THE SYNTHESIS OF CYCLIC HEXAPEPTIDE HOST MOLECULES

by

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ABSTRACT

In recent years, the field of host-guest chemistry has become a large and rapidly advancing area of research with a vast number of new host molecules being reported. Many of these developments are outlined in Chapter 1. The aim of this project was to achieve the synthesis and isolation of seven host molecules based upon a cyclic hexapeptide structure. Within this cyclic peptide, two glycine residues were incorporated to encourage the stabilization of the structure *via* the formation of β-turns. Four lysine residues made up the complement of six amino acid residues in each target molecule. In three of the synthetic targets, these lysines were to be crosslinked with different units to form polycyclic peptides with a cavity. In the other four synthetic targets, the lysines were to be used to allow the capping of a porphyrin unit onto the cyclic hexapeptide.

With the aim of synthesising the three polycyclic peptides, it was necessary to prepare and isolate a tripeptide crosslinked with an appropriate crosslinker. As described in Chapter 2, successful crosslinking was achieved when an N^{ϵ}-tosyl protected tripeptide was reacted with 1,2-bis(2-tosylethoxy)ethane, 1,6-dibromohexane and 1,4-α,α'-dibromo-*p*-xylene.

In Chapter 3, the final stages in the attempted synthesis of two of these polycyclic peptides are described *via* the successful cyclodimerisation of a crosslinked tripeptide and its resultant detosylation. Evidence from FAB mass spectrometry indicated that following a crosslinking reaction with 1,4-α,α'-dibromo-*p*-xylene, a polycyclic peptide was successfully formed. However, whereas the ^{1}H NMR spectra were consistent with the desired molecule, they could not be used to conclusively prove its synthesis.

The attempts made to synthesise a porphyrin capped cyclic peptide are described in Chapter 4. However, none of these attempts were successful, either due to problems encountered in the purification of advanced intermediates or unsuccessful capping of the cyclic peptide by the appropriate porphyrin.

CONTENTS

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My greatest thanks, however, are reserved for my Mum, without whose love, encouragement, wisdom and inspiration this project would never have been completed. It is a source of great sadness that her untimely death prevents me from placing a copy of this thesis into her hands. Nevertheless, it is to her memory and to the memory of my Dad, that this thesis is dedicated.

In loving memory of

Mum and Dad

Of making many books there is no end,

and much study wearies the body.

Ecclesiastes 12:12b

ABBREVIATIONS

Ac	acetyl
Adom	adamantyloxymethyl
ADP	adenosine diphosphate
AIDS	acquired immune deficiency syndrome
Ala	alanine
aq	aqueous
Ar	aromatic
Asp	aspartic acid
ATP	adenosine triphosphate
AZT	3'-azido-2',3'-dideoxythymidine
Boc	^{t}butyloxycarbonyl
Bom	benzyloxymethyl
BOP	benzotriazolyloxy-tris(dimethylamino)-phosphonium hexafluorophosphate
Bn	benzyl
bp	boiling point
Bt	benzotriazole
Bu	butyl
^{n}Bu	$CH_2CH_2CH_2CH_3$
^{t}Bu	*tert*-butyl (1,1-dimethylethyl)
Bz	benzyl
cm	centimetre(s)
COSY	correlation spectroscopy
DCC	dicyclohexylcarbodiimide
DCU	dicyclohexylurea
DDQ	2,3-dichloro-5,6-dicyanobenzo-1,4-quinone
DIBAL-H	diisobutylaluminium hydride
DIPEA	N,N-diisopropylethylamine
dm	decimetre(s)
DMA	N,N-dimethylacetamide
DMAP	4-N,N-dimethylaminopyridine

DMF	N,N-dimethylformamide
DMSO	dimethylsulphoxide
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
e.e.	enantiomeric excess
EI MS	electron impact mass spectrometry
EM	effective molarity
Et	ethyl
FAB MS	fast atom bombardment mass spectrometry
Fmoc	9-fluorenylmethyloxycarbonyl
FTIR	fourier transform infra red spectroscopy
g	gram(s)
G	Gibbs free energy (kJ mol^{-1})
Glu	glutamic acid
Gly	glycine
GMP	guanosine 5'-monophosphate
h	hour(s)
His	histidine
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
Hz	hertz
IR	infra red spectroscopy
J	coupling constant
kJ	kilojoule
l	liquid
Leu	leucine
Lys	lysine
M	molar (mol dm^{-3})
Me	methyl
mg	milligram(s)
MHz	megahertz
min	minute(s)
mM	millimolar (1×10^{-3} mol dm^{-3})
mmol	millimole(s)

mol		mole(s)
mp		melting point
MS		mass spectrum
m/z		mass/charge
nm		nanometre(s)
NMR		nuclear magnetic resonance spectroscopy:
	br	broadened
	m	multiplet
	s	singlet
	d	doublet
	t	triplet
	q	quartet
NOBA		3-nitrobenzylalcohol
NOE		nuclear Overhauser enhancement
NOESY		nuclear Overhauser enhancement spectroscopy
Nps		2-nitrophenylsulphonyl
PFP		pentafluorophenyl
Ph		phenyl
Phe		phenylalanine
Phg		phenylglycine
PP		phenylporphyrin
ppm		parts per million
Pr		propyl
^{i}Pr		*iso*-propyl (1-methylethyl)
Pro		proline
PyBOP		benzotriazolyloxy-tris(pyrrolidino)-phosphonium hexafluorophosphate
R		alkyl or phenyl group
R_f		retention factor
RT		room temperature
Ser		serine
SPPS		solid phase peptide synthesis

TBTU	benzotriazolyloxy-1,1,3,3-tetramethylammonium tetrafluoroborate
TCQ	tetrachloro-1,4-benzoquinone
TFA	trifluoroacetic acid
TFE	2,2,2-trifluoroethanol
THF	tetrahydrofuran
Tips	triisopropyl
TLC	thin layer chromatography
TMEDA	N,N,N',N'-tetramethylethylenediamine
TMS	tetramethylsilane
TPP	thiamin pyrophosphate
Trityl	triphenylmethyl
Trp	tryptophan
Ts	4-methylphenylsulphonyl (toluenesulphonyl) (tosyl)
µmol	micromole(s)
UV	ultra-violet
Val	valine
Z	benzyloxycarbonyl

CHAPTER 1:

INTRODUCTION

1.1 Host-guest chemistry

Few scientists who have studied the biochemistry of biological systems at the molecular level could fail to be inspired. The specificity of binding in enzymes and antibodies has led many chemists to speculate as to the feasibility of mimicking such systems using synthetic organic species[1]. The Athenian Poet-Dramatist, Aeschylus wrote 2500 years ago, "Pleasantest of all ties is the tie of host and guest[2]." It has been the aim of many chemists for 30 years, to study this tie between host and guest at the molecular level.

The area of host-guest (or supramolecular) chemistry is concerned with the chemistry of the intermolecular bond and, in particular, the chemistry of entities formed by the association of two or more species. This new field is a rapidly expanding area of research and one of its particular attractions is that it draws together chemists from many disciplines - from organic synthesis through to inorganic, physical and biochemistry[3]. It was in recognition of the significance of host-guest chemistry that the 1987 Nobel Prize for Chemistry was awarded to three of its pioneers, Charles Pedersen[4], Donald Cram[1] and Jean-Marie Lehn[3]. Pedersen's discovery in 1967 of dibenzo-18-crown-6 and its ability to form stable complexes with alkali and alkaline-earth metals, can be considered to mark the beginning of synthetic supramolecular chemistry[5,6]. Cram and Lehn have done much to develop this work by the design, synthesis and study of cryptands, podands, carcerands and many other kinds of novel host molecules.

Such is the size of the area of host-guest chemistry[5,7], that it is clearly not feasible to give a comprehensive review within the confines of this chapter: many reviews have already been published in this field[5,7-12]. Instead, it is the aim of this chapter to describe some of the fundamental principles, to show the development and to highlight some of the key recent successes in host-guest chemistry. Particular reference will be made to those host molecules containing amide bonds and porphyrins as key units in their construction.

1.1.1 Basic concepts of host-guest chemistry

A prerequisite to the understanding of the area of host-guest chemistry is a familiarity with the basic terminology and definitions. The partners of a supramolecular species have been named the *molecular receptor* and *substrate*[3,13,14]. Alternatively, many articles refer to them as the *host* and *guest*[15,16]. The latter terminology will be used in this thesis. The

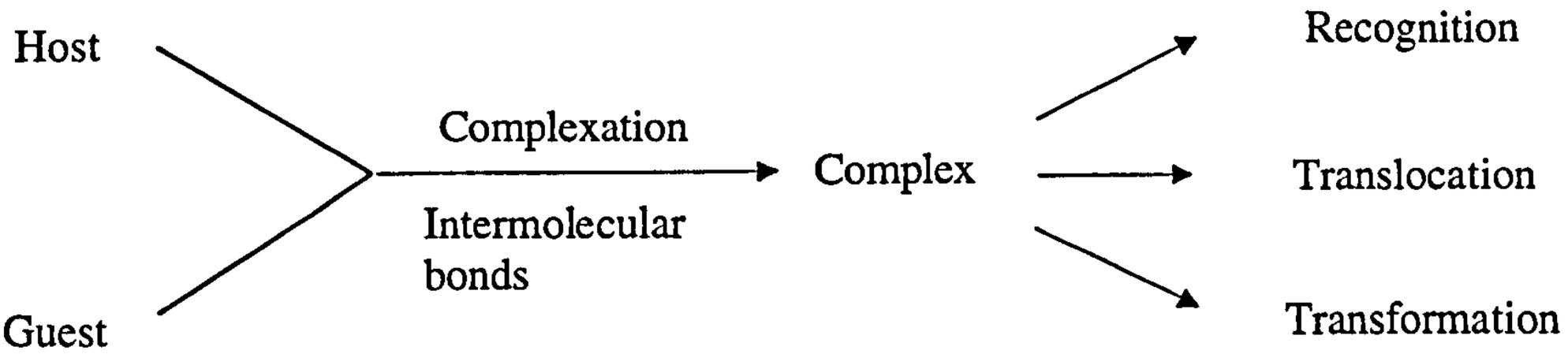
association of the host and guest are said to form a *supermolecule*[3] or *complex*. Such complexes often possess features as well defined as molecules in that they are distinct and frequently reasonably stable entities.

The host molecule is often defined as being the larger of the two species in the complex. In addition, one can say that the host molecule presents a more concave binding site whereas the guest binds over a more convex surface[15,16]. It is often the precise shape or electronic structure of these two surfaces that determines the specificity of the binding interaction[1].

Selective molecular interactions are crucial to many processes that occur in biochemistry. Vital processes such as substrate binding to a receptor protein, enzymatic reactions, translocation and transcription of DNA and immunological antigen-antibody association all depend upon selective molecular recognition processes in which key interactions mostly take the form of intermolecular bonds. It is the aim of many supramolecular chemists to mimic the selectivity of such natural recognition events by the design, synthesis and study of synthetic host molecules[3].

Molecular recognition, *translocation* and *transformation* represent the basic functions of host-guest complexes[3]. The binding of a guest to a host involves a *molecular recognition* process. A situation where this occurs in nature is in the irreversible inhibition of an enzyme[17]. In many enzymes, the binding is mostly permanent but in other systems, if the host-guest complex is transported to another environment, the guest can be released. A particularly famous example is the uptake of O_2 by hemoglobin in the oxygen-rich environment of the lungs and its release in the more oxygen-deficient muscles[17]. Thus the *translocation* of a guest has occurred. Finally, if the host contains not only binding sites but also reactive functional groups, chemical *transformation* of the bound substrate may occur. An example is the specific hydrolysis of peptide bonds on the carboxyl side of aromatic residues by the enzyme chymotrypsin[18]. The relationship of these functions to complexation is illustrated in the diagram in Fig 1.1.

Fig 1.1 : Relationship between host, guest and complex and the basic functions of host-guest complexes



1.1.2 Lock and key principle

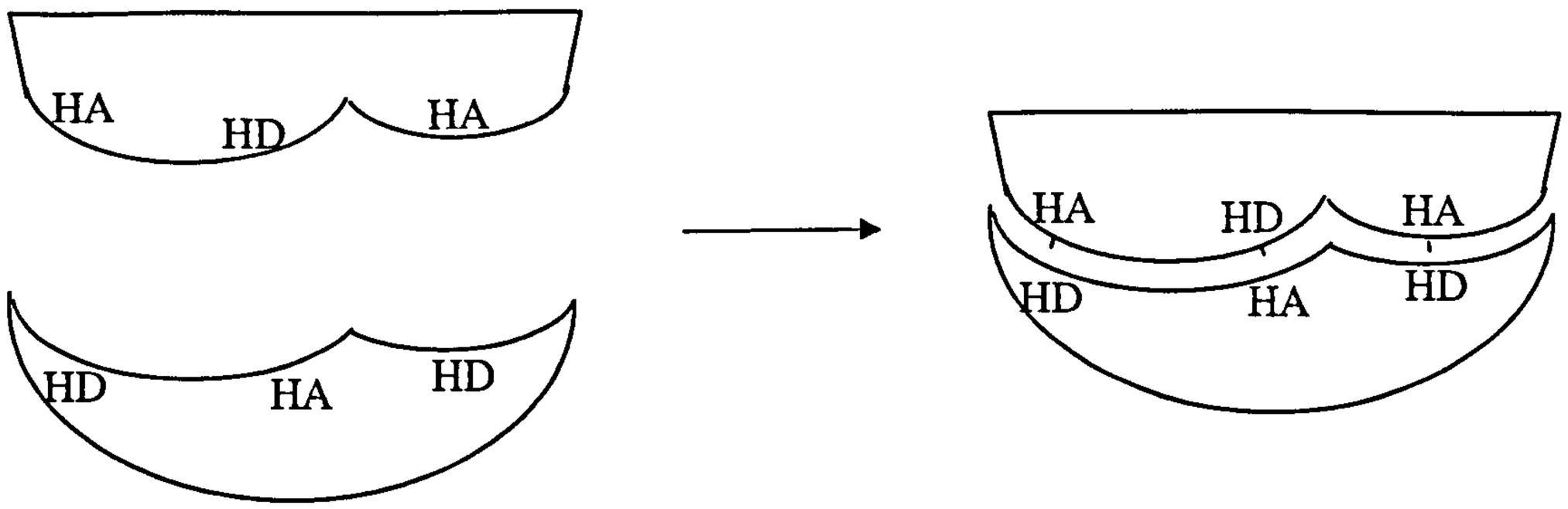
One of the earliest and most enduring contributions to the study of the selective binding of guests by hosts was made in 1894 by Emil Fischer[19]. In describing the interaction of enzymes with glucosides, he stated that host and guest fit together like a "lock and key"[20]. He extended the applicability of this so called "Schlussel-Schloss-Prinzip," to the action of enzymes in general[21] and explained that the selectivity of binding of a guest and a host is dependent upon the complementarity of the "geometrical structure" of the guest (key) with the host (lock). Thus, if the guest and host have complementary *shapes*, they will bind.

Related to this idea of the lock and key is Cram's *principle of complementarity* [1]. Cram argues that if a host and guest are to bind, then not only must the two have complementary shapes but they must also possess complementary functional groups.

Complexes are produced by the formation of such intermolecular bonds as van der Waals forces, hydrophobic interactions and also hydrogen bonds. If this intermolecular bonding, particularly hydrogen bonding is to be effective, it is vital that within the complex, groups capable of forming such forces are in complementary positions. Thus Cram states that "to complex, hosts must have binding sites which co-operatively contact and attract binding sites of guests without generating strong non-bonded repulsions"[22].

Both Fischer's lock and key principle and Cram's principle of complementarity are summarised in the diagram in Fig 1.2.

Fig 1.2 : Schematic diagram showing the binding of a complementary host and guest



HA = Hydrogen bond acceptor

HD = Hydrogen bond donor

1.1.3 Induced-fit theory

As knowledge of the functions and properties of enzymes increased, it became clear that the *basic* lock and key principle as described by Fischer could not account for every enzymatic action[23]. A classic example is *non-competitive inhibition* of an enzyme. This phenomenon has been explained by saying that an inhibitor prevents enzyme action but does not affect the binding of the substrate[17]. This result cannot, however, be explained by the classical lock and key principle, since if the inhibitor does not block the binding site, the binding site or *lock* should still remain open to and complementary to the guest or *key*.

This anomalous result neccessitated the modification of the lock and key principle. Thus in 1958, Koshland introduced his induced-fit theory[24]. Using the example of enzymes, Koshland explained his theory in the following terms;

"a) the precise orientation of catalytic groups is required for enzyme action,

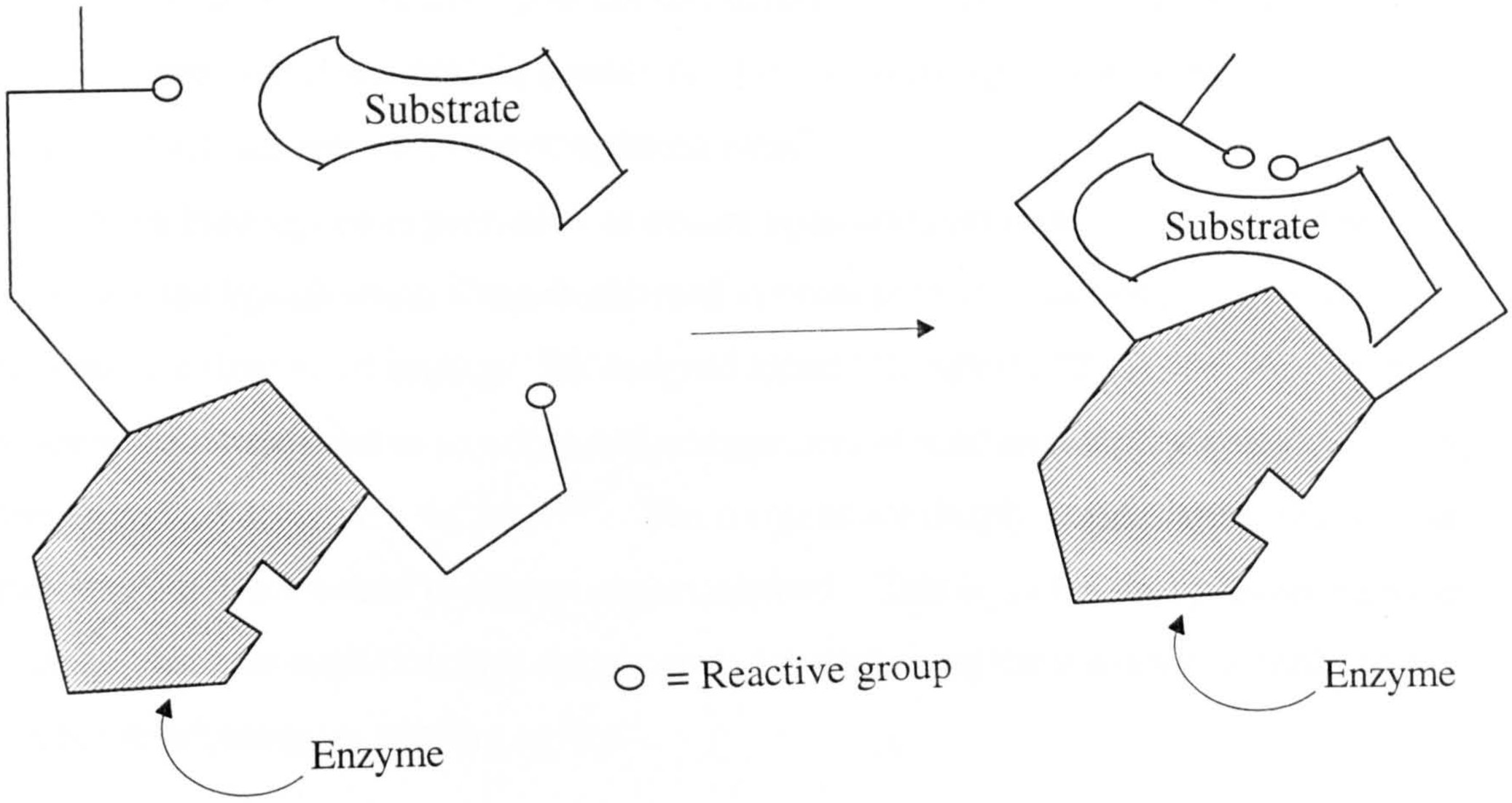
b) the substrate causes an appropriate change in the three-dimensional relationship of the amino acids at the active site and

c) the changes in the protein structure caused by the substrate will bring the catalytic groups into the proper alignment whereas a non-substrate will not."

Thus, whilst a host may not initially possess a complementary binding surface to a guest, its conformation can change on binding in such a way as to become complementary to the guest to which it evolved to bind. This has been likened to the image of a "hand-in-glove," thus

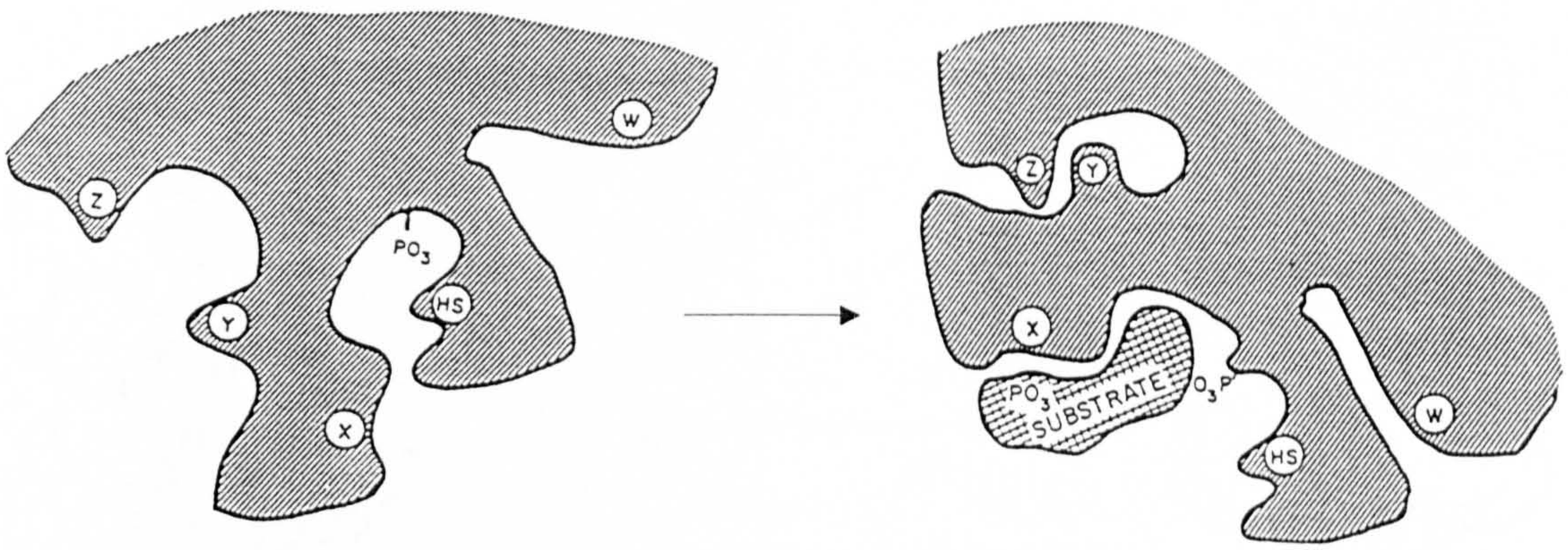
combining the idea of a selective fit together with the concept of flexibility[23]. A schematic model illustrating the induced-fit mechanism is illustrated in Fig 1.3.

Fig 1.3 : Schematic diagram illustrating the binding of a substrate by an enzyme following the induced-fit mechanism



Many experiments were performed in order to test this theory, a classic example of which is illustrated in Fig 1.4. The enzyme phosphoglucomutase was chosen and the reactivity of its SH group used as a test. Ligand binding induced the exposure of the SH groups, an observation which is inexplicable using the *basic* lock and key theory[25].

Fig 1.4 : Binding of a substrate by phosphoglucomutase showing the exposure of an SH group upon binding, as explained by the induced-fit mechanism

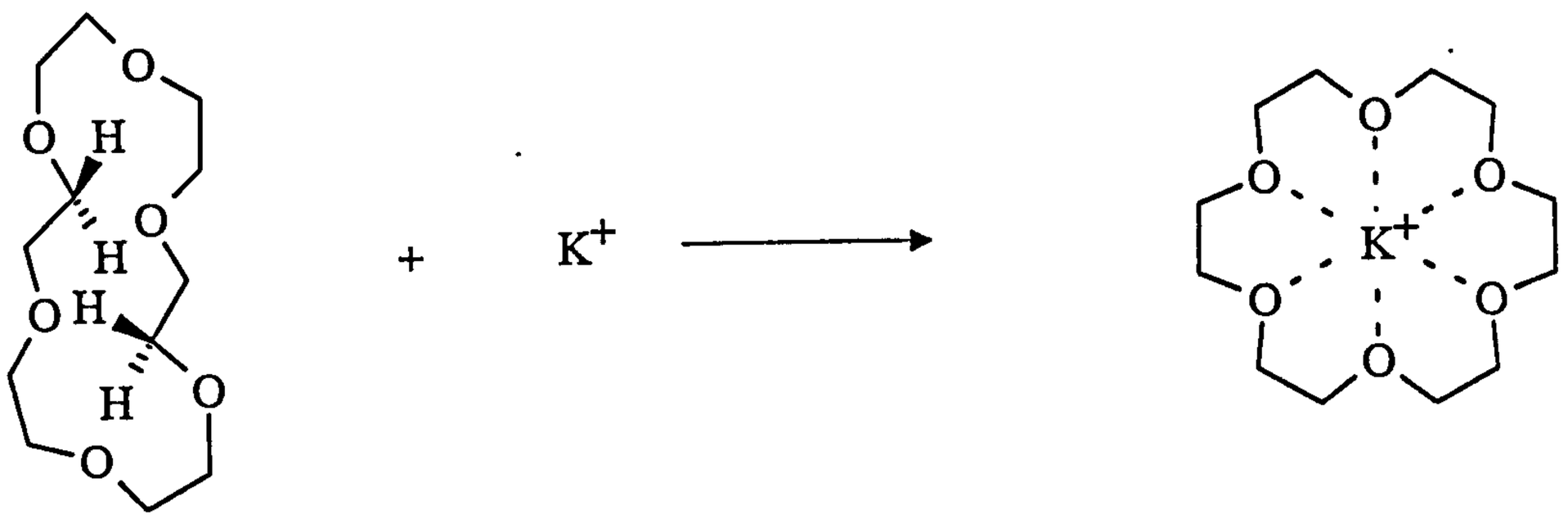


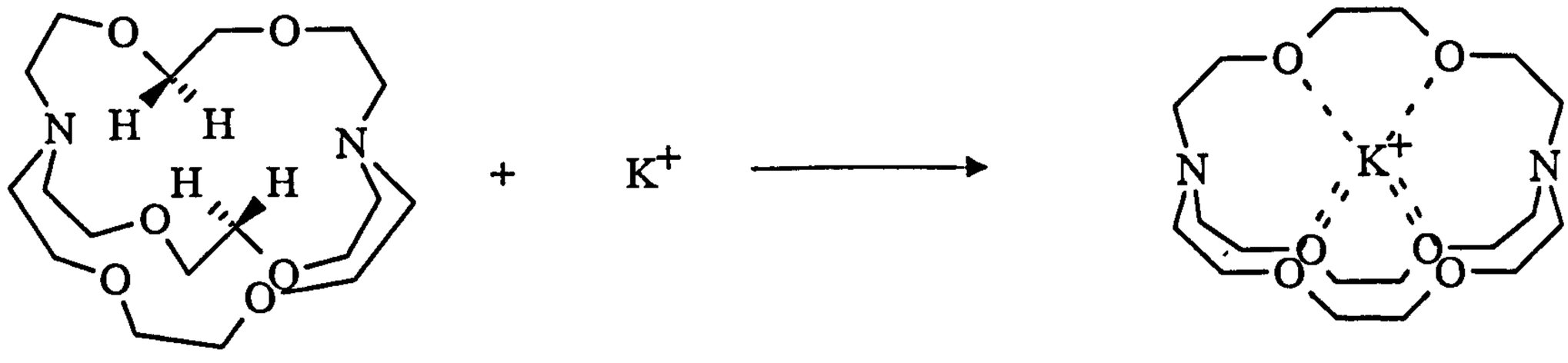
1.1.4 Principle of preorganisation

An additional theory describing binding between host and guest can be seen by making comparisons between the crystal structures of uncomplexed 18-crown-6 and 2,2,2-cryptand with their respective K^+ complexes. In both cases considerable host reorganisation and desolvation needs to occur upon complexation. As illustrated in Fig 1.5, prior to complexation, the hosts contain neither cavities nor convergently arranged binding sites, both of which are present in the complexed form[1].

Such binding site organisation as occurs upon complexation is not uncommon. Fig 1.6 shows the ligands which Cram synthesised in order to study what effect preorganisation has upon the strength of binding. He designed ligand (1), called a "Spherand," in which the oxygens are predisposed to an octahedral arrangement around an enforced spherical cavity, complementary to Li^+ and Na^+ ions[26,27]. The oxygens are deeply buried in the hydrocarbon shell and when not bound to a guest are unsolvated. This ligand is thus *preorganised* for binding - there are negligible free energy costs for organising the ion binding conformation and for desolvating the binding cavity.

Fig 1.5 : Binding of a potassium ion by 18-crown-6 and 2,2,2-cryptand



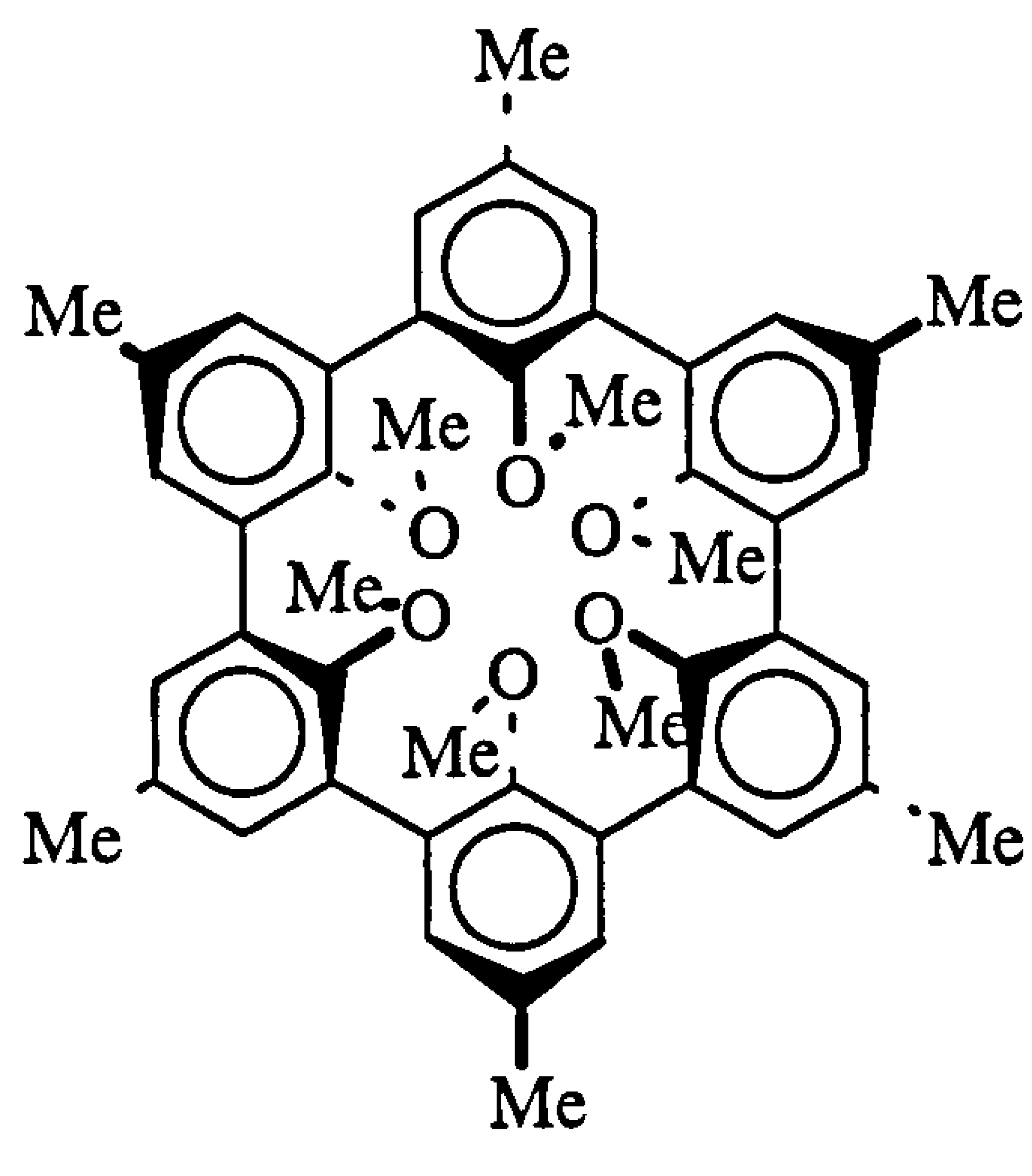


The spherand's open chain counterpart, podand (2), is by contrast, not preorganised. It differs constitutionally from spherand (1) solely in that it contains two Ar-H bonds as opposed to an Ar-Ar bond. It can exist in over 1000 conformations, only two of which can bind metal ions octahedrally. In addition, upon binding, free energy is required to desolvate the oxygens[1].

The difference in the relative strengths of binding of metal ions between the preorganised and unpreorganised ligands can be seen in the $-\Delta G^{o}$ values for the two complexes. Guest salts of lithium and sodium picrate were distributed between $CDCl_3$ and D_2O at $25^{0}C$ in the presence and absence of host. Spherand (1) binds Li^+ with 96.3 kJ mol^{-1} and Na^+ with 80.8 kJ mol^{-1}[22]. Podand (2) binds Li^+ and Na^+ with $-\Delta G^{o} < 25.1$ kJ mol^{-1}[28].

From these and other results, Cram has formulated his *"Principle of Preorganisation,"* emphasising it as a central factor in the strength of binding. He states that, "the more highly hosts and guests are organised for binding and the lower their solvation prior to their complexation, the more stable will be their complexes[1]."

Fig 1.6 : Diagrams showing the relative rigidity of spherand (1) compared to the flexibility of podand (2)



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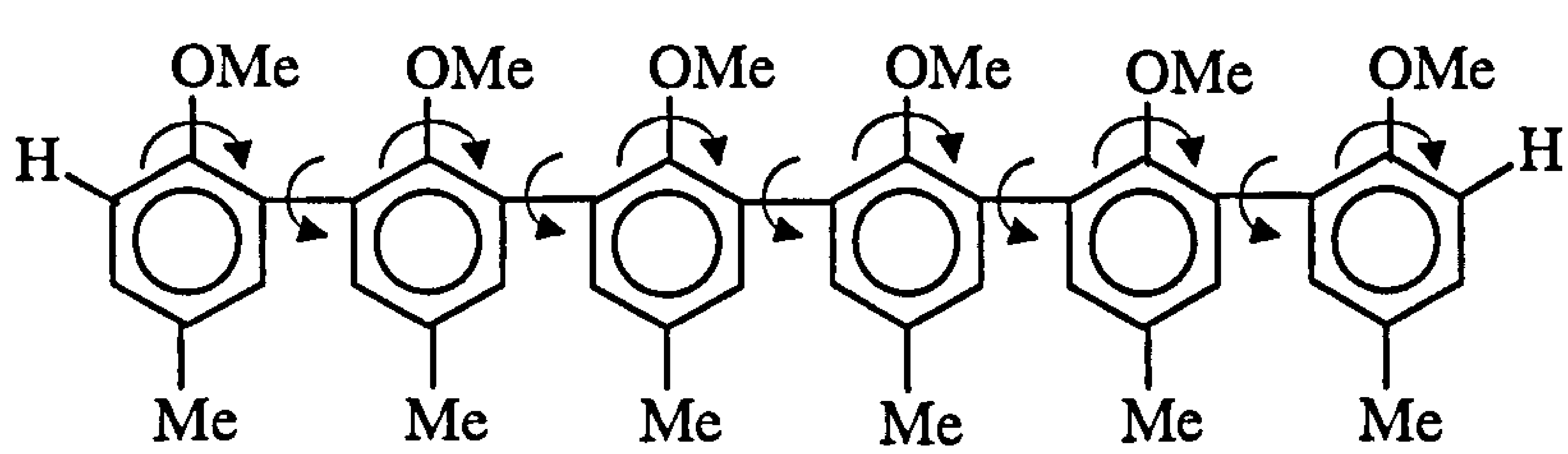

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1.1.5 Recent developments in host-guest chemistry

In the preceding pages, some of the key principles governing the binding of guests by hosts have been outlined. In the following pages of this chapter, recent progress in the field of host-guest chemistry will be described. Many of the simple functions of host molecules can be classified into the areas of *recognition*, *transport* and *catalysis*[3]. Each of these areas will be summarised in turn, comparing examples of natural hosts with some of the myriad of synthetic host molecules that have been synthesied over the past few years. Particular emphasis will be given to examples of hosts containing amide bonds, peptides and porphyrin structures. A brief mention will then be made concerning the general synthetic methods available for forming a variety of host molecules before the practical aims of my research are described.

1.2 Molecular recognition

1.2.1 Natural host molecules

One of the guiding inspirations behind much of the work carried out in the area of synthetic host-guest chemistry has been the desire to mimic the degree of guest binding specificity achieved by a large number of natural binding systems. Two examples of such natural hosts will be described, illustrating the guest binding principles outlined in Section 1.1, before a brief survey is made of the kinds of synthetic hosts that have been prepared.

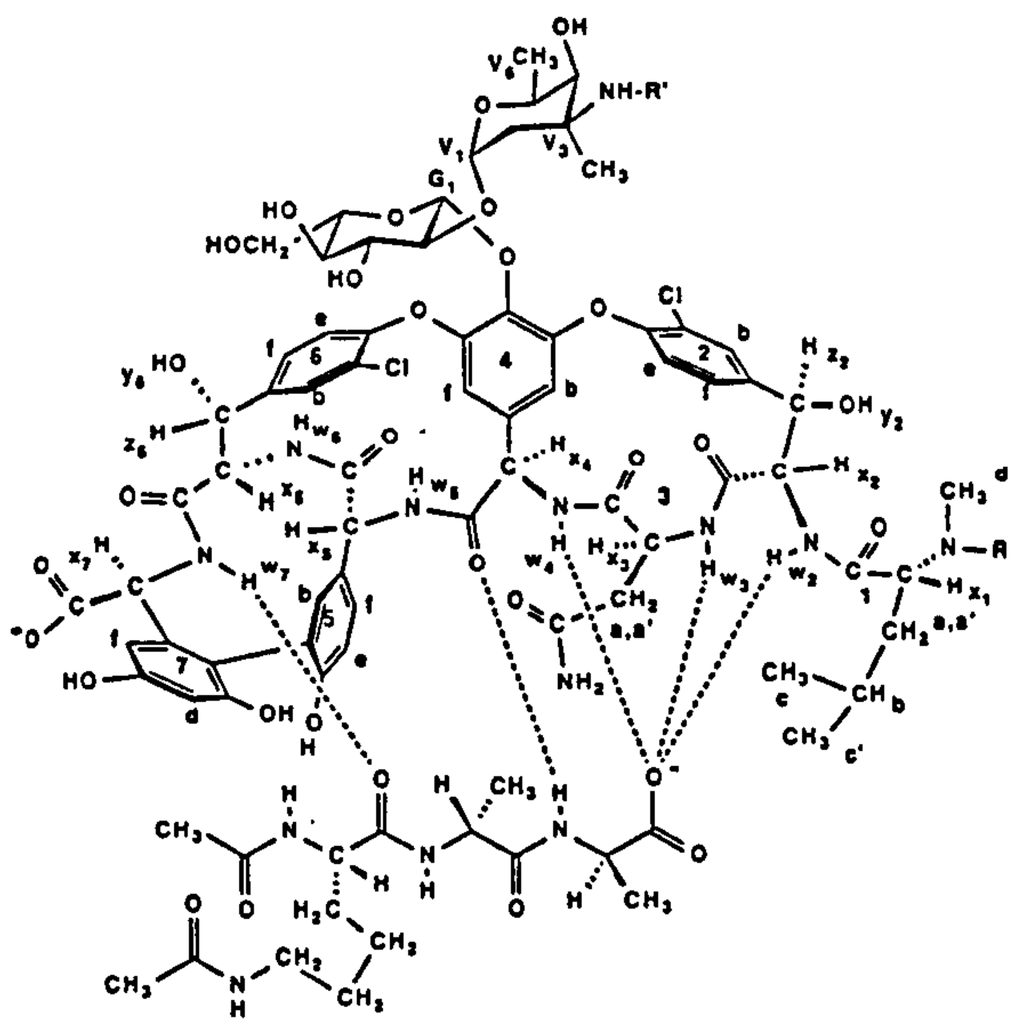
1.2.1.1 Vancomycin

The *vancomycin* group of antibiotics offer excellent systems for the study of host-guest interactions[29]. These natural antibiotics, all based upon a structure containing seven amino acids, exert their antibacterial activity by preferentially binding to peptide intermediates involved in cell-wall synthesis. They selectively bind to peptides terminating in D-Ala-D-Ala, their binding constants having values up to $10^6\ M^{-1}$ [30-33].

The binding of vancomycin to the dipeptide Ac-D-Ala-D-Ala and to the tripeptide Ac_2-L-Lys-D-Ala-D-Ala has been studied principally by the use of high-field ^1H NMR[31]. By the use of NOE studies, it has been found that the above mentioned principle of

complementarity of size, shape and charge distribution operates efficiently. Fig 1.7 describes the vancomycin-tripeptide complex and reveals the complexity of the interactions required for strong, selective binding. Not only does the host cavity have the correct size and shape but it also contains hydrogen bonding acceptor and donor sites in complementary positions to those of the guest. Recent research has also indicated that the formation of vancomycin dimers contributes to the formation of the correctly shaped binding site and thus strengthens the complex[32,33]. Such a dimer-guest complex is of necessity formed within a dilute aqueous solution so as to correspond to physiological conditions.

Fig 1.7 : Binding of a tripeptide substrate by the antibiotic vancomycin

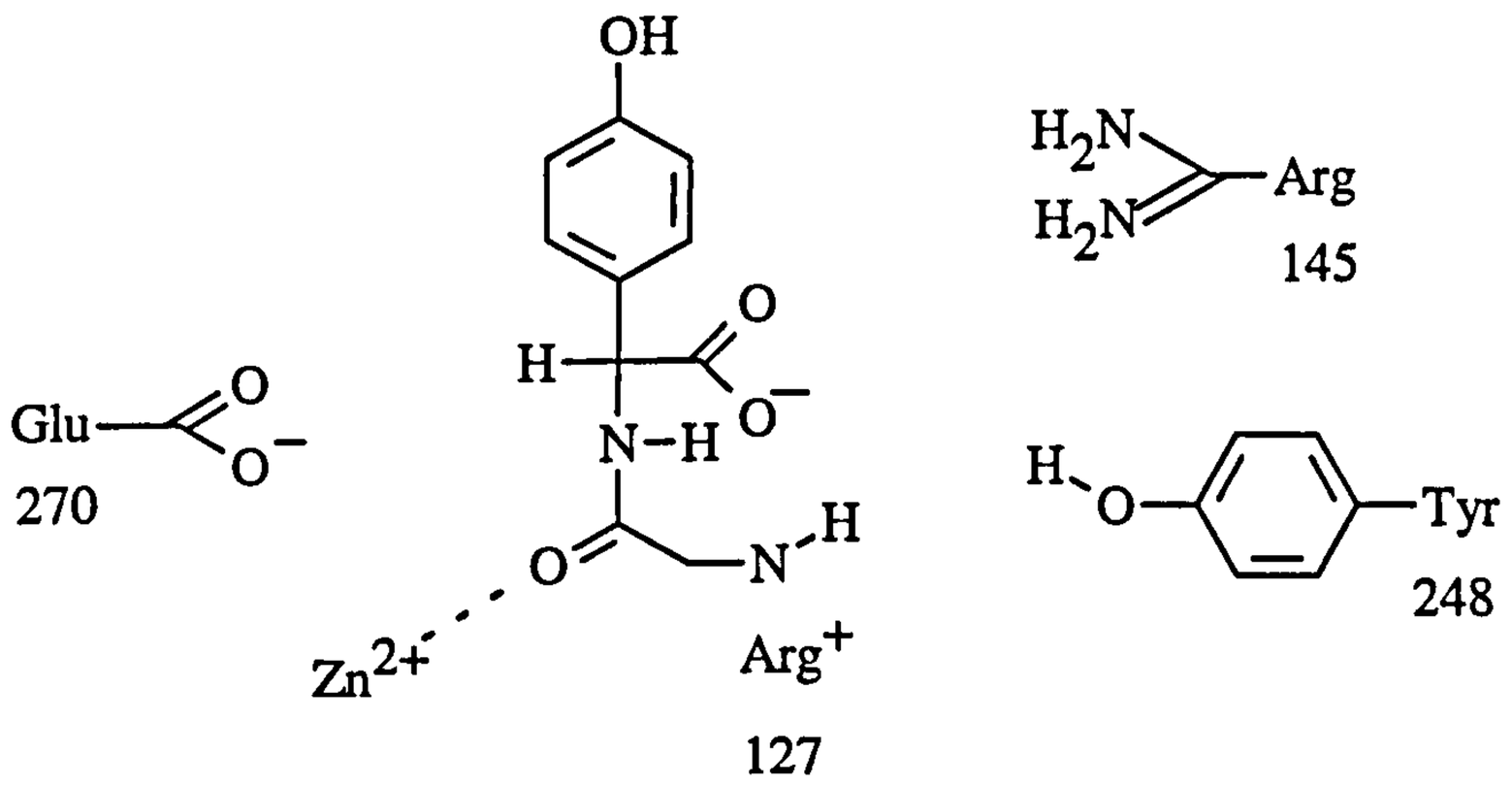


1.2.1.2 Carboxypeptidase A[17]

Some of the most impressive binding selectivities achieved in natural systems are those of enzymes. An example is *carboxypeptidase A*, a digestive enzyme that hydrolyses the carboxyl-terminal peptide bond in polypeptide chains. Hydrolysis is particularly efficient if the carboxyl-terminal residue has an aromatic or bulky aliphatic side chain as the enzyme has binding selectivity for these guests or substrates.

The structure of carboxypeptidase A is based upon a single polypeptide chain of 307 amino acid residues. This folds to present a binding site containing tyrosine, arginine and glutamic acid residues, together with a co-ordinated zinc ion. A schematic diagram of the binding of a guest such as glycyl tyrosine is given in Fig 1.8. Before the binding of such a guest, these residues are widely spaced. However, upon binding, considerable structural rearrangement occurs, with the binding groups being brought together to surround the guest. Indeed, the largest such movement is that of the phenolic hydroxyl group of the tyrosine, which moves 12 Å, a distance equal to about a quarter of the diameter of the protein. Consequently, this enzyme provides an example of the induced-fit model of binding described in Section 1.1.3. Following this conformational change, the enzyme has formed a binding site of the appropriate shape for binding. Again, the importance can be seen of complementarity of size, shape and binding interactions between host enzyme and guest peptide.

Fig 1.8 : Schematic diagram of the binding of glycyltyrosine to the active site of carboxypeptidase



1.2.2 Synthetic host molecules

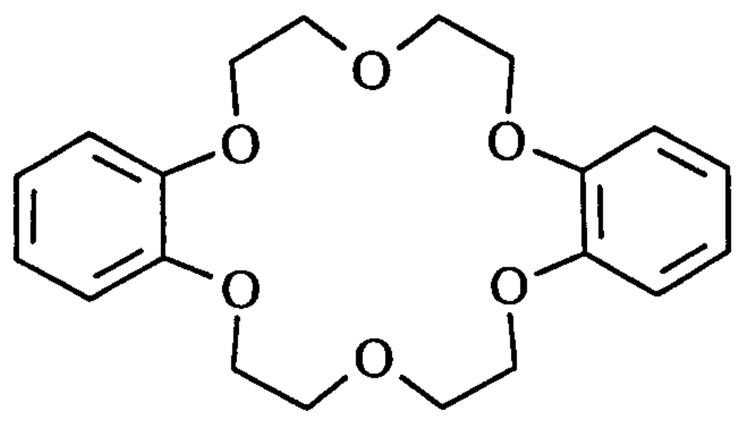
1.2.2.1 Early host molecules

One of the earliest synthetic host molecules to be developed was dibenzo-18-crown-6 (3). It was synthesised by Pederson in 1967 and was found to be the first neutral compound to complex alkali metal ions[6] (Fig 1.9). Moreover, it was found to have partial selectivity for K^+ over other alkali metals. Smaller and larger crown ethers were found to have partial selectivity for alkali metal ions higher up or lower down the periodic table, respectively[4].

In synthesising cryptands, Lehn enhanced the relative selectivities achievable for alkali metal ions[3]. Cryptands, eg. (4), contain a three dimensional, spheroidal cavity which entirely surrounds the bound ion. Consequently, the higher selectivity for a given guest of cryptand over crown ether is explained by the former's greater rigidity and preorganisation (Fig 1.9).

An efficient host for binding tetrahedral guests such as ammonium ions has also been developed (5). It contains a tetrahedral recognition site and is able to bind NH_4^+ rigidly by the formation of hydrogen bonds[34,35]. Further developments related to crown ethers have resulted in the formation of receptors that bind guests with two appropriate functional groups. Such hosts are given the name "ditopic hosts." Thus diammonium cations have been bound by cylindrical molecules - for example (6)[36]. One of the most unusual molecules of this type is (7), in which a porphyrin moiety is incorporated within the host molecule[37].

Fig 1.9 : A selection of host molecules based upon the crown ether and cryptand structures



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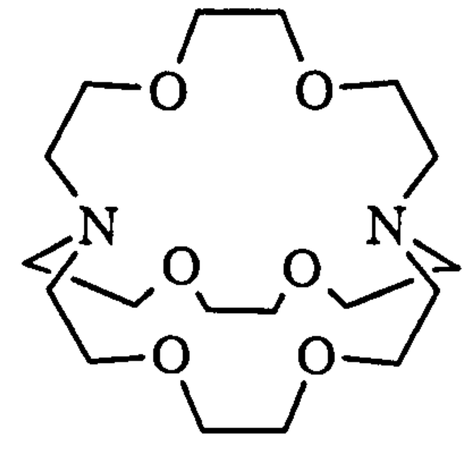

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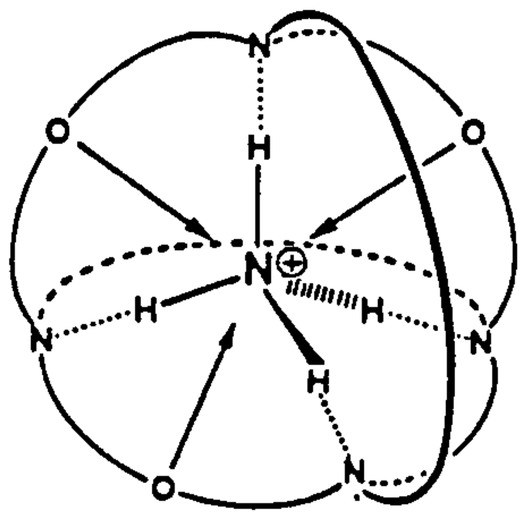

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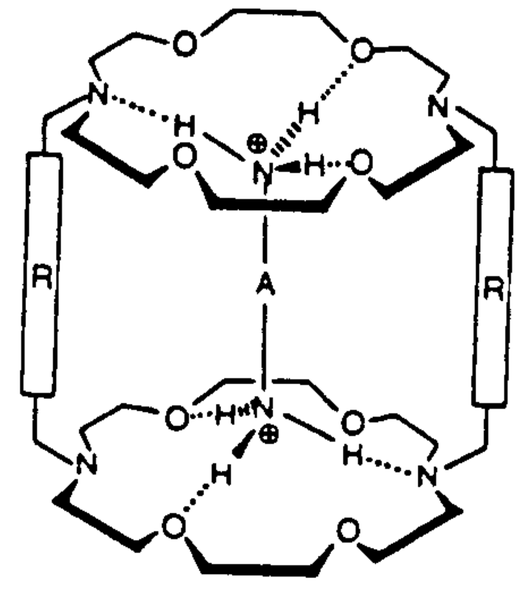

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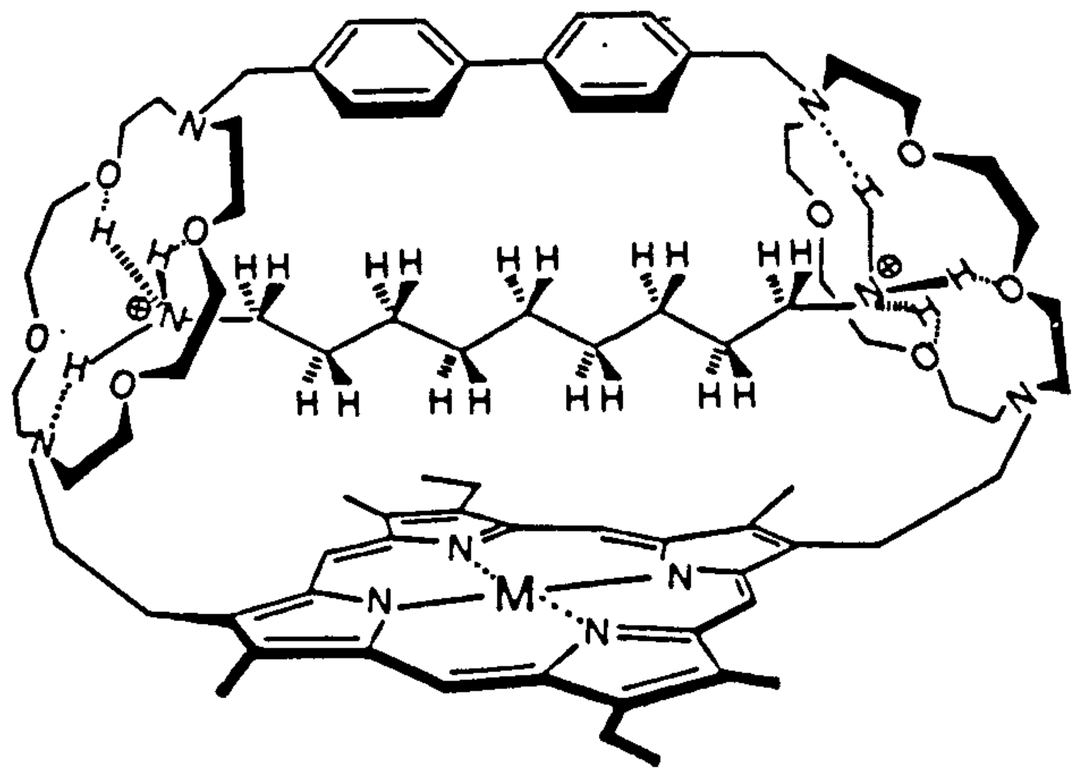


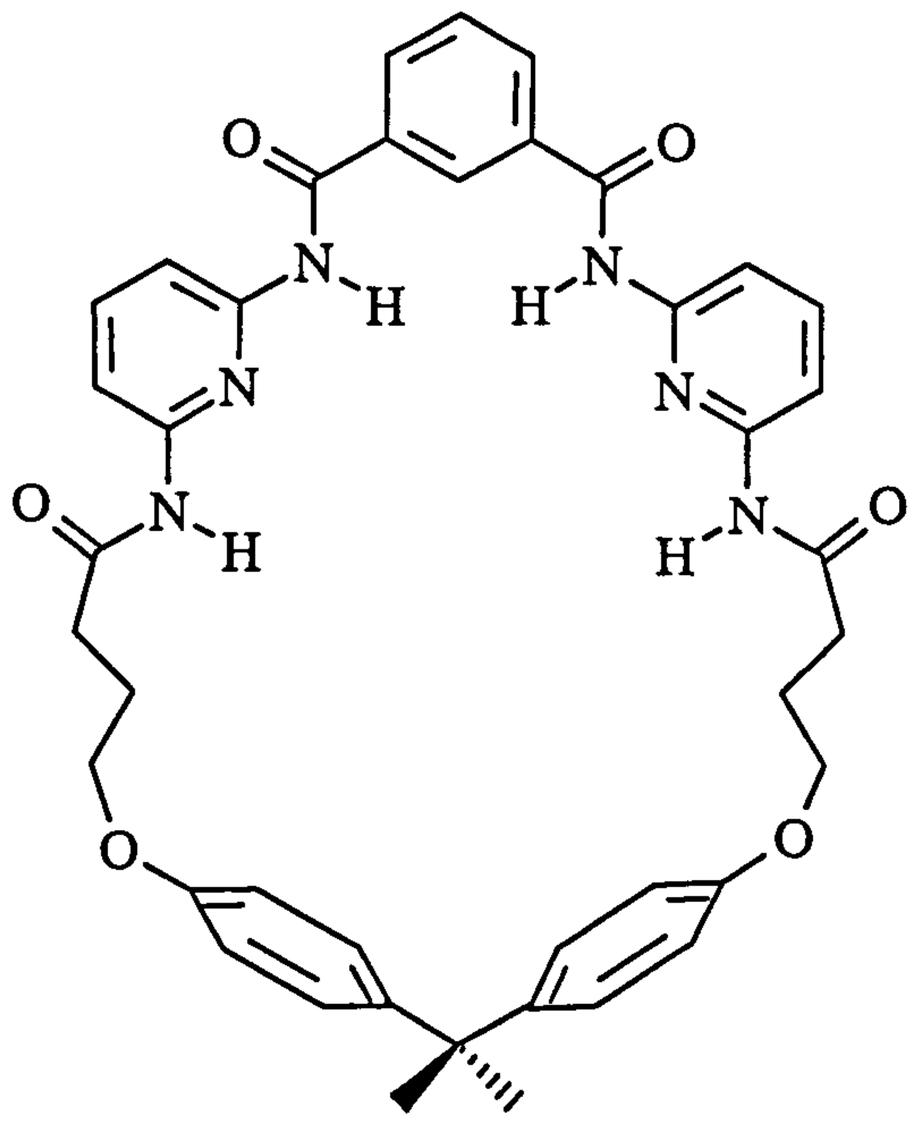
(7)

1.2.2.2 <u>The binding of barbiturates</u>

The use of hydrogen bonds to bind ammonium derivatives is just one example of their widespread use in host-guest chemistry. In another case, Hamilton has synthesised hosts capable of binding barbiturates[38,39]. Barbiturates are widely used as sedatives and anticonvulsants and a possible application for artifical hosts is to facilitate their removal from biological systems. Hosts (8) and (9) contain two 2,6-diaminopyridine units which complement and complex the imide NH's and carbonyls of the barbiturate. The pyridine

units are themselves linked together by suitable spacer groups to form the structures shown.

^{1}H NMR binding studies of (9) in $CDCl_3$, in which host was titrated against guest, indicates 1:1 binding and provides evidence for the hydrogen bonding. Thus, for example, large downfield shifts of the host amide (1.65 and 1.63 ppm) and guest imide (4.38 ppm) resonances are revealed. Further evidence for this binding comes from X-ray crystallography of the bound and unbound host. This technique also reveals the conformational change which occurs in the host on binding, placing all 6 hydrogen bonding groups into one plane. Thus, the host illustrates, for a synthetic system, the induced-fit theory described in Section 1.1.3. Overall, the binding is very strong ($K_a = (6.0 \pm 1.4) \times 10^5$ M^{-1}), due principally to the complementarity of host and guest[39].



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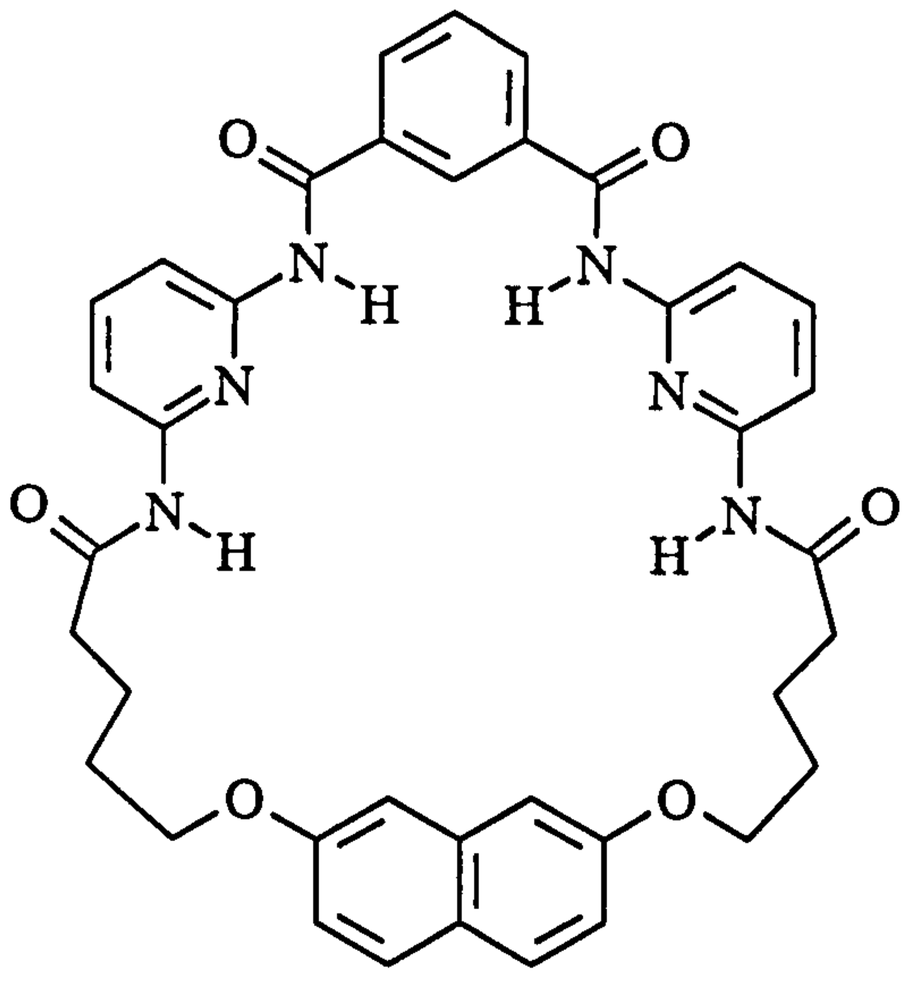
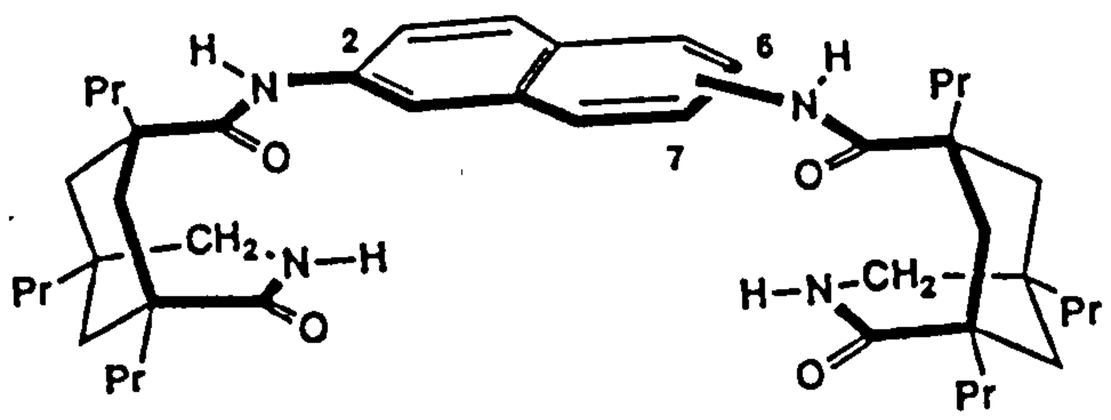


(9)

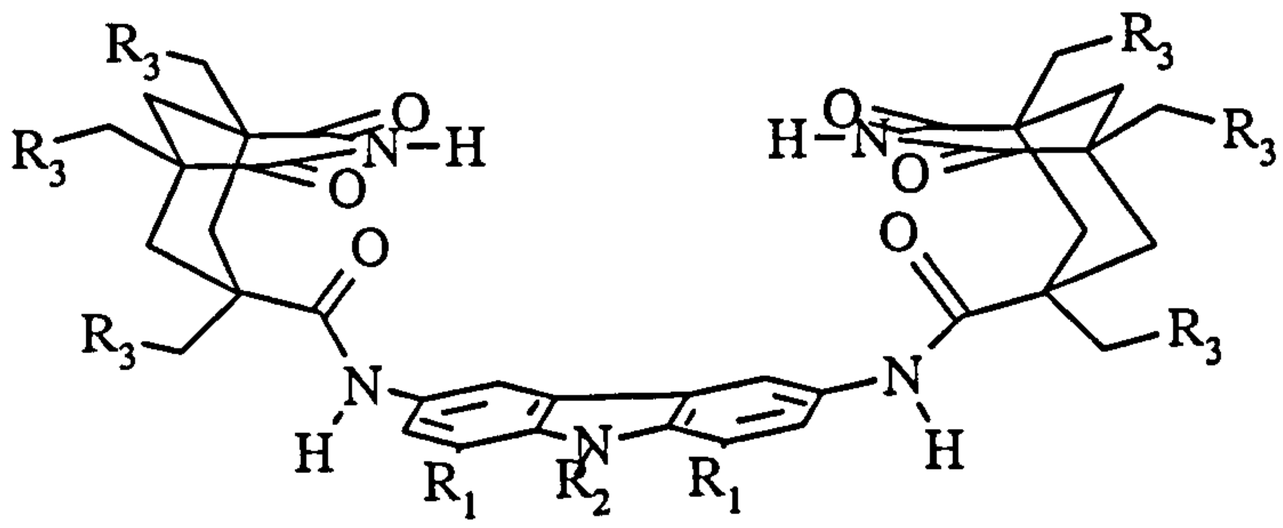
Rebek has also synthesised hosts which are complementary to barbiturates. He has designed an acyclic host molecule in which two derivatised cyclohexane moieties are linked by a disubstituted naphthalene spacer (10). A barbiturate derivative has been bound in $CDCl_3$[40]. The importance of the complementarity of the host to the guest is shown by the fact that whereas the 2,7-disubstituted naphthalene derivative bound the guest strongly (K_a = 32400 M^{-1}), the 2,6-derivative did so 36 times less strongly, K_a = 890 M^{-1}.

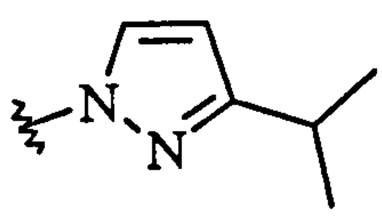


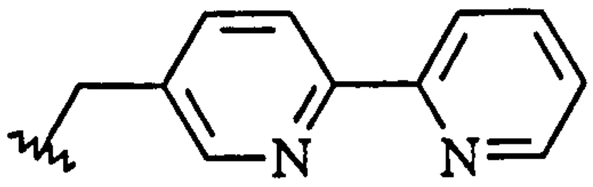
(10)

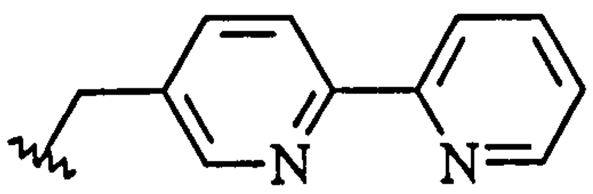
1.2.2.3 The binding of nucleic acid bases

Amongst the many species that Rebek has been able to bind using his cleft like hosts, nucleic acid bases such as ethyl adenine have figured prominently[40,41]. Hydrogen bonding again plays a key role in the intermolecular binding, but π-π interactions are also significant. With hosts (11) to (13), such π-π interactions increase the more polar the solvent used. Conversely, ^{1}H NMR evidence shows that hydrogen bonding between host and guest decreases to negligible as more hydrogen bonding solvents are used. Thus, from $CDCl_3$ to acetone-d_6 to D_2O, binding decreases from 1.5 x 10^5 M^{-1} to 1.3 x 10^3 M^{-1} to 85 M^{-1} respectively.



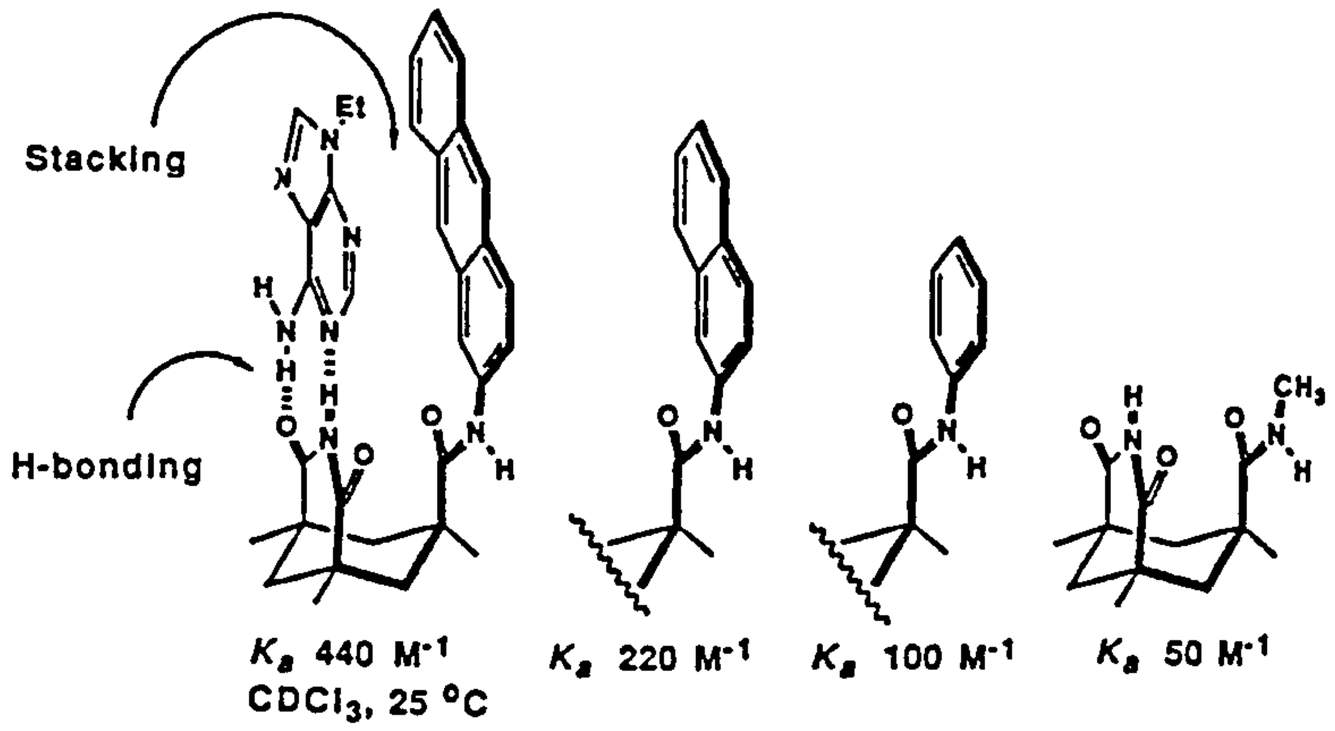
(11) in $CDCl_3$ R_1 =  R_2 = H R_3 = H

(12) in acetone-d_6 R_1 = H R_2 =  R_3 = OBn

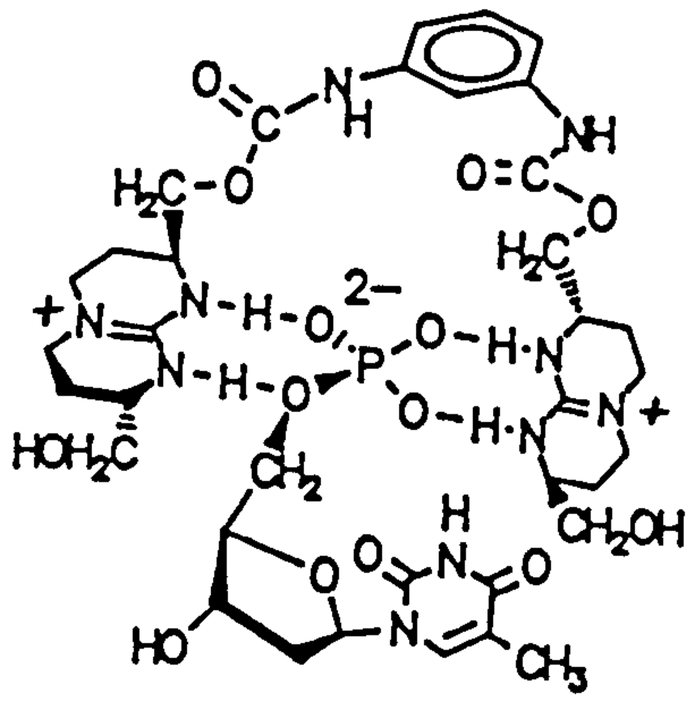
(13) in D_2O R_1 = H R_2 =  R_3 = OH

The importance of π-stacking in the binding of ethyl adenine is illustrated by a study in which Rebek synthesised related host molecules[42]. Scheme 1.1 shows the results of this analysis, in which a difference of 5 kJ mol^{-1}, separates the binding of ethyl adenine to anthracyl versus a methyl derivative.

Scheme 1.1 : Binding of ethyl adenine by the related host molecules of Rebek



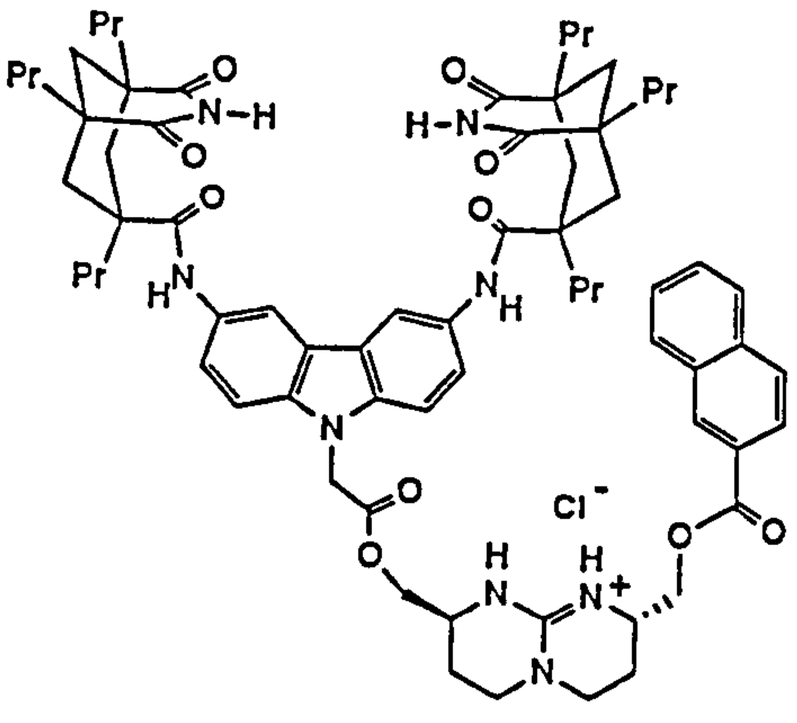
Schmidtchen has synthesised host molecule (14), capable of binding nucleotides as a whole[43]. It contains two guanidines linked by an aromatic urethane unit and binds specifically to the phosphate group on the nucleotide. In his report on this host, the author calculates an association constant of 10.6 M^{-1} in water and claims that it is the first example of the binding of a mononucleotide to an artificial host in water.



(14)

However, Rebek and de Mendoza have synthesised cleft host molecules (15) and (16) capable of binding mononucleotides by forming intermolecular bonds with the phosphate

group as well as the base[44,45]. Both use the guanidine unit of Schmidtchen, to bind the phosphate and use imide groups to bind the base. However, whereas Rebek uses two imides from the two Kemp triacid derivatives in his host, de Mendoza only uses one imide.



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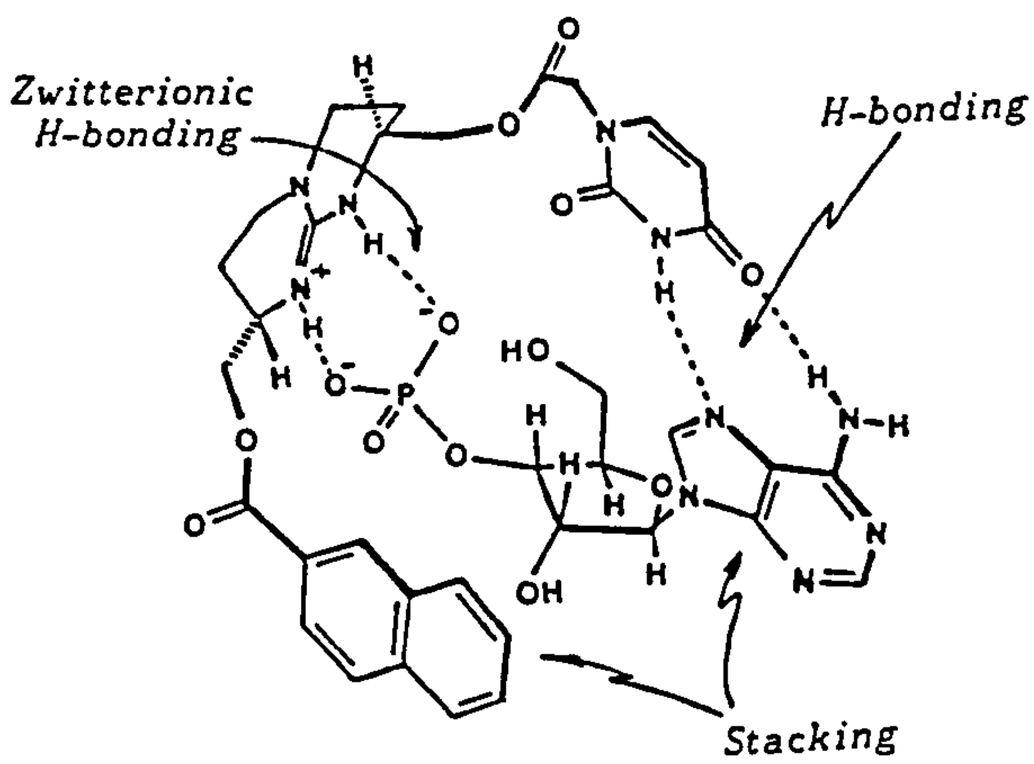
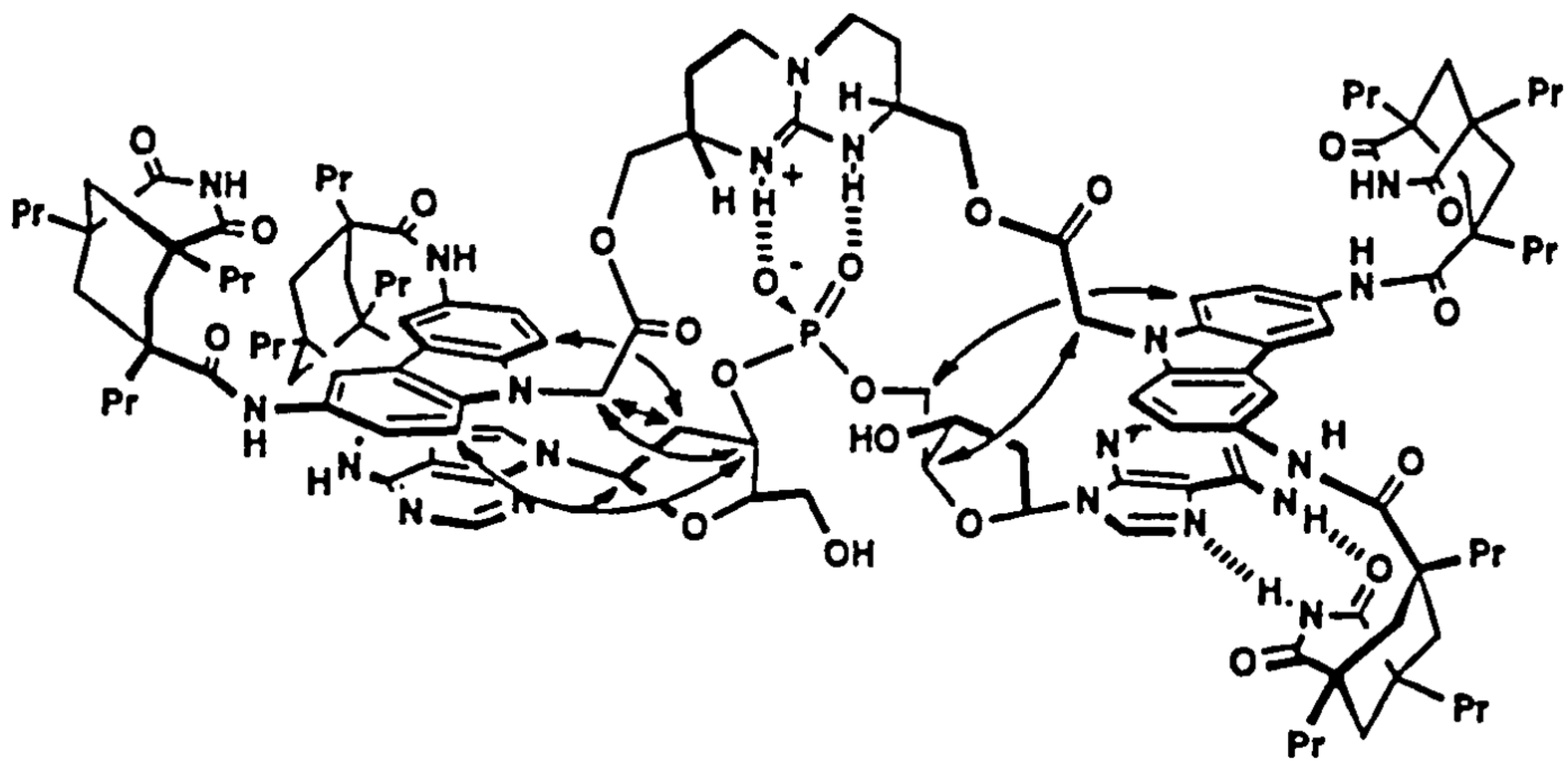


(16)

Finally, Rebek has designed host (17) for binding dinucleotides[46]. This host molecule contains the usual Kemp triacid derivative functionality as well as the guanidine unit.

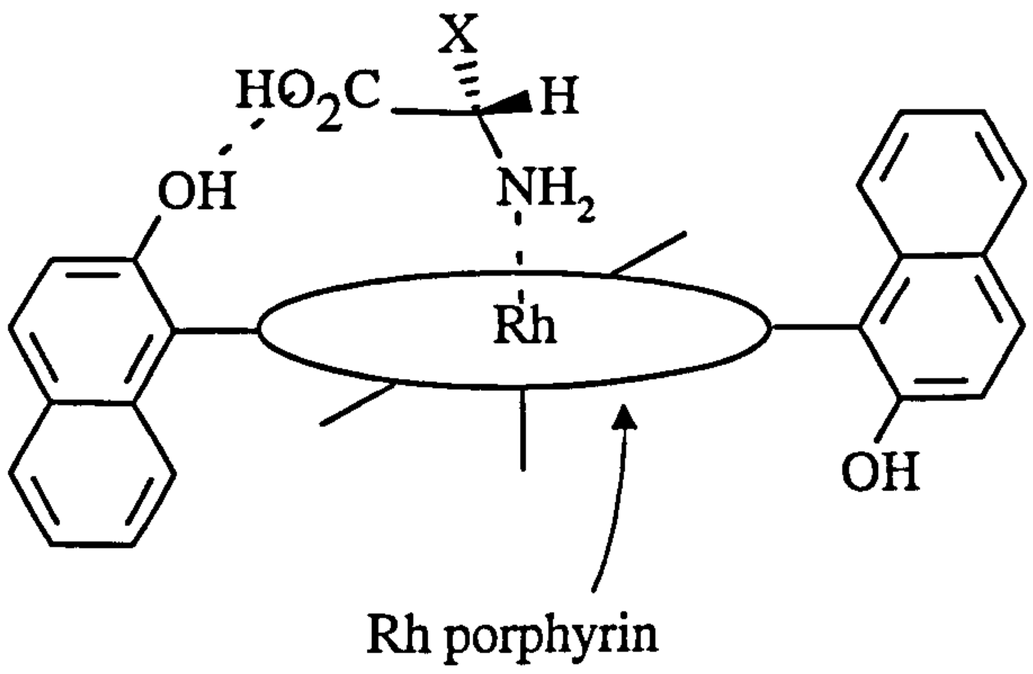


(17)

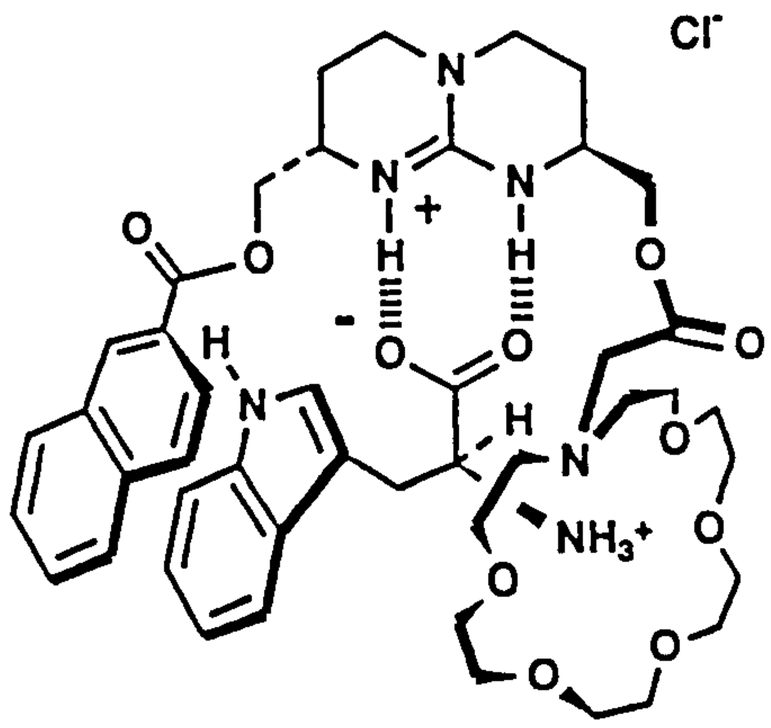
1.2.2.4 The binding of amino acids and peptides

Peptides and amino acids are amongst the most common kinds of guests to be complexed in biological systems. Enzymes, antibodies and antibiotics are examples of host species capable of binding peptides with high selectivity. Thus a great deal of work has been undertaken in recent years to try to mimic these binding interactions[47].

An early example of such a host was synthesised by Ogoshi[48]. This host (18) was based upon bifunctional rhodium porphyrins and was capable of binding amino acids at the amino and carboxyl functions, with binding constants in $CHCl_3$ of up to $5 \times 10^6\ M^{-1}$. The lack of enantioselectivity, however, was a problem.



(18)

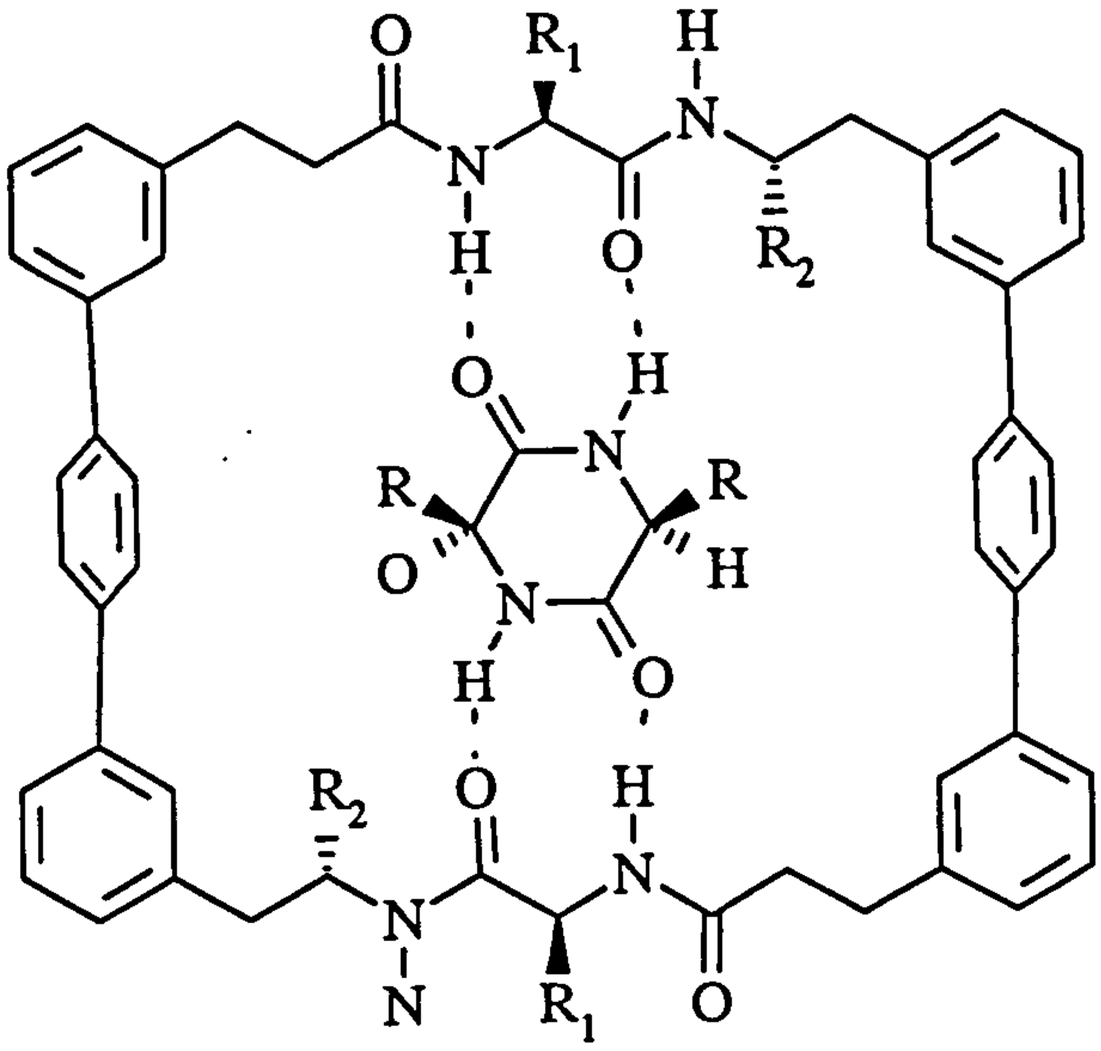


(19)

The enantioselective complexation of phenylalanine and tryptophan was achieved, however, by de Mendoza[49]. The S,S diastereoisomer of his host (19), is capable of selectively complexing L-tryptophan. As can be seen, the amino acid is bound in 3 places with 3 different kinds of interaction - electrostatic, hydrogen bonding and π-π interactions. In the above interaction, only 0.5% of the D-Trp was detected, whilst for D-Phe, the figure was 2%.

Chamberlin has synthesised a receptor (20), which as shown is capable of binding cyclic dipeptides or diketopiperazines[50]. This enantioselective host binds *cyclo*-L-Leu-L-Leu with a binding constant of $K = 606\ M^{-1}$ compared to $K = 74\ M^{-1}$ for *cyclo*-D-Leu-D-Leu, this giving an enantioselectivity of $\Delta\Delta G = 5.15\ kJ\ mol^{-1}$. Chamberlin's host complexes *cyclo*-Gly-L-Leu most strongly with $K = 2260\ M^{-1}$ and an enantioselectivity of

a similar order of magnitude to the previous cyclic dipeptide ($\Delta\Delta G = 4.06$ kJ mol^{-1}). Such enantioselectivity is remarkable for such a flexible host molecule and as shown, binding occurs *via* hydrogen bonding.

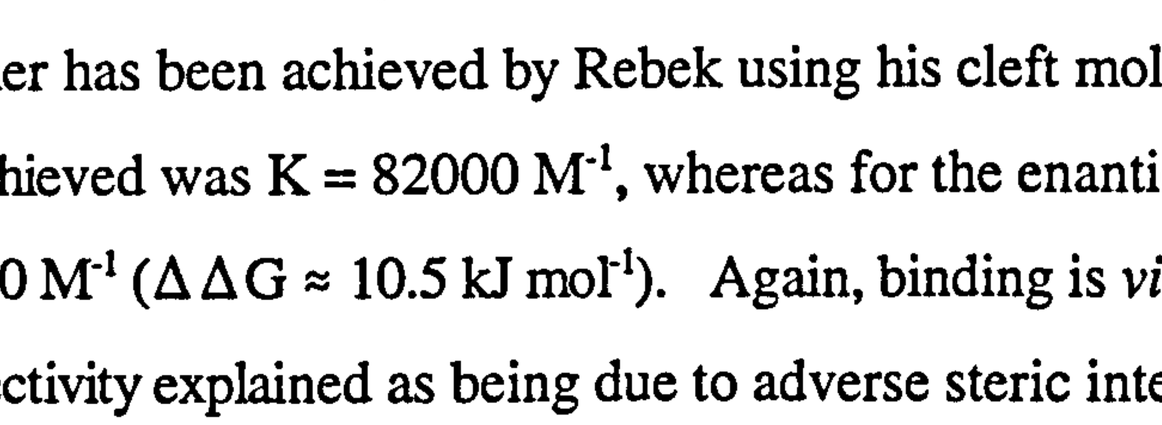


$R_1 = {}^{i}Bu$

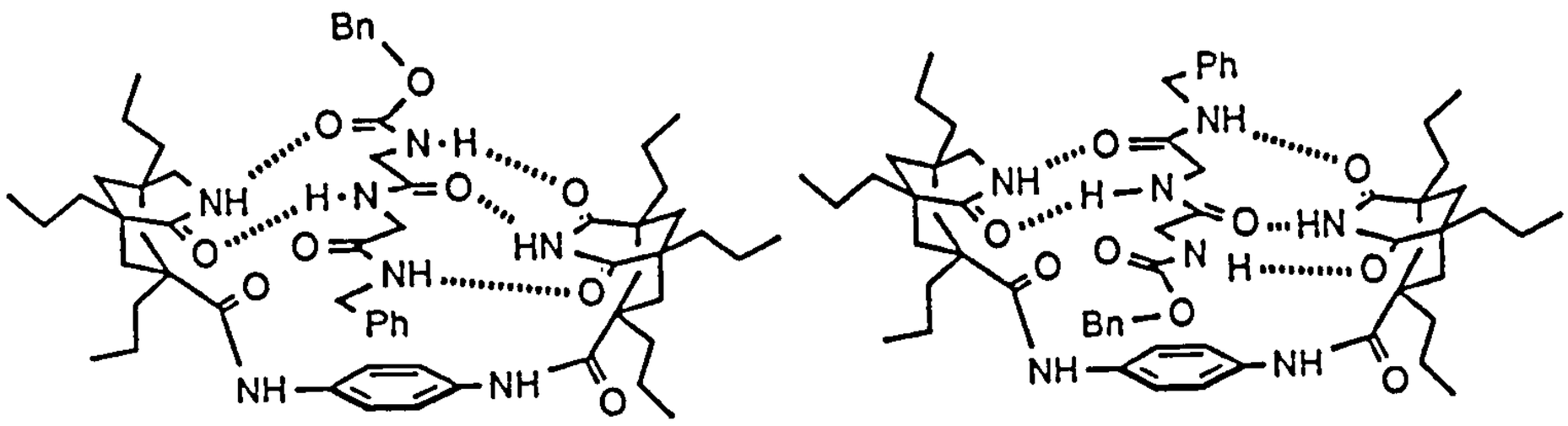
$R_2 = -CONMe_2$

(20)

Much greater enantioselectivity, combined with considerably stronger binding for the favoured enantiomer has been achieved by Rebek using his cleft molecule (21)[51]. In this case, the binding achieved was $K = 82000$ M^{-1}, whereas for the enantiomer of the host, the binding was $K = 840$ M^{-1} ($\Delta\Delta G \approx 10.5$ kJ mol^{-1}). Again, binding is *via* hydrogen bonding, with the enantioselectivity explained as being due to adverse steric interactions towards the unfavoured enantiomer.

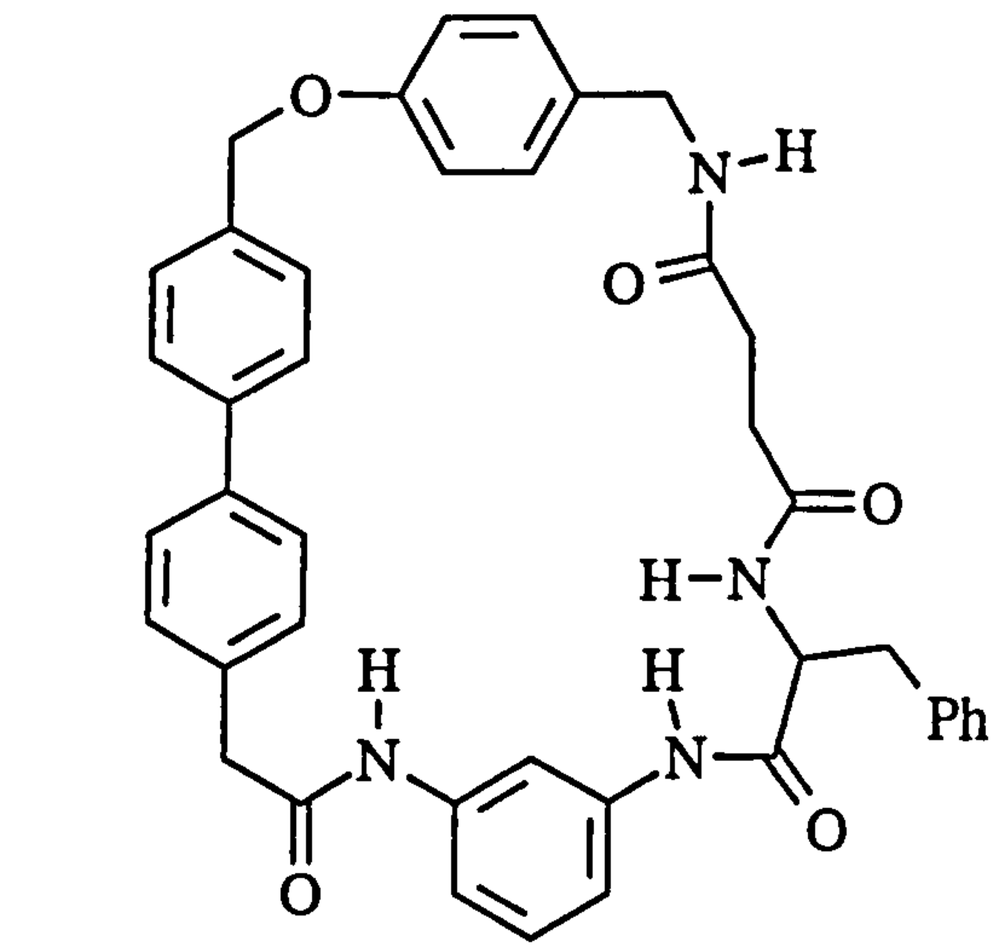


A selection of linear dipeptides have also been bound within related molecules, although this time with reduced enantioselectivity[52]. One enantiomer of the host (22), binds N-Z-L-Leu-Gly-NHBn with a binding constant of K = 4736 M^{-1}. However, in the case of the opposite enantiomer, the binding is only halved, at K = 2340 M^{-1} This comparatively poor enantioselectivity is explained as being due to the flexibility inherent in both host and guest. When more rigid guests such as the cyclic dipeptide piperazines are bound, enantioselectivity is correspondingly greater.



