1.1- Antimicrobial agents

Compounds that eliminate or inhibit the development of bacteria, fungi, parasites or viruses are called antimicrobials and respectively referred to as antibacterial, antifungal, antiprotozoal and antiviral agents.[1] These chemicals used to treat infectious diseases fall into two main categories, namely natural products and chemotherapeutic agents. Natural products are secondary metabolites that are only generated by certain microorganisms and are usually large, elaborated organic molecules that require complex enzymatic synthesis.[2] Alternately the group of chemotherapeutic agents comprise of compounds that have been synthesised chemically. A hybrid of these two categories exists, in which natural products that have been chemically modified to alter particular characteristics and is referred to as semi-synthetic antimicrobials.

Numerous antimicrobial agents are known; however, not all of them can be used *in vivo* as in addition to their antimicrobial activity they can be toxic to human beings.[3] Antimicrobials have to be non-toxic, non-allergenic, effective and selective, chemically stable, active against possibly more than one bacterium and inexpensive.[1] The ratio between the therapeutic effect and the toxic effect in the human body is described by the drugs therapeutic index (TI).[4]

Antimicrobial drugs (Figure 1.1) can have several different modes of action. Penicillins (**1**) and cephalosporins (**2**) act by interfering with the synthesis of cell wall, while tetracyclines (**3**), macrolides (**4**), aminoglycosides (**5**) and phenicols (**6**) interrupt the synthesis of proteins and sulfonamides (**7**) and fluoroquinolones (**8**) obstruct DNA functions.[5]

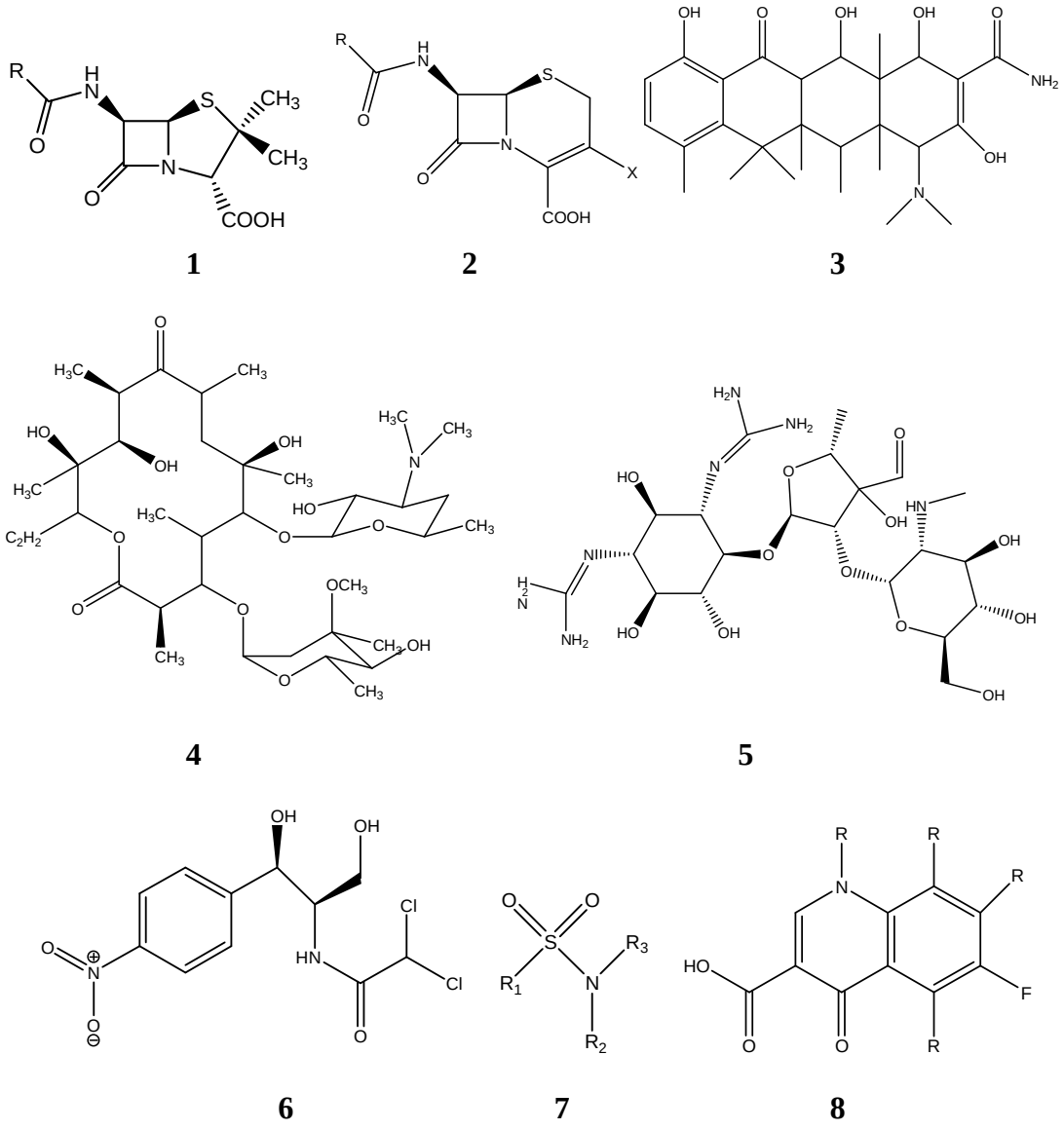


*Figure 1.1: Antimicrobial drugs: penicillin (**1**); cephalosporin (**2**); the 4 rings of the basic tetracycline structure (**3**); clarithromycin, example of macrolide (**4**); streptomicyn, example of aminoglycoside (**5**); chloramphenicol (**6**); sulfonamine structure (**7**); fluoro-quinolone (**8**).*

Antibacterial agents usually act by modifying the structure or the metabolic pathways of bacteria. This may be achieved *via* the creation of a bond with the cytoplasmic membrane, through inhibition of protein or nucleic acid biosynthesis or by interference with cell wall synthesis.[6]

The group of antibacterial agents comprise of antibiotics, disinfectants and antiseptics. Unlike disinfectants and antiseptics, antibiotics exhibit selective toxicity and exploit the biochemical features which differ in the host and in the parasites, damaging the microorganism, but leaving the host undamaged.[7] Disinfectants kill

bacteria but are unselective and can be toxic for mammals and are not used *in vivo*. Antiseptics are less poisonous: they are used topically but are toxic if ingested.

Bacteria are classified as either Gram-positive or Gram-negative as determined by the result of the Gram stain. Gram-positive bacteria have a thick peptidoglycan layer within the cell wall, so upon staining they became dark blue or violet. Conversely Gram-negative bacteria have only a thin peptidoglycan layer and a further outer membrane so they are rapidly decolorised by the stain becoming red or pink. This additional outer membrane gives Gram-negative bacteria a higher resistance to antibiotics.[2] Antibacterial agents can be classified on the basis of the number of bacteria they are active against. Those active against both Gram-positive and Gram-negative bacteria are named "broad spectrum", while those active against only one kind of bacteria are named "narrow spectrum" and those active against only an organism are named "limited spectrum".[8]

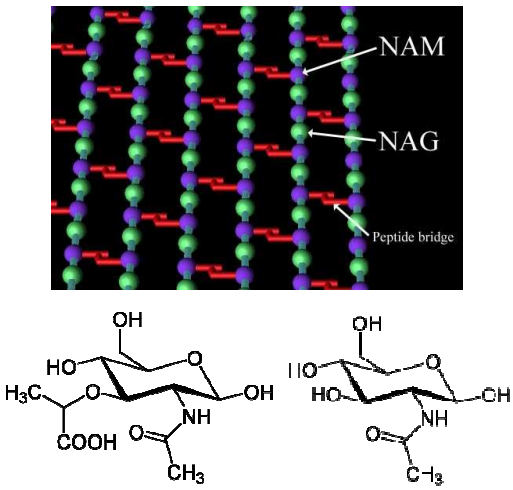
There are two possible modes of action of antibiotics. Bactericidal antibiotics, for example penicillin (**1**), act by either killing (or cleaving) the invading organism, while bacteriostatic antibiotics, for example chloramphenicol (**8**), are only able to limit bacterial growth and replication.[3,5]

In the 19th century the study of antibacterial agents became a topic of great interest and the most important breakthrough was the discovery of penicillin (**1**). Several researchers had observed that aerated LB media in the presence of penicillium mould became much less turbid than that which was not exposed to the mould. The Scottish bacteriologist Alexander Fleming was the first to understand that this was due to the antibacterial property of penicillin (**1**).

In the 1920s, Fleming realised that the penicillium mould modified the shape of the colonies of *Staphylococcus sp*. and from this deduced that penicillium mould had antimicrobial activity. He purified and studied the substance isolated from the mould, penicillin (**1**), and found that it inhibited certain bacterial cultures. Importantly it including *Staphylococcus sp.*, *Streptococcus pyrogenes* and *Pneumococcus sp*. cultures, was non toxic if used on human tissues. Penicillin (**1**) was administered as antibacterial agent for the first time in 1940s.[9]

Penicillin (**1**) and Cephalosporin (**2**), isolated from *Cephalosporium* moulds, contain planar 4-membered beta-lactam compounds, joined to another larger ring. They inhibit the synthesis of bacterial cell walls, which are fundamental for the growth of

bacteria. These cell walls mainly comprise of peptidoglycan,[6,2] formed by polysaccharide cross-linked chains of alternated *N*-acetyl-muramic acid (NAM, **9**) and *N*-acetyl-glucosamine (NAG, **10**) providing chemical stability, mechanical strength and rigidity, as shown in Figure 1.2. The beta-lactams, irreversibly bind to the enzyme as a "suicide inhibitor". Eukaryotes do not have peptidoglycans in their cell walls, so they cannot bind these drugs, which have little or no toxicity for human beings.

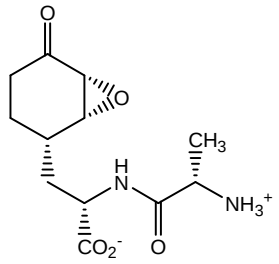


9 **10**

*Figure 1.2: Peptidoglycan structure of bacterial cell wall with N-acetyl-muramic acid (NAM, **9**) and N-acetyl-glucosamine (NAG, **10**).*[10]

1.2- Bacilysin & anticapsin

The dipeptide antibiotic bacilysin (**11**), shown in Figure 1.3, also referred to as tetaine or L-alanyl-(2,3-epoxycyclohexanone-4)-L-alanine, was discovered in 1949 in Oxford. It was one of the first identified examples of a natural pro-drug and one of the simplest peptide antibiotics known. In 1987 Woynarowska reported on the anticancer activity of bacilysin (**11**). The reduced growth of tumor cells was presumed to be the effect of impairing DNA and RNA synthesis.[11]

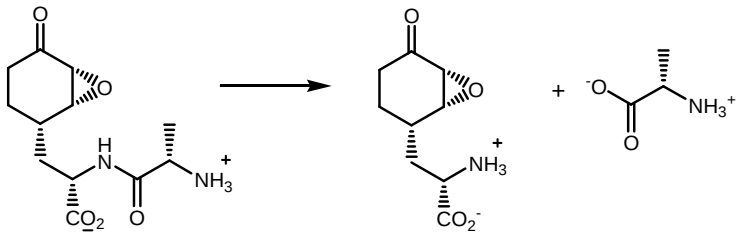


11

*Figure 1.3: Bacilysin (**11**), a simple dipeptide antibiotic.*

Bacilysin (**11**) is produced and excreted by certain strains of *Bacillus subtilis A14*, a Gram-positive, catalase-positive bacterium commonly found in soil.[12, 13, 14,15] Bacilli produce a broad spectrum of secondary metabolites, the majority of which are small peptides with unusual components, synthesised non-ribosomally.[16,17]

The structure of bacilysin (**11**) in conjunction with its broad spectrum antibacterial and antifungal activity was established by Walker and Abraham in 1970.[17-19] A dipeptide transport system carries bacilysin (**11**) into the microorganism, where intracellular peptidases hydrolyse it to produce L-anticapsin (**12**). and L-alanine (**13**) as shown in Scheme 1.1.[12,17]

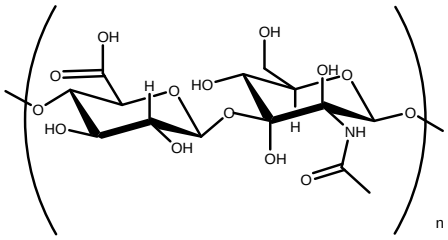


11 **12** **13**

*Scheme 1.1: The intercellular hydrolysis of bacilysin (**11**) from which L-anticapsin (**12**) and L-alanine (**13**) are produced.*

Anticapsin (**12**) is a fermentation product and biologically active metabolite isolated from the culture filtrate of *Streptomyces griseoplanus*. It was discovered in 1970 by the Lilly Research Laboratories (Eli Lilly and Co., Indianapolis, *USA)* as an epoxy-keto-amino acid, C-terminal residue of bacilysin (**11**).[13, 20, 21]

Anticapsin (**12**) limits the formation of the hyaluronic acid capsules (**14**), shown in Figure 1.4, in *Streptococcus pyogenes*.



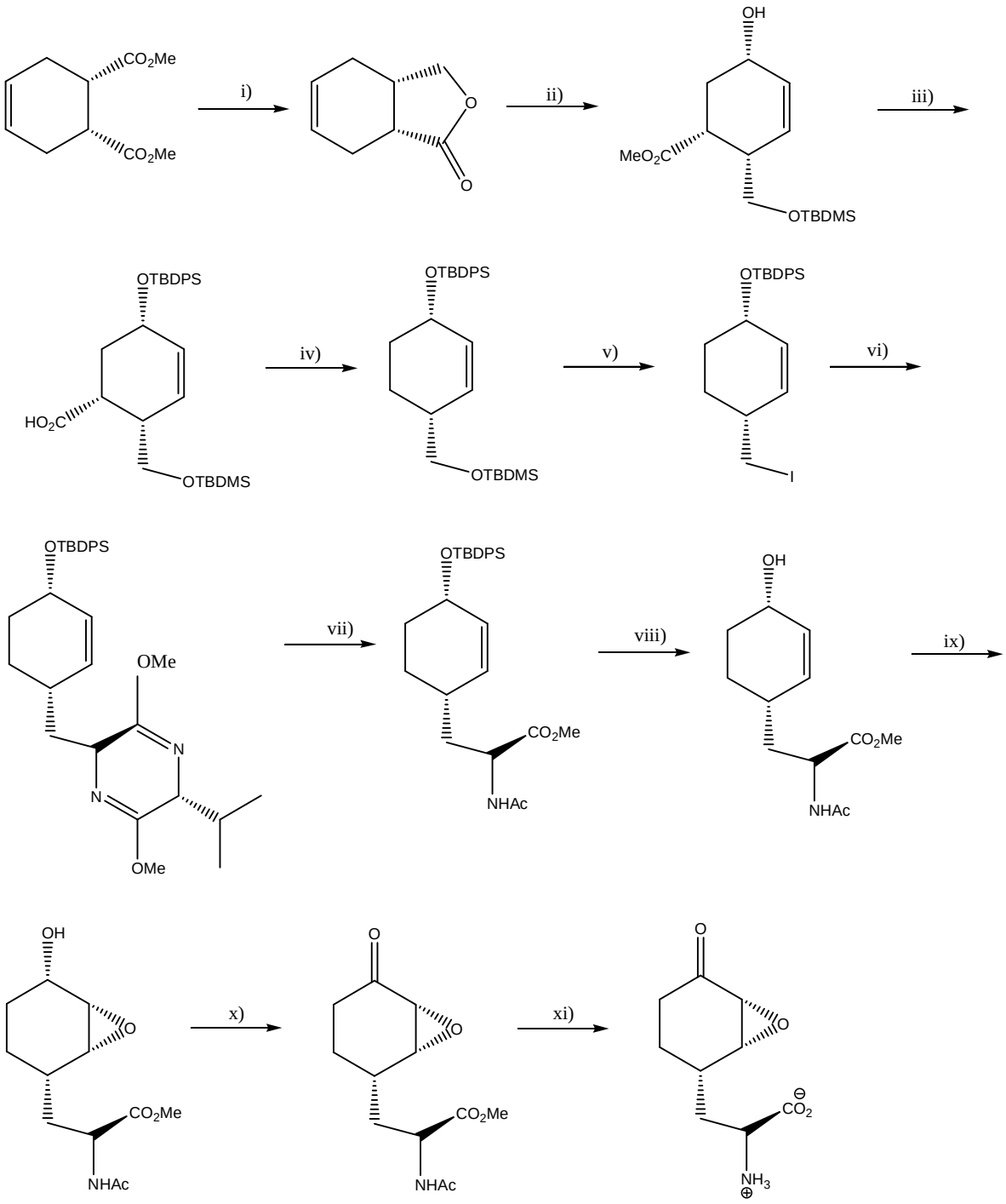
14

*Figure 1.4: Hyaluronic acid (**14**)*

Streptococcus pyogenes, shown in Figure 1.5, is a group A streptococcus (GAS) and Gram-positive bacterium. It is a very important human pathogen that causes a particularly wide spectrum of infections. GAS colonises the skin and mucosal surfaces and can lead to several diseases, skin or throat infections as well as more dangerous, life-threatening disorders, including necrotizing fasciitis, streptococcal toxics and Hock syndrome. These lead to complications including rheumatic fever, rheumatic heart disease and acute glomerulo nephritis. The capacity of GAS to adapt and persist at a numerous tissue sites means this pathogen can be very dangerous.[22]

Figure 1.5: Streptococcus pyogenes: .a) Colonies of Streptococcus pyogenes on blood agar exhibiting beta (clear) hemolysis; and b) Streptococcus pyogenes bacteria at 900x magnification.

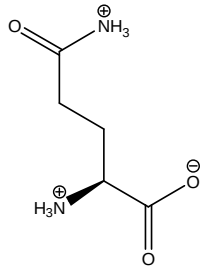
A synthetic route to synthesise anticapsin (**12**) was reported by Baldwin *et al.*, and is shown in Scheme 1.2:[23]



Reagents and conditions: i) TBDPSCI, imidazole, DMF; ii) $KOSiMe_3$, benzene, reflux, 1.5 h, acidic work-up [NH_4CI (sat. aq. soln.)]; iii) oxalyl chloride, DMF, toluene -5 °C to 10 °C 45 mins; sodium 2-mercapto pyridine-N-oxide, DMAP, benzene, 30 mins at r.t., addition of tert-dodecanethiol, hv (200 W tungsten lamp) 20 °C-3 °C 2.5 h; iv) Ts-H_20, THF-H_20, 24 h, r.t.; v) MsCI, pyridine, 19 h, r.t.; vi) Nal, acetone, reflux, 21 h; vii) (2R)-2,5-dihydro-2-isopropyl-3,6-dimethoxypyrazine, nBuLi, THF, -78°C; CuCN, 2 mins, 0 °C, -78°C then -21°C; -21 °C 24 h; viii) 0.25 M HCI (aq), CH_3CN 100 mins, r.t.; acetic anhydride, pyridine 1.75 h, r.t.; ix) NH_4F, MeOH, 50 °C 18 h; x) mCPBA, $CHCI_3$, 2 h, r.t.; xi) TPAP, N-methyl morpholine-N-oxide, CH_3CN , 1 h, r.t; xii) pronase E, phosphate buffer [~2:3 ratio of 0.1 M KD_2PO_4 and 0.1 M Na_2DPO_4 in D_20] pH 7.5, 30 °C, 3 h; acylase I from sp. aspergillus immobilised on eupergrit C, phosphate buffer [~2:3 ratio of 0.1 M KD_2PO_4 and 0.1 M Na_2DPO_4 in D_2O] pH 7.5, 30 °C 30 h then cellulose chromatography (80 % aqueous propan-2-ol as eluent).

*Scheme 1.2: Stereocontrolled enantiospecific synthesis of the revised structure of anticapsin (**12**):*

Anticapsin (**12**) acts as L-glutamine (**15,** figure 1.6) analogue, inhibiting Glucosamin-6-phosphate synthase (GlcN-6-PS), an enzyme also referred to as L-glutamine-D-fructose-6-phosphate amidotransferase. This enzyme is essential in the synthesis of peptidoglycan cell walls in bacteria and chitin cell walls in fungi.[17,19,24,25] Inhibition of this enzyme results in weakening of the cell walls. High internal osmotic pressure leads to lysis of the cell.[23] Anticapsin (**12**) has been reported to strongly inhibit the synthesis of GluN-6-PS enzyme in *E. coli* and *Staphylococcus aureus*. Anticapsin (**12**) inhibition has also been demonstrated in the cell-free extracts from bacteria, yeast and tumor cells.[12,24]

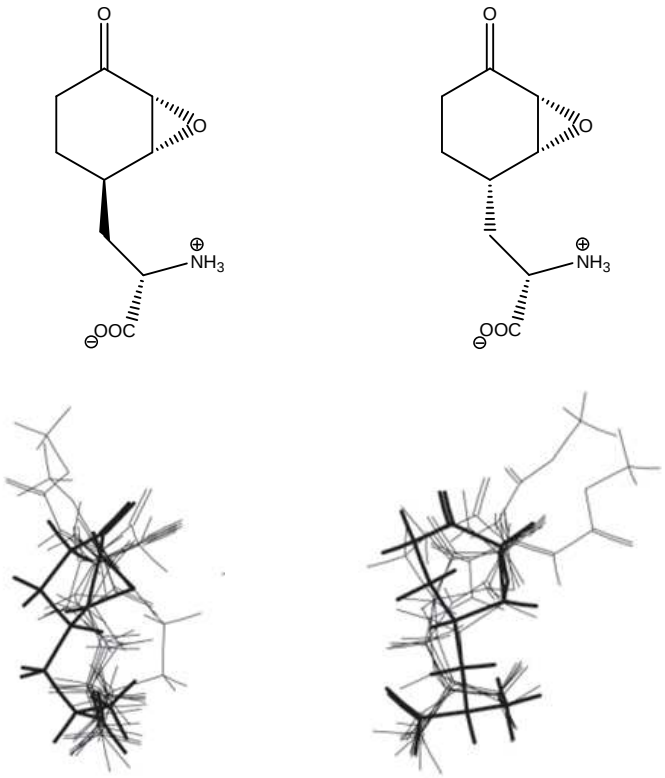


15

*Figure 1.6: L-glutamine (**15**)*

Wojciechowski *et al*., superimposed two diasteroisomers of anticapsin (**12**) differing only at the Cγ-carbon in order to understand the importance of the stereochemistry at the γ position, as shown in Figure 1.7. The *R*-isomer (**12-*R***) did not show any significant activity with GlcN-6-PS, while the *S*-isomer (**12-*S***) has shown to be a potent inhibitor of the enzyme.

To date, there have been no studies involving the altering of the stereochemistry of the epoxide or at the amine moiety to see if the change affects the activity.



