SYNTHESIS OF VIRIDAMINE ANALOGUES FOR USE IN SELECTIVE METAL COMPLEXATION STUDIES

by

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Inspiration

Col.3:22 – 4:1.

"[22]Slaves obey your human masters in all things, not only when they are watching you because you want to gain their approval; but do it with a sincere heart because of your reverence for the Lord. [23]Whatever you do, work at it with all your heart, as though you were working for the Lord and not for men. [24]Remember that the Lord will give you as a reward what He has kept for His people. For Christ is the real master you serve. [25] And every wrongdoer will be repaid for the wrong things he does, because God judges everybody by the same standard.

4 Masters, be fair and just in the way you treat your slaves. Remember that you too have a Master in heaven.

CONTENTS

CHAPTER 2: RESULTS AND DISCUSSION

SYNOPSIS

The aim of this project was the synthesis of viridamine analogues, to be used for selective metal complexation. This, therefore, required the synthesis of diketopiperazines, containing an imidazole-type side chain. The imidazole functionality was introduced into the synthesis *via* peptide acid coupling reaction between histidine and another amino acid. Before any coupling reactions were possible it was necessary to protect the carboxylic acid functionality of one of the two amino acids being used and the amine functionality of the other. This was to prevent mixed products forming.

Owing to the continued difficulty at achieving selective *N*-protection of histidine, it was decided to make use of the corresponding methyl ester instead. After some initial attempts, it was found that the methyl ester of histidine, which was bought as the dihydrochloride salt, could be readily coupled to a variety of Boc protected amino acids. The Boc protected amino acids could be prepared under various conditions using di-tert-butyl dicarbonate.

A range of conditions was investigated for the coupling of the two amino acids, *i.e.* the histidine methyl ester dihydrochloride and a Boc protected amino acid. Successful coupling was finally achieved using tetrahydrofuran as solvent, *N*-hydroxy benzotriazole as reaction promoter, *N*-methyl morpholine as a base and dicyclohexylcarbodiimide as the coupling agent. After varying the reaction conditions the optimised reaction conditions gave yields in the region of 76%.

Once coupling had been achieved, it remained to cyclise the dipeptide. Cyclisation was preceded by the removal of the Boc protecting group either *in situ* or in a two step process. In the absence of the imidazole functionality, removal of the Boc group was readily achieved using trifluoroacetic acid. However, attempted deprotection of dipeptides containing the imidazole functionality led to decomposition of the dipeptide under identical conditions. It was therefore necessary to find an alternative form of deprotection. This was found in the form of formic acid, which proved to be successful

in removing the Boc group and in effecting cyclisation to the analogous diketopiperazine. This particular form of *in situ* cyclisation proved to be very low yielding. This problem was circumvented by following the formic acid treatment by a period of reflux in a toluene / 2-butanol mixture. Cyclisation was effected with pure products being obtained in high yield, after column chromatography.

Complexation reactions were initiated with the synthesised diketopiperazines but unfortunately no X-Ray diffraction studies could be carried out, due to the formation of amorphous solids instead of crystalline materials.

OPSOMMING

Viridamien analoë kan gebruik word vir selektiewe metaal-kompleksering, en hierdie projek het die sintese van verskillende viridamien-analoë behels. Dit was dus nodig om diketopiperasiene wat imidasool-tipe sykettings bevat, te sintetiseer. Hierdie is bewerkstellig deur peptiedkoppeling van histidien met verskei ander aminosure. Die karboksielsuur funksionele groep van een van die twee aminosure, en die amien funksionele groep van die ander aminosuur moes egter eers beskerm word voordat koppelingsreaksies uitgvoer kon word. Beskerming van dié groepe voorkom dat gemengde produkte vorm.

Selektiewe *N*-beskerming van histidien kon nie verkry word nie, en die ooreenstemmende metiel-ester is eerder gebruik. Daar is bevind dat die metiel-ester van histidien, wat aangekoop is in die vorm van 'n di-hidrochloried sout, geredelik aan verskeie aminosure wat deur 'n Boc-groep beskerm is kan koppel. Sulke beskermde aminosure kan onder verskeie kondisies berei word deur gebruik te maak van di-*tert*-butiel-dikarbonaat.

'n Reeks van kondisies is ondersoek vir die koppeling van die twee aminosure, nl. die histidien metiel ester di-hidrochloried, en 'n aminosuur beskerm deur 'n Boc-groep. Die

gebruik van tetrahidrofuraan as oplosmiddel, *N*-hidroksiebenzotriasool as reaksie-inisieerder, *N*-metielmorfolien as 'n basis, en disikloheksielkarbodiimied as koppelings-reagens het kondisies vir suksesvolle koppeling daar gestel. Optimering van die reaksiekondisies het opbrengste in die omgewing van 76% gelewer.

Vervolgens is gepoog om die dipeptied te sikliseer. Dit is voorafgegaan deur die verwydering van die beskermende Boc-groep óf *in situ* óf in 'n twee-stap proses. In die afwesigheid van die imidasool funksionele groep is ontskerming maklik verkry deur middel van trifluoroasynsuur. Daarteenoor het pogings om dipeptiede wat die imidasool funksionele groep bevat, geëindig in afbraak van die dipeptied onder dieselfde kondisies. Dit was dus nodig om 'n alternatiewe vorm van ontskerming te vind. Die oplossing daarvoor is gevind in die vorm van mieresuur, wat nie aleenlik die Boc-groep suksesvol verwyder het nie, maar ook die siklisering na die analoë diketopiperazien begunstig het. Hierdie vorm van *in situ* sikliseering het baie lae opbrengs gegee en daarom was 'n ander metode gesoek. Die nuwe metode het behels dat die mieresuur proses opgevolg is met 'n tyd van kook in 'n tolueen / 2-butanol mengsel. Sikliseering is behaal met die verkry van suiwer produkte in hoë opbrengs, na kolomkromatografie.

Komplekseeringsreakies met die gesintetiseerde diketopiperaziens was geinisieer, maar as gevolg van die vorming van amorfiese vastestowwe kon geen X-straal diffraksie eksperimente uitgevoer word nie.

LIST OF ABBREVIATIONS

Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
Boc	*t*-butoxycarbonyl
Boc_2O	di-*tert*-butyldicarbonate
CD	circular dichroism
CDI	1,1-carbonyl diimidazole
cyclo(L-Histidyl-L-histidyl)	3,6-Bis(4-Imidazolylmethyl)-2,5-diketopiperazine
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-quinone
DMAP	dimethylaminopyridine
DMF	dimethylformamide
Et_3N	triethylamine
FCC	flash column chromatography
GeeHCl	glycine ethyl ester hydrochloride
Hme	histidine methyl ester
Hme.2HCl	histidine methyl ester dihydrochloride
HOBt	hydroxybenzotriazole
Me	methyl
MeOH	methanol
NaOMe	sodium methoxide
Nmm	*N*-methyl morpholine
NMR	nuclear magnetic resonance
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

CHAPTER 1

A SELECTIVE LITERATURE STUDY ON METAL COMPLEXATION

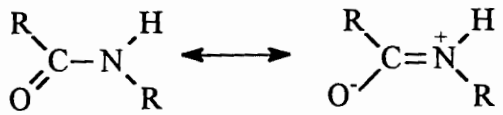
1.1 AN INTRODUCTION TO COMPLEXATION

Various studies are related to the complexation of metal ions. Metal complexation is of great importance not only to the mineral industry but also has an effect on our everyday life. One particular example is in the form of water purification. Linear hydrosoluble poly(acrylic acids) have been tested for their use as flocculants in the water treatment field. The elimination of low concentrations of metal ions present as pollutants depends greatly on their binding properties towards the organic or inorganic substances present in the bulk water and in particular with the organic molecules of a polymeric nature. Two metal ions of interest are Cd(II) and Pb(II). Both cadmium and lead have been removed during the flocculation step of contaminated water treatment by complexation with specifically designed poly(acrylic acids).[1] Similar treatments were successfully carried out for copper(II) and nickel(II).[2] Complexation reactions of diketopiperazines are of particular interest to our studies. I will therefore start from the smallest building blocks and expand to the structures of interest. Selectivity in complexation is of great interest and will also be addressed in this chapter.

1.2 AMIDE BONDS – DEPROTONATION AND PROTONATION

Amide bonds or groups provide the linkage between adjacent amino acid residues in proteins. When condensation of two amino acids yields a dipeptide, the resulting bond is often referred to as a peptide bond or group. In aqueous systems, hydrolysis of the peptides to the constituent amino acids is thermodynamically favoured. Chemically, the formation of peptide bonds involves a condensation of an amino group of one amino acid with an activated ester of another, with the elimination of an alcohol. This particular

ester function exhibits a higher activity than ordinary esters, especially under biological conditions where they are most commonly found, primarily owing to the more acidic carboxylic acid of the amino acids. The higher acidity is seen in the following example; [3] glycine $pK_a = 2.5$, compared to acetic acid having a pK_a of 4.7. The C-N and C-O bonds in an amino acid possess comparable amounts of both single and double bond character, but the *trans* form of the amide is strongly favoured (**Scheme 1.1**).



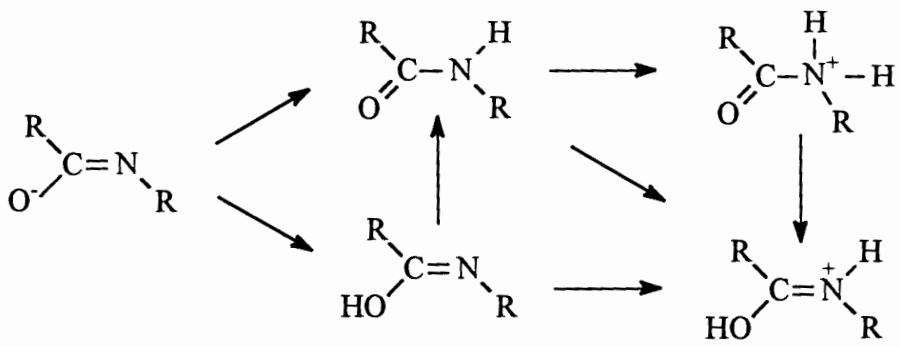
60% 40%

Scheme 1.1 The C-N and C-O bonds in an amino acid

The tetrahedral amino nitrogen ($pK_a \approx 9.7$) in an amino acid loses its basicity upon reaction to give a trigonal nitrogen in the amide bond. The trigonal nature of the nitrogen atom results from the planarity of amide groups. This planarity is due to the approximately 40% double-bond character in the carbon-nitrogen bond. More recent molecular orbital calculations agree well with the above description.[4]

Before the recognition of the primary structure of a protein as a homogeneous linear chain polymer of defined length and amino acid sequence, one test for proteins used the biuret colour produced by the reaction of Cu^{2+} with peptide bonds in alkaline solutions.[5] Greater appreciation of protein structure has increased interest in the metal-ion binding capabilities of the amide group, which is so abundant in nature.

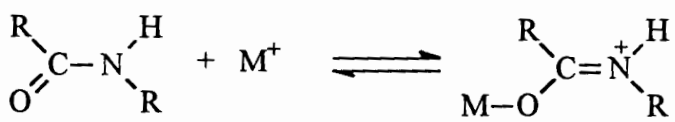
An amide group offers two potential binding sites for complexation of protons and metal ions; the oxygen and nitrogen atoms, respectively. Throughout most of the *p*H range, in the absence of metal ions, the amide group is neutral. It is only a very weak acid for proton loss from the trigonal nitrogen and upon loss of a proton gives a negatively charged species (**Scheme 1.2**). The prototypic amide group, in which we are most interested, has a pK_a value of approximately 15.[6]



Scheme 1.2 Protonic equilibria in the amide group. The major species in basic solutions is on the left, in neutral solutions at the top centre and in strongly acidic solutions at the bottom right.

Looking at amide group protonation we can anticipate the results for metal ion complexation. When the amide is anionic, protonation and metalation occur at the nitrogen, as shown on the top of **Scheme 1.2**. Just as protonation of a neutral amide occurs at the amide oxygen so does complexation of most metal ions.

On protonation or complexation at a neutral amide significant changes occur to the resonance forms: the C-O bond becomes longer and weaker and the C-N bond becomes shorter and stronger. Both experimental and theoretical results agree with the increased double bond character of the C-N bond upon protonation and metalation at the amide oxygen (**Scheme 1.3**).[7] In contrast, metalation at a neutral amide nitrogen weakens the C-N bond.



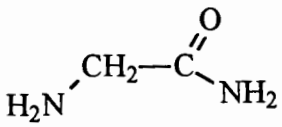
Scheme 1.3 Metalation at the amide oxygen

In acid-catalysed amide group hydrolysis water is the attacking nucleophile, while with metalation at the amide oxygen both water and hydroxide ion serve as potential nucleophiles. Metal ions promote amide group hydrolysis in neutral solutions because they introduce a positive charge at the amide oxygen under conditions where the much more nucleophilic hydroxide ion exists in significant concentrations. With such a weakly basic amide oxygen atom strong metal ion coordination will not occur at that site. In aqueous solutions, water provides a potent competitive oxygen donor. On the other hand, substitution of a nitrogen-bound hydrogen atom by a metal ion should create a strong bond, but the very low acidity of this hydrogen ($pK_a \approx 15$) implies that alkali- and alkaline earth metal ions will not be efficient in M-H substitution reactions. Transition-metal ions, however, promise to be more effective in substituting for a nitrogen bound amide hydrogen, but will potentially suffer competitive metal ion hydrolysis and precipitation in neutral and basic solutions.[8]

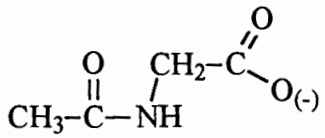
It can be concluded that the weak coordinating capabilities of an unbonded amide group points to a need for chelate ring formation to bring out the full metal ion binding capabilities of the amide functionality.

1.3 SINGLE PRIMARY LIGATING GROUP LIGANDS

To coordinate strongly to the amide group, metal ions must be capable of substituting for a nitrogen-bound amide hydrogen. To do this in neutral solutions, metal ions require an effective anchor to inhibit metal ion hydrolysis. The single-ligating groups that serve as effective anchors in neutral solutions can form, *e.g.*, 5-membered chelate rings between the deprotonated amide nitrogen and another nitrogen of an amide, for example on glycinamide (**1.1**). The two terminal groups of peptides and proteins differ markedly in their serviceability as primary ligating groups, or anchors, for metal ion interactions with an amide group. The two simplest model compounds are glycinamide, for the amino terminus, and *N*-acetyl-glycinate (**1.2**) for the carboxylate end.



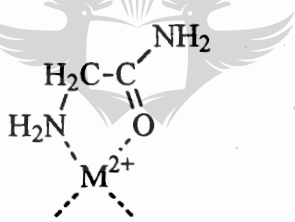
1.1



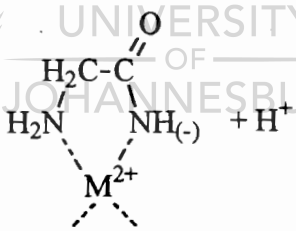
1.2

N-Acetyl-glycinate (**1.2**) acts only as a unidentate ligand through the carboxylate group. It is a weakly acidic ligand and has very low stability constants with most metals.[9] There is no hint of amide hydrogen deprotonation before metal hydroxide precipitation, which is expected to be as a result of the ligand's low acidity. Crystal structures with copper show only unidentate coordination *via* the carboxylate group.[10]

Glycinamide, however, chelates metal ions weakly *via* the amine nitrogen and the carbonyl oxygen (**1.3**), but a stronger chelation occurs upon substitution of an amide nitrogen bound hydrogen by some metal ions such as Cu^{2+} and Ni^{2+} (**1.4**).



1.3



1.4

The differences in coordination between the aminated and acetylated glycine re-emphasise general principles useful for metal ion chelation in peptides. An amine group anchor provides 5-membered ring chelation opportunities at both the amide oxygen and nitrogen, both of which are present in the glycinamide (**1.1**). In *N*-acetyl-glycinate (**1.2**), on the other hand, chelation between a carboxylate oxygen and the amide oxygen requires the unlikely 7-membered ring, mentioned earlier. The favourable 5-membered chelate ring remains possible, but the weak basicity of the carboxylate group renders it an ineffective anchor for competition with metal ion hydrolysis.

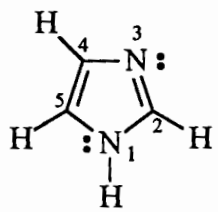
1.4 THE IMIDAZOLE GROUP

Before we can look at how the imidazole group functions as an effective anchor, it is important to understand how and why certain interactions take place between imidazole and other functionalities.

The imidazole ring as side chain is of particular interest and provides an effective anchor for chelation. The imidazole ring, as a histidine moiety, functions as a ligand toward metal ions in various biologically important molecules including Iron-Haem systems, vitamin B_{12} and its derivatives, and several metalloproteins. It is therefore important to understand the ligand-metal interactions in complexes containing imidazole rings and where possible the relationship between the properties of this heterocyclic ligand and its function in biological systems.

1.4.1 Properties of the Imidazole Ring

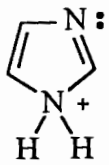
The parent molecule falls into the class of aromatic heterocycles and its unique structural features are easily explained with reference to pyridine and pyrrole, due to the structural similarities. Aromaticity in completely conjugated monocyclic systems requires a planar array of atoms with $(4n + 2)\pi$ electrons.[11] The presence of aromaticity in imidazole can then be seen if imidazole is considered to be constructed from a trigonal nitrogen with two electrons in the unhybridised p-orbital (N-1, "pyrrole nitrogen"), a trigonal nitrogen with a lone pair in a hybrid orbital and a single electron in the p-orbital (N-3, "pyridine nitrogen") and three trigonal carbons each with one electron in a p-orbital. An aromatic sextet is then available (**1.5**).



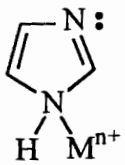
1.5

Imidazole is generally regarded as an aromatic, planar molecule. The N-3 nitrogen of imidazole is aptly termed the pyridine nitrogen while the N-1 nitrogen is referred to as the pyrrole nitrogen. The pyridine nitrogen displays fractional negative π and σ electronic charges indicating that this nitrogen is a modest σ acceptor and a weak π acceptor. The pyrrole nitrogen is involved in a two-way charge transfer. It donates substantial fractional electronic charge to the π system but withdraws an even greater amount of charge from the σ orbitals, resulting in a total gain of charge at the pyrrole N-1 nitrogen of the imidazole. These charge transfers were deduced from *ab initio* calculations carried out on pyrrole,[12] pyridine[13] and imidazole.[14] The calculations also indicated that the σ electrons of pyrrole and imidazole are strongly delocalised and polarised about the rings. It was further found that all the hydrogen atoms were σ donors, as is C-2 to a lesser extent. Carbon atoms 4 and 5 are σ acceptors, while all the carbon atoms are weak π acceptors, but polarisation of the σ electrons dominates the overall charge distribution.

An important structural feature with respect to the coordination site of imidazole is clarified when the aromatic nature of the molecule is recognised. There is only one pair of electrons that can be properly described as an unshared pair: the pair on N-3. The π electrons of N-1 are part of the aromatic sextet. Bonding of a proton or metal ion at N-1 is expected to be unfavourable since it would compromise the aromaticity of the ring. The important conclusion to be made is that the neutral imidazole molecule presents a single energetically favourable coordinating site for a proton or metal ion, namely the unshared pair on the N-3 nitrogen. Structures (**1.6**) and (**1.7**) are of very high energy. The transfer of the N-1 proton to N-3 would be very favourable energetically, but structures such as **1.6** and **1.7** would not be expected because of their high energy state. The structures (**1.8**) and (**1.9**) of the protonated and metal ion complexes of imidazole are aromatic cations, and at this point the distinction between N-1 and N-3 is lost since the cation is symmetrical, H-4 and H-5 are equivalent.

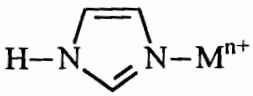


1.6



1.7

1.8



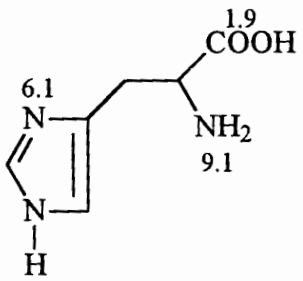
1.9

In addition to the protonation at N-3 to give the imidazolium cation, neutral imidazole undergoes deprotonation at N-1 in strongly basic solutions with reported pK_a values from 14.2 – 14.6.[15] The resulting anionic imidazole, also aromatic, possesses two equivalent sites for coordination and is a potential bridging ligand.

Imidazole is amphoteric, being a moderately strong base capable of accepting a proton at N-3, as well as a very weak acid capable of loosing a proton from N-1. In solutions near neutrality, the unprotonated imidazole molecule usually functions as a ligand *via* the unshared pair of electrons on N-3 (the "pyridine" nitrogen).

1.4.2 The Imidazole Ring as a Side Chain

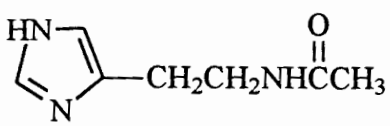
Now that we have looked at the how and why interactions take place between imidazole and other functionalities, we can look at its function as an anchor. The imidazole nitrogen of the histidine residue, provides one of the primary means by which metal ions may coordinate to the histidine molecule. The histidine molecule presents three potential coordination sites in aqueous solution. The carboxyl group (pK_a = 1.9), the imidazole nitrogen (pK_a = 6.1), and the amino nitrogen (pK_a = 9.1), which becomes available for complexation as the *p*H increases (**1.10**).



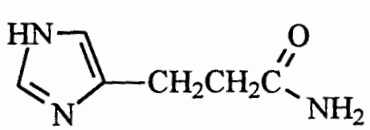
1.10

The imidazole ring provides an anchor for chelation with the amide nitrogen of the same histidine residue only, in a 6-membered chelate. When it is to function as an anchor with its amide (carboxyl) oxygen, the imidazole group of a histidine residue would have to participate in an unlikely 7-membered chelate ring. The different preferences for complexation sites can be seen by the examples discussed below. Crystal studies have shown though that histidine can use each of the three potential coordination sites for bonding to metal ions, depending on which coordination geometry the metal ion can adopt.[16]

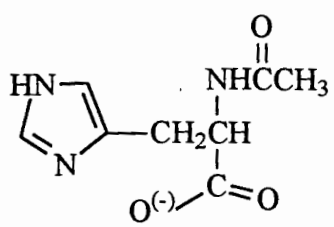
Acetylhistamine (**1.11**) and imidazole-4-propionamide (**1.12**) are appropriate models for single primary ligating groups of the imidazole side chain of histidine. The latter requires an unfavourable 7-membered chelate, and its metal ion binding does not seem to have been studied. Conversely, Cu^{2+} coordinates at the amide nitrogen of the smaller imidazole-4-acetamide in neutral solutions, forming a 6-membered ring.[17] Stability constants for metal ions with imidazole-4-propionate suggest primary complexation at the imidazole ring and little chelation at the carboxylate group.[18]



1.11



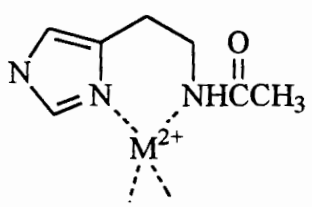
1.12



1.13

Acetylhistamine (**1.11**) requires an unfavourable 8-membered ring for imidazole nitrogen-amide oxygen chelation. Substitution of Cu^{2+} for the amide hydrogen to form a 6-membered chelate to the amide nitrogen does not occur in neutral solutions of acetylhistamine (**1.11**) or of neutral solutions of acetylhistidine (**1.13**).[19] It was noted, though, that the latter, a potentially tridentate ligand, does undergo deprotonation at *p*H values in the region of 10. Deprotonation of acetylhistamine seems to be very difficult. The difficulty experienced could be because of the steric hindrance that is caused by two ligands about a tetragonal Cu^{2+}. It was noted though, that if excess acetylhistamine (**1.11**) ligand was used in a ligand / copper solution at a *p*H of 9, an absorption maximum at 590 nm was recorded, which is commonly observed after amide deprotonation. The small difference between the two equivocal ligands seems to be a result of the difficulty to deprotonate the more basic amide nitrogen of acetylhistamine[20] and also the tendency of Cu^{2+} to prefer ligands with mixed imidazole nitrogen and oxygen donors.[21]

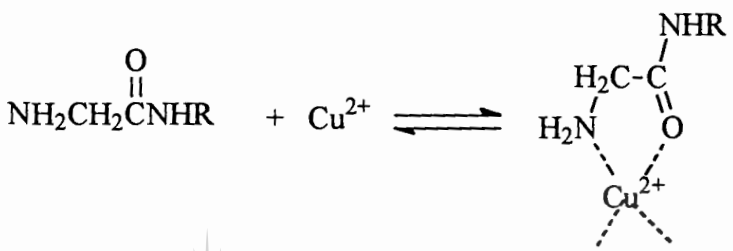
From the discussion it would seem that the most favourable chelation of a metal ion bound to a histidyl residue would be *via* the non-protonated nitrogen on the imidazole ring and the deprotonated amide nitrogen of the histidyl residue to give a 6-membered ring (**1.14**). This form of coordination is confirmed by the crystal structure obtained when histidine is complexed with zinc(II). A di-(L-histidino)-zinc(II) dihydrate complex was obtained when two histidine molecules chelated to zinc(II) through the amino group and an imidazole nitrogen atom as shown in **1.14**.[22] If deprotonation of the amide nitrogen does not occur, a 7-membered ring is possible *via* a weak interaction with the histidyl residue amide oxygen.



1.14

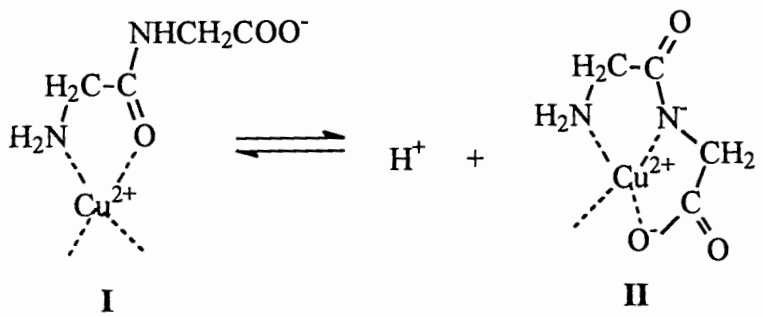
1.5 DIPEPTIDES AND THEIR ROLE IN METAL ION COMPLEXATION

The next part of the discussion shall shift from simple amino acids to peptides. Almost fifty years ago, investigations were carried out to determine the coordination of Cu^{2+} with glycyl peptides, on the basis of potentiometric investigations.[23] Similar to the manner of coordination observed for glycinamide (**1.1**), Cu^{2+} anchors at the amino terminus and chelates to the peptide oxygen of the amino terminal residue. A comparison was carried out between the stability constants for the complexation reaction (**Scheme 1.4**) of five peptides: glycinamide, diglycine, diglycinamide, triglycine and tetraglycine.



Scheme 1.4 The basic complexation reaction under consideration

The comparison showed that the slight variation in the stability constants was paralleled by the amino group basicity, which is a moiety common to all five peptides. Thus, all complexes showed a stability constant in the region of 7.90 – 8.15.[24] Distinction between the peptides became apparent upon substitution of the peptides hydrogen by Cu^{2+}, *e.g.* for glycylglycine (**Scheme 1.5 II**). Certain ligands, *e.g.* glycylsarcosine, failed to deprotonate, demonstrating that a proton is removed from the peptide nitrogen and not from the coordination sphere of the metal ion.[25]



I II

Scheme 1.5 Substitution of the peptides hydrogen by Cu^{2+}

Now that we have introduced the concept of dipeptides and we have seen, very briefly, the two ways in which complexation normally takes place, we can look more closely at trends in stability and amide hydrogen deprotonation constants of various dipeptides. We shall initially be looking at dipeptides with weakly coordinating or noncoordinating side chains and from here expand to more complex systems.

1.5.1 Dipeptides with Weakly Coordinating or Noncoordinating Side Chains

As discussed before, in comparing five peptides, the initial complex forms when the metal ion anchors to the amino terminus and chelates the amide oxygen of the amino terminal residue (**Scheme 1.5 I**). Hence, the nature of the amino terminal residue in a peptide shows the greatest influence on the stability of the *N,O*-chelated metal ion. A stability comparison between glycine-X and X-glycine dipeptides was carried out and the results compared by a plot of the log stability constant for bidentate Cu^{2+} chelation by the amino nitrogen and amide oxygen of peptides ($\log K^{Cu}_{CuL}$; **Scheme 1.4**) vs. *p*K for ammonium group deprotonation (pK^{H}_{HL}). The Gly-X dipeptide series formed a straight line plot with a relatively steep slope of 2.0±0.1.[26] In contrast, the X-Gly dipeptides, with side chains other than hydrogen in the amino terminal residue, deviate from the solid line and exhibit no apparent regularity. The negative deviations, for example where X = leucine or isoleucine, are a result of decreased stability, where the decrease can be attributed to steric hindrance of the branched side-chains.[27] The positive deviations, for example where X = *S*-methyl-L-cysteinyl, L-seryl or L-threonyl, are a sign of enhanced stability due to apical involvement of electronegative side-chain atoms.[28] When the order

of the amino acids in these five peptides is reversed, to give Gly-X dipeptides, all five stability constants for complex formation are close to the straight line as was the case for the first glycyl-peptides, where only the Gly part of the Gly-X dipeptides participates in the *N,O*-chelation: the X side chain part is not involved in steric or apical interactions.

The Gly-X ligands show few apical or steric interaction effects compared to the X-Gly systems where steric and apical interactions result in decreases and increases, respectively, of the stabilities of the complexes formed.[29]

The overall conclusion that can be drawn from these discussions of apical and steric interaction effects is that an alkyl substituent at the X residue of a X-Gly dipeptide facilitates the ionisation of the amide proton, while the same substituent at the X residue in Gly-X decreases the tendency for ionisation.

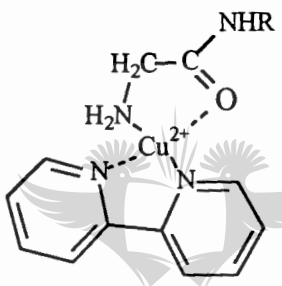
1.5.2 Ternary Complexes containing Peptides

After establishing the interactions taking place in the above mentioned dipeptide series, it is of great interest to see if the same interactions are still present in mixed complexes, involving the above mentioned dipeptides.

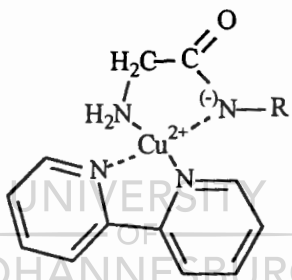
In the past 20 years, it has become increasingly apparent that several transition metal ions favour coordination with mixed ligand systems to a greater extent than would have been expected on a statistical basis. Most of the information on mixed ligand complexes of imidazole and derivatives with transition metal ions has been gained by potentiometric solution investigation along with a fair amount of information obtained from X-Ray crystal structures.