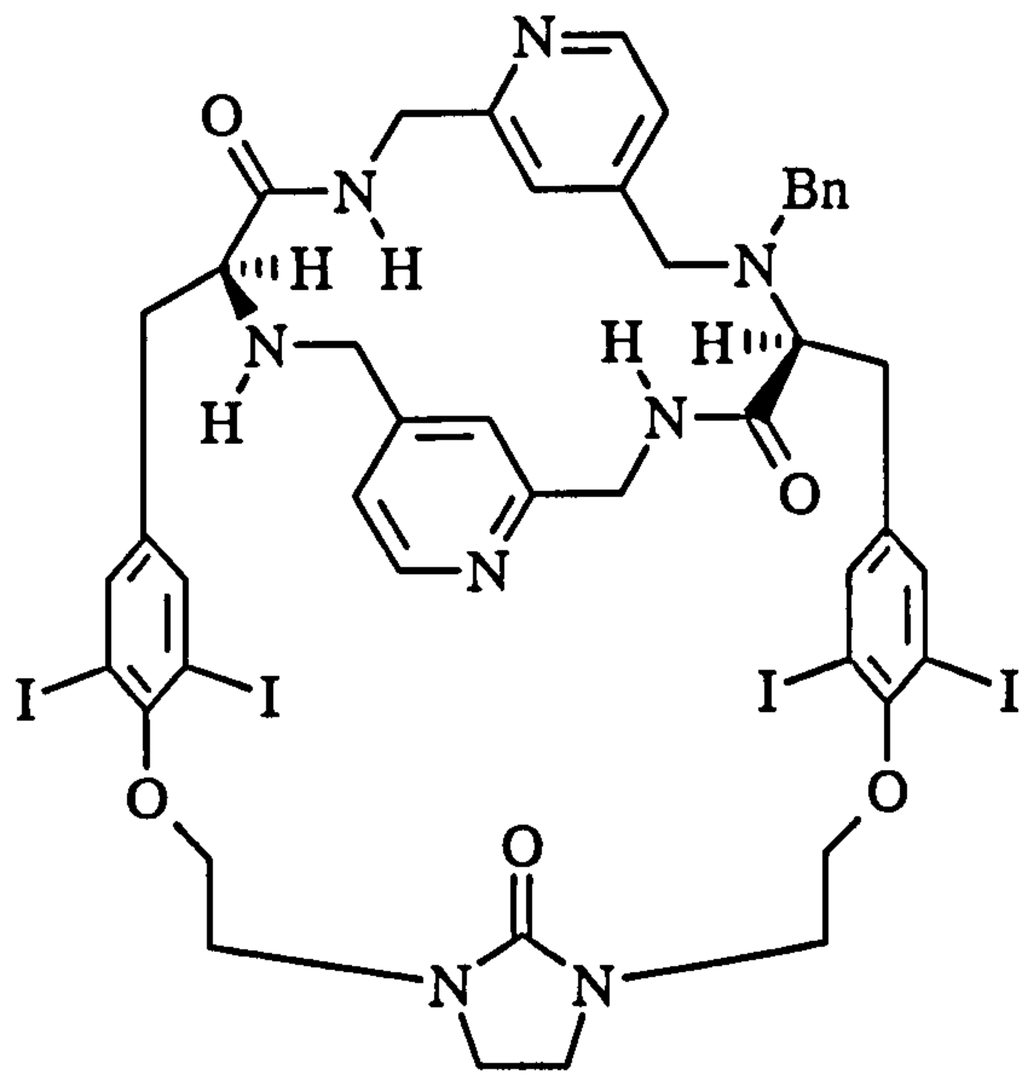
(22)

Particularly impressive enantioselective binding has been achieved by Kilburn. He has synthesised a selection of host molecules capable of binding amino acids and peptides[53,54]. Host (23) is a recent example and has been used to achieve a binding enantioselectivity of 80:20 or $\Delta\Delta G = 3.2$ kJ mol^{-1} using N-benzyloxycarbonyl-β-alanyl-L-alanine as guest[55]. As with Rebek's host molecules (21) and (22), Kilburn's macrocycle was synthesised in racemic form - the racemate being bound to a homochiral guest. The solvent was $CDCl_3$, with binding constants of $-\Delta G_{assoc} = 19.4$ and 16.2 kJ mol^{-1}, being calculated using a ^1H NMR titration experiment.



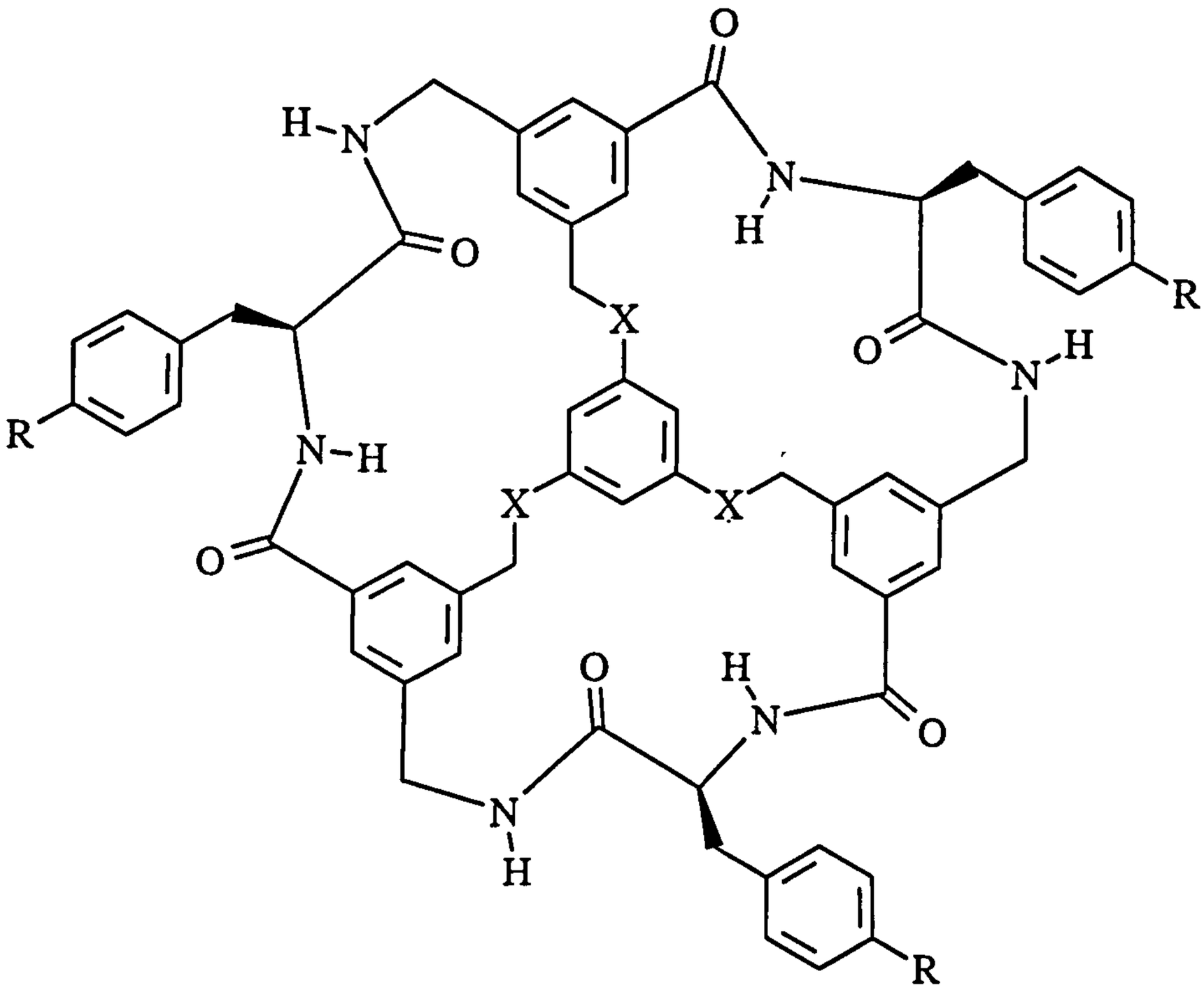
(23)

Some of the best enantioselective binding, however, has been achieved by Still[56-58]. One of his earliest peptidic host molecules (24) contains an amide binding site and additional functionality designed to distinguish between different guest enantiomers *via* steric and hydrogen bonding interactions. Enantioselectivities of 70 % have been achieved as in the case of the strong binding in $CDCl_3$ of Ac-L-Ala-NH-*tert*-butyl ($-\Delta G = 9.84$ kJ mol^{-1}) over the weaker binding of its enantiomer Ac-D-Ala-NH-*tert*-butyl ($-\Delta G = 4.35$ kJ mol^{-1}). In this case, the difference of 5.49 kJ mol^{-1} is quite large. A difference of 3.8 kJ mol^{-1} was recorded for Ac-L-Ala-L-Ala-OBn against Ac-D-Ala-L-Ala-OBn, also in $CDCl_3$.



(24)

More recently a selection of novel C_3-symmetric host molecules ((25) - (28)) have been synthesised[59-61]. Each of these molecules possesses a deep apolar binding cavity with alternate hydrogen bond donor (D) and acceptor (A) sites and conformational stability. These features enable hosts to bind amino acids and also octyl glycosides with high selectivity.



(25) X = O, R = H

(26) X = S, R = H

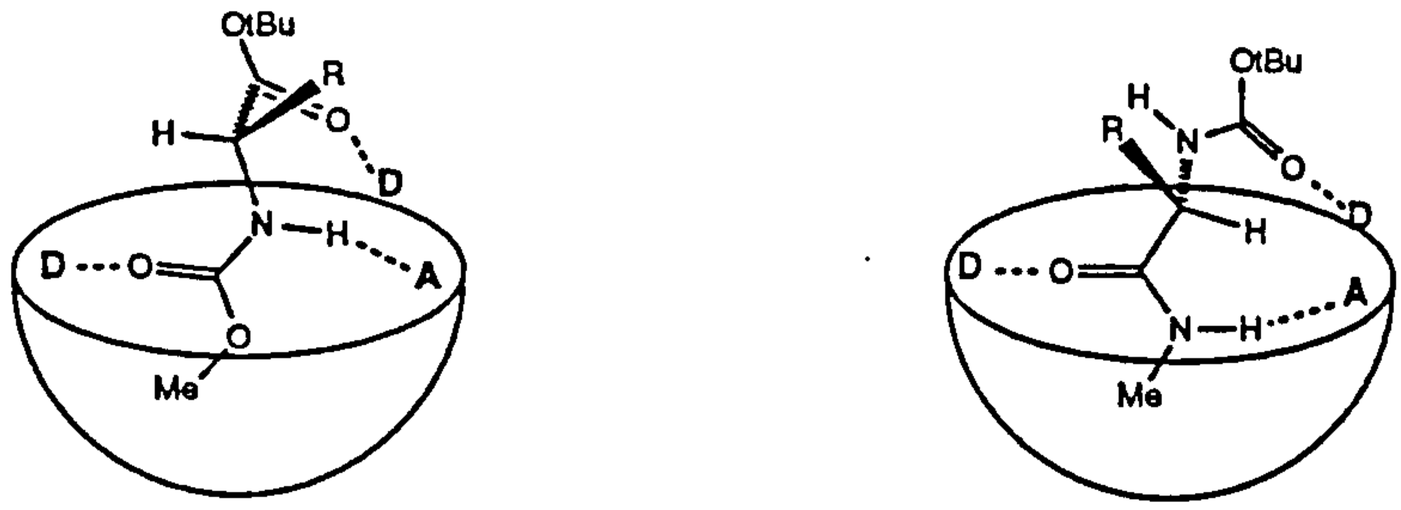
(27) X = S, R = $OCH_2CH=CH_2$

(28) X = S, R = CH_2CH_2-N(Et)-Dye

Host (26) is particularly appropriate for the binding, with high selectivity, of Boc protected N-methylamides of α-amino acids such as Boc-Val-NHMe[61]. In chloroform, the highest enantioselectivities were found when derivatives of alanine, valine, leucine, serine and threonine were used as guests. In these cases, enantioselectivity corresponded to 8-12 kJ mol^{-1} or 90-99% ee, in favour of the L-enantiomer. It was also found that such

derivatives of serine and threonine with their hydroxylated side chains bind ≥ 8 kJ mol^{-1} more tightly than equivalent amino acids with simple alkyl side chains such as alanine, valine and leucine. Those amino acids with an N-terminal methoxycarbonyl group generally bind more strongly. For example, L-MeO_2C-Ala-O^{t}Bu binds 3.8 kJ mol^{-1} more strongly than L-Boc-Ala-NHMe. However, enantioselectivities are similarly high in the range of 90-99%. The manner in which many of these guest amino acids bind is illustrated in Fig 1.10.

Fig 1.10 : Schematic diagram of the binding of amino acid derivatives by host molecule (26)



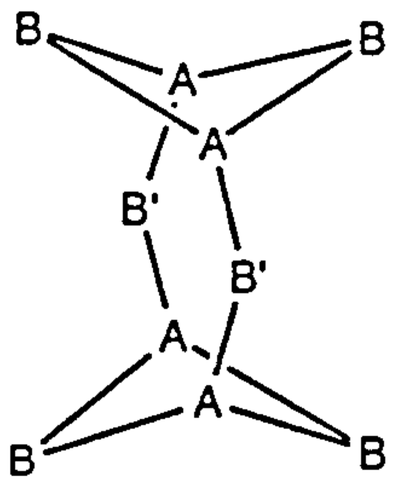
Practical applications for this host molecule have been devised whereby it is covalently bound to a solid phase support in order to effect the resolution of amino acid derivatives. Thus (27), which has the same enantioselective binding characteristics as (26), has been designed to bind to such a support[62].

Similarly, host (28) can be synthesised by binding the host to a dye such as Disperse Red. Using Combinatorial Chemistry to synthesise the guests, up to 50000 different potential peptide guests can be screened for binding[63,64]. A Combinatorial Library of different substrates is attached to Merrifield beads such that there is only one substrate per bead. Different chemical tags are also attached which following the binding experiment can be readily removed by photolysis and analysed by gas chromatography so as to rapidly distinguish the structure of each bound guest. It is clear from the experiment, which beads one needs to analyse in order to determine the most strongly bound dyed host molecule since they are the ones with the most intense red colour. By this method, one of the highest artifical enantioselectivities known was identified. Following large scale synthesis of the

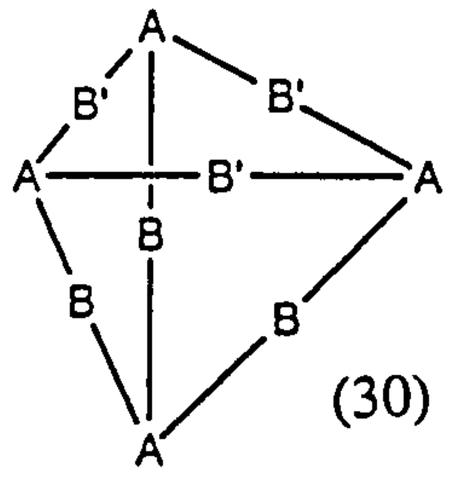
guest, cyclopropanoyl-L-Ala-L-Pro-L-Ala-$NHC_{12}H_{25}$ was found to bind with $\Delta G = 23.9$ kJ mol^{-1}, whereas its diastereomer cyclopropanoyl -D-Ala-L-Pro-L-Ala-$NHC_{12}H_{25}$ was not observed to bind at all[63].

A final example of a host molecule designed by Still (29) likewise binds peptides with high enantioselectivity[65]. However, its major advantage is its ease of synthesis. A 13% yield has been achieved simply by mixing the acid chloride of A with B at 3 mM concentration with Honig's base in dry THF.

As before, when a dye is attached to the substrate (30), Combinatorial Library binding is possible, with the result that 50000 acylated tripeptides have been analysed for their binding potential[66,67].



(29)



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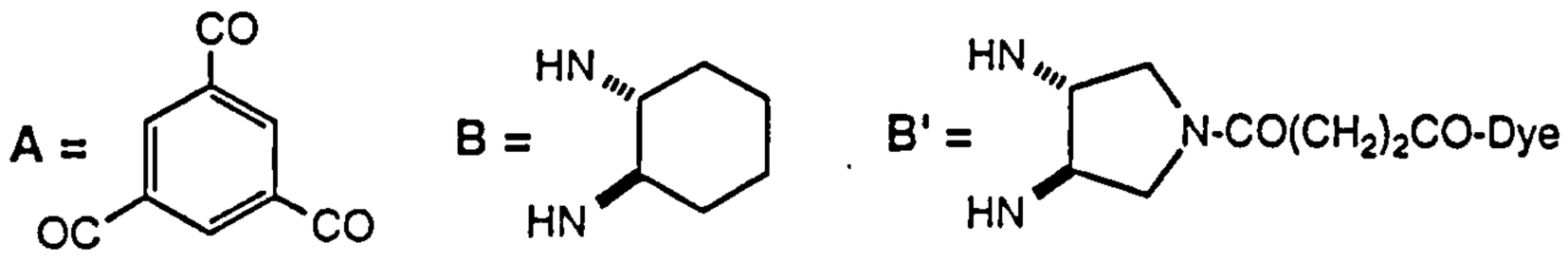


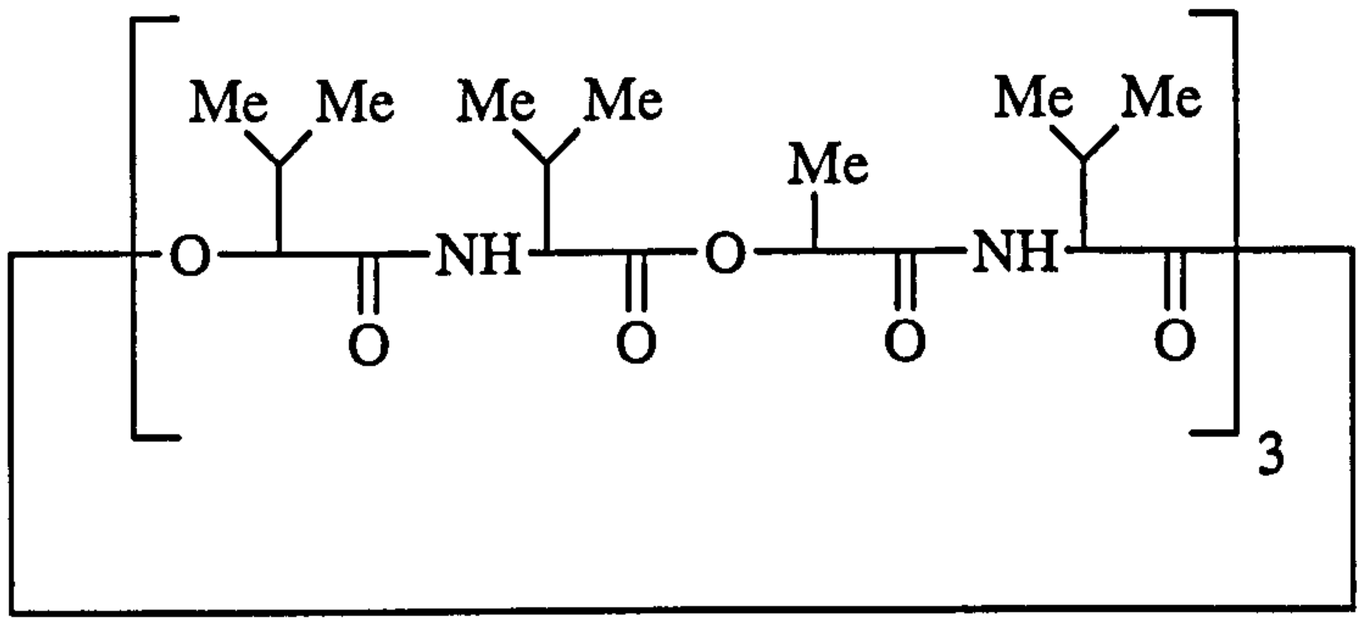
1.3 Transport

1.3.1 Natural transport systems

In addition to the binding of guests within a particular solvent environment a variety of host molecules are able to transport them and then release their guests into a different medium. A well known example of a natural host molecule which transports guests across a membrane in biological systems is *valinomycin* This molecule has been a model for many synthetic host molecules. In the following pages its structure and mode of action will be described followed by examples of two synthetic transport systems.

1.3.1.1 Valinomycin[68]

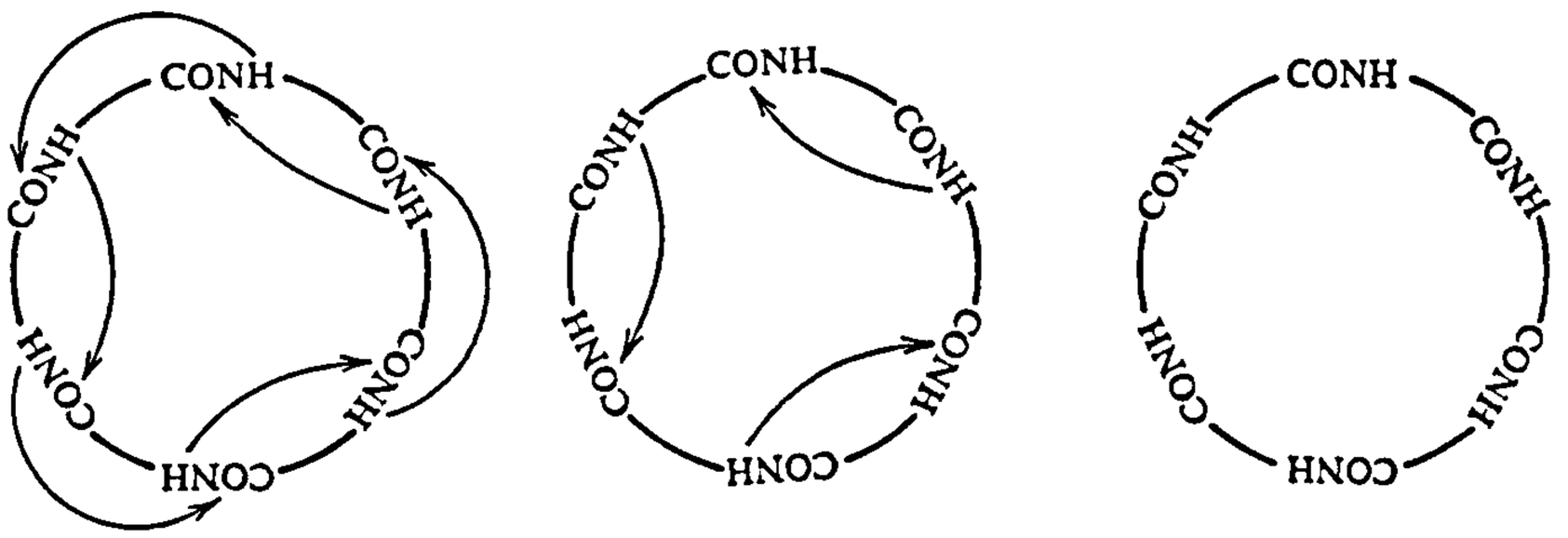
Valinomycin is a cyclic dodecadepsipeptide, an unusual peptide having alternate amino and hydroxycarboxylic acids linked by amide and ester bonds; [L-Valine-D-hydroxyisovaleric acid-D-valine-L-lactic acid]$_3$ (31). Its physiological function is the selective transport of K^+ ions across cell membranes from the outside to the inside of cells.



(31)

Valinomycin was isolated from *streptomyces fulvissimus* by Brockmann and Schmidt-Kastner in 1955[69] and synthesised in 1963 by Shemyakin[70]. As such it played an important part in the birth of the chemistry of molecular recognition. Its conformation in solution depends upon the solvent polarity (Fig 1.11)[71].

Fig 1.11 : The different hydrogen bonds present in valinomycin depending upon the nature of the solvent.



A ⇌ B ⇌ C

A non-polar solvent, eg. heptane, CCl_4

B medium polarity solvent, eg. $CHCl_3$

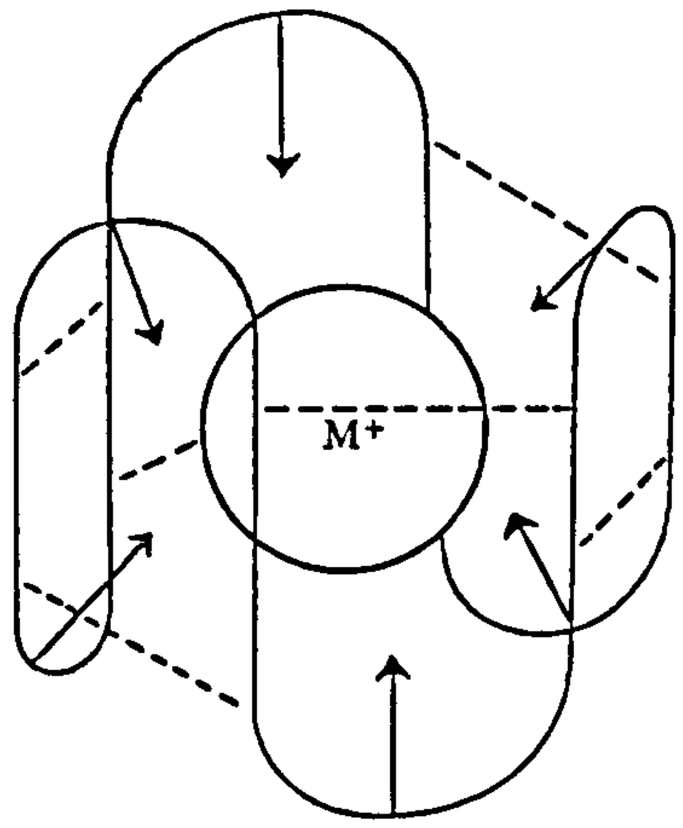
C Highly polar solvent, eg. water

In non-polar solvents such as heptane or CCl_4, the bracelet-like structure A is mainly present. In this structure, all the NH groups participate in hydrogen bonding. In solvents of medium polarity, hydrogen bonding breaks down such that only D-valyl NHs are involved - as represented by structure B. At elevated temperatures and in highly polar solvents, the NH groups are hydrogen bonded to the solvent - structure C.

On complexation with K^+ a structure having three-fold symmetry is formed, with the cation located at the centre of a 36 membered macrocycle and octahedrally co-ordinated to six ester carbonyl groups[72-74]. All the carbonyl oxygen atoms of the amide atoms of the amide groups take part in hydrogen bonding thus establishing a ring of limited flexibility. The diameter of the cavity corresponds to the size of K^+ or Rb^+ and thus valinomycin is specific for K^+ over Na^+.

The presence of the lipophilic side chains pointing towards the exterior of the molecule contribute to the solubility of the complex in solvents and media of low polarity, such as membranes. Thus the complex is able to travel through lipid membranes such as cell walls. A complex involving Na^+, in which the ion is bound weakly to the outside of valinomycin[75] would be unable to do this. A schematic diagram of the mode of binding of K^+ is given in Fig 1.12.

Fig 1.12 : Schematic diagram of the binding of K^+ by valinomycin

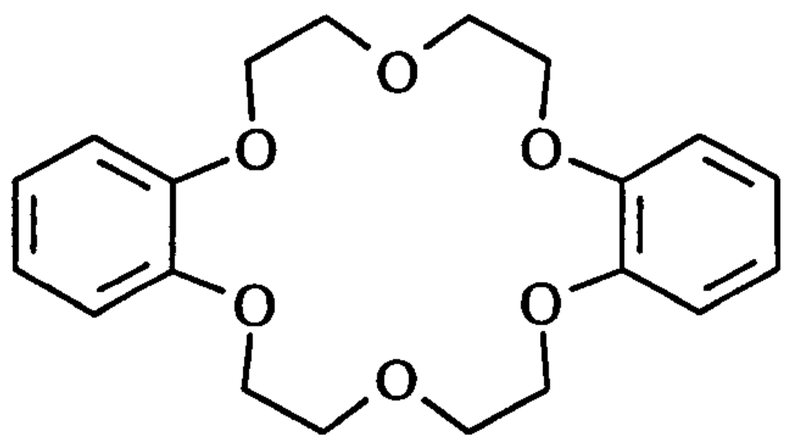


1.3.2 Synthetic transport systems

In designing a host for membrane transport, such a molecule should ideally possess different properties in each of three, chemically different media - the source phase, lipid membrane interior and the receiving phase. In the source phase, binding should be fast and strong[76]. In the hydrophobic membrane the cation should be strongly bound. In the receiving or exit side of the membrane, binding should be weak and release of the guest fast. Just some of the ways in which this is achieved synthetically are described in the next few pages.

1.3.2.1 Transport of amino acid anions

Tsukube has studied the transport of amino acid anions across a chloroform membrane[77]. His apparatus consisted of a U tube in which was placed a solution of the host in chloroform. Buffered aqueous solutions of equal concentrations of the amino acid anion at the same pH were placed in the arms of the U tube. These aqueous solutions floated on the chloroform membrane. The concentrations of the amino acid anions in both aqueous phases were recorded at steady state, usually after 24 h.



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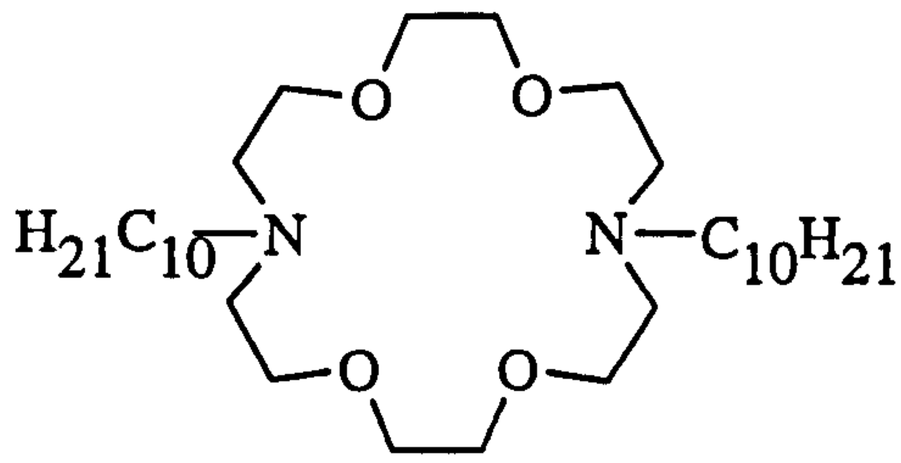

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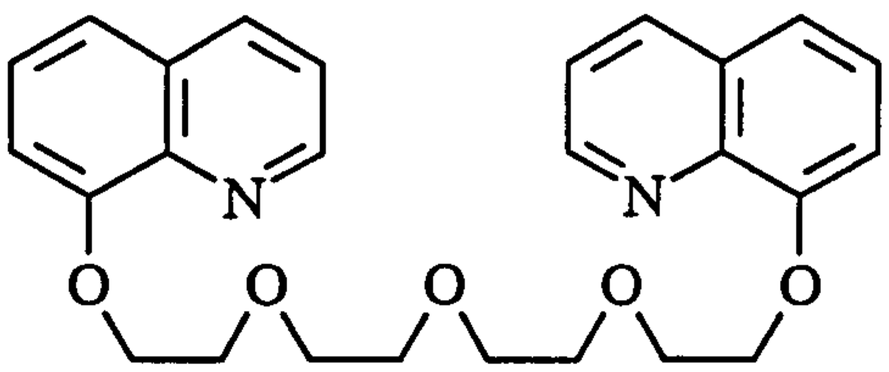
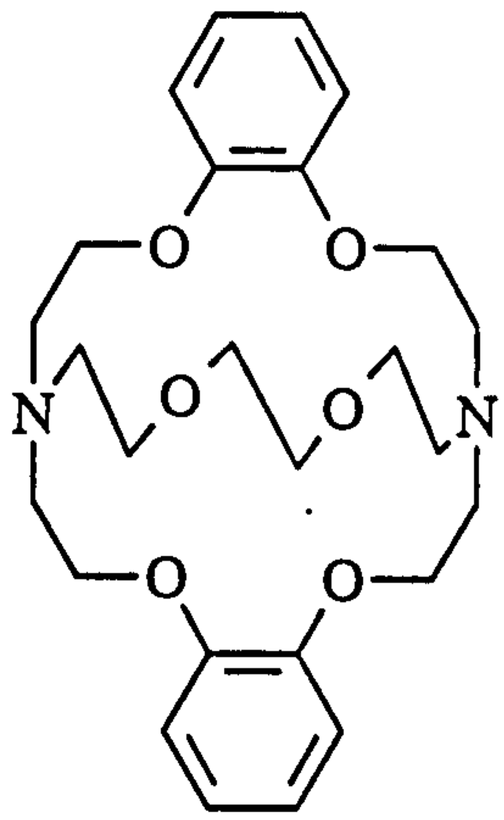


(34)

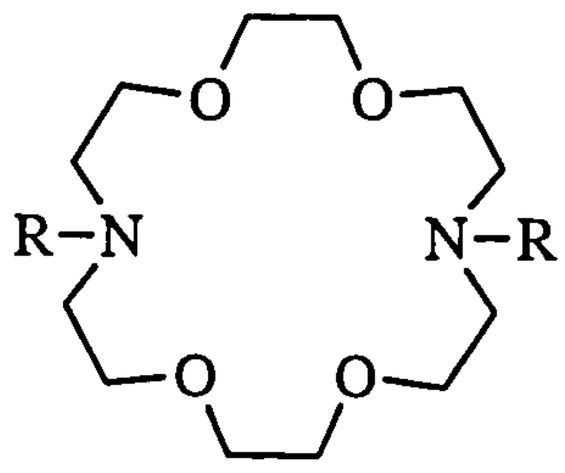


(35)

In the presence of an alkali-salt such as KCl in the aqueous source phase, the amino acid derivatives were successfully transported against their concentration gradients by the aid of artifical hosts (32) to (35). Each host proved to have differing abilities as a carrier of amino acids across the membrane. Thus crown ether (32) > diaza-crown ether (33) > non-cyclic crown ether (34) ≈ cryptand (35). In addition, each host had its own guest specificity. Thus, for example, crown ether (32) transported Bz-Phe more readily than Bz-Gly. With Bz-Phe as the guest, the steady state concentrations suggested that the receiver aqueous phase contained 70 % of the available Bz-Phe. With Bz-Gly as the guest, however, the receiver phase contained only 50 %.

However, such selectivities are small. Each of these hosts form complexes selectively with K^+, the hosts recognizing this metal only. The Z-amino acid carboxylate anions are co-transported, owing to ion pairing with cationic host - K^+ complex. Thus the differing rates of amino acid transport are probably due to the differing guest lipophilicities rather than the specific recognition ability of each host[78].

Gokel has used a similar system to that of Tsukube, again using a chloroform membrane[79]. The rates (mol h^{-1}) at which Z-protected amino acid anions were transported through the membrane were measured. (36) and (37), called lariat ethers, are examples of the kinds of host molecules used. Such hosts are derived from diaza-crown ethers and dipeptides.



(36) R = Z-PhgGly

(37) R = MeOPhgGly

$Z = PhCH_2OCO-$

Phg = D-α-phenylglycine

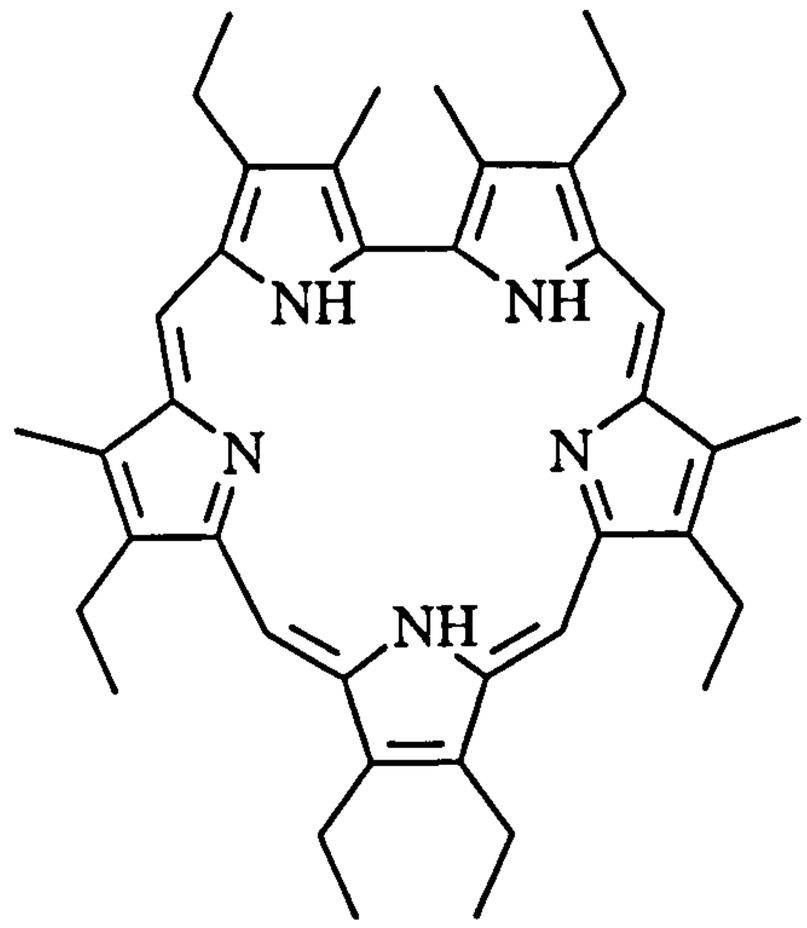
Chiral recognition of enantiomeric guests provides strong evidence of direct recognition of guests by the host's chiral pendant arms. Thus, for example, 0.15 mmol Z-L-Phe-OK was transported by 0.0372mmol (37) across the 3 cm^3 membrane at a rate of 20.5 μmol h^{-1}, compared to 14.9 μmol h^{-1} for Z-D-Phe-OK. The existence of hydrogen bonding interactions between the lariat pendant arms and bound Z-PheOK was shown by specific NH proton shifts in the ^1H NMR spectra of 1:1 mixtures of (37) and Z-PheOK in $CDCl_3$.

1.3.2.2 Transport of nucleotides and nucleosides

Nucleotide analogues have recently been the focus of considerable attention due to their potential antiviral activity. However, because of their charged nature, many nucleotides are poor at penetrating through the cell walls and into cells. Lipophilic host molecules, capable of masking this charged nature might have utility in aiding the passage of nucleotides through cell walls and delivering them to the point where they are required[80].

Sessler has used a host system to mediate the transport of guanosine 5'-monophosphate (GMP) through a lipophilic membrane[80]. The system used was a simple

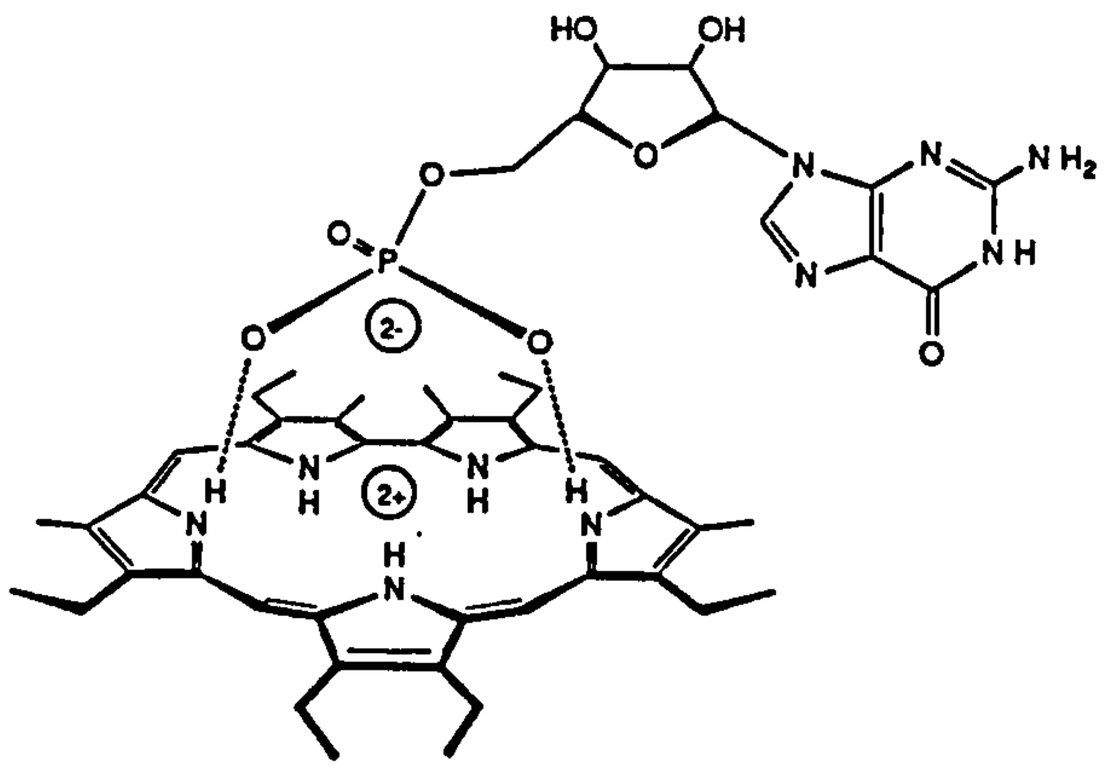
three phase (H_2O - CH_2Cl_2 - H_2O) model membrane. The host was a 22π-electron pentapyrrolic "expanded porphyrin" called a sapphyrin (38)[81]. Such a molecule can exist as H_3Sap, H_4Sap^+ and H_5Sap^{2+} according to its degree of protonation.



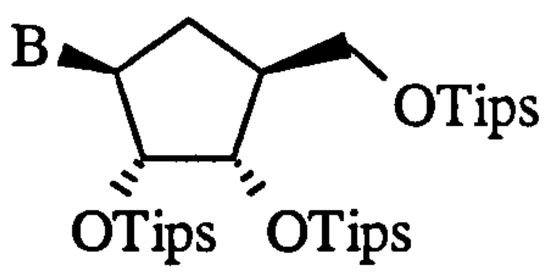
(38)

Initial transport experiments were carried out at pH 2.5 with GMP^{2-} diacid in the initial aqueous phase (Aq I) and H_5Sap^{2+} in the CH_2Cl_2 phase. A steady build up of GMP in Aq II was observed, corresponding to an initial transport rate of 86.9 x 10^{-3} μmol cm^{-2} h^{-1}. By contrast, when sapphyrin is not present in the organic phase, no transport occurs, even after 3 days. When the pH is increased to pH 4, initial transport rates decrease dramatically as the H_4Sap^+ form of Sapphyrin gains stability. Thus a 1:1 complex between sapphyrin and GMP diacid is proposed, as illustrated in Fig 1.13.

Fig 1.13 : The binding of a nucleotide by sapphyrin (38)



Nucleoside transport is also possible and by using triisopropyl (Tips) substituted nucleoside derivatives as the host molecules (39) to (42), other nucleoside analogues have enhanced transport in a simple (H_2O - $CHCl_3$ - H_2O) liquid membrane system[82]. Amongst these nucleoside analogues is 3'-azido-2',3'-dideoxythymidine (AZT) (43), an approved drug in the treatment of AIDS. AZT has high intrinsic lipophilicity but a small enhancement of AZT transport across the chloroform membrane was observed using (39), (40) and (42) as transport agents. It is thus possible that analogous transport agents might encourage the transport of AZT through a cell membrane.



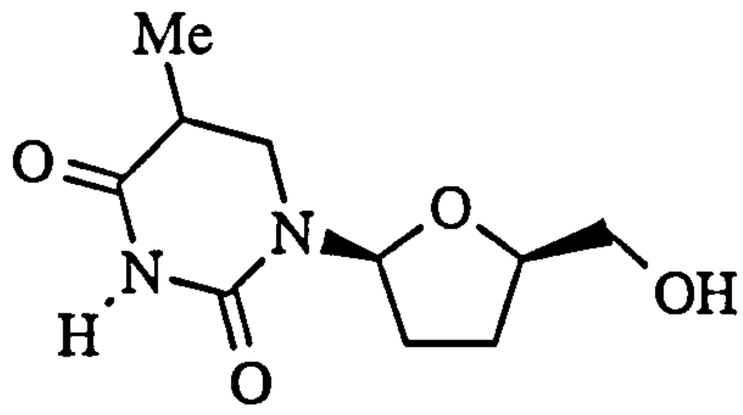
Tips = triisopropyl

(39) B = adenine

(40) B = uracil

(41) B = cytosine

(42) B = guanine



(43)

1.4 Catalysis

1.4.1 Natural catalytic systems

One of the key aims in the synthesis of host molecules is to produce systems which not only bind guests specifically but also catalyse reactions between them. If this can be done efficiently with high turnover and with minimal degradation of the host molecule, then an enzyme mimic will have been produced[83]. In the pages which follow, the catalytic processes occurring in a digestive enzyme called *chymotrypsin* will be outlined, following which some examples of synthetic hosts involved in the catalysis of both bond fission and bond fusion reactions will be described.

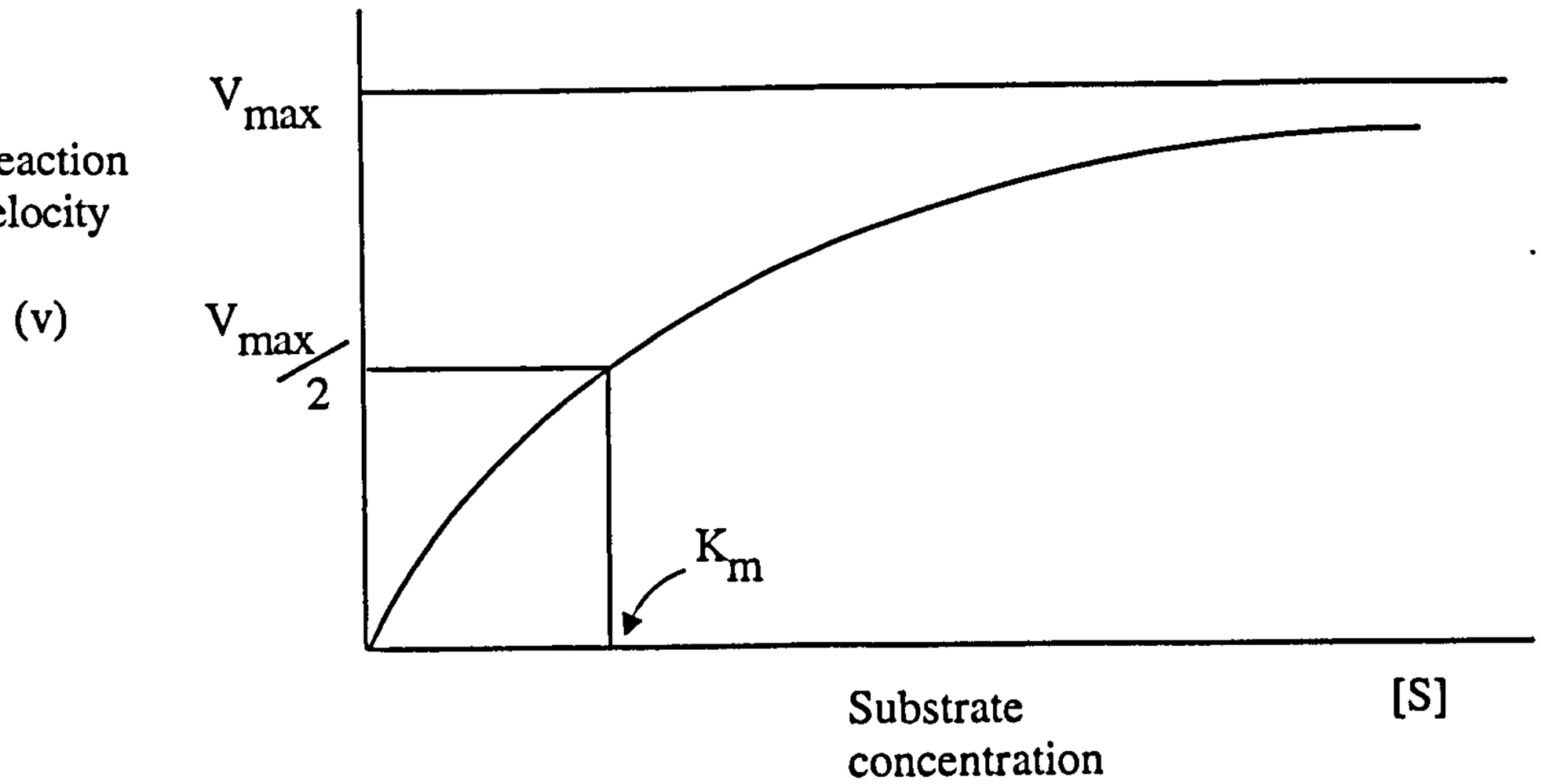
1.4.1.1 Chymotrypsin[17]

This enzyme consists of three polypeptide chains connected by two interchain disulphide bonds folded to produce a compact ellipsoid shaped molecule. Produced in the pancreas, its biological function is to catalyse the cleavage of peptide bonds specifically at the carboxyl side of aromatic side chains tyrosine, tryptophan and phenylalanine and of large hydrophobic residues such as methionine.

The mechanism of the reaction requires 3 key residues which act together as a *"catalytic triad"*. The three residues which form this triad, Ser 195, His 57 and Asp 102, are positioned close together in space and the latter two amino acids activate Ser 195 to react with the amide bond to be cleaved. The mechanism, which is illustrated in Scheme 1.2, involves attack by the Ser 195 hydroxyl group onto the amide bond to form a tetrahedral transition state and then an acyl enzyme intermediate. Acyl transfer thus occurs - a reaction which is mimicked in some of the synthetic systems described in Section 1.4.2.1. The acyl enzyme intermediate is then hydrolysed by water.

As with many enzymes, the reaction it catalyses follows Michaelis-Menten kinetics. Thus the rate of catalysis V, varies with the substrate concentration [S] in a manner shown in Fig 1.14. V is defined as the number of moles of product formed per second. Thus at a fixed concentration of enzyme, V is almost proportional to [S] when [S] is small. With a high value of [S], V is nearly independent of [S].

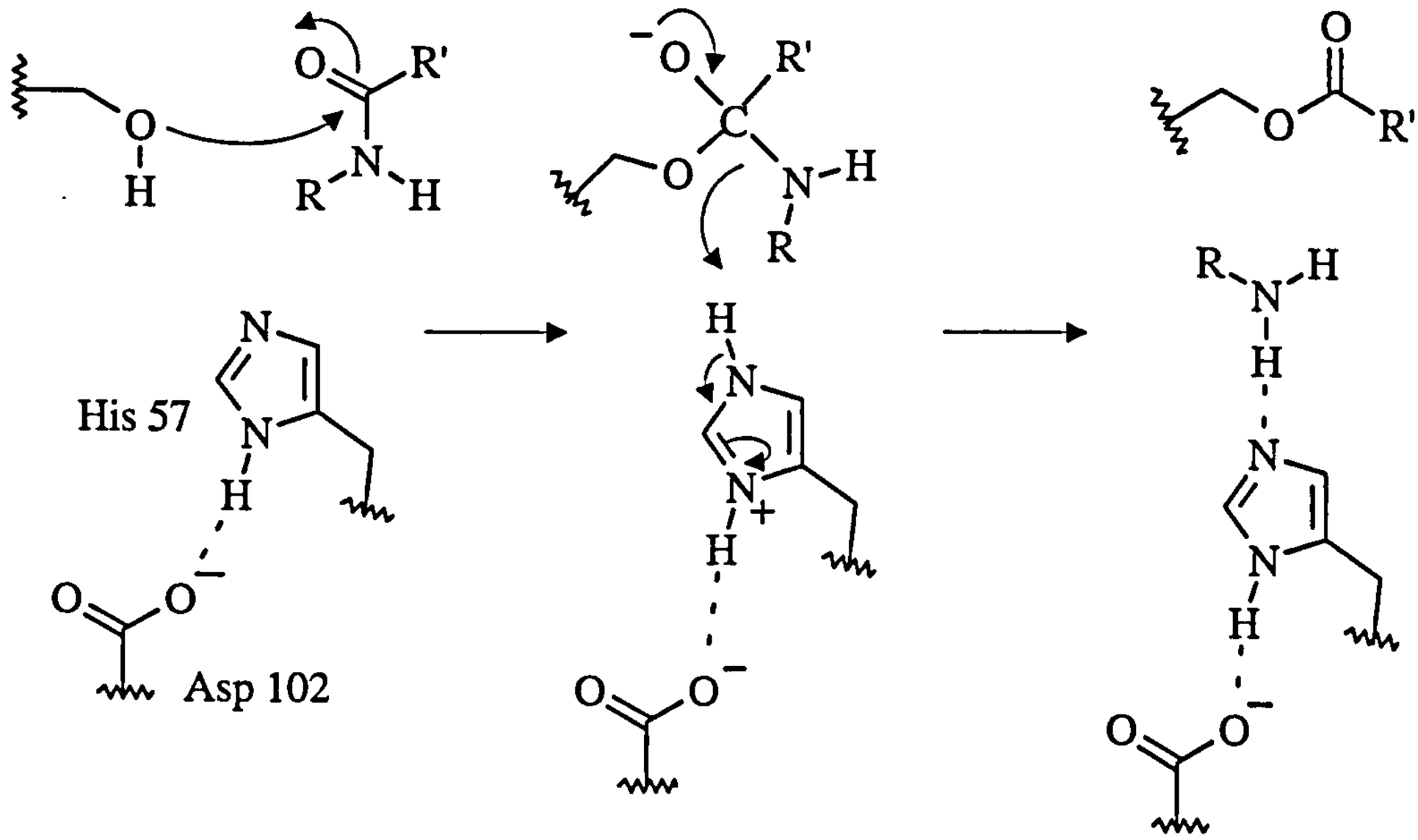
Fig 1.14: Graph illustrating the Michaelis-Menten model of enzyme kinetics



Scheme 1.2 : Mechanism of peptide bond cleavage by chymotrypsin

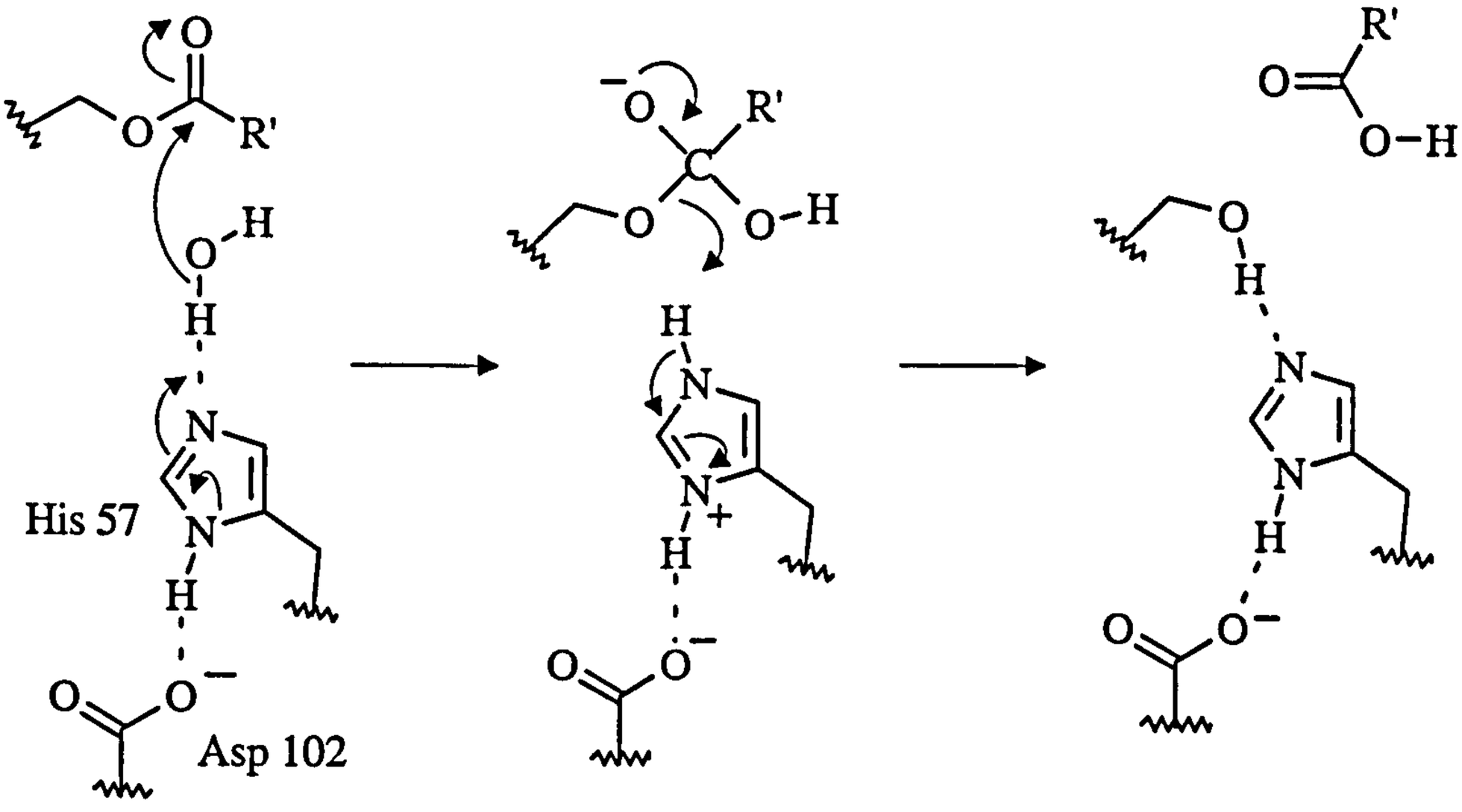
Acylation

Ser 195 Substrate Tetrahedral transition state Acyl enzyme intermediate



Deacylation

Acyl enzyme intermediate Tetrahedral transition state Acid component of the substrate



In 1913, Michaelis and Menten proposed a simple model to account for these kinetics. Crucial to this was a specific enzyme-substrate (ES) complex, which acted as an intermediate in the catalysis. Their model was as shown in Scheme 1.3.

Scheme 1.3 : Equation of the Michaelis-Menten model of enzyme kinetics

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

The Michaelis constant K_M, found from the graph in Fig 1.14, is defined as the concentration of substrate at half the maximum reaction rate. Its significance is that it is related to the rate constants of the individual steps in an enzyme reaction. Thus if, for example, in Scheme 1.3, k_2 is much greater than k_3, a high K_M would indicate weak binding. Conversely, a low K_M indicates strong binding.

1.4.2 Synthetic catalytic systems

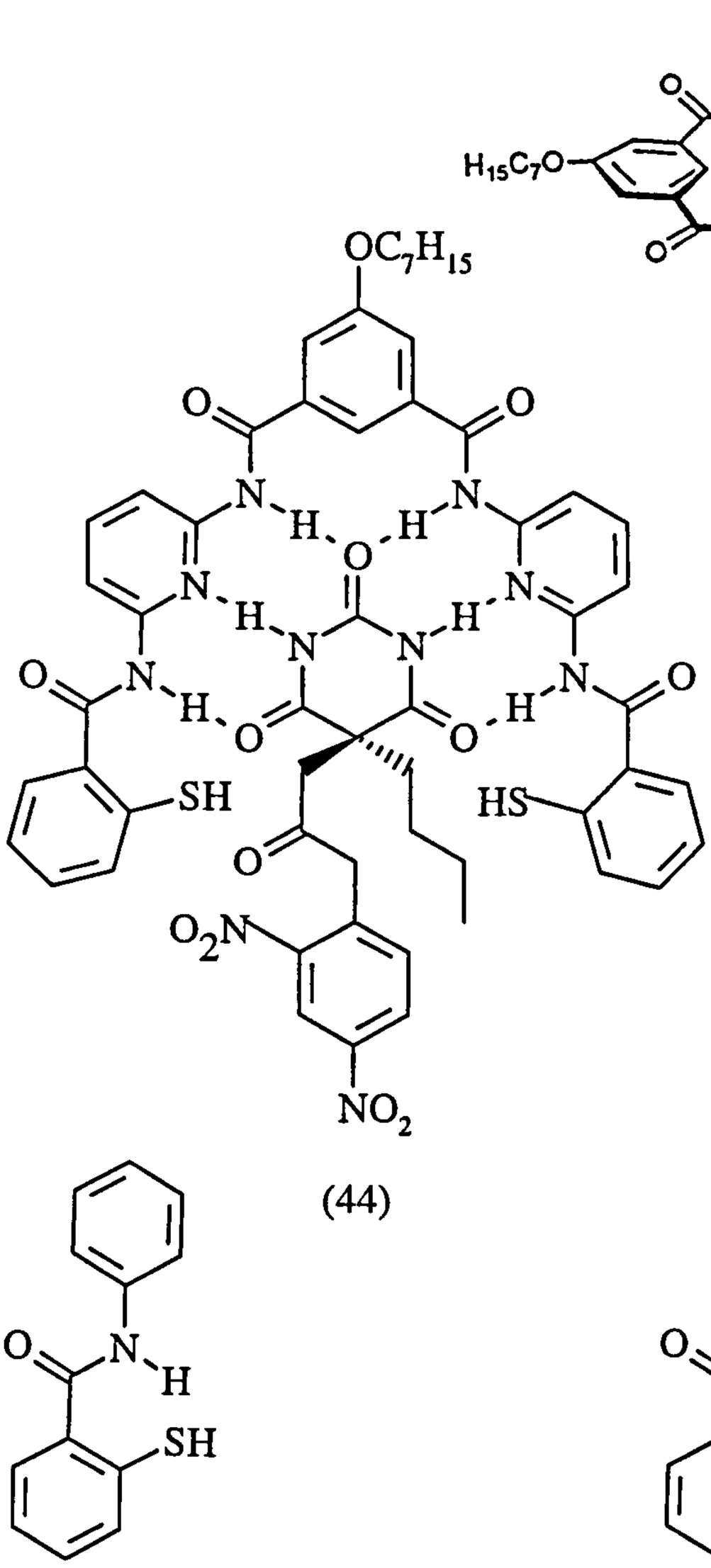
1.4.2.1 Acyl transfer catalysts

In Section 1.2.2.2, a host molecule was described which was capable of binding barbiturate guests. Hamilton has taken this work a stage further by the synthesis of the host (44)[84]. Fig 1.15 shows how the host binds to a guest containing an ester functionality. Molecular modelling studies on (44) show a minimum energy conformation in which the thiol is directed into the cavity and positioned ca. 3.5 Å from the ester carbonyl on the guest. This system is thus ideally designed to allow thiolysis or an acyl-transfer reaction to occur.

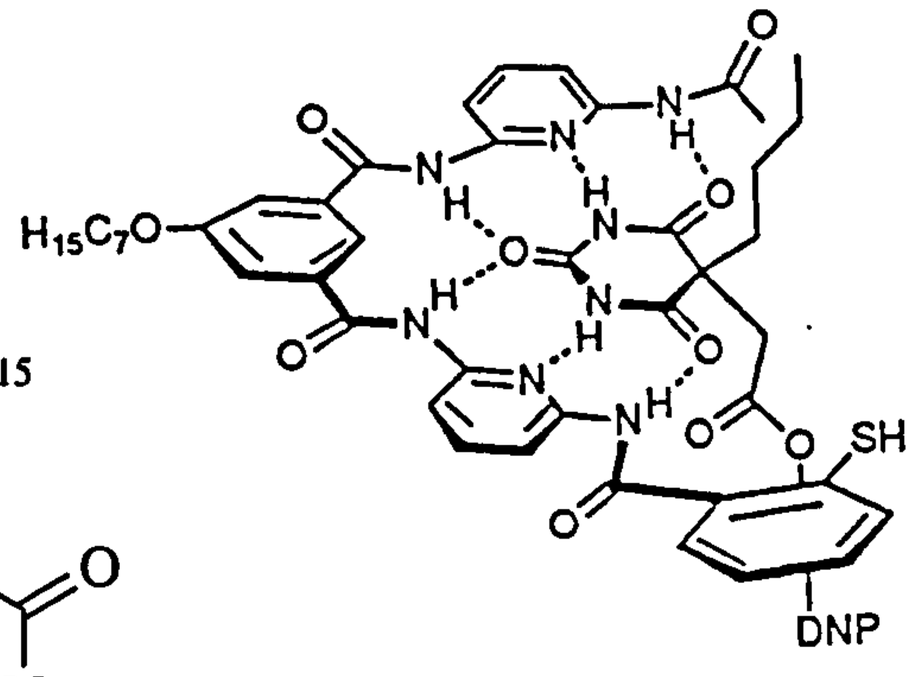
When compared to a standard uncatalysed reaction between the guest (45) and (46), the presence of the host catalyst in CH_2Cl_2, accelerates the reaction by a factor of more than 10^4. This effect is due to the precise hexa-hydrogen bond complementarity between host and guest as evidenced by the 8-fold rate increase when the reaction is carried out in the presence of (47). In addition, the reaction follows Michaelis-Menten kinetics, showing

saturation behaviour as the host concentration is increased and a Michaelis-Menten constant $K_M = 6.89 \pm 0.20 \times 10^{-5}$ mol dm^{-3}. However, a key drawback is the lack of turnover in the reaction, an effect caused by product inhibition.

Fig 1.15 : Binding of a guest to host molecule (44)

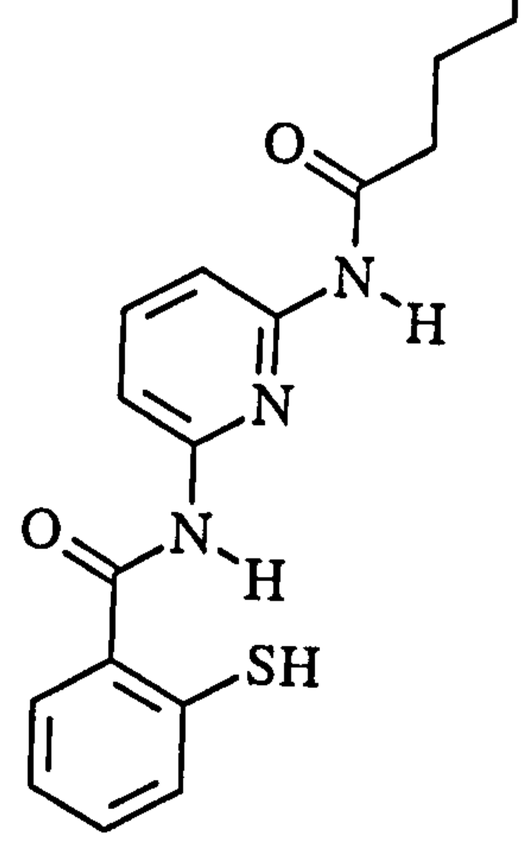


(44)



(45)

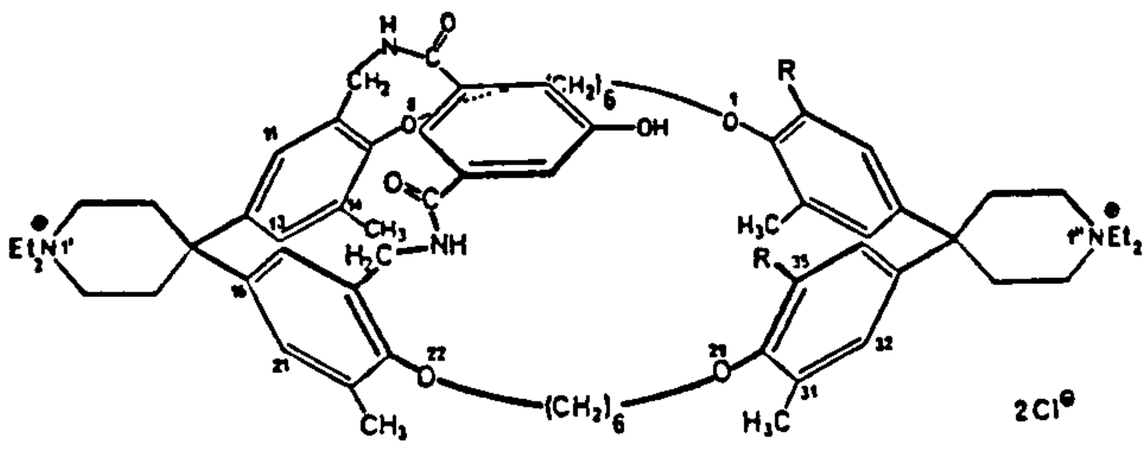
(46)



(47)

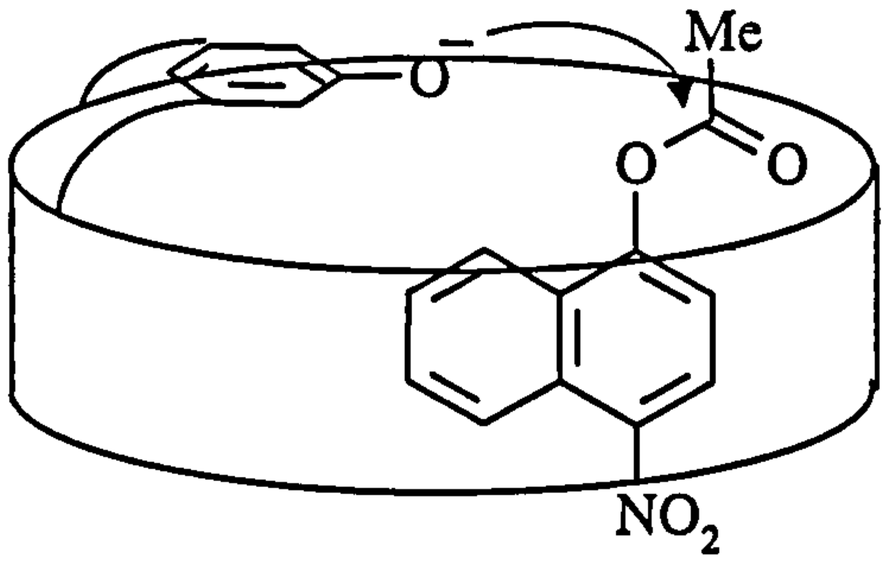
Diederich has also designed molecules capable of catalysing acyl transfer[85]. An example of such a host molecule (48) is illustrated in Fig 1.16 alongside a schematic diagram of the binding of the guest.

Fig 1.16 : Catalysis of acyl transfer by host molecule (48)



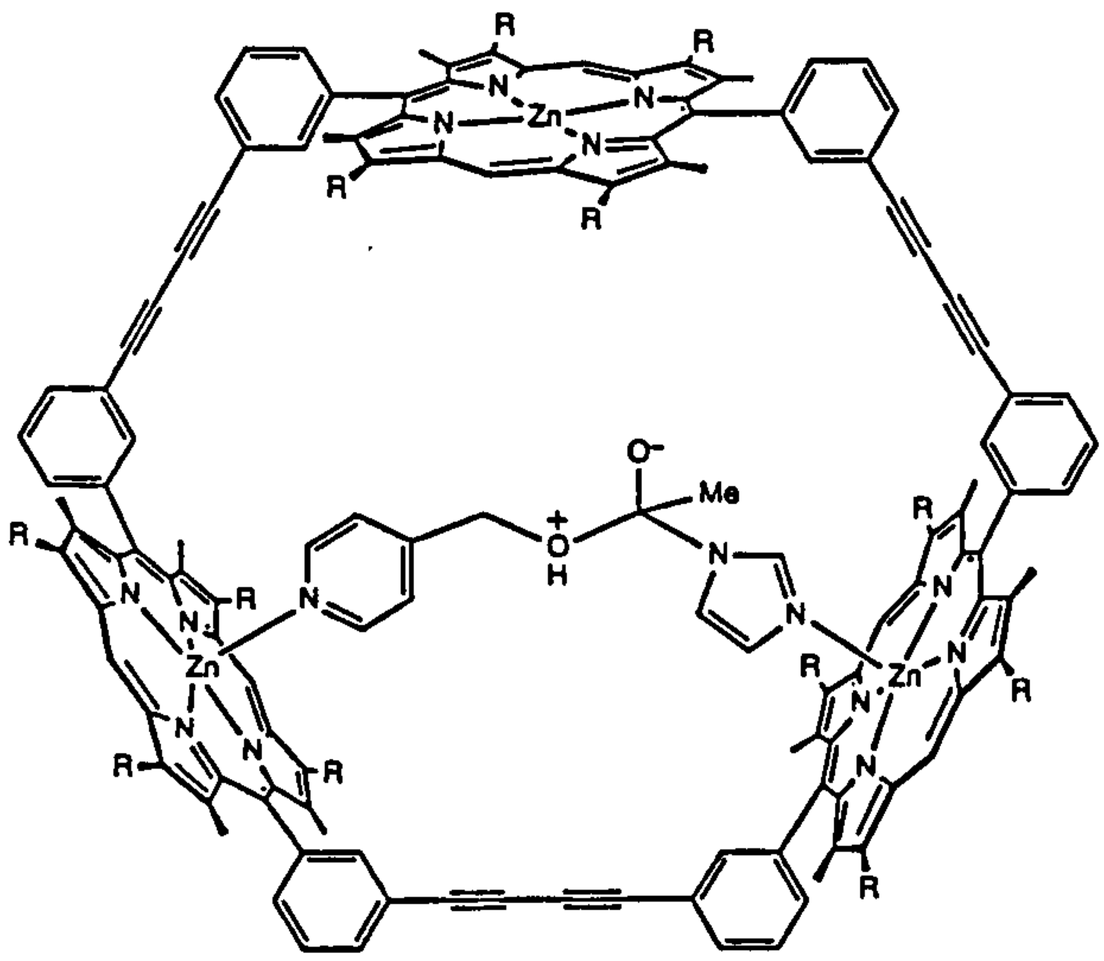
R = H

(48)

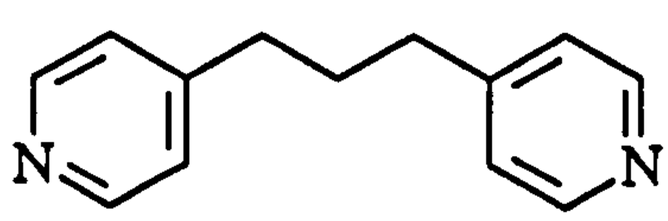


The guest in (48) is bound by hydrophobic forces when the solvent is an aqueous buffer solvent system. Due to the relative sizes of the cavity in the host and the size of the guest, binding occurs in the orientation shown, allowing supramolecular acyl transfer to occur. The rate of reaction was found to be fastest in phosphate buffer at pH8, being 472 times faster than the hydrolysis in the buffer when the host molecule was not present. "Weak but significant turnover" is also claimed, however, a figure for the number of catalytic turnovers is not given.

A final example of a system capable of catalyzing acyl transfer is (49)[86].



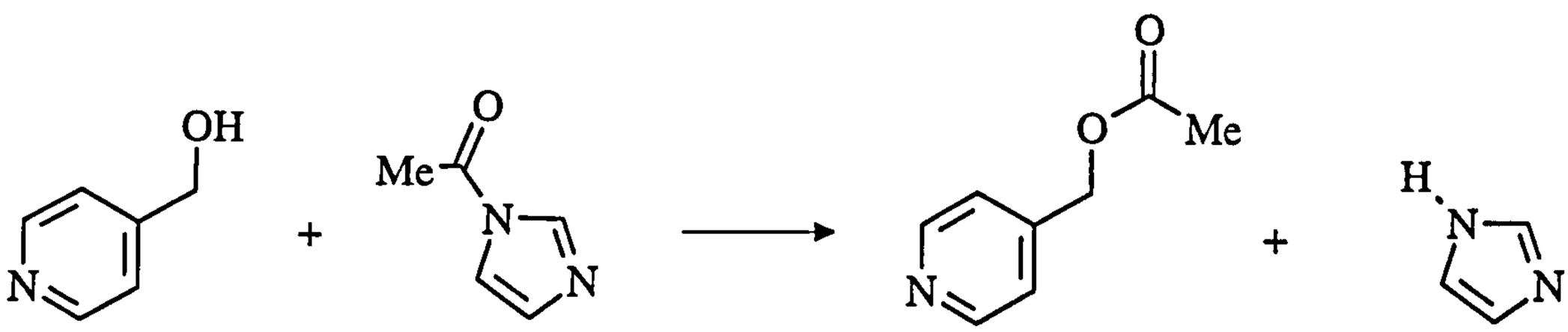
(49)



(50)

Acyl transfer occurs as shown in Scheme 1.4. In the uncatalysed reaction in toluene at 70^0 C, the apparent second order rate constant is 3.4×10^{-5} dm^{-3} mol^{-1} s^{-1}. With 9×10^{-3} mol dm^{-3} concentrations of each substrate and 0.45×10^{-3} mol dm^{-3} of host, the rate was increased 16-fold.

Scheme 1.4 : Acyl transfer catalysed by host molecule (49)



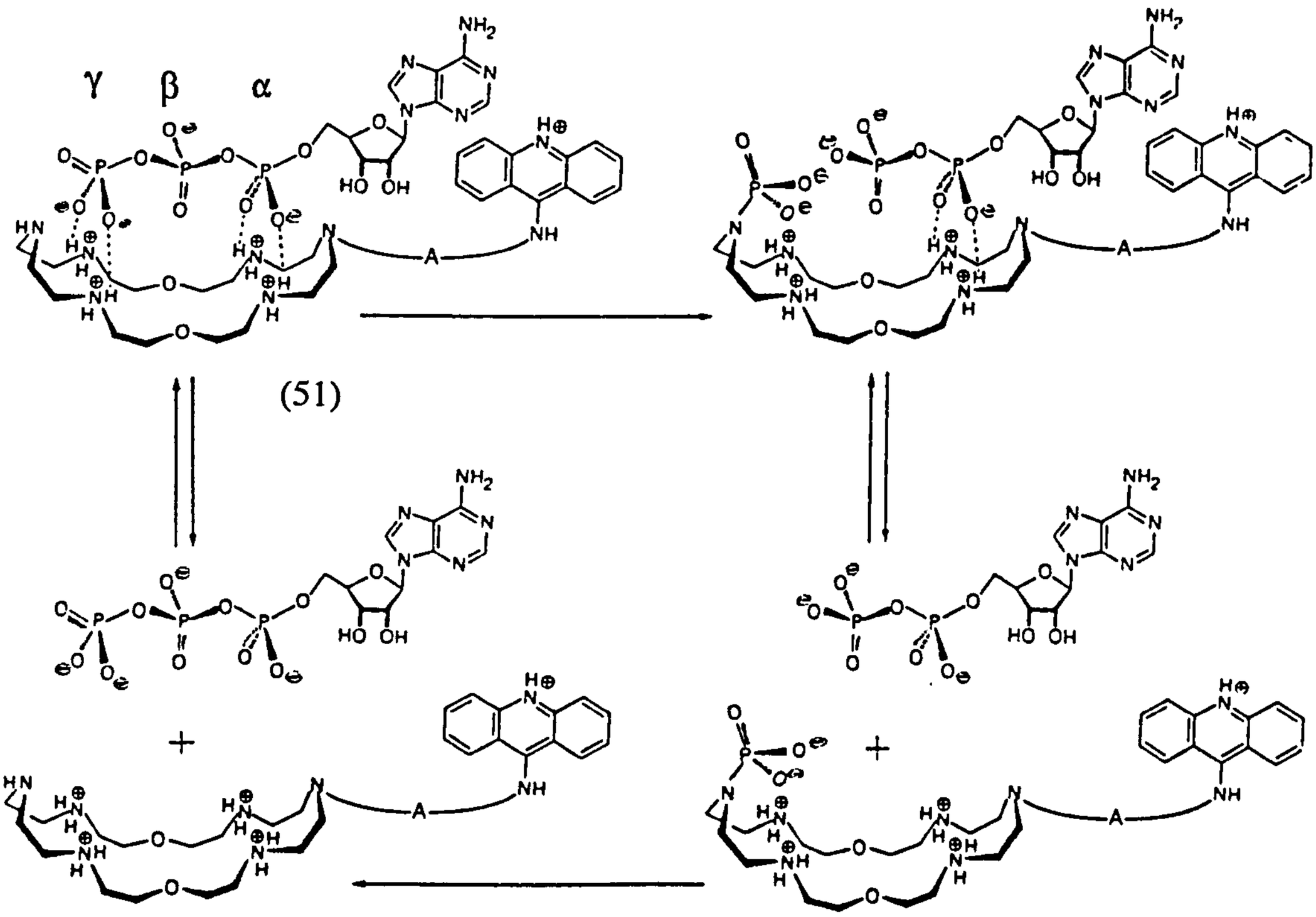
Amongst the evidence that this transformation occurs within the cavity is the fact that competitive inhibition is affected by the introduction of a molecule possessing a similar size and shape to the expected transition state. Such a molecule is (50).

1.4.2.2 Catalysis of ATP and ADP hydrolysis[87]

The hydrolysis of both adenosine triphosphate (ATP) and adenosine diphosphate (ADP) is catalysed by the macrocycle (51) designed by Lehn - Scheme 1.5 [87]. The reaction rate is accelerated by factors of 3 for the hydrolysis of ADP and 9 in the case of ATP, the difference being due to the better fit of ATP for the macrocycle over ADP. The binding of ATP with (51) was studied by ^{31}P NMR Spectroscopy using a 1:9 D_2O:H_2O solvent system at pH 7. The addition of 1 mole equivalent of (51) to a 10^{-2} M solution of ATP caused a a slight downfield shift of the P_α signal (-0.07 ppm) whereas both the central P_β signal and the terminal P_γ signal were shifted upfield by 1.32 ppm and 2.22 ppm respectively. The addition of 1 mole equivalent of ATP to a 10^{-3} M solution of (51) at pH 7 in D_2O caused significant upfield shifts of aromatic protons of the acridine moiety of (51). These results have been interpreted as suggesting that ATP is bound by hydrogen bonding between the phosphate and the macrocycle and by π-π hydrophobic interactions between adenosine and acridine groups. Such hydrogen bonding within water is extremely unusual due to the

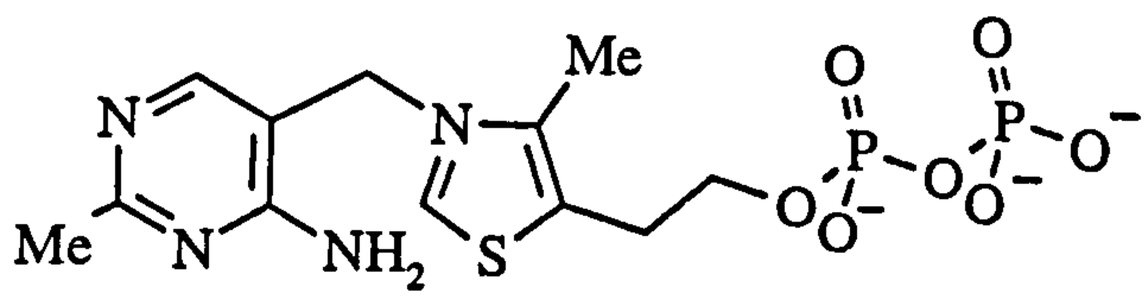
competition for hydrogen bonds by the presence of the water, but it is probably facilitated by the opposite charges of the phosphate groups and the macrocycle. Scheme 1.5 gives a representation of the mechanism of the reaction.

Scheme 1.5 : Catalysis of ATP hydrolysis by host molecule (51)



1.4.2.3 Catalysis of the benzoin condensation[88,89]

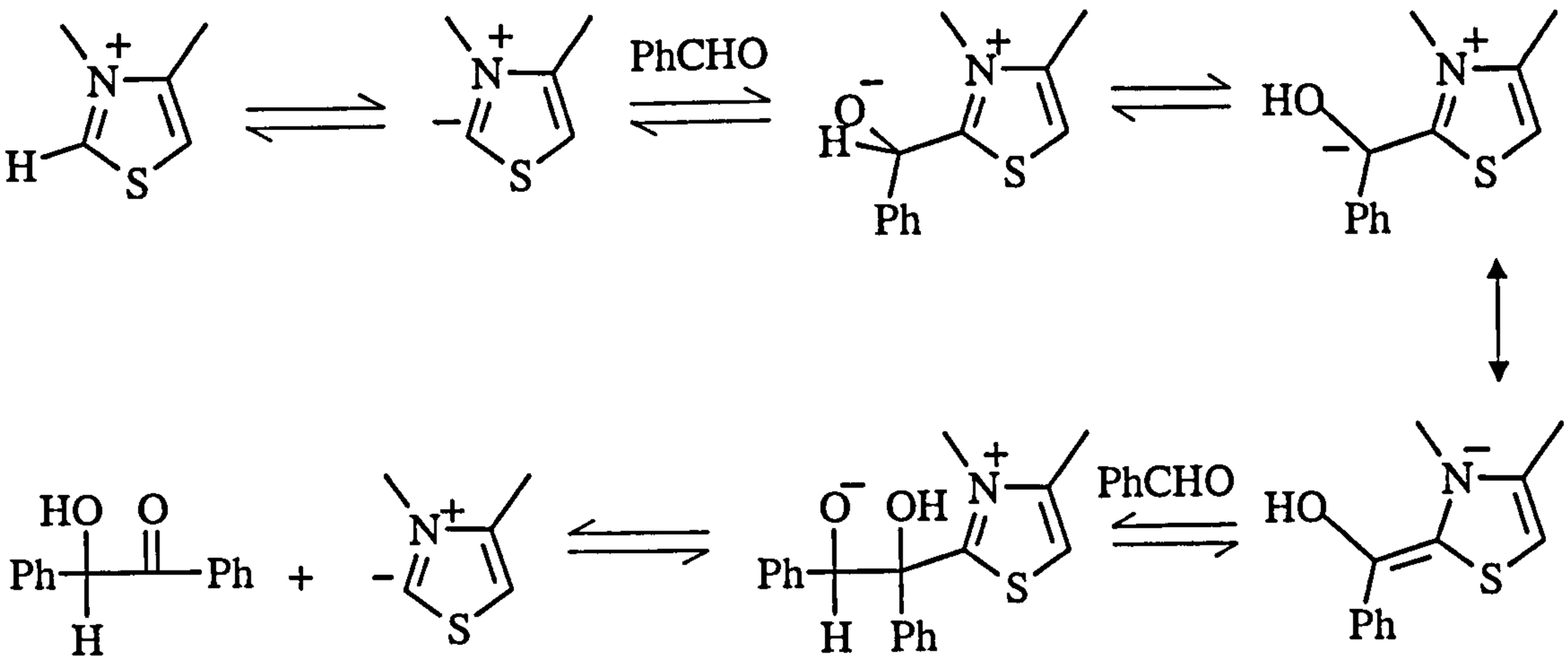
Ever since the work of Breslow in the 1950's [90,91], it has been established that, in the absence of enzymes, the thiazolium ring acting as a thiazolium ion in such coenzymes as thiamin pyrophosphate (TPP) (52), catalyses many enzymatic reactions such as the acetoin condensation[92,93].



(52)

Diederich has incorporated TPP into a macrobicycle, designed to catalyse the benzoin condensation. The macrocycle (53) and the mechanism of reaction are illustrated in Scheme 1.6.

Scheme 1.6 : The mechanism of the benzoin condensation as catalysed by host molecule (53)



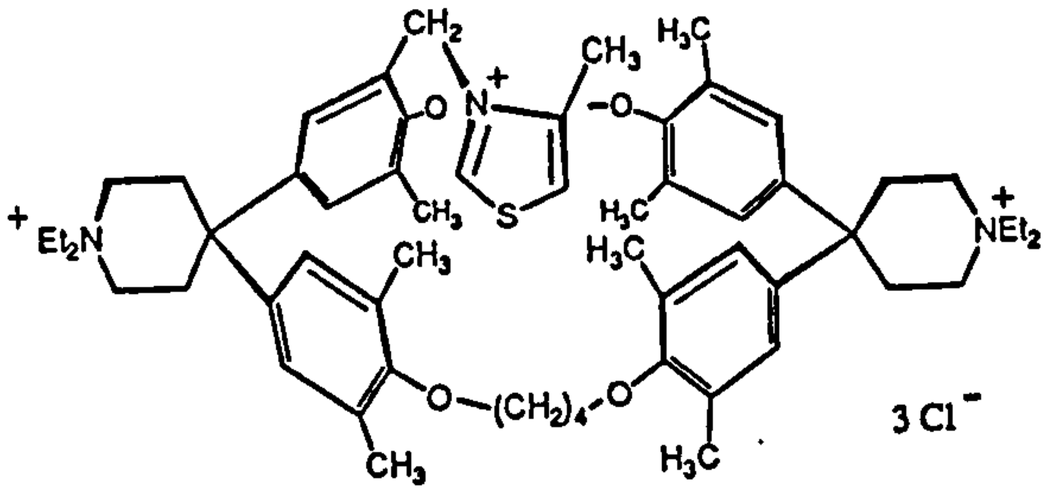
(53)

As with reactions described earlier, the reaction follows Michaelis-Menten kinetics with catalysis of both forward and reverse reactions.

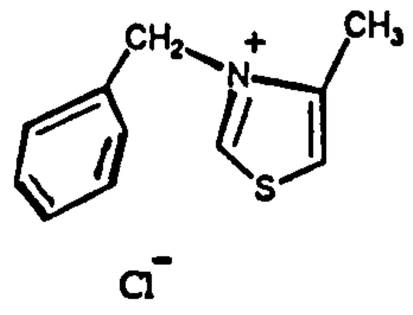
In addition, rates of reaction increase with decreasing solvent polarity and with increasing concentration of triethylamine base. These solvent effects are perhaps contrary to expectation, since one would expect increasing solvent polarity to result in guests being bound by stronger hydrophobic forces, thus increasing the effective molarity of the reagents within the cavity. However, increasing base concentration agrees with the expected result in that it would readily encourage reaction.

1.4.2.4 Oxidation of aromatic aldehydes[94]

Diederich has designed macrocycle (54) which is related to (53) and which is capable of catalysing the oxidation of aromatic aldehydes to carboxylic acids. The ^1H NMR spectrum of the macrocycle in D_2O compared with that of (55), reveals considerable upfield shifts of all the thiazolium protons (0.27 - 0.70 ppm), indicating that the thiazolium ring is located within the cavity.



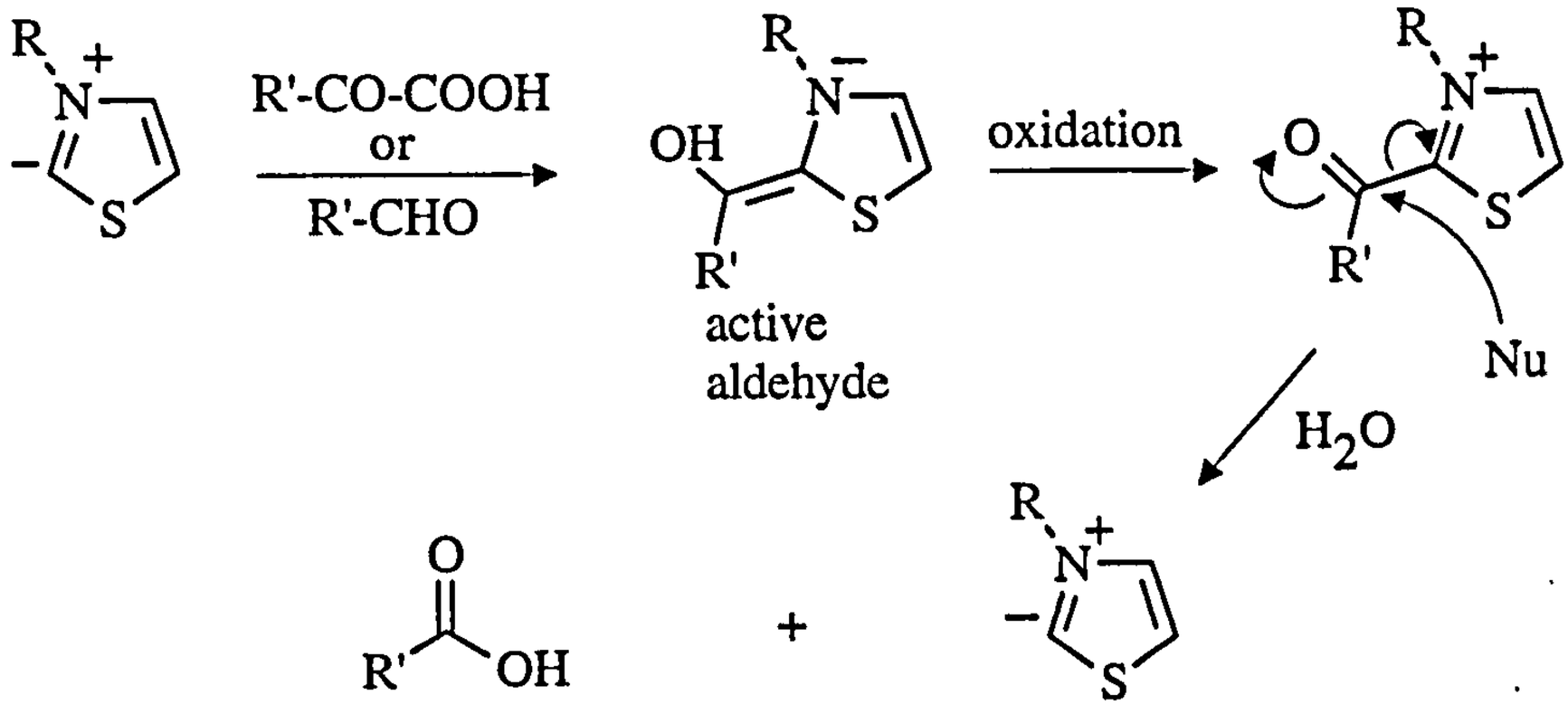
(54)



(55)

Oxidation of 2-naphthaldehyde is catalysed by (54), in the presence of potassium ferricyanide in 60:40 (v/v) Me_2SO : aqueous phosphate buffer (pH = 7.5) at 303K. Compared to its non-macrocyclic derivative (55), the rate of reaction is 6.2 times greater in the presence of macrocycle, due to the binding of the aldehyde in the cavity by hydrophobic forces. The mechanism of reaction is given in Scheme 1.7.

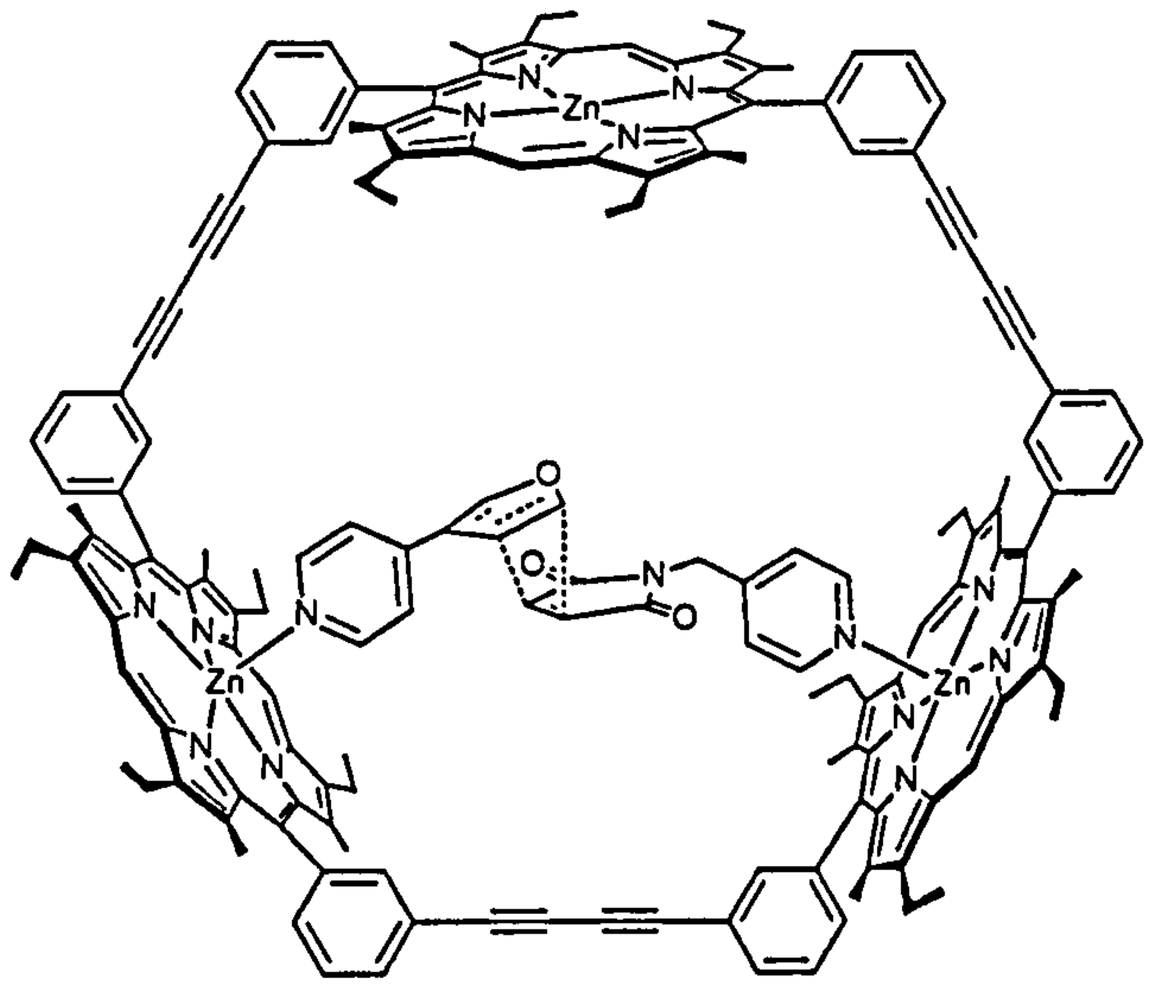
Scheme 1.7 : Mechanism of the oxidation of aromatic aldehydes as catalysed by host molecule (54)



1.4.2.5 Catalysis of the Diels-Alder reaction[95-97]

As well as catalysing an acyl transfer reaction, the cyclic porphyrin trimer of Sanders (56) has been shown to catalyse the Diels-Alder reaction between a furan derived diene and maleimide derived dienophile. In this reversible reaction, the guests were bound to the metallated porphyrins by the attachment of pyridine moieties. These pyridine units bind very strongly to the hosts and this, in addition to the fact that the reaction products bind at two locations to the host giving a strong chelate effect, causes product inhibition and thus a lack of reaction turnover. However, due to the reversible nature of the reaction[97], its kinetics and transition state energies can be studied. Scheme 1.8 shows the nature of the Diels-Alder catalysis.

Scheme 1.8 : Catalysis of the Diels-Alder reaction by host molecule (56)



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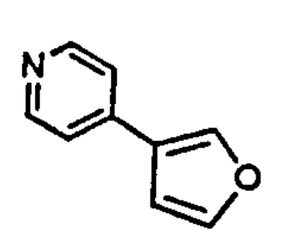

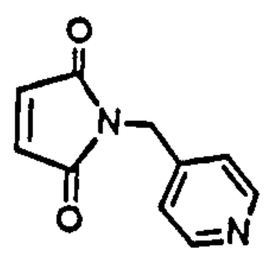

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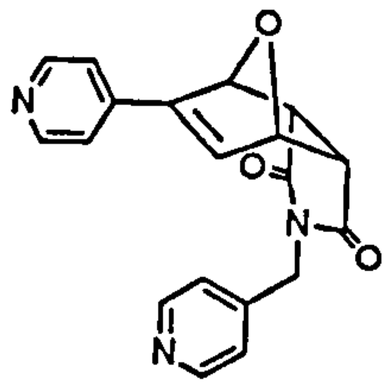
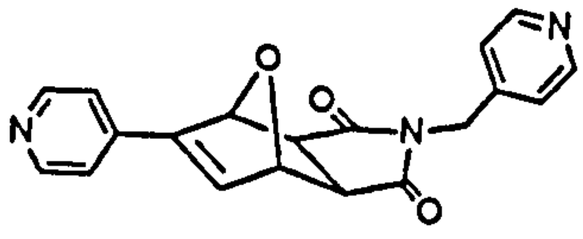




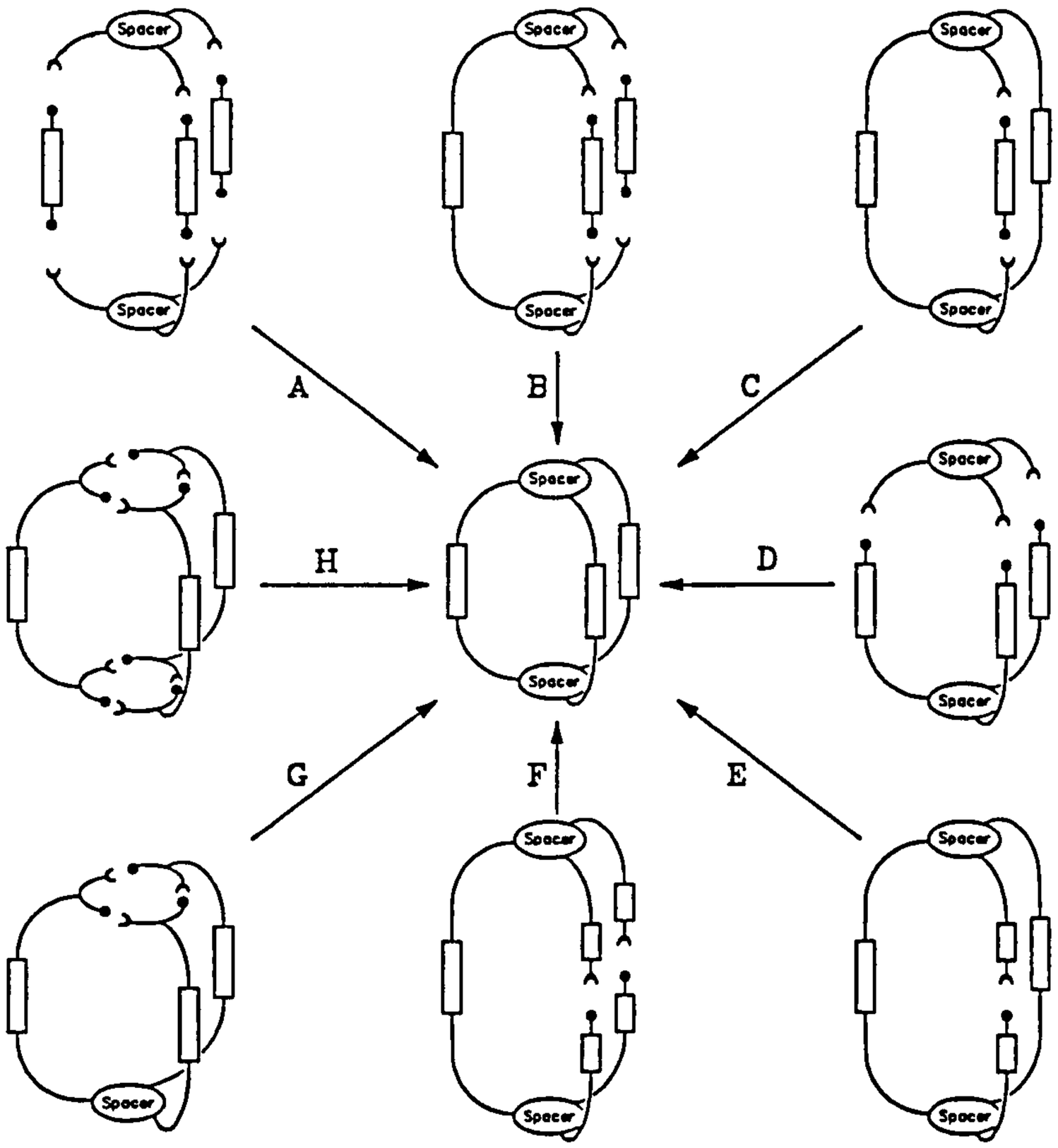
endo *exo*

In the absence of porphyrin trimer, the *endo* adduct obtained by kinetic control is a significant product at low temperature, whilst the *exo* adduct obtained by thermodynamic control is the only product detected at high temperatures. At 60^0 C, the *exo/endo* ratio is initially about 4, but due to the reversibility of the reaction, the *endo* adduct gradually disappears[97]. However, on addition of one equivalent of the trimer to the two reactants, the forward diels-alder reaction is accelerated 200 times at 60^0 C, yielding the *exo* adduct as the only detectable product. The stereochemistry of the product is drastically altered by the trimer, due to its increased stabilisation of the *exo* transition state with respect to the *endo* transition state. On addition of trimer, the energies of the bound transition states correspond to an increase in the activation energy of the forward *endo* reaction from 110 to 114 kJ mol^{-1} and a decrease in activation energy for the *exo* reaction from 105 to101 kJ mol^{-1} [97]. Thus, due to the reversibility of this reaction, the trimer effectively encourages conversion of the *endo* adduct to its *exo* isomer. Consequently, whilst a 0.9 $x10^{-3}$ mol dm^{-3} solution of *endo* adduct in the absence of trimer is quickly converted to an almost completely dissociated diene-dienophile pair, in the presence of trimer it is converted into about 55 % *exo* adduct in 100 h.