12-*R* **12-*S***

*Figure 1.7: Orthogonal view of the superimposition of two diasteroisomers of anticapsin (**12**) CγR-isomer (**12-R**) and CγS-isomer (**12-S**).*

Glucosamine-6-phosphate synthase

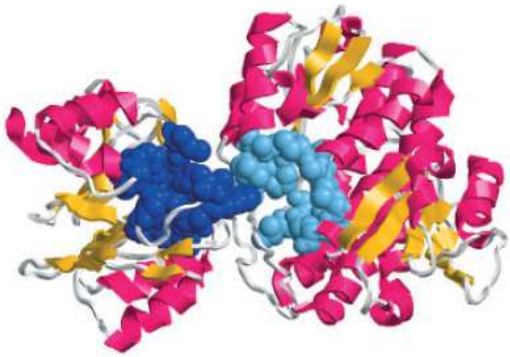
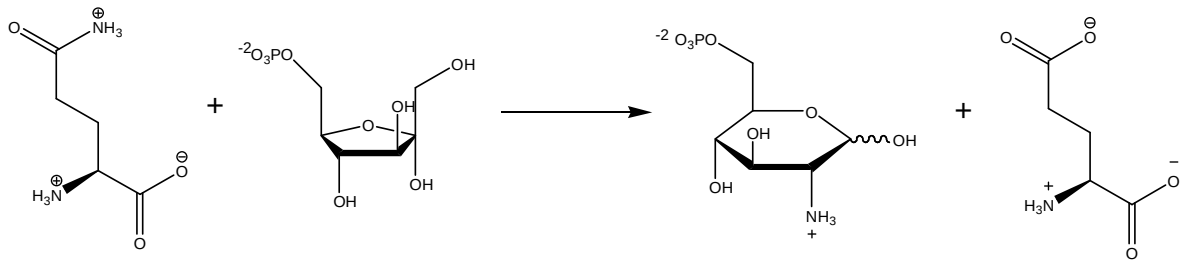


Figure 1.8: Glucosamine 6-phosphate synthase (GlcN-6-PS).[26]

GlcN-6-PS, shown in Figure 1.8, is essential in the synthesis of peptidoglycan cell walls in bacteria and chitin cell walls in fungi.[17,19,24,25] GlcN-6-PS catalyses the conversion of fructose 6-phosphate (Fru-6-P, **16**) into glucosamine 6-phosphate (Glu-6-P, **17**) where glutamine (**15**), utilised as nitrogen source, is converted into glutamic acid (**18**), as shown in Scheme 1.3.[27]



15 **16** **17** **18**

*Scheme 1.3: The GlcN-6-PS catalysed conversion of glutamine (**15**) and fructose 6-phosphate (**16**) into glucosamine 6-phosphate (**17**) and glutamic acid (**18**) respectively.*

Amidotransferases, shown in Figure 1.9, use the nitrogen of the amide of glutamine (**15**) molecule in several biosynthetic reactions (example of substrates are amino acids, carbohydrates and nucleotides). Amidotransferases contain two principal domains, namely a glutaminase domain, responsible of the hydrolysis of glutamine (**15**) to glutamic acid (**18**) and an ammonia, and synthetase (or synthase) domain, responsible of the amination of the substrate.[28,29]

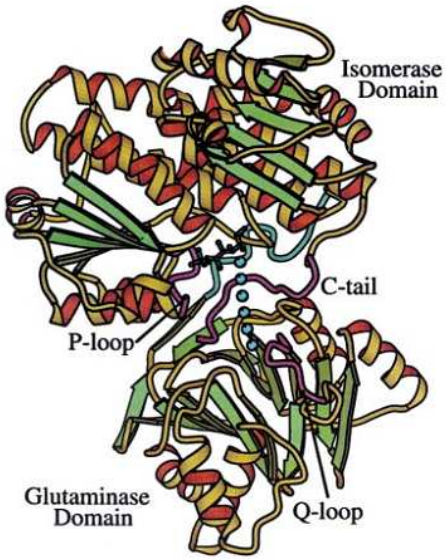


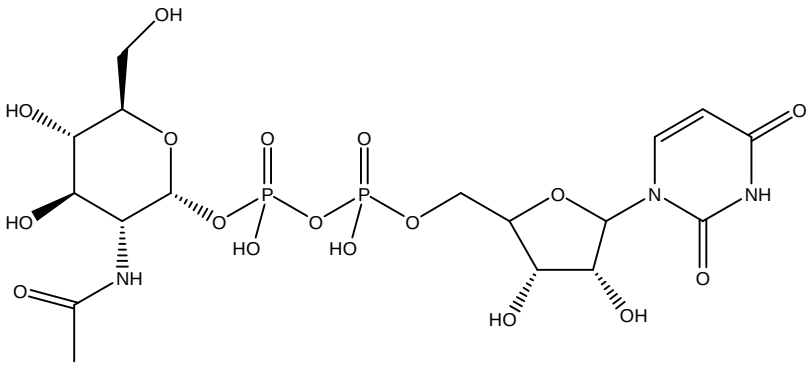
Figure 1.9: General amidotransferase structure.[27]

In *E. coli* the enzyme structure confirms the usual homodimeric organization of the molecule. A 18 Å hydrophobic channel joins the domains and allows the passage of ammonia. This transfer is also permitted by a C-terminal decapeptide between the domains.[27]

Mechanism of action of anticapsin

As previously mentioned, anticapsin (**12**) inhibits GlcN-6-PS in *Streptococcus Pyogenes*, by acting as an analogue of glutamine (**15**). This enzyme is implicated in the initial step of the biosynthesis of a precursor of the hyaluronic acid (**14**), named uridine diphosphate-*N*- acetyl glucosamine (UDP-*N*AcGlc, **19**), shown in Figure 1.10. Anticapsin (**12**), inhibiting the enzyme, inhibits the formation of hyaluronic acid (**14**) capsules resulting in bacterial cell death.[24]

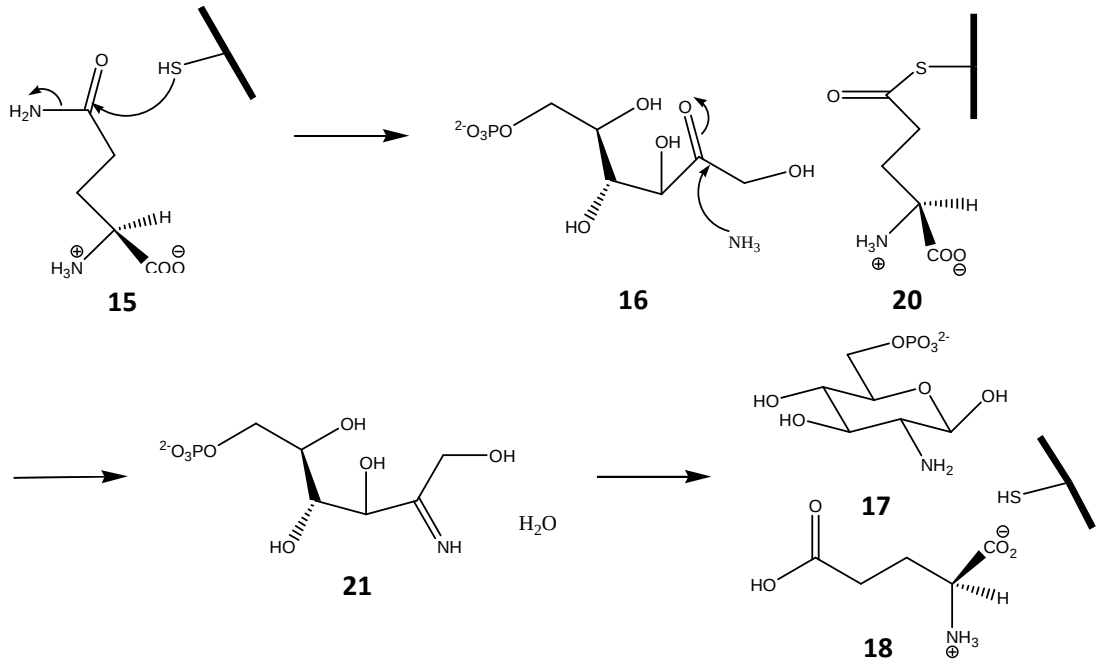
Hyaluronic acid (**14**) is a polysaccharide composed by D-glucuronic acid and D-*N*-acetyl-glucosamine, alternating β-1,4 and β-1,3 glycosidic bonds (Figure 1.4). The hyaluronic acid (**14**) capsules are involved in the virulence of the organism and make the bacteria resistant to attack by bacteriophage, allowing it to go unnoticed by the immune system.[21,30]



19

*Figure 1.10: Uridine diphosphate-N-acetyl glucosamine (**19**).*

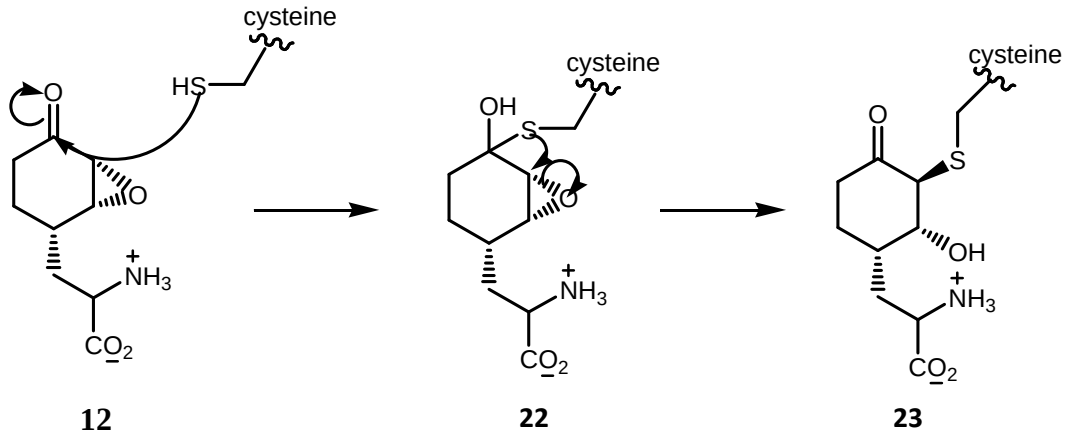
The main step in the biosynthesis of Glu-6-P (**17**), shown in Scheme 1.4, is the attack of the thiol side-chain of an active site cysteine of glucosamine synthase, to the carbonyl carbon of the glutamine (**15**). The ammonia generated from this reaction then reacts with the keto-group of Fru-6-P (**16**). After rearrangement of the imine (**21**), Glu-6-P (**17**) is formed and the thioester (**20**) is hydrolyzed to regenerate the active site and glutamic acid (**18**).



*Scheme 1.4: Mechanism of formation of Glu-6-P (**17**).*

The thiol group of active site cysteine of glucosamine synthase attacks its carbonyl carbon through nucleophilic addition, resulting in an irreversible alkylation after

migration of the sulfur onto the α-carbon (**22**) and an intramolecular opening of the epoxide ring (**23**), as shown in Scheme 1.5.[23,31]

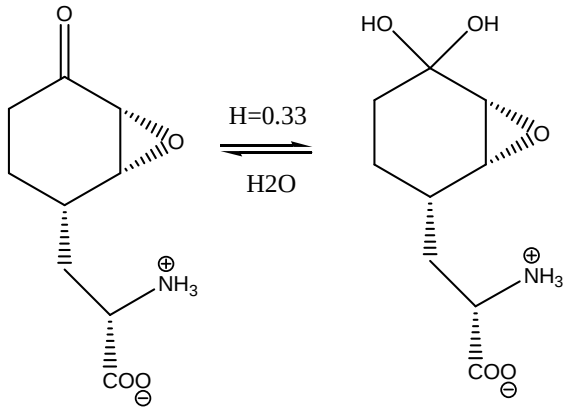


12 **22** **23**

Scheme 1.5: Mechanism of inactivation of GlcN-6-PS.

This hypothetical mechanism was derived from theoretical studies on non-biological systems but not definite conclusion was drawn on the reactivity of the epoxyketone containing natural products in the presence of thiophenes derivatives.

It has been shown that an equilibrium between anticapsin (**12**) and the hydrate form (*S*)-2-ammonio-3-((1*R*,2*S*,6*R*)-5,5-dihydroxy-7-oxa-bicyclo[4.1.0]heptan-2-yl)propanoate (**24**) in aqueous solution exists (Scheme 1.6),[23] which has been used to rationalize the ketone as most likely site of nucleophilic attack in other epoxyketones.[31]



12 **24**

*Scheme 1.6: Equilibrium between anticapsin (**12**) and its hydrate form (**24**).*

1.3- Antibacterial resistance

Antibiotics are still an important topic in research spanning several different fields, including human and veterinary medicine; animal feeds; agriculture; and the food industry.[1] The process to develop an antibiotic can be expensive and arduous, so often large pharmaceutical companies abandon the market. Actions have to be taken against the spreading of infections. It is desirable to improve the yields of synthetic pathways to existing antibacterial agents but it is also worth to try to find new agents. This is especially true for those microorganisms for which no antibacterial agent has yet been developed or for those that have developed antibacterial resistance. The problem of the antibacterial resistance requires the continuous discovery of new compounds as it depends on numerous variables, including genetics factors, hormone levels, nutritional status.[32]

100,000 tons of antibiotics are synthesised worldwide annually.[2] The overuse of antibiotics, especially in the last two decades, has influenced the ecology of bacteria and over time bacteria have become resistant to them. In the beginning of the 1960s only 10 % of the bacterial strains were resistant to penicillin G, while in 2010 that number has come close to 100 %.[6] Resistance may occur because of modification of the active site of the target enzyme, incapability for the antibiotic to access and creation of enzymes that inactivate or destroy the antibiotic.

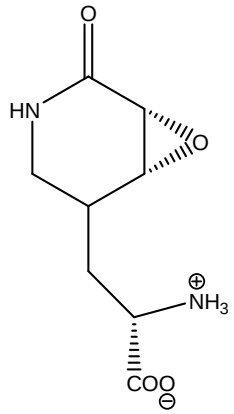
One of the causes of antibacterial resistance is the modification that can occur at the gene level, resulting in a greater affinity for the substrate or a smaller affinity for the antibiotic. The access of a substrate can be denied because of structural changes that altered for instance the permeability of the antibiotic through the bacterial membrane or because the alteration of the gene code provoke a mechanism of expulsion of the antibiotic. Another reason can be the lack of a particular structural moiety that the antibiotic needs in order to interact with the microorganism. Additionally, there is a strong possibility that the bacteria can evolve active mechanisms to destroy or inactivating antibiotics. This has happened in the case of beta-lactams.[2,6]

The random modification starts when a very tiny number of bacteria become genetically resistant to a given antibiotic. If even in 100,000,000 cells, then that single resistant bacterium will not die, but will multiply, generating a resistant progeny. With time, this new resistant population will take the place of the previous one.[33] This capability of surviving increase significantly with the amount of bacteria treated and with the quantity of antibiotic utilised.

Sometimes, resistance is not generated from a random mutation, but from the random acquisition of ‘resistance genes’.[34]

1.4- Analogues of anticapsin

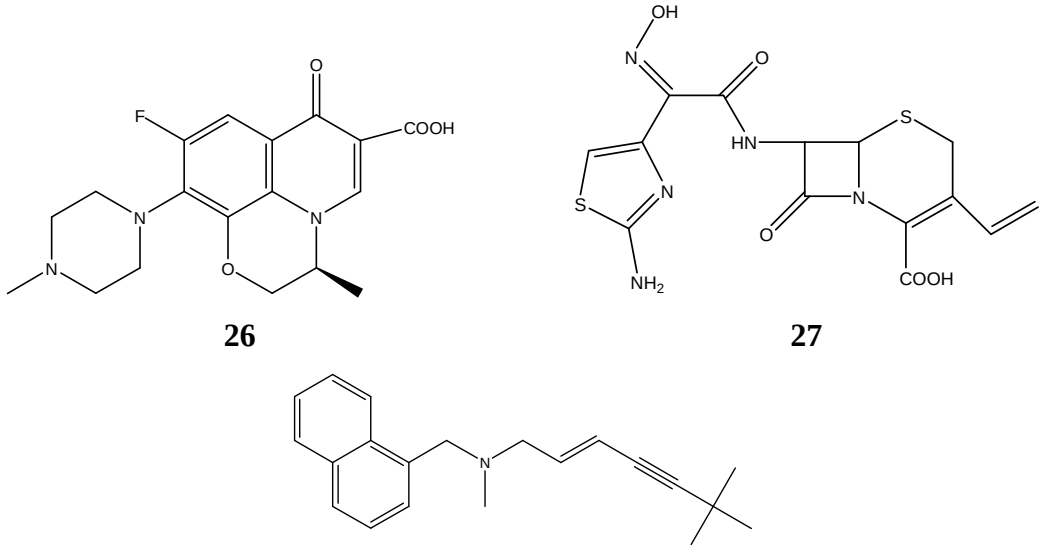
For the reasons mentioned above (Section 1.3), this thesis will discuss and explain the synthesis of a novel antibacterial agent, (*S*)-2-ammonio-3-((1*R*,6*R*)-2-oxo-7-oxa-3-aza-bicyclo[4.1.0]heptan-5-yl)propanoate (**25**), shown in Figure 1.11, an analogue of anticapsin (**12**).



25

*Figure 1.11: (S)-2-ammonio-3-((1R,6R)-2-oxo-7-oxa-3-aza-bicyclo[4.1.0]heptan-5-yl)propanoate (**25**), analogue of anticapsin (**12**).*

The main difference between anticapsin (**12**) and the novel molecule (**25**) will be the introduction of a nitrogen atom into the ring. Nitrogen has been chosen to be inserted into the ring as it is a constituent of molecules in almost every major drug class used in pharmacology and medicine, including Levaquin® (**26**), Omnicef® (**27**) and Lamisil Oral® (**28**), shown in Figure 1.12. Levaquin® (**26**), a fluoroquinolones drug, is used to treat bacterial infections of the skin, sinuses, kidneys, bladder, or prostate and also to treat bacterial infections that cause bronchitis or pneumonia, and to treat people who have been exposed to anthrax. Omnicef® (**27**) is a cephalosporin antibiotic, used against acute flare-ups of chronic bronchitis, middle ear infections, throat and tonsil infections, pneumonia, sinus infections and skin infections. Lamisil Oral® (**28**), is an antifungal antibiotic and it works against infections caused by fungus that affect the fingernails or toenails.[35]

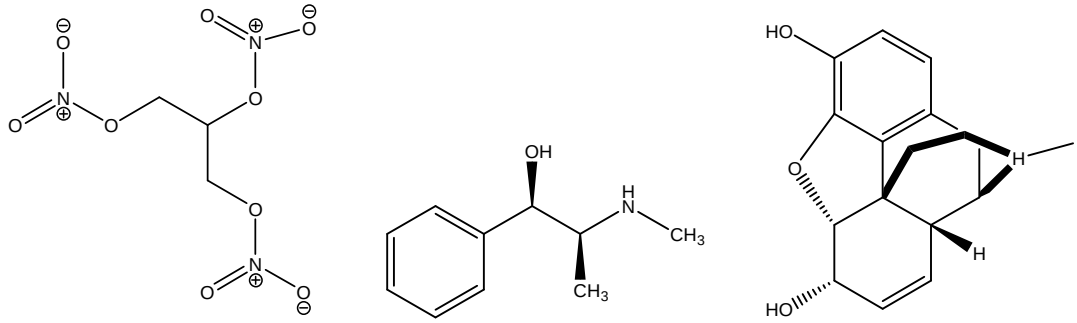


26 **27**

28

*Figure1.12: Levaquin® (**26**); Omnicef® (**27**); Lamisil Oral® (**28**).*

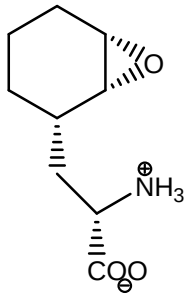
Nitrogen is present, not only in organic nitrate drugs like nitroglycerin (**29**), which is utilized in cardiovascular medicine,[36] but also in drugs derived also from plant, such as alkaloids (**30**) and morphine (**31**), shown in Figure 1.13.



29 **30** **31**

*Figure 1.13: Nitroglicerine (**29**), ephedrine, example of alkaloid (**30**) and morphine (**31**).*

An example of analogue of anticapsin, (*S*)-3-((1*R*,2*S*,6*S*)-7-oxa-bicyclo[4.1.0]heptan-2-yl)-2-ammoniopropanoate (**32**), shown in Figure 1.14, has been synthesized by Borowski and co-workers.[37] This is the anticapsin molecule (**12**) without the ketone moiety.



32

*Figure 1.14: (S)-3-((1R,2S,6S)-7-oxa-bicyclo[4.1.0]heptan-2-yl)-2-ammoniopropanoate (**32**), analogue of anticapsin without the ketone moiety.*

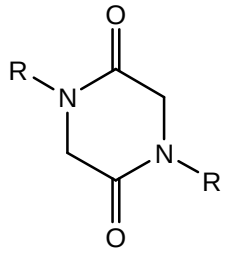
A comparison of the activity of this analogue (**32**) with anticapsin (**12**), in *E. coli*, *S. cerevisae* and *C. albicans,* found that the keto group is not essential to inactivate the amidotransferase enzyme, but without it, the activity decreases significantly. So it is possible that the proposed mechanism of inhibition, shown in Scheme 1.5, is not accurate. The ketone may be essential for binding but not involved directly in the inhibition. Another possibility is that the hydrate (**24**) stabilises binding in the active site prior to inhibition or that this hydrate form does not exist within the active site.

The target analogue (**25**) in Figure 1.11 was chosen with the aim of making an attack on the carbonyl and hydrolysis to form hydrate (**24**) less likely.

The anticapsin analogue (**25**) is a non-natural amino acid that could be biologically tested and compared to anticapsin (**12**).

This kind of amino acid, even if not present in nature, can play a vital role in understanding important protein conformations and interactions, really useful to design peptide-based therapeutic drugs. Once in the protein, natural amino acid analogues can help in identifying structural and functional domains, conformational changes and protein-protein interactions within the protein.

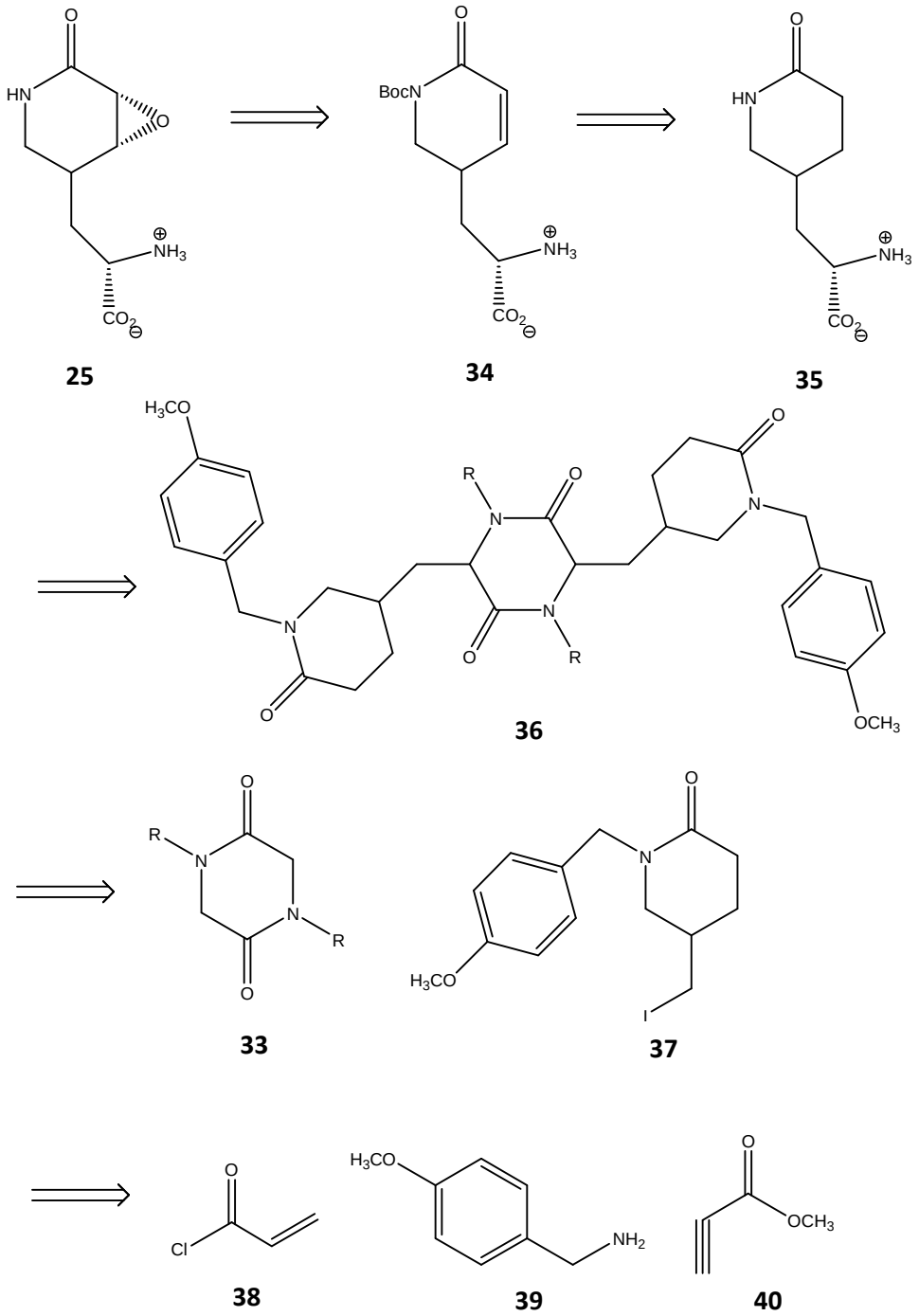
In order to synthesise the proposed molecule (**25**), the capability of the diketopiperazine scaffold (1,4-disubstituted diketopiperazine 2,5-dione, DKP, **33**), shown in Figure 1.15, has been exploited to generate amino acid.



