EXPLORATORY STUDIES OF NOVEL LIGAND SYSTEMS.

THESIS

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by

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ABSTRACT.

A range of novel ligand systems have been developed in three distinct phases and preliminary studies have been initiated to evaluate their complexation potential. Phase I incorporated the synthesis of single strand ligand systems, which were mainly based on amino acid residues. Techniques have been developed for the attachment of these ligand systems onto, firstly, a styrene monomer, and then later onto a pseudo-styrene linking group, *viz.* the *p*-toluoyl group. The linking reactions were based on the formation of amides or esters by the reaction of an acid chloride system with an amine or alcohol.

Phase II involved the synthesis of bis-chain ligand systems and their attachment onto the *p*-toluoyl linking group. A further linking group was also developed at this stage, *viz.* the xylyl group. In the preparation of phase II ligand systems, use was made of malonic ester and iminodiacetic acid derivatives.

Phase III has involved the synthesis of cyclic ligand systems, with skeletons based upon the structures used in phase I and phase II and two crown ether type systems have been prepared.

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For my parents, for all their help and guidance throughout my education. Many thanks for all the sacrifices you had to make over the years.

1. INTRODUCTION.

1.1 GENERAL SURVEY OF LIGAND DESIGN FOR SELECTIVE COMPLEXATION OF METAL IONS.

Metal ion complexes have many varied applications in the world today. Ethylenediaminetetraacetic acid (EDTA) (see

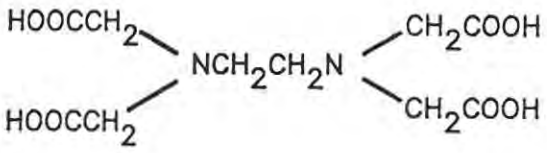


Figure 1

EDTA

figure 1) is perhaps one of the most useful and widely known ligands. It was first synthesised in 1935 as a calcium-sequestering agent in the textile industry. Its ability to form stable metal ion complexes has led to its becoming a highly investigated ligand. It has been widely used in the treatment of metal toxicity in the body and it has many non-medical uses as well, such as water softening and complexiometric titrations.

Other examples include penicillamine (figure 2) which

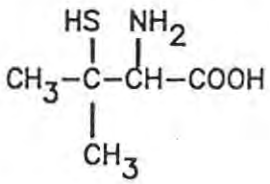


Figure 2

Penicillamine

was introduced in 1956 to combat Wilson's disease,[1] and it also had a limited use in treating lead poisoning. Recently it has found applications as an agent for the

control of rheumatoid diseases and as a decuprinating agent in primary biliary cirrhosis.[2]

Cisplat, a platinum containing complex, (see figure 3)

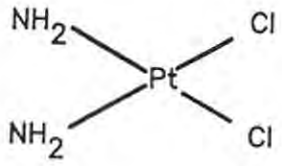


Figure 3 Cisplat

has been found to have high anti-cancer activity. Another recent development in the fight against cancer has been the development of the drug Razoxane which has shown high antitumour activity.

Many examples of metal containing complexes can be found throughout the world today and their usefulness accounts for the intense interest shown in all forms of metal complexes.

1.1.1 Introduction.

The need for ligands which selectively complex with specific metal ions has led to an increased interest in the area of ligand design. Areas which have received much attention include:-

the development of therapeutic reagents for the treatment of metal intoxication[1,3] and antibiotics which owe their antibiotic action to specific metal

complexation;[2]
the design of complexes to act as imaging agents in the body;[4]
the design of functional groups for the formation of functionalized polymers[5] and
the development of chelating ion-exchange resins,[6] selective metal extractants[7] and metal ion sequestering agents in detergents.[8,9]

Investigation of the processes involved in selective complexation continue to provide a greater understanding of some of the principles of metal selectivity shown by examples such as the biological cation transport system and ion binding in proteins like metallothionein.[10]

Donor atoms can be selected by using such tools as the hard and soft acid and base principle of Pearson[11] or the A and B type acids of Schwarzenbach[12]. The effects of incorporating these atoms into a ligand for selective complexation has not been fully studied. Investigations have generally been limited to the somewhat misleading principle of size-matched selectivity in macrocycles where the size of the "hole" in the ring of the macrocycle influences the size of the metal ion which can bind with it.

Hancock and Martell[5] have used molecular mechanics (MM) to show that the role of steric strain in complex

formation has a far greater effect on selectivity than just that of simple dimensions. This approach treats a molecule or complex as an assembly of atoms which are held together by classical forces. It models reality by assuming that bonds have ideal lengths and that any deformation of these bond lengths away from the ideal can be modelled using Hooke's law, as used in infrared spectroscopy. Bond angles, torsional distortions and all non-bonded interactions are treated in a similar way. Simple expressions for the various types of interactions are used to calculate the forces present within the molecule. These factors may all be combined to give a so-called strain energy, which gives some idea of the steric efficiency attained by complex formation. The lower the value for the steric strain (the higher the steric efficiency), the more favourable will be the complex formation reaction. Increased selectivity of a ligand for a particular metal ion will be achieved by designing the ligand with the greatest posible steric efficiency when complexed with that metal ion.

