with ninhydrin solution, by phosphomolybdic acid hydrate solution in ethanol or by iodine when appropriate.

The preparative plates UNIPLATE, Analtech in Silica gel GF with dimensions, 20x20cm, and particle size, 0.5 or 2 mm, were purchased from Merck.

The majority of reactions were performed under argon atmosphere using anhydrous solvents. The following anhydrous solvents were bought from Aldrich with sure seal stoppers:

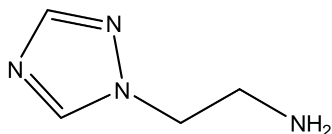
tetrahydrofuran, dichloromethane, *N,N*-dimethylformamide and pyridine. Tetrahydrofuran was also dried by distillation over sodium metal using benzophenone as indicator.

All reagents were obtained from Aldrich and used directly as supplied without further purification.

All solid reagents were weighed in an Explorer Ohaus or Metler Toledo PB303-S scale with four digits for decimal numbers.

3.2 – Synthesis of organometallic analogues of Maraviroc

3.2.1 – Attempted synthesis of 2-(1H-1,2,4-triazol-1-yl)ethanamine (1)



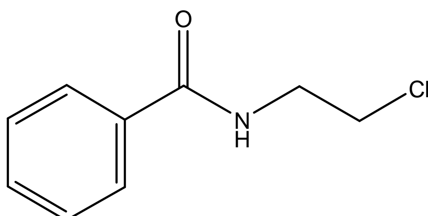
Potassium *tert*-butoxide (1.23 g, 10.96 mmol) was dissolved in DMF (~30 mL) in a round bottom flask. When a clear solution was obtained 1*H*-1,2,4-triazole (0.55 g, 7.96 mmol) was added and left stirring for 15 min. Next ethanamine-2-chloride hydrochloride (0.52 g, 3.60 mmol) was added to the mixture in three portions and left stirring for 18 h at r.t., where a white solid was formed. The reaction was followed by TLC using as system 1:1 methanol/EtOAc and stained by iodine. Water (~150 mL) and diethyl ether (~50 mL) were added and both layers were separated. The aqueous layer was washed with 3 x 50 mL Et_2O. All organic layers were combined, dried over magnesium sulphate and filtered. The filtrate was concentrated under vacuum affording a yellow oil; a sample of this residue was submitted to ^{1}H NMR. No presence of product **1** was detected.

3.2.1.1 – Attempted alternative route of synthesis of 2-(1H-1,2,4-triazol-1-yl)ethanamine

To a solution of potassium *tert*-butoxide (1.20 g, 10.70 mmol) in THF (30 mL) was added 1*H*-1,2,4-triazole (0.52 g, 7.57 mmol) and left stirring for 30 min. Next ethanamine-2-chloride hydrochloride (0.42 g, 3.62 mmol) was added to the mixture in three portions and left stirring at r.t. for 20 h. The white solid formed was filtered and washed with Et_2O (2 x 15 mL). The filtrate was concentrated under vacuum affording a yellow oil and a sample of it was submitted to ^{1}H NMR. Again no presence of product was detected.

3.2.1.2 - Second alternative route of synthesis of 2-(1H-1,2,4-triazol-1-yl)ethanamine (via protection of 2-chloroethamine with benzoyl chloride)

- Synthesis of *N*-(2-chloroethyl)benzamide **(2)**



To a solution of ethanamine-2-chloride hydrochloride (5 g, 43.12 mmol) in THF (100 mL), potassium *tert*-butoxide (2.98 g, 21.56 mmol) was added and the reaction mixture was stirred

for 30 min. Next, benzoyl chloride (6.5 mL, 55.95 mmol) was added and left stirring at r.t. for 18h. A TLC was performed using EtOAc as eluent and no reaction had occurred; therefore more ethanamine-2-chloride hydrochloride (5 g, 43.12 mmol) was added to the reaction mixture, which was left stirring for 72h at r.t. affording a colourless solution. A saturated solution of sodium bicarbonate (~150 mL) and diethyl ether (~50 mL) was added and both layers were separated. The aqueous layer was washed with 3 x 50 mL Et_2O. All organic layers were combined, washed with saturated solution of sodium bicarbonate (~60 mL), dried over magnesium sulphate and filtered. A white solid precipitated. The suspension was concentrated *in vacuum* to yield directly the pure white solid **2**. (5.07 g, 43.71 mmol, 51%).

Mp 103-105 ^{o}C

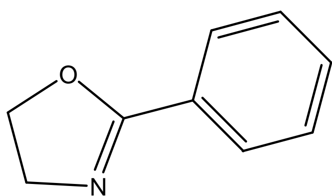
IR (KBr, cm^{-1}): 3302 (m, $\nu_{N\text{-}H}$, N-H of amide), 3066 (wk, $\nu_{C\text{-}H}$, C-H of CH_{Ar}), 2967, 2943 (wk, $\nu_{C\text{-}H}$, C-H of CH_2), 1636 (s, $\nu_{C=O}$, Ar(C=O)NH-), 1603 and 1578 [m, $\nu_{C\text{-}C}$,$(C=C)_{Ar}$], 1254 (m, $\nu_{C\text{-}N}$, (O=C)-NH), 690 (m, $\nu_{C\text{-}Cl}$, -H_2C-Cl)

^{1}H NMR (δ, $CDCl_3$, 400 MHz): 7.76 (d, J = 7.2 Hz, 2H, $C_{2,6}$ of C_6H_5), 7.51 (t, J = 7.2 Hz, 1H, C_4 of C_6H_5), 7.44 (t, J = 7.2 Hz, 2H, $C_{3,5}$ of C_6H_5), 6.63 (s, 1H, -N*H*), 3.75 (m, 2H, -$NHCH_2CH_2Cl$), 3.70 (m, 2H, -$NHCH_2CH_2Cl$).
^{13}C NMR (δ, $CDCl_3$, 300 MHz): 168.0 [C(O)], 134.4 (C_1 of C_6H_5), 132.1 (C_4 of C_6H_5), 129.0 (2C)[$C_{3,5}$ of C_6H_5], 127.3 (2C)[$C_{2,6}$ of C_6H_5], 44.4 (-$NHCH_2CH_2Cl$), 42.0 ($NHCH_2CH_2Cl$).

ESI-MS: *m/z* (%) = 186 (28) $[M+H]^+$(^{37}Cl), 184 (100) $[M+H]^+$(^{35}Cl), 148 (10) {$[M+H]^+$-HCl}, 105 (26) {$[M+H]^+$-$PhCO^+$}

- Attempted alkylation of 1*H*-1,2,4-triazole

Potassium *tert*-butoxide (0.31 g, 2.73 mmol) was dissolved in THF (~25 mL) in a round bottom flask. When a clear solution was obtained 1*H*-1,2,4-triazole (1.73 g, 25.00 mmol) was added and left stirring for 15 min. Next *N*-(2-chloroethyl)benzamide **(2)** (0.50 g, 2.72 mmol) was added to the mixture in three portions and left stirring for 22 h at r.t. affording a white suspension. The reaction was followed by TLC using EtOAc as eluent and stained by iodine where a new spot was detected. A small portion (~10 mL) of the reaction mixture was concentrated under vacuum affording a yellow oil.
The crude product was purified on a preparative plate coated by silica-gel. The eluent used was EtOAc. Four fractions were separated, concentrated and submitted to ^{1}H NMR. The first and the last fractions were impurities, the second fraction belonged to product **2** and the third fraction afforded a brown oil which corresponded to a sub-product, 2-phenyl-4,5-dihydrooxazole **(3)** due to an intramolecular reaction. (See characterization below)



(3)

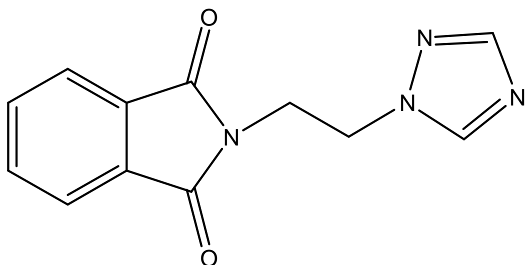
The same reaction was performed using different bases: sodium hydride, *n*-butyllithium and 1,8-diazabicyclo[5.4.0]undec-7-ene the intramolecular reaction predominated in all instances, affording the same brown oil of product **3**.

IR (thin-film, cm^{-1}): 3062 (wk, $\nu_{C\text{-}H}$, C-H of CH_{Ar}), 2929, 2878 (wk, $\nu_{C\text{-}H}$, C-H of CH_2), 1650 (m, $\nu_{C=N}$, -O-C=N-), 1604 and 1579 [m, $\nu_{C\text{-}C}$,$(C=C)_{Ar}$], 1260 (m, $\nu_{C\text{-}O}$).

^{1}H NMR (δ, acetone-d_6, 400 MHz): 7.79 (d, J = 9.2Hz, $C_{2,6}$ of C_6H_5), 7.55-7.50 (m, 1H, C_4 of C_6H_5), 7.50-7.40 (m, 2H, $C_{3,5}$ of C_6H_5), 4.47 (apparent t, J = 12.8 Hz, 2H, -OCH_2CH_2N-), 4.04 (t, J = 12.8 Hz, 2H, -OCH_2CH_2N-).

^{13}C NMR (δ, acetone-d_6, 300 MHz): 170.0 (C_1 of C_6H_5), 163.4 (C_2 of oxazole), 131.0 (2C) [$C_{2,6}$ of C_6H_5], 128.3 (2C) [$C_{3,5}$ of C_6H_5], 127.9 (C_4 of C_6H_5), 67.3 (-OCH_2CH_2N-), 59.6 (-OCH_2CH_2N-).

3.2.1.3 - Third alternative route of synthesis of 2-(1H-1,2,4-triazol-1-yl)ethanamine (via protection of ethanamine-2-chloride hydrochloride with 2-(2-bromoethyl)isoindoline-1,3-dione)

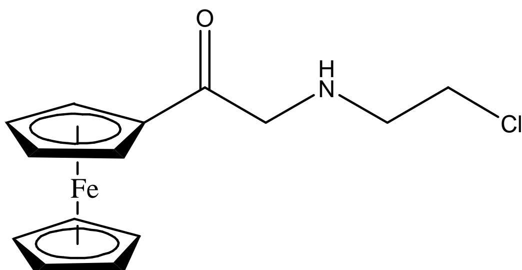


(not obtained)

Potassium *tert*-butoxide (1.07 g, 7.76 mmol) was dissolved in DMF (~50 mL) in a round bottom flask. When a clear solution was obtained 1*H*-1,2,4-triazole (0.42 g, 6.02 mmol) was added and left stirring for 30 min. Next 2-(2-bromoethyl)isoindoline-1,3-dione (1.03 g, 4.05 mmol) was added to the mixture in three portions and left stirring for 3 h at r.t. By analysis of TLC no reaction had occurred; so more potassium *tert*-butoxide (0.87 g, 6.32 mmol) was added. The reaction mixture was heated at reflux for 20h affording a white suspension. Water (~150 mL) and diethyl ether (~50 mL) were added and both layers were separated. The aqueous layer was washed with 3 x 50 mL Et_2O. All organic layers were combined, dried over magnesium sulphate and filtered. The filtrate was concentrated under vacuum affording a pale yellow oil and a sample of it was submitted to ^{1}H NMR. No presence of product **1** was detected. The aqueous

layer was washed with DCM and/or EtOAc (4 x 50mL) and all organic layers were combined, dried over magnesium sulphate and concentrated. Again no presence of product **1** was seen.