1.5 Synthesis of host molecules

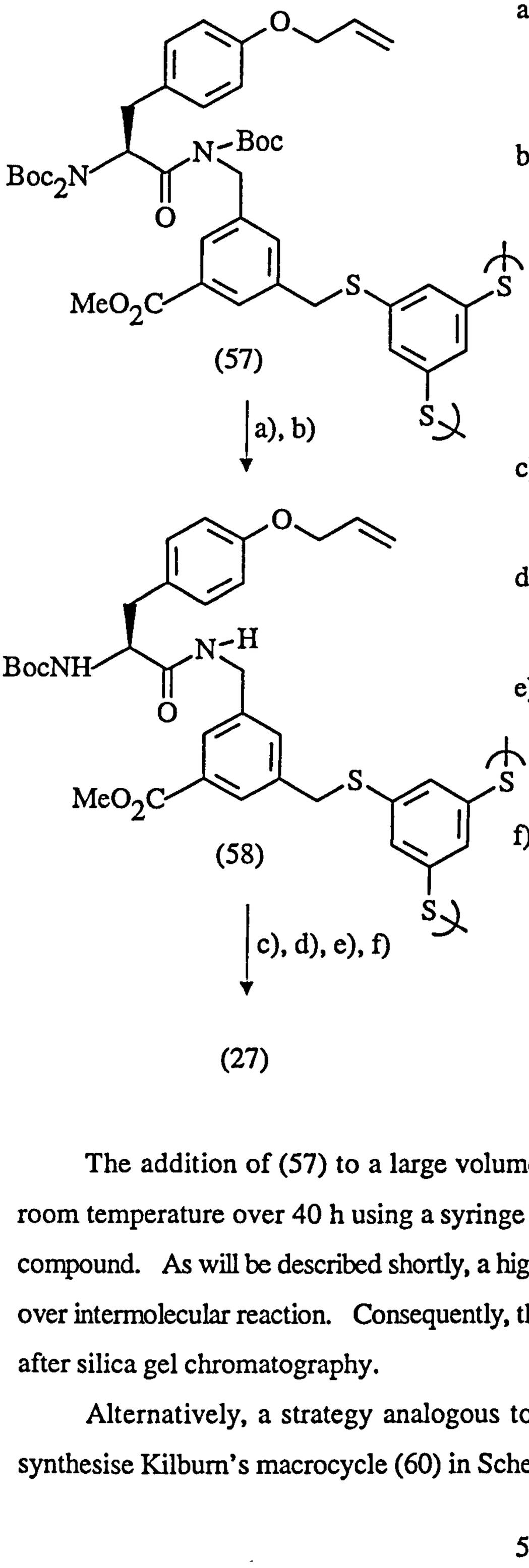
In order to achieve the synthesis of many of the host molecules described above, a wide variety of strategies have been described in the literature[98]. Common to all these strategies is the *"molecular building block principle"* - the stepwise assembly of easily accessible structural subunits. Scheme 1.9 gives a schematic diagram of the kinds of strategies possible in the synthesis of triply bridged cyclophane hosts. Thus it is possible to form such a molecule by six-fold bond formation using five components, namely two spacers and three bridge units (path A). Whilst this method is simple, it often gives low yields with difficult purification problems. Alternatively, a longer synthesis would give only two molecules in the final cyclisation step (paths C and D) or a situation where the final ring closure occurs intramolecularly (paths E, F and G). Frequently, the choice of synthetic strategy is dictated by the nature of the molecule being synthesised.

Scheme 1.9 : Schematic diagram showing possible strategies towards the synthesis of triply bridged cyclophane hosts



An example of a molecule synthesised according to the strategy outlined in path G is Still's C_3 symmetric host (27)[60]. The final stages in its synthesis are given in Scheme 1.10.

Scheme 1.10 : Final steps in the synthesis of host (27)



a) TFA, anisole, CH_2Cl_2, RT, 16 h, 100 %

b) Boc_2O, $^iPr_2NEt$, K_2CO_3, CH_2Cl_2, RT, 24 h, 86 %

c) THF, EtOH, H_2O, LiOH, 6 h, 100 %

d) PFP-OH, EDC, THF, RT, 4 h, 68 %

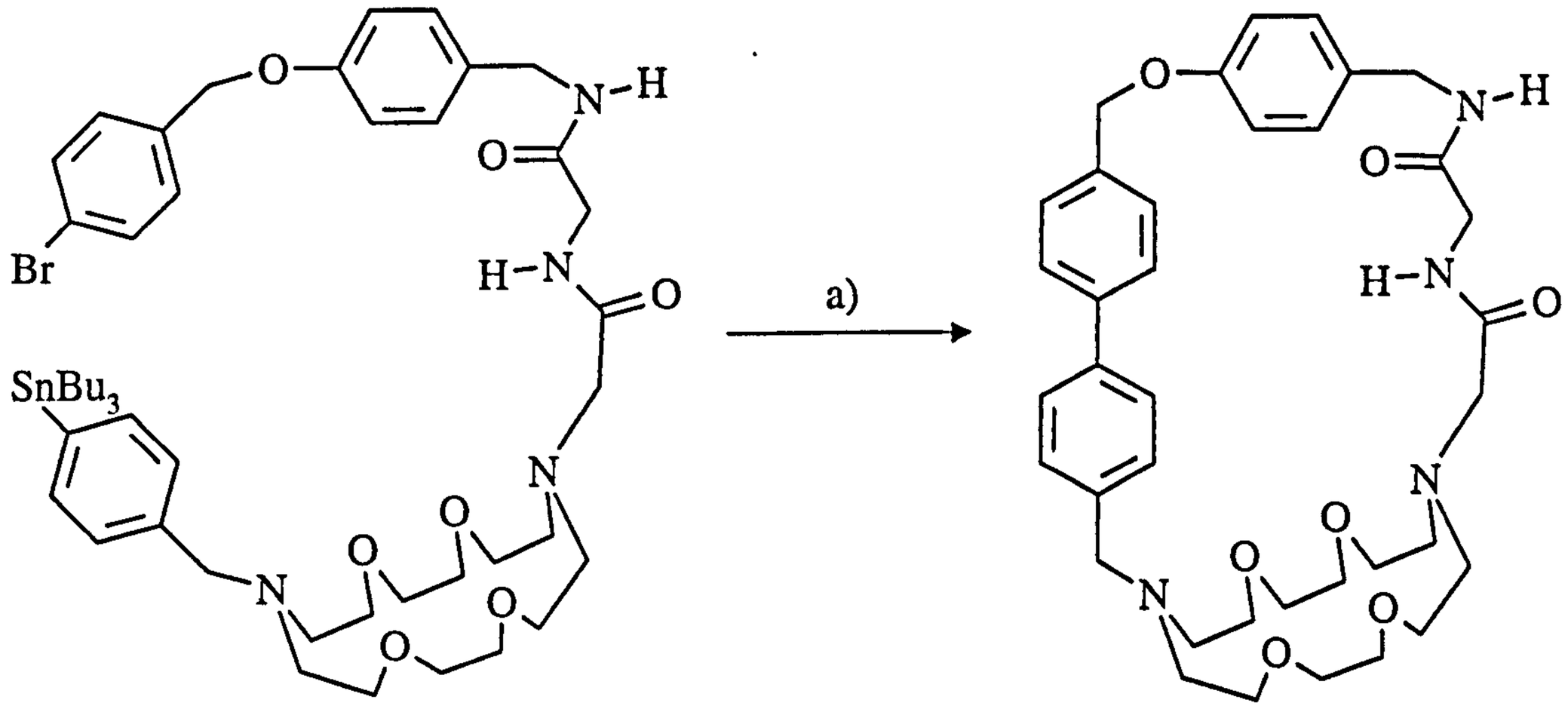
e) TFA, anisole, CH_2Cl_2, RT, 4 h, 100 %

f) TFA salt in DMA dropwise to $^iPr_2NEt$, THF, RT, 40 h, 78 %

The addition of (57) to a large volume of Honig's base in THF was carried out at room temperature over 40 h using a syringe pump so as to maintain a high dilution of the compound. As will be described shortly, a high dilution is necessary in order to ensure intra over intermolecular reaction. Consequently, the final cyclisation was achieved in 78 % yield after silica gel chromatography.

Alternatively, a strategy analogous to that illustrated in Path E has been used to synthesise Kilburn's macrocycle (60) in Scheme 1.11[54].

Scheme 1.11 : Final step in the synthesis of host (60)



(59) (60)

a) 5-10 mol % $Pd(PPh_3)_4$, K_2CO_3, DMF, reflux, 15 %
[(59) at a concentration of 10 mM]

The cyclisation precursor (59), is synthesised in 6 steps before the ring closure step is achieved in 15 % yield by means of a palladium catalysed biaryl formation. For this reaction, the cyclisation precursor (59) is kept at a high dilution of 10 x 10^{-3} mol dm^{-3}.

The synthesis of both (27) and (60) illustrate the importance of maintaining a high dilution of reactants in the final cyclisation. These conditions can be obtained by use of large quantities of solvent and also as in the synthesis of (27), by controlling the rate of addition of the reactants. In designing such cyclisations, the aim is to introduce reactants at a rate equal to the rate of the cyclisation.

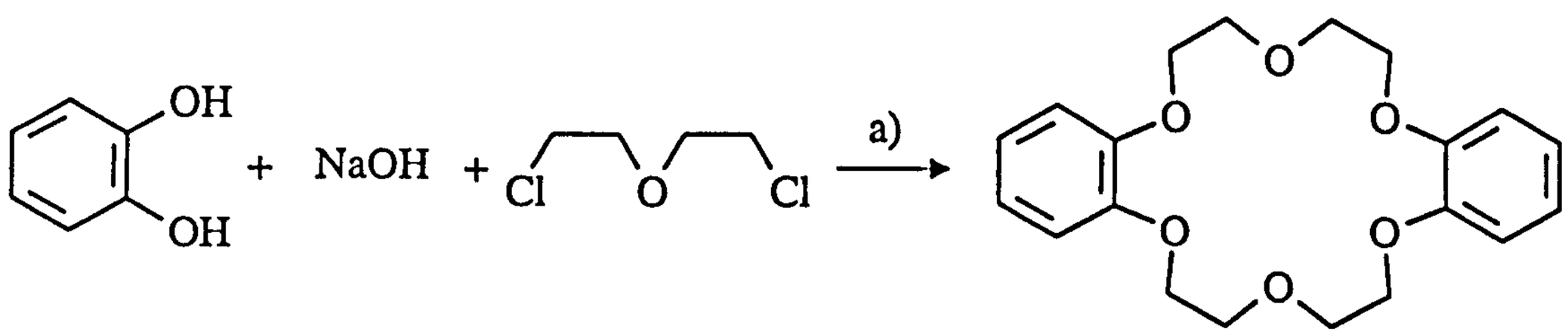
The ratio k_{intra} / k_{inter} has been established as important in determining the likelihood of a macrocyclisation occurring[99-101]. This ratio is known as the *"effective molarity" (EM)* and has units of mol dm^{-3}. It represents the reactant concentration at which the cyclisation (k_{intra}) and polymerisation (k_{inter}) of a given bifunctional monomer, occur at the same rate. Practically, it gives a guide as to the required rate of addition of reactants if cyclisation is to predominate over polymerisation. The reactants are infused continuously into the reaction mixture at such a rate as to prevent their accumulation. If the rate of feed v_f is less than EM x k_{intra}, then cyclisation predominates.

In addition to the high dilution principles, templates have also been used to achieve efficient macrocyclisations[102]. The use of cyclisation promoting templates can increase the

proportion of the conformers of a monomer that are suitable for cyclisation. Thus the k_{intra} value is enhanced relative to k_{inter}, EM is increased and the concentration of monomer required to achieve a particular yield of macrocycle is increased.

Template effects were used by Pedersen in the early days of host-guest chemistry in the synthesis of dibenzo-18-crown-6 (61)[6]. In the synthesis of this compound, outlined in Scheme 1.12, high dilution techniques were not required, due to the fact that the reactants became orientated correctly for synthesis, through ion-dipole interactions.

Scheme 1.12 : Pedersen's synthesis of dibenzo-18-crown-6

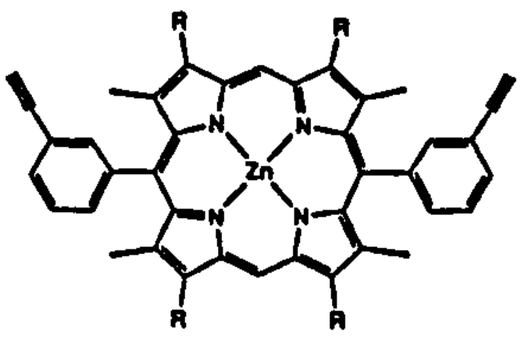


(61)

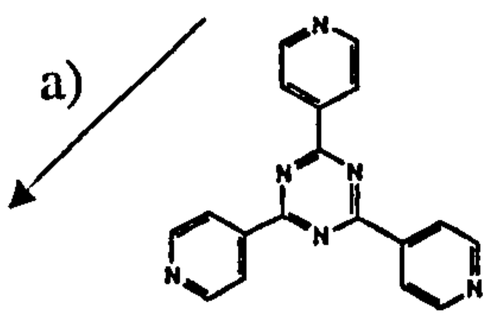
a) BuOH, 115^{o} C, 24 h

A more recent example of the use of the template effect in synthesis, is the construction of the porphyrin trimer (56) and also of porphyrin dimer (62)[103-105]. As can be seen in Scheme 1.13, when the porphyrin (63) is reacted under Glaser coupling conditions, the outcome of the reaction can be directed down either of two very different pathways, depending upon the use of a particular template.

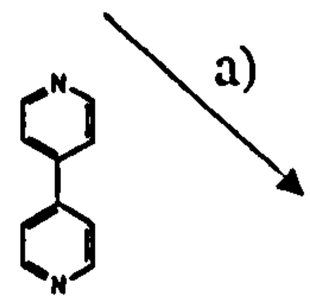
Scheme 1.13 : Template synthesis of porphyrin trimer (56) and porphyrin dimer (62)



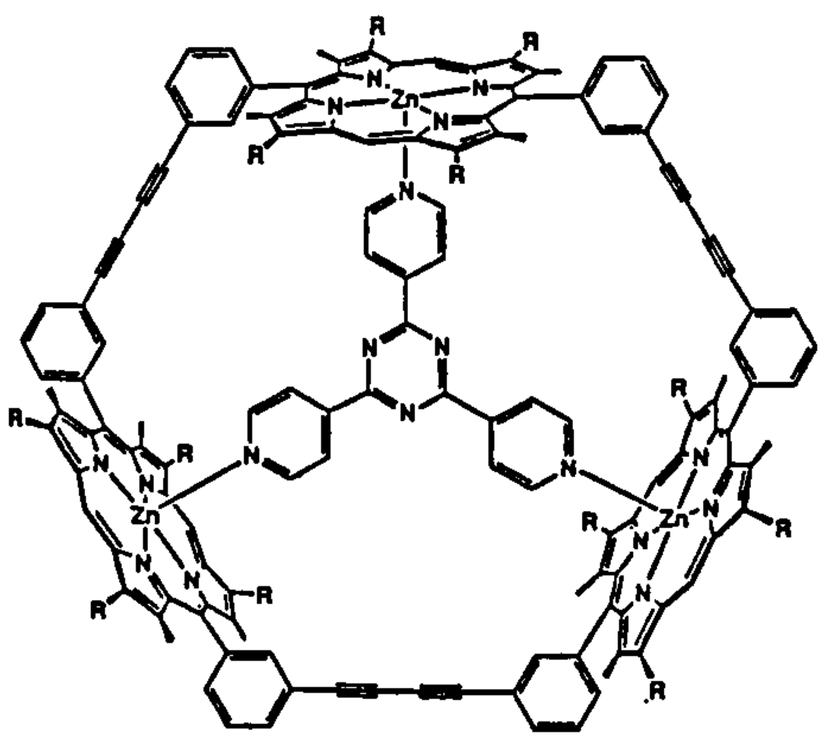
(63)



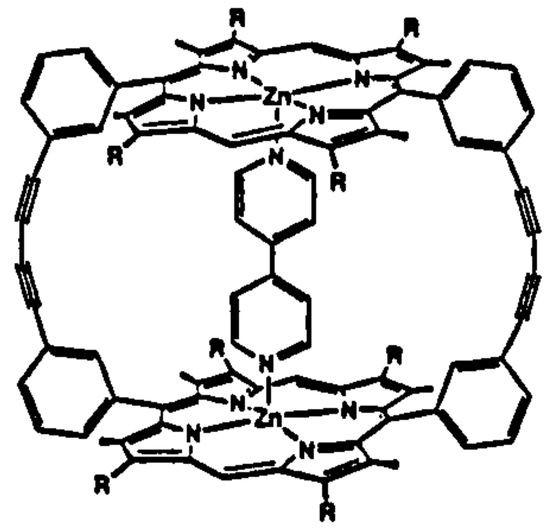
a)



a)



(56)



(62)

a) CuCl, TMEDA, CH_2Cl_2, air

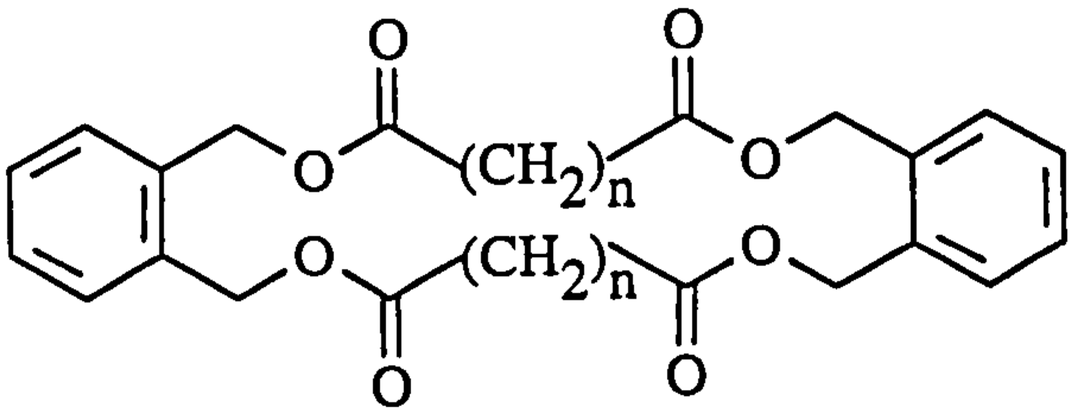
Thus, when 4,4'-bipyridyl is used as the template, the cyclic porphyrin dimer is formed, whereas template directed synthesis using 5-tri(4-pyridyl)triazine results in the formation of the trimer. The reason for the effectiveness of these templates is suggested as being due to the ability of their pyridyl groups to coordinate to the metallated porphyrins and thus orientate them to the appropriate conformation for cyclisation. Evidence for this suggestion is the fact that such product selection only occurs in non-coordinating solvents such as CH_2Cl_2. The use of a solvent such as pyridine, capable of competitive binding to the metallated porphyrin, results in mixed products.

The caesium effect has also been employed in many macrocyclisations, using Cs_2CO_3 in DMF solution[106-108]. Thus, for example, Kellogg has deprotonated a series of long-chain diamines as their bis-tosylated derivatives using Cs_2CO_3 in DMF[106]. The dicaesium salts were thus formed and reacted with various dibromides or dimesylates to give tosyl derived cyclic diamines. These cyclisations, which were *not* carried out under high dilution conditions, gave yields of the cyclic product of between 25 % and 95 % depending upon the

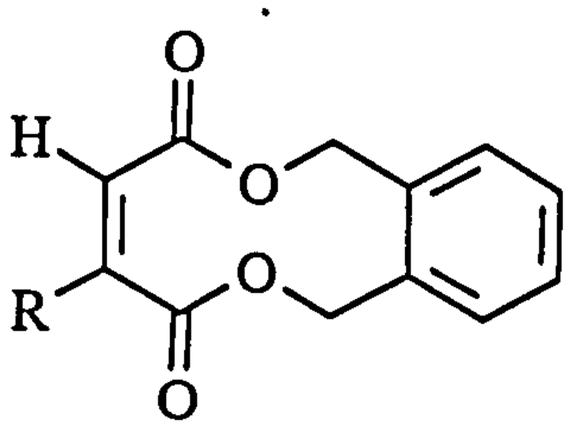
lengths of the alkyl chains.

There is much debate concerning the origin of this caesium effect. One idea is that the caesium ion collects the two ends of a monomer onto its surface thus encouraging a conformation which facilitates cyclisation over polymerisation. However, Galli and Mandolini have argued that caesium carbonate, compared to other alkyl metal bases, has no special ability to promote cyclisation[109]. In their experiments, performing macrolactonisations with a variety of alkali metal carbonates in DMF, yields of cyclised product were higher using Cs_2CO_3. However, the differences with K^+ and Na^+ were small and easily attributable to the change in cation size.

Amongst other methods used to encourage cyclisation is the use of the rigid-group principle[110-113]. This strategy has been used by Drewes to encourage the formation of cyclic esters in which the rigid group 1,4-α,α'-dibromo-*o*-xylene reacts to form the rings (64) to (75)[112,113]. Highest yields were obtained either when both reactants possessed "rigid groups" ((73) - (75)), or where one reactant had a "rigid group" and the other was a short chain dicarboxylic acid ((64) and (66)). (65) had an anomolous low yield.

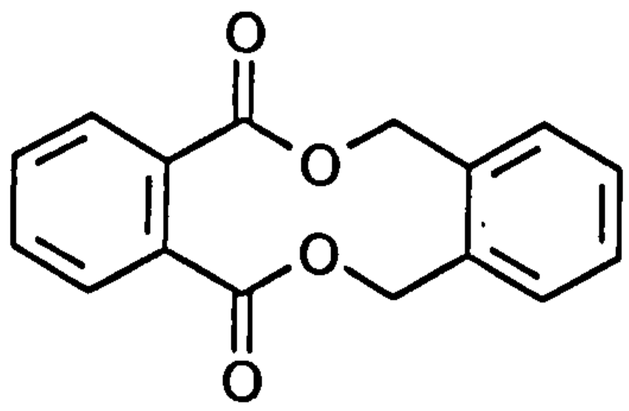


(64) n = 0
(65) n = 1
(66) n = 2
(67) n = 3
(68) n = 4
(69) n = 5
(70) n = 6
(71) n = 7
(72) n = 8



(73) R = H

(74) R = Me



(75)

It is suggested that rigid groups are effective in promoting cyclisation by limiting the conformational possibilities and orientating reactive groups of a linear precursor closer together. Thus, whilst the activation energies between conformers may not necessarily be decreased, the proportion of cyclic like conformations over linear ones are enhanced.

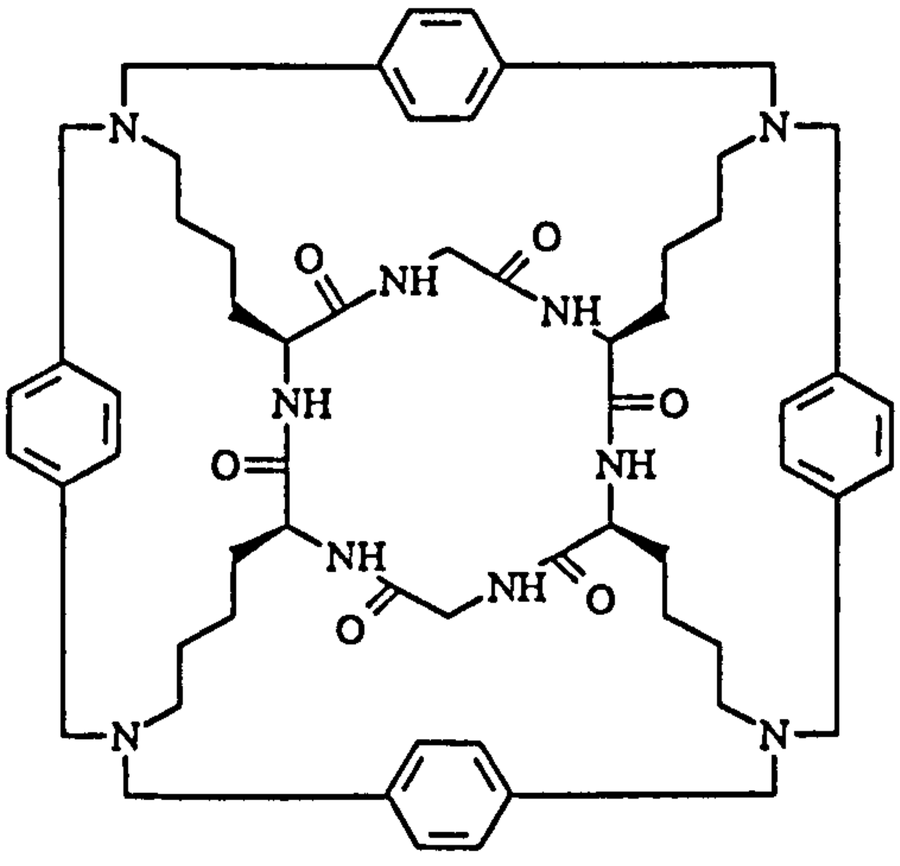
Finally, factors such as choice of solvents and temperature affect the rate of cyclisation of a macrocycle. The rate of cyclisation is increased if the two ends of a precursor are in close proximity to each other. This is encouraged or hindered depending upon how the solvent interacts with the solute and, as was seen with the synthesis of the porphyrin dimers and trimers above, how the solvent affects any templates. Increases in temperature can overcome activation energies involved in the interconversion of conformers although such an increase might make some cyclic-like conformers entropically disfavoured.

1.6 Polycyclic peptide host molecules

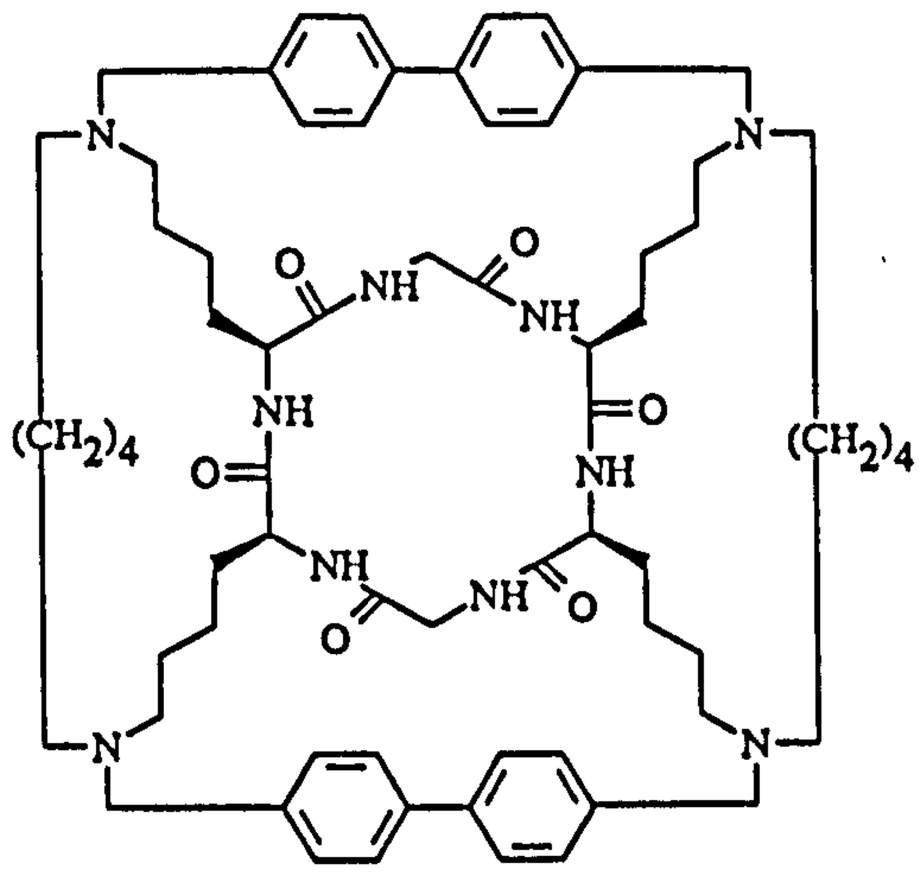
1.6.1 Early results

The pages above have described some of the many types of cavity like hosts that have been synthesised, the types of guest that they have bound and the applications to which they have been put.

Since 1988, work has been in progress within the group of P.D. Bailey to design and synthesise cyclic peptide based host molecules with significant 3-dimensional cavities. Two examples of such molecules, which have been successfully synthesised, are (76) and (77)[114-121].



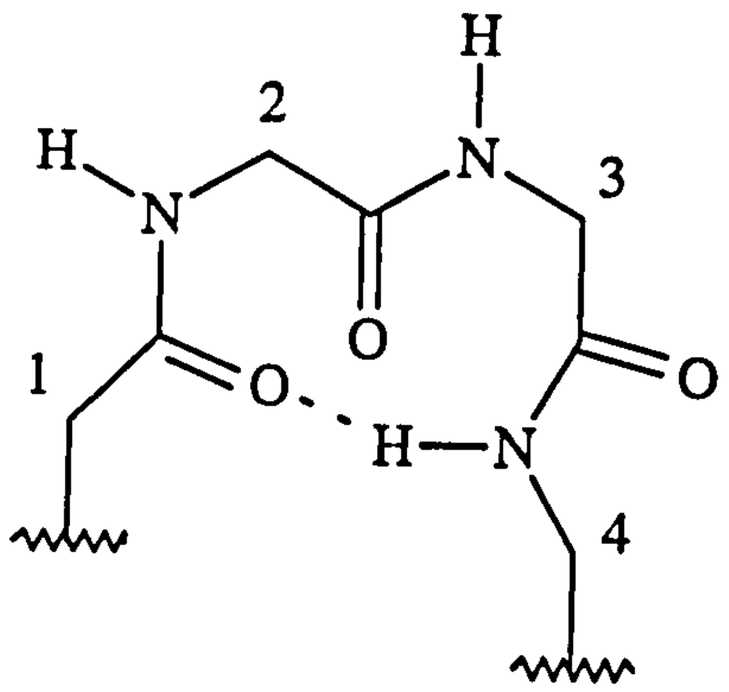
(76)



(77)

The common structural component of both these molecules is the cyclic hexapeptide *cyclo*-(Lys-Lys-Gly)$_2$. The use of such a cyclic peptide provides a good platform on which to build the cavities. Glycine was chosen for the cyclic peptide due to its ability to promote β-turns which stabilize cyclic peptide structures. β-turns are a form of secondary structure within the cyclic peptide which link those residues which are 1,4 to each other by hydrogen bonding (Fig 1.17).

Fig 1.17 : A β-turn in a cyclic peptide

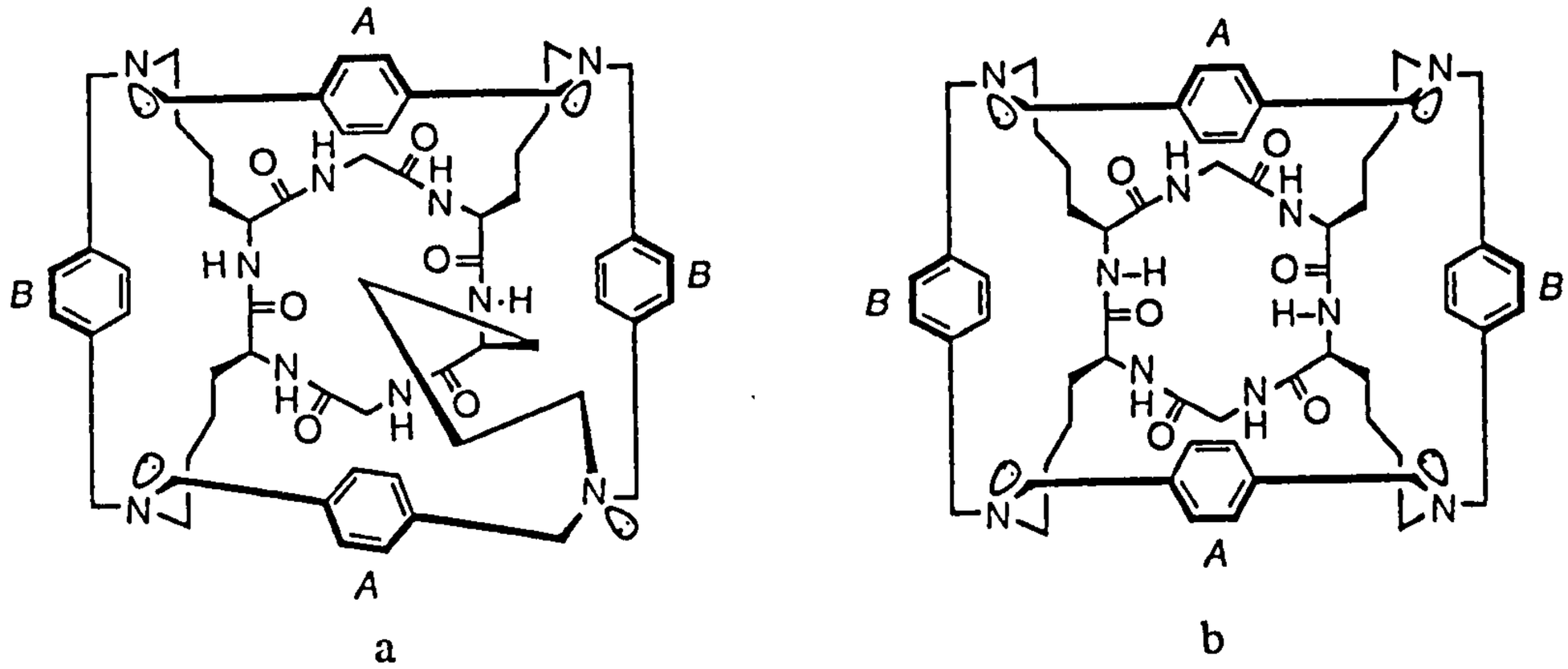


The use of L-lysine provides ϵ-nitrogens which are available for the crosslinking of groups between residues to form a cavity. In addition, the length of the side chain (4 carbons) gives a relatively deep cavity, the size of which can be varied by alternatively using homolysine (5 carbons) or ornithine (3 carbons) as required. Finally, because both (76) and (77) are symmetrical, a convergent synthetic strategy is feasible.

By using crosslinks between the lysine side chains, the conformations of the molecule can be further constrained. Varying the nature of the crosslinks, the size of the cavity produced can likewise be altered.

Performing a Monte Carlo conformational search on (76) and following its successful synthesis, extensive COSY and NOESY experiments have suggested that two low energy conformations are predominant (Fig 1.18).

Fig 1.18 : The two predominant low energy conformations of host (76) as found by a Monte Carlo conformational search

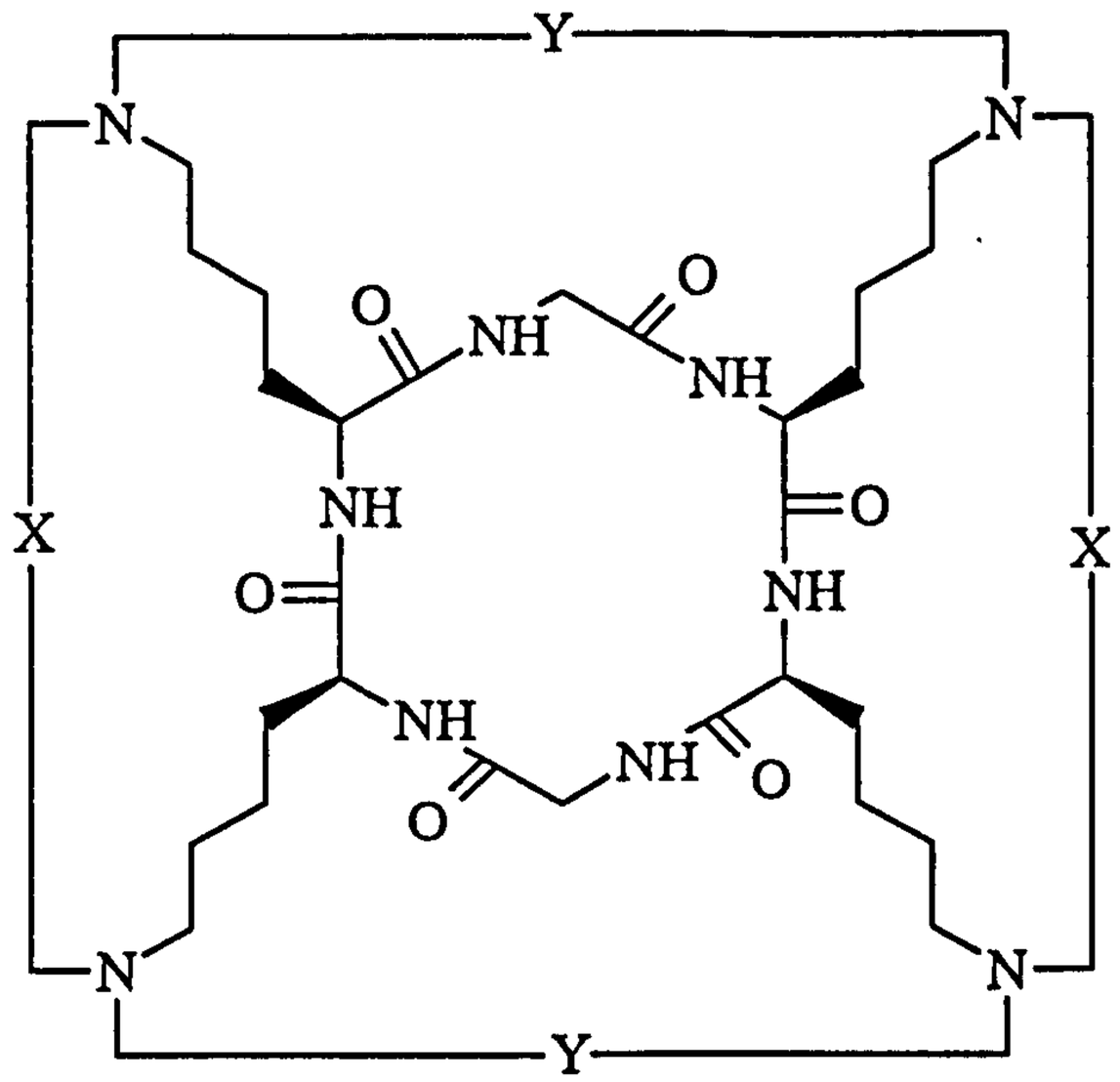


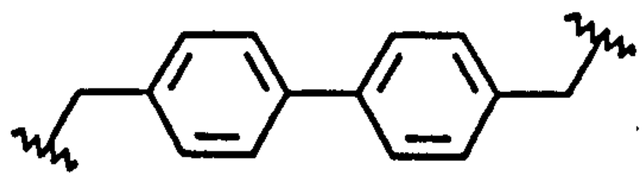
In both conformations, the cyclic peptide backbone shows approximate two-fold symmetry. The difference between the conformers lies in the configuration of the ϵ-nitrogens. For the lower energy structure (1.18a), three of the ϵ-nitrogen lone pairs are directed inwards whilst one lone pair is directed outwards. In the higher energy structure, all four lone pairs are directed inwards. The consequence is that whilst (1.18b) has an open cavity ready to receive guest molecules, that of (1.18a) is filled with a lysine side chain.

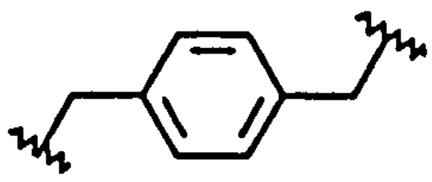
Preliminary results of binding experiments with N-Ac-L-Ala and host (76) have indicated that this amino acid is bound within the host cavity[118]. Bailey intends to test the binding of alternative potential guest amino acids so as to study any enantioselectivity in the cavity.

1.6.2 Project aims

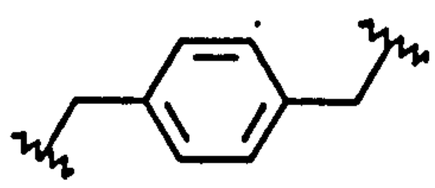
The aim of this project was to build upon the work achieved within the group of Bailey, described above, by the synthesis of molecules (78) to (81).

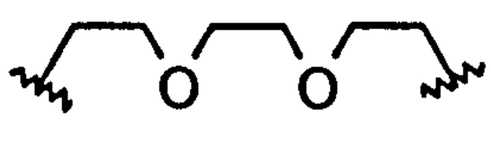


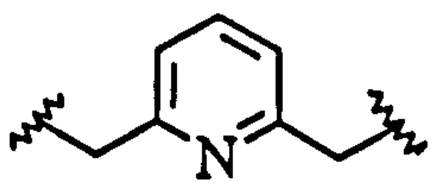
(78) X = 

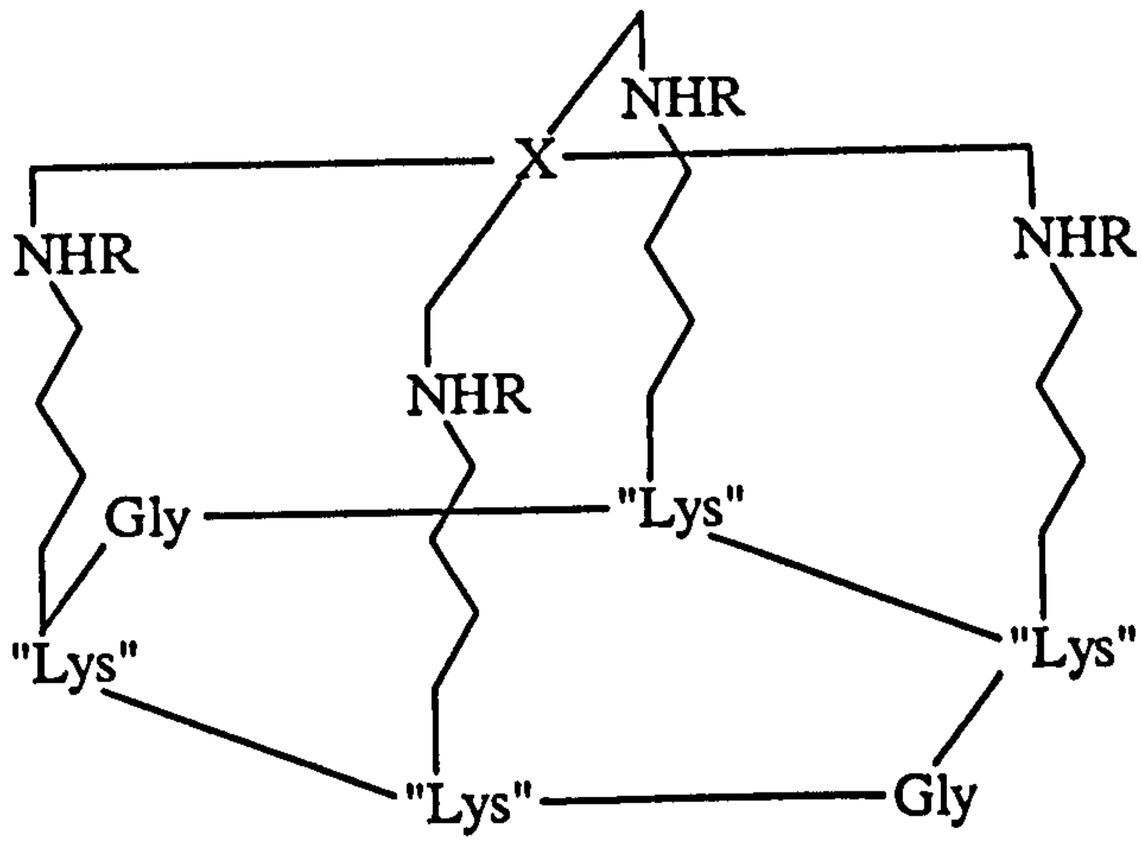
Y = 

(79) X = 

Y = 

(80) X = 

Y = 



X = porphyrin

R = H, Ts

(81)

The synthesis of polycyclic peptide (78) was to be attempted in order to study further the conformations of constrained cyclic peptides. In particular, it was important to discover whether the use of the biphenyl group as a crosslinker between adjacent lysine residues would affect the formation of the two low energy conformers analogous to (1.18a) and (1.18b). By potentially forming a larger cavity than (76), larger protected amino acids than N-Ac-L-Ala and potentially dipeptides may be bound.

It was intended that (79) would serve as a model for the synthesis of (80), which would itself act as a model for such binuclear metal complexes as hemerythrin and hemocyanin (see Chapter 3)[122,123]. Both of these molecules are oxygen transport agents for a variety of lower life forms and possess active sites analogous to (80). It was hoped that (80) would bind two iron or two copper ions and thence bind an oxygen molecule.

The porphyrin based structure (81) was designed to bind metal ions such as Fe^{2+} and Cu^{2+} and thus act as a stereoselective oxidising agent by virtue of the co-ordination of substrates between porphyrin and cyclic peptide (Chapter 4).

CHAPTER 2:

SYNTHESIS OF CROSSLINKED TRIPEPTIDES

2. Synthesis of crosslinked tripeptides

2.1 General solution-phase peptide synthesis

Ever since the idea of peptides and polypeptides was introduced by Emil Fischer in 1906[124], the synthesis of such compounds has been a long standing challenge to chemists[125]. The successful synthesis in 1953 by du Vigneaud of the cyclic octapeptide oxytocin - the first synthesis of a polypeptide hormone[126]- was a seminal achievement and represents the start of modern peptide synthesis[125]. Since that date a large number of new techniques for the racemisation free, specific coupling of amino acids have been developed[18,127-130], such that the solution phase synthesis of small peptides is now routine[131].

Small synthetic peptides have been widely used as, for example, antibiotics, neuropeptides and anti-cancer drugs[132]. The pages below will give a brief description of some of the techniques involved in solution-phase peptide synthesis. The use of these methods in the synthesis of a crosslinked tripeptide will then be described.

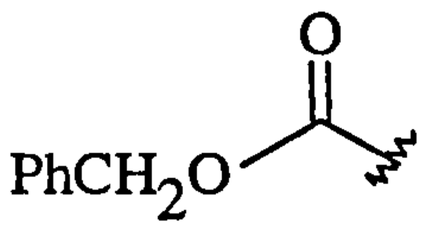
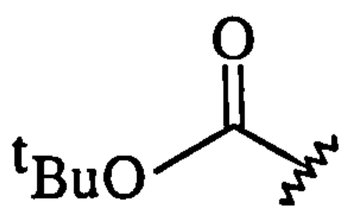
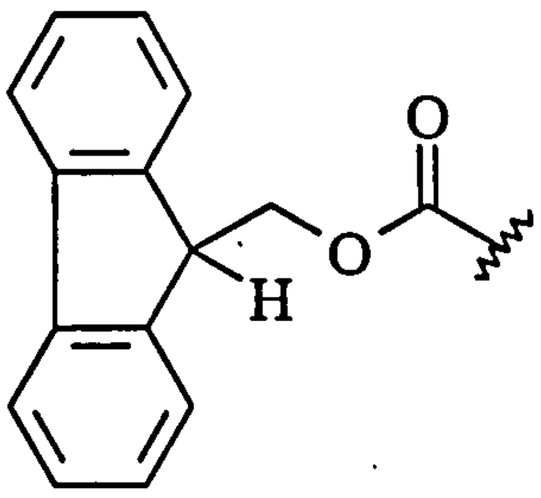
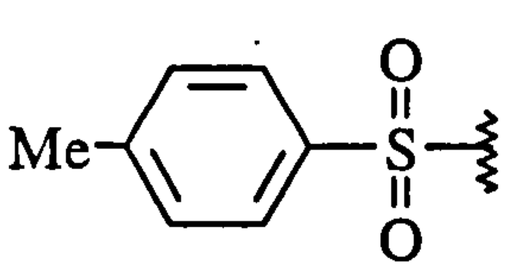
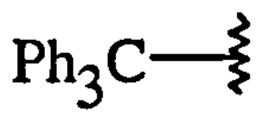
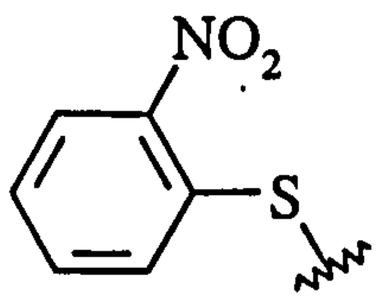
2.1.1 Protecting groups[18,125,130]

In order to prepare peptides by the coupling of amino acids, it is necessary to protect all those reactive functional groups that it is intended will not take part in the reaction. Consequently, one must protect the α-amino terminus, the carboxyl terminus and any potentially reactive side chains.

When protecting an α-amino group it is important to suppress its nucleophilicity either by withdrawing its electron density or by making it sterically inaccessible behind a bulky protecting group.

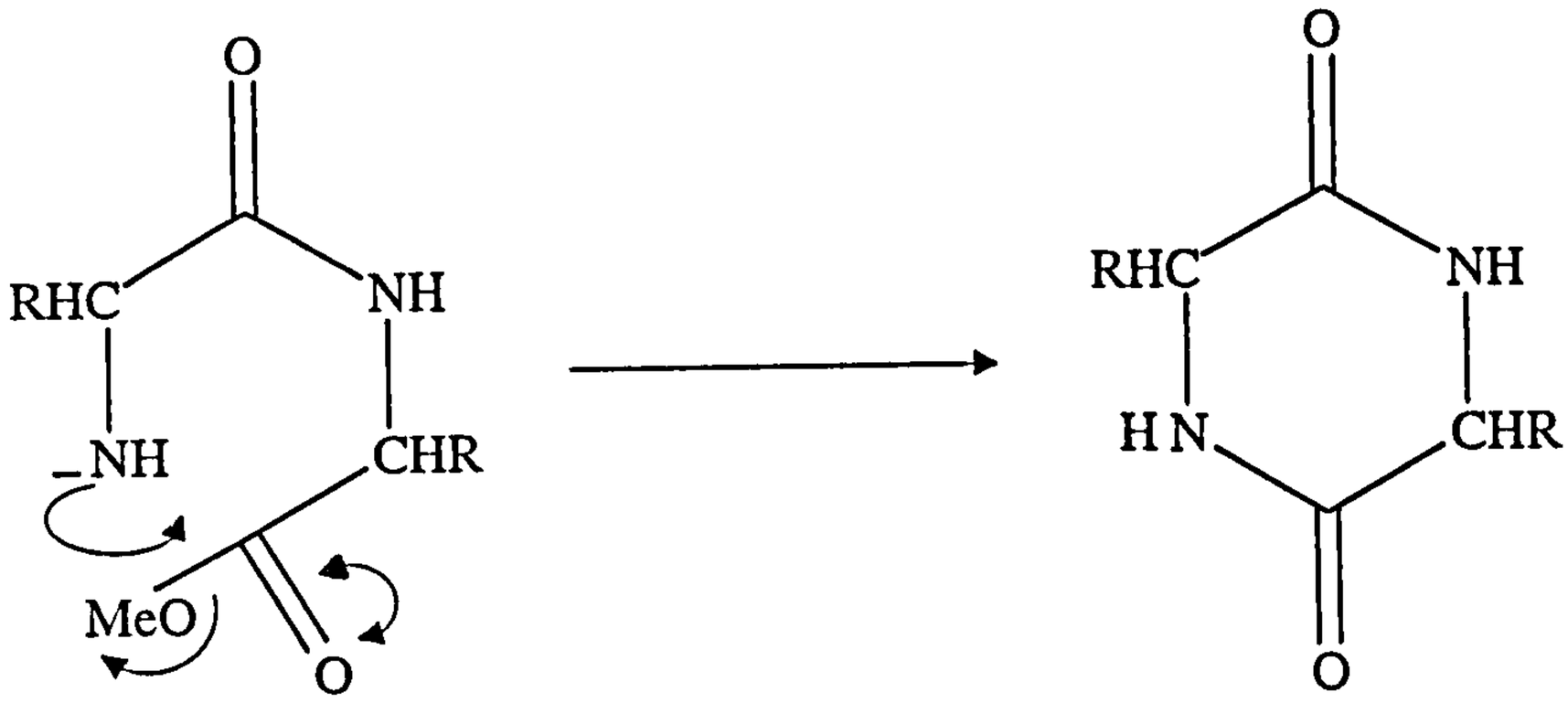
The α-amino function is frequently protected by alkoxycarbonyl groups since these groups commonly suppress racemisation in the peptide coupling (Section 2.1.3). Widely used alkoxycarbonyl protecting groups are benzyloxycarbonyl (Z)[133], t-butoxycarbonyl (Boc)[134,135] and 9-fluorenylmethoxycarbonyl (Fmoc)[136-138]. These three groups are described as *"orthogonal"* to each other in that each is stable to the conditions required to remove the other two (Table 2.1). However, other non-alkoxycarbonyl based protecting groups are also frequently used, among these being *p*-toluenesulphonyl (tosyl)[139], triphenylmethyl (trityl)[140] and 2-nitrophenylsulphenyl (Nps)[141].

Table 2.1: α-amino protection

Structure	Protecting group	Removal conditions
	Z	Catalytic hydrogenolysis
	Boc	Acid labile
	Fmoc	Base labile
	Tosyl	Strong reducing conditions or strong acid (Chapter 3)
	Trityl	Very acid labile, catalytic hydrogenolysis
	Nps	Acid labile, catalytic hydrogenolysis

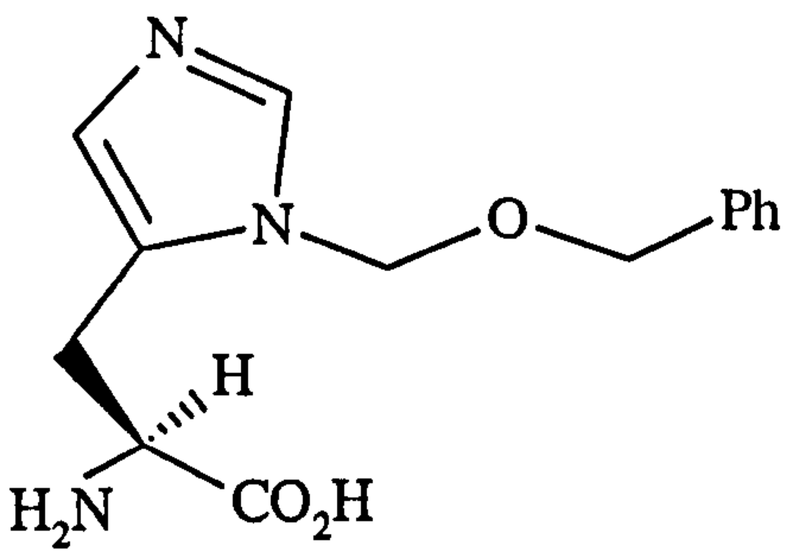
The most widely used means of protection of the α-carboxyl group is esterification. Simple methyl or ethyl esters can be used and survive most peptide bond forming procedures and most deprotective operations. Alkaline hydrolysis is the most common means of deprotection, although this can result in racemisation of the optically active α-carbon of the amino acid or the formation of a diketopiperazine as shown in Scheme 2.1.

Scheme 2.1 : Formation of a diketopiperazine

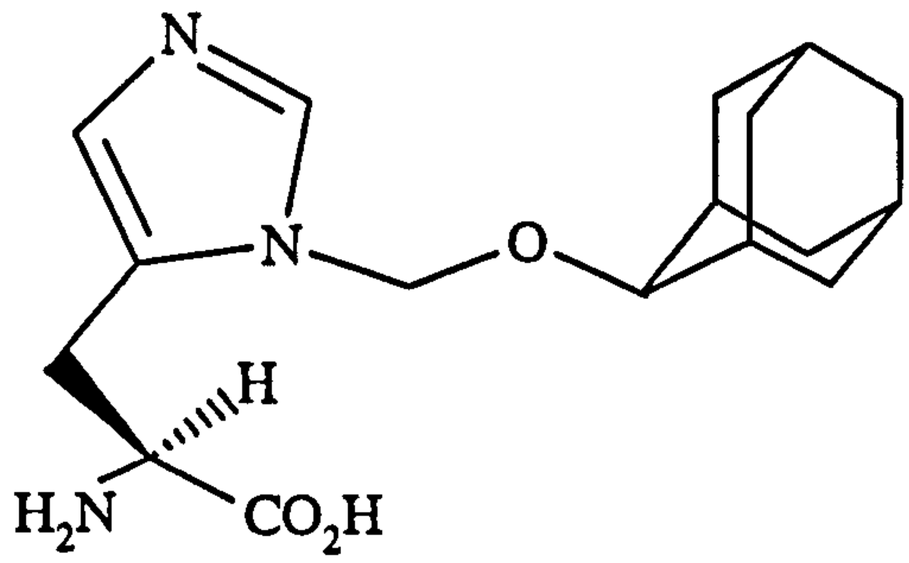


Benzyl and t-butyl esters are also frequently prepared, with these deprotections involving analogous conditions to those for the corresponding α-amino protection, i.e. catalytic hydrogenolysis or acidic conditions, respectively.

A variety of novel side chain protecting groups have also been developed. Carboxylic acids (eg. Glu, Asp), amino side chains (eg. Lys) or heteroaromatic side chains (eg. His) require full protection which is orthogonal to other protective groups being used. Thus, for example, in 1982 Jones developed the N^{π}-benzyloxymethyl protecting group which is cleaved from N^{π}-benzyloxymethyl histidine, His(N^{π}-Bom) (81)[125] by catalytic hydrogenolysis[142]. However, new protective groups are continuously being developed[130] with the N^{π}-2-adamantyloxymethyl group, used for example, to form N^{π}-2-adamantyloxymethyl histidine, His(N^{π}-2-Adom) (82) being a particularly recent example - this being cleaved under strong acidic conditions[143].



(82)



(83)

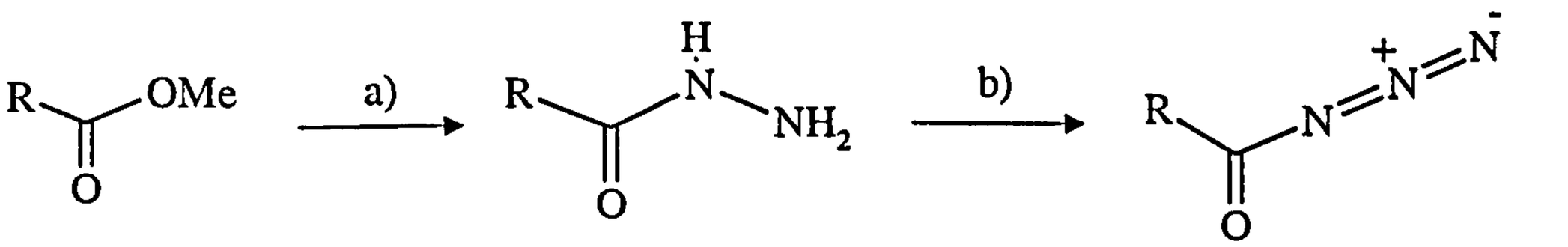
2.1.2 Amino acid coupling reactions[18, 125, 130, 144]

The mixing of carboxylic acids and amines does not lead spontaneously to the formation of an amide bond but rather to the production of a salt. It is therefore necessary to activate one of the components. To this effect, carboxylic acid activation has been most frequently employed in peptide synthesis. Acid catalysis is of no help in such reactions due to the basicity of the amino reagent; thus a variety of other different techniques have been developed in order to effect the activation of acylamino acids.

The classical method of activation, developed by Fischer, is to form the acid chloride[145]. However, whereas such species very efficiently form amide bonds on exposure to primary amines, they are also susceptible to significant side reactions. Consequently, most simple acylamino acids cyclise spontaneously to give oxazolones and thus racemic peptides (Section 2.1.3).

An alternative method, introduced by Curtius is the formation of acyl azides[146]. These can be made by the reaction of hydrazine with carboxylic esters and thence by treatment with nitrous acid (Scheme 2.2).

Scheme 2.2 : Formation of an acid azide



acyl hydrazide

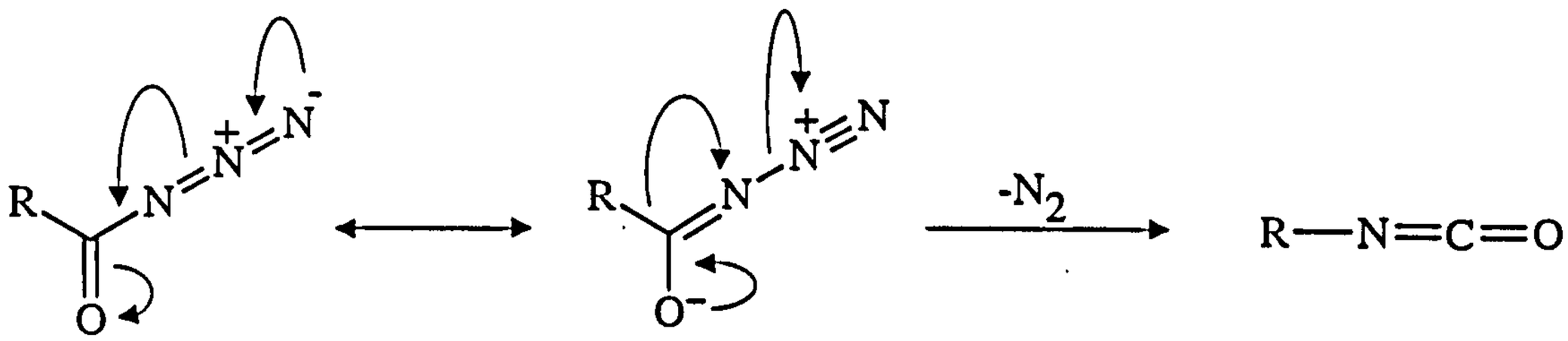
resonance stabilized acid azide

a) N_2H_4, MeOH

b) HNO_2 (NO^+)

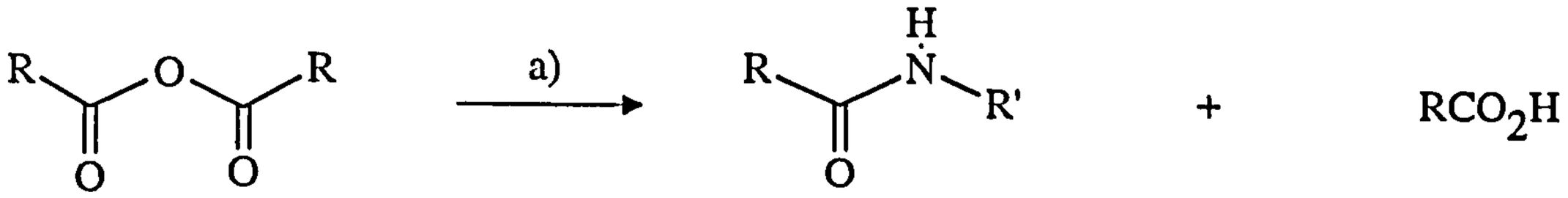
A key advantage of this method is that it is less susceptible to racemisation of the amino acid than other techniques. However, a number of side reactions can also be observed amongst which the Curtius rearrangement (Scheme 2.3) can give significant amounts of side products[147].

Scheme 2.3 : Curtius rearrangement



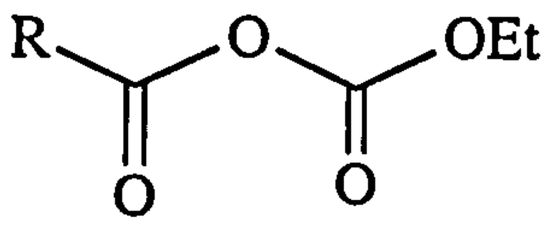
In the 1950s, the idea of using symmetrical and unsymmetrical acid anhydrides in peptide synthesis was introduced[148-150]. By dehydrating α-amino protected amino acids, symmetrical anhydrides can be formed. Such anhydrides are relatively reactive so peptide couplings are efficient and are also comparatively clean. The major disadvantage is the inevitable loss of half of the amino acid as carboxylate (Scheme 2.4). If the amino acid is protected, this can be expensive.

Scheme 2.4 : Formation of peptide bond using symmetrical anhydrides

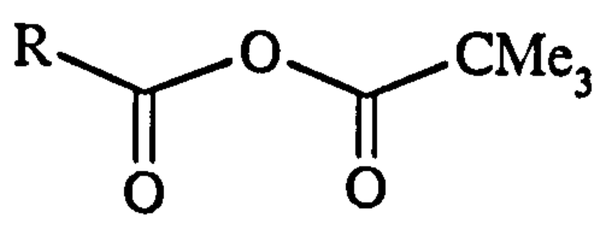


a) $R'\text{-}NH_2$

Alternatively, mixed anhydrides have been developed in which the challenge is to ensure reaction at the desired carbonyl group. Thus a mixed anhydride was formed by the reaction of the peptidic carboxylic acid with ethyl chloroformate. In the resultant anhydride (84) only the carbonyl of the amino acid is susceptible to nucleophilic attack, that of the carbonic acid being less electrophilic. Alternatively, steric interactions have been utilised in, for example, the formation of the pivalyl mixed anhydride (85).



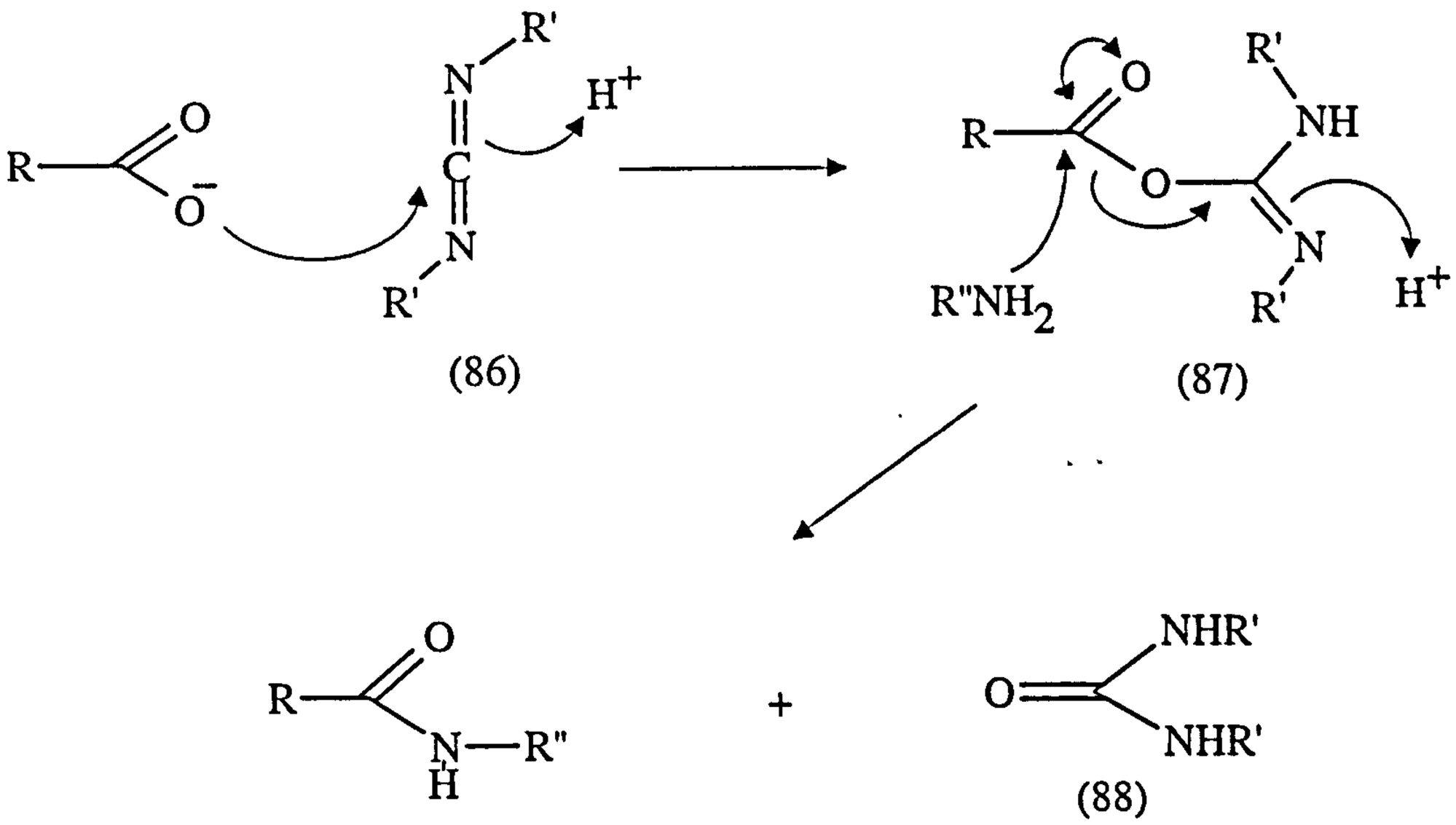
(84)



(85)

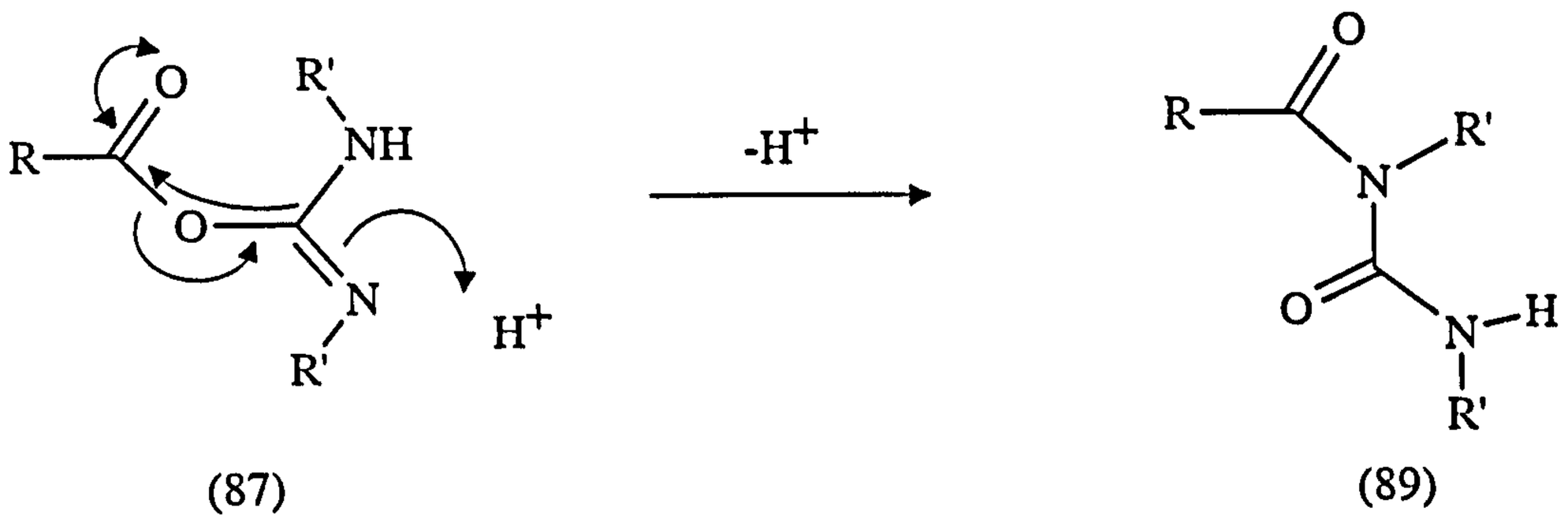
In 1955, the use of the coupling reagent dicyclohexylcarbodiimide (86) was recommended by Sheehan and Hess[151]. Scheme 2.5 shows the mechanism by which this facilitates the formation of an amide bond.

Scheme 2.5 : Formation of a peptide bond using dicyclohexylcarbodiimide



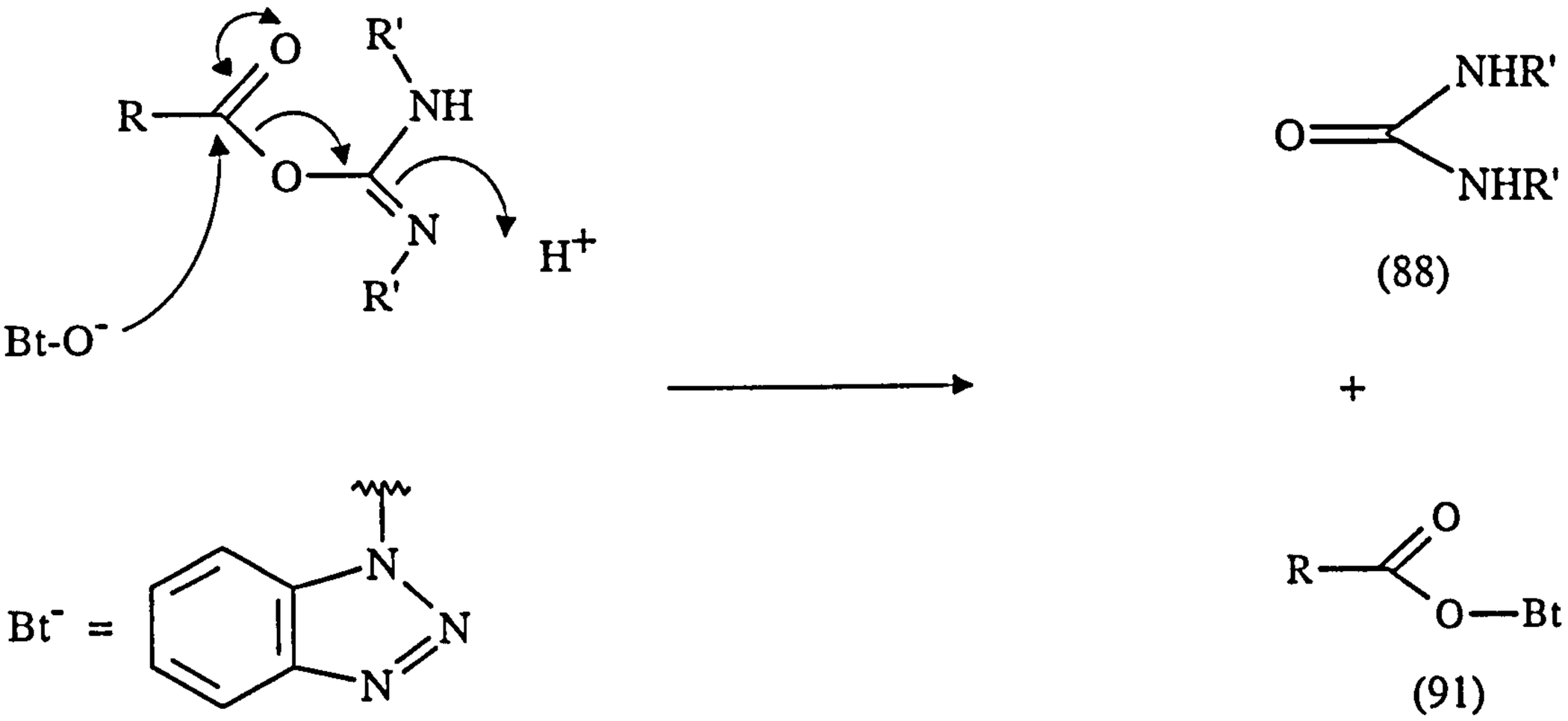
Unfortunately, this is not a clean reaction. Significant racemisation is known to occur and rearrangement of the intermediate O-acylisourea (87) can compete with peptide bond formation to produce an N-acylurea (89) (Scheme 2.6).

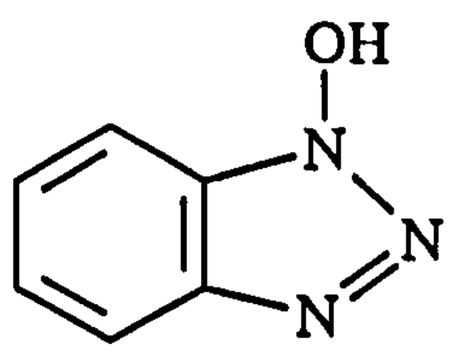
Scheme 2.6 : Rearrangement of an O-acylisourea to an N-acylurea



This problem has been countered by the catalysis of the amide bond forming reaction. Thus, as shown in Scheme 2.7, active esters have been generated *in situ* by the addition of reagents such as 1-hydroxybenzotriazole (HOBt) (90) [152]. HOBt, being a good nucleophile, reacts readily with the O-acylisourea (87) before formation of (89) can occur. An active benzotriazole ester (91) of lower reactivity is formed which is still active with respect to aminolysis but is less likely to take part in side reactions.

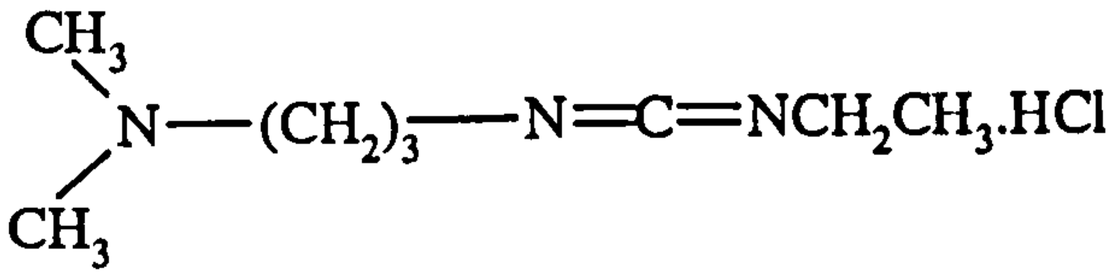
Scheme 2.7 : Formation of an active ester using 1-hydroxybenzotriazole

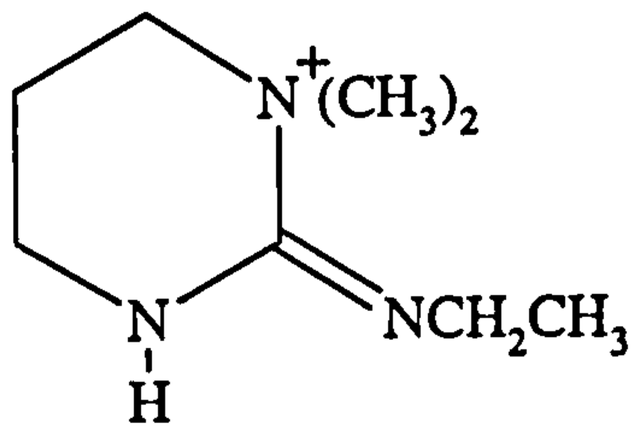




(90)

Difficulties in the removal of the sparingly soluble dicyclohexylurea (DCU) (88) have encouraged work to develop alternative carbodiimides which when used in peptide coupling reactions produce water soluble urea side products. An example of such a reagent is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (92) [153].



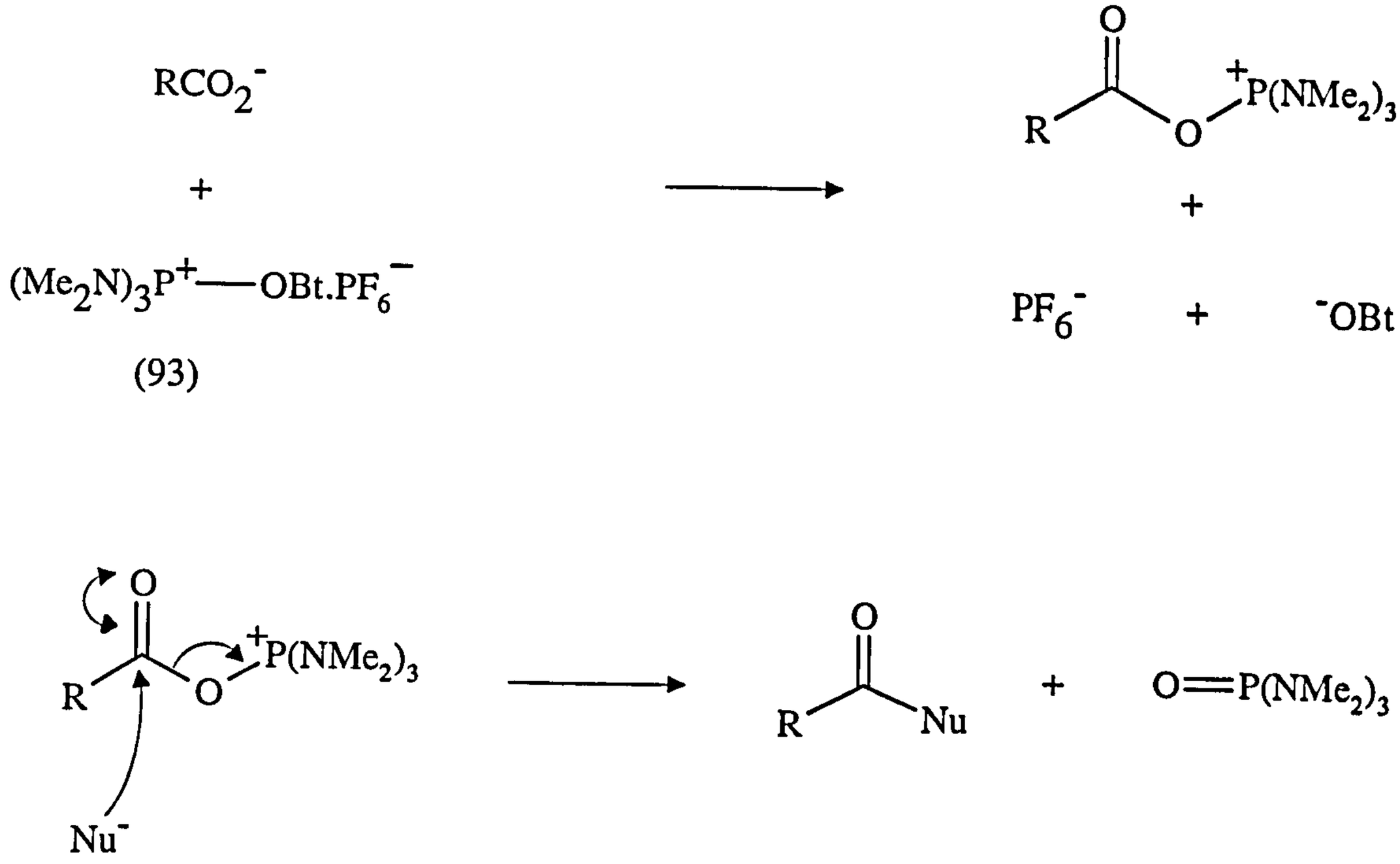


(92)

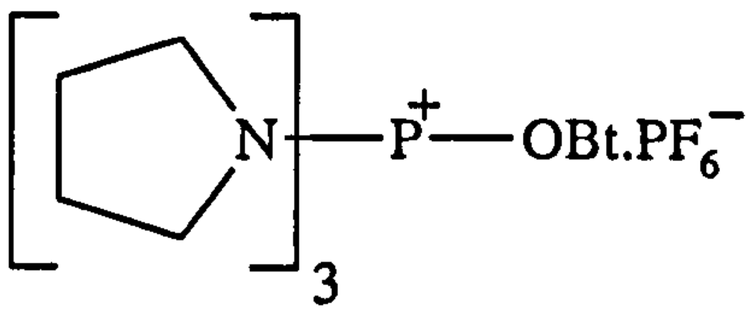
The formation of amide bonds by the reaction of amines with simple methyl or ethyl esters is a slow process. However, by using active esters with electron withdrawing substituents on the leaving group, these leaving groups can be stabilized and the amide bond can be formed more speedily. Other than the benzotriazole esters just mentioned, pentafluorophenyl esters, 4-nitrophenyl esters, 2,4,5-trichlorophenyl esters and succinimidyl esters are commonly used[130,154]. Such procedures are clean and relatively free from racemisation problems.

A final common technique is the use of phosphonium reagents. Castro has developed the benzotriazolyloxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) reagent (93) which forms the acyloxyphosphonium ester (Scheme 2.8)[155]. This ester is highly susceptible to nucleophiles.

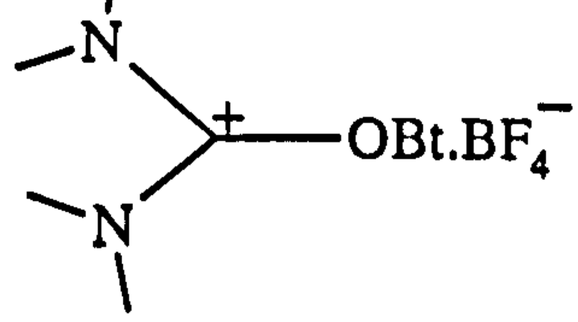
Scheme 2.8 : Activation of carboxylate anion to nucleophilic attack using the BOP phosphonium reagent



Nevertheless, whereas this reaction gives good yields of product and is a relatively clean procedure, racemisation can still be a problem. In addition, the formation of the highly toxic hexamethylphosphoramide is a serious concern. Thus alternative reagents such as benzotriazolyloxy-tris(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP) (94)[156] and benzotriazolyloxy-1,1,3,3-tetramethylammonium tetrafluoroborate (TBTU) (95) have also been developed.



(94)



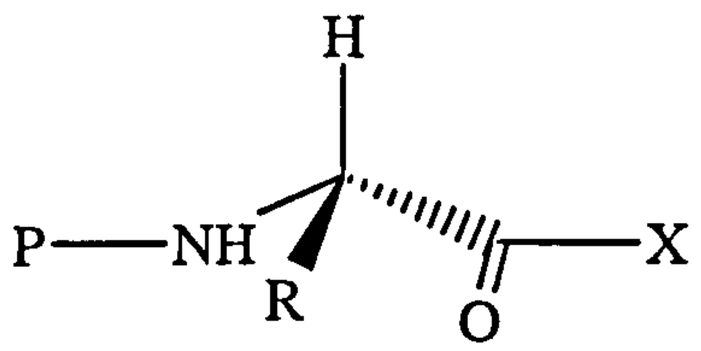
(95)

2.1.3 Racemisation[18,125,130]

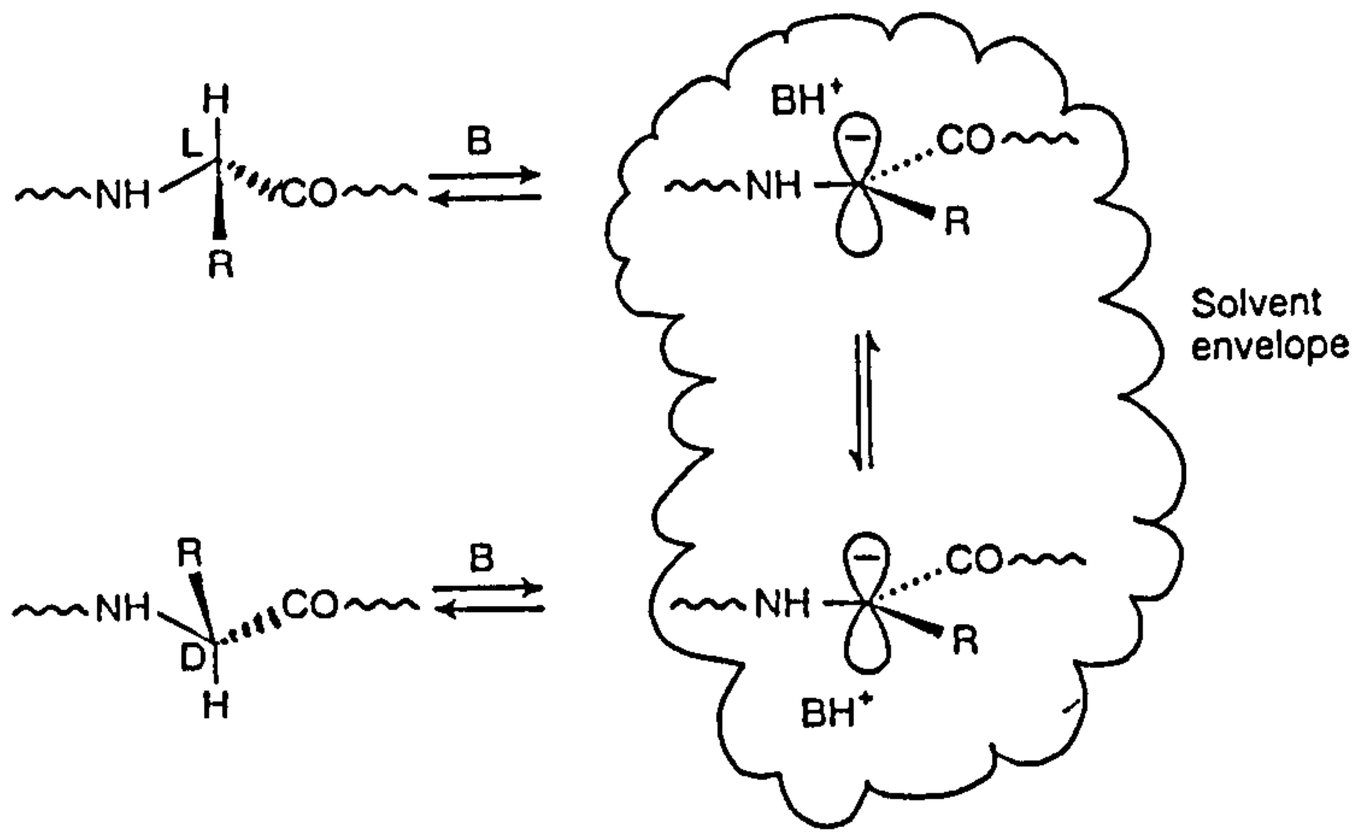
One of the most serious side reactions that can occur in peptide synthesis is the racemisation of the α-carbon of the amino acid. If such a complication is significant, a mixture of epimers can be formed leading to difficult purification problems.

There are two principle mechanisms by which racemisation can arise. As shown in Scheme 2.9, direct deprotonation of the α-carbon results in racemisation because the carbanion intermediate can gain a proton from either side. The rate of deprotonation is governed by the catalysing base, the solvent and the electron withdrawing effects of the groups P, R and X around the chiral centre (96). When X = NH-, O-alkyl or O^-, such racemisation is negligible. However, during activation and coupling, significant deprotonation can occur, particularly in the presence of strong unhindered bases in polar aprotic solvents such as DMF and DMSO. Deprotonation is a particular problem in the case of α-arylglycines due to the delocalisation and thus stabilisation of the negative charge of the deprotonated conjugate base. However, in the case of most peptide couplings, the rate of the coupling reaction is considerably greater than the rate of the deprotonation reaction just described, making racemisation problems by this pathway negligible.

Scheme 2.9 : Mechanism of racemisation by direct enolisation



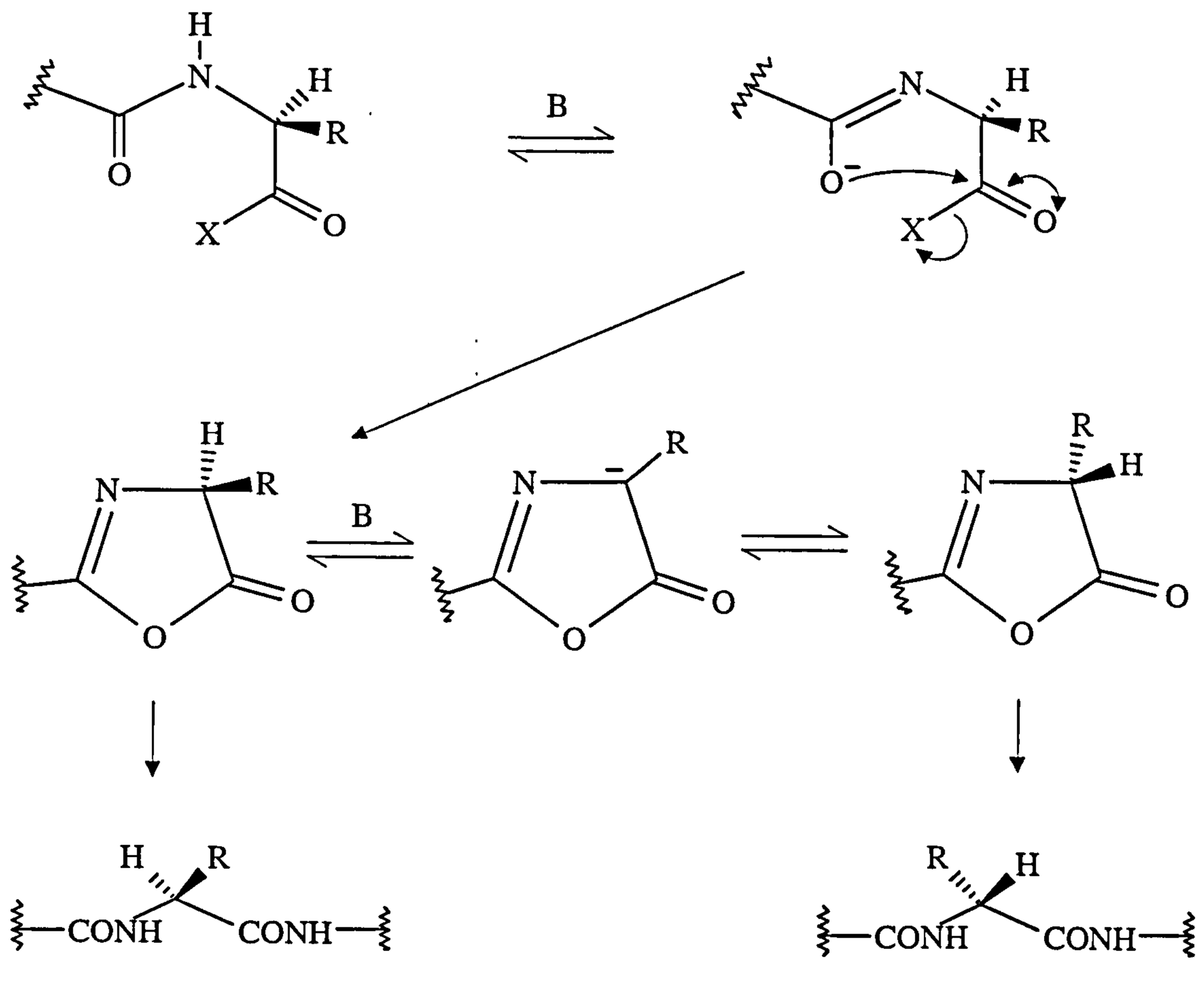
(96)



The key racemisation mechanism (see above Section 2.1.2) involves the formation of easily deprotonated oxazolones (Scheme 2.10). In many circumstances, with most good leaving groups, large scale or even total racemisation can occur.

However, oxazolone formation is considerably reduced when the amino terminus is protected by an alkoxycarbonyl group such as Z, Boc or Fmoc. Further, alkoxyoxazolones are both less readily racemised and more susceptible to nucleophiles than oxazolones derived from simple amino acids.

Scheme 2.10 : Mechanism of racemisation by the formation of oxazolones

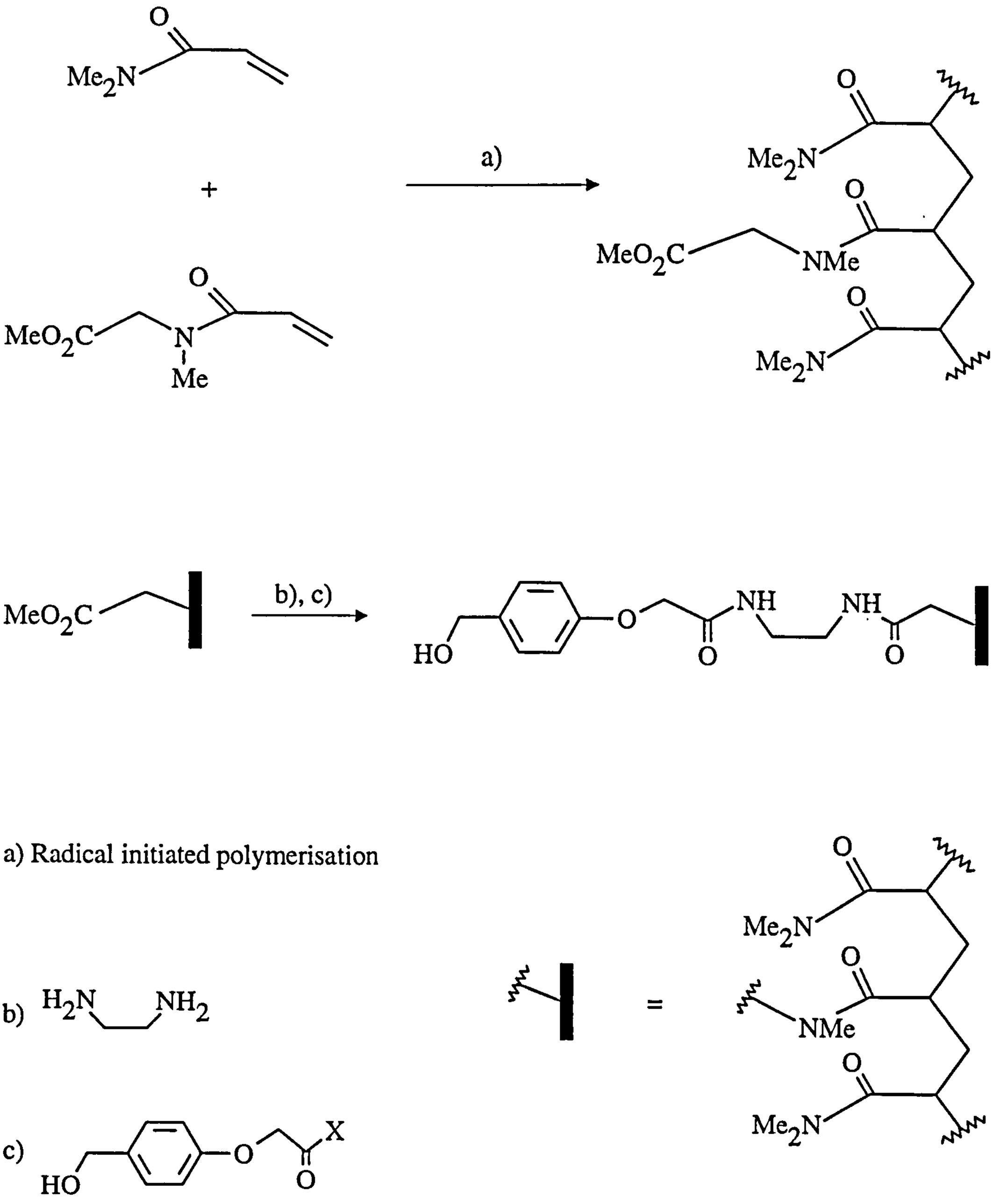


2.2 Solid phase peptide synthesis[18,157]

A variety of peptidic natural products including enzymes such as Ribonuclease A[158] have been synthesised by a different method, known as Solid Phase Peptide Synthesis (SPPS). This technique was pioneered in 1963 by R.B. Merrifield[159] who won the 1985 Nobel Prize for Chemistry for his work in this field. The basis of this technique is the covalent bonding of the carboxyl terminus onto a solid support from which the peptide chain can be constructed. Merrifield originally used a polystyrene resin[159], but many other polymers, for example, polydimethylacrylamide[18] (Scheme 2.11), have also been employed. The Fmoc group is frequently used for protection of the α-amino terminus, with active esters used for peptide activation. The advantage of this method is that by using a vast excess of each reagent for each peptide coupling, near quantitative yields can be obtained. By-products and excess reagent can then be removed by simple filtration and washing. The whole process can be readily automated to allow the efficient synthesis of long chain peptides in a period of days rather than weeks or months, as in solution phase peptide synthesis. The method of preparation of the polydimethylacrylamide polymeric support is illustrated in Scheme 2.11 and its use in SPPS is illustrated in Scheme 2.12.

However, although time consuming, solution phase peptide synthesis has a number of advantages over SPPS. Firstly, because each intermediate peptide is isolated during the synthesis, any problems with particular synthetic steps can be identified. Secondly, there is a limit to the quantity of peptide that a given resin can hold. Consequently, solution phase peptide synthesis is more suitable for the bulk synthesis of peptides.