1.1.2 **Donor Atoms.**

The nature of the donor atoms may also influence selectivity. Table I gives a few acids and bases which have been classified into three groups, hard, soft and intermediate (the HSAB principle).[11] It gives us some idea as to which atoms can be used in selective complexation. For example, designing a ligand to complex

Table I

A Classification of Acids and Bases According to the HSAB Principle Of Pearson[11]

Acids.

Hard.	Soft.
H^+, Li^+, Na^+, K^+	$Cu^+, Ag^+, Au^+, Tl^+, Hg^+$
$Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}$	$Pd^{2+}, Cd^{2+}, Pt^{2+}, Hg^{2+}$
$Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, La^{3+}$	$CH_3{}^+Hg, Co(CN)_5{}^{2-}, Pt^{4+}$
$Gd^{3+}, Lu^{3+}, Cr^{3+}, Co^{3+}, Fe^{3+}, As^{3+}$	Te^{4+}, Br^+, I^+
$Si^{4+}, Ti^{4+}, Zr^{4+}, Hf^{4+}, Th^{4+}, U^{4+}$	
$Pu^{4+}, Ce^{4+}, WO^{4+}, Sn^{4+}$	
$UO^{2+}, VO^{2+}, MoO^{3+}$	

Borderline.

$Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+},$
$Sn^{2+}, Sb^{3+}, Bi^{3+}, Rh^{3+}, Ir^{3+}, B(CH_3)_3$

Bases.

Hard.	Soft.
$H_2O, OH^-, F^-, CH_3CO_2{}^-, PO_4{}^{3-}$	$R_2S, RSH, RS^-, I^-, SCN^-$
$SO_4{}^{2-}, Cl^-, CO_3{}^{2-}, ClO_4{}^-, NO_3$-	$S_2O_3{}^{2-}, R_3P, R_3As, (RO)_3P$
$ROH, RO^-, R_2O, NH_3, RNH_2, NH_2NH_2$	$CN^-, RNC, CO, C_2H_4, H^-, R^-$

Borderline.

$C_6H_5NH_2, C_5H_5N, N_3{}^-, Br^-, NO_2{}^-, N_2, SO_3{}^{2-}$

with Fe^{3+} (hard acid) table I indicates that a hard base is required for complexation. Such a ligand would contain donor atoms such as negatively charged oxygen.

This classification is not complete however, as it gives no idea of the relative affinities of the various hard bases to hard acids. The neutral oxygen donor atoms, ROH and R_2O, are classified as hard, but it has been found that Fe^{3+} has little affinity for crown ethers which contain R_2O (ether oxygen) atoms. The same predicament is faced when predicting which donor will form the stronger bond to a metal ion in a ligand containing two or more

different donor atoms. It can be seen that it is not just a simple task of designing a ligand which contains hard donor atoms when complexation with hard metal ions is required. The various donor atoms need to be considered in more detail.

1.1.3 More Detailed Selection Criteria For Donor Atoms.

Five main groups of donor atoms are considered here. These include the most commonly occurring donor atoms which are in use in the synthesis of ligands today.

1.1.3.1 Neutral Oxygen Donor.

This group includes ligands such as water, alcohols, ethers, ketones and amides. It has been shown[13] that donor strength for sp^3-hybridised oxygen increases in the order $H_2O < ROH < R_2O$. There are also steric crowding effects which become more pronounced in complexes with smaller metal ions due to the bulk of the oxygen donor atoms. These steric effects tend to have a greater influence on complex stability than does the nature of the metal ion or the oxygen-metal ion bond strength. For the purposes of ligand design addition of neutral oxygen donor atoms to a ligand system increases selectivity for larger metal ions over smaller metal ions.

1.1.3.2 Negatively Charged Oxygen Donor.

This group includes ligands containing carboxylates, phenolates, hydroxamates and phosphonates as well as ligands like acetylacetonate and tropolonate. The complexation efficiency of this group depends upon the acidity (affinity for the OH^- ion) of the metal ions involved and the proton basicity of the oxygen atom. By increasing the number and basicity of the negatively charged donor atoms present in the ligand, the ligand will become more selective for acidic metal ions over less acidic ones. It should also be noted that steric effects can cause a drop in the stability of complexes of smaller metal ions such as Be^{2+}.

1.1.3.3 Neutral Saturated Nitrogen Donor.[14]

This group has the most wide spread application in coordination chemistry. The group includes amines and amides which are common throughout nature, occurring in such structures as proteins and enzymes. The amide group is found extensively in proteins, either as a free group or more commonly as a peptide bond. These bonds were first used for identifying the presence of proteins by employing the biuret colour reaction[15] which involves the reaction of Cu^{2+} ions with the amide group in alkaline medium. As interest in proteins grew, so too did interest in the amide group as a ligand system.

The amide group has two possible sites for binding, the oxygen or the nitrogen atoms. However, the neutral nitrogen atom displays stronger coordinating properties with many metal ions than does the neutral oxygen atom.[16] So for design purposes, only the effect of the nitrogen donor atom need be taken into consideration.