3.2.2 – Synthesis of 2-[(2-chloroethyl)amino]-1-ferrocenylethanone (4)

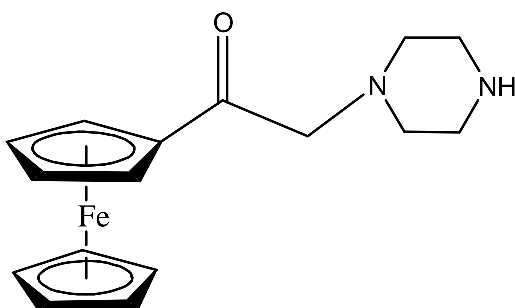


(not obtained)

Potassium *tert*-butoxide (0.22 g, 1.56 mmol) was dissolved in THF (~30 mL) in a round bottom flask. When a clear solution was obtained 2-chloroethanamine(0.09 g, 0.78 mmol) was added and left stirring for 15 min. Next 2-chloro-1-ferrocenylethanone (0.11 g, 0.42 mmol) was added to the reaction mixture slowly and left stirring for 3h at r.t., affording a red solution. The reaction was followed by TLC using 2:1 Hexane/DCM as eluent where only ferrocenyl starting material was detected. More ethanamine-2-chloride hydrochloride (0.09 g, 0.78 mmol) was added and left stirring at r.t. for 20h. TLC was performed in a different system (4:1 Hexane/DCM) and no presence of product was detected; so more ethanamine-2-chloride hydrochloride (0.18 g, 1.52 mmol) was added as well as potassium *tert*-butoxide (0.1 g, 0.76 mmol) and the reaction mixture was heated to reflux for 16h. Afterwards, the reaction mixture was filtered and the brown solid formed was washed with DCM (3x15 mL). The filtrate was concentrated affording a dark red oil and a sample was submitted to ^{1}H NMR. No presence of product **4** was seen.

3.2.3 – Synthesis of 1-ferrocenyl-2-(piperazin-1-yl)ethanone (5)

3.2.3.1 – First attempt



To a solution of piperazine (0.14 g, 1.59 mmol) in THF (30 mL), 2-chloro-1-ferrocenylethanone (0.10 g, 0.38 mmnol) was added slowly affording a red solution, which was stirred for 20 h. A

TLC was performed using 1:9 methanol/DCM as eluent and a new spot appeared on the silica plate. The reaction mixture was heated at reflux for 20h affording a dark red solution. Afterwards, the reaction mixture was filtered and the dark brown solid formed was washed with DCM (3x15 mL). The filtrate was concentrated affording a dark red oil and a sample was submitted to ^{1}H NMR.

The crude product was dissolved in DCM and applied on a preparative plate coated by silica-gel. The eluent used was 1:9 methanol/DCM. Three fractions were separated, concentrated and submitted to ^{1}H NMR. By analysis of NMR, the first fraction was 2-chloro-1-ferrocenylethanone, the second fraction corresponded to an impurity and third fraction afforded a red solid **5**. (0.09 g, 0.28 mmol, 74%). [For the characterization, see below].

3.2.3.2 – Second attempt

To a solution of piperazine (0.98 g, 11.38 mmol) in THF (100 mL), 2-chloro-1-ferrocenylethanone (1.00 g, 3.79 mmnol) was added slowly affording a red solution. The reaction mixture was heated at reflux for 20 h, whereupon the solution became darker. A TLC was performed using 1:9 methanol/DCM as eluent and a new spot appeared on the silica plate. The reaction mixture was filtered and the dark brown solid formed was washed with DCM (3x15 mL). The filtrate was concentrated affording a dark red oil and a sample was submitted to ^{1}H NMR.

The crude product was dissolved in DCM and purified by flash chromatography. The eluent used was 1:9 methanol/DCM. The third fraction afforded a red solid **5**. (0.83 g, 2.65 mmol, 70 %).

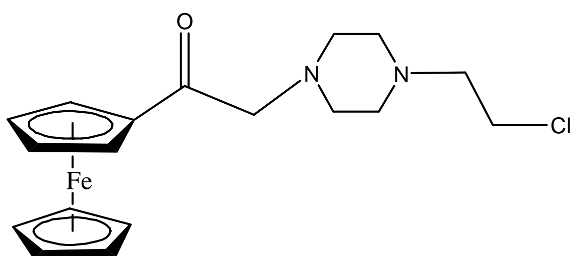
Mp 141-143 ºC

^{1}H NMR (δ, acetone-d_6, 400 MHz): 4.90 (t, J = 1.6 Hz, 2H, $C_{2,5}$ of -C(O)C_5H_4), 4.55 (t, J = 1.6 Hz, 2H, $C_{3,4}$ of C(O)C_5H_4), 4.23 (s, 5H, -C_5H_5), 3.54 (s, 2H, -C(O)CH_2-pip.), 3.04 (s, 1H, -N*H*), 2.80 (m, 4H, $C_{2,6}$ or $C_{3,5}$ of heterocycle), 2.50 (m, 4H, $C_{2,6}$ or $C_{3,5}$ of heterocycle).

^{13}C NMR (δ, acetone-d6, 400 MHz): 200.2 [C(O)], 78.4 (C1 of -C(O)C_5H_4), 71.8 (2C) [$C_{2,5}$ of -C(O)C_5H_4], 69.7 (5C) [-C_5H_5], 69.2 (2C) [$C_{3,4}$ of C(O)C_5H_4], 65.6 (-C(O)CH_2-pip.), 54.5 (2C)[$C_{3,5}$ of heterocycle], 45.8 (2C)[$C_{2,6}$ of heterocycle].

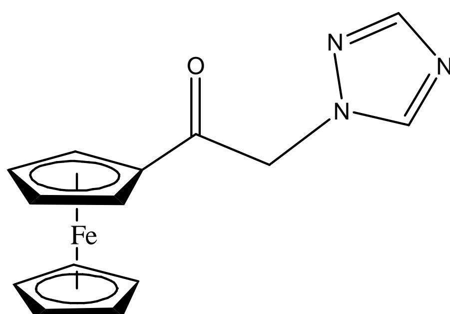
ESI-MS: *m*/z (%) = 313 (100) $[M+H]^+$

3.2.4 – Attempt of synthesis of 2-{4-(2-chloroethyl)piperazin-1-yl}-1-ferrocenyletanone (6)



1-ferrocenyl-2-(piperazin-1-yl)ethanone **(5)** (0.71 g, 2.30 mmol) was dissolved in THF (~40 mL) in a round bottom flask. When a red solution was obtained potassium *tert*-butoxide (0.64 g, 4.6 mmol) was added and left stirring for 15 min. Next, 1, 2-dichloroethane (2 mL, 25.38 mmol) was added to the mixture and left stirring for 18 h at r.t. The reaction was followed by TLC using as system 1:1 methanol/EtOAc and the plate was observed under U.V. light. No reaction occurred so more 1,2-dichloroethane was added (1 mL, 12.69 mmol). Again a TLC was done in the same system and a new spot appeared. Water (~120 mL) and DCM (~40 mL) was added and both layers were separated. The aqueous layer was washed with 3 x 50 mL DCM. All organic layers were combined, dried over magnesium sulphate and filtered. The filtrate was concentrated under vacuum affording a red solid and a sample of it was submitted to ^{1}HNMR. Despite having detected an apparent reaction product by TLC, only the starting material (product **5**) was recovered.

3.2.5 –Synthesis of 1-ferrocenyl-2-(1H-1,2,4-triazol-1-yl)ethanone (7)



To a solution of 1*H*-1,2,4-triazole (0.06 g, 0.88 mmol) and potassium *tert*-butoxide (0.06 g, 0.41 mmol) in THF (~ 30 mL), previously stirred for 30 min at r.t., was added slowly 2-chloro-1-ferrocenylethanone (0.11 g, 0.42 mmol). The reaction mixture was heated at reflux for 24 h affording a red suspension. The reaction mixture was filtered and the brown solid obtained was washed with DCM (3 x 15 mL). The filtrate was concentrated affording a red oil and a sample of it was submitted to ^{1}HNMR.

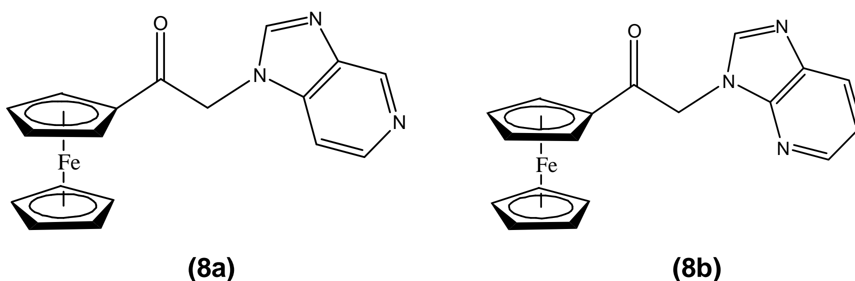
The crude product was dissolved in DCM and purified by preparative plate coated by silica-gel where the eluent used was EtOAc. Second fraction was separated and concentrated affording a red oil **(7)** (0.05 g, 0.15 mmol, 36 %).

Mp 128-130 ºC

^{1}H NMR (δ, acetone-d_6, 400 MHz): 8.42 (s, 1H, C_5 of heterocycle), 7.94 (s, 1H, C_3 of heterocycle), 5.64 (s, 2H, -C(O)CH_2-triazole), 4.97 (s, 2H, $C_{2,5}$ of –C(O)C_5H_4), 4.70 (s, 2H, $C_{3,4}$ of –C(O) C_5H_4), 4.39 (s, 5H, -C_5H_5),
^{13}C NMR (δ, acetone-d6, 400 MHz): 194.9 [C(O)], 151.4 (C_5 of heterocycle), 145.5 (C_1 of -C(O)CH_2-triazole), 145.4 (C_3 of heterocycle), 72.9 (2C)[$C_{3,4}$ of –C(O)C_5H_4)], 70.1 (5C)[-C_5H_5], 69.1 (2C)[$C_{2,5}$ of –C(O)C_5H_4], 55.1 (-C(O)CH_2-triazole).

ESI-MS: *m*/z (%) = 296 (91) $[M+H]^+$, 176 (100) $[M-H_2]^+$-FeC_5H_5

3.2.6 – Synthesis of 1-ferrocenyl-{2-[1*H*-imidazo(4,5*c*)pyridine]-1-yl}ethanone (8a) and 1-ferrocenyl-{2-[3*H*-imidazo(4,5*c*)pyridine]-1-yl}ethanone (8b)



(8a) **(8b)**

1*H*-Imidazo[4,5-*c*]pyridine (0.09 g, 0.79 mmol) was dissolved in THF (~30 mL) in a round bottom flask. When a clear solution was obtained potassium *tert*-butoxide (0.17 g, 1.19 mmol) was added and left stirring for 30 min. Next, 2-chloro-1-ferrocenylethanone (0.11 g, 0.41 mmol) was added slowly to the mixture and left stirring for 18 h at r.t. The reaction was followed by TLC using EtOAc as eluent and seen in the U.V. The reaction mixture was concentrated affording red oil.