We have already seen that, in the initially formed complex $Cu(L)^+$ between Cu^{2+} and glycinamide (**1**) (or related ligands), coordination occurs *via* the terminal amino group and the oxygen of the neighbouring amide group (**Scheme 1.5 II**). In agreement with this concept, the stabilities of ternary Cu^{2+} complexes of 2,2'-bipyridyl are largely

independent of the type of the second and following amino acid residues. The stabilities for glycinamide, glycylglycinate, glycylglycylamide, triglycine or tetraglycine were all similar[30] and this result agrees with the suggested structure for all mixed ligand complexes (**1.15**).[31] The stability value expected, on a statistical basis, for the present system differed from the experimentally determined value throughout, with the difference indicating that the mixed ligand complexes are more stable than expected. This result agrees with the observation generally made for ternary complexes of Cu^{2+}, a heteroatomic nitrogen base, and a second *N,O*-donor ligand.[32] However, the stability of binary Cu(L-H) complexes depends strongly on the ligating properties of the additionally involved groups. For mixed ligand complexes, the nearly consistent stabilities can be explained by structure (**1.16**).



1.15



1.16

In this structure, 2,2'-bipyridyl, the amino group and the ionized amide nitrogen coordinate to the square planar positions of Cu^{2+}. However, structure (**1.16**) contrasts the crystal structure analysis of the ternary (1,10-phenanthroline)(glycylglycinato)copper(II) complex.[33] In the solid state, the glycylglycine dianion co-ordinates with its three potentially binding heteroatoms, including the carboxylate group, in the square plane around Cu^{2+}. One of the phenanthroline nitrogen atoms completes the square, while the other occupies a tilted apical position, giving a distorted square-pyramidal geometry about Cu^{2+}. Both absorption spectrum[33] and calorimetric results[31] support the occurrence of the 5-coordinate structure in solution. The answer to this apparent contradiction between structure (**1.15**) and the crystal structure probably lies in an intramolecular equilibrium between different isomers in solution.

The stability in solution of the ternary $Cu(bpy)L^+$ complexes formed by dipeptides parallels the observation with the corresponding binary $Cu(L)^+$ complexes. For Gly-X dipeptides the stability constant for amino nitrogen and amide oxygen chelation was weaker in the ternary 2,2'-bipyridyl than in the binary complexes. For X-Gly dipeptides the difference was even greater. The Gly-X dipeptides thus show about 5 times greater tendency to form mixed complexes with bipyridyl ligands. The nearly constant difference in stability constants between the classes of dipeptides allows the same conclusion to be drawn for ternary complexes as that drawn for binary complexes: an alkyl substituent at the X residue of a X-Gly dipeptide facilitates the ionisation of the amide proton, while the same substituent at the X residue in Gly-X decreases the tendency for ionisation.

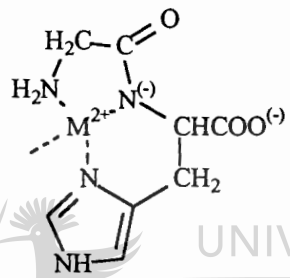
1.5.3 Peptides containing the Imidazole Functional Group

Discussions of the structure of the complexes containing the imidazole functional group concluded that one of the features of these complexes is that[34] "the tendency of the peptide and imidazole ligands ... to be coplanar suggests that an imidazole ring prefers to lie close to the plane of the strongest Cu-ligand bonds unless it is rotated out of the plane by steric hindrance or by hydrogen-bond formation". The Cu-N (imidazole) bond lengths are significantly shorter than those found in most other Cu^{2+} - imidazole complexes.[20] This bond shortening agrees with the expected π back-bonding from Cu^{2+} to the vacant π^* orbitals of heteroaromatic nitrogen bases, which was proposed to be important for the stability of such complexes.[35]

The imidazole side-chain of a histidine residue, when incorporated into peptides, offers a basic binding site, along with its own deprotonated peptide nitrogen, so as to allow the formation of a favourable 6-membered chelate, see structure **1.17**. Coordination of a metal ion occurs at the pyridine- rather than pyrrole-type nitrogen.[20] These two nitrogen atoms, when not metalated, undergo rapid tautomeric exchange.[26]

If we are to look at peptides containing the imidazole functionality, *i.e.* histidine-containing peptides, it would be best to look at the most simple type of peptides to get a basic understanding and expand from there. The simplest peptides in this respect are glycylhistidine, diglycylhistidine and derivatives thereof.

In neutral solutions, glycyl-L-histidine chelates to Cu(II),[36] Ni(II),[19] or Pd(II) [37] as a tridentate ligand with three nitrogen donor atoms: amino, deprotonated amide, and imidazole (**1.17**). The carboxylate group does not bind at the same time to tetragonal Cu^{2+} or to Pd^{2+}. In contrast to the Gly-Gly system, the third coordination site is stolen from the less basic carboxylic group by the imidazole nitrogen.



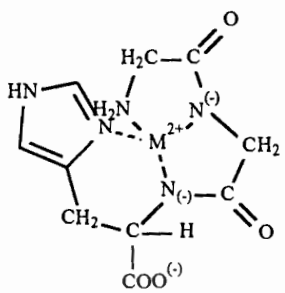
1.17

This structure, originally determined by potentiometric studies, has been confirmed in a variety of crystal structure determinations of complexes of histidine-glycine type peptides with Cu^{2+}, *i.e.* Gly-His,[38] Gly-His-Gly,[39] and Gly-His-Lys.[40] The carboxylate group may well be co-ordinated in the Ni^{2+} complex because the metal ion remains hexa coordinate in neutral solutions.[19] With Co^{2+} in oxygen-free solutions, the peptide nitrogen is deprotonated. A similar situation was observed for Zn^{2+} and the possibility that it represents the peptide proton was discussed in other sources.[41]

At about *p*H 9.6, a concentration-dependent deprotonation is observed for equimolar solutions of Gly-His and Cu^{2+}or Ni^{2+} or Pd^{2+}. An accompanying pronounced shift of the d-d absorption band to shorter wavelengths occurs for copper and palladium, while the pale green nickel complex turns yellow. These changes indicate substitution of oxygen by a nitrogen donor to the metal ions. The deprotonation was found to be a result of

metal ion substitution of the pyrrole hydrogen on the imidazole ring. The imidazole ring then bridges two metal ions. A detailed *p*H and spectrophotometric study of equilibria indicated the formation of a polymer, probably a tetramer.[37]

The tripeptide Gly-Gly-His chelates to Cu(II)[36] and Ni(II)[42] as a quadridentate ligand with four nitrogen donor atoms: amino, two deprotonated peptides, and imidazole (**1.18**). The titration curves for the four deprotonations occur over a narrow, highly buffered *p*H region, and it is difficult to resolve individual peptide deprotonations.[43]

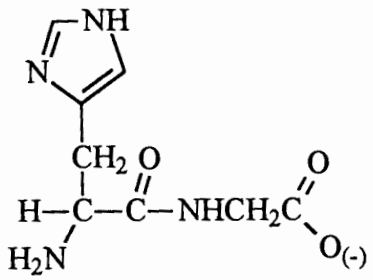


1.18

Oxidative decarboxylation has been suggested to occur in the Ni^{2+} complex of Gly-Gly-His.[44] The combination of an amino terminus with a histidyl residue in the third position presents the strongest peptide binding site for tetragonal metal ions capable of deprotonating peptide nitrogens. Zn^{2+} was also found to promote peptide nitrogen deprotonation in Gly-Gly-His, but binuclear complexes were also found to form.[45]

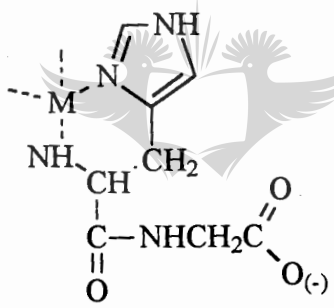
The corresponding amino-acetylated derivatives of Gly-His, Gly-Gly-His and Gly-Gly-Gly-His have also been investigated for their ability to bind Cu^{2+}. It was found that peptide deprotonations occurred with a greater amount of difficulty in the acetylated ligands, but can still take place if carried out in neutral solutions.[46]

Now to look at the histidine containing peptide just discussed above, but with the amino acids reversed, *i.e.* histidylglycine. In neutral solutions, L-histidylglycinate (**1.19**) presents four potential donor atoms.

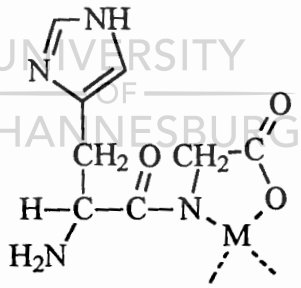


1.19

However, those from a single ligand cannot coordinate simultaneously to the four coordination sites about a single tetragonal metal ion. It is possible to imagine the dipeptide in (**1.19**) chelating as a substituted histidine through amino and imidazole nitrogens (**1.20**) or as a substituted glycylglycinate through amino and deprotonated amide nitrogens and a carboxylate oxygen (**1.21**).



1.20



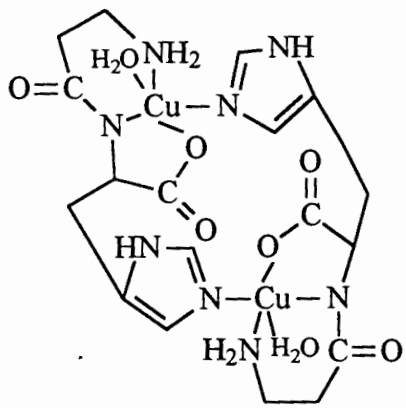
1.21

Neither of these chelation modes meets the requirements for induction of a diamagnetic planar Ni^{2+} complex. With copper, the former mode is favoured at a *p*H of 5.[47] Between *p*H 6 and 7 the peptide hydrogen undergoes substitution, and the latter substituted glycylglycinate mode results. A perfect fit is found between the four potential donors in His-Gly and the four tetragonal coordination sites in an equimolar neutral solution with Cu^{2+}, resulting in the formation of a dimer. This is very similar to the manner in which equimolar solutions of Gly-His and Cu^{2+} solved the problem of vacant coordination positions at *p*H 10 by tetramer formation. It becomes quite clear that the primary driving force makes use of available donors and fills vacant coordination sites by polymer

formation. The smallest polymer allowed by steric considerations seems to be favoured in each case.

If a second imidazole ring is added to His-Gly, the His-His peptide would be obtained. Cu(II)[48] and Ni(II)[19] substitute for the His-His peptide hydrogen in neutral solutions. In the systems with Cu^{2+} and Zn^{2+} binuclear complexes are the major species in the neutral *p*H range.[48] While amide ionisation was confirmed in the presence of copper it was not detected with zinc. For (histidylhistidinate)cobalt^{+} complex amide ionisation occurs with $pK_a \approx 7.8$.[41(b)]

L-Carnosine (β-alanyl-L-histidine) is an important dipeptide in the medical world and has many uses, *e.g.* treatment of surgical wounds, gastric ulcers, arthritis *etc.* L-Carnosine has various modes of complexation, depending on the metal ion to which it is complexing. L-Carnosine acts as a bridging dianionic ligand following removal of the amide and carbonyl protons in its copper(II) complex. Each copper is five coordinate. The ligand coordinates to one copper through its three donor atoms, namely the oxygen of the carboxylate group, the nitrogen of the NH_2 and another nitrogen of the deprotonated amide group. The same ligand then binds another copper centre through the pyridine nitrogen of the imidazole moiety. The fifth ligand on each copper atom, completing a square-pyramidal environment, is a water molecule (**1.22**).[49] A similar manner of complexation was also seen for cadmium(II), but where each cadmium atom was four coordinate, *i.e.* no water coordination (**1.23**).[50]



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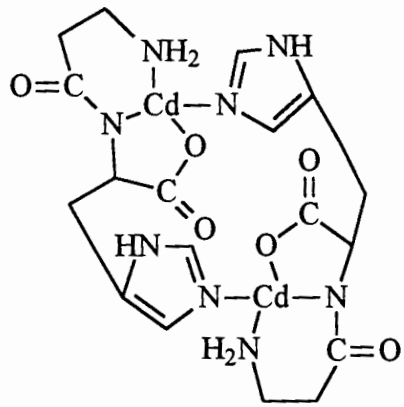


1.23

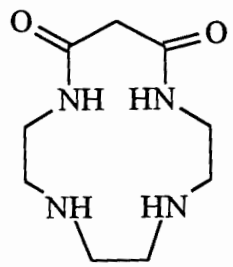
From the above discussion it can be seen that open chain dipeptides successfully complex Cu^{2+}, Ni^{2+}, Co^{2+}, Pd^{2+} and in some cases Zn^{2+} and Cd^{2+}. From this it could be seen that no differentiation was made between these metals during complexation and would therefore not allow separation of these metals by selective complexation.

1.6 CYCLIC LIGANDS WITH AMIDE FUNCTIONS

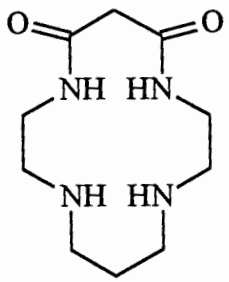
Cyclic ligands with amide functions permit the study of metal ion interactions with an amide linkage and with side-chain substituents under conditions where effects of the terminal functional groups of linear peptides are excluded.

1.6.1 Macrocycles with Amino and Amide Nitrogens

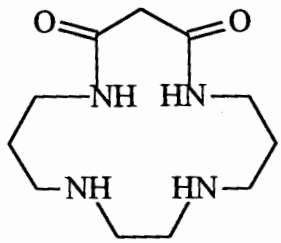
We first take a look at macrocycles with amino and amide nitrogens. The recent synthesis of the 13- to 15-membered macrocyclic dioxo tetraamines, 1,4,7,10-tetraazacyclotridecane-11,13-dione (**1.24**), 1,4,8,11-tetraazacyclotetradecane-12,14-dione (**1.25**), and 1,4,8,12-tetraazacyclopentadecane-9,11-dione (**1.26**) led to the study of their corresponding Cu^{2+} complexes.[51]



1.24



1.25



1.26

All three ligands form neutral amide ionised Cu(L-2H) species; a monodeprotonated $Cu(L\text{-}H)^{+}$ complex was not observed. In substitution reactions, the Cu^{2+} complex of the 14-membered macrocycle (**1.25**) is the most inert,[52] and the most stable among the three complexes.[53] Comparison with the open-chain diamide, *N,N'-bis*(2-aminoethyl)malon-diamide, the corresponding macrocycle (**1.25**) was much more stable, even though both complexes showed the same absorption maxima, *i.e.* 506 and 516 nm. From the absorption maxima it was possible to confirm that it was the ionisation of two amide nitrogens in both complexes which resulted in the greater stability. With Pb^{2+}, Cd^{2+} and Zn^{2+} the dioxo macrocycles (**1.24**)–(**1.26**) were deprotonated at a *p*H < 7, and precipitation of the hydrolysed metal ions occurred at *p*H > 7.

Complexes of the neutral 14-membered ligand (**1.25**) M(L-2H) were prepared with Ni^{2+} and Cu^{2+}. The Ni(L-2H) complex is orange-yellow and diamagnetic. Various reactions carried out on this nickel complex seem to point towards nickel acting as a protecting group for the amide functionality, as well as activating the methylene group towards C-alkylation.[54] The Ni(L-2H) and Cu(L-2H) complexes of macrocycle (**1.25**) are acid labile and may be oxidised to $Ni^{III}(L\text{-}2H)^{+}$ and $Cu^{III}(L\text{-}2H)^{+}$.[55] The brick red *trans*-$[Co^{III}(L\text{-}2H)(NH_3)_2]Cl$ complex has also been isolated.[56]

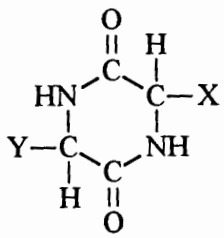
1.6.2 Cyclic Peptides

Now that we have a basic understanding as to how macrocycles complex, we can look at cyclic peptides. We will first look at cyclic dipeptides and then take a brief look at macrocyclic peptides.

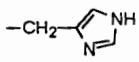
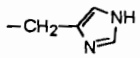
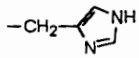
The advantage of cyclic dipeptides over their linear counterparts IS their "constrained" geometry and the absence of free $-COO^-$ and $-NH_2$ terminal groups. In cyclic dipeptides, the deprotonation of the peptide nitrogen occurs in the presence of transition-metal ions at high *p*H values only, unlike the case for linear peptides, and therefore under normal conditions the cyclic dipeptides coordinate *via* oxygen atoms only.

Cyclic peptides with amino acid residues, usually referred to as 2,5-piperazinediones or 2,5-diketopiperazines, containing complexing side-chain substituents such as imidazole, carboxylate, or thioether groups can coordinate to metal ions in a way that imitates that of enzyme coordination sites,[57] which makes their mode of complexation interesting. Side-chains of this kind can encourage the coordination of peptide nitrogens and are therefore of great importance to many fields of study.

The basic structure and a few examples of cyclic dipeptides, containing the imidazole functional group, are shown in (**1.27**). The cyclic peptides, which are of greatest interest to us, are those containing the imidazole functionality.



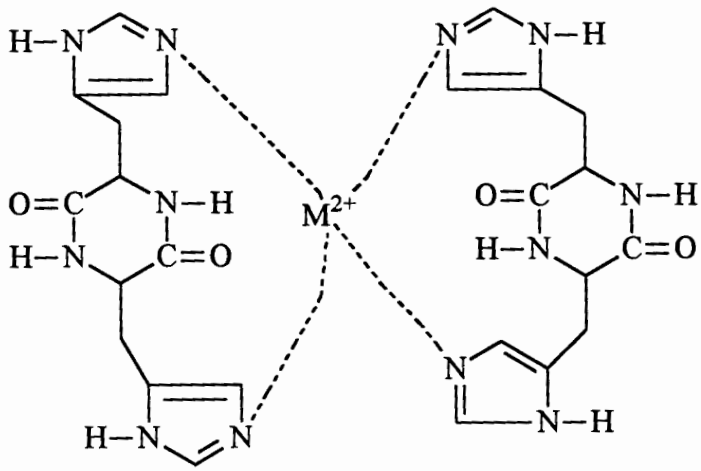
1.27

		X	Y
27a	*cyclo*- L-His-L-His		
27b	*cyclo*- L-Gly-L-His		H
27c	*cyclo*- L-Gly-L-Asp	$-CH_2COO^-$	H
27d	*cyclo*- L-Gly-L-Glu	$-CH_2CH_2COO^-$	H
27e	*cyclo*- L-Gly-L-Ser	$-CH_2OH$	H
27f	*cyclo*- L-Gly-L-Asn	$-CH_2CONH_2$	H
27g	*cyclo*- L-Gly-L-Gln	$-CH_2CH_2CONH_2$	H

Substitution of an amide hydrogen by Cu^{2+} in the cyclic dipeptides appearing in structures **1.27b-g** is less favourable than in their linear counterparts and occurs, if at all, well beyond the physiological *p*H range, where theses cyclopeptides are most commonly found.[58] Even in the presence of a potential anchor in **1.27b-d**, amide hydrogen ionisation does not occur until at least a *p*H greater than 10. Compounds **1.27e-g** do not undergo hydrogen ionisation at *p*H values less than 11.6. Cu^{2+} inhibits base hydrolysis of the diketopiperazine ring at that *p*H. Solvolysis of the "dangling" amide residue of the two peptides *cyclo*(Glycyl-L-Asn) and *cyclo*(Glycyl-L-Gln) is selectively promoted by Cu^{2+} in aqueous alkali to yield complexes identical to those found with **1.27c** and **1.27d**.

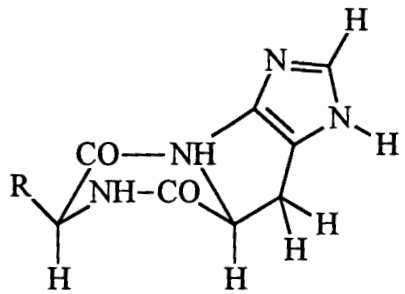
From the examples mentioned above, the one most widely studied is *cyclo*(L-Histidyl-L-histidyl) **(1.27a)**. This system was thought to be of interest to look at because (i) histidine-containing cyclic dipeptides are of great importance in many biological systems (ii) copper(II) enhances the catalytic action of histidine containing peptides and (iii) Cu(II)-imidazole bonding has been widely investigated.

Various modes of complexation have been observed for *cyclo*(L-Histidyl-L-histidyl). One involved the ligation of two *cyclo*(L-Histidyl-L-histidyl) to copper *via* two nitrogen atoms of the imidazole rings yielding two 13-membered chelate rings in solution (**1.28**).[59]



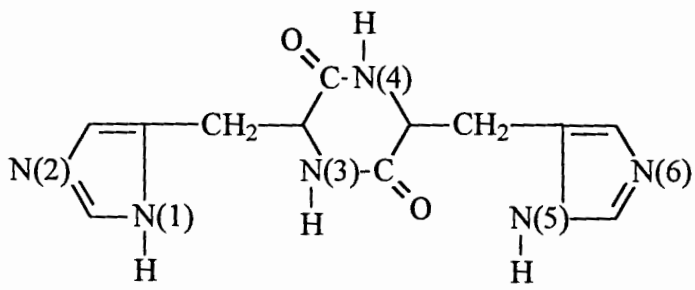
1.28

^{1}H NMR analysis, carried out in D_2O at 50 °C, showed equal broadening of the imidazole protons, while the α-methine and β-methylene protons were virtually unaffected. This study confirmed that only the imidazole groups were involved in the complex formation. In the NMR study a large excess of ligand was used because of the paramagnetism of Cu^{2+}. Consequently, the CD spectral method was used to confirm the predominant species when a 2:1 ligand:metal ratio was used and successfully confirmed the above-mentioned complex was predominant. X-Ray structure analysis showed that the copper atom was surrounded by four N atoms of the respective imidazolyl groups. The four N atoms do not lie planar, but are distorted toward a tetragonal configuration, with an interplanar angle of 29°. The diketopiperazine ring was found to be slightly buckled toward a boat conformation, similar to that found for *cyclo*(L-Alanyl-L-alanyl), which has the bowsprit-boat conformation (**1.29**).[60]



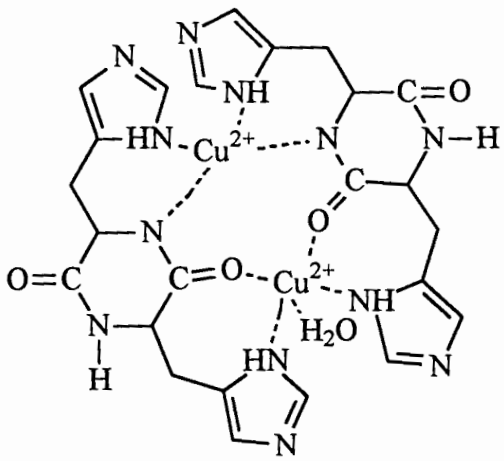
1.29

The two Cu-N bond lengths were compared to those in the sulfate[61] and perchlorate[62] of *tetrakis*(imidazole)copper(II), and with those of the aqua*bis*(*cyclo*(L-Histidyl-L-histidylato)dicopper(II) perchlorate hydrate[63], and were found to be identical. Aqueous solution studies of the above mentioned complex revealed three structures were possible, shown in **1.30**, involving the formation of 11-, 12- and 13-membered chelate rings through N(1) and N(5), N(1) and N(6), and N(2) and N(6), respectively:



1.30

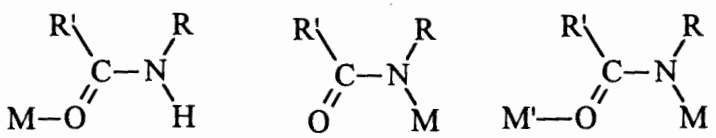
A second manner of complexation was detected when equimolar amounts of ligand and metal were used, with the addition of lithium hydroxide monohydrate, resulting in the aquabis(*cyclo*(L-Histidyl-L-histidylato)dicopper(II) perchlorate hydrate, mentioned earlier. The complex cation was found to be dimeric, with two copper atoms of different coordination modes (**1.31**).



1.31

The one copper atom was found to take a flattened tetrahedral form, coordinated by the nitrogen of the imidazole group and an anionic nitrogen of the diketopiperazine moiety of one ligand (DKPH-) from each of the two ligands. The diketopiperazine ring (DKPH-) was found to be nearly planar. The tetrahedral coordination of bivalent copper is rather unusual but can possibly be attributed to the dimerisation and strong affinity of copper(II) for N atoms. The second copper was found to have a coordination geometry that was an intermediate between the square pyramid and the trigonal bipyramid, being five coordinate, with donor atoms from a water plus an imidazole nitrogen and an amide carbonyl oxygen from each of the two ligands in the dimer. Electronic property studies[64] of this complex have been carried out and further confirm the structure discussed above. Brubaker *et al.*[58] suggest that the cyclopeptide nitrogen deprotonation assisted by copper(II) complexation may occur at relatively low *p*H values, which therefore allows us to assume that the structure remains the same in solution.

From the above findings it can be seen that the diketopiperazine ring ($DKPH_2$) can be regarded as a kind of secondary amide group which is capable of ligating in three ways (**1.32**): (I) the usual way, (II) uncommon and (III) deprotonation is necessary.



I II III

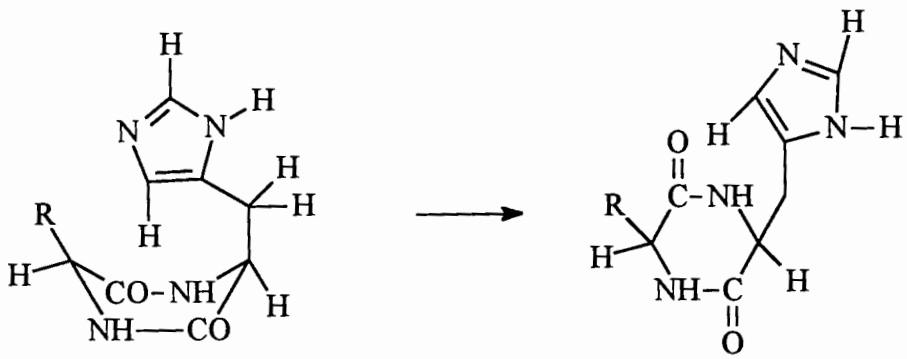
1.32

In another case, an X-ray structure analysis of the monomeric Cu(*cyclo*(L-Histidyl-L-histidyl))$_2$(ClO_4)$_2$.4H_2O complex[65] showed that Cu^{2+} adopted a distorted tetrahedral array and coordinated only to the four imidazole residues of the two ligands without involvement of the amide groups. Aqueous solution studies have also been performed on this complex[66] and stability constants of the corresponding complexes with Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} were determined.[67]

Cyclo(L-Histidyl-L-histidyl) coordination demonstrates unusual behaviour: (i) The formation of unusual-size chelate rings, driven by the peculiar conformational characteristics of the diketopiperazine ring. (ii) In the dimer species, the deprotonation of the peptide nitrogen, due to copper(II) complexation, is enhanced by the imidazole nitrogen coordination and by the interaction between the diketopiperazine and imidazole rings. These factors cumulatively account for the lowering of the deprotonation *p*H, compared to that for other cyclopeptides.[68] (iii) Thermodynamic parameters of complexation of *cyclo*(L-Histidyl-L-histidyl) with H^+ and with Cu^{2+} seem to be dependant on the degree of intramolecular interaction of the diketopiperazine ring and the imidazole residues and of the side-chains with each other.

Arena *et al.*[69] found that the decreased basicity in the above mentioned ligands can be attributed to the imidazole moiety in the diketopiperazine backbone. Further effects of the imidazole side chain were found when Koppel *et al.*[70] showed the influence of aromatic or pseudoaromatic substituents (such as phenylalanyl, tyrosyl and histidyl residues) on imidazole-containing cyclic dipeptides. The favoured conformer in solution is folded, with the aromatic ring facing or folded against the diketopiperazine ring. This favoured conformation is as a result of a dipole-induced dipole interaction between the

aromatic ring and the diketopiperazine ring. These interactions appear to have a great influence on the overall conformation of cyclic peptides. The diketopiperazine ring, as a result of these interactions between the aromatic side chains undergoes a change of conformation from the normal conformation of monosubstituted cyclic dipeptides, flagpole boat, to the planar conformation, more common for cyclic dipeptides with two aromatic side-chains (**Scheme 1.6**).



Scheme 1.6 The change of conformation from flagpole boat (left) to planar (right).

If both amino acids have the same configuration, the planar nature removes any interference that may be observed by the side-chains, but at the same time keeps the interaction between each substituent and the diketopiperazine ring at a maximum. In a series of studies of the metal complexes of cyclic dipeptides, it appears that the conformation of the cyclic dipeptide greatly affects the formation and conformation of the metal complex.

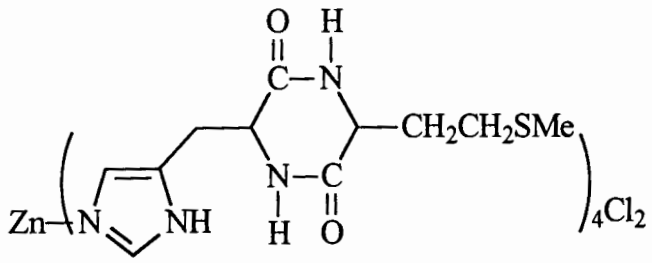
Looking again at *cyclo*(L-Histidyl-L-histidyl): There are three possible conformations of *cyclo*(L-Histidyl-L-histidyl) in solution: folded-unfolded (same as in solid state), folded-folded (both of the imidazole rings hang over the piperazine ring) and unfolded-unfolded (both imidazole rings are kept away from the piperazine ring). Cyclic dipeptides with aromatic rings usually prefer to be in a folded conformation than an unfolded one, in solution.

The effects of this conformation are seen in the complexation of *cyclo*(L-Histidyl-L-histidyl) with three metal ions: Cu^{2+}, Ag^{+} and Zn^{2+}.[71] The copper(II) complex with *cyclo*(L-Histidyl-L-histidyl) was discussed earlier and we saw the formation of a 13-membered chelate with both imidazole rings in the folded position. The silver(I) complex prefers two unfolded imidazole rings whose nitrogen linearly coordinates to the Ag^{+} to give a polymeric material. The zinc(II) complex consisted of a folded imidazole ring and an unfolded one. It was not determined if the coordination was taking place *via* the unfolded or folded imidazole ring and which nitrogen of the imidazole ring links to the Zn^{2+} ion.

Due to the apparent tendency of cyclopeptides to form large chelate rings, Arena *et al.*[72] carried out a detailed thermodynamic investigation of zinc(II)*cyclo*(L-Histidyl-L-histidyl) and potentiometric and calorimetric measurements on zinc(II)*cyclo*(L-Glycyl-L-histidyl). These systems were used to obtain evidence for the formation of macrochelate rings. To obtain a complete picture similar studies were carried out on copper(II)*cyclo*(L-Glycyl-L-histidyl). From their studies they were able to confirm that the ligand features control the geometric and electronic requirements of the two metal ions, leading to the formation of macrochelate rings in both cases.

