Attempted routes towards the synthesis of fluorinated analogues of ornithine as potential inhibitors of ornithine decarboxylase

by

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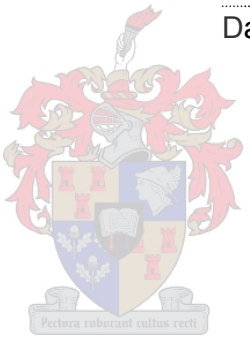
Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

Signature ..... Date .....



Summary

Human African Trypanosomiasis (HAT) is a disease that threatens more then 60 million men, woman and children in Africa. It is known that the inhibition of the enzyme, ornithine decarboxylase (ODC) leads to cell arrest and subsequent death of *Trypanosoma brucei*, the parasite that causes the disease. The fluorinated ornithine analogue, DFMO (difluoromethylornithine or eflornithine) is a known inhibitor of ODC. Although various syntheses for DFMO exist they have some practical drawbacks which prevent the cost effective production of this compound as a drug for HAT treatment. This work focuses on the synthetic preparation of the fluorinated ornithine analogue DFMO as well as the fluorinated ornithine analogues 2-MFMO, 3-fluoro-ornithine and 3,3-difluoro-ornithine.

Our chosen synthetic methodology focused on the introduction of the fluorine functionality using a simpler, safer and more convenient method than current direct fluorination techniques, or those that rely on the use of CFCs. Instead we decided to develop and optimise a fluorodehydroxylation method based on the transformation of hydroxylated ornithine analogues. The fluorodehydroxylation method substitutes a hydroxyl group to the corresponding fluorine and can also be used to transform an aldehyde or ketone to the corresponding difluoro group.

Application of this fluorination method requires the synthesis of appropriate hydroxylated precursors to be transformed to the corresponding fluorine analogues. The first synthetic section of this thesis discusses the synthesis of such precursors for the synthesis of both the α-methyl fluorinated analogues, DFMO/2-MFMO, and the analogue fluorinated on position three, namely 3-fluoro-ornithine and 3,3-difluoro-ornithine. The last synthetic section discusses the subsequent development of the fluorodehydroxylation method on the hydroxylated ornithine analogues as well as the results obtained from these reactions.

Opsomming

Afrika slaapsiekte (trypanosomiase) bedreig meer as 60 milljoen mans, vrouens en kinders in Afrika. Dit is bewys dat inhibisie van die ensiem ornitien dekarboksilase (ODC) lei tot die dood van *Trypanosoma brucei*, die parasiet wat die siekte veroorsaak. Die gefluorineerde ornitienanaloog DFMO (difluorometielornitien of eflornitien) is ’n bevestigde inhibitor van ODC. Alhoewel verskeie sinteses van DFMO bestaan, is daar verskeie praktiese probleme wat met hulle geassosieer word, en hulle as sulks ongeskik maak as ’n koste effektiewe vervaardigingsmetode vir die bereiding van DFMO as slaapsiektebehandeling. Hierdie studie fokus op die ontwikkeling en optimiseering van ’n vereenvoudige sintetiese bereiding van DFMO, asook die gefluorineerde ornitienanaloë 2-MFMO, 3-fluoro-ornitien and 3,3-difluoro-ornitien.

Ons gekose sintetiese metodologie is gefokus op die toevoeging van ’n fluoor funksionaliteit wat makliker, veiliger en meer geskik is as die metodes wat tans gebruik word, naamlik direkte fluorineringstegnieke of metodes wat gebruik maak van CFCs (chloorfluookoolwaterstowwe). In plaas van hierdie tegnieke het ons besluit om ’n metode te ontwikkel wat gebaseer is op die fluorodehidroksielasie van gehidroksileerde ornitienanaloë. Fluorodehidroksielasie is ’n metode waardeur ’n alkoholgroep na die ooreenstemmende fluoor verander kan word. Dieselfde metode kan ook gebruik word om ’n aldehied of ’n ketoon na die ooreenstemmende difluoorgroep te verander.

Die toepassing van hierdie fluorineringsmetode vereis die sintese van geskikte gehidroksileerde voorlopermolekules wat na die gefluorineerde analoë omgeskakel kan word. Eerstens word die sintese van die voorlopermolekules vir die bereiding van die α-metiel gefluorineerde analoë, d.i. DFMO/2-MFMO, asook die analoë wat gefluorineer is op posisie drie, naamlik 3-fluoro-ornitien and 3,3-difluoro-ornitien, bespreek. Tweedens word die ontwikkeling van die fluorodehidroksielasie metode wat die gehidroksileerde voorlopermolekules omskakel bespreek, asook die resultate van hierdie reaksies.

[24]A man can do nothing better than to eat and drink and find satisfaction in his work. This too, I see, is from the hand of God, [25]for without him, who can eat or find enjoyment?

Ecclesiastes 2:24-25

For the LORD gives wisdom,
and from his mouth come knowledge and understanding.

Proverbs 2:6

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In the last two years I've had some very good and some very trying times whilst doing my masters. A masters takes a lot of work and without the people in my life things would have been even harder. I am very glad to have finished it and still love what I'm doing.

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Table of contents

List of Abbreviations

Δ-MFMO	α-(fluoromethyl)dehydroornithine
2-MFMO	2-monofluoromethylornithine
CFC	Chlorofluorocarbon
^{13}C NMR	Carbon 13 Nuclear Magnetic Resonance Spectroscopy
Cys	Cysteine
CTACl	Cetyltrimethylammonium chloride
CTABr	Cetyltrimethylammonium bromide
DAST	(Diethylamino)sulfur trifluoride
Deoxo-Fluor	Bis(2-methoxyethyl)amino-sulfur trifluoride
DFMO	Difluoromethylornithine
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DNA	Deoxyribonucleic acid
ESI-MS	Electronspray Ionization Mass Spectroscopy
EtOAc	Ethyl acetate
Et_3N	Triethylamine
EtOH	Ethanol
eq	Equivalent
FDA	Food and Drug Administration
FGI	Functional group interconversion
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
HAT	Human African trypanosomiasis
HMPT	Hexamethyl phosphorous-triamide
I-PDGF	platelet-derived growth factor
K_i	Inhibition constant
LDA	Lithium diisopropylamide
Lys	Lysine
m-CPBA	*meta*-Chloroperbenzoic acid
MeOH	Methanol
ODC	Ornithine decarboxylase

PLP	Pyridoxal 5'-phosphate
RNA	Ribonucleic acid
Select-Fluor	(1-chloromethyl-4-fluoro–1,4-Diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)
t-BOC	*tert*-butoxycarbonyl
t-butyl	*tert* butyl
TLC	Thin layer chromatography
TPAP	Tetrapropylammonium perruthenate
YAR	2-chloro-1,1,2-trifluorethyldiethylamine

Pectora roborant cultus recti

Chapter 1

African sleeping sickness, a Background

1.1 African sleeping sickness

1.1.1 History of the disease

African trypanosomiasis (African sleeping sickness) is a disease that affects people in 36 countries in sub-Saharan Africa, of which 22 are among the least developed countries in the world. This includes countries such as Mozambique, Angola, Nigeria, Uganda, Zambia and the Democratic Republic of the Congo. The disease is considered to be "old"; it was already known to slave traders who rejected Africans with characteristic swollen glands. Human African trypanosomiasis (HAT) has had three severe epidemics, the first from 1896 to 1906 in the Congo and Uganda basin, the second in 1920 and the third from the early 1970s into the 21st century. In 1960, the disease was practically eradicated, an effort that took almost 40 years, starting just after 1920. HAT is particularly troublesome since it is concentrated in rural areas, which is often isolated from screening centres. This in part contributed to the fact that the countries in Africa did not – and some still do not – have the resources to control and monitor the disease. According to the World Health Organization, sleeping sickness is currently a threat to more than 60 million men, woman and children. Of these, only 3 to 4 million people are actively screened. In 1999, the screening process identified 45 000 cases of sleeping sickness, thus indicating an infection rate of ~1-2%. Current estimates of the total number of people infected with the disease range from 300 000 to 500 000, with newly infected cases in the region of 25 000 a year, with a staggering 55 000 deaths a year. Consequently sleeping sickness has a profound economic impact on the countries involved, primarily due to the

crippling of their labour force. The result is an obvious decrease in economic productivity, hampering the development of entire regions. This is exemplified by the 20-40% decrease in cattle production in areas that are epidemic, amounting to losses in the region of US$2.7 billion a year (*1*).

1.1.2 Causes and vectors of the disease

African sleeping sickness is caused by two protozoan parasites. Both strains are morphologically similar, but differ in immediate virulence. *Trypanosoma brucei gambiense*, also known as the Gambian sleeping sickness, causes a chronic disease with symptoms taking months or even years after infection to appear. Initial symptoms include high fever, swollen lymph nodes as well as a swollen face and hands, weakness and headache, joint pain and itching. When the parasite crosses the blood brain barrier the disease moves into the second stage characterized by neurological impairments such as slurred speech, progressive confusion, difficulty with waking and seizures. Patients become sleepy all the time and can't seem to stay awake until they fall into a coma and die. Symptoms of *Trypanosoma brucei rhodesiense*, a more virulent strain, are similar except that the initial symptoms appear three to four weeks after infection, followed by rapid onset of the second neurological stage. The vector of the disease is the tsetse fly from the genus *Glossina*. Tsetse flies are primarily found in habitats that are warm, shady and humid. The *gambiense* strain is found near cultivated human habitats, such as pools and lowland forests and as a result threatens a larger part of the population to be infected by this particular strain. The *rhodesiense* strains are found mainly in savannah woodland areas and occupy a smaller area compared to the *gambiense* strain and thus infect less people.

1.2 Drugs for the treatment of African sleeping sickness

There are no drugs or vaccines that can protect humans from contracting sleeping sickness. However, a few drugs are available for the treatment of the disease, but most of them are old and need to be replaced by safer, more effective and more

affordable drugs. The main reason why pharmaceutical companies have not developed a more effective drug to combat the disease is due to the fact that it is a 3rd world disease, which results in a lack of revenue for these companies. Another reason that no new drugs have been developed in the last decade is that basic research on the organism and the pathogenesis of the disease has been largely under funded. This is the case with most tropical diseases (*2*). The current anti-trypanosomal agents can broadly be divided into three groups: the amidine and non-organo-arsenate drugs, the organo-arsenates, and the fluorinated ornithine analogues.

1.2.1 The amidine and non-organo-arsenate drugs

Three drugs, namely suramin, pentamidine and berenil fall into the category of amidine and non organo-arsenate drugs that are being used to treat African sleeping sickness. None of these drugs can cross the blood brain barrier and is only effective in the treatment of African sleeping sickness before the onset of the neurological stage of the disease. Suramin and pentamidine are used for the treatment of African sleeping sickness in humans whereas berenil is used as a veterinary drug. Their individual structures are represented in Figure 1.1

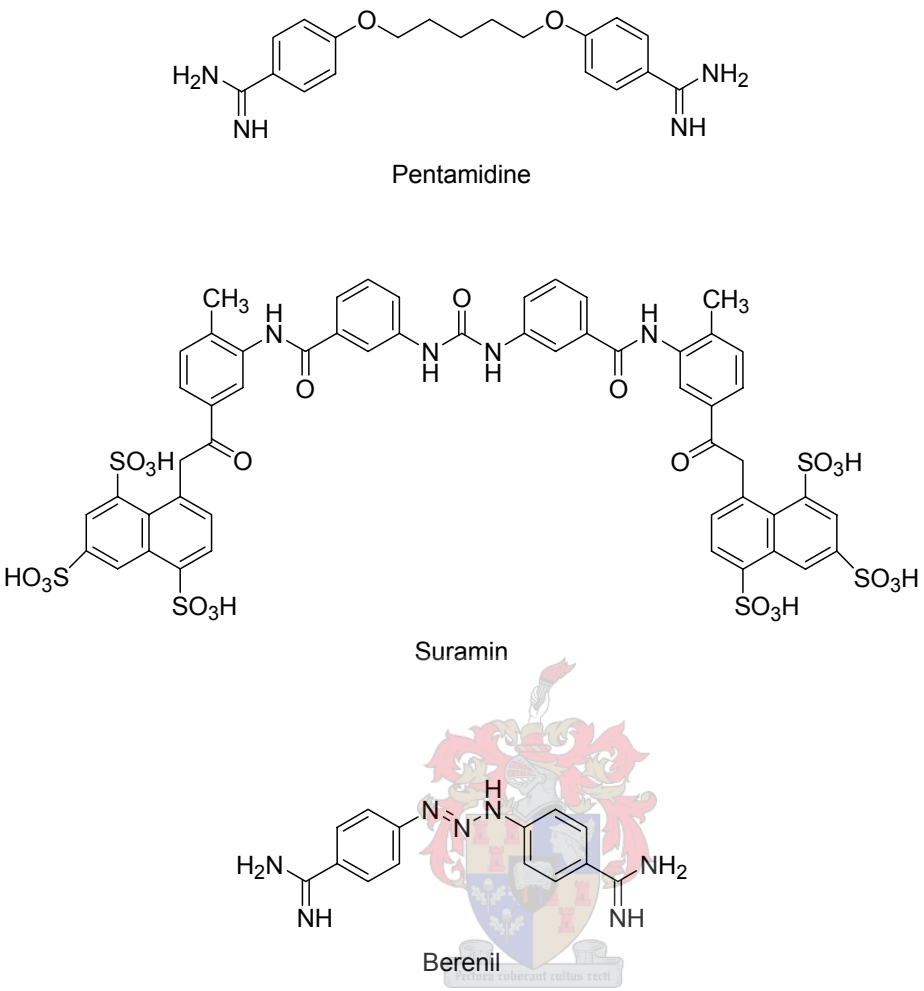


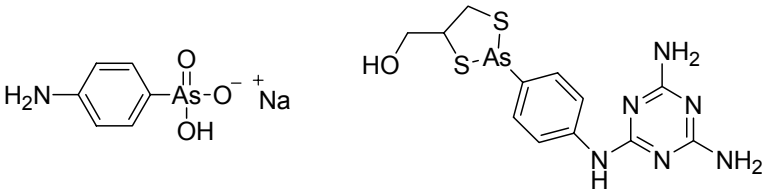
Figure 1.1: Drugs used for early stage treatment of the disease: pentamidine, suramin and the veterinary drug berenil

Suramin is intravenously administered in increasing doses over a period of a month. Suramin's effectiveness is explained by the ability shown by trypanosomes to absorb the drug into their cells. It is postulated that suramin binds to a host of enzymes such as dihydrofolate reductase, thymidine kinase and glycolytic enzymes by electrostatic interaction resulting in the inhibition of the enzymes and subsequent death of the parasite. It was also shown by Hosang that suramin inhibits the binding of platelet-derived growth factor (I-PDGF) to cell membranes inhibiting proliferation of the parasite (*3, 4*).

Pentamidine is typically given in doses of 4mg/kg per body weight seven to ten times daily or every other day intramuscularly. The exact mechanism of its anti-protozoal activity is not known. However, as it is a di-cation it is postulated to interact with intracellular polyanions in the parasite's kinoblast disrupting their structure, leading to cell degradation. It is also postulated that due to the millimolar concentrations of pentamidine in cells it could inhibit multiple cellular targets resulting in the parasite's death (*3*). Like pentamidine, the drug berenil is also di-amidine. However, it has been shown that berenil binds to RNA and DNA and as such is carcinogenic to humans. This is the reason why berenil is only used in the treatment of animals, most commonly cattle (*5*).

1.2.2 The organo-arsenic compounds

The first anti-trypanosomal agent that was found to cross the blood brain barrier is the organo-arsenic compound atoxyl (Figure 1.2). Atoxyl was originally used to treat skin diseases, but was found to be active against African sleeping sickness. However, it was found not to be very effective, as it required the administration of large doses as well as causing blindness in hundreds of patients. Subsequently the drug melarsoprol was developed as a replacement to atoxyl.



Atoxyl Melarsoprol

Figure 1.2: The arsene based drugs: atoxyl and melarsoprol

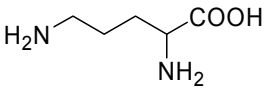
Melarsoprol is also an organo-arsenic compound with the ability to cross the blood brain barrier, and is mostly used to treat the later stages of the disease. Melarsoprol leads to the rapid lysis of trypanosomes although its mode of action is not fully understood. The inhibition of glycolytic enzymes, leading to the shut down of glycolysis and subsequent lysis of the cells, are postulated to be the most likely

mode of action. It is also postulated that the drug is a non-specific inhibitor of different enzymes while also forming adducts with intracellular thiols, such as trypanothione and dihydrolipolate. Adducts to these thiols explain the drug's serious side effects, most notably arsenic encephalopathy. Arsenic encephalopathy results in paralysis, brain damage or even death and between 5-10% of patients are affected. In spite of these serious side effects, melarsoprol is still the only available drug that can treat the later stages of both strains of the disease (*3, 6*).

1.2.3 The fluorinated ornithine analogues

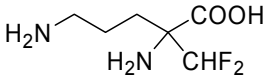
Eflornithine or difluoromethylornithine (DFMO, **1.2**) was originally pursued as a drug for the treatment of cancer (*7, 8*). The clinical trials were unsuccessful and it was subsequently discovered that DFMO was active against African sleeping sickness. In 1990 the FDA approved the drug for use as an anti-trypanosomal agent. DFMO is an ornithine (**1.1**) analogue with a difluoromethyl group added to the α-carbon. DFMO can cross the blood brain barrier and is used in the treatment of the neurological stage of the disease. The drug is particularly effective against the *gambiense* strain, even in the later stages of the disease, and has come to be known as the resurrection drug for its remarkable ability to revive comatose patients. In contrast to its effectiveness against the *gambiense* strain DFMO is not very effective against the *rhodesiense* strain. DFMO has some side effects but these are not as serious as the previously mentioned drugs. The side effects include nausea, vomiting, diarrhoea, convulsions, bone marrow toxicity and hearing loss, although the latter two are only seen in patients that receive large doses over prolonged periods of time. The hearing loss is a reversible side-effect as hearing returns when treatment is stopped. DFMO is an expensive drug that is not widely available on the market. Most of the current DFMO stock comes in the form of donations from pharmaceutical companies. A recent example is the collaboration between the World Health Organization, Bristol-Meyers-Squibb, Dow Chemical, Akron Manufacturing and the French-German company Aventis. In March 2001 they reached an agreement to produce and donate 60 000 doses of

eflornithine a year to help combat the disease. DFMO is normally used as a first line course of treatment for HAT, but due to its high cost of synthesis and low availability its role is primarily restricted to use as a combination drug with melarsoprol, especially in cases where drug resistance to melarsoprol is prominent.



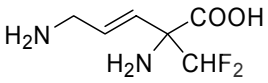
Ornithine

1.1



DFMO (Elfornithine)

1.2



Δ-MFMO

1.3

Figure 1.3: Structures of ornithine (1.1), DFMO (1.2) and Δ-MFMO (1.3)

In regards to mode of action, DFMO acts as an inhibitor of the ornithine decarboxylase (ODC) enzyme of the trypanosome parasite. The specifics of this mechanism of action will be discussed later in the chapter.

Another fluorinated ornithine analogue that also acts as an inhibitor of ODC is α-(fluoromethyl) dehydroornithine (Δ-MFMO, **1.3**). It has been shown to be an even more effective inhibitor of ODC with a K_I of 2.7 μM compared to a value of 39 μM for mammalian DFMO (*9, 10*). However, Δ-MFMO has never been used in the clinical treatment of HAT.

1.3 ODC as an anti-trypanosomal target

1.3.1 Background

ODC is a pyridoxal 5'-phosphate (PLP) -dependant enzyme that catalyses the first committed step in the polyamine biosynthesis, which is the transformation of L-

ornithine to the diamine putrescine (*11, 12*). This pathway occurs in all eukaryotes. Polyamines are ubiquitous to all cells and have been shown to be important for cell growth and differentiation. Thus inhibition of ODC would lead to arrest of cell growth and subsequent death.

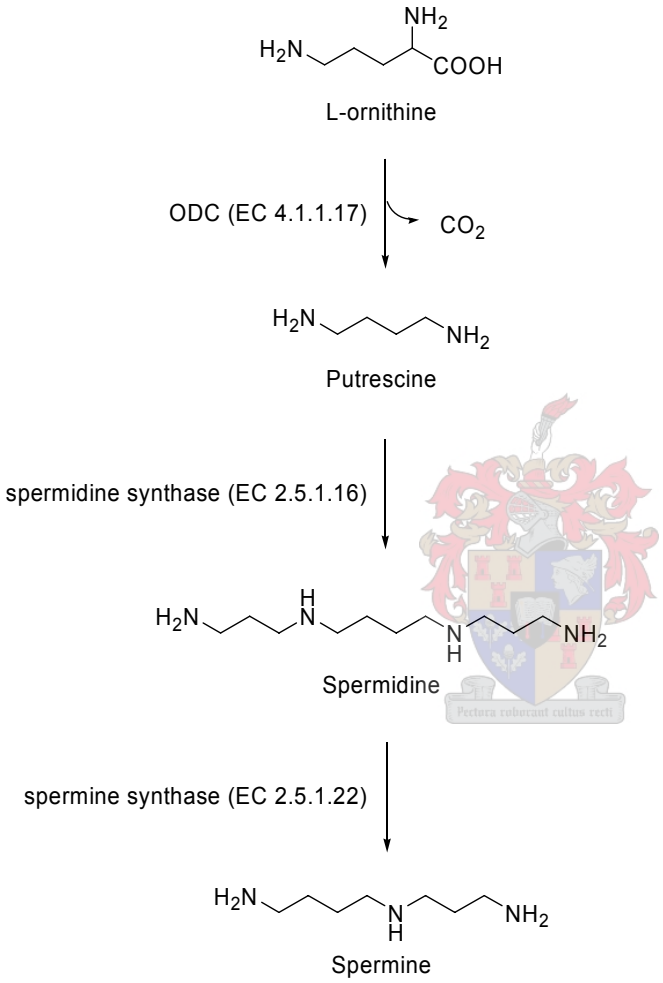


Figure 1.4: Polyamine metabolism from L-ornithine to spermine (*13*)

Ornithine is a non-canonical amino acid existing as two enantiomers, L- and D-ornithine. ODC is stereo-selective for L-ornithine, with a selectivity of 1 in 10 000. However, selective inhibition of the parasite ODC enzyme is not achieved through differential binding, but is believed to arise through metabolic differences between host and parasite. There are three such differences. First, the human ODC enzyme has a high turnover rate when compared with the parasite ODC, in other

words a short half-life. Thus, inhibition of the enzyme does not have a lasting effect in humans due to a constant recycling of the inhibited enzyme in the cells. This is one of the reasons why DFMO failed as anti-cancer drug in human cells. Second, the parasite is dependant on the formation of the polyamine spermidine. Spermidine is a polyamine metabolite in the polyamine metabolism from ornithine as shown in figure 1.4 above. Thus inhibition of ODC results in a shortage of spermidine. The parasite uses spermidine to synthesise the novel cofactor trypanothione. Trypanothione is used by the parasite to maintain reduced pools of thiols in the cell to keep the intracellular redox-balance. This is different from most mammals in that they use glutathione to maintain an intracellular redox-balance (*14-17*). Ultimately, if the parasite cannot synthesise trypanothione it will not survive. Lastly, the ODC enzyme plays a more important role in the parasite than in human cells. In the blood stage of the parasites' life cycle the environment has a shortage of polyamines which causes the parasite to be dependant on ODC activity for its requirement of polyamines (*18*). In support of this observation it was shown that *T. brucei* has essential requirement on ODC activity. In the study to show the essential requirement of ODC activity, a knockout cell line was unable to grow in the absence of polyamine putrescine (*19*).

1.3.2 Mechanism of ODC catalysis

As previously mentioned, ODC is dependant of the cofactor PLP. This cofactor catalyses a wide range of reactions ranging from decarboxylation, transamination, racemisation, β- or γ-elimination and carbon-carbon bond formation (*20, 21*). All enzymes that use PLP as cofactor bind it in transient fashion via a Schiff base with an active site Lys residue. The substrate is subsequently bound by exchanging this internal aldimine for an external aldimine by forming a Schiff base with the substrate. PLP-dependant enzymes use the PLP cofactor as an electron sink to stabilise the C_α carbanion that forms during the course of nearly all the types of reactions these enzymes catalyse. Reaction specificity of the enzyme is determined by the specific nature of the active site and not by the cofactor. The specific nature of the active site controls the orientation of the substrate inside it

and the relation of the substrate to the cofactor. This allows the enzyme to have control over whether it will act as a decarboxylase, racemase or transaminase, since decarboxylases cleave the C_α-carboxylate bond, while transaminases and racemases cleave the C_α-H bond. Also, the specific orientation of a substrate allows correct protonation to either give the decarboxylated product (protonation of the C_α carbon) or the transamination product (protonation of C4' of the PLP cofactor). These two routes are outlined with L-ornithine used in the reaction (figure 1.5).

In the case of ODC the enzyme transforms its substrate L-ornithine to the di-amine putrescine by means of a decarboxylation reaction. When entering the active site the side chain amino group (N_δ) occupies a conserved binding site directing L-ornithine into a specific position inside the active site. The PLP forms a Schiff base with the amino group of L-ornithine, allowing it to act as an electron sink. Decarboxylation takes place with carbon dioxide being set free followed by protonation of the C_α to form the di-amine putrescine. It has been shown that specific factors such as the position of the Cys 360 which protonates the C_α together with the dynamics of the active site increases the reaction specificity of ODC (*22*).

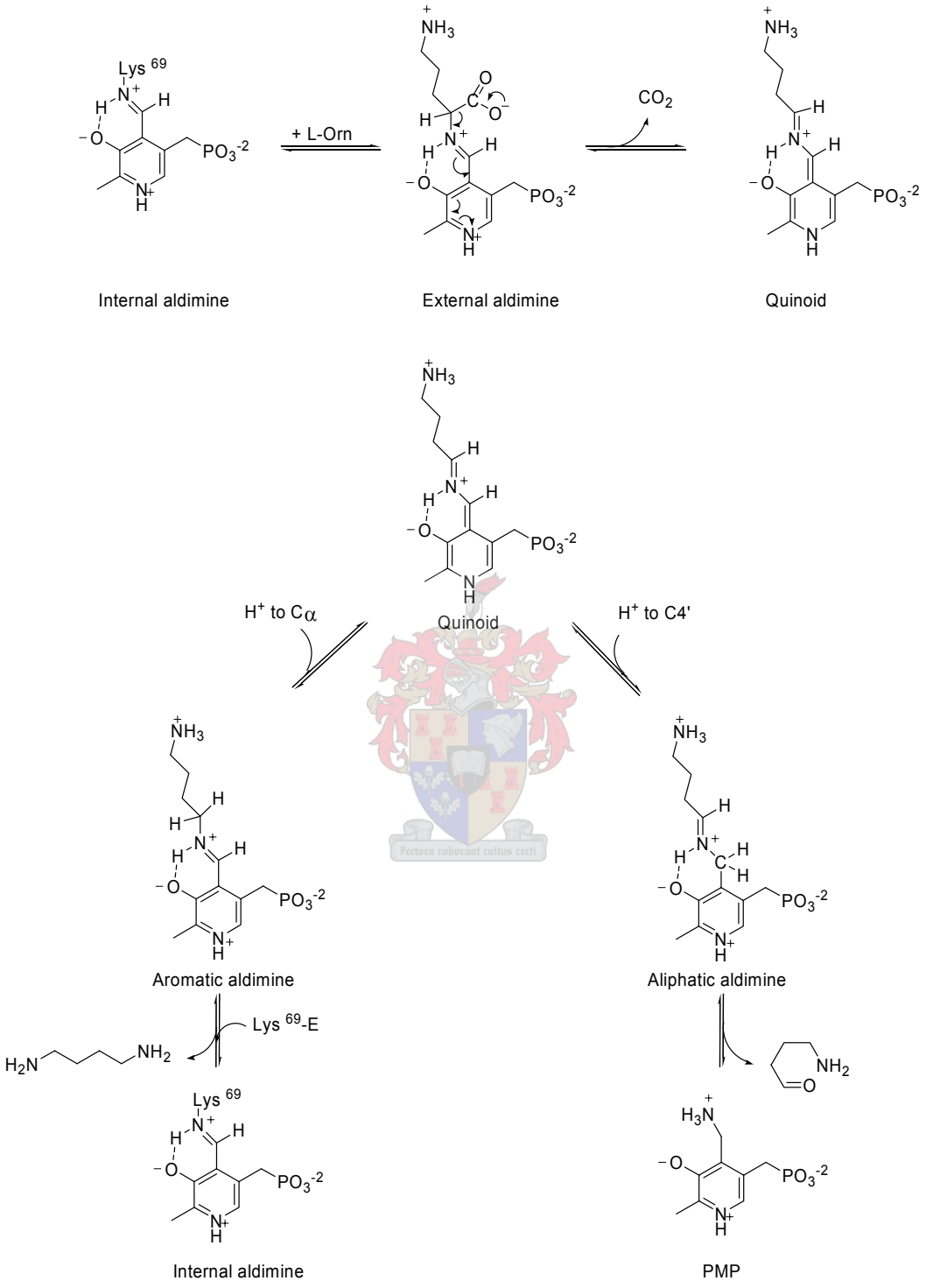


Figure 1.5: Different reactions of PLP. The internal aldimine Schiff base is cleaved in a transamination reaction to form a Schiff base with the substrate (L-Ornithine) and decarboxylation to form the quinoid structure. The reaction can now proceed in two different paths, protonation at the Cα and deamination or protonation at C4' and hydrolysis.