The crude product was dissolved in DCM and purified by preparative plate coated by silica-gel where the system used was 1/20 methanol/DCM. Four fractions were separated and concentrated. The first highest fraction was starting material and the lower one last was an impurity. The second and third highest fractions had rf´s very close to one another but it was possible to separate them. These fractions afforded brown oils and corresponded to different isomers in proportion 1:1 of product **8** (isomer a: 0.04 g, 0.10 mmol, 25%, isomer b: 0.04 g, 0.10 mmol, 24%)

Isomer a:

Mp 146-150 ºC

^{1}H NMR (δ, acetone-d_6, 300 MHz): 8.93 (s, 1H, C_4 of heterocycle), 8.40 (d, J = 5.4 Hz, 1H, C_6 of heterocycle), 8.35 (s, 1H, C_2 of heterocycle), 7.68 (d, J = 5.4 Hz, 1H, C_7 of heterocycle), 5.85 (s,

2H, -C(O)CH_2-heterocycle), 5.05 (s, 2H, $C_{2,5}$ of –C(O)C_5H_4), 4.74 (s, 2H, $C_{3,4}$ of –C(O)C_5H_4), 4.02 (s, 5H, -C_5H_5).

^{13}C NMR (δ, acetone-d6, 400 MHz): 196.0 [C(O)], 149.4 (C_{7a}), 148.6 (C_2 of heterocycle), 142.2 (C_6 of heterocycle), 134.9 (C_4 of heterocycle), 133.9 (C_{3a}), 115.2 (C_7 of heterocycle), 77.0 (C_1 of –C(O)C_5H_4), 73.9 (2C)[$C_{3,4}$ of –C(O)C_5H_4], 71.0 (5C)[-C_5H_5], 70.1 (2C)[$C_{2,5}$ of –C(O)C_5H_4],)], 51.9 (-C(O)CH_2-heterocycle).

ESI-MS: *m*/z (%) = 346 (100) $[M+H]^+$

<u>Isomer b</u>:

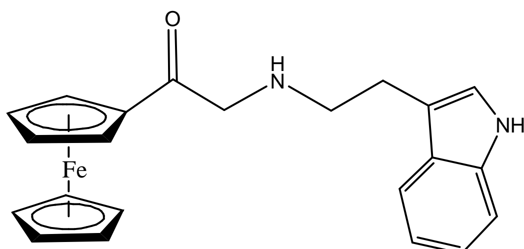
IR (thin-film, cm^{-1}): 3082 (wk, $\nu_{C\text{-}H}$, C-H of CH_{Ar}), 2960, 2895 (wk, $\nu_{C\text{-}H}$, C-H of CH_2), 1662 (s, $\nu_{C=O}$, -O=C-CH_2-), 1620 (s, $\nu_{C=N}$, $(C=N)_{Ar}$), 638 (s, $\nu_{C\text{-}Fe}$, -C_5H_5-Fe-C_4H_4-)

^{1}H NMR (δ, acetone-d_6, 300 MHz): 8.98 (s, 1H, C_7 of heterocycle), 8.38 (s, 1H, C_5 of heterocycle), 8.26 (s, 1H, C_2 of heterocycle), 7.54 (s, 1H, C_6 of heterocycle), 5.76 (s, 2H, -C(O)CH_2-heterocycle), 5.02 (s, 2H, $C_{2,5}$ of –C(O)C_5H_4), 4.72 (s, 2H, $C_{3,4}$ of –C(O)C_5H_4), 4.38 (s, 5H,-C_5H_5).

^{13}C NMR (δ, acetone-d6, 400 MHz): 196.8 [C(O)], 147.1 (C_2 or C_4 of heterocycle), 143.3 (C_2 or C_4 of heterocycle), 142.7 (C_6 of heterocycle), 142.0 (C_{3a} or C_{7a} of heterocycle), 140.4 (C_{3a} or C_{7a} of heterocycle), 106.5 (C_7 of heterocycle), 77.0 (C_1 of –C(O)C_5H_4), 74.1 (2C)[$C_{3,4}$ of –C(O)C_5H_4], 70.2 (5C)[-C_5H_5], 68.5 (2C)[$C_{2,5}$ of –C(O)C_5H_4],)] 51.5 (-C(O)CH_2-heterocycle).

ESI-MS: *m*/z (%) = 346 (80) $[M+H]^+$, 226 (100) $[M+2H\text{-}FeC_5H_5]^+$.

3.2.7 – Synthesis of 1-ferrocenyl-2-[(1H-indol-3-yl)ethanmino]etanone (9)



2-(1*H*-indol-3-yl)ethanamine (0.13 g, 0.78 mmol) was dissolved in THF (~30 mL) in a round bottom flask. When a pale suspension was obtained, potassium *tert*-butoxide (0.16 g, 1.12 mmol) was added and left stirring for 30 min. Next, 2-chloro-1-ferrocenylethanone (0.10 g, 0.38 mmol) was added slowly to the mixture and left stirring for 18 h at r.t. The reaction was followed by TLC using 1:10 metanol/DCM as eluent and seen in the U.V. The reaction mixture was concentrated affording a red oil.

The crude product was dissolved in DCM and purified by preparative plate coated by silica-gel where the system used was 1/10 methanol/DCM. Two fractions were separated and concentrated. The hightest Rf fraction was the starting material. The second fraction afforded a brown oil and corresponded to product **9**, which crystallised from acetone.

IR (thin-film, cm^{-1}): 3399 (s, ν_{N-H}, N-H of $–CH_2NH$-), 2922 (wk, ν_{C-H}, C-H of CH_2), 1657 (s, $\nu_{C=O}$, $-O=C-CH_2$-), 743 (s, ν_{C-Fe}, $-C_5H_5-Fe-C_4H_4$-)

^1H NMR (δ, acetone-d_6, 400 MHz): 10.13 (s, 1H, -N*H*), 7.64 (d, J = 7.6Hz, 1H, C_4 of heterocycle), 7.42 (d, J = 8.0 Hz, 1H, C_7 of heterocycle), 7.23 (s, 1H, C_2 of heterocycle), 7.13 (t, J = 7.6 Hz, 1H, C_6 of heterocycle), 7.05 (t, 1H, J=7.6 Hz, C_5 of heterocycle), 4,84(bs, 2H, $C_{2,5}$ of $–C(O)C_5H_4$), 4.55 (bs, 2H, $C_{3,4}$ of $–C(O)C_5H_4$), 4.23 (s, 5H,$-C_5H_5$), 3.93 (bs, 1H, -$CH_2NHCH_2CH_2$-), 3.00 (bs, 2H, -$NHCH_2CH_2$-), 2.84 (bs, 2H, -$NHCH_2CH_2$-), 1.98 (s, 2H, -$C(O)CH_2$-heterocycle).

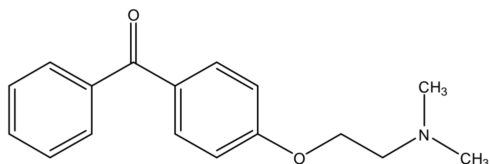
^{13}C NMR (δ, acetone-d6, 400 MHz): 196.8 [C(O)], 170.0 (C_3 of heterocycle), 136.9 (C_{7a} of heterocycle), 127.8 (C_{3a} of heterocycle), 122.4 (C_2 of heterocycle), 121.1 (C_6 of heterocycle), 118.5 (C_4 of heterocycle), 118.4 (C_5 of heterocycle), 111.3 (C_7 of heterocycle), 71.9 (2C)[$C_{3,4}$ of $–C(O)C_5H_4$], 69.7 (5C)[$-C_5H_5$], 68.8 (2C)[$C_{2,5}$ of $–C(O)C_5H_4$],)], 50.2 (-$NHCH_2CH_2$-), 46.0 (-$NHCH_2CH_2$), 20.0 (-$C(O)CH_2$-heterocycle).

3.3 - Synthesis of Tamoxifen analogues

All general procedures were as described in pages 71 and 72 (Section 3.1). In addition, gas chromatography with mass spectrometry detection was recorded using an Agilent 5975C Series apparatus with a 0.25µm HP5-MS column (15m x 0.25mm) x, 1uL injection split, injector at 280ºC, with a heating ramp of 50-300ºC at 20ºC/min using He as the carrier gas at constant flow rate of 1.5 mL/min. The 3-(trichlorogermyl)propanoyl chloride was bought from Gelest, and used without further purification.

3.3.1 - Synthesis of substituted 4-hydroxybenzophenone [47] [48]

3.3.1.1 – Synthesis of {4-[2-(Dimethylamino)ethoxy]phenyl}-(phenyl)methanone (1)



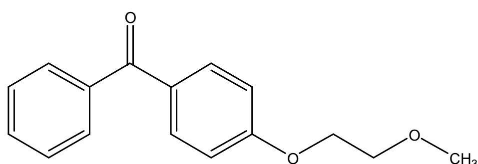
4-Hydroxybenzophenone (5.06 g, 25.53 mmol) was dissolved in anhydrous DMF (~120 mL) under argon atmosphere. When a yellow solution was obtained anhydrous potassium carbonate (10.25 g, 75.61 mmol) was added and left stirring for 15 min. Next 2-(dimethylamino)ethylchloride hydrochloride (8.46 g, 58.73 mmol) was added to the mixture and then heated up to 80ºC. The yellow suspension was refluxed for 72h. A solid was formed and the mixture was cooled to r.t. Water (~150 mL) and diethyl ether (~50 mL) was added and both layers were separated. The aqueous layer was washed with 3 x 50 mL Et_2O. All organic layers were combined, dried over magnesium sulphate and filtered. The filtrate was concentrated under vacuum affording yellow oil (4.84 g) and a sample of it was submitted to ^{1}HNMR.
The crude product was dissolved in DCM and purified in column chromatography (silica gel). At first, the eluent was composed only by ethyl acetate in order to flush all 4-hydroxybenzophenone from the column present in the oil and then the system was changed to 1:1 methanol/EtOAc to afford viscous oil which corresponded to the pure product **1** (1.19 g, 4.42 mmol, 17 %).

IR (KBr, cm^{-1}): 3010 (m, ν_{C-H}, C-H of CH_{Ar}), 2769 (s, ν_{C-H}, C-H of CH_2 and CH_3), 1645 (s, $\nu_{C=O}$, Ar(C=O)Ar), 1602 and 1505 [m, ν_{C-C},$(C=C)_{Ar}$], 1257 (s, ν_{C-0}, Ar-O-CCH_2), 1234 [m, ν_{C-N}, C-N(CH_3)].

^{1}H NMR (δ, $CDCl_3$,, 249,87 MHz): 7.78 (d, J = 6.2 Hz, 2H, C-2,6 of C_6H_5), 7.71 (d, J = 7.5, 2H, C-2,6 of C_6H_4O), 7.55-7.50 (m, 1H, C-4 of C_6H_5), 7.40-7.35 (m, 2 H, C-3,5 of C_6H_5), 6.95 (d, J= 10.0 Hz, 2H, C-3,5 of C_6H_4O), 4.09 [t, J= 5.0 Hz, 2H, $-OCH_2CH_2N(CH_3)_2$), 2.71 (t, J= 5.0 Hz, 2H, $-CH_2N(CH_3)_2$), 2.30 [s, 6H, $-N(CH_3)_2$].