Scheme 2.11 : Preparation of the polydimethylacrylamide solid support in solid phase peptide synthesis

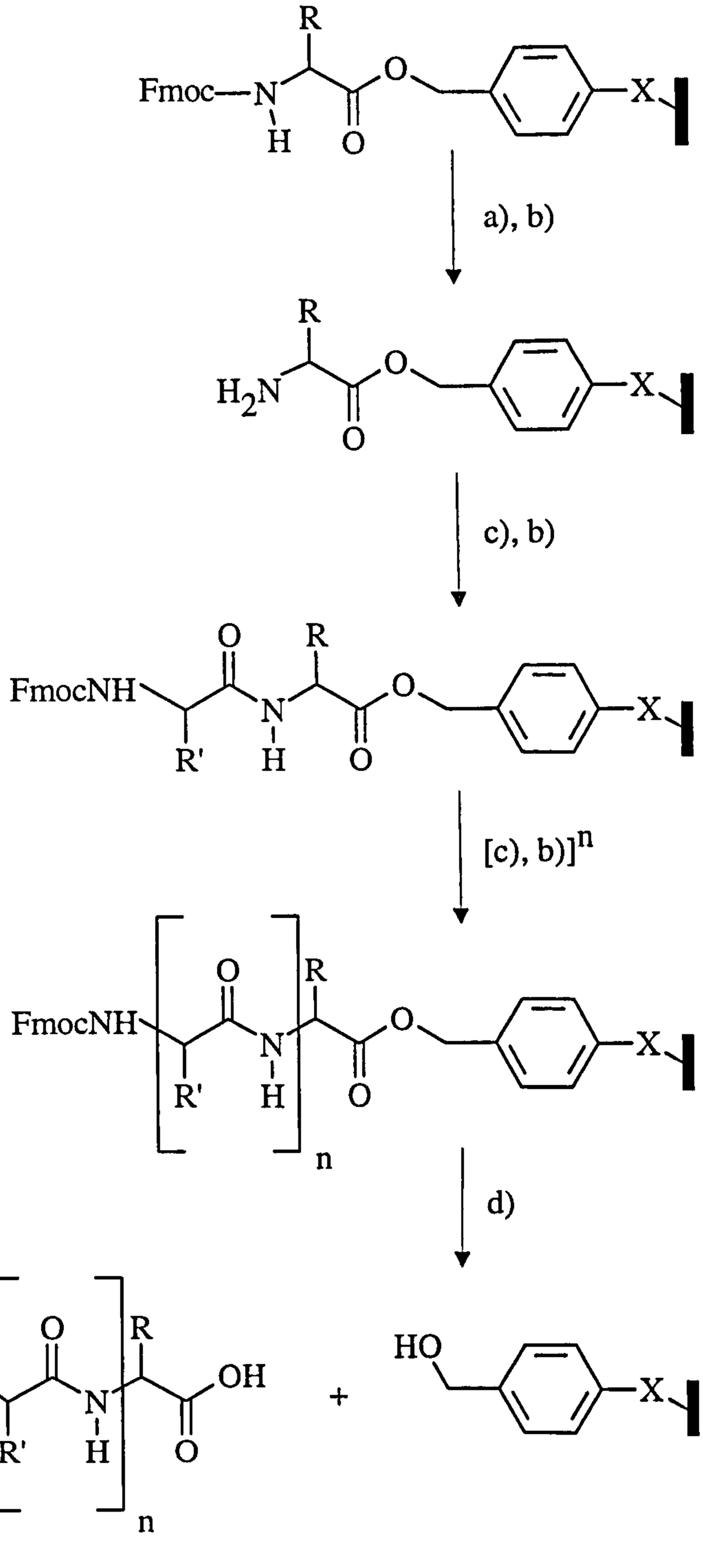


a) Radical initiated polymerisation

b) H_2N NH_2

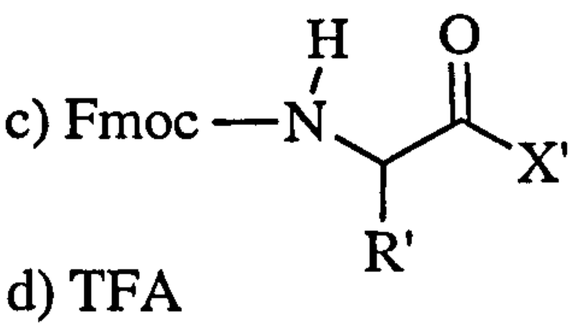
c) HO, O, X, O

Scheme 2.12 : A typical reaction sequence using solid phase peptide synthesis



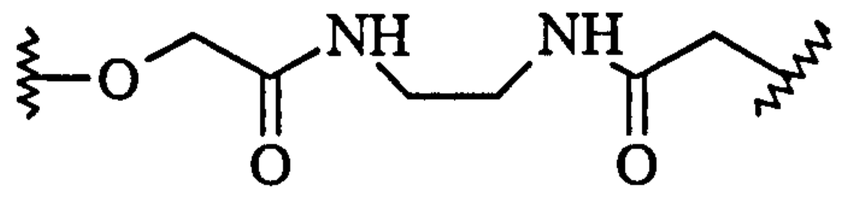
a) 20 % piperidine in DMF

b) Wash with DMF



d) TFA

X =



2.3 Synthesis of crosslinked tripeptide precursors

2.3.1 Synthetic strategy for the synthesis of polycyclic peptides

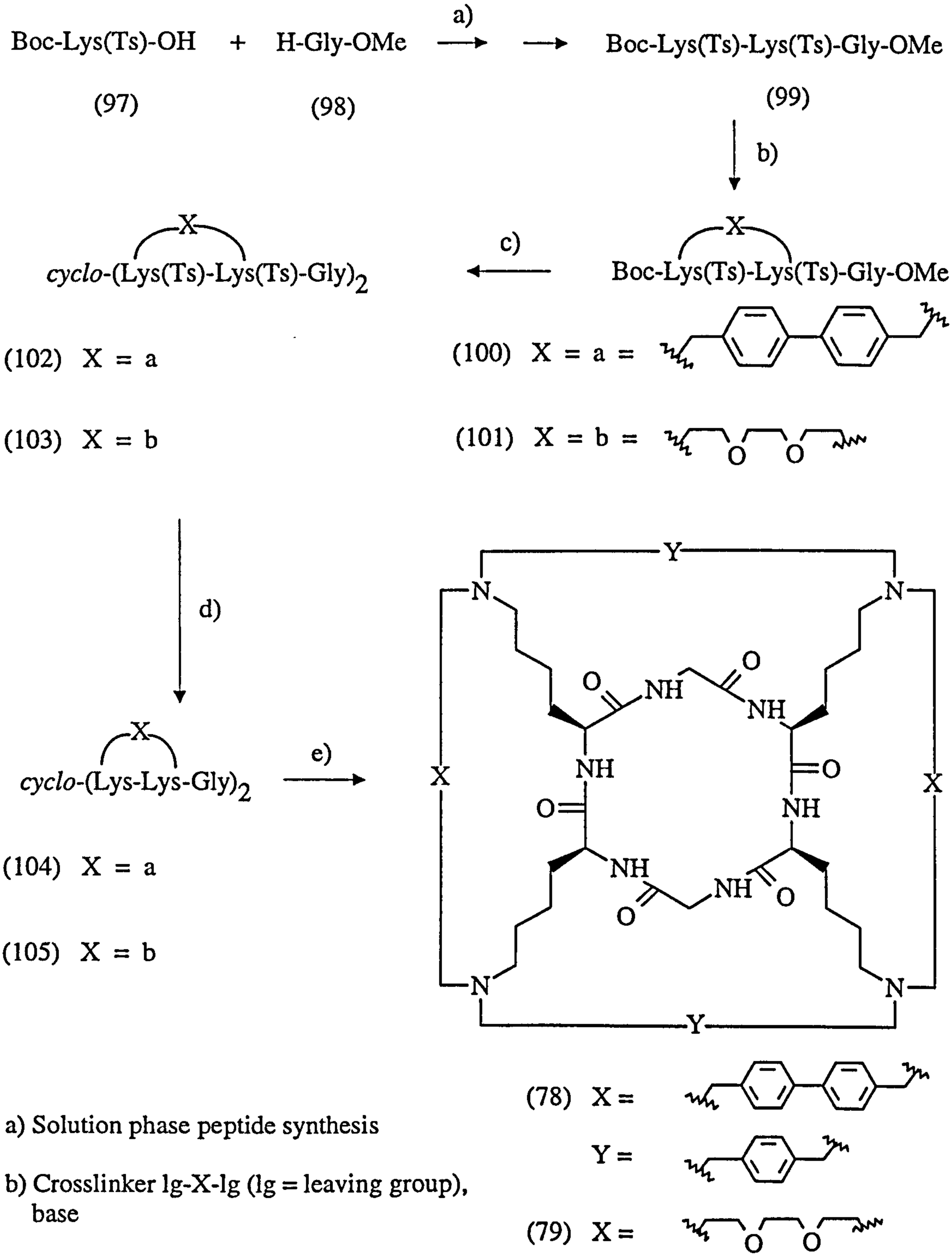
As described in Section 1.6.2, three polycyclic peptide synthetic targets (78)-(80) were chosen as potential host molecules. Following experience gained from previous workers within the group[115-117], the strategy shown in Scheme 2.13, involving 5 key stages, was considered as being most suitable for their synthesis:

a) The first step involved the synthesis of Boc-Lys(Ts)-Lys(Ts)-Gly-OMe (99) by solution phase methodology. The advantage of solution phase over solid-phase peptide synthesis[157] was primarily its suitability for the production of relatively large quantities of this tripeptide precursor.

b) Crosslinking of the lysine side chains to give the desired 4,4'-bis-methylbiphenyl or 1,2-bis(2-ethoxy)ethane crosslinks was the second stage. Because of the nature of the ϵ-amino groups on the lysyl side chains, there would be potential for three kinds of reaction to form the crosslinks - imine formation with carbonyl groups followed by reduction, amide formation and then reduction or nucleophilic displacement by the ϵ-amine of a leaving group. The last of these three techniques was chosen for this project. In order to encourage efficient crosslinking to occur, it was vital that once one end of the crosslinker had reacted, the correct conformation for cyclisation was readily accessible. It was considered that the linear geometry of the imine bonds and the planar nature of the amide bonds would cause constraints that might inhibit easy crosslink formation. Conversely, nucleophilic reaction would allow greater conformational flexibility for cyclisation to occur. In addition, by substituting the ϵ-nitrogen of each lysine side chain with an electron withdrawing tosyl group, the base catalysed deprotonation of the ϵ-amine would occur more readily, thus encouraging efficient nucleophilic displacement.

c) Cyclodimerisation of the resulting crosslinked tripeptides was to be attempted with a view to forming a cyclic hexapeptide which would be pre-crosslinked at the "1,2" and "4,5" residues. An advantage of such a cyclodimerisation[160] procedure over the cyclisation of the appropriate linear hexapeptide[161], was that it is easier to form the cyclic peptide with the crosslinks in the specific locations required. In addition, due to the C_2 symmetry of the cyclic peptide, cyclodimerisation was particularly appropriate so as to reduce the number of steps in the synthesis. Scheme 2.14 shows routes previously followed within the group

Scheme 2.13 : Strategy for the synthesis of polycyclic peptides



a) Solution phase peptide synthesis

b) Crosslinker lg-X-lg (lg = leaving group), base

c) Cyclodimerisation

d) De-tosylation

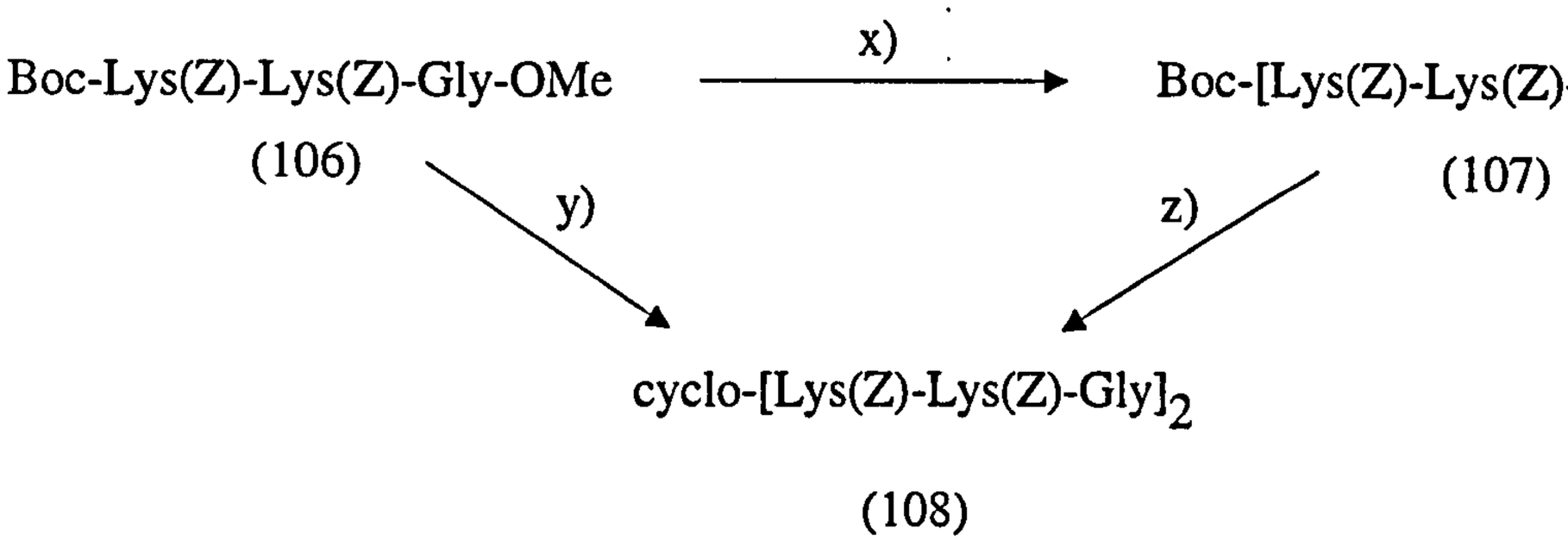
e) Crosslinker lg-Y-lg

with the aim of synthesising a cyclic hexapeptide. Cyclodimerisation of the linear tripeptide (106) [step y)] proved to be a more efficient route to the cyclic peptide (108) than the formation and then cyclisation of linear hexapeptide (107) [steps x) and z)][115]. The cyclodimerisation route gave a 38 % yield of cyclic peptide (108) from linear tripeptide (106), whereas the best reported yield following the linear pathway was 20.5 % from the same tripeptide starting material.

d) Deprotection of the ϵ-amino groups of the lysyl side chains by removal of the four tosyl groups was the fourth stage.

e) The synthesis was to conclude with the final crosslinking reactions at the "2,4" and "1,5" residues to form the appropriate final target molecule.

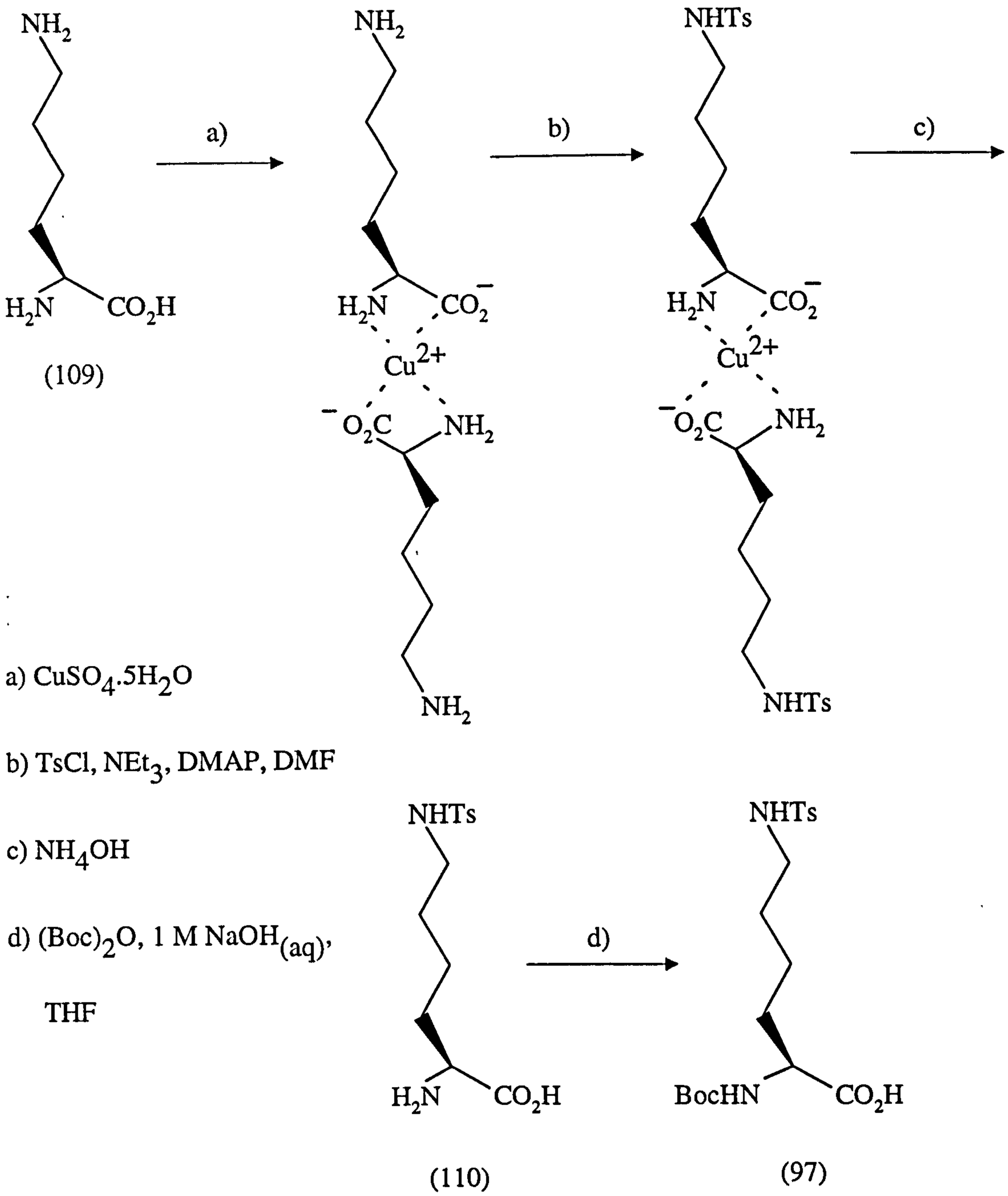
Scheme 2.14 : Formation of the Z protected cyclic hexapeptide (108)



2.3.2 Synthesis of Boc-Lys(Ts)-OH

N-ϵ-toluenesulphonyl-lysine (110) was synthesised from deprotected lysine (109) by others within the group of P.D. Bailey by the procedure outlined in Scheme 2.15 [128].

Scheme 2.15 : Synthesis of Boc-Lys(Ts)-OH (97)



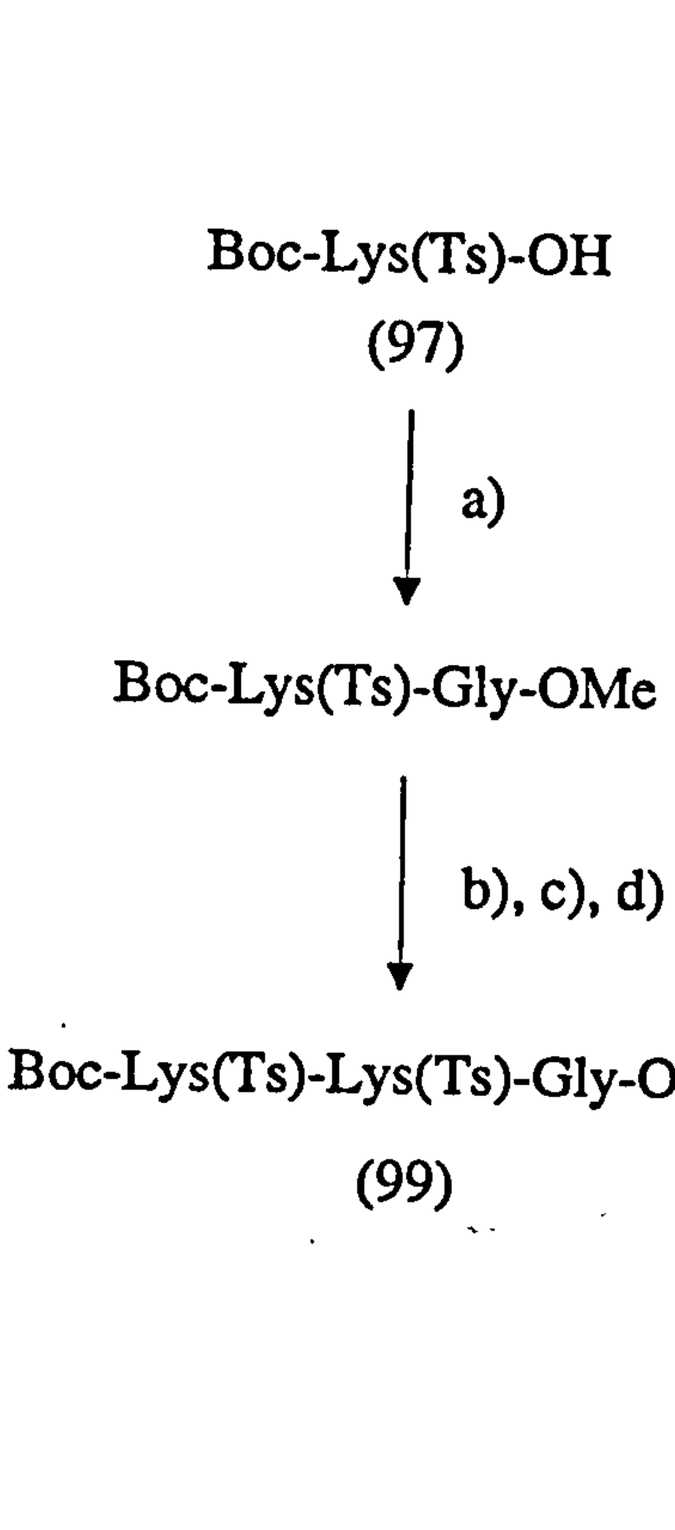
Following the generous donation of 10 g of (110) this was reacted with di-*tert*-butyl dicarbonate in ethoxyethane with dilute aqueous sodium hydroxide as base[128]. Following the work-up procedure, a 33 % yield of Boc-Lys(Ts)-OH (97) was obtained.

2.3.3 Synthesis of protected tripeptide

The tripeptide (99) was synthesised *via* the standard solution phase peptide synthesis methodology described earlier. Standard DCC/HOBt peptide couplings were used giving an overall yield of 63 %. However, due to an allergic reaction to DCC by one member of the research group, alternative peptide couplings using EDC (92) and HOBt (90) were used (Scheme 2.16). Due to the high solubility of EDC and its urea in water compared with DCU which is sparingly soluble in most common organic solvents, peptide couplings using EDC were easier to work up (simple water washes removed EDC and its urea) and gave slightly higher overall yields of product (67 %).

The protective groups used in the synthesis of tripeptide (99) were chosen due to their orthogonal protective properties. Thus t-butyloxycarbonyl (Boc) is acid labile, the methyl ester is hydrolysed by the use of methanolic base and tosyl (Ts) is commonly removed by either strongly reductive methods (eg. Na in liquid ammonia) or by the use of strong acid (Chapter 3).

Scheme 2.16 : Synthesis of the protected tripeptide precursor (99)



a) H-Gly-OMe,
DIPEA, DMF
DCC/HOBt or EDC/HOBt

b) 9:1 TFA:H_2O

c) DIPEA, DMF

d) Boc-Lys(Ts)-OH,
DCC/HOBt or EDC/HOBt, DMF

Yields:

Dipeptide using:

DCC 81 % EDC 83 %

Tripeptide using:

DCC 78 % EDC 81 %

Overall using:

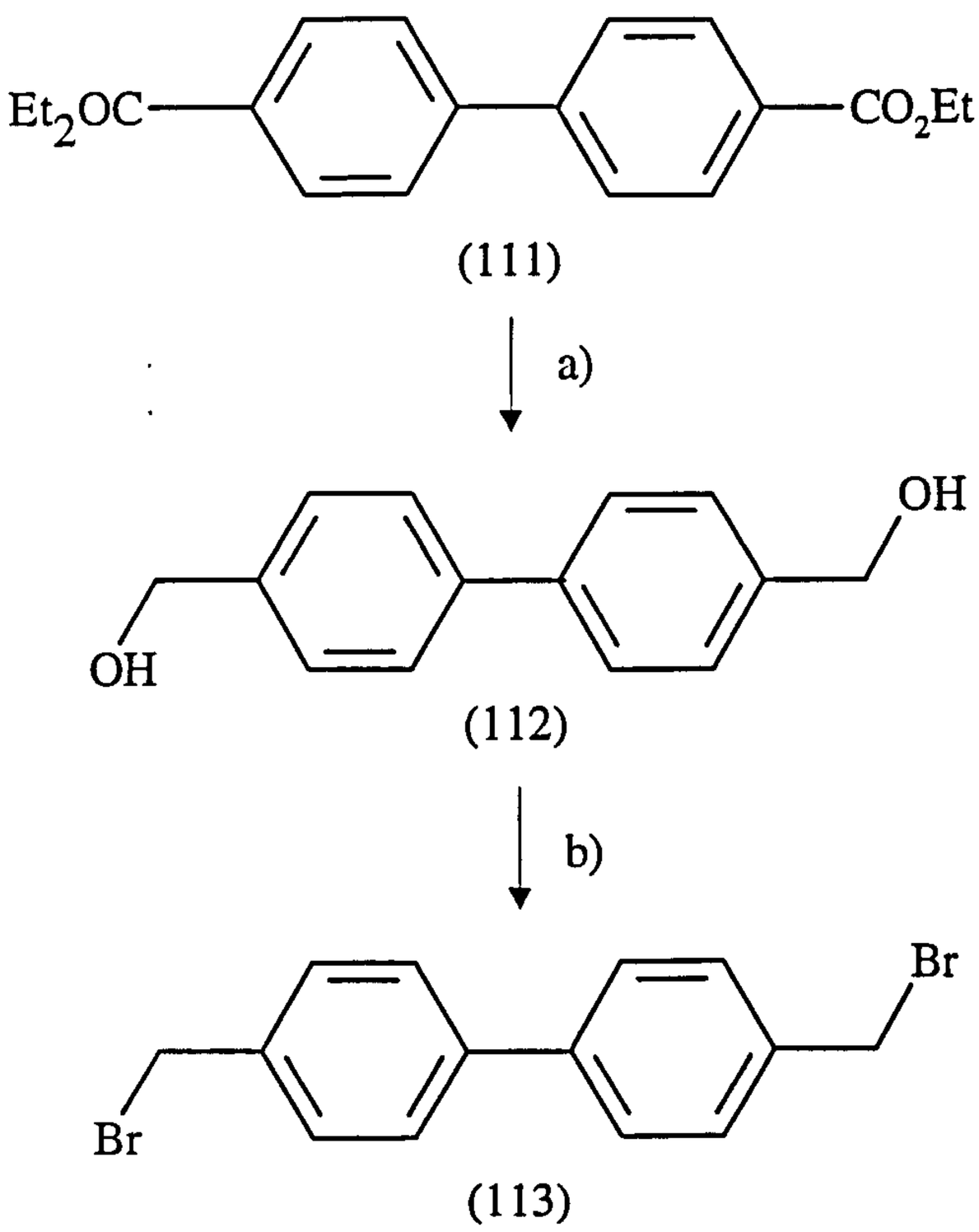
DCC 63 % EDC 67 %

2.3.4 Synthesis of crosslinked tripeptide

2.3.4.1 Crosslinking with 4,4'-bis-bromomethylbiphenyl

In order to form the crosslinked tripeptide (110) it was first necessary to synthesise the crosslinker 4,4'-bis-bromomethylbiphenyl (113). This was achieved as shown in Scheme 2.17.

Scheme 2.17 : Synthesis of 4,4'-bis-bromomethylbiphenyl (113)



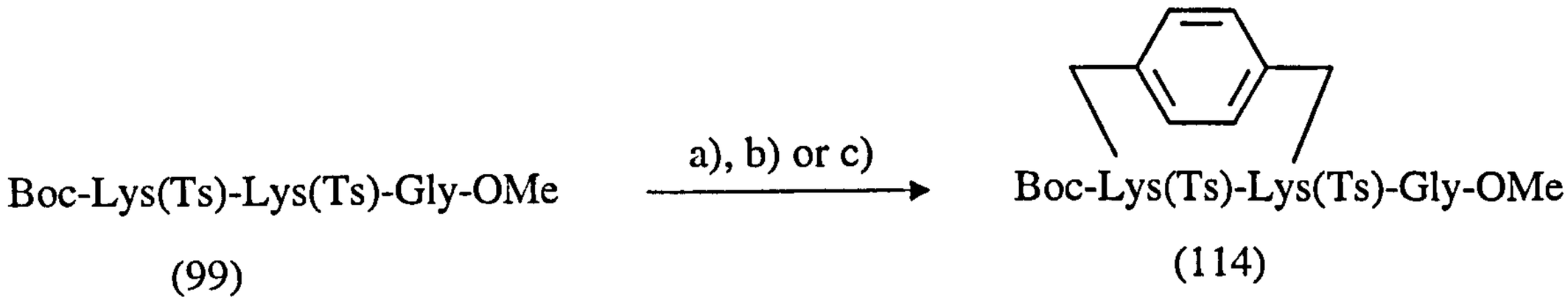
Following the treatment of diethyl-4,4'-biphenyldicarboxylate (111) with lithium aluminium hydride in dry THF, a quantitative yield of 4,4'-dihydroxymethylbiphenyl (112) was produced. Treatment of (112) with PPh_3 and CBr_4 under standard Mitsunobu conditions[162] led to a 75 % yield of (113) following column chromatography on silica gel using ethyl acetate : petroleum ether (40:60) as the eluant. The ^1H NMR spectrum of

(113), showed two doublets centred at $\delta = 7.46$ and $\delta = 7.55$, each with the same coupling constant of 8.24 Hz. Together with a CH_2Br singlet at $\delta = 4.54$, the spectrum was as expected[120]. FAB mass spectrometry showed the anticipated molecular ions at m/z = 338, 340, 342 in a 1:2:1 intensity pattern. This pattern is due to there being two equally abundant ^{79}Br and ^{81}Br isotopes.

The conditions initially used to crosslink tripeptide (99) were based upon experience gained by earlier crosslinking reactions within the group[120,121]. The solvent used was DMF, the base was Cs_2CO_3 and all reactions were performed under rigorously dry conditions. The Cs^+ ion was thought to act as a template for cyclisation[106-108] and the dry conditions were required to prevent displacement of leaving groups on the crosslinkers by OH^-. In the first attempt at this crosslinking reaction, the crosslinker was added slowly using a syringe pump to a solution of the tripeptide and base in dry DMF under argon. Unfortunately, none of the desired product (100) was detected. A second attempt at this reaction under analogous conditions gave evidence of the correct material in the FAB mass spectrum of the crude reaction mixture in that peaks at 1064 $[M + Cs]^+$, 932 $[M + H]^+$, 832 $[M - Boc]^+$ and 776 $[M - Ts]^+$ were prominent. However, evidence for this compound was lost following silica gel column chromatography using 5-20 % methanol in dichloromethane as the eluant.

In order to ascertain the most suitable model conditions for the crosslinking of aromatic bromomethyl compounds onto the tripeptide (99), 1,4-α,α'-dibromo-*p*-xylene was added using a syringe pump to stirred tripeptide and Cs_2CO_3 in DMF, both with and without 3 Å molecular sieves. This reaction is illustrated in Scheme 2.18.

Scheme 2.18 : Synthesis of *p*-xylyl crosslinked tripeptide (114)



a) 1,4-α,α'-dibromo-*p*-xylene, Cs_2CO_3 (5 mol. equiv.), DMF, 3 Å molecular sieves

b) 1,4-α,α'-dibromo-*p*-xylene, Cs_2CO_3 (5 mol. equiv.), DMF

c) 1,4-α,α'-dibromo-*p*-xylene, Cs_2CO_3 (5 mol. equiv.), DMF, H_2O (ca. 35 mol. equiv.)

FAB mass spectrometry showed that when the reaction was carried out in dry conditions in the presence of molecular sieves (method a)), the required *p*-xylyl crosslinked tripeptide (114) was successfully synthesised. Thus, two relevant peaks, m/z = 855 corresponding to the molecular ion of (114) and m/z = 755 due to the loss of the t-butoxycarbonyl (Boc) protecting group from (114) were obtained. Unfortunately, however, following silica gel column chromatography (9:1 dichloromethane : methanol), only a 2 % yield of (114) was isolated. Conversely, when the reaction was run without molecular sieves (method b)), the absence of these peaks in the FAB mass spectrum suggested that none of the desired product was formed. The absence of a common spot in the TLCs of each reaction mixture (in which 19:1 dichloromethane : methanol was used as the eluant) and the co-incidence of a TLC spot from the former reaction with authentic (114), produced by a previous group member[120] confirmed the FAB mass spectrometry results.

Because the use of molecular sieves in the crosslinking of (99) with 1,4-α,α'-dibromo-*p*-xylene had been shown to be marginally more successful than the equivalent reaction without molecular sieves, an attempt was made to crosslink (99) with 4,4'-bis-bromomethylbiphenyl (113) also with the use of molecular sieves. Unfortunately, however, FAB mass spectrometry showed that this reaction was unsuccessful and that instead the starting tripeptide (99) had been recovered. Peaks at m/z = 886 due to $[M + Cs]^+$, m/z = 754 due to $[M + H]^+$ and m/z = 654 from $[M - Boc]^+$ were prominent. Caesium iodide was added in two later reactions at room temperature in order to catalyse the nucleophilic

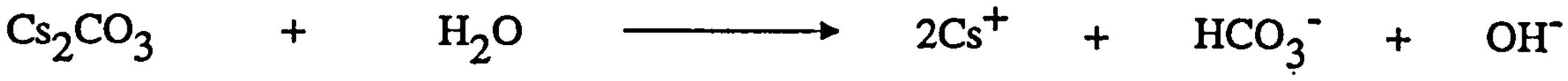
displacement of the bromide leaving groups and thus aid the cyclisation. Evidence of the required product (100) in the FAB mass spectrum came from a peak at m/z = 932 due to $[M + H]^+$ and m/z = 832 due to $[M - Boc]^+$. However, starting material was also still present as shown by a peak at m/z = 654. Two dominant spots were observed by TLC using 19:1 dichloromethane : methanol surrounded by other fainter, closely running, uncharacterised impurities. One of the dominant spots, at $R_f = 0.30$, co-ran with authentic starting tripeptide (99). The other spot ran at $R_f = 0.35$, in a similar location to the *p*-xylyl crosslinked tripeptide (114), as would be expected from their related polarities. It was thus assumed that the latter spot was due to the crosslinked tripeptide (100). Silica gel column chromatography using 5-20 % methanol in dichloromethane was used to attempt to isolate the desired product, but this was unsuccessful.

It was thought that a possible reason why the reaction was not going to completion was that once one end of the crosslinker (113) had reacted with one of the lysyl side chains, there was a high energetic barrier for the tripeptide to be orientated into the correct conformation for ring closure. Although this would not explain the reason for the presence of starting material, such a situation would increase the number of by-products.

Thus, a new caesium iodide catalysed reaction was heated to 65^0 C in an attempt to overcome any such energetic barriers and accelerate the ring closure reactions. Unfortunately, none of the desired product was shown to be produced by the FAB mass spectrum of the crude product. The attempted purification by silica gel column chromatography was again unsuccessful and FAB mass spectrometry of the impure fractions produced also showed no evidence either of the desired product or of starting material. The use of analogous conditions at room temperature, with a catalytic amount of caesium iodide and in which acetonitrile was used as solvent instead of DMF, was likewise shown by FAB mass spectrometry to be unsuccessful. However, the spectrum of the crude product did show peaks at m/z = 872 and m/z = 1004, peaks which were also to be observed later in the crosslinking of tripeptide (99) with 1,2-bis(2-chloroethoxy)ethane, described in Section 2.3.4.2. Although neither of these peaks were ever unambiguously assigned, it is pertinent to note that the difference between them - 132 - is also the molecular mass of caesium. Following reactions with peptides in which caesium carbonate was present, as was the case in all of the above reactions, it was frequently the case that a mass ion plus caesium was observed. It is thus possible that these peaks at m/z = 872 and m/z = 1004 are from the same species.

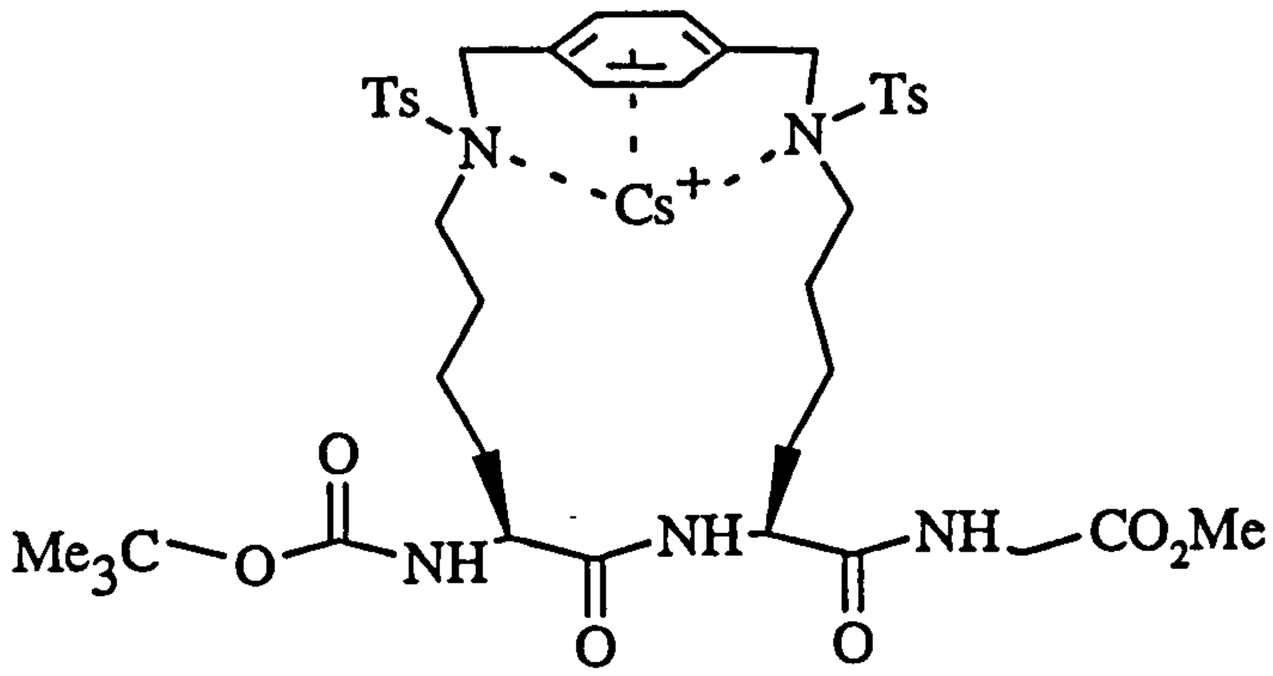
Previously, as explained above, it was thought that the presence of water would discourage the desired crosslinking reaction from occurring. However, it was found from other crosslinking reactions (Section 2.3.4.2) that the presence of a small amount of water catalysed crosslinking reactions. As shown by the equation in Scheme 2.19, it was assumed that the presence of water encouraged the formation of stronger basic conditions thus facilitating nucleophilic reactions between tripeptide and crosslinker. However, it was thought that the addition of too much water would produce a basic solution of such strength as to hydrolyse the methyl ester of tripeptide (99). In such a circumstance, the isolated yield of crosslinked tripeptide (100) would be reduced. Thus it was assumed that only a small amount of water was required to be added to the crosslinking reactions.

Scheme 2.19 : Reaction of caesium carbonate with water



The effect played by the addition of water to a crosslinking reaction was explored in the reaction of tripeptide (99) with 1,4-α,α'-dibromo-*p*-xylene. This is illustrated in Scheme 2.18 as method c). In order that the quantity of water present in the reaction flask was known, it was felt to be necessary to ensure that prior to the addition of the dry reagents, all glassware employed was thoroughly dried. Thus to a flame dried reaction flask, dried caesium carbonate and tripeptide (99) were added to dry DMF under argon. One drop of water (ca. 35 mol. equiv.) was added as an arbitrary but known quantity followed by the addition of 1,4-α,α'-dibromo-*p*-xylene. In a further change to previous experiments, it was decided to add the crosslinker in one batch rather than slowly using a syringe pump. Previously, it had been thought that with a low concentration of crosslinker with respect to tripeptide, the rate of cyclisation would be increased over the rate of polymer formation. However, it was now thought possible that the caesium from the caesium carbonate might act as a template encouraging cyclisation (Fig 2.1)[106-108]. If this were the case, then a syringe pump was not thought to be required. Following these new reaction conditions and after column chromatography (1 - 10 % methanol in dichloromethane), a 40.5 % yield of the desired product (114) was achieved, higher than the 35 % best quoted yield of (114) achieved by a previous group member[120].

Fig 2.1 : Possible role of caesium as a template for the formation of crosslinked tripeptide (114)



Similarly, ca. 35 mol. equiv. of water were added to the crosslinking reactions involving 4,4'-bis-bromomethylbiphenyl (113), tripeptide (99) and caesium carbonate in DMF. In order to test the effect of the rate of addition of crosslinker on the yield of the desired product, the crosslinker was added under three different conditions; slowly over 24 h using a syringe pump, adding the crosslinker in stages or adding all the crosslinker in one batch at the start of the reaction. In each case an excess of crosslinker was used in order to drive the reaction to completion. In a reaction involving the addition of crosslinker (113) and tripeptide (99) in DMF by syringe pump to a suspension of Cs_2CO_3 in DMF with ca. 35 mol. equiv. of water, evidence that the desired product (100) had been synthesised came from FAB mass spectrometry in which a peak at m/z = 832 indicated a fragment ion involving the loss of the Boc group from (100). Comparison of the integrals of the aromatic protons (16H) against those of the methyl ester (3H) and the methyls of the tosyl groups (6H) in the 80 MHz ^{1}H NMR spectrum, gave additional evidence of the desired product. Although for the other related attempted crosslinking reactions involving the addition of water, molecular ions or recognisable fragment ions of the desired product were not observed in the FAB mass spectra, 80 MHz ^{1}H NMR spectroscopy indicated the presence of the desired product in all the cases described above. However, at no time could it be purified by silica gel column chromatography, due to the presence of a large number of unidentified by-products which TLC showed ran very closely to the desired product.

In an attempt to overcome any energetic barriers to cyclisation and thus achieve a cleaner product (100), the crosslinker was added in aliquots to tripeptide, DMF and Cs_2CO_3 and a trace amount of water at 50^0 C. However, FAB mass spectrometry showed that no product (100) was formed. Similarly, the room temperature addition of crosslinker in aliquots to tripeptide (99), Cs_2CO_3 and a trace amount of water in acetonitrile failed to produce the desired product.

The failure of these biphenyl crosslinking reactions to produce the desired compound (100) cleanly compared with the success of the crosslinking of 1,4-α,α'-dibromo-*p*-xylene is notable. It is possible that the biphenyl moiety is too long and rigid to synthesise, cleanly and efficiently, a high yield of crosslinked tripeptide. Whilst one end of the biphenyl group would react with the ϵ-nitrogen of a lysyl side chain, the formation of the correct conformation for cyclisation would involve considerable strain in the tripeptide molecule. Thus, the rate of cyclisation would be reduced allowing competing straight chain polymer forming reactions to predominate. The presence of a large quantity of baseline material in the TLCs of the crude reaction material is evidence for the presence of polymers.

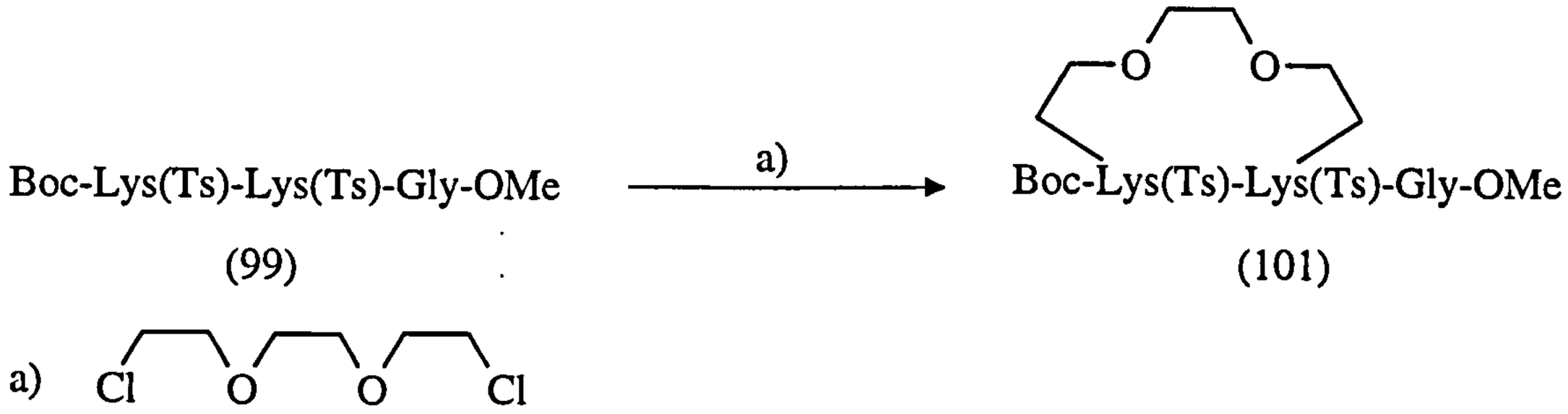
2.3.4.2 Synthesis of a 1,2-bis(2-ethoxy)ethane crosslinked tripeptide

At the same time that the first attempts were being made to synthesise the 4,4'-bis-bromomethylbiphenyl crosslinked tripeptide (100), experiments were started with a view to synthesising the 1,2-bis(2-ethoxy)ethane crosslinked tripeptide (101). Thus a solution of 1,2-bis(2-chloroethoxy)ethane was added by syringe pump to a solution of tripeptide (99) and Cs_2CO_3 in DMF. As with the attempted crosslinking of the biphenyl moiety, the conditions used were based upon previous experience within the group[120,121]. Unfortunately, no peaks corresponding to the desired tripeptide (101) were detected by FAB mass spectrometry.

Despite the fact that the addition of water was shown in Section 2.3.4.1 to encourage the crosslinking of 1,4-α,α'-dibromo-*p*-xylene with tripeptide (99), at the time when the first attempts were made to synthesise a 1,2-bis(2-ethoxy)ethane crosslinked tripeptide, it was thought that the presence of water caused the displacement of the leaving groups on the crosslinker and thus prevented efficient and successful crosslinking. Thus a reaction in which molecular sieves were added to the solution was attempted (Scheme 2.20). The FAB mass spectrum of the crude product indicated the presence of the desired crosslinked

tripeptide (101) by a peak at m/z = 868, due to $[M + H]^+$. However starting material (99) was also shown to be present by the presence of a peak at m/z = 654 due to the loss of Boc from its molecular ion. The desired product (101) was successfully separated from the crosslinked tripeptide starting material by silica gel column chromatography (19:1 dichloromethane : methanol). However, attempts to separate (101) from other uncharacterised impurities, which TLC showed to be closely running to the desired product, were unsuccessful. The FAB mass spectrum, despite showing an intense amount of background noise and a large number of peaks due to uncharacterised impurities, gave distinct peaks due to (101) which are tabulated and assigned in Table 2.2.

Scheme 2.20 : Reaction of tripeptide (99) with 1,2-bis(2-chloroethoxy)ethane



3 Å molecular sieves, Cs_2CO_3, DMF

Table 2.2: FAB Mass Spectrum of the purified product (101) from the reaction in Scheme 2.20.

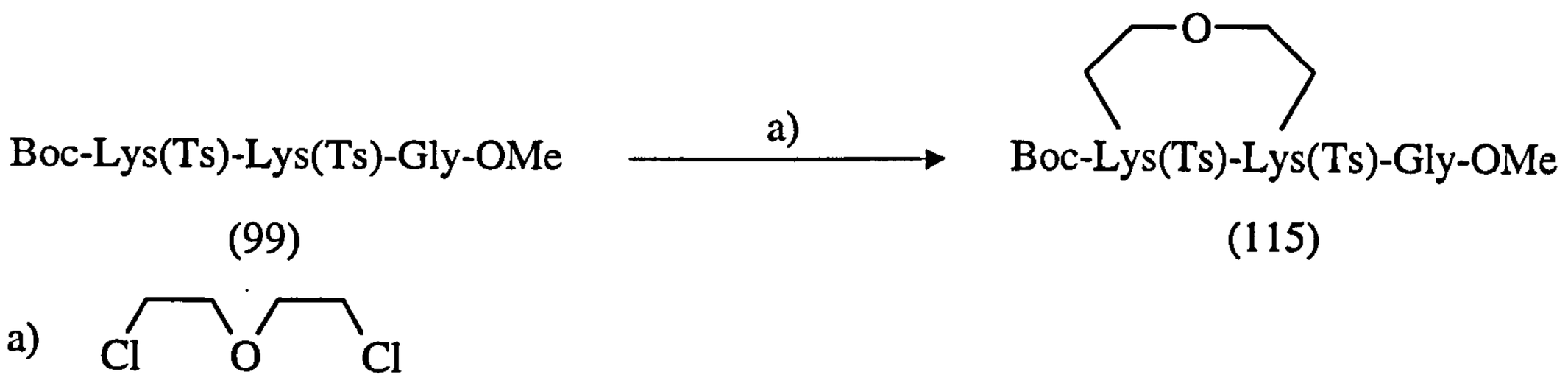
m/z	Assignment
1000	$[M + Cs]^+$
768	$[M - Boc]^+$
711	$[M - Ts]^+$
611	$[M - Boc - Ts]^+$

As explained in Section 2.3.4.1, it was thought that by increasing the rate of the cyclisation reaction, tripeptide (99) would react more efficiently with the crosslinker and the number of side products would be reduced. However, an attempt to increase the rate of any cyclisation reaction by heating the mixture to 65^0 C failed. Indeed, FAB mass spectrometry of the crude product failed to show any peaks that could be assigned to the desired products. High mass peaks at m/z = 1004 and m/z = 872 were observed (as they had also been observed in a reaction to make the crosslinked tripeptide (100) - see Section 2.3.4.1). Both these peaks were possibly due to the same unknown compound since the difference between them - 132 - is the same value as the Cs^+ ion. As was seen in Table 2.2, peaks assigned as $[M + Cs]^+$ were sometimes observed in the crude reaction products when Cs_2CO_3 was involved in the reaction. However, whilst this suggests that the m/z = 872 peak is the molecular ion of a compound, its structure could not be determined and neither peak could be assigned. If this peak were peptidic, one might expect a peak at m/z = 772 due to the loss of the Boc protecting group. This, however, was not observed. An attempted catalysis of the crosslinking using sodium iodide also failed to produce the required product. Instead, FAB mass spectrometry following silica gel column chromatography showed peaks at m/z = 776 (starting material plus sodium) and m/z = 654 (starting material minus Boc) suggesting that the starting material (99) had been recovered. A possible reason for the failure of this reaction was that sodium competed with any template effect of caesium thus reducing the chance of any product (101) being produced.

In a further attempt to synthesise (101), acetonitrile was used as the solvent instead of DMF, molecular sieves and caesium carbonate were added and the reaction was heated to 65^0 C. The crosslinker was again added slowly using a syringe pump. However, this reaction was shown by FAB mass spectrometry to have failed. An attempt at room temperature catalysis of the crosslinking in acetonitrile using caesium iodide also failed. By using caesium iodide rather than sodium iodide, the problem of sodium competing with the template effect of caesium should have been overcome. However, in the FAB mass spectra of the crude samples from both reactions, the peaks observed in previous reactions at m/z = 872 and m/z = 1004 were again prominent. In the case of the latter reaction, m/z = 872 was still present following silica gel column chromatography. An attempt to obtain an accurate mass of the m/z = 872 peak, with a view to characterisation, was, however, unsuccessful, due to the small size of the peak in the FAB mass spectrum of the columned material.

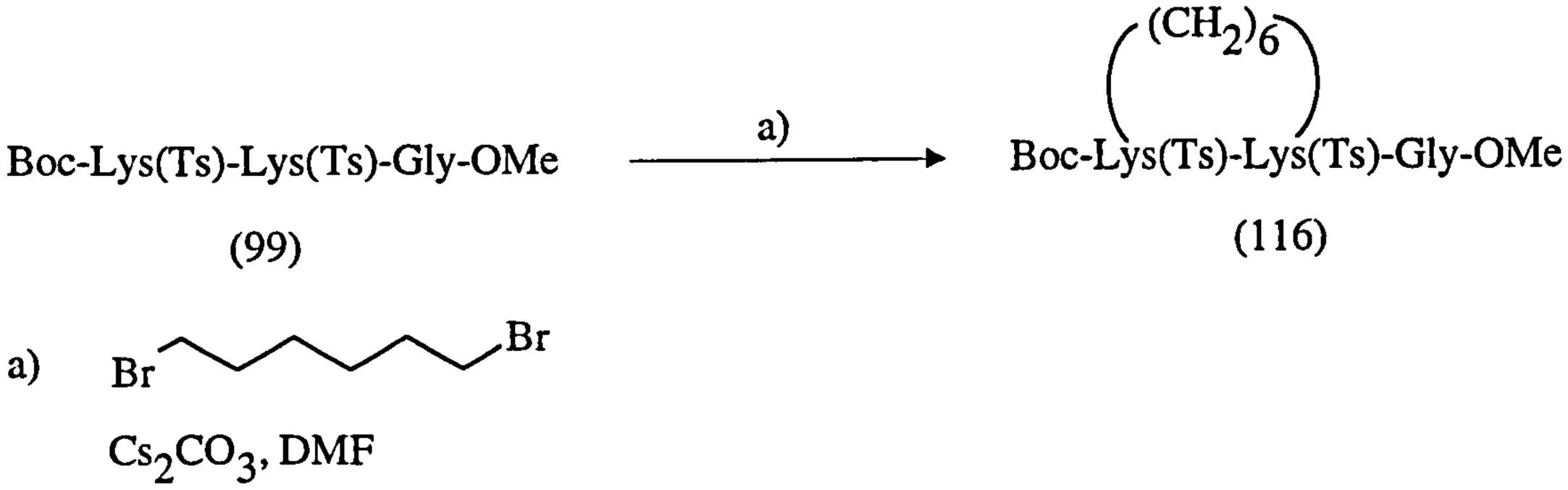
It was argued that 1,2-bis(2-chloroethoxy)ethane could be potentially too long for efficient ring closure. Its length and flexibility were possibly so great as to make the entropy cost on cyclisation prohibitive, ie., it had low preorganisation for binding[1]. Consequently, in order to test this theory, the shorter crosslinker, 2-chloroethyl ether was used in DMF together with caesium iodide and molecular sieves (Scheme 2.21). This reaction was carried out at 65^0 C in order to increase the rate of nucleophilic displacement of the Cl^- leaving groups and thus hopefully encourage cyclisation. FAB mass spectrometry of the crude product did not, however, provide any evidence of the desired product (115). Peaks at m/z = 824 (corresponding to the molecular ion plus hydrogen and m/z = 724 (molecular ion minus the t-butoxycarbonyl protecting group) were expected but were not observed. High mass peaks at m/z = 1136, 1004, 904, 872 and 771 were present but were not assigned.

Scheme 2.21 : Reaction of tripeptide with 2-chloroethyl ether



In order to model the desired crosslinking reaction, 1,6-dibromohexane was added by syringe pump to the tripeptide (99), in which as before, caesium carbonate was used as base and molecular sieves were used to ensure dryness. This reaction is shown in Scheme 2.22.

Scheme 2.22 : Reaction of tripeptide with 1,6-dibromohexane

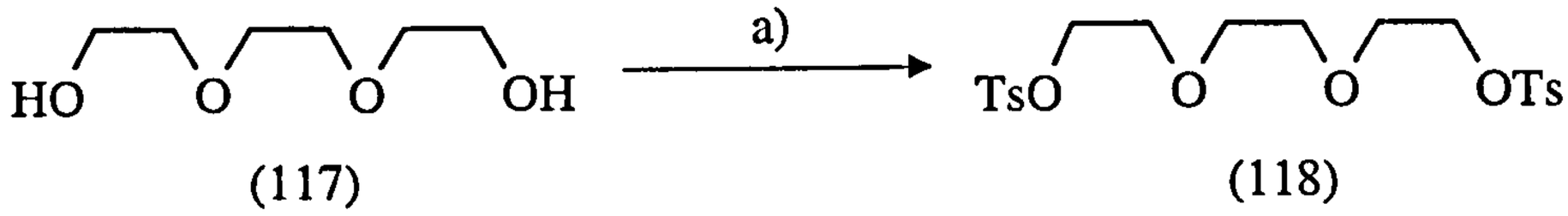


Unfortunately, FAB mass spectrometry showed that this reaction had been unsuccessful. However, as explained in Section 2.3.4.1, it was then thought that the addition of water to the crosslinking reaction would encourage efficient and clean formation of the desired crosslinked tripeptide. Thus following analogous conditions to the crosslinkings using 1,4-α,α'-dibromo-*p*-xylene and 4,4'-bis-bromomethylbiphenyl, one drop or ca. 35 mole equivalents (with respect to tripeptide) of water were added to dried Cs_2CO_3 and dry tripeptide (99) in dry DMF. The 1,6-dibromohexane crosslinker in DMF was added by syringe pump over a period of 48 h. The addition of water was, as in the case of the formation of the *p*-xylyl crosslinked tripeptide (114), found to be critical to the success of the reaction and following silica gel column chromatography using 19:1 dichloromethane : methanol as the eluant, a 45 % yield of the desired hexyl crosslinked tripeptide was obtained. The FAB mass spectrum showed peaks at m/z = 858, 836 and 736 corresponding to the molecular ion plus sodium, the molecular ion plus hydrogen and the molecular ion minus the Boc protecting group, respectively. The accurate mass of the $[M + H]^+$ ion (m/z = 836) was consistent with that expected. The ^1H NMR spectrum is illustrated in Fig 2.2. Singlets due to the Boc protecting group (δ = 1.253), the tosyl methyls (δ = 2.391, 2.412) and the methyl ester (δ = 3.671) are clearly present. Two doublets from each of the *para* substituted tosyl groups are also observed (centred at δ = 7.287, 7.638). Alkyl CH_2 protons from the lysine side chains and the alkyl crosslinker are also observed as a broad multiplet (δ = 1.2, 2.1). It would be expected that for this crosslinked tripeptide (116), the respective integrations of the combined Boc and alkyl peaks against those for the tosyl

aromatics, methyl ester and tosyl methyls would be 29H to 8H to 3H to 6H respectively. This indeed is found to be the case. The ^{13}C NMR was likewise as expected with the correct number of each kind of carbon expected, present. Thus, for example, 15 CH_2 peaks would be expected from the lysyl side chains, the alkyl crosslinker (δ = 20 - 32) and the glycine residue (δ = 41). Four peaks due to CH_2 adjacent to a nitrogen would also be anticipated (δ = 46 - 50). All these peaks are observed.

Before these reaction conditions were applied to the synthesis of a 1,2-bis(2-ethoxy)ethane crosslinked tripeptide (101), a new crosslinker, 1,2-bis(2-tosylethoxy)ethane (118) was synthesised. Following treatment of triethylene glycol with tosyl chloride, triethylamine and catalytic amounts of 4-N,N-dimethylaminopyridine (DMAP) in dichloromethane[163] a 79 % yield of 1,2-bis(2-tosylethoxy) ethane (118) was achieved (Scheme 2.23).

Scheme 2.23 : Synthesis of 1,2-bis(2-tosylethoxy)ethane (118)



(117) (118)

a) tosyl chloride, NEt_3, DMAP, dichloromethane

It was considered that instead of using a crosslinker with Cl^- leaving groups, the use of tosyl leaving groups might be more suitable. Due to their greater ability to delocalise and thus stabilise negative charge following their nucleophilic displacement, tosyl groups are better leaving groups than Cl^-. It was thus hoped that the nucleophilic substitution reactions involved in the crosslinking of the tripeptides would be more efficient using a ditosylated crosslinker rather than the dichloride. The use of a catalytic amount of water in the crosslinking of tripeptide (99) with 1,2-bis(2-tosylethoxy)ethane (118) again using Cs_2CO_3 in DMF gave a consistent 45 % yield of the desired tripeptide (101) in two separate reactions. As with the successful 1,6-dibromohexane crosslinking reaction described above, crosslinker (118) in DMF was added by syringe pump to the basic suspension of Cs_2CO_3 and solution of tripeptide (99) in DMF and water. Table 2.3 lists the high mass peaks in the FAB mass spectrum of the crude material.

Table 2.3: High mass peaks in the FAB Mass Spectrum of the unoptimised crosslinking of tripeptide (99) with 1,2-bis(2-tosylethoxy)ethane (118).

m/z	**Assignment**
940	unknown
868	$[(101) + H]^+$
768	$[(101) - Boc]^+$
711	$[(101) - Ts]^+$
654	$[\text{starting tripeptide (99)} - Boc]^+$

The TLC of the crude product from the reaction in DMF (for which the FAB mass spectrum was tabulated in Table 2.3), showed two closely running spots corresponding to starting material and the desired product. The presence of starting material on the TLC plate was shown by the co-running of authentic starting material. Although TLC evidence and the production of a clean ^1H NMR spectrum of the desired product showed that successful separation of the product and starting material had been achieved, it was clearly important to ensure reaction of all of the starting material. Consequently, a two-fold excess of the crosslinker 1,2-bis(2-tosylethoxy)ethane (118) was added. In a further change to the reaction conditions, it was decided that addition of the crosslinker should be in three stages without the use of a syringe pump to a mixture of tripeptide and caesium carbonate in DMF and catalytic amounts of water. Previously, it had been thought that these crosslinking reactions would give higher yields if the crosslinker was added slowly to the tripeptide and base. By this means, it had been thought that with a low concentration of crosslinker with respect to tripeptide, the rate of cyclisation would be increased over the rate of polymer formation. However, as with the *p*-xylyl crosslinked tripeptide (114) it was later thought possible that the caesium carbonate might act as a template encouraging cyclisation (Fig 2.3)[106-108]. If this were the case, then a syringe pump was not thought to be required. These were found to be the optimal conditions for the synthesis of (101). No tripeptide starting material was observed in the crude reaction product by TLC or by FAB mass spectrometry. Following silica gel column chromatography using 5-20 % methanol in dichloromethane, a yield of 54 % of the desired crosslinked tripeptide (101) was isolated. In a later reaction, a yield of 62 % was achieved. The FAB mass spectrum and a typical ^1H NMR spectrum

of the reaction product (101) are illustrated in Figs 2.4 and 2.5. The peaks in the FAB mass spectrum are tabulated in Table 2.4. The ^1H NMR spectrum of crosslinked tripeptide (101) shows a broad multiplet (δ = 1.2 - 1.9) due to the lysyl CH_2 groups as well as singlets due to Boc (δ = 1.293), tosyl (δ = 2.428, 2.447) and the methyl ester (δ = 3.711). Two sets of two superimposed doublets due to the two *para* substituted tosyl groups are also present (centred at δ = 7.32 and δ = 7.70). A broad multiplet (δ = 3.2 - 3.45) due to CH_2 groups next to nitrogen is present as well as a multiplet (δ = 3.5 - 3.8) due to the crosslinker protons (CH_2O). The integration of this CH_2O multiplet is 8H, corresponding, as expected, to the 8H of the tosyl aromatics, the 3H of the methyl ester, the 6H of the tosyl methyls and the combined 21H of the lysyl CH_2s and the Boc group.

Fig 2.3 : Possible role of caesium as a template for the formation of crosslinked tripeptide (101)

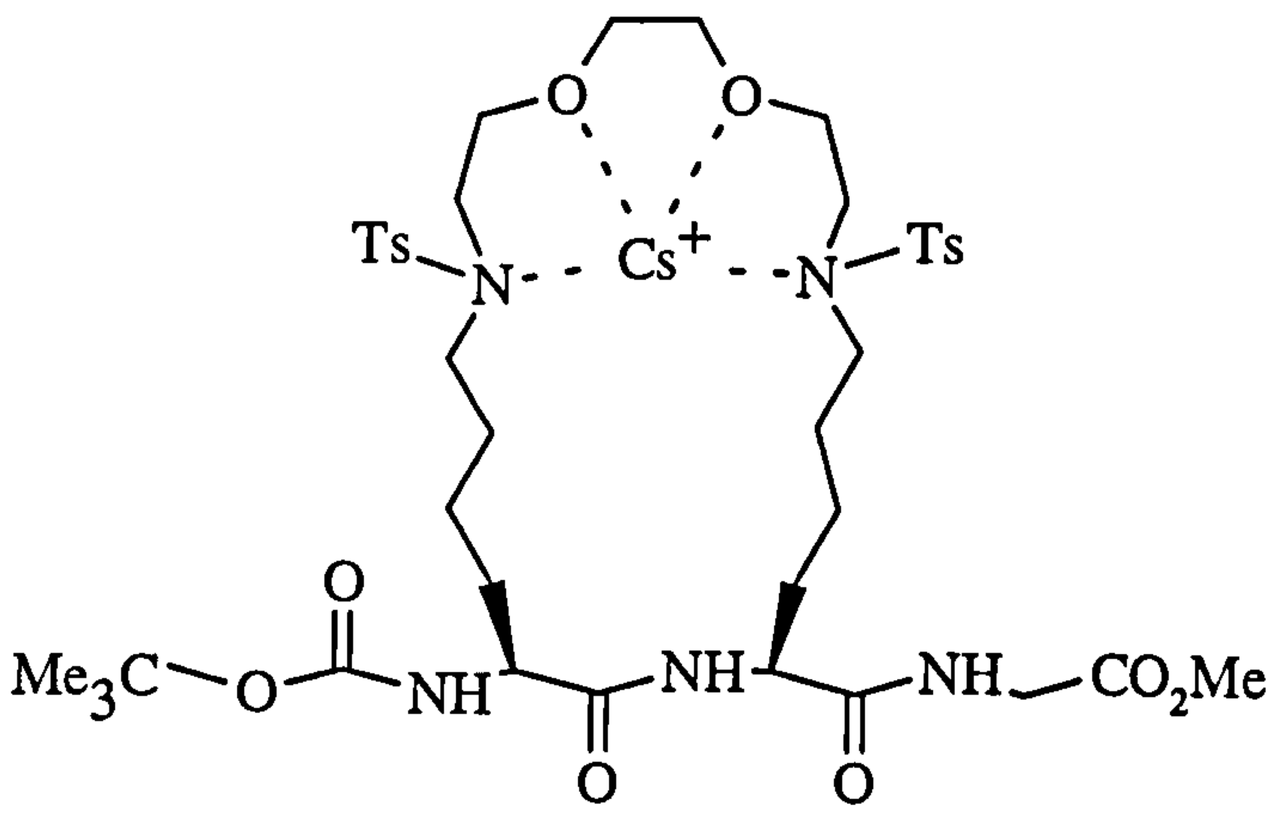


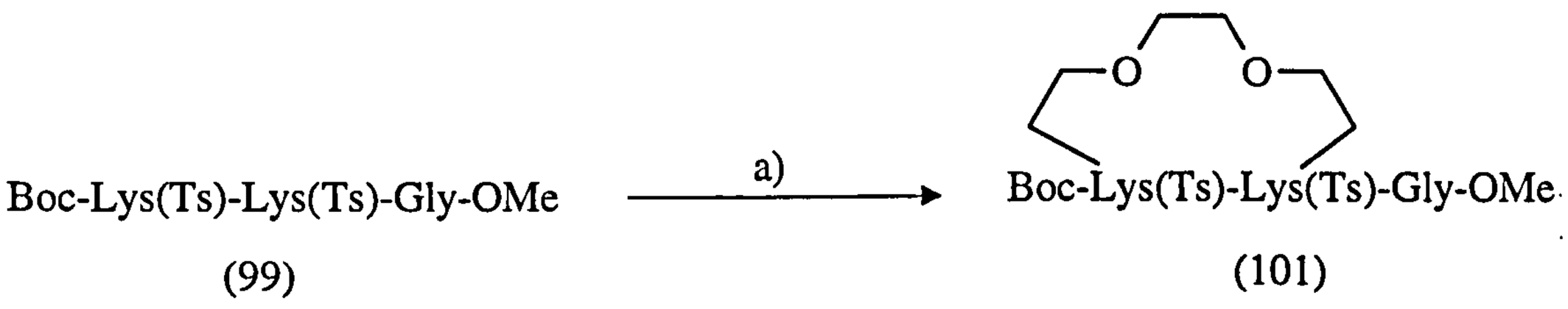
Table 2.4 : High mass peaks in the FAB mass spectrum shown in Fig 2.4 of the optimised crosslinking of tripeptide (99) with 1,2-bis(2-tosylethoxy)ethane (118)

m/z	**Assignment**
890	$[(101) + Na]^+$
868	$[(101) + H]^+$
768	$[(101) - Boc]^+$
712	$[(101) - Ts]^+$
651	unknown
612	$[(101) - Boc - Ts]^+$

The use of acetonitrile as solvent instead of DMF was shown by TLC and FAB mass spectrometry to result in a failure to produce (101). The TLC of the crude reaction product using 19:1 dichloromethane : methanol as eluant, showed that the majority of the material did not move from the baseline. It is thus possible that mainly polymeric material had been formed.

Following the scale up of the reaction in DMF, such that 5 g of tripeptide (99) was crosslinked in one batch, yields of 50-60 % were maintained. The conditions found optimal for the formation of crosslinked tripeptide (101) are shown in Scheme 2.24. Thus, with the successful synthesis of glycol crosslinked tripeptide (101), the synthesis of target polycyclic peptides (79) and (80) could proceed. This is explained in Chapter 3.

Scheme 2.24 : Optimised conditions for the synthesis of the 1,2-bis(2-ethoxy)ethane crosslinked tripeptide (101)



(99) (101)

a) Batchwise addition of $TsO[(CH_2)_2O]_2(CH_2)_2OTs$ (1 mol. equiv. initially; after 24 h, 1/2 mol. equiv. and after another 24 h, 1/2 mol. equiv.)

Cs_2CO_3 (5 mol. equiv.),

H_2O (ca. 35 mol. equiv.), DMF [to give 0.01 M concentration of (99)], RT

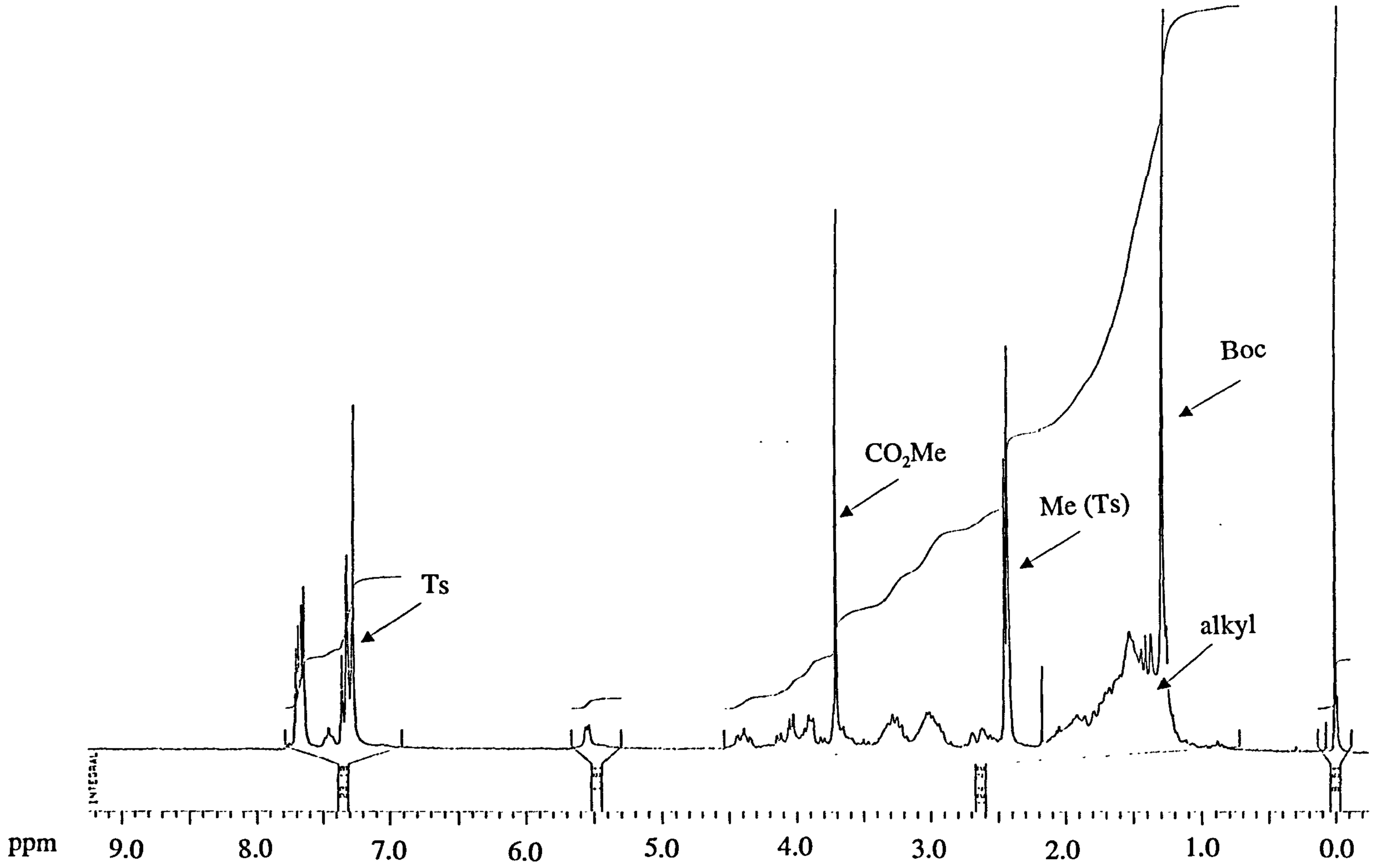


Fig 2.2 : 200 MHz ^1H NMR spectrum of hexyl crosslinked tripeptide (116) in $CDCl_3$

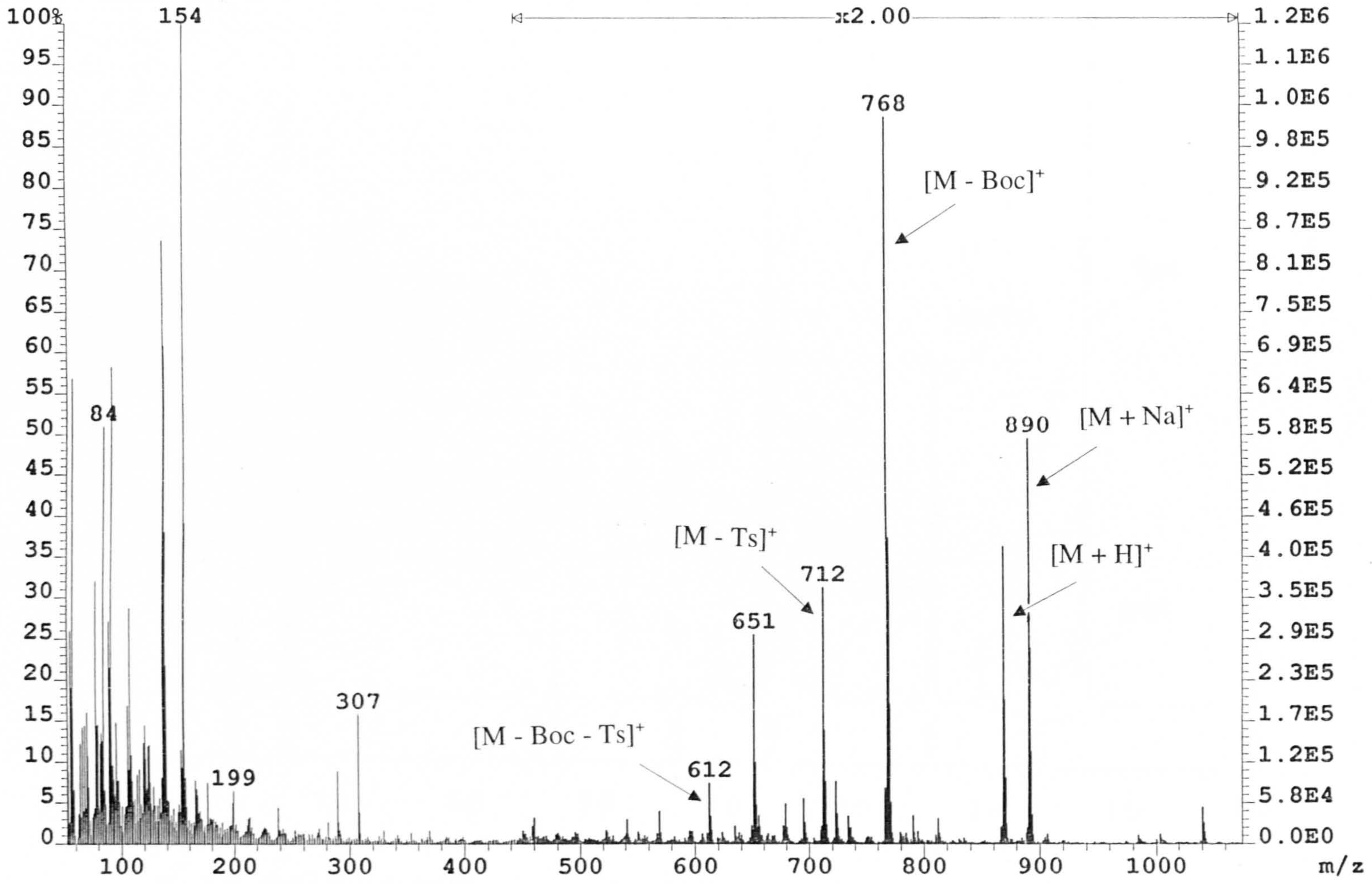


Fig 2.4 : FAB mass spectrum of 1,2-bis(2-ethoxy)ethane crosslinked tripeptide (101)

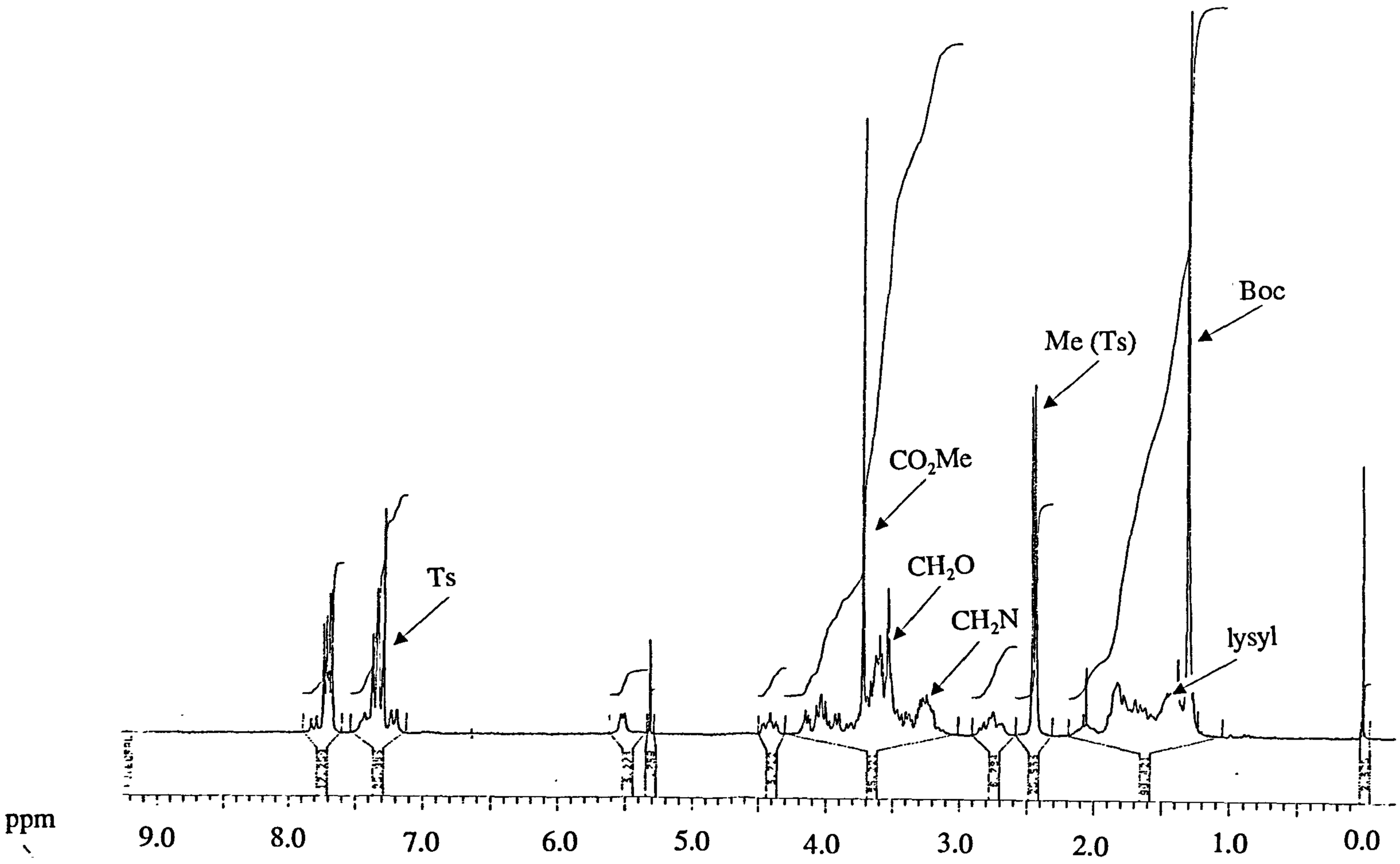


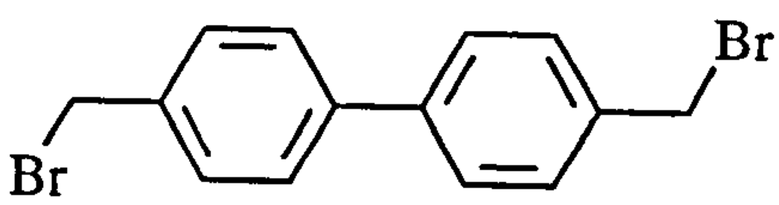
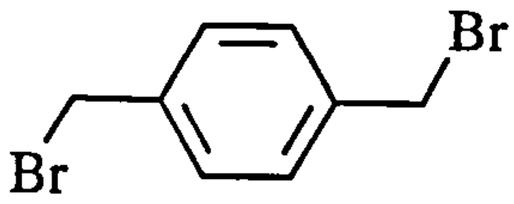
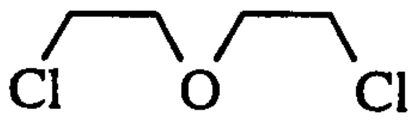
Fig 2.5 : 200 MHz ^1H NMR spectrum of 1,2-bis(2-ethoxy)ethane crosslinked tripeptide (101) in $CDCl_3$

2.4 Summary

The tripeptide (99) was successfully synthesised by solution phase methodology. Tables 2.5 and 2.6 provide a summary of the results from the various crosslinking experiments described above. Evidence from FAB mass spectrometry and ^{1}H NMR spectroscopy indicated that in the presence of catalytic amounts of water, the crosslinking of the tripeptide (99) with 4,4'-bis-bromomethylbiphenyl (113) was successful. The use of a syringe pump or addition of (113) in aliquots all gave evidence of the formation of the desired product (100). However, purification of the desired product (100) by column chromatography was unsuccessful due to the presence of a large quantity of uncharacterised reaction by-products which were found to run closely to (100) in the column TLC. Crosslinking with 1,4-α,α'-dibromo-*p*-xylene was more successfully achieved but again required the presence of catalytic amounts of water. Isolation of the desired product (114) was achieved to give a yield of 40.5 %. Only in one reaction was the crosslinking with 1,2-bis(2-chloroethoxy)ethane successful, however, in this case, the presence of closely running uncharacterised by-products prevented the isolation of crosslinked tripeptide (101). Two possible reasons for this lack of success are that the chlorides are poor leaving groups and in all these reactions care was taken to ensure that a minimal amount of water was present. Crosslinking with 2-chloroethyl ether in these conditions was also unsuccessful. However, syringe pump addition of 1,6-dibromohexane to tripeptide (99) in the presence of catalytic amounts of water, gave a 45 % yield of crosslinked tripeptide (116). Addition of 1,2-bis(2-tosylethoxy)ethane by syringe pump to tripeptide (99) in the presence of catalytic amounts of water gave a 45 % yield of (101), whereas addition in aliquots gave the optimised yield of 62 %, following silica gel column chromatography.

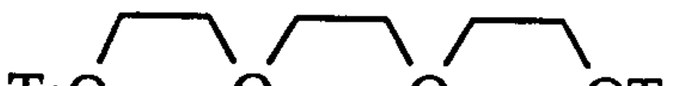
In conclusion, with the exception of the addition of the long rigid crosslinker 4,4'-bis-bromomethylbiphenyl (113), all crosslinkings in DMF in the presence of catalytic amounts of water were successful. Those in acetonitrile, or in dry conditions in DMF were unsuccessful.

Table 2.5 : Summary of results from the crosslinking of tripeptide (99) with various crosslinkers

Crosslinker	Solvent	Base	H_2O added?	Addition*	Result†
	DMF, RT	Cs_2CO_3	×	S	(✓)
	DMF, RT	Cs_2CO_3	mol. sieves	S	(✓)
	DMF, RT	Cs_2CO_3, CsI	mol. sieves	S	×
	DMF, (65^0 C)	Cs_2CO_3, CsI	mol. sieves	S	×
	CH_3CN, RT	Cs_2CO_3, CsI	mol. sieves	S	×
	DMF, RT	Cs_2CO_3	mol. sieves	S	×
	DMF, RT	Cs_2CO_3	✓	S	(✓)
	DMF, RT	Cs_2CO_3	✓	B	(✓)
	DMF, RT	Cs_2CO_3	✓	Aliq	(✓)
	CH_3CN, RT	Cs_2CO_3	✓	Aliq	×
	DMF, (50^0 C)	Cs_2CO_3	✓	Aliq	×
	DMF, RT	Cs_2CO_3	×	S	×
	DMF, RT	Cs_2CO_3	mol. sieves	S	✓ 2 %
	DMF, RT	Cs_2CO_3	✓	B	✓ 40.5 %
	DMF, (60^0 C)	Cs_2CO_3, CsI	mol. sieves	S	×

*† See bottom Table 2.6

Table 2.6 : Summary of results from the crosslinking of tripeptide (99) with additional crosslinkers

Crosslinker	Solvent	Base	H_2O added?	Addition*	Result	
	DMF, RT	Cs_2CO_3	×	S	×	
	DMF, RT	Cs_2CO_3	mol. sieves	S	(✓)	
	DMF, (65^0 C)	Cs_2CO_3	mol. sieves	S	×	
	DMF, RT	Cs_2CO_3, NaI	mol. sieves	S	×	
	CH_3CN, (65^0 C)	Cs_2CO_3	mol. sieves	S	×	
	CH_3CN	Cs_2CO_3, CsI	mol. sieves	S	×	
$Br(CH_2)_6Br$	DMF, RT	Cs_2CO_3	mol. sieves	S	×	
	DMF, RT	Cs_2CO_3	✓	S	✓	45%
	DMF, RT	Cs_2CO_3	✓	S	✓	45 %
	CH_3CN, RT	Cs_2CO_3	✓	Aliq	×	
	DMF, RT	Cs_2CO_3	✓	Aliq	✓	62 %

* S = Addition of crosslinker by syringe pump
B = Addition of crosslinker in one batch
Aliq = Addition of crosslinker in aliquots

† × = reaction failed
(✓) = evidence of product but could not be purified
✓ = reaction successful

CHAPTER 3:

TOWARDS THE SYNTHESIS OF POLYCYCLIC PEPTIDE HOST MOLECULES

3. Towards the synthesis of polycyclic peptide host molecules

3.1 Hemerythrin and hemocyanin

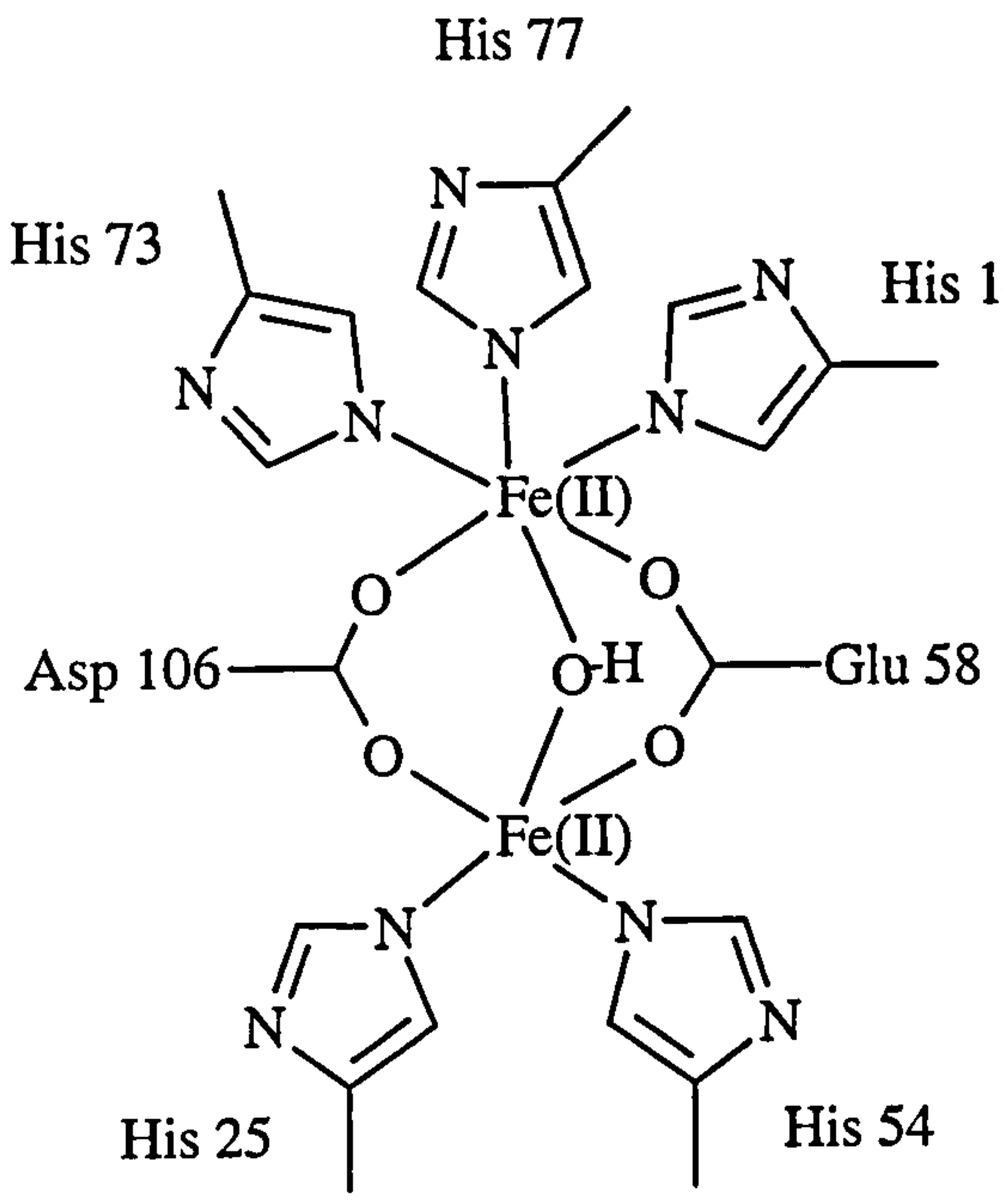
The transport of dioxygen around the body is a key biological process in multicellular organisms[164]. Three major classes of metalloproteins have evolved which are capable of performing this function by the reversible binding of oxygen; hemoglobins, hemerythrins and hemocyanins[122,123,164]. Hemoglobin, with its porphyrin ring prothstetic group surrounded by a polypeptide chain is widely found in mammalian species[164]. However, this chapter will describe an attempt to synthesise a polycyclic peptide which possesses the theoretical potential to model the active sites of hemerythrin and hemocyanin. In the following discussion, the nature of the active sites of these two molecules will be indicated, a brief overview of attempts by other researchers to mimic these oxygen carriers will be presented before my own attempted synthesis of polycyclic peptides is described.

3.1.1 Hemerythrin[122,164,165]

Hemerythrins form a family of related oxygen carrying proteins. Contrary to what might be expected from the name of this family, no heme group porphyrin rings are present in these proteins. Instead, they consist of a folded polypeptide chain containing an active site which itself possesses two iron atoms. Depending upon the species of organism for which the molecule is an oxygen carrier, it can exist in various oligomeric forms up to an octamer, as in the case of the sipunculid worm *phascolopsis gouldii*. The molecular weight of a subunit of this molecule is ca.13500 (108000 for the octamer)[122].

The structure of the active site of deoxyhemerythrin, based upon X-ray crystallographic analysis is illustrated in Fig 3.1. This active site structure is common to all species using hemerythrin as an oxygen carrier[122].

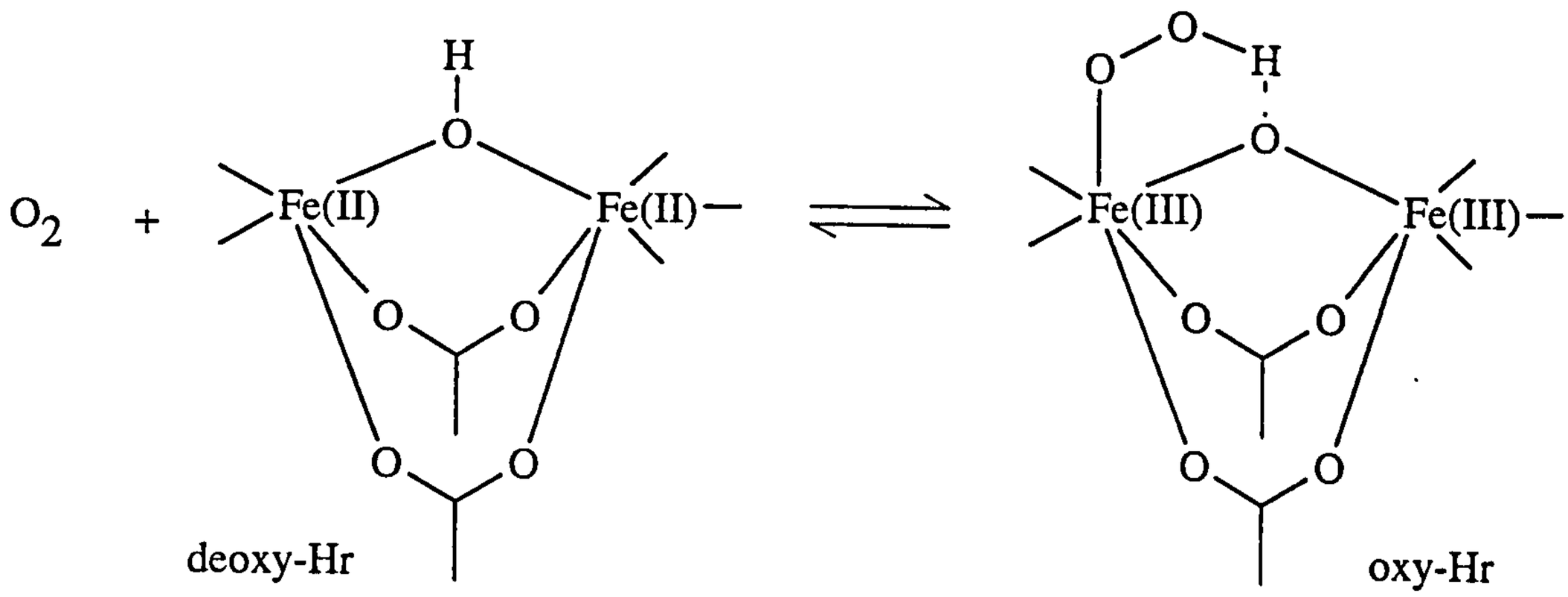
Fig 3.1 : Structure of the active site of hemerythrin



The two iron atoms in the active site are inequivalent. One iron atom is octahedrally ligated to the ϵ-nitrogen atoms of three histidine residues, two oxygen atoms from aspartic acid and glutamic acid as well as an oxygen from a μ-hydroxo bridge. The other iron is at the centre of a distorted trigonal bipyramidal moiety. It is bound to the ϵ-nitrogen atoms of two histidine residues, the remaining two oxygens from Asp and Glu and an oxygen from the same hydroxyl bridge.

A schematic diagram showing the uptake and release of dioxygen is shown in Scheme 3.1. In oxyhemerythrin, dioxygen has been shown by X-ray diffraction studies to bind as a hydrophobic group end on to the previously pentacoordinate iron(II) ion. Both iron(II) ions are oxidised to iron(III), so the addition of dioxygen may be formally considered to involve the addition of O_2^{2-}. However, the mechanism by which this occurs is unknown[122].

Scheme 3.1 : A schematic diagram of the uptake and release of dioxygen by hemerythrin

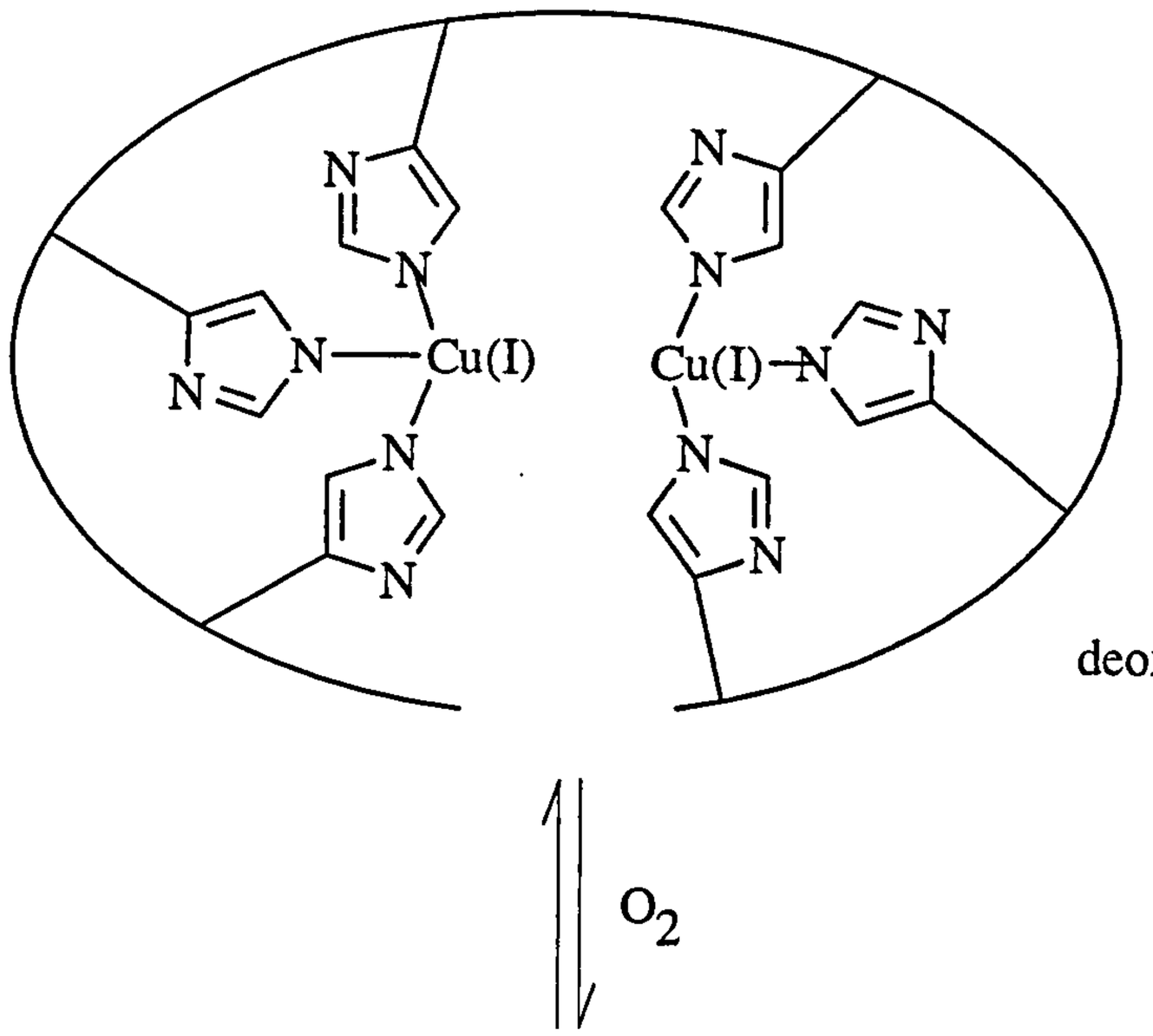


In the case of the *themiste dyscrita* oxyhemerythrin, five residues with atoms within 4 Å of the dioxygen molecule are present. All of these, two leucines, one isoleucine, one phenylalanine and one tryptophan, are hydrophobic. Similarly, most hemerythrin dioxygen binding sites are surrounded by hydrophobic residues. The detailed role of these surrounding residues is, however, not fully understood at present. It is hoped that by constructing small molecules which model the Fe-μ-O-Fe unit, a clearer understanding of the means by which dioxygen is bound may be ascertained.

3.1.2 Hemocyanin[123, 164, 165]

Hemocyanins are found in many kinds of arthropods and molluscs. These are large, multisubunit proteins that are likewise used to transport dioxygen. *Limulus polyphemus* hemocyanin is made up of 48 subunits of which subunit II is the best characterised with a molecular weight of about 73 kDa. The nature of the active site of subunit II of arthropod deoxyhemocyanin is illustrated in Scheme 3.2. The fundamental difference between this protein and hemerythrin is that hemocyanin possesses an active site based upon two Cu atoms rather than on two Fe atoms. Each copper atom is at the centre of a trigonal complex and is ligated to the protein by the ϵ-nitrogens of three histidine residues. Each copper is formally in the Cu(I) oxidation state.