In general it can be stated that complex stability depends upon the relative acidity and basicity of the metal ion and nitrogen atom respectively. The order of basicity towards metal ions is $NH_3 < RNH_2 < R_2NH < R_3N$,[17] but addition of R- groups will not only increase the nitrogen's basicity but also the steric strain involved in complex formation. So, although these groups are the most commonly occurring coordination centres in nature, they cannot be used readily to improve selectivity since steric hindrance tends to overwhelm any increase in the basicity due to inductive effects.

1.1.3.4 **Unsaturated Nitrogen Donors.**

The unsaturated nitrogen donor pyridine is a stronger base than any saturated nitrogen donor in the gas phase,[17] but its inability to disperse a charge from a Lewis acid to the solvent, by hydrogen bonding, means that in solution it is a weaker base than other saturated nitrogen donors.[10]

The main advantage of unsaturated nitrogen donor atoms

is their ability to impart rigidity to the ligand.[18] This can give the system an effective pre-organised structure which can increase complex stability.[19] The effect of pre-organisation will be discussed in more detail later.

1.1.3.5 Heavier Donor Atoms.[20]

When donor atoms such as sulphur, phosphorus, selenium and arsenic are neutral, they can coordinate well with soft metal ions. They suffer the disadvantage of being bulky and are ineffective in hydrogen bond formation.

The most important of these heavy atom donors is the thiol group. This group is slightly acidic and can form very stable complexes with soft metal ions. It occurs throughout nature, especially in proteins and it is this fact that has caused considerable interest in its propensity for selective complexation.

1.1.4 The Use Of Sterically Crowding Groups To Improve Selectivity.[21]

It has been shown earlier (sections 1.1.3.3 and 1.1.3.4) that the addition of N-alkyl or C-alkyl groups has two opposing effects. The first is an increase in the nitrogen's donor strength by induction which is often overshadowed by the increase in steric strain which is associated with the presence of the bulky alkyl groups.[22]

It is very difficult to predict which effect will be the greater and, although some interesting selectivity effects may be observed, their unpredictability makes the use of such groups a very hit and miss affair. Obviously more research will need to be done in this field.

1.1.5 The Use Of Rotational Restriction To Increase Selectivity.

It has been found[23] that the addition of alkyl groups to the ethylene bridge in ethylenediaminetetraacetic acid (EDTA) causes an increase in complex stability. This could be due to the inductive effect, but is more likely to be due to the decrease in the energy required for EDTA to change from its stable anti-conformation to the skewed conformation required for complex formation. The replacement of alkyl groups by a cyclohexane ring causes even greater pre-organization in the skewed conformation so increasing complex stability still further. This effect will probably be of great help where there is a need to overcome the electrostatic repulsion caused by the presence of similarly charged atoms within a ligand. For example, in siderophores, which have large numbers of mutually repelling charged oxygen atoms, high energies need to be overcome to permit a conformation in which complexation can occur.

1.1.6 The Chelate Effect.

The term "chelate", first introduced by Morgan and Drew,[24] was taken from the Greek term *chele*, meaning crab's claw. Metal chelate complexes may be simply defined as complexes in which the donor atoms are attached to each other as well as to the metal ion. The factors which contribute to the stability of chelate ligands are not yet fully understood. It has been proposed[25] that the chelate effect can be thought of in terms of restricted volumes, where the second donor atom could bind once the first donor atom has coordinated with the metal ion. Another approach[26] deals with this effect by expressing the formation constants for the complexes in terms of mole fractions and thus setting the translational entropy to zero. This has the effect of making the reactants fill, completely, the space of the standard reference state. Both approaches effectively do the same thing, but the latter seems to be the simpler to use as it does not take into consideration ligand structure and geometry.

Prediction of the formation constants of chelate complexes (and thus their stability) can be based on donor atom additivity.[27] In this approach, the formation constant of a ligand containing two different types of donor atom is described by the average of the formation constants for a pair of similar ligands each containing only one of the two donor types. For example, the

formation constant for glycine will be the average of the formation constants for ethylenediamine and oxalate.

Most of the work on these compounds has been done using chelates which form five-membered chelate rings. Larger chelate rings seem to have lower stability than their five-membered counterparts. This could be due to the greater difficulty of bringing together dipoles and charges on donor atoms (a statistical effect, where the greater the number of atoms between two donor atoms, the less likely will be the probability of the two atoms being close enough together in space to allow for formation of a chelate ligand). The effect of steric strain can be predicted to a certain extent by the use of molecular mechanics (MM) calculations (as shown in an example by Hancock *et al.*[28]). From collected data,[29] it can be shown that an increase in chelate ring size leads to a larger drop in complex stability for larger metal ions than for smaller ones. This principle seems to hold for all examples, and so can be used in the design of ligands for selective complexation. This principle also seems to hold for macrocyclic ligands. This is, in fact, contrary to the theory of size-match selectivity, which suggests that the size of the macrocyclic "hole" is related to the strength of complexation to a metal ion. The size-match selectivity effect seems to be overshadowed by the preference of chelates to form five-membered rings rather than larger rings.

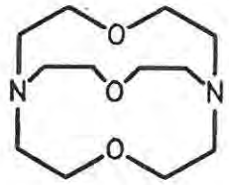
1.1.7 The Macrocyclic Effect.