$^{13}C\{^1H\}$ NMR (δ, $CDCl_3$, 249.87 MHz): 195.4 [*C*(O)], 162.5 (*C*O), 138.2(C–1 of C_6H_5), 132.5 (C-4 of C_6H_5), 131.5 (C-1 of C_6H_4O), 130.1 (2C)(C-2,6 of C_6H_4O), 129.7 (2C)(C-2,6 of C_6H_5), 128.2 (2C)(C-3,5 of C_6H_5), 114.1 (2C)(C-3,5 of C_6H_4O), 66.5 ($-OCH_2CH_2$), 58.0 ($CH_2N(CH_3)_2$), 45.8 (2C) [$N(CH_3)_2$].

3.3.1.2 – Synthesis of {4-[2-(methoxy)ethoxy]phenyl}(phenyl)methanone (2)



4-Hydroxybenzophenone (5.01 g, 25.27 mmol) was dissolved in anhydrous DMF (~60 mL) under argon atmosphere. When a yellow solution was obtained anhydrous potassium carbonate (12.13 g, 87.76 mmol) was added and left stirring for 15 min. After that 2-chloroethyl methyl ether (7 mL, 76.63 mmol) was added and anhydrous DMF (~60 mL) was also added to promote dissolution of the mixture, which was refluxed up to 80ºC. Afterwards the reaction was left reluxing for 96h affording a solid and the mixture was cooled down to r.t. Water (~150 mL) and diethyl ether (~50 mL) was added and both layers were separated. The aqueous layer was washed with 3 x 50 mL Et_2O. All organic layers were combined, dried over magnesium sulphate and filtered. A white solid crushed out of the filtrate. The suspension was concentrated *in vacuo* to yield directly the pure product **2** (5.26 g, 205.41 mmol, 81%), which was demonstrated by a sample submitted to ^{1}HNMR. On the aqueous layer that was separated diethyl ether was added and after a week white thin crystals were formed which led us to submit them for X-Ray analysis.

IR (KBr, cm^{-1}): 3005 (w, ν_{C-H}, C-H of CH_{Ar}), 2938 (m, ν_{C-H}, C-H of CH_2, CH_3 and $C-H_{Ar}$), 1652 (s, $\nu_{C=O}$, Ar(C=O)Ar), 1600 and 1508 [m, ν_{C-C},$(C=C)_{Ar}$], 1260 (s, ν_{C-0}, Ar-O-CCH_2).

^{1}H NMR (δ, $CDCl_3$,, 249.87 MHz): 7.84-7.81 (d, J = 2.5 Hz, 2H, C-2,6 of C_6H_5), 7.77-7.74 (d, J = 2.5 Hz, 2H, C-2,6 of C_6H_4O), 7.60-7.50 (m, 1H, C-4 of C_6H_5), 7.50-7.40 (m, 2H, C-3,5 of C_6H_5), 7.01-6.98 (d, J = 5.0 Hz, 2H, C-3,5 of C_6H_4O), 4.20-4.10 (m, 2H, $-OCH_2CH_2$), 3.75-3.70 (m, 2H, $-CH_2OCH_3$), 3.40 (s, 3H, $-OCH_3$).

$^{13}C\{^1H\}$ NMR (δ, $CDCl_3$, 249.87 MHz): 196.0 [*C*(O)], 162.8 (*C*O), 138.7 (C-1 of C_6H_5), 133.0 (2C)[C-2,6 of C_6H_5], 132.3 (C-4 of C_6H_5), 130.7 (2C)[C-2,6 of C_6H_4O], 128.6 (2C)[C-3,5 of C_6H_5), 114.5 (2C)[C-3,5 of C_6H_4O], 71.2 (-O*C*H_2CH_2), 67.9 (-*C*H_2OCH_3), 59.7 (O*C*H_3), 59.7 (-O*C*H_3).

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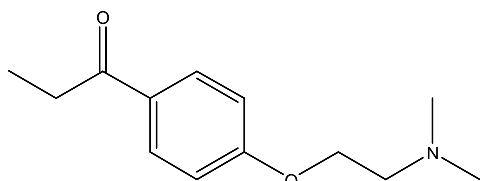
3.3.2 - Synthesis of substituted 4-hydroxypropiophenone [47] [48]

3.3.2.1 – First approach of synthesis of 1-{4-[2-(Dimethylamino)ethoxy]phenyl}propan-1-one (3)



1-(4-Hydroxyphenyl)propan-1-one (5.01 g, 33.37 mmol) was dissolved in anhydrous DMF (~60 mL) under argon atmosphere. When a yellow solution was obtained anhydrous potassium carbonate (16.11 g, 116.53 mmol) was added and the mixture was stirred for 15 min. Next 2-(dimethylamino)ethylchloride hydrochloride (14.49 g, 100.59 mmol) was added to the mixture and anyhdrous DMF (~60 mL) was also added to promote dissolution of the mixture, which was refluxed up to 80ºC. Afterwards the reaction was left refluxing for 72h affording a white solid, and the mixture was cooled down to r.t. Water (~150 mL) and diethyl ether (~50 mL) were added and both layers were separated. The aqueous layer was washed with 3 x 50 mL Et_2O. All organic layers were combined, dried over magnesium sulphate and filtered. The filtrate was concentrated under vacuum affording yellow oil (2.30 g) and a sample of it was submitted to ^{1}HNMR.

The crude product was dissolved in DCM and purified in column chromatography (silica gel). At first, the eluent was composed only by ethyl acetate in order to flush all 4-hydroxybenzophenone from the column present in the oil and then the system was changed to 1:1 methanol/DCM to afford white solid which corresponded to the pure product **3** (1.32 g, 5.98 mmol, 18 %).

IR (KBr, cm^{-1}): 2938 and 2789 (m, $\nu_{C\text{-}H}$, C-H of CH_2 and CH_3), 1674 (s, $\nu_{C=O}$, Ar(C=O)Ar), 1598 and 1506 [s, $\nu_{C\text{-}C}$,$(C=C)_{Ar}$], 1270 (m, $\nu_{C\text{-}0}$, Ar-O-CCH_2), 1227 [s, $\nu_{C\text{-}N}$, C-N(CH_3)].

^1H NMR (δ, $CDCl_3$,, 249.87 MHz): 7.96 (q, J= 2.5 Hz, 2H, C-3,5 of OC_6H_4), 6.97 (q, J= 2.5 Hz, 2H, C-2,6 of OC_6H_4), 4.15 (t, J=6.2 Hz, 2H,-OCH_2CH_2), 3.00 [q, J= 7.5 Hz, 2H, -C(O)CH_2CH_3], 2.78 [t, J=6.2 Hz, 2H, -$CH_2N(CH_3)_2$], 2.35 (s, 6H, -$N(CH_3)_2$), 1.24 [t, J=7.5 Hz, 3H, -C(O)CH_2CH_3).

$^{13}C\{^1H\}$ NMR (δ, $CDCl_3$, 249,87 MHz): 199.5 [*C*(O)], 162.6 (*C*O), 130.2 (2C)(C-3,5 of OC_6H_4), 130.1 (C-4 of OC_6H_4), 114.2 (2C)(C-2,6 of OC_6H_4), 66.2 (-OCH_2CH_2), 58.2 ($CH_2N(CH_3)_2$), 46.0 (2C)[-N($CH_3)_2$], 31.4 [C(O)CH_2CH_3), 8.7 [C(O)CH_2CH_3].

3.3.2.2 - Second approach of synthesis of (3)

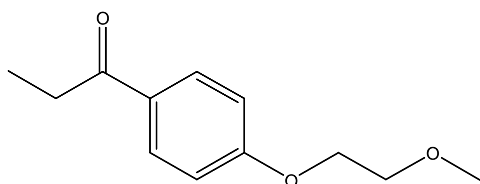
Another reaction was performed according to the same procedure described previously, although the solvent in this case was dry acetone. Under argon atmosphere anhydrous potassium carbonate (6.46 g, 46.71 mmol) was added to a yellow solution of 1-(4-Hydroxyphenyl)propan-1-one (2.02 g, 13.40 mmol) in anhydrous DMF (~60 mL) and left stirring for 15 min. After adding 2-(dimethylamino)ethylchloride hydrochloride (5.89 g, 40.90 mmol) the mixture was refluxed at 80ºC for 48h. After a solid was formed the mixture was cooled to r.t. and all the solvent was evaporated under vacuum before the extraction. The workup was performed accordingly to the previous reaction. Water (~120 mL) and DCM (~40 mL) were added to the mixture and organic and aqueous layer were separated. The aqueous layer was washed with 6 x 30 mL EtOAc, dried over magnesium sulphate and filtered under vacuum. The filtrate obtained was concentrated affording yellow oil and a sample was submitted to ^{1}HNMR.

The crude product was distillate in order to separate 4-hydroxypropiophenone from the product and at 125ºC it was possible to obtain a small fraction. Analysis of ^{1}HNMR of a sample of this fraction was similar to the crude.

From the crude product an amine salt was formed by adding Et_2O•HCl and different bases (NaH, NH4•OH and pyridine) were used to regenerate the product from the amine salt isolated although none of them worked.

In both attempts for purification of the crude product analysis of ^{1}HNMR demonstrated presence of some impurities and 4-hydroxypropiophenone within the product.

3.3.2.3 – Synthesis of 1-{4-[2-(Methoxy)ethoxy]phenyl}propan-1-one (4)



1-(4-Hydroxyphenyl)propan-1-one (5.08 g, 33.82 mmol) was dissolved in anhydrous DMF (~60 mL) under argon atmosphere. When a yellow solution was obtained anhydrous potassium carbonate (16.05 g, 116.14 mmol) was added and left stirring for 15 min. After that 2-chloroethyl methyl ether (9.2 mL, 100.72 mmol) was added and anhydrous DMF (~60 mL) was also added to promote dissolution of the mixture which was refluxed up to 80ºC. Afterwards the reaction was left refluxing for 48h affording a pale yellow solid and the mixture was cooled down to r.t. Water (~150 mL) and diethyl ether (~50 mL) were added and both layers were separated. The

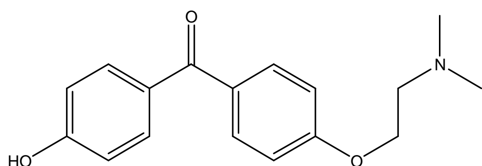
aqueous layer was washed with 3 x 50 mL Et_2O. All organic layers were combined, dried over magnesium sulphate and filtered. A white solid crushed out of the filtrate. The suspension was concentrated under vacuum to yield directly pure product **4** (6.1004 g, 27.58 mmol, 82%), which was demonstrated by a sample submitted to ^{1}HNMR.

IR (KBr, cm^{-1}): 3020 (m, ν_{C-H}, C-H of CH_{Ar}), 2879 and 2818 (m, ν_{C-H}, C-H of CH_2, CH_3 and C-H_{Ar}), 1682 (s, $\nu_{C=O}$, Ar(C=O)Ar), 1603 and 1511 [m, ν_{C-C},$(C=C)_{Ar}$], 1270 (s, $\nu_{C-0,}$ Ar-O-CCH_2).