1.3.2 Mechanism of inhibition of ODC by DFMO

DFMO acts as a mechanism-based inhibitor of the enzyme ornithine decarboxylase. When it is taken up as substrate by the enzyme, DFMO binds covalently to the enzyme, thus rendering it inactive. Initially it was thought that only L-DFMO inhibits the ODC enzyme, but in a recent study it was shown that the D-enantiomer irreversibly inhibits the enzymes as well. However, the L-enantiomer is more effective as an inhibitor as the equilibrium constant for the absorption of the isomers into the active site shows that the L-enantiomer is absorbed into the active site >20 times more readily than the D-enantiomer (*23*). DFMO enters the active site of ODC in the same manner as L-ornithine would and is directed by the active site into a specific orientation. The Schiff base between Lys 69 of the enzyme and the PLP is broken to form the external aldimine with DFMO. Due to the initial orientation of DFMO in the active site, the formed Schiff base between DFMO and the cofactor places the PLP in the correct position for decarboxylation. A schematic representation of the inhibition by DFMO is given in figure 1.6.

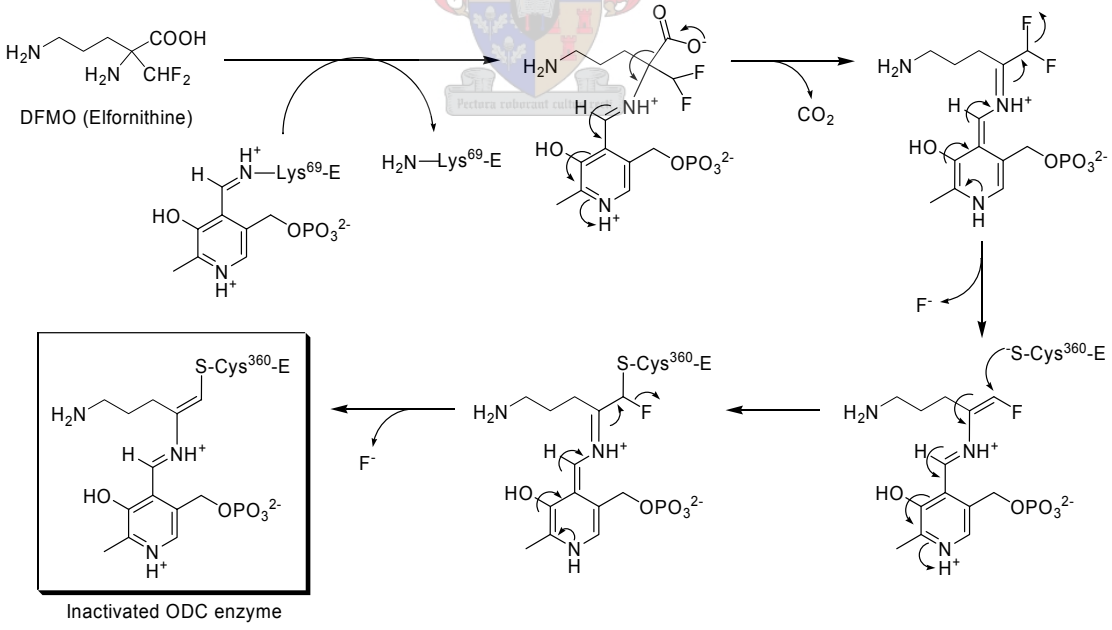


Figure 1.6: Mechanism of inhibition of ODC by DFMO

It is postulated that decarboxylation is favoured due to the carboxyl group that is buried in a hydrophobic pocket which would stabilise the neutral transition state. Decarboxylation of DFMO takes places with delocalisation of the electrons into the cofactor PLP. However, instead of being followed by protonation as in the case of the native substrate, the decarboxylation of DFMO is followed by the elimination of the first fluoride to form a neutral transition state. At this point, Cys 360 attacks the C_α carbon with elimination of the second fluorine to form a covalent bond between the Cys 360 and the inhibitor. This covalent bond formation of the enzyme with the substrate is the essential step which inactivates the ODC enzyme.

1.4 Conclusion

In the preceding pages the context and threat of African sleeping sickness have been elaborated upon. No new drugs for the treatment of African sleeping sickness have been produced in the last two decades, nor has the current drugs been improved on. In the next chapter the use of the fluorinated ornithine analogues as a potential drug against African sleeping sickness will be elaborated upon. We believe that the fluorinated ornithine analogues as a drug against African sleeping sickness holds great promise.

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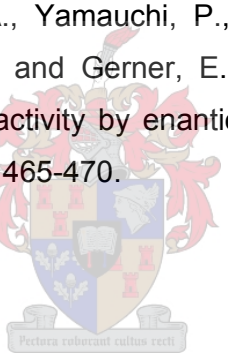

Chapter 2

Synthesis of Fluorinated Ornithine Analogues - An overview

2.1 Fluorine in Organic Chemistry

Fluorination of organic molecules has been a focus of organic synthesis for a long period of time. The value of fluorinated molecules have especially been demonstrated by their effectiveness as drugs, as in the case of DFMO for treatment of trypanosomiasis. The synthesis of fluorinated molecules can be approached in two ways: In the first, which is also the more classical approach, a fluorinated molecule or fluorinated building block is used as starting material to which other functional groups are added. In the second strategy the requisite fluorine atoms are only introduced at a later stage in the synthesis through some fluorination technique.

In industry the synthesis of fluorinated amino acids still largely rely on direct fluorination. Direct fluorination techniques use chemicals such as HF and SF_4 which are dangerous to work with and involve the use of specialised equipment. Apart from the expensive equipment and the danger involved in working with these chemicals, some of these chemicals also pose a threat to the environment (*1*). As a result industrial companies are now focusing on the synthesis of fluorinated building blocks to avoid the use of direct fluorination techniques.

2.2 Synthetic preparation of DFMO

2.2.1 The industrial preparation of DFMO

The synthesis of DFMO was originally done by Bey *et al.* (*2*). Although there is more than one patented synthesis of DFMO, all of them are based on this original procedure. The US patent No. 4,309,442 describes a synthesis of DFMO starting

from ornithine whereas the Swiss patent CH 672 124 describes a synthesis of DFMO from malonic acid esters. However, the most recent patent, US patent 7012158, starts with a glycine equivalent as a route to the synthesis of DFMO. This route is an improvement over the other patented routes and is shown in figure 2.1.

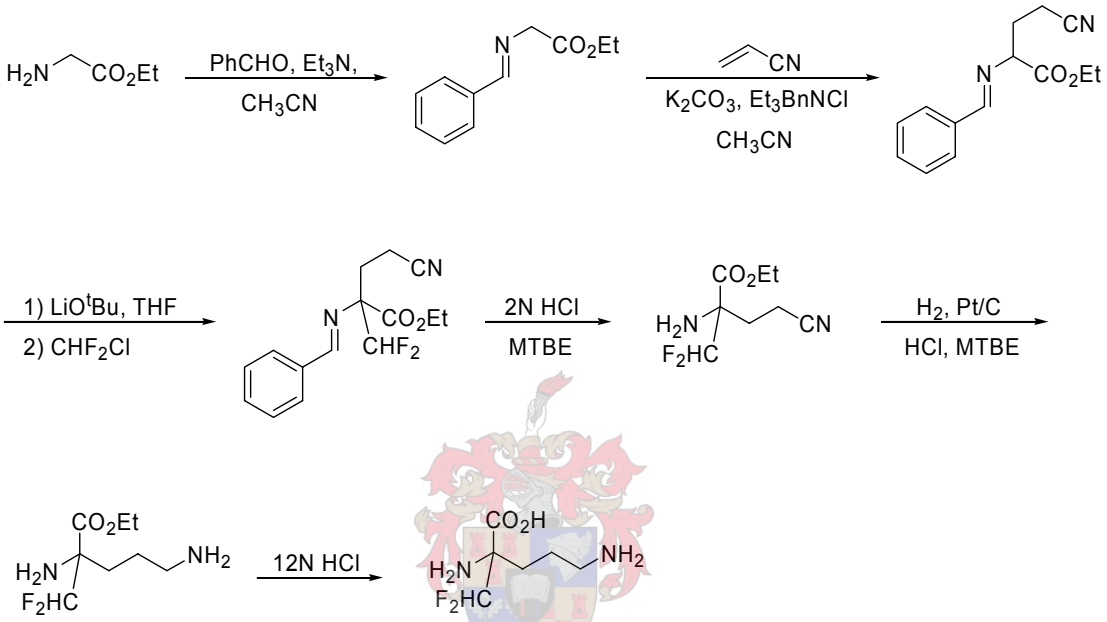

Figure 2.1: The most recent patented synthesis of difluoromethylornithine (DFMO)

The first step involves the formation of a Schiff base between the glycine ester with benzaldehyde in acetonitrile to give the protected amine product. Michael addition to acrylonitrile using potassium carbonate as base and triethylbenzylammonium chloride as phase transfer catalyst affords the addition of a cyanoethyl side chain α to the ester group. Fluorination is accomplished using as strong base, such as LDA or lithium tertiary butyl oxide, to generate the nucleophile and chlorodifluoromethane is used as the difluoromethyl electrophilic alkylation reagent. Hydrolysis of the Schiff-base protecting group in acid media provides the α-amine group followed by reduction of the nitrile moiety to yield the δ-amine group. Final deprotection of the ethyl ester gives the final fluorinated ornithine analogue product, DFMO.

2.2.2 Drawbacks of the industrial synthesis of DFMO

The main problems associated with the current synthesis of DFMO relates to three aspects: the nature of the base used in the introduction of the difluoromethyl group, the source of the difluoromethyl group and the lack of stereocontrol. In the first case, the use of a strong base is necessary for deprotonation to take place. However, strong bases such as LDA or lithium tertiary butyl oxide are difficult to work with due to the requirement of low temperatures and also the corrosive nature of the bases. The use of specialised equipment is needed when performing these reactions on large scale. The second problem relates to the introduction of the the difluoromethyl group. This group is introduced by use of chlorodifluoromethane as the electrophile. Chlorodifluoromethane is a CFC gas and as such strict measures have to be implemented to ensure that the use of the gas in the syntheses does not pollute the environment. Introduction of the chlorodifluormethane gas into the reaction also requires the use of high pressures. In combination these two factors contribute to the expenses involved in the syntheses of DFMO. In the third case it might be problematic that the synthesis produces a racemic mixture of enantiomers. Although it has been shown that both the enantiomers do act as an inhibitor of ornithine decarboxylase enzyme (ODC), it has also been shown that the L-enantiomer is more effective (*3*).

We would like to address the problems associated with the current industrial synthesis of DMFO. We believe that an improved synthesis of DFMO will increase the availability of the drug for distribution to the countries affected by African sleeping sickness. The first problem to address in the synthesis of DFMO is the introduction of the fluorine functionality. It is our aim to introduce the fluorine functionality by using fluorodehydroxylation as a fluorination method.

2.3 Fluorodehydroxylation as fluorination technique

Fluorodehydroxylation is the transformation of an alcohol functionality to the corresponding fluorine group. The fluorodehydroxylation method can also be used to convert a ketone/aldehyde to a difluoro group. Fluorodehydroxylation as

fluorination method is much safer than the direct fluorination methods currently in use and not as difficult to perform. Another advantage of fluorodehydroxylation is that the protection of primary and secondary amines is not always necessarily required. Taken together, the use of fluorodehydroxylation as fluorination technique is easier, more affordable and safer than those used by industry. An overview of the possible fluorodehydroxylation reagents is given below.

2.3.1 YAR as fluorodehydroxylating agent

The Yarvenko reagent (2-chloro-1,1,2-trifluorethyldiethylamine, YAR, **2.1**) was used as a fluorodehydroxylating reagent to synthesise 4-fluoroglutamic acid. However, the use of YAR is only suitable for the exchange of alcohols to fluorides and carboxylic acid to carbonyl fluorides. The YAR reagent cannot transform either an aldehyde or ketone into the corresponding difluoro-group. The other disadvantage is that this reagent does not have a significant shelf life and degrades upon storage (*4*). With regards to the mechanism of the YAR reagent, it is similar to the mechanism of the next fluorodehydroxylation reagent and will be discussed in the next section.

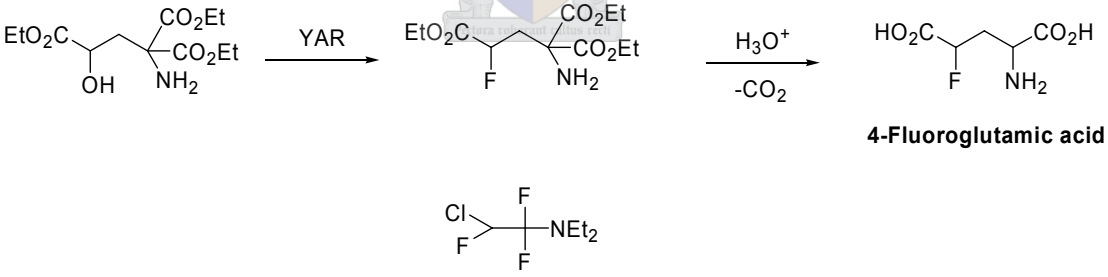

Figure 2.2: Use of the Yarvenko reagent (2.1) in the synthesis of 4-fluoroglutamic acid.

2.3.2 DAST and Deoxo-Fluor as fluorodehydroxylating agents

A reagent that is more versatile than the Yarvenko reagent is SF_4. SF_4 has been used for a large number of fluorodehydroxylation reactions. However, SF_4 is a very

hazardous and difficult reagent to work with. Furthermore since SF_4 is a gas, reactions of SF_4 have to be done at low temperatures and under high pressures and frequently require the use of liquid HF as solvent. These conditions do not make it an attractive option as fluorodehydroxylating reagent.

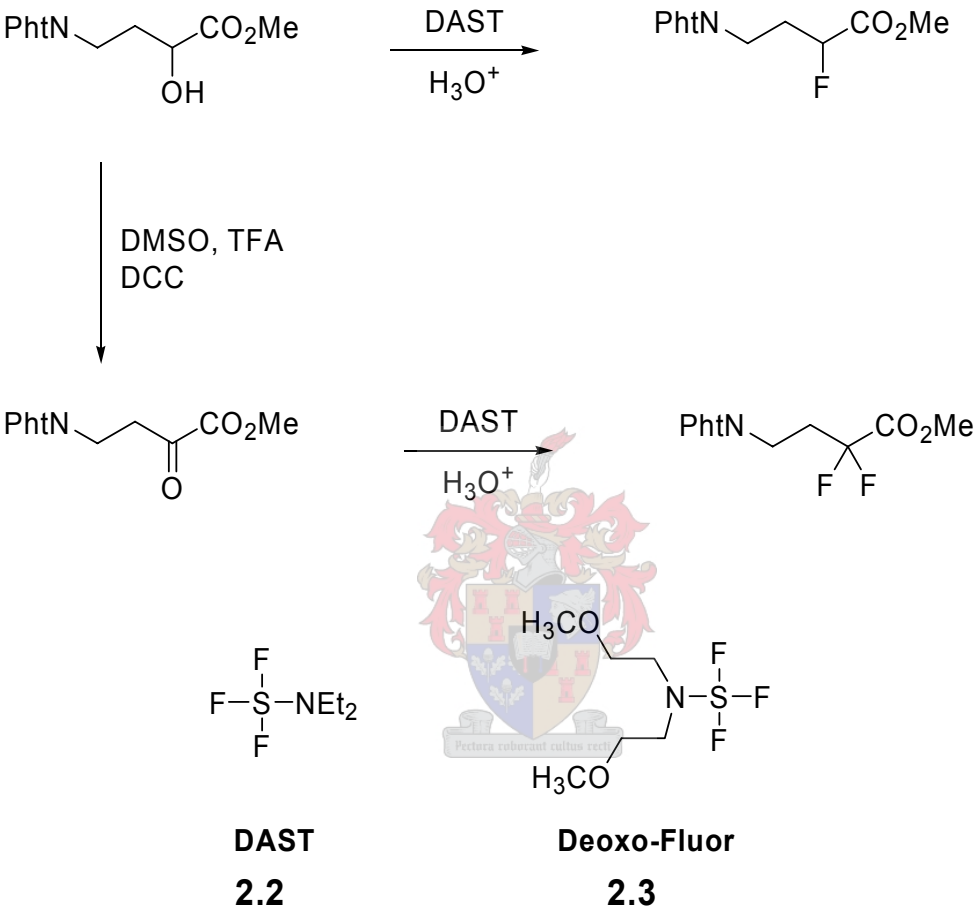

Figure 2.3 Use of DAST (2.2) as fluorodehydroxylation reagent with the structure of Deoxo-Fluor (2.3)

The reagent (diethylaminosulfur) trifluoride (DAST, **2.2**) was developed as a replacement for SF_4 (figure 2.3). In contrast to SF_4, DAST is easily handled and performs the fluorination reaction under mild conditions. DAST is also capable of converting aldehydes and ketones to the corresponding difluoro-groups, although aldehydes are more easily converted. Reactions performed with DAST proceed with inversion of configuration thus allowing stereocontrol of the reaction. Disadvantages of DAST are its preparation from highly reactive sulfur

tetrafluorides with dimethylaminotrimethylsilane, and its thermal instability. As a replacement to DAST, *bis*(2-methoxyethyl)amino-sulfur trifluoride (Deoxo-Fluor) was developed. Deoxo-Fluor is thermally more stable than DAST and is just as effective as a fluorodehydroxylation reagent. This makes it more amendable as a reagent for large scale reactions (*4, 5*).

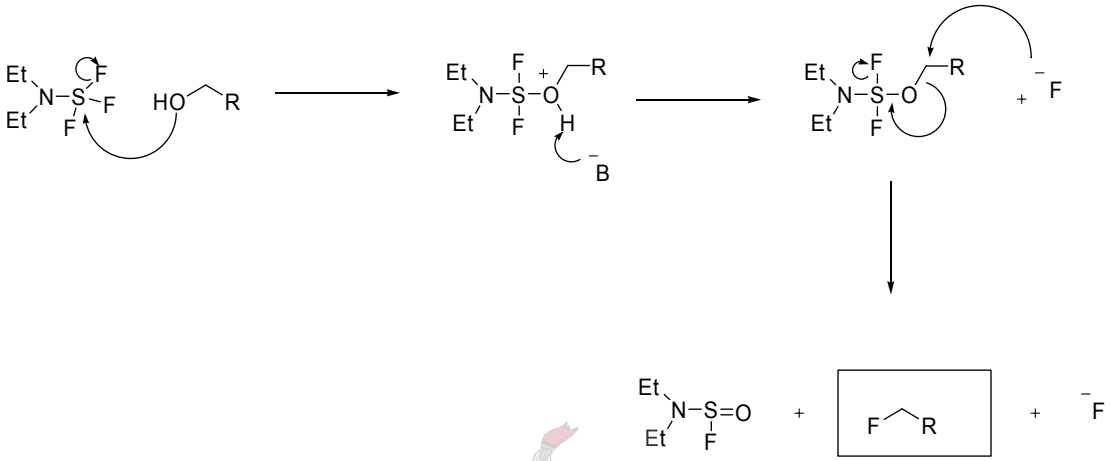

Figure 2.4 Reaction mechanism of DAST as fluorodehydroxylation reagent

With regards to the mechanism of these reactions, both activate the hydroxyl group to act as leaving group in a nucleophilic substitution reaction with fluorine. The reaction mechanism of DAST as fluorodehydroxylation reagent is shown in figure 2.4.

2.3.3 Selectfluor as fluorodehydroxylating agent

The use of Selectfluor, (1-chloromethyl-4-fluorodiazoniabicyclo[2.2.2]octane bis(tetrafluoroborate), **2.4**) as an electrophilic fluorinating reagent has also found widespread application due to its simple and safe use. The use of Selectfluor as a fluorodehydroxylation reagent has been demonstrated by the synthesis of glycosyl fluorides from anomeric hemi-acetals by using the reagent together with dimethylsulfide. The proposed reaction mechanism is shown in figure 2.5.

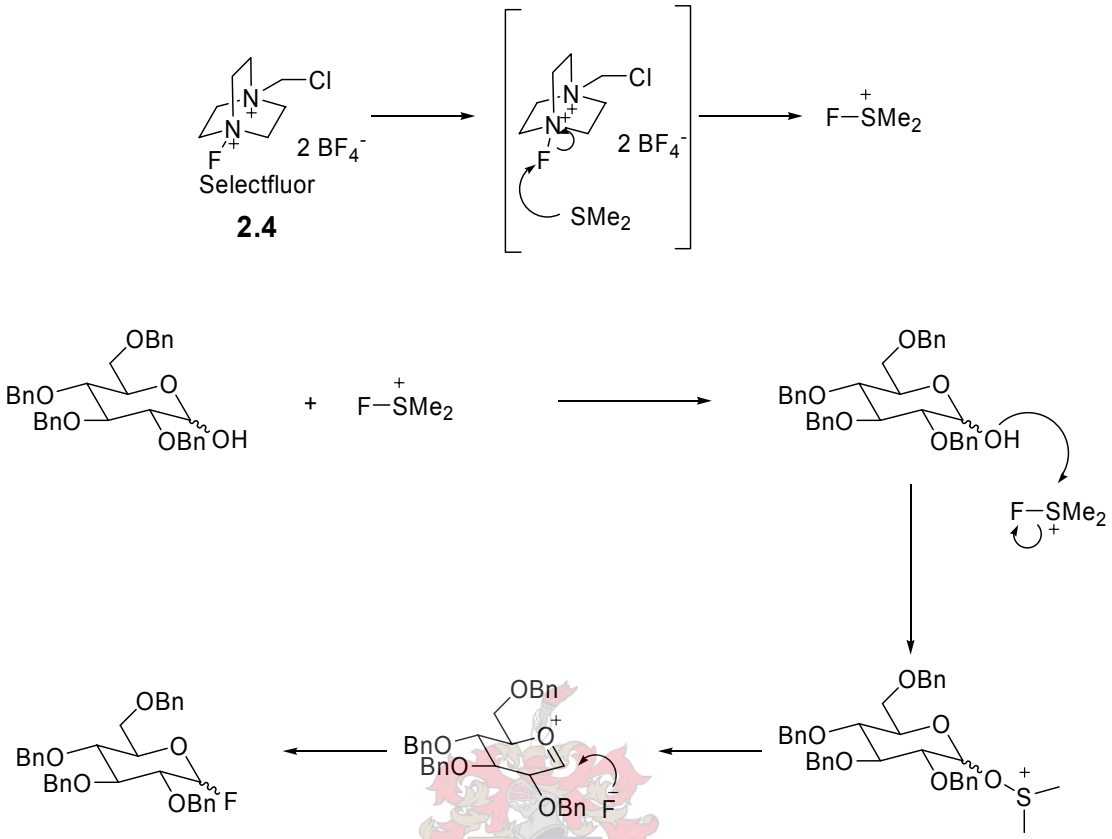

Figure 2.5: Proposed reaction mechanism of Selectfluor as fluorodehydroxylation reagent

2.3.4 Fluorodehydroxylation by halide exchange

Fluorodehydroxylation can also be achieved by halide exchange. This implies that the hydroxyl group is first replaced with a halogen atom (especially bromine) which is then subsequently replaced by fluorine (figure 2.6). In this manner fluorine is introduced into the reaction from an inorganic source, such as KF or CsF. The use of CsF as source of fluorine has been successful in the replacement of a hydroxyl group via a bromine intermediate as demonstrated by Lafargue *et al.* (*7*). The advantages of this method are considerable: First, it does not require harsh conditions or low temperatures for the reaction to take place, second, the source of fluorine is not hazardous or difficult to work with and third, very affordable to acquire. The only disadvantage of this method is that you can only insert a single fluorine atom into the molecule which renders this method unsuitable for the synthesis of the difluoro analogues.

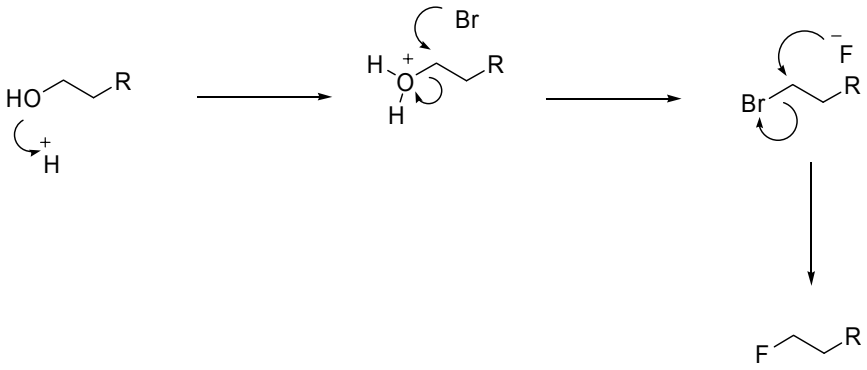

Figure 2.6 :Reaction mechanism of halide exchange as fluorodehydroxylation reagent.

2.4 Objectives of this study

The primary objective at the outset of this study was to develop a simpler, cheaper and more convenient synthetic procedure for the preparation of DFMO. However, this objective was subsequently refined as outlined below:

2.4.1 Synthesis of 2-(hydroxymethyl)-ornithine (2.5)

In an effort to synthesise DFMO using fluorodehydroxylation as fluorination method we needed to synthesise a precursor containing an appropriate hydroxyl group. As shown in figure 2.7 (2-Hydroxymethyl)ornithine (**2.5**) is an appropriate precursor molecule for the synthesis of DFMO. The hydroxyl group is oxidised to the aldehyde which is then transformed to DFMO. Together with the synthesis of DFMO the monofluorinated analogue, 2-monofluoromethylornithine, can also be synthesised by direct fluorodehydroxylation of 2-hydroxymethylornithine. A detailed retrosynthesis of DFMO will be done in chapter 3.