Macrocycles exhibit several effects which make them useful in complex formation. Their complexes with metal ions have greater thermodynamic stability and kinetic inertness towards de-metallation than either straight chain ligands or chelate ligands. This gives them the unusual ability to form complexes with metal ions of unusual oxidation states, e.g. Ni(III),[30] Cu(III)[31] and Hg(III).[32]

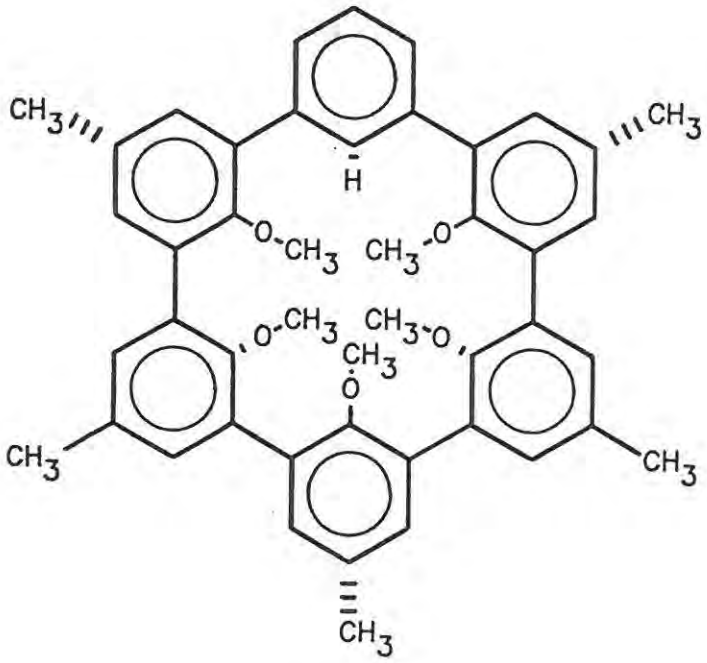
The macrocyclic effect can be broken down into several important contributing factors :-

pre-organization of the ligand;[19]
desolvation of the donor atoms in the confined space of the macrocyclic cavity;[33]
intrinsic basicity effects (inductive effects);[34]
dipole-dipole repulsion in the cavity of the ligand.[19,34]

The macrocycle's ability to selectively complex to specific metal ions has always been attributed to the so-called size-match selectivity principle. This concept can be misleading as true size-match selectivity only appears to occur with macrocycles which have a very rigid cavity. Common macrocycles such as the crown ethers and the tetraazamacrocycles are relatively flexible and selectivity is due to the same effects which influence selectivity in straight chain ligands (described earlier). With the tetraazamacrocycles in



Cryptand–111



Spherand

Figure 4

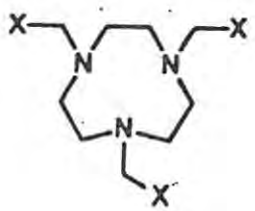
particular, the dominant effect is the ligand's ability to form five- or six-membered chelate rings. In order to investigate the effect of true size-match selectivity, greater rigidity and pre-organization must be built into the ligand. Examples of such compounds are found in cryptates and spherates (see figure 4).

1.1.8 The Cryptate Effect.

The greater rigidity found in cryptates enhances their metal-ion size selectivity. Cryptates still have some degree of flexibility and can fold in upon themselves and, consequently, they are not fully pre-organized. However, greater pre-organization leads to greater selectivity for specific metal ions when compared with equivalent open-chained analogues.[35]

1.1.9 Concluding remarks on ligand design.

The trend for future investigations will thus be towards the development of more pre-organized ligands such as spherands,[19] sepulchrates[36] and pre-organized cyclic systems (see figure 5). There are several examples (such as the compound *N,N′,N″-tris*(3-hydroxy-6-methyl-2-pyridylmethyl)-1,4,7-triazacyclonane) of highly pre-organised cyclic molecules which are still being investigated.[37] These cyclic systems have great rigidity



X = 3-hydroxy-6-methyl-2-pyridyl

N,N′,N″-tris(3-hydroxy-6-methyl-2-pyridylmethyl)-1,4,7-triazacyclonane

Figure 5

and a relatively small internal cavity and,

consequently, are very specific for metal ions of a certain size, (in this case iron III) showing that to achieve selectivity one does not need to always investigate very complex and large ligand systems.[38]

1.2 BIOMIMETIC LIGANDS.

Biomimetic compounds have generated much interest in both medicine and industry. Many of these compounds have been developed from simplified models of naturally occurring molecules (such as proteins), although many are a result of the original natural molecule being chemically modified in order to enhance certain chemical attributes. Synzymes (or synthetic enzymes) are synthetic compounds which model the catalytic activity of enzymes. These compounds have greatly increased our understanding of the mechanisms of enzyme catalysis.

Enzymes which are used as biocatalysts have enormous potential. Their main advantage is their ability to vastly increase the rate at which reactions take place; some can effect transformations between 10^6 and 10^{14} times faster than the corresponding uncatalysed reaction. Enzymes also tend to be very specific in the reactions they will catalyse and they are particularly useful in their ability to effect regiospecific and steriospecific transformations.