Scheme 3.2 : Schematic diagram of the uptake of dioxygen by the active site of hemocyanin



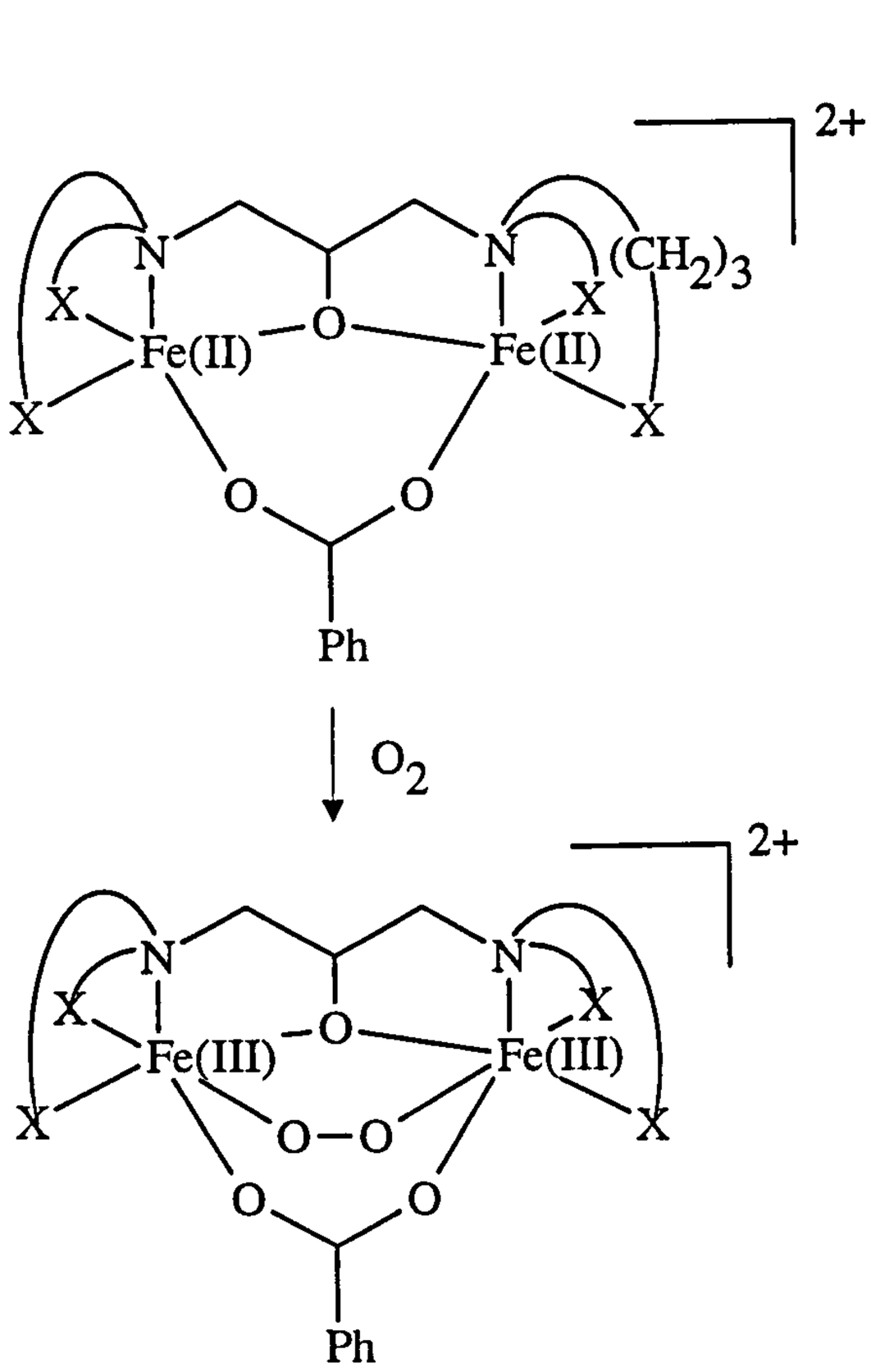
As with hemerythrin, the oxygenation of arthropod hemocyanins may be formally considered to involve the oxidation of Cu(I) to Cu(II) and the binding of dioxygen in the O_2^{2-} oxidation state. Upon dioxygen binding each Cu(II) ion becomes bound to the N^{ϵ} atoms of the two closest histidine residues and also the two oxygen atoms in a square planar geometry (Scheme 3.2). The distance between the N^{ϵ} atom of the third histidine and each copper is considerably extended compared with the other two (2.5 Å over 2.1 Å) and the nature of the bonding is not clearly understood. Thus dioxygen is bound in a η^2-μ geometry. This binding mode is consistent with the observed change in the Cu-Cu distance in subunit II of the *limulus polyphemus* hemocyanin. In its deoxygenated form, X-ray diffraction indicated a Cu-Cu distance of 4.6 ± 0.2 Å. In the oxygenated form, however, this was reduced to 3.6 ± 0.2 Å.

3.2 Synthetic biomimetic dioxygen complexes

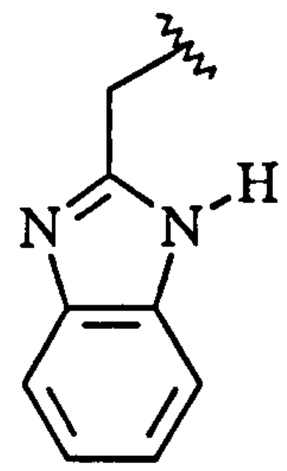
Several researchers have attempted to mimic the oxygen binding activity of hemerythrin and hemocyanin using small molecule complexes[166-168]. A large number of such dimetal complexes are known of which diiron and dicopper complexes feature prominently[169-170].

Four analogous alkoxo-bridged diiron(II) complexes have been synthesised by Lippard[167, 171]. These are derived from N,N,N',N'-X_4-2-hydroxy-1,3-diamino propane, where X is one of several nitrogenous bases (119)-(122) (Scheme 3.3).

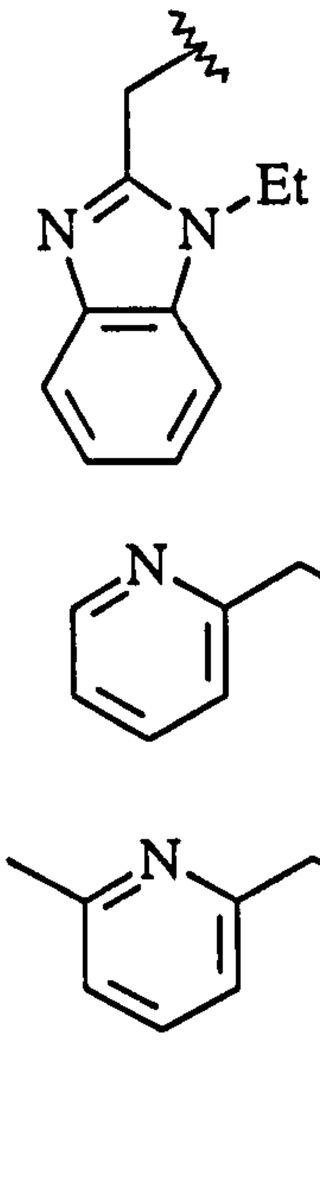
Scheme 3.3 : Dioxygen binding by four alkoxo-bridged diiron(II) complexes derived from N,N,N',N'-X_4-2-hydroxy-1,3-diamino propane



(119) X =



(120) X =



(121) X =

(122) X =

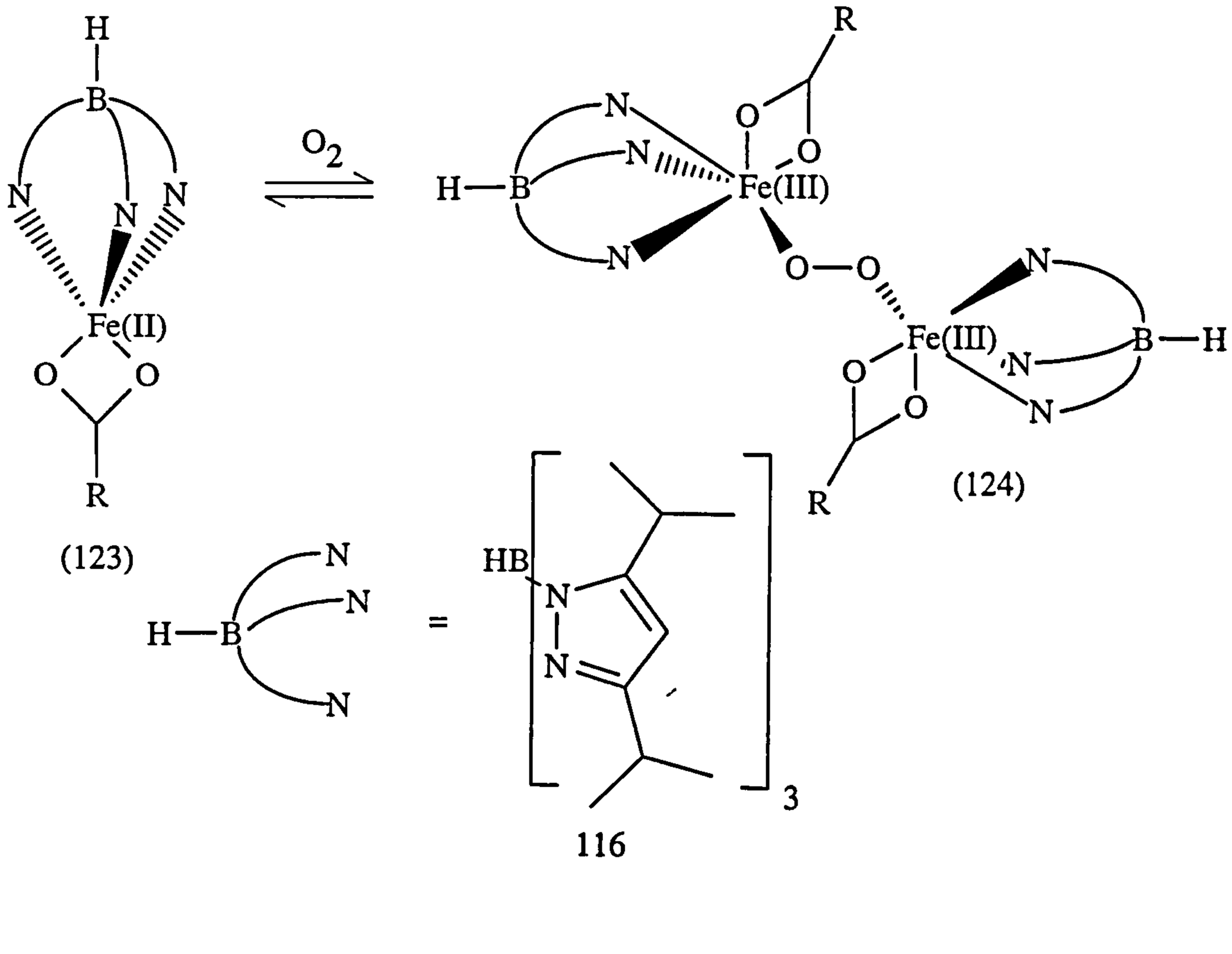
These compounds are related to hemerythrin in that they possess a carboxylate bridging group. However, whereas hemerythrin possesses two such carboxylate groups steric factors prevent the incorporation of a second carboxylate into these synthetic

molecules. In addition, an alkoxo bridging group has not thus far been observed in a non-heme protein.

The first of these compounds to be shown to form a dioxygen adduct was (119). At low temperature (T < -80° C), (120)-(122) likewise form stable dioxygen adducts. However, only complex (122) was found to show reversible binding to O_2. Whereas (119)-(121) are relatively symmetric, each having two 5-coordinate square pyramidal Fe(II) ions, complex (122) has only one square pyramidal ion - the other being coordinated to water and thus having octahedral geometry[172]. It is possible that the resultant steric crowding is alleviated by the observed lengthening of the Fe-N bonds. This bond lengthening indicates greater flexibility in the complex and facilitates the more reversible binding of small molecules to the metal centre.

In another attempt to mimic the dioxygen binding of hemerythrin, the mononuclear complex (123) was synthesised[173]. In this 5-coordinate iron(II) complex, the metal is bound to a tridentate, facially coordinating ligand hydrotris(diisopropylpyrazolyl)borate, HB(3,5-$^{i}Pr_2pz)_3$, and a chelating carboxylate ligand. On addition of dioxygen in non-coordinating solvents at temperatures below -20^{0}C, a dinuclear peroxide adduct (124) is formed (Scheme 3.4).

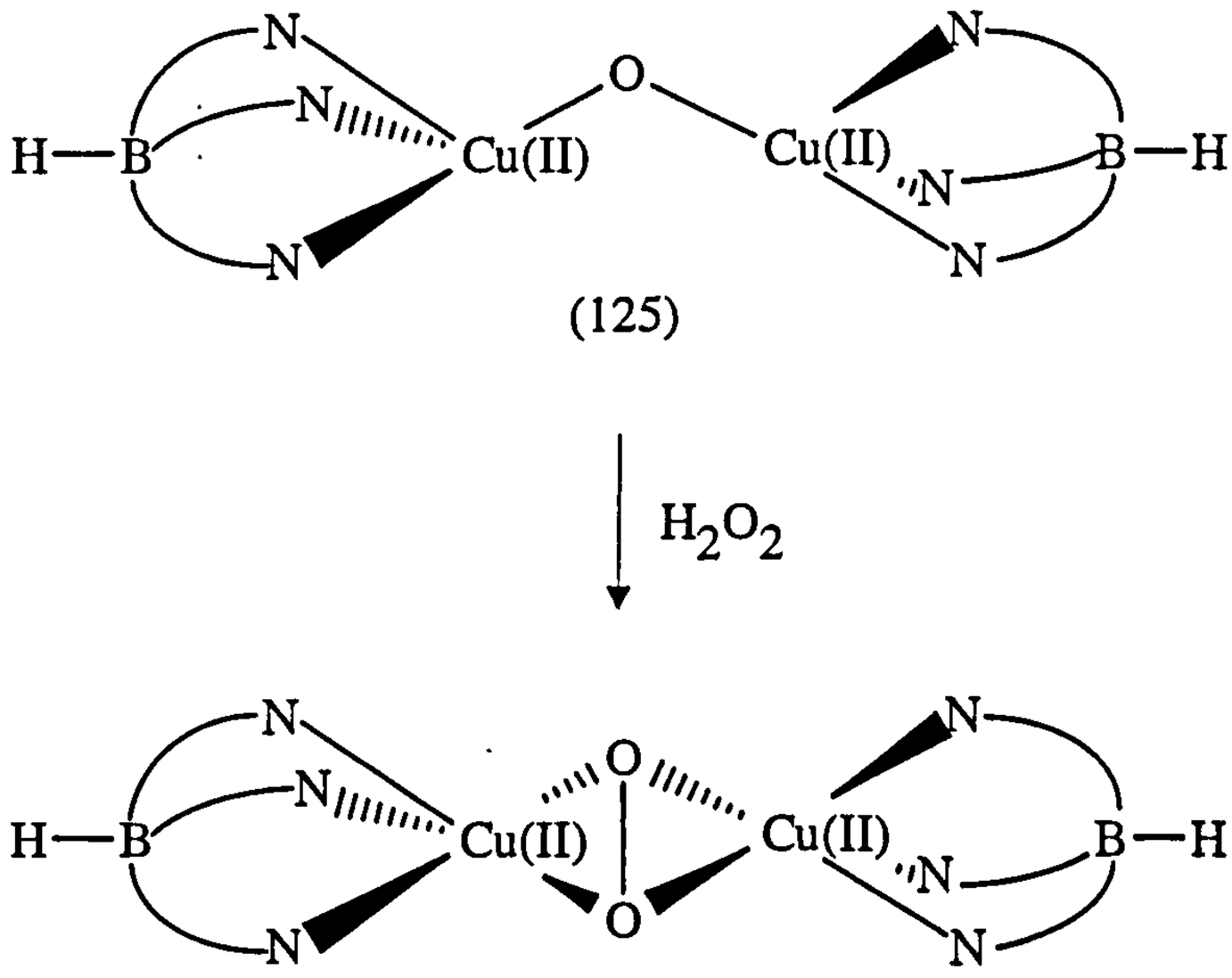
Scheme 3.4 : Addition of dioxygen to a hydrotris(diisopropylpyrazolyl)borate iron(II) complex (123)

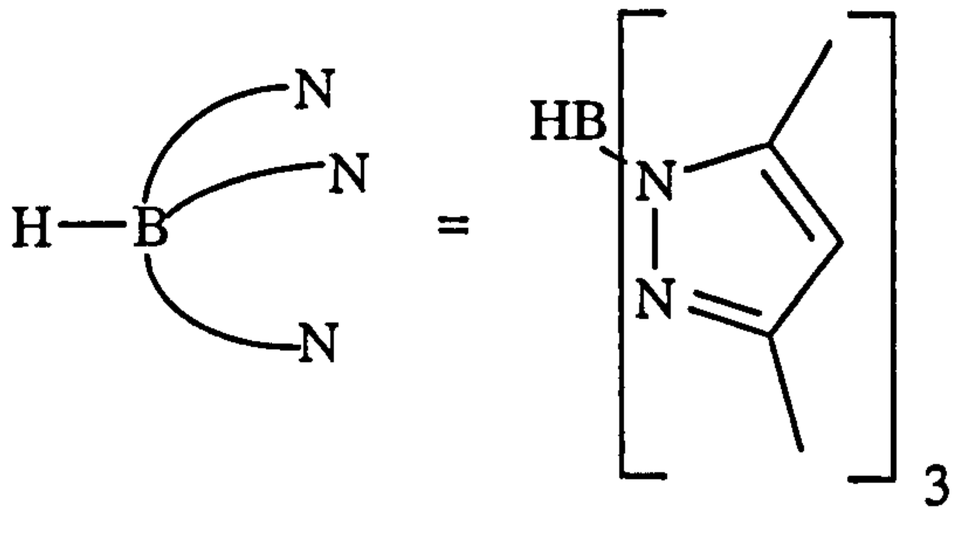


Below -50^0 C the binding is irreversible but between -50^0 C and -20^0 C, reversibility is shown by the exposure of (124) to an inert atmosphere. In such conditions, the starting material (123) is regenerated.

In an attempt to model the oxygen binding activity shown by hemocyanin[166] a dicopper complex has been synthesised which possesses a related tridentate facially coordinating ligand hydrotris(dimethylpyrazolyl)borate, HB(3,5-Me_2pz)$_3$ [174]. Initially, the synthesis of this oxyhemocyanin model involved the formation of a μ-oxo dinuclear copper(II) complex [Cu(HB(3,5-Me_2pz)$_3$)]$_2$O (125). Upon exposure to hydrogen peroxide in CH_2Cl_2 the desired μ-peroxo dinuclear copper(II) complex (126) was formed. The binding of peroxide shown by this complex is analogous to that shown by oxyhemocyanin in that it is a η^2-μ peroxo complex (Scheme 3.5).

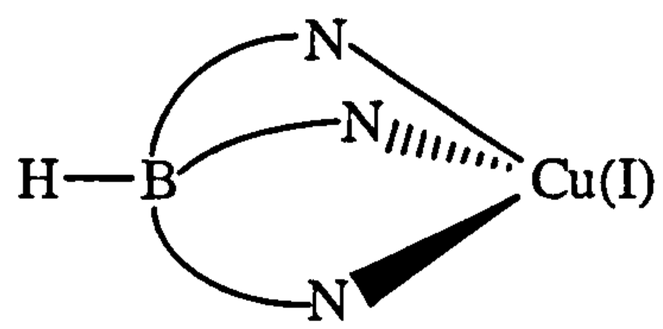
Scheme 3.5 : Formation of a model of hemocyanin by the addition of hydrogen peroxide to μ-oxo dinuclear copper(II) complex (125)



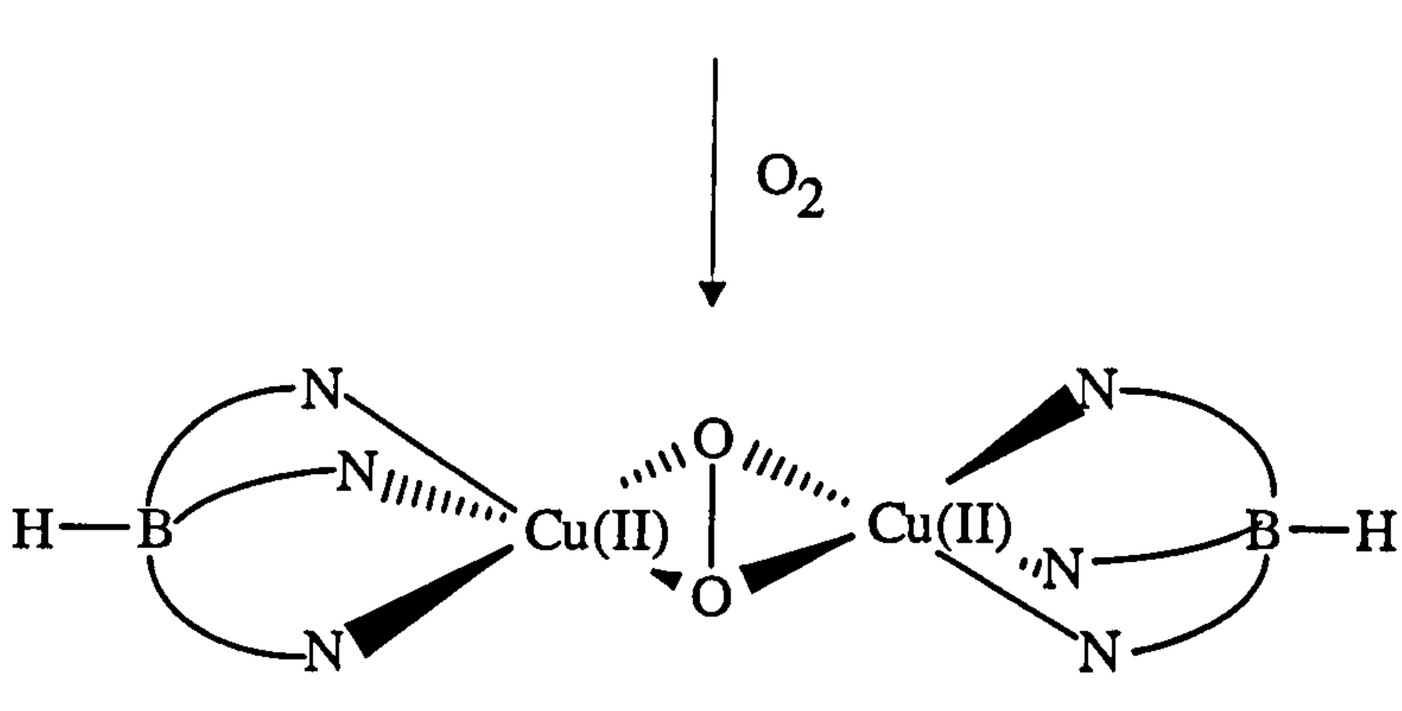


An alternative route to this complex was later developed in which more hindered hydrotris(pyrazolyl)borate ligands were used to enhance the stability and crystallinity of the complexes[175]. Upon addition of dioxygen to mono-nuclear copper(I) complex (127) the μ-peroxo copper(I) complex $[Cu(HB(3,5\text{-}^{i}Pr_2pz)_3)]_2(O_2)$ (128) was formed (Scheme 3.6). Following crystallisation of the product, X-ray diffraction was used to demonstrate the presence of the anticipated planar η^2-μ peroxide binding mode and also to show a Cu-Cu separation of 3.56 Å - which compares favourably with the 3.6 ± 0.2 Å Cu-Cu distance found in hemocyanin (Section 3.1.2).

Scheme 3.6 : Formation of a model of hemocyanin by the addition of dioxygen to mono-nuclear copper(I) complex (127)



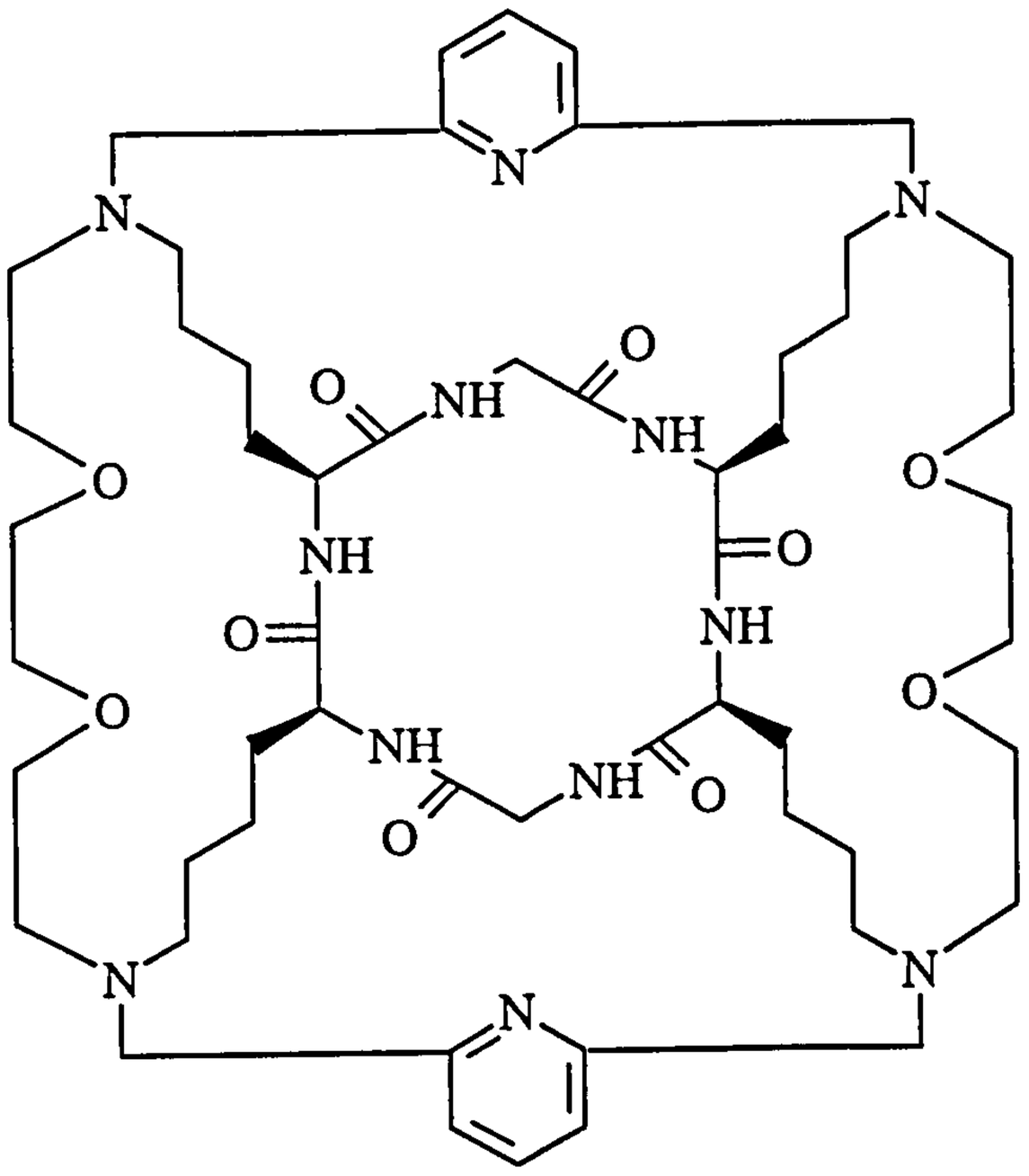
(127)



(128)

3.3 The structure of polycyclic peptide metal chelator (80)

With a view to mimicking the oxygen binding activity of hemerythrin and hemocyanin, the polycyclic peptide (80) was designed as an appropriate target molecule. It was thought that two features of this molecule would play a key role in the formation of a dimetal complex of either iron or copper thus allowing it to mimic the oxygen binding activity of these enzymes.

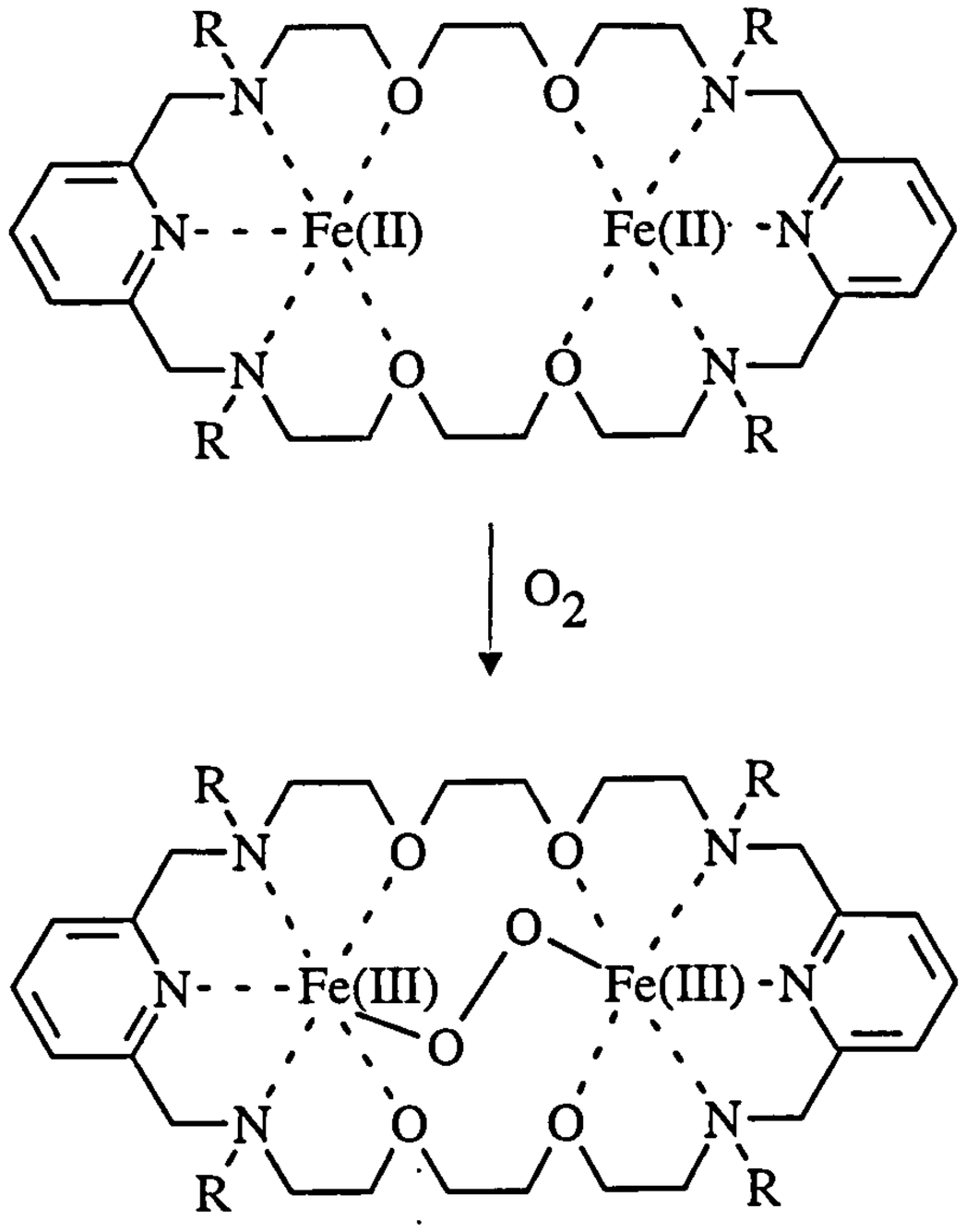


(80)

3.3.1 The macrocyclic ring of polycyclic peptide (80)

A key feature of this molecule is the macrocyclic ring containing polyether as well as nitrogen functionality which was to be constructed above the plane of the cyclic peptide. As shown in Scheme 3.7, it was this ring which it was hoped would bind either two iron atoms to form a hemerythrin model or two copper atoms to form a hemocyanin model.

Scheme 3.7 : The binding of dioxygen by both the diiron and the dicopper complexes of the macrocylic ring of the polycyclic peptide (80)



In the deoxyhemerythrin models described above, both iron atoms are 5-coordinate and are bonded to three nitrogen and two oxygen functionalities. Likewise, if a diiron complex of (80) were to form, each iron atom would be able to bind to three nitrogens, from two amines and a pyridyl group, as well as to two oxygens, each from a polyether. Upon addition of dioxygen it was hoped that the μ-O_2 complex would form.

With a view to constructing a hemocyanin model, however, it was envisaged that each copper atom of a dicopper complex would only bind to the three nitrogen functionalities. This expectation is based upon the observed coordination patterns of copper in both hemocyanin and the model described in Section 3.2. As before, dioxygen complexation would hopefully take place in the η^2-μ-O_2 mode.

3.3.2 The cyclic peptide moiety of polycyclic peptide (80)

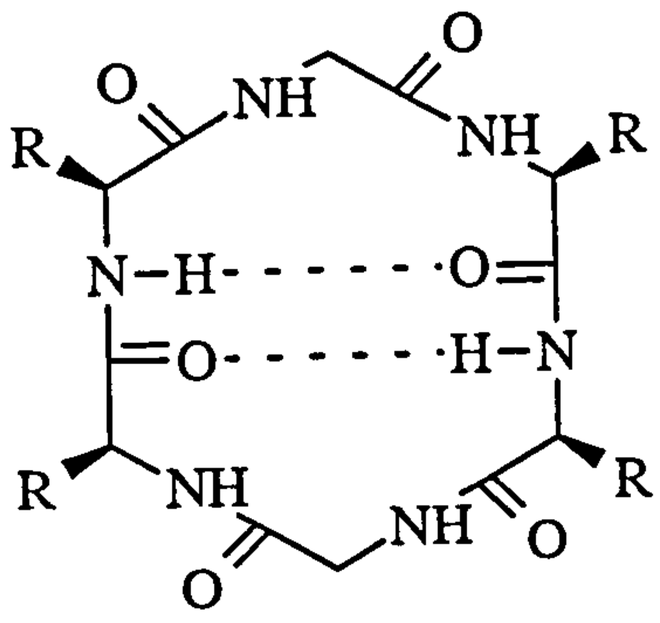
The cyclic peptide was expected to play a role in binding by providing a hydrophobic pocket inside which the above complexation could occur. The above discussion of the structure of hemerythrin and hemocyanin described how their active sites are surrounded by hydrophobic residues. The use of hydrophobic amino acids such as glycine and lysine would potentially mimic these residues by forming a synthetic hydrophobic pocket within which oxygen binding could occur.

The use of glycine and lysine confers a number of advantages to the structure of the molecule. Their use also assists in maximising the yields of the final molecule. The use of glycine in the cyclic peptide would encourage the formation of β-turns[176], thus providing a relatively rigid base to the host molecule (Scheme 3.8). Such a conformationally stable structure would thus be more preorganised for the binding of metal ions. In addition, two β-turns stabilize the target cyclic peptide encouraging its efficient synthesis.

Upon the coupling of peptide fragments, it is frequently a problem that racemisation can occur at the C-terminal residue. In Chapter 2, it was explained that the cyclic peptide synthesis would be attempted by a cyclodimerisation procedure using derivatised linear tripeptide fragments H-Lys-Lys-Gly-OH. The use of achiral Gly or N-alkylated Pro in the C-terminal position would overcome this problem of racemisation. However, the bulky amino acid Pro would be likely to reduce the yield of cyclic peptide due to steric inhibition of the peptide coupling. Consequently, the use of the small amino acid glycine would be more suitable for cyclodimerisation.

Finally, in the synthesis of the polycyclic peptide, chirality can be introduced by the use of chiral building blocks such as lysine. The four lysine amino acids incorporated allow the construction of the appropriate "bucket-shaped molecule" and provide the four covalent links required to connect this cyclic peptide structure with the metal-chelating macrocycle.

Scheme 3.8 : The cyclic peptide base of the polycyclic peptide (80) showing the presence of intramolecular hydrogen bonding between lysyl residues and the formation of β-turns

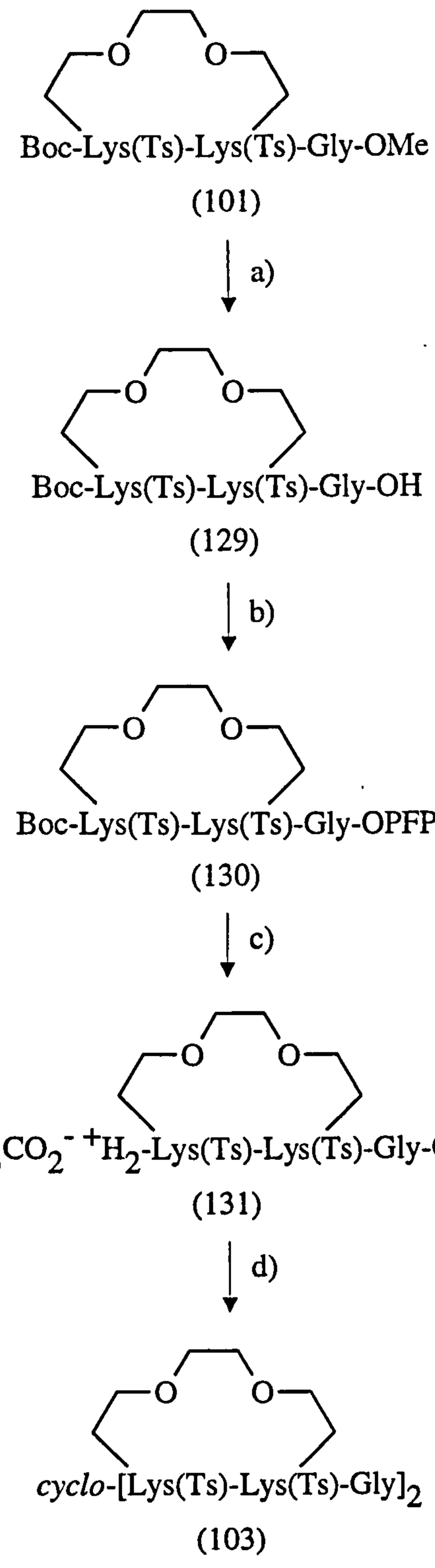


3.4 Towards the synthesis of polycyclic peptide metal chelators

3.4.1 Cyclodimerisation studies

Following the results described in Chapter 2 the crosslinked tripeptide (101) was synthesised. This molecule was used in the attempt to construct the host molecule (80) described above.

Scheme 3.9 : Synthesis of crosslinked cyclic peptide (103)



Initial conditions

a) KOH, MeOH, H_2O

b) DCC, PFP-OH, DMF

c) 1:1 TFA:CH_2Cl_2

d) Cs_2CO_3, DMF, (concentration of (131) = 5mM)

Optimised conditions

a) KOH, MeOH, H_2O

b) EDC, PFP-OH, EtOAc

c) 1:1 TFA:CH_2Cl_2

d) Cs_2CO_3, DMF, (concentration of (131) = 5mM)

The initial conditions used to effect the cyclodimerisation of (101) were based upon those developed previously within the group[115-117]. These are shown in Scheme 3.9. The process by which the optimised conditions for cyclodimerisation were determined is explained below.

Of the four steps involved in the cyclodimerisation, the initial hydrolysis step consistently gave a quantitative yield of the tripeptide carboxylic acid (129). Table 3.1 gives the main high mass peaks of the observed FAB mass spectrum together with their assignments. The ^{1}H NMR and ^{13}C NMR spectra were consistent with the desired compound.

Table 3.1: High mass peaks in the FAB mass spectrum of tripeptide carboxylic acid (129)

m/z	**Assignment**
892	$[M + K]^+$
876	$[M + Na]^+$
854	$[M + H]^+$
776	$[M - Boc + Na]^+$
754	$[M - Boc]^+$

Applying the conditions previously developed within the group[115-117], step b) involved the formation of a pentafluorophenyl (PFP) active ester. Following filtration of the reaction mixture - to remove precipitated dicyclohexylurea - and evaporation of the solvent, the brownish-white oil formed was triturated with hexane and ether to give the PFP ester (130). Step c) involved the removal of the Boc-nitrogen protecting group to give a similar brownish-white oil. Residual trifluoroacetic acid (TFA) was removed by washing in toluene and then residual toluene was removed by triturating with ether. In both cases, the products were not characterised but rather were used immediately for the next stage in the reaction. The TFA salt (131) was cyclodimerised following the conditions in step d) and following silica gel column chromatography using 5-20 % methanol in dichloromethane as eluant, an overall yield of cyclic peptide (103) of 9 % was achieved. FAB mass spectrometry of the crude product shows a distinct peak at m/z = 1472 corresponding to the $[M + H]^+$ ion of (103) as well as a peak at m/z = 1493 due to $[M + Na]^+$. In the FAB mass

spectrum of the columned material a peak at m/z = 1472, corresponding to the molecular ion plus hydrogen was observed. The reasonable purity of this material was suggested by the fact that it was a single spot by TLC. The 80 MHz ^{1}H NMR spectrum was as expected with a singlet at δ = 2.4 due to the tosyl methyl groups and a broad multiplet at δ = 1.3 - 2.1 due to the lysyl side chains. Multiplets due to CH_2N and CH_2O were present at δ = 3.0 - 3.3 and δ = 3.5, respectively. Finally, doublets due to the tosyl groups were observed at δ = 7.3 and δ = 7.7.

It is believed that pure cyclodimerisation precursors (130) and (131) are crucial to the efficient formation of the desired product. The presence of a small amount of hydrolysed PFP ester (131), for example, would theoretically promote straight chain oligomer and polymer formation. Indeed, following step b), hydrolysed PFP ester (129) had been observed on the baseline of the TLC when 19:1 dichloromethane : methanol had been used as the eluant. Compound (129), however, was situated at R_f = 0.25 in the more polar TLC eluant, 2:17:1 methanol: chloroform : acetic acid. TLC showed that by means of column chromatography on silica gel, using 1:1 chloroform : ethyl acetate as eluant, the PFP ester (130) could be isolated from (129). In later reactions, more polar ethyl acetate was used as eluant in order to improve the efficiency and speed of purification. In addition, by changing the reaction solvent in step b) from DMF to another dipolar aprotic solvent, dry ethyl acetate (stored over 4 Å molecular sieves under argon), the reaction products were found to be easier to handle and could be isolated as a white solid rather than a brownish-white oil. The reason for this is presumed to be because residual ethyl acetate with a boiling point of 77^0 C is easier to remove under reduced pressure than DMF which has a boiling point of 153^0 C. An indication that the PFP ester (130) was present came from the reaction TLCs in which those following the use of ethyl acetate were similar to those when DMF had been used as the reaction solvent.

Following these changes to the conditions employed in step b), the consistent overall cyclodimerisation yields were increased to ca. 40 %.

The use of EDC rather than DCC as a coupling reagent was a final modification to the reaction conditions. By using EDC rather than DCC, purification was assisted in that unlike residual DCC and its by-product DCU, which were removed by filtration, residual EDC and its by-product could be easily removed by a simple water wash. Using these new conditions, the optimal yield from the four cyclodimerisation steps increased to 63 % after column chromatography. The FAB mass spectrum of this compound showed the expected

$[M + H]^+$ ion at m/z = 1472 and an accurate mass determination confirmed the predicted molecular formula. The ^1H NMR and ^{13}C NMR spectra were as expected and are illustrated in Figs 3.2 and 3.3.

The ^1H NMR spectrum showed the expected multiplet at $\delta = 1.3 - 2.1$ due to the CH_2s in the lysyl side chains. The singlet at $\delta = 2.4$ was due to the tosyl methyl groups. The two multiplets at $\delta = 3.0 - 3.3$ and $\delta = 3.5$ were due to the CH_2N and CH_2O protons, respectively, whilst the multiplets at $\delta = 4.1$ and $\delta = 4.42$ were due to glycine and lysine α-CHs. The four superimposed doublets in the aromatic region ($\delta = 7.3 - 7.7$) were due to the two kinds of *para*-substituted tosyl aromatics.

The ^{13}C NMR showed peaks at $\delta = 21.50$ and $\delta = 21.54$ due to tosyl methyls and the expected number of 15 carbons from the CH_2 groups. These 15 peaks are due to the 6 CH_2s of the lysine side chains ($\delta = 22.30 - 30.53$), 1 CH_2 from glycine ($\delta = 43.88$), 4 due to CH_2N ($\delta = 48.43 - 49.78$) and 4 from CH_2O ($\delta = 70.31 - 70.87$). Fifteen CH_2 peaks were expected because although there are 30 CH_2 carbons in cyclic peptide (103), the molecule possesses 2-fold rotational symmetry. Two peaks due to lysine α-CH were present at $\delta = 52.67$ and $\delta = 55.57$ whilst 3 resolved tosyl CH peaks ($\delta = 127.10 - 129.92$) and 3 resolved tosyl C peaks ($\delta = 136.37 - 143.35$) were observed. Finally, three carbonyl carbons ($\delta = 170.47 - 172.89$) completed the complement of carbons in the molecule. Overall, the spectrum showed the sample to be relatively clean with no significant peaks due to impurities.

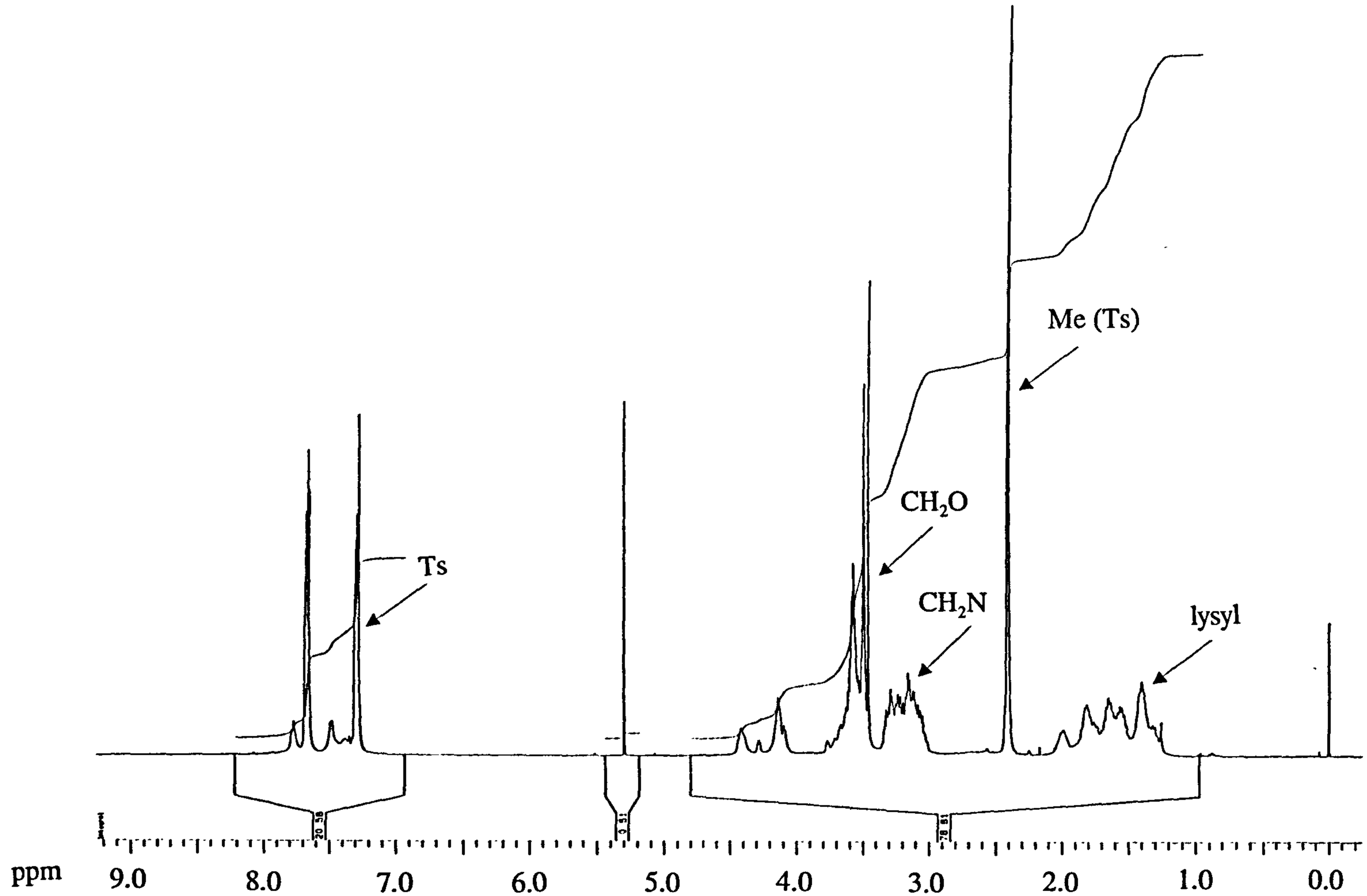


Fig 3.2 : 400 MHz ^{1}H NMR spectrum of cyclic peptide (103) in $CDCl_3$ with trace CD_3OD

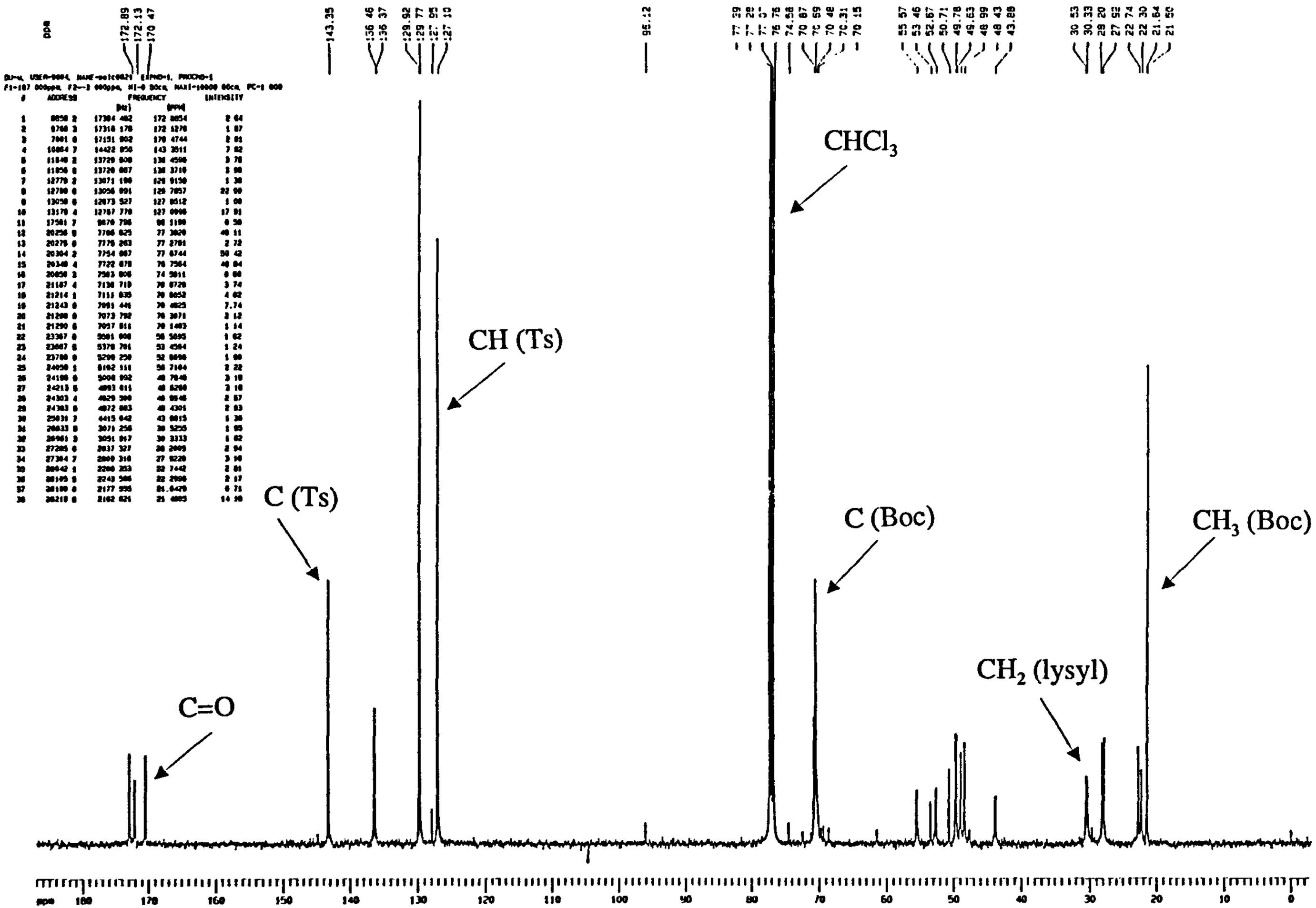


Fig 3.3 : 100 MHz ^{13}C NMR spectrum of cyclic peptide (103) in $CDCl_3$ with trace CD_3OD

Unfortunately, following the scale up of this reaction from 500 mg of starting tripeptide (101) to 3.5 g of (101), the overall cyclodimerisation yield following the optimised conditions and after column chromatography was reduced to 16 %. It is not clear what the reason is for this reduction in yield.

In order to test the effect upon the overall yield of using different alkali metal bases in step d), the base was varied from Cs_2CO_3 to K_2CO_3 and Na_2CO_3. These were tested having used DCC rather than EDC as the coupling agent in step b). Previously[115], it had been suggested within the group that the use of caesium ions in related cyclodimerisations enabled high yields to be obtained by a template effect[106-108]. However, overall yields were not significantly affected by the base used, remaining at ca. 40 %. It is thus suggested that a template effect is not a significant factor in determining the yield in this cyclodimerisation. This would be expected in view of the poor chelating ability of the C=O and N-H groups in the amide bonds of the cyclic peptide being synthesised.

A summary of the optimised conditions for cyclodimerisation is given in Scheme 3.9.

3.4.2 Detosylation of the cyclic peptide

Following the successful synthesis of cyclic peptide (103) it was important to bring about the removal of the four tosyl protecting groups as the penultimate step in the target molecule synthesis. It was vital that all four tosyl groups were removed cleanly in order to avoid complex purification problems in the separation of tri-, di-, and mono-tosylated material from the desired detosylated product. In addition, it was important to ensure the maximum possible yield of the target molecule ready for the final step in the synthesis.

A great variety of different reagents have been used in the deprotection of tosyl protected amines[177]. Strongly acidic conditions such as the use of HF[178,179] and HBr/AcOH[180] have been used. More frequently, powerful basic conditions have also been employed. Thus $Na/NH_{3(l)}$ [139,181], sodium amalgam [182,183], sodium naphthalenide [184-187] and anthracenide[188] and sodium bis(2-methoxyethoxy)aluminium hydride[189] have all been used to remove tosyl groups. More unusual conditions such as the use of electrolysis in tetramethylammonium chloride[190] and even photolysis[191] conditions have also been employed. However, the literature is clear that the optimum method is highly dependent upon the nature of the tosylated substrate and that conditions suitable for one molecule are not neccessarily suitable for another. Thus, whereas Mazur and Plume used HF to remove

N-tosyl groups from arginine residues in a nonapeptide[178], Okada and Sugihara found N-tosyl to be stable to HF[192]. Fujii observed HF to be useful for removing N-tosyl from histidine but not from arginine[179].

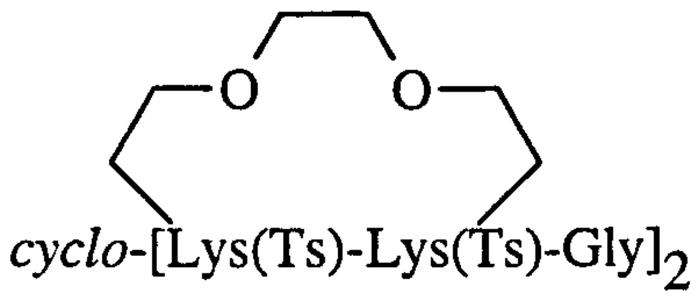
The initial conditions used in the attempt to the remove the tosyl group from cyclic peptide (103) was to heat with HBr/AcOH in phenol[180]. However, this only produced a black tar which due to the lack of a molecular ion peak at m/z = 855 in the FAB mass spectrum was assumed not to contain the desired product. Greater success was achieved by treating (103) with sodium anthracenide in dry THF under argon. The resultant blue solution was stirred for 1 h under argon. After purification of the product using analytical HPLC at pH 5 with a potassium phosphate aqueous buffer, the FAB mass spectrum of one of the three isolated products showed two high mass peaks which could be assigned to the desired product. These peaks were m/z = 894, assigned as the molecular ion of (105) plus potassium from the buffer and m/z = 856, due to the molecular ion plus hydrogen. Unfortunately, however, due to the lack of a suitable preparative column for purification of the product following any attempted scale up of the reaction, this procedure was not taken any further.

Experiments by others within the group[193] indicated that successful detosylation of cyclic peptides could be achieved using a 50 % sodium dispersion in paraffin using liquid ammonia solvent. Thus to a blue solution of the sodium dispersion in liquid ammonia was added a solution of cyclic peptide (103) in dry THF under argon. The resultant blue solution was stirred for 2 h. The use of acetic acid as a sodium quenching agent produced a large quantity of sodium acetate. A FAB mass spectrum of this crude product showed a peak at m/z = 856, potentially corresponding to the molecular ion of (105) plus hydrogen. Many other similarly sized high mass peaks between m/z = 715 and m/z = 1071 were also present but could not be assigned. Following removal of the sodium acetate by its dissolution in water, a second FAB mass spectrum again showed a peak at m/z = 856, but again this peak was not significantly above the general background noise of the spectrum. In view of these rather ambiguous results and the small quantity (ca. 2 mg) of material isolated, further attempts to isolate the desired product (105) were not undertaken.

Success in the production and isolation of (105) was achieved by using sodium metal in liquid ammonia. To a blue solution of sodium in ammonia was added a solution of (103) in dry THF under argon. This blue reaction mixture was stirred for 2 h and the sodium quenched with ammonium chloride. The FAB mass spectrum of the crude reaction

products revealed a significant $[M + H]^+$ peak at m/z = 856, with quadruple the intensity of the background noise. Silica gel column chromatography in which the eluant was 19:1 methanol : liquid ammonia was used to purify the desired product. By the addition of liquid ammonia to the methanol eluant, the polarity was increased and it was found that the deprotected amine cyclic peptides were eluted more efficiently from the column. The above reaction and purification procedure was attempted on two occasions. In the first, a yield of 36 % of deprotected cyclic peptide (105) was achieved. The second reaction gave a yield of 69 % of the desired product (Scheme 3.10). The reaction TLC (19:1 methanol : liquid ammonia) showed a spot which whilst being stained brown on exposure to ninhydrin was not UV active. This was considered to be consistent with (105) in that the desired product has no UV active aromatic or conjugated chromophores. The ability of ninhydrin to stain this TLC spot is indicative of free amines. The FAB mass spectrum with its peaks at m/z = 855 and m/z = 856 due to $[M]^+$ and $[M + H]^+$ is consistent with that expected. It is reproduced in Fig 3.4. The lack of any aromatic protons and the loss of the tosyl methyl singlet in the ^1H NMR spectrum (Fig 3.5), in which the sample is dissolved in $CDCl_3$ with trace CD_3OD to aid solubility, clearly shows that all the tosyl groups have been removed. As would be expected, there is a broad multiplet at $\delta = 1.15 - 1.95$ due to the lysyl CH_2s as well as multiplets at $\delta = 2.45 - 2.6$ and $\delta = 2.6 - 2.75$ due to CH_2N, a multiplet at $\delta = 3.4 - 3.55$ due to CH_2O and a multiplet at $\delta = 4.0 - 4.2$ due to the glycine and lysine α-CHs - the integrations being 3:1:1:2:1, respectively.

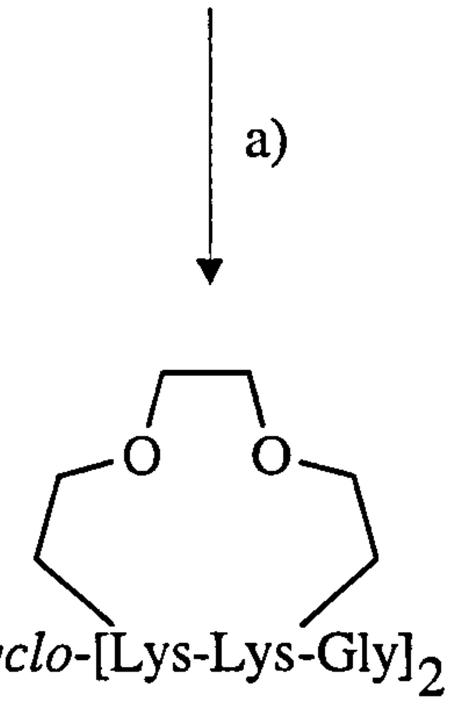
Scheme 3.10 : Synthesis of polycyclic peptides from cyclic peptide advanced intermediates



cyclo-[Lys(Ts)-Lys(Ts)-Gly]$_2$

(103)

a)

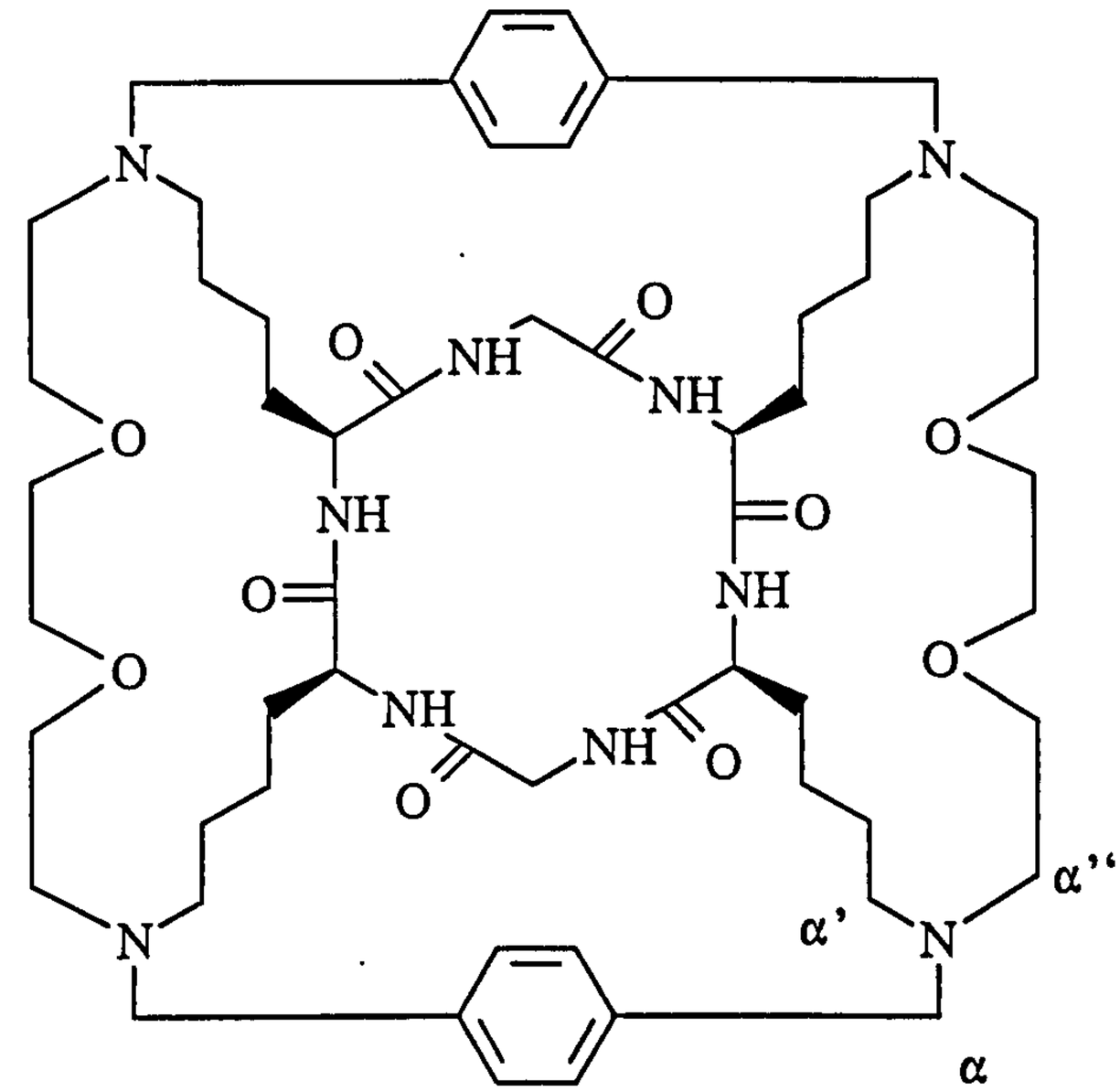


cyclo-[Lys-Lys-Gly]$_2$

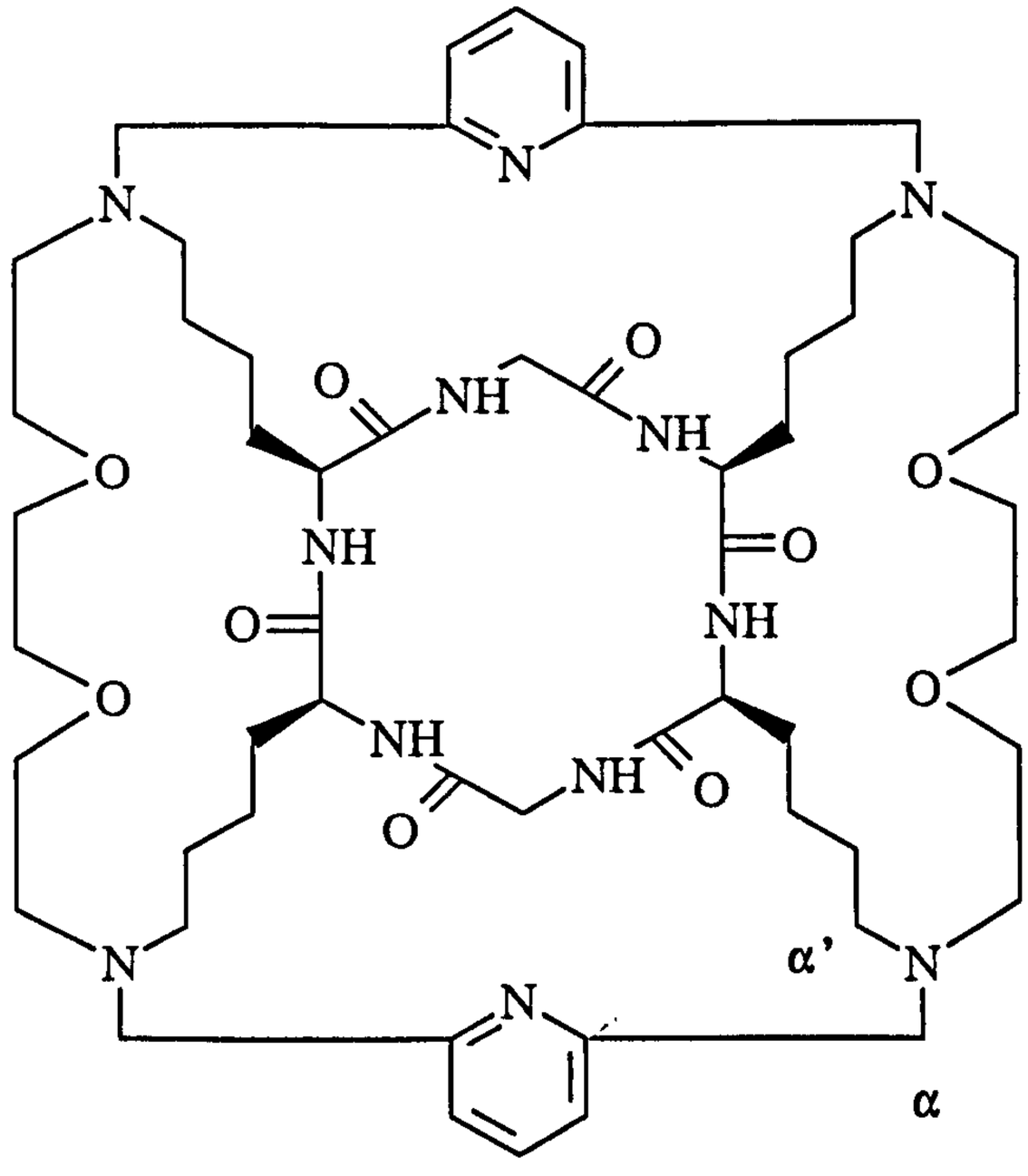
(105)

b)

c)



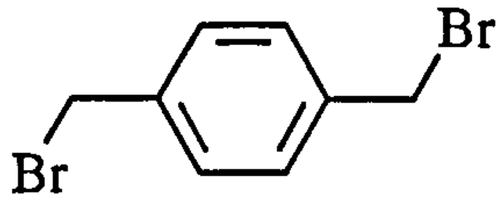
(79)



(80)

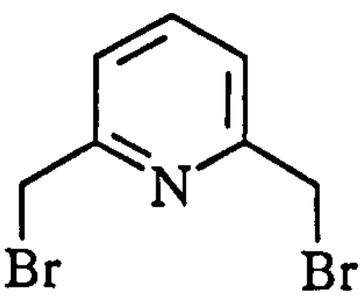
a) Na/NH_3

b)



DIPEA, TFE, (concentration of (105) = 5mM)

c)



DIPEA, TFE, (concentration of (105) = 5mM)

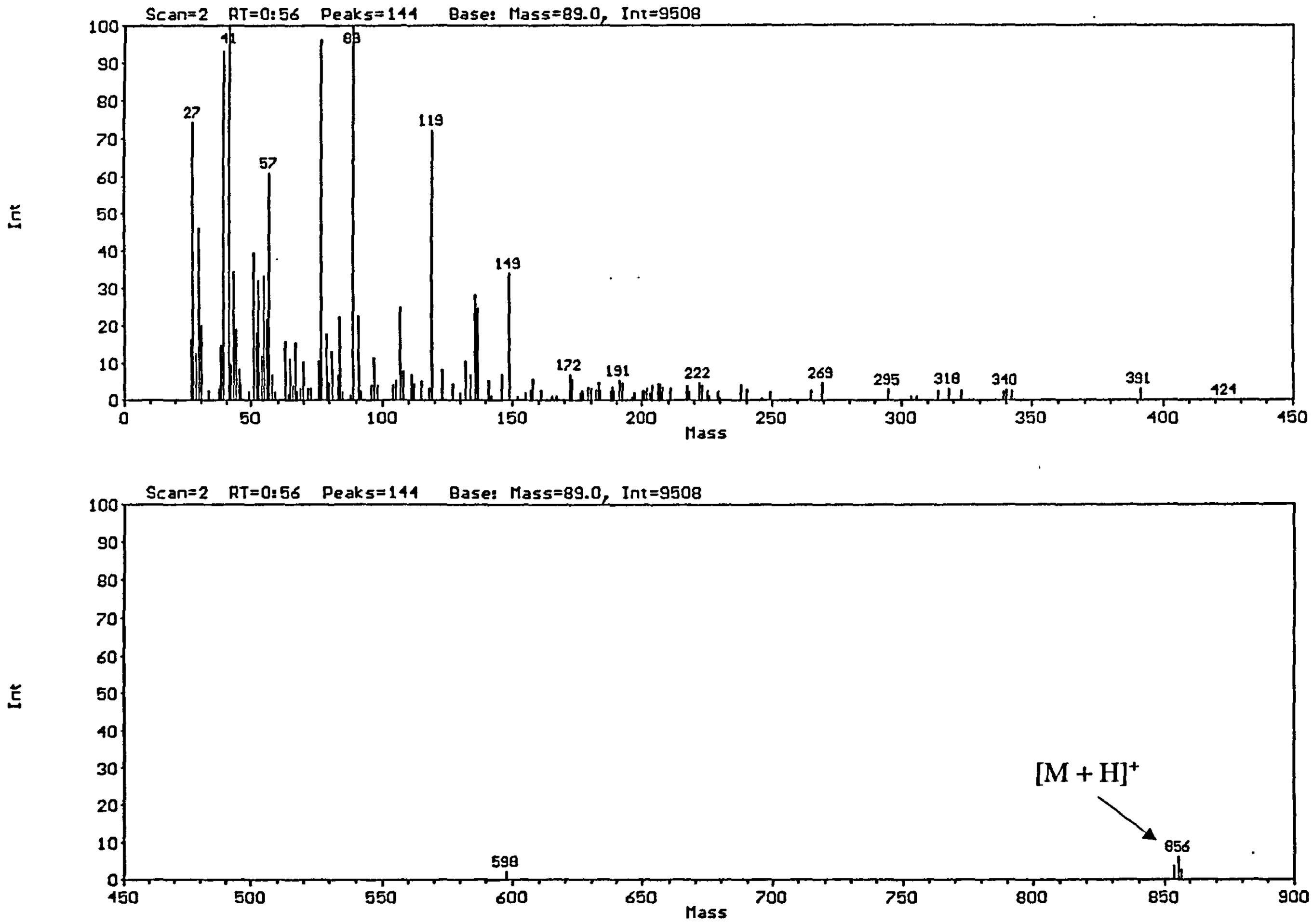


Fig 3.4 : FAB mass spectrum of deprotected cyclic peptide (105)

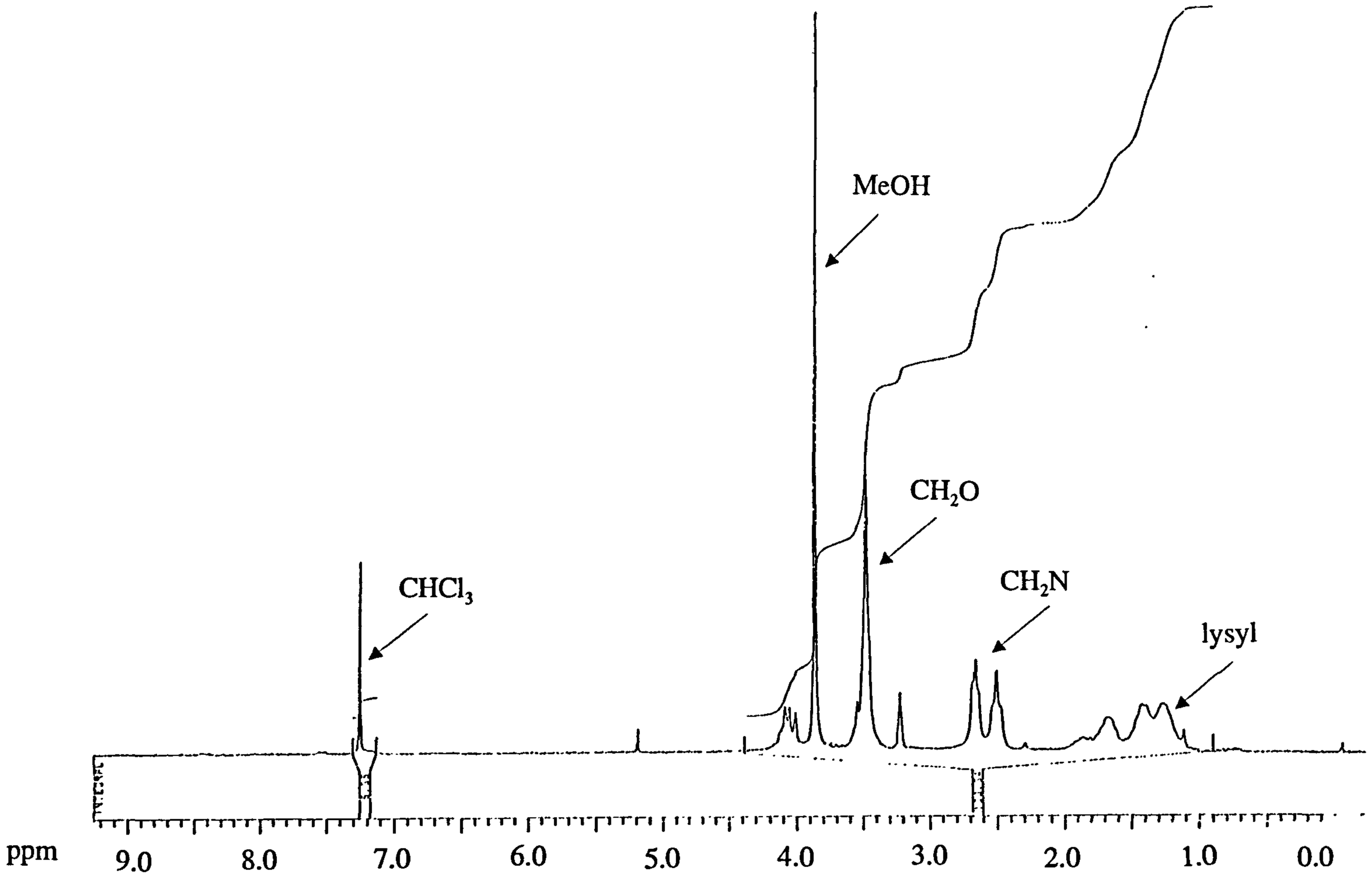
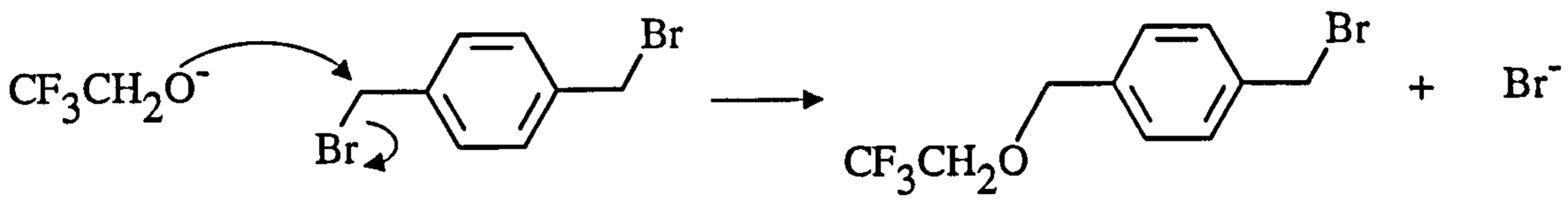


Fig 3.5 : 200 MHz ^1H NMR spectrum of deprotected cyclic peptide (105) in $CDCl_3$ with trace CD_3OD

3.4.3 Final crosslinking reactions

In order to model the final step in the synthesis of the desired polycyclic peptide (80), a double crosslinking reaction between 1,4-α,α'-dibromo-*p*-xylene and the deprotected cyclic peptide (105) was attempted (Scheme 3.10). It was thus hoped to synthesise another polycyclic peptide (79) in addition to the hemerythrin and hemocyanin mimic (80). Following the precedent established within the group[118], the deprotected cyclic peptide (105) was dissolved in 2,2,2-trifluoroethanol (TFE) under argon. It was stirred at 40^0 C, a temperature which was maintained throughout the reaction. To this solution were added by syringe pump over 12 h, DMF solutions of the 1,4-α,α'-dibromo-*p*-xylene crosslinker and N,N-diisopropylethylamine (DIPEA) as base. The polar, aprotic DMF was used as the solvent rather than TFE because as shown in Scheme 3.11, the presence of DIPEA would deprotonate the TFE, the conjugate base of which would compete with the ϵ-nitrogens of the cyclic peptide (105) to displace the Br^- ions. As a consequence, the crosslinker would possess only one efficient leaving group preventing it from forming the desired compound.

Scheme 3.11 : Displacement of bromide leaving groups by the TFE anion



A DMF solution of crosslinker was initially added followed by a solution of equal equivalents of crosslinker and base, with the final addition being the remaining base solution. Each of these three solutions were added by syringe pump over a period of 12 h. This method was chosen so as to ensure that an excess of base compared to crosslinker was at no time present in the reaction mixture. The intended role of the base was to neutralise the HBr produced following the nucleophilic displacement of the Br^- ions on each crosslinker molecule. However, if an excess of base were present, there would be an increased

likelihood of the TFE reaction solvent being deprotonated and the displacement illustrated in Scheme 3.11 occurring. It was hoped that the above reaction procedure would prevent this from resulting.

Following these syringe pump additions, the mixture was stirred for a further 3 days in order to encourage the completion of the reaction. Silica gel column chromatography was attempted using 180:19:1 chloroform : methanol : liquid ammonia as eluant. As with the deprotected cyclic peptide (105), it was found that the addition of a small quantity of ammonia to the eluant aided the efficient elution of the products. TLC (95:5 methanol : ammonia) showed the presence of a dominant spot at $R_f = 0.55$ surrounded by closely running but minor (less intense) spots. As would be expected from the presence of aromatic chromophoric groups on the desired product (79), this dominant spot was UV active. The FAB mass spectrum revealed high mass peaks at m/z = 1060 and m/z = 780 - the former being assigned as the $[M + H]^+$ ion of the desired product (79). This mass spectrum is displayed in Fig 3.6.

The ^1H NMR spectrum of the 10 mg sample, however, using $CDCl_3$ as the solvent with a trace of CD_3OD to aid solubility, did not conclusively prove that (79) had been successfully synthesised. Whilst the presence of aromatic peaks were suggested by a broad band at $\delta = 7.2 - 7.6$ and lysyl protons were possibly observed by the presence of a large broad multiplet at $\delta = 1.1 - 2.0$, additional features could not be determined due to the presence of a large quantity of solvent peaks and sample impurities. Repeated attempts to purify the sample by silica gel column chromatography were unsuccessful since, as explained above, the sample impurities ran very closely to the main product spot by TLC (95:5 $MeOH:NH_3$ and 180:19:1 $CHCl_3:MeOH:NH_3$). Following each attempt to purify the compound by column chromatography, ^1H NMR experiments were attempted using either the $CDCl_3$: CD_3OD mixture or d_7-DMF as solvents. However, no improvements were obtained. A typical ^1H NMR spectrum in d_7-DMF is illustrated in Fig 3.7, again showing the broad aromatic peak and the lysyl protons mentioned above. Whilst being consistent with the expected ^1H NMR of the desired product it does not conclusively prove its identity. The sharp *para*-substituted aromatic proton peaks observed at $\delta = 7.6$ are not believed to be due to the desired product because identical peaks to these were also observed in the ^1H NMR spectra of the reaction products from the attempted synthesis of (80).

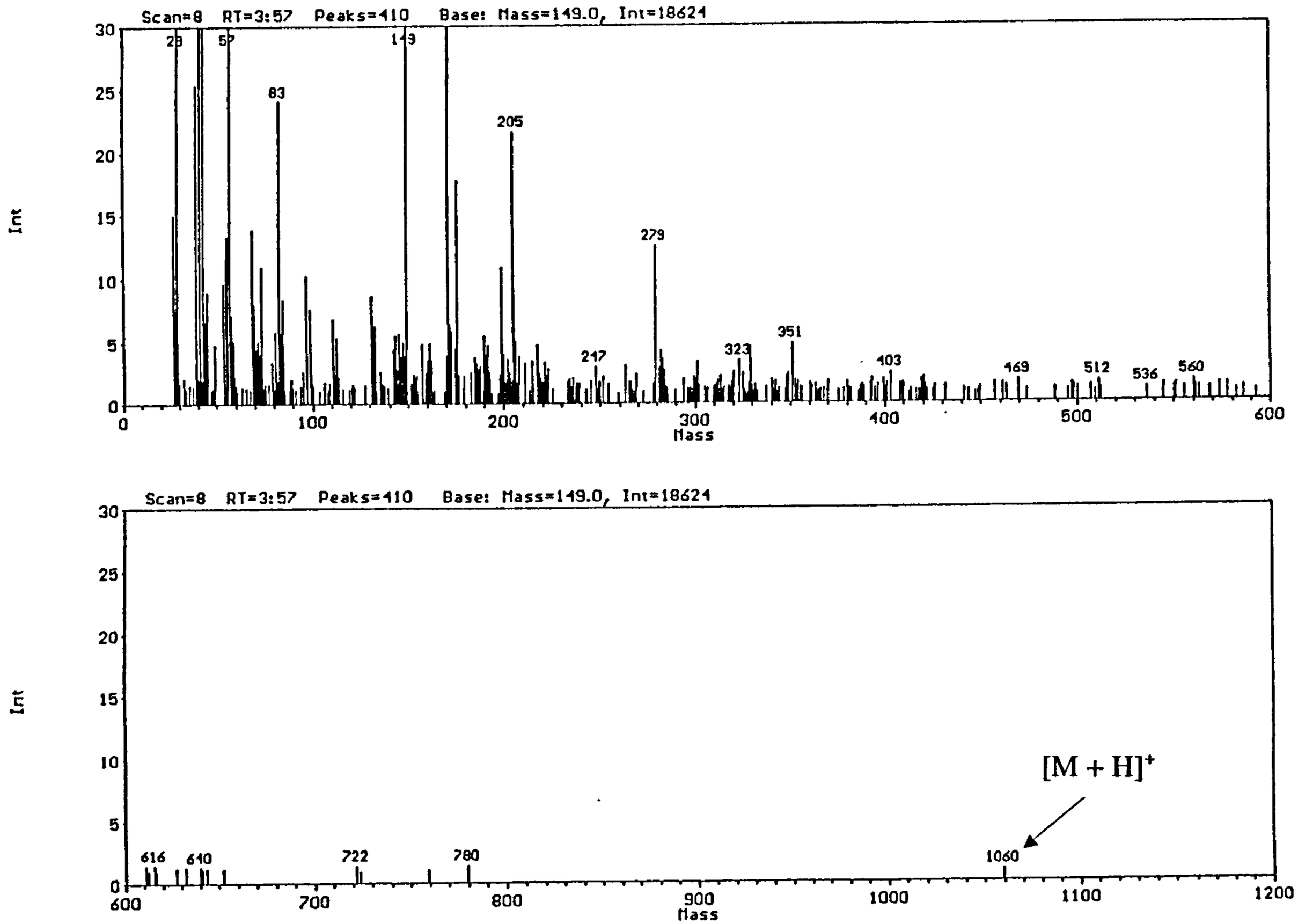


Fig 3.6 : FAB mass spectrum of the columned reaction products from the attempted synthesis of polycyclic peptide (79)

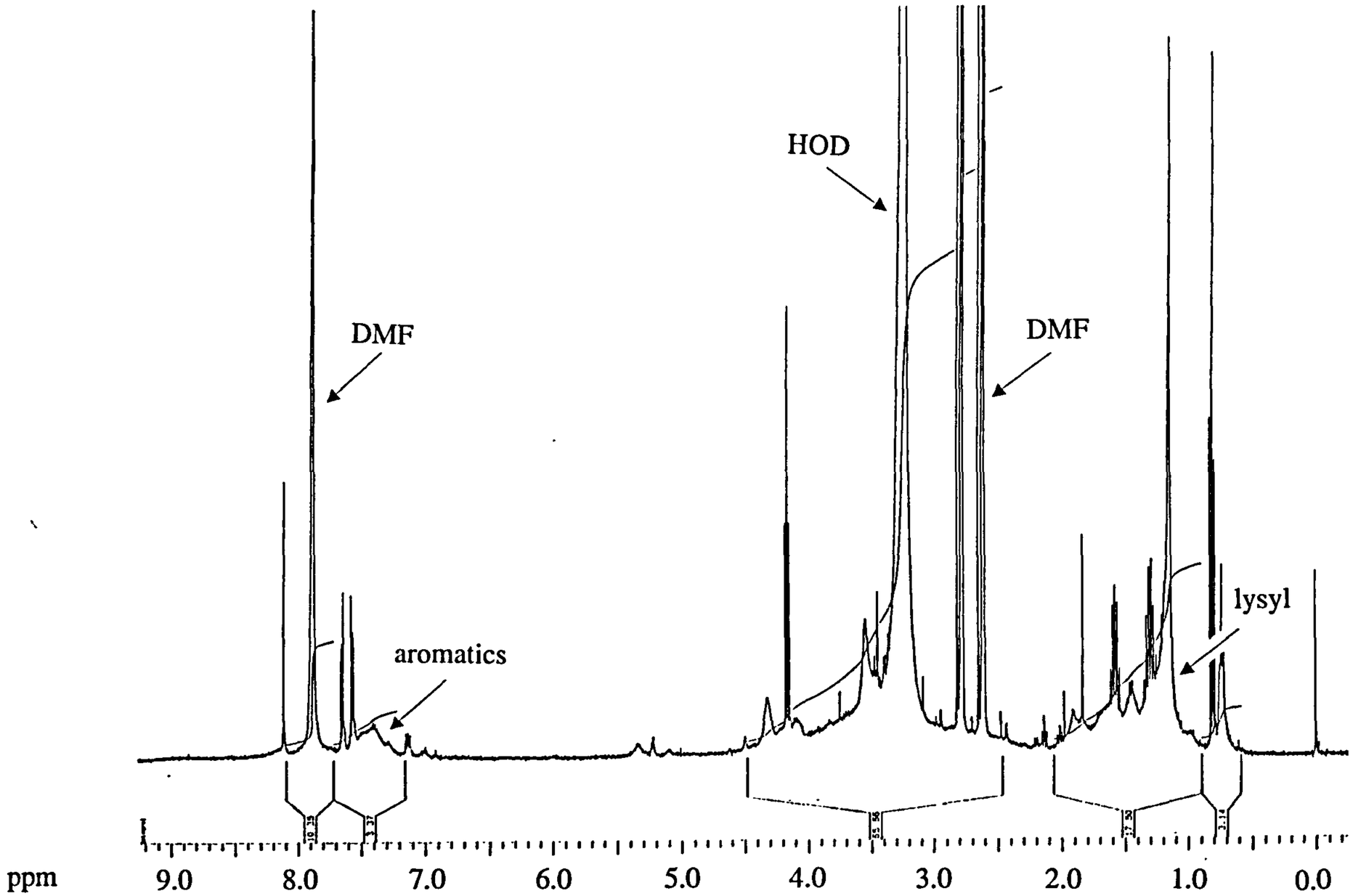


Fig 3.7 : 400 MHz ^{1}H NMR spectrum in d_7-DMF of the columned reaction products from the attempted synthesis of polycyclic peptide (79)

It had been hoped that COSY ^{1}H NMR spectroscopy would have aided the characterisation of any (79) that had been formed. This technique would also be useful if the polycyclic peptide (80) was to be isolated. By using this technique, the CH_2 protons α, α' and α'', on (79) and (80) in Scheme 3.10 could be correlated and shown to couple to each other. It was also hoped to produce a NOESY ^{1}H NMR spectrum to determine if any through space NOE interactions between, for example, the *para*-xylyl and the triethyleneglycol based crosslinks were observed. If this had been achieved, along with the production of clean, well resolved ^{1}H NMR and ^{13}C NMR spectra then strong evidence for the successful crosslinking of the 1,4-α,α'-dibromo-*p*-xylene crosslinker would have been produced. However, the poor quality of the spectra precluded this attempt at characterisation.

The conclusion that must be drawn from these spectroscopic experiments is that whilst from mass spectrometric evidence, the cyclic peptide (79) was formed in this reaction, proof of this from ^{1}H NMR spectroscopy was not forthcoming. Nevertheless, the ^{1}H NMR spectrum was consistent with the desired product.

With a view to synthesising the polycyclic peptide (80), the above crosslinking reaction, in which 1,4-α,α'-dibromo-*p*-xylene was replaced by an equivalent amount of 2,6-dibromomethylpyridine, was repeated. The crosslinker and base were added using a syringe pump in an identical manner to the attempted synthesis of (79).

Two attempts at this reaction were made, each using 70 mg of deprotected cyclic peptide (105). However, as with the attempted synthesis of (79), TLC using the same solvent system showed a dominant UV active spot surrounded by small, less intense spots. The compounds producing these spots were uncharacterised. Despite repeated attempts to purify the reaction products by silica gel column chromatography using 180:19:1 chloroform : methanol : liquid ammonia as eluant, TLC in the same solvent system showed that the product had not been purified.

Following the first attempted synthesis of (80), FAB mass spectra were recorded after each attempt at column chromatography. It was expected that if cyclic peptide (80) had been formed, these spectra would show a peak at m/z = 1061 corresponding to the molecular ion or m/z = 1062 due to the molecular ion plus hydrogen. Unfortunately, no such peaks were observed. Following column chromatography of the reaction product from the second attempted synthesis of (80), a peak at m/z = 1062 was observed but was not significantly more intense than the general spectrum background noise. In addition,

because this peak was only observed in 1 out of 20 scans, it was not considered to be definitive evidence for the formation of the desired product.

In order to aid the characterisation of any compound (80) that it was hoped had been produced, a ^{1}H NMR spectrum of the crosslinker 2,6-dibromomethylpyridine was recorded in $CDCl_3$. This was compared with the ^{1}H NMR spectra of the product from the crosslinkings. Thus, in the spectrum of the crosslinker, a singlet at $\delta = 4.5$ due to the CH_2Br protons was present. The 3 aromatic protons gave the expected doublet at $\delta = 7.35$ coupled to a triplet at $\delta = 7.7$. Unfortunately, however, the ^{1}H NMR spectra of the 4 mg of the columned reaction products, using a $CDCl_3$ and CD_3OD mixture, CD_3OD and d_7-DMF failed to reveal such a peak distribution. Indeed, with the exception of the *para*-substituted aromatic impurities described earlier, no aromatic protons were observed in any of the spectra. However, the presence of peptidic material in the samples was suggested by the presence of a broad multiplet at $\delta = 1.2 - 1.7$. Thus, as with FAB mass spectrometry, ^{1}H NMR spectroscopy failed to reveal any evidence for the successful synthesis of (80) in either attempted synthesis. The small amount of material available for each ^{1}H NMR spectrum (4 mg) precluded an analysis by ^{13}C NMR spectroscopy.

A possible reason for the failure to observe the desired product (80) was that a large quantity of the 70 mg of cyclic peptide (105) used in each reaction may have been converted to polymeric material. This could occur by the reaction of one ϵ-nitrogen on a lysyl side chain with one bromomethyl group on a crosslinker molecule. However, instead of the other bromomethyl group on the crosslinker reacting with the ϵ-nitrogen on the adjacent lysine, it would conceivably be possible for it to react with an ϵ-nitrogen on another cyclic peptide molecule. Such a process could occur many times, thus preventing the formation of (80) and instead leading to the formation of polymers. It was hoped that such a situation would be avoided by the use of a syringe pump and by using a large amount of reaction solvent in each reaction to produce a low concentration (5×10^{-3} M) of the cyclic peptide. However, it is possible that polymeric material was still formed. It would be expected that such material would not move from the baseline in a TLC experiment, even using such a polar eluant as 180:19:1 chloroform : methanol : liquid ammonia. This was indeed found to be the case in that a very intense baseline spot on the reaction TLCs was observed.

3.5 Summary

Tetratosylated cyclic peptide (103) was successfully synthesised and was then successfully detosylated using Birch reduction conditions. Mass spectrometry indicated that the resultant deprotected cyclic peptide (105) was successfully crosslinked using a 1,4-α,α'-dibromo-*p*-xylene crosslinker to form the target molecule (79) but this could not be proven by ^{1}H NMR spectroscopy. Both attempts to crosslink (105) using 2,6-dibromomethylpyridine, in order to synthesise target molecule (80), were shown by FAB mass spectrometry and ^{1}H NMR spectroscopy to be unsuccessful.

CHAPTER 4:

TOWARDS THE SYNTHESIS OF A PORPHYRIN CAPPED CYCLIC PEPTIDE

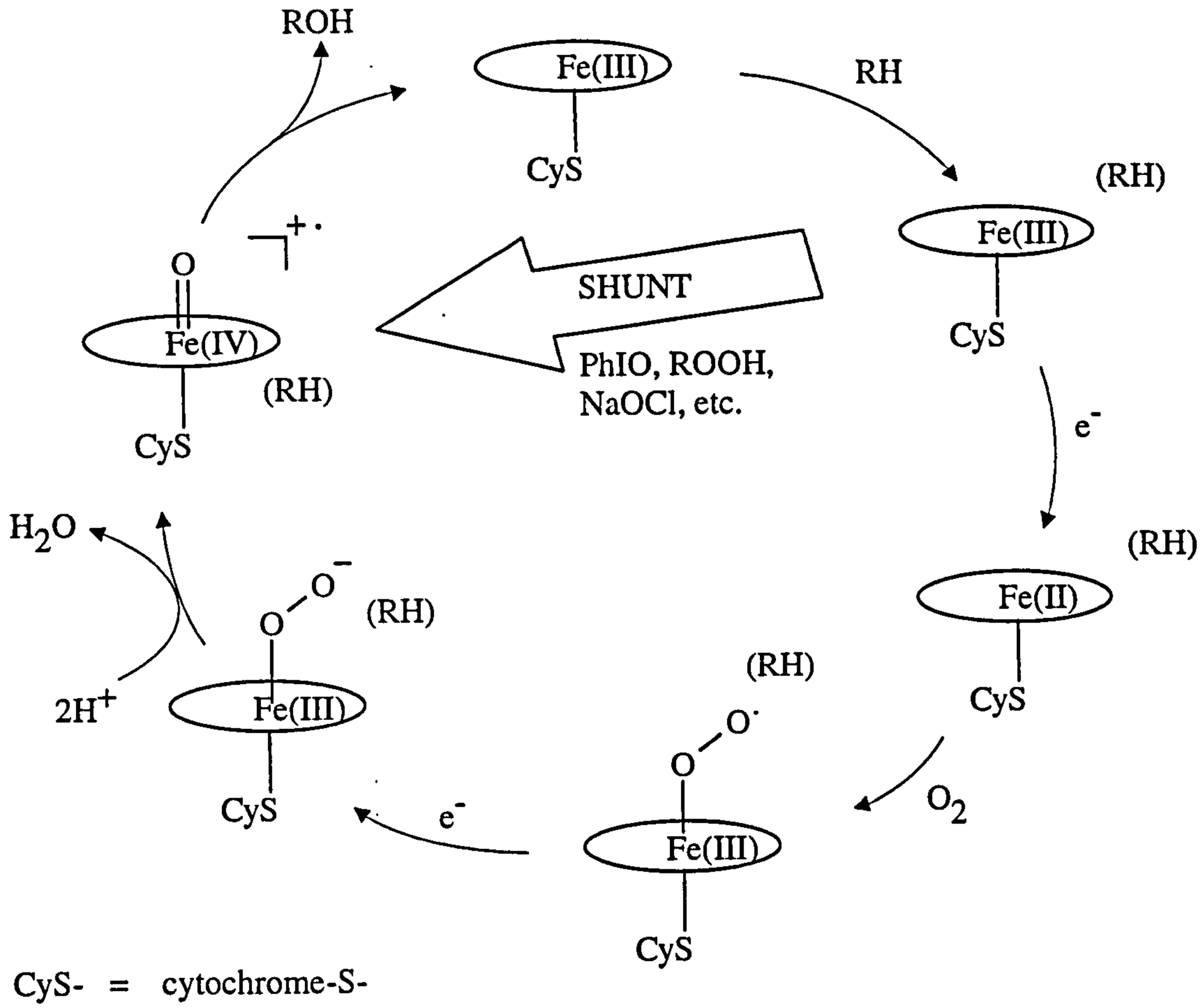
4. Towards the synthesis of a porphyrin capped cyclic peptide

4.1 Oxidations catalysed by metalloporphyrins

Heme enzymes such as cytochrome P-450 monooxygenases are capable of catalysing an array of intrinsically difficult reactions such as the regioselective epoxidation of alkenes and the hydroxylation of alkanes[194]. Their role in nature is to oxidise inert and poorly water soluble substrates such as steroids, fatty acids, leukotrienes and prostaglandins. Cytochrome P-450 monooxygenases are also beneficial for the non-specific oxidation of toxic substances such as chemical carcinogens, enhancing their polarity and solubility and facilitating their excretion[195]. Such enzymes contain a metal ion surrounded by a porphyrin ligand, itself bound inside a protein cavity. As such, the porphyrin ligand acts as the prosthetic group of the enzyme and is the centre at which all the catalytic activity of the enzyme occurs. Frequently, this metalloporphyrin group is bound at the Fe ion by a single *proximal* amino acid residue. The nature of the catalytic activity of the enzyme is governed by the proximal ligand, the distal amino acid residues and the protein itself.

Oxidation occurs in a catalytic cycle in which a single enzyme molecule can catalyse the oxidation of a large number of substrates. The *"turnover"* of substrates, defined as the number of substrate molecules converted to product following catalysis by a single catalyst molecule, can be many thousands. The catalytic cycle is illustrated in Scheme 4.1 and shows that the key intermediate in the cycle is thought to contain a metal atom in a high oxidation state that is bonded to an oxygen atom. This oxygen atom is then transferred to the substrate regenerating the enzyme for another catalytic cycle.

Scheme 4.1 : Catalytic cycle of cytochrome P-450 monooxygenase

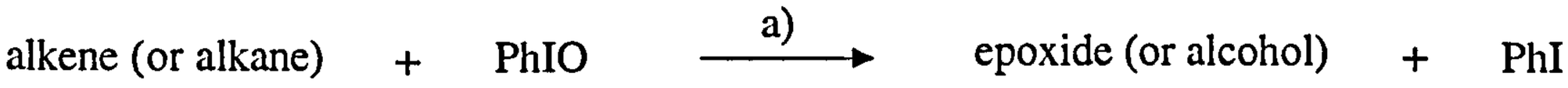


CyS- = cytochrome-S-

Much progress has been made since 1979, in the mimicking of cytochrome P-450 monooxygenases and in the understanding of the nature of their activity[196]. Under anaerobic conditions, it is possible to bypass much of the biological pathway shown in Scheme 4.1 by the addition of oxygen transfer agents or *"shunt reagents"* such as PhIO, H_2O_2, $NaIO_4$, NaOCl and RO_2OH [197]. Such shunt reagents can also be used to catalyse oxygen transfer using synthetic metalloporphyrins. The following discussion will briefly describe a number of oxidation reactions catalysed by metalloporphyrins before giving a short overview of the methods used to synthesise porphyrins and capped porphyrins.

In 1979, Groves published the first paper describing the use of iodosylbenzene as a simple oxygen donor in olefin epoxidations and alkane hydroxylations (Scheme 4.2)[198]. The catalyst used was chloro-5,10,15,20-tetraphenylporphyrinatoiron(III).