^{1}H NMR (δ, $CDCl_{3,}$, 249.87 MHz): 7.97-7.91 (ddd, J = 2.5 Hz, 2H, C-3,5 of OC_6H_4), 6.99-6.93 (ddd, J = 2.5 Hz, 2H, C-2,6 of OC_6H_4), 4.20-4.15 (m, 2H, -OCH_2CH_2), 3.80-3.75 (m, 2H, -CH_2OCH_3), 3.46 (s, 3H, -OCH_3), 3.00 [q, J= 7.5 Hz, 3H, -C(O)CH_2CH_3] , 1.24 [t, J= 7.5 Hz, 3H, -C(O)CH_2CH_3].
^{13}C{^{1}H} NMR (δ, $CDCl_3$, 249.87 MHz): 220.5 [C(O)], 197.5 (CO), 161.5 (C-4 of OC_6H_4), 130.6 (2C)(C-3,5 of OC_6H_4), 114.6 (2C)(C-2,6 of OC_6H_4), 71.2 (CH2OCH_3), 67.8 (-OCH_2CH_2), 59.7 (-OCH3), 31.8 [-C(O)CH_2CH_3), 8.84 [C(O)CH_2CH_3].

3.3.3 - Synthesis of substituted bis(4-hydroxyphenyl)methanone

3.3.3.1 – Synthesis of {4-[2-(dimethylamino)ethoxy]phenyl}(4-hydroxyphenyl)methanone (5)



Bis(4-hydroxyphenyl)methanone (3.03 g, 14.12 mmol) was dissolved in anhydrous DMF (~40 mL) under argon atmosphere. When a yellow solution was obtained sodium hydride (1.08 g, 28.16 mmol) was added in small portions for 15 min and the solution was left refluxing for 1 h. Afterwards, a solution of 2-(dimethylamino)ethylchloride hydrochloride (2.12 g, 14.75 mmol) in anhydrous DMF (~40 mL) was added to the previous mixture and left refluxing for 1 h affording an orange solution.
The mixture was cooled to r.t. and quenched with saturated ammonium chloride solution (~125 mL) and extracted first with DCM (~4x30 mL) and then with EtOAc (2x30 mL). All organic layers were combined, dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording yellow oil and a sample was submitted to ^{1}HNMR.

The crude product was dissolved in EtOAc and purified in column chromatography (silica gel). The eluent was 1:1 methanol/DCM to afford off white solid which corresponded to the pure product **5** (0.24 g, 0.85 mmol, 6 %).

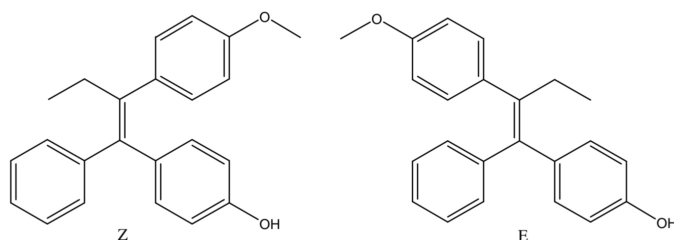
3.3.3.2 -Alternative route of synthesis of product 5 [54]

Bis(4-hydroxyphenyl)methanone (4.05 g, 18.91 mmol) was dissolved in anhydrous DMF (~40 mL) under argon atmosphere. When a yellow solution was obtained cesium carbonate (18.38 g, 56.42 mmol) was added and heated in an oil bath at 80ºC. To the yellow mixture 2-methylaminoethylchloride hydrogen chlorine (3.00 g, 20.83 mmol) was added in three portions for over 2 h and left refluxing for 65 h affording orange/brown solution.
The mixture was cooled to r.t., quenched with saturated ammonium chloride solution (~120 mL) and extracted with EtOAc (4x60 mL). All organic layers were combined, washed with Brine (4x60 mL), dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording yellow oil and a sample was submitted to ^{1}HNMR.
The crude product was dry loaded in EtOAc and purified in column chromatography (silica gel). The eluent was composed by 1:9 methanol/EtOAc to afford off white solid. After 1:9 DCM/hexane was added in order to dissolve remaining impurites which were not eliminated by column and filtered on a filter paper affording off white solid **5** (2.35 g, 8.24 mmol, 44 %).

IR (KBr, cm^{-1}): 3475 (s, $\nu_{O\text{-}H}$, O-H), 2957 (m, $\nu_{C\text{-}H}$, C-H of CH_{Ar}), 2567 and 2368 (m, $\nu_{C\text{-}H}$, C-H of CH_2 and CH_3), 1640 (s, $\nu_{C=O}$, Ar(C=O)Ar), 1602 and 1506 [m, $\nu_{C\text{-}C}$,$(C=C)_{Ar}$], 1257 (s, $\nu_{C\text{-}0}$, Ar-O-CCH_2), 1248 [m, $\nu_{C\text{-}N}$, C-$N(CH_3)$].

^{1}H NMR (δ, CD_3OD, 249,87 MHz): 7.78 (d, J = 10.0 Hz, 2H, C-2,6 of C_6H_4O), 7.71 (d, J = 10.0, 2H, C-2,6 of C_6H_5O), 7.11 (d, J = 10.0 Hz, 2H, C-3,5 of C_6H_4O), 6.91 (d, J = 10.0 Hz, 2 H, C-3,5 of C_6H_5O), 4.25 [t, J = 5.0 Hz, 2H, $-OCH_2CH_2N(CH_3)_2$], 2.87 (t, J= 5.0 Hz, 2H, $-CH_2N(CH_3)_2$), 2.30 [s, 6H, $-N(CH_3)_2$].
$^{13}C\{^{1}H\}$ NMR (δ, $CDCl_3$, 249.87 MHz): 163.3 [C(O)], 162.0 (C-4 of C_6H_5O), 161.0 (C–4 of C_6H_4O), 132.9 (2C)(C-2,6 of C_6H_5O), 132.6 (2C)(C-2,6 of C_6H_4O), 115.8 (2C)(C-3,5 of C_6H_5O), 114.3 (2C)(C-3,5 of C_6H_4O), 66.0 ($CH_2N(CH_3)_2$), 58.4 ($-OCH_2CH_2$), 48.7 (2C) [$N(CH_3)_2$].

3.3.4 - Synthesis of (E)(Z)-4-[2-(4-methoxyphenyl)-1-phenylbut-1-enyl]phenol (6) [51]



Zinc powder (4.09 g, 62.55 mmol) was dissolved in anhydrous THF (~40 mL) under argon atmosphere. The grey solution was cooled down to 0ºC and a solution of titanium tetrachloride in toluene (1M, 31 mL, 30.50 mmol) was added slowly. The mixture was left stirring at r.t. for 30min. The reaction mixture was heated up to 80ºC and left refluxing for 2.5h affording a black suspension. Afterwards, the suspension was allowed to cool to 0ºC and anhydrous pyridine (1.2 mL, 15.20 mmol) was added. In a separate schlenk flask a solution of 4-hydroxybenzophenone (1.22 g, 6.15 mmol) and 1-(4-methoxyphenyl)propan-1-one (1.10 g, 6.70 mmol) in anhydrous THF (~40 mL) was stirred for 15 min. This solution was transferred slowly to the black suspension which contained the titanium solution. After addition, the mixture was heated at reflux for 48 h.

10% of potassium carbonate aqueous solution (~120 mL) and Et_2O (~40 mL) was added to the reaction mixture, where both layers were mixed and the organic layer was decanted off from the aqueous layer. Et_2O (3x 40 mL) was used to extract the remaining reaction mixture from the aqueous layer. All organic phases were combined, dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording bright yellow oil and a sample was submitted to ^{1}HNMR.

The crude product was dissolved in DCM and purified in column chromatography (silica gel) using 1:10 methanol/toluene as eluent. First fraction was concentrated and it was pure product 6 (t_r = 12.41 min) by TLC but ^{1}HNMR showed that it contained a sub-product. A sample of this fraction was submitted to GC-MS which indicated the nature of the sub-product [(E)(Z)-3,4-bis(4-methoxyphenyl)hex-3-ene] (t_r = 10.17 min) **(7)**. GC-EI-MS: t_r: 10.17 min; m/z (%): 296 (100) (M^+), 267 (28) (M^+-CH_2CH_3), 252 (7) (M^+-CH_2CH_3,-CH_3), 221 (5) (M^+-CH_2CH_3,-CH_3,-OCH_3), tr: 12.41 min; 330 (100) (M^+), 314 (22) (M^+-OH or M^+-CH_3), 257 (5) (M+-OCH_3, -OH, -CH_2CH_3), 207 (12) (M^+-OC_6H_5 or M^+-$OCH_3C_6H_5$), 121 (8) (M+-$C_6H_5CH_2C_6H_5$) The side product obtained was a result of homo-coupling reaction of 1-(4-methoxyphenyl)propan-1-one.

Attempt to purify first fraction by quenching it to concentrated aqueous solution of hydroxypotassium and extracting this solution with DCM and/or EtO_2 enabled the separation of the product from the sub-product.

3.3.4.1 - Alternative route of synthesis of product 6 [53]

Under argon atmosphere a solution of titanium tetrachloride in toluene (1M, 31 mL, 30.71 mmol) was added slowly to a stirred suspension of zinc powder (4.45 g, 68.03 mmol) in anhydrous THF (~60 mL) at 0ºC. Afterwards the mixture was allowed to warm to r.t. for 30min. The resulting blue/green mixture was heated up to 80º C and left refluxing for 2h affording a black suspension, which was cooled to r.t. In a separate schlenk flask a solution of 4-hydroxybenzophenone (1.00 g, 5.06 mmol) and 1-(4-methoxyphenyl)propan-1-one (2.70 g, 16.43 mmol) in anhydrous THF (~40 mL) was stirred for 15 min. This solution was transferred slowly to the cooled black titanium suspension in THF at 0ºC and after refluxing again the mixture for 2h in the dark, the suspension was kept stirring for 16h.

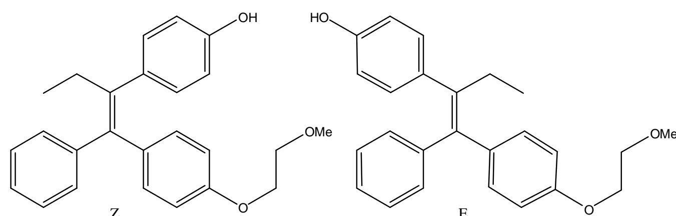
The reaction mixture was quenched with 10% of potassium carbonate aqueous solution (~50 mL) and extracted with EtOAc (3 x 50 mL). All organic phases were combined, washed with Brine 3 x 30 mL, dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording pale yellow oil and a sample was submitted to ^{1}HNMR.

The crude product was dry loaded in MeOH and purified in column chromatography (silica gel), which afforded three fractions. The eluent was composed by 3:7 EtOAc/Hexane. The second fraction was concentrated yielding an incolor oil which corresponded to the mixture 1:1 of product **6** (1.2742 g, 3.86 mmol, 77 %).

IR (KBr, cm^{-1}): 3378 (s, ν_{O-H}, O-H), 2957 (m, ν_{C-H}, C-H of CH_{Ar}), 1608 and 1504 [s, ν_{C-C},$(C=C)_{Ar}$], 1244 (s, $\nu_{C-0,}$ Ar-O-CCH_2).