However, enzymes have several major disadvantages which have limited their widespread use. The first of these is cost. For a reaction to be commercially viable, cheap, readily available reagents and catalysts are necessary. To produce enzymes in useful quantities and of

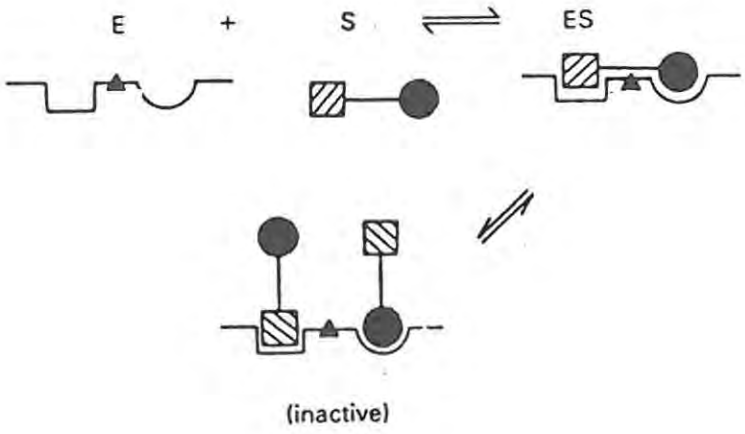
sufficiently high purity is very expensive, and it is this cost factor which has limited industrial applications. Moreover, many chemical substrates are soluble in organic media, whereas most enzymes are only effective in aqueous media, resulting in the problem of phase incompatibility, which is often difficult to overcome. In order for a reaction to be efficient and industrially useful it must be capable of converting large quantities of reagents to products very quickly. However, enzymes tend to bind strongly to both products and reactants and this causes two types of inhibition :- product inhibition and substrate inhibition.

1.2.1 **Product inhibition.**

This is where the presence of the product in the reaction medium causes the blocking of the active sites on the enzyme and so inhibits enzyme activity. This means that the industrial process will have to have some way of removing the product in order to enable the reaction to go to completion, and this can be difficult to achieve.

1.2.2 **Substrate inhibition.**

This occurs when high concentrations of reactants are employed. This effect can be considered simplistically in terms of an active site which has two different ends



Scheme 1

(see scheme 1). When there is a high concentration of reactants in the reaction medium then there will be competition for the two ends of the reactive site. If two reactants each bind with an end of a single reactive site, then the reactive site will effectively be blocked and no reaction will take place. This can be very limiting as industrial applications tend to employ high concentrations of reactants in order to speed up reactions and drive them to completion.

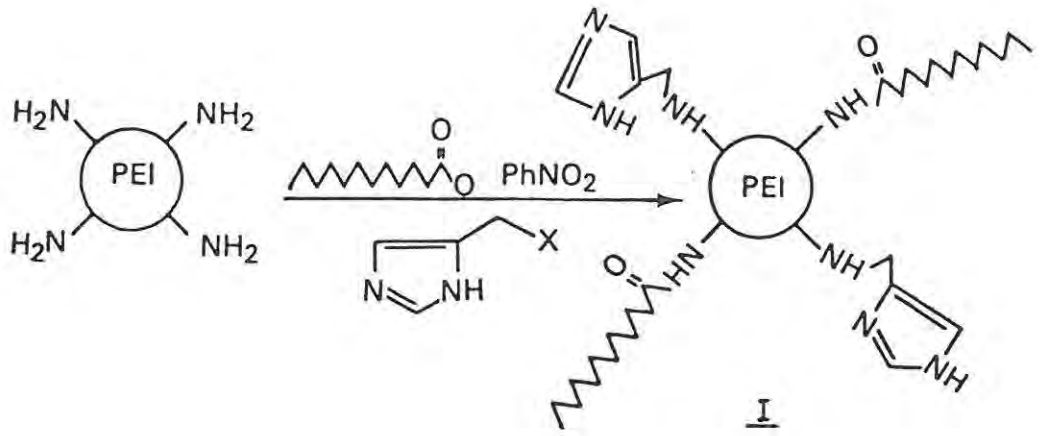
1.2.3 Modelling of enzymes.

Enzymes tend to be very large and complex molecules. This makes the exact duplication of the reactive sites very difficult. However, there are ways in which this problem can be overcome. Firstly, smaller coenzymes can bind to suitable polymer substrates which will provide the correct dielectric environment. This approach may be

difficult and is still subject to similar problems as are encountered with the use of enzymes, *viz.* denaturing at high temperatures and to a certain extent cost.

The second approach involves a simplification of the task by lowering expectations of rate enhancement. While the turnover rate for many enzymes is huge (for carbonic anhydrase it is in the order of 6 x 10^5 molecules per s.), Sir John Cornforth[39] in his study of the hydration of olefins with non-enzymatic catalysts, proposed that for 1kg of a catalyst which was 1/1000th as effective as the enzyme fumarase there would be 500 metric tons of product produced per year. This shows that the enzyme does not have to be modelled exactly to produce huge increases in catalytic activity.