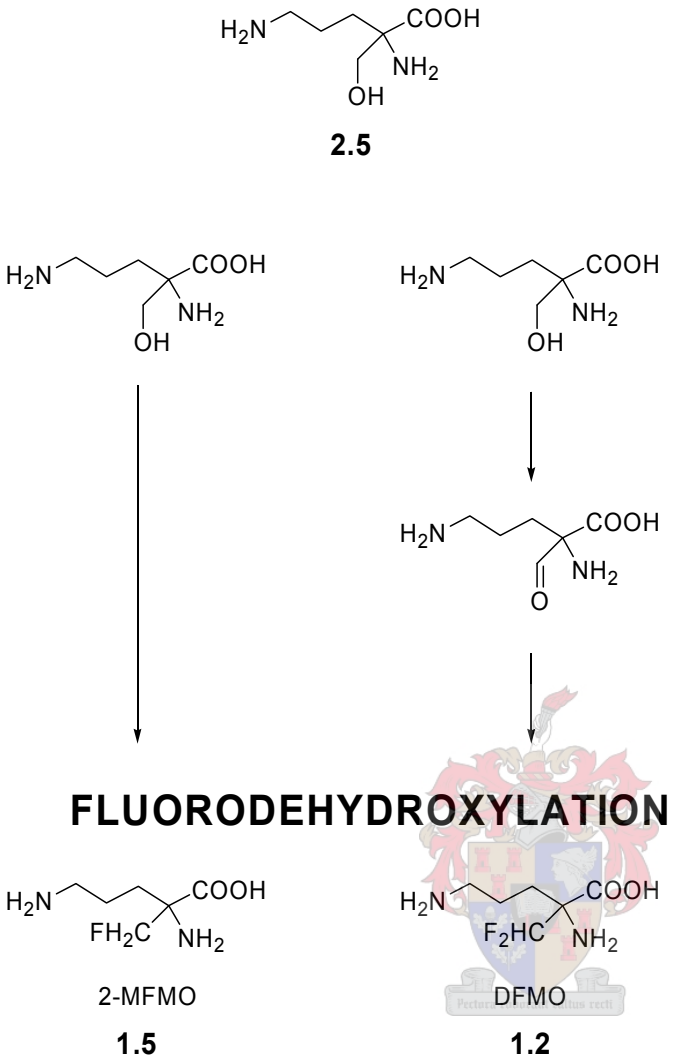

Figure 2.7: (2-Hydroxymethyl)ornithine (2.5) as a suitable hydroxyl precursor to the synthesis of both DFMO and 2-MFMO using fluorodehydroxylation as fluorination method.

2.4.2 Synthesis of 3-hydroxy-ornithine (2.7)

Fluorine analogues of ornithine that have to date not been synthesised include 3-fluoro-ornithine (**2.7**) and the difluoro-analogue, 3,3-difluoro-ornithine (**2.8**). Both these analogues can be synthesised from the appropriate hydroxyl precursor in the same manner as DFMO (**1.2**) and 2-MFMO (**1.5**) as shown above. The appropriate precursor in this case is 3-hydroxyornithine (**2.6**) which can be used to synthesise both the fluorinated analogues.

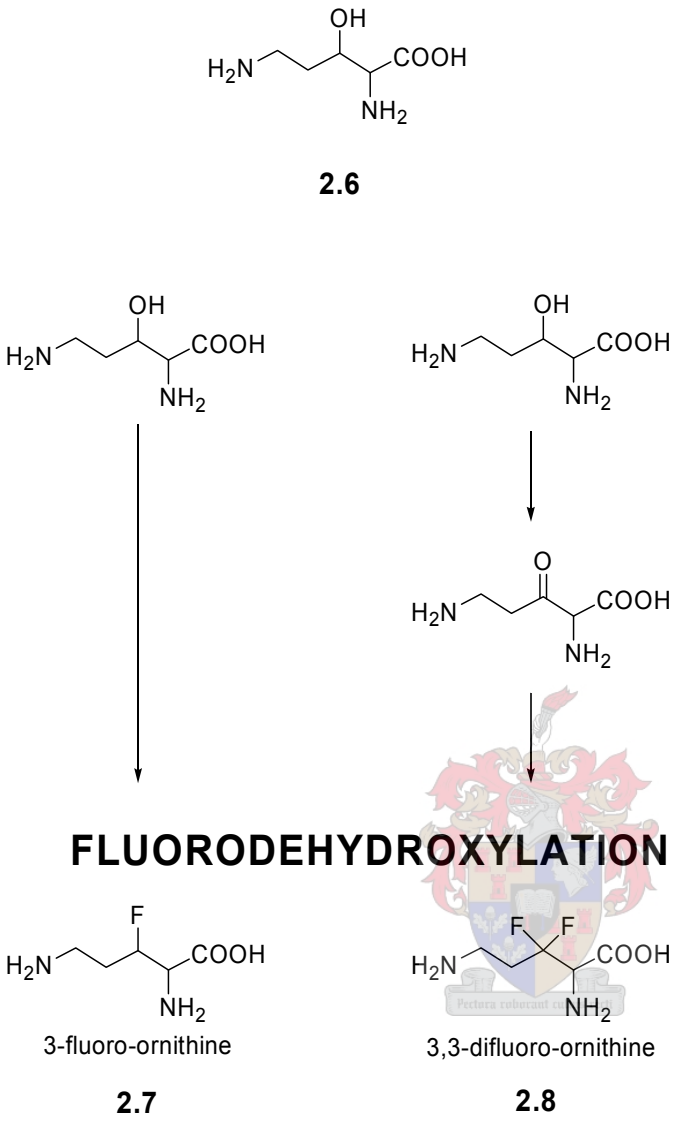

Figure 2.8: 3-Hydroxyornithine (2.6) as a precursor in the synthesis of 3-fluoro-ornithine (2.7) and 3,3-difluoro-ornithine (2.8) using fluorodehydroxylation as fluorination technique

Together with 2-MFMO (**1.5**), neither 3-fluoro-ornithine (**2.7**) nor 3,3-difluoro-ornithine (**2.8**) have been tested as inhibitors of ODC. All three of these analogues could be potential drugs for the treatment of HAT as they are also fluorinated analogues of ornithine and share similarities with DFMO and Δ-MFMO which has already been shown to be inhibitors of the ODC enzyme. These analogues will hopefully be effective against HAT and as such the synthesis and testing of these analogues was incorporated as a part of this thesis.

2.4.3 Evaluation of fluorodehydroxylation strategies on these compounds

In the synthesis of the fluorinated ornithine analogues using fluorodehydroxylation as fluorination method, we will have to ensure that the total synthesis of these compounds is indeed an improvement on the current industrial methods. The use of the reagents described above already ensures that our synthesis of these analogues will be safer and easier to perform. We would also like to optimize the use of halide exchange as fluorodehydroxylation method as it is the simplest and most affordable method. Lastly, we would like to incorporate stereocontrol into the synthesis, whether it is via the hydroxyl compounds or by the inherent stereocontrol given by the fluorodehydroxylation reagents.

2.5 Conclusion

In an effort to find a more simple and effective way of introducing fluorine into an organic molecule, the use of the fluorodehydroxylation method holds great promise, especially for the preparation of DFMO and the other fluorinated ornithine analogues from the required hydroxyl precursors.

2.5 References

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Chapter 3

Synthesis of 2-(hydroxymethyl)ornithine as MFMO/DFMO precursor

3.1 Introduction

In chapter two we discussed our chosen strategy for the development of a new, simple synthetic protocol for the preparation of DFMO (**1.2**) and its monofluoromethyl analogue, 2-MFMO (**1.3**). This strategy relies on the preparation of an appropriate hydroxylated precursor, which would subsequently be transformed into the fluorinated compounds through fluorodehydroxylation. In this chapter we will discuss the synthesis of the required precursor molecule, 2-(hydroxymethyl)ornithine (**2.5**) and how it will be used in the fluorodehydroxylation reactions. To find the suitable precursor molecule we performed a retrosynthetic analysis on DFMO, which is shown in figure 3.1.

The first two functional group interconversions in the retrosynthetic figure show that the difluoromethyl group can be obtained from an aldehyde by means of a fluorodehydroxylation reaction, and the aldehyde is in turn formed by oxidation of the primary hydroxyl group of **2.5** which thus makes this the precursor molecule. Further retrosynthetic analysis of 2-(hydroxymethyl)ornithine, as indicated in figure 3.1, shows the various possible synthons from which this precursor may be assembled. One possibility is the disconnection between the α- and β-carbons of the main chain to yield serine and an aminoalkyl chain synthon. Another is the disconnection of the hydroxymethyl group which gives ornithine and a hydroxymethyl synthon. Finally both of these disconnects may be combined to give glycine and both a hydroxymethyl and aminoalkyl chain synthon.

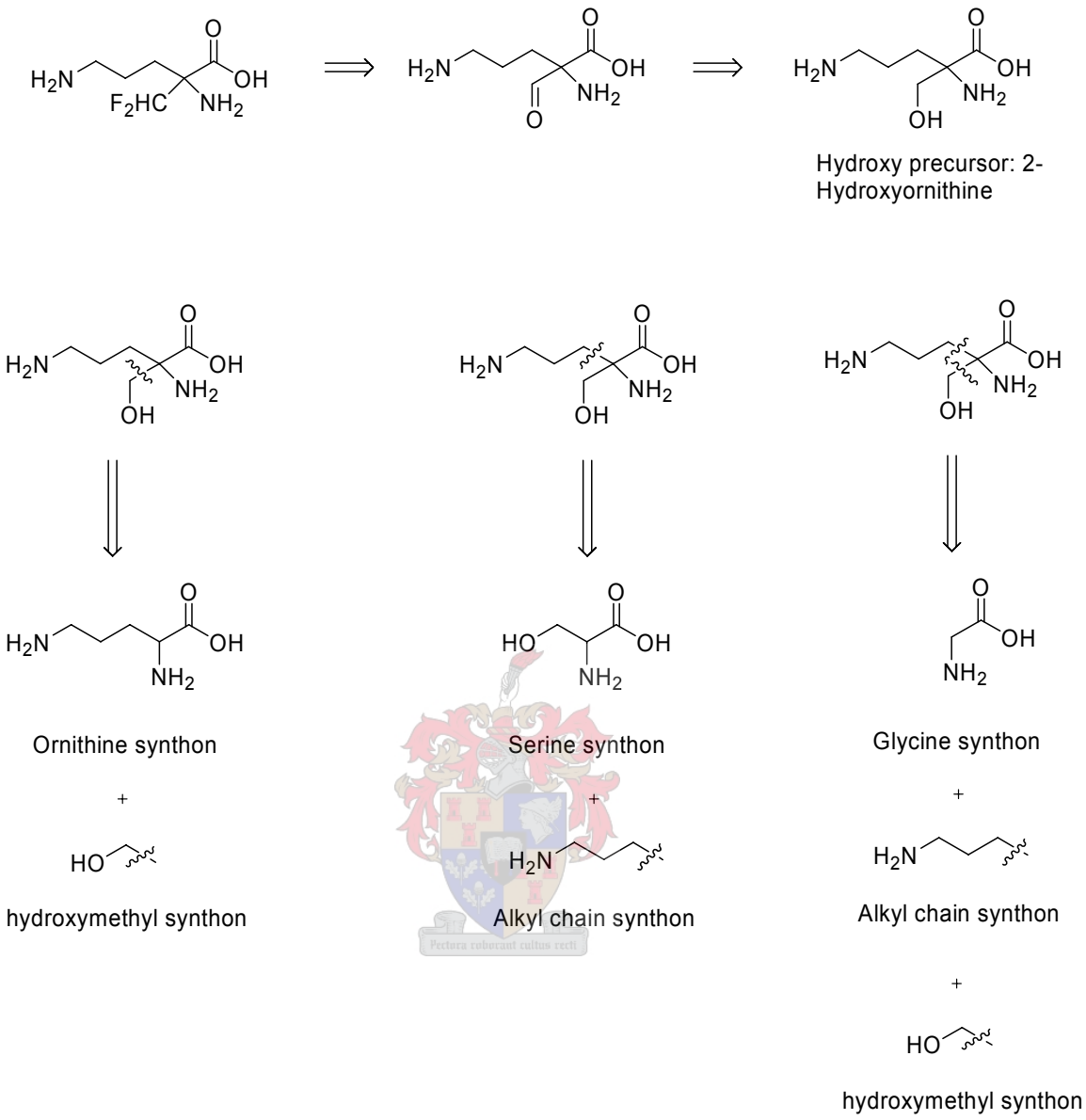


Figure 3.1: Retrosynthetic synthesis of DFMO

This retrosynthetic analysis suggested three strategies to synthesise 2-(hydroxymethyl)ornithine. Strategy one focused on the addition of the aminoalkyl chain to serine to provide the precursor molecule. Strategy two focused on the addition of *both* the hydroxymethyl and aminoalkyl synthons to a glycine equivalent to yield compound **2.5**. The third and last strategy focused on the addition of the hydroxymethyl group to a ornithine equivalent as published by Bey *et al.* (*1*). The method proposed by Bey *et al.* (*1*) gave a overall yield of 60% over 4

steps, but has as the disadvantage that it uses ornithine as starting material, which may be prohibitively expensive on large scale. Although we were aware of this method from the start, the advantages and possibilities afforded by the other methods prompted us to attempt them before the synthesis proposed by Bey *et al.* (*1*).

3.2 Strategy 1: Addition of the aminopropyl chain to serine equivalents

A thorough literature search pointed us to two different methods to introduce the aminoalkyl chain to a serine equivalent. Both of these methods employs chemistry that are α-alkylation reactions and as such require that the serine equivalent act as nucleophile and the aminoalkyl chain as the electrophile. The first method is chemistry introduced by Seebach and his group, while the second makes use of oxazoline ring chemistry. Both of these methods enable the serine equivalent to be deprotinated and to then act as a nucleophile.

The main advantage of the Seebach approach is that there is stereocontrol over the alkylation. This is achieved through the inherent properties of the oxazolidines which are used as serine equivalents in the alkylation reactions, as shown in Figure 3.2.

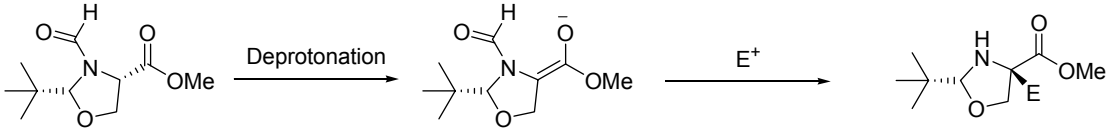


Figure 3.2: Stereocontrol by use of Seebach chemistry.

In the Seebach method, a serine methyl ester is condensed with pivaldehyde to form an oxazolidine. The stereochemistry of the parent amino acid influences the stereoselectivity of the ring-closure reaction by forcing the resulting tertiary butyl substituent on position 2 of the oxazolidine to take on the energetically most favoured conformation. This influence is borne out by the spatial interaction of the substituents on the ring, the most favoured conformation will always be the one in which there are the least amount of clashes between substituents. Another factor

that influences the stereoselectivity of the ring formation is the stabilisation of the resulting 1,3-allyl strain after deprotonation.

Upon deprotonation of the acidic α-proton the parent amino acid loses its original stereochemical configuration as the C-5 becomes trigonal. The only stereogenic centre left in the ring is the one introduced at the tertiary butyl position during ring formation. However, the reaction of the formed enolate is influenced by the stereochemistry of the *t*-butyl group, and only occurs on the opposite face of the *t*-butyl group (figure 3.3). In this way the tertiary butyl group relays the stereochemical information of the parent amino acid so that alkylation happens with retention of stereochemistry. This is referred to as self reproduction of chirality and is the main feature of the chemistry introduced by Seebach (*2-4*).

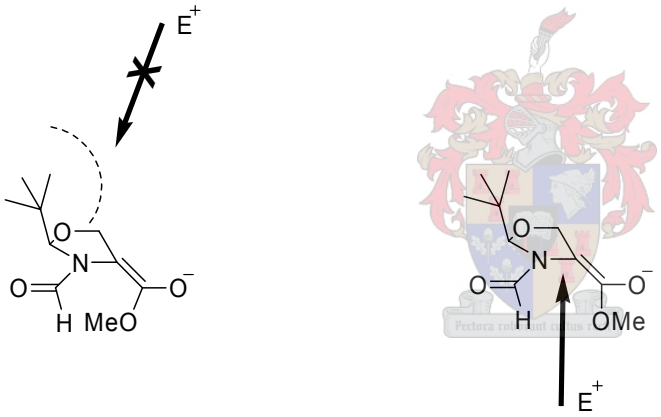


Figure 3.3: Addition of the electrophile only occurs from the opposite face of the *tert*-butyl group allowing the alkylation to proceed with retention of stereochemistry.

The disadvantage of this chemistry is two fold: First, the synthesis of the oxazolidine is tedious taking 3 steps. Second, the enolate that is formed upon deprotonation is not well-stabilised, as only the ester carbonyl on position 5 of the oxazolidine offers resonance stabilisation. This relatively poor stabilisation lowers the acidity of the α-hydrogen and thus requires the use of a strong base such as LDA to deprotonate it.

The second method to α-alkylate serine found in the literature makes use of 2-phenyloxazolines as serine equivalents. The advantage of this system is that the synthesis of the precursor is relatively simple, requiring a single step. The starting materials needed to synthesise the oxazoline are also less expensive than those used for the oxazolidine used in Seebach's approach. Furthermore the formed enolate is stabilised to a greater degree by the anion being delocalised both into the adjacent ester as well as the aryl ring at C-2, as shown in Figure 3.4.

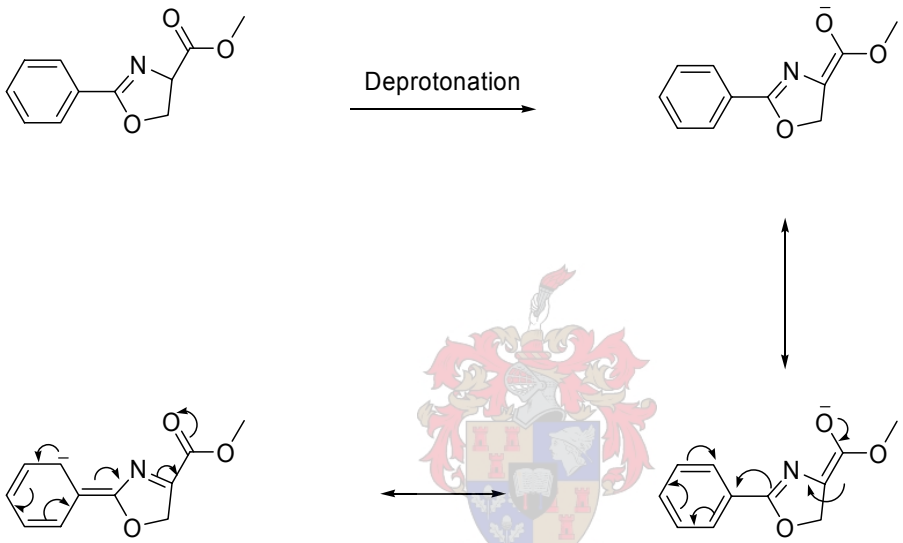


Figure 3.4: Deprotonation of the oxazoline and the stabilisation afforded by the phenyl ring

The disadvantage of the oxazoline chemistry is that the phenyl ring does not have the ability as the tertiary butyl group to relay information about the stereochemistry of the parent amino acid and as such there is no stereocontrol in the reaction.

We thus set out to investigate both these synthetic methods as possible ways whereby the aminoalkyl chain could be introduced to serine equivalents.

3.2.1 Seebach's oxazolidine chemistry

3.2.1.1 Synthesis of 2*R*,4*R*-methyl 2-*tert*-butyl-1,3-oxazolidine-3-formyl-4-carboxylate (**3.4**)

An alkylation strategy using Seebach's oxazoline chemistry required that we synthesised the oxazolidine (**3.4,** figure 3.5); this was initially done starting with racemic serine because of its lower cost and because we first wanted to establish the alkylation chemistry before becoming concerned with stereocontrol.

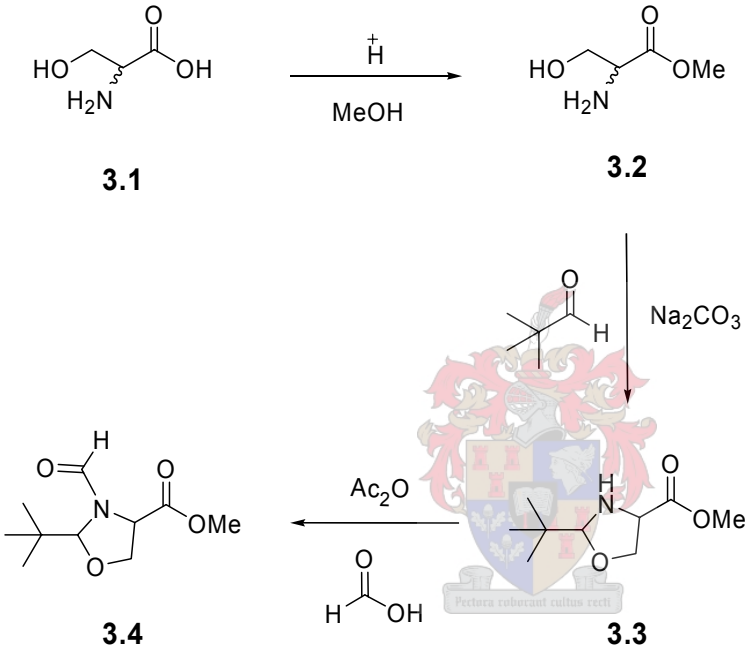
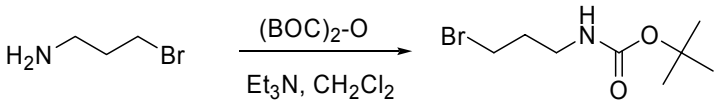


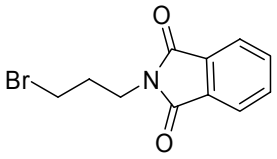
Figure 3.5: Synthesis of Seebach intermediate

The serine was protected with a methyl group using Fischer esterification conditions to afford the methyl ester **3.2** in good yield. After condensation of the ester with pivaldehyde and protection of the nitrogen with a formyl group according to the literature procedure the oxazolidine **3.4** was obtained in 42% overall yield (*3, 5*). ^{1}H NMR data of **3.4** agreed with published data.

3.2.1.2 Synthesis of electrophile



3.5b



3.5a

Figure 3.6: Protection of 3-bromopropylamine with a BOC group and as a phthalimide.

We decided to use two different aminoalkyl chains as electrophiles, each differing only in the protecting group on the amine. The two protecting groups used are a phthalimide and BOC derivative (figure 3.6). The phthalimide derivative (**3.5a**) was obtained from commercial sources. The BOC derivative was synthesised according to a published method starting with 3-bromopropylamine to give **3.5b** in good yield of 71%. ^{1}H NMR data correlated with those published (*6, 7*).

3.2.1.3 Synthesis of alkylated products **3.6a** and **3.6b**

In the alkylation reactions reported by Seebach's group benzyl, allyl, ethyl and methyl halides were used as electrophiles. From these reactions we were confident that alkylation would succeed using our proposed aminoalkyl chain.

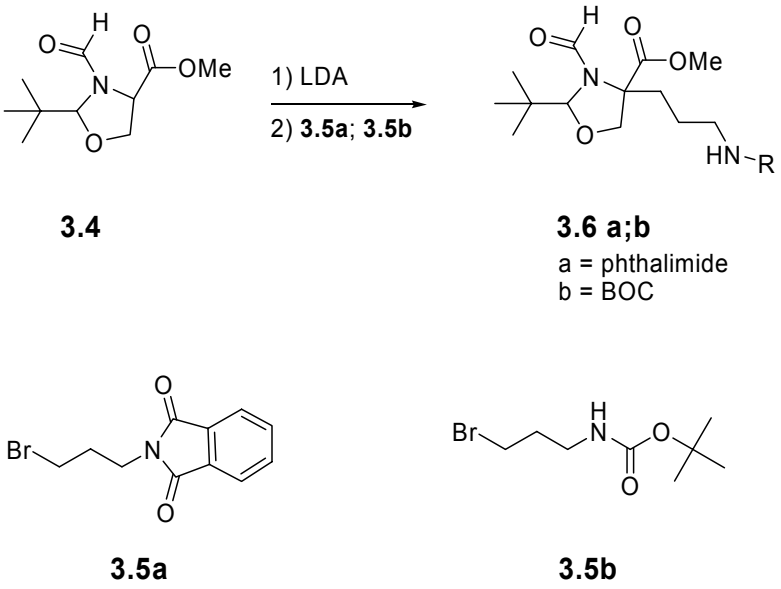


Figure 3.7: Alkylation of Seebach intermediate

Using the oxazolidine **3.4** we attempted alkylation with the two electrophiles to introduce the ornithine side chain (figure 3.7). Deprotonation was done with LDA at -78ºC in CH_2Cl_2 according to literature procedures (*3, 8, 9*). After formation of the enolate the electrophile was added to the reaction mixture. However, we failed to identify the formation of any alkylation products (**3.6a; 3.6b**) with either of the two electrophiles (**3.5a; 3.5b**).

3.2.1.4 Synthesis of α-methylated oxazolidine

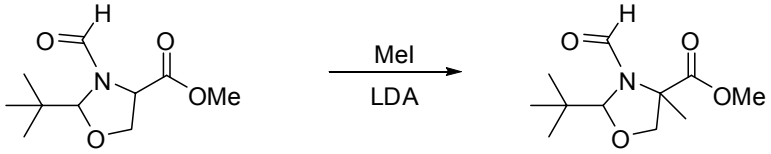


Figure 3.8: Methyl alkylation of Seebach intermediate

α-Methyl serine and α-methyl cysteine have previously been synthesised (*3, 5, 7*). We decided to alkylate the oxazolidine with methyl iodide to confirm that the reaction worked in our hands (figure 3.8). The reaction with methyl iodide was attempted twice and the second attempt succeeded based on TLC analysis of the reaction mixture. However, purification by flash chromatography was largely unsuccessful and a mixture of products was obtained. ^{1}H NMR analysis of the

crude mixture confirmed that the reaction was successful. The use of DMPU and HMPT as co-solvents were reported to increase the yields obtained in the reaction and as such was attempted. Unfortunately neither of the co-solvents increased the yields significantly.

Since the Seebach oxazolidine chemistry chemistry was unsuccessful in our hands we decided to focus on the second method for α-alkylations found in the literature, *ie*. the use of the oxazoline chemistry as explained earlier.

3.2.2 Oxazoline-mediated chemistry

3.2.2.1 Synthesis of 2-phenyl-2-oxazoline-4-carboxylate methyl ester (**3.8**)

Literature pointed to two different methods for the preparation of oxazoline **3.8** and is shown in figure 3.9. Both methods require the use of protected serine **3.2** which was synthesised previously.