Scheme 4.2 : Oxidation of alkanes and alkenes using a porphyrin catalyst and iodosylbenzene



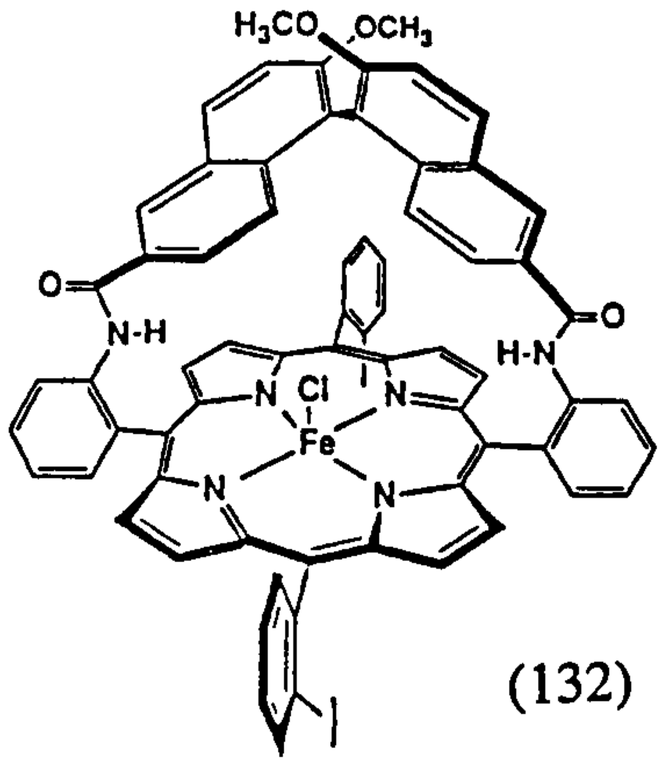
a) chloro-5,10,15,20-tetraphenylporphyrinatoiron (III)

Groves showed that by the use of this porphyrin catalyst cyclohexene could be oxidised to give cyclohexene oxide and the allylic oxidation product cyclohexanol and that adamantane could be oxidised to adamantan-1-ol and adamantan-2-ol.

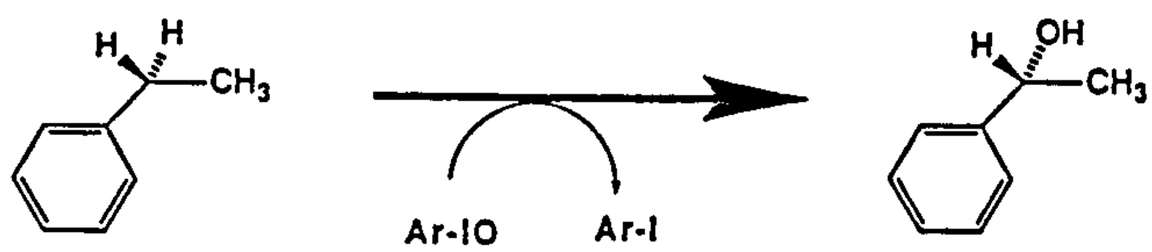
Since this paper was published, much work has been undertaken to study these oxidation reactions and in particular to determine to what extent they can be used to synthesise stereochemically pure compounds[196]. Thus, Groves and Viski reported the use of chiral iron porphyrin catalyst (132) in the asymmetric hydroxylation of a variety of unactivated hydrocarbons (Scheme 4.3)[199,200]. These hydrocarbon substrates are oxidised within the chiral cavity of the catalyst. The chirality of the bridging dinaphthyl group of the catalyst induces chirality in the reaction products. Ethyl benzene gave a 40 % yield of 1-phenylethanol with a 71:29 ratio of the R and S enantiomers respectively - ie 42 % e.e.

Asymmetric epoxidation reactions have also been catalysed by chiral porphyrin ligands incorporating optically active bitetralins and binaphthyls[201-203]. Naruta and Maruyama have synthesised a porphyrin containing four such groups to form a chiral "twin coronet" porphyrin (133). In this system, the bitetralins bridge adjacent *meso*-phenyl groups. Again using iodosylbenzene as the oxygen transfer agent, styrene has been oxidised to its epoxide in 485 turnovers and with 58 % e.e.

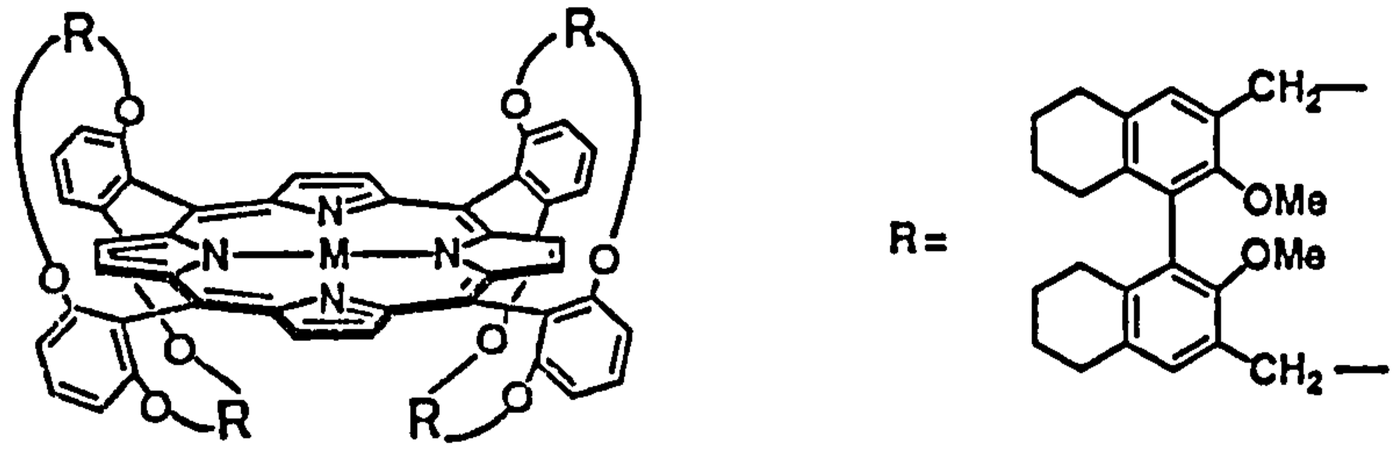
Scheme 4.3 : Oxidation of ethylbenzene using a chiral iron porphyrin catalyst[199,200]



(132)



However, the highest observed enantioselectivities were obtained in the epoxidation of electron deficient alkenes. In the case of 3,5-dinitrostyrene, despite a low turnover of only 36, an e.e. of 96 % was achieved.



(133)

It is hypothesised that the reason for the impressive enantioselectivity of the 3,5-dinitrostyrene is that the substrate is more strongly bound within the cavity than other substrates such as styrene. When styrene and its derivatives are bound within the cavity of the porphyrin catalyst, effective π-π^* interactions can develop with the aromatic rings of the naphthyl groups. These interactions are stronger in the case of the more electron deficient 3,5-dinitrostyrene which it is also suggested has the correct orbital symmetry for efficient π-π^* orbital overlap with the naphthyl groups. With such strong binding, catalysis of epoxide formation can occur with good stereoselectivity.

4.2 Tetraarylporphyrin synthesis

It will be noticed that all of the porphyrin oxidation catalysts above possess *meso*-aryl groups. Indeed, in order to produce a cavity of sufficient size in a porphyrin host and for ease of synthesis, many such catalysts are based upon the 5,10,15,20-tetraphenylporphyrin group.

The synthesis of 5,10,15,20-tetraphenylporphyrin was first reported by Rothemund in 1935[204-207]. In this process, benzaldehyde and pyrrole were reacted together in a sealed bomb at 150^0 C for 24 h. Yields were about 10 % and the conditions employed were so severe that few substituted pyrroles or benzaldehydes could be converted to the corresponding porphyrin[208]. Modifications to these conditions were reported in the 1960s by Adler and Longo[209,210]. By refluxing benzaldehyde and pyrrole in propionic acid for 30 min, a 20 % yield of tetraphenylporphyrin was obtained. With these milder conditions, derivatives of tetraphenylporphyrin have also been synthesised by the use of alternative aromatic aldehydes. Thus, for example, replacing benzaldehyde with 4-chlorobenzaldehyde gives a 20 % yield of *meso*-tetra(4-chlorophenyl)porphyrin.

A key development in tetraarylporphyrin synthesis was reported by Lindsey in 1987[208]. The new reaction conditions reported in the synthesis of tetraphenylporphyrin were based upon three hypotheses:

a) Tetraphenylporphyrinogen was expected to be the thermodynamically favoured product when benzaldehyde and pyrrole were condensed under acidic conditions.

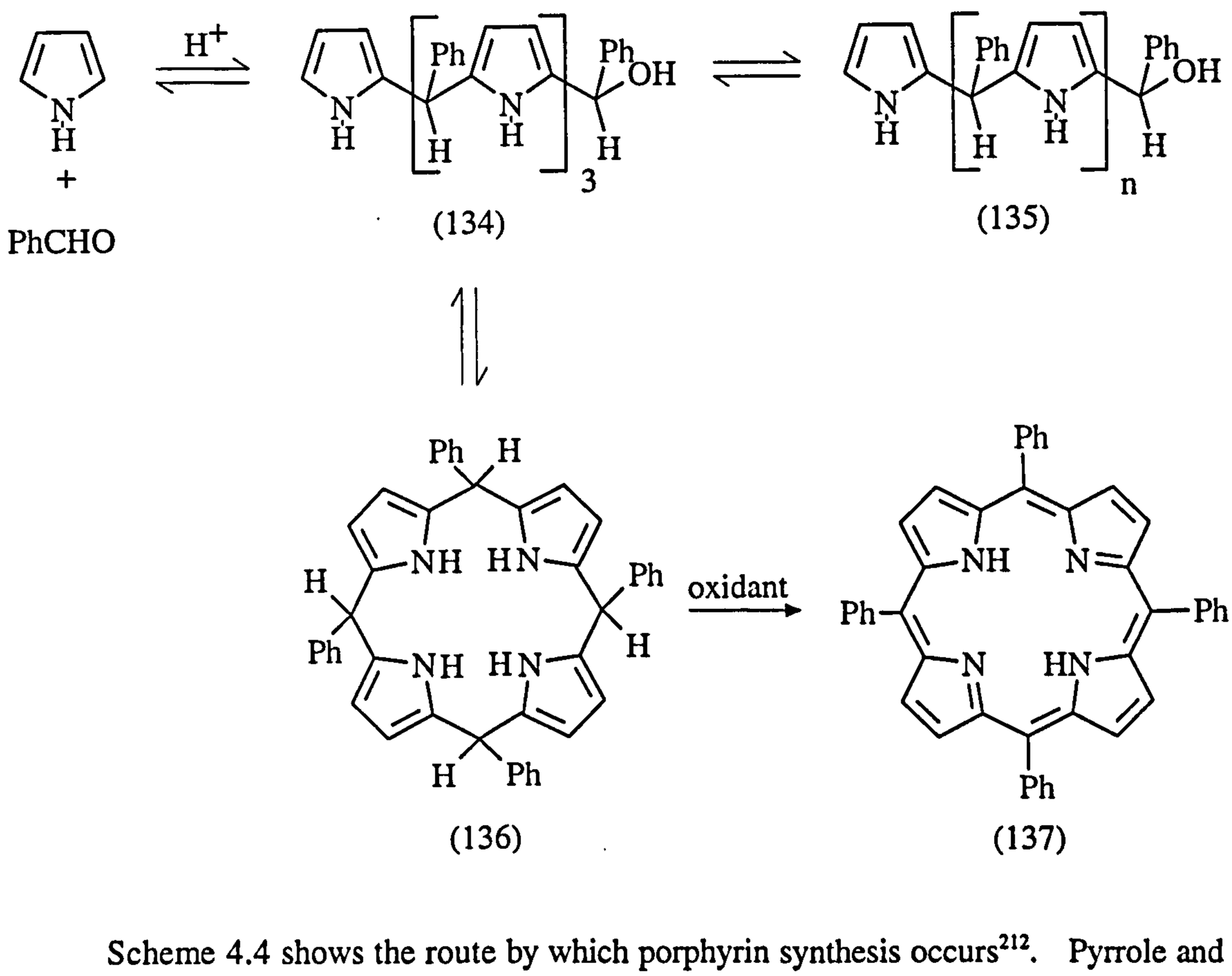
b) Benzaldehyde and pyrrole are such reactive molecules that high temperatures should not be necessary to effect their reaction.

c) By employing mild reaction conditions in their synthesis, a large variety of substituted

porphyrins should be accessible.

Thus by reacting a 1:1 mixture of pyrrole and benzaldehyde with a trace acid catalyst (eg. $BF_3.Et_2O$)[211] at room temperature in chloroform, tetraphenylporphyrinogen was formed. After 1 h, an excess of an oxidant such as tetrachloro-1,4-benzoquinone (TCQ) or 2,3-dichloro-5,6-dicyanobenzo-1,4-quinone (DDQ) was added and the mixture refluxed. This irreversibly converted porphyrinogen to porphyrin. The reaction yield was found to be dependent upon the initial concentration of reactants - a moderate concentration of 10^{-2} M enabled yields of 50-55 % to be achieved.

Scheme 4.4 : Reaction scheme of the Lindsey method of porphyrin synthesis[212]

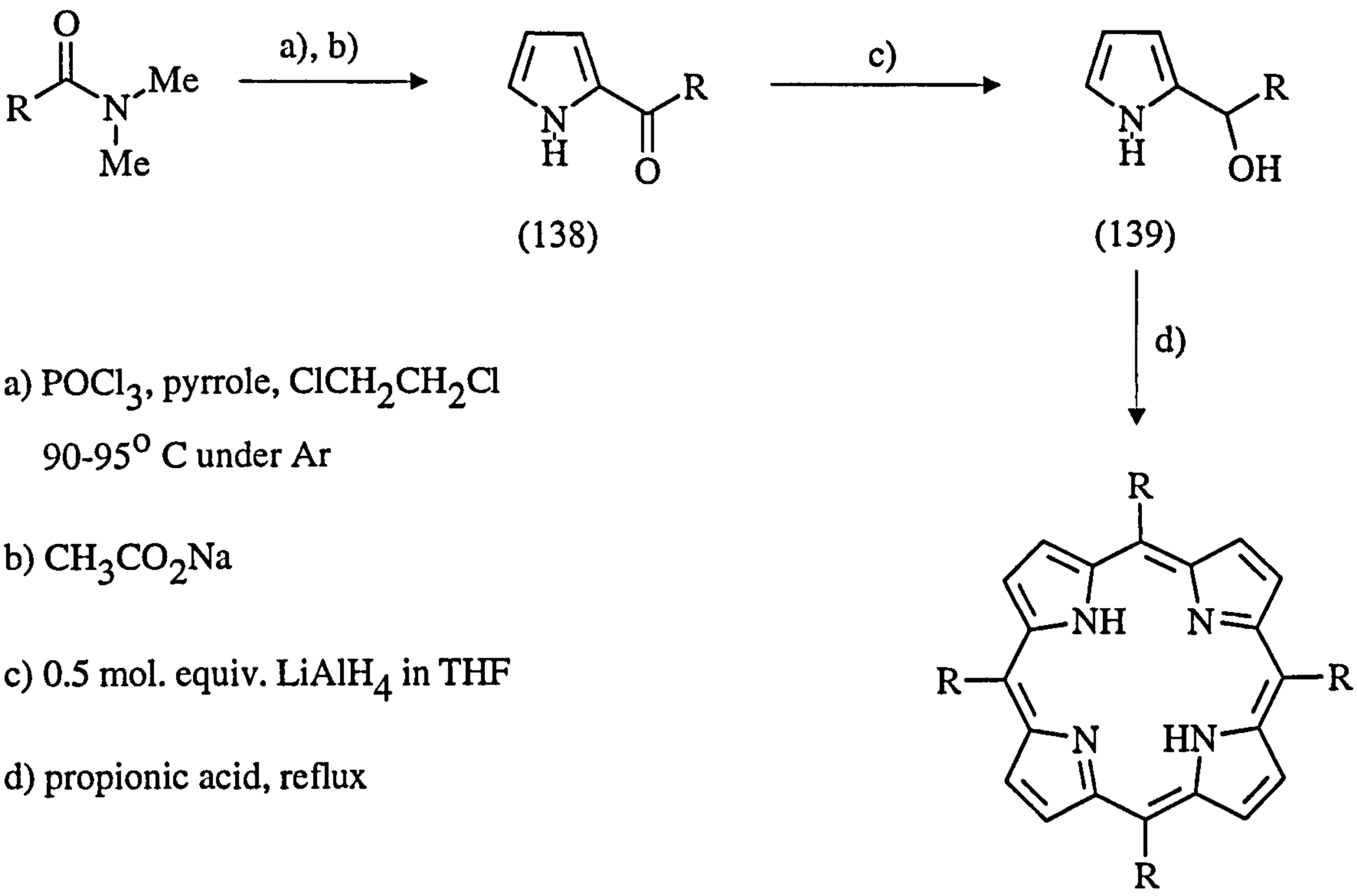


Scheme 4.4 shows the route by which porphyrin synthesis occurs[212]. Pyrrole and benzaldehyde condense reversibly to form straight chain tetrapyrromethane (134), which can either cyclise to the porphyrinogen (136) or continue polymerisation to give higher molecular weight polypyrromethanes (135). Porphyrinogen (136) is oxidised to give porphyrin (137).

The above method of Lindsey is widely used in synthesis[213,214]. Nevertheless, further

advances have been reported. Kuroda has prepared 2-acylpyrroles (138) by heating pyrrole with N,N-dimethylamides at 90-95^0 C [215]. These were reduced quantitatively by a half equivalent of $LiAlH_4$ and the resultant acid sensitive alcohols (139) were refluxed in propionic acid to give the target porphyrin (Scheme 4.5).

Scheme 4.5 : Kuroda's method of porpyhrin synthesis from 2-acylpyrroles[215]

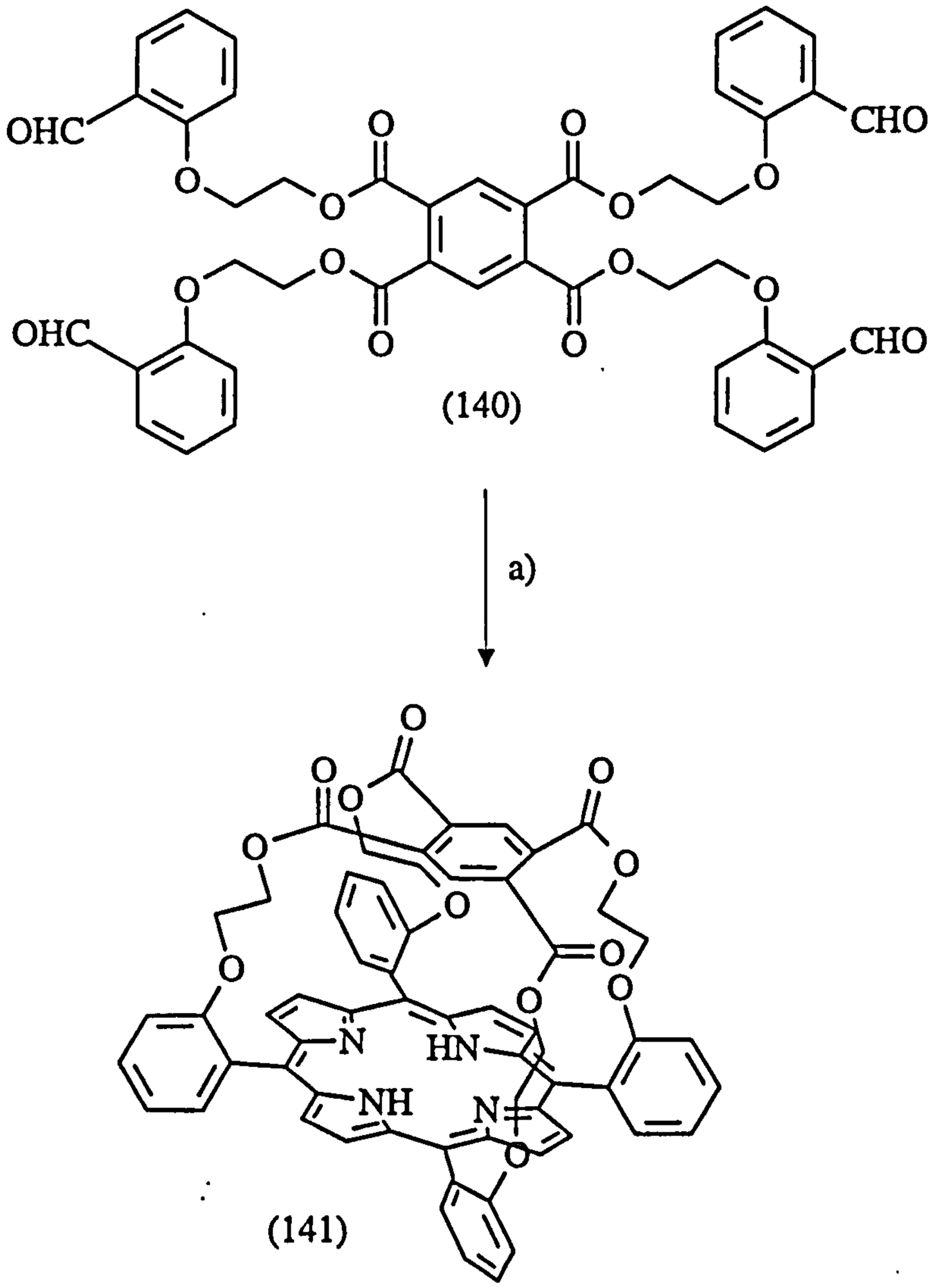


Very recently, a new synthesis of tetraphenylporphyrin has been described in which high-valent transition metal salts (eg. $TiCl_4$, $VOCl_3$, $VO(OEt)Cl_2$, $VO(O^iPr)Cl_2$, $Mn(OAc)_3$) are used as aromatizing agents[216]. Since these species also act as Lewis acid catalysts in porphyrinogen formation, pyrrole and benzaldehyde can in this new procedure be stirred with the above salts to form porphyrin directly. In the case of $TiCl_4$ and the V^V species, reactions are at room temperature. Depending upon the salt used, yields are generally comparable to or higher than those observed with the Lindsey method. In addition, higher yields have been obtained for derivatives of tetraphenylporphyrin such as *para*-methoxy-tetraphenylporphyrin and *para*-bromo-tetraphenylporphyrin.

4.3 Synthesis of capped porphyrins

The Adler-Longo method of porphyrin synthesis described in Section 4.2 was exploited by Baldwin in the synthesis of the capped porphyrin (141)[217,218]. The aldehyde (140) was refluxed with pyrrole for 1.5 h in propionic acid to yield crude porphyrin. Any traces of dihydroporphyrin (chlorin) were oxidised by treating with DDQ. Purification gave a 2 % yield of capped porphyrin (141) (Scheme 4.6)

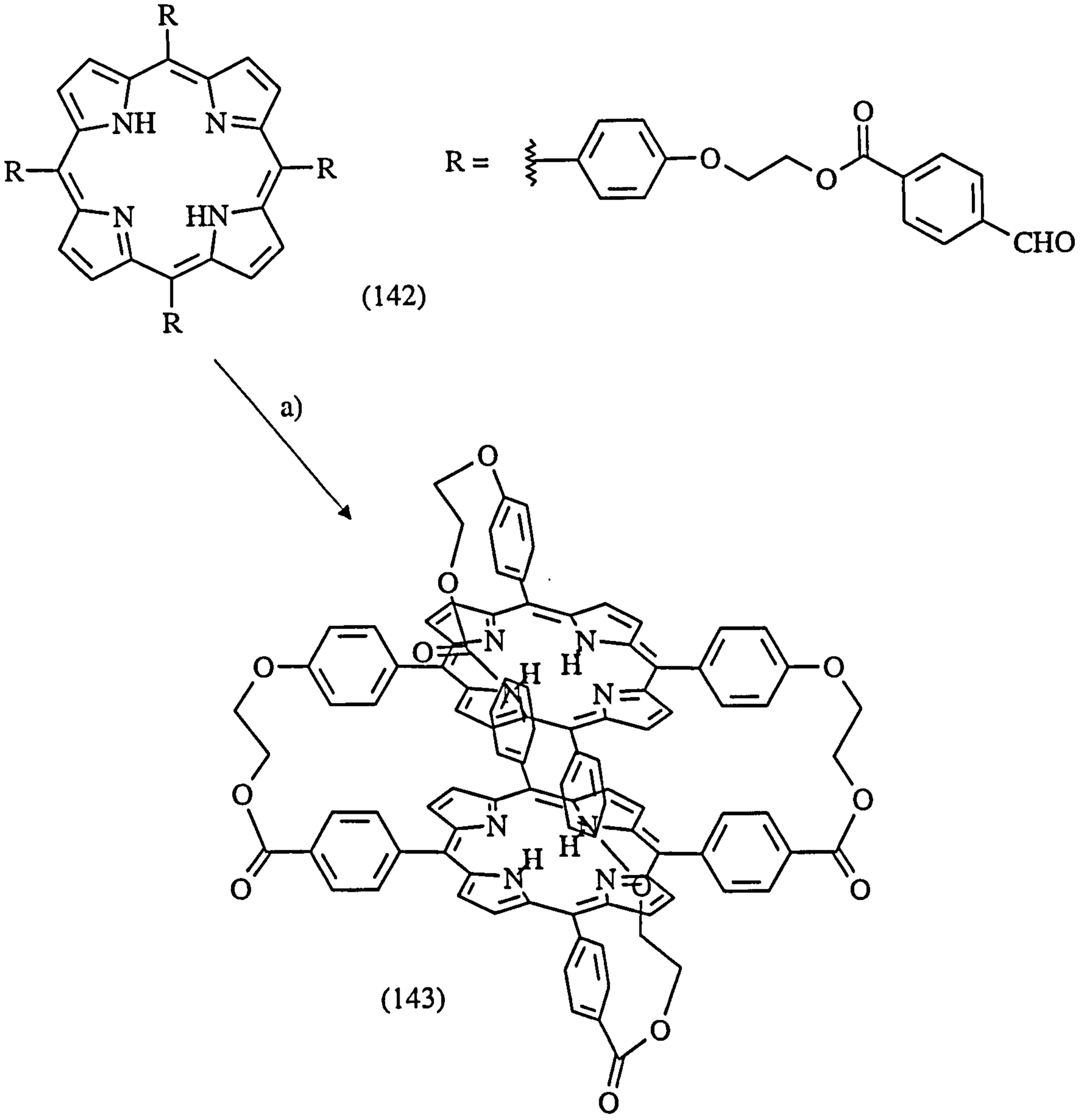
Scheme 4.6 : Baldwin's synthesis of a capped porphyrin[217,218]



a) pyrrole, propionic acid, reflux

Kagan has synthesised a four-fold bridged bis-(5,10,15,20-tetraphenyl)porphyrin by a similar procedure, again using the Adler-Longo methodology[219]. By refluxing tetraaldehyde (142) and pyrrole in propionic acid, a yield of up to 6 % of product (143) was achieved (Scheme 4.7).

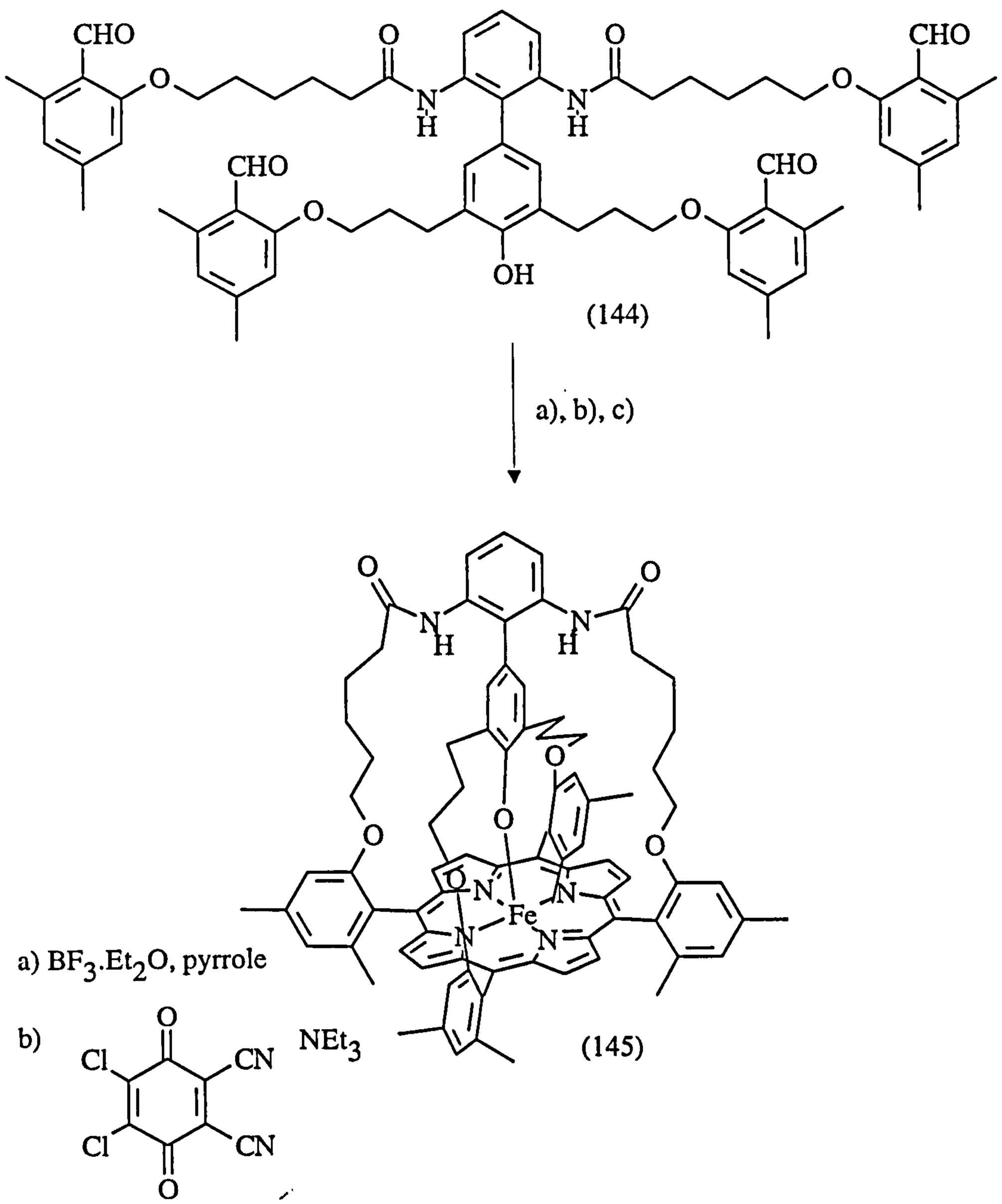
Scheme 4.7 : Kagan's synthesis of a four-fold bridged bis-(5,10,15,20-tetraphenyl)porphyrin[219]



a) pyrrole, propionic acid, reflux

A similar strategy was employed more recently by Bruice in the synthesis of his pendant capped porphyrin (145)[220]. However, refluxing with pyrrole in propionic acid gave only a negligible yield of the desired product. Optimal conditions were found to be based upon the Lindsey method of porphyrin synthesis in which tetraaldehyde (144) was condensed with pyrrole in the presence of $BF_3.Et_2O$ and oxidised using TCQ (Scheme 4.8). Product (145) was isolated in 7 % yield.

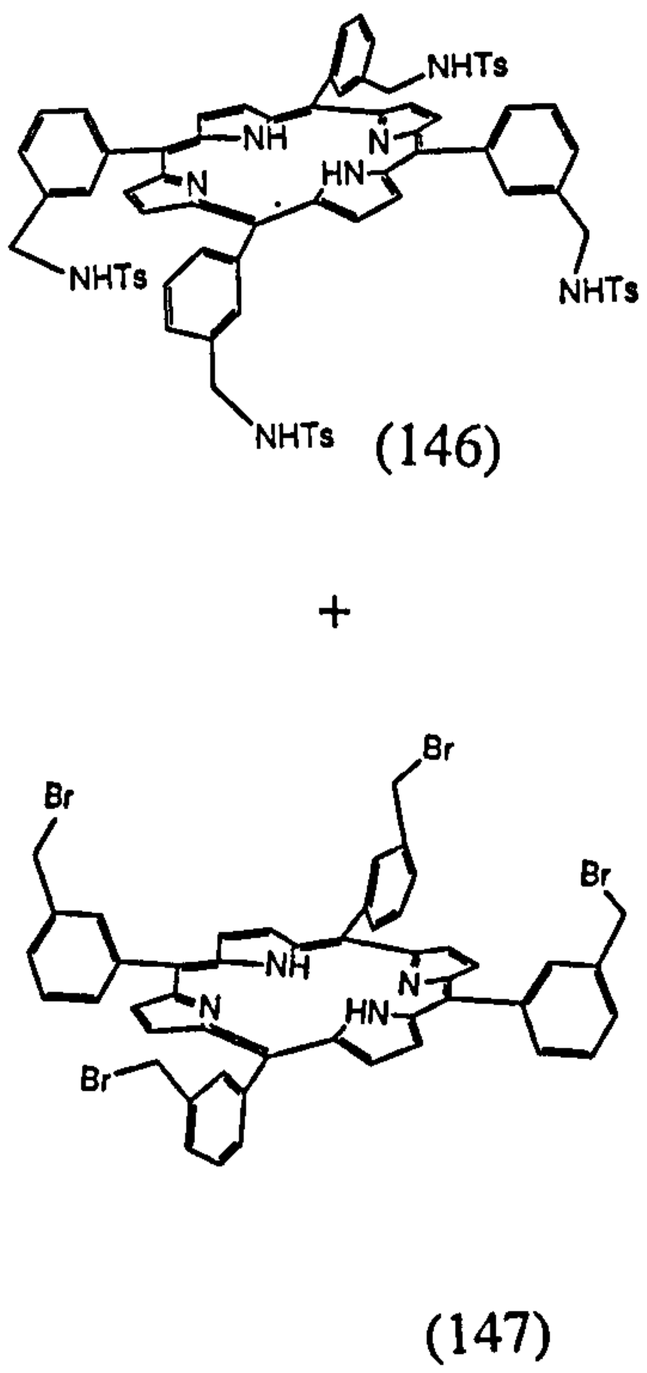
Scheme 4.8 : Bruice's synthesis of a pendant capped porphyrin[220]



Such a pendant capped porphyrin was synthesised in order to mimic iron(III) porphyrin enzymes such as catalase. This enzyme has a phenolic hydroxyl, tyrosine residue, ligated to a metalloporphyrin. The pendant capped porphyrin (145) mimics catalase by featuring a phenolic group ligated *via* its oxygen to the Fe(III) and isolated from the other functional groups by the capping structure.

An alternative, convergent strategy for the synthesis of quadruply bridged co-facial bis-porphyrins has been developed by Bruice (Scheme 4.9)[214]. The two porphyrins (T3-$TsNHCH_2PP)H_2$ (146) and (T3-$BrCH_2PP)H_2$ (147) were synthesised by the Lindsey method of porphyrin synthesis described in Section 4.2 and then coupled in the presence of Cs_2CO_3 under high dilution conditions in DMF (10^{-3} M). The desired dimer (148) was obtained in 38 % yield.

Scheme 4.9 : Bruice's synthesis of a four-fold bridged bis-(5,10,15,20-tetraphenyl)porphyrin[214]

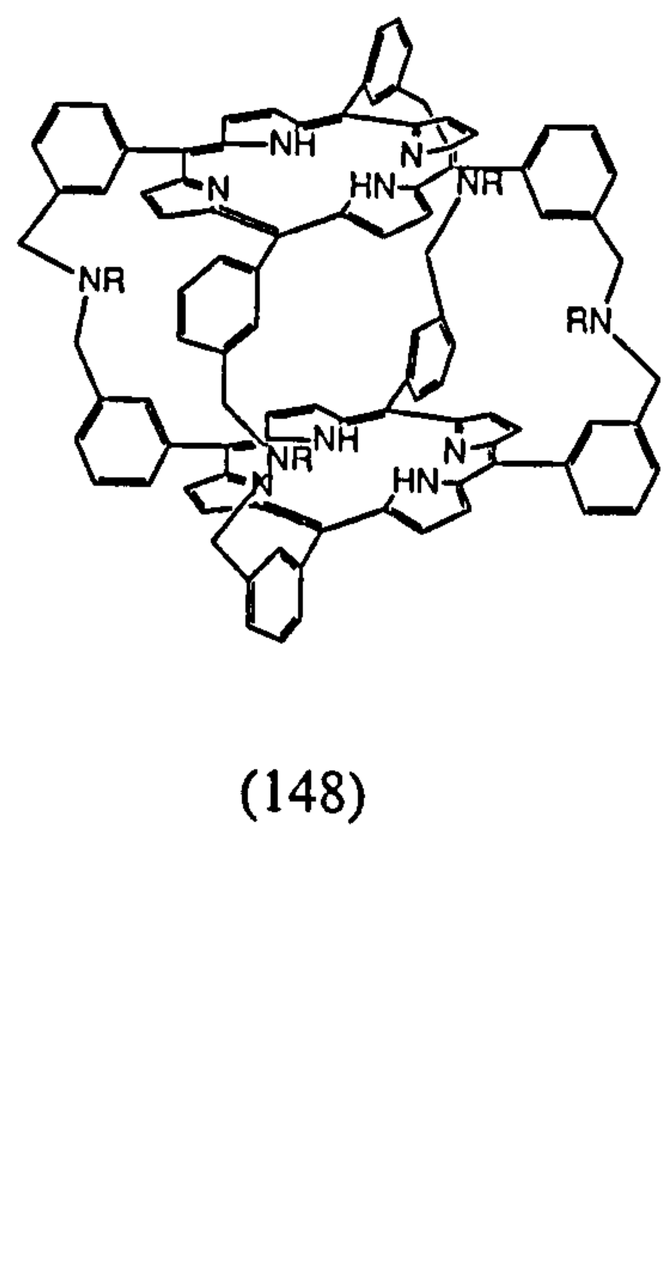


(146)

+

(147)

a)



(148)

a) Cs_2CO_3, DMF, 38 % yield

4.4 Synthesis and derivatisation of porphyrins

Experience gained from the synthesis of capped porphyrins and double-decker porphyrin dimers outlined in Section 4.3 indicated two potential synthetic strategies appropriate to the formation of porphyrins capped with cyclic peptides. These are outlined in schematic form in Scheme 4.10:

a) A convergent strategy in which the cyclic peptide and the porphyrin are synthesised independently. In the presence of an appropriate base, the cyclic peptide and porphyrin can then be added together under high dilution conditions encouraging the efficient closure of the 4 connecting bonds between porphyrin and cyclic peptide. This procedure is related to the synthesis of double-decker porphyrin dimers as described by Bruice[214]. Its main advantage is that if both the cyclic peptide and the porphyrin components can be synthesised in high yield, then only one coupling step is required to afford the target molecule. However, despite the 38 % yield for a related coupling reaction mentioned in Section 2.3, such reactions in which four new bonds are formed would be expected to be highly dependent upon either the correct high dilution conditions or the presence of an appropriate template, if straight chain oligomer and polymer formation were to be avoided.

b) A linear strategy in which benzaldehyde derivatives are coupled to a cyclic peptide to give a tetraaldehyde. Thence, following the procedure of Baldwin in Section 4.3[217,218], this could be treated with pyrrole under Adler and Longo, Lindsey or other appropriate conditions to yield the porphyrin capped cyclic peptide. By following this strategy, the potentially low yielding final coupling reaction of strategy a) above, is avoided. However, it is vital to ensure the pristine purity of the tetraaldehyde intermediate prior to final cyclisation, otherwise during this final "intermolecular" porphyrin forming reaction, any mono-, di- or tri-aldehydes present will encourage intermolecular porphyrin formation, leading to the production of oligomers and polymers.

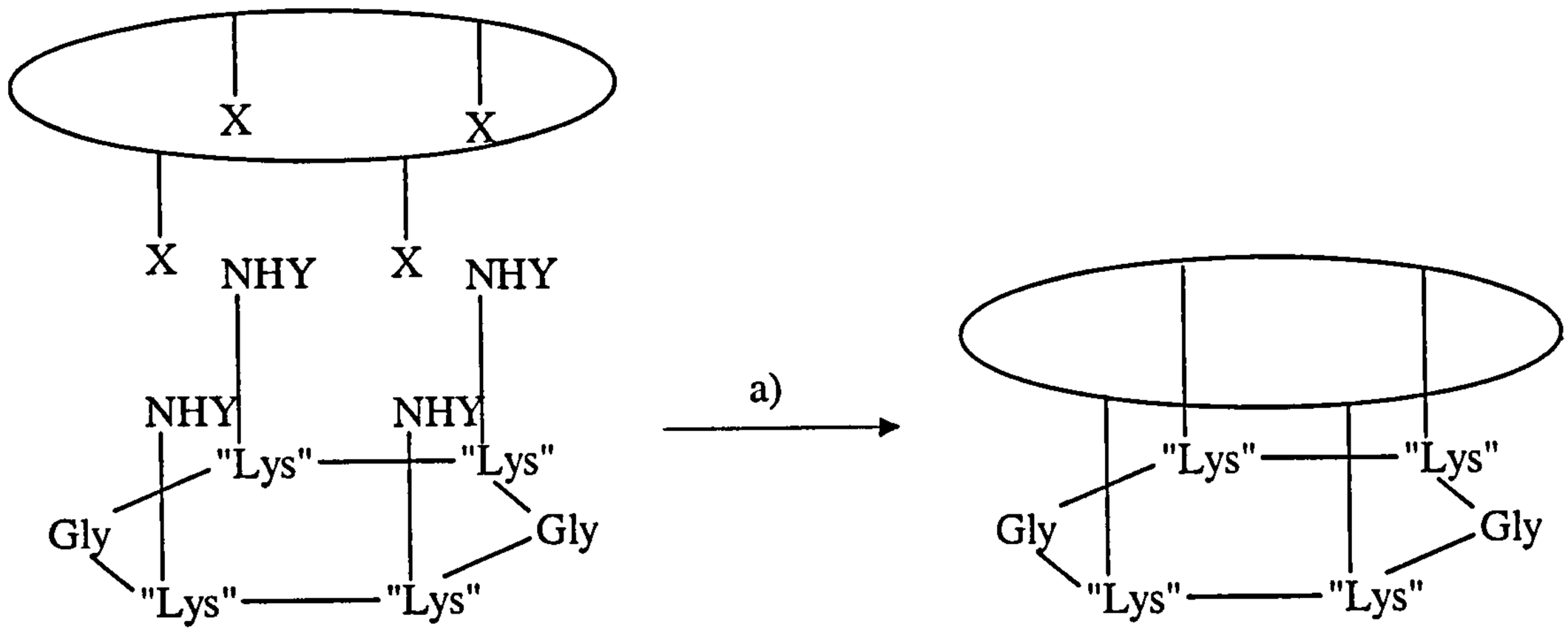
With a view to synthesising a porphyrin capped cyclic peptide by both of these synthetic strategies, the four molecules illustrated in Fig 4.1 were envisaged as being viable targets. It was hoped that following their syntheses and metallation, a proximal ligand such as a chloride ion could be bound to the open face of the porphyrin. Thus a porphyrin cavity would be formed capped by a chiral and - as was seen in Chapter 3 - a rigid cyclic peptide.

The rigidity of the cavity would allow good recognition and strong binding of guests. Because the cavity possesses chirality, it would potentially act as a chiral epoxidation and

hydroxylation catalyst in a manner related to the porphyrin catalysts described above.

Scheme 4.10 : Schematic diagram of the strategy for the synthesis of a porphyrin capped cyclic peptide

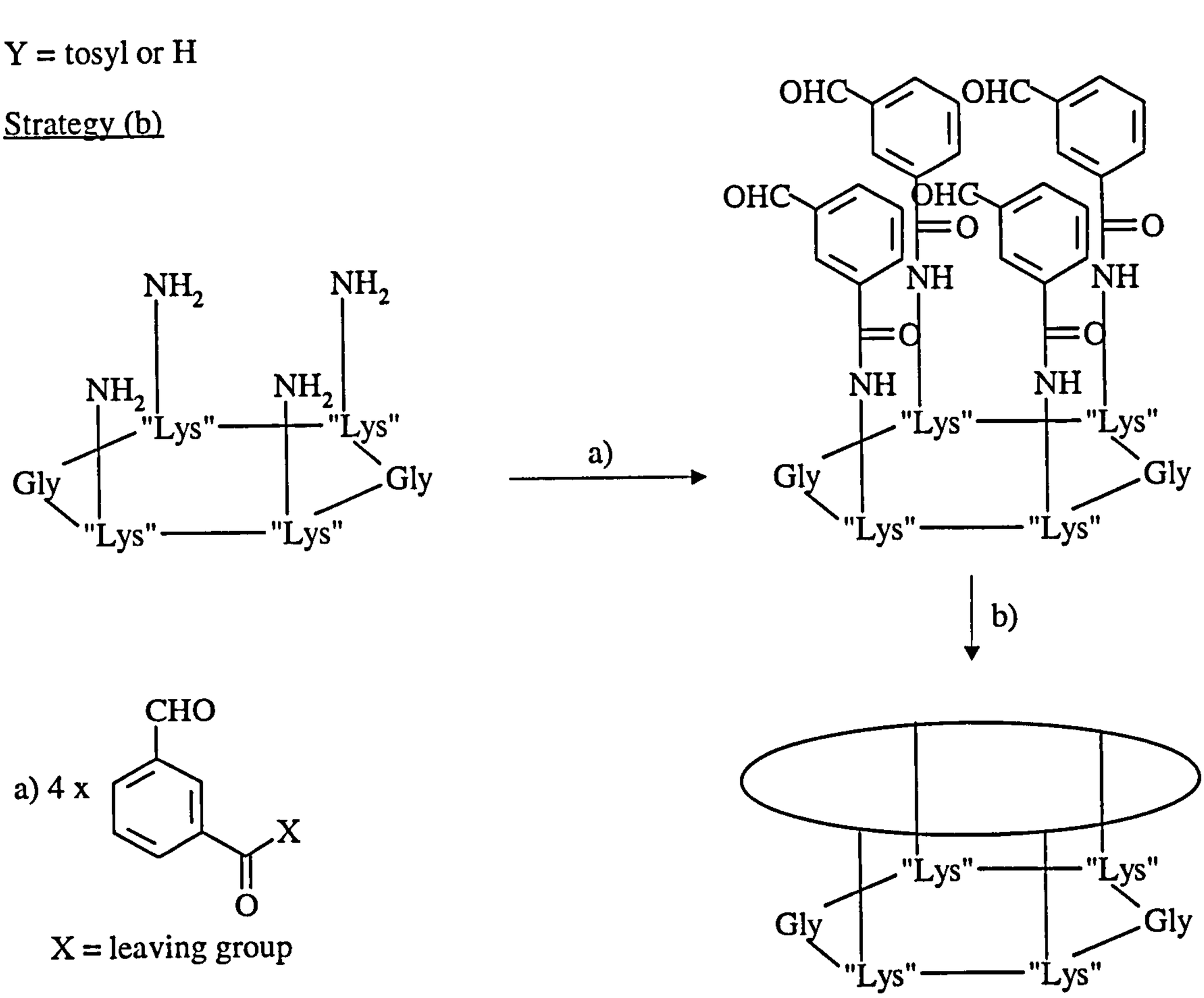
Strategy (a)



X = leaving group

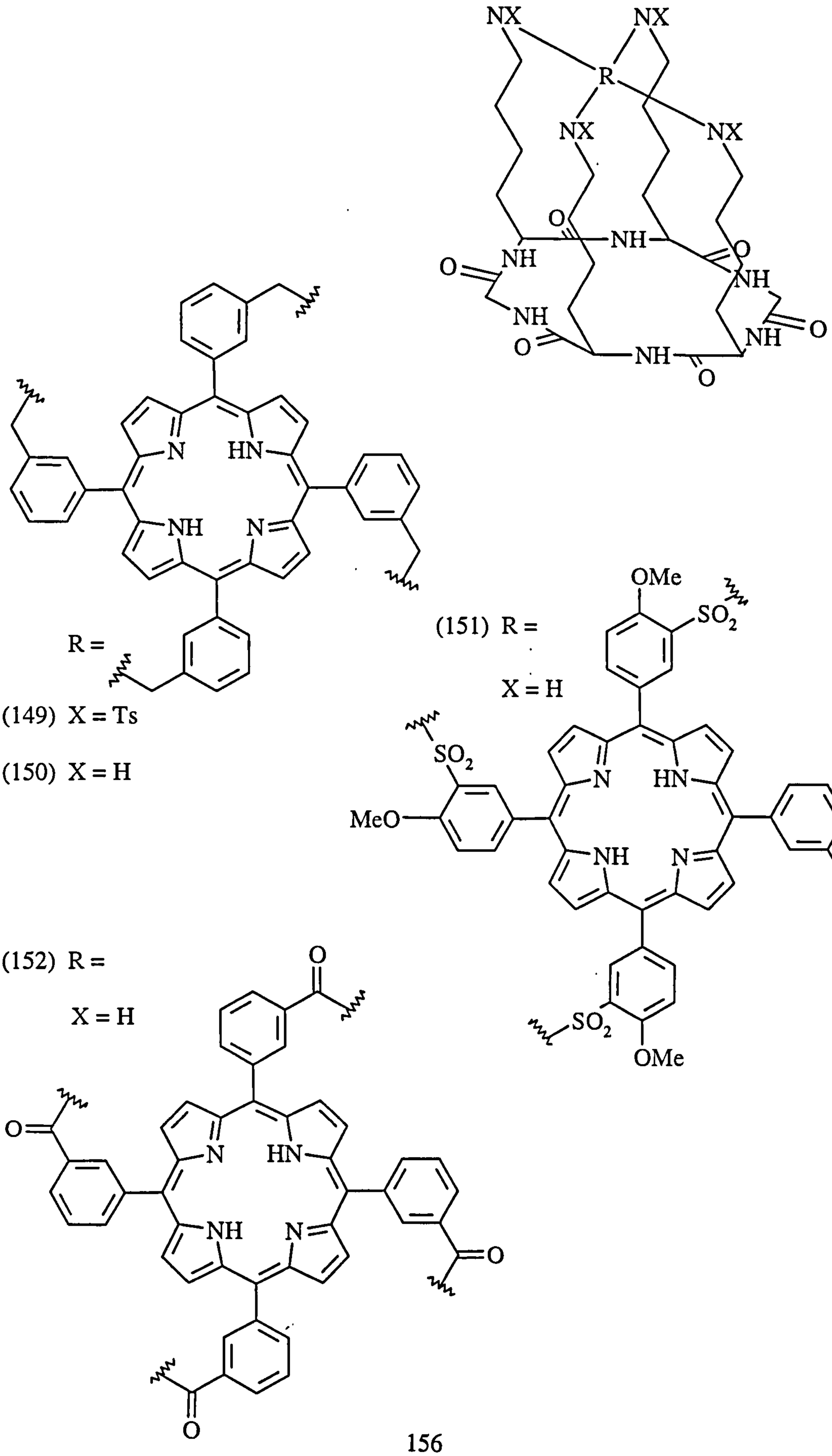
Y = tosyl or H

Strategy (b)



b) pyrrole, acidic conditions

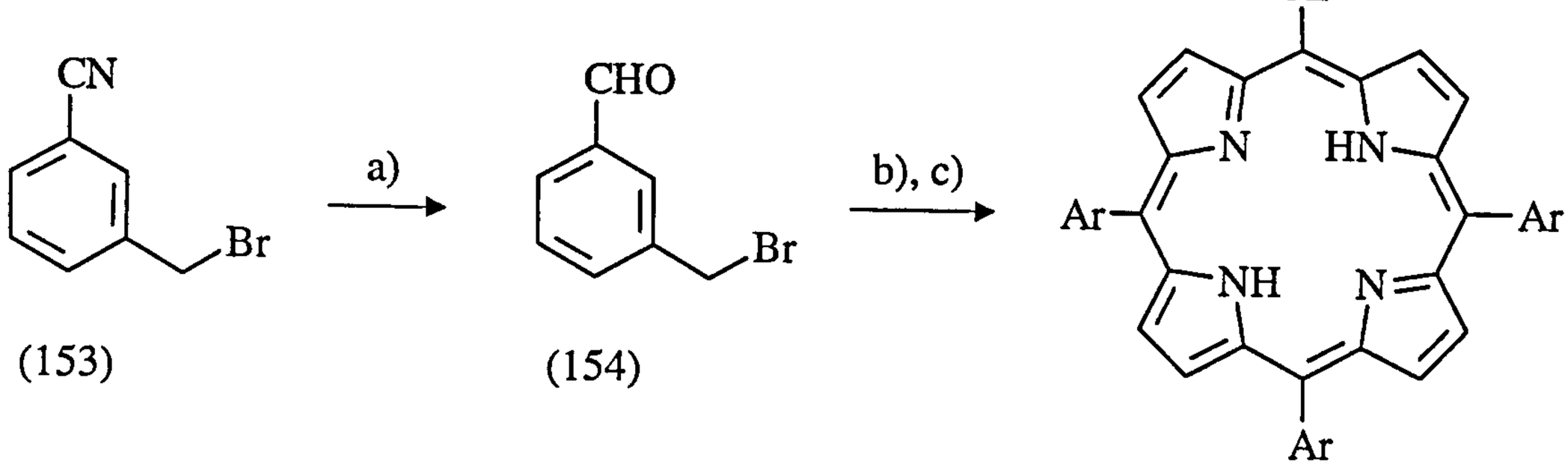
Fig 4.1 : Porphyrin capped cyclic peptide target molecules



4.4.1 Attempted capping of 5,10,15,20-tetrakis(3-bromomethylphenyl)porphyrin

The syntheses of (149) and (150) were attempted *via* the convergent strategy (a). The reaction scheme for the synthesis of (149) is outlined in Scheme 4.11.

Scheme 4.11 : Reaction scheme for the syntheses of the porphyrin capped cyclic peptide molecules (149) and (150)



(153) (154) (155)

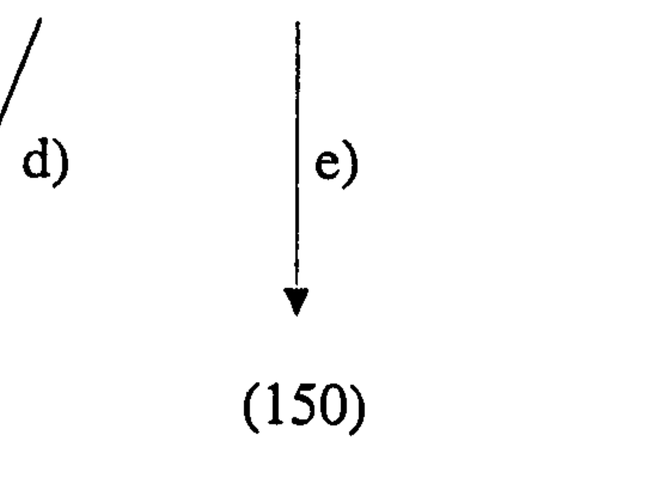
a) DIBAL-H, THF, 0^{o} C

b) pyrrole, $BF_3.Et_2O$, $CHCl_3$, RT

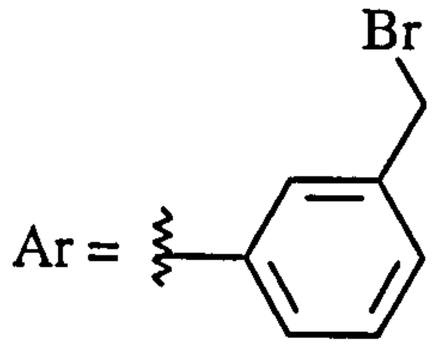
c) TCQ, NEt_3, $CHCl_3$, reflux

d) *cyclo*-[Lys(Ts)-Lys(Ts)-Gly]$_2$, Cs_2CO_3, DMF

e) *cyclo*-[Lys-Lys-Gly]$_2$ (159), DIPEA, DMF

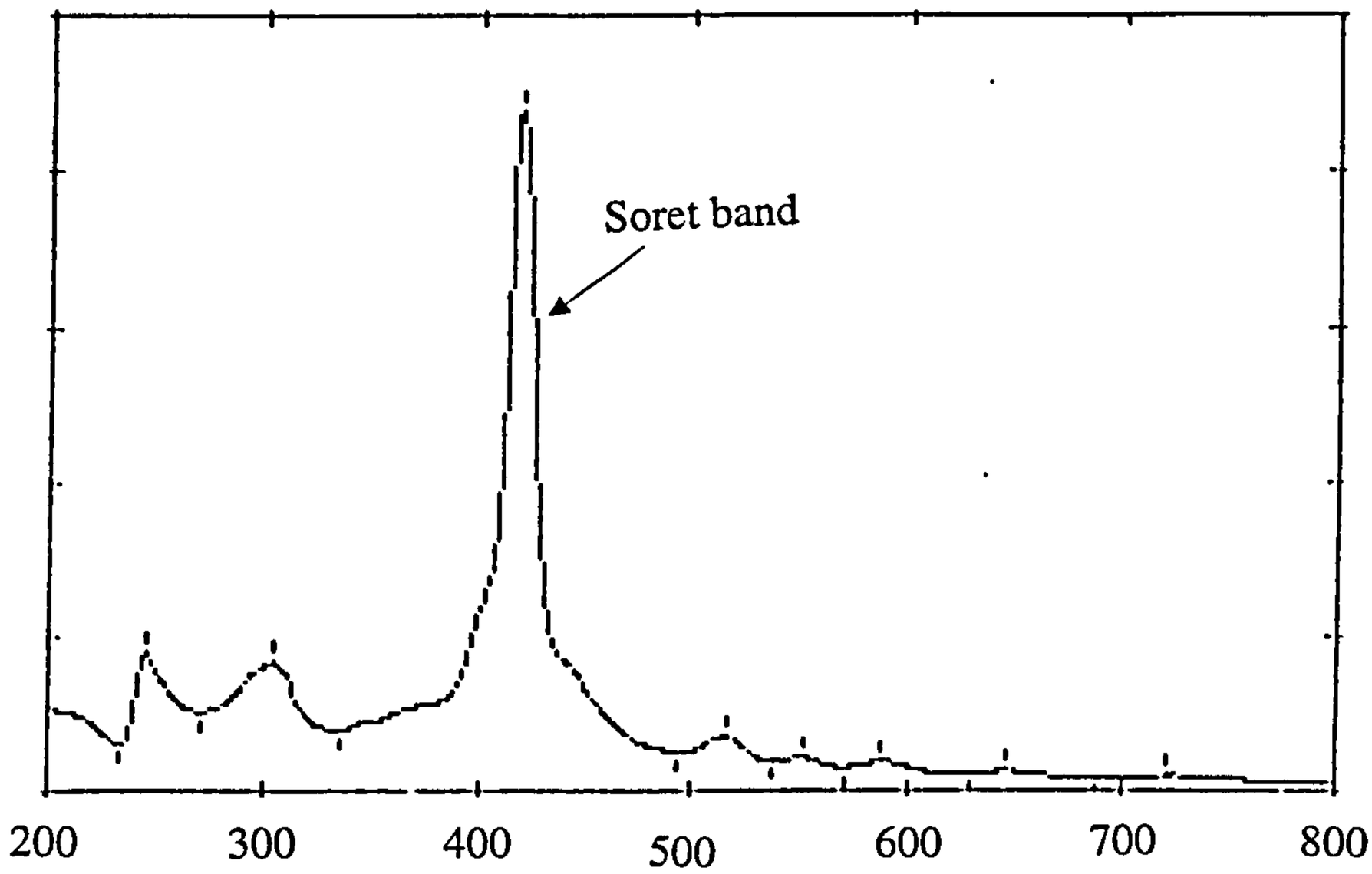


(149) (150)



The first step in the synthesis of porphyrin (155) was the formation of aldehyde (154) as the precursor to porphyrin formation. This was produced by treating nitrile (153) with DIBAL-H. Initially, the solvent used was chlorobenzene as suggested in the literature[214]. However, due to the high boiling point of chlorobenzene (bp = 132^{0} C) and the resultant difficulties experienced in its removal under reduced pressure, dichloromethane (bp = 40^{0} C) was used as an alternative solvent. Using this solvent, aldehyde (154) was isolated in 83 % yield, similar to the 82 % value quoted in the literature[214].

Fig 4.2 : UV-Vis spectrum of porphyrin (155)



An attempt to synthesise porphyrin (155) was made by the Lindsey procedure using aldehyde (154) and pyrrole with $BF_3.Et_2O$ as the Lewis acid catalyst[208, 211, 212]. The mixture was then refluxed using TCQ as oxidant and NEt_3 as base. This procedure followed the literature precedent given by Bruice's synthesis of the identical porphyrin[214]. Evidence for the successful formation of porphyrin (155) was given by the presence of the expected Soret band in the UV-Vis spectrum at 418 nm. The smaller α,β-peaks also had the expected values of 451, 514, 549 and 648 nm. A typical UV-Vis spectrum of the sample is shown in Fig 4.2. In addition, the compound gave the predicted $[M + H]^+$ (m/z = 987) molecular ion in the FAB mass spectrum. A peak at m/z = 907 indicated loss of Br from the molecular ion. However, despite this evidence for its successful production, all attempts at its isolation as evidenced by the TLC and UV-Vis spectroscopy were unsuccessful. The literature procedure[214] suggested a series of solvent extractions using ether and dichloromethane followed by crystallisation of the compound from dichloromethane solution using methanol. This extraction procedure was repeatedly attempted but could not be

successfully reproduced. TLC evidence following each stage of these attempted purifications showed that a large number of products were still present in the sample mixture. Following the attempted precipitation and then filtration of the porphyrin from dichloromethane using methanol, the quantity of porphyrin in the precipitate and the filtrate were calculated. The literature value for the molar decadic absorptivity factor (ϵ) of the Soret band of the porphyrin is $\epsilon = 3.24 \times 10^{-3}$ m^{-1} M^{-1}. Based upon this figure, the concentrations of porphyrin in the precipitate and the solution were calculated as being 30 % and 23 %, respectively - not significantly different from the 33 % observed in the crude reaction mixture. Both precipitate and filtrate show peaks at m/z = 987 in their FAB mass spectra. Varying the ratios of dichloromethane and methanol and cooling the mixture to 0^0 C all failed to precipitate the desired pure porphyrin. Extraction into a selection of other solvents such as benzene, toluene, xylene, acetone, ethyl acetate, isopropanol and ethanol were attempted, but all failed. Similarly, silica gel column chromatography using 5-20 % methanol in dichloromethane as eluant was unsuccessful. Although the desired porphyrin was shown by UV-Vis spectroscopy of the column fractions and the presence of an m/z = 987 peak in the eluted fraction, to be separated from the high proportion of non-porphyrinic baseline material, it could not be separated from other uncharacterised material running closely to it on the column TLC. The use of alternative column chromatography solvent systems such as chloroform : methanol, ether : dichloromethane and ethyl acetate : 40-60 petroleum ether was equally unsuccessful in further separating these closely running compounds.

An attempt was made to test if changing the oxidant in the synthesis from TCQ to the more powerful DDQ oxidant would result in a greater quantity of the desired porphyrin being synthesised. If any of the impurities in the above reactions were chlorins, then hopefully DDQ would oxidise them to the desired porphyrin. It was hoped that with fewer impurities, purification problems would be easier to overcome. In an additional experiment, ethanol (0.75 % v/v) was added with the boron trifluoride etherate catalyst. A literature precedent[212] suggested that the Brønsted acid proton releasing activity of the ethanol, further catalysed the reaction of the aldehyde and pyrrole. However, the literature solvent extraction method[214] described above did not result in successful isolation of the porphyrin following either experiment and the same problems as before were encountered in the column chromatographic isolation of the required porphyrin (155).

In order to determine the reason for the failure to isolate porphyrin (155), it is

important to know the structures of the inseparable impurities in the reaction mixture. However, the use of FAB mass spectrometry and UV-Vis spectroscopy did not allow their characterisation. Other than the assigned peaks at m/z = 987 and m/z = 907, no high mass peaks were consistently observed in the various FAB mass spectra recorded following the different stages of the attempted purification of porphyrin (155).

The synthesis of the cyclic peptide *cyclo*-(Lys-Lys-Gly)$_2$ (159) was more successful. The cyclic peptide *cyclo*-(Lys(Z)-Lys(Z)-Gly)$_2$ in which the ϵ-nitrogens of the lysine side chains were protected by carbonyl benzyl groups had already been synthesised within the group by Dr. John Heffernan[221]. Following its generous provision, it was treated with a palladium on activated carbon catalyst suspended in 2,2,2-trifluoroethanol under a hydrogen atmosphere. The resultant product (159) was isolated in quantitative yield and gave a single spot by TLC - indicating its purity. The solvent system used for this TLC - 7:7:5:1 isopropanol : ethanol : water : ammonia - was used because it had been shown by others in the laboratory to be effective at moving similar deprotected cyclic peptides on silica gel TLC plates[222]. The FAB mass spectrum of this product revealed a significant peak at m/z = 627, corresponding to the molecular ion of the desired compound (159) plus hydrogen. The ^{1}H NMR spectrum of *cyclo*-(Lys-Lys-Gly)$_2$ is illustrated in Fig 4.3. Despite the presence of solvent peaks from 2,2,2-trifluoroethanol and methanol, coupled doublets from the two inequivalent glycyl protons can be seen at δ = 3.6 and δ = 3.95. Although due to the two-fold rotational symmetry of (159), both glycine residues are magnetically equivalent, the two α-hydrogens on each glycine are in different environments due to the chirality of the lysine side chains. Because of these splittings, the fact that all four lysyl side chains are located on one side of the plane of the cyclic peptide can be shown to be the case. In addition, due to their location on a chiral carbon, the α-lysyl hydrogens induce magnetic inequivalence in the two adjacent hydrogens on the respective lysyl side chains. Thus each α-lysyl hydrogen is split into a doublet of doublets rather than the triplet which would have been expected if the α-lysyl carbon had been racemic. These doublets are located at δ = 4.051, 4.064, 4.204 and 4.218. The fact that two such lysyl multiplets are present, giving 4 doublets, is again due to the two-fold rotational symmetry of the molecule. These splittings are clearly observed in the expansion of the ^{1}H NMR spectrum of (159) shown in Fig 4.4.

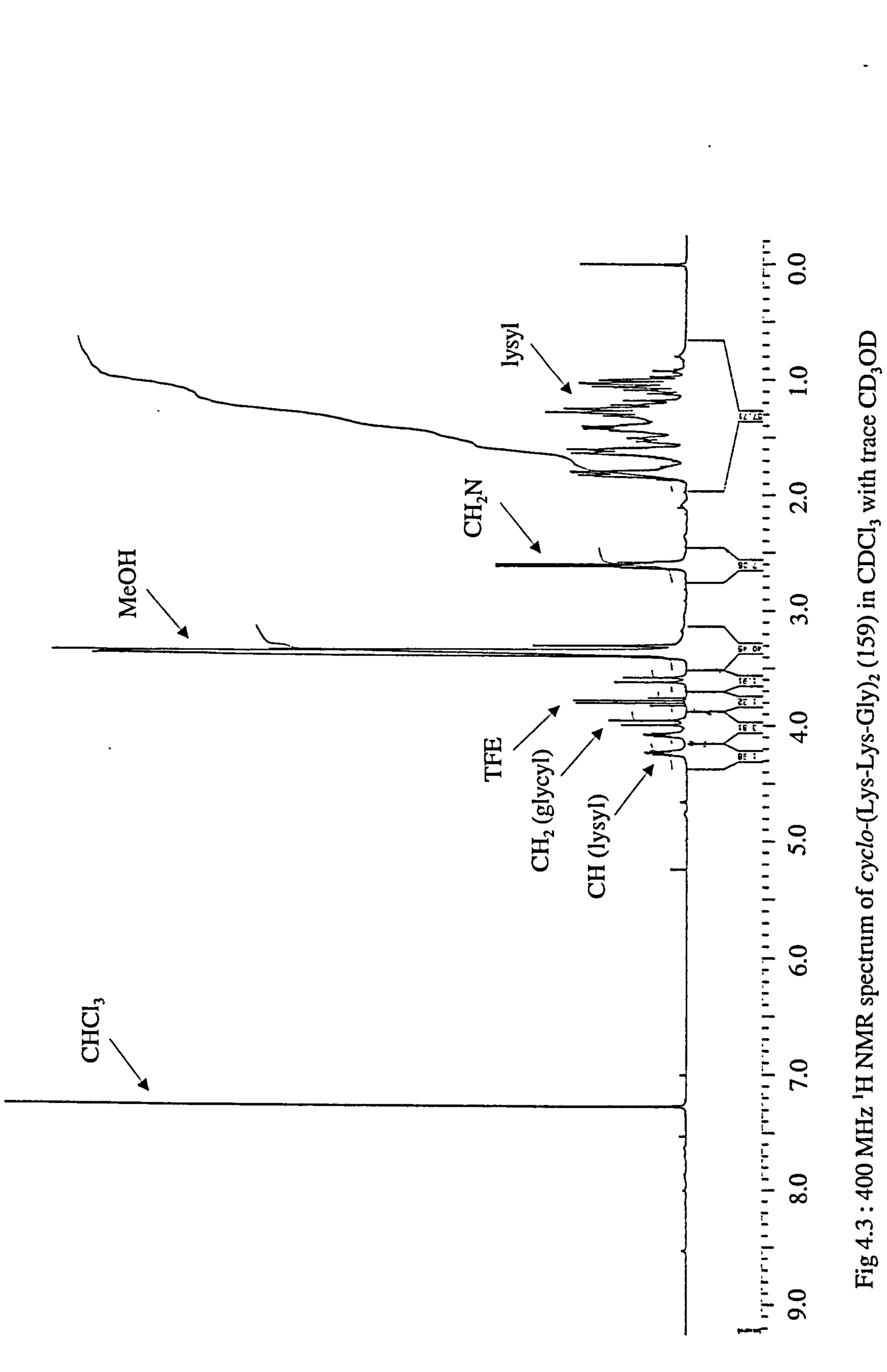


Fig 4.3 : 400 MHz ^{1}H NMR spectrum of *cyclo*-(Lys-Lys-Gly)$_2$ (159) in $CDCl_3$ with trace CD_3OD

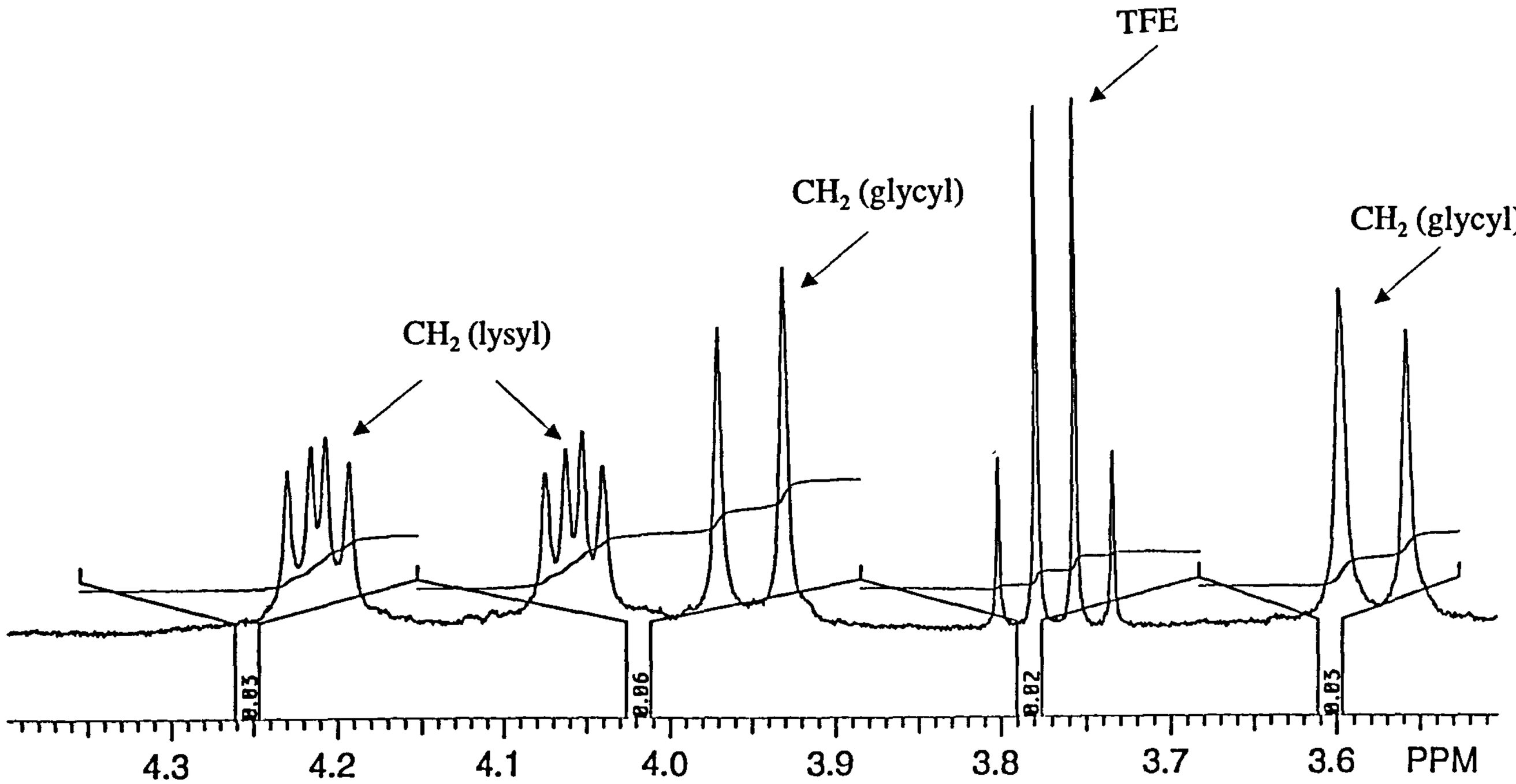
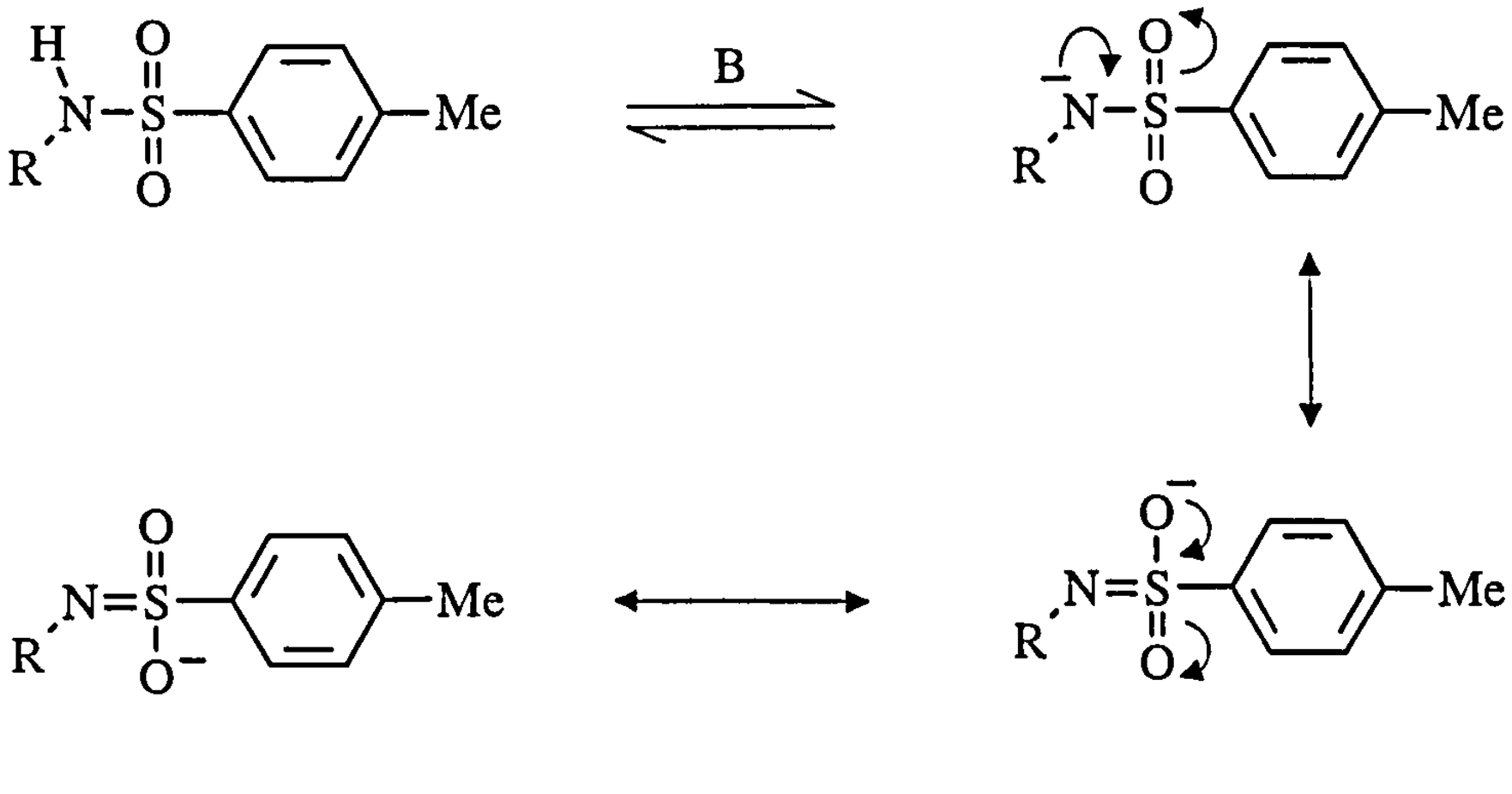


Fig 4.4 : Expansion of the 400 MHz ^{1}H NMR spectrum of *cyclo*-(Lys-Lys-Gly)$_2$ (159) shown in Fig 4.3

Despite the fact that all attempts to isolate porphyrin (155) had failed, 10 mg of authentic (155) was available, having been very generously donated by Professor Bruice in California. With the aim of synthesising (150), 5 mg of this porphyrin was reacted with 1 mol. equiv. of *cyclo*-(Lys-Lys-Gly)$_2$ in the presence of Cs_2CO_3 in 1.5 cm^3 DMF (1 x 10^{-3} M). However, none of the desired porphyrin capped cyclic peptide (150) was detected by FAB mass spectrometry. An ion at m/z = 1289 due to $[M + H]^+$ was expected but not observed. The reaction mixture was dissolved in 3-nitrobenzylalcohol (NOBA)[223] and filtered in an effort to remove any polymeric material. However, likewise, no $[M + H]^+$ ion corresponding to (150) was observed. A possible cause of the failure of this reaction was the inefficient deprotonation of the lysyl primary amines, leading to sluggish nucleophilic attack by these amines on the carbon adjacent to each bromide leaving group (Scheme 4.10). A slow nucleophilic attack would lead to an increased chance of oligomer and polymer formation.

It was felt that a tosyl group might stabilise any anion formed at the ϵ-nitrogen of the lysyl side chains (Scheme 4.12), thus encouraging efficient ring closure reactions.

Scheme 4.12 : Stabilisation of negative charge by the tosyl group



Thus *cyclo*-(Lys(Ts)-Lys(Ts)-Gly)$_2$ was synthesised following a similar cyclodimerisation procedure to that described in Chapter 3. The four steps of this strategy shown in Scheme 4.13, gave an overall yield of this cyclic peptide of 30 %. FAB mass spectrometry gave the expected $[M + H]^+$ peak at m/z = 1243. The ^{1}H NMR spectrum was identical with that of the same compound made by another group member[121].

Scheme 4.13 : Synthesis of *cyclo*-[Lys(Ts)-Lys(Ts)-Gly]$_2$

Boc-Lys(Ts)-Lys(Ts)-Gly-OMe

↓ a)

Boc-Lys(Ts)-Lys(Ts)-Gly-OH

↓ b)

Boc-Lys(Ts)-Lys(Ts)-Gly-OPFP

↓ c)

$CF_3CO_2^-$ $^+H_2$-Lys(Ts)-Lys(Ts)-Gly-OPFP

↓ d)

cyclo-[Lys(Ts)-Lys(Ts)-Gly]$_2$

a) KOH, MeOH, H_2O

b) EDC, PFP-OH, EtOAc

c) 1:1 TFA:CH_2Cl_2

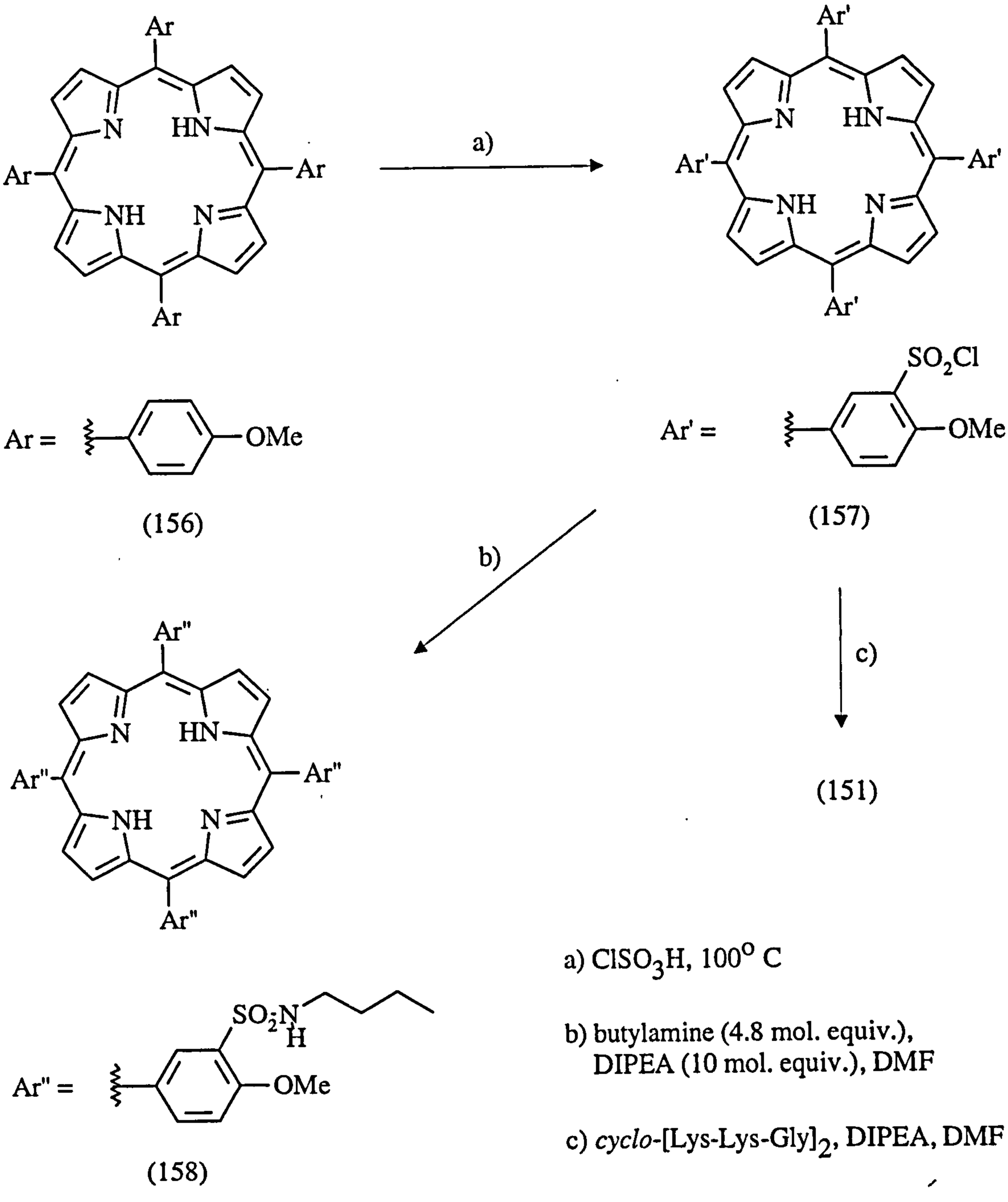
d) Cs_2CO_3, DMF (sufficient for 5mM concentration of activated tripeptide precursor)

In an attempt to form porphyrin capped cyclic peptide (149), 1 mg of porphyrin (155) was reacted with 1 mol. equiv. of *cyclo*-[Lys(Ts)-Lys(Ts)-Gly]$_2$ in the presence of Cs_2CO_3 base. Cyclic peptide and porphyrin were maintained at a concentration of 1 x 10^{-3} M. These were similar conditions to those used by Bruice in the formation of his double-decker dimeric porphyrins described in Section 4.3[214]. However, no peak at m/z = 1609, due to the $[M + H]^+$ peak of the desired porphyrin capped cyclic peptide (149) was detected in the FAB mass spectrum, possibly due to the very small scale of the reaction. Any product formed would be present in extremely small quantities and might be masked by the presence of any polymeric impurities.

4.4.2 Attempted capping of 3-chlorosulphonyl-4-methoxyphenylporphyrin

A convergent strategy was likewise attempted for the synthesis of porphyrin capped cyclic peptide (151). The reaction scheme followed is shown in Scheme 4.14.

Scheme 4.14 : Reaction scheme for the synthesis of the porphyrin capped cyclic peptide (151)



Porphyrin (157) was chosen as a suitable substrate with which to cap the cyclic peptide *cyclo*-(Lys-Lys-Gly)$_2$, for three reasons:

1) The expected ease of formation of a sulphonamide link with the deprotected primary amines of the lysine side chains[224].

2) Methodology was already well established for the formation of the tetrakis-chlorosulphonyl porphyrin[225].

3) The presence of the *ortho-para* directing 4-methoxy group substituted on each *meso*-phenyl group of porphyrin (156) would encourage the substitution of the chlorosulphonyl group in the 3-position. Molecular modelling shows this to be the best substitution for the group to achieve a relatively strain free capping of the cyclic peptide. In addition, because the group would not be *ortho* to the porphyrin ring, the *meso*-phenyl would be freely spinning at room temperature, thus equilibrating any atropisomers[226-228]. The methoxy group would also activate the ring to electrophilic substitution.

Thus porphyrin (156) - purchased from Aldrich - was dissolved in chlorosulphonic acid and was heated to 100^{0}C for 1 h. Following dilution of the acidic solution with ice and water, porphyrin (157) was extracted using dichloromethane. During the course of this extraction procedure it is common for an emulsion to form. It was found to be important that emulsions were broken up using a polar aprotic solvent such as acetonitrile rather than a polar, protic solvent such as ethanol. The use of ethanol was shown by FAB mass spectrometry to lead to the formation of the ethyl sulphonyl ester . The FAB mass spectrum of the desired product showed the expected $[M + H]^+$ peak at m/z = 1129 as well as a fragment ion at m/z = 1029 due to the loss of the chlorosulphonyl group. TLC using dichloromethane eluant showed the compound to be pure with one purple spot present at $R_f = 0.5$. The ^{1}H NMR, although broad, was as expected with a singlet at δ = -2.5 due to the 2 NH protons, a singlet at δ = 4.5 due to the methoxy group, a singlet at δ= 8.5 due to the β-pyrrole protons and the aromatic protons at δ = 7.8, 9.0 and 9.1 with the splittings expected for the phenyl substituents of the desired porphyrin (157).

In order to model the proposed cyclic peptide capping reaction, porphyrin (157) was reacted with butylamine. This amine is related to those of the lysyl side chains in that both are primary amines with a C_4 straight chain moiety. After optimisation, it was found that by stirring 1.2 mol. equiv. of butylamine and 2.5 mol. equiv. of DIPEA (equivalents measured per chlorosulphonyl group), in DMF at room temperature, porphyrin (158) was synthesised in 11.2 % yield following work up and column chromatography (1-5 %

methanol in dichloromethane). Porphyrin (158) gave a $[M + H]^+$ peak at m/z = 1275 in the FAB mass spectrum and as such provided confirmation of the identity of its precursor (157). The ^1H NMR spectrum in $CDCl_3$ was likewise as expected with the anticipated splittings in the aromatic region. Nevertheless, although these splitings were quite distinct, some of the peaks were broader than would be desired. It was thought that this broadness was due to the formation of stacked porphyrin aggregates. Such species would cause line broadening as the magnetic ring current of different porphyrins interacted with each other. Previously, such porphyrin aggregates had been broken up and the ^1H NMR spectra sharpened by the addition of deuterated trifluoroacetic acid to the $CDCl_3$ solvent[229]. The spectrum of the starting material, 4-methoxyphenylporphyrin (156), using this solvent system also gave sharp peaks. In the spectrum of (158), however, although the expected splittings, for example, those due to the methyl groups at the end of the n-butyl side chains, were clear and distinct, those due to the aromatic protons remained relatively broad.

The cyclic peptide used to attempt the synthesis of (151) was the deprotected cyclic peptide *cyclo*-(Lys-Lys-Gly)$_2$ (159), itself produced as described in Section 4.4.1. Cyclic peptide (159) (1 mol. equiv.), porphyrin (157) (1 mol. equiv.) and DIPEA (8 mol. equiv.) were initially stirred together at RT at a concentration of 0.001 M. The lack of a $[M + H]^+$ ion in the FAB mass spectrum showed that this reaction produced none of the desired product. It was felt that if the reaction rate was increased, this would favour porphyrin capped cyclic peptide formation over the formation of polymers. It was hypothesised that once one link between the porphyrin and cyclic peptide had been made, intramolecular substitution would rapidly occur before the resultant desired product could encounter other molecules. Thus, in order to increase this reaction rate, a reaction with similar concentrations of reactants to the above was initiated, with the temperature maintained constantly at 50^0 C. However, this likewise failed to produce the desired product. The FAB mass spectrum of the crude product failed to show the expected molecular ion at m/z = 1609.

In a further reaction, a DMF solution of porphyrin (157) was added by syringe pump to a DMF solution of cyclic peptide (159) (1 mol. equiv.) and DIPEA over 12 h. By this means it was hoped to reduce the concentration of porphyrin with respect to deprotonated cyclic peptide and thus further reduce the likelihood of the formation of polymeric material. The reaction mixture was stirred for a further 2 days at 5×10^{-3} M, but again FAB mass spectrometry failed to show the $[M + H]^+$ ion peak of the desired compound (151). A large

amount of polymeric material is believed to have been formed - there being an intense baseline spot present when the reaction mixture was submitted to TLC with dichloromethane as eluant. This baseline, possibly polymeric material was removed by the addition of 3-nitrobenzylalcohol and filtration of the mixture. However, the remaining material, a faint collection of closely running spots by TLC and amounting to only 4 mg, failed to show any reproducible high mass peaks. Further purification of this material by column chromatography was not considered feasible in view of the small quantities available. In view of the disappointing results described above, it was assumed that none of the required product was present. Due to the absence of any reproducible high mass peaks in any of the capping reactions using porphyrin (157), none of the material produced from the reaction could be characterised.

In the case of all of the above attempted capping reactions, FAB mass spectrometry was a key tool towards detecting (151). However, it is possible that with the large quantities of polymeric material formed, any tiny amount of (151) produced would be masked by these side products. Unfortunately, the removal of polymeric material by the use of NOBA, failed to allow the production of the required molecular ion peak, suggesting that at no stage was the desired porphyrin capped cyclic peptide (151) formed.

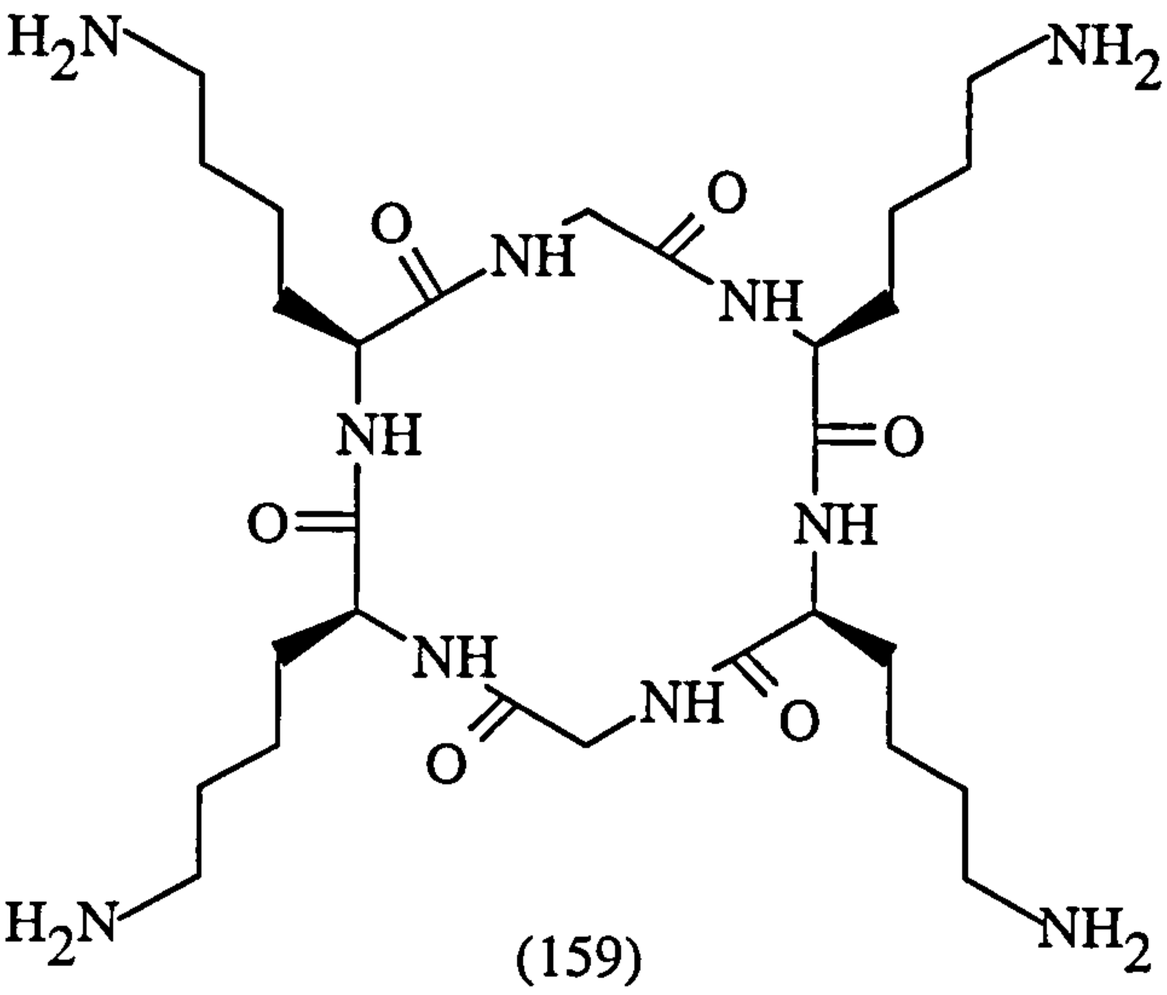
A key reason for the failure of these reactions and also the capping reactions in Section 4.4.1 is possibly due to the rigidity of the porphyrin ring. Because of the rigid, planar nature of the porphyrin ring, the cyclic peptide would need to adopt a precise complementary conformation in order to form any porphyrin-capped cyclic peptide. This would severely reduce the probability of forming such a product. It is also pertinent to note that thus far, no attempt to cap the cyclic peptide *cyclo*-(Lys-Lys-Gly)$_2$ has succeeded[118]. The conformational restrictions required of the cyclic peptide in order to successfully achieve such a reaction may be too great to synthesise a significant amount of the required product.

4.4.3 Attempted porphyrin synthesis from a cyclic peptide tetraaldehyde

In a final attempt to synthesise a porphyrin capped cyclic peptide, the derivatisation of cyclic peptide *cyclo*-(Lys-Lys-Gly)$_2$ (159) was attempted so as to form a tetraaldehyde. Such a product could theoretically be reacted with a pyrrole by an appropriate method of porphyrin synthesis to form the desired porphyrin capped cyclic peptide (Scheme 4.15).

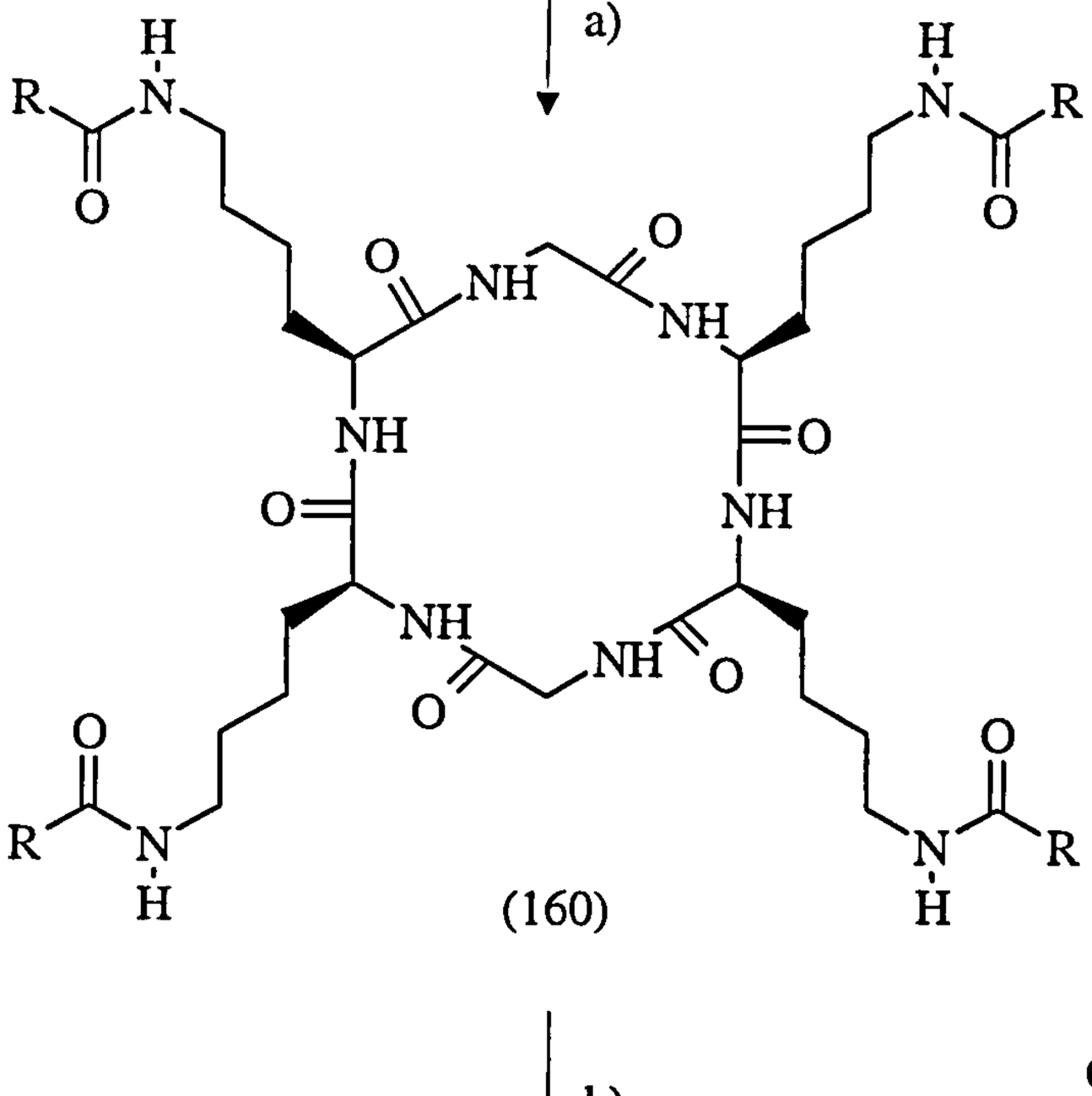
This strategy is analogous to that performed by Baldwin in his synthesis of a capped porphyrin (Section 4.3)[217, 218].