^{1}H NMR (δ, $CDCl_{3,}$, 249,87 MHz): 7.35-6.45 (m, 13H, C_6H_5, C_6H_4OH, $C_6H_4OCH_3$), 4.86, 4.64 (s, 1H, O*H*), 3.76, 3.75 (s, 3H, -OCH_3), 2.50-2.40 (m, 2H, -CH_2CH_3), 1.00-0.90 (m, 3H, -CH_2CH_3)
^{13}C NMR (δ, $CDCl_{3,}$, 249,87 MHz): 158.2 (C-1 of $OCH_3C_6H_4$), 154.6 (C-1 of OC_6H_4), 141.8 (C-1 of C_6H_5), 139.0 (C-4 of $OCH_3C_6H_4$), 138.1 (C-4 of OC_6H_4), 136.9 (C-C_6H_5), 136.1 (C-CH_2CH_3), 132.1, 130.9, 130.8, 130.7, 129.5, 128.1, 127.4, 126.5, 125.5, 114.9, 114.3, 113.3, 113.2 (13C) (OC_6H_5, C_6H_5, $OCH_3C_6H_4$), 55.1 (-OCH3), 28.9 (-CH_2CH_3), 14.1 (-CH_2CH_3).

3.3.5 - Synthesis of (E)(Z)-4-{1-[4-(2-methoxyethoxy)phenyl]-1-phenylbut-1-en-2-yl}phenol (8) [51]



Zinc powder (4.36 g, 66.70 mmol) was dissolved in anhydrous THF (~40 mL) under argon atmosphere. The grey solution was cooled to 0ºC and a solution of titanium tetrachloride in toluene (1M, 33 mL, 33.33 mmol) was added slowly. The mixture was left stirring at r.t. for 30 min. The reaction mixture was heated up to 80ºC and left refluxing for 2.5h affording a black suspension. Afterwards, the suspension was allowed to cool to 0ºC and anhydrous pyridine (1.5 mL, 16.64 mmol) was added. In a separate schlenk flask a solution of [4-(2-methoxyethoxy) phenyl]phenylmethanone **(2)** (2.07 g, 8.08 mmol) and 1-(4-hydroxyphenyl)propan-1-one (1.02 g, 6.79 mmol) in anhydrous THF (~40 mL) was stirred for 15 min. This solution was transferred slowly to the black suspension which contained the titanium solution. After addition, the mixture was heated at reflux for 63h.

10% of potassium carbonate aqueous solution (~80 mL) and DCM (~40 mL) was added to the reaction mixture, where both layers were mixed and the organic layer was decanted off from the aqueous layer. DCM (3x 40 mL) was used to extract the remaining reaction mixture from the aqueous layer. All organic phases were combined, dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording bright yellow oil and a sample was submitted to ^{1}HNMR.

A small sample of crude product (100 mg) was purified on a preparative plate coated by silica-gel. The eluent used was 3:7 EtOAc/DCM. Three fractions were separated and concentrated but none of them were pure by ^{1}H NMR. So another small sample of crude product (50 mg) was purified on a preparative plate in a different system (1:5 EtOAc/Toluene). Seven fractions were separated with a small separation between them. All fractions were concentrated and submitted to ^{1}H NMR. Second fraction corresponded to a white pure solid mixture of 2:1 of isomers **8**.

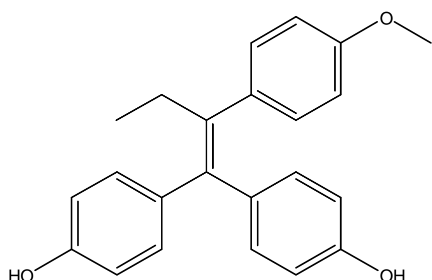
^{1}H NMR (δ, $CDCl_3$,, 249,87 MHz): 7.30-7.10 (m, 5H, $C_6\mathit{H}_5$), 7.05-7.00 (m, 2H, C-3,5 of $OC_6\mathit{H}_4$), 6.90-6.80 (m, 2H, C-2,6 of $OC_6\mathit{H}_5$), 6.70-6.65 (m, 2H, C-2,6 of $OC_6\mathit{H}_4$), 6.55-6.50 (m, 2H, C-3,5 of $OC_6\mathit{H}_5$), 5.20, 4.95 (s, 1H, O*H*), 4.17-4.09 (m,2H,-$OC\mathit{H}_2CH_2$) 3.84-3.78 (m, 2H, -$C\mathit{H}_2OCH_3$), 3.37 (s, 3H, -$OC\mathit{H}_3$), 2.40, 2.35 (q, J = 7.5 Hz, 2H, -$C\mathit{H}_2CH_3$), 1.30, 0.90 (t, J = 7.5 Hz, 3H, -$CH_2C\mathit{H}_3$).

^{13}C NMR (δ, $CDCl_3$,, 249,87 MHz): 157.2 (C-1 of $O\mathit{C}_6H_5$), 155.8 (C-1 of $O\mathit{C}_6H_4$), 149.2 (C-1 of C_6H_5), 140.8 (C-4 of $O\mathit{C}_6H_5$), 139.1 (C-4 of $O\mathit{C}_6H_4$), 135.2 (*C*-C_6H_5), 135.2 (*C*-CH_2CH_3), 132.3,

131.0, 129.8, 128.5, 127.0, 121.3, 113.9 (13C) (OC_6H_5, C_6H_5, OC_6H_4), 71.4 (-CH_2OCH_3), 67.3 (-OCH_2CH_2), 59.6 (-OCH3), 19.9 (-CH_2CH_3), 14.1 (-CH_2CH_3).

Two different system of solvents (pentane and petroleum ether) were used for recrystallisation of product from the crude was found unsuccessful.
The protection of the OH group of the product that was present in the crude was tried in order to improve the separation between the fractions obtained in a column chromatography (silica gel) was not possible by using acetic anhydride in pyridine using 4-dimethylaminopyridine (DMAP) as catalyst.

3.3.6 - Synthesis of [4-(1-hydroxyphenyl)-2-(4-methoxyphenyl)but-1-enyl]phenol (9) [53]



Under argon atmosphere a solution of titanium tetrachloride in toluene (1M, 29 mL, 28.42 mmol) was added slowly to a stirred suspension of zinc powder (4.15 g, 62.92 mmol) in anhydrous THF (~40 mL) at 0ºC. Afterwards the mixture was allowed to warm to r.t. for 30min. The resulting blue/grey mixture was heated up to 80º C and left refluxing for 2h affording a black suspension, which was cooled to r.t. In a separate schlenk flask a solution of 4,4´dihydroxybenzophenone (1.00 g, 4.67 mmol) and 1-(4-methoxyphenyl)propan-1-one (2.56 g, 15.60 mmol) in anhydrous THF (~60 mL) was stirred for 15 min. This solution was transferred slowly to the cooled black titanium suspension in THF at 0ºC and after refluxing again the mixture for 2h in the dark, the suspension was kept stirring for 14h.
The reaction mixture was quenched with 10% of potassium carbonate aqueous solution (~50 mL) and extracted with EtOAc (3 x 50 mL). All organic phases were combined, washed with Brine 3 x 30 mL, dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording orange-red oil and a sample was submitted to ^{1}HNMR.
The crude product was dissolved in DCM and purified in column chromatography (silica gel). At first, the eluent was composed by 2:8 EtOAc/Hexane to remove the first five fractions and then the system was changed to 100% of EtOAc to flush from the column the sixth fraction. All fractions were concentrated and submitted to ^{1}HNMR.
The first three fractions corresponded to the product **7**. In the first fraction it was possible to isolate isomer E affording colourless oil. In the second fraction a mixture of isomers E and Z plus the product **9** was obtained. In the third fraction a mixture of two isomers of product **7** was

isolated. By analysis of ^1H NMR of these three fractions it was possible to characterize 7, ratio 1:1 (0.86 g, 2.89 mmol, 28%).

IR (KBr, cm^{-1}): 3545 (s, ν_{O-H}, O-H), 2967, 2885 (m, ν_{C-H}, C-H of CH_{Ar}), 1605 and 1508 [m, ν_{C-C},$(C=C)_{Ar}$], 1259 (s, ν_{C-0}, Ar-O-CCH_2).

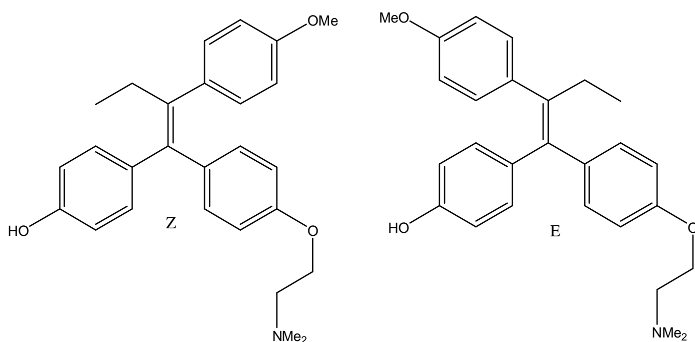
^1H NMR (δ, $CDCl_3$,, 249,87 MHz): 7.20-7.10 (m, 4 H, C-3,5 of $H_3COC_6H_4$), 6.90-6.80 (m, 4H, C-2,6 of $H_3COC_6H_4$), 3.81 (s, 1H, -OCH_3), 2,56, 2.36 (q, J = 7.5 Hz, 2H, -CH_2CH_3), 0.98, 0.69 (t, J = 7.5 Hz, 3H, -CH_2CH_3).
^{13}C NMR (δ, $CDCl_3$,, 249,87 MHz): 212.3 (2C) [C-1 of $H_3COC_6H_4$], 158.6 (2C) [C-4 of $H_3COC_6H_4$], 134.0 (2C) [C-CH_2CH_3], 130.9 (2C) [C-3,5 of $H_3COC_6H_4$], 113.9 (2C) [C-2,6 of $H_3COC_6H_4$], 55.7 (-OCH3), 32,2, 30,2 (-CH_2CH_3), 9.5,9.2 (-CH_2CH_3).

The sixth fraction corresponded to the product pretended and contained EtOAc, which was removed first by adding DCM followed by EtO_2 affording a red solid **9** (1.28 g, 3.70 mmol, 79%).

IR (KBr, cm^{-1}): 3545 (s, ν_{O-H}, O-H), 2954 (m, ν_{C-H}, C-H of CH_{Ar}), 1609 and 1507 [m, ν_{C-C},$(C=C)_{Ar}$], 1240 (s, ν_{C-0}, Ar-O-CCH_2).