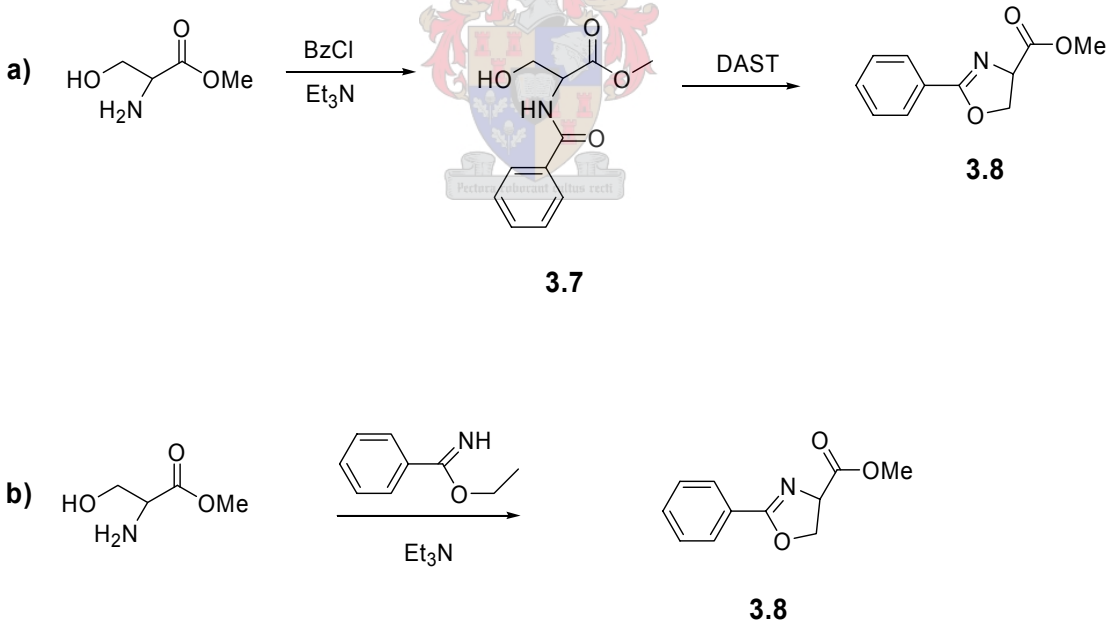


Figure 3.9: Synthesis of 2-phenyl-2 oxazoline-4-caboxylate

In the first method (**a**) the amino group is protected as the benzoyl amide using benzoyl chloride and triethylamine as base (*10, 11*). The benzoyl protected serine can now be cyclised with (diethylaminosulfur) trifluoride (DAST). DAST activates

the hydroxyl group, which is then subsequently attacked by the carbonyl oxygen of the benzoyl group to form the oxazoline (*10, 12*). In the second method the methyl ester of serine is reacted with ethyl benzimidate to give the oxazoline in a single step reaction (*13*). In our hands the second method afforded better yields and was easier to perform. The oxazoline **3.8** was synthesised in 44% yield and the ^{1}H NMR data correlated with those published (*10, 12*).

3.2.2.2 Synthesis of the alkylated product of the oxazoline 3.8.

While deprotonation of the oxazoline can be done with a milder base than LDA, we decided to follow literature procedures and used LDA as base at -78ºC in CH_2Cl_2 to deprotonate the α-hydrogen to form the enolate.

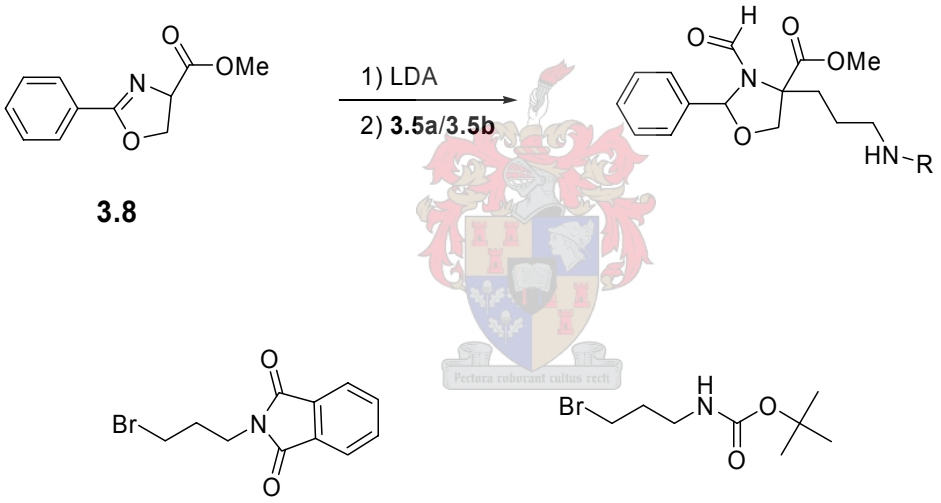


Figure 3.10: Alkylation of 2-phenyl-2-oxazoline-carboxylate methyl ester

Alkylation with both electrophiles (**3.5a**; **3.5b**, figure 3.10) was attempted separately but NMR analysis revealed that no product formed. From the NMR data no product formation could be identified but a mixture of starting reagents. As was the case with the alkylation of the oxzaolidine the reaction was not successful. However, previous studies had shown that the use of LDA as base together with low temperatures is required because the methyl ester of the oxazoline has the propensity to undergo β-elimination with subsequent Michael addition (*13, 14*). One way to circumvent this possible side reaction is to use the tertiary butyl ester

as protecting group for the serine carboxylic acid instead of the methyl ester as successfully used by Park *et al.* (*13*). This method requires the use of the milder base KOH together with a phase transfer catalyst to deprotonate the oxazoline to form the enolate. However, investigation into the synthesis of serine *t*-butyl ester showed it to be non-trivial as the ester has to be formed in preference to the *t*-butyl ether. We thus decided to rather focus on the strategy based on the double alkylation of a glycine equivalent.

3.3 Strategy 2: Addition of hydroxymethyl and alkyl chain to glycine synthon: (Synthesis of 2-phenyl-5-oxazolone (3.9))

The double alkylation strategy is based on the use of a glycine equivalent to which both the hydroxymethyl and aminoalkyl synthons will be added. The main advantages of this strategy would be the affordability of the starting materials as well as a supposed increase in alkylation efficiency. A suitable glycine equivalent is hippuric acid which can be cyclised using Ac_2O to form an oxazolone (or azlactone).

This ring can then be deprotonated using a very mild base such as triethylamine to form the enolate; this is due to the good stabilisation of the negative charge by both the phenyl ring and the adjacent carbonyl group. Since there are two hydrogens at the α-position, deprotonation can take place twice to allow the addition of both the hydroxymethyl and aminoalkyl synthons. This strategy is shown in figure 3.11.

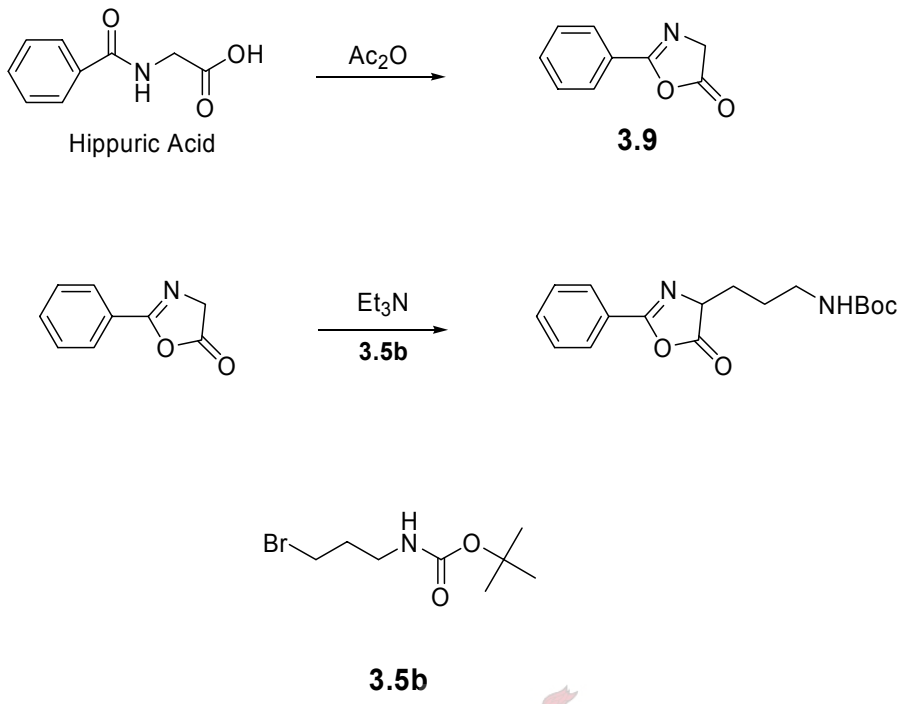


Figure 3.11: Synthesis of 2-phenyl-5-oxazolone and alkylation with the aminoalkyl chain synthon

The azlactone **3.9** was synthesised from hippuric acid according to literature using an excess Ac_2O to give **3.9** in 40% yield (*15*). Deprotonation with triethylamine to yield the enolate was done followed by alkylation with both the electrophiles (**3.5a**; **3.5b**) separately. The reactions did not give any alkylation product and the ^1H NMR analysis showed traces of the original glycine equivalent hippuric acid. The presence of hippuric acid in the reaction mixture prompted us to investigate the stability of the azlactone, **3.9**.

According to the literature, some saturated azlactones, especially the azlactone 3.9 which do not have any alkyl substituents at the α-position, are prone to ring-opening reactions which return the original starting materials. This is true of the saturated compound with only a methyl subtituent as well. The structure of saturated and unsaturated azlactones is shown in figure 3.12 (*16, 17*).

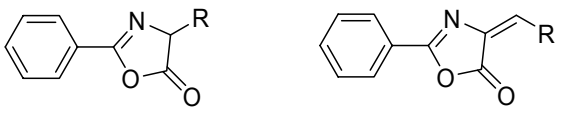


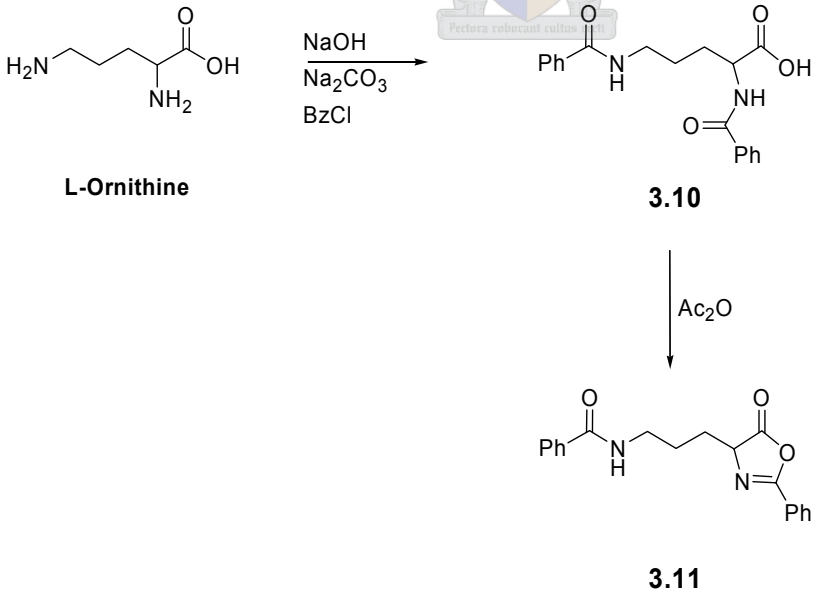
Figure 3.12: Structure of saturated and unsaturated azlactones.

To avoid this situation a one pot synthesis where both the electrophiles are added sequentially after the formation of the enolate could be attempted, but the apparent instability of the formed azlactone convinced us that the yields would be very low even if this strategy as successful. This reasoning prompted us to move on to our last strategy.

3.4 Strategy 3: Addition of the hydroxymethyl synthon to an ornithine equivalent

3.4.1 Synthesis of *N*-(3-(5-oxo-2-phenyl-4,5-dihydrooxazol-4-yl)propyl)benzamide (**3.11**)

Our last strategy is the published method used by Bey *et al.* to synthesise 2-(hydroxymethyl)ornithine (*1, 18*).

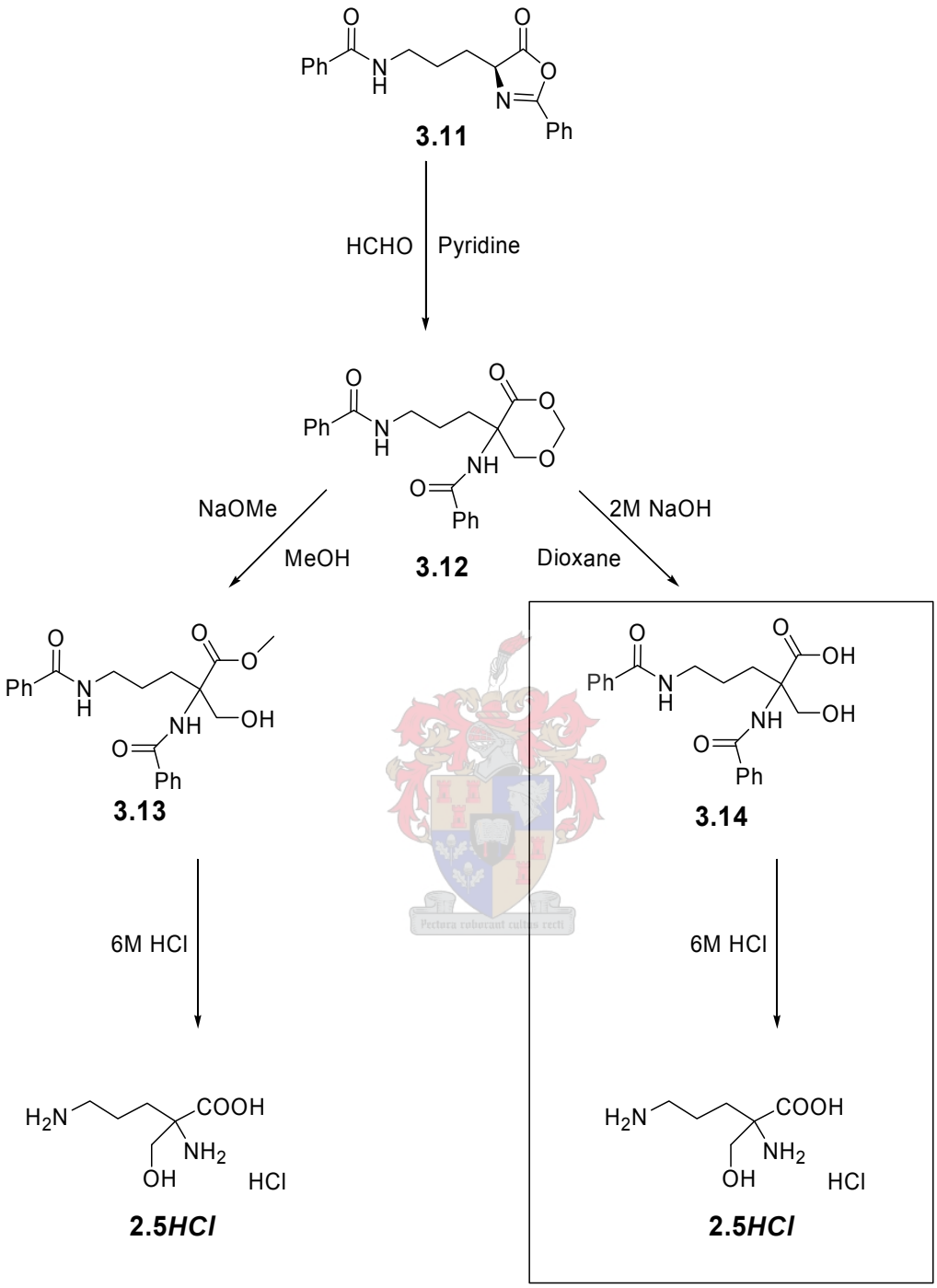


Scheme 3.13: Synthesis of *N*-(3-(5-oxo-2-phenyl-4,5-dihydrooxazol-4-yl)propyl)benzamide (2.11) from L-ornithine

The oxazolone **3.11** was synthesised as in the literature by first preparing ornithuric acid **3.10** by reaction of L-ornithine with benzoyl chloride. Ornithuric acid was cyclised with an excess of Ac_2O to form the oxazoline **3.11** in 90% yield. ^1H NMR data confirmed the correct shifts for both ornithuric acid and the oxazolone **3.11** (figure 3.13).

3.4.2 Synthesis of 2-(hydroxymethyl)ornithine (**2.5**)

The next step in the synthesis was to add the hydroxymethyl group. The addition of the hydroxymethyl group is accomplished by using pyridine to deprotonate the oxazolone and by addition of formaldehyde to introduce the hydroxymethyl functionality.



Scheme 3.13: Addition of hydroxymethyl group and synthesis of 2-(hydroxymethyl)ornithine

The synthesis of **3.12** was successful with an overall yield of 83% being obtained from the starting material L-ornithine. ^{1}H NMR data correlated with the reported shifts. The compound **3.12** was ring-opened to form the methyl ester **3.13**

according to the method of Bey *et al.* to give **3.13** in a good yield (70%). We also decided to attempt to open **3.12** to get the free carboxylic acid instead of the methyl ester in order to determine wheter its reactivity in the fluorodehydroxylation reactions would be different. The compound **3.12** was ring-opened to afford the free carboxylic acid according to the method of Witskona *et al.* to afford **3.14** in a yield of 90% (*19*). ^{1}H NMR data correlated with the expected shifts for both **3.13** and **3.14.**

The synthesis of 2-(hydroxymethyl)ornithine monohydrochloride **2.5*HCl*.** was completed according to the method of Bey *et al.* in excellent yield. The free carboxylic acid **3.14** was deprotected by refluxing in 6M HCl and gave **2.5*HCl*** in 95% yield in less time than needed to hydrolyse **3.13** to form **2.5*HCl***. The hydroxymethyl protons are represented by two doublets (3.8ppm) which are diagnostic for this molecule. In our hands the synthesis of **2.5*HCl*** which was the most successful and gave the best yield followed the path highlighted in scheme 313.

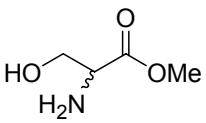
3.4 Conclusion

We attempted the synthesis of 2-(hydroxymethyl)ornithine by using three strategies. Strategies one and two, which rely on the addition of the ornithine side chain and the addition of both the hydroxymethyl and aminoalkyl chain respectively, were unsuccessful. However, the third strategy, the addition of the hydroxymethyl group to a ornithine equivalent was successful and we thus synthesised the required 2-(hydroxymethyl)ornithine.

3.5 Experimental

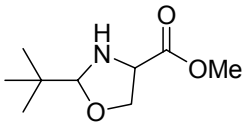
3.5.1 Synthesis of 2*R*,4*R*-Methyl 2-*tert*-butyl-1,3-oxazolidine-3-formyl-4-carboxylate (**3.4**)

DL-Serine methyl ester **3.2**



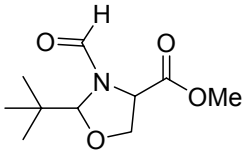
To MeOH (15ml) in an ice bath was added dropwise acetal chloride (3.39ml, 48mmol). In a separate flask DL-serine (5.07g, 48mmol) was dissolved in MeOH (5ml) and was subsequently added dropwise to the acidic MeOH solution in the ice bath. The reaction mixture was heated to reflux for 2 hours after which it was cooled to room temperature. The solvent was removed under reduced pressure leaving a clear oil which formed a white solid upon cooling to give **3.2** (4.4g, yield 77%). ^{1}H NMR (300 MHz, D_2O) δ 3.38 (s, 3H); 4.05-4.10 (dd, 2H); 4.2 (t,1H)

2R,4R-Methyl 2-tert-butyl-1,3-oxazolidine-4-carboxylate **(3.3)**



To a solution of **3.2** (2.65g, 22.27mmol) in pentane (30ml), was added triethylamine (6.13ml, 44mmol) and pivaldehyde (4.86ml, 44mmol). The reaction mixture was heated to reflux with continuous removal of H_2O using a Dean Stark trap. The resulting reaction mixture was allowed to cool to room temperature and the white precipitate removed by filtration. The reaction mixture washed with diethyl ether (10ml) and the solvent removed *in vacuo* to afford **3.3** as a colorless oil (3.2g yield 77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (s, 9H); 3.70 (s, 3H); 3.86 (d, 2H, *J* = 7.0Hz); 4.05 (s, 1H); 4.28 (s, 1H)

2R,4R-Methyl 2-tert-butyl-1,3-oxazolidine-3-formyl-4-carboxylate **(3.4)**

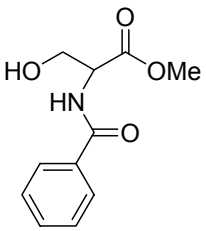


To a solution of **3.3** (2.57g,13.74mmol), formic acid (15ml) and sodium formate (952mg, 14.70mmol) was added acetic anhydride (4.25ml, 45mmol) was added dropwise. The reaction mixture was stirred at 0˚C for an hour after which it was warmed to room temperature and stirred overnight. The reaction solvent was removed *in vacuo* and the resulting residue neutralized with aq $NaHCO_3$ solution. The aqueous layer was extracted with diethyl ether (3x10ml) and the organic layer

dried over $MgSO_4$. Filtration followed by removal of solvent *in vacuo* provided **3.4** as a white solid (1.26g, yield 43%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (s, 9H); 3.75 (s, 3H), 4.0 (t, 1H, *J* = 8.0Hz); 4.45 (dd, 1H, *J* = 6.0, *J* = 3.3Hz); 4.88 (s, 1H); 4.90 (dd, 1H *J* = 4.0, *J* = 3.3Hz); 8.4 (s, 1H)

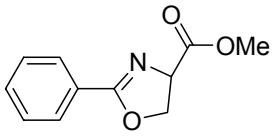
3.5.2 Synthesis of 2-phenyl-2-oxazoline-4-carboxylate methyl ester (**3.8**)

N-Benzyl DL serine methyl ester **(3.7)**



3.2 (693mg, 4.46mmol) was dissolved in H_2O (10ml) and the resulting solution neutralized with $NaHCO_3$ solution until the pH ~ 7.7. The dissolved amino acid was added dropwise to the flask containing benzoyl chloride (431µl, 3.72mmol) whilst stirring vigorously. The reaction mixture was left to stir for 2h at room temperature after which it was extracted with CH_2Cl_2 (3x10ml). The combined organic layers were dried over $MgSO_4$. Filtration followed by removal of solvent under reduced pressure provided the crude product as a white solid. The crude product was purified by flash chromatography (silica gel; ethyl acetate/hexane 1:2) to give **3.7** as a white solid (260mg, yield 26%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.75 (s, 3H); 4.05 (qd, 2H); 4.85 (p, 1H); 7.4 (t, 2H); 7.5 (t, 1H); 7.80 (d, 2H).

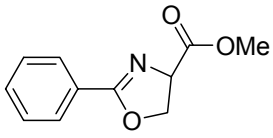
2-phenyl-2oxazoline-4-carboxylate methyl ester **(3.8)** from **3.7**



To a solution of **3.7** (260mg, 1.165mmol) in CH_2Cl_2 (6ml) at -78ºC was added (diethylamino)sulfur trifluoride (DAST) (184µl, 1.39mmol) was added dropwise over a period of 20min. The reaction mixture was stirred for 1h at -78ºC after which K_2CO_3 (240mg, 1.74mmol) was added in one portion. The reaction mixture was allowed to warm to room temperature after which it was quenched by pouring into sat. $NaHCO_3$ solution (11ml). The aqueous layer was extracted with CH_2Cl_2 (3x15ml) and the combined organic phases dried over Na_2SO_4. Flitration followed by removal of solvent *in vacuo* provided the crude product which was purified by flash chromatography (silica gel; ethyl acetate/hexane 1:2) to give **3.8** as a yellow oil (142mg, yield 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.75 (s, 3H); 4.56 (t, 1H, *J* =

8.8Hz) 4.65 (td, 2H, *J* = 13, *J* = 10.85Hz); 4.95 (dd, 1H, *J* = 2.8, *J* = 7.7Hz); 7.4 (t, 2H); 7.5 (t, 1H); 7.80 (d, 2H)

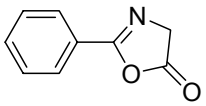
2-phenyl-2-oxazoline-4-carboxylate methyl ester **(3.8)**



To a solution of **3.2** (1.00g, 6.45mmol) in anhydrous CH_2Cl_2 (15ml) was added triethylamine (1.11ml, 8.00mmol) and the resulting reaction mixture stirred until it became homogenous. Benzimidate (1.21g, 6.45mmol) was added in one portion to the flask and the reaction mixture refluxed for 2h after which it was allowed to cool to room temperature. The atmosphere was replaced with Ar and the reaction mixture stirred overnight. The resulting reaction mixture was washed with sat. $NaHCO_3$ (3x5ml) after which the combined $NaHCO_3$-layers were washed with CH_2Cl_2 (2x7ml). The combined organic layers were pooled, washed with brine (2x7ml) and dried over $MgSO_4$. Filtration followed by *in vacuo* solvent removal provided the crude product which was purified by flash chromatography (silica gel; diethyl ether/petroleum ether 2:1) to afford **3.8** as a colorless oil (583mg, yield 44%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.75 (s, 3H); 4.56 (t, 1H, *J* = 8.8Hz) 4.65 (td, 2H, *J* = 13, *J* = 10.85Hz); 4.95 (dd, 1H, *J* = 2.8, *J* = 7.7Hz); 7.4 (t, 2H); 7.5 (t, 1H); 7.80 (d, 2H)

3.5.3 Synthesis of *N*-(3-(5-oxo-2-phenyl-4,5-dihydrooxazol-4-yl)propyl)benzamide (**3.11**)

2-phenyl-5-oxazolone **(3.9)**

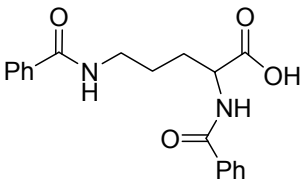


a) To a heated flask (100ºC) containing hippuric acid (5.00g, 27.90mmol) was added Ac_2O (13.2ml, 139,52mmol) in one portion. The reaction mixture was stirred vigorously until the reaction mixture became homogenous (~ 10-20min). The reaction mixture was allowed to cool down to room temperature. Excess Ac_2O was removed on a high vacuum pump (3-4.5mm Hg @ ≤ 55ºC). The resulting straw colored oil crystallized to give **3.9** as orange crystals (600mg, yield 67%).

b) To a solution of hippuric acid (1.00g, 5.58mmol) in CH_2Cl_2 (20ml) at -78ºC was added (diethylamino)sulfur trifluoride (DAST) (811µl, 6.14mmol) dropwise over a period of 20min. The reaction mixture was stirred for 1h at -78ºC after which

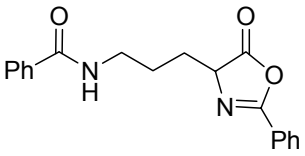
K_2CO_3 (1.15g, 8.37mmol) was added in one portion. The reaction mixture was allowed to warm to room temperature after which it was quenched by pouring into sat. $NaHCO_3$ solution (40ml). The aqueous layer was extracted with CH_2Cl_2 (3x15ml) and dried over $MgSO_4$. Filtration followed by removal of solvent *in vacuo* provided the crude product which was purified by flash chromatography (silica gel; ethyl acetate/hexane 1:1) to be give **3.9** as a orange solid (364mg, yield 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.4 (s, 2H); 7.4 (t, 2H); 7.5 (t, 1H); 7.80 (d, 2H)

2,5-Bis(benzamido)pentanoic acid **(3.10)**



To a solution of L-Ornithine monohydrochloride (5.00g, 29,6mmol) in H_2O (20ml) at 0ºC was added NaOH (4M, 30ml) dropwise over a period of 10min followed by addition of Na_2CO_3 (1.2M, 50ml) dropwise over a period of 20min. The reaction mixture was stirred for 5min after which benzoyl chloride (8.0ml, 69mmol) was added dropwise over 20min.The reaction mixture was stirred for an hour at 0ºC after which is was allowed to warm to room temperature and stirred for another hour. White precipitate removed by filtration after which filtrate was added to a HCl solution (3M, 80ml).The resulting white precipitate was cooled down to 0ºC for a hour after which it was removed by filtration and recrystallized from EtOH/H_2O to give **3.10** as a fine white crystal (8.75g, yield 87%). ^{1}H NMR (400 MHz, $DMSO_{d6}$): δ 1.60 (m, 2H); 1.90 (m, 2H); 3.20 (q, 3H, *J* = 6Hz); 7.4-7.6 (m, 6H); 7.8 (dd, 4H, *J* = 16.4, *J* = 8.5Hz)

N-(3-(5-oxo-2-phenyl-4,5-dihydrooxazol-4-yl)propyl)benzamide **(3.11)**

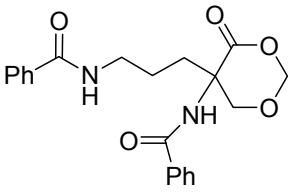


To a solution of **3.10** (1.00g, 2.93mmol) was added Ac_2O (4.6ml, 48.98mmol) and the resulting reaction mixture refluxed for an hour. The reaction mixture was cooled to room temperature and removal of solvent *in vacuo* provided a thick colourless oil which formed a white solid upon cooling. Recrystallization of white the solid from absolute EtOH provided **3.11** as a white solid (750mg, yield 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.8 (m, 2H); 2.2 (m, 1H); 3.55 (t, 2H); 4.45 (d, 1H); 6.6 (bs, 1H); 7.4 (t, 2H, *J* = 7Hz); 7.5 (t, 3H, *J* = 7Hz); 7.6

(t, 1H, *J* = 8.5Hz); 7.75 (d, 2H, *J* = 7Hz); 8.0 (d, 2H, *J* = 7Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24, 29.65, 30.25, 60.4, 66.25, 75.4, 125.4, 126.8, 128.1, 128.5, 128.8, 131.45, 133.05, 134.4, 167.5, 179.0

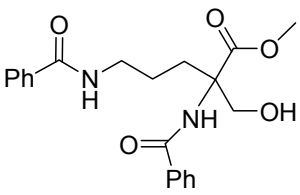
3.5.4 Synthesis of 2-hydroxymethylornithine monohydrochloride (**2.5**)

N-(3-(5-Benzamido-4-oxo-1,3-dioxan-5-yl)propyl)benzamide **(3.12)**



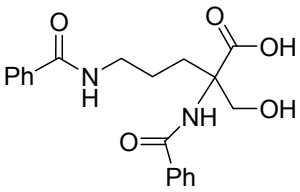
To a flask containing **3.11** (3.0g, 8.69mmol) was added pyridine (3.5ml, 43.48mmol) and formaldehyde (37%) (11.26ml, 408.7mmol) and the resulting reaction mixture stirred for 20h at room temperature. The reaction was quenched by the addition of H_2O upon which white precipitate formed. The white precipitate was removed by filtration and dried over P_2O_5. The white solid was recrystallized from acetone/pentane to give **3.12** as a white solid (2.75g, yield 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.90 (m, 2H); 2.15 (m, 2H); 3.4 (m, 1H); 3.75 (m, 1H) 3.98 (dd 1H, *J* = 9.5, *J* =1.5Hz) 4.30 (d, 1H, *J* = 10.9Hz); 5.44 (dd, 2H, *J* = 3.4, *J* = 1.7Hz); 6.6 (bs, 1H); 7.4-7.6 (m, 6H); 7.8 (dd, 4H).