Further investigations of the zinc(II) complexes with histidine-containing cyclopeptides were carried out. When *cyclo*(L-Methionyl-L-histidyl) (CMH), see **1.27b** where Y = $CH_2CH_2SCH_3$, was studied the crystal structure showed that the Zn^{2+} ion was coordinated tetrahedrally by four N atoms, *i.e.* the pyrrole nitrogen, of the imidazolyl groups of the histidyl residues of peptides exclusively in a 4:1 ligand:metal ratio in the complex (**1.33**). The N-Zn-N angles were all within 3.5° of 109.5° and the average distance of four independent Zn-N bonds was 2.01A°.[50,73] Similarly, it was found that the same coordination took place when nickel(II) and copper(II) were used. From this it was possible to conclude that these metal ions (Ni^{2+}, Cu^{2+} and Zn^{2+}) interact exclusively with the imidazolyl group of CMH.[74] The same studies were carried out on **1.27b**, where Y = H, and again these metal ions (Ni^{2+}, Cu^{2+} and Zn^{2+}) appear to interact exclusively with

the imidazolyl group of the ligand.[74] Note that in all the above cases perchlorate salts were used.



1.33

On attempting the above mentioned complexation with chloride salts a variety of complexes were formed with different ligand to metal ratios, and some even involved metal-sulphur interactions.[75] These complexes are discussed in a later chapter.

Complexation studies were also carried out using silver(I) with CMH and **1.27b**.[76] In the case of CMH, complexation was noted to take place with coordination of one of the imidazole nitrogens as well as the S atom, but in the case of **1.27b**, only the imidazole nitrogen was involved in the coordination.

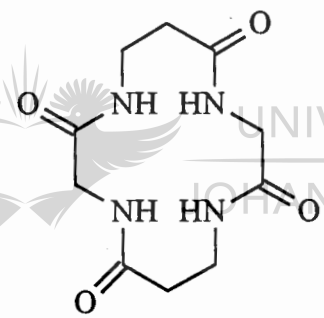
1.6.3 Ternary Complexes of Cyclic Peptides with Simple Amino Acids

The thermodynamic stereoselectivity and spectroscopic characteristics of copper(II) ternary complexes with L- or D-amino acids (Xaa) and *cyclo*(L-Histidyl-L-histidyl) have been investigated. As was the case with linear peptides a much greater stability for mixed ligand complexes was observed, along with other interesting characteristics of the mixed complex. The stability constants revealed that when the amino acid contains aromatic residue, the ternary complexes of the L enantiomer are significantly more stable than the D. Differences were observed between the different aromatic amino acids, which suggested involvement of the aromatic ring-stacking interactions between one of the dipeptide imidazoles and the Xaa aromatic side chain in the mixed-ligand complexes. Arena *et al.*[77] also found that non-covalent interactions or secondary bonds, which

include Coulomb and van der Waals forces, hydrogen bonds and hydrophobic interactions, determine the stereoselectivity of the complex forming to a large extent. It was then proposed that hydrophobic and ring-stacking interactions, responsible for the stereoselectivity, are favoured by the two imidazole nitrogen donors of *cyclo*(L-Histidyl-L-histidyl) and the nitrogen and carboxylate oxygen donors of the L- and D-amino acids.

1.7 MACROCYCLIC PEPTIDES

A further expansion of cyclic peptides results in macrocyclic peptides. An example of a macrocyclic peptides is that of the 14-membered macrocyclic tetrapeptides *cyclo*(β-alanylglycyl-β-alanylglycyl) (**1.34**).



1.34

This macrocyclic peptide reacts with Cu^{2+} to form a quadruply deprotonated peptide complex with alternating 5- and 6-membered rings.[68] Rather drastic conditions are needed to prepare a complex of this tetrapeptide, which is not surprising since it has no terminal groups which might act as primary ligating sites. The preparation involves the treatment of a slurry of the ligand with freshly precipitated copper(II) hydroxide at a *p*H greater than 13. The resulting complex is stable in a *p*H range down to 8. The $Cu(L\text{-}4H)^{2-}$ complex is square planar, with an absorption maximum at 488 nm; this maximum remains unaltered in the *p*H range 8.2 –14. This copper(II) complex is easily oxidised to the copper(III) complex, which is stable in neutral solution. This macrocyclic complex

has been found to be thermodynamically and kinetically more stable than the corresponding complexes with linear peptides.[78]

1.8 WHY COPPER(II)?

In the above discussion you would have noticed that the complexation of various ligands with copper has been discussed. Of all the transition metals, copper has been the most widely studied because of copper's importance in a number of vital biological functions.[79] Copper ions are found in many active sites of a number of important proteins and enzymes. These proteins are associated with various important biological processes such as oxygen transport, electron transfer, superoxide dismutation, and many more. In many cases they were found to consist of three amino acids, with histidine imidazoles frequently occupying one or more of the coordination sites. As a result of our main interest being these histidine-containing peptides and their ease of complexation with copper, we have looked at various forms of complexation with copper and expanded from there to the remaining transition metals of interest.

1.9 SELECTIVITY

It now remains to look at the selectivity of the pre-mentioned ligands.

Determination of the relationship of a ligand structure to the chemical and physical properties of derived metal complexes has become of great interest and various approaches of investigation have been initiated. One approach of particular interest is the use of combinatorial systems. Combinatorial systems allows one to look at a variety of structural features simultaneously. The stability and activity of metal complexes are dependent on many intertwined variables, such as the coordination geometry required by the metal and the steric and electronic characteristics of the ligand.

The use of combinatorial systems was investigated by Still *et al.*[80] where they prepared libraries of ligands on solid-phase. These solid-phase libraries contained cyclen units with short peptidic appendages. Cyclen is a macrocyclic tetraamine known to tightly bind any divalent transition metal ions.[81]

The approach for construction of these cyclen libraries was the use of one of the ring nitrogens for attachment to the resin and to use the remaining three for substituent elaboration using encoded split synthesis[82] with Fmoc amino acids. These ions were found to bind with selectivity imparted by the variable library elements. This approach may prove useful for the fine-tuning and optimisation of properties of known ligands.

Francis *et al.*[83] have investigated selective transition metal binding by ligands prepared through solid-phase combinatorial synthesis but by an alternative approach. They have looked at the design of potential ligands without predefined binding sites, but rather with a diverse set of functional groups and conformational restrictions, that leave a variety of potential coordination environments. The complexing ability of members of this library was tested by exposing samples of beads to homogenous solutions of the selected metal ions. Known qualitative tests for the metal ions were used to determine the degree of complexation. Thereafter, changing a variety of variables and making use of tag photolysis[84] and GC-ECD analysis it became possible to ascertain which library member had the highest affinity for the metal ion being tested at the time. From the results obtained it was possible to synthesise the desired ligand and test it for selectivity. In comparing the tag analysis obtained for Ni(II) and Fe(III) it was found that there was no structural overlap between the binders of Ni(II) and Fe(III) which suggests that the library might selectively complex one metal in the presence of the other.

A variety of compounds were synthesised by Otsuka *et al.*[85] and proved to show no selectivity, even with their differing functionalities. On comparison to our ligands, hope remained for selectivity of our ligands; the diketopiperazine systems are much more strained, which is expected to make the mode in which they complex much more selective.

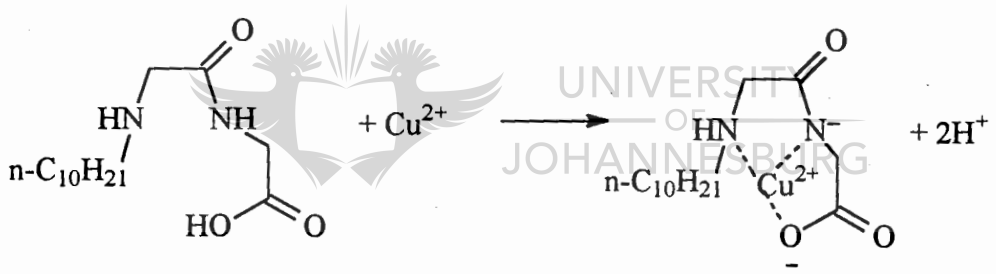
1.9.1 The Role of Selectivity in Dipeptides and their Double Bond

A comparison was carried out on the effect of introducing unsaturation into dipeptides. Potentiometric and spectroscopic measurements and theoretical calculations revealed that the introduction of a double bond into a dipeptide, resulting in the formation of an α,β-dehydroamino acid residue, had a considerable effect on the coordination ability of the dehydropeptide ligand. The two effects compiled from various studies,[86] that seemed to have the largest effect on the speciation and stability of the complexes formed by Δ-dipeptides can be summarised as follows. The stable extended structure allows for π-electronic configuration of the amide bond with the $C^{\alpha}=C^{\beta}$ double bond, which acidifies the amide hydrogen making it more available for metal-ion coordination than that of the saturated common analogues. The second effect is of steric nature and relies on the tendency of the α,β-dehydroamino acid residue to bend the peptide chain towards a folded conformation, which affects the positioning of the carboxylate group. This may result in the destabilisation of the involvement of this group in α,β-dehydropeptide in the formation of some complexes. This in turn may introduce selectivity in the complexation taking place.

1.9.2 Selective Complexation in Chemosensors

Selective metal complexation is currently being investigated in many realms of our world today. One particularly interesting use is chemosensors. Chemosensors are small abiotic molecules that signal the presence of an analyte and generally combine two components. These two components are a recognition site that binds the target substrate and a readout system that signals binding.[87] Selectivity is of paramount importance for a chemosensor. In the case of metal ion sensors the recognition site is a metal-chelating ligand designed to selectively complex the ion of interest. The readout system in metal ion sensors is often a fluorophore. These two components are usually covalently linked and on metal ion complexation a variation in the position and or intensity of the emission band of the fluorophore will occur. This set-up has been used to devise a variety of molecular

sensors for the detection of alkali and transition metal ions in solution. Once again copper has been of particular interest.[88] Metal ion sensors for copper(II) have been widely studied and a large number of sensors have been devised by connecting fluorescent probes with different types of selective Cu(II) ligand subunits.[89] Recent developments in this field made use of dipeptides as ligands. Instead of the two components being covalently linked (which requires a proper design and synthetic efforts), Grandini *et al.*[90] developed a system in which the active components assemble spontaneously to give a sensor. This was achieved by simply mixing, under suitable conditions, commercially available surfactants and fluorophores with a dipeptide-based ligand. The glycylglycine dipeptide was used to achieve strong and highly specific binding of copper(II) ions in neutral water. The glycylglycine dipeptide is known to coordinate copper(II) ions by means of its amino group, the deprotonated amido nitrogen atom, and the carboxylate group(**Scheme 1.7**).[91]



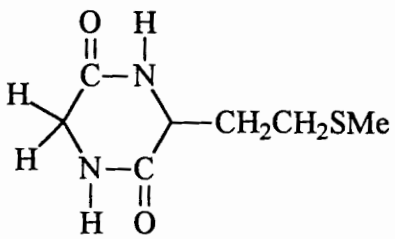
Scheme 1.7 Coordination of the glycylglycine dipeptide to copper(II).

The key feature for the selective formation of a strong complex is the deprotonation of the amido nitrogen just under slightly acidic or neutral *p*H conditions exclusively with copper(II) ions, and not with other metal ions such as zinc(II), nickel(II) or cobalt(II). The neutral complex is easily retrieved, due to its low solubility above *p*H 5, compared to the dipeptide ligand dissolving easily in the *p*H range of 2 - 9. Further selectivity tests showed that even in the presence of other 3d series divalent metal ions, except for Fe(II), the sensor not only did not respond to other metal ions but was also selective towards copper(II) ions in their presence.

1.9.3 Selectivity in Cyclopeptides

Bressan *et al.*[92] have studied *cyclo*(L-methionyl-L-glycine) (CMG) (**1.35**) and its coordination ability with a variety of metal ions. They previously reported the ability of CMG to form stable complexes with soft metal ions, like palladium(II), platinum(II), mercury(II), silver(I), gold(I) and (III). Most of these complexes were isolated in the solid state and in all cases the sulfur end of the ligand was the primary ligating site of coordination and further coordination of the metals to deprotonated amide nitrogen atoms could occur. When the same complexation reactions were attempted with hard or borderline divalent metal ions, like zinc(II), calcium(II) and copper(II), no solid products could be isolated, both in water and in alcoholic solutions. It was found that the coordinating ability of the methionine sulfur of the ligand CMG appears to be rather scarce towards hard or borderline divalent metal ions, the more stable complex being formed with copper(II).[93] From this it can be seen that selective complexation is possible, if soft metal ions are the ones of interest.

Introduction of the imidazole functionality to a ligand can increase coordination ability of the ligand because of the excellent ability of the imidazole group to coordinate metal ions. When complexation reactions were carried out with *cyclo*(L-Methionyl-L-histidyl) (CMH) (**1.36**) the CMH ligand was found to coordinate both hard and soft metal ions. Different manners of coordination were seen to occur with different metal ions. When complexation was carried out with divalent transition metal ions coordination took place *via* the imidazole nitrogens,[50, 74, 73] compared to complexation with a soft metal ion, *e.g.* silver(I), coordination took place with one of the imidazole nitrogens as well as the S atom.[76]



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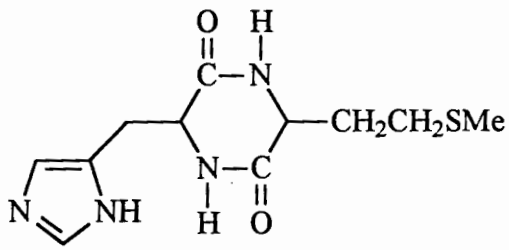


1.36

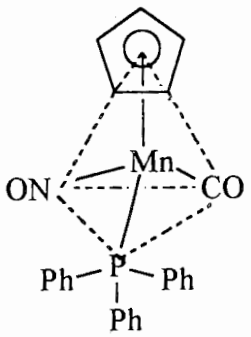
From the two discussions above it can be seen that selective complexation with hard divalent metal ions with CMG / CMH is possible, but only through a very round about route, *i.e.* CMG could be used to remove the soft metal ions and thereafter extract the hard divalent transition metal ions with CMH. This approach is definitely not an option as the great expenses that would be incurred, along with the losses of material, would not make the project viable to industry.

1.10 OPTICALLY ACTIVE ORGANOMETALLIC COMPOUNDS OF TRANSITION ELEMENTS WITH CHIRAL METAL ATOMS

Many studies have been discussed above on how and why complexation reactions take place and whether or not selective complexation is possible. I have included this small section, for interest, on studies that have been carried out on pre-complexed metal ions.

Organometallic chemistry of the transition metals is an intermediate between organic and inorganic chemistry and has manifold structures and a diversity of possible reactions. Transition metal compounds are diamagnetic and are mainly low-spin complexes in which the metal atoms adopt a noble gas (18 VE) configuration. Organometallic compounds of the transition metals are famous for their excellent ability to crystallise. This therefore allowing the absolute configurations at the metal atom, of these optically active complexes, to be determined by X-Ray crystallography, either by means of anomalous X-Ray scattering or by internal comparison with a known absolute configuration in the ligand. Along with X-Ray structures, NMR spectroscopy can be a useful tool of investigation.

About thirty years ago, the first optically active transition metal complex with four different substituents was achieved with the manganese complex **1.37**.



1.37

These complexes can not only be used in reactions that occur at the chiral metal atom but also in reactions that occur at the ligands. They thus find applications in enantioselective organic synthesis.[94]

Optically active organometallic compounds with chiral transition metal atoms are configurationally stable in the solid state but in many cases a change has been noted to occur in solution.[95] If, during the change of configuration, intermediates form more information can be gained on the compounds and the interactions that are possible. Racemisation and epimerisation experiments can be used, with the aid of labeling by optical activity, to obtain complimentary information to that obtained when using NMR spectroscopy.[96]

A variety of reactions of complexes with chiral metal atoms exist. One type of reaction that has taken the lime light in this field involves the hydrogenation of dehydroamino acids.[97] A specific chiral conformation in the chelating skeleton of the ligand is responsible for the manner of oxidative addition of hydrogen to the square-planar complex. This manner of oxidative addition results in the formation of an octahedral complex.[98] This particular active use of chirality at the metal atom in enantioselective catalysts remains a challenge for future research.

1.11 ANALYSING TECHNIQUES

1.11.1 Absorption Spectra

Spectroscopy can be defined in physics and physical chemistry as the study of spectra. The basis of spectroscopy is that each chemical element has its own characteristic spectrum. Various spectroscopy techniques exist but one of particular interest is that of atomic absorption spectroscopy. Atomic absorption spectroscopy is the process involving the absorption of radiation by atoms.

Absorption spectroscopy is a very useful technique for analysing changes in compounds. If we look at the compounds of interest to us, we see that there are many characteristic peaks that allow us to determine what interactions are taking place. The longest wavelength absorption band of the amide group itself occurs near 220 nm and is a result of an $n \rightarrow \pi^*$ transition. Two kinds of transitions to longer wavelengths appear upon coordination of transition metal ions. Peptide complexes of copper(II) and nickel(II) with deprotonated amide nitrogen give rise to intense charge-transfer absorption near 250 nm.[99] Peptides with aromatic side chains also absorb in this wavelength region.

The ligand field (d-d) transitions for Pd^{2+} occur between 290 to 330 nm, from 410–450 nm for the yellow, tetragonal, diamagnetic Ni^{2+} and from 520–650 nm for violet to blue for Cu^{2+} complexes. The most useful diagnostic information concerning the structures of transition metal ion-peptide complexes has been obtained from the ligand-field bands in the visible region for Cu^{2+} and Ni^{2+}.[100] For example, substitution of equatorial water molecules in aqueous Cu^{2+} by other oxygen or nitrogen donor atoms moves the absorption maximum to shorter wavelengths, with nitrogen having a stronger effect. Quantifying the blue shift has its limitations, because three transitions reside under the absorption band and the same donor atom yields differing effects, depending upon the chelate ring size and substituents on the ligand. Even with these limitations useful generalisations are possible.

1.11.2 Circular Dichroism Spectra

Circular dichroism (CD) result from unequal absorption of the left and right circularly polarised components of plane-polarised light. No differential absorption appears in optically inactive substances. Even in a strongly optically active material the absorption difference between the two components reaches only about 1% of the total absorption. The molar absorptivity (ε) and the differential molar absorptivity (Δε) between the left and right circularly polarised light for CD are usually measured in $M^{-1}cm^{-1}$. The general shape of a CD band due to a single transition resembles that of an absorption band and may possess a negative sign in addition.

With optically active compounds CD spectra often form a valuable addition to absorption spectra. When looking at peptides and related compounds with transition metal ions, CD discussions often refer to CD through the ligand field (d-d) bands of tetragonal transition metal ion complexes. CD signs and intensities for ligand field transitions have been recorded for a variety of simple systems and can be used as source for further interpretation of more complex systems.[101] Typical CD for tripeptide complexes with Cu^{2+}, Ni^{2+}, and Pd^{2+} occur at about 560, 480 and 335 nm, respectively. Another point of interest is the effect of the presence of an aromatic side chain. The aromatic side chain in the amino terminal position of a dipeptide copper(II) complex results in a positive CD, compared to the usual negative CD observed.[102] These and other interesting conclusions can be drawn from CD spectra making microscopic interactions easier to identify.

1.11.3 Liquid-Liquid Extraction Systems

Most studies of complexation are carried out on a macroscopic scale, where crystals are studied and manners of coordination are determined. Another interesting side to complexation is the kinetic process of the solvent extraction. Watarai[103] has published an interesting article in which he discusses interfacial adsorption and complexation in liquid-liquid extraction systems. From this study, rates of complexation were obtained and from this it was possible to determine principal adsorption equilibria and rate-determining

steps in the extraction mechanism. Furthermore the rate of extraction of the metal of interest and reactivity at the interface were determined.

Now all that is left to do is to take all of this information and to allow it to expand our understanding of complexation reactions and therefore give us the necessary insight to develop better and more pertinent ligands in the future.

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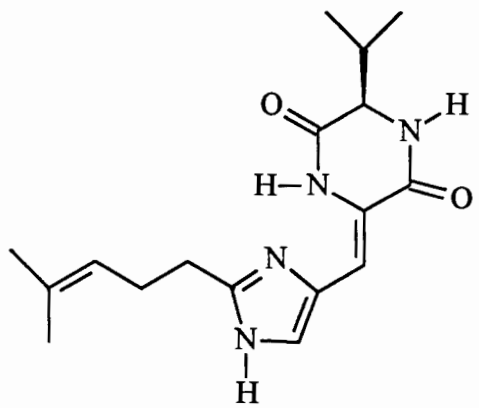
CHAPTER 2

RESULTS AND DISCUSSION

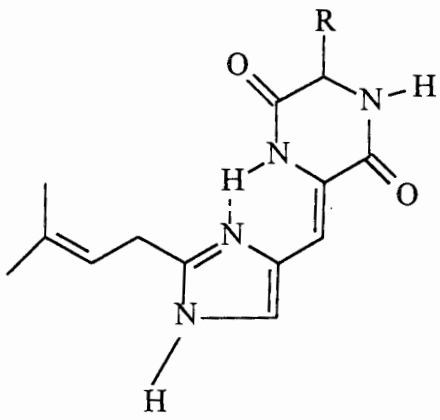
2.1 THE BROADER PICTURE

A continual problem facing the mineral industries today is that of selective metal ligation and extraction. It is this problem of selectively removing a single metal in the presence of others that this project intends to address. The ultimate aim of this investigation is the development of viridamine **(2.1)** analogues, to be used as selective metal complexing agents. This project is based on a prior investigation, carried out by Holzapfel and Marsh,[1] and later Dippenaar, Holzapfel and Boeyens.[2] Viridamine was found to have different complexing properties for different metals. It was noted to complex in a distorted tetrahedral manner with copper, nickel and cobalt, but would not complex iron(II), iron(III) or palladium(II). The rate of complexation was also affected remarkably depending on the metal in question, *i.e.* Ni(II)>Cu(II)>Co(II). Furthermore, it was found that both the metal and ligand were quantitatively recovered by an acidic treatment of the metal-viridamine complex.



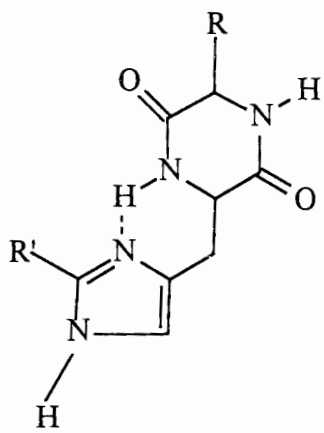
2.1

A focus of this project is to zoom in on the structure-selectivity relationship of viridamine analogues and hereby understand the manner of complexation of different metals. Analogues similar to **2.2 - 2.4** were identified as possible target structures that would be able to be prepared from simple starting materials, e.g. amino acids.



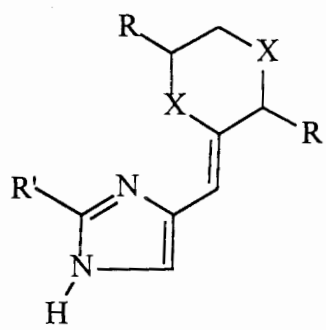
R = alkyl, H

2.2



R and R' = alkyl, H

2.3



R = alkyl, H, O-alkyl
R' = alkyl, H
X = NH, O

2.4

It was decided that the first type of structure to be pursued would revolve around the diketopiperazine structure **2.3**. Such structures can be prepared from amino derivatives. The amino acids were initially protected and thereafter peptide coupling was carried out. After deprotection of the dipeptide, cyclisation afforded the desired diketopiperazine. Fine-tuning of the diketopiperazine structure was then possible allowing us to refine the synthetic route to obtain the best selectivity possible.

The sections that follow will discuss in more detail the routes of protection, coupling, deprotection, cyclisation and fine-tuning that have been undertaken.

2.2 PROTECTION OF THE AMINO ACID AMINE GROUP

2.2.1 INTRODUCTION

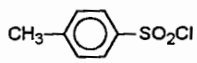
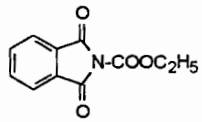
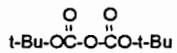
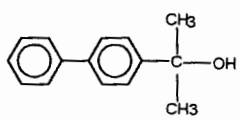
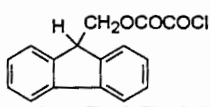
Due to amino acids having two reactive sites, coupling reactions between amino acids yield a mixture of products, unless one of the reactive sites is protected. In this particular instance, it is desirable for the amino acid to react through its carboxylic acid group. Therefore, the amine functionality of one of the reacting partners should be protected and it is this form of protection that we shall focus on in this chapter.

Protecting Groups Available for *N* Protection.

There are various protecting groups available for amine protection. These protecting groups vary from a *p*-tosyl to the Boc group. A few of these groups are listed below:

TABLE 1

Protecting groups useful in amino acid *N*-protection

Name of protecting group	Abbr.	Compound used for protection
p-Toluenesulfonyl (*p*-Toluenesulfonyl chloride)	*p*-Tosyl	
Phthaloyl (*N*-ethyloxycarbonyl phthalimide)	Pht	
tert-Butyl carbonate (Di-*t*-butyl dicarbonate)	Boc	
Biphenylisopropyloxycarbonyl (*p*-Biphenyl-dimethyl-carbinol)	Bpoc	
9-Fluorenylmethyloxycarbonyl (9-Fluorenylmethyl chlorocarbonate)	Fmoc	

The aim of this protection is to deactivate the nitrogen in question by the addition of a protecting group. At a later stage, the protecting group can be removed either by acidification, the addition of a base or by some alternative procedure. Deprotection involves an attack on the weakness of each specific protecting group, allowing the possibility of further reactions.

Due to the ease of attachment and removal of the *t*-butyloxycarbonyl group (Boc), it is often favoured over other protecting groups for the amine moiety of amino acids in peptide synthesis. Of particular interest to us is the Boc protection of histidine. In time gone by, Boc-azide was used to obtain the diBoc protected histidine.[3] Since then Boc-azide has been found to be very dangerous, due to the need for strict *p*H control, in the absence of which the reaction may become too hot and result in an explosion, and therefore alternative Boc protecting agents have been developed and investigated. These reagents include *t*-butyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate,[4] 2-*t*-butyloxycarbonyl-oxyimino-2-phenylacetonitrile (Boc-ON),[5] propargyl chloroformate,[6] Boc anhydride /di-*t*-butyl dicarbonate ($(Boc)_2O$) and many more.

Recent studies have provided an interesting Boc surrogate. The *t*-butanesulfinyl group serves as an excellent chiral directing group and Boc surrogate for asymmetric synthesis and application of β-amino acids. Tang *et al.* demonstrated that the *t*-butanesulfinyl moiety serves not only as an imine activating and chiral directing group but also as a versatile amine protecting group for subsequent synthetic transformations.[7]

The *p*-toluenesulfonyl (*p*-Tosyl) group is another attractive group for the protection of the imidazole residue in histidine and histidine derivatives.[8] It is easily introduced and is stable under various conditions. Moreover, it lowers the basicity of the imidazole, unlike the benzyl protecting group which enhances the basicity. This can be very useful because histidine and its derivatives are prone to base-induced racemisation. The *p*-Tosyl group is easily removed with a strong base such as liquid ammonia or with hydrogen fluoride.[9] 1-Hydroxybenzotriazole has also been known to remove the *p*-Tosyl group.[10]

An interesting protecting group that has recently been investigated with respect to nitrogen is that of 1*H*-benzotriazol-1-yl methanesulfonate (BMS).[11] In a molecule with both primary and secondary amino groups, mesylation will only occur at the primary amino group. When a compound contains both amino and hydroxy groups, the reagent selectively mesylates at the amino group. This reagent is of great importance when selective protection is needed on a multifunctional compound.

t-Butyl dimethyl silane (TBDMS) is yet another popular protecting group for nitrogen and is still used widely today and is easily removed under acidic conditions.[12]

The imidazole ring of histidine is a constant problem in peptide synthesis, therefore protecting groups are needed that will remain intact on the imidazole ring, even during side chain deprotection. The trityl group has been found to remain intact at N^{im} during N^{α}-Trt deprotection.[13] The trityl group has also been found to bring about a decrease in the basicity of the imidazole ring through electronic effects; its bulk helps to prevent the base catalysed rearrangement which results in racemisation and it is totally stable to nucleophilic attack.[14] All of these characteristics make it an attractive group for protection.

Different *N* Protecting Groups on One Compound

Many synthetic substrates rely on the incorporation of two different protecting groups, of different sensitivities, onto the histidine molecule. Such an approach makes it possible to introduce different functionalities of the histidine at different stages of the reaction sequence, by removal of the protecting groups at different stages. Colombo *et al.*[15] made use of the acid labile *t*-butoxymethyl group (Bum) to protect the nitrogen on the imidazole ring and then made use of base sensitive groups to protect the histidine side chain. This form of protection proved to be interesting as the protecting groups could be removed at different stages of the synthesis without great difficulty, allowing multiple reactions to be carried out without too many side products forming.

The *p*-methoxyphenylsulphonyl group (MPS) is another useful group that can be used in conjunction with acid sensitive protecting groups.[16] MPS attaches itself to the N^{im} atom of histidine and can be easily removed using basic conditions. The N^{im}-MPS group is not removed under the acidic conditions that are required for N^{α}-deprotection of the Boc, making it a nice group to use if deprotection of the imidazole nitrogen is required at a different time to that of the side chain nitrogen.

Reaction Conditions

Various reaction conditions exist for Boc protection and these conditions vary according to the amino acid being protected. Ponnusamy *et al.*[17] carried out their Boc protection using $(Boc)_2O$ and found that in most cases it is necessary to use a base and, depending on the solvent used, different temperatures and times were necessary. Tarbell *et al.*[18] proposed much more general reaction conditions using $(Boc)_2O$, using water as solvent and carrying the reaction out at room temperature, they obtained their Boc protected amino acids in 10-30 minutes, in yields of 75-95%. Kocieński discussed the basic conditions needed for a variety of Boc protecting agents in his book but he too said that $(Boc)_2O$ is one of the more common and easier to use Boc protecting agents.[19] In some cases, where the protection is very slow, (dimethylamino)pyridine (DMAP) has been used, both in catalytic[20] and equimolar amounts.[21] These slower protections were normally in the case of secondary amines and pyrrole nitrogen protection and are normally carried out in dichloromethane.[22] The addition of DMAP was successful in increasing the reaction rate, as well as improving the yield of the reactions.