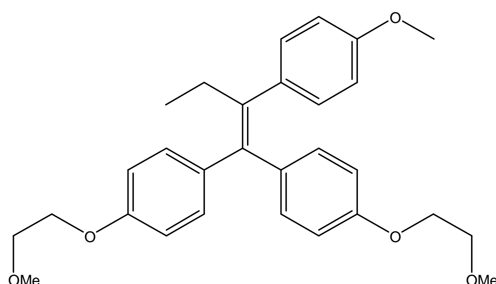
^1H NMR (δ, $CDCl_3$,, 249,87 MHz): 7.10-7.00 (m, 4H, C-3,5 of OC_6H_5), 6.83 (d, 2H, C-3,5 of $H_3COC_6H_4$), 6.80-6.75 (m, 4H, C-2,6 of OC_6H_5), 6.50-6.45 (m, 2H, C-2,6 of $H_3COC_6H_4$), 5.20 (s, 1H, OH), 4.95 (s, 1H, OH), 3.76 (s, 3H, -OCH_3), 2.49 (q, J = 7.5 Hz, 2H, -CH_2CH_3), 0.95 (t, J = 7.5 Hz, 3H, -CH_2CH_3).
^{13}C NMR (δ, $CDCl_3$,, 249,87 MHz): 158.1 (C-1 of $H_3COC_6H_4$), 154.7, 153,8 (2C) [C-1 of OC_6H_4], 140.9 (C-4 of $H_3COC_6H_4$), 137.6 [$C(OC_6H_4)_2$], 137.0, 136.6 (2C) [C-4 of OC_6H_5), 135.2 (C-CH_2CH_3), 132,6 (4C) [C-3,5 of OC_6H_5], 131.2 (4C) [C-2,6 of OC_6H_5], 115.4 (2C) [C-3,5 of $H_3COC_6H_4$], 114.7 (2C) [C-2,6 of $H_3COC_6H_4$], 21.9 (-CH_2CH_3), 14.6 (-CH_2CH_3).

3.3.7 - Synthesis of (E)(Z)-4-{1-[4-(2-(dimethylamino)ethoxy)phenyl]-2-(4-methoxyphenyl) but-1-enyl}phenol (10) [54]



[4-(1-hydroxyphenyl)-2-(4-methoxyphenyl)but-1-enyl]phenol (**9**) (1.23 g, 3.55 mmol) was dissolved in anhydrous DMF (~17 mL) under argon atmosphere. When a red solution was obtained cesium carbonate (2.83 g, 8.70 mmol) was added and heated in an oil bath at 80ºC. To the red mixture a solution of 2-(dimethylamino)ethylchloride hydrochloride (1.91 g, 13.23 mmol) in DMF (~10 mL) was added and the reaction mixture was left refluxing for 1.5h.
The mixture was cooled to r.t., quenched with saturated ammonium chloride solution (~30 mL) and extracted with EtOAc (4x15 mL). All organic layers were combined, washed with brine (4x15 mL), dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording orange-red oil and a sample was submitted to ^{1}HNMR. The crude product obtained contained starting material (**9)** and some impurites which were removed by column chromatography (silica-gel) 2:8 EtOAc/Hexane (0.59 g, 1.70 mmol). Some product (**9**) was not possible to recover.

3.3.8 - Synthesis of 1-(2-methoxyethoxy)-4-{1-[4-(2-methoxyethoxy)phenyl]-2-(4-methoxyphenyl)but-1-enyl}benzene (11) [54]

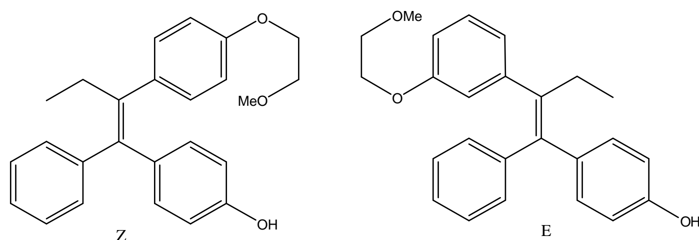


[4-(1-hydroxyphenyl)-2-(4-methoxyphenyl)but-1-enyl]phenol (**9**) (0.59 g, 1.70 mmol) was dissolved in anhydrous DMF (~10 mL) under argon atmosphere. When a red solution was obtained cesium carbonate (1.67 g, 5.10 mmol) was added and heated in an oil bath at 80ºC. To the red mixture 2-chloroethyl methyl ether (170 µl, 1.84 mmol) was added in three portions for over 2 h and left refluxing for 20h. The reaction was followed by TLC and it seemed that no reaction occurred so more 2-chloroethyl methyl ether (1 mL, 10.95 mmol) was added in two portions and the mixture was left refluxing for 2h affording orange/brown solution.
The mixture was cooled to r.t., quenched with saturated ammonium chloride solution (~30 mL) and extracted with EtOAc (4x15 mL). All organic layers were combined, washed with brine (4x15 mL), dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording pale yellow oil and a sample was submitted to ^{1}HNMR.
The crude product was dissolved in DCM and purified in column chromatography (silica gel). The eluent was composed at first to be by 3:7 EtOAc/Hexane to remove the first two fractions and then the column was flushed with EtOAc in order to obtain fraction 3.
First fraction was concentrated affording viscous yellow oil **11** (0.6282 g, 1.36 mmol, 80%).

IR (cm^{-1}): 2928 (m, ν_{C-H}, C-H of CH_{Ar}), 1606 and 1508 [m, ν_{C-C},$(C=C)_{Ar}$], 1243 (s, ν_{C-0}, Ar-O-CCH_2).

^{1}H NMR (δ, $CDCl_3$,, 249,87 MHz): 7.15-7.10 (m, 2H, C-3,5 of $H_3COC_6H_4$), 7.00-6.90 (m, 4H, C-3,5 of -$CH_2OC_6H_4$), 6.80-6.70 (m, 4H, C-2,6 of -$CH_2OC_6H_4$), 6.60-6.55 (m, 2H, C-2,6 of $H_3OC_6H_4$), 4.10-4.15, 4.00-3.50 (m, 4H, -OCH_2CH_2), 3.80-3.75, 3.70-3.65 (m, 2H, -CH_2OCH_3), 3.46, 3.41 (s, 3H, -OCH_3), 2.49 (q, J = 7.5 Hz, 2H, -CH_2CH_3), 0.95 (t, J = 7.5 Hz, 3H, -CH_2CH_3).
^{13}C NMR (δ, $CDCl_3$,, 249,87 MHz): 158.1 (C-1 of $H_3COC_6H_4$), 157.8, 156,9 (2C) [C-1 of –$CH_2OC_6H_4$], 140.9 (C-4 of $H_3COC_6H_4$), 137.1, 136.7 (2C) [C-4 of CH $_2OC_6H_4$], 135.1 [C-($CH_2OC_6H_4)_2$], 132.3 (2C) [C-3,5 of –$H_3COC_6H_4$], 131.2, 130.2 (4C) [C-3,5 of -$CH_2OC_6H_4$], 114.5 (2C) [C-2,6 of –$H_3COC_6H_4$], 113.8,113.7 (4H) [C-2,6 of -$CH_2OC_6H_4$], 71.5, 71.4 (2C) [-CH_2OCH3], 67.2, 66.9 (2C) [-OCH_2CH_2], 21.1(-CH_2CH_3), 14.2 (-CH_2CH_3).

3.3.9 - Synthesis of (E)(Z)-4-{2-[3-(2-methoxyethoxy)phenyl]-1-phenylbut-1-enyl}phenol (12) [51]



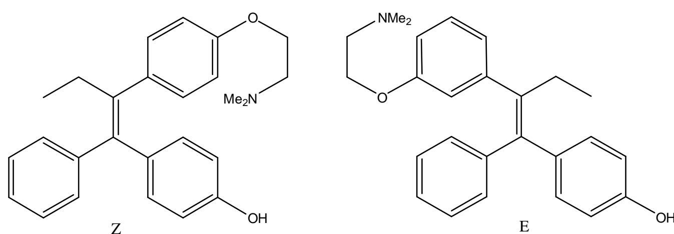
Zinc powder (3.19 g, 66.70 mmol) was dissolved in anhydrous THF (~60 mL) under argon atmosphere. The grey solution was cooled to 0ºC and a solution of titanium tetrachloride in toluene (1M, 25 mL, 25.00 mmol) was added slowly. The mixture was left stirring at r.t. for 30 min. The reaction mixture was heated up to 80ºC and left refluxing for 2.5h affording a black suspension. Afterwards, the suspension was allowed to cool to 0ºC and anhydrous pyridine (1.5 mL, 14.40 mmol) was added. In a separate schlenk flask a solution of 4-hydroxybenzophenone (1.15 g, 5.80 mmol) and 1-[4-(2-Methoxy-ethoxy)-phenyl]-ethanone **(4)** (1.02 g, 4.88 mmol) in anhydrous THF (~25 mL) was stirred for 15 min. This solution was transferred slowly to the black suspension which contained the titanium solution. After addition, the mixture was heated at reflux for 38h.
10% of potassium carbonate aqueous solution (~40 mL) and DCM (~40 mL) was added to the reaction mixture, where both layers were mixed and the organic layer was decanted off from the aqueous layer. DCM (3x 35 mL) was used to extract the remaining product from the aqueous layer. All organic phases were combined, dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording pale yellow oil and a sample was submitted to ^{1}HNMR.

The crude product was dissolved in DCM and purified in column chromatography (silica gel) using 3:7 EtOAc/Hexane as eluent. Four fractions were separated from the column, concentrated and analysed by NMR. The second fraction corresponded to the product pretended and contained EtOAc, which was stripped by adding DCM affording a light grey solid mixture of isomers (2:1) **12** (1.18 g, 3.16 mmol, 65%).

IR (KBr, cm^{-1}): 3475 (s, $\nu_{O\text{-}H}$, O-H), 2957 (m, $\nu_{C\text{-}H}$, C-H of CH_{Ar}), 1602 and 1506 [m, $\nu_{C\text{-}C}$,$(C{=}C)_{Ar}$], 1248 (s, $\nu_{C\text{-}0}$, Ar-O-CCH_2).

^{1}H NMR (δ, $CDCl_3$,, 249,87 MHz): 7.35-6.40 (m, 13H, C_6H_5, OC_6H_5, -$CH_2OC_6H_4$), 5.19, 4.89 (s, 1H, O*H*), 4.10-4.00 (m, 2H, -OCH_2CH_2), 3.75-3.70 (m, 2H, -CH_2OCH_3), 3.52, 3.51 (s, 3H, -OC*H*3), 2.50-2.40 (m, 2H, -CH_2CH_3), 0.95-0.90 (m, 3H, -CH_2CH_3).
^{13}C NMR (δ, $CDCl_3$,, 249,87 MHz): 157.4, 156.7 (C-1 of OC_6H_5), 154.7, 153.8 (C-1 of -$OCH_2C_6H_4$), 144.4, 144.0 (C-1 of C_6H_5), 141.8, 141.2 (C-4 of –$OCH_2C_6H_5$), 138.3, 138.3 (C-4 of OC_6H_4), 136.7, 136.2 (*C*-C_6H_5), 135.2 (*C*-CH_2CH_3), 132.5, 131.3, 131.2, 131.1, 129.9, 128.5, 127.8, 126.9, 125.9, 115.4, 114.7, 114.3, 113.9 (13C) (OC_6H_5, C_6H_5, $OCH_2C_6H_4$), 71.5 (-CH_2OCH_3), 67.4 (-OCH_2CH_2), 59.6 (-OCH_3), 28.9 (-CH_2CH_3), 14.1 (-CH_2CH_3).