Methyl 2,5-bis(benzamido)-2-(hydroxymethyl)pentanoate **(3.13)**



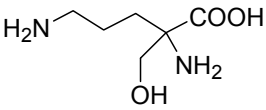
3.12 (500mg, 1.309mmol) was dissolved in MeOH (5ml) to form a white suspension to which NaOMe (15.3mg, 0.284mmol) was added in one portion. The reaction mixture was stirred until homogenous (~15min) after which the pH were adjusted to 7 with HCl solution (1M). Removal of the solvent under reduced pressure afforded the crude residue which was dissolved in H_2O (1ml). The aqueous layer was extracted with CH_2Cl_2 (5x2ml) and the combined organic layers dried over $MgSO_4$. Filtration followed by solvent removal *in vacuo* provided **2.13** as a white solid (463mg, yield 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.6 (m, 2H); 2.0 (m, 1H); 2.55 (m, 1H); 3.5 (m, 2H); 3.85 (s, 3H); 3.95 (q, 2H, *J* =5Hz); 4.35 (q, 2H, *J* =5Hz); 6.35 (bs, 1H); 7.4-7.6 (m, 6H); 7.8 (dd, 4H).

2,5-Bis(benzamido)-2-(hydroxymethyl)pentanoic acid **(3.14)**



3.12 (1.00g, 2.61mmol) was dissolved in dioxane (20ml) and stirred until a uniform reaction mixture formed. NaOH (2M, 20ml) was added to the reaction mixture and stirred until the reaction mixture became homogenous. The dioxane was removed under reduced pressure and the residue acidified with a HCl (2M) solution until the pH ~ 2. The white precipitate that formed was dissolved in EtOAc (20ml) and the organic layer washed with H_2O (3x10ml) after which it was dried over Na_2SO_4. Filtration followed by solvent removal *in vacuo* provided **3.14** as a yellow oil which formed a white solid over time (870mg, yield 90%). ^{1}H NMR (400 MHz, D_2O):δ 1.60 (m, 2H); 2.10 (m, 2H); 3.4 (t, 2H, *J* = 6.5Hz); 4.0 (d, 2H, *J* = 10.2Hz); 4.10 (d, 2H, *J* = 10.2Hz); 7.4-7.6 (m, 6H); 7.8 (dd, 4H).

2-Hydroxymethyl ornithine monohydrochloride **(2.5*HCl*)**



a) To a flask containing **3.13** (770mg, 2.00mmol) was added HCl (6M, 5ml) and the reaction mixture was heated to reflux for 48h. The reaction mixture was allowed to cool to room temperature and the white precipitate removed by filtration. The filtrate was washed with diethyl ether (3x5ml) after which the aqueous layer was lyophilised until constant weight was obtained to provide **2.5*HCl*** as a orange solid (344mg, yield 90%). ^{1}H NMR (400MHz, D_2O): δ 1.60 (m, 1H); 1.70-1.95 (m, 3H); 2.90 (t, 2H, *J* = 7.6Hz); 3.8 (dd, 2H, *J* =12.2Hz)

b) To a flask containing **3.14** (1.094g, 2.95mmol) was added HCl (6M, 10ml) and the reaction mixture heated to reflux for 24h. The reaction mixture was allowed to cool to room temperature and the white precipitate removed by filtration. The filtrate was washed with diethyl ether (3x10ml) after which the aqueous layer were lyophilized (x 2) to provide **2.5** as an orange solid (344mg, yield 90%). ^{1}H NMR (400MHz, D_2O): δ 1.60 (m, 1H); 1.70-1.95 (m, 3H); 2.90 (t, 2H, *J* = 7.6Hz); 3.8 (dd, 2H, *J* =12.2Hz)

3.6 References

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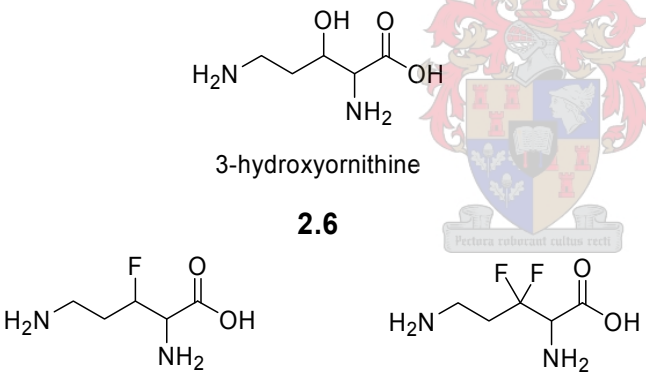
Pectora roborant cultus recti

Chapter 4

Synthesis of 3-hydroxyornithine as precursor to 3-fluoro-ornithine and 3,3-difluoro-ornithine

4.1 Introduction

To the best of our knowledge both 3-fluoro-ornithine (**2.8**) and 3,3-difluoro-ornithine (**2.9**) are fluorinated ornithine analogues that have neither previously been synthesised nor tested as inhibitors of *T. brucei's* ODC. However, since these molecules should act as mechanism-based inhibitors of this enzyme in a similar manner to DFMO, they may well hold promise as new ODC inhibitors to combat African sleeping sickness. As such, we decided to synthesise the fluorinated analogues and test them as inhibitors of ODC.



3-hydroxyornithine **2.6**

3-fluoro-ornithine **2.7**

3,3-difluoro-ornithine **2.8**

Figure 4.1: The structures of 3-hydroxyornithine (2.6), 3-fluoro-ornithine (2.7) and 3,3-difluoro-ornithine (2.8).

A retrosynthetic analysis (Figure 4.2) of 3,3-difluoro-ornithine (**2.7**) suggests 3-hydroxyornithine (**2.7**) as a possible synthetic precursor. This follows after two functional group interconversions, represented by a fluorodehydroxylation reaction and the oxidation of an alcohol, of the target molecule 3-hydroxyornithine (**2.6**). The molecule **2.6** may also be fluorodehydroxylated directly to give the

monofluorinated analogue 3-fluoro-ornithine (**2.7**). A further retrosynthesis on the hydroxy amino acid **2.6** suggests two strategies for its synthesis, each of which will be discussed below. Additionally, 3-hydroxy-ornithine is an ornithine analogue that is important in various other areas of research. These include its use as an intermediate of important natural products like β-lactams and amino polyols, as well as biosynthetic precursor to both the β-lactamase inhibitor clavulanic acid and the anticancer agent, activin (*1-4*). It is possible that the fluorinated analogues that we want to synthesise might be valuable in those areas as well.

The first proposed strategy to synthesise 3-hydroxyornithine is based on an aldol condensation (figure 4.2). Aldol reactions are normally easy to perform, generally give moderate to high yields of product and, most importantly, the formation of a hydroxyl group is inherent to the reaction. The disconnect that suggests an aldol reaction is made between the α- and β-carbons, giving a glycine equivalent and an appropriate aldehyde as the two starting materials. In our case, the appropriate aldehyde would be 3-*N*-aminopropionaldehyde with a suitable protecting group on the amine functionality. The commercially available 3–[(benzyloxycarbonyl)-amino] propionaldehyde (**4.2**) was obtained to be used as the appropriate aldehyde.

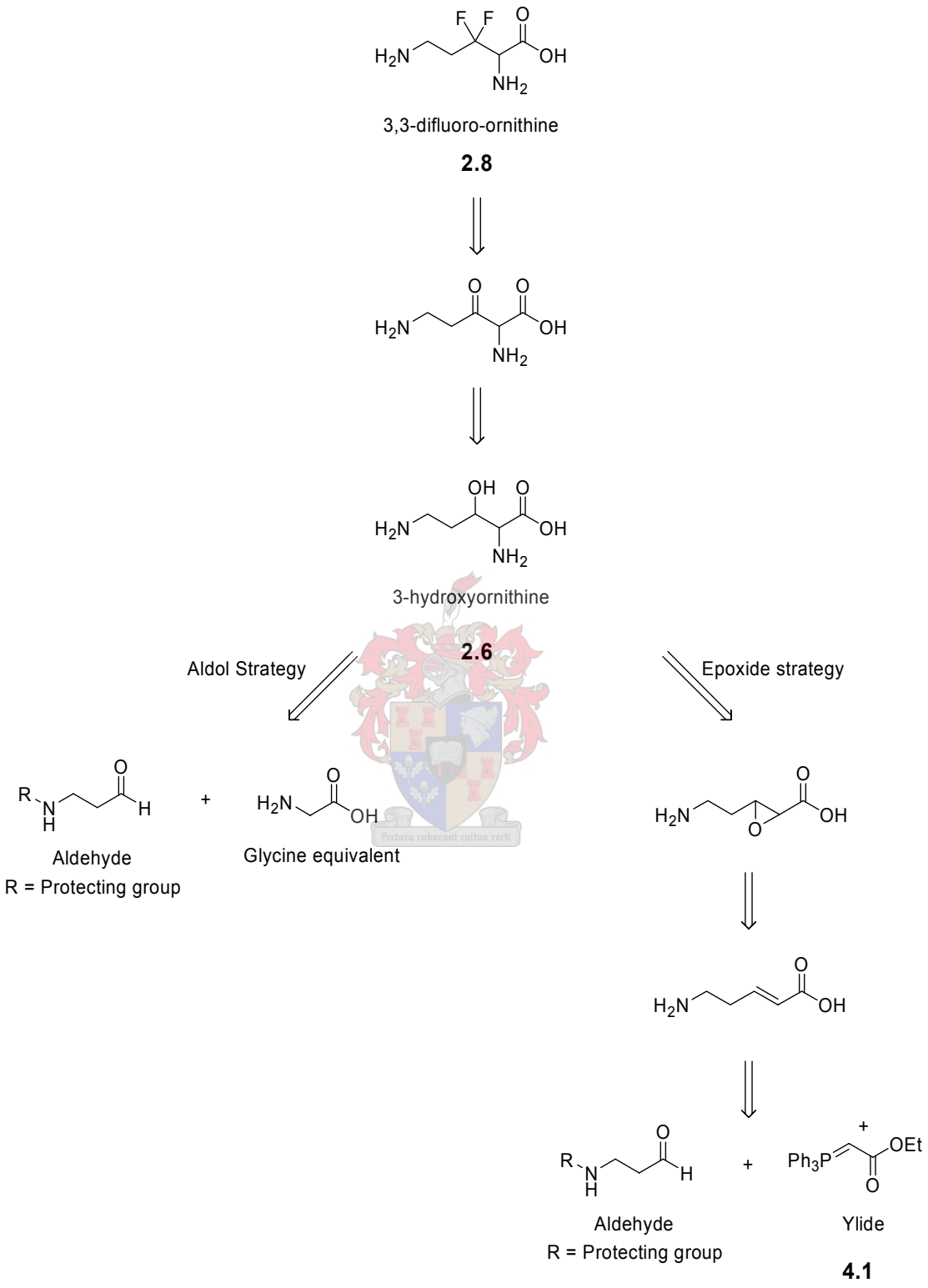


Figure 4.2: Retrosynthesis of 3,3-difluoro-ornithine to yield synthons for both the aldol- and epoxide synthetic strategies for the synthesis of 3-hydroxyornithine.

There are a large number of available glycine equivalents that can be used in the synthesis of 3-hydroxyornithine. These differ from equivalents which are cheap and simple to use (and which do little more than act as a stabilising unit for the glycine enolate) to glycine equivalents that are more expensive but also more versatile and allows for the controlled introduction of chirality into the formed aldol product. As an example three different glycine equivalents and their representing reactions are shown in figure 4.3.

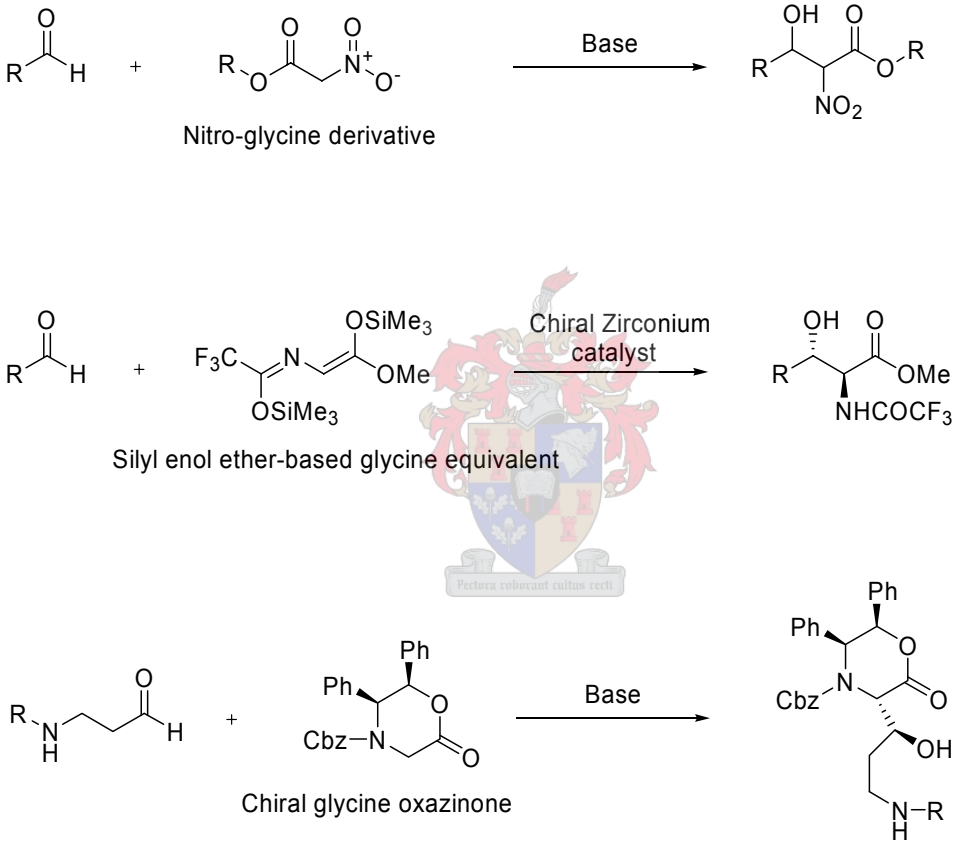


Figure 4.3: Three different glycine equivalents; nitro-glycine, silicon-based with chiral auxiliary and the chiral glycine oxazinone

As a first example, the nitroaldol reaction is a common reaction for the formation of C-C linkages with the introduction of hydroxyl- and nitro-functional groups together with the possible formation of one or two stereogenic centres (*5*-*7*). The use of the nitro-aldol reaction and its appropriate nitro-glycine equivalent is an example of an affordable glycine equivalent. The second and third examples are more expensive

and versatile glycine equivalents. In some cases the glycine equivalent themselves do not induce specific chirality but need a chiral catalyst such as the silyl enol ether based glycine equivalent used by Kobayashi *et al.* together with a chiral catalyst to synthesise *anti*-β-hydroxy-α-amino acids (*8*). Other glycine equivalents do induce specific chirality by themselves, for example the chiral oxazinone glycine equivalent used by Demong *et al*. This glycine equivalent was specifically used to synthesise 3-hydroxyornithine, the target molecule of our intended synthesis (*9*).

The second proposed strategy to prepare 3-hydroxyornithine (figure 4.2) is by ring-opening of an epoxide with an appropriate amine; the epoxide in turn is formed by epoxidation of an alkene which is obtained from a Wittig reaction. In this case the formal disconnect on the precursor molecule is also between the α- and β-carbons, although the disconnect now relates to the Wittig coupling as depicted in the retrosynthesis (figure 4.2). The Wittig reaction is accomplished by using the same commercially available aldehyde **4.2**, in reaction with the ylide 2-(ethoxy-2-oxoethyl) triphenylphosphorane (**4.1**).

We thus set out to investigate both these strategies for the synthesis of 3-hydroxyornithine (**2.7**) as precursor to the fluorinated ornithine analogues 3-fluoroornithine (**2.8**) and 3,3-difluoro-ornithine (**2.9**).

4.2 Strategy 1: The use of an aldol condensation to synthesise 3-hydroxyornithine (2.7).

As a first attempt at using an aldol condensation we decided to synthesise 3-hydroxyornithine using a nitro-aldol reaction. Soengas *et al.* used ethyl nitroacetate as a glycine equivalent to synthesise a β-hydroxy-α-amino acid as product (*6*). The second attempt at using an aldol condensation will be based on the method proposed by Demong *et al.* for the synthesis of 3-hydroxyornithine.

4.2.1 Synthesis of 5-Benzyloxycarbonylamino-3-hydroxy-2-nitro-pentanoic acid ethyl ester (**4.3**)

The advantage of the nitro-aldol reaction is that the starting materials are cheap and the reaction is easy to perform. The disadvantage is that the yield of the reaction is not always very high and that the products are obtained as racemic mixtures. An extensive literature search revealed other reaction conditions as alternatives to those used by Soengas *et al.* (Route A). First, Ballini and Bosica (*10*) performed a large number of nitroaldol reactions in aqueous media, with 0.025 M NaOH as base in the presence of cetyltrimethylammonium chloride (CTACl) as a phase transfer catalyst (Route B). Second, reaction conditions used by Corey *et al. (11)* in a similar nitroaldol reaction were also attempted. The reaction conditions used by Corey *et al.* (*11*) are very similar to those used by Soengas *et al.*; however instead of 2-propanol as solvent Corey *et al.* used THF and finely powdered KF (Route C).

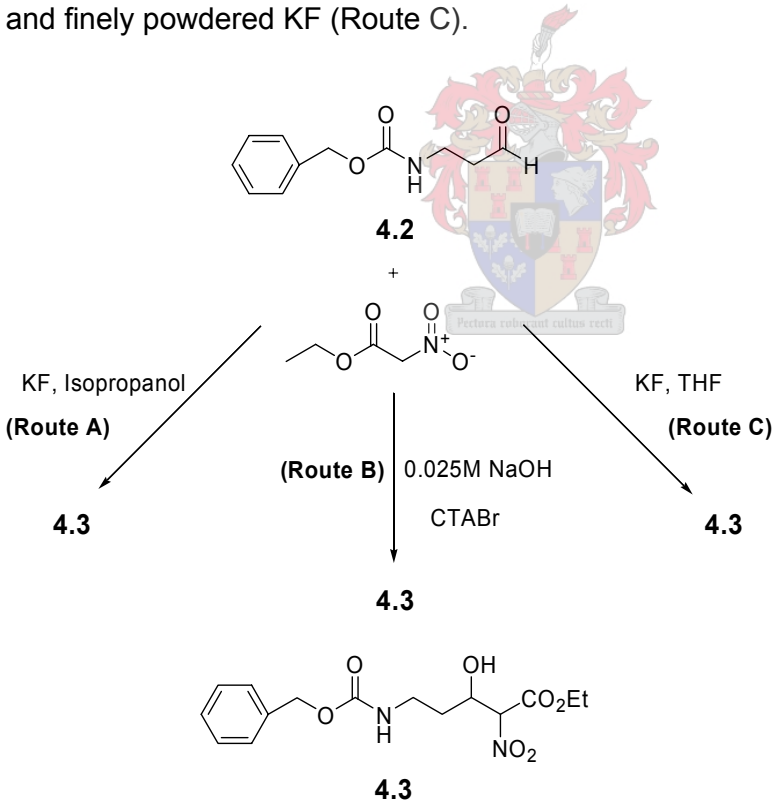


Figure 4.4: Nitro-aldol reaction with ethyl nitroacetate to synthesise 4.3

Initial attempts at the synthesis of **4.3** from nitro-ethyl acetate and **4.2** using the reaction conditions proposed by Soengas *et al.* (see figure 4.4 above) was

unsuccessful, as no product formation could be discerned by TLC analysis. Unfortunately, attempts at the synthesis of **4.3** using the reaction conditions prescribed by Corey *et al.*, using THF as solvent and finely powdered KF, and those by Ballini *et al.*, using cetyltrimethylammonium bromide (CTABr) instead of (CTACl) as a phase transfer catalyst, also proved unsuccessful. Monitoring the reactions by TLC indicated the presence of unreacted starting material and even after longer reaction times the situation did not improve. ^{1}H NMR data of crude reaction mixtures also failed to indicate any product formation.

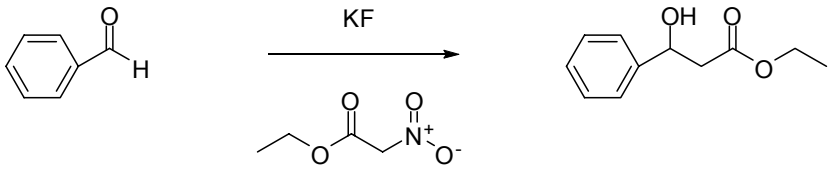


Figure 4.5: Reaction of benzaldehyde with ethyl nitroacetate

In order to test the reaction conditions we decided to perform the nitro-aldol reaction with benzaldehyde, another aldehyde that is commonly used in aldol reactions and which ought to give an aldol condensation product. The nitro-aldol reaction with benzalhyde was also attempted using all three different reaction conditions as for the reactions with **4.2**. Once again the reaction in our hands did not give any product, but only returned a mixture of starting materials. As we were unable to synthesise 3-hydroxyornthine using the nitro-aldol reaction, we decide to attempt the published synthesis of Demong *et al.* (*9*).

4.2.2 Synthesis of 3-(3-Benzyloxycarbonylamino-1-hydroxy-propyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylic acid benzyl ester (**4.5a/4.5b**)

The synthesis done by Demong *et al.* focused on the preparation of the specific (2*S*,3*S*)- and (2*R*,3*R*)- stereoisomers of 3-hydroxyornithine (*9*). To achieve this they used the commercially available chiral glycine oxazinone **4.4a** and **4.4b** to synthesise the different stereoisomer's. The glycine enolate was formed using di-*n*-butyl boron triflate as base after which the aldehyde (**4.2)** was added to the reaction mixture. The advantage gained by the use of this synthetic strategy is the

stereoselectivity of the reaction. However, the disadvantage of the reaction is the low yield obtained of the product (46% overall).

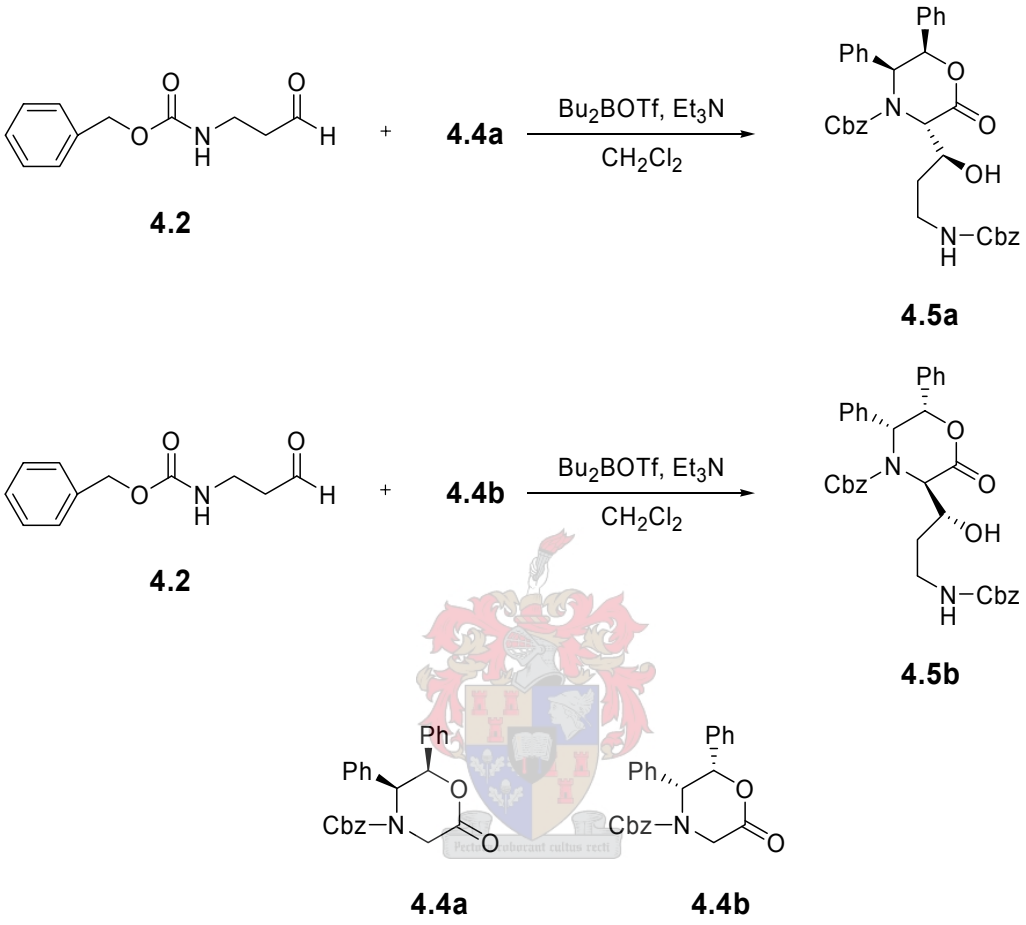
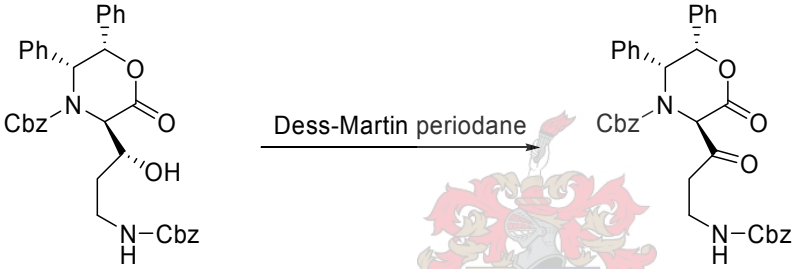


Figure 4.6: Synthesis of 4.6 and 4.7 from chiral boron glycine enolate.