An additional point of interest is that of protection of sterically hindered amino acids. Khalil *et al.*[23] carried out a variety of Boc protection reactions on sterically hindered amino acids. In some cases the protection took several days[24] and large amounts of $(Boc)_2O$ were needed. The majority of the reactions were low yielding. The researchers eventually found that if tetramethylammonium hydroxide (TMAH) was added the reaction proceeded smoothly and high yields were obtained. Similarly, addition of

NaHMDS in tetrahydrofuran allows direct protection of aryl amines with $(Boc)_2O$, which was not possible previously.[25]

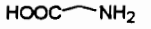
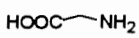
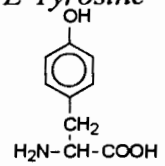
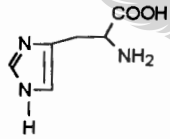
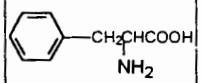
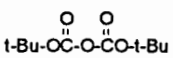
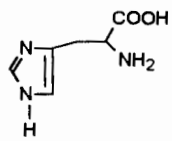
McNulty and Still[26] carried out an interesting conversion of an amino acid to its Boc protected ester. This one-pot conversion involved a treatment of the amino acid with methanol and hydrochloric acid, followed by $(Boc)_2O$ and triethylamine in dimethyl formamide. This one-pot conversion proved to be high yielding.

A diverse manner of protection reactions were carried out by Einhorn *et al.*[27] where they made use of ultrasonication. This manner of protection allowed for an easy work-up and gave the protected compounds in high yield. The amino acid (or salt) and $(Boc)_2O$ are dissolved in either ethanol or methanol, in the presence of sodium bicarbonate, and exposed to ultrasonic irradiation and in a period of up to 5 hours the Boc protected product is obtained in yields of 80-100%.

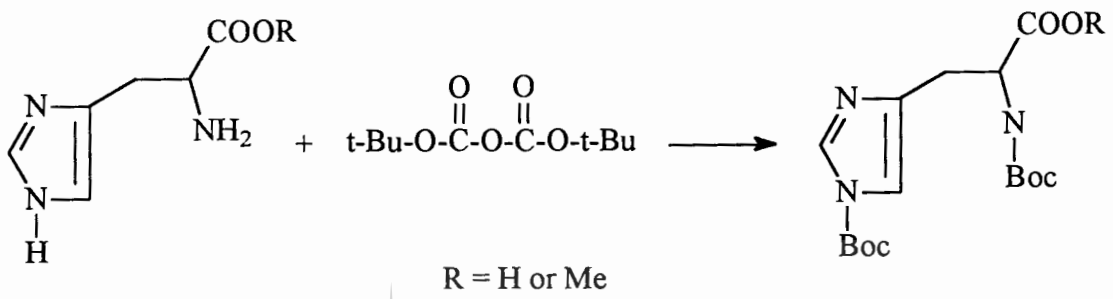
In some cases, the amine group may already be protected, but with an sensitive group. Sakaitani *et al.*[28] report a procedure for the conversion of *N*-benzyloxycarbonyl group to the *N*-*t*-butoxycarbonyl group. The transformation is carried out, in ethanol, with triethylsilane and $(Boc)_2O$, in the presence of a catalytic amount of palladium acetate. This transformation reaction can take anything from 5-40 hours, is substrate dependant, and offers yields $\geq$ 90%. This becomes useful if the basicity of the imidazole ring must be lowered or a smaller protecting group is needed.

2.2.2 DISCUSSION

We initially attempted Boc protection on the simplest amino acid, glycine, in order to eliminate any other possible interfering effects. Some other protecting groups were also employed for comparative purposes (see **Table 2**).

TABLE 2				
Initial attempts at protecting the amine group				
Entry no.	**A: Amino Acid**	**B: Protecting Group**	**Product (yield)**	**Conditions**
1	*Glycine* 	*Boc anhydride*	*N*-Boc-Gly (0%)	1) NaOH (*p*H 10.2) 2) HCl (*p*H 6).[29]
2	*Glycine* 	*Boc anhydride*	*N*-Boc-Gly (0%)	1) NaOH (*p*H 10.2) 2) HCl (*p*H 6).
3	*L-Tyrosine* 	*p*-TosCl	*N*-Tos-Tyr (0%)	1) NaOH (*p*H 10.2) 2) HCl (*p*H 3).[30]
4	*Histidine* 	*N-Ethyloxy-carbonyl phthalimide*	N_α-Phth-His (0%)	**A**, MeOH, addition of **B** to the vigorously stirring sol.[31]
5	*L-Phenyl-alanine* 	*Boc anhydride* 	*N*-Boc-Phe (78%) **2.5**	1M NaOH, H_2O t-BuOH 20-40 °C [32]
6	*Histidine* 	1. N-*Ethyloxy-carbonyl phthalimide* 2. *Boc anhydride*	N_α-Phe-N_τ-Boc-His (0%)	Three solvents: i. DMF ii. DMF / H_2O iii. THF / H_2O 1. **A,** solvent **B** was added under vigorous stirring. 2. $KHCO_3$, H_2O t-BuOH, 20-40 °C

Various attempts were made at protecting histidine, since the imidazole moiety was the core of our desired structure. Due to histidine possessing two active nitrogen atoms, a di-protection was necessary, to allow the peptide coupling reaction to take place through the carboxylic acid. The multifunctional nature of histidine rendered it a particularly difficult amino acid on which to perform Boc protection. Part of the problem lies in the formation of a zwitterion, which makes the free acid derivatives of histidine water-soluble. The desired reaction is shown in **Scheme 2.1**, followed by a list (**Table 3**) of the attempts to Boc protect histidine.



Scheme 2.1 General reaction for the protection of the amino group of histidine, as the free acid or as a methyl ester, using Boc anhydride.

TABLE 3				
Protection of the amine group of Histidine (free acid / methyl ester)				
Entry no.	**HIS**	**$(Boc)_2O$**	**Yield**	**Conditions**
1	R = H	1 equiv.	0%	1M NaOH, H_2O,t-BuOH, 20-40 °C [4]
2	R = H	2 equiv.	Recovery of $Boc_2(O)$	NaOH or Na_2CO_3 [33]
3	R = H	2 equiv.	0%	$KHCO_3$, t-BuOH, 20-40 °C
4	As above, but at 2× / 3× concentration: 0% yield.			

Table 3 continued...				
Entry no.	**HIS**	**$(Boc)_2O$**	**Yield**	**Conditions**
5	R = H	2 equiv.	0%	$KHCO_3$, t-BuOH, 60 °C
6	R = H	3 equiv.	$N^{\alpha,im}$-Boc-His 2% **2.6**	$KHCO_3$, t-BuOH, 60 °C
7	R = H	2 equiv.	0%	$(CH_3)_3COK$, DMF, t-BuOH
8	R = H	2 equiv.	0%	THF or PYRIDINE NEt_3 and DMAP [34]
9	R = H	2 equiv.	0%	2 equiv. NaH in pyridine

Due to the low yields obtained when using the histidine free acid, it was decided to attempt nitrogen protection of the histidine methyl ester. The histidine methyl ester was successfully Boc protected, affording the N_α-N_τ-diBoc protected amino ester. Two isomers were obtained, where the N_α (side chain nitrogen) and then either the N_τ or N_π nitrogen of the imidazole ring were protected (**Table 4**).

TABLE 4				
Entry no.	**HIS**	**$(Boc)_2O$**	**Yield**	**Conditions**
1	R = Me	2 equiv.	$N^{\alpha,\tau}$-Boc-Hme 42% **2.7**	1) NaOMe, 2) $KHCO_3$, t-BuOH, 20-40 °C
2	R = Me	2 equiv.	$N^{\alpha,\tau}$-Boc-Hme 76% **2.8** $N^{\alpha,\pi}$-Boc-HIS 7% **2.7**	Et_3N/MeOH [35]
3	R = Me	2 equiv.	$N^{\alpha,\tau}$-Boc-Hme 74% **2.8** $N^{\alpha,\pi}$-Boc-Hme 6% **2.7**	Na_2CO_3 in aqueous THF. [36]

One pot conversions

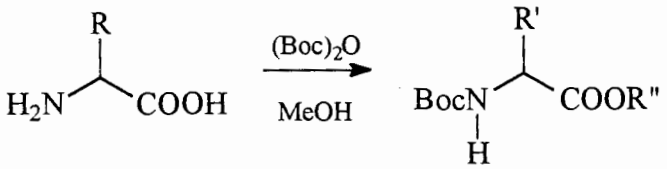
Due to the successful protection of the histidine methyl ester, it was decided to carry out the protection of the histidine amino acid under the same conditions. After work-up of the reaction mixture that had been subjected to standard reaction conditions (Histidine, MeOH, Boc_2O) it was found that an unexpected conversion of the acid to the methyl ester had occurred, in addition to Boc protection. The diBoc-histidine methyl ester was obtained in 41% yield (**Table 5**). This was unexpected as no acid was added to promote esterification, as has been deemed necessary by the studies carried out by McNulty and Still.[26]

Due to this unexpected conversion, during the diBoc protection, it was decided to attempt this "one-pot" conversion on other amino acids. This procedure was carried out on L-phenyl alanine and glycine. In both cases the amino acid was dissolved in methanol and one equivalent of $(Boc)_2O$ was added. The reactions were left to stir at ambient

temperature overnight. In both cases the Boc protected amino acids were obtained, but the reactions did not continue through to the ester formation (**Table 5**).

TABLE 5

Boc protected products obtained



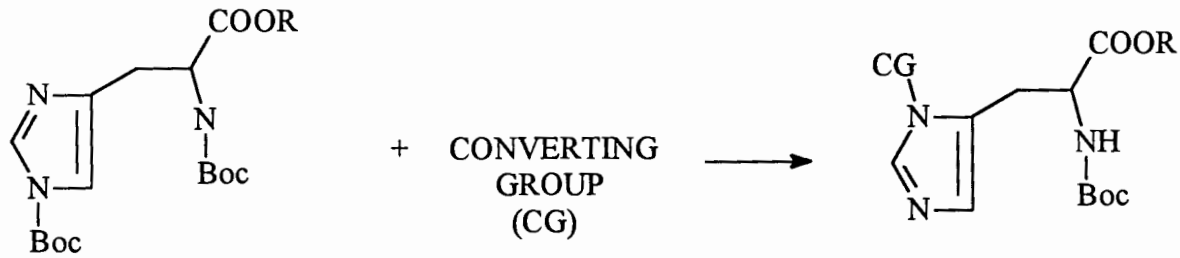
Entry no.	R	R'	R"	Product
1	-CH_2-Im	-CH_2-Boc-Im	-Me	**2.8**
2	-CH_2Ph	-CH_2Ph	-H	**2.5**
3	-2H	-2H	-H	**2.9**

The Importance of N^{τ} vs. N^{π} Protection

Among the α-amino acids, which commonly occur in proteins and peptides, histidine is uniquely prone to racemisation when activated for peptide bond formation. This even occurs when its α-amino group has alkoxycarbonyl protection, which normally ensures the preservation of chiral integrity. It has been found that dicyclohexylcarbodiimide activation of *N*(α)-alkoxycarbonyl-L-histidines, which are protected at the N_{τ} of the imidazole ring, still undergo gross racemisation[37] when compared to the N_{π} protected histidine, which can be coupled to give pure peptides.[38] Two mechanistic questions were investigated, as to whether the π-nitrogen brings about racemisation by acting as an internal base or whether it acts as an internal nucleophile giving an optically labile heterocycle. Jones *et al.* [39] showed that the racemisation of N^{α},N^{τ}-protected histidine, which occurs on activation with dicyclohexylcarbodiimide, takes place by action of the π-nitrogen as an intramolecular base catalyst.

When we attempted diBoc-protection of histidine the major product obtained was the N^{α},N^{τ}-protected histidine. It was therefore necessary to convert the N(τ)-protection to the N(π)-protection, to avoid racemisation during the next stage of the synthesis, *i.e.* coupling

with dicyclohexylcarbodiimide. The general reaction for conversion is shown in **Scheme 2.2**.



A

Scheme 2.2 General reaction for the conversion of N^{α},N^{τ}-diBoc protected histidine methyl ester to the $N^{\alpha}N^{\pi}$-diBoc protected histidine methyl ester.

The converting group used is quite important, as it should introduce a chemically simple group to the histidine but at the same time solve the problem of racemisation. Brown *et al.*[35] investigated a range of simple substituents, which could in principal, be positioned at the π-nitrogen and so prevent racemisation, but which would at the same time be likely to give easily prepared derivatives, with effortless solubility and have good resistance to nucleophilic attack. All of these characteristics would be combined with easy removal under mild conditions at the end of the synthesis. They found that the π-benzyloxymethyl- and π-4-bromobenzyloxymethyl groups appeared to meet these conditions.

Due to the availability and similarity, we carried out our reactions using chloromethyl methyl ether (MOMCl). However, various conditions failed to afford the desired N^{π}-protected histidine. A similar lack of success faced these attempts when substituting benzyl bromide for the MOMCl in analogous reactions.

Hanford *et al.*[40] provided an interesting stepwise approach to the diBoc protected histidine. They discussed the preparation of the L-histidine methyl ester dihydrochloride from L-histidine. They then went on to prepare the α-Boc-protected amino acid of the

histidine methyl ester, followed by imidazole ring protection, to obtain the diBoc protected product. No reference was made as to which *N*-protected product was obtained, but on comparison to the characterisation data obtained it could be concluded that the product contained τ-nitrogen protection on the imidazole ring. They also went on to prepare the Boc protected free acid of histidine from the Boc protected methyl ester. We did not attempt this reaction on basis of the expected racemisation during dicyclocarbodiimide activation, which was the next step of our synthesis.

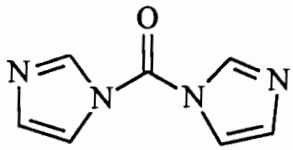
Investigations have shown that in most cases the protecting groups for the heterocyclic ring result in *N*(τ)-protection.[41] It is therefore necessary to convert the diprotected product if it is to be used in coupling reaction. Alternatively one can make use of the Boc protected amino acid partner to be coupled to histidine and use the methyl ester of histidine. This approach is discussed in the following section.

Another form of *N*-protection exists, and that is to make use of a reagent that has two active sites to effect both protections at once. Not only should it protect both the α and N^{im} nitrogens of the histidine at once but also force a conformation that makes the τ position unfavorable. This was achieved using 1,1-carbonyl diimidazole (CDI).

2.2.3 DIPROTECTION USING CDI

2.2.3.1 INTRODUCTION

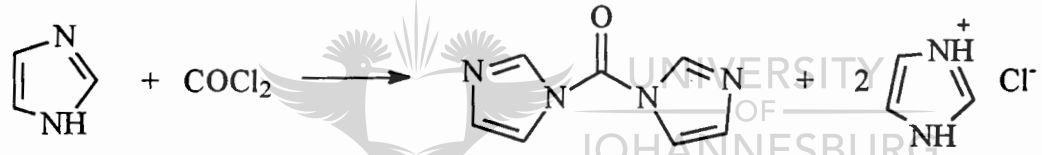
1,1-Carbonyl diimidazole (CDI) (**2.10**) was first described by Staab[42] in 1957 as a versatile protection reagent and since then it has been used in many areas of organic synthesis. Some of these applications include the esterification of carboxylic acids with chloroform,[43] as an aid in coupling reactions,[44] as means of introducing a carbonyl group in the preparation of five-membered heterocyclic systems,[45] as a peptide forming reagent, [46] and many more, as discussed below.



2.10

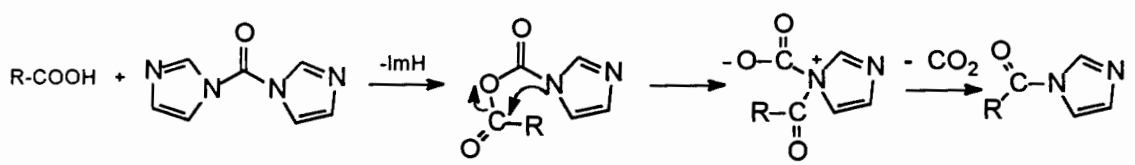
Reagent **2.10** is an excellent compound for small-scale laboratory preparations since it is a non-toxic, stable solid, which can be easily and accurately weighed out and often leads to nearly quantitative yields of easily purified products.

CDI can be prepared in anhydrous benzene from imidazole and phosgene (**Scheme 2.3**).[47] Paul *et al.*[46] found that it was important to use a slight excess of imidazole to prevent contamination of the product by unreacted phosgene. They also found it to be important to remove all of the imidazole hydrochloride from the reagent, as contaminated CDI gave poor yields in later reactions. Importantly, CDI is now commercially available.



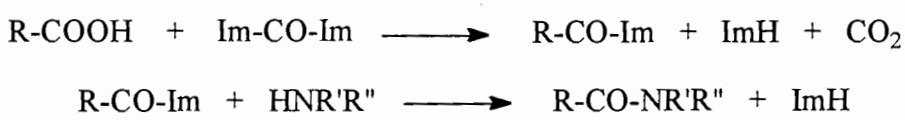
Scheme 2.3 Preparation of CDI from imidazole and phosgene, in anhydrous benzene.

Reactions of CDI with carboxylic acids usually result in the formation of imidazolides (**Scheme 2.4**). These particular reactions are normally carried out in tetrahydrofuran (THF) or chloroform ($CHCl_3$), at room temperature making use of an equimolar amount of CDI.



Scheme 2.4 Formation of imidazolides from the reaction between CDI and carboxylic acids.

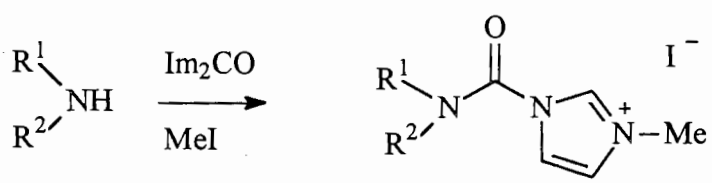
When CDI is used in the synthesis of amides, peptides, hydrazides and hydroxamic acids, the preferred solvents are THF, $CHCl_3$ and dimethyl formamide (DMF) (**Scheme 2.5**). Again an equimolar amount of CDI is used and the reactions are carried out at room temperature.



Scheme 2.5 The synthesis of amides, peptides, hydrazides and hydroxamic acids, using CDI.

It was previously established that making use of DMF, at -10 °C, minimises racemisation of the amino acids reacting to less than 0.5%.[48] (It is essential, though, that during any of the reactions mentioned above absolute dryness is maintained since the reagent decomposes almost instantly on contact with water.) CDI has also been used to form cyclic peptides.

Recent studies have looked at the use of CDI in the preparation of carbamoyl imidazolium salts. Carbamoyl imidazolium salts act as useful carbamoylation reagents, in reactions with alcohols, phenols, thiols and thiophenols to form carbamates, under relatively mild conditions. The carbamoyl imidazolium salts are stable and easily prepared from the corresponding amine and CDI (**Scheme 2.6**).[49]



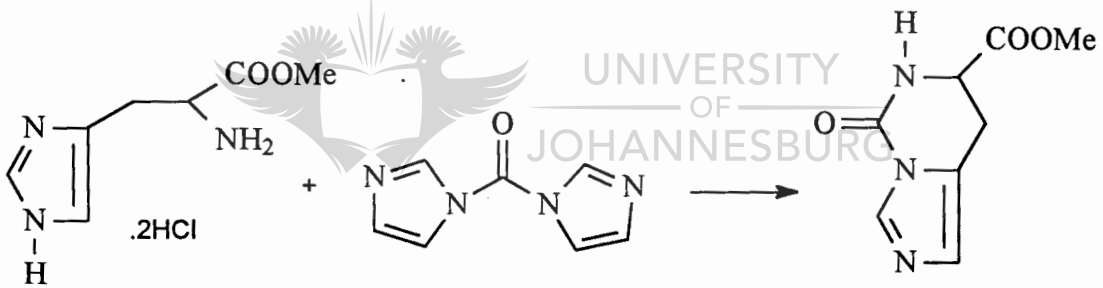
Scheme 2.6 Preparation of carbamoyl imidazolium salts.

2.2.3.2 DISCUSSION

Due to the wide variety of uses of CDI, and based on the principals used by Wright,[45] it was decided to try using this compound as a protecting group for histidine. It was predicted that this compound would selectively protect the N^{α} and N^{π} nitrogen atoms, forming a six-membered ring, (of histidine) rather than the N^{α} and N^{τ} nitrogen atoms, which would form a bridged compound.

Diprotection of Histidine Methyl Ester Dihydrochloride

It was initially decided to attempt protection of the histidine methyl ester, as a preventative to any effects that the free acid may have on the protection reaction. The general reaction that was expected to take place is shown in **Scheme 2.7**.



Scheme 2.7 General reaction equation for the diprotection of histidine methyl ester dihydrochloride using CDI

Various conditions were tested and it was finally found that a dry environment, a non-competing nucleophilic solvent (dimethyl formamide (DMF)) and an acidic workup were necessary to form and recover the desired product. The yield was further improved by elimination of the acidic work-up and by making use of flash chromatography. The various attempts are tabulated below.

TABLE 6				
Diprotection of histidine methyl ester dihydrochloride				
Entry no.	**CDI**	**Additional reagents**	**Yield**	**Conditions**
1	2 equiv.	None	0%	MeOH Water / ether workup
2	1 equiv.	Et_3N 3 equiv.	0%	MeOH Ether / $NaCO_3$ workup
3	2 equiv.	NaH 4 equiv.	0%	MeOH Ether / $NaHCO_3$ workup
4	2 equiv.	None	30% **2.11**	DMF DCM / pH 5 HCl workup.
5	2 equiv.	Apparatus flaming out.	0% (workup too excessive)	DMF DCM / pH 5 HCl / $NaHCO_3$ / brine workup.
6	2 equiv.	Apparatus flaming out.	30% **2.11**	DMF DCM / pH 5 HCl workup.
7	2 equiv.	Nmm 4 equiv.	68% **2.11**	DMF DCM / pH 5 HCl workup.
8	2 equiv.	None	83% **2.11**	DMF Flash chromatography.
9	1 equiv.	None	83% **2.11**	DMF Flash chromatography.

From the tabulated data it can be seen that the diprotected histidine methyl ester was obtained in an 83% yield, using simple reaction conditions. Further literature studies provided other methods by which to obtain the diprotected product. Yuan *et al.*[50] first neutralised the histidine methyl ester dihydrochloride with sodium methoxide. Thereafter they carried out their reaction in a tetrahydrofuran / chloroform mixture, using 1

equivalent of CDI. After work-up, the diprotected histidine methyl ester crystalline product was obtained in a yield of 60%. Chivikas *et al.*[51] also prepared the diprotected methyl ester along a similar route to Yuan, making use of triethyl amine and chloroform followed by the CDI, obtaining a yield of 64%. Both of the pre-mentioned routes involved a two step reaction compared to our high yielding one-pot synthesis of the desired product. This work also confirmed that DMF is the preferred solvent and also showed that pre-neutralisation is not necessary.

Diprotection of Histidine as its Free Acid

Diprotection of histidine methyl ester using CDI results in all of the active sites of the compound being deactivated. To allow for reactions to take place, it is necessary to make one of the reactive sites available. Two options are available: (i) to rather diprotect the histidine as a free acid and not as the methyl ester or (ii) to remove the ester group from the diprotected histidine methyl ester. We decided to initially attempt protection of histidine as its free acid.

Numerous attempts failed to afford the requisite protected compound. These attempts included the addition/omission of a base, use of excess CDI and various work-up procedures.[52]

Conversion of the Diprotected Histidine Methyl Ester to its Free Acid.

No success was attained in trying to form the CDI protected product of histidine as the free acid. If we proceeded with this protocol, and making use of this methyl ester, it would be necessary to deprotect the ester to prepare the substrate for peptide coupling. To carry out this deprotection it was very important to take into account all of the other functionalities present in the compound. The deprotection was partially achieved using a triethyl amine / water mixture.[53] This resulted in the formation of the amine salt. It proved to be difficult to neutralise the amine salt and therefore attempts were made to couple straight from the amine salt. This attempt was unsuccessful.

An alternative form of ester removal involved the use of sodium hydroxide and aqueous ethanol, followed by recrystallisation from hot acetone.[54] However this approach also proved to be unsuccessful. Due to the large amount of time spent working with CDI, and the paucity of positive results emanating from these studies, it was decided not to carry out any other attempts at the removal of the ester group but to rather look at another approach to protect histidine. This approach will be discussed in the following section.

2.3 AMINO ACID COUPLING REACTIONS

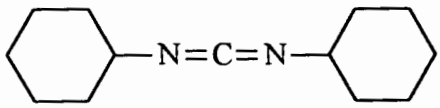
2.3.1 INTRODUCTION

With the increasing importance of synthetic peptides in chemical and biomedical research, a variety of methods and reagents have been developed for peptide bond formation. Ideally, the reagent for such a reaction should allow rapid product formation under mild conditions and in good yield, not compromise the diastereomeric purity, avoid side reactions, and generate easily removable co-products. Applicability to both solution and solid phase synthesis should be an additional criterion.

Various coupling reagents exist, each with their own reactivity and optimal conditions. In this discussion I hope to introduce to the reader some new coupling agents and to briefly mention some well known and extensively used reagents.

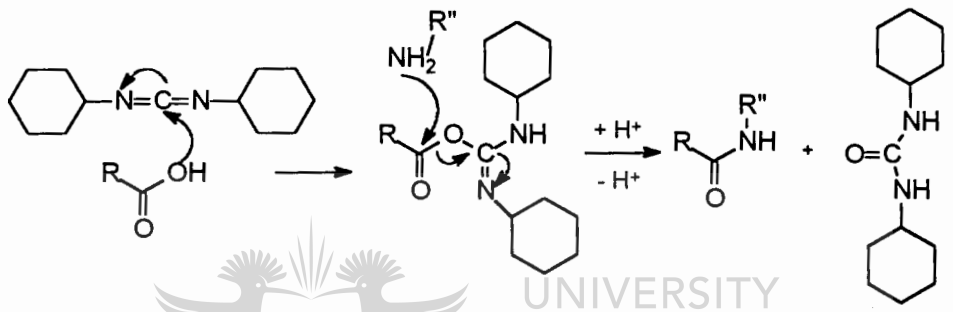
Coupling Agents

The coupling of two amino acids is often referred to as Peptide Coupling. Two amino acids are coupled with the aid of a coupling agent. One of the most common coupling agents used is dicyclohexyl carbodiimide (DCC) (**2.12**):



2.12

DCC readily reacts with water to form a stable product, dicyclohexyl urea (DCU) (**2.13**). Due to the mechanism of activation of acids with DCC, **Scheme 2.8**, one would expect that it is necessary to add DCC and the acid first, then add the amine at a later stage. However, this is not the case and a one-pot reaction is possible. This is because the DCC and the amine cannot form stable DCU and therefore provides no competition.



2.13

Scheme 2.8 General reaction for the activation of an amino acid using DCC.

Various conditions have been reported, under which DCC has been used.[55] The reactions have been carried out in DMF, THF, DCM and many more solvents. Activation with DCC is normally carried out at 0 °C, but several researchers claim to obtain better yields when the activation is carried out at –10 °C. DCC has been used in the presence of various reaction promoters and bases. All of these conditions are determined by the substrates to be coupled.

2-Chloro-1,3-dimethyl-2-imidazolinium hexafluorophosphate (CIP) (**2.14**) has recently been recognised for its effectiveness as a coupling agent for *N*-methylamino acids in solution and on solid support. CIP-mediated activation, in the presence of 1-hydroxy-7-azabenzotriazole (HOAt), was found to be efficient enough for hindered couplings in solution. Furthermore, it was found to minimise racemisation to practically nothing and

coupling reactions were found to be complete within 1 to 3 hours, at 25 °C. On comparison to DCC / DMAP activation, a well-known and highly efficient peptide coupling procedure, which resulted in 37% isomerisation after a 24 hour reaction. When using CIP it was found that DMF was a better solvent than DCM, resulting in higher yields and less D-isomer content.[56]

Another newly developed coupling agent for coupling hindered *N*-methyl amino acids is 2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate (BEMT) (**2.15**). BEMT was sythesised from inexpensive and non-toxic materials and was a great improvement on the DCC/HOBt and PyBroP[157] systems used. The efficiency of BEMT was shown by Li *et al.*,[58] where a variety of solvents was used, including DMF, THF, DCM and acetonitrile and in most cases high yields, fast reactions and low degrees of racemisation were observed. Strong hindered bases (DIEA) were preferred to bases like triethylamine.

Li and Xu[59] also developed a coupling reagent based on HOBt, benzotriazol-1-yloxy-*N*,*N*-dimethylmethaniminium hexachloroantimonate (BOMI) (**2.16**). This coupling reagent, prepared from DMF, HOBt and bis(trichloromethyl)carbonate (BTC), was shown to be very efficient for peptide coupling in both solid and solution phase in terms of yield, rate and low racemisation.

A novel coupling reagent ethyl-1-hydroxy-1*H*-1,2,3-triazole-4-carboxylate (HOCt) (**2.17**) was developed to be used in conjunction with diisopropyl carbodiimide (DIC) in solid phase peptide synthesis. Robertson *et al.*[14] carried out a systematic study into racemisation upon activation and coupling of single amino acids and dipeptides with this reagent, *constantly pointing out the problems experienced with histidine*. As mentioned before, N^{α} protected histidine, when activated, is especially prone to racemisation, as a result of the reactivity of the imidazole ring. The imidazole ring is a weak base but strong enough to cause intramolecular proton abstraction to form an intermediate which can protonate to either the D- or L-amino acid. Masking the π-nitrogen in the imidazole is highly desirable but most protecting groups block the τ-nitrogen. One of the only commercially available analogues with π-nitrogen protection is Fmoc-His(Bum),[15] the

synthesis of which is a multi-step procedure and low yielding, making it very expensive. In Robertson's studies,[14] racemisation was completely eliminated for 18 Fmoc-protected amino acids, *and to 50% for Fmoc-histidine(Trt)*. Further investgations were carried out for Fmoc-histidine(Trt) and milder conditions were found to be necessary, *i.e.* 0 °C instead of room temperature during activation with CtOH/DIC, which reduced racemisation to zero. This is comparable to when *N*-hydroxybenzotriazole is used (8%), making this reagent system very important when histidine is to be coupled.

A simple coupling reagent that I have not yet mentioned is that of ethyl-2-ethoxy-1,2-dihydroquinoline-1-carboxylate (EEDQ) (**2.18**).[60] This coupling reagent has been widely used in coupling reactions. It does not provide any special features for complicated reactions but is a convenient reagent for simple coupling reactions.

Trimethyl aluminium ($AlMe_3$) is an interesting coupling agent because it is much less expensive than the commonly used carbodiimide coupling reagents and it is non toxic. It is normally used for the coupling of a protected amino acid or peptide ester with a zwitterionic amino acid or peptide.[61]

Yet another interesting reagent is (trimethylsilyl)ethoxyacetylene (TSEA) (**2.19**).[62] TSEA is a dehydrating agent used in conjunction with mercury oxide, triethylamine and dichloroethane, in either dichloromethane or acetonitrile. The reactions are carried out at temperatures between 40-60 °C. TSEA has been used to effect intramolecular cyclisations between an acid and an alcohol or between an amine and acid, as well as being used as the coupling agent for peptide coupling. Its versatile use and easy reaction conditions make it an asset to synthetic chemistry.