33

*Figure 1.15: 1,4-disubstituted diketopiperazine 2,5-dione (**33**).*

The retrosynthetic route is shown in the Scheme 1.7.



*Scheme 1.7: Retrosynthetic approach to the synthesis of the anticapsine analogue (**25**).*

The starting materials (**38**, **39**, **40**) are commercially available and lead to the target activated compound after six steps. Once activated, compound (**37**) can be alkylated to the DKP scaffold (**33**) selectively. After deprotection and hydrolysis, the resulting amino acid can be selectively epoxidised, obtaining the desired heterocyclic analogue.

This work will be discussed in more detail in the following chapters.

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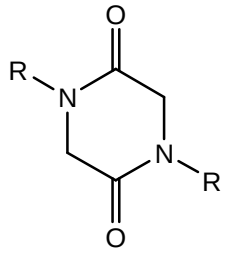
2.1- Aim

The aim of this first part of the project is to synthesise 1,4-disubstituted diketopiperazine 2,5-dione core (DKP, **33**), shown in Figure 2.1.



33

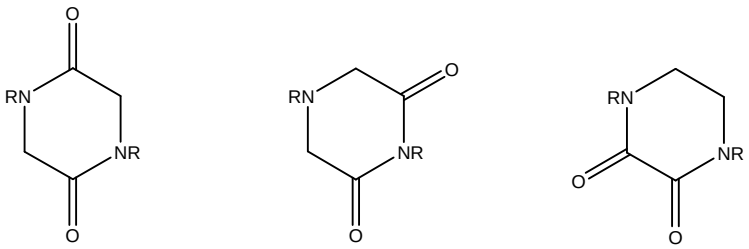
*Figure 2.1: General structure of 1,4-disubstituted diketopiperazine 2,5-dione (**33**).*

2.2- Introduction

Diketopiperazines, the smallest cyclic peptides known, are very important in biology and drug discovery as it is possible to obtain amino acids from them, rendering them a possible substitute for common peptides. DKPs can be used to stereoselectively incorporate an amino acid functionality into a molecule and be selectively deprotected under mild condition using cerium ammonium nitrate (CAN).[1-3]

These compounds have been found in both microorganisms[4] and plants.[5] Like many products of microbial secondary metabolism they show antimicrobial activity against some organisms[6,7] and in nature they are usually synthesised from a small library of primary metabolites.

In nature there are three DKP isomers, 2,5 DKP (**33**), 3,5 DKP (**41**) and 5,6 DKP (**42**) with the most prevalent being 2,5-DKP , Figure 2.2.[8,5,9] 2,5-DKP are also an unwanted side products in the synthesis of oligopeptides.[10,11,12]

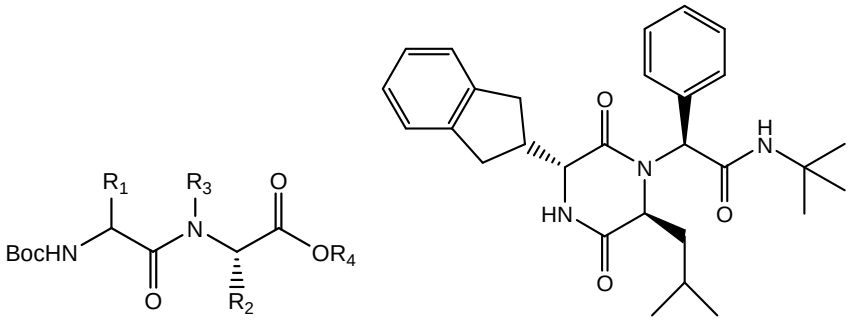


33 **41** **42**

*Figure 2.2: Three structural DKP isomers, 2,5 DKP (**33**), 3,5-DKP (**41**) and 5,6-DKP (**42**).*

DKPs can generate amino acids or peptides through hydrolysis in appropriated conditions, Figure 2.3.[13,1]

They are also important building blocks for other chiral compounds and they have potential applications in industry and medicine.[14,15,1] Research in this area is ongoing.[3,16] For instance Pèrez-Picaso *et al.* recently reported an efficient microwave assisted synthesis using a one-pot cyclisation of *N*α-Boc-dipeptidyl tert-buthyl esters (**43**)[17] and Sollis *et al.* describing the multicomponent Ugi reaction to synthesize trisubstituted DKPs (**44**), Figure 2.3.[18]



43 **44**

*Figure 2.3: Example of Nα-Boc-dipeptidyl ester (**43**) and example of chiral trisubstituted 2,5-diketopiperazine (**44**).*

The synthesis of the 1,4 disubstituted DKP template has also been used in the synthesis of 2,6-diaminopimelic acid (**45**) and derivatives of this amino acid (**46**, **47**), shown in Figure 2.4, as well as in the synthesis of several non-natural amino acids.[19-21]

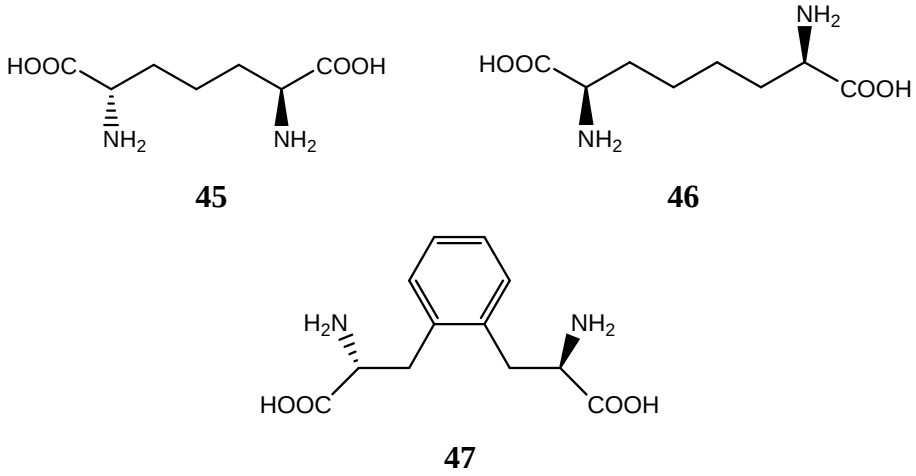
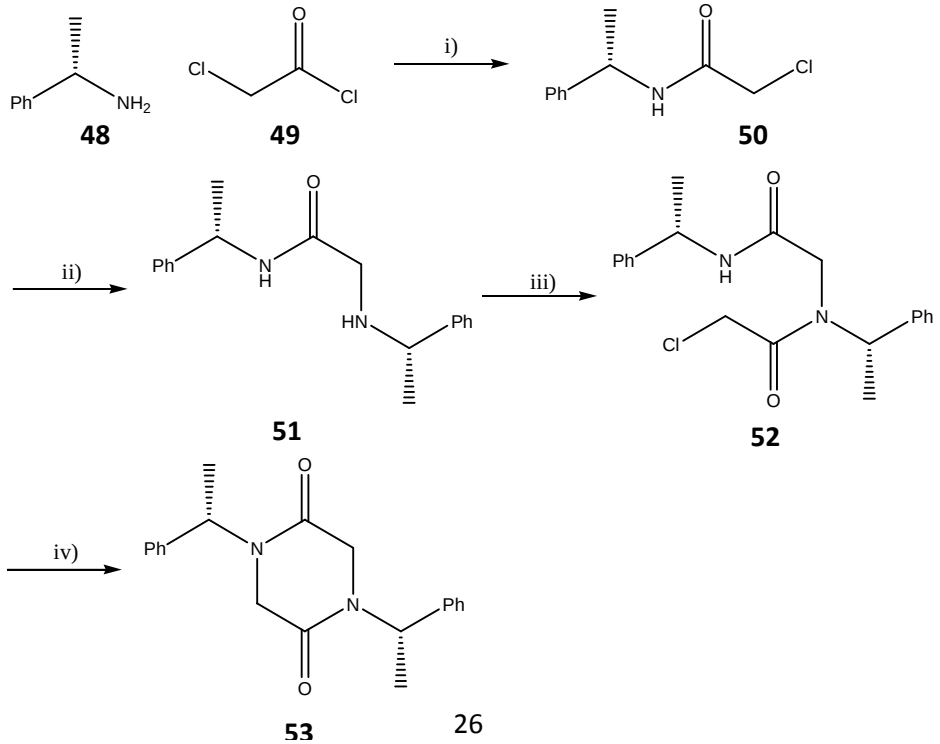


*Figure 2.4: Structure of (2R, 6R) diaminopimelic acid (**45**); (2R, 7R) diaminosuberic acid (**46**) and(S,S) ortho-phenylene-bis-alanine (**47**) .*

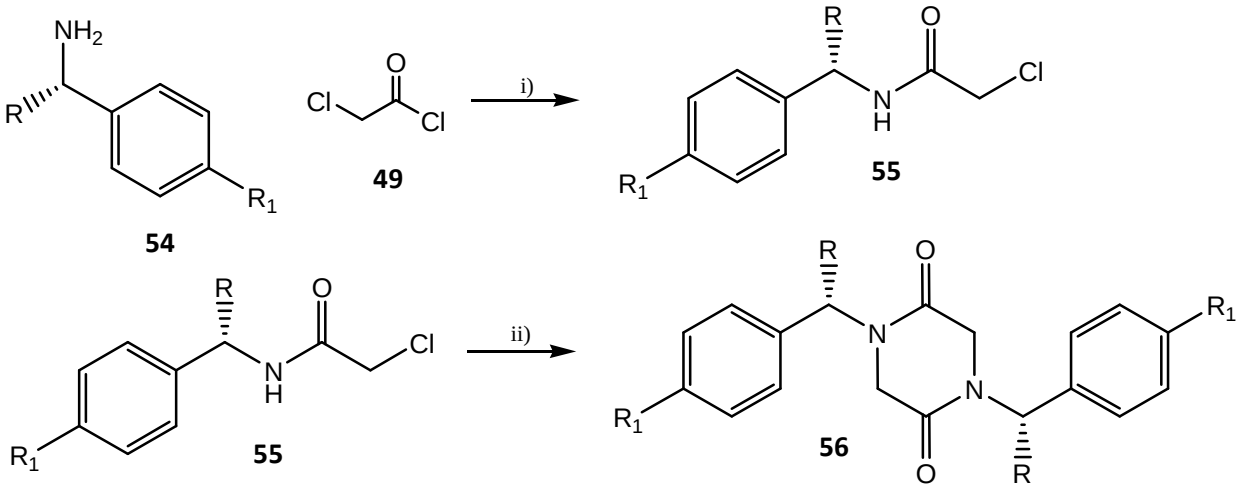
The synthesis of DKP is widely described in literature and is usually reported as a four-steps "head to tail" synthesis, in which the first step is the Shotten-Baum reaction between phenylethyl amine (**48**) and chloroacetyl chloride (**49**) as shown in Scheme 2.1. The substitution of the other chlorine with a second molecule of phenylethyl amine is followed by the attack of the amine to a second molecule of chloroacetyl chloride. The cyclisation is complete when the amide nitrogen attacks the electropositive carbon in α to the carbonyl substituting the chlorine in the same position.



Reagents and conditions: i) Na_2CO_3, Acetone/H_2O; ii) Phenylethyl amine, K_2CO_3, EtOH; iii) chloroacetyl chloride, Na_2CO_3, Acetone/H_2O; iv) n-BuLi, THF, 0 °C.

*Scheme 2.1: "Head to tail" four steps DKP (**33**) synthesis.*

In Paradisi's laboratory DKP is the main scaffold used to synthesize amino acids, as shown in Scheme 2.2.[3] This alternate synthetic strategy involves the acylation of the commercially available amine (**54**) using chloro acetylchloride (**49**) leading to the chloroacetamide (**55**) followed by the cyclisation of the latest to achieve the final DKP (**33**).

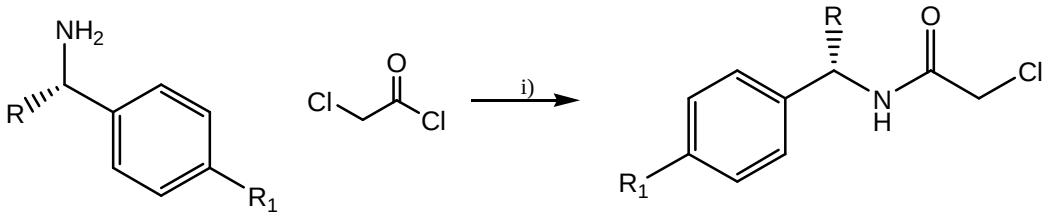


Reagents and conditions: i) DCM, TEA, -10 °C-r.t. 2 h; ii) TEBA (10 mol %), NaOH, DCM, r.t., 48 h.

*Scheme 2.2: Synthesis of DKP (**33**) used in Paradisi's laboratory.*

2.3- Synthetic approach and discussion

The first step in the synthesis of substituted benzyl DKP (**56**) is the preparation of chloroacetamide (**55**) *via* the Schotten-Baumann reaction, the acylation of the amine with the carboxylic acid chloride, as shown in Scheme 2.3. During the course of the reaction one molecule of hydrochloric acid is produced and so one equivalent of base is used to neutralise it and to avoid the formation of the amine salt driving the equilibrium. The reaction is very effective and proceeds with high yields. The crude product of the reaction seems quite clean, even without purification, but it has been noticed that after silica gel chromatography column the yield of the successive step in this sequence, namely the cyclisation with TEBA is higher.



54(a, b) **49** **55**

a R = H; R_1 = H

b R = CH_3; R_1 = OCH_3

Reagents and conditions: i) DCM, TEA, -10 °C-r.t., 2 h.

*Scheme 2.3: Acylation of the amine (**54**) chloroacetamide (**55**)*

The problem with bifunctional molecules such as chloroacetamides is their tendency to polymerise under certain conditions, in fact they are often isolated as side products of peptide synthesis.[19,20] Usually high concentrations favour polymerisation and high dilutions favour cyclisation.[21] Reaction described in Scheme 2.4 seem to be totally selective towards the cyclisation.

The product (**55**) is recognisable from the ^{1}H-NMR spectrum (for instance for compound **55a**, Figure 2.5) because of the characteristic peaks of the benzylic protons at 7.39 and 7.26 ppm, of the nitrogen 6.86 ppm, of the benzylic methylene at 4.50 ppm and of the methylene in α to the carbonyl at 4.11 ppm.

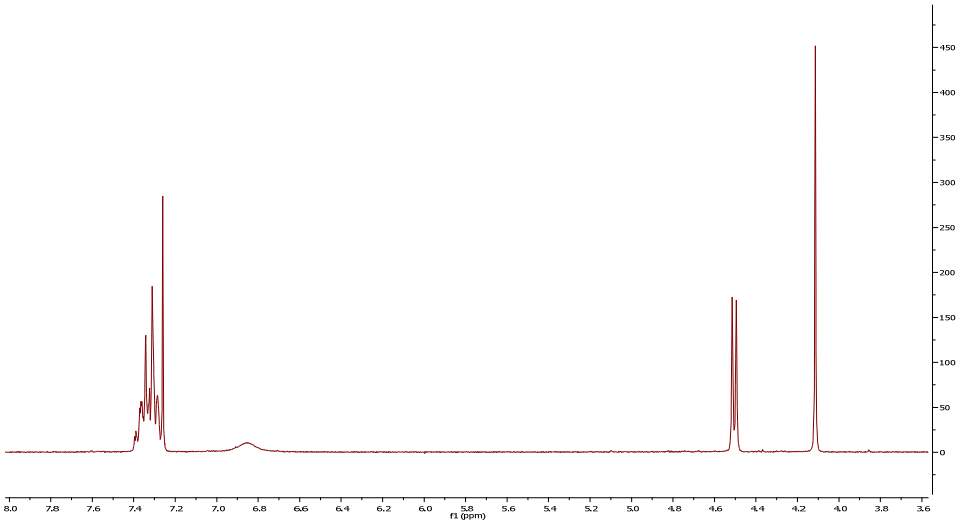
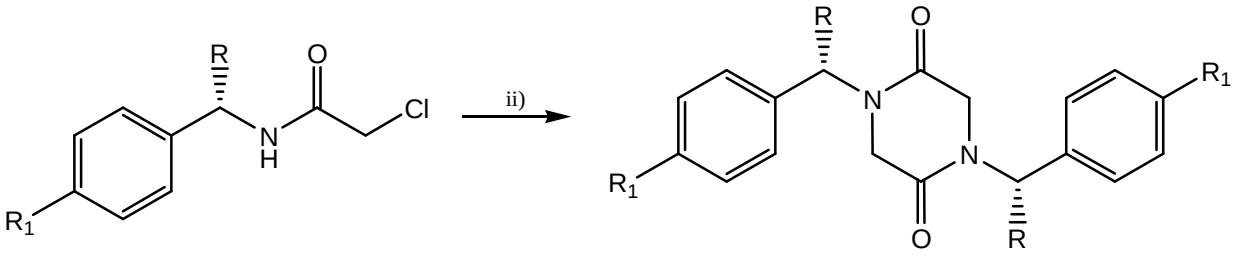


*Figure 2.5: ^{1}H-NMR in $CDCl_3$ of the benzyl-acetamide (**55a**).*

The cyclisation of chloroacetamide (**55**) shown in Scheme 2.4, was performed in the presence of a phase transfer catalyst (PTC) to produce DKPs (**56**) in very high yields (70-90 %).[3]



55 **56**

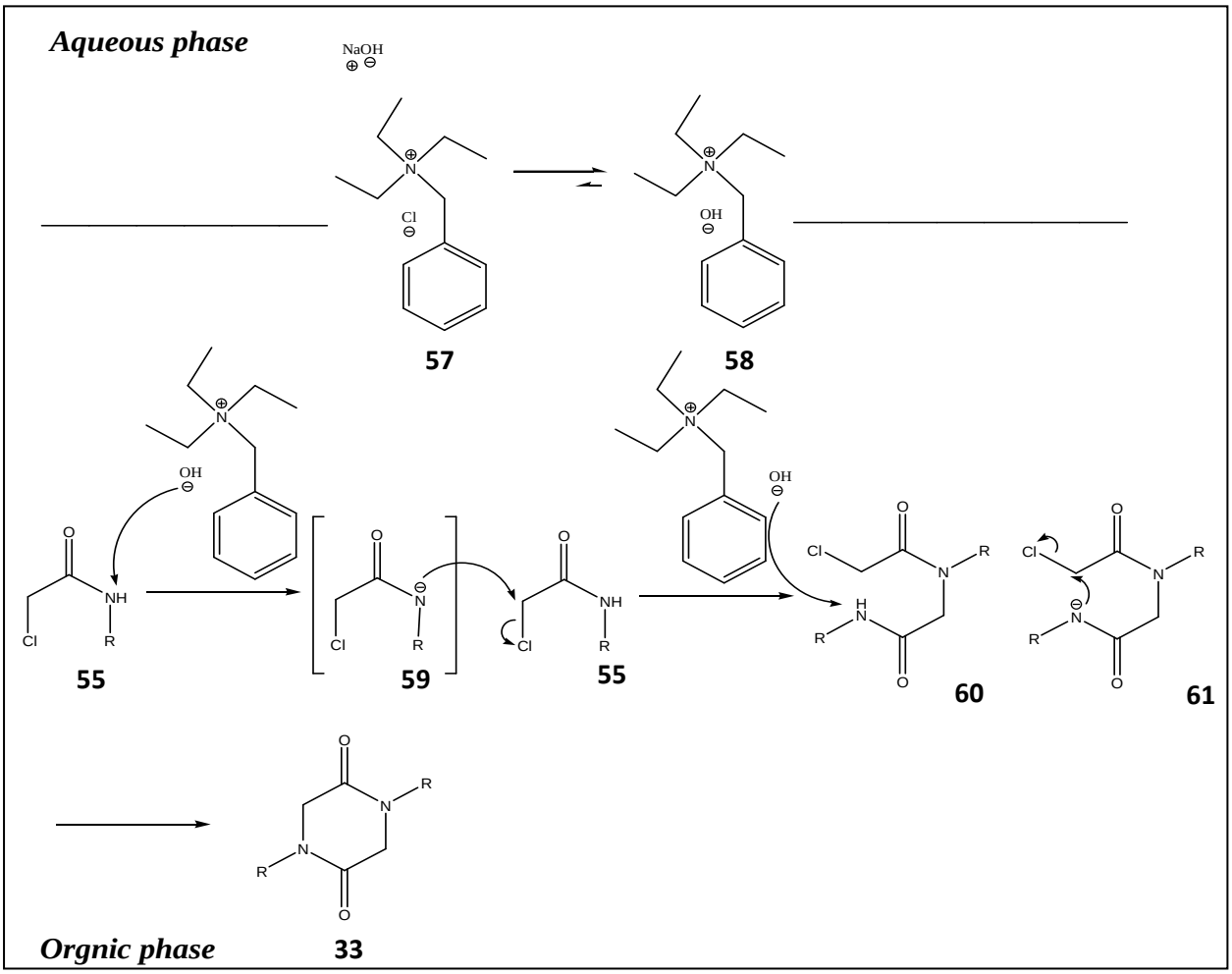
a R = H; R_1 = H

b R = CH_3; R_1 = OCH_3

Reagents and conditions: i) DCM, acryloyl chloride, TEBA (10 mol %), r.t., 48 h.

*Scheme 2.4: Cyclisation of the amide (**55a,b**).*

PTCs increase the rate of the reaction under mild conditions. The PTC employed in this reaction was benzyltriethylammonium chloride (TEBA). As it is soluble in both aqueous and organic phases it allows the interaction between the two phases in a heterogeneous system. Hydroxide ions (OH^-), the reactive anion of sodium hydroxide (NaOH), the inorganic base, are continuously introduced into the organic phase in the form of lipophilic ion pairs, where the lipophilic cations are supplied by the catalyst.[25,26] The concentration of the reacting anions in the organic phase cannot exceed the concentration of the catalyst (10 mol %). The catalyst (**57**) forms a partial covalent bond with the hydroxyl ion of the base, forming the active catalyst (**58**) which, once in the organic phase, can deprotect the amide nitrogen (**59**) which nucleophilically attacks the carbon in α to another molecule of chloroacetamide forming compound (**60**). The second deprotonation of the nitrogen (compound **61**) and nucleophylic attack produce the DKP (**33**) as shown in Scheme 2.5.