The reaction of both of **4.4a** and **4.4b** with **4.2** to give **4.5a** and **4.5b** respectively was performed as prescribed in the literature. The reactions are depicted in figure 4.6. The synthesis of both **4.5a** and **4.5b** were successful according to ^{1}H NMR data that correlated with those published. The yields reported were higher than those achieved by us as we only managed to synthesise **4.5a** and **4.5b** in a yield of 23% and 32% respectively. We decided not to deprotect these precursors to 3-hydroxyornithine but to rather perform the fluorodehydroxylation reactions directly on **4.5a** and **4.5b**.

4.2.3 Synthesis of 3-(3-Benzyloxycarbonylaminopropionyl)-2-oxo-5,6-diphenyl-morpholine-4-carboxylic acid benzyl ester.

The synthesis of 3,3-difluoro-ornithine (**2.9**) is based on the transformation of a ketone functional group to the difluoro group. The oxidation of the hydroxyl group from the previously synthesised precursors to 3-hydroxyornithine, **4.5a** or **4.5b** would yield the appropriate ketone. We decided to attempt the oxidation of the hydroxyl group by using the Dess-Martin periodinane method. The use of the Dess-Martin periodinane as oxidation agent has two advantages, the mild conditions needed for the reaction to take place and the good yields of product normally obtained.



4.7

Figure 4.7: Oxidation of the hydroxyl-group of 4.5b to from the corresponding ketone using Dess-Martin periodinane

The oxidation reaction was done similar to reaction conditions for Dess-Martin periodinane oxidation in the literature. The reaction was done in CH_2Cl_2 overnight after which TLC analysis did not reveal any product formation. However, the polarities of both the hydroxyl compound as well as the ketone would be very similar and as such might not show a big difference on TLC. The formation of a carbonyl peak as shown by ^{13}C NMR analysis would determine if any product formed. The ^{13}C NMR data was not positive for the formation of a carbonyl peak and thus negative for product formation. Although other oxidation methods are available, such as Swern oxidation or the use of the TPAP oxidant we decided to postpone oxidation attempts till after successful fluorination was preformed which would indicate reactivity of the hydroxyl group (*12*).

4.3 Strategy 2: Epoxide chemistry to synthesise to 3-hydroxyornithine.

Two recent publications used epoxide chemistry to synthesise 3-hydroxyornithine and cyclic derivatives of 3-hydroxyornithine (*13, 14*). The use of an epoxide to introduce the hydroxyl group seemed like an attractive option since the epoxide can be utilised in a variety of ways. As a first attempt we decided to synthesise the epoxide as performed in the literature but open the epoxide with dibenzylamine instead of NaN_3 (*13*). The reason for opening the epoxide with dibenzylamine is illustrated by a similar synthesis preformed by Charvillon and Amouroux. They used dibenzylamine to open an epoxide to synthesise 3-hydroxy-aspartic acid. The hydroxyl group was subsequently transformed to the fluorine by use of a fluorodehydroxylation reaction. In the fluorodehydroxylation reaction the amino group had to be protected as a dibenzylamine group to prevent elimination of the fluorine and formation of a double bond (*15*). We will follow the same synthetic strategy to introduce the fluorine functionality after which deprotection will yield 3-fluoro-ornithine. This strategy is shown in figure 4.8

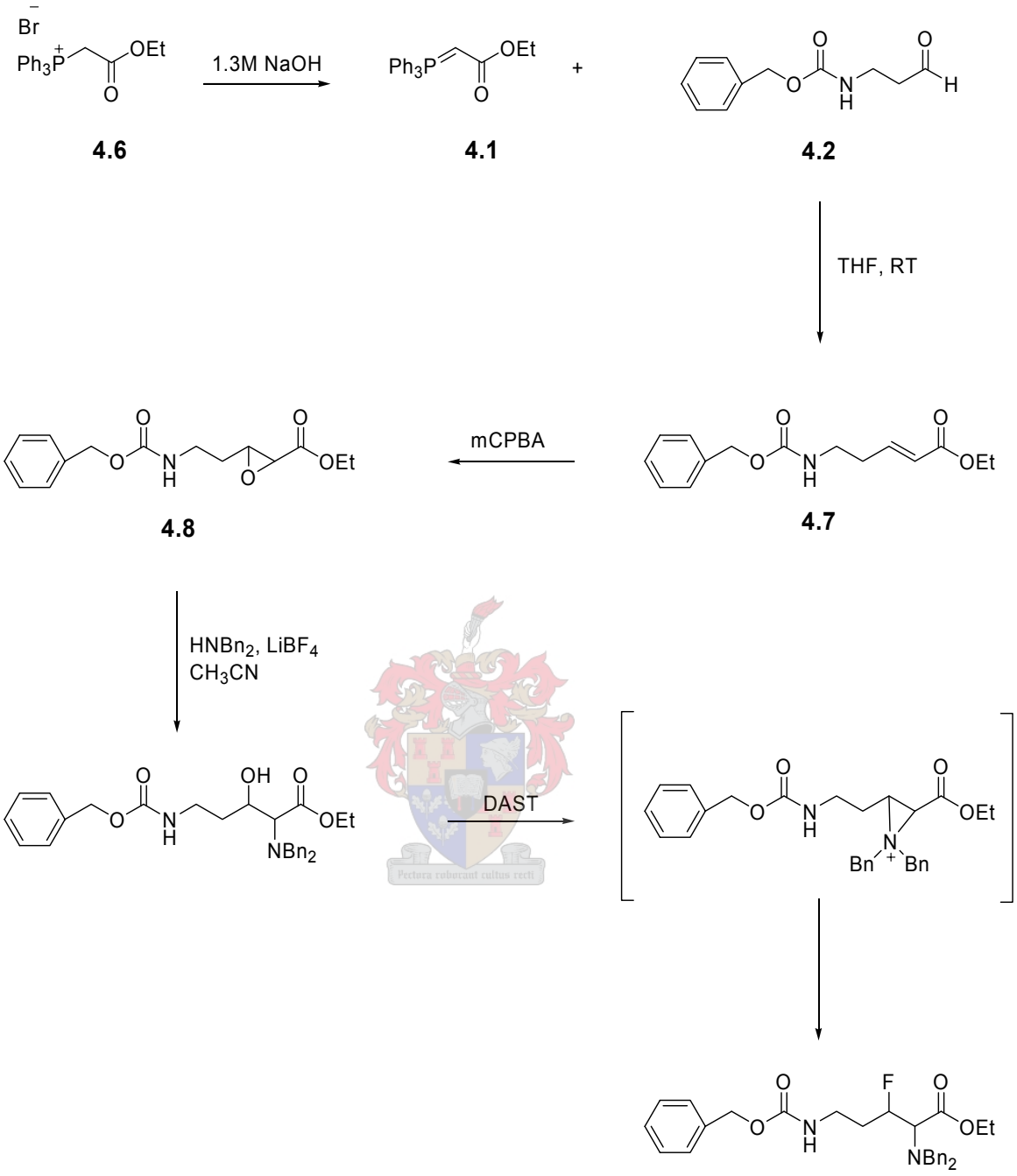


Figure 4.8: Synthesis of 3-fluoro-ornithine via epoxide chemistry

4.3.1 Synthesis of (*E*)-ethyl 5-(benzyloxycarbonylamino)pent-2-enoate (**4.10**)

The precursor for the ylide was available as the phosphonium bromide salt [(2-ethoxy-2-oxoethyl)triphenylphosphonium bromide] (**4.6**). **4.6** was crystallized from

toluene to afford pure colourless crystals. ^{1}H NMR data together with melting point determination confirmed the purity of the crystals; MP 155ºC (Lit. 150ºC).

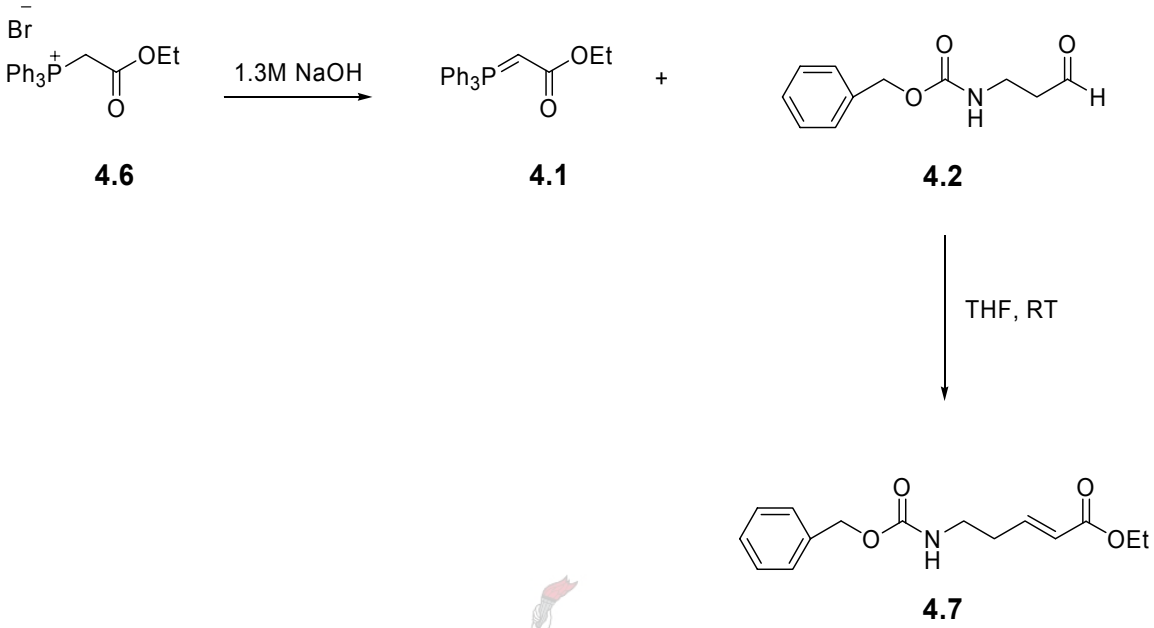
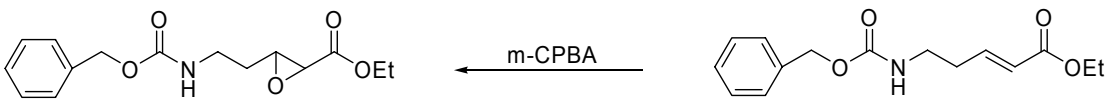


Figure 4.8: Synthesis of Wittig product 4.7

The phosphorane, (2-ethoxy-2-oxoethyl)idenetriphenylphosphorane (**4.1**) was synthesised by dissolving the bromide salt in CH_2Cl_2 to which a NaOH solution was added. The product crystallised from toluene/EtOH (9:1) as white crystals. ^{1}H NMR data confirmed the correct shifts for the product. The reaction of **4.2** with **4.1** was performed in THF at room temperature for 24h. The product was purified by flash chromatography to yield **4.7** in 78%. ^{1}H NMR data confirmed the Wittig product.

4.3.2 Synthesis of epoxide (**4.11**)

As a first attempt at synthesising the epoxide from the double bond we decided to use *m*-chloroperbenzoic acid (m-CPBA). A literature search revealed three different reaction conditions by which the epoxide could be formed. Firstly at low temperature (-55ºC) as done by Srinivasan *et al.* (*16*), secondly at room temperature and lastly at reflux in CH_2Cl_2 as done by Shen *et al.* (*17*).



4.7 **4.8**

Figure 4.9: Synthesis of epoxide from 4.10 using *m*-CPBA

No product formation could be detected for both the reactions at low temperature and at room temperature according to TLC analysis. The attempt at epoxide formation using the reflux reaction conditions only resulted in product degradation according to ^{1}H NMR analysis. Various other reactions to introduce the epoxide were found in the literature and will be attempted in the future (*18-20*).

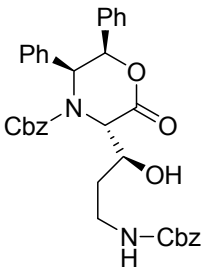
4.3 Conclusion

The attempted syntheses of 3-hydroxyornithine using the nitro-aldol reaction was unsuccessful in our hands. However, the synthesis of 3-hydroxyornithine as done by Demong *et al.* was successfully preformed to yield protected forms of 3-hydroxyornithine **4.6** and **4.7**. Although a first attempt at oxidation of the 3-hydroxyornithine precursor **4.7** with the Dess-Martin periodinane was unsuccessful various other oxidation methods may be employed. Synthesis of the Wittig product was successful although initial attempts at epoxide formation failed. The synthesis of 3-hydroxyornithine using the epoxide chemistry will be pursued in future work.

4.4 Experimental

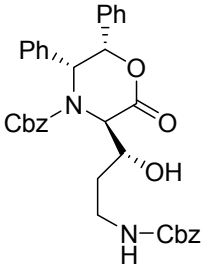
4.4.1 Synthesis of (3*R*,5*S*,6*R*)-benzyl 3-((*S*)-3-(benzyloxycarbonylamino)-1-hydroxypropyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate (**4.5a**) and (3*S*,5*R*,6*S*)-benzyl 3-((*R*)-3-(benzyloxycarbonylamino)-1-hydroxypropyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate (**4.5b**)

(3S,5S,6R)-benzyl 3-((S)-3-(benzyloxycarbonylamino)-1-hydroxypropyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate **(4.5a)**



4.4a (311.4mg, 0.803mmol) was dissolved in anhydrous CH_2Cl_2 (9.5ml) under an Ar atmosphere. The reaction was cooled to -5ºC and di-*n*-butyl boron triflate (1M in CH_2Cl_2, 1.606ml) was added dropwise via a syringe to the reaction mixture followed by the addition of triethylamine (335µl, 2.409mmol). The reaction mixture was stirred for 15min at -5ºC after which it was cooled to -78ºC and stirred for another 5min. In a separate flask 3–[(Benzyloxycarbonyl)-amino]propionaldehyde (**4.2**)(200mg, 0.965mmol) was dissolved in anhydrous CH_2Cl_2 (1.75ml) under Ar atmosphere until reaction mixture became homogenous. This solution was added drop wise to the boron enolate via a syringe. The reaction mixture was now stirred for 1h and subsequently quenched by the addition of potassium phosphate buffer (0.025M, pH 7) at -78ºC after which the reaction was allowed to warm to room temperature. The aqueous layer was extracted with CH_2Cl_2 (2x10ml) and dried over Na_2SO_4. Filtration and *in vacuo* removal of solvent provided the crude products as an oily residue. Two successive flash chromatography columns (silica gel; ethyl acetate/hexane 1:2; silica gel; CH_2Cl_2/ethyl acetate/hexane 3:1:1) followed by and recrystallization from hexane/ethyl acetate gave **4.5a** as a white solid (130mg, yield 23%) ^{1}H NMR (600MHz, $CDCl_3$): δ 1.91-2.35 (m, 2H); 3.45 (d, 1H, *J* = 10.9Hz); 3.68 (d, 1H, *J* = 10.9Hz); 4.29 (d, 1H, *J* = 10.7Hz); 4.87 (d, 1H, *J* = 12.5Hz); 5.02 (d, 1H, *J* = 12.5Hz); 5.12 (s, 2H); 5.30 (d, 1H, *J* = 3.3Hz); 5.20 (bs, 1H); 6.55 (d, 1H, *J* = 7.5Hz); 6.70 (d, 1H, *J* = 7.5Hz); 6.97-7.41 (m, 20H).

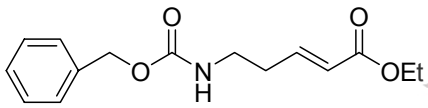
(3R,5R,6S)-benzyl 3-((R)-3-(benzyloxycarbonylamino)-1-hydroxypropyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate **(4.5b)**



4.5b was prepared in analogous way, **4.4b** was used instead of **4.4a** to give **4.5b** as white solid (184mg, yield 34%) ^{1}H NMR (600MHz, $CDCl_3$): δ 1.91-2.35 (m, 2H); 3.45 (d, 1H, *J* = 10.9Hz); 3.68 (d, 1H, *J* = 10.9Hz); 4.29 (d, 1H, *J* = 10.7Hz); 4.87 (d, 1H, *J* = 12.5Hz); 5.02 (d, 1H, *J* = 12.5Hz); 5.12 (s, 2H); 5.30 (d, 1H, *J* = 3.3Hz); 5.20 (bs, 1H); 6.55 (d, 1H, *J* = 7.5Hz); 6.70 (d, 1H, *J* = 7.5Hz); 6.97-7.41 (m, 20H).

4.4.2 Synthesis of (*E*)-ethyl 5-(benzyloxycarbonylamino)pent-2-enoate (**4.7**)

Synthesis of (E)-ethyl 5-(benzyloxycarbonylamino)pent-2-enoate **(4.7)**



To a solution of **4.1** (268mg, 0.772mmol) in dry THF (2ml) was added **4.2** (160mg, 0.772mmol) in one portion. The reaction mixture was stirred for 24h at room temperature after which the excess solvent was removed *in vacuo* to afford the crude product. The crude product was purified by flash chromatography (silica gel; ethyl acetate/hexane 3:1) to afford **4.7** as yellow oil (167mg, yield 78%) ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.3 (t, 3H, J = 7.3Hz); 2.40 (dq, 2H, *J* = 6.6, *J* = 6.2Hz); 3.35 (dq, 2H, *J* = 6.6, *J* = 6.4Hz) 4.2 (q, 2H, *J* = 7.2); 5.10 (s, 2H); 5.9 (d, 1H, *J* = 15.9Hz); 6.9 (m, 1H); 7.35 (bs, 5H).

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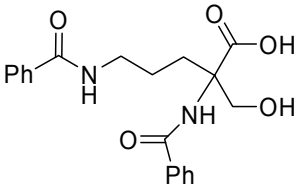
Chapter 5

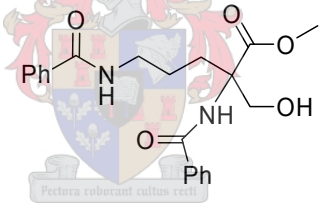
Attempts at fluorination of the hydroxylated precursors

5.1 Introduction

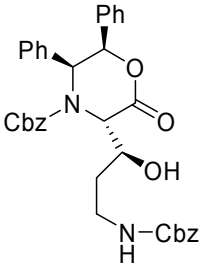
The final step in the preparation of the proposed fluorinated ornithine analogues is the optimization of the fluorodehydroxylation reaction, as set out in chapter 2. In chapters 3 and 4 the synthesis of the appropriately hydroxylated precursors were discussed. 2-(Hydroxymethyl)ornithine, the precursor of DFMO and 2-MFMO, was prepared with both the amine and carboxylate functionalities protected (**3.13**) as well as with only the amine groups protected (**3.14**). Chapter 4 showed the synthesis of 3-hydroxy-ornithine, the precursor to 3-fluoro-ornithine in protected form as the molecules **4.5a** and **4.5b** (figure 5.1). In this chapter the attempts at fluorodehydroxylation of these compounds will be discussed.



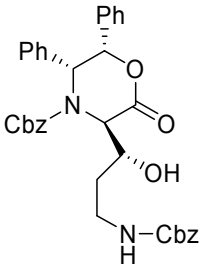
3.14



3.13



4.5a



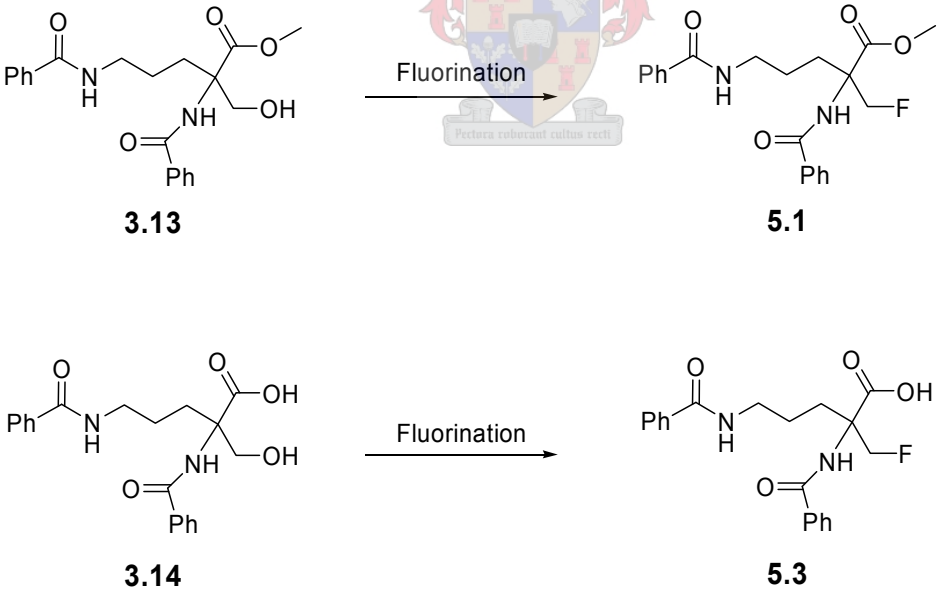
4.5b

Figure 5.1: Protected hydroxylated ornithine analogues on which fluorodehydroxylation were attempted

We decided to attempt two fluorodehydroxylation strategies. The first strategy will make use of the DAST and Deoxo-Fluor fluorodehydroxylation reagents to introduce the fluorine functionality into the precursor molecules. The second strategy will aim to make use of the halide replacement method to introduce the fluorine functionality. Both of these fluorodehydroxylation strategies were discussed in detail in chapter 2. Attempts at fluorodehydroxylation using both strategies were made on all the protected hydroxylated ornithine analogues shown above.

5.2 Strategy 1 on 2-hydroxymethyl precursors: Attempted introduction of a monofluoromethyl group using DAST

The first attempt at fluorination was directed at the 2-hydroxymethyl precursors **3.13** and **3.14**; the synthesis of which was discussed in chapter 3. Both of these precursors are supposed to give the monofluorinated products **5.1** and **5.3** respectively (figure 5.2).



3.13 **5.1**

3.14 **5.3**

Figure 5.2: Proposed fluorination reactions on 2-(hydroxymethyl)ornithine precursors 3.13 and 3.14

5.2.1 Synthesis of 2,5-bis-benzoylamino-2-fluoromethyl-pentanoic acid methyl ester **(5.1)**

Reaction conditions for the reaction of **3.13** with DAST using 1.2 eq were obtained from the literature (*1, 2*). The reaction was monitored by TLC and after completion the crude reaction mixture was purified by flash chromatography. The ^{1}H NMR data showed a shift in the signals for hydroxymethyl protons, which is to be expected due to the influence of the fluorine atom. A mass spectrum was also obtained to confirm the introduction of the fluorine. However, the mass spectrum indicated that the introduction of the fluorine was not successful, but that the main product formed instead is the oxazoline **5.2** in 94% yield, as shown in figure 5.3.

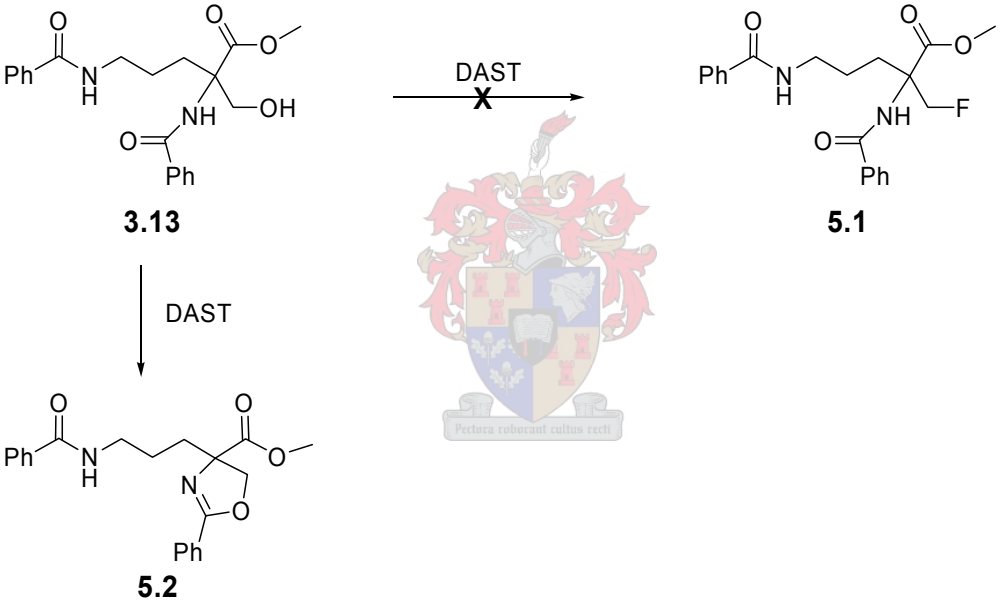


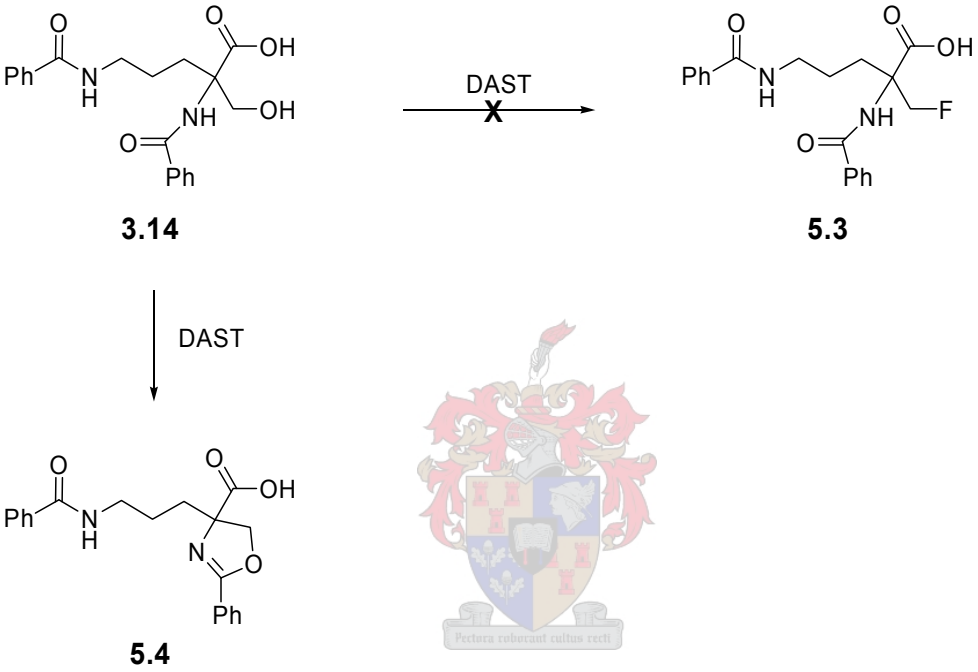
Figure 5.3: Fluorination using DAST on 3.13 to give oxazoline 5.2

This result shows that while the DAST reactant activates the hydroxyl group as expected, subsequent ring closure takes place in preference to substitution with fluorine.