Enzymes tend to be globular proteins with compact structure. The use of branched polymers to mimic the enzyme's compact structure has met with encouraging success. Klotz *et al.*[40] have investigated the importance of the shape of the polymer and they have found that mimicking the active site of an enzyme would be more effective if globular or branched polymers are used. Klotz *et al.* studied the use of poly(ethyleneimine) (PEI) and by substituting the PEI nitrogens with lauroyl groups and imidazoylmethyl groups (see scheme 2) they achieved a rate enhancement of between 10^2 and 10^3 for the solvolysis of nitrophenylesters.



I

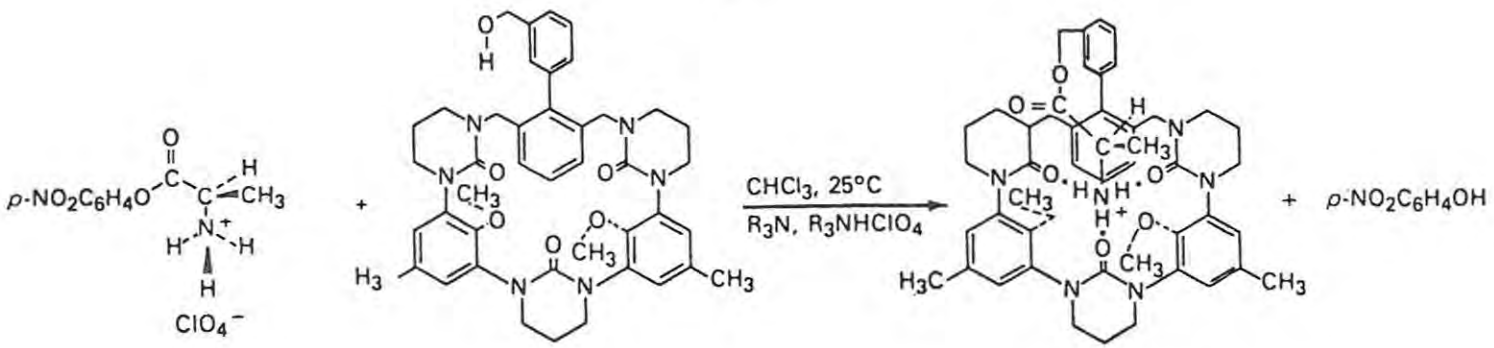
Scheme 2

This result is thought to have been brought about, in part, by the binding of the hydrophobic nitrophenyl ester onto the surface of the polymer in the vicinity of the imidazole groups.[41]

1.2.4 Synthetic Macrocycles (cavitands).

Cram[42] defined the term "cavitand" as "a synthetic organic compound that contains an enforced cavity of dimensions at least equal to those of the smaller ions, atoms or molecules" (complexed to it). Cram and Katz[43] have described the synthesis of the macrocyclic compound

III



Alanine ester + Model catalyst → Acylated product

Scheme 3

(scheme 3), which is an interesting model for serine protease. Such enzymes (hydrolases) are characterised by the presence of a nucleophilic serine moiety in the active centre. In the first step of the hydrolysis the amino or hydroxyl groups in the serine group are acylated, and the amino or alcohol moiety of the substrate is displaced. Hydrolysis of the acyl serine intermediate completes the reaction. The complexation of the L-alanine nitrophenyl ester by III (scheme 3) precisely aligns the carbonyl carbon for attachment by the phenolic hydroxyl, which is analogous to the serine hydroxyl in the enzyme. Acylation of III (scheme 3) is faster than the acylation of a reference compound (3-phenylbenzyl alcohol) by a factor of 10^{11}. Although the resulting ester intermediate does not turn over, this model system is interesting in that an enormous rate enhancement is observed in a rigid, well-defined system. Follow-up work on this catalyst is continuing and the aim is to achieve a catalyst which can turn over.[43]

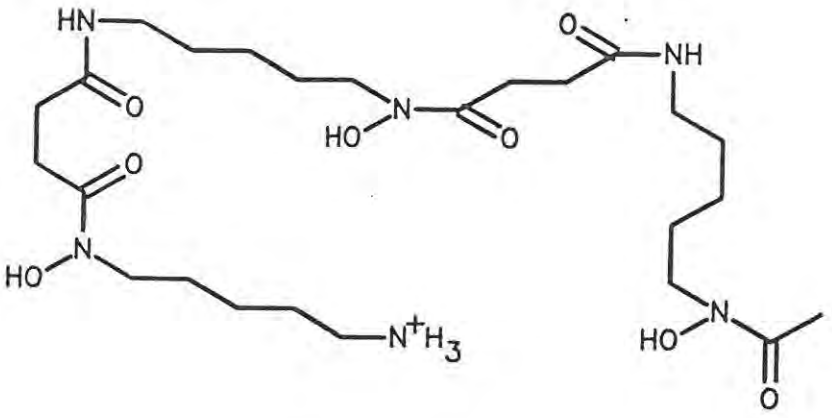
Enzyme engineering has a bright future. The combination of specificity and degree of rate enhancement of enzymes as biocatalysts is unique and applications of modified enzymes and synzymes which exploit these properties will continue to appear and compete with conventional catalysts.