*Scheme 2.5: Phase transfer catalysed DKP (**33**) synthesis.*

The ^{1}H-NMR spectrum of compound (**56a**, Figure 2.6) shows the characteristics peaks of the benzylic protons at 7.26-7.35, the singlet of the benzylic methylene is now shifted to 4.6 ppm respect the previous spectrum and the one of the methylene in α to the carbonyl is now shifted to 1.6 ppm. In the case of the *para*-methoxybenzyl-diketopiperazine there is also the peak of the methoxy group at 3.9 ppm

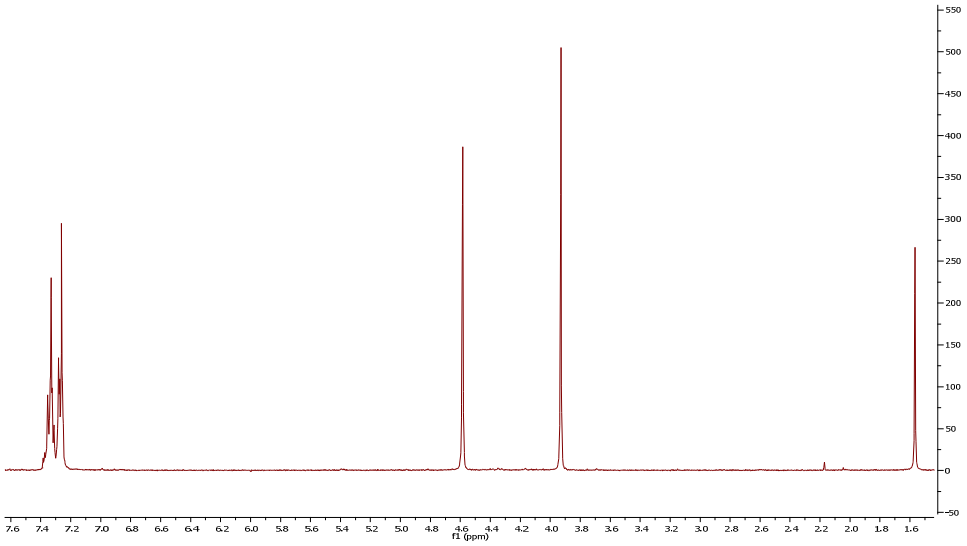
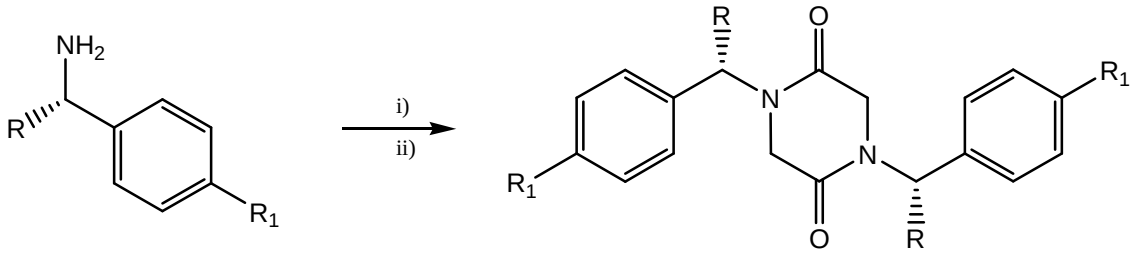


*Figure 2.6: ^{1}H-NMR in $CDCl_3$ of the DKP (**56a**).*

Further investigations have lead to the development of a new procedure for the one-pot synthesis of substituted benzyl DKP (**56**), in which the PTC (**57**) is added to the reaction from the beginning with NaOH as base, avoiding the use of TEA, as shown in Scheme 2.6.[27] Chloroacetamides can be easily prepared from the corresponding amine, as shown in Scheme 2.2, but they need purification before the cyclisation step, which lengthens the overall synthetic process and inevitably affects the overall yield. The original method has been extended to incorporate the synthesis of the chloroacetamide and its selective cyclisation in one-pot under PT conditions.



54 **56**

a R = H; R_1 = H

b R = CH_3; R_1 = OCH_3

c R = CH_3; R_1 = H

Reagents and conditions: i) $C_2H_2Cl_2O$, DCM, NaOH (50 %),TEBA (10 mol %) 48 h.

*Scheme 2.6: Phase transfer catalysed one-pot DKP synthesis (**56**).*

2.4- Conclusions

As already mentioned, DKPs (**33**) are important in numerous research fields, especially for their capability to generate amino acids.

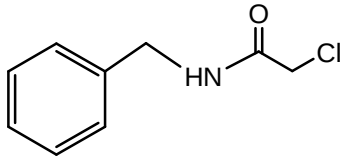
In this research project a one-pot synthesis of 1,4-disubstituted piperazine-2,5-dione (**33**) has been developed employing a PTC (**57**), starting from a suitable amine and chloroacetyl chloride (**49**).

The development a one-pot procedure for the synthesis of organic compound is an important achievement. It allows the desired product to be obtained in a shorter time period and without expensive purification which is usually required in a step-by-step synthesis. In this specific case, the one-pot procedure avoids the purification of the on a silica gel chromatography column, because the DKP core (**33**) is reached in only one step using PTC (**57**) and with a reaction selectivity towards the cyclisation rather than the polymerisation of chloroacetamide (**55**).

With this easy procedure, starting from different commercially available amines (**54**), it is possible to obtain different typology of 1,4-disubstituted piperazine-2,5-dione (**56**), which can be hydrolysed to the corresponding amino acid. Amino acids, both natural and non natural, are useful building blocks for numerous biological active molecules and in the case of this research project the goal is the synthesis of a molecule with potentially antimicrobial activity, (*S*)-2-ammonio-3-((1*R*,6*R*)-2-oxo-7-oxa-3-aza-bicyclo[4.1.0]heptan-5-yl)propanoate (**25**).

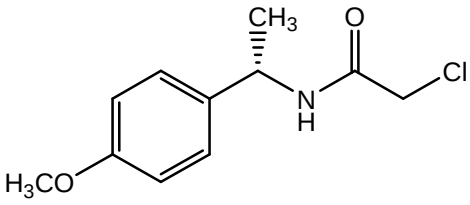
2.5- Experimental section

- N-benzyl-2-chloroacetamide (**55a**)[3]



To a stirred solution of benzyl amine (**54a**, 1 g, 9.3 mmol) and triethylamin (TEA, 1.41 g, 13.6 mmol) in dichloromethane (DCM, 15 mL) at -10 °C was added chloroacetyl chloride (**49**, 1,26 g, 11.2 mmol) dropwise. The solution was allowed to reach room temperature and after 2 hours was cooled to 0 °C before water (10 mL) and 10 % HCl until neutralisation were added. The organic phase was removed *in vacuo* after extracting the aqueous phase with EtOAc (3 x 15 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to produce a crude brown solid that was purified by silica gel chromatography column (EtOAc:Cyclohexane, 50:50) to produce a white solid in 76 % yield. Rf: 0.8, Cyclohexane:EtOAc, 50:50; δ_H (400 MHz; $CDCl_3$): 7.39-7.26 (m, 5H, Ph), 6.86 (br. s, 1H, NH), 4.51-4.49 (d, *J* = 5.8, 2H, CH_2Ph), 4.11 (s, 2H, CH_2Cl); 166.00 (CO), 137.47 (Ph), 129.23 (Ph), 127.94 (Ph), 44.45 (CH_2N), 43.09 (CH_2Cl).

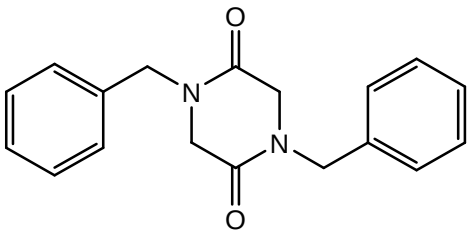
- (S)-2-Chloro-N-(1-(4-methoxyphenyl)ethyl)acetamide (**55b**)[3]



To a stirred solution of (*S*) 1-(4-methoxyphenyl)ethanamine (**54b**, 0.7 g, 4.6 mmol) and triethylamin (TEA, 0.97 mL, 6.9 mmol) in dichloromethane (DCM, 20 mL) at -10 °C was added chloroacetyl chloride (**49**, 0.45 mL, 5.6 mmol) dropwise. The solution was allowed to reach room temperature and after 2 hours was cooled to 0 °C

before water (10 mL) and 10 % HCl until neutralization were added. The aqueous phase was washed with EtOAc (3 x 15 mL), the organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo* to produce a crude brown solid that was purified by silica gel chromatography column (EtOAc:Cyclohexane, 50:50) obtaining a white solid in 76 % yield:. Rf: 0.63, Cyclohexane:EtOAc, 50:50; δ_H (600 MHz, $CDCl_3$) 7.29–7.24 (d, *J* = 6.8 Hz, 2H, Ph), 6.90–6.88 (d, *J* = 8.7, 2H, Ph), 6.73 (br. s, 1H, NH), 5.14-5.05 (m, 1H, CH), 4.05-3.99 (d, *J* = 7.1 Hz, 2H, CH_2), 3.80 (s, 3H, OCH_3), 1.53-1.51 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CH); δ_C (151 MHz, $CDCl_3$) 164.80 (CO), 159.06 (Ph), 134.42 (Ph), 127.31 (Ph), 114.15 (Ph), 55.29 (CH_2Cl), 48.70 (CH_3O), 42.63 (CH), 21.51 (*C*H_3CH); 50:50; HRMS (EI): m/z calcd for $C_{11}H_{14}ClNO_2$: 227.0713, found: 227.0711; $[\alpha]^{20}_D$ – 89 (c 0.35, $CHCl_3$); mp: 243–245 °C.

- <u>1,4-dibenzylpiperazine-2,5-dione (**56a**)</u>[3],[27]

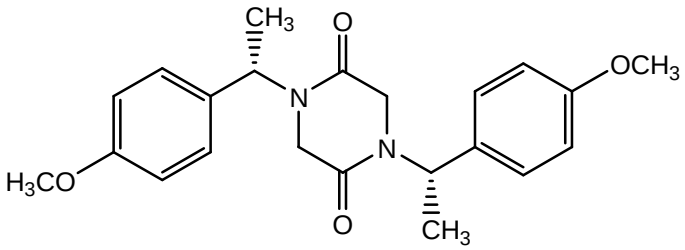


To a stirring solution of 2-chloro-N-(benzyl)acetamide (**55a**, 1.61 g, 7.0 mmol) in DCM (20 mL) were added NaOH 50% (4.5 mL) and TEBA over time (10 % mol). The reaction was left for 48 hours, cooled down to 0 °C and water (20 mL) and HCl until neutralization were added. The aqueous phase was washed with DCM (3 x 20 mL), the organic phases were combined, dried with $MgSo_4$ and concentrated *in vacuo*. The product was purified *via* silica gel chromatographic column (EtOAc:cyclohexane from 15:85 to 60:40) in 85 % yield as a white solid.

Alternate strategy consists in starting from a stirring solution of benzyl amine (**54a**, 0.51 mL, 4.66 mmol) in DCM (8 mL). NaOH 50% (3 mL, 37.3 mmol) and drop-wise chloroacetyl chloride (0.45 mL, 5.6 mmol) were added, after cooling the temperature to -10 °C. The solution was allowed to warm slowly to room temperature. The formation of the amide was monitored by TLC and once the amine had been consumed, TEBA (10 % mol) was added gradually over 48 hours to the vigorous

stirred solution. The reaction was quenched firstly with water (20 mL) and then brought to pH 7 by the addition of 10 % HCl at 0 °C. The DCM was removed *in vacuo*, the aqueous phase was washed with EtOAc (3 x 20 mL), the organic phases were combined, dried with $MgSo_4$ and concentrated *in vacuo*. The product was purified *via* silica gel chromatographic column (EtOAc:cyclohexane from 20:80 to 60:40) in 90 % yield as a white solid. Rf: 0.39, Cyclohexane:EtOAc, 50:50; δ_H (300 MHz, $CDCl_3$) 7.38-7.23 (m, 10 H, Ph), 4.58 (s, 4H, CH_2Ph), 3.93 (s, 4H, CH_2N); δ_C (75 MHz, CDCl3) 163.2 (CO), 134.9 (Ph), 128.9 (Ph), 128.5 (Ph), 128.2 (Ph), 49.3 (CH_2N), 49.2 (CH_2Ph); mp: 175–176 °C; Anal.Calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.38; H, 6.27; N, 9.43.

- 1,4-Bis[(*S*)-1-(4-methoxyphenyl)ethyl]piperazine-2,5-dione (**56b**)[3]

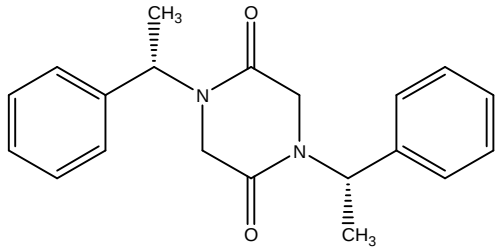


To a stirring solution of (S)-2-Chloro-N-(1-(4-methoxyphenyl)ethyl)acetamide (**55b**, 1.45 g, 6.4 mmol) in DCM (10 mL) were added NaOH 50% (4.0 mL) and TEBA over time (10 % mol). The reaction was left for 48 hours, cooled down to 0 °C and water (20 mL) and HCl until neutralization were added. The aqueous phase was washed with DCM (3 x 20 mL), the organic phases were combined, dried with $MgSo_4$ and concentrated *in vacuo*. The product was purified *via* silica gel chromatographic column (EtOAc:cyclohexane from 15:85 to 60:40) in 40 % yield as a white solid.

Rf: 0.18, Cyclohexane:EtOAc, 50:50; δ_H (500 MHz; $CDCl_3$): 7.20–7.15 (m, 4H, Ph), 6.89–6.82 (m, 4H, Ph), 5.88 (q, *J* = 7.1 Hz, 2H, CH), 3.81 (d, J = 16.5 Hz, 2H, CH_2N), 3.78 (s, 6H, CH_3O), 3.49 (d, *J* = 16.5 Hz, 2H, CH_2N), 1.50 (d, *J* = 7.2 Hz, 6H, C*H*$_3$CH); δ_H (126 MHz, $CDCl_3$) 163.67 (CO), 159.25 (Ph), 130.19 (Ph), 128.52 (Ph), 114.05 (Ph), 55.20 (CH_2N), 49.61 (CH), 44.45 (*C*H_3CH), 15.21 (CH_3O); Anal.

Calcd for $C_{22}H_{26}N_2O_4$: C, 69.09; H, 6.85; N, 7.32. Found: C, 68.95; H, 6.93; N, 7.13. α^{20}_D - 383.5 (c 0.65, $CHCl_3$); mp: 98–99 °C.

- 1,4-Bis[(*S*)-1-phenylethyl]piperazine-2,5-dione (**56c**)[27]



To a stirring solution of (*S*)-phenylethyl amine (**54c**, 1 mL, 7.86 mmol) in DCM (15 mL) were added NaOH 50% (5.3 mL), chloroacetyl chloride (**49**, 0.75 mL, 9.4 mmol) dropwise, after cooling the temperature to -10 °C. The solution was allowed to warm slowly to room temperature. The formation of the amide was monitored by TLC and once the amine had been consumed, TEBA (10 % mol) was added gradually over 48 hours to the vigorous stirred solution. The reaction was quenched firstly with water (20 mL) and then brought to pH 7 by the addition of 10 % HCl at 0 °C. The DCM was removed *in vacuo*, the aqueous phase was washed with EtOAc (3 x 20 mL), the organic phases were combined, dried with $MgSo_4$ and concentrated *in vacuo*. The product was purified *via* silica gel chromatographic column (EtOAc:cyclohexane from 20:80 to 60:40) in 40 % yield as a white solid. Rf: 0.15; δ_H (500 MHz, $CDCl_3$) 7.56-7.16 (m, 10 H, Ph), 5.95 (q, 2H, *J* = 7.1 Hz, CH), 3.86 (d, 2H, *J* = 16.7 Hz, CH_2), 3.52 (d, 2H, *J* = 16.7 Hz, CH_2), 1.54 (d, *J* = 7.1 Hz, 6H); δ_C (126 MHz, CDCl3) 163.8 (CO), 138.3 (Ph), 128.8 (Ph), 128.1 (Ph), 127.3 (Ph), 50.1 (CH_2N), 44.7 (CH); 15.1 (CH_3); mp: 108–110 °C; HRMS (EI): m/z calcd for $C_{20}H_{22}N_2O_2$: 322.1681, found: 322.1693; $[\alpha]^{20}_D$ – 319.1 (c 2.2, $CHCl_3$).

2.6- Bibliography

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    Shabbir S. S., Woollard P. M., Price M. A., *J. Med. Chem.*, **2006**, 49, 4159–4170.
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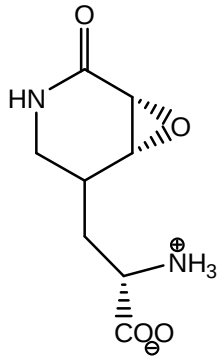
3.1- Aim

The aim of this second part of the project is to synthesise the side chain of the (*S*)-2-ammonio-3-((1*R*,6*R*)-2-oxo-7-oxa-3-aza-bicyclo[4.1.0]heptan-5-yl)propanoate (**25)**.



25

*Figure 3.1: (S)-2-ammonio-3-((1R,6R)-2-oxo-7-oxa-3-aza-bicyclo[4.1.0]heptan-5-yl)propanoate (**25**).*

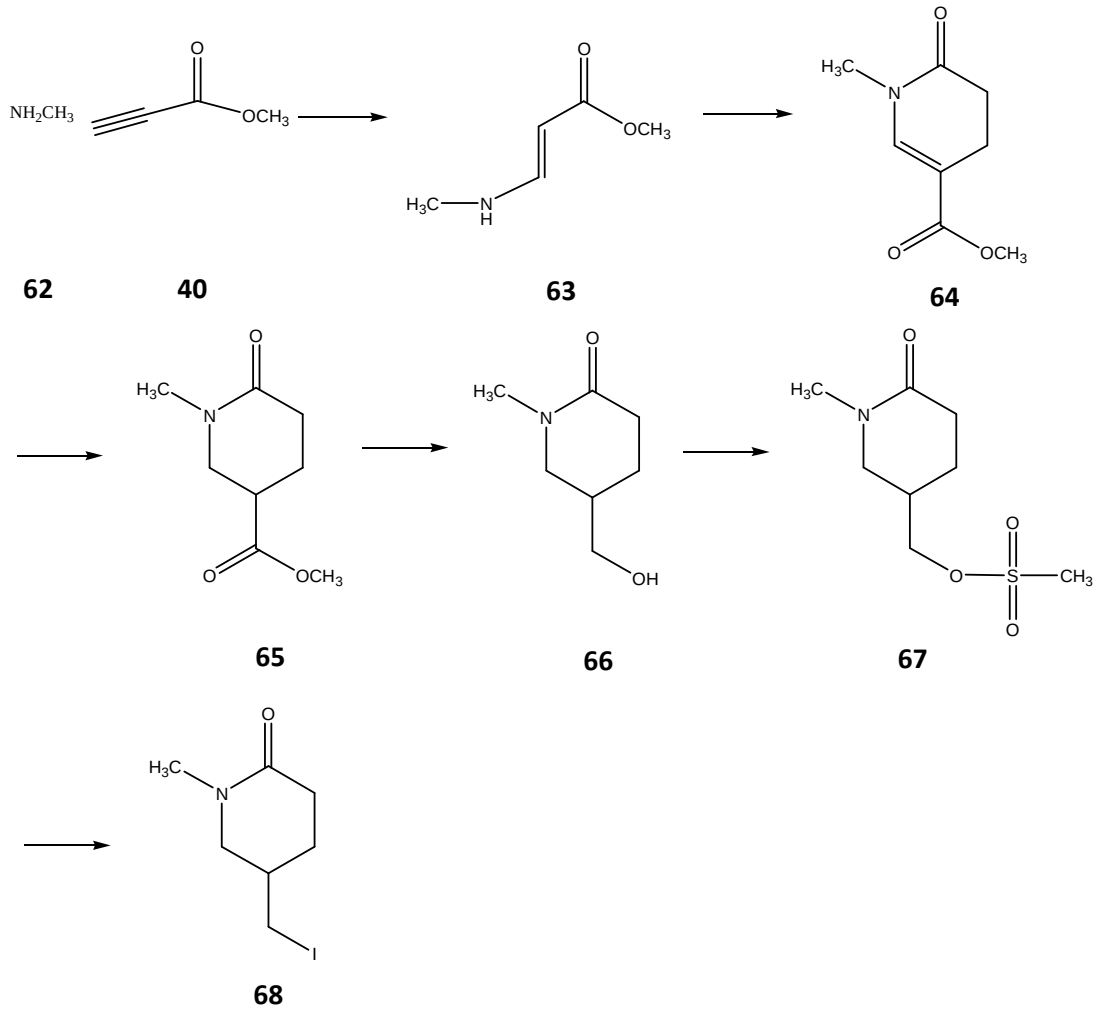
3.2- Introduction

This side chain of our target analogue (**25**) was chosen in order to render the attack on the carbonyl carbon and the hydrolysis to form hydrate (**24**) less likely, as explained in the Section 1.4. Methyl amine (**62**) and methyl propiolate (**40**) were chosen as starting materials as they are commercially available and enabled the synthesis of a six-member ring with an amide functionality (δ-lactame like). Many pharmaceuticals contain a nitrogen atom, it was postulated that the introduction of a nitrogen into this six-member ring would potentially lead to interesting novel activity.

The reaction pathway initially attempted involved 6 steps, as shown below in Scheme 3.1.

This synthetic strategy involves the conjugate addition of the commercially available methyl amine (**62)** to methyl propiolate (**40)** to produce compound (**63**). This was followed by an aza-anulation with acryloyl chloride (**38**) to product the δ-lactone (**64**). Reduction of the heterocyclic olefin leads to the production of compound (**65**) which was then further reduced to the corresponding alcohol (**66**). The next step

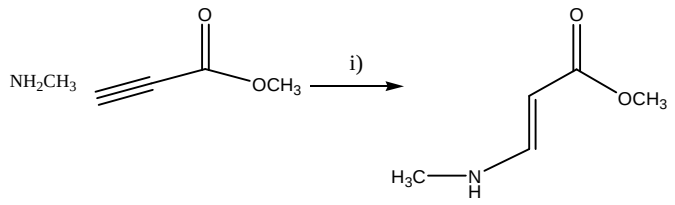
involved the substitution of the alcoholic moiety with the iodine one, leading to the compound (**68**) passing through the mesylated compound (6**7**).



*Scheme 3.1: Pathway for the synthesis of 5-(iodomethyl)-1-methylpiperidin-2-one **(68)**.*

3.3- Synthetic approach and discussion

The first step of the synthetic route was the formation of the (*E*)-methyl 3-(methylamino)acrylate (**63**) obtained by the conjugate addition of methylamine (**62**) to methyl propiolate (**40**). This reaction gave a mixture of diasteroisomers in a ratio of approximately 70:30 (Scheme 3.2), with a yield of 75 %. At this point it was not possible to accurately determine which diasteroisomer had been formed in majority, however the specifics of the ratio are discussed below. The methyl amine (**62**) is very volatile, with a boiling point of -6 °C, so the reaction is performed at -10 °C. The reaction was carried out under nitrogen to avoid the preferential addition of water to the triple bond.

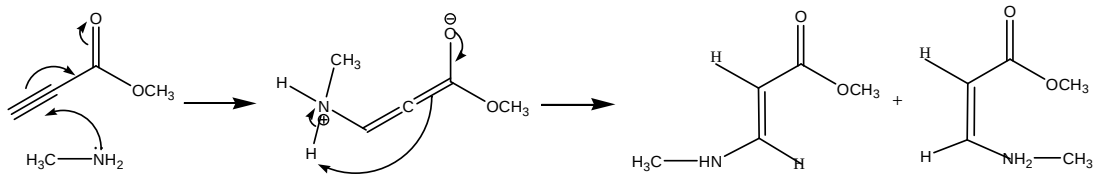


62 **40** **63**

Reagents and conditions: i) THF, -10 °C, 2 h.

*Scheme 3.2: Conjugate addition of methyl amine (**62**) to methyl propiolate (**40**) to obtain enamine (**63**)*

The proposed mechanism of this reaction is shown below, Scheme 3.3: The lone pair of the amine (**62**) attacks the triple bond of methyl propiolate (**40**) and after rearrangement to a diene intermediate (**69**), this leads to the formation of the two diesteroisomers of enamine [E-(**63**)] and [Z-(**63**)].



62 **40** **69** **E-63** **Z-63**

*Scheme 3.3. Mechanism of the reaction of methyl amine (**62**) and methyl propiolate (**40**),* via *diene (**69**) producing enamine [E-(**63**)] and [Z-(**63**)].*

The product of this reaction (**63**) degrades on silica gel but the conversion is quantitative as shown by the ^{1}H-NMR spectrum of the crude and no purification is required (Figure 3.2). The spectrum shows the olefinic protons as a quartet for those α to the nitrogen at approximately 7.55 ppm and 6.59 ppm for diastereoisomers *a* and *b* respectively. The other alkene protons in the position α to the carbonyl carbon are shown as a doublet of doublets at approximately 4.74 ppm and 4.49 ppm for diastereoisomers *a* and *b* respectively. The recognisable broad peak of the amine group occurs at 4.55 ppm. The characteristic singlets of the methoxy protons are visable at 3.67 ppm (diastereoisomer *a*) and 3.64 ppm (diastereoisomer *b*), with a doublet for the methyl group bonded to the nitrogen, at around 2.97 ppm (diastereoisomer *b*) and 2.78 ppm (diastereoisomer *a*).