5.2.2 Synthesis of 2,5-bis-benzoylamino-2-fluoromethyl-pentanoic acid **(5.3)**

The same reactions conditions that were used for the DAST reaction with **3.13** were also used for the reaction with **3.14**. After completion of the reaction the crude product was purified by flash chromatography.. The sample was sent for

both NMR and MS analysis to confirm that the product (**5.3**) formed. NMR analysis once again showed a shift in the signal for hydroxymethyl protons, but the MS spectrum did not correlate with the introduction of a fluorine atom. Instead, the formed product was the oxazoline **5.4** (Figure 5.4), indicating that in this case the oxazoline product also forms in preference. The yield of the reaction was low at 31%.



3.14 **5.3** **5.4**

Figure 5.4: Fluorination using DAST on 3.14 resulting in the formation of the oxazoline 4.2

Our finding that it was possible to purify the free carboxylic acid on silica gel was surprising, as it is generally very difficult to chromatograph a free carboxylic acid on a silica column. While it is postulated that the oxazoline and the phenyl rings give enough non-polar character to the molecule so that some of **5.4** could be eluted, the low yield can probably be ascribed to a loss of product during purification.

5.3 Strategy 2 on 2-hydroxymethyl precursors: Introduction of a monofluoromethyl by halogen replacement

Lafargue *et al.* reported a halogen replacement reaction where the hydroxyl group was substituted with bromine, after which the bromine is exchanged with fluorine (*3*). In this synthesis the hydroxyl group was substituted for bromine using CBr_4 and PPh_3 in CH_2Cl_2. The fluorine was introduced using an inorganic source of fluorine, namely CsF. They achieved an overall yield of 72% for the fluorination step which held good promise for our attempts at the introduction of the fluorine atoms in our hydroxylated precursors.

5.3.1 Attempted syntheses of 2,5-bis-benzoylamino-2-bromomethylpentanoic acid methyl ester (**5.5**) and 2,5-bis-benzoylamino-2-bromomethylpentanoic acid (**5.6**) from the bromine intermediate using CBr_4/PPh_3

The reaction of CBr_4/PPh_3 with **3.13** was performed according to the reaction conditions described in the literature, but instead of the brominated product we once again obtained the oxazoline **5.2** in 84% yield as deduced form the NMR and MS data. The reactions are shown in figure 5.5 below.

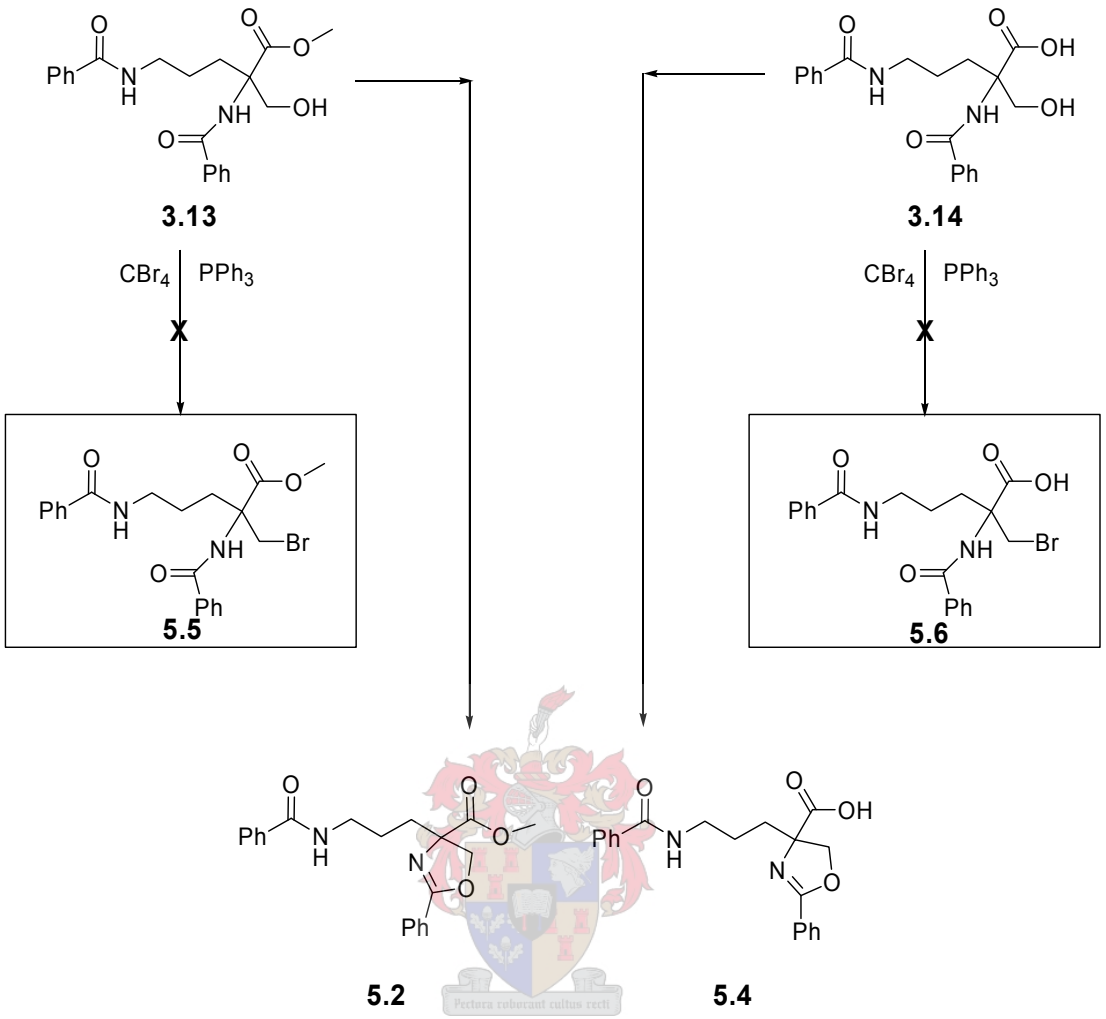


Figure 5.5: Reaction of CBr_4/PPh_3 with the methyl ester (3.13) and the free acid (3.14) to form 5.2 and 5.4 instead of 5.5 and 5.6.

The oxazoline (**5.2**) is probably formed by direct attack of the amide carbonyl on either the bromine (**5.5**) or phosphonium intermediate. This is also true for the reaction of CBr_4/PPh_3 with **3.14**. The main reaction product of CBr_4/PPh_3 with **3.14** was the oxazoline **5.4** according to TLC analysis by comparison to the previously purified compound. Since no other discernible products were formed, compound **5.4** was not purified from this reaction mixture.

5.3.2Attempted synthesis of *N*-[3-(4-Fluoromethyl-5-oxo-2-phenyl-4,5-dihydro-oxazol-4-yl)-propyl]-benzamide (**5.13**) using HBr/AcOH/Ac_2O and CsF

All attempts at fluorination using the fluorodehydroxylation methods thus far only yielded the formation of the oxazoline rings **5.2** and **5.4**. A literature search was performed to determine if we can use the formation of the oxazoline to our advantage. A publication by Obrecth *et al.* proposed the trans-ring substitution of an oxazoline to a azlactone using HBr/AcOH/Ac_2O together with the transformation of the hydroxyl group to a bromine (*4*). The proposed reaction mechanism is shown in figure 5.6.

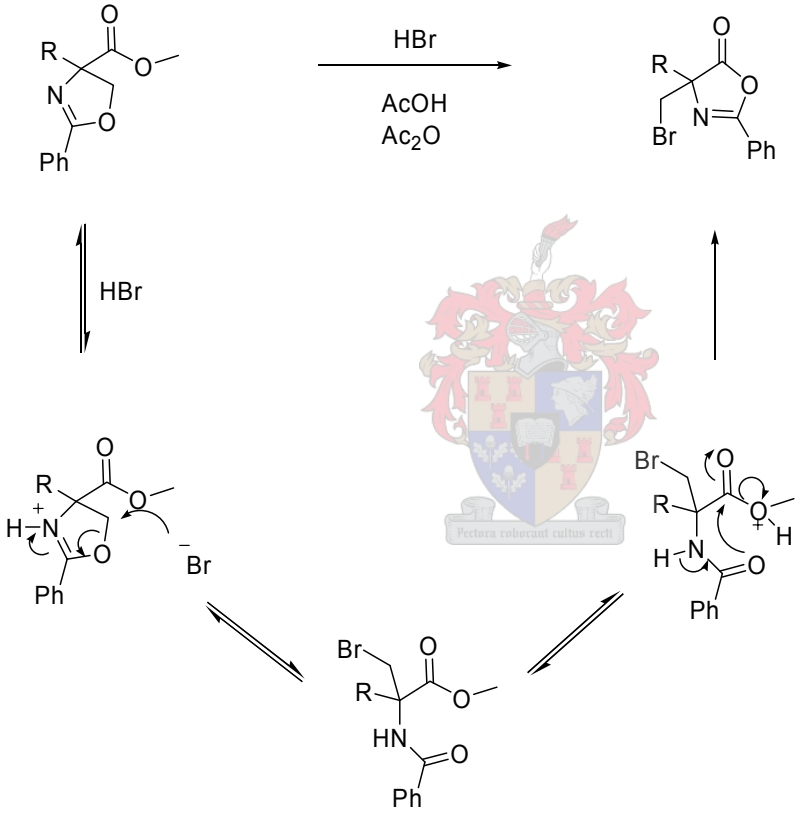
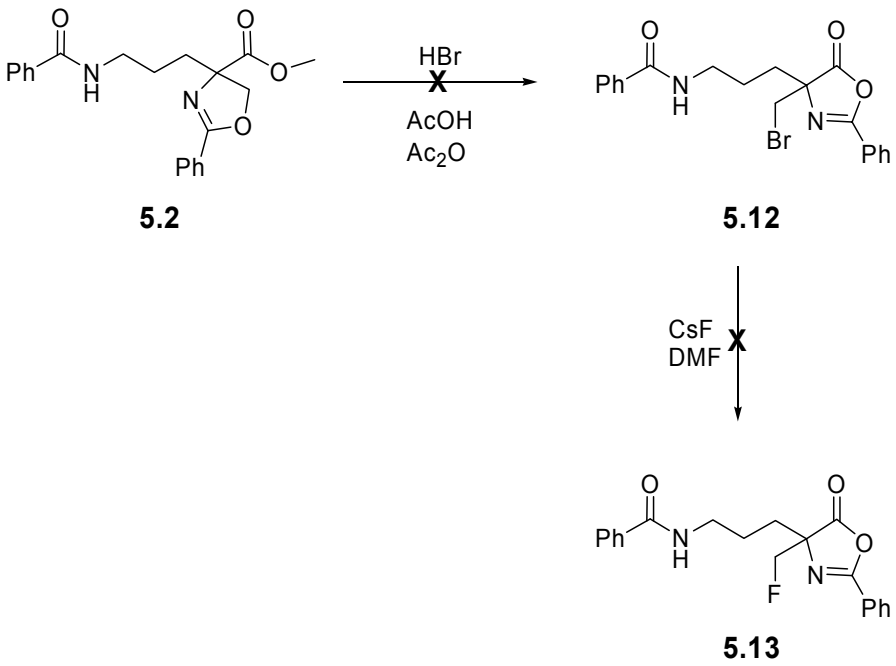


Figure 5.6: Proposed mechanism by which HBr opens the oxazoline and closes it as an azlactone with a bromomethyl substituent.

If the same trans-ring substitution could take place in our case, we would be able to use the formed oxazoline **5.2** to form the azlactone **5.12**. Fluorination of **5.12** with CsF as prescribed in the literature would then yield the mono-fluorinated product **5.13**, as proposed in figure 5.7.



5.2 **5.12** **5.13**

Figure 5.7: Proposed HBr/CsF reaction to yield the fluorinated product.

The reaction of **5.2** with HBr/AcOH/Ac_2O was performed according to the reaction conditions as prescribed in the literature (*4*). The reaction mixture was found to be a mixture of products and attempts at purification were unsuccessful. We subsequently decided to rather perform the reaction in one pot, with addition of the CsF immediately after formation of the bromine azlactone **5.12**. However, a second attempt at the reaction of **5.2** and HBr/AcOH/Ac_2O was also unsuccessful. MS analysis of the crude HBr/AcOH/Ac_2O reaction showed that the bromo-azlactone only formed in trace amounts, with the main components of the HBr/AcOH/Ac_2O reaction mixture being starting material **3.13** as well as some **3.14**. Formation of **3.14** can be explained due to the acidic nature of the reaction mixture which hydrolysed the methyl ester to the carboxylic acid. MS analysis of the reaction after addition of CsF showed only trace amounts of the fluorinated product together with the starting materials **3.13** and **3.14**. While we believe that synthesis of the fluorinated product using the trans-ring substitution may be possible, it is clear that the reaction conditions will have to be optimised if this is to be pursued.

5.3.3 Synthesis of 2,5-bis-benzoylamino-2-chloromethylpentanoic acid (**5.7**) using $SOCl_2$ to introduce chlorine as halogen intermediate.

Since the exchange of the hydroxyl with a bromo group always resulted in the formation of the oxazoline, we decided to introduce chlorine instead, as it is not as good a leaving group as bromine. The reaction would follow the same rationale as before with introduction of the fluorine by halide replacement using CsF. We decided to introduce the chlorine derivative with thionylchloride with reaction conditions obtained from the literature (*5*). The reaction was attempted on **3.14** because of a large amount of **3.14** available at that time

The chlorination reaction was followed to completion by TLC and the crude reaction mixture purified by flash chromatography. The NMR and MS analysis showed that the product formed was neither the chlorinated derivative nor the oxazoline, but rather the spiro compound **5.8** in which the 5-membered oxazoline is joined to a 6-membered ring at the same carbon (Figure 5.8). The product was obtained in a yield of 50%. A detailed analysis of the carbon and proton spectra is given in tabulated form in the experimental section.

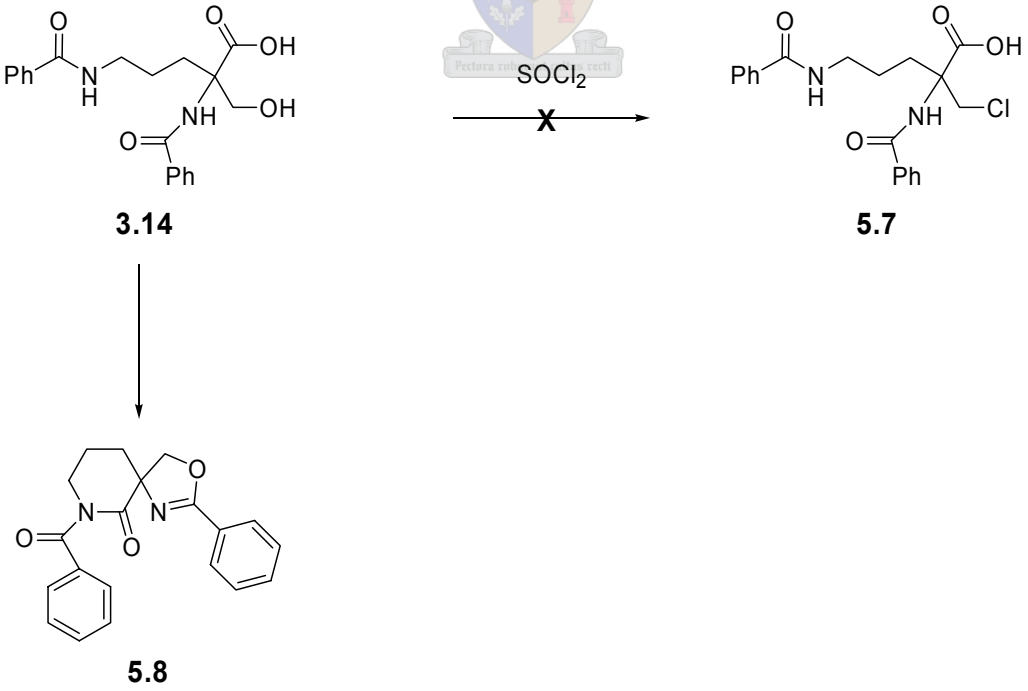
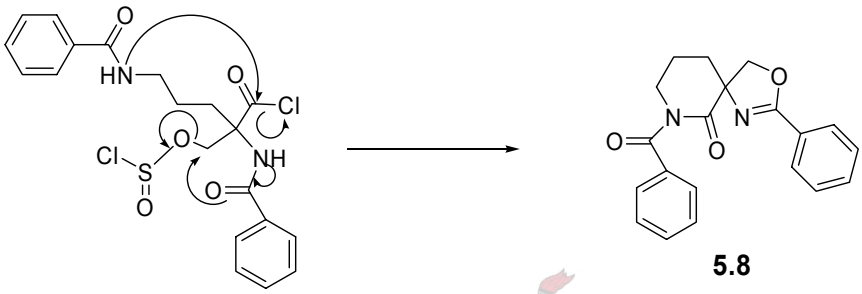


Figure 5.8: Ring formation after reaction of $SOCl_2$ with 3.14

At first this result was somewhat puzzling; however we had not taken into consideration the possible formation of the acid chloride of **3.14**. Reaction of $SOCl_2$ with **3.14** activated the carboxylic acid as the acid chloride which was subsequently attacked by the δ amine to form the 6-membered imide ring. The $SOCl_2$ also activated the hydroxyl group for nucleophilic attack by the amide carbonyl to form the oxazoline 5-membered ring. The postulated mechanism of ring formation is shown in figure 5.8.



5.8

Figure 5.9: Mechanism of ring formation of the spiro-ring 5.8

The isolated product also crystallized from EtOAc/Hexane; a solvated sample was subsequently sent for single crystal X-ray diffraction. The postulated product structure was thus also confirmed by the crystal data. Figure 5.10 shows the crystal structure along the plane of the six membered ring showing the five membered ring on the right with the two phenyl groups facing upwards.

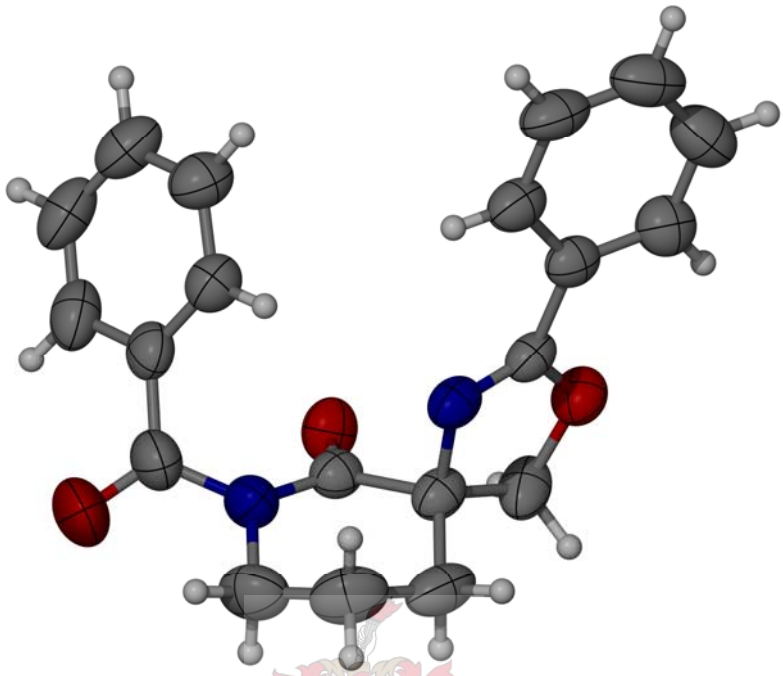


Figure 5.10: ORTEP diagram: View of crystal structure looking into the 6-membered imide ring with the attached 5-membered oxazoline on the right hand side. Grey atoms (carbon), blue atoms (nitrogen), red atoms (oxygen).

The molecule can be seen as U shaped with the phenyl groups forming the arms and the six-membered ring at the bottom. The phenyl rings are flat and the bond lengths and angles are within accepted values for the ring closure. The phenyl ring attached to the five membered ring has a plane difference of 12.24˚ (0.15) to each other. The two phenyl rings are in planes at 74.62˚ (0.08) to each other.

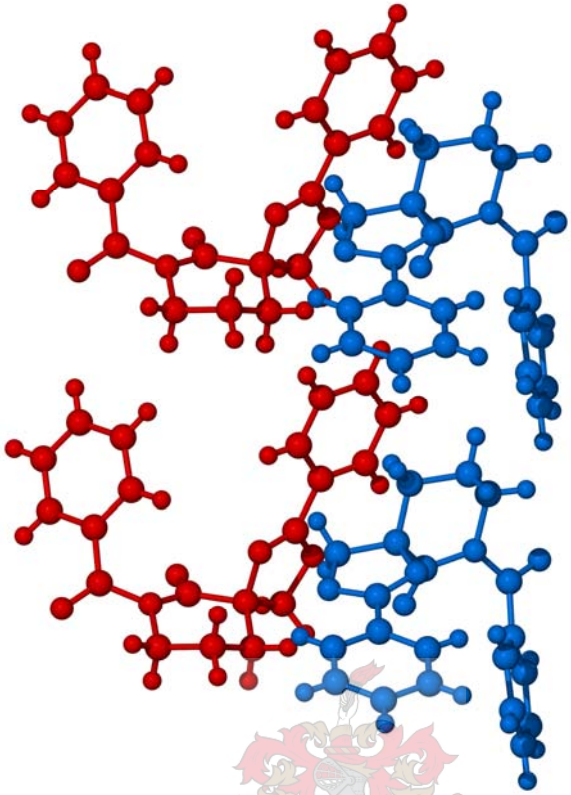


Figure 5.11: Crystal packing of the ring structure.

The crystal packing of the molecule is shown in figure 5.11. There are no clear cut distinctions such as hydrogen bonds explaining the specific packing although a few interesting facts can be mentioned. The U-shaped molecule (red) is stacked along the a-axis upon each other in a translational manner. The adjacent layer (blue) is related by a 2-fold screw axis parallel to the b-axis. The rest of crystal structure layers are translational repetitions of these charactaristics. Also, there is a close contact between the imide carbonyl group and the adjacent phenyl ring.

5.4 Selective azlactone closure with AcCl

Thus far, the 2-hydroxymethyl precursors **3.13** and **3.14** both formed the corresponding oxazoline rings whether fluorination was attempted by either of the fluorodehydroxylation strategies. Phillips *et al.* confirmed this finding, showing that the treatment of β-hydroxy amides with DAST and Deoxo-Fluor leads to the

formation of oxazolines and oxazoles (*1*). To eliminate the presence of the β-hydroxy amide we decided to rather focus on the initial formation of the azlactone **5.9** which leaves the hydroxymethyl group free to react without side reactions taking place. To form **5.9**, we postulated that the carboxylic acid of **3.14** could be selectively activated in the presence of the hydroxymethyl group for formation of the azlactone and would occur by preference if only one equivalent of cyclising agent were added.

5.4.1 Synthesis of *N*-[3-(4-Hydroxymethyl-5-oxo-2-phenyl-4,5-dihydro-oxazol-4-yl)-propyl]benzamide (**5.9**)

To synthesise the azlactone **5.9**, we treated **3.14** with one equivalent of acetyl chloride to activate the carboxylic acid as the mixed anhydride.

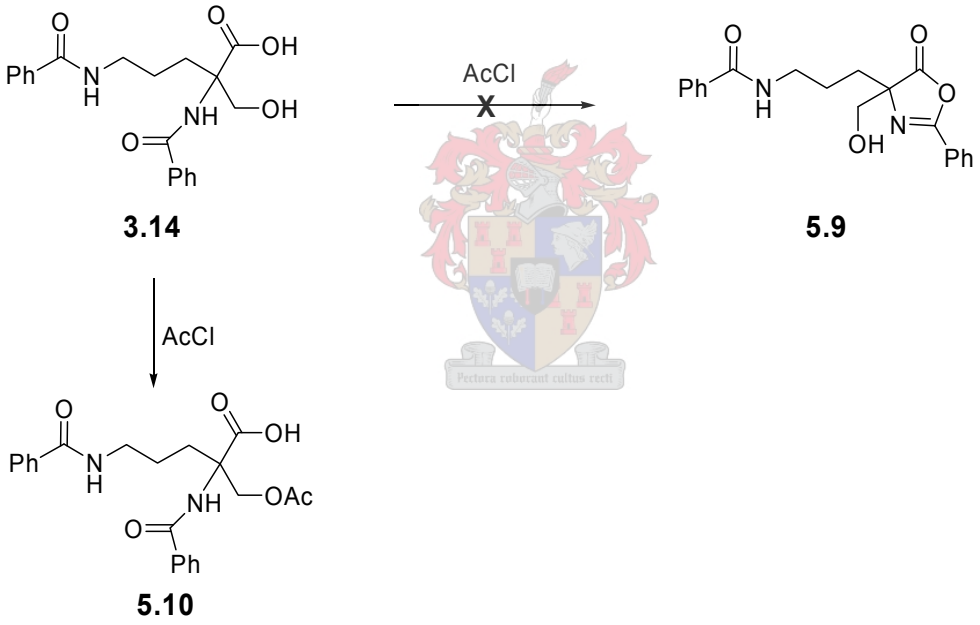


Figure 5.12: Attempted selective azlactone formation with AcCl

TLC analysis showed the formation of two products. A sample of the crude reaction mixture was sent for MS analysis to determine if the azlactone **5.9** had formed. The MS data revealed that two products which were formed were the acetylated starting material **5.10** and either the azlactone **5.9** or the oxazoline **5.4**. Since the NMR data could not distinguish between these two possibilities, we

decided to do a DAST reaction using 1.2 eq on the crude reaction mixture. The possible products that can form from the DAST reaction are shown in figure 5.13.

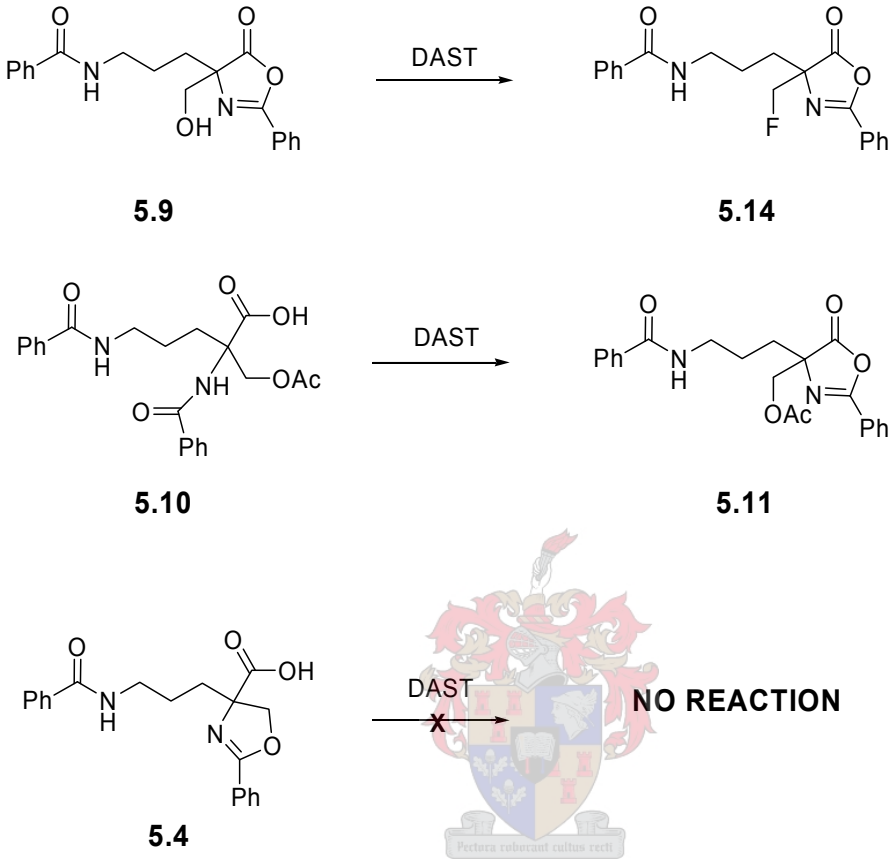


Figure 5.13: Possible product formation of the reaction of DAST with the crude reaction mixture of 3.14 treated with one equivalent of acetyl chloride

We expected that the reaction of **5.9** with DAST would yield the fluorinated analogue **5.14**, while its reaction with **5.10** (the presence of which is confirmed) should give the azlactone **5.11** as final product. The reaction of **5.4** with DAST should not give any product. A DAST reaction with the crude reaction mixture was attempted using the same reaction conditions as discussed before. MS and NMR analysis data of the DAST product mixture revealed the formation of two products: the expected azlactone **5.11** and the azlactone **5.4**. This reaction showed that the carboxylic acid is not more activated than the hydroxymethyl group and cannot be used to facilitate selective azlactone formation.