1.2.5 Metallothionein.

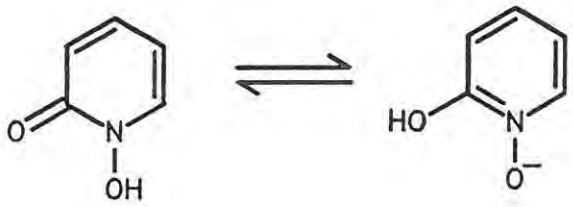
Metallothioneins are naturally occurring proteins that are present in the liver and kidneys of various animal species including human beings.[44] These proteins contain a large amount of cysteine[45] and strongly bind metal ions, especially Cd^{2+}, Hg^{2+} and Zn^{2+}.[46] The action of this protein is thought to be a protective one in metal poisoning but is not yet fully understood.[47] The complete amino-acid sequence of these proteins for human beings,[48] horses[49] and mice[50] have been reported and in all cases the polypeptide chain contains 60 - 61 amino-acid residues, among which 20 are cysteine.

Oligopeptides were synthesized by Yoshida *et al.*[51] (using the solid phase method, see section 1.3), containing three cysteinyl residues and having amino acid sequences analogous to portions of this protein. Strong affinity of the synthetic peptides to Cd^{2+} and Hg^{2+} were observed and the dissociation constants of the peptide-metal complexes were 2-4 orders of magnitude lower than those of cysteine-metal and dithioerythritol-metal complexes. The oligopeptides proved very effective as detoxification agents against Cd^{2+} because they bound strongly with Cd^{2+} while only relatively weakly with Zn^{2+}, which is an essential ion in the body.

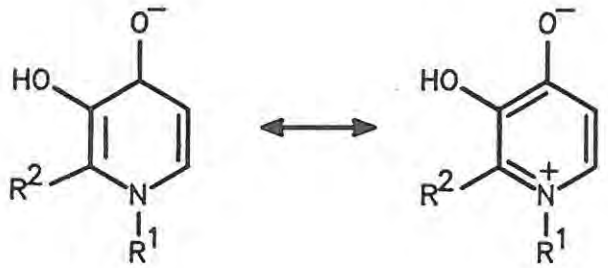
Other biomimetic ligands which have been developed for metal toxicity treatment are the 3-hydroxypyridine-4-



6.1 Desferrioxamine



6.2 2-hydroxypyridine-*N*-oxide



6.3 3-hydroxypyridin-4-one

6.4 3-hydroxypyridin-2-one

Figure 6

ones. These have been developed by modelling the fungal siderophore (a growth promoting agent secreted by microorganisms to scavenge iron from the environment),[52] desferrioxamine (see figure 6.1). This was used to treat the iron overload the body acquires after multiple blood transfusions. However, it breaks down fairly quickly in the body and so treatement has to be given six times a week.

Siderophores[53] are low molecular weight compounds (500 - 1000 mass units) with incredibly high affinities for iron (III) ($K_f = 10^{30} - 10^{50}$ M^{-1}). Coordination of the metal occurs, in most cases, via the oxygen of either the hydroxamate or catechol moieties. The investigation searched for compounds which would form neutral iron complexes (for better absorption into the body) and having either a hydroxamate or catechelate-like structure. It was found that 2-hydroxypyridine-*N*-oxide,[54] figure 6.2, (which can be considered to be an aromatic hydroxamate) has a high affinity for iron (III). This led to a consideration of 3-hydroxypyridin-4-ones, figure 6.3, and 3-hydroxypyridin-2-ones, figure 6.4, as possible iron chelaters. Investigations revealed that these two compounds had great potential for use in iron toxicity treatement, and further development is continuing.

1.2.6 Concluding remarks about biomimetic compounds.

There are many naturally occurring compounds which perform certain reactions very effectively. These compounds are often very difficult and expensive to obtain for laboratory and industrial use and so there will be an ongoing investigation into ways of modelling or mimicking these compounds. Synthetic models of natural compounds have proved to have most of the advantages of the natural compound while lessening the disadvantages.