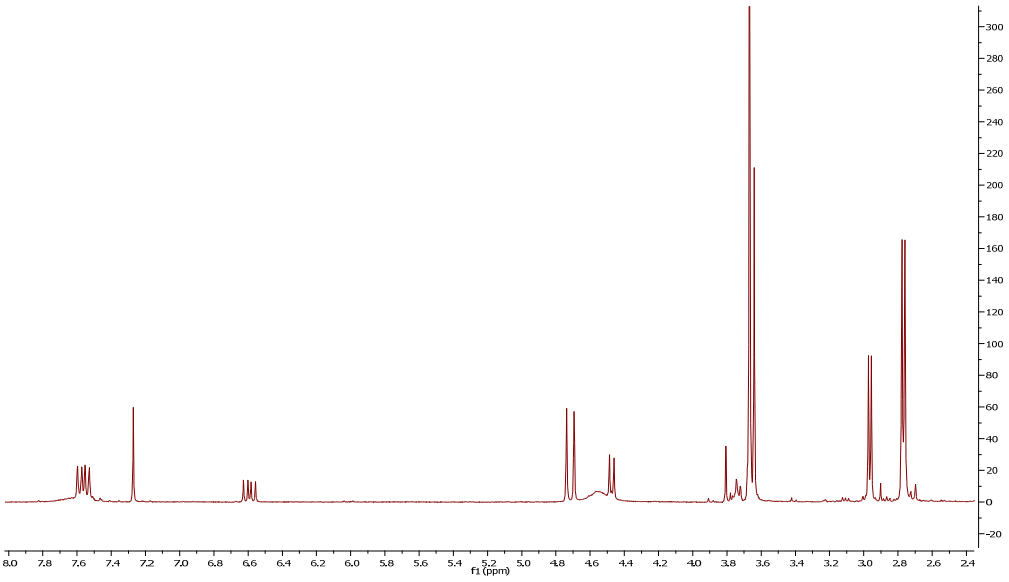
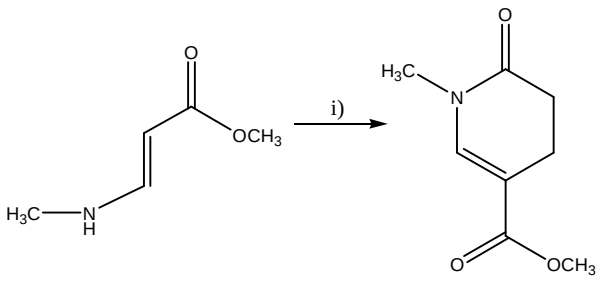


*Figure 3.2: ^{1}H-NMR in $CDCl_3$ f the enamine (**63**).*

The creation of the methyl 1-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylate (**64**) proceeds *via* an aza-annulation reaction of the enamine (**63**) and acryloyl

chloride (**38**),[1,2] as shown in Scheme 3.4. The presence of the electron withdrawing ketone and ester functionalities enhance significantly the efficiency and selectivity of the annulations.[1]

The overall yield of this reaction was relatively low, 47 %. This was possibly due to only one of the two diasteroisomers reacting; sterics dictate that the *cis* isomer (***Z*-63**) is more likely to cyclise than the *trans* isomer (***E*-63**). From this speculation it was postulated that it was the *cis* siomer (***Z*-63**) that had been synthesised in approximately 70% previously (Schemes 3.2 and 3.3, Figure 3.2) Furthermore, TLC evaluation of the reaction showed several side products, denoted by the visualisation of many different spots meaning that the reaction was not very clean. To avoid the addition of water to the double bond of enamine (**63**) and its nucleophilic attack on the carbonyl functionality of the acryloyl chloride (**38**), the reaction was performed under nitrogen.

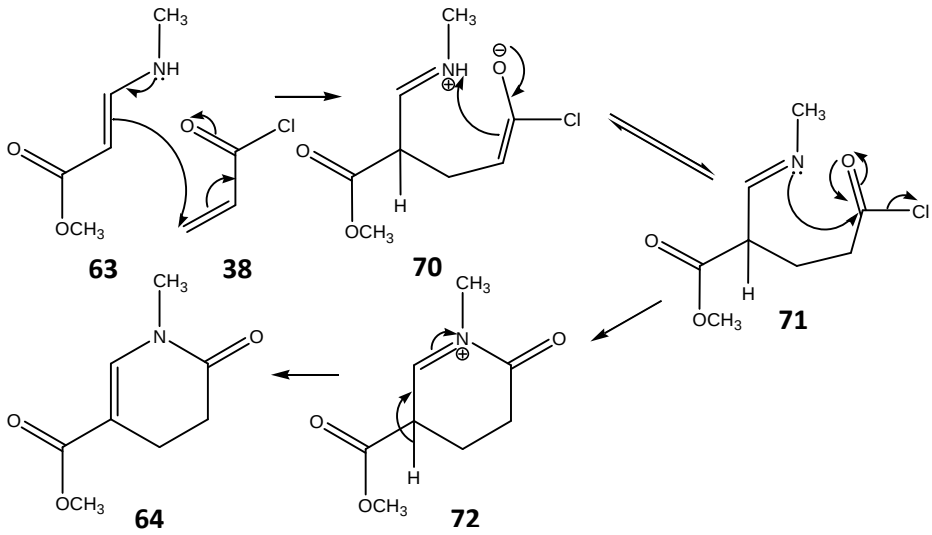


63 **64**

Reagents and conditions: i) THF, $CH_2CHCOCl$, reflux, 2 h.

*Scheme 3.4: Aza-annulation reaction between enamine (**63**) and acryloyl chloride (**38**).*

The mechanism of the aza-annulation reaction has not been fully elucidated but a proposed mechanism is shown below (Scheme 3.5). Initially, the double bond of the enamine (**63**) attacks the alkene functionality of acryloyl chloride (**38**), which consequently rearranges to give the form (**70**) and then (**71**). The chlorine is a good leaving group and the lone pair of the nitrogen easily attacks the carbonylic carbon generating the etherocycle (**72**). The last rearrangement of (**71**) allows the formation of (**64**).



Scheme 3.5: Mechanism of aza-annulation.

The spectrum shows the distinctive singlet of the olefinic proton at 7.27 ppm, in part hidden by the peak of chloroform. The singlet of the methoxy group is observed at 3.75 ppm, with the singlet of the methyl group, bonded to the nitrogen, at 3.15 ppm. The four aliphatic protons of the heterocycle appear as a multiplet at 2.61 ppm, as shown in Figure 3.3.

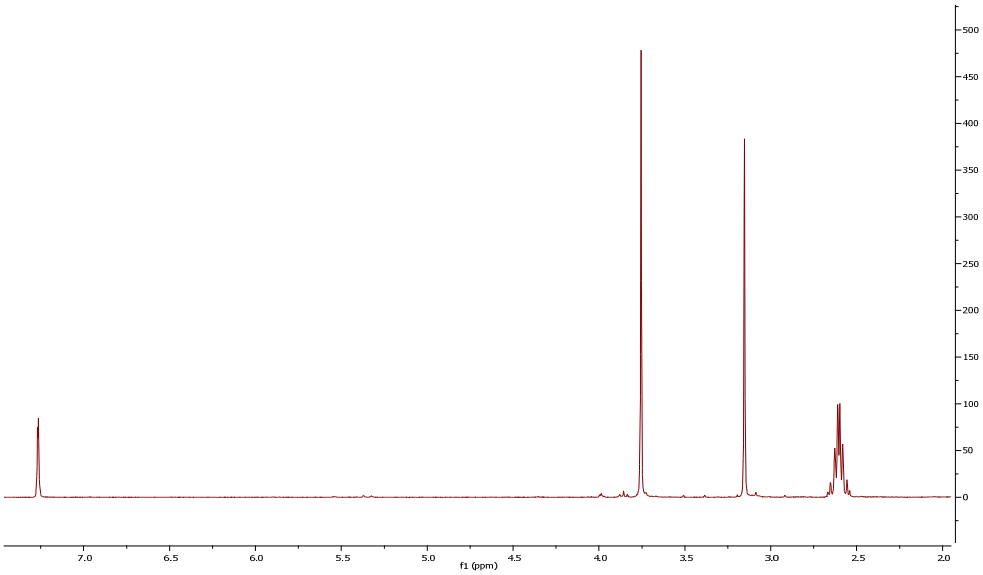
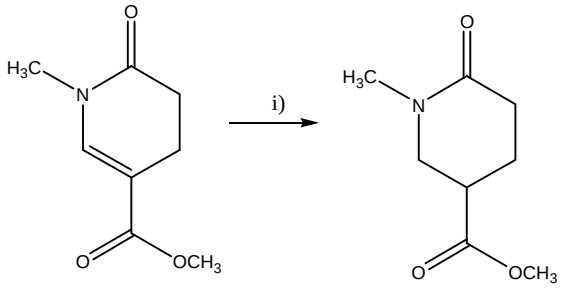


*Figure 3.3: ^{1}H-NMR in $CDCl_3$ of the product of the aza-annulation(**64**).*

Compound (**64**) was then reduced *via* palladium catalysed hydrogenation to produce the racemic methyl 1-methyl-6-oxopiperidine-3-carboxylate (**65**), as shown in Scheme 3.6, in 85 % yield. This reaction has to be performed very carefully under strictly anhydrous conditions as both hydrogen and palladium can react violently with water. This reduction was first attempted using $NaBH_4$ (and then $LiBH_4$) as reducing agents. This was performed in an endeavour to simultaneously reduce the double bond of the heterocycle and of the ester function, to produce compound (**65**). Neither of these hydrides was strong enough to reduce the double bond, so this methodology was not further pursued and the reduction was carried out in two separate steps.



64 **65**

Reagents and conditions: i) EtOH, H_2, Pc/C, r.t., 12 h.

*Scheme 3.6: Hydrogenation of compound (**64**) to give compound (**65**).*

The ^{1}H-NMR spectrum of compound (**65**) is now much more complicated than the starting material of the reaction, compound (**64**). The reduction of the olefin means that the heterocycle looses the planar characteristics associated with sp^2 hybridised carbons. The interactions of axial and equatorial protons round the flexible ring mean that all the signals, apart from the methyl and methoxy group (singlets), are multiplets (Figure 3.4).

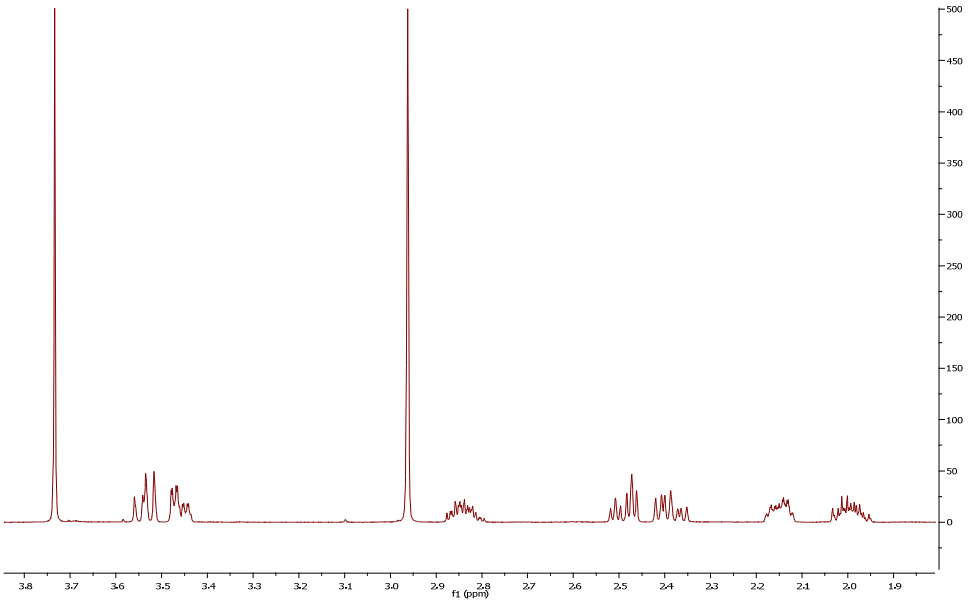
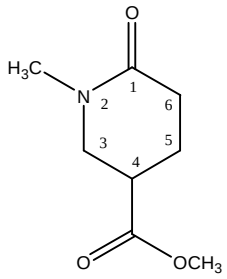


*Figure 3.4: ^{1}H-NMR in $CDCl_3$ of the product of the hydrogenation (**65**).*

For an easy identification of the signals, numbers have been assigned to the heterocycle atoms (**73**), as shown in Figure 3.5. Starting from the lower frequencies we find two multiplets relative to the CH_2 5, two multiplets of the methylene 6, a

multiplet of the proton 4 and around 3.5 ppm the multiplets of the protons 3 (less shielded because next to the nitrogen)

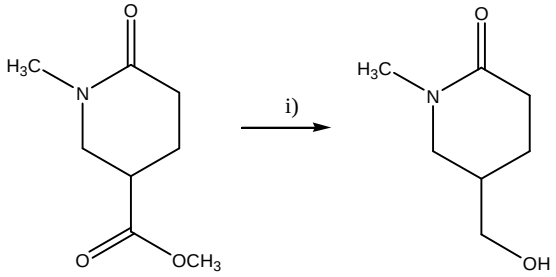
As expected, the peak of the alkene proton is not present anymore.



73

*Figure 3.5: Compound (**65**) in which heterocycle atoms have been assigned numbers for ease of identification.*

Lactame (**65**) was then reduced further. $LiBH_4$ was used to reduce the ester function to 5-(hydroxymethyl)-1-methylpiperidin-2-one (**66**) in 60 % yield, as shown in Scheme 3.7. Again the reaction was carried on under nitrogen flow, because hydride reacts violently with water.



65 **66**

Reagents and conditions: i) THF,$LiBH_4$, 0 °C-r.t., 12 h.

*Scheme 3.7: Reduction of the ester function to alcohol (**66**).*

Comparing the spectrum of this purified alcohol (**66**), Figure 3.6, to that of the ester (**65**), figure 3.4, it is observed that the signal of the methoxy group (3.6 ppm) is no longer present. Additionally the generation of CH_2OH in place of the ester has led to the creation of new multiplets at approximately 3.3 and 3.4 ppm. The large peak at 3.4 is due to residual methanol in the sample.

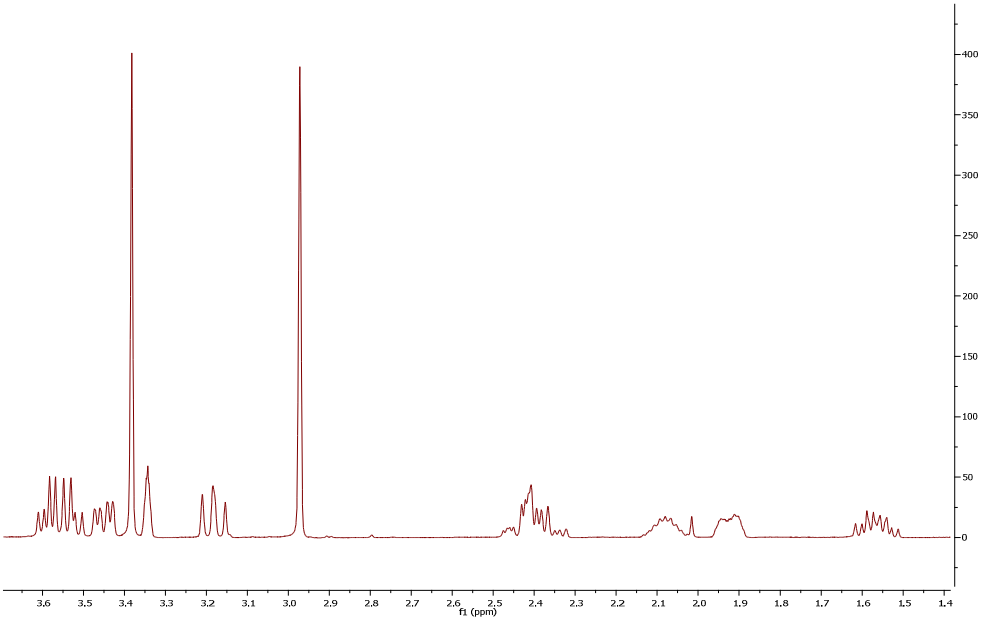
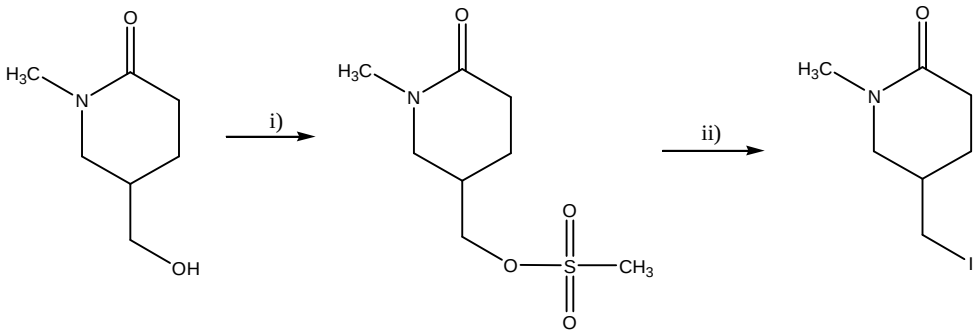


*Figure 3.6: ^{1}H-NMR in CD_3OD of the alcohol (**66**) .*

The next step in the proposed synthesis was the exchange of the alcohol functionality in (**66**) with iodine to create compound (**68,** Scheme 3.8). The alcohol functionality is not exchanged directly with iodine, but the reaction proceeds via the mesylate intermediate (**67**)**.** The reactions are performed in this way as mesylate is a better leaving group than the hydroxyl and it activates the alcohol (**66**) thus increasing the final yield of compound (**68**). Mesylation is usually carried out with TEA in DCM. In this instance compound (**66**) was insoluble in DCM so pyridine was used as both base and solvent for the reaction leading to 80 % yield. Mesyl chloride reacts readily with water, producing the methanesulfonic acid; therefore the reaction is performed under anhydrous conditions.

The mesylate compound (**67**) did not require purification before iodination to produce 5-(iodomethyl)-1-methylpiperidin-2-one (**68**) in 83 % yield.



66 **67** **68**

Reagents and conditions: i) Py,CH_3ClO_2S, 0 °C- refluz,2 h; ii) acetone, NaI, reflux, 5 h.

*Scheme 3.8: Exchange of the alcohol (**66**) with the iodide (**68**).*

The ^{1}H-NMR spectrum of compound (**68**, Figure 3.7) does not differ significantly from the alcohol spectrum (**66**), Figure 3.6, but the peaks are slightly shifted to higher frequencies, due to a difference in the electronegativity of the iodine compared to the alcoholic moiety

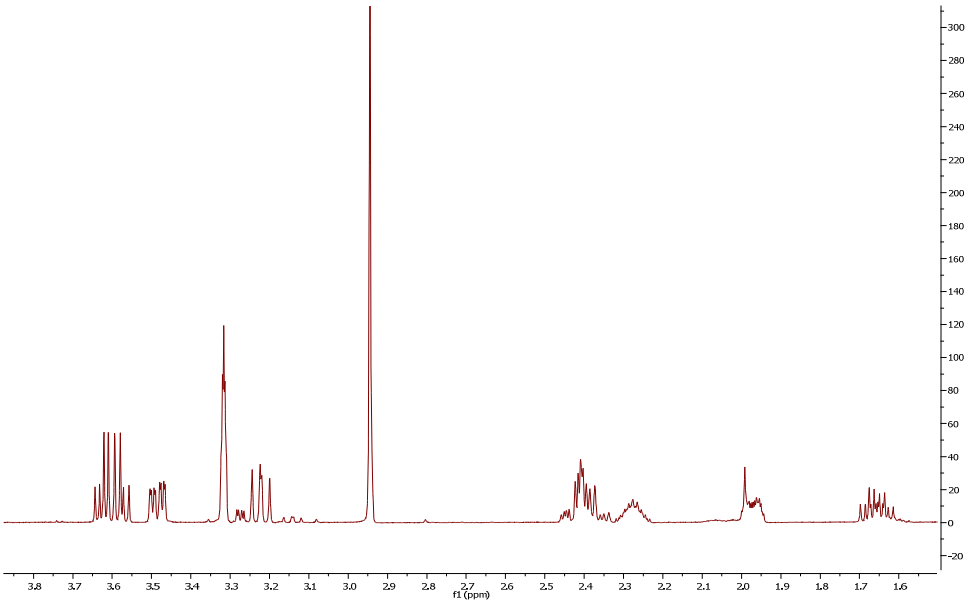
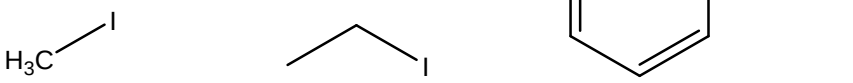


*Figure. 3.7: ^{1}H-NMRin CD_3OD of the iodo-compound (**68**) .*

At this stage the side chain (**68**) was ready to be coupled with the previously prepared substituted benzyl diketopiperazine core (**56**), as shown in Scheme 1.7 in Section 1.4, however this coupling, with LHMDS, was unsuccessfully and only starting materials and a complex mixture of side-products were isolated. This unsuccessful coupling was unexpected as similar reactions had been performed successfully many times using several different alkylating agents including methyl iodide (**74)**, ethyl iodide (**75**) and benzyl iodine (**76**), Figure 3.8.

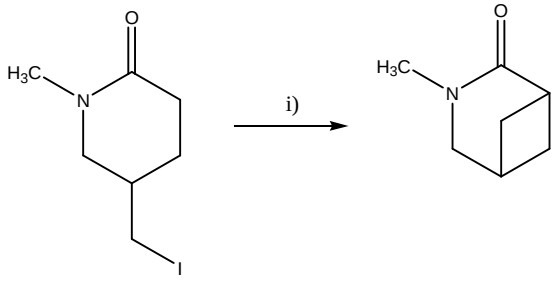


74 **75** **76**

Figure 3.8: Alkilating agents.

When investigating the possible reasons for this unsuccessful coupling, the reaction of the iodo-compound (**68**) and LHMDS was examined, as shown in Scheme 3.9. After purification by silica gel column chromatography compound (**77**) was isolated, derived from an intramolecular cyclisation and elimination of iodine.

The isolation of this compound (**77**) from the reaction is not entirely surprising as the protons in the α position to the carbonyl carbon are very acidic and the iodine is very reactive. However, the strain of a four-member ring fused to a six-member ring was thought to be unfavoured. Interestingly, this product was not isolated during the coupling with the diketopierazine moiety.

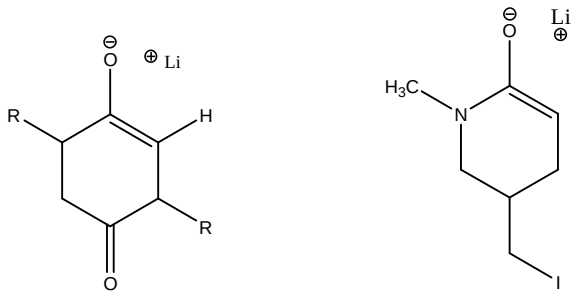


68 **77**

Reagents and conditions: i) THF, LHMDS,.

*Scheme 3.9: Reaction of (**68**) in presence of the only base.*

It is possible that the problems observed during the coupling are due to the fact that the enolate on the diketopiperazine (**78**) is quenched by the formation of a second enolate on the alkylating group (**79**), as shown in Figure 3.9 and as will be discussed more in detail in chapter 4.