All attempts at the fluorination of our hydroxyl precursors resulted in unwanted side reactions. The fluorodehydroxylation reagents did activate the hydroxyl group as was expected, but the presence of the β-hydroxy amide resulted in the formation of the oxazoline. To solve this problem we will have to remove the protecting group or substitute it for a more appropriate one. We now turned our attention to the preparation of 3-fluoro-ornithine analogues.

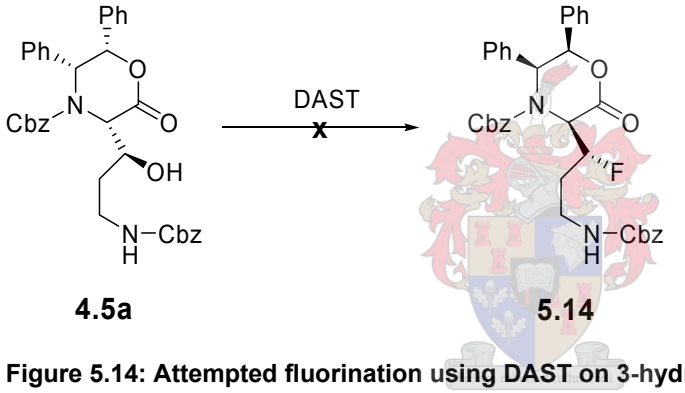
5.5 Strategy 1 on 3-hydroxy precursors: Fluorodehydroxylation using DAST and Deoxo-Fluor

A synthesis of (2*S*,3*R*,6*S*)-3-fluoro-2,6-diaminopimelic acid was preformed by Sutherland *et al.* (*2*). In the synthesis they also used DAST to transform a 3-hydroxyl group to the corresponding fluorine. Initial attempts at fluorination were unsuccessful with the only product formation the dehydrated compound. They accidentally succeeded in synthesis of the fluorinated compound by contamination of the DAST reaction with water which suppressed the dehydration of the activated hydroxyl group. In our fluorination attempts of the 3-hydroxy precursors using DAST and Deoxo-Fluor we followed the reaction procedure as described by Sutherland *et al.* with contamination of the reaction mixture with water (*2*).

We decided to try both fluorodehydroxylation reagents, namely DAST and Deoxo-Fluor, on the protected 3-hydroxyornithine precursors **4.5a** and **4.5b** to determine if the reagents differ in regards to yield and purity of the product obtained. While both reagents perform the same reaction, Deoxo-Fluor is a milder reagent than DAST and is less prone to side reactions or to the degradation of starting material. Since **4.5a** and **4.5b** only differ with regards to the stereochemistry of the hydroxyl group, it was assumed that they would react similarly to the fluorodehydroxylation reagents. For this reason the reaction with DAST was tested with **4.5a**, while **4.5b** was used to test Deoxo-Fluor.

5.5.1 Attempted synthesis of (3*R*,5*S*,6*R*)-benzyl 3-((*S*)-3-(benzyloxycarbonylamino)-1-fluoropropyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate (**5.14**)

Precursor **4.5a** was subsequently treated with DAST using 1.2 eq which was deliberately contaminated with water according to procedures published in the literature (*1, 6*) as shown in figure 5.14. According to TLC analysis no product formation could be discerned from reaction of **4.5a** with DAST, even after an extra equivalent of DAST was added. A sample of the reaction was sent for ^{1}H NMR and MS analysis, which also confirmed that the fluorination reaction had not occured. MS data confirmed the presence of unreacted **4.5a** as well as some unidentified degradation products of **4.5a**.

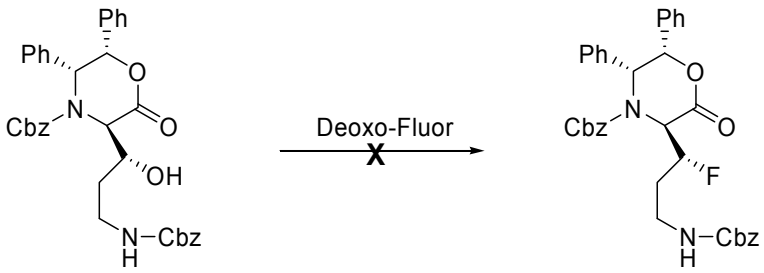


4.5a **5.14**

Figure 5.14: Attempted fluorination using DAST on 3-hydroxy-ornithine precursors 4.5a

5.5.2 Attempted Synthesis of (3*S*,5*R*,6*S*)-benzyl 3-((*R*)-3-(benzyloxycarbonylamino)-1-fluoropropyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate (**5.15**)

The precursor **4.5b** was treated with Deoxo-Fluor (1.2 eq) as was the case with **4.5a**. TLC analysis of the reaction mixture looked promising; however, purification together with ^{1}H NMR and MS analysis of the product revealed that it was an unidentifiable degradation product of **4.5b** and not the fluorination product (figure 5.15).



4.5b **5.15**

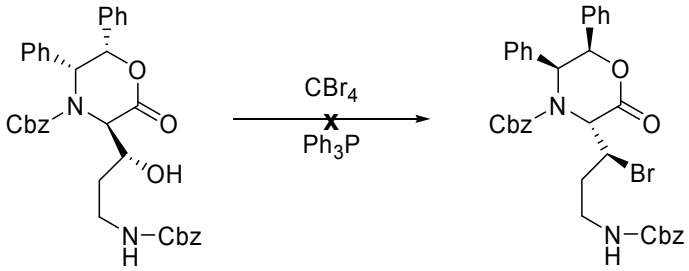
Figure 5.15: Attempted fluorination using Deoxo-Fluor on 3-hydroxy-ornithine precursors 4.5b

To our surprise the use of DAST/Deoxo-Fluor to fluorinate the precursors of 3-hydroxyornithine did not result in the desired fluorinated analogues. We now focused our attention to fluorination by halide replacement.

5.6 Strategy 2 on 3-hydroxy precursors: Fluorodehydroxylation using halogen exchange

5.6.1 Synthesis of (3S,5*R*,6*S*)-benzyl 3-((*R*)-3-(benzyloxycarbonylamino)-1-bromopropyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate (**5.16**)

To test whether it would be possible to introduce the required fluorine atom by a halogen exchange reaction, the substitution of the hydroxyl group with bromine was attempted by the reaction of **4.5b** with CBr_4/PPh_3 (figure 5.16). The same reaction conditions were used as for the previous CBr_4/PPh_3 reactions (section 5.3).



4.5b **5.16**

Figure 5.16: Reaction of 3-hydroxyornithine with CBr_4/PPh_3

TLC analysis of the crude reaction mixture looked promising. However, MS analysis of a sample of the crude reaction mixture did not show formation of the brominated product, but instead only indicated a number of indiscernible degradation peaks of the precursors **4.5b**.

A possible reason for the poor reactivity observed with the 3-hydroxyornithine precursors could be that the precursor molecule is unstable to the fluorodehydroxylation conditions.

5.7 Conclusion

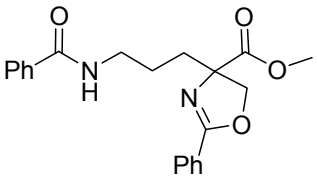
It is clear from the reactions performed on the 2-hydroxymethyl precursors that the benzoyl protecting group on the α-amine favoured the azlactone closure products as soon as the hydroxymethyl group was activated. Attempts to use the protecting group to our advantage only proved that the hydroxymethyl group was more reactive that the carboxylic acid. As such the protecting group on the amino group will have to be modified for the fluorodehydroxylation reactions to be successful.

The attempted fluorination reactions on the 3-hydroxyornithine precursors were all unsuccessful. The performed reactions did not give any indication as to why they failed.

5.8 Experimental

5.8.1 Synthesis of methyl-4-(3-benzoylamino-propyl)-2-phenyl-2-oxazoline-4-carboxylate **(5.2)**

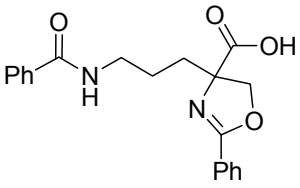
methyl-4-(3-benzoylamino-propyl)-2-phenyl-2-oxazoline-4-carboxylate ***(5.2)***



To a solution of **3.13** (200mg, 0.521mmol) in CH_2Cl_2 (6ml) at -78ºC was added (diethylamino)sulfur trifluoride (DAST) (76µl, 0.573mmol) drop wise over a period of 5min. The reaction mixture was stirred for 1h keeping the temperature at -78ºC after which K_2CO_3 (108mg, 0.782mmol) was added in one portion. The reaction mixture was allowed to warm to room temperature and poured into a sat. aq $NaHCO_3$ solution (8ml). The aqueous layer was extracted with CH_2Cl_2 (3x5ml) and the organic layers dried over $MgSO_4$. Filtration and *in vacuo* removal of the solvent provided **5.2** as a white solid (180mg, yield 94%). ^{1}H NMR (400MHz, $CDCl_3$): δ 1.70 (m, 2H); 1.95 (m, 1H); 2.15 (m, 1H); 3.45 (q, 1H); δ 3.65 (q, 1H); 3.75 (s, 3H); 4.27 (d, 1H, *J* = 9.1Hz); 4.76 (d, 1H, *J* = 1.9Hz); 6.9 (bs, 1H); 7.30-7.50 (m, 6H); 7.80 (d, 2H, *J* = 7.30Hz); 7.95 (d, 2H, *J* = 7.30Hz)

5.8.2 Synthesis of 4-(3-benzoylaminopropyl)-2-phenyl-2-oxazoline-4-carboxylic acid **(5.4)**

4-(3-Benzoylamino-propyl)-2-phenyl-4,5-dihydro-oxazole-4-carboxylic acid **(5.4)**

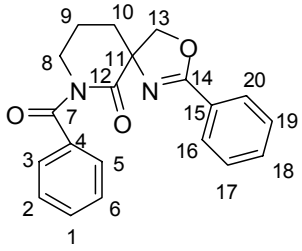


To a solution of **3.14** (119mg, 0.352mmol) in CH_2Cl_2 (2ml) at -78ºC was added (diethylamino)sulfur trifluoride (DAST) (53,5µl, 0.405mmol) drop wise over a period of 5min. The reaction mixture was stirred for 30min and then another portion of DAST (15µl) was added maintaining the temperature at -78ºC. The reaction mixture was stirred for 15min after which K_2CO_3 (70mg, 0.507mmol) was added in one portion. The reaction mixture was allowed to warm to room temperature and poured into a sat. aq $NaHCO_3$ solution (8ml). The aqueous layer was extracted with CH_2Cl_2 (3x5ml) and the organic layers dried over $MgSO_4$. Filtration and *in vacuo* removal of the solvent provided the crude product which was purified by flash chromatography (silica gel; CH_2Cl_2/ethyl acetate/hexane 2:3:1) to afford **5.4** as white oily residue (37mg, yield 33%) ^{1}H NMR (400 MHz,

$CDCl_3$): δ 1.6 (m, 2H); 1.95 (m ,2H); 3.45 (q, 2H); 3.95 (q, 2H, *J* = 6.6); 4.10 (q, 2H, *J* =7.1); 6.40 (bs, 1H); 7.4-7.6 (m, 6H); 7.75 (d, 2H, *J* = 7.3Hz); 8.0 (d, 2H, *J* = 7.1Hz)

5.8.3 Unintentional synthesis of 7-Benzoyl-2-phenyl-3-oxa-1,7-diazaspiro[4.5]dec-1-en-6-one (**5.8)**

7-Benzoyl-2-phenyl-3-oxa-1,7-diazaspiro[4.5]dec-1-en-6-one **(5.8)**



To a solution of **3.14** (81mg, 0.218mmol) in CH_2Cl_2 (533µl) at 0ºC was added thionyl chloride (160µl, 2.18mmol) in one portion and the reaction mixture stirred for 20h maintaining the temperature at 0ºC. The excess thionyl chloride was removed under reduced pressure after which the residue was dissolved in CH_2Cl_2 (1ml) and washed with 10% aq Na_2CO_3 (2x750µl) and H_2O (3x1ml). The combined organic layers were dried over $MgSO_4$. Filtration and *in vacuo* removal of solvent provided a yellow solid. The crude product was purified by flash chromatography (silica gel, ethyl acetate/hexane 1:2) to yield **5.8** as fine white crystals (32mg, yield 48%). ^{1}H NMR (400MHz, $CDCl_3$): δ 1.97 (m, 1H); 2.04 (dt; 1H, *J* = 11.6, *J* = 2.5); 2.2 (m, 1H); 2.6 (m ,1H); 3.8 (dt, 1H, *J* = 7.5, *J* = 3.9Hz); 4.0 (d, 1H, *J* = 8.6Hz); 4.15 (m, 1H); 5.10 (d, 1H, *J* = 8.5Hz); 7.25 (t, 2H, *J* = 7.3Hz); 7.37 (t, 1H, *J* = 7.5Hz); 7.4 (t, 2H, *J* = 7.0Hz); 7.52 (t, 1H, *J* = 7.3Hz); 7.67 (dd, 2H, *J* = 6.6, *J* = 1.9Hz); 8.02 (dd, 2H, *J* = 7.1Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.0 (C9), 35.5 (C10), 47.0 (C8), 74.0 (C13), 76.0 (C11), 127.25 (C15), 128.1 (C17/19), 128.15 (C2/6), 128.35 (C16/20), 128.55 (C3/5), 131.65 (C18), 131.8 (C1),135.2 (C4), 164.7 (C14), 173.7 (C7), 174.5 (C12).

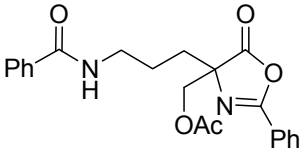
No.	**^{1}H ppm and *J***	**^{13}C ppm**
1	7.52 (1H, t; *J* = 7.3Hz)	131.8

2	7.25 (2H, t; *J* = 7.3Hz)	128.15
3	8.02 (2H, d of d; *J* = 7.1Hz)	128.55
4	-	135.2
5	8.02 (2H, d; *J* = 7.1Hz)	128.55
6	7.25 (2H, t; *J* = 7.3Hz)	128.15
7	-	173.7
8	2.6 (1H, m); 4.15 (1H, m)	47.0
9	1.97 (1H, m); 2.2 (1H, m)	20.0
10	2.04 (1H, d of t; J^1 = 11.6Hz, J^2 = 4Hz); 3.8 (1H, d of t; J^1 = 7.5Hz; J^2 = 3.9Hz)	35.5
11	-	76.0
12	-	174.5
13	4.0 (1H, d of d; *J* = 8.6Hz); 5.10 (1H, d of d; *J* = 8.5Hz)	74.0
14	-	164.7
15	-	127.25
16	7.67 (2H, d of d; *J* = 6.6Hz)	128.35
17	7.4 (2H. t; *J* = 7.0Hz)	128.1
18	7.37 (1H, t; *J* = 7.5Hz)	131.65
19	7.4 (2H. t; *J* = 7.0Hz)	128.1
20	7.67 (2H, d; *J* = 6.6Hz)	128.35

Table 1: ^{1}H- and ^{13}C-shifts of 5.8

5.8.4 Synthesis of L-acetoxymethyl-4-(3-benzoylaminopropyl)-5-oxo-2-phenyl-2-oxazoline **(5.11)**

L-acetoxymethyl-4-(3-benzoylaminopropyl)-5-oxo-2-phenyl-2-oxazoline ***(5.11)***



To a solution of **3.14** (119mg, 0.352mmol) in CH_2Cl_2 (2ml) at -78ºC was added (diethylamino)sulfur trifluoride (DAST) (53,5µl, 0.405mmol) drop wise over a period of 5min. The reaction mixture was stirred for 30min and then another portion of DAST (15µl) was added maintaining the temperature at -78ºC. The reaction mixture was stirred for 15min after which K_2CO_3 (70mg, 0.507mmol) was added in one portion. The reaction mixture was allowed to warm to room temperature and poured into a sat. aq $NaHCO_3$ solution (5ml). The aqueous layer was extracted with CH_2Cl_2 (3x5ml) and the combined organic layers dried over $MgSO_4$. Filtration and *in vacuo* removal of solvent provided the crude product which was purified by flash chromatography (silica gel; CH_2Cl_2/ethyl acetate/hexane 2:3:1) to afford **5.11** as a white oily residue (37mg, yield 33%)

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.6 (m, 2H); 1.95 (s, 3H); 2.00 (m, 2H); 3.50 (q, 2H, *J* = 6.2); 4.30 (d, 1H, *J* =11Hz); 4.55 (d, 1H, *J* =11Hz); 6.25 (bs, 1H); 7.40-7.55 (m, 6H); 7.75 (d, 2H, *J* =7.0Hz); 8.05 (d, 2H, *J* =7.0Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ 20.5, 23.85, 30.8, 66.15, 72.5, 125.2, 126.8, 128.1, 128.55, 128.9, 131.5, 133.2, 134.35, 167.55, 169.9, 177.9.

5.9 References

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Chapter 6

Future Work and Conclusion

6.1 Overview of Achievements

The aim of this thesis was to synthesise DFMO as well as several other fluorinated analogues of ornithine, namely 2-MFMO, 3-fluoro-ornithine and 3,3-difluoro-ornithine. To synthesise these precursors we wanted to employ a fluorination technique that was simpler, safer and more affordable than the currently used fluorination techniques used by industry. An overview of current fluorination methods convinced us to use the fluorodehydroxylation technique to insert the fluorine functionality. The fluorodehydroxylation method requires a hydroxyl group to be transformed to the corresponding fluorine. Fluorodehydroxylation reactions are also capable of transforming an aldehyde or ketone to the corresponding difluoro-group. As such our synthesis would thus have to incorporate the preparation of a precursor molecule with an appropriate hydroxyl functionality.

The 2-(hydroxymethyl)ornithine precursor for DFMO/2-MFMO as well as the 3-hydroxyornithine precursor for 3-fluoroornithine/3,3-difluoroornithine were synthesised successfully. The individual hydroxyl precursors were subjected to fluorination reactions using various fluorodehydroxylation strategies. For the 2-(hydroxymethyl)ornithine precursors of DFMO/2-MFMO, unwanted side reactions occurred as major products instead of the fluorinated products. We attempted several different strategies to overcome these side reactions but they proved unsuccessful. The fluorination attempts on the 3-hydroxyornithine precursor for 3-fluoroornithine/3,3-difluoroornithine did not yield the fluorinated analogues. The reactions perfomed on these precursors gave no indication at all as to why they did not give the fluorinated products.

6.2 Future work

The side reactions observed with the 2-(hydroxymethyl)ornithine precursor is due to the benzoyl protecting group on the amino group of the molecule. As soon as the hydroxyl group is activated by the fluorodehydroxylation reagents, ring formation occurs by attack of the carbonyl amide. Future work on the synthesis of DFMO/2-MFMO will involve the removal and/or change of the amine protecting group from the hydroxyl precursor. Several possibilities will be considered after which fluorodehydroxylation reactions will be attempted to yield the fluorinated precursors (*1*-*3*).

The formation of the spiro-ring may not have been the product that we expected; however this ring structure can still prove to be very useful. A search on the Cambridge database revealed that the structure has not previously been crystallized. This specific spiro-ring was also not found as part of a scifinder search and has to date not been synthesised. The spiro-ring can possibly be used as a screening or selection compound and together with derivation could open up new doors for its use. A possible example of the use of the spiro-ring structure is oulinde below in figure 6.1. The desired product may furthermore be obtained by acid hydrolysis of the lactimide.

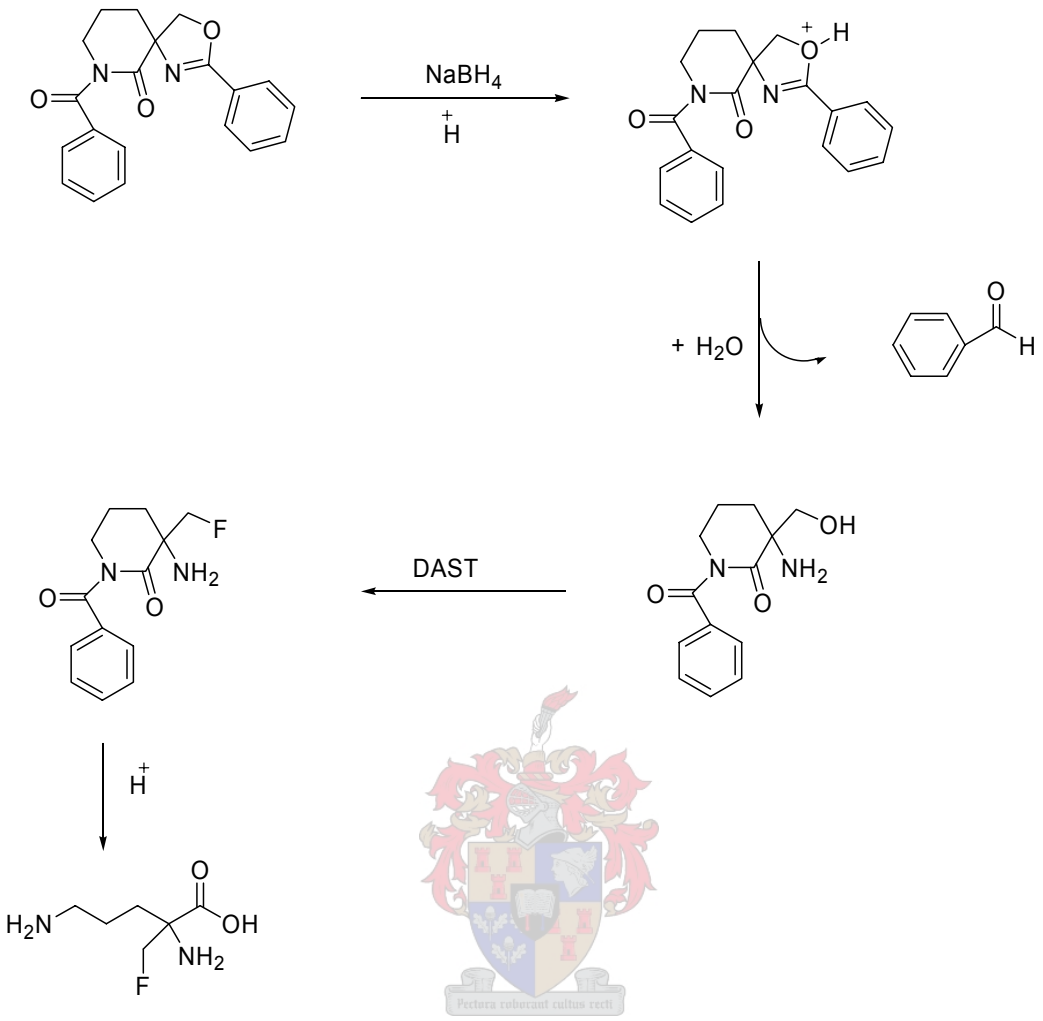

Figure 6.1: Reduction of the spiro-ring structure to yield the free alcohol group and fluorination with DAST to yield the fluorinated precursor.

Reduction of the double bond of the oxazoline in acidic medium will protonate the oxygen, which will result in the formation of the free amine and alcohol group with loss of benzaldehyde as shown in figure 6.1. This will yield a product where the hydroxymethyl group is free for a fluorodehydroxylation reaction without the possibility of interaction of the carbonyl amide as before. If reduction of the oxazoline yields the proposed structure, the synthesis of the fluorinated analogue should be successful.

As mentioned in chapter 4, the synthesis of a new hydroxyl precursor for 3-fluoro-ornithine/3,3-difluoro-ornithine will be attempted. This strategy will use the opening

of an epoxide to introduce the hydroxyl group. One possible problem to overcome is the fact that the epoxide could possibly open on the wrong carbon, introducing the hydroxyl group at position 2 and not position 3 (figure 6.2). However, if the opening of the epoxide is successful, further attempts at fluorination will be preformed using our fluorodehydroxylation strategies. Together with the fluorination attempts we will also focus on the synthesis of different stereoisomers (*4-6*).

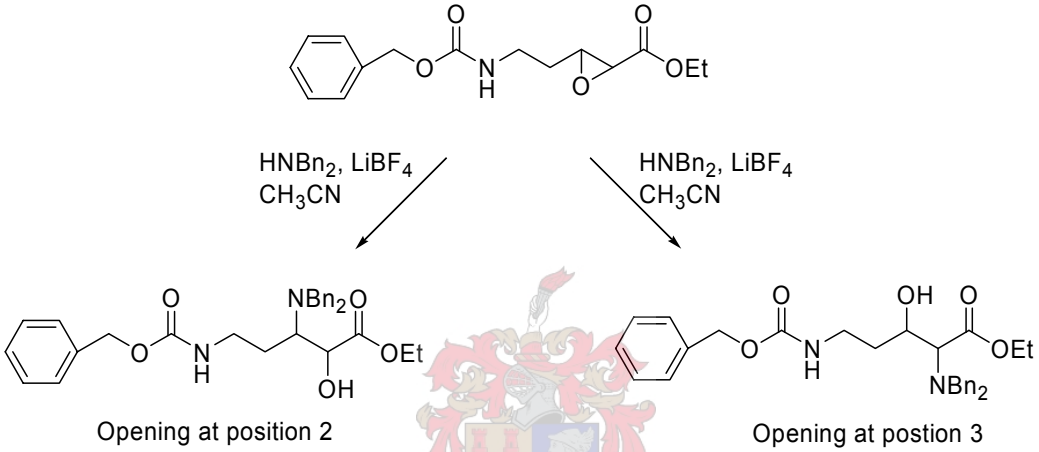

Figure 6.2: Opening of the epoxide to result in the hydroxyl group at position 2 or 3.

6.2 Conclusion

In chapter one the disease African sleeping sickness and the threat it poses to Africa has been elaborated upon. The need for new, affordable and more effective drugs to treat the disease is apparent. The need for new drugs to treat all of the parasitic diseases in Africa have been emphasised by the review article published by Renslo *et al.* (*7*). It has also been shown that DFMO is an important and effective drug for the treatment of African sleeping sickness, especially the *gambiense* strain of the disease. A new synthesis of DFMO in which the introduction of the fluorine functionality is easier and more cost effective could make the drug more readily available to the people that need it. Our focus in this thesis was to develop a new fluorination method to synthesise DFMO.

As mentioned in the overview we decided to use the fluorodehydroxylation method to introduce the fluorine functionality. Synthesis of the required hydroxyl precursors was very successful, however the attempts at fluorination were not. Even so, great progress have been made towards the synthesis of the fluorinated ornithine analogues and our findings thus far have shown us new directions to follow.

We are more than hopeful that we will be able to synthesise the fluorinated ornithine analogues as laid out at the start of this thesis. As soon as we have successfully synthesised the fluorinated analogues, inhibition studies will proceed to determine if any of the new fluorinated analogues are potential inhibitors of the ODC enzyme.

6.3 References

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