Scheme 4.15 : Reaction scheme for the synthesis of the porphyrin capped cyclic peptide (152)



(159)

a)

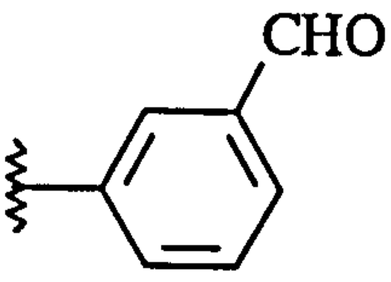


(160)

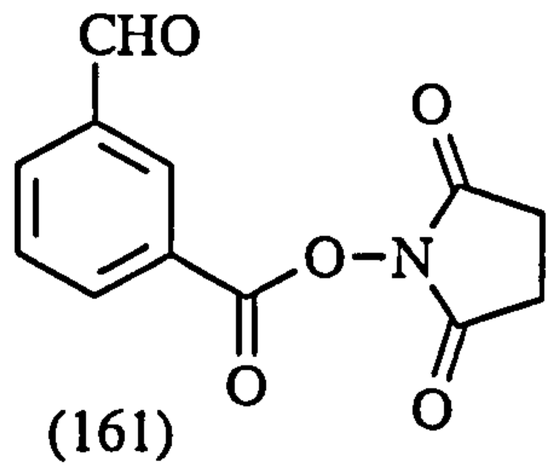
b)

(152)

R =



a)



, DMF

(161)

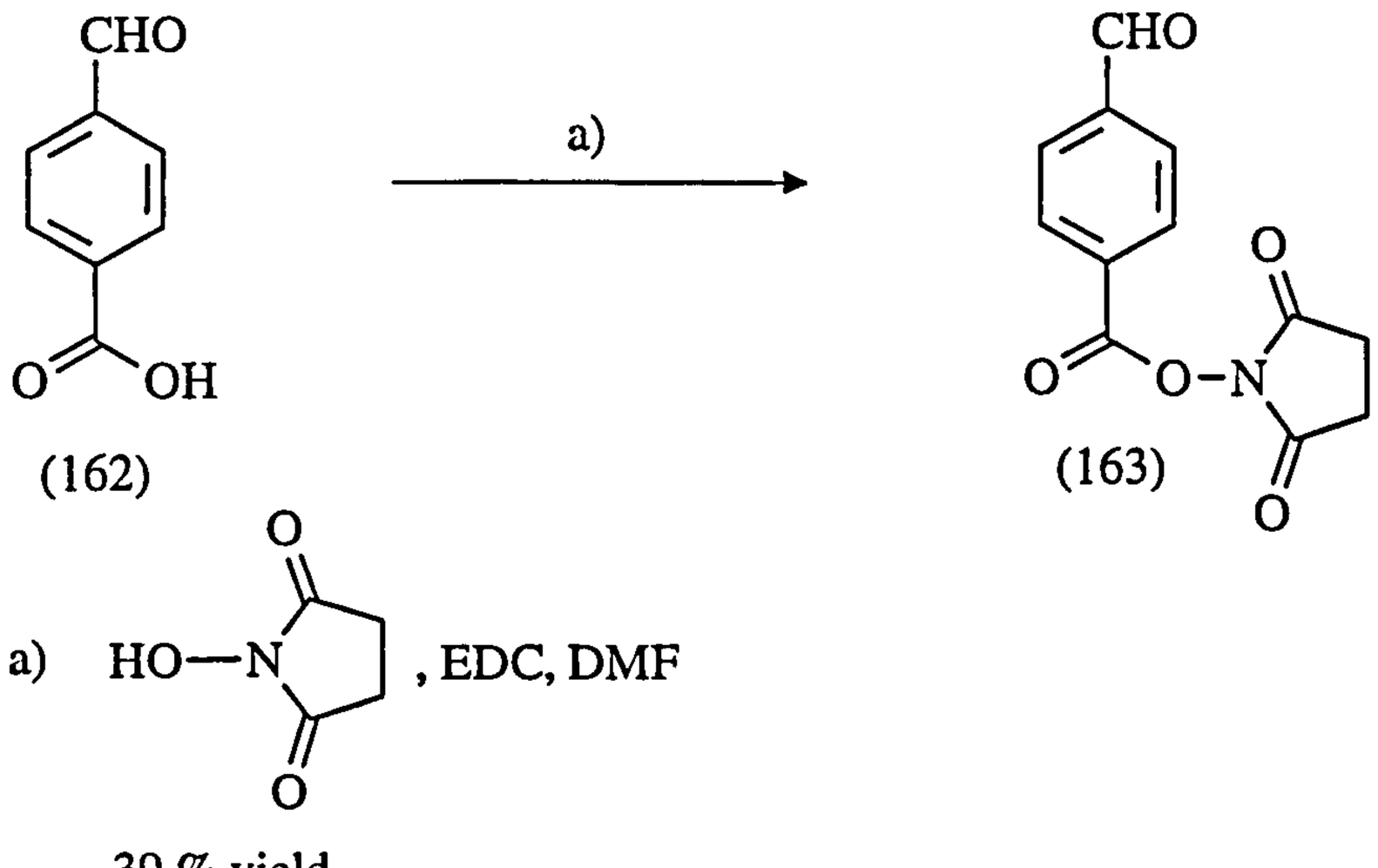
b) propionic acid, pyrrole, reflux

The aldehyde chosen to react with the cyclic peptide and thus form tetraaldehyde (160) was succinimidyl-3-formyl benzoate (161). The succinimidyl ester was found by other members of the group[193] to be a sufficiently active ester to react efficiently with primary amines but not so active as to form significant side products (see Section 2.1.2). The 3-isomer of this compound was used in the synthesis because molecular models indicated that unlike the 2- and 4-isomers, this isomer would encourage the synthesis of porphyrin (152) without undue conformational strain.

Prior to the attempted synthesis of (160), a model reaction was performed in chloroform in which 4.8 mol. equiv. of the cheaper succinimidyl-4-formyl benzoate (163) was reacted with *cyclo*-(Lys-Lys-Gly)$_2$. By using 4.8 mol. equiv. of (163), a slight excess of the reagent corresponding to 1.2 mol. equiv. for each of the cyclic peptide's four primary amines was employed with a view to driving the reaction to completion. No additional base was required as the N-hydroxysuccinimide produced in the reaction acted as the base.

Succinimidyl-4-formyl benzoate (163) had itself been synthesised according to the reaction shown in Scheme 4.16.

Scheme 4.16 : Synthesis of succinimidyl-4-formyl benzoate (163)



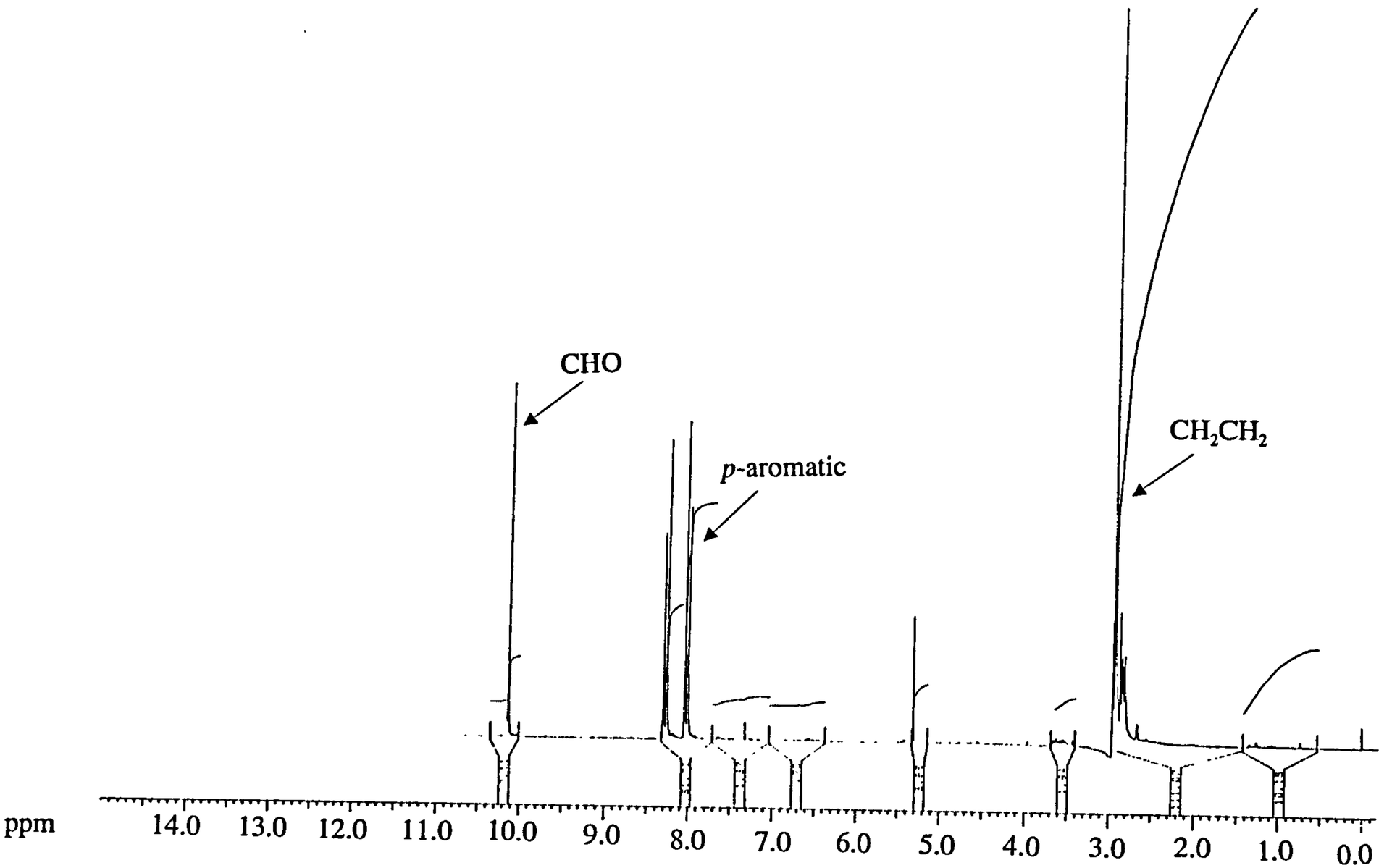


Fig 4.5 : 200 MHz ^{1}H NMR spectrum of succinimidyl-4-formyl benzoate (163) in $CDCl_3$

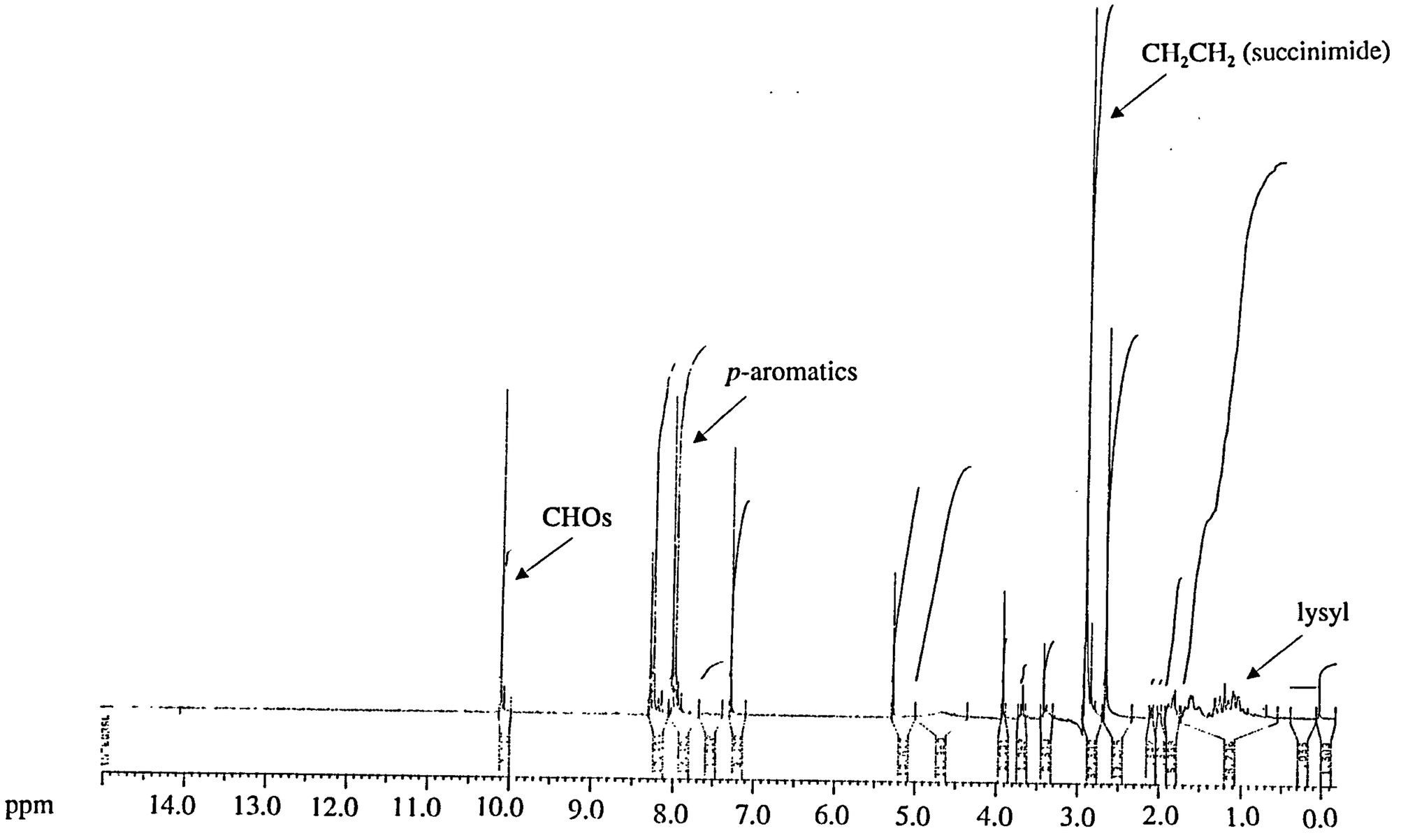
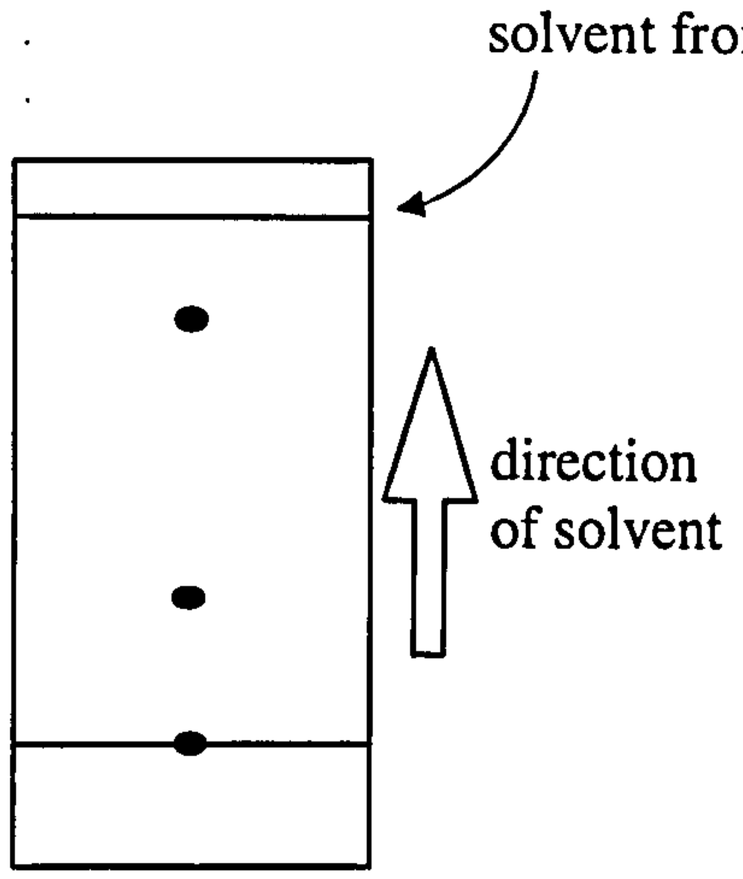


Fig 4.7 : 200 MHz ^{1}H NMR spectrum of the products from the reaction of *cyclo*-(Lys-Lys-Gly)$_2$ (159) and succinimidyl-4-formyl benzoate (163) in $CDCl_3$

Using the peptide coupling reagent, EDC, in DMF, the carboxylic acid group of 4-carboxybenzaldehyde was activated to nucleophilic substitution from the hydroxyl group of N-hydroxysuccinimide. A 39 % yield of the desired succinimidyl-4-formyl benzoate was isolated and was characterised by electron impact (EI) mass spectrometry and ^{1}H NMR spectroscopy. The EI mass spectrum gave the expected molecular ion at m/z = 247 and a larger fragment ion at m/z = 246 due to loss of hydrogen from the aldehyde group to give an acylium ion. A large peak at m/z = 133 was probably due to the acylium ion formed from the loss of the N-oxysuccinimide group. Both the ions m/z = 247 and 246 gave the appropriate accurate mass values. The ^{1}H NMR was as expected for (163) and is shown in Fig 4.5. Singlets due to the CH_2 protons (δ = 2.89) and the aldehyde (δ = 10.06) were present along with the two mutually coupled doublets (δ = 7.96 and δ = 8.22) due to the *para*-substituted aromatic group.

Fig 4.6 : TLC of the products from the reaction of *cyclo*-(Lys-Lys-Gly)$_2$ (159) and succinimidyl-4-formyl benzoate (163) using 19:1 dichloromethane : methanol as eluant



Following the model reaction between (163) and *cyclo*-(Lys-Lys-Gly)$_2$ described above, TLC analysis (19:1 dichloromethane : methanol) indicated the presence of two aldehydic compounds in the product mixture together with aldehydic baseline material (Fig 4.6). The aldehydic nature of all these compounds was determined by staining with 2,4-dinitrophenylhydrazine ethanolic solution. The dominant spot on the TLC plate ran at R_f = 0.8 and also co-ran with succinimidyl-4-formyl benzoate, suggesting the presence of a large quantity of unreacted starting material in the compound. Indeed, FAB mass spectrometry of the crude product showed the presence of a large peak at m/z = 133 due

to (163). The molecular ion and $[M - H]^+$ peaks of (163) at m/z = 247 and m/z = 246 were unfortunately too small to see above the background noise of the FAB mass spectrum. It was hoped that the lower running aldehydic spot at $R_f = 0.3$, which was much fainter than the spot due to (163), would be the desired tetraaldehyde product and indeed a small but distinct $[M + H]^+$ peak at m/z = 1155 was observed in the FAB mass spectrum. The baseline material possibly contained some of the unreacted cyclic peptide tetraamine, although the molecular ion of this was not observed in the FAB mass spectrum of the crude reaction material. In addition, no molecular ions due to the mono-, di- or tri-substituted cyclic peptide were observed.

The ^1H NMR spectrum of the crude product in $CDCl_3$ is shown in Fig 4.7. Two features are notable:

a) Aldehyde peaks are present at $\delta = 10.1$ and $\delta = 10.0$, the former having an integration 8 times that of the latter.

b) Two *para*-substituted aromatic patterns are present. The larger one is centred at $\delta = 8.1$ and the smaller is centred at $\delta = 8.0$. As with the aldehyde peaks, the peaks centred at $\delta = 8.1$ have an integration eight times those at $\delta = 8.0$.

A comparison of the spectrum in Fig 4.7 with that of (163) shown in Fig 4.5, indicates that the larger aldehyde and aromatic peaks in the product ^1H NMR spectrum are due to the starting material (163). It was hoped that the smaller aldehyde and aromatic peaks were due to the desired cyclic peptide tetraaldehyde.

Silica gel column chromatography, with 19:1 dichloromethane : methanol as eluant, was used in the attempt to isolate the desired tetraaldehyde. Unfortunately, separation of the two products was not successfully achieved. A large proportion of both aldehydic products was adsorbed onto the silica and in addition, the small amount of material that was removed from the column was shown by ^1H NMR spectroscopy to contain the same ratio of aldehydic and aromatic peaks as the crude product. Even the use of the polar methanol : liquid ammonia solution as eluant failed to extract this bound product from the column. It is possible that some of the benzaldehyde groups on each compound were autoxidised to carboxylic acids, due to their prolonged exposure to air whilst the column was run. These carboxylic acid groups would potentially bind to the hydroxyl groups on the silica gel. Proof that both aldehydic compounds do slowly bind to the silica gel came from a 2-dimensional TLC experiment, in 19:1 dichloromethane : methanol. The TLC plate was run in the normal way in one direction and then was run a second time at right angles to the

previous direction. Following the second TLC, baseline material from each compound was observed - this material having successfully moved up the TLC plate during the first run.

Altogether, three such model experiments were attempted, two using chloroform as the reaction solvent and a third using DMF. However, in all these reactions, similar results and problems to those described above were encountered and the cyclic peptide tetraaldehyde required was not isolated.

Despite the failure to isolate the cyclic peptide derivative of (163) described above, an attempt was made to synthesise and isolate the cyclic peptide tetraaldehyde (160). A batch of succinimidyl-3-formyl benzoate (161) had been synthesised within the group[193] and following its generous provision, this was used to attempt the preparation of (160).

In the first attempt to prepare cyclic peptide (160), *cyclo*-(Lys-Lys-Gly)$_2$ (159) was reacted with 4.8 mol. equiv. of (161). The solvent used was d_7-DMF. After stirring the mixture for 3 days under argon, the ^{1}H NMR spectrum of the crude reaction mixture showed, as with the model reactions, two aldehydic peaks at δ = 10.0 and δ = 10.1. However, unlike the model reactions, the peak at the lower chemical shift, δ = 10.0, was larger than that at δ = 10.1 by a factor of 3. This suggests that, if the peaks are assigned in the same way as in the earlier reactions described above, with the peak at δ = 10.0 being due to the cyclic peptide tetraaldehyde and that at δ = 10.1 being due to the starting aldehyde (161), the reaction equilibrium has been driven further towards the products. However, because the δ = 10.0 peak is due to a species containing four aldehydic groups, there is still a larger amount of (161) than (160).

The reaction TLC, using 19:1 dichloromethane : methanol as eluant, was similar to those of the model reactions, in that two aldehydic spots at R_f = 0.8 and 0.3 as well as an aldehydic baseline spot were present. Again, because the reaction was incomplete, it was assumed that any cyclic peptide starting material (159) or mono-, di- or tri-substituted cyclic peptide would remain on the TLC baseline due to their possession of polar primary amine groups.

An attempt was made to isolate the desired tetraaldehyde from the starting material (161) by washing the mixture three times with toluene. It was previously observed that (161) could be dissolved in toluene and it was assumed that in a similar manner to other peptidic compounds, cyclic peptide (160) would remain insoluble in toluene. Unfortunately, this attempted purification method was unsuccessful. The TLCs of the toluene washings as well as the product residue, showed that both aldehydic compounds at R_f = 0.8 and 0.3

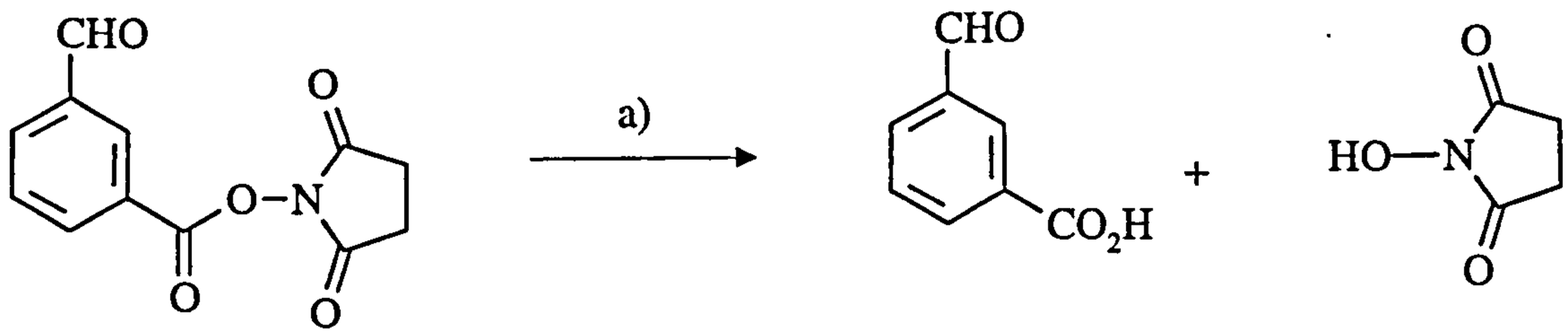
had been removed by the toluene. An attempt to drive this reaction to completion by the addition of caesium carbonate base was likewise unsuccessful. It was thought that the base would deprotonate the primary amines of the cyclic peptide (159), enhancing their nucleophilicity and encouraging their reaction with (161). This reaction was allowed to stir under argon for 2 days. However, the reaction mixture TLC showed no significant depletion of the spot at $R_f = 0.8$ due to (161) with respect to that at $R_f = 0.3$. Likewise, following removal of the base by filtration, the ratios of the aldehydic and aromatic peaks in the ^{1}H NMR remained unaltered.

In a second attempt to prepare cyclic peptide (160), *cyclo*-(Lys-Lys-Gly)$_2$ (159) was again reacted with 4.8 mol. equiv. of (161). However, unlike in the earlier reaction, the solvent used was $CDCl_3$, with 5 mol. equiv. of DIPEA base added to try to drive the reaction to completion.

Unfortunately, the ^{1}H NMR spectrum, on the basis of the aldehydic peaks at $\delta = 10.0$ and $\delta = 10.1$, again suggested the presence of both the desired product (160) and the aldehydic starting material (161). However, unlike the earlier attempt to prepare (160), the integrations of the peaks were equal, suggesting a reduction in the amount of (160) prepared. Despite the presence of extra base, the reason for this is possibly due to reduced polarity of the $CDCl_3$ solvent compared to d_7-DMF, reducing the likelihood of the polar by-product N-hydroxysuccinimide from forming.

A repeated attempt to remove the starting material by washing with toluene was, as before, unsuccessful. Aqueous sodium hydrogen carbonate solution was used to try to selectively remove starting material. It was hoped that by using this basic aqueous solution, the ester bonds of the starting material would be hydrolysed (Scheme 4.17). The acidic products from this hydrolysis, 3-carboxybenzaldehyde (164) and N-hydroxysuccinimide (165) would, hopefully, be soluble within the $NaHCO_3$ solution and would be selectively removed from a solution of cyclic peptide in dichloromethane by a simple aqueous wash. The amide bonds of any (160) that had been formed would be stable to this weak aqueous base.

Scheme 4.17 : Hydrolysis of succinimidyl-3-formyl benzoate (161)



(161) (164) (165)

a) HCO_3^- (aq)

The reaction mixture was washed three times with saturated sodium hydrogen carbonate solution for 45 min, after a previous experiment had shown that it takes such a length of time for (161) to dissolve in this solution.

Following the aqueous wash, however, TLC (19:1 dichloromethane : methanol) of the residual product material as well as TLC of the aqueous washings showed that all the material had remained on the baseline and that the spots at $R_f = 0.3$ and 0.8 had been lost. ^1H NMR spectroscopy in $CDCl_3$ (with a trace of CD_3OD to aid solubility) confirmed that most of the aldehydic material had been lost. It is not clear why this occurred, but it is possible that whilst in solution the benzaldehyde derivatives were autoxidised to benzoic acid derivatives. The high polarity of this material would explain why it remained on the baseline of the TLC plate. The lack of carboxylic acid peaks in the ^1H NMR would be explained by the presence of CD_3OD in the ^1H NMR solvent.

A reaction was tried with remaining unpurified tetraaldehyde (160) to see if any of the desired porphyrin capped cyclic peptide (152) could be formed despite the presence of the starting aldehyde (161). It was felt that because the formation of (152) involves the linking of aldehydes on the same molecule ("intramolecular") as opposed to the intermolecular porphyrin synthesis involving (161), if the reaction was performed at high dilution, some of the desired porphyrin (152) might form. Thus 50 mg of the mixture of tetraaldehyde (160) and aldehyde (161) were heated in the presence of 12 mg of pyrrole in 5 ml propionic acid to 141^0 C. Unfortunately, the lack of a Soret band at about 420 nm in the UV-Vis spectrum, showed that no porphyrin had been formed.

As a result of these failures and due to lack of time, the attempt to synthesise the porphyrin capped cyclic peptide by the above procedure was likewise abandoned.

4.5 Summary

None of the target molecules (149) to (152) were successfully synthesised. In the case of (149) and (150), this was due to problems encountered in the purification of the required porphyrin precursor (155). With (151), failure was due to unsuccessful capping of the cyclic peptide by the porphyrin (157). Synthesis of (152) failed due to problems in the purification of the required tetraaldehyde precursor (160).

CHAPTER 5:

EXPERIMENTAL

5. Experimental

5.1 General

5.1.1 Instrumentation/physical methods

NMR spectra were recorded at the University of York using Bruker MSL 300 (300 MHz, ^{1}H; 75 MHz, ^{13}C), JEOL EX 270 (270 MHz, ^{1}H; 67.5 MHz, ^{13}C), JEOL FX90Q (90 MHz, ^{1}H; 22.5 MHz, ^{13}C), Bruker WP80SY (80 MHz, ^{1}H) or Varian EM360A (60 MHz, ^{1}H) spectrometers. At Heriot-Watt University, NMR spectra were recorded using Bruker DPX400 (400 MHz, ^{1}H; 100 MHz, ^{13}C), Bruker AC200 (200 MHz, ^{1}H; 50 MHz, ^{13}C), Bruker WP200 (200 MHz, ^{1}H; 50 MHz, ^{13}C) or Bruker WP80SY (80 MHz, ^{1}H) spectrometers. Chemical shifts were measured on the δ scale using tetramethylsilane as the internal standard.

Infra-red spectra were recorded on a Perkin-Elmer 1605 FTIR machine. Melting points were measured using a Gallenkamp melting point apparatus and are uncorrected.

Mass spectra were recorded at York using a VG AUTOSPEC spectrometer (both EI and FAB) and at Heriot-Watt using a VGMS9 spectrometer (again both EI and FAB).

UV-Vis spectra were recorded at room temperature on a Hewlett Packard 8452A diode array spectrometer and analysed using a Hewlett Packard HP9500 UV-Vis ChemStation. A 1 cm pathlength quartz cuvette was used.

Elemental analyses were conducted at Napier University and at Zeneca Pharmaceuticals Ltd.

Syringe pump additions were conducted using a B. Braun Perfusor Securor FT instrument and a machine designed and built by Mr. Bill Stirling, Technical Services, Department of Chemistry, Heriot-Watt University.

5.1.2 Chromatography

Analytical TLC was carried out on Merck aluminium sheet silica gel 60 F_{254} plates.

A variety of solvent systems were used and are indicated in the text with the relevant compounds. Visualisation was generally achieved under UV light (254 nm), treating with

iodine vapour or by using one of the following dyeing reagents.

a) Ninhydrin. A solution of ninhydrin (0.3 g) in butan-1-ol (100 cm^3) and glacial ethanoic acid (3 cm^3) was used. Free amino groups gave purple or brown spots following the heating of the TLC plate.

b) Phosphomolybdic acid. A filtered solution of phosphomolybdic acid (5 % v/v) in absolute ethanol was used. Compounds gave blue spots on yellow backgrounds.

c) 2,4-Dinitrophenylhydrazine. A solution of 2,4-dinitrophenylhydrazine (1 g) in sulphuric acid (37 %, 10 cm^3) and ethanol (1000 cm^3) was used. Compounds with aldehydic groups gave orange, brown spots following heating of the TLC plate.

Flash chromatography was performed using silica gel 60 (purchased from Camlab). Approximately 20 g of silica was used for every gram of material to be purified[230].

HPLC was performed on a Bio-rad 1330 HPLC with UV detection. The column used was a Spherisorb 50D52C_{18} reverse-phase column.

5.1.3 Solvents and Reagents

When purified solvents were used, they were purified as described below. Otherwise standard laboratory grade solvents were used except for methanol (AR), acetonitrile (HPLC grade), acetone (AR), N,N-dimethylformamide (anhydrous), ethanol (AR) and ethyl acetate (AR).

Dichloromethane and chloroform were pre-dried over calcium chloride and distilled from phosphorus pentoxide.

Toluene and diethyl ether were dried over sodium.

Tetrahydrofuran was distilled from sodium under argon gas.

Deuterated N,N-dimethylformamide was supplied by Goss Scientific Instruments Ltd.

Reagents were used as purchased unless preliminary TLC indicated the need for distillation or recrystallisation from appropriate solvents.

5.2 Experimental relating to chapter 2

5.2.1 Addition of t-butoxycarbonyl protecting group to lysine

Preparation of *N-α-t-butoxycarbonyl-N-ϵ-toluenesulphonyl-lysine (97)*

Boc-Lys(Ts)-OH

A solution of N-ϵ-toluenesulphonyl-lysine (1.12 g 3.73 mmol) in tetrahydrofuran (7.5 cm^3), water (3.7 cm^3) and 1 M NaOH (3.7 cm^3) was cooled in an ice-water bath. Di-*tert*-butyl pyrocarbonate was added and the stirring was continued at room temperature for 1 h. The solution was concentrated under reduced pressure to 3.5 cm^3, cooled in an ice-water bath, covered with a layer of ethyl acetate (20 cm^3) and acidified with a dilute solution of $KHSO_4$ to pH 2-3. The aqueous phase was extracted with ethyl acetate (20 cm^3 x 2). The ethyl acetate extracts were combined, washed with water (20 cm^3 x 2) dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. Flash column chromatography (1 - 10 % methanol in dichloromethane) yielded 434 mg (33 %) of a clear oil.

^{1}H NMR ($CDCl_3$, RT, 200 MHz);

δ/ppm 1.3 - 1.8 (m, 6H, $CH_2CH_2CH_2CH_2N$), 1.649 (s, 9H, $C(CH_3)_3$), 2.408 (s, 3H, CH_3, Ts), 2.903 (m, 2H, CH_2NHTs), 4.263 (m, 1H, α-CH, Lys), 5.391 (m, 1H, NH), 5.537 (m, 1H, NH), 7.29 (d, 2H, J = 8.15 Hz, CH, Ts), 7.73 (d, 2H, J = 8.15 Hz, CH, Ts), 9.3-10.7 (s, br, 1H, COOH)

^{13}C NMR ($CDCl_3$, RT, 50 MHz);

δ/ppm 21.389 (CH_3, Ts), 22.092 (CH_2, Lys), 28.196 (CH_3, Boc), 28.715, 31.647, (CH_2, Lys), 42.593 (CH_2NHTs), 53.091 (CH, Lys), 80.076 (C, Boc), 126.947, 129.592 (CH, Ts), 136.673, 143.222 (C, Ts), 155.765 (C=O, Boc), 176.465 (C=O)

MS FAB+ve (NOBA matrix) $C_{18}H_{28}N_2O_6S$ M^+ calculated as 400

m/z = 401 $[M + H]^+$, 301 $[M - Boc]^+$, 255 $[M - Boc - COOH]^+$

IR, υ_{max}, cm^{-1} (KBr disc)

3288.2 (br), 1725.9, 1517.5, 1368.3, 1322.5, 1157.5, 1093.1, 857.3, 815.1, 733.7, 662.2

R_f 2:1:17 (methanol : acetic acid : chloroform): 0.35

mp (^{0}C): oil

5.2.2 General methods in peptide synthesis

5.2.2.1 General method for peptide couplings using EDC/HOBt peptide coupling reagents

The N-α-protected amino acid/peptide together with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1 mol. equiv.) and 1-hydroxybenzotriazole (1 mol. equiv.) were dissolved in N,N-dimethylformamide under argon and the mixture stirred for 1 h at 0^0 C followed by 40 min at room temperature. After re-cooling to 0^0 C, the amino component and N,N-diisopropylethylamine (10 % v/v of DMF, pH 9) in N,N-dimethylformamide were added and the stirred mixture allowed to warm up slowly to room temperature. Once the reaction had gone to completion as shown by TLC, the N,N-dimethylformamide was removed under reduced pressure with residual solvent being removed by forming an azeotrope with toluene (5 cm^3 x 3). The resultant solid was dissolved in dichloromethane and the solution washed with 2 x saturated $NaHCO_3$, 0.7 M citric acid, distilled water and 2 x saturated brine. The organic layer was then dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The resultant solid was triturated in hexane and diethyl ether and the solvents removed under reduced pressure to yield the required product as a white solid.

5.2.2.2 N-t-butoxycarbonyl deprotection

The protected peptide was dissolved in 90 % trifluoroacetic acid in 10 % water and stirred at room temperature for approximately 1 h or until the starting material had disappeared as shown by TLC. The solvent was removed under reduced pressure and any residual solvent removed by forming an azeotrope 3 times with toluene. Each time toluene was removed under reduced pressure. The oily product was triturated with diethyl ether until a white solid was formed. This was not characterised but was used immediately. For general coupling the trifluoroacetate salt was dissolved in N,N-dimethylformamide with sufficient N,N-dimethylformamide to give an apparent pH 9. This solution was then added at 0^0C to the relevant reaction mixture.

5.2.3 Peptide couplings

Preparation of *N-α-t-butoxycarbonyl-N-ϵ-toluenesulphonyl-lysyl-glycine-methyl-ester*

Boc-Lys(Ts)-Gly-OMe

Using DCC/HOBt as peptide coupling reagents

N-α-t-Butoxycarbonyl-N-ϵ-toluenesulphonyl-lysine (550 mg, 1.375 mmol), 1-hydroxybenzotriazole (186 mg, 1.375 mmol) and dicyclohexylcarbodiimide (283 mg, 1.375 mmol) were dissolved in N,N-dimethylformamide (3 cm^3) and the mixture stirred for 1 h at 0^0C followed by 40 min at room temperature. The mixture was re-cooled to 0^0 C before the addition of glycine methyl ester (172 mg, 1.375 mmol) dissolved in N,N-dimethylformamide (3 cm^3) (with N,N-diisopropylethylamine added to give an apparent pH 9). The mixture was then allowed to slowly warm up to room temperature. Once the reaction had gone to completion as shown by TLC, the solvent was removed under reduced pressure with residual solvent being removed by forming an azeotrope with toluene (5 cm^3 x 3). The toluene was removed under reduced pressure. The dipeptide was dissolved in

ethyl acetate (30 cm^3) and undissolved dicyclohexylurea removed by filtration. The solution was washed with 2 x sat. $NaHCO_3$, 0.7 M citric acid, distilled water and 2 x sat. brine. The organic layer was then dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The resultant solid was triturated in hexane and diethyl ether and then the solvents removed under reduced pressure to yield 525 mg (81 %) of the required dipeptide as a white solid.

Using EDC/HOBt as peptide coupling reagents

N-α-t-Butoxycarbonyl-N-ϵ-toluenesulphonyl-lysine (550 mg, 1.375 mmol), 1-hydroxybenzotriazole (186 mg, 1.375 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (264 mg, 1.375 mmol) were dissolved in N,N-dimethylformamide (3 cm^3) and the mixture stirred for 1 h at 0^0 C followed by 40 min at room temperature. The mixture was re-cooled to 0^0 C before the addition of glycine methyl ester (172 mg, 1.375 mmol) dissolved in N,N-dimethylformamide (3 cm^3) (with N,N-diisopropylethylamine added to give an apparent pH 9). The mixture was allowed to slowly warm up to room temperature. Once the reaction had gone to completion as shown by TLC, the solvent was removed under reduced pressure with residual solvent being removed by forming an azeotrope with toluene (5 cm^3 x 3). The toluene was removed under reduced pressure. The dipeptide was disssolved in dichloromethane (50 cm^3) and the solution was washed with 2 x sat. $NaHCO_3$ 0.7 M citric acid, distilled water and 2 x sat. brine. The organic layer was then dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The resultant solid was triturated in hexane and diethyl ether and then the solvents removed under reduced pressure to yield 536 mg (83 %) of the required dipeptide as a white solid. Data agreed with the literature values given in brackets [ ][120].

^1H NMR ($CDCl_3$, RT, 200 MHz); [($CDCl_3$, RT, 90 MHz)];

δ/ppm 1.3-1.8 [1.20-1.80] (m, 6H, $CH_2CH_2CH_2CH_2N$), 1.645 [1.411] (s, 9H, $C(CH_3)_3$), 2.417 [2.417] (s, 3H, CH_3, Ts), 2.892 [2.915] (t, 2H, J = 6.14 Hz, CH_2NHTs), 3.738 [3.718] (s, 3H, OCH_3), 4.0-4.2 [4.0-4.1] (m, 3H, CH_2, Gly, α-CH, Lys), 5.40 [5.46] (m, 2H, NH), 6.99 (m, br, 1H, NH), 7.29 [7.30] (d, 2H, J = 8.27 Hz [8.2], CH, Ts), 7.73 [7.72] (d, 2H, J = 8.27 Hz [8.2], CH, Ts)

^{13}C NMR ($CDCl_3$, RT, 50 MHz); [($CDCl_3$, RT, 22.5 MHz)];

δ/ppm 21.410 [21.210] (CH_3, Ts), 22.106 [22.224] (CH_2, Lys), 28.201 [28.186] (CH_3, Boc), 28.775, 31.835 [28.947, 31.887] (CH_2, Lys), 40.965, 42.572 [41.101, 42.895] (CH_2 Gly; CH_2NHTs), 52.320 [52.212] (OCH_3), 53.889 [54.224] (CH, Lys), 80.029 [80.460] (C, Boc), 128.937, 129.573 [127.001, 129.652] (CH, Ts), 136.859, 143.148 [136.945, 143.189] (C, Ts), 155.835 [155.460] (C=O, Boc), 170.243, 172.498 [170.002, 172.614] (C=O)

MS FAB+ve (NOBA matrix) $C_{21}H_{33}N_3O_7S$ M^+ calculated as 471.2

m/z: 472 $[M + H]^+$, 372 $[M - Boc]^+$

MS FAB+ve (NOBA matrix) $[M + H]^+$ accurate mass

Calculated 472.2117

Found 472.2113

IR, υ_{max}, cm^{-1} (KBr disc);

3412.4, 2933.3, 1753.5, 1666.5, 1617.6, 1525.5, 1438.4, 1367.5, 1325.9, 1211.9, 1159.6, 1093.9, 816.0, 661.8

R_f 2:17:1 (methanol : chloroform : acetic acid) 0.57 [0.57]

19:1 (dichloromethane : methanol): 0.4

mp (^{0} C): 140-145 [142-146]

Preparation of *N-α-t-butoxycarbonyl-N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl-glycine-methyl-ester (99)*

Boc-Lys(Ts)-Lys(Ts)-Gly-OMe

<u>Using DCC/HOBt as peptide coupling reagents</u>

N-α-t-Butoxycarbonyl-N-ϵ-toluenesulphonyl-lysyl-glycine-methyl-ester (517 mg, 1.1 mmol) was dissolved in 90 % trifluoroacetic acid in 10 % water and stirred at room temperature for approximately 1 h or until the starting material had disappeared as shown by TLC. The solvent was removed under reduced pressure and any residual solvent removed by forming an azeotrope 3 times with toluene. Each time the toluene was removed under reduced pressure. The oily product was triturated with diethyl ether until the trifluoroacetic acid salt was formed as a white solid. This was dissolved in N,N-dimethylformamide (3 cm^3), N,N-diisopropylethylamine was added to give an apparent pH 9 and the solution cooled to 0^0 C.

N-α-t-Butoxycarbonyl-N-ϵ-toluenesulphonyl-lysine (440 mg, 1.1 mmol), 1-hydroxybenzotriazole (149 mg, 1.1 mmol) and dicyclohexylcarbodiimide (226 mg, 1.1 mmol) were dissolved in N,N-dimethylformamide (3 cm^3) and the mixture stirred for 1 h at 0^0 C followed by 40 min at room temperature. The mixture was re-cooled to 0^0 C and then the above solution of N-ϵ-toluenesulphonyl-lysyl-glycine-methyl-ester trifluoroacetic acid salt was added. The mixture was allowed to slowly warm up to room temperature. Once the reaction had gone to completion as shown by TLC the solvent was removed under reduced pressure with residual solvent being removed by forming an azeotrope with toluene (5 cm^3 x 3). The toluene was removed under reduced pressure. The tripeptide was dissolved in ethyl acetate (30 cm^3) and undissolved dicyclohexylurea removed by filtration. The solution was washed with 2 x sat. $NaHCO_3$, 0.7 M citric acid, distilled water and 2 x sat. brine. The organic layer was then dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The resultant solid was triturated in hexane and diethyl ether and then the solvents removed under reduced pressure to yield 646 mg (78 %) of the required tripeptide as a white solid.

Using EDC/HOBt as peptide coupling reagents

N-α-t-Butoxycarbonyl-N-ϵ-toluenesulphonyl-lysyl-glycine-methyl-ester (517 mg, 1.1 mmol) was dissolved in 90 % trifluoroacetic acid in 10 % water and stirred at room temperature for approximately 1 h or until the starting material had disappeared as shown by TLC. The solvent was removed under reduced pressure and any residual solvent removed by forming an azeotrope 3 times with toluene. Each time the toluene was removed under reduced pressure. The oily product was triturated with diethyl ether until the trifluoroacetic acid salt was formed as a white solid. This was dissolved in N,N-dimethylformamide (3 cm^3), N,N-diisopropylethylamine was added to give an apparent pH 9 and the solution was cooled to 0^0 C.

N-α-t-Butoxycarbonyl-N-ϵ-toluenesulphonyl-lysine (440 mg, 1.1 mmol), 1-hydroxybenzotriazole (149 mg, 1.1 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (211 mg, 1.1 mmol) were dissolved in N,N-dimethylformamide (3 cm^3) and the mixture stirred for 1 h at 0^0 C followed by 40 min at room temperature. The mixture was re-cooled to 0^0 C and then the above solution of N-ϵ-toluenesulphonyl-lysyl-glycine-methyl-ester trifluoroacetic acid salt was added. The mixture was allowed to slowly warm up to room temperature. Once the reaction had gone to completion as shown by TLC, the solvent was removed under reduced pressure with residual solvent being removed by forming an azeotrope with toluene (5 cm^3 x 3). The toluene was removed under reduced pressure. The tripeptide was dissolved in dichloromethane (50 cm^3) and the solution was washed with 2 x sat. $NaHCO_3$, 0.7 M citric acid, distilled water and 2 x sat. brine. The organic layer was then dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The resultant solid was triturated in hexane and diethyl ether and then the solvents removed under reduced pressure to yield 671 mg (81 %) of the required tripeptide as a white solid in 81 % yield. Data agreed with the literature values given in brackets [ ][120].

^{1}H NMR ($CDCl_3$, RT, 270 MHz); [($CDCl_3$, RT, 300 MHz)]

δ/ppm 1.2-1.9 [1.32-1.60] (m, 12H, $CH_2CH_2CH_2CH_2N$), 1.392 [1.412] (s, 9H, $C(CH_3)_3$), 2.414 [2.401] (s, 6H, CH $_3$ Ts), 2.90 [2.90] (m, br, 4H, CH $_2$NHTs), 3.707 [3.695] (s, 3H, OCH_3), 4.016 [4.018] (m, 2H, CH_2, Gly), 4.153 [4.175] (m, 1H, Lys α-CH), 4.507 [4.500] (m, 1H, Lys α-CH), 5.581 [5.618] (m, br, 1H, NH), 5.722 [5.836] (m,

br, 1H, NH), 5.819 [5.910] (m, br, 1H, NH), 7.26 [7.223] (m, br, 1H, NH), 7.287 [7.281] (d, 4H, J = 7.9 Hz [8.2], CH, Ts), 7.419 [7.426] (m, br, 1H, NH), 7.727 [7.735] (d, 4H, J = 7.9 Hz [8.2], CH, Ts)

^{13}C NMR ($CDCl_3$, RT, 67.90 MHz); [($CDCl_3$, RT, 75 MHz)]

δ/ppm 21.518 [21.750] (CH_3, Ts), 21.839, 22,273 [22.157, 22.710] (CH_2, Lys), 28.294 [28.610] (CH_3, Boc), 28.671, 30.502, 31.559, 33.352 [28.655, 31.108, 31.901, 33.892] (CH_2, Lys), 41.204, 41.959, 42.714 [41.161, 42.113, 42.810] (CH_2, Gly; CH_2NHTs), 52.260 [52.242] (OCH_3), 53.190, 54.851 [53.110, 54.701] (CH, Lys), 80.333 [80.014] (C, Boc), 126.954, 127.049, 129.710 [126.928, 129.810] (CH, Ts), 136.882, 136.996, 143.300 [137.002, 143.003] (C, Ts), 156.267 [156.004] (C=O, Boc), 170.291, 172.500, 173.104 [170.165, 172.310, 172.996] (C=O)

MS FAB+ve (NOBA matrix) $C_{34}H_{51}N_5O_{10}S_2$ M^+ calculated as 753.3

m/z: 886 $[M + Cs]^+$, 776 $[M + Na]^+$, 754 $[M + H]^+$, 654 $[M - Boc]^+$

MS FAB+ve (NOBA matrix) $[M + H]^+$ accurate mass

Calculated 754.3156

Found 754.3153

Elemental analysis (%)

Calculated	C 54.17	H 6.82	N 9.29
Found	C 54.29	H 6.80	N 9.27

IR, υ_{max}, cm^{-1} (KBr disc);

3413.0, 2930.2, 1638.6, 1617.0, 1526.3, 1367.8, 1324.1, 1158.2, 1093.9, 816.1

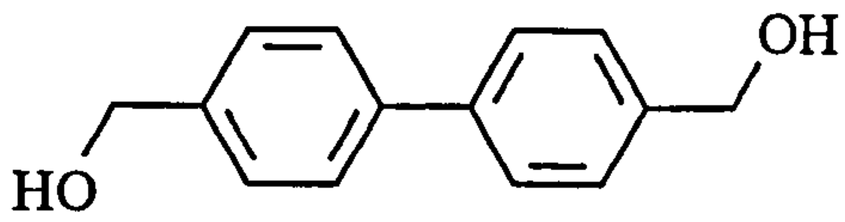
R_f 2:37:1 (methanol : chloroform : acetic acid) 0.68 [0.68]

19:1 (dichloromethane : methanol): 0.3

mp (^{0} C): 148-152 [151-154]

5.2.4 Synthesis of crosslinkers

Preparation of *4,4'-bis-hydroxymethylbiphenyl (112)*



Diethyl biphenyl-4,4'dicarboxylate (5 g, 16.8 mmol) in tetrahydrofuran (120 cm^3) was stirred at room temperature under argon. The reaction mixture was cooled to 0^0 C and lithium aluminium hydride (2.55 g, 67.2 mmol) was added. Stirring was continued for 16 h until shown to be complete by TLC. The reaction was quenched with H_2O (3 cm^3) and 2 M NaOH (10 cm^3). More water (10 cm^3) was added and the precipitated inorganic by-products removed by filtration. The solvent was removed under reduced pressure and any residual water was removed by forming an azeotrope with toluene (5 cm^3 x 3). The toluene was removed under reduced pressure to yield 3.52 g (98 %) of a white solid. Data agreed with the literature values given in brackets [ ][120].

^{1}H NMR (d_6-DMSO, RT, 270 MHz); [(d_6-DMSO, RT, 250 MHz)]

δ/ppm 4.63 [4.56] (s, 4H, CH_2OH), 5.35 (s, br, 2H, CH_2OH), 7.48 [7.49] (d, 4H, J = 8.01 Hz, **CH**, biphenyl), 7.70 [7.61] (d, 4H, J = 8.01 Hz, **CH**, biphenyl)

^{13}C NMR (d_6-DMSO, RT, 67.90 MHz); [(d_6-DMSO, RT, 62.5 MHz)]

δ/ppm 68.641 [68.191] (CH_2OH), 126.250 [130.186] (CH, Ar), 127.024 [131.895] (CH, Ar), 128.481 [130.002] (C, Ar), 131.614 [132.011] (C, Ar)

MS EI $C_{14}H_{14}O_2$ M^+ calculated as 214

m/z: 214 $[M]^+$, 197 $[M - OH]^+$, 183 $[M - CH_2OH]^+$; 165 $[M - CH_2OH - OH]^+$; 77 $[C_6H_5]^+$

MS EI M^+ accurate mass

Calculated 214.0994

Found 214.0999

IR υ_{max}, cm^{-1} (Nujol mull)

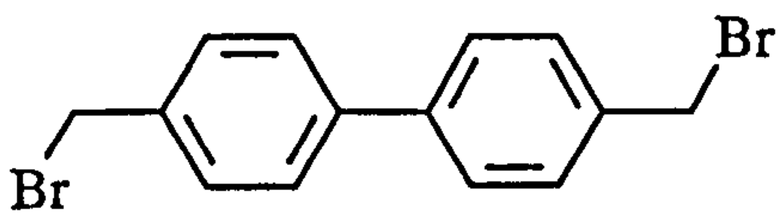
3270, 2956, 2855, 1661, 1562, 1463, 1378, 1208, 1048, 1012, 848, 803

R_f 3:2 (40:60 petroleum ether : ethyl acetate) 0.30 [0.30]

1:1 (40:60 petroleum ether : ethyl acetate) 0.40

mp (^{0} C): 113-115 [114-116]

Preparation of *4,4'-bis-bromomethylbiphenyl (113)*



4,4'-Bis-hydroxymethylbiphenyl (3.60 g, 16.8 mmol) and carbon tetrabromide (11.17 g, 33.6 mmol) in acetonitrile (250 cm^3) were stirred at room temperature under argon. Triphenylphosphine (8.81 g, 33.6 mmol) was added and the stirring continued for 24 h, until shown to be complete by TLC. The solvent was removed under reduced pressure and flash column chromatography (using gradient elution from 19:1 40-60 petroleum ether : ethyl acetate to 3:2 40-60 petroleum ether : ethyl acetate) yielded 4.23 g (75 %) of a white solid. Data agreed with that obtained from a previously prepared authentic sample [ ][120].

^{1}H NMR ($CDCl_3$, RT, 270 MHz); [(d_6-DMSO, RT, 200 MHz)]

δ/ppm 4.54 [4.67] (s, 4H, CH_2Br), 7.46 [7.56] (d, 4H, **CH**, biphenyl, J = 8.24 Hz), 7.55 [7.69] (d, 4H, **CH**, biphenyl, J = 8.24 Hz)

^{13}C NMR ($CDCl_3$, RT, 67.90 MHz); [(d_6-DMSO, RT, 50 MHz)]

δ/ppm 33.239 [39.163] (CH_2), 127.502 [133.085] (CH, biphenyl), 129.578 [133.476] (CH, biphenyl), 137.109 [134.947] (C, biphenyl), 140.563 [134.624] (C, biphenyl)

MS EI $C_{14}H_{12}Br_2$ M^+ calculated as 339.9 (^{79}Br, ^{81}Br)

m/z 342 $[M]^+$ ($2^{81}Br$), 340 $[M]^+$ (^{81}Br,^{79}Br), 338 $[M]^+$ ($2^{79}Br$), 261 $[M - {}^{79}Br]^+$, 259 $[M - {}^{81}Br]^+$, 180 $[M - 2Br]^+$

MS EI $C_{14}H_{12}{}^{79}Br_2$ M^+ accurate mass

Calculated 337.9306

Found 337.9313

IR, υ_{max}, cm^{-1} (KBr disc);

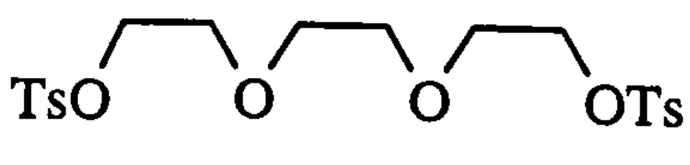
1636.9, 1497.3, 1436.4, 1398.1, 1227.4, 1204.0, 1120.0, 1002.8, 825.9, 797.6, 751.1, 721.4, 697.1

R_f 5:2 (40:60 petroleum ether : ethyl acetate) 0.80 [0.78]

19:1 (40-60 petroleum ether : ethyl acetate) 0.65

mp (^{0} C) 89-91 [89-92]

Preparation of *1,2-(bis-2-tosylethoxy)ethane* (118)



Triethylene glycol (4 g, 26.7 mmol), tosyl chloride (15.24 g, 0.08 mmol), triethylamine (16.16 g, 0.16 mmol) and a catalytic amount of 4-N,N-dimethylaminopyridine (DMAP) were stirred in dichloromethane (200 cm^3) at 0^0 C under argon. The reaction mixture was allowed to warm up to room temperature and stirred for 16 h until shown to be complete

by TLC. The reaction mixture was washed with 2 M hydrochloric acid (100 cm^3), sat. $NaHCO_3$ (100 cm^3), distilled water and sat. brine. The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Flash column chromatography (1:1 40-60 petroleum ether : ethyl acetate) of the resultant solid gave 9.65 g (79 %) of a white solid.

^1H NMR ($CDCl_3$, RT, 270 MHz);

δ/ppm 2.45 (s, 6H, CH_3, Ts), 3.53 (s, 4H, $TsOCH_2CH_2OCH_2$-), 3.65 (t, 4H, J = 4.7 Hz, $TsOCH_2CH_2O$-), 4.14 (t, 4H, J = 4.7 Hz, $TsOCH_2CH_2O$-), 7.34 (d, 4H, CH, Ts, J = 8.3 Hz), 7.79 (d, 4H, CH, Ts, J = 8.3 Hz)

^{13}C NMR ($CDCl_3$, RT, 67.90 MHz);

δ/ppm 21.631 (CH_3, Ts), 68.724, 69.196, 70.668, (CH_2, glycol), 127.955, 129.861 (CH, Ts), 132.919, 144.867 (C, Ts)

MS FAB+ve (NOBA matrix) $C_{20}H_{26}O_8S_2$ M^+ calculated as 458

m/z 481 $[M + Na]^+$, 459 $[M + H]^+$, 155 $[Ts]^+$, 91 $[PhCH_2]^+$, 77 $[Ph]^+$

MS FAB+ve (NOBA matrix) $[M + H]^+$ accurate mass

Calculated 459.1147

Found 459.1143

IR, υ_{max}, cm^{-1} (KBr disc)

2897.2, 1596.4, 1352.7, 1261.4, 1172.0, 1132.6, 1016.1, 982.4, 911.9, 851.1, 804.8, 778.8, 665.1

R_f 1:1 (40-60 petroleum ether : ethyl acetate) 0.5

mp (^{0} C) 80-82

5.2.5 General procedure for crosslinking reactions

The methods described below are the optimised conditions developed for the crosslinking of tripeptide (99)

5.2.5.1 Using syringe pump

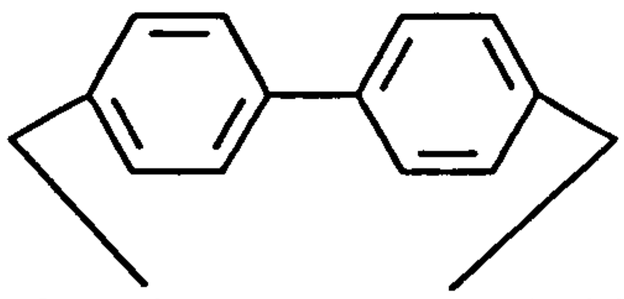
Caesium carbonate (5 mol. equiv.) was stirred in N,N-dimethylformamide under argon. Water (ca. 35 mol. equiv.) was added followed by tripeptide (1 mol. equiv.). The crosslinker (1 mol. equiv.) in N,N-dimethylformamide was added over approximately 24 h using a syringe pump. In order to ensure completeness, more crosslinker (2 x 0.5 mol. equiv.) in N,N-dimethylformamide was added over approximately 12 h using a syringe pump. The reaction mixture was stirred for a further 24 h before being filtered to remove the caesium carbonate. The solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography.

5.2.5.2 Without syringe pump

The caesium carbonate (5 mol. equiv.) was stirred in N,N-dimethylformamide under argon. Water (ca. 35 mol. equiv.) was added followed by tripeptide (1 mol. equiv.) and crosslinker (1 mol. equiv.). After 24 h, more crosslinker (0.5 mol. equiv.) was added followed by a further batch (0.5 mol. equiv.) after 12 h. Both batches were dissolved in N,N-dimethylformamide. Following the addition of all the crosslinker, the mixture was stirred for a further 24 h before being filtered to remove the caesium carbonate. The solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography.

5.2.6 Preparation of crosslinked tripeptides

Attempted preparation of *N-α-t-butoxycarbonyl-[(N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl)-4(N^{ϵ}),4'(N$^{ϵ'}$)-(bis-methylphenadiyl)]-glycine-methyl-ester (100)*



Boc-Lys(Ts)-Lys(Ts)-Gly-OMe

Many attempts were made to synthesise the above compound. Representative examples of these experiments are described below:

Method A

Dry caesium carbonate (216 mg, 0.167 mmol) and N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (50 mg, 0.067 mmol) were stirred in dry N,N-dimethylformamide (10 cm^3) under argon at RT. 4,4'-Bis-bromomethylbiphenyl (22.6 mg, 0.067 mmol) in N,N-dimethylformamide (5 cm^3) was added by syringe pump over a period of 36 h and the mixture stirred for a further 3 days to ensure completion. The solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Repeated flash column chromatography (gradient elution from 99:1 dichloromethane : methanol to 90:10 dichloromethane : methanol) failed to produce a pure sample of the desired product, but FAB MS suggested the successful synthesis of the desired compound.

Method B

Dry caesium carbonate (216 mg, 0.67 mmol), N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (50 mg, 0.067 mmol) and 3 Å molecular sieves were

stirred in dry N,N-dimethylformamide (10 cm^3) under argon at RT. 4,4'-Bis-bromomethylbiphenyl in N,N-dimethylformamide (5 cm^3) was added by syringe pump over a period of 36 h and the mixture was stirred for a further 3 days to ensure completion. The mixture was worked up as above but FAB MS indicated that only the starting tripeptide had been isolated.

Method C

Dry caesium carbonate (216 mg, 0.67 mmol), N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (50 mg, 0.067 mmol), 3 Å molecular sieves and catalytic amounts of caesium iodide were stirred in dry N,N-dimethylformamide (10 cm^3) under argon at RT. In an alternative reaction, dry acetonitrile (10 cm^3) was used as the solvent under identical reaction conditions. 4,4'-Bis-bromomethylbiphenyl (22.6 mg, 0.067 mmol) in N,N-dimethylformamide (5 cm^3) (or acetonitrile (5 cm^3) in the parallel reaction) was added by syringe pump over a period of 36 h and the mixture stirred for a further 3 days to ensure completion. The mixture was worked up as above. Evidence was available by FAB MS in the DMF reaction for the successful synthesis of the desired compound but it could not be purified by repeated flash column chromatography (using gradient elution from 99:1 dichloromethane : methanol to 90:10 dichloromethane : methanol). No evidence was available for the successful synthesis of the target molecule by FAB MS in the acetonitrile reaction.

Method D

Dry caesium carbonate (216 mg, 0.67 mmol), N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (50 mg, 0.067 mmol), 3 Å molecular sieves and catalytic caesium iodide were stirred in dry N,N-dimethylformamide (10 cm^3) under argon at 65^0 C. 4,4'-Bis-bromomethylbiphenyl (22.6 mg, 0.067 mmol) in N,N-dimethylformamide (5 cm^3) was added by syringe pump over a period of 36 h and the mixture stirred for a further 3 days to ensure completion. The mixture was worked up as above, however, no evidence was available from FAB MS or ^{1}H NMR spectroscopy for the successful synthesis of the desired compound.

Method E

Dry caesium carbonate (648 mg, 2 mmol) was stirred in dry N,N-dimethylformamide (30 cm^3) under argon in a flame dried flask. Water (ca. 250 mg, 14 mmol) was added followed by N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (300 mg, 0.4 mmol). 4,4'-Bis-bromomethylbiphenyl (48.5 mg, 0.44 mmol) in N,N-dimethylformamide (10 cm^3) was added by syringe pump over a period of 48 h at RT and the mixture was stirred for a further 48 h at RT to ensure completion. The mixture was worked up as above. FAB MS and ^1H NMR spectroscopy indicated the successful synthesis of the desired compound but flash column chromatography failed to achieve its isolation.

Method F

In two reactions, dry caesium carbonate (648 mg, 2 mmol) was stirred in dry N,N-dimethylformamide (40 cm^3) under argon in a flame dried flask. Water (ca. 250 mg, 14 mmol) was added followed by N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (300 mg, 0.4 mmol) and 4,4'-bis-bromomethylbiphenyl (148.5 mg, 0.44 mmol - in the second reaction the quantity was 270 mg, 0.8 mmol). The mixture was stirred for 24 h under argon at RT. The mixture was worked up as above. FAB MS and ^1H NMR spectroscopy of the reaction products from each reaction indicated the successful synthesis of the desired compound but flash column chromatography failed to achieve its isolation.

Method G

Dry caesium carbonate (108 mg, 0.34 mmol) was stirred at RT in dry acetonitrile (7 cm^3) under argon in a flame dried flask. Water (ca. 250 mg, 14 mmol) was added followed by N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (50 mg, 0.067 mmol) and 4,4'-bis-bromomethylbiphenyl (22.5 mg, 0.067 mmol). After 24 h, more 4,4'-bis-bromomethylbiphenyl (22.5 mg, 0.067 mmol) was added. The mixture was stirred for a further 24 h to ensure completion. However, FAB MS and ^1H NMR spectroscopy showed this reaction to have been unsuccessful towards the synthesis of the desired compound.

Method H

Dry caesium carbonate (648 mg, 2 mmol) was stirred in dry N,N-dimethylformamide (40 cm^3) under argon in a flame dried flask. Water (ca. 250 mg, 14 mmol) was added followed by N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (300 mg, 0.4 mmol) and 4,4'-bis-bromomethylbiphenyl (135 mg, 0.4 mmol) and the mixture stirred at 50^0 C. After 24 h, more 4,4'-bis-bromomethylbiphenyl (67.5 mg, 0.2 mmol) was added followed by the addition of another batch of 4,4'-bis-bromomethylbiphenyl (67.5 mg, 0.2 mmol) after a further 12 h. The reaction mixture was stirred for a further 24 h under argon at 50^0 C to ensure completion. The mixture was worked up as above but FAB MS and ^1H NMR spectroscopy failed to show the presence of the desired compound.

Optimal method

Dry caesium carbonate (648 mg, 2 mmol) was stirred in dry N,N-dimethylformamide (40 cm^3) under argon in a flame dried flask. Water (ca. 250 mg, 14 mmol) was added followed by N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (300 mg, 0.4 mmol) and 4,4'-bis-bromomethylbiphenyl (135 mg, 0.4 mmol) and the mixture stirred under argon at RT. After 24 h, more 4,4'-bis-bromomethylbiphenyl (67.5 mg, 0.2 mmol) was added followed by the addition of another batch of 4,4'-bis-bromomethylbiphenyl (67.5 mg, 0.2 mmol) after a further 12 h. The reaction mixture was stirred for a further 24 h at RT to ensure completion before being filtered to remove the caesium carbonate. The solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Repeated flash column chromatography (gradient elution from 99:1 dichloromethane : methanol to 90:10 dichloromethane : methanol) failed to produce a pure sample of the desired product, but evidence was available for the successful formation of the target compound.

^{1}H NMR ($CDCl_3$, RT, 80 MHz);

δ/ppm 1.1-1.9 (m, 12H, $CH_2CH_2CH_2CH_2N$), 1.35 (s, 9H, $C(CH_3)_3$), 2.4 (s, 6H, CH_3, Ts), 2.8-3.2 (m, 8H, CH_2NTs), 3.7 (s, 3H, OCH_3), 4.0 (m, br, 3H, CH_2, Gly, Lys α-CH), 4.3 (m, br, 1H, Lys α-CH), 4.7 (m, br, 1H, NH), 7.2-7.8 (m, 16H, CH, Ts, biphenyl)

MS FAB+ve (NOBA matrix) $C_{48}H_{61}N_5O_{10}S_2$ M^+ calculated as 931.3

m/z 1064 $[M + Cs]^+$, 932 $[M + H]^+$, 832 $[M - Boc]^+$, 776 $[M - Ts]^+$

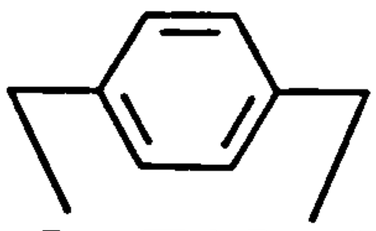
MS FAB+ve (NOBA matrix) $[M + H]^+$ accurate mass

Calculated 932.3938

Found 932.3943

R_f 19:1 (dichloromethane : methanol) 0.35

Preparation of *N-α-t-butoxycarbonyl-[(N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl)-α(N^ϵ), α'(N$^{ϵ'}$)-para-xyl-diyl]-glycine-methyl-ester (114)*



Boc-Lys(Ts)-Lys(Ts)-Gly-OMe

Three attempts were made to synthesise the above compound. These experiments are described below:

Method A

In each of two separate reactions, dry caesium carbonate (216 mg, 0.67 mmol) and N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (50 mg, 0.067 mmol) were stirred in dry N,N-dimethylformamide (10 cm^3) under argon at RT. In one

reaction, 3 Å molecular sieves were added whilst in the other they were not present. 1,4-α,α'-Dibromo-*p*-xylene (17.5 mg, 0.067 mmol) in N,N-dimethylformamide was added by syringe pump over a period of 24 h and the mixture stirred for a further 3 days to ensure completion. The solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove the molecular sieves and any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. FAB MS of the crude samples indicated the presence of the desired product from the reaction containing molecular sieves but not from the reaction without molecular sieves. Flash column chromatography of the former reaction mixture (9:1 dichloromethane : methanol), yielded 1.2 mg (2 %) of the desired compound as shown by FAB MS and TLC

<u>Optimised method</u>

Dry caesium carbonate (648 mg, 2 mmol) was stirred in dry N,N-dimethylformamide (40 cm^3) under argon in a flame dried flask. Water (ca. 250 mg, 14 mmol) was added followed by N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (300 mg, 0.4 mmol) and 1,4-α,α'-dibromo-*p*-xylene (95.6 mg, 0.4 mmol) and the reaction stirred at RT. After 24 h, more 1,4-α,α'-dibromo-*p*-xylene (47.8 mg, 0.2 mmol) was added followed by the addition of another batch of 1,4-α,α'-dibromo-*p*-xylene (47.8 mg, 0.2 mmol) after a further 12 h. The reaction mixture was stirred for a further 24 h under argon at RT to ensure completion before being filtered to remove the caesium carbonate. The solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Flash column chromatography (gradient elution from 99:1 dichloromethane : methanol to 90:10 dichloromethane : methanol) yielded 138 mg (40.5 %) of a white solid. Data agreed with the literature values given in brackets [ ][120].

^{1}H NMR ($CDCl_3$, RT, 270 MHz); [($CDCl_3$, RT, 300 MHz)];

δ/ppm 1.0-1.7 [1.2-1.5] (m, 12H, $CH_2CH_2CH_2CH_2N$), 1.351 [1.356] (s, 9H, $C(CH_3)_3$), 2.459 [2.482] (s, 6H, CH_3, Ts), 2.6-3.4 [2.7-3.4] (m, 8H, CH_2NTs), 3.694 [3.702] (s, 3H, OCH_3), 3.783 [4.026] (m, 2H, CH_2, Gly), 4.167 [4.261] (m, 1H, Lys α-

CH), 4.41 [4.80] (t, br, 1H, NH), 4.672 [4.433] (m, 1H, Lys α-CH), 5.17 [5.29] (d, br, 1H, NH), 6.89 [6.78] (d, br, 1H, NH), 7.300 [7.366] (d, 4H, J = 8.1 Hz [8.4], CH, Ts), 7.346 [7.291] (s, 4H, CH, xylyl), 7.733 [7.748] (d, 4H, J = 8.1 Hz [8.4], CH, Ts)

^{13}C NMR ($CDCl_3$, RT, 67.90 MHz); [($CDCl_3$, RT, 75 MHz)]

δ/ppm 21.121 [21.230] (CH_2, Lys), 21. 537 [21.882] (CH_3, Ts), 22.197, 24.896, 25.897 [22.452, 23.172, 26.012] (CH_2, Lys), 28.162 [28.211] (CH_3, Boc), 29.653, 33.730 [29.021,30.610] (CH_2, Lys), 41.148 [41.117] (CH_2, Gly), 47.490, 47.943, 51.907 [47.642, 48.111] (CH_2NHTs), 52.152 [52.101] (OCH_3), 52.511 [52.211] (CH, Lys), 53.643 [55.377] (CH_2NHTs), 56.607 [52.715] (CH, Lys), 80.635 [80.810] (C, Boc), 127.049, 127.124 [127.001, 127.073] (CH, Ts), 128.502, 128.993, 129.295 [128.880,129.002,129.220] (CH, xylyl), 129.899, 129,936 [130.002, 131.002] (CH, Ts), 135.580 [135.501] (C, xylyl), 135.901, 136.260 [136.302,136.970] (C, Ts), 136.694 [135.915] (C, xylyl), 143.564, 143.734 [143.601] (C, Ts), 156.909 [156.930] (C=O, Boc), 170.027, 172.217, 172.707 [170.112,172.020, 172.940] (C=O)

MS FAB+ve (NOBA) matrix $C_{42}H_{57}N_5O_{10}S_2$ M^+ calculated as 855.3

m/z 878 $[M + Na]^+$, 856 $[M + H]^+$, 756 $[M - Boc]^+$, 711 $[M - Ts]^+$

MS FAB+ve (NOBA) matrix $[M + H]^+$ accurate mass

Calculated 856.3625

Found 856.3623

IR, υ_{max}, cm^{-1} (Nujol mull);

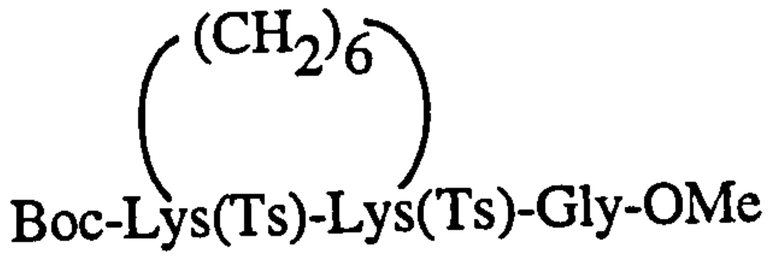
3386.6, 2924.5, 2855.2, 2362.2, 1660.7, 1456.1, 1377.4, 1333.6, 1156.2, 849.0, 815.6

R_f 2:37:1 (methanol : chloroform : acetic acid) 0.70 [0.69]

19:1 (dichloromethane : methanol) 0.35

mp (^0C) 177-180 [179-181]

Preparation of *N-α-t-butoxycarbonyl-[(N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl)-1(N^{ϵ}),6($N^{E'}$)-hexadiyl]-glycine-methyl-ester (116)*



Two attempts were made to synthesise the above compound. These experiments are described below:

Method A

Dry caesium carbonate (432 mg, 1.33 mmol), N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (100 mg, 0.13 mmol) and 3 Å molecular sieves were stirred in dry N,N-dimethylformamide (25 cm^3) under argon at RT. 1,6-Dibromohexane (35.6 mg, 0.15 mmol) in N,N-dimethylformamide (20 cm^3) was added by syringe pump over a period of 36 h and the mixture stirred for another 48 h to ensure completion. The solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. However, FAB MS and TLC failed to show any evidence of the desired compound.

Optimised method

Dry caesium carbonate (648 mg, 2 mmol) was stirred in dry N,N-dimethylformamide (30 cm^3) under argon in a flame dried flask. Water (ca. 250 mg, 14 mmol) was then added followed by N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine methyl-ester (300

mg, 0.4 mmol). 1,6-Dibromohexane (97.2 mg, 0.4 mmol) in N,N-dimethylformamide (10 cm^3) was added by syringe pump over a period of 48 h and the mixture stirred under argon at RT for a further 48 h to ensure completion. The mixture was filtered to remove the caesium carbonate and the solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Flash column chromatography (gradient elution from 99:1 dichloromethane : methanol to 90:10 dichloromethane : methanol) yielded 151.4 mg (45 %) of a white solid. Data agreed with that previously found[120]. Literature values are given in brackets [ ].

^{1}H NMR ($CDCl_3$, RT, 200 MHz); [($CDCl_3$, RT, 300 MHz)]

δ/ppm 1.2-2.1 [1.2-1.9] (m, br, 20H, CH_2, alkyl), 1.253 [1.317] (s, 9H, $C(CH_3)_3$), 2.391 [2.439] (s, 3H, CH_3, Ts), 2.412 [2.475] (s, 3H, CH_3, Ts), 2.9-3.1 [2.6-2.7] (m, br, 4H, CH_2NRTs), 3.1-3.35 [2.9-3.3] (m, br, 4H, CH_2NRTs), 3.671 [3.731] (s, 3H, OCH_3), 3.85-4.10 [3.93-4.12] (m, 3H, CH_2, Gly, α-CH, Lys), 4.354 [4.417] (m, 1H, α-CH, Lys), 5.519 [5.590] (m, 1H, NH), 7.287 [7.317] (m, 4H, CH, Ts [d, J = 8.5 Hz]), 7.428 [7.501] (m, 1H, NH), 7.638 [7.643] (m, 4H, CH, Ts [d, J = 8.5 Hz]), 7.71 (m, 1H, NH)

^{13}C NMR ($CDCl_3$, RT, 50 MHz); [($CDCl_3$, RT, 75 MHz)]

δ/ppm 20.739 [21.120] (CH_2, alkyl), 21.297 [21.910] (CH_3, Ts), 22.235, 24.415, 24.552, 24.752, 26.553, 26.857 [22.811, 24.911, 24.996,26.956, 27.118] (CH_2, alkyl), 27.905 [28.131] (CH_3, Boc), 28.189, 29.321, 31.489 [28.772, 29.815, 31.902] (CH_2, alkyl), 40.989 [41.354] (CH_2, Gly), 46.527, 47.821, 48.320, 50.013 [46.872, 48.171, 48.860, 50.510] (CH_2NHTs), 51.853 [57.002] (OCH_3), 53.114, 56.571 [52.160, 53.611] (CH, Lys), 80.052 [80.502] (C, Boc), 126.573, 126.843, 129.462, 129.662 [126.720,127.001, 129.410,129.910] (CH, Ts), 135.238, 136.385, 142.901, 143.487 [135.650, 136.612] (C, Ts), 156.418 [156.701] (C=O, Boc), 169.662, 172.317, 173.378 [169.985, 172.714, 173.510] (C=O)

MS FAB+ve (NOBA matrix) $C_{46}H_{61}N_5O_{10}S_2$ M^+ calculated as 835.4

m/z 858 $[M + Na]^+$, 836 $[M + H]^+$, 736 $[M - Boc]^+$, 680 $[M - Ts]^+$, 580 $[M - Boc - Ts]^+$

MS FAB+ve (NOBA matrix) $[M + H]^+$ accurate mass

Calculated 836.3938

Found 836.3935

IR, υ_{max}, cm^{-1} (nujol mull)

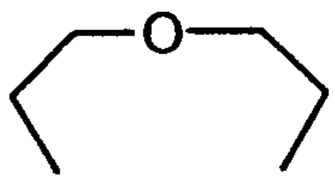
3386.5, 2925.0, 2855.6, 1661.4, 1463.3, 1377.9, 1338.1, 1158.3, 1090.9, 1020.7, 848.8, 721.8

R_f 2:37:1 (methanol : dichloromethane : acetic acid) 0.7 [0.71]

19:1 (dichloromethane : methanol) 0.33

mp (^{0} C) 182-185 [183-186]

Attempted preparation of *N-α-t-butoxycarbonyl-[(N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl)-(1-ethoxy)($N^ϵ$),(2-ethoxy')($N^{ϵ'}$)-oxy]-glycine-methyl-ester (115)*

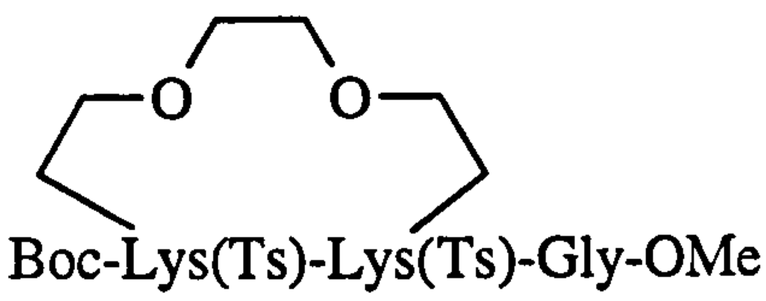


Boc-Lys(Ts)-Lys(Ts)-Gly-OMe

Dry caesium carbonate (216 mg, 0.67 mmol), N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (50 mg, 0.067 mmol), 3 Å molecular sieves and catalytic amounts of caesium iodide in dry N,N-dimethylformamide (10 cm^3) were stirred under argon at RT. 2-Chloroethyl ether (10.4 mg, 0.074 mmol) in N,N-dimethylformamide (5 cm^3) was added by syringe pump over a period of 36 h and the mixture stirred for a further 24 h to ensure completion. The solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over

$MgSO_4$, filtered and the solvent removed under reduced pressure. However, neither a peak at m/z = 824 due to $[M + H]^+$ nor a peak at m/z = 724 due to $[M - Boc]^+$ were observed in the FAB MS, indicating that the desired compound was not synthesised.

Preparation of *N-α-t-butoxycarbonyl-[(N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl)-(1-ethoxy)($N^ϵ$),(2-ethoxy')($N^{ϵ'}$)-1,2-ethyl]-glycine-methyl-ester (101)*



Many attempts were made to synthesise the above compound. Representative examples of these experiments are described below:

Method A

Dry caesium carbonate (432 mg, 1.33 mmol) and dry N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (100 mg, 0.133 mmol) were stirred in dry N,N-dimethylformamide (20 cm^3) under argon at RT.

1,2-Bis(2-chloroethoxy)ethane (27.3 mg, 0.146 mmol) in N,N-dimethylformamide (5 cm^3) was added by syringe pump over a period of 24 h and the mixture stirred for a further 3 days to ensure completion. The solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. However, FAB MS did not reveal any evidence of the desired product.

Method B

Dry caesium carbonate (432 mg, 1.33 mmol), dry N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (100 mg, 0.133 mmol) and 3 Å molecular sieves were stirred in dry N,N-dimethylformamide (20 cm^3) under argon at RT. 1,2-Bis(2-chloroethoxy)ethane (27.3 mg, 0.146 mmol) in N,N-dimethylformamide (5 cm^3) was added by syringe pump over a period of 24 h and the mixture stirred for a further 3 days to ensure completion. The reaction was worked up as above. FAB MS of the crude product showed the presence of the desired compound. Flash column chromatography (19:1 dichloromethane : methanol) separated the desired product from the starting tripeptide (99), but failed to produce a pure sample of product. However, FAB MS of the relevant column fractions indicated the presence of the desired product.

Method C

In two separate experiments dry caesium carbonate (216 mg, 0.067 mmol), dry N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (50 mg, 0.067 mmol) and 3 Å molecular sieves were stirred in dry N,N-dimethylformamide (10 cm^3) (or dry acetonitrile (10 cm^3)) under argon at 65^0 C. 1,2-Bis(2-chloroethoxy)ethane (13.7 mg, 0.073 mmol) in N,N-dimethylformamide (5 cm^3) was added by syringe pump over a period of 24 h and the mixture stirred for a further 3 days to ensure completion. The reaction was worked up as above but FAB MS failed to show any evidence of the desired compound in either reaction.

Method D

Dry caesium carbonate (432 mg, 1.33 mmol), dry N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (100 mg, 0.133 mmol), 3 Å molecular sieves and catalytic amounts of sodium iodide were stirred in dry N,N-dimethylformamide (20 cm^3) under argon at RT. 1,2-Bis(2-chloroethoxy)ethane (27.3 mg, 0.146 mmol) in N,N-dimethylformamide (5 cm^3) was added by syringe pump over a period of 24 h and the mixture stirred for a further 3 days to ensure completion. The reaction was worked up as above. However, following flash column chromatography (19:1 dichloromethane :

methanol), FAB MS showed that only the starting materials were isolated.

Method E

Dry caesium carbonate (216 mg, 0.067 mmol), dry N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (50 mg, 0.067 mmol) and catalytic amounts of caesium iodide were stirred in dry acetonitrile (10 cm^3) under argon at RT. 1,2-Bis(2-chloroethoxy)ethane (27.3 mg, 0.146 mmol) in acetonitrile (5 cm^3) was added by syringe pump over a period of 24 h and the mixture stirred for a further 3 days to ensure completion. The reaction was worked up as above. However, FAB MS of the crude sample and each of the fractions following flash column chromatography (19:1 dichloromethane : methanol) failed to reveal any evidence of the desired compound.

Method F

Dry caesium carbonate (648 mg, 2 mmol) was stirred in dry N,N-dimethylformamide (30 cm^3) under argon in a flame dried flask. Water (ca. 250 mg, 14 mmol) was then added followed by dry N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (300 mg, 0.4 mmol). 1,2-Bis(2-tosylethoxy)ethane (201 mg, 0.44 mmol) in N,N-dimethylformamide (10 cm^3) was added by syringe pump over a period of 48 h and the mixture stirred for a further 48 h under argon at RT to ensure completion. The mixture was worked up as above and following flash column chromatography (gradient elution from 98:2 dichloromethane : methanol to 95:5 dichloromethane : methanol), 155 mg (45 %) of the desired compound as a white solid was isolated.

Method G

Dry caesium carbonate (108 mg, 0.34 mmol) was stirred in dry acetonitrile (7 cm^3) under argon in a flame dried flask. Water (ca. 250 mg, 14 mmol) was added followed by dry N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (50 mg, 0.067 mmol) and 1,2-bis(2-tosylethoxy)ethane (12.4 mg, 0.067 mmol). After 24 h, more 1,2-bis(2-tosylethoxy)ethane was added. The mixture was stirred under argon at RT for a further 24 h to ensure completion. However, FAB MS and ^1H NMR spectroscopy

showed this reaction to have been unsuccessful in the synthesis of the desired compound.

Optimised method

Dry caesium carbonate (648 mg, 2 mmol) was stirred in dry N,N-dimethylformamide (40 cm^3) under argon in a flame dried flask. Water (ca. 250 mg, 14 mmol) was added followed by N-α-t-butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-methyl-ester (300 mg, 0.4 mmol) and 1,2-bis(2-tosylethoxy)ethane (182.5 mg, 0.4 mmol). After 24 h, more 1,2-bis(2-tosylethoxy)ethane (91.3 mg, 0.2 mmol) was added followed by the addition of another batch of 1,2-bis(2-tosylethoxy)ethane (91.3 mg, 0.2 mmol) after a further 24 h. The reaction mixture was stirred for a further 24 h to ensure completion before being filtered to remove the caesium carbonate. The solvent was removed under reduced pressure. Ethyl acetate was added and the mixture filtered to remove any remaining caesium carbonate. The solution was washed with water (x 2) and sat. brine (x 2), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Flash column chromatography (gradient elution from 99:1 dichloromethane : methanol to 90:10 dichloromethane : methanol) yielded 345.4 mg (62 %) of a white solid.

^{1}H NMR ($CDCl_3$, RT, 200 MHz);

δ/ppm 1.2-1.9 (m, br, 12H, $\mathbf{CH_2CH_2CH_2CH_2N}$), 1.293 (s, 9H, $C(\mathbf{CH_3})_3$), 2.428 (s, 3H, $\mathbf{CH_3}$, Ts), 2.447 (s, 3H, $\mathbf{CH_3}$, Ts), 3.2-3.45 (m, br, 8H, $\mathbf{CH_2}$NRTs), 3.5-3.8 (m, br, 8H, CH_2O), 3.711 (s, 3H, OCH_3), 3.85-4.18 (m, br, 3H, CH_2, Gly; α-CH, Lys), 4.40 (m, 1H, α-CH, Lys), 5.50 (d, 1H, J = 5.39 Hz, NH), 7.20 (d, 1H, J = 5.91 Hz, NH), 7.32 (m, 4H, CH, Ts), 7.70 (m, 4H, CH, Ts), 7.80 (d, 1H, J = 7.89 Hz, NH)

^{13}C NMR ($CDCl_3$, RT, 50 MHz);

δ/ppm 20.782 (CH_2, Lys), 21.426 (CH_3, Ts), 22.273, 28.581 (CH_2, Lys), 28.023 (CH_3, Boc), 28.334, 31.127 (CH_2, Lys), 41.096 (CH_2, Gly), 48.512, 48.707, 49.502, 49.893 (CH_2NHTs), 51.983 (OCH_3), 53.152, 56.277 (CH, Lys), 70.508, 71.188 (OCH_2), 80.156 (C, Boc), 126.798, 126.989, 129.578, 129.785 (CH, Ts), 135.348, 138.863, 143.057, 143.694 (C, Ts), 156.685 (C=O, Boc), 169.776, 172.374, 173.086 (C=O)

MS FAB+ve (NOBA matrix) $C_{40}H_{61}N_5O_{12}S_2$ M^+ calculated as 867.4

890 $[M + Na]^+$, 868 $[M + H]^+$, 768 $[M - Boc]^+$, 712 $[M - Ts]^+$, 612 $[M - Boc - Ts]^+$

MS FAB+ve (NOBA matrix) $[M + H]^+$ accurate mass

Calculated	868.3836
Found	868.3838

Elemental Analysis: (%)

Calculated	C 55.35	H 7.08	N 8.07
Found	C 55.43	H 7.10	N 8.06

IR, υ_{max}, cm^{-1} (KBr disc)

3411.1, 2931.0, 1640.1, 1616.5, 1521.0, 1455.2, 1336.2, 1157.4, 1089.8, 816.8, 728.6, 652.9

$[\alpha]_D^{20}$ -32.04

R_f 19:1 (dichloromethane : methanol) 0.4

mp (^{0} C) 58-64

5.3 Experimental relating to chapter 3

5.3.1 General method for the cyclodimerisation of tripeptides

The tripeptide was dissolved in 30 % 1 M potassium hydroxide solution in methanol and the solution stirred at room temperature until the reaction had gone to completion as judged by TLC. The methanol was removed under reduced pressure. The remaining solution was acidified using 1 M HCl to pH 3 (universal indicator paper) the solution diluted with water and the desired product extracted (x 5) with dichloromethane. The organic extracts were combined and the solvent removed under reduced pressure. The resultant solid was dried to yield the free acid as a white solid.

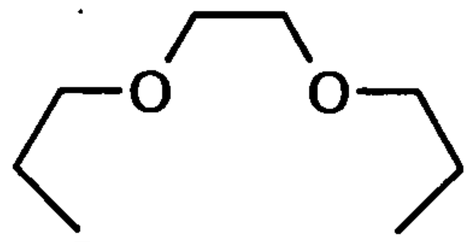
The tripeptide free acid, pentafluorophenol (1.5 mol. equiv.) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (2 mol. equiv.) were dissolved in dry (stored over 4 Å molecular sieves) ethyl acetate under argon. The mixture was stirred for 24 h until shown to be complete by TLC. The mixture was filtered and the solution washed with sat. brine to remove residual EDC. The crude product was purified through a short column by flash column chromatography in order to remove unreacted tripeptide carboxylic acid.

N-t-butoxycarbonyl deprotection was achieved by dissolving the pentafluorophenyl ester in 1:1 trifluoroacetic acid : dichloromethane. The mixture was stirred for approximately 1 h or until the starting material had disappeared as shown by TLC. The solvent was removed under reduced pressure and any residual solvent removed by forming an azeotrope 3 times with toluene. Each time the toluene was removed under reduced pressure. The oily product was triturated with diethyl ether until a white solid was formed.

Assuming 100 % deprotection, caesium carbonate (or sodium or potassium carbonate) (5 mol. equiv.) and N,N-dimethylformamide (0.005 M) were added under argon and the reaction mixture left to stir for approximately four days. The solution was filtered to remove caesium carbonate and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography

5.3.2 Cyclodimerisation of tripeptides

Preparation of *N-α-t-butoxycarbonyl-[(N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethyl]-glycine (129)*



Boc-Lys(Ts)-Lys(Ts)-Gly-OH

N-α-t-butoxycarbonyl-[(N-ϵ-tosyl-N-ϵ-lysyl-N-ϵ-tosyl-lysyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethane]-glycine-methyl-ester (1.35 g, 1.56 mmol) was dissolved in 30 % 1 M potassium hydroxide solution in methanol (70 cm^3) and the solution stirred at room temperature until the reaction had gone to completion as judged by TLC. The methanol was removed under reduced pressure and the remaining solution was acidified using 1 M HCl to pH 3 (universal indicator paper). The solution was diluted with water and the desired product was extracted (x 5) with dichloromethane. The organic extracts were combined and the solvent removed under reduced pressure. The resultant solid was dried to yield 1.3 g (98 %) of the free acid as a white solid.

^{1}H NMR ($CDCl_3$, RT, 200 MHz);

δ/ppm 1.2-2.1 (m, br, 12H, $CH_2CH_2CH_2CH_2N$), 1.273 (s, 9H, $C(CH_3)_3$), 2.386 (s, 3H, CH_3, Ts), 2.401 (s, 3H, CH_3, Ts), 2.8-3.3 (m, br, 8H, CH_2NRTs), 3.3-3.7 (m, 8H, CH_2O), 3.97 (m, 2H, CH_2, Gly), 4.08 (m, 1H, α-CH, Lys), 4.44 (m, 1H, α-CH, Lys), 5.6 (m, 1H, NH), 7.27 (m, 4H, CH, Ts), 7.38 (d, 1H, J = 8.0 Hz, NH), 7.66 (m, 4H, CH, Ts), 7.75 (d, 1H, J = 8.2 Hz, NH), 8.5-9.2 (s, br, COOH)

^{13}C NMR ($CDCl_3$, RT, 50 MHz);

δ/ppm 21.056 (CH_2, Lys), 21.423 (CH_3, Ts), 22.501, 26.795 (CH_2, Lys), 28.043 (CH_3, Boc), 28.595, 28.853, 31.177 (CH_2, Lys), 41.497 (CH_2, Gly), 48.487, 48.836, 49.183, 49.703 (CH_2NHTs), 53.013, 56.144 (CH, Lys), 70.395, 70.836, 70.992 (CH_2O), 80.359 (C, Boc), 128.844, 128.973, 129.591, 129.781 (CH, Ts), 135.455, 136.741, 143.088, 143.835 (C, Ts), 156.407 (C=O, Boc), 171.759, 172.901, 173.524 (C=O)

MS FAB+ve (NOBA matrix) $C_{39}H_{59}N_5O_{12}S_2$ M^+ calculated as 853.3

m/z 892 $[M + K]^+$, 876 $[M + Na]^+$, 854 $[M + H]^+$, 776 [M - Boc + Na]$^+$, 754 [M - Boc]$^+$

MS FAB+ve (NOBA matrix) $[M + Na]^+$ accurate mass

Calculated 876.3495

Found 876.3499

IR, υ_{max}, cm^{-1} (KBr disc);

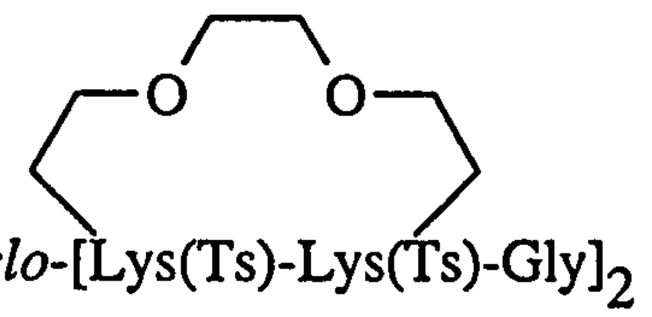
3413.7, 2934.0, 1654.2, 1522.3, 1457.8, 1335.4, 1157.1, 1089.8, 816.3, 729.6, 654.1

R_f 2:1:17 (methanol : acetic acid : chloroform) 0.25

19:1 (dichloromethane : methanol)

mp (^{0} C) 67-70

Preparation of *cyclo-[N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethyl-(N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethane] (103)*



cyclo-[Lys(Ts)-Lys(Ts)-Gly]$_2$

Initial conditions for synthesis

N-α-t-butoxycarbonyl-[(N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethyl]-glycine (450 mg, 0.53 mmol), pentafluorophenol (146 mg, 0.79 mmol) and dicyclohexylcarbodiimide (DDC) (220 mg, 1.07 mmol) were dissolved in dry N,N-dimethylformamide (10 cm^3) under argon. The mixture was stirred for 24 h until shown to be complete by TLC. The reaction mixture was filtered to remove the by-product, dicyclohexylurea. The solvent was removed under reduced pressure and the resultant brown oil was triturated in hexane (x 3) and diethyl ether (x 2), to give a brownish-white solid which was not characterised but was used for the next stage of the procedure.

N-α-t-butoxycarbonyl-[(N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethyl]-glycine-pentafluorophenyl ester (537.6 mg, 0.53 mmol) was dissolved in 1:1 trifluoroacetic acid : dichloromethane (30 cm^3) and stirred at room temperature for approximately 1 h, when TLC showed the starting material to have disappeared. The solvent was removed under reduced pressure and any residual trifluoroacetic acid removed by forming an azeotrope three times with toluene. The toluene was removed under reduced pressure. The oily product was triturated three times with diethyl ether to form a white solid. This was not characterised but used immediately.

Assuming 100 % deprotection, caesium carbonate (859 mg, 2.64 mmol) and N,N-dimethylformamide (105 ml, 0.005 M) were added under argon and the mixture stirred for four days. In other experiments, sodium carbonate (279 mg, 2.64 mmol) or potassium carbonate (369 mg, 2.64 mmol) were also used as the base and gave similar yields of cyclic peptide. The solution was filtered to remove the alkali metal carbonate and the solvent removed under reduced pressure. The crude product was dissolved in ethyl acetate, filtered to remove residual caesium carbonate, washed with water (100 cm^3 x 2) and sat. brine (100 cm^3) and dried over $MgSO_4$. The mixture was filtered and the solvent removed under reduced pressure. Flash column chromatography (gradient elution from 99:1 dichloromethane : methanol to 90:10 dichloromethane : methanol) yielded 35 mg (9 %) of a white solid.

Optimised condtions for synthesis

N-α-t-butoxycarbonyl-[(N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethyl]-glycine(450 mg, 0.53 mmol), pentafluorophenol (146 mg, 0.79 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were dissolved in dry (stored over 4 Å molecular sieves) ethyl acetate (10 cm^3) under argon. The mixture was stirred for 24 h until shown to be complete by TLC. The solution was filtered and washed with sat. brine to remove any residual EDC. The solvent was removed under reduced pressure. Flash column chromatography using a short silica gel column and using ethyl acetate as the eluant, followed by removal of the solvent under reduced pressure and washing with diethyl ether gave a white solid which was not characterised but was used for the next stage in the procedure.

N-α-t-butoxycarbonyl-[(N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethyl]-glycine-pentafluorophenyl ester (537.6 mg, 0.53 mmol) was dissolved in 1:1 trifluoroacetic acid : dichloromethane (30 cm^3) and stirred at room temperature for approximately 1 h, when TLC showed the starting material to have disappeared. The solvent was removed under reduced pressure and any residual trifluoroacetic acid removed by forming an azeotrope three times with toluene. The toluene was removed under reduced pressure. The oily product was triturated three times with diethyl ether to form a white solid. This was not characterised but was used immediately.

Assuming 100 % deprotection , caesium carbonate (859 mg, 2.64 mmol) and N,N-

dimethylformamide (105 cm^3, 0.005 M) were added under argon and the reaction mixture stirred for four days. The solution was filtered to remove the caesium carbonate and the solvent removed under reduced pressure. The crude product was dissolved in ethyl acetate, filtered to remove residual caesium carbonate, washed with water (100 cm^3 x 2) and sat. brine (100 cm^3) and dried over $MgSO_4$. The mixture was filtered and the solvent removed under reduced pressure. Flash column chromatography (gradient elution from 99:1 dichloromethane : methanol to 90:10 dichloromethane : methanol) yielded 244 mg (63 %) of a white solid.

^{1}H NMR ($CDCl_3$ trace CD_3OD, RT, 400 MHz);

δ/ppm 1.3-2.1 (m, br, 24H, $CH_2CH_2CH_2CH_2N$), 2.404 (s, 6H, CH_3, Ts), 2.411 (s, 6H, CH_3, Ts), 3.0-3.3 (m, br, 16H, CH_2NRTs), 3.5 (m, 16H, CH_2O), 4.1 (m, br, 6H, CH_2; Gly, α-CH, Lys), 4.42 (m, br, 2H, α-CH, Lys), 7.286 (d, 4H, J = 8.0 Hz, CH, Ts), 7.295 (d, 4H, J = 8.0 Hz, CH, Ts), 7.659 (d, 4H, J = 8.0 Hz, CH, Ts), 7.670 (d, 4H, J = 8.0 Hz, CH, Ts)

^{13}C NMR ($CDCl_3$ trace CD_3OD, RT, 100 MHz);

δ/ppm 21.50, 21.54 (CH_3, Ts), 22.30, 22.74, 27.92, 28.20, 30.33, 30.53 (CH_2, Lys), 43.88 (CH_2, Gly), 48.43, 48.99, 49.63, 49.78 (CH_2NRTs), 52.67, 55.57 (CH, Lys), 70.31, 70.48, 70.69, 70.87 (CH_2O), 127.10, 129.77, 129.92 (CH, Ts), 136.37, 136.46, 143.35 (C, Ts), 170.47, 172.13, 172.89 (C=O)

MS FAB+ve (NOBA matrix) $C_{68}H_{98}N_{10}O_{18}S_4$ M^+ calculated as 1470.6

m/z 1472 $[M + H]^+$, 1316 $[M - Ts]^+$, 1162 $[M - 2Ts]^+$

MS FAB+ve (NOBA matrix) $[M + H]^+$ accurate mass

Calculated 1471.6022

Found 1471.5956

Elemental analysis: (%)

Calculated	C 55.49	H 6.71	N 9.52
Found	C 55.30	H 6.69	N 9.48

IR, υ_{max}, cm^{-1} (KBr disc);

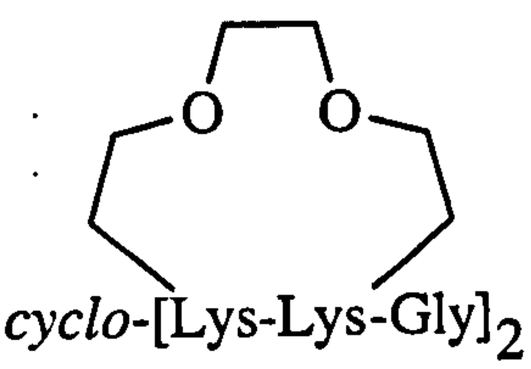
3332.1, 3063.6, 2931.6, 2361.3, 2336.7, 2244.9, 1923.7, 1649.6, 1597.5, 1530.0, 1454.9, 1336.1, 1144.0, 990.3, 816.0, 728.6, 654.6

R_f 19:1 (dichloromethane : methanol) 0.20

mp (^{0} C) 118-121

5.3.3 Detosylation of the cyclic peptide

Preparation of *cyclo-[lysyl-lysyl-glycyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethyl-(lysyl-lysyl-glycyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethane] (105)*



cyclo-[Lys-Lys-Gly]$_2$

Method A: Sodium anthracenide reduction

A 1.0 M stock solution of sodium anthracenide was prepared by dissolving anthracene (1.000 g, 5.60 mmol) and sodium (0.115 g, 5.00 mmol) in dry tetrahydrofuran (50 cm^3) under a stream of nitrogen. After stirring for 2 h, dissolution was complete and a dark blue colour persisted. *Cyclo*-[N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethyl-(N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethane] (100 mg, 0.068 mmol) in dry tetrahydrofuran (50 cm^3) was stirred at 0^0 C under argon. The sodium anthracenide solution (27.5 ml, 2.72 mmol) was added and stirring continued for 1 h. The reaction was quenched with sat. sodium chloride solution. The solvent was removed under reduced pressure and the sample dried to give a brown residue.

A sample of this residue (10.3 mg) was dissolved in pH 5 potassium phosphate aqueous buffer (1 cm^3) and the sample analysed using a reverse phase analytical HPLC column. Three distinct fractions were observed and isolated and each analysed by FAB MS. Whereas the second and third fractions showed no evidence of the presence of the desired product, the first fraction gave peaks at both m/z = 854 $[M]^+$ and 893 $[M + K]^+$, indicating the presence of the desired product.