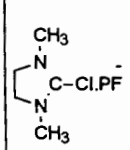
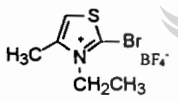
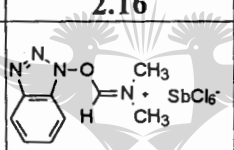
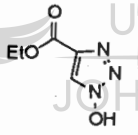
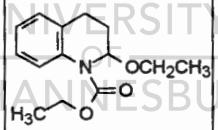
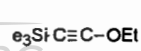
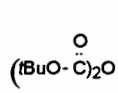
A very interesting concept is the use of di-*t*-butyl dicarbonate ($(Boc)_2O$, Boc anhydride) (**2.20**) as a coupling agent. Boc anhydride is an extensively used reagent for clean and rapid introduction of acid-labile Boc-protection for the amine functionality. It is also an effective *t*-butoxycarbonylating agent, it has been used to convert amines to isocyanates, carbamates and urea derivatives. Mohapatra *et al.*[63] found Boc-anhydride to be an

effective reagent for the activation of carboxylic acid carbonyls and from this it was decided to look at its ability as a coupling reagent. Coupling was successfully achieved in the presence of DMAP, using pyridine and THF as solvent, in 6 hours at room temperature, and yields of up to 77% were obtained.

Halpern and Chew carried out a comparison of the racemisation that occurred when different coupling reagents were used.[44] They found that, in the case of carbodiimide reagents such as DCC and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDC), a greater amount of racemisation occurred compared to when CDI or potassium 2-ethyl-5-phenylisoxazolium-3'-sulfonate were used. They went on to discuss the effects of protecting groups and solvents on the amount of racemisation.

TABLE 7

Coupling Reagent Structures

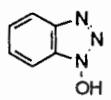
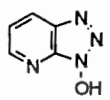
CIP **2.14**	BEMT **2.15**	BOMI **2.16**	HOCt **2.17**	EEDQ **2.18**	TSEA **2.19**	$Boc(O)_2$ **2.20**
						

Reaction Promoters

Cases can arise where the coupled amino acid rearranges and thereby becomes less open to nucleophilic attack, resulting in the incoming amino acid not having enough of a driving force to displace the coupling agent from the acid. In these cases, it is necessary to add a reaction promoter, such as *N*-hydroxy phthalimide or *N*-hydroxybenzotriazole.[64] These promoter groups pre-complex the acid in a manner such as to reduce rearrangement of the amino acid on addition of the coupling agent and hereby decrease racemisation.[13,65]

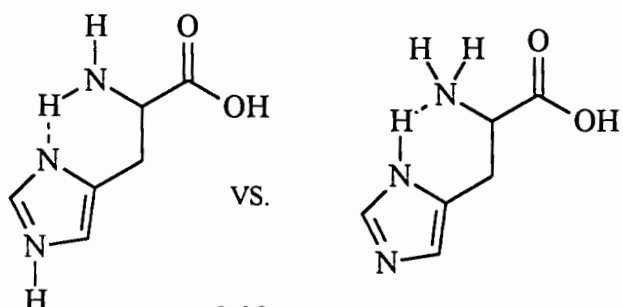
The most common peptide coupling additive is *N*-hydroxybenzotriazole (HOBt) (**2.21**), used either in combination with a carbodiimide or another coupling agent, or built into a

stand-alone reagent such as 1-benzotriazolyloxytris(dimethylamino)phosphonium hexafluoro-phosphate (BOP). *N*-Hydroxybenzotriazole can also been used in conjunction with 1-ethyl-3-(3-dimethylamino)propyl carbodiimide (EDCl) in DMF, effecting high yields.[66] The pre-mentioned additives have been noted to inhibit side chain reactions and reduce racemisation. A further adaptation of HOBt is 1-hydroxy-7-azabenzotriazole (HOAt) (**2.22**), which has been noted for its ability to speed up reactions, reduce the loss of chiral integrity and provides a visual indication of reaction end point (yellow to colourless).[67]

TABLE 8	
Reaction Promoter Structures	
HOBt **2.21**	HOAt **2.22**
	

Many other reaction promoters exist, *i.e.* KOBt, NaOBt, NH_4OBt and *N,O*-bis(trimethylsilyl)-acetamide.[68] In each case, their use is dependant upon which amino acids are being coupled, what solvent is being used and if a base is being used.

The use of promoters is particularly popular when carrying out peptide coupling with histidine. Histidine has two possible (N---H---N) bonding positions (**2.23**), resulting in a decrease in its ability to displace the coupling reagent. Therefore, once activation of the acid has taken place, a reaction promoter is needed to aid histidine to couple to the activated acid, *i.e.* to displace the coupling agent.



2.23

2.3.2 DISCUSSION

In our studies, various reaction conditions have been attempted to couple two amino acids. These conditions include the use of DCM as solvent, as well as dry THF. It was found that THF was a better ligating solvent and therefore played an important role in the formation of our desired dipeptide. Heating of the reaction mixture was also attempted. This proved to be successful, but in the particular reaction a very low yield was obtained.

In the earlier stages of the coupling attempts, it was found that the DCC remained coupled to the product, preventing complete peptide coupling. Reaction promoters were introduced to prevent this. *N*-Hydroxyphthalimide was tested but was found to have no influence on the outcome of the reaction. The use of HOBt not only ensured complete peptide coupling but also increased the yield, with only one equivalent being needed.

In the instances where the hydrochloride salts of the amino acid esters were used, it was found that a base was necessary. *N*-Methyl morpholine (Nmm) proved to be a good base for this purpose, with one equivalent being needed for each hydrochloride present.

The workup of the reaction required the removal of the DCU that had separated, which was simply achieved by filtration, removal of excess THF and partitioning between ethyl acetate and saturated sodium bicarbonate. The organic layer was washed with a saturated salt solution and dried over magnesium sulfate.

Due to the low yield obtained for the coupling reaction of Boc-L-glycine with histidine methyl ester, three different solvents were used to test their effect on the yield. DMF proved to increase the yield only slightly. Due to the removal of DMF being so difficult, especially on large scale reactions, THF was used with further optimisation. Additional optimisation attempts found that if the aqueous work up was omitted and the crude product was purified directly by column chromatography the yield was increased from

26% to 79%. This was expected to be as a result of no loss of product in the form of a zwitterion, which was possible during the aqueous workup.

The tables that follow include the coupling reactions attempted and the successes and failures encountered. The tables highlight the process by which peptide coupling was eventually achieved, as discussed above. The reactions represented by entries 4, 7, 9 and 10 were particularly encouraging, in the light of our previously encountered difficulties at attempted peptide coupling. From here we were able to use the established reaction conditions to couple more amino acids to the histidine methyl ester. Confirmation of the identity of the products obtained was achieved initially by making use of characteristic peaks in the ^{1}H-NMR, *i.e.* the two low field imidazole singlets, the two AB systems showing the coupling was achieved, and in each case the additional side chain peaks were observed. The experimental data were later confirmed by comparison with similar compounds prepared by Cleij *et al.*[54] and by making use of other analytical techniques.

Formation of the histidine dimer coupled product proved to be quite troublesome, involving lengthy chromatography columns for purification and ultimately not being able to obtain the coupled product totally clean. This impurity problem can be explained by the racemisation of histidine on activation with DCC, as explained in the discussion on amine group protection. This situation has been improved by making use of a one-pot dimerisation / cyclisation sequence.

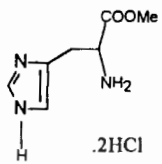
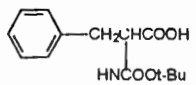
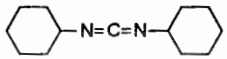
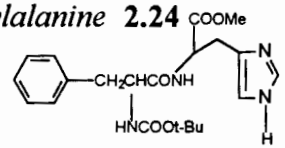
TABLE 9				
Amino Acid coupling				
Entry no.	**NH Amino Acid**	**COOH Amino Acid**	**Coupling Agent**	**Desired Product**
	Histidine methyl ester Dihydrochloride 	*Boc-DL-phenylalanine* 	*Dicyclohexyl carbodiimide* (DCC) 	*Histidine* methyl ester-*Boc-phenylalanine* **2.24** 
1	THF, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, H_2O / $NaHCO_3$, Flash column chromatography (FCC).[69] (0%)			
2	Triethylamine, THF, as above.[70] (23%)			
3	DCM, as above.[71] The use of DCM was for comparison with THF, *i.e.* to ascertain the effect of a less polar solvent. From NMR analysis, it could be concluded that DCC was still present, possibly still attached to the amino acid. This resulted in the reaction not running to completion. It was decided to add a promoter in subsequent reactions. (Shown later in this table)			
4	DCM, DCC, 0 °C → 40 °C (5 h) → 25 °C (14 h). (62%)			

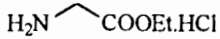
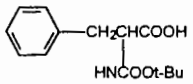
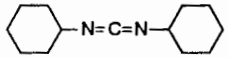
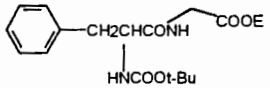
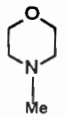
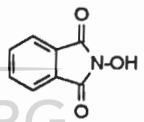
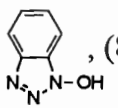
Table 9 continued...				
Entry no.	**NH Amino Acid**	**COOH Amino Acid**	**Coupling Agent**	**Desired Product**
	Glycine ethyl ester hydrochloride 	*Boc-L-phenylalanine* 	*Dicyclohexyl carbodiimide* (DCC) 	*Glycine ethyl ester-Boc-phenylalanine* **2.25** 
5	THF, *N*-methyl morpholine (Nmm, ), 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, H_2O / $NaHCO_3$, FCC. (42%)			
6	The above reaction was repeated using a reaction promoter, *N*-hydroxy phthalimide  , (37%).			
7	Repetition of the above reaction with *N*-hydroxybenzotriazole (HOBt),  , (82%).			

Table 9 continued...

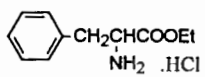
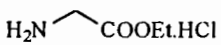
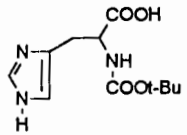
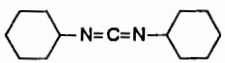
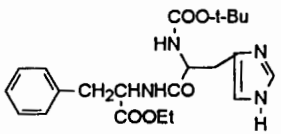
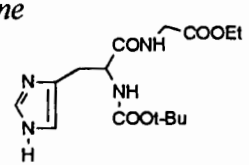
Entry no.	NH Amino Acid		COOH Amino Acid	Coupling Agent	Desired Product
	A1 *L-Phenylalanine ethyl ester hydrochloride* 	A2 *Glycine ethyl ester hydrochloride* 	*N^{α}-Boc-Histidine* 	*Dicyclohexyl carbodiimide* (DCC) 	A1 L-Phenylalanine ethyl ester - *N^{α}-Boc-Histidine* 
					A2 Glycine ethyl ester - *N^{α}-Boc-Histidine* 
8.A1 8.A2	THF, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, H_2O / $NaHCO_3$, FCC. This reaction was carried out twice, first with A1 and then with A2. In both cases very low yields were obtained, making NMR analysis very difficult. From literature[1] it was concluded that coupling is difficult if the N^{im} is not protected.				

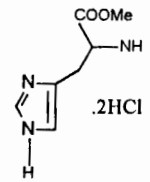
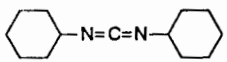
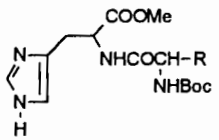
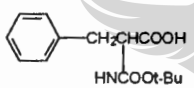
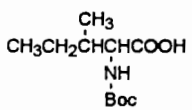
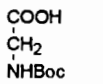
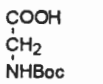
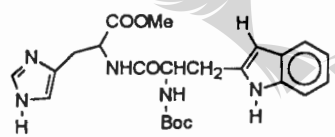
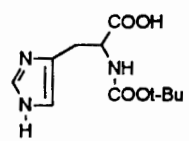
Table 9 continued...				
	NH Amino Acid	**COOH Amino Acid**	**Coupling Agent**	**Desired Product**
	Histidine methyl ester dihydrochloride 	*Boc protected amino acid*	*Dicyclohexyl carbodiimide* (DCC) 	*Histidine methyl ester-Boc-protected amino acid* 
Entry no.	**Boc protected amino acid**	**Comments / Conditions**		
9	*Boc-L-phenylalanine* 	THF, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, $NaCl_{aq}$ / $NaHCO_3$, FCC. (76%). **2.24b**		
10	*Boc-L-isoleucine* 	THF, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, $NaCl_{aq}$ / $NaHCO_3$, FCC. (55%). **2.26**		

Table 9 continued...		
11	*Boc-L-alanine* $CH_3CHCOOH$ NH Boc	THF, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, $NaCl_{aq}$ / $NaHCO_3$, FCC. (37%). **2.27** *This reaction was repeated using 1.5 eq. boc-alanine but there was no improvement on the yield.*
12	*Boc-L-leucine* CH_3 CH_3 $CHCH_2CHCOOH$ NH Boc	THF, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, $NaCl_{aq}$ / $NaHCO_3$, FCC. (69%). **2.28**
13	*Boc-L-glycine* COOH CH_2 NHBoc	THF, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, $NaCl_{aq}$ / $NaHCO_3$, FCC. (26%). **2.29**
14	*Boc-L-glycine* COOH CH_2 NHBoc	DMF, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, $NaCl_{aq}$ / $NaHCO_3$, FCC. (39%). **2.29**

Table 9 continued...		
15	*Boc-L-glycine*  	EtOAc, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, $NaCl_{aq}$ / $NaHCO_3$, FCC. (16%). **2.29**
16	*Boc-L-glycine*  	THF, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, FCC. (76%). **2.29**
17	*Boc-L-tryptophan*  	THF, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, $NaCl_{aq}$ / $NaHCO_3$, FCC. (50%). **2.30**
18	*N^{α}-Boc-Histidine*  	THF, Nmm, HOBt, 0 °C, DCC, 0 °C (1 h) → 25 °C (14 h), filtered, FCC. (76%, not totally clean). **2.31**

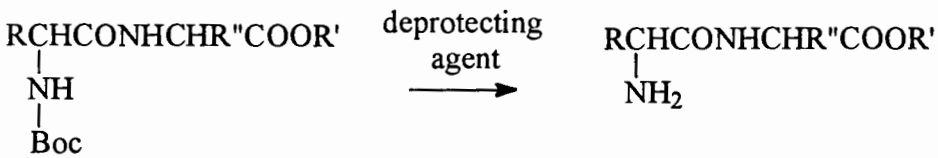
From the data recorded above it can be seen that seven histidine-coupled products were synthesised, in good yield. The basic reaction conditions required *N*-hydroxy-benzotriazole as a reaction promoter, dicyclohexyl carbodiimide as a coupling reagent, tetrahydrofuran as a solvent, *N*-methyl morpholine as a base, and flash column chromatography for purification. It therefore remained for us to deprotect and cyclise these products to obtain the desired diketopiperazines, which is discussed in the following section.

2.4 *N*-DEPROTECTION AND DIKETOPIPERAZINE SYNTHESIS

2.4.1 INTRODUCTION

Before successful cyclisation can be achieved, it is necessary to deprotect the amine functionality of the dipeptide ester. This can (in principle) be achieved by a variety of methods. These methods vary according to the complexity of the dipeptide in question and according to the protecting group to be removed.

Due to all of our dipeptides being Boc-protected, I will discuss the various conditions available for Boc-deprotection. The *t*-butoxycarbonyl (Boc) group is an acid sensitive protecting group and therefore removal of the Boc group is achieved by an acidic treatment. The general reaction that takes place during deprotection is shown in **Scheme 2.9.**



Scheme 2.9 General reaction for *N*-Deprotection

Trifluoroacetic acid is one of the most common reagents used to effect Boc deprotection.[72] It has been found that in certain instances the use of neat TFA is too harsh for the dipeptide undergoing deprotection; under these circumstances DCM can be used in conjunction with TFA, in a suitable ratio to still afford deprotection but prevent decomposition. The *N*-deprotection of Boc-L-phenylalanyl glycine ethyl ester was achieved using TFA:DCM (2:1),[73] in a high yielding reaction (71%). The exact reaction conditions are included in the discussion below (**Scheme 2.12**, pg 40). In certain cases, the dipeptide undergoing deprotection may have an acid sensitive functionality present. It is then necessary to add an acid scavenger, such as triethylsilane[74] or thiophenol[75], to prevent decomposition of the entire dipeptide.

In the case of neutral reaction conditions, ceric ammonium nitrate (CAN) can be used to afford deprotection. CAN acts as a catalyst and is therefore used in small amounts, usually with acetonitrile as solvent.[76]

Selective removal of the *N*-Boc protective group can be achieved by using silica gel at a low pressure. This is accomplished by simple adsorption onto the silica gel, followed by gentle heating under reduced pressure. In this instance, the Boc group on an indole or conjugated to an aromatic or carbonyl can be removed selectively, leaving the side chain Boc-protection intact.[77]

Another form of selective deprotection can be attained using $Mg(ClO_4)_2$ in acetonitrile. This mild condition is effective enough to remove the Boc group from amides or carbamates but at the same time leaving simple Boc-protected amines unaffected.[78]

The list of reagents for the removal of the Boc group is comprehensive; it remains for me to list a few of the more frequently used reagents, *i.e.* hydrochloric acid,[79] sulphuric acid,[80] hydrogen fluoride,[81] trimethylsilyl triflate,[82] boron trifluoride etherate,[83] trimethylsilyl iodide,[84] tin tetrachloride,[85] aluminium chloride.[86] In some cases attachment to a solid support is desired.[87] Apart from this abbreviated list, there are still many more reagents and conditions available with which to effect this transformation.

Subsequent to deprotection, it is necessary to cyclise the dipeptide to obtain the desired diketopiperazine. The cyclisation process is shown **Scheme 2.10**.

Scheme 2.10 General reaction equation for the formation of a 2,5-diketo piperazine:

Cyclisation can be achieved using a cyclisation agent. The cyclisation agent normally consists of a mixture of solvents, having specific characteristics, or it may be a particular reagent. In some cases the simple organic solvent in which the dipeptide is dissolved can effect cyclisation. Several articles have referred to the use of methanol to afford cyclisation.[88] Recently it has been found that spontaneous cyclisation occurs upon amine deprotection, as was the case for the deprotection of Fmoc-*N*-allyl glycine with piperidine in DCM at room temperature.[89]

Janus *et al.*[90] reported a simple cyclisation involving acetic acid and 2-butanol. It is important to note the presence of acetic acid, as it was found that a small amount of acid was necessary to catalyse cyclisation: the source of the acid was a catalytic amount of added acetic acid or residue acid in the form of the formate salt, as will become clear from the discussion below.[91] It has been found that the concentration of acetic acid used varies, depending on the dipeptide in question. It was further established that, if the hydrochloride salt of the dipeptide was to be cyclised, it was necessary to add *N*-methyl morpholine.[92]

With reference to the formate salt mentioned above:[91] if the dipeptide was deprotected with formic acid, the deprotected product was obtained as the formate salt. In this

instance, cyclisation was carried out using a toluene / 2-butanol mixture under reflux for 3-4 hours. Cyclisations have also been effected in neat toluene under reflux.[93]

Another simple cyclisation procedure was developed by Kopple *et al.*[94] who carried out the cyclisation reaction in phenol, heating just below the boiling point of phenol. This particular procedure was carried out on unblocked dipeptides or their hydrobromide salts.

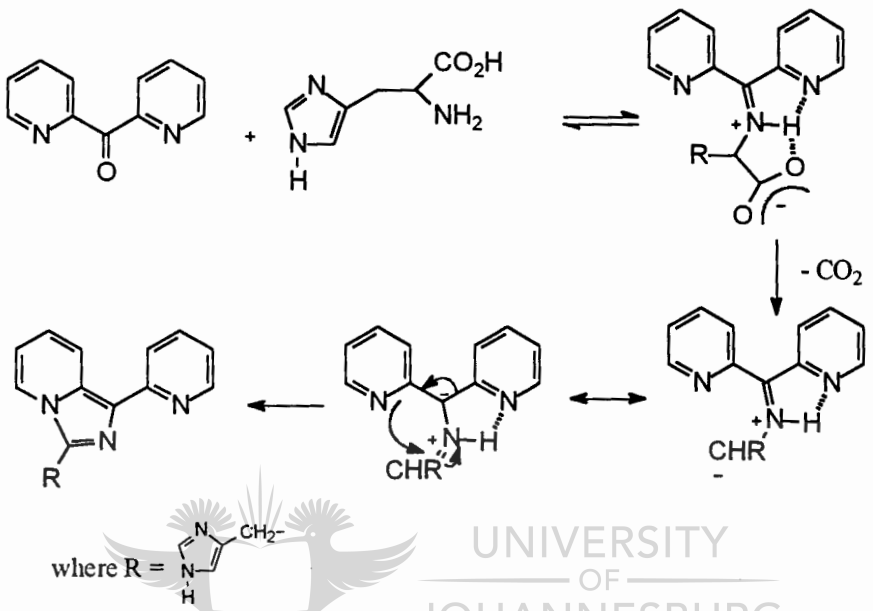
An interesting cyclisation involved the use of a coupling agent, diphenylphosphoryl azide (DPPA). A linear dipeptide was treated with triethyl amine (TEA) prior to the addition of DPPA. The ring formation was effected at a low temperature in DMF. TEA was added periodically throughout the reaction to maintain a neutral *p*H.[95]

Another interesting method to form the desired diketopiperazine was achieved by making using of a solid support. Since the trityl moiety is known to be a protective group for the NH of the imidazole ring of histidine, Sabatino *et al.*[96] made use of a trityl resin to anchor a Fmoc-histidine-O-allyl residue at the nitrogen, and from here carried out a variety of transformations on the histidine, *e.g.* peptide chain elongation. After Fmoc-deprotection, using piperidine, the peptide was suspended in a solution of *N*-ethyldiisopropylamine (DIPEA) and TBTU (O-benzothiazol-1-yl-N,N,N',N'-tetramethyl-uronium tetrafluoro-borate) in DMF, to afford cyclisation and later cleaved from the resin using TFA-H_2O, obtaining *cyclo*(L-Histidyl-L-glycyl)$_3$. This procedure has been repeated on other dipeptides, with diketopiperazine formation taking place directly after Fmoc-deprotection, whilst still being attached to the solid support.[97]

Merrifield *et al.*[98] carried out an attractive combination of the two pre-mentioned points, *i.e.* making use of solid support and a coupling agent. Cyclisation was carried out using EDDQ while the linear peptide was attached to the solid support. It is important to note in most cases cyclisation takes place in solution after removal from the solid support.

Grigg *et al.*[99] investigated the cyclisation reaction taking place between histidine and 2,2-dipyridyl ketone. It is not directly related to diketopiperazine formation, but the

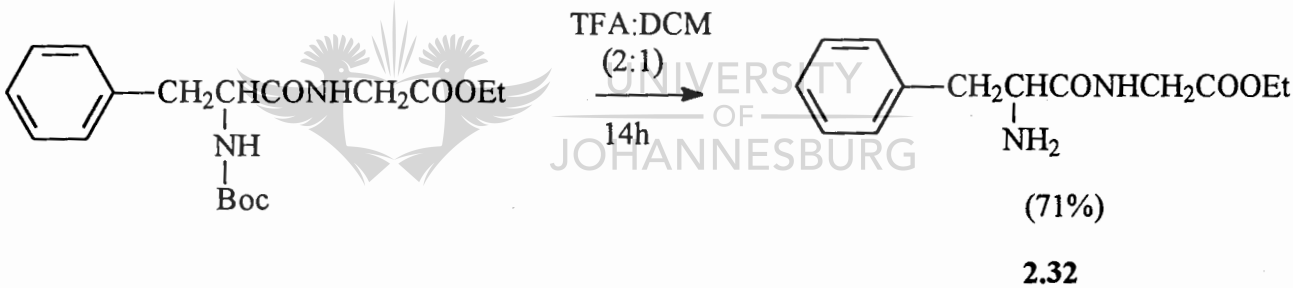
procedure followed to obtain the end product, a result of a 1,5-electrocyclisation and subsequent aromatisation, could be of great interest. The 1,3-disubstituted-2-azaindolozones was obtained by refluxing in methanol, in the presence of a catalytic amount of acetic acid. Again here we see the use of methanol to afford cyclisation and we are reminded of the importance of the presence of an acid.



Scheme 2.11 Formation of 1,3-disubstituted-2-azaindolozones

2.4.2 DISCUSSION

There are various methods available to deprotect the amine functionality, as discussed above. We initially carried out our deprotection reaction on a simple dipeptide, *i.e.* one not containing histidine. It was found that the use of a trifluoroacetic acid / dichloromethane mixture (TFA/DCM) was successful in affording the deprotected product (**Scheme 2.12**). For our purposes, with peptides containing the histidine group, various reaction times and TFA/DCM ratios were tested without success: in each case the peptide was found to decompose. Even under mild conditions, *i.e.* treatment of the dipeptide with very dilute TFA and for 25 minutes, decomposition was found to occur. This can be explained by the possible formation of a carbene, which results in side reactions, such as *t*-butylation.[100] Another explanation for this decomposition, is an acid-base reaction between the strongly acidic TFA and the basic imidazole ring, which might inhibit the desired reaction.



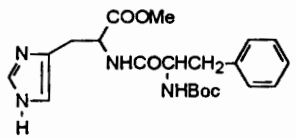
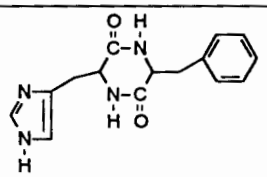
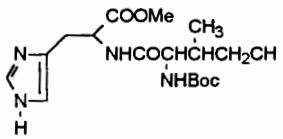
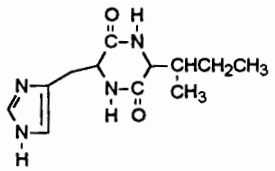
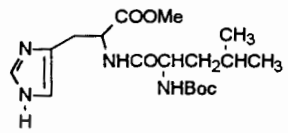
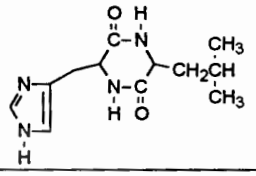
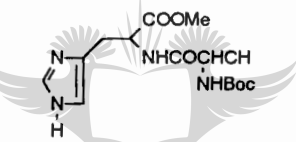
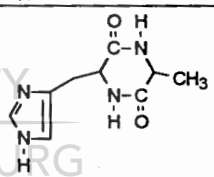
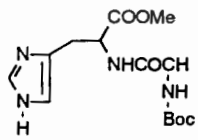
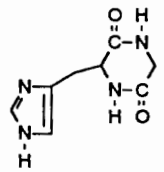
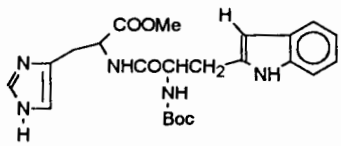
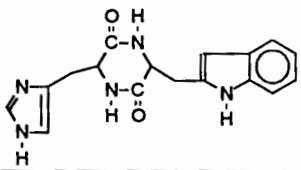
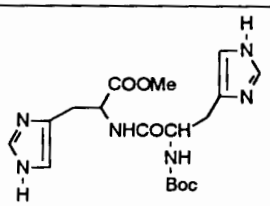
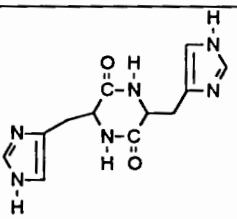
Scheme 2.12 Deprotection using TFA:DCM mixture.[101]

After some experimentation, it was established that the use of formic acid,[102] a weaker acid, led directly to the diketopiperazine (*i.e.* deprotection and *in situ* cyclisation). This particular reaction was carried out on Boc-L-phenylalanyl histidine methyl ester. This success led us to extend the scope of the reaction to other substrates. The use of formic acid for deprotection and cyclisation steps was repeated on the other histidine-coupled products. Analytical data indicated that the required diketopiperazines were produced, but in a mixture with their respective deprotected-uncyclised analogues (**2.33 – 2.36**). Various attempts at the purification of the crude material failed to provide the desired diketopiperazine of satisfactory purity. As a result it was decided to heat the formic acid reaction mixture in an attempt to effect a more complete cyclisation process. Initially, the

reaction was carried out at room temperature for 2 hours followed by heating at 67 °C for fourteen hours. A second set of reactions was heated to 100 °C. Neither of these reaction conditions was successful in effecting a one-pot procedure. Therefore, an alternative route was sought.

Subsequent to successfully deprotecting Boc-L-phenylalanyl glycine ethyl ester (an easily synthesised model compound), various reaction conditions were attempted to afford cyclisation. These conditions included heating in ethanol, the use of a grignard reagent,[103] heating in various mixtures of HOAc / 2-butanol,[90,92] toluene / 2-butanol,[88c,92] or by heating in methanol (**2.37**, 65%).[88b] Most of these reaction conditions failed altogether to afford the requisite diketopiperazine. However, success was found in the use of the toluene / 2-butanol mixture at 120 °C for 3 hours, followed by column chromatography to afford the product in a yield of 79% (**2.37**) (various attempts at a crystallisation afforded a maximum product yield of 28%).

Based on this success the improved reaction conditions were used to effect cyclisation on various histidine-coupled products. In general the reaction conditions can be summarised as follows: the dipeptide of interest was dissolved in formic acid and allowed to stir for 4 hours at room temperature. The excess formic acid was then removed under vacuum and the residue formate salt was dissolved in a toluene / 2-butanol mixture (1:1 v/v). This mixture was allowed to stir at reflux, at 120 °C, for 4 hours. The volume of the reaction mixture was reduced and the reaction cooled to 0 °C. The resulting crystals were filtered and dried. Due to a mixture of products forming, *i.e.* the deprotected-uncyclised compound and the cyclised compound, the product was purified by lengthy flash chromatography (6% H_2O, 12% MeOH in EtOAc). In some cases, crystals did not separate out of the reaction solution after concentrating the reaction mixture, in these cases the product mixture was subjected directly to column chromatography. In carrying out this reaction procedure yields of up to 92%, of the desired diketopiperazine, were obtained. **Table 10** shows the marked improvement realised by this process, *i.e.* refluxing in toluene / 2-butanol and purifying the product mixture by column chromatography.