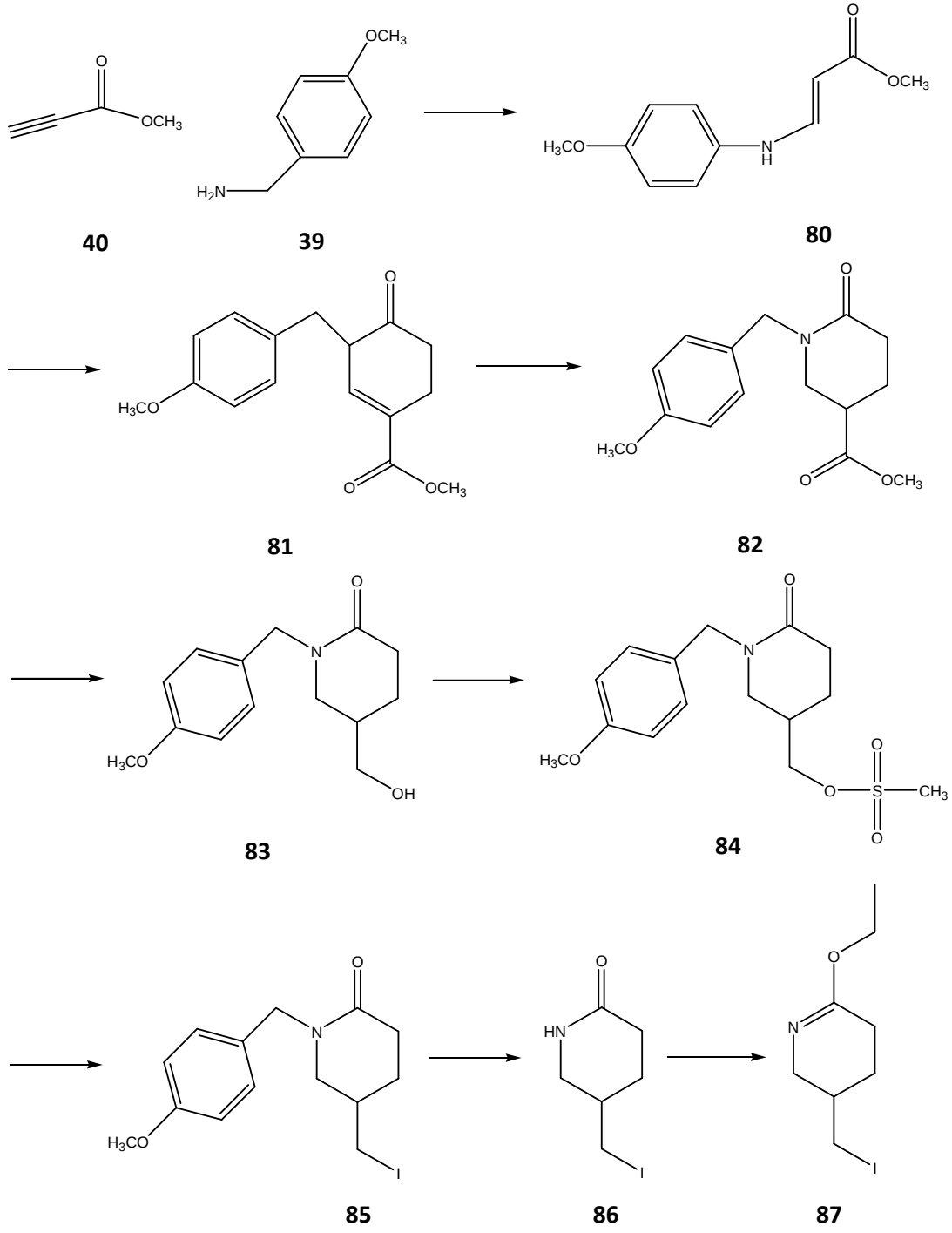
78 **79**

*Figure 3.9: Competitive enolate forms in the reaction of coupling between compound (**68**) and DKP (**33**).*

If this happens faster than the alkylation reaction, no product is isolated. At this stage, after the unsuccessful coupling it was decided to investigate a new synthetic 8 step strategy, as shown below in Scheme 3.10.

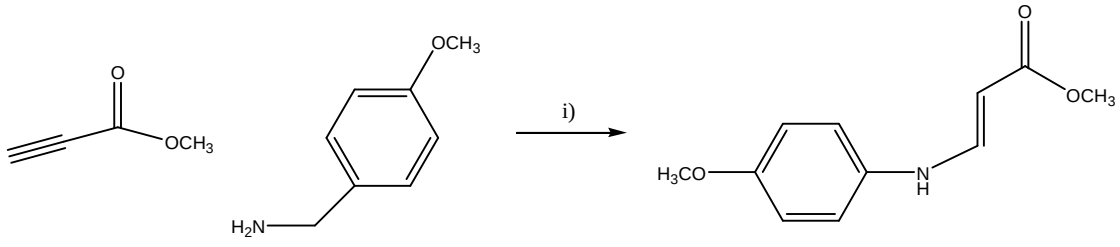
By protecting the nitrogen with an easily removable *para*-methoxy benzyl group, instead of the methyl group, it was envisaged that the amide carbon could be protected as lactim (**87**) thus making the protons of the α position significantly less reactive, shown in Scheme 3.10.

This synthetic strategy up to the formation of the iodinated compound (**85**) is identical to what previously reported in Scheme 3.1. It involves the conjugate addition of the commercially available methoxyphenyl amine (**39)** to methyl propiolate (**40**) to produce the enamine form (**80**) followed by an aza-anulation with acroyl chloride (**38**) to produce the heterocycle (**81**). Reduction of the heterocyclic olefin leads to compound (**82**) which was then further reduced to the relative alcohol (**83**). The next step involved the substitution of the alcoholic moiety with the iodide, leading to the compound (**85**) passing through the mesylated compound (**84**). The iodo-compound (**85**) was deprotected at the amide nitrogen (**86**) using cerium ammonium nitrate (CAN) to allow in the next step the protection of the carbonyl (**87**).



*Scheme 3.10: Alternative pathway for the synthesis of the lateral chain (**87**).*

The first step of this new synthetic route is again a conjugate addition, this time between the more hindered amine, methoxyphenyl amine (**39**)**,** and methyl propiolate (**40**).



40 **39** **80**

Reagents and conditions: i) THF, -10 °C, 2h.

*Scheme 3.11: Reaction of formation of the enamine (**79**) through conjugate addition.*

In the ^{1}H-NMR spectrum (Figure 3.10) again are visible the peaks of both the diasteroisomers of methyl 3-(4-methoxybenzylamino)acrylate, [*E*-(**80**)] and [*Z*-(**80**)], formed by the reaction (ratio *E:Z* is again approximately 70:30). This reaction proceeds very well, with 99 % yield and does not require purification. In the spectrum the characteristic protons of the phenyl appear at approximately 7 ppm, split in two groups because of the *para*-methoxy group that shields the two protons that are in the ortho position relative to the methoxy group. At 7.6 ppm and 6.8 ppm we can see the peaks of the protons of the double bond closer to the carbonyl carbon (*cis* and *trans*), with the alkene protons closer to the nitrogen occurring as doublets at 4.8 ppm (*trans*) and 4.6 ppm (*cis*). The methylene protons of the benzyl group are observed at 4.4 ppm and 4.2 ppm. Finally the characteristic singlets of the methoxy groups appear at 3.9 ppm and 3.7 ppm., for the methoxy moieties of the benzylic and ester groups respectively.

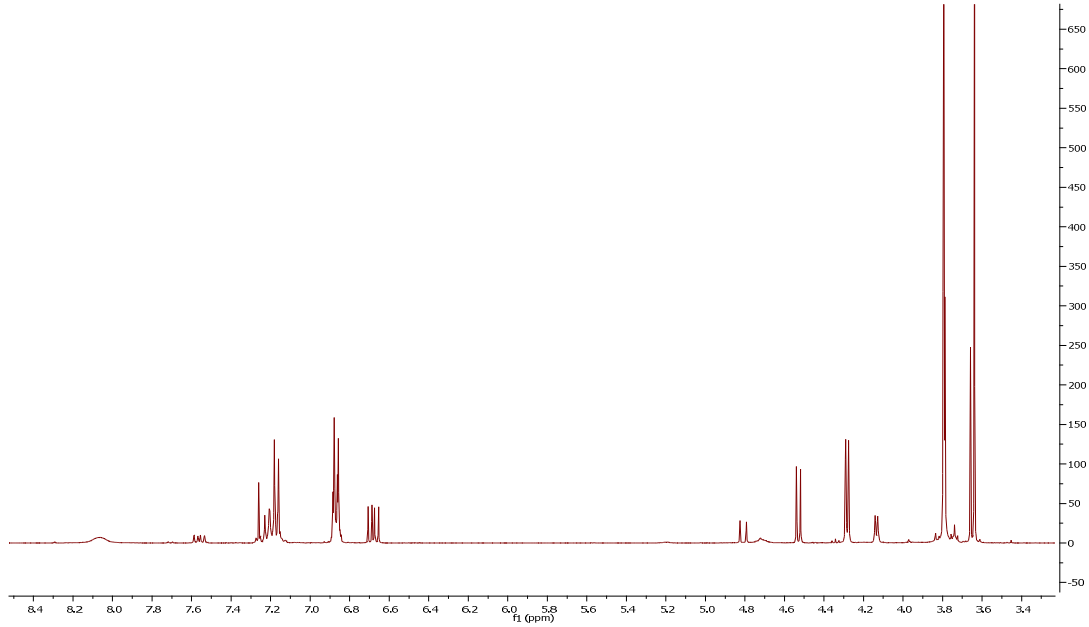
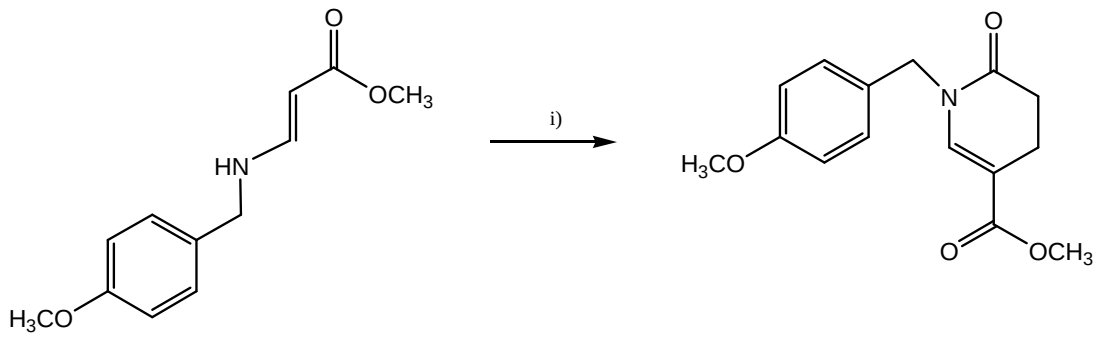


*Figure 3.10: ^{1}H-NMR in $CDCl_3$ of the enamine (**80**).*

Methyl 1-(4-methoxybenzyl)-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylate (**81**) was produced by a reaction between enamine (**80**) and acryloyl chloride (**38**), as shown in Scheme 3.12.



80 **81**

Reagents and conditions: i) THF, $CH_2CHCOCl$, r.t., 12h.

Scheme 3.12 Aza-annulation reaction.

The reaction between enamine (**80**) and acryloyl chloride (**38**) is not as clean as that previously described for enamine (**63**). The six-membered ring δ-lactam-like (**81**) is isolated in only 33 % yield. Several side products were observed by TLC and again it is suggested that only one of the two isomers, the *cis*, was reactive. The ^{1}H-NMR spectrum, Figure 3.11, shows the distinctive signal of the alkene proton at 7.3 ppm, as a singlet, with the two groups of the phenyl protons at approximately 7.3 ppm.

The singlet of the protons of the benzylic methylene group is observed at 4.7 ppm. The singlets of the methoxy groups, respectively for the one bonded to the phenyl ring and for that which forms the ester group are at 3.9 ppm and 3.7 ppm respectively. Finally at 2.6 ppm the signal of the four aliphatic protons of the heterocycle can be recognised.

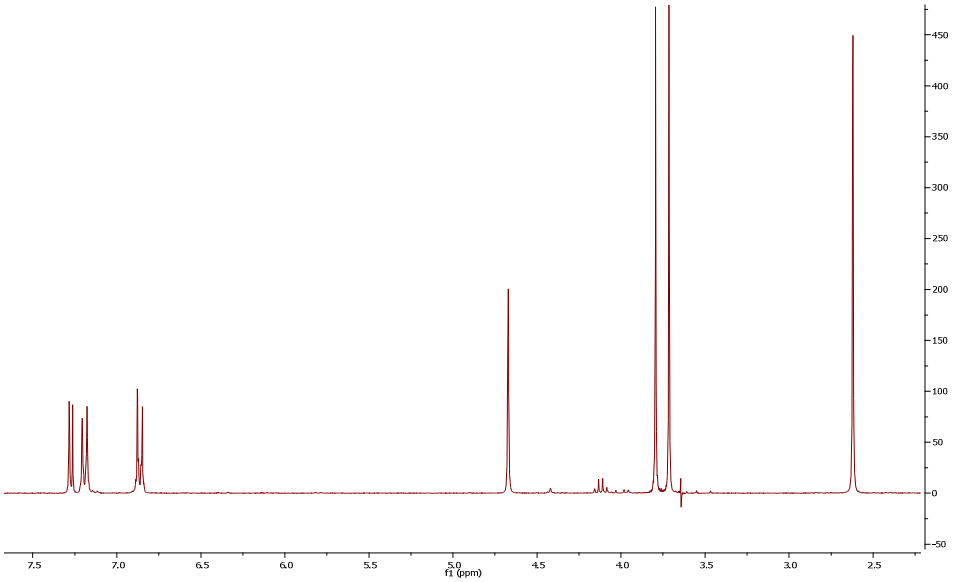
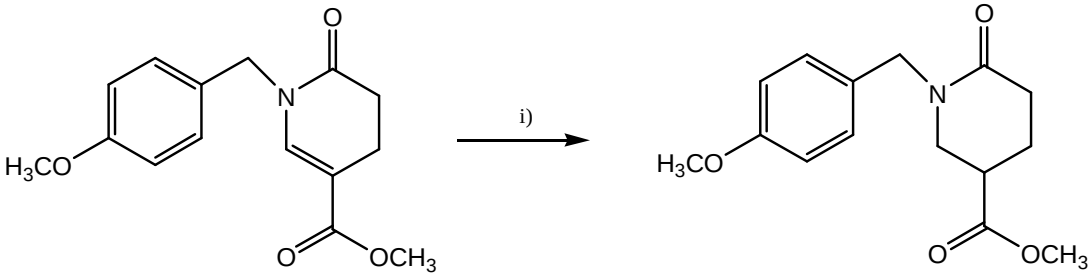


*Figure 3.11: ^{1}H-NMR in $CDCl_3$ of the six-membered ring with (**81**).*

The double bond of the six-membered ring delta lactam like (**81**) was then reduced *via* palladium catalysed hydrogenation to produce methyl 1-(4-methoxybenzyl)-6-oxopiperidine-3-carboxylate (**82**), as shown in Scheme 3.13, with a yield of 86 %.



81 **82**

Reagents and conditions: i) EtOH,, H_2, Pd/C,r.t., 12h.

*Scheme 3.13: Hydrogenation of compound (**80**) to give compound (**81**).*

The ^{1}H-NMR spectrum for methyl 1-(4-methoxybenzyl)-6-oxopiperidine-3-carboxylate (**82**) again had become more complicated than the starting material of this reaction (**81**) as the reduction of the olefin has decreased the planar characteristics of the molecule and the axial and equatorial protons interact more producing more multiplets (signals between 2 and 4 ppm). This was a previously observed phenomenon, with compound (**65**), as shown in Figure 3.12.

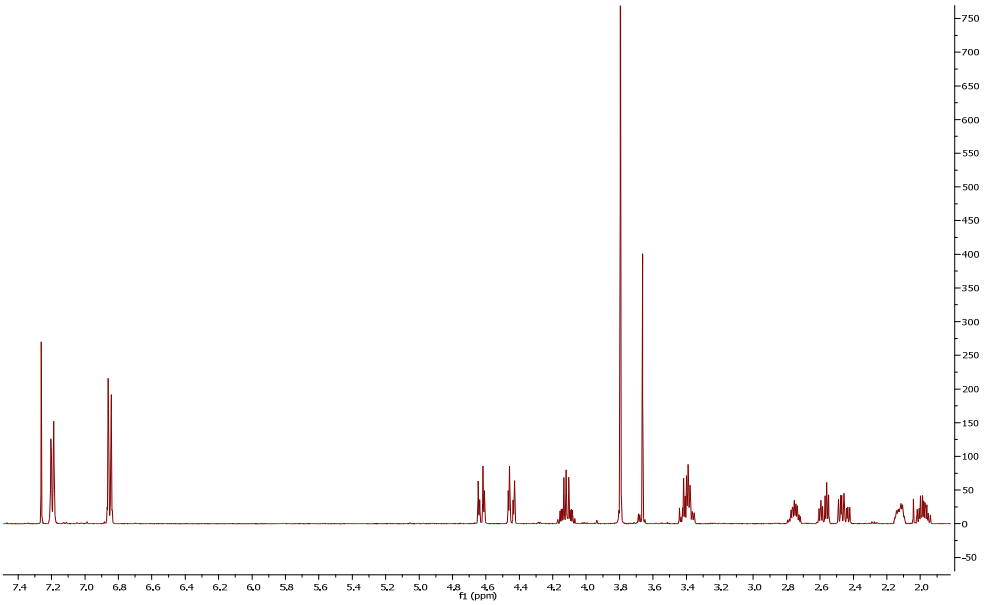
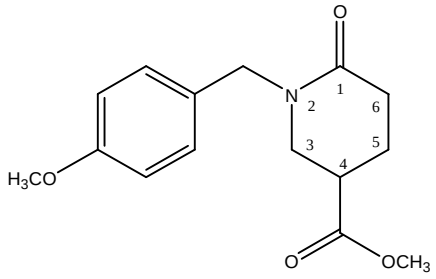


*Figure 3.12: ^{1}H-NMR in $CDCl_3$ of the six-membered ring without after reduction of the double bond (**82**).*

For an easy identification of the signals, numbers have been assigned to the heterocycle atoms (**88**), as shown in Figure 3.13. Starting from the lower frequencies we find two multiplets relative to the methylene 5, two multiplets of the protons 6, a multiplet of the proton 4 and around 3.4 ppm the multiplets of the protons 3, more shielded because closest to the nitrogen. Around 4.6 ppm there are the signal (doublet of doublet) of the benzylic CH_2.

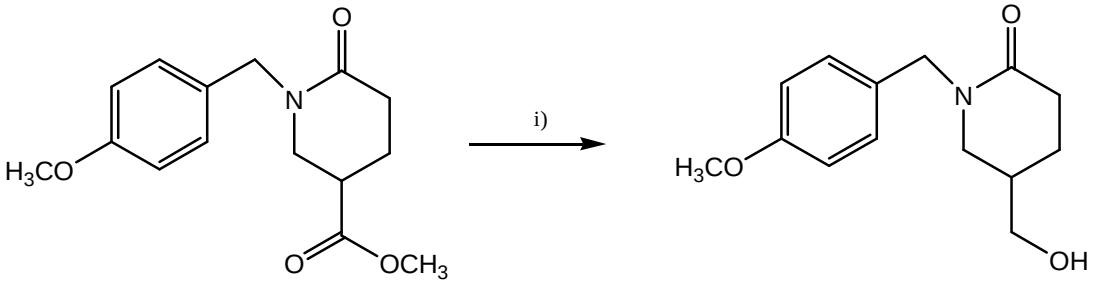
The signal of the olephinic proton is no more visible.



88

*Figure3.13: Compound (**82**) in which heterocycle atoms have been assigned numbers for ease of identification.*

The ester function was reduced with $LiBH_4$ to produce 5-(hydroxymethyl)-1-(4-methoxybenzyl)piperidin-2-one (**83**) in 85 % yield. This shows a significant improvement in yield compared to the reduction of compound (**66**) that was 60 % of yield. The reason of the improvement was probably due to the better solubility of compound (**83**).



82 **83**

Reagents and conditions: i) THF, $LiBH_4$, 0 °C-r.t., 16 h.

*Scheme 3.14: Reduction of the ester function (**82**) to alcohol (**83**).*

Several differences can be observed when comparing this ^{1}H-NMR spectrum in Figure 3.14 with the previous one. The protons of the benzylic methylene (4.6 ppm) now appear as a singlet, whereas they previously occurred as a doublet of doublet. Furthermore the large singlet of the ester methoxy moiety of compound (**82**) has disappeared and in its place two new multiplets at approximately 4.6 ppm can be seen. These are representative of the new methylene created by the reduced ester's carbonyl carbon.

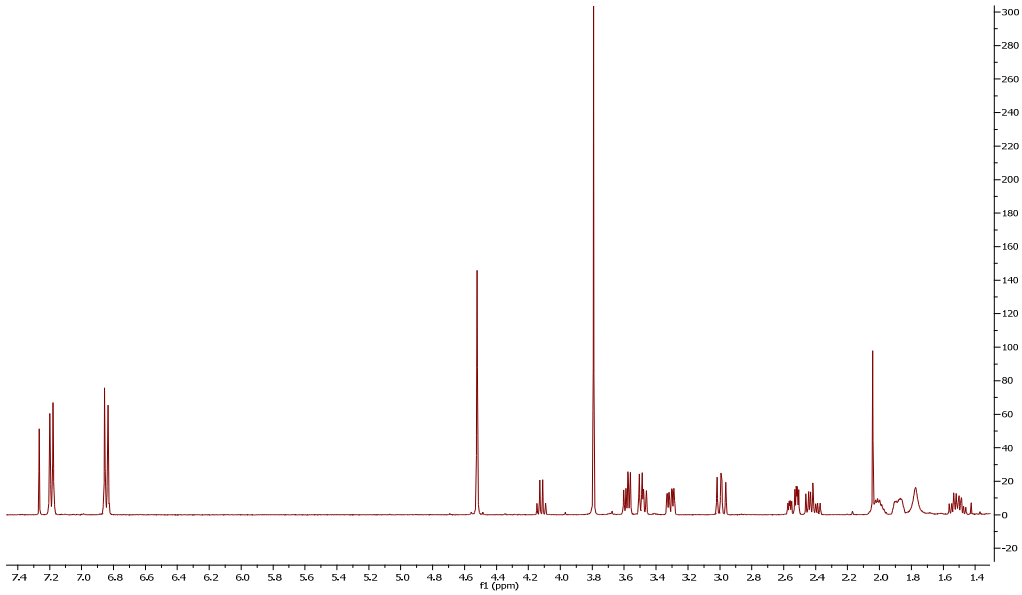
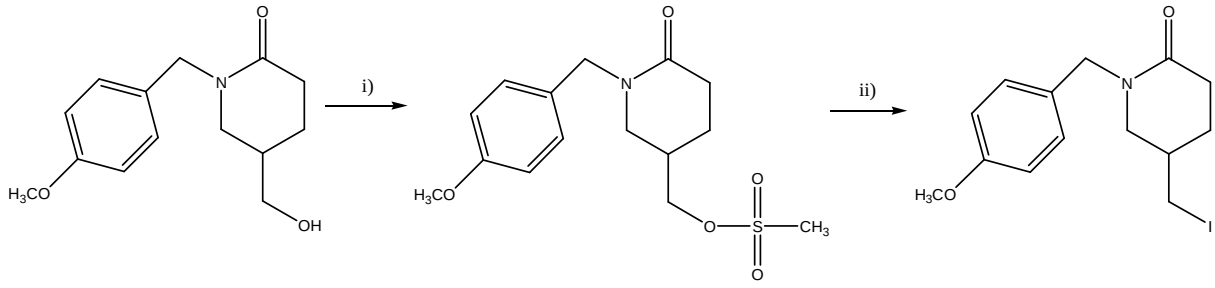


*Figure 3.14: ^{1}H-NMR in $CDCl_3$ of the alcohol (**83**).*

Before the exchange of the alcohol of compound (**83**) with the iodine, it was first activated by substituting with a better leaving group, namely mesylate to produce compound (**84**), as shown in Scheme 3.15. This reaction proceeded well, with 93 % yield, better than that of the previously synthesised compound (**67**, 80 %). The iodination of compound (**85**) was performed using sodium iodide in acetone. Again this reaction gave a high yield (80 %), and the overall yield for these two steps was 78 %.



83 **84** **85**

Reagents and conditions: i) DCM, TEA, -10 °C-r.t., 16 h; ii) acetone, NaI, reflux, 4 h.

Scheme 3.15: Exchange of the alcohol with the iodine.

The ^{1}H-NMR spectrum of compound (**85**) does not differ significantly from the spectrum of the alcohol (**83**, Figure 3.14**),** as shown in Figure 3.15.