3.3.10 Synthesis of (E)(Z)-4-{2-[4-(2-(dimethylamino)ethoxy)phenyl]-1-phenylbut-1-enyl}phenol (13) [51]

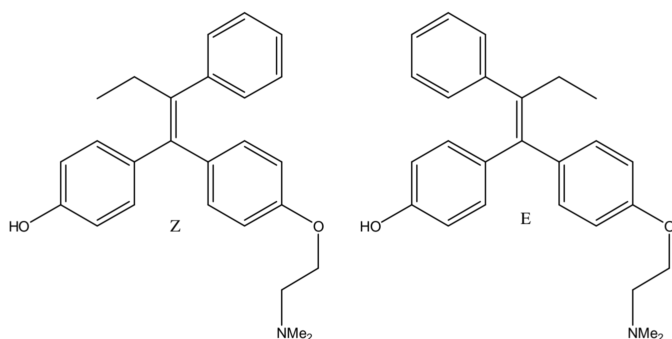


Zinc powder (2.99 g, 45.79 mmol) was dissolved in anhydrous THF (~60 mL) under argon atmosphere. The grey solution was cooled to 0ºC and a solution of titanium tetrachloride in toluene (1M, 23 mL, 23 mmol) was added slowly. The mixture was left stirring at r.t. for 30 min. The reaction mixture was heated up to 80ºC and left refluxing for 2.5h affording a black suspension. Afterwards, the suspension was allowed to cool to 0ºC and anhydrous pyridine (0.95 mL, 11.38 mmol) was added. In a separate schlenk flask a solution of 4-hydroxybenzophenone (1.12 g, 5.65 mmol) and 1-[4-(2-Dimethylamino-ethoxy)-phenyl]-ethanone **(3)** (1.00 g, 4.55 mmol) in anhydrous THF (~25 mL) was stirred for 15 min. This solution was transferred slowly to the black suspension which contained the titanium solution. After addition, the mixture was heated at reflux for 66h.

10% of potassium carbonate aqueous solution (~40 mL) and DCM (~40 mL) was added to the reaction mixture, where both layers were mixed and the organic layer was decanted off from the aqueous layer. DCM (3x 35 mL) was used to extract the remaining reaction mixture from the aqueous layer. All organic phases were combined, dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording pale yellow oil and a sample was submitted to ^{1}HNMR.

The crude product was dissolved in EtOAc and purified in column chromatography (silica gel) using 1:9 MeOH/EtOAc as eluent. Three fractions were separated but none believed to be the product.

3.3.11 Synthesis of (E)(Z)-4-{1-[4-(2-(dimethylamino)ethoxy)phenyl]-2-phenylbut-1-enyl}phenol (14) [55]



Under argon atmosphere a solution of potassium *tert*-butoxide (1.98 g, 17.68 mmol) and propylbenzene (2.3 mL, 16.46 mmol) in anhydrous THF (~10 mL) was stirred for 15 min in a schlenk flask. Afterwards the solution was cooled to 0ºC and *n*-butylitium solution in hexane (1.6 M, 10 mL, 16.00 mmol) was added followed by TMEDA (5.1 mL, 33.79 mmol), which was previously purified by distillation after drying over hydroxypotassium. After addition of n-butylitium solution a dark red suspension was obtained and left stirring for 30 min at r.t. In a separate schlenk flask a solution of (4-(2-(dimethylamino)ethoxy)phenyl)(4-hydroxyphenyl)methanone (**5**) (0.80 g, 2.81 mmol) in anhydrous THF (~20 mL) was stirred for 15 min. This solution was transferred slowly to the dark red suspension which contained the propylbenzene anion at -70ºC. The red solution was left stirring for 3h affording a yellow solution. Afterwards, aqueous solution of H_2SO_4 (32%, 6 mL) was added slowly to the reaction mixture to promote the elimination step of the carbinol formed affording a white suspension, which was left stirring for 16h. Though the TLC demonstrated no reaction the mixture was quenched with saturated ammounium chloride solution (60 mL) in order to increase pH of the mixture to 7. Then, the mixture was extracted with DCM (4x30 mL). All organic layers were combined, dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording brown oil. Analysis of a sample of crude product by NMR proved the presence of the

two starting materials (propylbenzene and (4-(2-(dimethylamino)ethoxy)phenyl)(4-hydroxyphenyl)methanone (**5**).

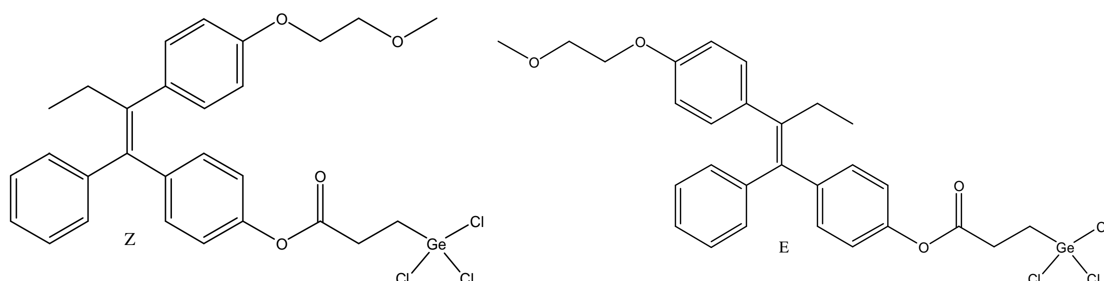
The crude product was purified by column chromatography and two fractions were isolated using 1:9 MeOH/EtOAc as eluent. The first fraction corresponded to propylbenzene and the second fraction was product **5**.

3.3.11.1 - Alternative route of synthesis of product 14

Under argon atmosphere a solution of potassium t-butoxide (1.98 g, 17.68 mmol) and propylbenzene (2.3 mL, 16.46 mmol) in anhydrous THF (~15 mL) was stirred for 15 min in a schlenk flask. Afterwards the solution was cooled to 0ºC and n-butylitium solution in hexane (1.6 M, 12 mL, 19.20 mmol) was added followed by TMEDA (5.1 mL, 33.79 mmol), which was previously purified by distillation using palets of hydroxypotassium that retained all the impurites present in TMEDA. After addition of n-butylitium solution a dark red suspension was obtained and left stirring for 30 min at r.t. In a separate schlenk flask a solution of (4-(2-(dimethylamino)ethoxy)phenyl)(4-hydroxyphenyl)methanone **(5)** (0.80 g, 2.81 mmol) in anhydrous THF (~25 mL) was stirred for 15 min. This solution was transferred slowly to the dark red suspension which contained the propylbenzene anion at -70ºC. The red solution was left stirring for 20h affording a yellow solution.

The crude product was quenched with saturated ammounium chloride solution (60 mL) in order to increase pH of the mixture to 7. Then, the mixture was extracted with DCM (4x30 mL). All organic layers were combined, dried over magnesium sulphate and filtered. The filtrate obtained was concentrated affording brown oil. Analysis of a sample of crude product by NMR proved the presence of the two starting materials

3.3.12 - Synthesis of (E)(Z)-4-{2-[4-(2-methoxyethoxy)phenyl]-1-phenylbut-1-enyl}phenyl 3-(trichlorogermyl)propanoate (15) [43]



Under argon atmosphere a purple solution of the mixture of isomers of product **12** (0.25 g, 0.67 mmol) and triethlyamine (103 µl, 0.73 mmol) in anhydrous DCM (~10 mL) was added dropwise to a solution of 3-(trichlorogermyl)propanoyl chloride (104 µl, 0.67 mmol) in anhydrous DCM (~5 mL) at 0ºC. The reaction mixture was left stirring for 1h at r.t. and heated for 4h at 38ºC affording pale white suspension. The mixture was then filtered and the filtrate was concentrated.

The residue was dissolved in THF and cooled to 0ºC affording a white solid. The suspension was filtered and the solid was washed with cold THF. A sample of white solid was analysed by ^{1}HNMR, which demonstrated that the solid was triethylamine.
The filtrate that remained from the filtration was concentrated affording pale yellow-greenish oil, which corresponded to the pure product **15** (0.23 g, 0.38 mmol, 57%).

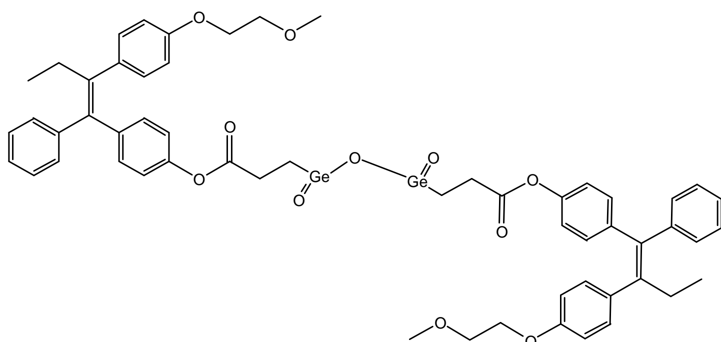
IR (KBr, cm^{-1}): 2963 (m, ν_{C-H}, C-H of CH_{Ar}), 1607 and 1508 [m, ν_{C-C},$(C=C)_{Ar}$], 1239 (s, $\nu_{C-0,}$ Ar-O-CCH_2), 586 (m, ν_{Ge-C}, Ge-C).

^{1}H NMR (δ, $CDCl_{3,}$, 249,87 MHz): 7.35-6.40 (m, 13H, C_6H_5, OC_6H_5, -$CH_2OC_6H_4$), 4.10-4.00 (m, 2H, -OCH_2CH_2), 3.75-3.70 (m, 2H, -CH_2OCH_3), 3.52, 3.51 (s, 3H, -OC*H*3), 2.98, 2.88 (t, J = 7-5 Hz, 2H, -$OCH_2CH_2GeCl_3$), 2.36 (t, J = 7.5 Hz, 2H, -$OCH_2CH_2GeCl_3$), 2.50-2.40 (m, 2H, -CH_2CH_3), 0.95-0.90 (m, 3H, -CH_2CH_3).

^{13}C NMR (δ, $CDCl_{3,}$, 249,87 MHz): 157.4, 156.7 (C-1 of OC_6H_5), 154.7, 153.8 (C-1 of -$OCH_2C_6H_4$), 144.4, 144.0 (C-1 of C_6H_5), 141.8, 141.2 (C-4 of –$OCH_2C_6H_5$), 138.3, 138.3 (C-4 of OC_6H_4), 136.7, 136.2 (C-C_6H_5), 135.2 (C-CH_2CH_3), 132.5, 131.3, 131.2, 131.1, 129.9, 128.5, 127.8, 126.9, 125.9, 115.4, 114.7, 114.3, 113.9 (13C) (OC_6H_5, C_6H_5, $OCH_2C_6H_4$), 71.5 (-CH_2OCH_3), 67.4 (-OCH_2CH_2), 59.6 (-OCH_3), 28.9 (-CH_2CH_3), 27.6 (-$OCH_2CH_2GeCl_3$), 25.6 (-$OCH_2CH_2GeCl_3$), 13.7 (-CH_2CH_3).

ESI-MS: *m*/z (%) = 537 [MH^+-H_2O], 577 $[M+Na]^+$, 397 [M-$Ge(OH)_3$-CH_3OH] (~ 95) (with M corresponding to compound 17)

3.3.12.1 - Attempt of synthesis of $[Ge(CH_2)_2C(O)OC_{25}H_{18}O_2)]O_3$ (16) [43]



Product **15** (35 mg, 0.06 mmol) was dissolved in H_2O (2 mL) and added dropwise in acetone (~10 mL) at r.t. The reaction mixture was left stirring for 3h affording a clear solution. Some acetone was evaporated and afterwards the mixture was kept in the freezer for 41h. The remaining acetone was evaporated and a sample of the crude product was submitted to NMR which demonstrated that no reaction had occurred.

Bibliography

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