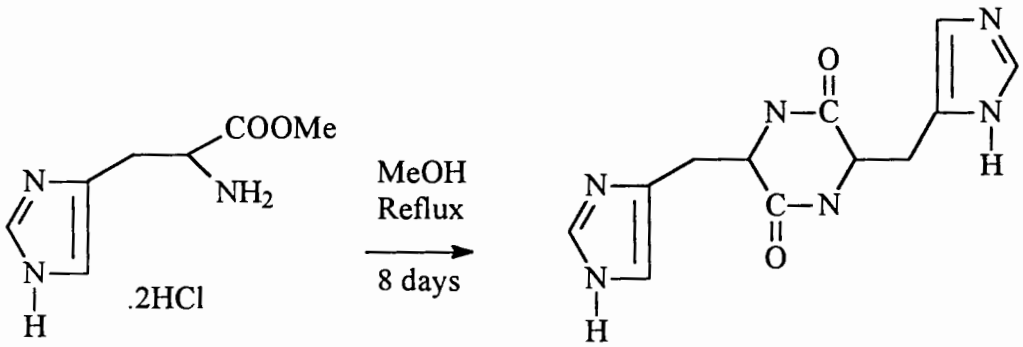
TABLE 10			
Dipeptides and their corresponding diketopiperazines			
Product no.	**Dipeptides**	**Product no.** (yield %)	**Diketopiperazines**
2.24b		**2.38** (93%)	
2.26		**2.39** (70%)	
2.28		**2.40** (50%)	
2.27		**2.41** (85%)	
2.29		**2.42** (92%)	
2.30		**2.43** (54%)	
2.31		**2.44** (74%)	

2.4.3 DIMER FORMATION

As an alternative route to the formation of the desired diketopiperazines it was decided to try to form homo dimers from the respective amino acid esters. Various methods exist but the most common is that of dissolving the amino acid ester and allowing it to reflux for a period of time.

Histidine Dimer Formation

The histidine dimer had already been formed by an alternative method discussed earlier, *via* coupling followed by cyclisation. Some other approaches to this dimer were attempted, in an effort to telescope the synthesis of that product. These methods included heating the histidine methyl ester dihydrochloride in MeOH at 37 °C and at 60 °C for various lengths of time, and in the absence or presence of a base or an acid scavenger in the form of epichlorohydrin.[104] Additionally, more forcing conditions, *i.e.* making use of acetic acid in 2-butanol under reflux or toluene and 2-butanol under reflux, were attempted. Most of these reaction conditions failed to provide the cyclic product, and allowed the quantitative recovery of the starting material. However, it was later established that the dimer of histidine could be prepared in a yield of 30%, when making use of two equivalents of NaOMe in MeOH at 37 °C for eight days.[105]



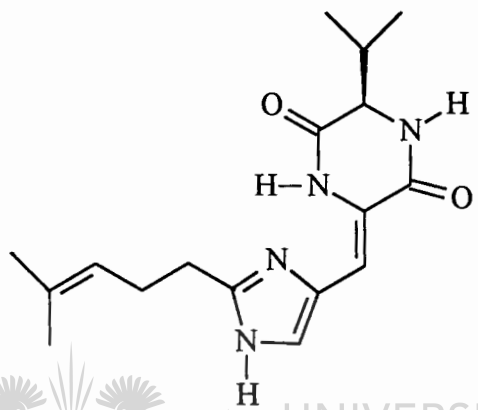
2.44

Scheme 2.13 Histidine dimer formation, *via* a one-pot synthesis from histidine methyl ester dihydrochloride

2.5 COMPLEXATION

2.5.1 INTRODUCTION

The initial idea behind this project was based on a publication by Holzapfel *et al.*,[2] in which a fungal metabolite, viridamine (**2.1**) was described.[1] In this article the authors discussed the manner of complexation that takes place between viridamine and various metal ions.



2.1

They found that viridamine forms ML_2 (L = viridamine) complexes with copper, cobalt and nickel. The metal ions are coordinated in a forced tetrahedral array, through four nitrogen atoms. The central chelate rings are coplanar with respect to adjacent diketopiperazine and imidazole rings. The structure is made up of an extensive framework, which includes various stabilised hydrogen bonds. This structure has several voids, which are occupied by clathrated water. The water is not chemically bound to the structure and therefore on loss of water the structure remains intact. Intramolecular steric repulsions between the carbonyl oxygen of the diketopiperazine ring and the isopentenyl side-chains prevent square planar geometry, rather enforce a distorted tetrahedral array. The copper complex has a strong tendency to form a square planar complex but as a result of the steric repulsion between the two ligands this is not possible. Out-of-plane displacement of the two coordinating nitrogen atoms allows for complexation to take place with copper(II). The cobalt complex forms with the chelate ring in a planar

arrangement. NMR analysis confirms the formation of a tetrahedral nickel complex. In comparison, the d^8 Pd(II) system, where the tendency to form a square planar complex is so much greater, the tetrahedral arrangement would result in such great instability that the complex would not be formed. This, therefore, introduced the idea of selective complexation.

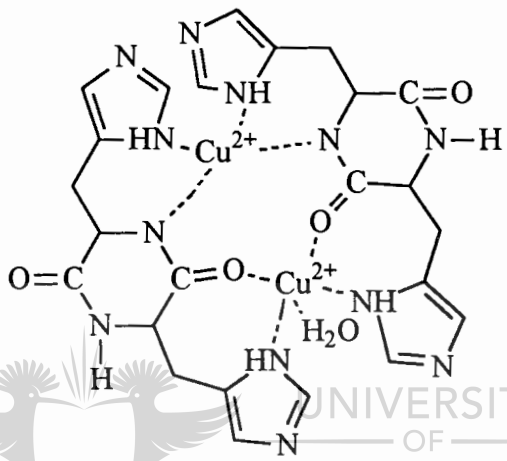
Selective complexation is of great importance to many different areas of our everyday life, *e.g.* in the purification of water,[106] as well as in a variety of industrial operations, *e.g.* in the synthesis of chemosensors.[107]

For this particular project the main interest was in the manner of complexation of diketopiperazines. These diketopiperazines are expected to exert a greater selectivity in complexation than their linear counter-parts because of their constrained geometry, and due to no ligating side chains being present, *i.e.* no free $-COO^-$ or $-NH_2$ terminal groups.[108] In addition to these structural features, specific basic conditions are necessary to allow for deprotonation of the peptide nitrogen to occur, unlike with their linear counter-parts. This, therefore, makes it possible to prevent coordination through the amide nitrogen and allow the normal coordination to take place, *i.e. via* the amide oxygen atom only.

Of particular interest to us are diketopiperazines with imidazole side chains. Side-chains of this kind can encourage the coordination of peptide nitrogens and are therefore of great importance if selective complexation is necessary.

Various conditions have been attempted for the complexation of divalent metal ions to these diketopiperazines. In most cases, a base is necessary to initiate deprotonation after which complexation takes place. In the case of viridamine[2] sodium acetate was sufficient to effect deprotonation and allow complexation. The complexation was later noted to be selective, as mentioned above.

Most studies involving imidazole-containing diketopiperazines have been carried out using *cyclo*(L-Histidyl-L-histidyl). Two main coordination geometries have been recorded. One type results after the addition of lithium hydroxide to a 1:1 metal / ligand mixture. This reaction mixture affords a dimeric complex, with two copper atoms of different coordination modes.[109] This complex was discussed in great detail in the literature chapter and was referred to as the aquabis complex (**1.31**). This aquabis complex was also obtained when sodium hydroxide was used as a base.[110]



1.31

The second manner of complexation, which is of greater interest to us because of the possibility of selective complexation, involves the use of a 2:1 ligand:metal mixture in methanol.[111] The crystals that are eventually obtained display a distorted tetrahedral coordination geometry. This complex was discussed at great length earlier and it was shown that the coordination takes place *via* the four nitrogen atoms of the imidazole ring (two from each ligand). Similar complexation reactions were carried out with $CuSO_4.5H_2O$ and $Cu(NO_3)_2.H_2O$, *i.e.* using a 2:1 ratio and no base. The same tetrahedral coordination was observed.[112]

Interestingly enough it has been found that the counterion of the metal salt used can affect the manner of complexation that takes place. In the case of the complexation of copper with *cyclo*(L-Methionyl-L-histidyl), the perchlorate salt provided crystals with

lower metal to ligand ratios, compared to when the chloride salt was used. A similar trend was observed for cobalt and nickel.[113]

2.5.2 DISCUSSION

Once the diketopiperazines were synthesised, it remained to test their selectivity in metal complexation. Complexation was initially tried under similar conditions to those used by Holzapfel.[1] These conditions involved a 2:1:2 ligand : metal (II) : sodium acetate (NaOAc) ratio. The complexation studies were initially aimed at complexation reactions of Ni^{2+}, Cu^{2+} and Co^{2+}.

The complexation reactions were carried out with the six prepared diketopiperazines; at that stage the *cyclo*(L-Histidyl-L-histidyl) had not yet been prepared. Complexation with the histidyl-phenyl alanine ligand initially looking promising with nickel, as a purple precipitate appeared once all of the methanol solvent had evaporated. With each of the ligands, a colour change was noted and complexation was assumed to be occurring. It was later found that the same colour change occurred when the metal and NaOAc were added together, *i.e.* in the absence of the ligand. This made a conclusion on whether complexation was taking place or not impossible.

Exclusion of the NaOAc proved to be futile as no reaction took place. It was decided to attempt a stronger base, *i.e.* sodium methoxide (NaOMe).

Histidyl-phenyl alanine ligand and copper were dissolved in methanol and left to stir for approximately 30 minutes. This stirring period was hoped to allow for precomplexation of the imidazole moiety with the metal ion. Sodium methoxide was added and immediately precipitation occurred. A few drops of HCl (1 M) were added and the precipitate was recrystallised from water, in an acetone environment. Lovely crystals formed and are discussed below:

Appearance under microscope: white / clear needle-like crystals overlapping each other; surrounded by deep blue square crystals.

Stability: A few of the blue crystals, surrounded by white needles, which were removed from the solvent and left out in open air. After two days, the white needle-like crystals appeared to have absorbed water from the air and were no longer crystalline. The blue crystals remained stable at room temperature for a further two weeks.

Melting Point: 192-194 °C (decomp.)

Note: $CuCl_2.2H_2O$ (the salt we used to prepare this complex) has a lighter blue colour and melts at 100 °C. This is a positive indication that we may have obtained a complex.

Comments: Disappointingly, on leaving the crystals in solutions, while waiting for a X-Ray diffractometer to become available, they eventually decomposed thereby precluding further analysis to be carried out. Numerous subsequent attempts at obtaining the crystals a second time, including making use of seed crystals, have been met with mute failure.

Due to the immediate precipitation on addition of the base it was decided to try to layer the base onto a cooled ligand / metal mixture, thereby extending the mixing period. The ligand and metal were dissolved in methanol and cooled to freezing point with liquid nitrogen. The base and layering solvent were then layered onto the cooled solution. Two different base / solvent systems were used: distilled diisopropyl amine / methanol; diisopropyl amine / acetone. These two systems were tested with copper and nickel in combination with histidyl-phenyl alanine and histidyl-glycine as ligands. However, all of the complexes formed amorphous solids rather than crystalline material. We also attempted the addition of a small amount of a 25% ammonia solution in a bid to enhance the solubility of the complexes, without success to date.

Further literature studies (using other ligands) pointed towards the use of copper (II) perchlorate.[109,110,111] Copper (II) perchlorate complexation studies were carried out with all prepared diketopiperazines, including the newly prepared histidine cyclised dimer. Two conditions were tested on each ligand: (i) M:L 1:1 in methanol, with the later addition of lithium hydroxide monohydrate ($LiOH.H_2O$); (ii) M:L 1:2 in methanol. In reactions based on method (i), precipitation of the complexes occurred with all of the

ligands. Even during recrystallisation from hot water, it was difficult to prevent precipitation. A small amount of 25% ammonium hydroxide solution was added to each and all are currently in the process of being recrystallised from water, and will form part of continuing studies in this context. In method (ii), crystals were expected to separate out after a day and these crystals were then to be recrystallised from hot water. A precipitate formed once all the methanol had evaporated. This precipitate is a subset of current crystallisation studies.

Various solvent systems were attempted to promote the formation of crystals during complexation with copper (II) perchlorate. Very dilute ligand / methanol solutions were prepared before adding the metals salt and thereafter the $LiOH.H_2O$, but precipitation still occurred. Even addition of the $LiOH.H_2O$ as a very dilute water solution still resulted in precipitation. In each case the precipitate was forced into solution by the addition of a small amount of 25% ammonium hydroxide solution.

In a further attempt to prevent precipitation on addition of $LiOH.H_2O$, reaction vessels were made with a very narrow neck (ca. 1mm diameter and 20 mm long neck) connecting the metal / ligand mixture to the dilute $LiOH.H_2O$ / water mixture. This was hoped to allow for slow dispersion of the base solution into the metal / ligand mixture. This resulted in precipitation of the complexes in the neck of the vessel. Precipitation still occurred even on addition of less $LiOH.H_2O$.

A variety of solvent systems were also attempted for the metal:ligand 1:2 ratio. These complexation reactions were carried out using a large excess of solvent. Slow evaporation of the solvents used was achieved by sealing the reaction vessels with parafilm. As yet no results have been forthcoming.

In some of the attempts, needle-shaped crystals have been obtained. However, these were very small and brittle and therefore no X-Ray studies have yet been carried out.

UV studies were carried out on the complexation reaction in the absence of NaOAc. A series of metal:ligands ratios were prepared: *i.e.* $C_L = 4C_m$; $C_L = 2C_m$; $C_L = C_m$; $2C_L = C_m$; $4C_L = C_m$ (c = concentration). Spectra were initially run of the metal (0.0085 M) alone and thereafter of the series for each ligand with each metal. In each of the ligand / metal absorption spectra, a large absorption was noticed in the region of 200 nm. After running spectra of the ligands alone it was found that this absorption resulted from the ligand. This large absorption is presumed to stem from the imidazole group.

Looking specifically at the absorption spectra for *cyclo*(L-Histidyl-L-phenylalanyl) and copper: from the metal salt to the metal / ligand spectra a blue shift was observed, accompanied by an increase in absorption, with the largest absorption being with the $4C_m = C_L$. When cobalt was used, the absorption was subsequently weaker than with copper but a subtle increase in absorption was noted. A subtle extra peak was noticed in the $4C_m = C_L$ absorption spectrum and this can be accounted for by the colour change from pink to a pale purple. The absorption with nickel was very low but a small increase in intensity was noticed compared to when the absorption for the metal alone was analysed.

The absorption spectra for *cyclo*(L-Histidyl-L-alanyl) showed similar trends to that of *cyclo*(*L*-Histidyl-*L*-phenylalanyl), with copper showing the largest increase in absorption. The shift in position for the alanine containing ligand was less than that seen for the analogous phenylalanine ligand. This was expected on the basis of visual changes, *i.e.* the colour change on addition of the metal to the *cyclo*(L-Histidyl-L-phenylalanyl) ligand was a lot more than that for *cyclo*(L-Histidyl-L-alanyl).

The general trend in terms of the complexing ability of the metals with all of the ligands was noted to be: $Cu^{2+} > Co^{2+} > Ni^{2+}$, concluded simply by absorption maxima, with no particular trend noted for any particular ligand.

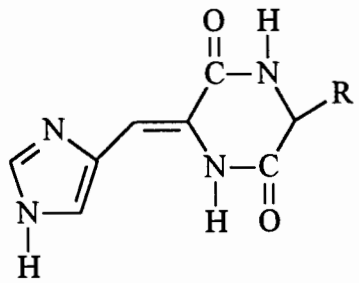
Once crystals of sufficient size have been obtained, the crystals will be analysed by X-Ray diffraction to determine their structure and composition.

2.6 DEHYDROGENATION

2.6.1 INTRODUCTION

It seemed, from the difficulties experienced in the present study with regard to complexation studies, and taking into account the apparent ease with which viridamine formed high quality crystals with various metal salts, that the inclusion of the unsaturation in the diketopiperazine molecule is essential. Having recognised this fact, it was attempted to incorporate unsaturation in our diketopiperazine in a position analogous to that found in viridamine.[1,2] Further reference to the necessity of this particular form of unsaturation for selective complexation was reported by Dyba *et al.*[114] They reported on two effects that proved to be critical for the speciation and stability of complexes formed with this type of unsaturated peptide. One was an electronic effect of the double bond on the increase in charge on the amide nitrogen and the concurrent polarisation of the N-H bond, thus making metal-ion binding to the amide nitrogen easier when compared with the saturated analogues. The second effect is of a steric nature and relies on the tendency of the side chain to bend towards a turn conformation.[114]

The following discussion deals with the various pathways attempted to obtain the desired unsaturated diketopiperazine (**2.45**). There are a manifold of ways in which this particular functionality can be incorporated, *e.g.* dehydrogenation of the saturated diketopiperazine, introducing the double bond at an earlier stage of the synthesis and many more. These miscellaneous routes will be expanded on, as will some of the difficulties experienced.



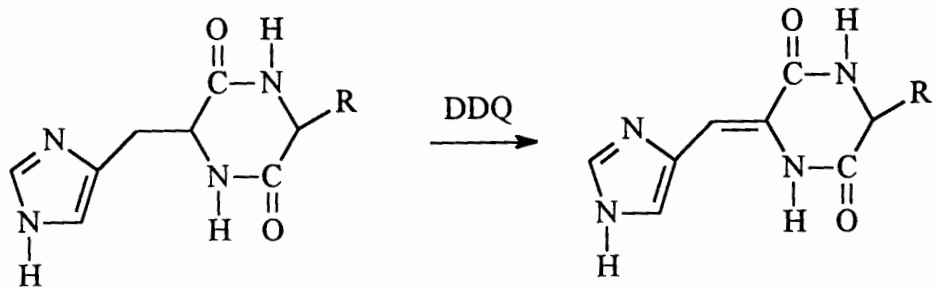
2.45

Where R is the second amino acid functionality.

2,3-Dichloro-5,6-dicyano-1,4-quinone (DDQ) is one of the most common reagents used for introducing a double bond. As a result of DDQ's popularity I will discuss DDQ in detail and just mention a few other routes that are used in this scope. DDQ is a bright yellow solid, which is indefinitely stable in a dry atmosphere, but decomposes in the presence of water with the evolution of HCN. DDQ should therefore be stored under nitrogen in a sealed container.[115]

The introduction of a double bond by hydride ion abstraction and proton elimination constitutes the primary reaction of DDQ, although a few examples are recorded where radicals are produced by this quinone. DDQ has been used in the dehydrogenation of hydrocarbons[116] and carbonyl compounds,[117] oxidation of alcohols, benzylic oxidations,[118] phenolic cyclisation and coupling reactions,[119] silylation promoted oxidation[120] and many more. The ability to effect selective dehydrogenation in the presence of sensitive substituents, such as alcohols and phenols, illustrates the mildness of this method and the further advantage it offers.[121]

The basic DDQ reaction involves the reduction of the DDQ to obtain 2,3-dichloro-5,6-dicyanohydroquinone (DDQH), with the main driving force being the gaining of aromaticity. The desired introduction of the double bond into our diketopiperazine is shown in **Scheme 2.14**.



Scheme 2.14 General reaction taking place when using DDQ.

From the widespread study of DDQ reactions, a set of basic conditions have been reported for optimal reactivity and product yield.[122] Dioxane and benzene are recognised as the preferred solvents, being used in concentrations ranging from 5 to 100 parts of solvent per part of reactant. The advantage of using either of these two solvents is that the DDQH formed during the reaction is insoluble and can therefore be filtered off. Other solvents that have been used are THF, *t*-BuOH, acetic acid, methanol and mixtures of ethyl acetate and trichloroethylene.

These dehydrogenation reactions are most frequently run at reflux using 1.1 to 2 moles of DDQ. Reaction times vary from a few hours to extended periods. Acid catalysts are frequently used in the dehydrogenation process to initiate or increase the reaction rate. Three catalysts reported to be the best are *p*-nitrophenol, thymoquinol and picric acid. Other acid catalysts used include maleic acid, trichloroacetic acid, oxalic acid, anhydrous sulphuric or hydrochloric acid, *p*-toluenesulfonic acid and 3,5-dinitrobenzoic acid. A nitrogen blanket is frequently used, but this is considered to be more of a precaution than a necessity.

A variety of work-up procedures exist for DDQ reactions. Most workers do not bother to destroy excess DDQ present at the end of a reaction, but this can easily be done using sodium bisulfite or sodium hydrosulfite.[123] The most general procedure used is to filter the DDQH at the end of the reaction, often after drowning with ether, DCM, benzene, ethyl acetate or some other solvent. Base-products are removed from the product by

extraction and a final purification was effected by chromatography, usually on alumina. Other adsorbents are also used, such as silica, magnesium silicate and florisil.

Another reagent used as a dehydrogenation protocol is *tert*-butyl hypochlorite (*t*-BuOCl).[124] This reagent was not as opportune to work with as DDQ, as it is light sensitive and therefore required a more involved reaction set-up.

Condensation provides an alternative method to obtain the unsaturated diketopiperazine. To carry out the condensation reaction it is necessary to start from simpler starting materials than was necessary for DDQ, *i.e.* taking the total synthesis of the diketopiperazine a few steps back from cyclisation. One particular reaction involves the condensation of an *N*-protected amino acid and an aromatic aldehyde. To effect this condensation acetic anhydride is used as solvent, in conjunction with sodium acetate.[125] During condensation reactions, a cyclic intermediate was formed. Wong *et al.*[126] discussed an acidic treatment to convert the cyclic intermediate to the desired open-chain unsaturated product. In contrast, Shinkai, H. *et al.*[127] described a basic treatment that resulted in the same ring opening to the desired open-chain unsaturated product. Filler *et al.* also reported on a basic ring opening treatment but they went on to discuss the need for other tertiary amines to be present, *e.g.* aniline hydrochloride.[128] The condensation reaction employed by Wong as well as attempted modifications on it are discussed in more detail in a subsection below.

In agreement to the condensation, carried out by Wong, Raap *et al.* reported on the use of glacial acetic acid as an alternative solvent that can be used in conjunction with sodium acetate to afford the same unsaturated product.[129]

Another condensation reaction that introduces unsaturation into its product involves the condensation of phosphorylglycine esters, in the presence of DBU in DCM.[130] Again this condensation resorts to the very first step of the synthesis of the aimed for ligands, *i.e.* where a phosphorylglycine ester would be used as the starting material.

2.6.2 DISCUSSION

Obtaining the desired unsaturated diketopiperazine structure **(2.45)** has proved to be quite problematic. Various pathways have been attempted and are discussed in detail, in subsections below:

2.6.2.1. DDQ Chemistry

DDQ chemistry involves the use of 2,3-dichloro-5,6-dicyano-1,4-quinone (DDQ) as a dehydrogenating agent. The initial idea behind this pathway was to introduce the double bond directly to the diketopiperazine, already containing the imidazole functionality, as shown in **Scheme 2.14** (pg 52).

It was decided to carry out this series of reactions on the simplest diketopiperazine prepared, namely 3-(4-imidazolylmethyl)-2,5-piperazinedione. A fair amount of literature is available about DDQ but not many have researched the introduction of a double bond in the position we desire. It was initially decided to use methanol[131] and acetic acid [115] as solvents, primarily because of the difficulty involved in dissolving our diketopiperazines.

The reaction was initially attempted in methanol, under reflux. However, a multitude of products was detected by TLC. The reaction was repeated using acetic acid, also under reflux. Once again, numerous reactions appeared to be taking place. In both cases only 1.1 equivalents of DDQ were used.

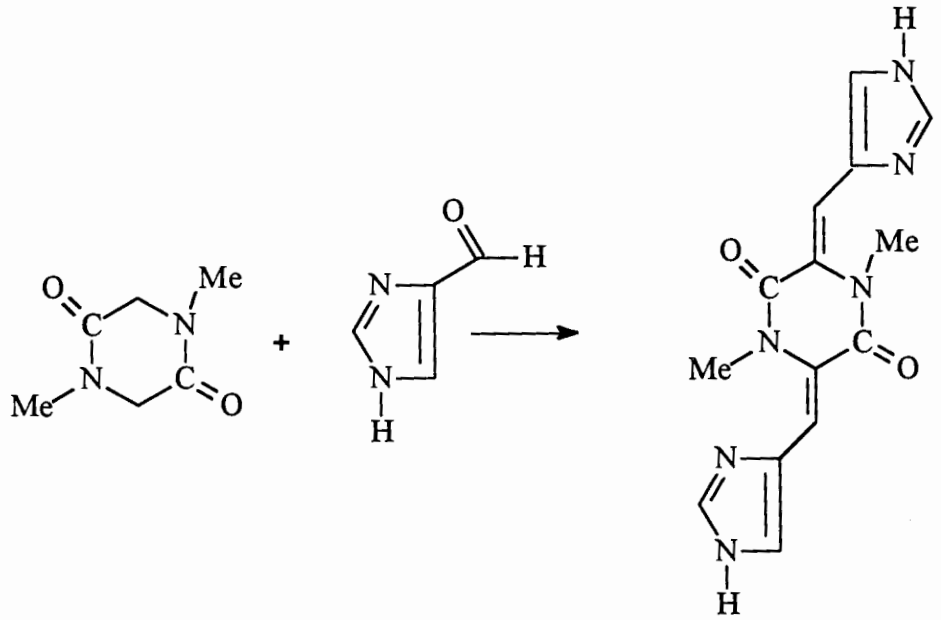
In an attempt to decrease the number of side reactions taking place, both reactions were carried out at low temperatures, *i.e.* methanol (10 °C) and acetic acid (salt/ice bath). In order to eliminate moisture effects the reactions were carried out in flamed out flasks and under nitrogen. In both cases, column chromatography provided only the starting material, indicating that the DDQ possessed insufficient activity at that temperature.

Due to over-oxidation taking place at high temperature and no oxidation at colder temperatures, it was decided to carry out reactions at room temperature and 40 °C. Both of these reactions were carried out with acetic acid as solvent. From TLC and column chromatography no definite conclusion could be drawn, although it appeared as if many products had formed in these reactions.

In order to test our DDQ and find suitable conditions it was decided to carry out a test reaction of similar methodology. After consulting the literature [122] the test reaction was carried out on a 3-ketosteriod, using 1.1 equivalents of DDQ, in dioxane, under reflux, for 30 hours. The test reaction was successful, (confirmed by NMR analysis – olefinic signals down field) and therefore a similar reaction was carried out on our substrate, 3-(4-imidazolylmethyl)-2,5-piperazinedione. Unfortunately only starting material was obtained after column chromatography. Addition of an acid catalyst, 2-nitrophenol[132], was also unsuccessful. This set of reactions seemed to indicate that DDQ was too reactive at elevated temperatures and inactive at lower temperatures, and that a happy medium would be elusive. For this reason, further attempts focused on other approaches.

2.6.2.2. Introduction of the double bond *via* a condensation reaction

Due to two acidic protons existing between the nitrogen and carbonyl in the diketopiperazine ring it was decided to introduce functionality at this position, in an aldol type reaction. This methodology was tested with sarcosine and 4(5)-imidazole carboxaldehyde. The general reaction expected to take place is shown in **Scheme 2.15**.

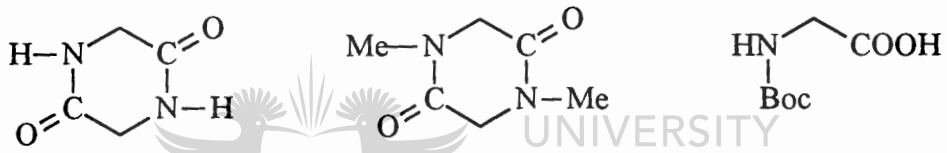


Scheme 2.15 General Reaction expected to take place between sarcosine and 4(5)-imidazole carboxaldehyde

Various reaction conditions were tested based on work by Ishikawa *et al.*[133]; *i.e.* neutral, basic (0.25 equivalents of pyridine), acidic (1.25 equivalents of toluene sulfonic acid) conditions. In each case, dry benzene was used as solvent and two equivalents of the aldehyde were used to one equivalent of sarcosine. All three of these reaction conditions proved to be unsuccessful. It was decided to try deprotonation with sodium hydride before trying to incorporate the aldehyde. Two equivalents of sodium hydride were used, after which the aldehyde was added, but this also proved to be unsuccessful. Further test reactions were carried out using benzaldehyde, instead of 4(5)-imidazole carboxaldehyde, for cost reasons. The next series of reactions made use of various ratios of TFA (trifluoroacetic acid) to benzene. Initially, the sarcosine was dissolved in a 25% TFA / benzene solution and stirred at room temperature. Due to no reaction taking place after several hours, the reaction mixture was heated to 80 °C and a further equivalent of TFA was added. Column chromatography showed no sign of the desired product. More demanding conditions were attempted where all glassware was flamed out under nitrogen, the TFA and benzene were degassed by the freeze-pump-thaw method, the benzaldehyde was freshly distilled and the reactions were carried out under nitrogen.

Three reactions were attempted using the following sensitive conditions: 5% TFA / benzene; 1:1 TFA:benzene and neat TFA. All three reaction conditions failed to give any of the desired product.

After an intense literature search, two condensation reactions became of particular interest,[125,126,127] as they involved the condensation of an aromatic aldehyde and "amino acid type" compound, an *N*-acylglycine, using acetic anhydride and freshly fused sodium acetate. The reaction formed a cyclic intermediate and after a sodium acetate, water / acetone workup the desired dehydrogenated product was obtained. It was hoped that it would be possible to carry out this type of reaction on three different starting materials, *i.e.* glycine anhydride (**2.46**), *N*-protected sarcosine anhydride (**2.47**) and Boc-protected glycine (**2.48**).

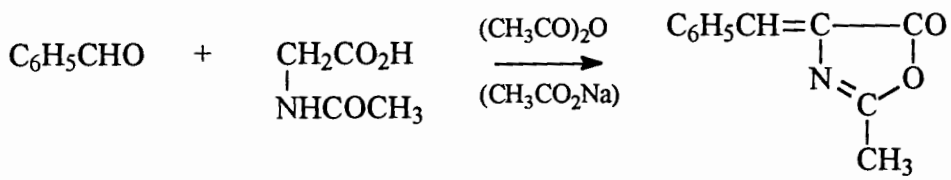


2.46 **2.47** **2.48**

All three reactions were carried out according to Shinkai, H. *et al.*,[127] using an excess of the "amino-acid-type" compound. The test reactions were initially carried out using benzaldehyde, with the ultimate aim of using 4(5)-imidazole carboxaldehyde, which would allow for the introduction of the double bond and the imidazole functionality simultaneously. Despite various attempts, none of the requisite condensation product could be detected.

The next most probable "amino-acid" type compound to carry this reaction out on would be the dipeptides formed and discussed in coupling discussion, **2.24 – 2.31**. To allow for this type of reaction to take place it would initially be necessary to convert the ester to a carboxylic acid, to allow for the expected intermediate to form, as discussed by Wong *et al.*[126] Carrying out the condensation reaction on the free acid dipeptide, with benzaldehyde, proved to be unsuccessful as multiple products formed. In order to test the

reaction conditions discussed by Wong *et al.* acetyl glycine (**2.49**) was prepared[134] and the condensation reaction carried out with benzaldehyde, as per publication.[125] The expected cyclic intermediate (**2.50**) was obtained in a 41% yield (lit.[125a] 67%).