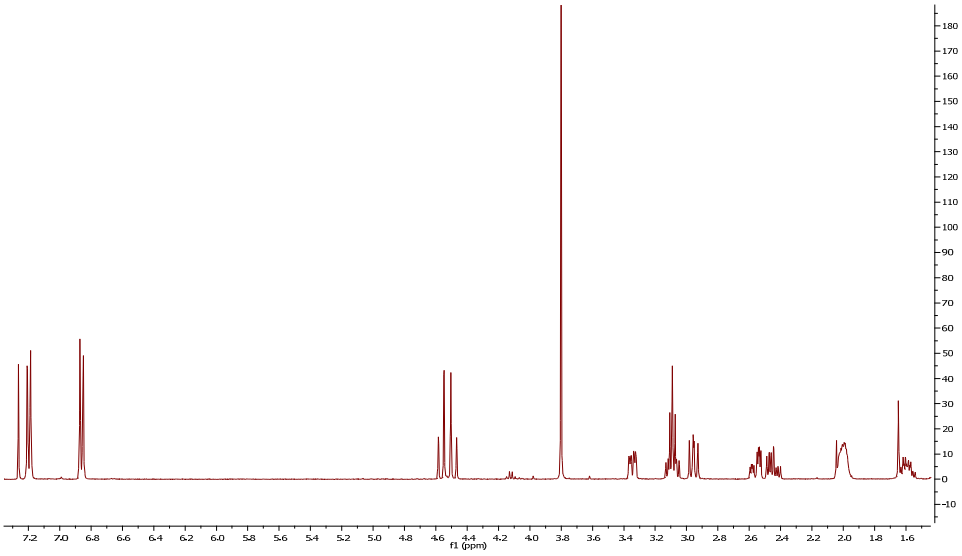
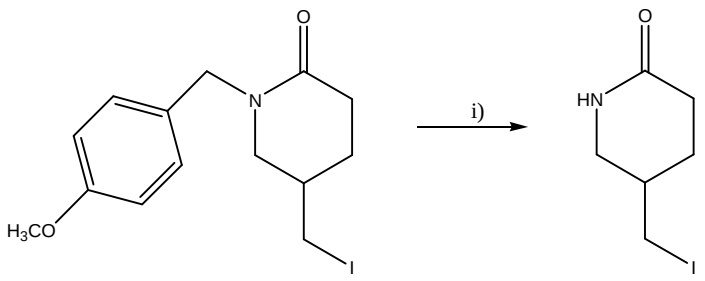


*Figure 3.15: ^{1}H-NMR in $CDCl_3$ of the iodo-compound (**85**).*

Cerium ammonium nitrate (CAN), in acetonitrile and water (3:2), was used to deprotect the amide functionality of compound (**85**) as a method by which to obtain (**86**), as shown in scheme 3.16. This is one of the most used reactions of deprotection for the methoxy-benzyl group. This reaction proceeded in 41 % yield. After 2 hours starting material was still present. It has been tried to leave the reaction over night in order to increase the yield, but the product decomposed.

Because of its high oxidative ability, its good solubility in a wide number of organic solvents and its low toxicity, CAN is largely used as oxidative reagent also to deprotect ethers,[3] chemoselective catalytic deprotection of acetals and ketals,[4,5] TBMDS and TIPS groups.[6] The mechanism seems to proceed through radicals intermediate, but it's not completely known.



85 **86**

Reagents and conditions: i) CAN, CH_3CN/H_2O, r.t.,2.30 h.

*Scheme 3.16: Reaction of deprotection of compound (**84**) to give compound (**86**).*

The ^{1}H-NMR spectrum proves that the compound is the one expected (**86**) since the broad peak of the amine is visible in Figure 3.16 at 5.8 ppm and the peaks of the methoxybenzyl group (previously present at7.2, 6.8, 4.5 and 3.8 ppm, Figure 3.16) are disappeared.

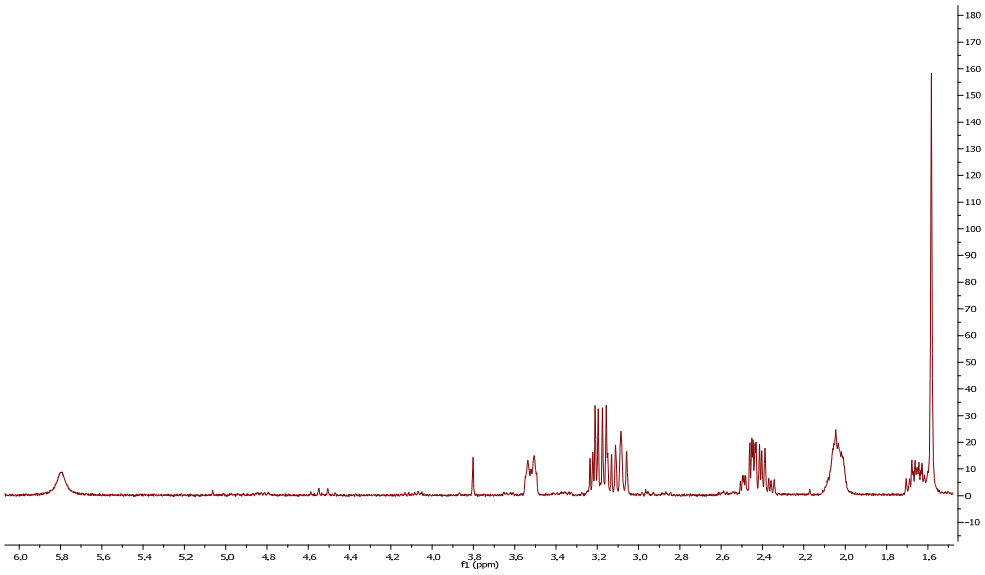
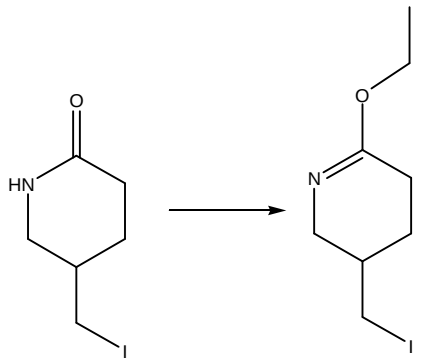


*Figure 3.16: ^{1}H-NMR in $CDCl_3$ of the iodo-compound (**86**).*

The protection of the amide functionality of the iodo-compound (**86**) has been made *via* formation of the relative ethyl ether through triethyloxonium tetrafluoroborate, as shown in Scheme 3.17 achieving compound (**87**). The aim is to avoid the formation of enolate, possible side product in the reaction of coupling with the DKP (**33**, see chapter 4).



86 **87**

Reagents and conditions: i) Et_2OBF_3, DCM, r.t.,16 h.

Scheme 3.17: Reaction of protection of the carbonyl.

After purification in chromatography column starting material was recovered. Probably the reaction can be pushed further increasing the yield leaving the reaction stirring for more than 16 hours.

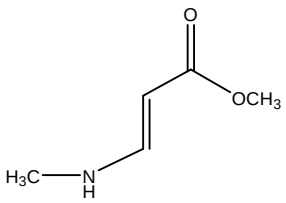
3.4- Conclusions

The side chain (**87**) has been synthesised with the aim of coupling it to the diketopiperazine core (**33**), reaction that will be discussed in the next chapter. Iodine is fundamental for this reaction as it is a good leaving group that is suitable for alkylating electropositive carbons, such as the one in the α position to the carbonyl of DKP (**33**).

The methoxyphenyl ethyl group bonded to the nitrogen of the heterocycle (**85**) was selected due to its ease of cleavage *via* the CAN reaction. The final step in this synthetical strategy was the protection of the amide carbonyl functional group (**86**) which avoids the possible formation of the enolate (**79**) during the coupling. The synthesis of the iodo-compound (**79**) has been successful, but further research is necessary to improve the yields, especially the cyclisation and the protection of the carbonyl (**87**).

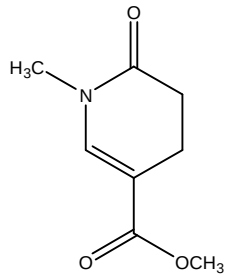
3.5- Experimental section

- (*E*)-Methyl-3-(methylamino)acrylate (**63**)



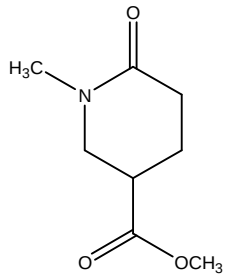
To a stirred solution of methyl propiolate (**40**, 2.5 mL, 29.9 mmol) in anhydrous THF (15 mL) was added methyl amine (**62**, 16.5 mL, 2M, 33 mmol) at -10 °C. After 2 hours the reaction was concentrated *in vacuo*. to yield a viscous yellow liquid (**63**) in 75 % yield, which was used in subsequent reactions without further purification. Rf: 0.36, (Cyclohexane:EtOAc, 50:50); δ_H (400 MHz; $CDCl_3$): 7.60-7.53 (m, 1H, CHN, diasteroisomers Z) and 6.63-6.56 (m, 1H, CHN, diasteroisomers E), 4.74 (d, 1H, *J* = 13.2 Hz, *C*HC, diasteroisomers Z) and 4.49 (d, 1H, *J* = 8.0 Hz, *C*HC, diasteroisomers E), 4.55 (br. s, 1H, NH), 3.67 (s, 3H, CH_3O, diasteroisomers Z) and 3.64 (s, 3H, CH_3O, diasteroisomers E); 2.97 (d, 3H, *J* = 5.0 Hz, CH_3N, diasteroisomers Z) and 2.78 (d, 3H, *J* =5.1 Hz, CH_3N, diasteroisomers E); δ_C (100 MHz, $CDCl_3$): 171.2 (CO, diasteroisomers Z), 169.9 (CO, diasteroisomers E), 154 (*C*HCO, diasteroisomers Z), 150 (*C*HCO, diasteroisomers E), 85.0 (*C*HNH, diasteroisomers Z), 81.4(*C*HNH, diasteroisomers E), 50.4(CH_3O, diasteroisomer E), 50.0 (CH_3O, diasteroisomer Z), 34.7 (CH_3NH, diasteroisomer Z) 30.0 (CH_3NH, diasteroisomer E); HRMS: calculated for $C_5H_9NO_2$ 115.0633, found 115.0636.

- Methyl 1-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylate (**64**)



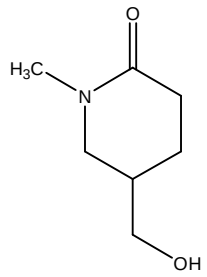
To a stirred solution of (*E*)-methyl-3-(methylamino)acrylate (**63**, 0.99 g, 8.6 mmol) in anhydrous THF (10 mL) under nitrogen was added acryloyl chloride (**38**, 0.77 mL, 9.47 mmol). After 5 hours reflux the reaction mixture was washed with saturated $NaHCO_3$ (10 mL) before EtOAc (10 mL) was added. The fractions were separated and the aqueous phase was extracted with EtOAc (4 x 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo* to yield a crude yellow oil, purified by silica gel column chromatography (EtOAc:hexane, range 85:15 to 70:30) to afford the bright yellow crystal (**64**) in 47% yield. Rf: 0.36, Cyclohexane:EtOAc, 50:50; δ_H (400 MHz; $CDCl_3$): 7.27 (s, 1H, CH), 3.75 (s, 3H, CH_3O), 3.15 (s, 3H, CH_3N); 2.65-2.55 (m, 4H, CH_2CH_2); δ_C (100 MHz, $CDCl_3$): 170.0 (COO), 166.7 (CO), 140.8 (CHN), 108.3 (*C*HC), 51.5 (CH_3O), 34.6 (CH_3N), 30.7 (CH_2CO), 20.0 (CH_2CH_2); mp: 74-76 °C; HRMS: calculated for $C_8H_{11}NO_3$ 169.0739, found $C_8H_{11}NO_3$ 169.0739.

- Methyl 1-methyl-6-oxopiperidine-3-carboxylate (**65**)



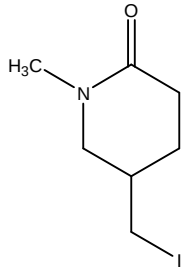
To a stirred solution of methyl 1-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylate **(64,** 2.1 g, 12.4 mmol) in EtOH (30 mL) under nitrogen were added Na_2CO_3 (3.12 g, 37.3 mmol) and Pd/C (1.3 g, 12.2 mmol). The hydrogen was added as a gas through hydrogen balloon. The reaction was left overnight and the solid Pd/C was removed by filtration in fluted paper. The solution was concentrated *in vacuo*, the resulting crude oil was purified by silica gel column chromatography (cyclohexane:EtOAc, 60:40) to afford a clear oil in 85 % yield. Rf: 0.15, EtOAc 100%; δ_H (400 MHz; CDCl3) 3.73 (s, 3H, CH_3O), 3.56-3.44 (m, 2H, CH_2N), 2.96 (s, 1H, CH_3N), 2.88-2.80 (m, 1H, CH), 2.52 -2.46 (m, 2H, *C*H_2CO), 2.42-2.35 (m, 2H, *C*H_2CO), 2.18-2.12 (m, 2H, *C*H_2CH_2), 2.03-1.96 (m, 2H, *C*H_2CH_2); δ_C(100 MHz, $CDCl_3$) 172.7 (COO), 169.0 (CO), 52.3 (CH_3O), 50.5 (CH_2N), 38.8 (CH), 34.8(CH_3N), 30.6 (*C*H_2CO), 24.0 (*C*H_2CH_2); HRMS: calculated for $C_8H_{13}NO_3$ 171.0895, found 171.0895.

- 5-(hydroxymethyl)-1-methylpiperidin-2-one (**66**)



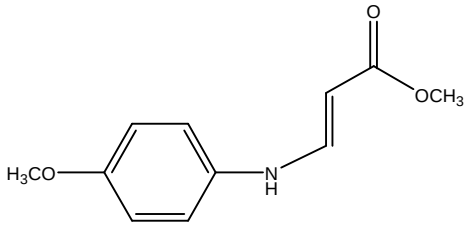
To a stirring solution of methyl 1-methyl-6-oxopiperidine-3-carboxylate (**65**, 0.52g, 2.9 mmol) in anhydrous THF (8 mL) under nitrogen, was added $LiBH_4$ (2.9 mL, 5.8 mmol) at -10 °C. The reaction was allowed to reach the room temperature and was stirred overnight. Water and HCl 1M were added in order to quench the reduction agent and to neutralise the solution. Water was taken off at the freezing drier machine and the powder obtained was purified *via* silica gel chromatographic column (MeOH:DCM 1:10) achieving a slightly yellow liquid in 60% yield. Rf: 0,43 (MeOH:EtOAc 1:3); δ_H (400 MHz; CD_3OD) 3.58-3.51 (m, 2H, CH_2OH), 3.44-3.40 (m, 1H, CH_2N), 3.22-3.14 (m, 1H, CH_2N), 2.94 (s, 3H, CH_3N), 2.44-2.29 (m, 2H, CH_2O), 2.10 -2.00 (m, 1H, CH), 1.91-1.86 (m, 1H, CH_2CH_2), 1.57-1.47 (m, 1H, CH_2CH_2); δc (100 MHz; CD_3OD), 169.0 (CO), 52.3 (CH_2N), 50.5 (CH_2OH), 38.8 (CH), 34.8 (CH_3N), 30.6 (CH_2O), 24.0 (CH_2CH_2); HRMS: calculated for $C_7H_{13}NO_2$ 143.0946, found 143.0944.

- 5-(iodomethyl)-1-methylpiperidin-2-one (**68**)



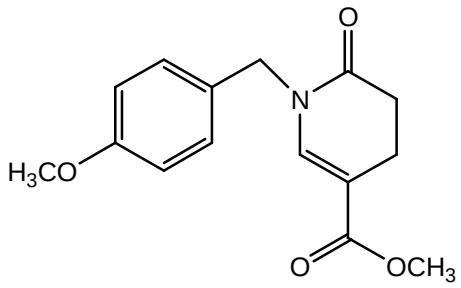
To a stirring solution of 5-(hydroxymethyl)-1-methylpiperidin-2-one (**66**, 0.2 g, 1.4 mmol) in anhydrous pyridine (15 mL) was added slowly and at 0 °C mesyl chloride (0.21 mL, 1.82 mmol). After allowing the solution to reach the room temperature it was brought to reflux for 3 hours and concentrated *in vacuo*. The crude brown oil (0.25 g, 1.13 mmol) was dissolved in acetone (15 mL) and was added NaI (0.51 g, 3.39 mmol). The solution was brought to reflux for 4 hours, the solvent was removed *in vacuo*, brine was added (10 mL) and the mixture was extracted with DCM (4 x 15 mL). The crude brown oil was purified by silica gel chromatography column (DCM:MeOH 10:1) achieving 62 % yield over two steps. Rf: 0,5 (MeOH:EtOAc 1:3); δ_H (400 MHz; $CDCl_3$) 3.59-3.49 (m, 1H, CH_2I), 3.48-3.41 (m, 2H, CH_2N), 3.25-3.14 (m, 1H, CH_2I), 2.96 (s, 3H, CH_3N), 2.55-2.34 (m, 2H, *C*H_2CO), 2.32-2.21 (m, 1H, *C*H), 2.00-1.93 (m, 1H, C*C*H_2C), 1.73-1.57 (m, 1H, *C*H_2CH_2); δ_c (100 MHz; $CDCl_3$) 169 (CO), 52.3 (CH_2N), 50.5 (CH_2I), 38.8 (CH), 34.8 (CH_3N), 30.6 (*C*H_2CO), 24.0 (*C*H_2CH_2); HRMS: calculated for $C_7H_{12}NOI$ 252.9964, found 252.9973.

- (*E*)-Methyl 3-(4-methoxybenzylamino)acrylate (**80**)



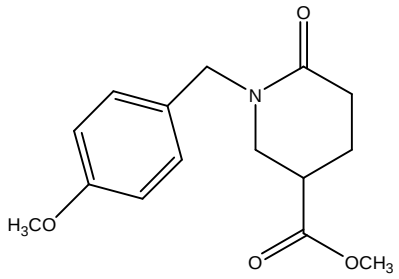
The procedure was the same used to synthesise compound (**63**), employing methyl propiolate (**40**, 1.5 mL, 17.8 mmol) this time with 4-methoxybenzyl amine (**39**, 2.32 mL, 17.84 mmol) instead of methyl amine (**62**), achieving (*E*)-Methyl 3-(4-methoxybenzylamino)acrylate (**80**) as a white solid in 99 % yield. Rf: 0.35, Cyclohexane:EtOAc, 50:50; δ_H (400 MHz; CDCl3) 8.07 (br. s, 1H, NH), 7.59-7.54 (dd, *J* = 13.3 Hz, *J* = 8.0 Hz, 1H, CH-CO, diasteroisomers E), 7.23-7.15 (m, 2H, Ph), 6.89-6.86 (m,2H, Ph), 6.71-6.65 (dd, *J* = 13.1 Hz, *J* = 8.1 Hz, 1H, CH-CO, diasteroisomers Z), 4.83-4.79 (d, *J* = 13.3 Hz, 1H, CH-N, diasteroisomers E), and 4.54-4.52 (d, *J* = 8.1 Hz, 1H, CH-N, diasteroisomers Z), 4.29-4.28 (d, *J* = 5.9 Hz, 2H, CH_2-Ph, diasteroisomer Z), 4.13-4.14 (d, *J* = 7.7 Hz, 2H, CH_2-Ph, diasteroisomer E); 3.8 (t, *J* = 1.8 Hz, *J* = 3.79 Hz, 3H, CH_3O), 3.66 (s, 3H, CH_3CO, diasteroisomer E), 3.64 (s, 3H, CH_3CO, diasteroisomer Z); δ_C (100 MHz, $CDCl_3$) 171.1 (COO), 170.1 (CO), 159.3 (Ph), 152.3 (*C*HNH), 130.6 (Ph), 128.7 (Ph), 114.3 (Ph), 86.6 (*C*HCO, disteroisomer E), 82.6 (*C*HCO, disteroisomer Z), 55.4 (CH_3OPh), 52.0 (CH_3OCO) , 50.3 (CH_2NH);. HRMS: calculated for $C_{12}H_{15}NO_3$ 221.1052, found 221.1043.

- Methyl 1-(4-methoxybenzyl)-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylate (**81**)



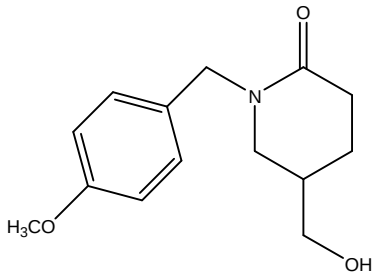
The procedure followed is the same used to synthesise compound (**64**), using as starting material acryloyl chloride (**38**, 1.59 mL, 19.6 mmol) and (*E*)-Methyl 3-(4-methoxybenzylamino)acrylate (**80**, 3.94g, 17.82 mmol) achieving after silica gel chromatography column (EtOAc:Cyclohexan from 15:85 to 20:80) a yellow oil in 33 % yield. Rf: 0.58, Cyclohexane:EtOAc, 50:50;.; δ_H (400 MHz; CDCl3) 7.27 (s, 1H, CH), 7.21-7.18 (d, *J* = 10.2 Hz, *J* = 8.2 Hz, 2H, Ph), 6.88-6.85 (m, 2H, Ph), 4.67 (s, 2H, CH_2Ph) 3.79 (s, 3H,CH_3O), 3.72 (s, 3H, CH_3N); 2.62 (s, 4H, CH_2CH_2); δ_C (100 MHz, $CDCl_3$) 169.9 (COO), 167.0 (CO), 159.3 (Ph), 139.5 (CHN), 129.3 (Ph), 128.7 (Ph), 114.2 (Ph), 108.9 (*C*HCO), 55.5 (CH_2Ph), 51.8 (CH*3*OPh), 49.5 (CH_3OCO), 30.9 (CH_2CO), 20.2 (CH_2CH_2); HRMS: calculated for $C_{15}H_{17}NO_4$ 275.1158; found $C_8H_{11}NO_3$ 275.1171.

- Methyl 1-(4-methoxybenzyl)-6-oxopiperidine-3-carboxylate (**82**)



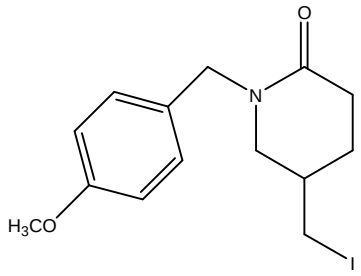
The procedure used to achieve methyl 1-(4-methoxybenzyl)-6-oxopiperidine-3-carboxylate (**82**) was the same used to synthesise compound (**65**), but starting from methyl 1-(4-methoxybenzyl)-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylate (**81**, 1.59 g, 5.7 mmol). The obtained clear oil was purified by silica gel chromatography column (EtOAc 100%) in 86 % yield. Rf: 0.34, EtOAc, 100%; δ_H (400 MHz; CDCl3) 7.20-7.19 (d, *J* = 7.0 Hz, 2H, Ph), 6.86-6.84 (d, *J* = 8.6 Hz, 2H, Ph), 4,65-4,61 (dd, *J* = 14.4, Hz, 4.1 Hz, 2H, CH_2Ph), 4,46-4,43 (dd, *J* = 14.4 Hz, 3.8 Hz, 2H, CH_2Ph), 3.79 (s, 3H,CH_3O), 3.66 (s, 3H, CH_3N); 3.44-3.36 (m, 4H, CH), 2.80-2.72 (m, 2H, CH_2N), 2.61-2.55 (m, 1H, CH_3OH), 2.49-2.42 (m, 2H, CH_2CO), 2.14-2.10 (m, 1H, CH_2CH_2), 2.02-1.94 (m, 1H, CH_2CH_2), δ_C (100 MHz, $CDCl_3$) 172.8 (COO), 169.0. (*C*O), 159.1 (Ph), 129.6 (Ph), 128.9 (Ph), 114.1 (Ph), 55.3 (CH_3OPh), 52.3 (CH_2Ph), 49.6 (CH_3O), 48.0 (CH_3N), 39.1 (CH), 30.8 (CH_2CO), 24.0 (CH_2CH_2); HRMS: calculated for $C_{15}H_{19}NO_4$ 277.1314, found 277.1325.