Method B: Hydrogen bromide/acetic acid

To *cyclo*-[N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl)-(2-ethoxy)($N^ϵ$),(2-ethoxy')($N^{ϵ'}$)-1,2-ethyl-(N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl)-(2-ethoxy)($N^ϵ$),(2-ethoxy')($N^{ϵ'}$)-1,2-ethane] (100 mg, 0.068 mmol) in phenol (76.6 mg, 0.815 mmol) was added 1:1 hydrogen bromide : acetic acid in a 5 cm^3 Wheaton vial. The vial, fitted with a superseal under a screw cap was heated in a sand bath to 70^0 C for 16 h. The vial was then cooled by placing in ice. However, work up was not deemed to be appropriate as the above procedure gave only a black tar. FAB MS of this crude product failed to show any peaks that could be assigned to the desired product.

Method C: Sodium dispersion in liquid paraffin/liquid ammonia reduction

To a 3-necked flask fitted with a cold-finger condenser and drying tube was added liquid ammonia (ca. 100 cm^3) under argon. The liquid was cooled to -70^0 C and 50 % sodium dispersion in paraffin (422 mg, 9.17 mmol) was added with stirring to give a dark blue solution. *Cyclo*-[N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl)-(2-ethoxy)($N^ϵ$),(2-ethoxy')($N^{ϵ'}$)-1,2-ethyl-(N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl)-(2-ethoxy)($N^ϵ$),(2-ethoxy')($N^{ϵ'}$)-1,2-ethane] (450 mg, 0.305 mmol) was dissolved in dry tetrahydrofuran (25 cm^3) and added dropwise to the reducing solution. Stirring was continued for 2 h and the reaction quenched with glacial acetic acid (2 cm^3). The liquid ammonia was allowed to evaporate and the residue suspended in 1:1 methanol : water (20 cm^3). Kieselguhr was added, the suspension filtered and the solvent removed under reduced pressure. Residual acetic acid was removed by forming an azeotrope with toluene and by leaving the sample in a vacuum desicator containing sodium hydroxide pellets.

The residue was dissolved in 2,2,2-trifluoroethanol and the sodium acetate salt

removed by filtration. A sample of the residue from the 2,2,2-trifluoroethanol solution was analysed by FAB MS. Although a peak at m/z = 856 due to $[M + H]^+$ was observed it was not significantly more intense than the background noise of the spectrum.

Method D: Sodium/liquid ammonia reduction

To a 3-necked flask fitted with a cold-finger condenser and drying tube was added liquid ammonia (ca. 100 cm^3) under argon. The liquid was cooled to -70^0 C and sodium (300 mg, 13.04 mmol) was added with stirring to give a dark blue solution. *Cyclo*-[N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl)-(2-ethoxy)($N^ϵ$),(2-ethoxy')($N^{ϵ'}$)-1,2-ethyl-(N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl)-(2-ethoxy)($N^ϵ$),(2-ethoxy')($N^{ϵ'}$)-1,2-ethane] (400 mg, 0.271 mmol) was dissolved in dry tetrahydrofuran (15 cm^3) and added dropwise to the reducing solution. Stirring was continued for 2 h and the reaction quenched with ammonium chloride (800 mg, 15 mmol). The liquid ammonia was dissolved in 2,2,2-trifluoroethanol, the mixture filtered to remove sodium chloride and the solvent was removed under reduced pressure. Flash column chromatography (19 : 1 methanol : liquid ammonia) yielded 160 mg (68.8 %) of a white solid.

^{1}H NMR ($CDCl_3$, RT, 200 MHz);

δ/ppm 1.15-1.95 (m, br, 24H, $CH_2CH_2CH_2CH_2N$), 2.45-2.6 (m, 8H, CH_2NHR), 2.6-2.75 (m, 8H, CH_2NHR), 3.4-3.55 (m, 16H, CH_2O), 4.0-4.2 (m, 8H, $2CH_2$, Gly; 4α-CH, Lys)

MS FAB+ve (NOBA matrix) $C_{40}H_{74}N_{10}O_{10}$ M^+ calculated as 854.6

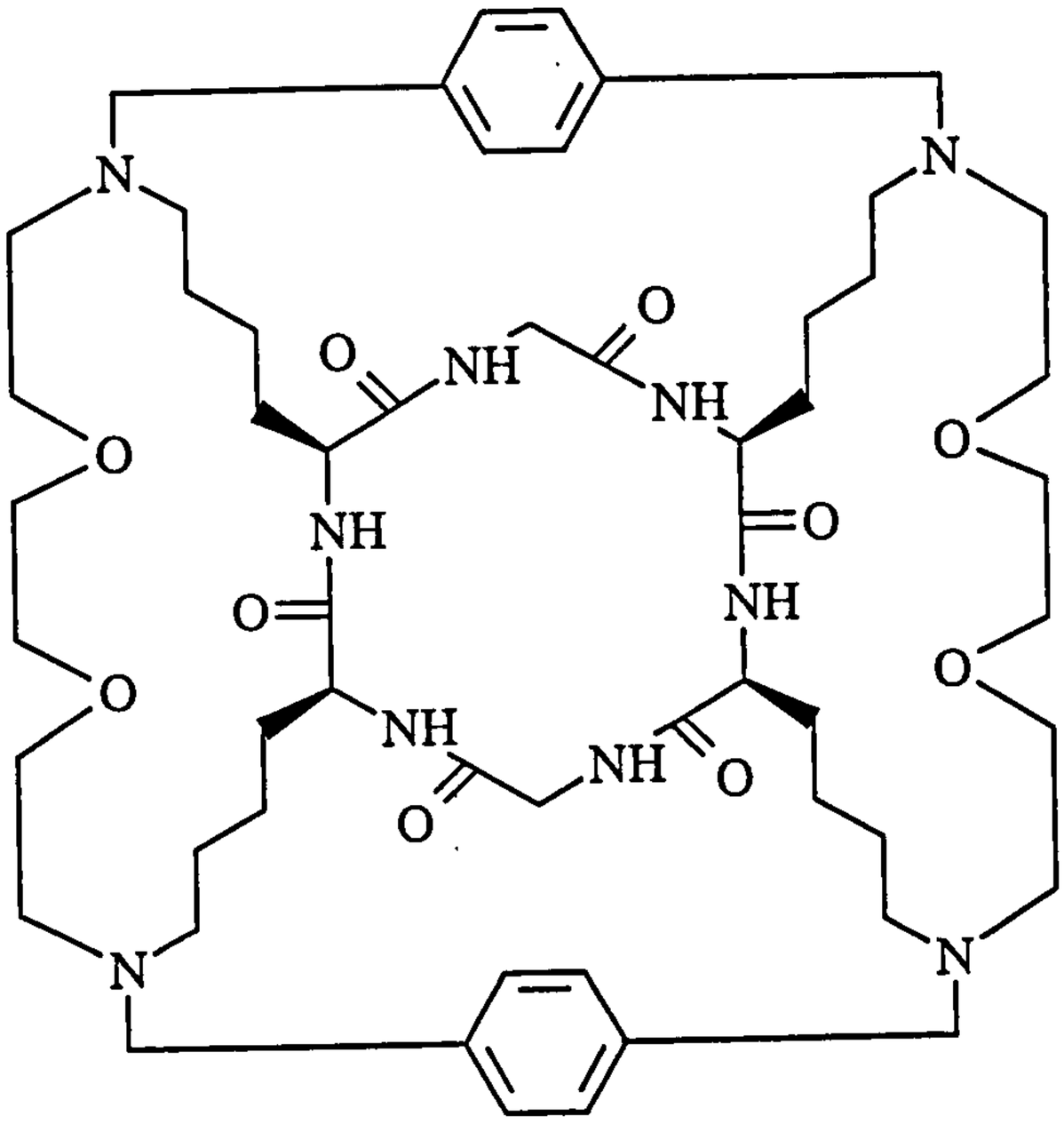
m/z 856 $[M + H]^+$

R_f 19:1 (methanol : ammonia) 0.15

19:1 (dichloromethane : methanol) 0.00

5.3.4 Final crosslinking reactions

Attempted synthesis of *(79)* by slow addition of *1,4-α,α'-dibromo-p-xylene* to *cyclo-[lysyl-lysyl-glycyl)-(2-ethoxy)(N$^{\epsilon}$),(2-ethoxy')(N$^{\epsilon'}$)-1,2-ethyl-(lysyl-lysyl-glycyl)-(2-ethoxy)(N$^{\epsilon}$),(2-ethoxy')(N$^{\epsilon'}$)-1,2-ethane] (105)*



Cyclo-[lysyl-lysyl-glycyl)-(2-ethoxy)(N$^{\epsilon}$),(2-ethoxy')(N$^{\epsilon'}$)-1,2-ethyl-(lysyl-lysyl-glycyl)-(2-ethoxy)(N$^{\epsilon}$),(2-ethoxy')(N$^{\epsilon'}$)-1,2-ethane] (84.4 mg, 0.0987 mmol) was dissolved in 2,2,2-trifluoroethanol (14 cm^3) and the mixture stirred at 40^0 C under argon. 1,4-α,α'-dibromo-*p*-xylene (26.1 mg, 0.0987 mmol) in N,N-dimethylformamide (2 cm^3) was added by syringe pump over a period of 12 h and stirring continued. 1,4-α,α'-dibromo-*p*-xylene (26.1 mg, 0.0987 mmol) and N,N-diisopropylethylamine (12.76 mg, 0.0987 mmol) in N,N-dimethylformamide (2 cm^3) was then added by syringe pump over a period of 12 h and stirring continued. Finally, N,N-diisopropylethylamine (12.76 mg, 0.0987 mmol) in N,N-dimethylformamide (2 cm^3) was added by syringe pump over a period of 12 h and stirring continued. The mixture was stirred for a further 72 h before the solvent was removed under reduced pressure. Residual solvent was removed by forming an azeotrope three times with toluene and removing the toluene under reduced pressure. Flash column

chromatography (180:19:1 chloroform : methanol : ammonia) yielded 29 mg of a white solid. FAB MS analysis of this product gave a distinct peak at m/z = 1060, indicative of the mass ion of the desired product. However, TLC evidence suggested it was still impure. Repeated flash column chromatography failed to satisfactorily purify the desired product. ^{1}H NMR spectroscopy of the 10 mg sample, in CD_3OD, $CDCl_3$, D_2O and d_7-DMF, although consistent with the desired product, failed to prove its identity.

^{1}H NMR (d_7-DMF, RT, 400 MHz);

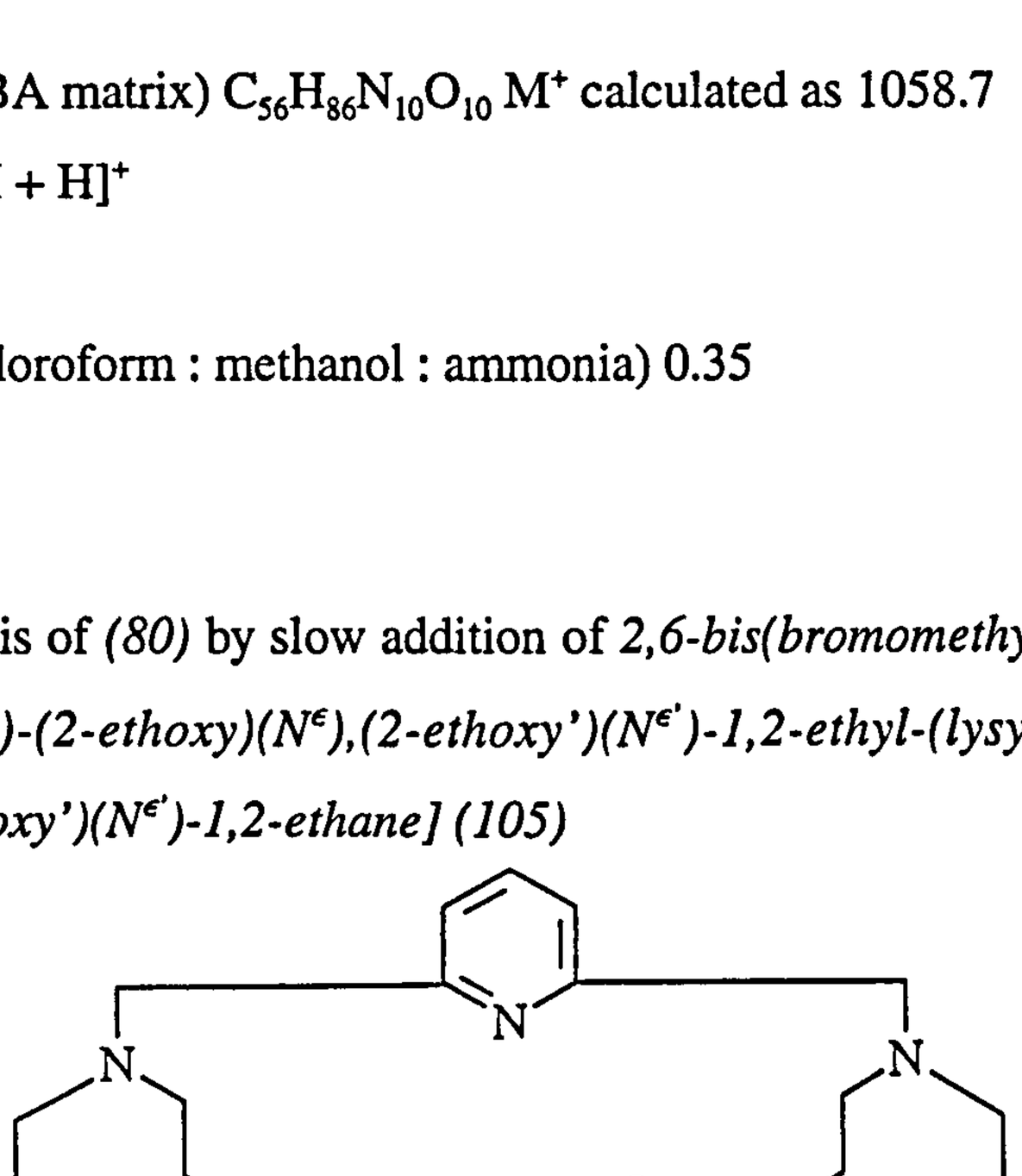
δ/ppm 1.1-1.9 (m, br, 24H, $CH_2CH_2CH_2CH_2N$), 3.1-3.5 (HOD), 3.5-3.6 (m, 16H, CH_2O), 4.05-4.35 (m, 8H, 2 x CH_2, Gly; 4 x α-CH, Lys), 7.2-7.6 (m, br, 8H, CH, xylyl)

MS FAB+ve (NOBA matrix) $C_{56}H_{86}N_{10}O_{10}$ M^+ calculated as 1058.7

m/z 1060 $[M + H]^+$

R_f 180:19:1 (chloroform : methanol : ammonia) 0.35

Attempted synthesis of *(80)* by slow addition of *2,6-bis(bromomethyl)pyridine* to *cyclo-[lysyl-lysyl-glycyl)-(2-ethoxy)(N^ϵ),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethyl-(lysyl-lysyl-glycyl)-(2-ethoxy)(N^ϵ),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethane] (105)*



220

Cyclo-[lysyl-lysyl-glycyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethyl-(lysyl-lysyl-glycyl)-(2-ethoxy)(N^{ϵ}),(2-ethoxy')($N^{\epsilon'}$)-1,2-ethane] (90 mg, 0.105 mmol) was dissolved in 2,2,2-trifluoroethanol (16 cm^3) and the mixture stirred at 40^0 C under argon. 2,6-Bis(bromomethyl) pyridine (27.9 mg, 0.105 mmol), in N,N-dimethylformamide (2 cm^3) was added by syringe pump over a period of 12 h and stirring continued. 2,6-Bis(bromomethyl)pyridine (27.9 mg, 0.105 mmol) and N,N-diisopropylethylamine (13.6 mg, 0.105 mmol) in N,N-dimethylformamide (2 cm^3) was then added by syringe pump over a period of 12 h and stirring continued. The mixture was stirred for a further 72 h before the solvent was removed under reduced pressure. Residual solvent was removed by forming an azeotrope three times with toluene and removing the toluene under reduced pressure. Analysis of the crude product by FAB MS failed to indicate the presence of the desired product. Following repeated flash column chromatography (180:19:1 chloroform : methanol : ammonia) and analysis of the fractions by FAB MS, a peak at m/z = 1062 due to $[M + H]^+$ was observed in 1 out of 20 scans but its intensity was not significantly above those of surrounding uncharacterised peaks. No evidence of the desired product was observed when the 4 mg sample was submitted to ^1H NMR spectroscopy using CD_3OD, $CDCl_3$, D_2O and d_7-DMF as the solvents.

MS FAB+ve (NOBA matrix) $C_{54}H_{84}N_{12}O_{10}$ M^+ calculated as 1060.7

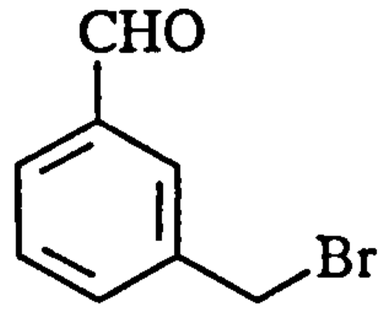
m/z 1062 $[M + H]^+$

R_f 180:19:1 (chloroform : methanol : ammonia) 0.35

5.4 Experimental relating to chapter 4

5.4.1 Porphyrin synthesis

. Preparation of *3-bromomethylbenzaldehyde (154)*



To a solution of 3-bromomethylbenzonitrile (1 g, 5.10 mmol) in dichloromethane (1 cm^3) at 0^0 C under argon, a 1 M solution of diisobutylaluminium hydride in dichloromethane (6.1 cm^3) was added over 20 min. The resulting solution was stirred for 1.5 h at 0^0 C. It was then diluted with dichloromethane (25 cm^3) and the reaction quenched by the addition of 2 M hydrochloric acid (25 cm^3) and shaking the mixture for 10 min. The organic and aqueous layers were separated and the aqueous layer extracted with dichloromethane (20 cm^3 x 3). The organic solutions were combined, washed with water (25 cm^3) and sat. brine (25 cm^3), dried over $MgSO_4$ and then filtered. The solvent was removed under reduced pressure and the resultant oil solidified by cooling to 0^0 C. The solid was triturated with diethyl ether and the solvent removed under reduced pressure to yield 842 mg (83 %) of the desired product as shiny-white crystals.

Note: The above are optimised conditions of synthesis. The literature precedent suggested the use of chlorobenzene as the solvent with diisobutylaluminium hydride in hexanes as the reagent. However, due to its high boiling point, chlorobenzene (bp = 132^0 C) was difficult to remove under reduced pressure even using toluene to form an azeotrope. Thus dichloromethane (bp = 40^0 C) was used as the solvent. Data agreed with the literature values given in brackets [ ][214].

^{1}H NMR ($CDCl_3$, RT, 270 MHz);

δ/ppm 4.538 [4.54] (s, 2H, CH_2), 7.522 [7.52] (dd, 1H, 7.52 Hz [8], 7.76 Hz [8], **CH**, C_5), 7.665, 7.813 [7.67, 7.82] (2d, 2H, J = 7.52 Hz [8], J = 7.76 Hz [8], **CH**, C_4, C_6); 7.901 [7.90] (s, 1H, **CH**, C_2), 10.103 [10.02] (s, 1H, CHO)

^{13}C NMR ($CDCl_3$, RT, 67.90 MHz);

δ/ppm 32.1 [32.0] (CH_2), 129.6, 129.7, 129.8 [129.6, 129.7, 129.8] (3CH, Ar), 134.9 [134.8] (CH, Ar), 136.8, 138.3 [136.8, 138.9](C, Ar), 191.6 [191.6] (CHO)

MS EI $C_8H_7O^{79}Br$ M^+ calculated as 198

m/z 201 $[M + H]^+$ (^{81}Br), 200 $[M]^+$ (^{81}Br), 199 $[M - H]^+$ (^{81}Br), $[M + H]^+$ (^{79}Br), 198 $[M]^+$ (^{79}Br), 197 $[M - H]^+$ (^{79}Br), 171 $[PhCH_2{}^{81}Br]^+$, 169 $[PhCH_2{}^{79}Br]^+$, 119 $[M - Br]^+$, 91 $[PhCH_2]^+$

MS EI $[M]^+$ (^{79}Br) accurate mass

Calculated 197.9680

Found 197.9675

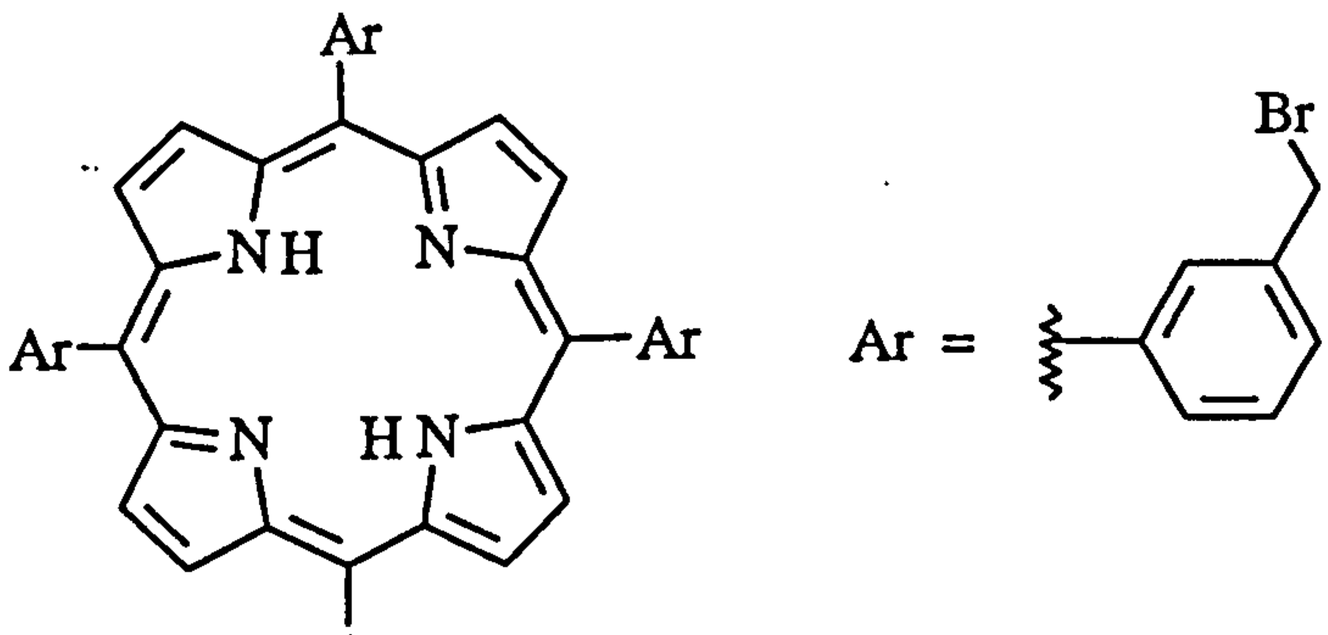
IR, υ_{max}, cm^{-1} (nujol mull)

2956.3, 2925.8, 2854.6, 1713.6, 1690.5, 1586.1, 1465.0, 1301.8, 1211.7, 1156.8, 810.2, 784.1, 695.6

R_f 19:1 (40-60 petroleum ether : ethyl acetate) 0.22

mp (^{0} C) 45-48 [46-49]

Attempted preparation of *5,10,15,20-tetrakis(3-bromomethylphenyl)porphyrin (155)*



To a mixture of 3-bromomethylbenzaldehyde (1 g, 5.03 mmol) and pyrrole (0.34 g, 5.03 mmol) in chloroform (500 cm^3) was added a 2.5 M solution of boron trifluoride etherate in chloroform (0.9 cm^3). The resulting solution was stirred at room temperature for 1 h. Triethylamine (0.22 g, 2.18 mmol) and then tetrachloro-1,4-benzoquinone (TCQ) (0.923 g, 3.75 mmol) were added and the mixture refluxed for 1 h. 2,3-Dicyano-5,6-dichloro-1,4-benzoquinone (DDQ) (0.851 g, 3.75 mmol) was also used as an oxidising agent in place of TCQ but without significant improvement in the reaction yield. Following a precedent[212], ethanol (3.75 cm^3, 0.75 % v/v) was also added at the reflux stage but again without any noticeable improvement in the reaction. The crude product was analysed by FAB MS and UV/Vis spectroscopy.

In order to effect the purification of the desired porphyrin, three different methods were attempted.

a) The solvent was removed under reduced pressure and the residue triturated with diethyl ether and then filtered. The filtrate was evaporated and the residue triturated with dichloromethane and then filtered. The filtrate was concentrated to ca. 15 cm^3 and methanol (20 cm^3) was added. However, unlike the description in the literature[214], the porphyrin could not be precipitated despite different ratios of dichloromethane and methanol being used and despite the cooling of the mixture to 0^0 C in an ice-water bath.

b) The solvent was removed under reduced pressure and the residue triturated with a variety of solvents, ie., acetone, benzene, dichloromethane, ethanol, ethyl acetate,

isopropanol, methanol, toluene, xylene, N,N-dimethylformamide, acetonitrile. However, none of these could be used to isolate or solubilise successfully the desired porphyrin.

c) Flash column chromatography using dichloromethane, 95:5 dichloromethane : methanol, 50:50 40-60 petroleum ether : dichloromethane or 95:5 40-60 petroleum ether : ethyl acetate as the eluants was performed on the crude sample. However, a great deal of product was adsorbed onto to the column. In addition none of the desired porphyrin could be adequately separated from the large number of by-products observed by TLC; many of which ran very closely to the desired porphyrin using each of the solvent systems above. Data agreed with the literature values given in brackets[214].

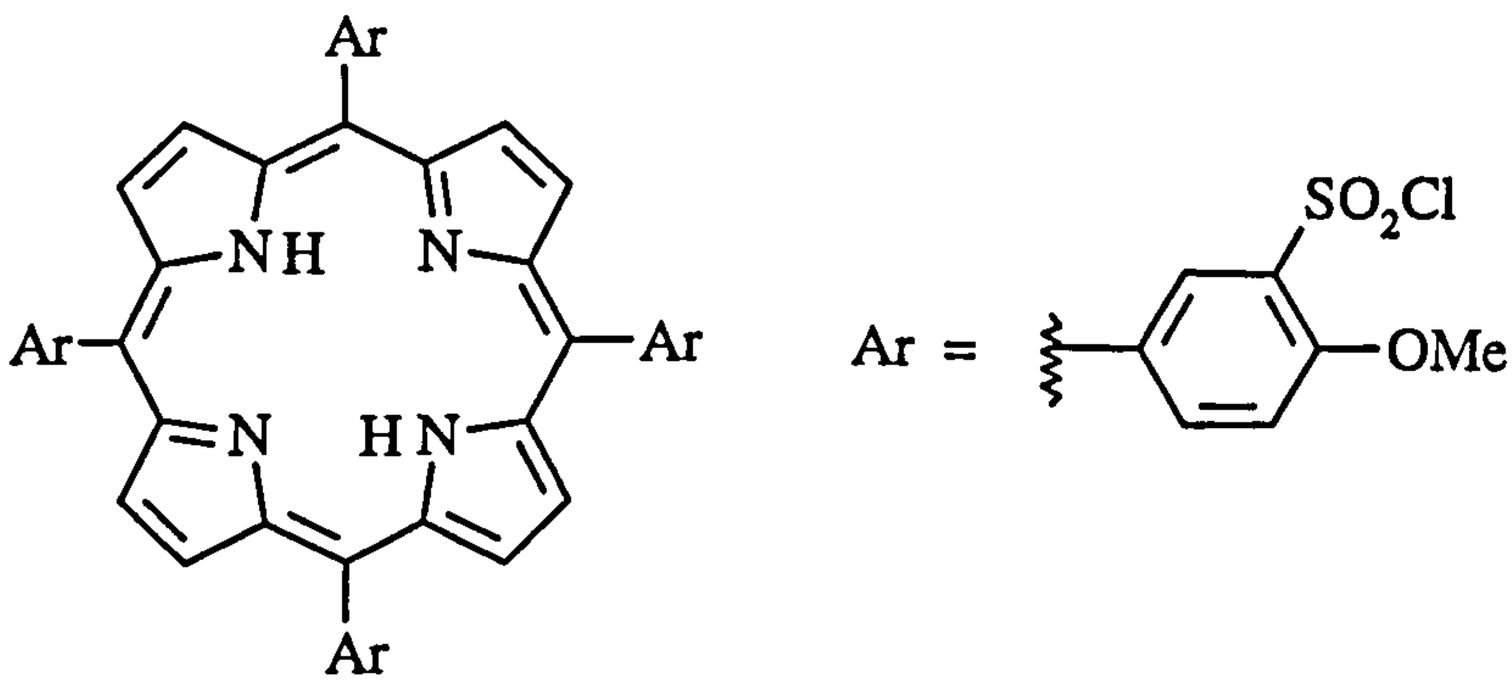
MS FAB+ve (NOBA matrix) $C_{48}H_{34}Br_4N_4$ M^+ calculated as 986 ($2^{79}Br$, $2^{81}Br$)

m/z 991 $[M + H]^+$ ($4^{81}Br$), 989 $[M + H]^+$ ($3^{81}Br$, ^{79}Br), 987 $[M + H]^+$ ($2^{81}Br$, $2^{79}Br$), 985 $[M + H]^+$ (^{81}Br, $3^{79}Br$), 983 $[M + H]^+$ ($4^{79}Br$), 909 $[M - Br]^+$ ($3^{81}Br$), 907 $[M - Br]^+$ ($2^{81}Br^{79}Br$), 905 $[M - Br]^+$ (^{81}Br, $2^{79}Br$), 903 $[M - Br]^+$ ($3^{79}Br$)

UV-Vis, chloroform, λ_{max} (% relative height of Soret band), nm;

419 (100 %) [419.5 (100 %)], 515 (8.1 %) [515 (5.0 %)], 551 (5.0 %) [549 (2.2 %)], 588 (4.4 %) [589 (1.7 %)], 645 (3.0 %) [646 (1.1 %)], 721 (2.3 %)

Preparation of *5,10,15,20-tetrakis(3-chlorosulphonyl-4-methoxyphenyl)porphyrin (157)*



5,10,15,20-Tetrakis(4-methoxyphenyl)porphyrin (100 mg, 0.316 mmol) was stirred with chlorosulphonic acid (2 cm^3) at 100^0 C for 3 h. The mixture was cooled, ground ice (30 - 40 g, caution!) was added and the green mixture diluted with water (50 cm^3). The desired product was extracted with chloroform (50 cm^3 x 5). Any emulsions that formed were broken up using liberal amounts of acetonitrile. It is important not to use alcoholic solvents to break up emulsions so as not to convert the chlorosulphonyl groups on the desired product to sulphonyl esters. The organic extracts were combined and the solvent removed under reduced pressure to yield 126 mg (80 %) of the desired product as a purple solid.

^{1}H NMR ($CDCl_3$, RT, 300 MHz);

δ/ppm -2.492 (s, br, 2H, **NH**), 4.463 (s, 12H, OCH_3), 7.863 (d, 4H, J = 8.7 Hz, **CH**, C_5 or C_6, Ar), 8.534 (s, 8H, **CH**, β-pyrrole), 9.007 (d, 4H, J = 8.7 Hz, **CH**, C_5 or C_6, Ar), 9.147 (s, 4H, **CH**, C_2, Ar)

MS FAB+ve (NOBA matrix) $C_{48}H_{34}O_{12}N_4S_4{}^{35}Cl_4$ M^+ calculated as 1126

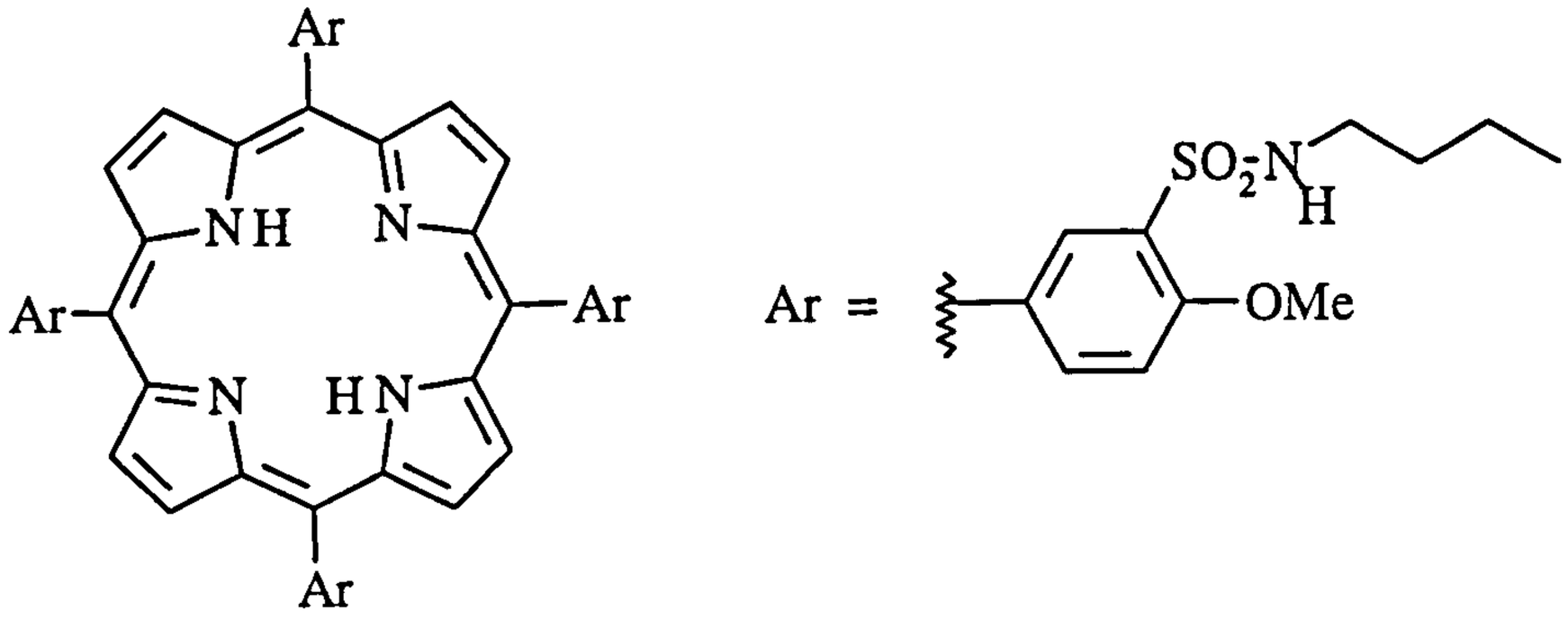
m/z 1135 $[M + H]^+$ (4 ^{37}Cl), 1133 $[M + H]^+$ (3 ^{37}Cl, ^{35}Cl), 1131 $[M + H]^+$ (2 ^{37}Cl, 2 ^{35}Cl), 1129 $[M + H]^+$ (^{37}Cl, 3 ^{35}Cl), 1127 $[M + H]^+$ (4 ^{35}Cl), 1033 $[M - SO_2Cl]^+$ (3 ^{37}Cl), 1031 $[M - SO_2Cl]^+$ (2 ^{37}Cl, ^{35}Cl), 1029 $[M - SO_2Cl]^+$ (^{37}Cl, 2 ^{35}Cl), 1027 $[M - SO_2Cl]^+$ (3 ^{35}Cl)

UV-Vis, chloroform, λ_{max} (% relative height of Soret band), nm;

422 (100 %), 518 (4.5 %), 554 (3.4 %), 596 (2.9 %), 650 (2.1 %)

R_f (dichloromethane) 0.5 purple/pink spot

Preparation of *5,10,15,20-tetrakis(3-butylsulphonamide-4-methoxyphenyl)porphyrin (158)* [Model reaction for capping of cyclic peptide onto 5,10,15,20-tetrakis(3-chlorosulphonyl-4-methoxyphenyl)porphyrin]



Freshly prepared 5,10,15,20-tetrakis(3-chlorosulphonyl-4-methoxyphenyl)porphyrin (156.7 mg, 0.139 mmol), butylamine (48.7 mg, 0.667 mmol), N,N-diisopropylethylamne (144 mg, 1.111 mmol) in N,N-dimethylformamide (46.3 cm^3) were stirred at room temperature under argon for 20 h. The solvent was removed under reduced pressure and residual solvent removed by forming an azeotrope with toluene (x 3). The toluene was removed under reduced pressure. The purple solid was dissolved in dichloromethane (150 cm^3) and washed with 1 M HCl (50 cm^3) and sat. brine (50 cm^3). Flash column chromatography (gradient elution from 99:1 dichloromethane : methanol to 95:5 dichloromethane : methanol) yielded 20 mg (11.2 %) of a purple solid.

In order to obtain as sharp a ^1H NMR as possible, three ^1H NMR spectra were recorded using the conditions described below. The sharpest spectrum was obtained using $CDCl_3$ as the solvent.

^{1}H NMR ($CDCl_3$, RT, 300 MHz);

δ/ppm -2.51 (s, br, 2H, NH), 0.881 (m, 12H, $CH_2CH_2CH_2CH_3$), 1.2-1.8 (m, 16H, $CH_2CH_2CH_2CH_3$), 3.2 (m, br, 8H, CH_2NHSO_2), 4.381 (s, 12H, OCH_3), 7.77 (d, br, 4H, CH, C_5 or C_6, Ar), 8.48 (s, br, 8H, CH, β-pyrrole), 8.91 (d, br, 4H, CH, C_5 or C_6, Ar), 9.15 (s, 4H, CH, C_2, Ar)

^{1}H NMR (3:1 d-TFA : $CDCl_3$, 45^0 C, 300 MHz, from δ = -0.1 - 10.0);

δ/ppm 0.988 (t, 12H, J = 7.3 Hz, $CH_2CH_2CH_2CH_3$), 1.3-1.6 (m, br, 16H, $CH_2CH_2CH_2CH_3$), 3.23 (m, br, 8H, CH_2NHSO_2), 4.373 (s, 12H, OCH_3), 7.86 (m, br, 4H, CH, Ar), 8.73 (s, br, 8H, CH, β-pyrrole), 8.94 (m, br, 4H, CH, Ar), 9.25 (m, br, 4H, CH, Ar)

^{1}H NMR (3:1 d-TFA : $CDCl_3$, RT, 300 MHz, from δ = -0.1 - 10.0);

δ/ppm 0.981 (t, 12H, J = 7.3 Hz, $CH_2CH_2CH_2CH_3$), 1.3-1.8 (m, br, 16H, $CH_2CH_2CH_2CH_3$), 3.21 (m, br, 8H, CH_2NHSO_2), 4.367 (s, 12H, OCH_3), 7.86 (m, br, 4H, CH, Ar), 8.72 (s, br, 8H, CH, β-pyrrole), 9.02 (m, br, 4H, CH, Ar), 9.31 (m, br, 4H, CH, Ar)

MS FAB+ve (NOBA matrix) $C_{64}H_{74}N_8O_{12}S_4$ M^+ calculated as 1274.4

m/z 1275 $[M + H]^+$, 1220 $[M - C_4H_8]^+$

MS FAB+ve (NOBA matrix) $[M + H]^+$ accurate mass

Calculated 1275.439

Found 1275.439

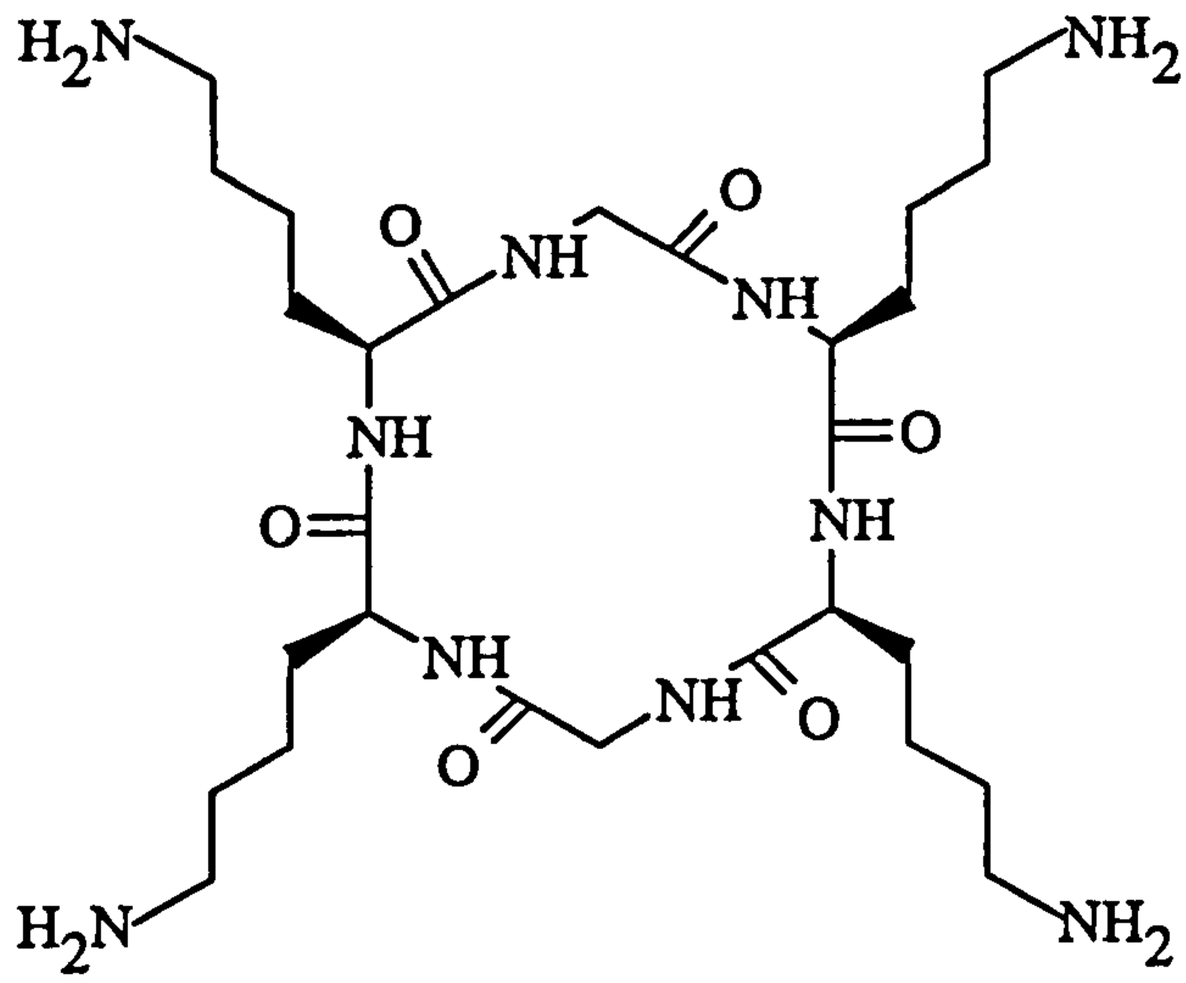
UV-Vis, chloroform, λ_{max} (% relative height of Soret band), nm;

420 (100 %), 521 (4.4 %), 556 (3.2 %), 589 (3.0 %), 646 (2.0 %)

R_f 19:1 (dichloromethane : methanol) 0.30

5.4.2 Preparation of cyclic peptides

Preparation of *cyclo-[lysyl-lysyl-glycyl-lysyl-lysyl-glycine-] (159)*



Palladium on activated carbon (wet degussa type, E101 NE/W) (5 mg) was preactivated with hydrogen in 2,2,2-trifluoroethanol (5 cm^3). To this solution was added *cyclo*-[N-ϵ-benzyloxycarbonyl-lysyl-N-ϵ-benzyloxycarbonyl-lysyl-glycyl-N-ϵ-benzyloxycarbonyl-lysyl-N-ϵ-benzyloxycarbonyl-lysyl-glycine-] (100 mg, 0.086 mmol) and the mixture was stirred under an H_2 atmosphere for 5 days. The mixture was filtered under suction through Kieselguhr and the solvent removed under reduced pressure from the filtrate. The residue was triturated with diethyl ether to yield 53.8 mg (100 %) of a hydroscopic white solid. The material was made as required for the next stage in the synthesis of the porphyrin capped cyclic peptide and used promptly. Data agreed with the literature values given in brackets [ ][118].

^1H NMR ($CDCl_3$: trace CD_3OD, RT, 400 MHz); [(d_6-DMSO, RT, 300 MHz)]

δ/ppm 0.9-1.9 [1.2-1.9] (m, 24H, $CH_2CH_2CH_2CH_2NH_2$), 2.585 [2.507] (t, 8H, CH_2NH_2, J = 6.9 Hz), 3.578 [3.558] (d, 2H, J = 15.8 Hz [15.0], CH, Gly), 3.951 [3.867] (d, 2H, J = 15.8 Hz [15.0], CH, Gly), 4.051 (d, 1H, J = 9.06 Hz, α-CH, Lys), 4.064 (d, 1H, J = 8.94 Hz, α-CH, Lys), [4.032 (m, 2H, α-CH, Lys)], 4.204 (d, 1H, J = 9.06 Hz, α-CH, Lys), 4.218 (d, 1H, J = 8.94, α-CH, Lys), [4.174 (m, 2H, α-CH, Lys)]

MS FAB+ve (NOBA matrix) $C_{28}H_{54}O_6N_{10}$ M^+ calculated as 626

m/z 627 $[M + H]^+$

R_f 7:7:5:1 (isopropanol : ethanol : water : ammonia) 0.2 (not UV active but stains with ninhydrin)

Preparation of *N-α-t-butoxycarbonyl-N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl-glycine*

Boc-Lys(Ts)-Lys(Ts)-Gly-OH

N-α-t-Butoxycarbonyl-N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl-glycine-methyl-ester (500 mg, 0.664 mmol) was dissolved in 30 % 1 M potassium hydroxide solution in methanol (28 cm^3) and the solution stirred at room temperature until the reaction had gone to completion as judged by TLC. The methanol was removed under reduced pressure and the remaining solution was acidified using 1 M HCl to pH 3 (universal indicator paper), the solution diluted with water and the desired product extracted (x 5) with dichloromethane. The organic extracts were combined and the solvent removed under reduced pressure. The resultant solid was dried to yield 491 mg (100 %) of the free acid as a white solid.

^1H NMR ($CDCl_3$, RT, 270 MHz);

δ/ppm 1.2-2.0 (m, 12H, $CH_2CH_2CH_2CH_2N$), 1.423 (s, 9H, $C(CH_3)_3$), 2.430 (s, 6H, CH_3, Ts), 2.9 (m, br, 4H, CH_2NHTs), 4.184 (m, 2H, CH_2, Gly), 4.136 (m, 1H, α-CH, Lys), 4.609 (m, 1H, α-CH, Lys), 5.782 (m, br, 1H, NH), 6.1 (m, br, 3H, NH), 7.346 (d, 4H, J = 8.0 Hz, CH, Ts), 7.593 (m, br, 1H, NH), 7.795 (d, 4H, J = 8.0 Hz, CH, Ts)

^{13}C NMR ($CDCl_3$, RT, 67.90 MHz);

δ/ppm 21.441 (CH_3, Ts), 22.106, 22.307 (CH_2, Lys), 28.250 (CH_3, Boc), 28.491, 28.673 (CH_2, Lys), 41.623, 42.391, 42.693 (CH_2, Gly; CH_2NHTs), 53.068, 54.926 (CH, Lys), 80.203 (C, Boc), 129.638, 126.979 (CH, Ts), 136.850, 136.729, 143.196 (C, Ts), 156.108 (C=O, Boc), 172.285, 172.949, 173.272 (C=O)

MS FAB+ve (NOBA matrix) $C_{33}H_{49}N_5O_{10}S_2$ M^+ calculated as 739.3

m/z 779 $[M + K]^+$, 762 $[M + Na]^+$, 740 $[M + H]^+$, 640 $[M - Boc]^+$

MS FAB+ve (NOBA matrix) $[M + K]^+$ accurate mass

Calculated 778.2558

Found 778.2553

MS FAB+ve (NOBA matrix) $[M + Na]^+$ accurate mass

Calculated 762.2819

Found 762.2825

IR, υ_{max}, cm^{-1} (nujol mull)

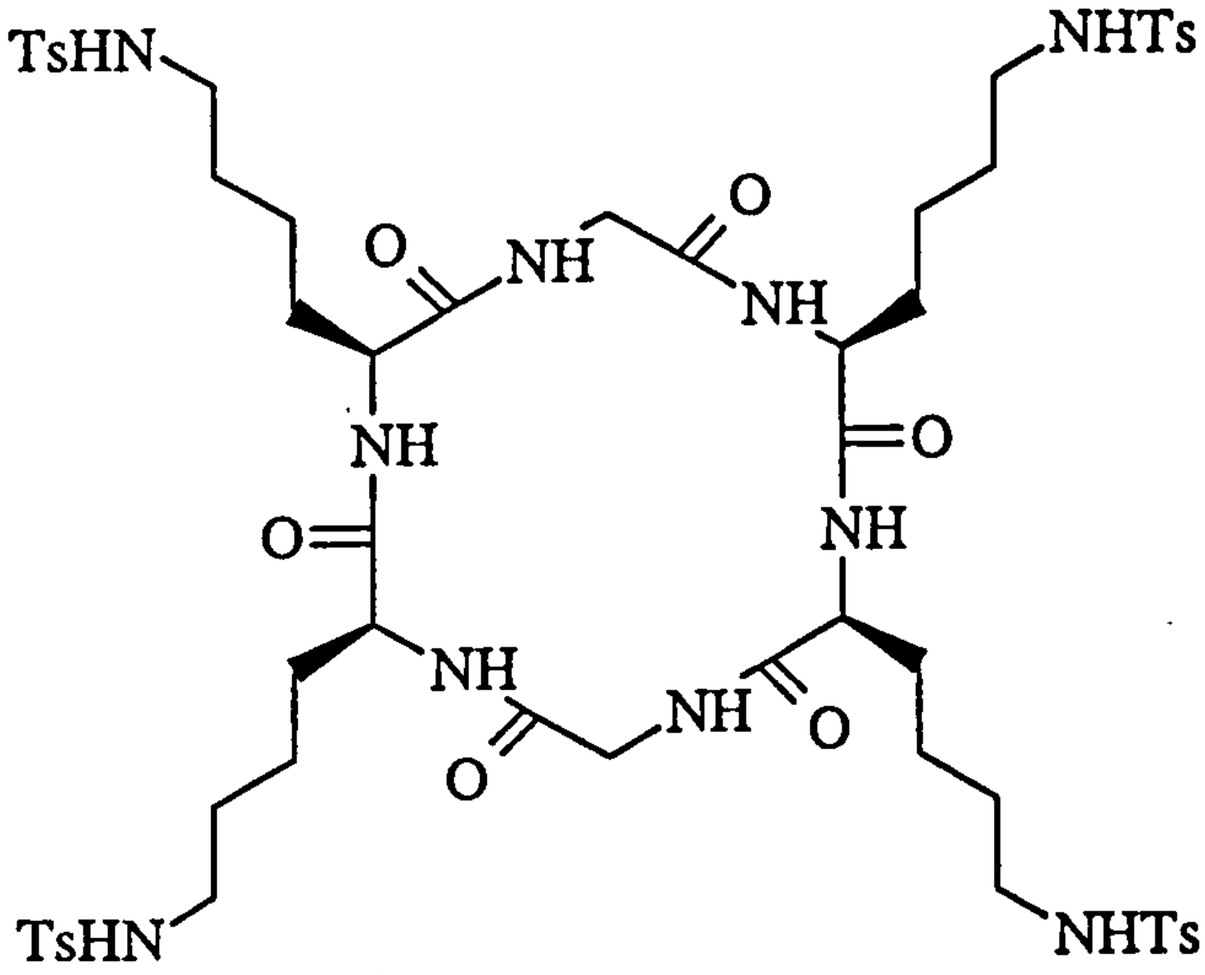
3288.5, 2925.5, 2855.5, 1659.2, 1599.5, 1526.7, 1460.8, 1377.9, 1323.2, 1250.0, 1157.7, 1093.9, 861.1, 815.8, 722.4

R_f 2:1:17 (methanol : acetic acid : chloroform) 0.4

19:1 (dichloromethane : methanol) 0.0

mp (^{0} C) 75-80

Preparation of *cyclo-[N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl-glycyl-N-ϵ-toluenesulphonyl-lysyl-N-ϵ-toluenesulphonyl-lysyl-glycine-]*



N-α-t-Butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine (491 mg, 0.664 mmol), pentafluorophenol (244 mg, 1.33 mmol) and dicyclohexylcarbodiimide (274 mg, 1.33 mmol) were dissolved in dry (stored over 4 Å molecular sieves) ethyl acetate (30 cm^3) under argon. Previously, the solvent used was dry N,N-dimethylformamide. The mixture was stirred for 24 h before more dicyclohexylcarbodiimide (274 mg) was added. The mixture was stirred for 48 h until shown to be complete by TLC. The mixture was filtered to remove precipitated dicyclohexylurea and the solvent removed under reduced pressure. Flash column chromatography (gradient elution from 4:1 dichloromethane : ethyl acetate to 1:1 dichloromethane : ethyl acetate) followed by removal of the solvent under reduced pressure gave a white solid which was not characterised but was used immediately for the next stage in the procedure.

N-α-t-Butoxycarbonyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-pentafluorophenyl-ester (539 mg, 0.596 mmol) was dissolved in 1:1 trifluoroacetic acid : dichloromethane (50 cm^3) and stirred at room temperature for approximately 1 h, when TLC showed the starting material to have been consumed. The solvent was removed under reduced pressure and any residual trifluoroacetic acid removed by forming an azeotrope three times with toluene. The toluene was removed under reduced pressure. The oily product was triturated twice with diethyl ether to form a brownish-white solid. This was not characterised but was used immediately.

Assuming 100 % deprotection, potassium carbonate (411 mg, 2.97 mmol) or caesium carbonate (968 mg, 2.97 mmol) and N,N-dimethylformamide (59.5 cm^3, 0.01 M) were added under argon and the reaction mixture stirred for 5 days. The solvent was removed under reduced pressure, ethyl acetate (50 cm^3) was added and the mixture filtered. The solution was washed with water (50 cm^3 x 2) and sat. brine (50 cm^3) and dried over $MgSO_4$. The mixture was filtered and the solvent removed under reduced pressure. Flash column chromatography (95:5 dichloromethane : methanol) yielded 111 mg (30 %) of a white solid. Data agreed with the literature values given in brackets [ ][121].

^{1}H NMR ($CDCl_3$, RT, 270 MHz); [($CDCl_3$, RT, 300 MHz)]

δ/ppm 1.0-2.0 [1.06-1.82] (m, 24H, $CH_2CH_2CH_2CH_2N$), 2.368 [2.41] (s, 12H, CH_3, Ts), 2.857 [2.86] (m, 8H, CH_2NHTs), 4.271 [4.13](m, 4H, CH_2, Gly), 4.40 [4.26] (m, 4H, α-CH, Lys), 5.02 [5.40] (d, br, 1H, NH), 6.24 (m, br, 1H, NH), 7.247 [7.30] (d, 8H, J = 8.0 Hz, CH, Ts), 7.712 [7.71] (d, 8H, J = 8.0 Hz, CH, Ts), 7.50 [7.51] (m, 1H, NH), 7.98 (m, 1H, NH)

MS FAB+ve (NOBA matrix) $C_{56}H_{78}N_{10}O_{14}S_4$ M^+ calculated as 1242.5

m/z 1243 $[M + H]^+$

IR, υ_{max}, cm^{-1} (nujol mull)

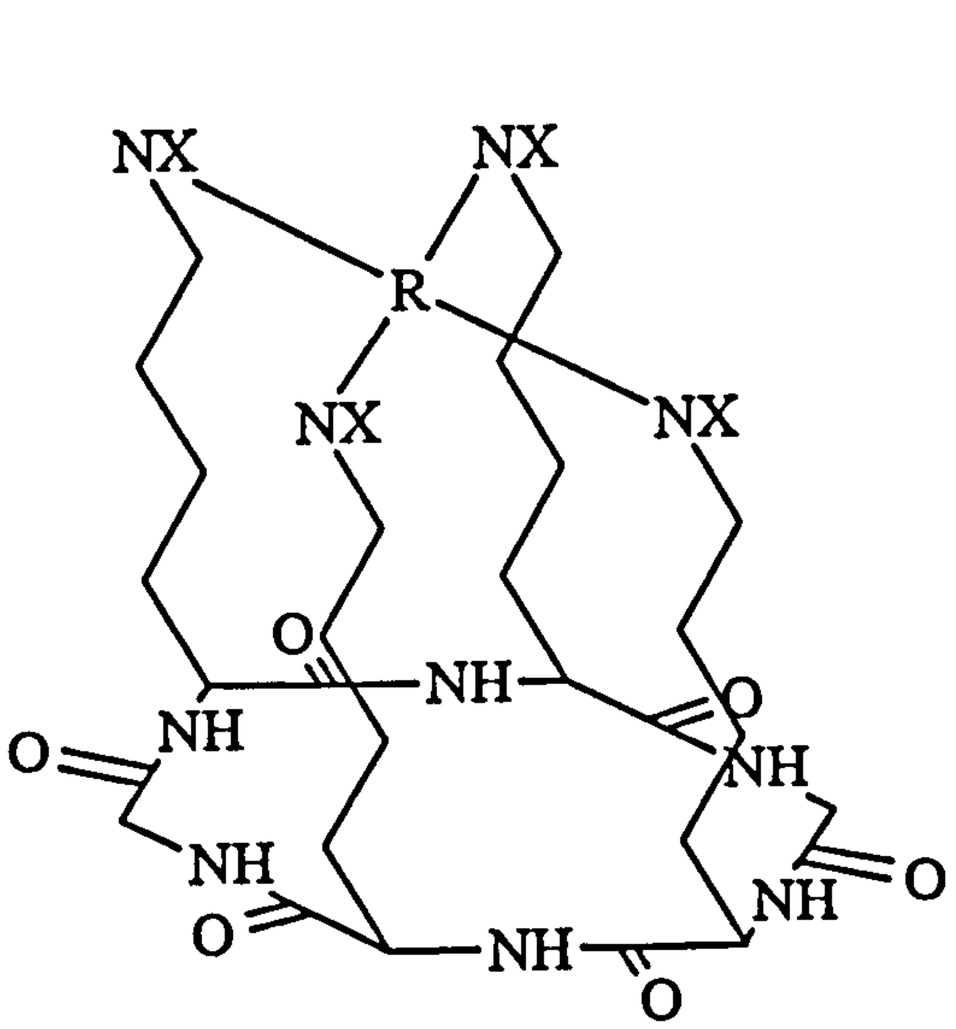
2926.0, 2855.2, 2362.7, 2343.7, 1659.8, 1520.2, 1463.3, 1377.9, 1322.9, 1158.0, 1093.4, 892.9, 848.5, 815.1, 722.7

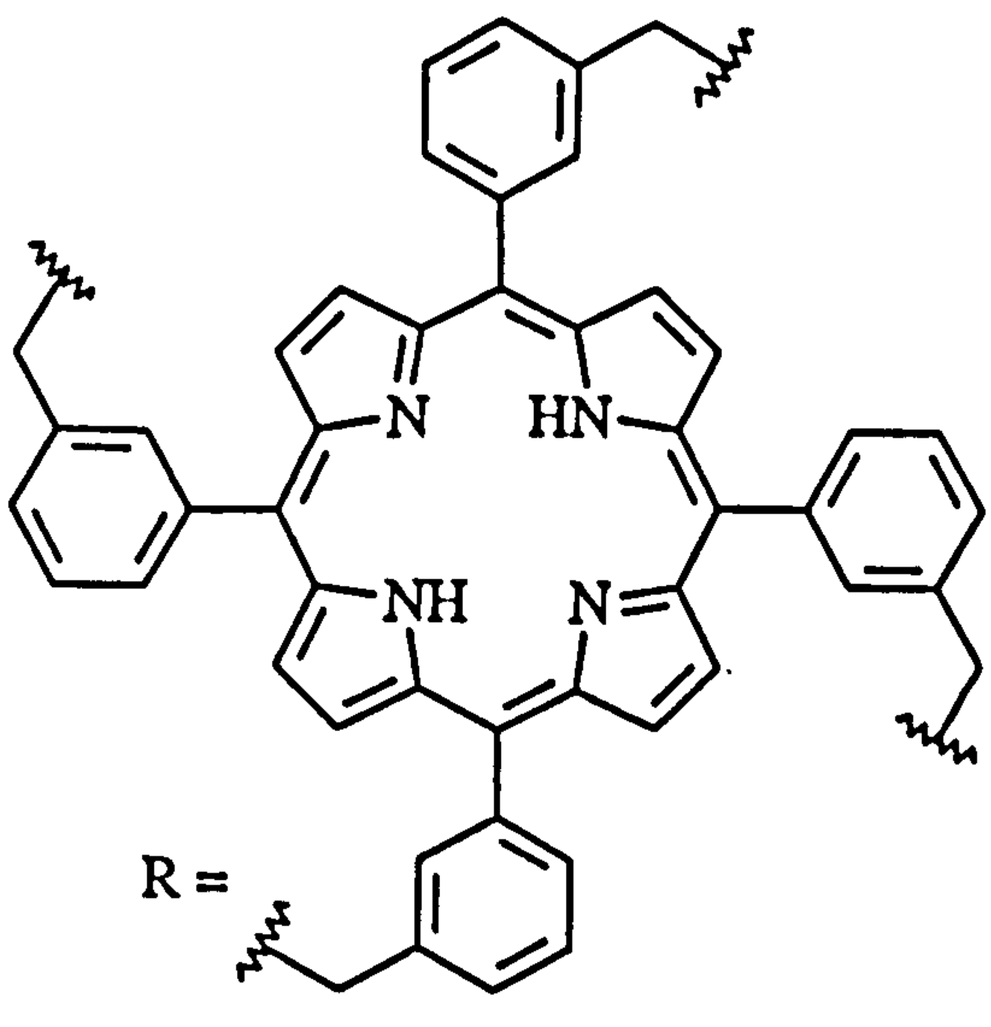
R_f 2:17:1 (methanol : chloroform : acetic acid) 0.55 [0.55]

19:1 (dichloromethane : methanol) 0.40

5.4.3 Attempted capping of a cyclic peptide onto a porphyrin

Attempted synthesis of *(150)* by the reaction of *5,10,15,20-tetrakis(3-bromomethylphenyl)porphyrin* and *cyclo-[lysyl-lysyl-glycyl-lysyl-lysyl-glycine-]*

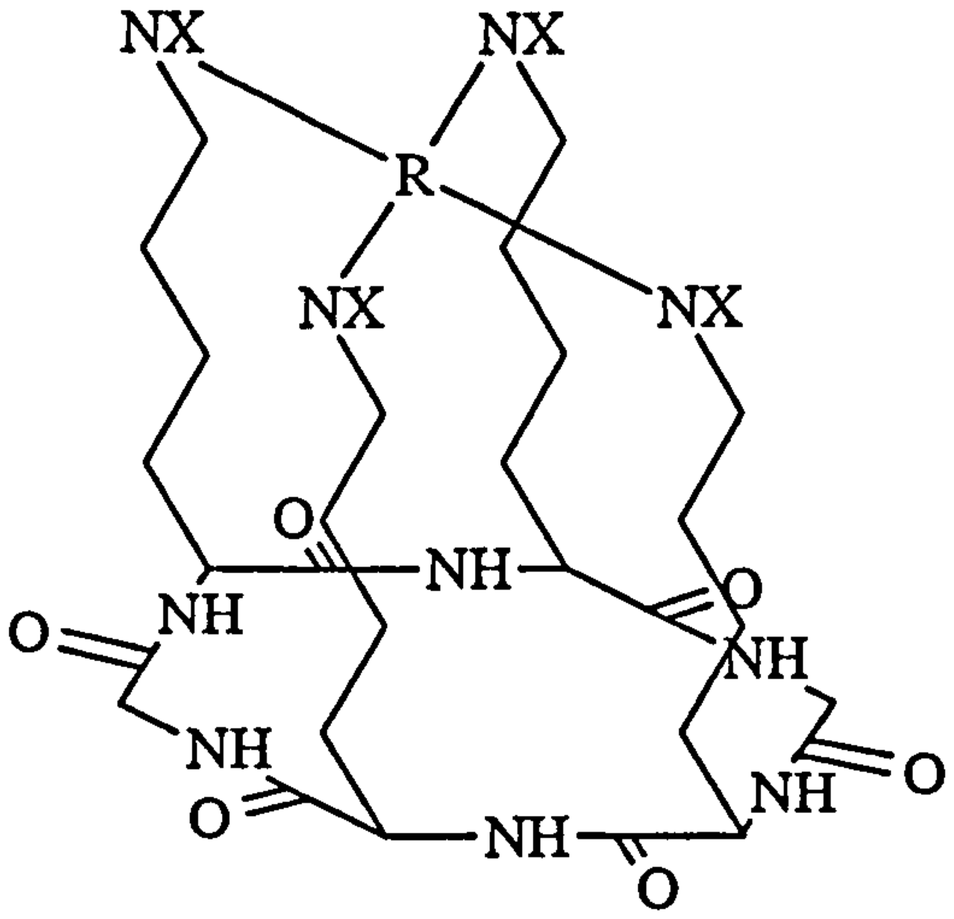


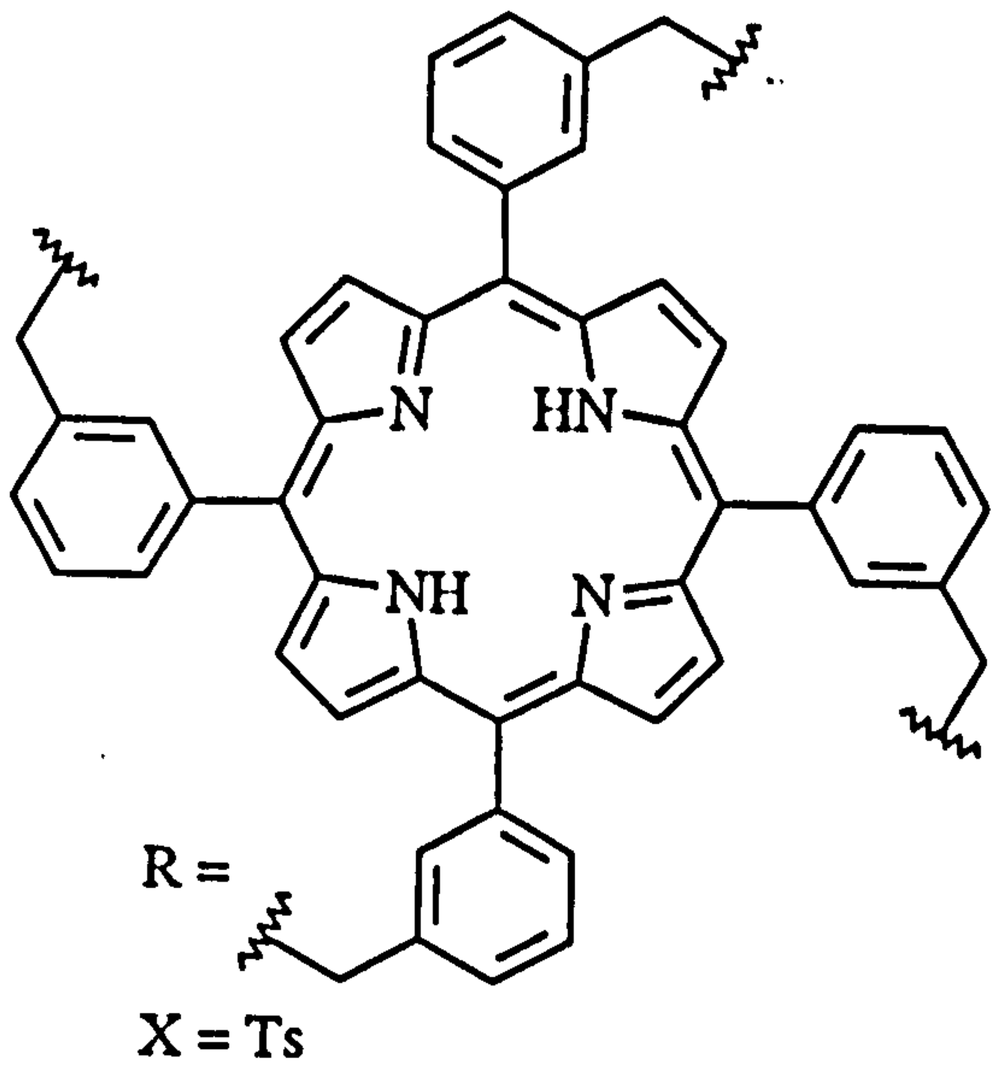


X = H

5,10,15,20-Tetrakis(3-bromomethylphenyl)porphyrin (8 mg, 0.008 mmol), *cyclo*-[lysyl-lysyl-glycyl-lysyl-lysyl-glycine-] (5 mg, 0.008 mmol) and N,N-diisopropylethylamine (8.3 mg, 0.062 mmol) in N,N-dimethylformamide (1.28 cm^3) were stirred for 3 days under argon. The solvent was removed under reduced pressure and residual N,N-dimethylformamide removed by forming an azeotrope three times with toluene. The toluene was removed under reduced pressure. The crude product was analysed by FAB MS but failed to show evidence of the desired product (ie. no peak at m/z = 1289 due to $[M + H]^+$. The sample was dissolved in 3-nitrobenzylalcohol and filtered to remove polymeric material, before analysis by FAB MS. However, again, no evidence of the desired product was obtained.

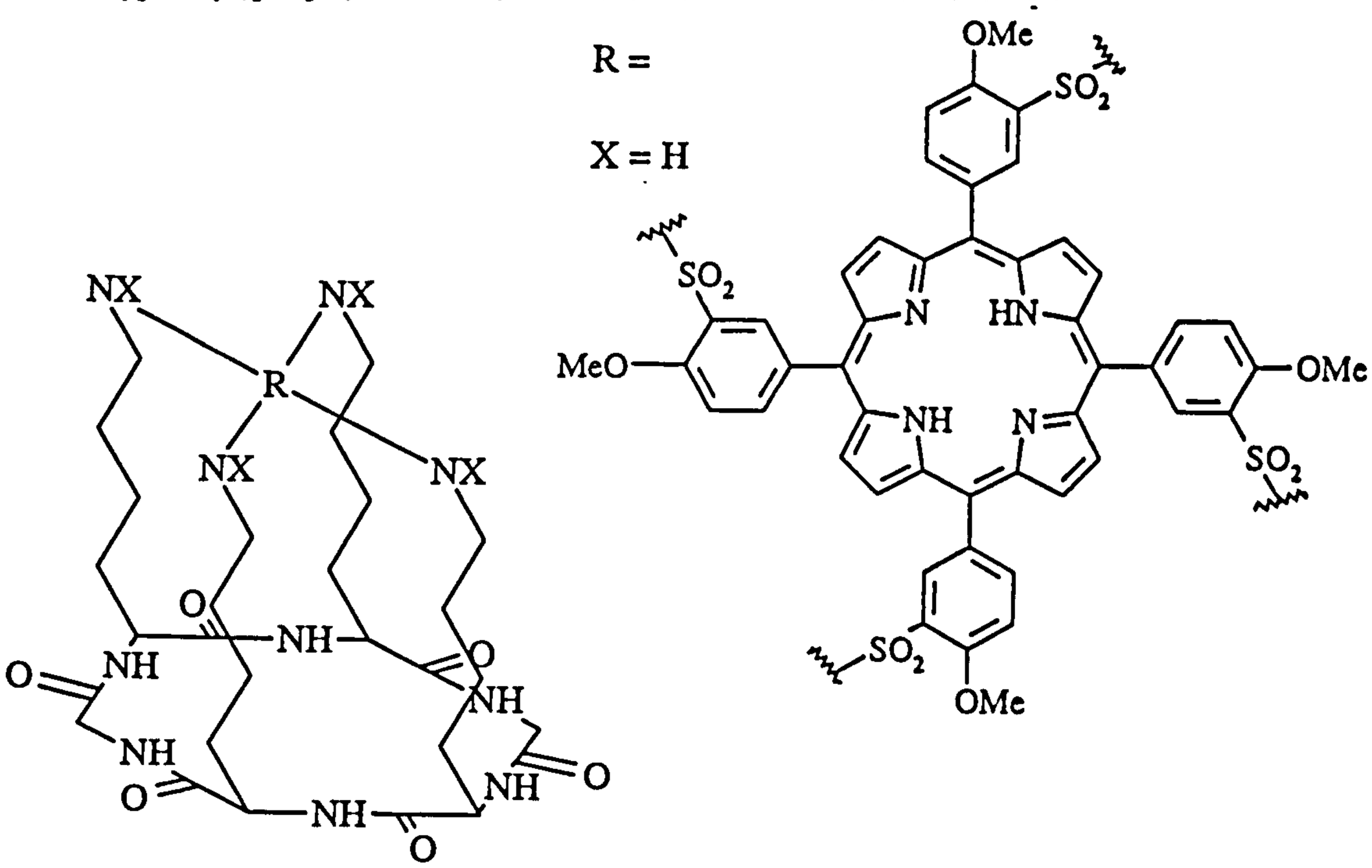
Attempted synthesis of *(149)* by the reaction of *5,10,15,20-tetrakis(3-bromomethylphenyl)porphyrin* and *cyclo-[N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-]*





5,10,15,20-Tetrakis(3-bromomethylporphyrin)porphyrin (1 mg, 0.001 mmol), *cyclo-*[N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycyl-N-ϵ-tosyl-lysyl-N-ϵ-tosyl-lysyl-glycine-] (1.26 mg, 0.001 mmol) and caesium carbonate (1.98 mg, 0.006 mmol) in N,N-dimethylformamide (1 cm^3) were stirred for 2 days under argon. The solvent was removed under reduced pressure and residual N,N-dimethylformamide removed by forming an azeotrope three times with toluene. The toluene was removed under reduced pressure. The crude product was analysed by FAB MS but no peak at m/z = 1905 due to $[M + H]^+$ and no recognisable fragment ions due to the desired product were observed.

Attempted synthesis of *(151)* by the reaction of *5,10,15,20-tetrakis(3-chlorosulphonyl-4-methoxyphenyl)porphyrin* and *cyclo-[lysyl-lysyl-glycyl-lysyl-lysyl-glycine-]*



Three methods were used in the attempt to cap the above cyclic peptide with the above porphyrin.

Method A

5,10,15,20-Tetrakis(3-chlorosulphonyl-4-methoxyphenyl)porphyrin (60 mg, 0.96 mmol), *cyclo*-[lysyl-lysyl-glycyl-lysyl-lysyl-glycine-] (108 mg, 0.096 mmol) and N,N-diisopropylethylamine (99.1 mg, 0.767 mmol) in N,N-dimethylformamide (95.8 cm^3) were stirred together at room temperature for 5 days. The solvent was removed under reduced pressure and residual solvent removed by forming an azeotrope three times with toluene. The toluene was removed under reduced pressure. The crude product was analysed by FAB MS but no peak at m/z = 1609 due to $[M + H]^+$ or any recognisable fragment ions of the desired product were observed. Flash column chromatography (90:10 dichloromethane : methanol) resulted in three products being isolated none of which gave evidence by FAB MS of the desired product. None of these fractions could be adequately characterised by FAB MS.

Method B

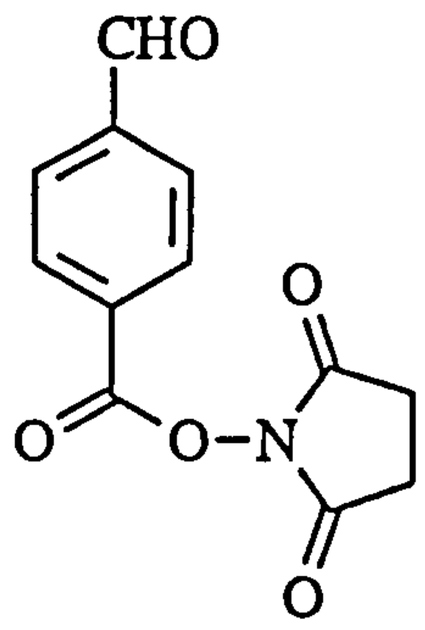
5,10,15,20-Tetrakis(3-chlorosulphonyl-4-methoxyphenyl)porphyrin (60 mg, 0.96 mmol), *cyclo*-[lysyl-lysyl-glycyl-lysyl-lysyl-glycine-] (108 mg, 0.096 mmol) and N,N-diisopropylethylamine (99.1 mg, 0.767 mmol) in N,N-dimethylformamide (95.8 cm^3) were stirred together at 50^0 C for 5 days. It was hoped that by increasing the temperature with respect to method A, the rate of the desired capping reaction would be increased over any side reactions such as the formation of polymeric material. The solvent was removed under reduced pressure. The reaction TLC (90:10 dichloromethane : methanol) was identical to that for Method A with three products being present. An identical work up procedure to Method A failed to provide any evidence of the desired product.

Method C

Cyclo-[lysyl-lysyl-glycyl-lysyl-lysyl-glycine-] (22 mg, 0.035 mmol) and N,N-diisopropylethylamine (36.3 mg, 0.281 mmol) in N,N-dimethylformamide (5 cm^3) were stirred together at room temperature under argon. 5,10,15,20-tetrakis(3-chlorosulphonyl-4-methoxyphenyl)porphyrin (39.6 mg, 0.035 mmol) in N,N-dimethylformamide (2 cm^3) was added by syringe pump over 24 h. The mixture was stirred for another 48 h. The solvent was removed under reduced pressure with any residual solvent being removed by forming an azeotrope twice with toluene. The toluene was removed under reduced pressure and the solid washed twice with diethyl ether. No evidence was found in the FAB MS of the crude sample of the desired product. A sample of crude product was dissolved in 3-nitrobenzylalcohol and filtered in order to remove any polymeric material, but FAB MS failed to show any evidence of the desired compound in the filtrate.

5.4.4 Attempted porphyrin synthesis from a cyclic peptide tetraaldehyde

Preparation of *succinimidyl-4-formyl benzoate (163)*



4-Carboxybenzaldehyde (1 g, 6.66 mmol), N-hydroxysuccinimide (843 mg, 7.32 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (1.4 g, 7.30 mmol) in N,N-dimethylformamide (25 cm^3) were stirred at room temperature under argon for 24 h. In order to ensure completion of the reaction, more N-hydroxysuccinimide (843 mg, 7.32 mmol) and EDC (1.4 g, 7.30 mmol) were added and the mixture stirred for a further 24 h. The solvent was removed under reduced pressure and residual solvent removed by forming an azeotrope twice with toluene - itself removed under reduced pressure. The resultant solid was dissolved in dichloromethane and washed with sat. sodium hydrogen carbonate solution (x 2), water and sat. brine. The solution was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to produce a white solid. The solid was dried to yield 643 mg (39 %) of a white solid.

^{1}H NMR ($CDCl_3$, RT, 200 MHz);

δ/ppm 2.89 (s, 4H, CH_2CH_2), 7.96 (d, 2H, J = 8.41 Hz, CH, Ar), 8.22 (d, 2H, J = 8.41 Hz, CH, Ar), 10.06 (s, 1H, CHO)

^{13}C NMR ($CDCl_3$, RT, 50 MHz);

δ/ppm 25.573 (CH_2), 129.639, 131.006 (C, Ar), 140.213, 160.981 (CH, Ar), 169.066 (C=O)

MS EI $C_{12}H_9NO_5$ M^+ calculated as 247.0

m/z 247 $[M]^+$, 246 $[M - H]^+$, 149 $[M - C_4H_4O_2N]^+$, 133 $[M - C_4H_4O_3N]^+$

MS EI $[M]^+$ accurate mass

Calculated 247.0481

Found 247.0465

MS EI $[M - H]^+$ accurate mass

Calculated 246.0403

Found 246.0416

IR, υ_{max}, cm^{-1} (nujol mull);

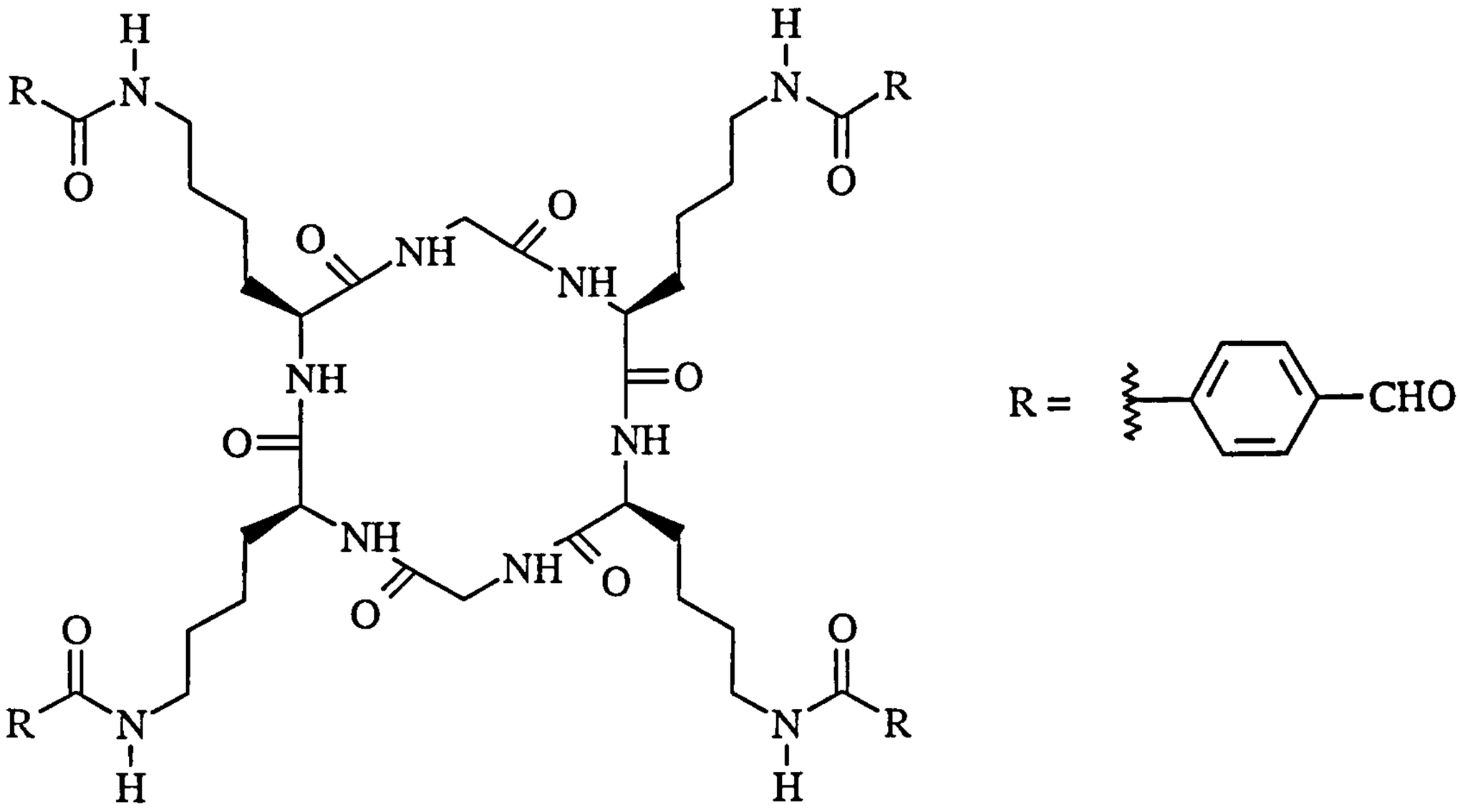
2924.9, 2854.8, 1776.2, 1726.6, 1703.4, 1661.7, 1464.9, 1377.9, 1203.7, 1076.3, 1001.8, 746.0

R_f 1:1 (40-60 petroleum ether : ethyl acetate) 0.5

19:1 (dichloromethane : methanol) 0.8

mp (^{0} C) 145-147

Attempted preparation of *cyclo-[N-ϵ-(4-carboxybenzaldehyde)-lysyl-N-ϵ-(4-carboxybenzaldehyde)-lysyl-glycyl-N-ϵ-(4-carboxybenzaldehyde)-lysyl-N-ϵ-(4-carboxybenzaldehyde)-lysyl-glycine-]*



Cyclo-[lysyl-lysyl-glycyl-lysyl-lysyl-glycine-] (27.7 mg, 0.044 mmol) and succinimidyl-4-formyl benzoate (54.6 mg, 0.221 mmol) in chloroform (4 cm^3) with a few drops of methanol to ensure solubility, were stirred under argon for 3 days. In alternative reactions, N,N-dimethylformamide was used as the solvent. The solvent was removed under reduced pressure. ^1H NMR spectroscopy indicated the presence of the desired product alongside the starting material succinimidyl-4-formyl benzoate.

Two attempts were made to effect the purification of the desired compound.

Method A

The solid residue from the reaction was washed three times with diethylether following observations that succinimidyl-4-formyl benzoate is selectively soluble in diethylether over peptidic compounds. However, ^1H NMR spectroscopy showed that the required product was not isolated.

Method B

Flash column chromatography (gradient elution from dichloromethane to 19:1

dichloromethane : methanol) failed to isolate the desired product, but rather most of the desired product remained bound to the column.

^{1}H NMR ($CDCl_3$, RT, 200 MHz);

δ/ppm *1.0-1.7 (m, 24H, $CH_2CH_2CH_2CH_2N$), 1.7-1.9 (m, 8H, CH_2N)*, 2.89 (s, 4H, CH_2CH_2, succinimidyl), *7.92 (d, 8H, J = 6.7 Hz, CH, Ar)*, 7.98 (d, 2H, J = 6.7 Hz, CH, Ar), *8.16 (d, 8H, J = 6.7 Hz, CH, Ar)*, 8.24 (d, 2H, J = 6.7 Hz, CH, Ar), *10.05 (s, 4H, CHO)*, 10.12 (s, 1H, CHO)

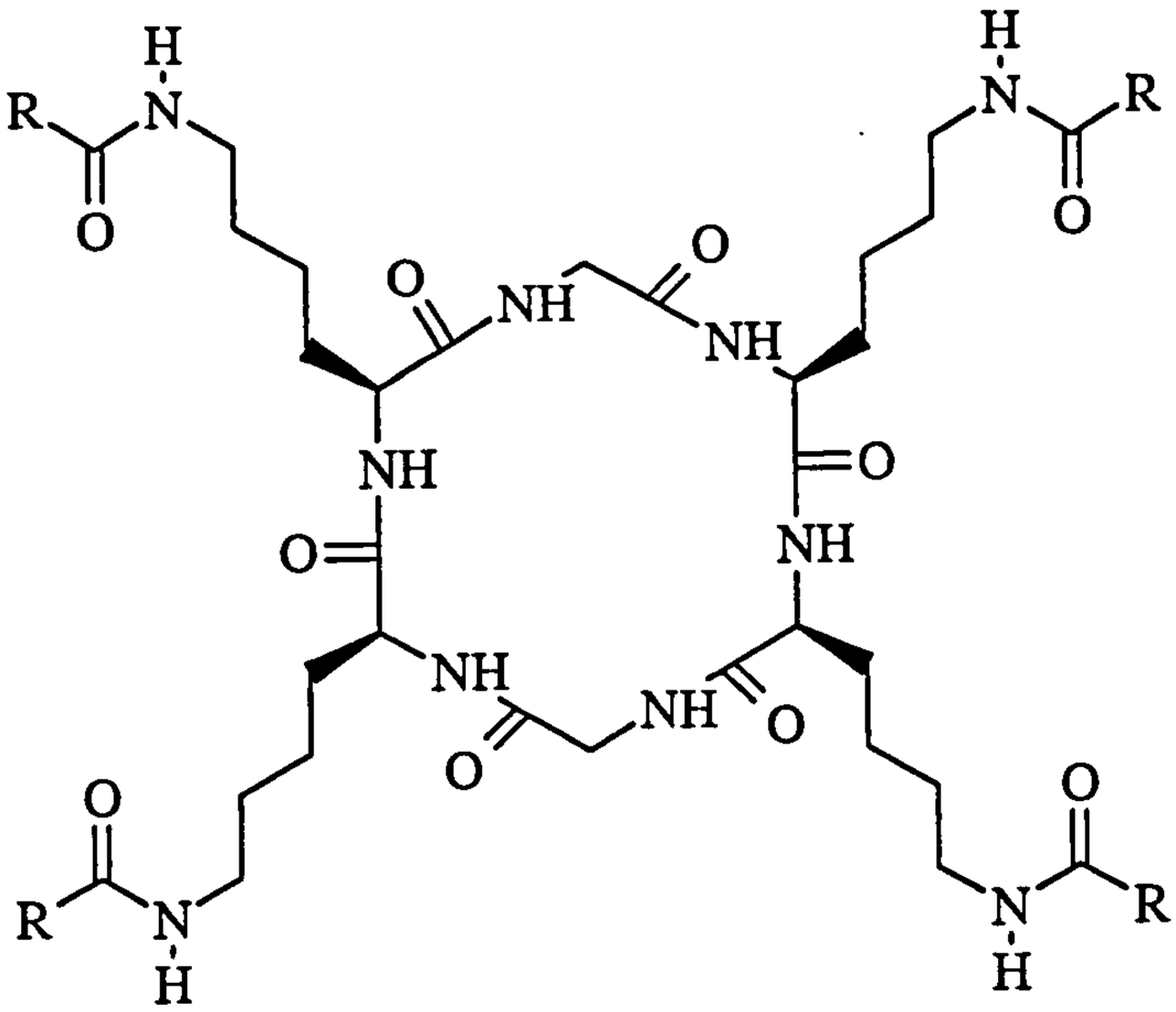
The above spectrum is of a mixture of two compounds. Those assignments in italics are due to the desired cyclic peptide tetraaldehyde. Their integrations are H per H, 32 times smaller than the other assignments, which are due to the starting material succinimidyl-4-formyl benzoate.

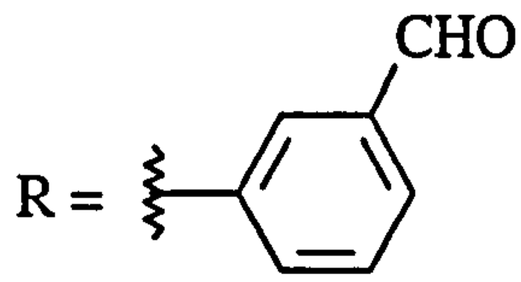
MS FAB+ve (NOBA matrix) $C_{60}H_{70}O_{14}N_{10}$ M^+ calculated as 1154

m/z 1155 $[M + H]^+$, 133 [succinimidyl-4-formyl benzoate - $C_4H_4O_3N]^+$

R_f 9:1 (dichloromethane : methanol) 0.3 (UV active and stains with 2,4-dinitrophenylhydrazine)

Attempted preparation of *cyclo-[N-ϵ-(3-carboxybenzaldehyde)-lysyl-N-ϵ-(3-carboxybenzaldehyde)-lysyl-glycyl-N-ϵ-(3-carboxybenzaldehyde)-lysyl-N-ϵ-(3-carboxybenzaldehyde)-lysyl-glycine-] (160)*





Two attempts were made to prepare this compound.

Method A

Cyclo-[lysyl-lysyl-glycyl-lysyl-lysyl-glycine-] (26 mg, 0.042 mmol) and succinimidyl-3-formyl benzoate (50 mg, 0.202 mmol) in deuterated N,N-dimethylformamide (1 cm^3) were stirred under argon for 3 days. ^1H NMR spectroscopy in the reaction solvent indicated the presence of the desired product together with starting material. The solvent was removed under reduced pressure and residual solvent was removed by forming an azeotrope twice with toluene - the toluene being removed under redcued pressure. An attempt was made to isolate the desired product by washing with toluene. However, ^1H NMR spectroscopy showed this to be unsuccessful.

Method B

Cyclo-[lysyl-lysyl-glycyl-lysyl-lysyl-glycine-] (35.6 mg, 0.057 mmol), succinimidyl-3-formyl benzoate (67.4 mg, 0.0273 mmol) and N,N-diisopropylethylamine (36.75 mg, 0.284

mmol) in deuterated chloroform with a few drops of deuterated methanol (to aid solubility) (2 cm^3) were stirred for 5 days under argon. The base was used in an attempt to drive the reaction to completion. ^1H NMR and TLC (19:1 dichloromethane : methanol) indicated the presence of the desired product as well as succinimidyl-3-formyl benzoate starting material. The solvent was removed under reduced pressure. An attempt was made to isolate the desired product by washing three times with toluene. It had been previously observed that succinimidyl-3-formyl benzoate was soluble in toluene and it was expected that the cyclic peptide tetraaldehyde, in common with many peptides, would be insoluble. Nevertheless, this was unsuccessful. Another attempt was made to isolate the desired product by washing three times with sodium hydrogen carbonate, stirring the mixture each time for 45 min. Theoretically, the ester bond of succinimidyl-3-formyl benzoate would be hydrolysed during this treatment to give 3-carboxybenzaldehyde and N-hydroxysuccinimide which could be removed by washing with water. The desired product should be untouched. The residue was washed with water and then dissolved in dichloromethane with trace methanol. TLC (19:1 dichloromethane : methanol), however, indicated the loss of most of the desired product at $R_f = 0.3$ to baseline material. ^1H NMR analysis indicated that this attempt to purify the desired product was unsuccessful and that the aldehyde peaks of the tetraaldehyde product (160) had been lost.

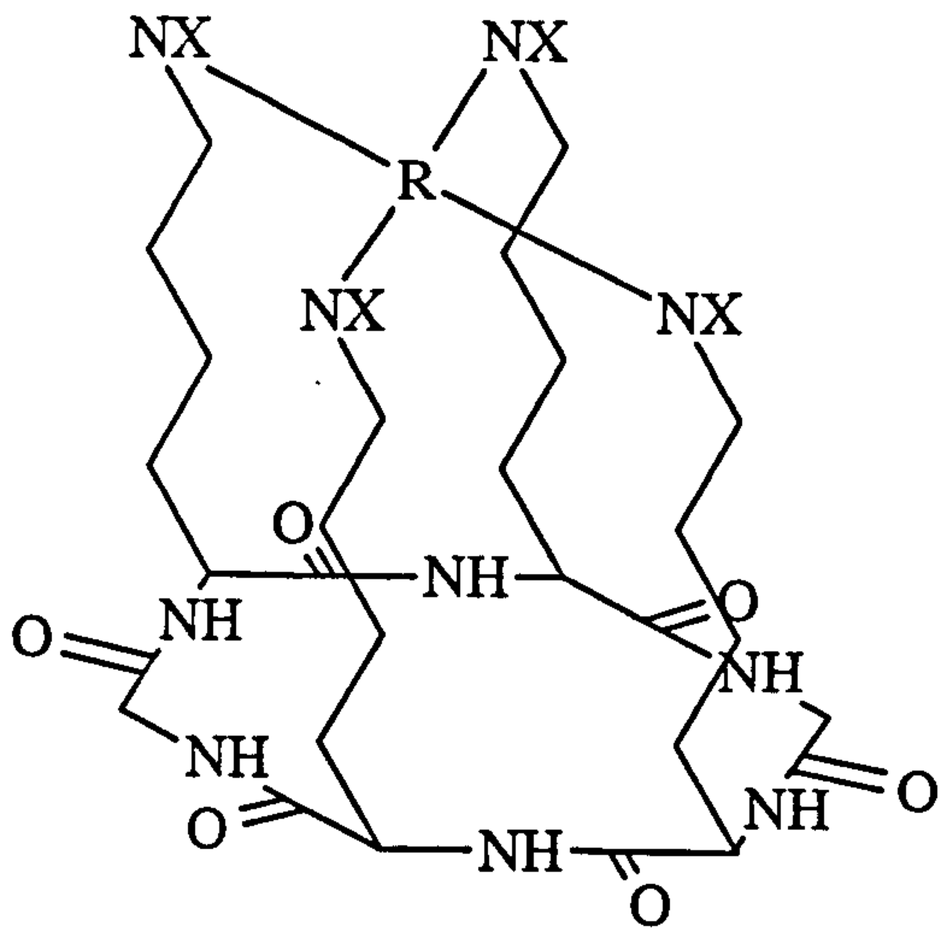
^1H NMR ($CDCl_3$, RT, 400 MHz);

δ/ppm *1.2-1.6 (m, 24H, $CH_2CH_2CH_2CH_2N$)*, *1.6-1.9 (m, 8H, CH_2N)*, 2.61 (s, 4H, CH_2CH_2, succinimidyl), 3.6 (s, **HOD**), 7.6-8.6 (m, 4H, Ar; *16H, Ar*), *10.0 (s, 4H, CHO)*, 10.1 (s, 1H, **CHO**)

The above spectrum is of a mixture of two compounds. Those assignments in italics are due to the desired cyclic peptide tetraaldehyde. Their integrations are H per H, 1.3 times smaller than the other assignments, which are due to the starting material succinimidyl-3-formyl benzoate.

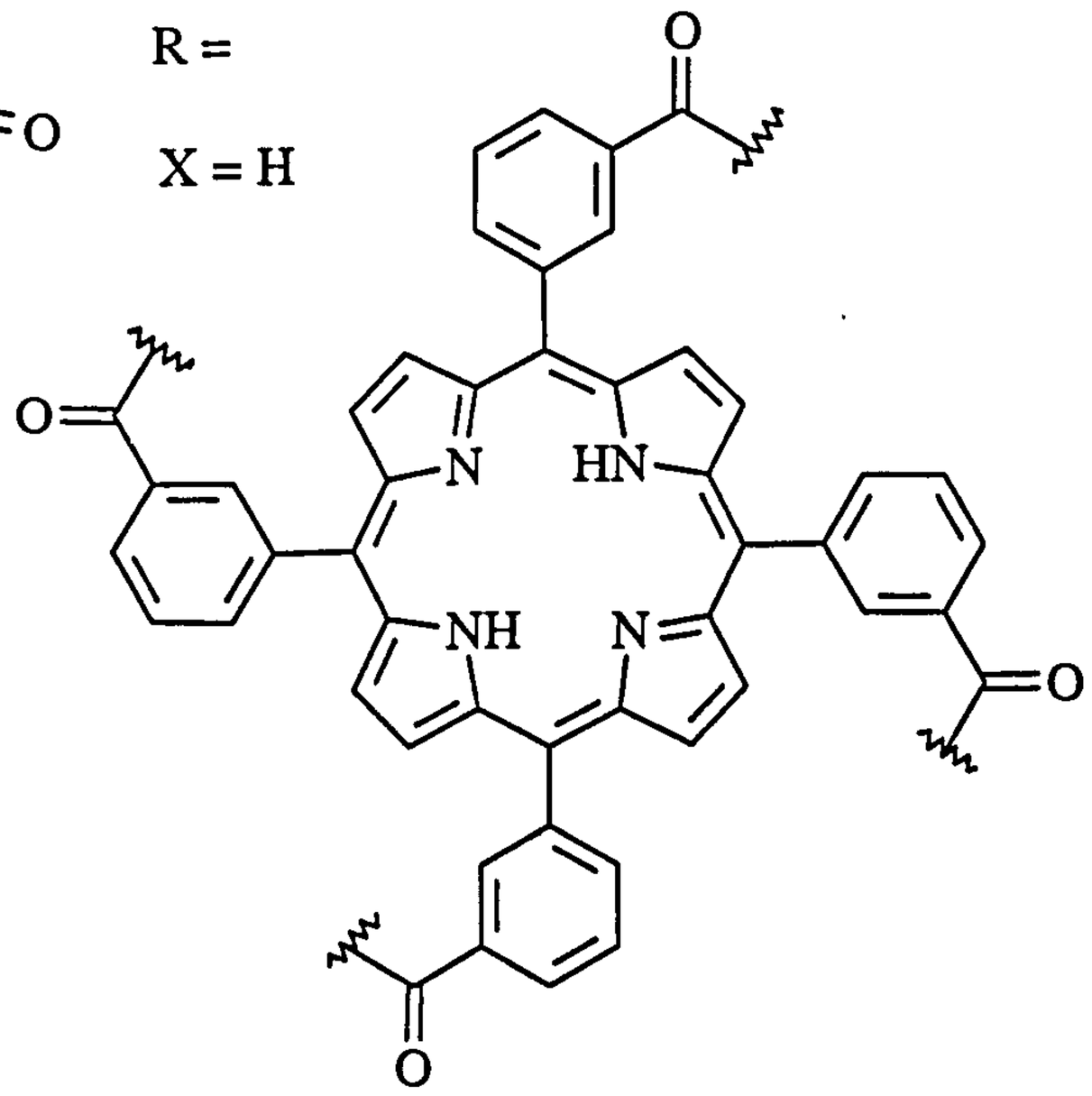
R_f 9:1 (dichloromethane : methanol) 0.3 (UV active and stains with 2,4-dinitrophenylhydrazine)

Attempted preparation of *porphyrin capped cyclic peptide (152)* from unpurified *cyclo-[N-ϵ-(3-carboxybenzaldehyde)-lysyl-N-ϵ-(3-carboxybenzaldehyde)-lysyl-glycol-N-ϵ-(3-carboxybenzaldehyde)-lysyl-N-ϵ-(3-carboxybenzaldehyde)-lysyl-glycine-] (160), pyrrole* and *propionic acid*



R =

X = H



To a solution of impure *cyclo*-[N-ϵ-(3-carboxybenzaldehyde)-lysyl-N-ϵ-(3-carboxybenzaldehyde)-lysyl-glycol-N-ϵ-(3-carboxybenzaldehyde)-lysyl-N-ϵ-(3-carboxybenzaldehyde)-lysyl-glycine-] (50 mg, 0.043 mmol), in propionic acid (5 cm^3) was added freshly distilled pyrrole (12 mg, 0.18 mmol). The solution was heated to 141^0 C and refluxed for 30 min. The reaction mixture was allowed to cool before it was diluted with dichloromethane. A UV-Vis spectrum of the solution showed that no porphyrin had been formed and thus that the attempted synthesis of the desired compound was unsuccessful.

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