2.50

Scheme 2.16 Formation of the cyclic intermediate

Further literature studies pointed towards the need for basic conditions during the condensation reaction. Fürstner *et al.*[135] discussed the need for two equivalents of aldehyde per active site, to compensate for loss of aldehyde in side reactions. They carried out their condensation reactions in DMSO (dimethyl sulfoxide), in the presence of 2M NaOH, at 60 °C for 24 hours. We attempted these reaction conditions on compounds (**2.47**) and (**2.48**), using benzaldehyde. Due to no reaction taking place we attempted the reaction a last time using 4(5)-imidazole carboxaldehyde and compound (**2.47**), under the pre-mentioned conditions, but still none of the desired product was formed. The continued failure to achieve positive results with a substrate of any different form from those discussed by Wong, may suggest a substrate specificity for this reaction.

2.6.2.3. *tert*-Butyl hypochlorite

Another method to introduce a double bond directly into the prepared diketopiperazine was the treatment of the diketopiperazine with *t*-butyl hypochlorite. This treatment was expected to result in the desired unsaturation.

2.6.2.3.1 Preparation of *t*-BuOCl

t-BuOCl (**2.51**) was prepared from sodium hypochlorite, acetic acid and *t*-butyl alcohol by the method described by Mintz and Walling.[136]

2.6.2.3.2 Using *t*-BuOCl as a dehydrogenating reagent

Once again, the introduction of the double bond into the prepared diketopiperazine was carried out on 3-(4-imidazolylmethyl)-2,5-piperazinedione, where histidine has been coupled to glycine and cyclised. The reaction was carried out as described by Hauptmann,[137] changing the reaction solvent to methanol and using sodium methoxide as a deprotonating agent. The reaction was carried out at 0 °C, using 2 equivalents of *t*-BuOCl. This reaction proved to be unsuccessful and it was decided to repeat the reaction using *t*-butanol as solvent and potassium *tert*-butoxide as base. This reaction also proved to be unsuccessful.

Disappointingly enough, no success was obtained in attempting to add the desired unsaturation to our synthesised diketopiperazine or to any other possible starting material, under the reaction conditions used. It was with regret that time constraints forced the termination of this portion of the structure-activity study relating to synthetic analogues of viridamine.

2.7 CONCLUSIONS AND FUTURE WORK

It has been established that the multifunctional nature of histidine renders it a particularly difficult amino acid on which to perform certain chemical transformations. Part of the problem lies in the formation of a zwitterion, which renders the free acid derivatives of histidine water-soluble. The problem has been resolved by using the histidine methyl ester instead of the free acid.

Neutralisation of histidine has proved to be a problem due to the formation of the zwitterion. Successful neutralisation (without isolation of the neutralised ester) has been achieved *in situ* by the addition of a base.

Coupling of the histidine moiety to the other amino acids *via* its carboxylic acid functionality proved to be difficult. This problem was successfully overcome by employing the amine functionality of the histidine for the peptide coupling instead. This approach led to the preparation of a small number of histidine-based dipeptides, with the use of *N*-hydroxybenzotriazole resulting in better yields. The coupled products were isolated by flash chromatography and characterised by NMR (300MHz) spectrometry, amongst other analytical techniques.

Once the dipeptide products had been prepared, it remained to remove the Boc protecting group to allow for cyclisation. As expected, the histidine functionality proved to be a problematic in this transformation. The addition of TFA for the removal of the Boc group resulted in decomposition of the dipeptide, even when used for as short a time as 25 minutes. Formic acid was then substituted for TFA, which allowed for the successful removal of the Boc protecting group. Isolation of the deprotected product proved to be difficult, as a result of the formation of the formate salt. *In situ* deprotection and cyclisation was initially achieved using the formic acid, but the products obtained were impure and the reactions low yielding. The addition of an extra step, *i.e.* heating the formate salt in a toluene / 2-butanol mixture under reflux, improved the yield but still resulted in the need for a tiresome purification of the cyclic compound. The desired diketopiperazines were finally purified by flash chromatography.

Various complexation studies were carried out on the respective diketopiperazines. It was initially thought that a base was essential for complexation of our diketopiperazines to the three divalent metal ions of interest; *i.e.* nickel, copper and cobalt. This was later found to be dependant on the desired manner of complexation and the ratio of metal:ligand, as discussed in section **2.5**.

NaOAc did not appear to be a strong enough base with which to effect complexation, when using a 1:2 metal:ligand ratio. The use of NaOMe appeared to be able to initiate complexation but resulted in immediate precipitation. This process rendered an X-Ray diffraction analysis of the complexes very difficult. Crystals were obtained but unfortunately were not stable in the solvent from which they crystallised and were lost before further analysis could be carried out.

Further complexation studies were carried out according to published procedures. Determination of the manner of complexation has not yet been possible, as a result of the formation of very small brittle crystals. Of particular interest was the complexation reactions carried out under the exact conditions reported by Hori *et al.*, *i.e.* involving the use of a 2:1 ligand:metal mixture in methanol. Small brittle crystals were obtain, which made X-Ray studies very difficult, but because of the analyses reported by Hori the possibility for selective complexation remained. From the UV analyses, it could be seen that the complexation was greatest for copper, then for cobalt and finally with nickel having the lowest absorption in the spectra.

Various approaches were attempted to introduce a double bond to the diketopiperazines, with no success. This was hoped to enhance selectivity during complexation, by the more highly conjugated structure that would result.

Due to a product mixture being obtained during cyclisation, it remains to optimise the cyclisation conditions so as to force the uncyclised intermediate to the desired diketopiperazine. Such optimisation reactions would be of great interest as they would eliminate the lengthy column chromatography, which is necessary at this stage.

The project remains open to further adaptations of the structure of the diketopiperazine, such as substitutions onto the imidazole ring, and test for selectivity with a variety of metal ions, as a result of the structure adaptations. It would furthermore be of utmost importance to be able to incorporate the unsaturation into the diketopiperazine structures. Such a move would allow a better structure-activity relationship. It has already been

shown in this study that the double bond is essential for successful and rapid complexation to take place.

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CHAPTER 3

EXPERIMENTAL

3.1 GENERAL

SOLVENTS

All solvents used for chromatography were analytically pure or pre-purified. Ethyl acetate (EtOAc) was distilled from anhydrous potassium carbonate. Hexane was purified by distillation. All other reagents were analytically or synthetically pure.

CHROMATOGRAPHY

Thin-layer chromatography (TLC) was performed using Merck GF_{254} silica gel plates with a 0.25 mm silica layer. TLC plates were developed in a variety of solvent systems. The solvent system ratios refer to v:v ratios. Development of the plate was accomplished using ultraviolet light (254 nm) followed by dipping the plate in ninhydrin and heating in an oven. In some cases, the ninhydrin was not necessary and the plates were placed in an iodine tank. Column chromatography was carried out on Merck Kieselgel 60 (70-230) and "flash chromatography" was carried out on Merck Kieselgel 60 (230-400 mesh), under positive nitrogen pressure.

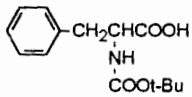
INSTRUMENTATION

NMR spectra were recorded on a Varian Gemini-300 or on a Varian VXR 200 spectrometer. Unless otherwise specified, all NMR spectra were obtained from solutions in deuterochloroform ($CDCl_3$) and are reported in parts per million (ppm, δ) and referenced to the relevant solvent peak. In some cases, it was necessary to employ proton-proton decoupling experiments to confirm the assignment of peaks. The peak multiplicity of the signals are abbreviated as follows: **s** - singlet, **d** – doublet, **dd** – doublet of doublets, **t** – triplet, **td** – triplet of doublets, **dt** – doublet of triplets, **q** - quartet and **m** – multiplet. The coupling constants (*J*) are calculated in Hz and reported to the nearest 0.1 Hz. Melting points were obtained using Reichert Kofler hot-stage apparatus

and are uncorrected. Electron-impact mass spectra were recorded on a Finnigan-MAT 8200 spectrometer at 70 eV, unless otherwise specified.

3.2 PROTECTION OF THE AMINO ACID AMINE GROUP

Boc-*L*-phenylalanine (**2.5**)



A round-bottomed flask equipped with a stirrer was charged with a sodium hydroxide solution (1.98 ml, 1 M).[1] To this solution were added L-phenylalanine (0.3 g, 1.82 mmol) and *tert*-butyl alcohol (1.36 ml, 14.47 mmol). After stirring for 5 minutes the solution had reached *p*H 11.5. Di-tert-butyl dicarbonate (0.414 ml, 1.82 mmol) was added drop-wise over approximately 30 minutes. The reaction was brought to completion by stirring overnight. At this stage the reaction mixture had reached a *p*H of 8. The solution was extracted twice with pentane (5 ml) and the organic phase was extracted three times with saturated aqueous sodium bicarbonate solution (5 ml). The combined aqueous layers were acidified with potassium hydrogen sulfate powder ($KHSO_4$), under vigorous stirring. Copious evolution of carbon dioxide was observed during the addition of $KHSO_4$. The reaction mixture was extracted four times with ether (50 ml). The combined organic layers were washed twice with water (30 ml), dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and the solvent was removed *in vacuo* to yield a yellow oil. Flash chromatography (2% MeOH in EtOAc) afforded **2.5** as a white sticky solid (308 mg, 64% yield).

Mp: 85-87 °C (Lit. 86-88 °C)[1]

TLC: R_f 0.51 (2% MeOH in EtOAc).

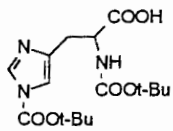
^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 7.30 - 7.14 (m, 5H), 6.10 (s, 1H), 4.98 (d, 1H, J = 7.2 Hz), 4.55 (dt, 1H, J = 7.2 and 6.3 Hz), 3.23 (dd, 1H, J = 13.7 and 5.3 Hz), 2.98 (dd, 1H, J = 13.7 and 6.3 Hz), 1.39 (s, 9H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 176.1, 155.4, 135.8, 129.3, 128.5, 126.9, 80.3, 54.6, 37.8, 28.3.

EIMS: 265 (M^+, 2%), 209 (M^+-C_4H_8, 56%), 192 (M^+-C_4H_9O, 11%), 164 (M^+-

$C_5H_9O_2$, 43%), 148 ($C_9H_{10}NO$, 96%), 120 ($C_8H_{10}N$, 55%), 91 (C_7H_7, 89%), 57 (C_4H_9, 100%).

N^{α},N^{im}-Bis(tert-butyloxycarbonyl)-L-Histidine (**2.6**)



A round bottomed flask equipped with a stirrer was charged with a potassium bicarbonate solution (1.82 ml, 1 M). To this solution were added L-Histidine (0.282 g, 1.82 mmol) and tert-butyl alcohol (1.36 ml, 14.47 mmol). The reaction solution was heated to 60 °C and left to stir at 60 °C. After 15 minutes. di-tert-butyl dicarbonate (Boc_2O) (0.827 ml, 3.60 mmol) was added drop-wise over approximately 15 minutes. The reaction mixture was allowed to stir for 5 hr at room temperature and a further 5 hr at 60 °C. A second portion of Boc_2O (0.414 ml, 1.82 mmol) was added and the solution was brought to completion by stirring overnight. At this stage the reaction solution had reached *p*H 8. The mixture was extracted twice with pentane (5 ml) and the organic phase was extracted three times with saturated aqueous sodium bicarbonate solution (5 ml). The combined aqueous layers were acidified with $KHSO_4$, under vigorous stirring. Copious evolution of carbon dioxide was observed during the addition of $KHSO_4$. The reaction mixture was extracted four times with ether (50 ml). The combined organic layers were washed twice with water (30 ml), dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo* to yield a yellow oil. Flash chromatography (2:1 hexane:EtOAc) afforded **2.6** (11 mg, 2% yield). TLC analysis indicated the same product was dissolved in the aqueous layer, but no amount of effort resulted in a successful extraction into an organic phase.

Mp: 169-171 °C (LIT 170 °C[1])

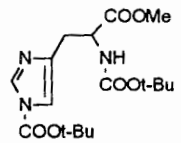
TLC: R_f 0.75 (2% MeOH in EtOAc).

^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 7.53 (s, 1H), 6.79 (s, 1H), 5.64 (d, 1H, J = 7.5 Hz), 4.37 (dt, 1H, J = 7.5 and 5.1 Hz), 3.04 (d, 2H, J = 5.1Hz), 1.41 (s, 9H), 1.40 (s, 9H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 171.1, 155.5, 145.1, 134.8, 132.9, 117.7, 81.9, 79.8, 53.9, 29.7, 28.3, 27.9.

EIMS: 355 (M^+, 2%), 338 (M^+ - OH, 0.1%), 311 (M+1 - COOH, 3%), 299 (M^+ - C_4H_8, 1%), 282 (M^+ - C_4H_9O, 1%), 255 (M+1 - $C_5H_9O_2$, 2%), 243 (M^+ - C_8H_{16}, 2%), 210 (M^+ - $C_6H_9O_4$, 8%), 182 (M^+ - $C_7H_{11}NO_4$, 17%), 154 ($C_6H_8N_3O_2$, 25%), 138 ($C_6H_6N_2O_2$, 13%), 110 ($C_4H_2N_2O_2$, 42%), 82 (C_3NO_2, 42%) 81 ($C_4H_5N_2$, 23%), 57 (C_4H_9, 100%), 41 (C_2H_3N, 26%).

IR ν_{max} ($CHCl_3$) / cm^{-1} 3700, 3440, 1710, 1560, 1500, 1400, 1380, 1300, 1260, 1160, 1100, 1060, 1020, 810.



N^{α},N^{τ}-Bis(tert-butyloxycarbonyl)-L-Histidine methyl ester (**2.7**)

Sodium (96 mg, 4.18 mmol) was dissolved in methanol (MeOH) (4 ml) to form a standardised sodium methoxide solution. L-Histidine (503 mg, 2.08 mmol) was dissolved in MeOH (*ca.* 2 ml). The sodium methoxide solution was added to the histidine / MeOH solution and the reaction mixture was allowed to stir for approximately 30 minutes. The reaction solution was brought to dryness *in vacuo*. To the residue was added aqueous $KHCO_3$ (2.09 ml, 1 M) and the mixture was diluted with t-BuOH (1.56 ml). Stirring was initiated and after 20 minutes two equivalents of Boc_2O (0.96 ml, 4.16 mmol) were added drop-wise. The reaction was brought to completion by stirring overnight. $KHSO_4$ was added to the reaction solution until a *p*H of 1 was obtained. Washing with ether (2 × 15 ml) and twice with water (10 ml) completed the workup. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. Flash chromatography (EtOAc) afforded **2.7** (324 mg, 42% yield) as a white powder.

Analytical data provided on next page.

N^{α},N^{τ}-Bis(tert-butyloxycarbonyl)-L-Histidine methyl ester (**2.7**) and

N^{α},N^{π}-Bis(tert-butyloxycarbonyl)-L-Histidine methyl ester (**2.8**)

Histidine methyl ester (100 mg, 0.413mmol) was dissolved in methanol (0.9 ml) with stirring.[2] Triethylamine (0.12 ml, 0.826 mmol) was added dropwise and the reaction mixture was allowed to stir for 10 minutes. Di-tert-butyl dicarbonate (0.19 ml, 0.826 mmol) was added dropwise. After a reaction period of 14 hours, the solvent was removed under reduced pressure to yield feathery white crystals. Purification by flash chromatography (2:1 hexane:EtOAc) yielded two fractions of **2** (major fraction: 116 mg, 76 % yield, minor fraction: 11 mg, 7 % yield) with a white waxy solid as the major product and the minor product as an oil.

Major Product (**2.7**):

Mp: 105 - 107 °C

TLC: R_f 0.38 (2:1 hexane:EtOAc).

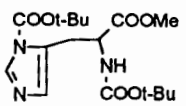
^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 7.92 (s, 1H), 7.08 (s, 1H), 5.68 (d, 1H, J = 8.0 Hz), 4.51 (dt, 1H, J = 8.0 and 4.9 Hz), 3.67 (s, 3H), 3.04 (dd, 1H, J = 14.5 and 5.4 Hz), 2.99 (dd, 1H, J = 14.5 and 5.0 Hz), 1.55 (s, 9H), 1.38 (s, 9H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 172.1, 155.3, 146.7, 138.5, 136.8, 114.4, 85.5, 79.6, 53.1, 52.2, 30.2, 28.3, 27.8.

EIMS: 369 (M^+, 3%), 313 (M^+ - C_4H_8, 42%), 257 (M^+ - C_8H_{16}, 64%), 226 (M^+ -$C_9H_{19}O$, 5%), 196 (M^+ - $C_{10}H_{20}O_2$, 50%), 152 ($C_7H_8N_2O_2$, 74%), 110 ($C_4H_2N_2O_2$, 28%), 82 ($C_4H_6N_2$, 74%), 57 (C_4H_9, 100%), 41 (C_2H_3N, 65%), 29 (CH_3N, 45%).

IR ν_{max} ($CHCl_3$) / cm^{-1} 3800, 3440, 1760, 1710, 1520, 1460, 1400, 1380, 1300, 160, 1160, 1020, 850.

Minor Product (**2.8**):



TLC: R_f 0.15 (2:1 hexane:EtOAc).

^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 8.00 (s, 1H), 6.81 (s, 1H), 5.07 (d, 1H, J = 8.1 Hz), 4.61 (dt, 1H, J = 8.1 and 6.5 Hz), 3.71 (s, 3H), 3.51 (dd, 1H, J = 14.3 and

6.2 Hz), 3.01 (dd, 1H, J = 14.3 and 8.0 Hz), 1.62 (s, 9H), 1.36 (s, 9H).

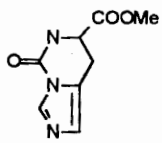
^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 172.2, 147.7, 138.7, 130.9,127.3, 114.4, 85.9, 79.9, 52.7, 52.4, 28.8, 28.3, 27.9.

N^{α},N^{τ}-Bis(*tert*-butyloxycarbonyl)-L-Histidine methyl ester (**2.7**)

A ONE POT CONVERSION

Histidine (100 mg, 0.644mmol) was dissolved in methanol (0.9 ml). Di-tert-butyl dicarbonate (1.29 ml, 1.288 mmol) was added dropwise and the reaction mixture was left to stir for two days. The reaction solution was brought to dryness *in vacuo*, purified by flash chromatography (2:1 hexane:EtOAc) to yield **2.7** (32 mg, 14% yield) as a white waxy solid.

DIPROTECTION USING CDI



5,6,7,8-Tetrahydro-7-methoxycarbonyl-5-oxoimidazo[1,5-c] pyrimidine (**2.11**)

All apparatus was flamed out under vacuum and flushed with nitrogen. Histidine methyl ester dihydrochloride (50 mg, 0.21 mmol) was dissolved in dimethyl formamide (DMF) (1 ml). Stirring was initiated and 1,1-carbonyl diimidazole (34 mg, 0.21 mmol) was added, after which the reaction was allowed to proceed for 14 hours at ambient temperature. The solvent was removed *in vacuo*, and the crude yellow oil was dissolved in DCM. An acidic workup was carried out with dilute HCl (*p*H 5). The organic and aqueous layers were separated and the organic layer was dried over Na_2SO_4. Pyrimidine **2.11** was obtained, after bringing the residue to dryness *in vacuo*, as white powdery crystals (12 mg, 30%). The yield was improved by the addition of N-methyl morpholine (0.09 ml, 0.83 mmol) at the start of the reaction (27 mg, 68%). The yield was further

improved by eliminating the acidic workup and running the product through a flash column (2% MeOH / EtOAc) (33 mg, 83%).

Mp: 164-166 °C (Lit. 166-168 °C)[3]

TLC: R_f 0.32 (2% MeOH / EtOAc).

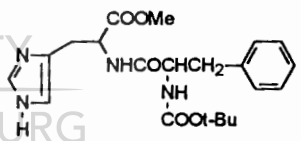
^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 8.14 (d, 1h, J = 0.9 Hz), 6.87 (d, 1H, J = 0.9 Hz), 6.31 (bs, 1H), 4.34 (ddd, 1H, J = 8.9, 5.3 and 1.8 Hz), 3.80 (s, 1H), 3.42 (ddd, 1H, J = 15.6, 5.4 and 1.2 Hz), 3.32 (ddd, 1H, J = 15.6, 8.3 and 1.2 Hz).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 169.6, 147.8, 135.1, 126.2, 123.8, 53.3, 52.7, 23.2.

EIMS: 195 (M^+, 27%), 152 (M^+ - CHNO, 33%), 136 ($C_2H_3O_2$, 47%), 81 ($C_4H_5N_2$, 100%), 28 (CO, 30%).

3.3. AMINO ACID COUPLING

Boc-L-phenylalanyl histidine methyl ester (**2.24**)



Boc-L-phenylalanine (78 mg, 0.29 mmol) and L-Histidine methyl ester (50 mg, 0.29 mmol) were dissolved in DCM (2 ml) and the solution was cooled in an ice / water bath while dicyclohexyl carbodiimide (DCC) (71 mg, 0.35 mmol) was added. Stirring was continued for an hour at 0 °C and for a further 5 hr at 40 °C. The reaction solution was allowed to reach room temperature and left to stir overnight. The *N,N'*-dicyclohexyl urea (DCU) that had formed was removed by filtration and the excess solvent removed *in vacuo*. The reaction mixture was extracted with four portions of DCM (30 ml) and the combined organic phase layers washed with two portions of $NaHCO_3$ (20 ml). The organic phase was dried over Na_2SO_4 and filtered and the solvent was removed *in vacuo*. The residue was purified by flash chromatography, which was gradient eluted with a series of solvent systems, (DCM, 1:1 hexane:EtOAc; 10% hexane in acetone, followed by pure acetone). On bringing the fractions to dryness *in vacuo* **2.24**, was obtained as white crystals (75 mg, 62% crude yield).

Mp: 136-138 °C

TLC: R_f 0.83 (acetone).

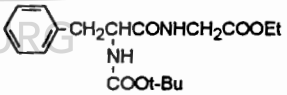
^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 8.05 (bs, 1H), 7.49 (s, 1H), 7.30 – 7.15 (m, 5H), 7.00 (d, 1H, J = 6. Hz), 6.70 (s, 1H), 5.17 (d, 1H, J = 6.6 Hz), 4.72 (dt, 1H, J = 6.9 and 4.5 Hz), 4.25 (dt, 1H, J = 7.6 and 6.9 Hz), 3.66 (s, 3H), 3.09 (dd, 2H, J = 19.0 and 4.5 Hz), 2.95 (dd, 2H, J = 14.0 and 7.6 Hz), 1.38 (s, 5H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 171.2, 170.9, 155.9, 136.2, 135.3, 132.4, 129.2, 128.6, 126.9, 121.2, 80.6, 56.4, 52.8, 52.5, 37.9, 29.7, 28.3.

EIMS: 416 (M^+, 65%), 385 (M^+ -CH_3O, 2%), 360 (M^+ - C_4H_8, 20%), 343 (M^+ - C_4H_9O, 23%), 325 (M^+ - C_7H_7, 15%), 196 ($C_{13}H_{18}NO_2$, 79%), 152 ($C_7H_8N_2O_2$, 77%), 120 ($C_6H_4N_2O$, 66%), 57 (C_4H_9, 100%).

IR ν_{max} ($CHCl_3$) / cm^{-1} 3445, 3304, 2992, 2741, 1741, 1692, 1498, 1456, 1440, 1396, 1377, 1342, 1297, 1249, 1169.

Boc-L-phenylalanyl glycine ethyl ester (**2.25**)



Boc-L-phenylalanine (100 mg, 0.38 mmol), glycine ethyl ester hydrochloride (53 mg, 0.38 mmol) and *N*-methyl morpholine (0.04 ml, 0.38 mmol) were dissolved in dry THF (2 ml). Stirring was initiated and the solution was cooled with an ice / water bath while DCC (82 mg, 0.40 mmol) was added. Stirring was continued for an hour at 0 °C and a further hour at room temperature. The reaction was left to stir and was then extracted with EtOAc (50 ml) and $NaHCO_3$ (25 ml, sat.). The organic phase was further washed with $NaHCO_3$ (20 ml, sat.) and water (10 ml). The aqueous layers were extracted with ether and the combined organic layers were dried over Na_2SO_4, filtered and the excess solvent removed *in vacuo*. Purification of the residue was achieved by flash chromatography (2:1 hexane:EtOAc), affording **2.25** (108 mg, 82% yield).

Mp: 89-92 °C (Lit. 89-91 °C)[4]

TLC: R_f 0.91 (2% MeOH in EtOAc).

^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 7.29 – 7.11 (m, 5H), 6.73 (t, 1H, J = 5.3 Hz), 5.20 (d, 1H, J = 8.10 Hz), 4.41 (dt, 1H, J = 8.1 and 6.3 Hz), 4.14 (q, 2H, J = 7.10 Hz), 3.99 (dd, 1H, J = 17.8 and 5.2 Hz), 3.87 (dd, 1H, J = 17.8 and 4.4 Hz), 3.15 (dd, 1H, J = 13.8 and 6.3 Hz), 2.93 (dd, 1H, J = 13.8 and 7.6 Hz), 1.33 (s, 9H), 1.23 (t, 3H, J = 7.2 Hz).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 171.6, 169.4, 155.4, 136.6, 129.3, 128.6, 126.8, 80.2, 61.4, 55.7, 41.3, 38.4, 28.2, 14.1.

EIMS: 350 (M^+, 1%), 294 (M^+ - C_4H_8, 12%), 277 (M^+ - C_4H_9O, 8%), 259 (M^+ - C_7H_7, 1%), 249 (M^+ - $C_5H_9O_2$, 7%), 233 (M^+ - $C_5H_{11}NO_2$, 14%), 220 (M^+ - $C_{13}H_{18}NO_2$, 9%), 164 ($C_9H_{12}N_2O$, 38%), 120 ($C_8H_{11}N$, 71%), 57 (C_4H_9, 100%).

IR ν_{max} ($CHCl_3$) / cm^{-1} 3440, 1750, 1740, 1710, 1690, 1660, 1530, 1510, 1500, 1380, 1170, 1030.

Boc-L-phenylalanyl glycine ethyl ester (**2.25**)

USING A REACTION PROMOTER

Experimental procedure as described above, but including the addition of the reaction promoter, *N*-hydroxy phthalimide (62 mg, 0.38 mmol). The crude residue was purified by flash chromatography (2:1 hexane:EtOAc). Compound **2.25** was the first fraction obtained from the column (49 mg, 37% yield).

Boc-L-phenylalanyl glycine ethyl ester (**2.25**)

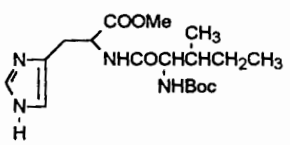
USING A REACTION PROMOTER

The experimental procedure was carried out as described above, but including the addition of the reaction promoter, *N*-hydroxybenzotriazole (51 mg, 0.38 mmol). The reaction residue was purified by flash chromatography (1:2 hexane:EtOAc). Compound **2.25** was obtained (108 mg, 82% yield) as fine white crystals.

Boc-L-phenylalanyl histidine methyl ester (**2.24**)

The coupling reactions were carried out as before (see pg 121) but with the following changes; using two equivalents of the reaction promoter, *N*-hydroxybenzotriazole (102 mg, 0.75 mmol) and using two equivalents of Nmm. The reaction residue was purified by flash chromatography (2% MeOH / EtOAc, later with 5% MeOH / EtOAc). Compound **2.24** was obtained (119 mg, 76% yield) as fine white crystals.

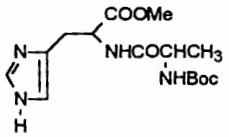
Boc-L-isoleucyl histidine methyl ester (**2.26**)



Synthesised as described above for **2.24**.

Yield:	56%
Mp:	144-146 °C
TLC:	R_f 0.42 (10% MeOH / EtOAc).
^{1}H NMR:	(300 MHz, $CDCl_3$) δ_H 8.00 (bs, 1H), 7.52 (d, 1H, J = 0.6 Hz), 7.20 (bs, 1H), 6.74 (s, 1H), 5.21 (d, 1H, J = 7.8 Hz), 4.78 (dt, 1H, J = 7.2 and 4.5 Hz), 3.86 (dd, 1H, J = 7.8 and 7.2 Hz), 3.68 (s, 3H), 3.13 (d, 2H, J = 4.5 Hz), 1.84 – 1.70 (m, 1H), 1.69 – 1.52 (m, 1H), 1.41 (s, 9H), 1.21 – 1.17 (m, 1H), 0.91 (d, 3H, J = 6.6 Hz), 0.88 (t, 3H, J = 7.4 Hz).
^{13}C NMR:	(75 MHz, $CDCl_3$) δ_C 171.4, 171.1, 156.2, 135.2, 129.2, 128.5, 80.3, 59.5, 52.6, 52.5, 36.9, 29.7, 28.4, 24.9, 15.4, 11.2.
EIMS:	383 (M+1, 4%), 351 (M^+ - CH_3O, 0.5%), 327(M+1 - C_4H_8, 2%), 310 (M+1 - C_4H_9O, 2%), 268 (M^+ - $C_5H_{10}NO_2$), 243 ($C_{12}H_{23}N_2O_3$, 2%), 226 ($C_9H_{14}N_4O_3$, 1%), 196 ($C_8H_{10}N_3O_3$, 14%), 168 ($C_7H_{10}N_3O_2$, 4%), 110 ($C_5H_8N_3$, 10%), 82 ($C_4H_6N_2$, 25%), 57 (C_4H_9, 46%), 44 (CO_2, 100%).
IR	ν_{max} ($CHCl_3$) / cm^{-1} 3445, 3292, 2992, 2973, 2935, 2884, 1747, 1695, 1504, 1460, 1449, 1373, 1338, 1297, 1173.

Boc-L-alanyl histidine methyl ester (**2.27**)



Synthesised as described above for **2.24**.

Yield: 40%

Mp: 118-120 °C

TLC: R_f 0.27 (10% MeOH / EtOAc).

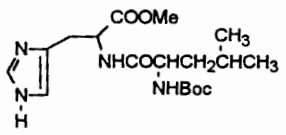
^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 7.84 (bs, 1H), 7.49 (s, 1H), 7.27 (s, 1H), 6.73 (s, 1H), 5.31 (d, 1H, J = 6.6 Hz), 4.75 (dt, 1H, J = 7.5 and 4.5 Hz), 4.09 (qd, 1H, J = 7.2 and 6.6 Hz), 3.68 (s, 3H), 3.13 (d, 2H, J = 4.5 Hz), 1.40 (s, 9H), 1.32 (d, 3H, J = 7.2 Hz).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 172.5, 171.2, 155.8, 135.3, 129.9, 120.5, 80.4, 52.8, 52.5, 50.6, 30.9, 28.3, 18.0.

EIMS: 341 (M+1, 73%), 310 (M+1-CH_3O, 3%), 285(M+1-C_4H_8, 41%), 268 (M+1-C_4H_9O, 34%), 254 (M+1-$C_5H_{11}O$, 4%), 225 (M+1-$C_5H_{10}NO_2$, 15%), 196 ($C_8H_{10}N_3O_3$, 100%), 152 ($C_7H_8N_2O_2$, 99%), 136 ($C_6H_6N_3O$, 26%), 110 ($C_5H_8N_3$, 40%), 82 ($C_4H_6N_2$, 96%), 57 (C_4H_9, 81%), 44 (CO_2, 48%).