- 5-(hydroxymethyl)-1-(4-methoxybenzyl)piperidin-2-one (**83**)



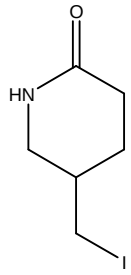
The same procedure used to synthesise (**66**) has been used also for compound (**83**) 5-(hydroxymethyl)-1-(4-methoxybenzyl)piperidin-2-one, using methyl 1-(4-methoxybenzyl)-6-oxopiperidine-3-carboxylate (**82**, 0.94 g, 3.4 mmol) as starting material, but this time with a different work out. Water and HCl 1M were added. The organic layer was separated and the water was extracted with EtOAc (4 x 20 mL). The organic layers were combined and dried over $MgSO_4$, concentrated *in vacuo* and purified by silica gel chromatography column (EtOAc:MeOH 95:5) obtaining a slightly yellow oil in 85 % yield. Rf: 0,4 (MeOH:EtOAc 5:95); δ_H (400 MHz; CD_3OD) 7.20-7.18 (d, *J* = 8.6 Hz, 2H, Ph), 6.86-6.83 (d, *J* = 8.5 Hz, 2H, Ph), 4.52 (s, 2H, CH_2-Ph), 3.79 (s, 3H, CH_3OPh), 3.60-3.56 (m, 2H, CH_2N), 3.51-3.46 (m, 2H, CH_2N), 3.33-3.29 (m, 1H, CH_2O), 3.02-2.96 (m, 1H, CH_2O), 2.58-2.51 (m, 2H, CH_2-CO), 2.46-2.37 (m, 2H, CH_2-CO), 2.05-1.98 (m, 1H, CH), 1.91-1.86 (m, 2H, CH_2CH_2), 1.56-1.46 (m, 2H, CH_2CH_2); δ_C (100 MHz; CD_3OD) 169.6 (CO), 158.7 (Ph), 129.4 (Ph), 129.3 (Ph), 114.0 (Ph), 64.7 (CH_3OPh), 55.1 (CH_2Ph), 49.8 (CH_2OH), 49.1 (CH_2N), 36.2 (CH), 31.1 (CH_2CO), 23.8 (CH_2CH); HRMS: calculated for $C_{14}H_{19}NO_3$ 249,1365, found 249.1376.

- 5-(iodomethyl)-1-(4-methoxybenzyl)piperidin-2-one (**85**)



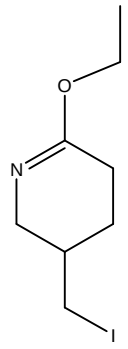
To a stirring solution of 5-(hydroxymethyl)-1-(4-methoxybenzyl)piperidin-2-one (**83**, 0.7 g, 2.8 mmol) in anhydrous DCM (10 mL) were added TEA (0,61 mL, 4.35 mmol) and Mesyl chloride (0.27 mL, 3.48 mmol), after cooling down the solution to -10 °C. The reaction was left over night, quenched with water and the organic layer was separated. Brine (10 mL) was added and the aqueous phase was washed with EtOAc (5 x 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo* achieving a dark yellow oil in 93 % yield. Compound (**84**) was dissolved in acetone (10 mL) and NaI (1 g, 6.75 mmol) was added. The reaction was refluxed for 2 hours and then stirred overnight; the day after it was refluxed for further two hours. Acetone was removed *in vacuo*, water was added (10 mL) and extracted with EtOAc (5 x 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude orange oil was purified by silica gel chromatography column (EtOAc 100%) achieving a dark yellow oil with a yield of 80 %. Rf: 0,65 (MeOH:EtOAc 5:95); δ_H (400 MHz; CD_3OD) 7.22-7.20 (d, *J*= 8.6 Hz, 2H, Ph), 6.88-6.86 (d, *J* = 8.6 Hz, 2H, Ph), 4.62-4.47 (q, *J* = 14.4 Hz, 2H, CH_2Ph), 3.81 (s, 3H, CH_3OPh), 3.38-3.34 (m, 2H, CH_2NH), 3.14-3.06 (m, 1H, CH_2I), 2.99-2.94 (m, 2H, CH_2NH), 2.61-2.54 (m, 2H, CH_2CO), 2.50-2.41 (m, 2H, CH_2CO), 2.06-1.99 (m, 2H, CH and CH_2CH_2), 1.66-1.55 (m, 1H, CH_2CH_2); δ_C (100 MHz; CD_3OD) 169.0 (CO), 159.0 (Ph), 129.6 (Ph), 128.9 (Ph), 114.0 (Ph), 55.2 (CH_3OPh), 52.0 (CH_2N), 49.4 (CH_2Ph), 36.1 (CH), 30.7 (CH_2CO), 28.0 (CH_2 CH_2), 7.9 (CH_2I); HRMS: calculated for $C_{14}H_{18}INO_2$ 359,0382, found 359,0391.

- 5-(iodomethyl)piperidin-2-one (**86)**



To a stirring solution of 5-(iodomethyl)-1-(4-methoxybenzyl)piperidin-2-one (**85**, 0.2 g, 0.56 mmol) in CH_3CN/H_2O 3:2 (10 mL) was added cerium ammonium nitrate (CAN, 0.92 g, 1.68 mmol). The reaction was stirred for 2:30 hours. CH_3CN was removed *in vacuo*, and the aqueous phase was washed with DCM (5 x 10 mL). The organic layers were combined, dried over $MgSO_4$, concentrated *in vacuo* and purified in silica gel chromatography column (from cyclohexan:EtOAc 20:80 to EtOAc:MeOH 95:5) in a yield of 41 % as a pale brown solid. Rf: 0,24 (MeOH:EtOAc 5:95); δ_H (400 MHz; CD_3OD) 5.80 (br. s, 1H, NH), 3.54-3.51 (d, *J* = 12.2 Hz, 1H, CH_2N), 3.24-3.13 (m, 2H, CH_2I), 3.10-3.06 (m, 1H, CH_2N), 2.51-2.34 (m, 2H, CH_2CO), 2.08-2.00 (m, 2H, CH and CH_2CH_2), 1.70-1.60 (m, 1H, CH_2CH_2); δ_C (100 MHz; CD_3OD) 171.3 (CO), 47.8 (CH_2N), 35.6 (CH), 29.9 (CH_2CO), 27.7 (CH_2 CH_2), 7.8 (CH_2I); mp: 90-92 °C; HRMS: calculated for $C_6H_{10}INO$ 238,9807, found 238,9809.

- 6-ethoxy-3-(iodomethyl)-2,3,4,5-tetrahydropyridine (**87**)



To a stirring solution of Boron trifluoride triethyl etharate (0.1 mL, 0.79 mmol) in anhydrous Et_2O (5 mL) and nitrogen flow epichlorohydrine was added dropwise (0.05 mL, 0.63 mmol). The reaction was refluxed for one hour and after cooling it to room temperature, it was stirred for a further hour allowing the formation of the pale brown salt triethyloxonium trifluoroborate. Under nitrogen flux, the solvent was filtered out and once the salt was dry compound (**86**, 0.87 g, 0.36 mmol) was added afrer been dissolved in anhydrous DCM (6 mL). The reaction was stirred over night, neutralized with a solution of Na_2CO_3 (8 mL). The organic layer was separated, the aqueous phase was washed with EtOAc (3 x 10 mL), the organic phases were combined, dried over $MgSO_4$, concentrated *in vacuo* and purified through silica gel chromatography (EtOAc) obtaining a brown liquid in a yield of 32 % . Rf: 0.63 (MeOH:EtOAc 5:95).

3.6- Bibliography

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3. Mark I. E., Ates A., Augustyns B., Gautier A., Quesnel Y., Turet L., Wiaux M., *Tetrahedron Letters*, **1999**, 40, 5613.
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5. Ates A., Gautier A., Leroy B., Plancher J.-M., Quesnel Y., Vanherck J.-C., Markó I. E., *Tetrahedron*, **2003**, 59, 8989.
6. Hwu J. R., Jain M. L. et al., *J. Org. Chem*., **2000**, 65, 5077.

4.1- Aim

The aim of this last part of the project is the coupling between the DKP core (**56c**), and the novel side chain (**68** and **87**) described in Chapters 2 and 3 respectively, followed by the hydrolysis of the substituted DKP core (**56c**) in an effort to synthesise amino acid (**25**).

4.2- Introduction

The treatment of bacterial or fungal infections is an ongoing challenge in modern medicine. Both fungi and mammals are eukaryotes and so achieving a good selective toxicity is not easy or straightforward. For this reason the enzymatic pathways leading to the formation of fungal and bacterial cell walls have became an important target for drugs.

An example of such a target is L-glutamine (**15**), involved in the transfer of ammonia in the isomerisation of Fru-6-P (**16**) to Glu-6-P (**17**), a reaction catalysed by GlcN-6-PS, shown previously in Scheme 1.3.[1]

This reaction is the first step in the pathway leading to the formation of the activated form of *N*-acetyl-D-glucosamine, namely 5'-diphospho-N-acetyl-D-glucosamine (**19**, UDP-GlcNAc, Figure 11).

This nucleotide sugar is essential for the biosynthesis of biomacromolecules such as bacteria peptidoglycan, fungal chitin and mammalian glycoproteins.

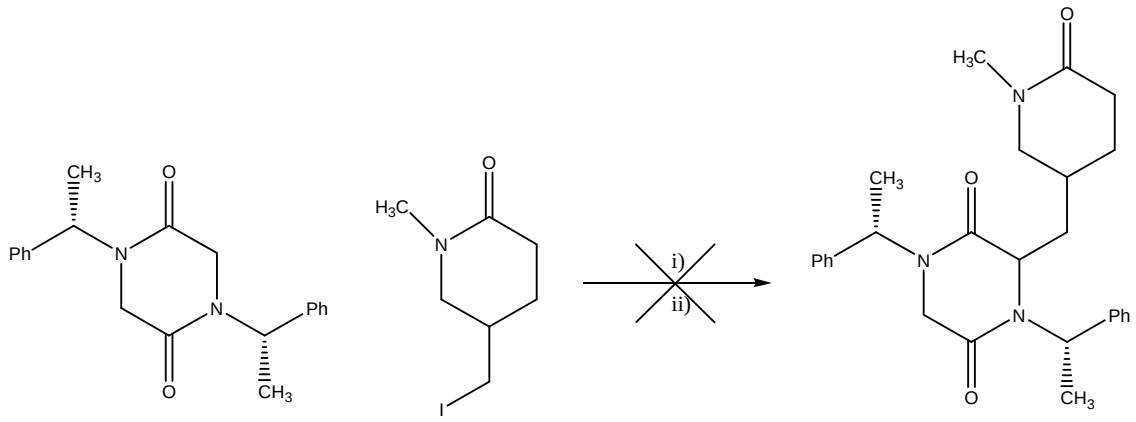
Many bacteria use the products of the biosynthetic pathway to synthesise hyaluronic acid capsules which surrounds their cell walls and which are connected with the infectious process.[2,3]

The absence of GlcN-6-PS has different consequences in human beings than in either fungi or bacteria. Mammals rapidly express this enzyme with a long half life and the relatively long life of mammalian cells means that a deficiency of the enzyme has no lasting effects. Conversely, the absence of GlcN-6-PS can be lethal to bacteria resulting in morphological changes and ultimately cell lysis.[4] These behavioural differences allow for a selective toxicity. GlcN-6-PS has already been a target for a potential drug to be used in the treatment of diabetes,[5] bowel disease[6] and fungal infections.[7]

Considering that high specificity is a fundamental aspect of a rational drug design, it is important to understand the mechanism by which an inhibitor operates and to determine the crucial functionalities and interactions to achieve selectivity and optimal activity.

4.3- Synthetic approach and discussion

The coupling between the substituted benzyl DKP core (**56c**) and the side chain (**68**) was made *via* alkylation using lithium bis(trimethyl)silyl amide (LHMDS, **90**) as base. The metallation of the DKP (**56c**) was carried out in THF at -20°C and then quenched with the iodo-derivative (**68**), as shown in Scheme 4.1, but unfortunately no coupling product (**89**) was recovered. TLC analysis of the crude reaction mixture showed four different spots, the two most intense corresponded to the starting materials recovered after silica gel chromatography column.



56c **68** **89**

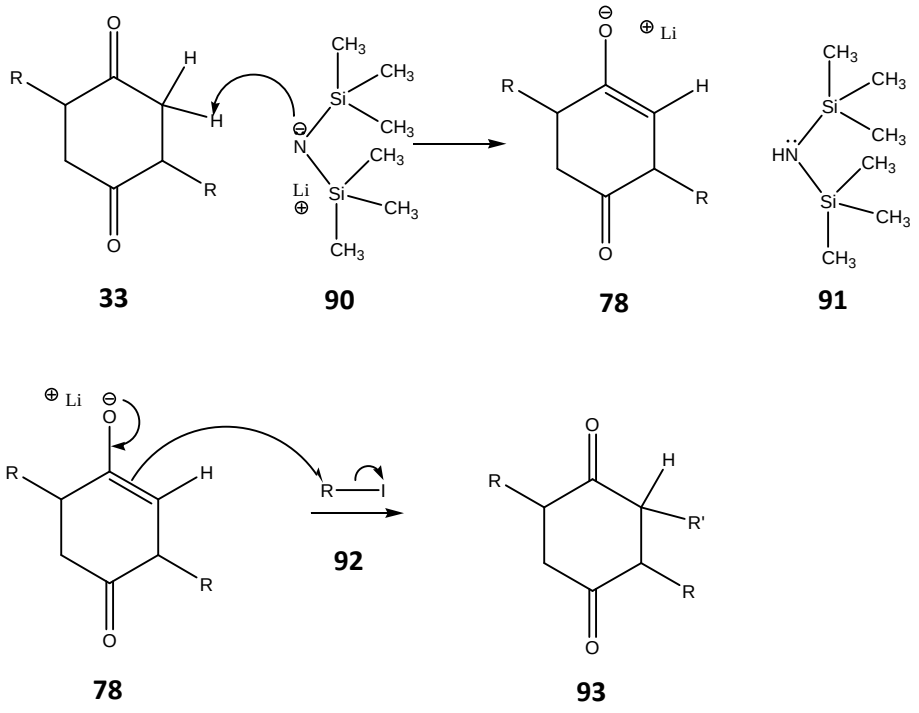
*Reagents and conditions: i) anhydrous THF, LHMDS, -78 °C, 1 h; ii) anhydrous THF, 3-((1-methyl-6-oxopiperidin-3-yl)methyl)-1,4-bis((S)-1-phenylethyl)piperazine-2,5-dione (**68**), -78 °C, 2 h.*

*Scheme 4.1: Reaction investigating the alkylation of the DKP (**56c**) with iodo-derivative **(68)**, in an effort to synthesise substituted DKP (**89**).*

The same reaction was attempted using an alternative, less hindered base, namely butyl lithium (BuLi), but this lead to the same result.

It is difficult to understand the reason why this coupling was unsuccessful. Similar reactions with other iodo-derivatives have been performed successfully many times in the Paradisi laboratory (Figure 3.8).

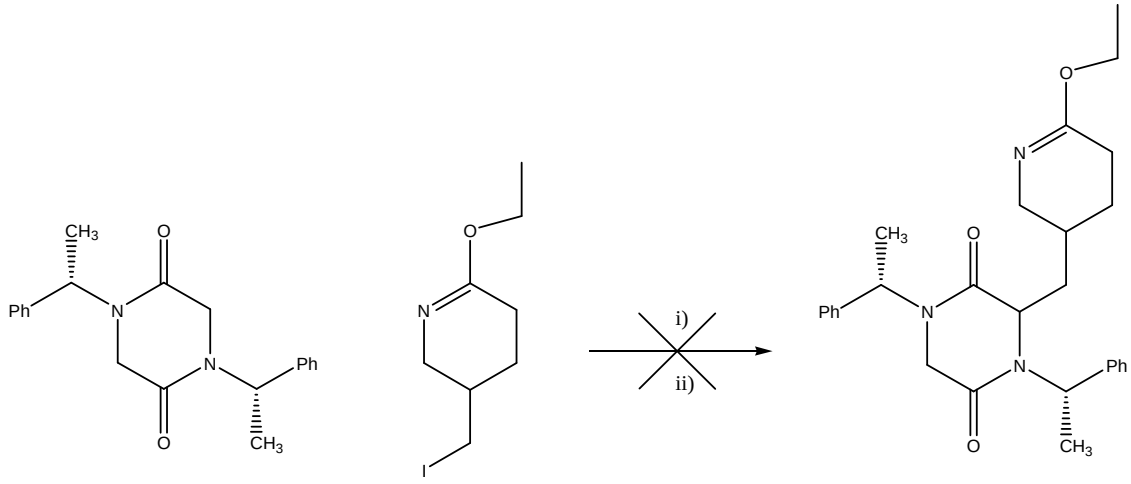
The general mechanism of the alkylation of DKP (**33**) is described in Scheme 4.2. The first step, the deprotonation of the DKP (**33**) proceeds very quickly, typically within 30 minutes and 1 hour. The second step, the alkylation, takes between 1 and 2 hours. In this instance, the coupling of the DKP (**56c**) with iodo- derivative (**68**), the reaction was left overnight to see if any production of product could be detected, unfortunately nothing was observed.



*Scheme 4.2: Mechanism of alkylation of DKP (**33**) with LHMDS (**90**).*

The protons in the α position to the carbonyl carbon of the iodo-compound (**68**) are very reactive and so in an effort to avoid the metallation at this site, it was decided to attempt to couple a substituted benzyl DKP (**56c**) with an alternative iodo-derivative (**87**) in which the carbonyl functionality was protected, as shown in Scheme

4.3.



56c **87** **94**

*Reagents and conditions: i) anhydrous THF, LHMDS, -78 °C, 1 h. ii) anhydrous THF, 3-((6-ethoxy-2,3,4,5-tetrahydropyridin-3-yl)methyl)-1,4-bis((S)-1-phenylethyl)piperazine-2,5-dione (**87**), -78 °C, 2 h.*

*Scheme 4.3: Reaction investigating the alkylation of the DKP (**56c**) with iodo- derivative **(87)**, in an effort to synthesise substituted DKP (**94**).*

TLC analysis of the reaction mixture after 2 and even after 16 hours showed only starting materials, which were recovered after silica gel chromatography column.

4.4- Conclusions

It is important to understand the mechanisms by which anticapsin (**12**) enters in the active site of GlcN-6-PS and inhibits this enzyme. These kind of studies are useful in the development of antimicrobial drugs. In the heterocyclic analogue of anticapsin (**25**), which has been the target molecule of this research project, the ketone moiety has been replaced with an amide functionality. It is envisaged that any attack on the carbonyl moiety and the subsequent hydrolysis to form the hydrate (**24**) are less likely in the heterocyclic analogue of anticapsin (**25**), compared to anticapsin (**12**). This analogue (**25**) could help to understanding whether the proposed mechanism, previously shown in Scheme 1.4, is correct.[8]

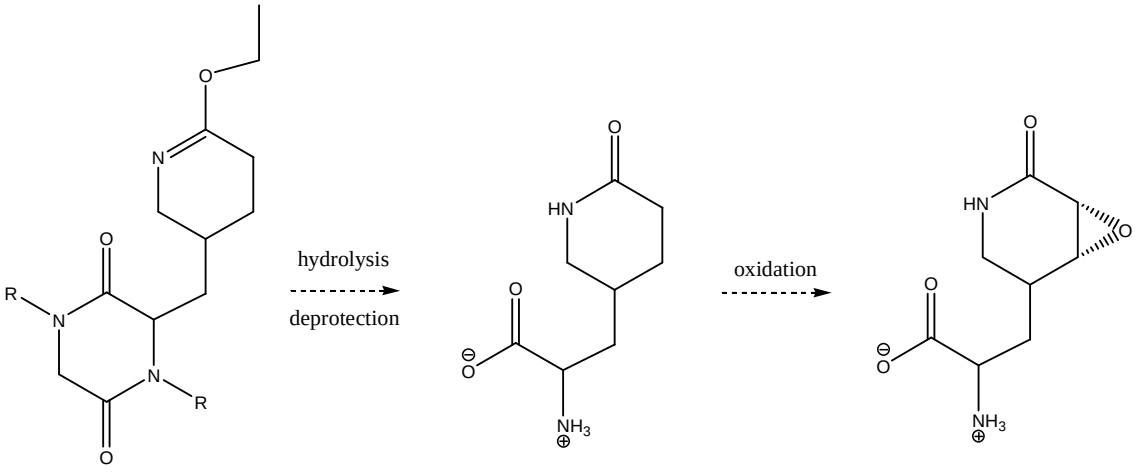
In order to synthesise the target molecule (**25**) the coupling of substituted benzyl diketopiperazine (**56c**) and two different kinds of iodo-dervied side chains (**68** and

87) were investigated. DKPs (**33**) are very interesting because of their ability to generate different kinds of amino acids after alkylaion and hydrolysis. Unfortunately the alkylations were unsuccessful and only starting materials were recovered.

4.5- Future work

Further investigations are required to explain why these reactions failed and to find alternative means by which to synthesise alkylated DKPs.

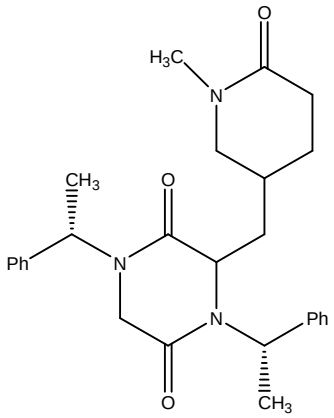
Upon production of the alkylated DKPs (**89** and **94**) from the coupling of a side chain to the DKP core (**56c**) a few subsequent steps shall be required to obtain the desired amino acid (**25**), shown in Scheme 4.4, and test its biological activity as an antimicrobial agent. Depending on the outcome of these biological tests it is envisaged that a series of different antaicapsin analogues will be synthesised in a similar manner, *via* coupling of a side chain with the DKP core, for further investigations.



94 **35** **25**

*Scheme 4.4: Hydrolysis of the alkylated DKP (**94**) to produce 2-ammonio-3-(6-oxopiperidin-3-yl)propanoate (**25**) via 2-ammonio-3-((1R,6R)-2-oxo-7-oxa-3-aza-bicyclo[4.1.0]heptan-5-yl)propanoate (**35**).*

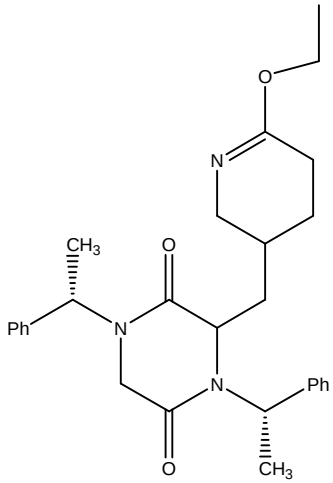
4.6- Experimental section



- 3-((1-methyl-6-oxopiperidin-3-yl)methyl)-1,4-bis((S)-1-phenylethyl)piperazine-2,5-dione (**89**)

To a stirred solution of DKP (**56c**, 0.2 g, 1.02 mmol) in anhydrous THF (15 mL) under nitrogen at -20 °C was added LHMDS (0.62 mL, 1.02 mmol) dropwise. After 1 hour the reaction was cooled to -78 °C before 5-(iodomethyl)-1-methylpiperidin-2-one (**68**, 0.16 g, 1.02 mmol) was added. After stirring overnight water (10 mL) was added and aqueous phase extracted with EtOAc (4 x 15 mL). The combined organic layers were dried over $MgSO_4$, concentrated *in vacuo* and purified *via* silica gel chromatography column (EtOAc/Cyclohexane from 10:90 to 50:50) to isolate only the starting materials (0.13 g of DKP **56c** and 0.094 of iodo-compound **68**) and minute quatities of two undentified compounds.

- 3-((6-ethoxy-2,3,4,5-tetrahydropyridin-3-yl)methyl)-1,4-bis((S)-1-phenylethyl)piperazine-2,5-dione (**94**)



To a stirred solution of DKP **(56c**, 0.087 g, 0.27 mmol) in anhydrous THF (5 mL) under nitrogen at -20 °C was added LHMDS (0.3 mL, 0.3 mmol) dropwise. After 1 hour the reaction was cooled to -78 °C before iodo compound NAME!(**97**, 0.072 g, 0.27 mmol) was added. After two hours water (10 mL) was added and the aqueous phase was extracted with EtOAc (4 x 15 mL). The combined organic layers were dried over $MgSO_4$, concentrated *in vacuo* and purified *via* silica gel chromatography column (EtOAc/Cyclohexane from 10:90 to 50:50) to isolate only the starting materials (0.076 g of DKP **56c** and 0.026 of iodo-compound **68**).

4.7- Bibliography

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