IR ν_{max} ($CHCl_3$) / cm^{-1} 3444, 3430, 3297, 2970, 2942, 2888, 1747, 1688, 1504, 1441, 1372, 1252, 1173, 1052, 1027.

Boc-L-leucyl histidine methyl ester (**2.28**)



Synthesised as described above for **2.24**.

Yield: 69%

Mp: 106-108 °C

TLC: R_f 0.43 (10% MeOH / EtOAc).

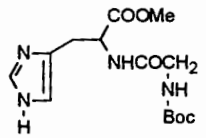
^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 8.78 (v bs, 1H), 7.51 (s, 1H), 7.19 (d, 1H, J = 7.2 Hz), 6.73 (s, 1H), 5.16 (d, 1H, J = 7.2 Hz), 4.76 (dt, 1H, J = 7.2 and 4.5

Hz), 4.03 (td, 1H, J = 8.4 and 7.2 Hz), 3.69 (s, 3H), 3.14 (d, 2H, J = 4.2 Hz), 1.74 – 1.45 (m, 3H), 1.41 (s, 9H), 0.91 (d, 3H, J = 5.7 Hz), 0.89 (d, 3H, J = 6.0 Hz).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 172.3, 171.0, 156.2, 135.4, 129.1, 121.5, 80.5, 53.8, 52.8, 52.5, 40.9, 28.4, 27.9, 24.7, 22.8, 22.1.

EIMS: 383 (M+1, 100%), 245 (M+1-$C_5H_9O_2$, 15%), 213($C_{10}H_{17}N_2O_3$, 3%), 196 ($C_8H_{10}N_3O_3$, 67%), 169 ($C_7H_9N_3O_2$, 17%), 152 ($C_7H_8N_2O_2$, 28%), 110 ($C_5H_8N_3$, 16%), 82 ($C_4H_6N_2$, 55%), 57 (C_4H_9, 59%), 28(CO, 45%).

IR ν_{max} ($CHCl_3$) / cm^{-1} 3432, 3298, 2992, 2973, 2941, 2864, 1747, 1692, 1504, 1460, 1441, 1373, 1338, 1313, 1252, 1166.



Boc-L-glycyl histidine methyl ester (**2.29**)

Synthesised as described above for **2.24**. Due to this reaction being a very low yielding reaction, three solvents were tried to see the effect on the yield.

Yield: 16% (EtOAc), 26% (THF), 39% (DMF), 79% (THF)(no work-up)

Mp: 124 - 126 °C

TLC: R_f 0.09 (10% MeOH / EtOAc).

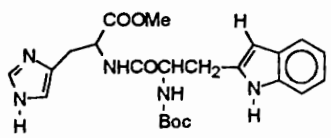
^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 9.00 (vbs, 1H), 7.75 (d, 1H, J = 7.1 Hz), 7.41 (s, 1H), 6.69 (s, 1H), 5.91 (t, 1H, J = 4.8 Hz), 4.68 (dt, 1H, J = 7.1 and 5.1 Hz), 3.70 (d, 2H, J = 4.8 Hz), 3.57 (s, 3H), 3.01 (d, 2H, J = 5.1 Hz), 1.33 (s, 9H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 170.9, 169.7, 156.2, 135.3, 132.3, 117.3, 79.9, 60.2, 52.5, 52.2, 43.9, 28.1.

EIMS: 327 (M+1, 1%), 296 (M+1 – CH_3O, 0.5%), 271 (M+1 – C_4H_8, 1%), 254 (M+1 – C_4H_9O, 1%), 196 ($C_8H_{10}N_3O_3$, 10%), 167 ($C_7H_{11}N_4O$, 2%), 152 ($C_7H_{10}N_3O$, 18%), 110 ($C_5H_8N_3$, 7%), 84 (C_4H_6NO, 100%), 82 ($C_4H_6N_2$, 17%).

IR ν_{max} ($CHCl_3$) / cm^{-1} 3560, 3530, 3300, 2990, 1749, 1720, 1700, 1660, 1650, 1640, 1560, 1510, 1440, 1370, 1250, 1170, 1080, 1050, 1030.

Boc-L-tryptophyl histidine methyl ester (**2.30**)



Synthesised as described above for **2.24**.

Yield: 50%

Mp: 132-134 °C

TLC: R_f 0.39 (10% MeOH / EtOAc).

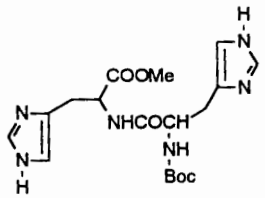
^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 8.87 (s, 1H), 7.58 (d, 1H, J = 7.6 Hz), 7.35 (s, 1H), 7.30 (d, 1H, J = 8.1 Hz), 7.16 – 7.06 (m, 3H), 6.95 (s, 1H), 6.56 (s, 1H), 5.31 (d, 1H, J = 6.6 Hz), 4.68 (dt, 1H, J = 6.6 and 4.7 Hz), 4.38 (dt, 1H, J = 6.9 and 6.6 Hz), 3.59 (s, 3H), 3.29 (dd, 1H, J = 14.7 and 6.6 Hz), 3.16 (dd, 1H, J = 14.7 and 6.9 Hz), 3.02 (d, 2H, J = 4.8 Hz), 1.41 (s, 9H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 171.8, 171.0, 155.8, 136.2, 135.2, 129.4, 127.3, 123.4, 121.9, 120.5, 119.5, 118.6, 111.3, 109.8, 80.5, 55.6, 52.8, 52.5, 33.9, 29.7, 28.3.

EIMS: 456 (M+1, 17%), 382 (M^+ - C_4H_9O, 2%), 339 (M^+ – $C_5H_{10}NO_2$, 19%), 327 (M+1 – C_9H_7N, 13%), 267 (M^+ - $C_{11}H_{12}N_2O$, 5%), 226 ($C_{13}H_{10}N_2O_2$, 37%), 170 ($C_{11}H_8NO$, 69%), 130 (C_9H_8N, 76%), 82, ($C_4H_6N_2$, 28%), 57 (C_4H_9, 26%).

IR ν_{max} ($CHCl_3$) / cm^{-1} 3490, 3430, 3320, 3300, 3000, 2940, 2870, 1760, 1750, 1745, 1710, 1690, 1550, 1520, 1500, 1470, 1440, 1380, 1180.

Boc-L-histidyl histidine methyl ester (**2.31**)



Synthesised as described above for **2.24**.

Yield: 76%

Mp: 246-249 °C

TLC: R_f 0.26 (6% H_2O / 12% MeOH / EtOAc).

^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 7.74 (d, 2H, J = 10.2 Hz), 6.92 (d, 2H, J = 4.2 Hz), 4.68 (dt, 1H, J = 5.3 and 2.4 Hz), 4.32 (dt, 1H, J = 5.6 and 2.4 Hz), 3.70(s, 3H) 3.17 – 2.82 (m, 4H), 1.38 (s, 9H).

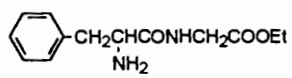
^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 173.7, 172.6, 157.5, 135.9, 135.7, 134.9, 133.0, 118.9, 118.4, 80.8, 56.3, 55.7, 53.0, 30.0, 29.7, 28.7.

EIMS: 409 (M+3, 31%), 308 (M+3 – $C_5H_9O_2$, 12%), 256 (M+3 – $C_9H_{14}N_2O_2$, 39%), 110 ($C_5H_8N_3$, 100%).

IR ν_{max} ($CHCl_3$) / cm^{-1} 3690, 3650, 2960, 2880, 1710, 1570, 1520, 1470, 1380, 1030.

3.4. *N*-DEPROTECTION AND DIKETOPIPERAZINE SYNTHESIS

L-Phenylalanyl glycine ethyl ester (**2.32**)



Boc-L-phenylalanine glycine ethyl ester (101 mg, 0.29 mmol) was dissolved in dichloromethane (1.26 ml).[5] Stirring was initiated and trifluoroacetic acid (TFA) (2.84 ml) was added. The reaction was left to stir overnight. The reaction mixture was brought to dryness *in vacuo*, and partitioned between DCM and $NaHCO_3$. The organic phase was dried over sodium sulfate, filtered and brought to dryness *in vacuo*, to yield **2.32**, (51 mg, 70%)

Mp: 196-200 °C

TLC: R_f 0.07 (1:2 Hexane:EtOAc).

^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 7.77 (s, 1H), 7.33 – 7.17 (m, 5H), 4.19 (q, 2H, J = 7.1 Hz), 4.06 (dd, 1H, J = 18.3 and 5.6 Hz), 3.97 (dd, 1H, J = 18.3 and 5.4 Hz), 3.63 (dd, 1H, J = 9.9 and 3.9 Hz), 3.29 (dd, 1H, J = 13.7 and 3.7 Hz), 2.65 (dd, 1H, J = 13.8 and 9.9 Hz), 1.43 (s, 2H), 1.27 (t, 3H, J = 7.1 Hz).

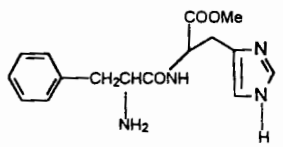
^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 174.5, 169.8, 137.8, 129.2, 128.6, 126.7, 61.4, 56.4, 41.0, 40.9, 14.2.

EIMS: 251 (M + 1, 2%), 205 (M^+ - C_2H_5O, 5%), 177 ($C_{10}H_{13}N_2O$, 4%), 146 (C_9H_8NO, 1%), 120 ($C_8H_{10}N$, 100%), 103 ($C_4H_9NO_2$, 22%), 77 (C_6H_5, 14%).

IR ν_{max} ($CHCl_3$) / cm^{-1} 3400, 1740, 1710, 1690, 1660, 1560, 1530, 1510.

In some cases, the isolation of the *N*-deprotected products was possible during purification of the diketopiperazine products by column chromatography. These intermediates were characterised by NMR spectrometry.

Optimisation of the diketopiperazine reaction was therefore necessary to force the reaction to completion, *i.e.* complete conversion to the cyclic diketopiperazine products.



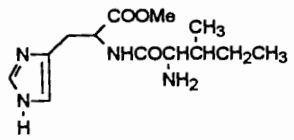
L-Phenylalanyl histidine methyl ester (**2.33**)

TLC: R_f 0.26 (4% H_2O / 12% MeOH / EtOAc).

^{1}H NMR: (300 MHz, CD_3OD) δ_H 8.33 (bs, 1H), 8.08 (s, 1H), 7.32 – 7.12 (m, 5H), 7.04 (s, 1H), 4.73 – 4.63 (m, 2H), 3.68 (s, 3H), 3.28 – 2.76 (m, 4H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 172.9, 172.2, 137.8, 135.6, 131.3, 130.2, 129.4, 127.8, 118.4, 54.6, 53.4, 52.9, 38.9, 34.8.

L-Isoleucyl histidine methyl ester (**2.34**)

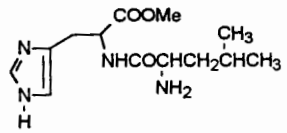


TLC: R_f 0.24 (4% H_2O / 12% MeOH / EtOAc).

^{1}H NMR: (300 MHz, CD_3OD) δ_H 7.63 (s, 1H), 6.89 (s, 1H), 4.67 (dd, 1H, J = 8.4 Hz), 4.33 (d, 1H, J = 7.2 Hz), 3.68 (s, 3H), 3.18 (dd, 1H, J = 14.9 and 5.6 Hz), 2.95 (dd, 1H, J = 14.9 and 8.4 Hz), 1.90 – 1.73 (m, 1H), 1.58 – 1.40 (m, 1H), 1.24 – 0.50 (m, 1H), 0.93 (d, 3H, J = 6.6 Hz), 0.88 (t, 3H, J = 7.2 Hz).

^{13}C NMR: (75 MHz, CD_3OD) δ_C 173.1, 172.8, 136.1, 133.9, 118.3, 57.5, 53.9, 52.7, 38.3, 29.8, 25.6, 15.8, 11.5.

L-Leucyl histidine methyl ester (**2.35**)

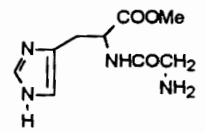


TLC: R_f 0.24 (4% H_2O / 12% MeOH / EtOAc).

^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 9.08 (bs, 1H), 8.12 (d, 1H, J = 7.2 Hz), 7.85 (s, 1H), 7.43 (d, 1H, J = 7.2 Hz), 6.87 (s, 1H), 4.77 (dd, 1H, J = 7.1 and 5.1 Hz), 4.53 (dd, 1H, J = 8.1 and 6.0 Hz), 3.69 (s, 3H), 3.21 (dd, 1H, J = 14.9 and 5.1 Hz), 3.05 (dd, 1H, J = 14.9 and 7.1 Hz), 1.67 – 1.48 (m, 3H), 0.89 (d, 3H, J = 6.3 Hz), 0.86 (d, 3H, J = 6.0 Hz).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 172.2, 171.0, 134.6, 130.3, 118.4, 52.6, 52.4, 50.8, 41.0, 27.8, 24.7, 22.8, 21.9.

L-Glycyl histidine methyl ester (**2.36**)

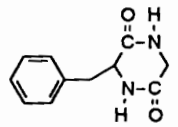


TLC: R_f 0.09 (4% H_2O / 12% MeOH / EtOAc).

^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 8.39 (s, 1H), 7.08 (s, 1H), 4.75 (dd, 1H, J = 8.3 and 5.1 Hz), 3.09 (s, 2H), 3.71 (s, 3H), 3.27 (dd, 1H, J = 15.0 and 5.1 Hz), 3.02 (dd, 1H, J = 15.0 and 8.3 Hz).

^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 172.5, 170.9, 135.7, 132.7, 118.3, 53.5, 53.0, 41.9, 28.9.

4-Phenylmethyl-2,5-diketopiperazine (**2.37**)



Phenylalanine glycine ethyl ester (54 mg, 0.22 mmol) was dissolved in methanol (0.5 ml). Stirring was initiated and the reaction was heated to 60 °C. The reaction was left to reflux at this temperature for approximately 5 hours. The residue was brought to dryness *in vacuo*. The white solid, which resulted, was recrystallised from ethanol to yield **2.37**, bulky white crystals (27 mg, 65%).

Mp: 273-275 °C (Lit. 273-275 °C)[6]

TLC: R_f 0.34 (2% MeOH / EtOAc)

^{1}H NMR: (300 MHz, DMSO) δ_H 8.14 (s, 1H), 7.88 (s, 1H), 7.28-7.14 (m, 5H), 4.05 (bs, 1H), 3.33 (dd, 1H, J = 17.4 and 6.9 Hz), 3.08 (dd, 1H, J = 13.5 and 4.5 Hz), 2.88 (dd, 1H, J = 13.5 and 4.8 Hz), 2.75 (d, 1H, J = 17.4 Hz).

^{13}C NMR: (75 MHz, DMSO) δ_C 166.9, 165.4, 135.8, 129.9, 127.9, 126.6, 55.4, 43.6, 38.7.

EIMS: 204 (M^+, 71%), 162 ($M^+ - C_2H_2O$, 1%), 132 ($M^+ - C_2H_4N_2O$, 3%), 120 ($M^+ - C_4H_6NO$, 5%), 113 ($M^+ - C_7H_7$, 11%), 91 (C_7H_7, 100%), 85 (C_4H_6NO, 42%), 57 (C_2H_3NO, 11%), 30 (C_2H_6, 13%), 28 (CO, 12%).

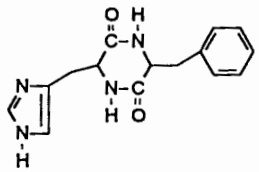
IR ν_{max} (KBr) / cm^{-1} (3600-2600 (vbr)), 1710, 1660, 1460, 1340, 1100, 1010, 870.

4-Phenylmethyl-2,5-diketopiperazine (**2.37**)

Boc-phenylalanine glycine ethyl ester (62 mg, 0.18 mmol) was dissolved in formic acid (6.2 ml) and the solution was allowed to stir at room temperature for 2 hours. The excess formic acid was removed *in vacuo* and the residue dissolved in a toluene / 2- butanol mixture (5 ml / 10 ml). The mixture was left stirring under reflux for 3 hours. The solvent level was reduced to approximately 3 ml, cooled to 0 °C, and placed in a

refridgerator overnight. A bulky white solid crystallised out, which was recrystallised from ethanol. The crystals were dried and characterised as **2.37** (23 mg, 64%).

3-(4-Imidazolylmethyl)-6-phenylmethyl-2,5-diketopiperazine (**2.38**)



Boc-phenylalanine histidine methyl ester (100 mg, 0.24 mmol) was dissolved in formic acid (10 ml). Stirring was initiated and the reaction mixture was allowed to stir for 4 hours at room temperature. The excess formic acid was removed *in vacuo* and the sticky gel obtained dissolved in a mixture of 2-butanol (6.2 ml) and toluene (3.1 ml). The reaction mixture was left to stir under reflux for 4 hours. The solvent was removed and the residue purified by column chromatography (4% H_2O / 12% MeOH in EtOAc). **2.38** was obtained in good yield (64 mg, 93%).

Mp: 264-268 °C (Lit. 267-268 °C)[7]

TLC: 0.08 (4% H_2O / 12% MeOH / EtOAc).

^{1}H NMR: (300 MHz, CD_3OD) δ_H 8.44 (vbr s, 1H), 7.71 (s, 1H), 7.42 - 7.11 (m, 5H) 6.69 (s, 1H), 4.24 (t, 1H, J = 4.5 Hz), 4.01 (dd, 1H, J = 8.1 and 3.6 Hz), 2.98 (dd, 1H, J = 13.7 and 4.5 Hz), 2.81 (dd, 1H, J = 13.7 and 5.5 Hz), 2.62 (dd, 1H, J = 14.7 and 3.6 Hz), 1.70 (dd, 1H, J = 14.7 and 8.1 Hz).
NH protons exchange in CD_3OD and are therefore not seen.
Occasionally they are seen as flat broad singlets in the CD_3OD.

^{13}C NMR: (75 MHz, CD_3OD) δ_C 168.8, 168.7, 136.9, 136.4, 133.1, 131.5, 129.7, 128.3, 118.9, 57.4, 55.9, 40.7, 32.7.

^{1}H NMR: (300 MHz, DMSO) δ_H 11.92 (bs, 1H), 8.09 (s, 1H), 7.76 (s, 1H), 7.51 (s, 1H), 6.62 (s, 1H), 7.28-7.13 (m, 5H), 4.12 (s, 1H), 3.83 (d, 1H, J = 6.0 Hz), 3.00-2.50 (2 × d, 2 × 1H), 1.76-1.42 (m, 1H), 1.30-0.96 (m, 1H).

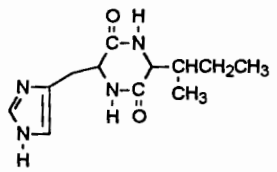
^{13}C NMR: (75 MHz, CD_3OD) δ_C 166.7, 166.0, 136.2, 134.9, 133.1, 130.1, 128.1, 126.6, 116.8, 55.4, 54.4, 38.6, 31.3.

EIMS: 284 (M^+, 76%), 204 ($C_{11}H_{11}N_2O_2$, 21%), 193 (M^+ - C_7H_7, 9%), 113

($C_4H_5N_2O_2$, 26%), 109 ($C_5H_7N_3$, 19%), 91 (C_7H_7, 49%), 82 ($C_4H_6N_2$, 100%).

IR ν_{max} (KBr) / cm^{-1} (3680-2000 (vbr)), 3480, 3210, 3140, 3000, 2920, 1690, 1460, 1350, 1105.

3-(4-Imidazolylmethyl)-6-isoleucyl-2,5-diketopiperazine (**2.39**)



Was prepared as described above for **2.38**.

Yield: 70%

Mp: 220-222 °C

TLC: R_f 0.08 (4% H_2O, 12% MeOH / EtOAc).

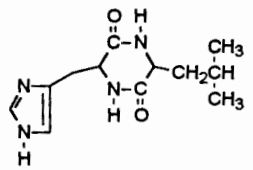
^1H NMR: (300 MHz, CD_3OD) δ_H 8.35 (s, 1H), 7.88 (d, 1H, J = 1.1 Hz), 6.99 (d, 1H, J = 1.1 Hz), 4.29 (ddd, 1H, J = 6.0, 4.5 and 1.5 Hz), 3.85 (d, 1H, J = 3.8 and 1.7 Hz), 3.28 (dd, 1H, J = 15.0 and 6.0 Hz), 3.07 (dd, 1H, J = 15.0 and 4.5 Hz), 1.75(m, 1H), 1.23 - 0.9 (m, 2H), 0.87 (d, 3H, J = 6.9 Hz), 0.83 (t, 3H, J = 6.9 Hz).

^{13}C NMR: (75 MHz, CD_3OD) δ_C 169.3, 169.2, 136.3, 132.7, 120.1, 60.8, 56.1, 39.9, 31.7, 25.0, 15.3, 12.2.

EIMS: 250 (M^+, 18%), 235 (M^+ - CH_3, 4%), 221 (M^+ - C_2H_6, 29%), 194 (M+1, C_4H_9, 5%), 170 ($C_8H_{14}N_2O_2$, 19%), 109 ($C_5H_7N_3$, 11%), 82 ($C_4H_6N_2$, 100%).

IR ν_{max} (KBr) / cm^{-1} (3680-2400 (vbr)), 3440, 3220, 3100, 3000, 2900, 1680, 1460, 1340, 1100.

3-(4-Imidazolylmethyl)-6-leucyl-2,5-diketopiperazine (**2.40**)



Was prepared as described above for **2.38.**

Yield: 50%

Mp: 205-206 °C

TLC: R_f 0.09 (4% H_2O, 12% MeOH / EtOAc).

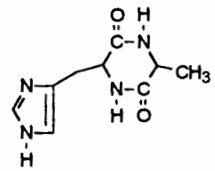
^{1}H NMR: (300 MHz, CD_3OD) δ_H 7.76 (s, 1H), 6.94 (s, 1H), 4.24 (dt, 1H, J = 4.9 and 1.1 Hz), 3.81 (ddd, 1H, J = 9.0, 4.5 and 1.1 Hz), 3.53 (dd, 1H, J = 14.9 and 5.3 Hz), 2.71 (dd, 1H, J = 14.9 and 4.4 Hz), 1.73 – 1.53 (m, 1H), 1.35 – 1.22 (m, 2H), 0.85 (d, 3H, J = 0.9 Hz), 0.83 (d, 3H, J = 0.9 Hz).

^{13}C NMR: (75 MHz, CD_3OD) δ_C 170.6, 169.1, 136.1, 132.4, 119.9, 56.5, 54.3, 45.2, 31.9, 24.9, 23.5, 21.7.

EIMS: 251 (M+1, 20%), 236 (M+1 – CH_3, 3%), 194 (M+1 – C_4H_9, 8%), 170 ($C_8H_{14}N_2O_2$, 22%), 109 ($C_5H_7N_3$, 9%), 82 ($C_4H_6N_2$, 100%).

IR ν_{max} (KBr) / cm^{-1} (3680-2200 (vbr)), 3200, 2990, 2900, 1680, 1460, 1350, 1100.

3-(4-Imidazolylmethyl)-6-methyl-2,5-diketopiperazine (**2.41**)



Was prepared as described above for **2.38**.

Yield: 85%

Mp: 251-253 °C (Lit. 251-253 °C)[9]

TLC: R_f 0.03 (4% H_2O, 12% MeOH / EtOAc).

^{1}H NMR: (300 MHz, CD_3OD) δ_H 8.42 (vbr s, 1H), 7.73 (s, 1H), 6.94 (s, 1H), 5.86 (vbr s, 1H), 5.44 (vbr s, 1H), 4.25 (td, 1H, J = 5.7 and 1.2 Hz), 3.91 (qd,

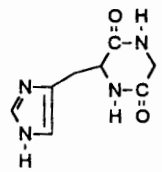
1H, J = 6.9 and 1.2 Hz), 3.26 (dd, 1H, J = 15.0 and 5.4 Hz), 2.99 (dd, 1H, J= 15.0 and 4.5 Hz), 0.99 (d, 3H, J = 6.9 Hz).

^{13}C NMR: (75 MHz, CD_3OD) δ_C 170.8, 168.9, 136.1, 132.4, 119.8, 56.4, 51.8, 31.7, 20.3.

EIMS: 208 (M^+, 22%), 193 (M^+ - CH_3, 1%), 128 ($C_5H_8N_2O_2$, 14%), 113 ($C_4H_5N_2O_2$, 7%), 109 ($C_5H_7N_3$, 12%), 82 ($C_4H_6N_2$, 100%).

IR ν_{max} (KBr) / cm^{-1} (3680-2200 (vbr)), 3460,3200, 3080, 2880, 1700, 1460, 1340, 1120.

3-(4-Imidazolylmethyl)-2,5-diketopiperazine (**2.42**)



Was prepared as described above for **2.38**.

Yield: 92%

Mp: 244-246 °C (Lit. 246-249 °C)[9]

TLC: R_f 0.02 (4% H_2O, 12% MeOH / EtOAc).

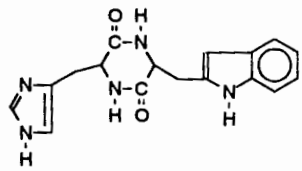
^{1}H NMR: (300 MHz, CD_3OD) δ_H 8.52 (bs, 1H), 7.66 (s, 1H), 6.91 (s, 1H), 5.71 (bs, 1H), 4.12 (t, 1H, J = 4.8 Hz), 3.68 (d, 1H, J = 17.7 Hz), 3.29 (d, 1H, J = 17.7 Hz), 3.22 (dd, 1H, J = 14.7 and 5.1 Hz), 2.99 (dd, 1H, J = 14.7 and 4.8 Hz).

^{13}C NMR: (75 MHz, CD_3OD) δ_C 169.9, 168.5, 136.4, 132.9, 119.4, 56.4, 45.0, 32.1.

EIMS: 195 (M+1, 2%), 109 ($C_5H_7N_3$, 15%), 82 ($C_4H_6N_2$, 100%).

IR ν_{max} (KBr) / cm^{-1} (3640-2080 (vbr)), 3500,3200, 3100, 2960, 1690, 1480, 1360, 1100.

3-(4-Imidazolylmethyl)-6-tryptophanyl -2,5-diketopiperazine (**2.43**)



Was prepared as described above for **2.38**.

Yield: 54%

Mp: 201-205 °C

TLC: R_f 0.05 (4% H_2O, 12% MeOH / EtOAc).

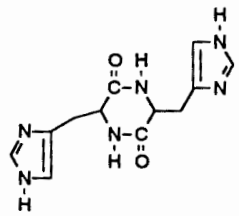
^{1}H NMR: (300 MHz, CD_3OD) δ_H 7.86 (bs, 1H), 7.63 (d, 1H, J = 7.8 Hz), 7.37 (d, 1H, J = 7.8 Hz), 7.20 – 7.04 (m, 5H), 5.76 (bs, 1H), 3.46 (dd, 1H, J = 14.4 and 3.8 Hz), 2.95 (dd, 1H, J = 14.4 and 4.4 Hz), 4.26 (t, 1H, J = 4.1 Hz), 3.89 (unresolved dd, 1H), 2.41 (unresolved dd, 1H), 1.10 (unresolved dd, 1H).

^{13}C NMR: (75 MHz, CD_3OD) δ_C 169.7, 168.7, 137.8, 136.8, 131.5, 129.7, 129.2, 128.3, 126.2, 122.6, 120.3, 112.5, 109.5, 57.3, 55.6, 32.5, 30.8.

EIMS: 324 (M + 1, 2%), 190 (M^+ - $C_9H_{11}N$, 1%), 123 ($C_6H_7N_2O$, 2%), 81 ($C_4H_5N_2$, 6%), 28 (CO, 100%).

IR ν_{max} (KBr) / cm^{-1} (3680-2200 (vbr)), 3440, 3210, 2940, 1710, 1510, 1470, 1340, 1100.

3,6-Bis(4-Imidazolylmethyl)-2,5-diketopiperazine (**2.44**)



Was prepared as described above for **2.38**.

Yield: 74%

Mp: > 315 °C (Lit. > 330 °C[9] and 328°C)[8]

TLC: R_f 0.05 (16% H_2O, 20% MeOH / EtOAc).

^{1}H NMR: (300 MHz, CD_3OD) δ_H 7.68 (d, 1H, J = 1.2 Hz), 6.85 (s, 1H), 4.17 (dd, 1H, J = 6.8 and 4.2 Hz), 3.11 (dd, 1H, J = 14.7 and 4.2 Hz), 2.32 (dd, 1H, J = 14.7 and 6.8 Hz).

^{13}C NMR: (75 MHz, CD_3OD) δ_C 169.0, 136.4, 133.1, 119.4, 56.2, 32.4.

EIMS: 274 (M^+, 7%), 193 (M^+ - $C_4H_5N_2$, 22%), 110 ($C_4H_4N_2O_2$, 20%), 82 ($C_4H_6N_2$, 49%).

IR ν_{max} (KBr) / cm^{-1} (3650-2200 (vbr)), 1745, 1660, 1580, 1530, 1470, 1350, 1270, 1185, 1100, 995, 850, 640.

DIMERS

3,6-Bis(4-Imidazolylmethyl)-2,5-diketopiperazine) (**2.44**)

Histidine methyl ester dihydrochloride (2.4 g, 9.92 mmol) was dissolved in methanol (25 ml).[9] A sodium methoxide solution (920 mg in 20 ml) was added. After a short period of standing (*ca.* 10 minutes) ether (20 ml) was added and the precipitated salt was filtered off. The filtrate was concentrated to a smaller volume (≈5 ml). After standing for 8 days, at 37 °C, the solvent was removed and the product recrystallised from hot water. **2.44** was obtained as white crystals, 30% yield.

3.5. COMPLEXATION.

Various methods were attempted during the complexation studies, with two general procedures dominating:

The first involved the addition of 1 equivalent of lithium hydroxide to a 1:1 metal / ligand mixture in methanol, where copper(II) perchlorate pentahydrate was used. This reaction mixture has been reported to result in a dimeric complex, with two copper atoms of different coordination modes.[10] This complex was discussed in detail in the literature chapter and was referred to as the aquabis complex. This aquabis complex is also expected to be obtained when sodium hydroxide is used as a base.[11] Both of these methods were attempted on the prepared diketopiperazines, but in all the cases only an amorphous solid formed, making X-ray analysis impossible.

The second procedure involved the use of a 2:1 ligand:metal mixture in methanol, where copper(II) perchlorate pentahydrate was used.[12] The expected crystals have been shown to display a distorted tetrahedral coordination geometry, on X-ray analysis. This complex was discussed at some length earlier and it was noted that the coordination takes place *via* the four nitrogen atoms of the imidazole ring, two from each ligand. Similar complexation reactions were carried out with $CuSO_4.5H_2O$ and $Cu(NO_3)_2.H_2O$, *i.e.* using the 2:1 ratio and no base. The same tetrahedral coordination was expected from literature observations.[13] Again in all cases no suitable crystals were obtained for X-Ray analysis.

3.6 REFERENCES

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