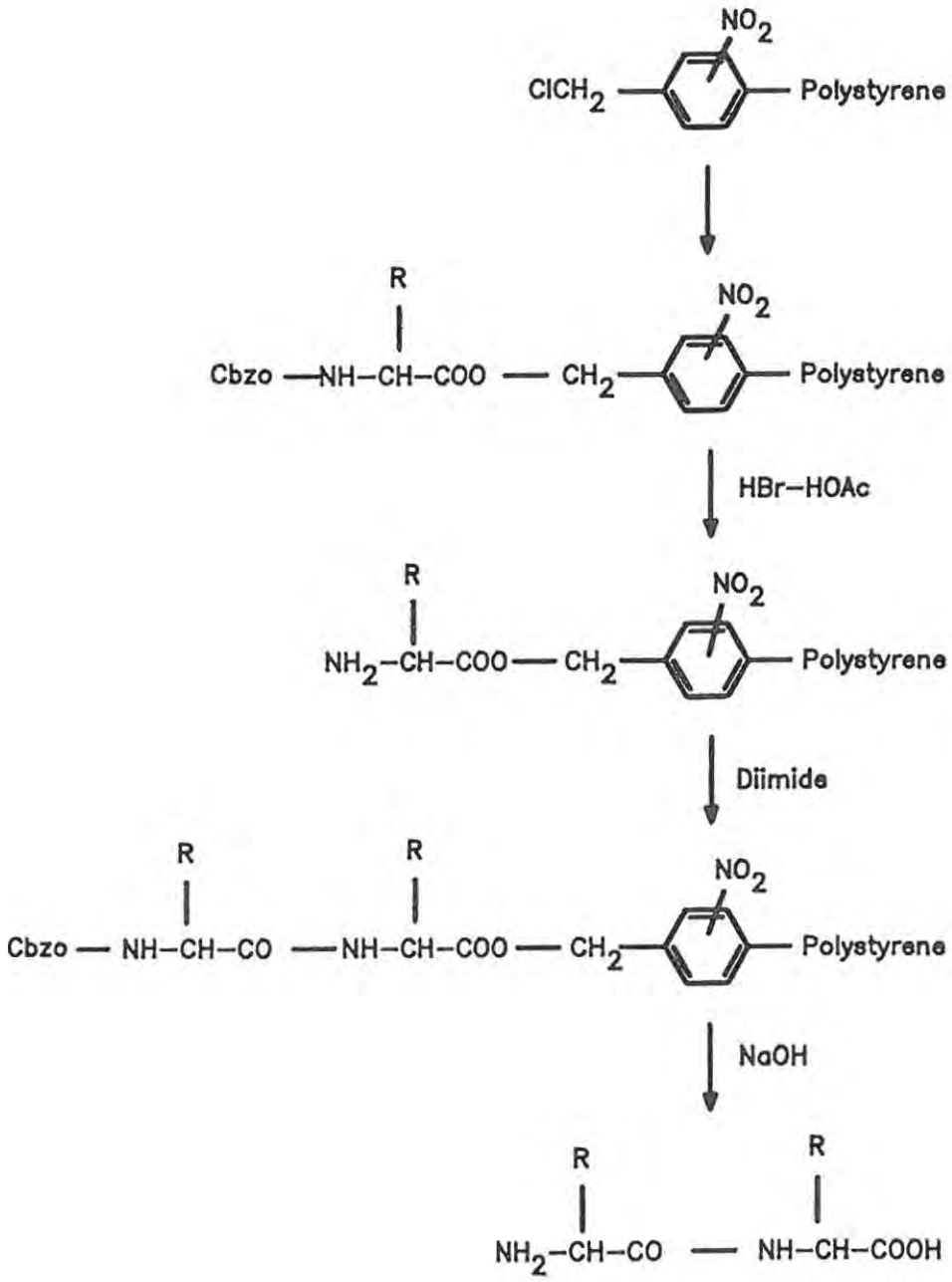
1.3 SURVEY OF THE DESIGN AND USES OF SYNTHETIC FUNCTIONALIZED POLYMERS.

1.3.1 Advances In Functionalized Polymers.

Prior to about 1920, polymers were not thought of as being covalently bonded macromolecules although much work had already been done with polymers nearly a century earlier. One of the earliest reports was that of Simon[55] who isolated and polymerised styrene. At the time polymers were thought of as "colloidal" or as relatively small cyclic compounds. However, it was Staudinger[56] who, in 1920, proposed long-chain formulae for polymers. His concept was not widely accepted until the mid-1930's. After this, techniques were developed to produce structurally useful polymers.

Until the mid-1960s, polymers were only of interest as materials rather than as organic molecules in their own right. Most of the research at the time was directed at improving the physical properties of the polymers using empirical methods, and there was very little research being done into the molecular reactions occurring in the formation of the polymers. However, this field has developed rapidly of late. By 1965, the nearest any research had come to treating polymers as reactive species was in the field of ion-exchange resins.[57] These resins had previously been examined as acid and base catalysts, although no technological applications

appeared to have been developed by that time.



Scheme 4

The first significant utilization of polymers as reactive organic molecules was said to have been made by

Professor Bruce Merrifield who, in 1963, developed the use of an insoluble macromolecule as a protecting group in the "solid-phase technique" for the synthesis of peptides [58] (see scheme 4). The polymer not only provided protection for the amino acids but it also provided a facile method for the isolation and purification of the product at each step. Since that development, the use of functionalized polymers has increased dramatically and, in 1984, Professor Merrifield was awarded the Nobel Prize for Chemistry for his work in this field. The variety of different uses to which functionalized polymers can be put seems almost limitless as chemists and biochemists find still more uses for these systems.[59]

Functionalized polymers usually comprise a reactive species which is tightly bound to a macromolecular support, which acts as a convenient "handle" for the manipulation of the species. This has numerous potential advantages in that the macroscopic "handle" can improve the ease of separation, isolation and purification. Reagents can now be used in excess without the complication of difficult separations further on in the process thus increasing yields and reducing the reaction times.[60]

Expensive or precious reactive species can be retained and possibly recycled more efficiently when immobilized, while corrosive, noxious or toxic species may be

rendered safe when bound to a macromolecule.[61] Such systems may lead to a general clean-up of the whole of chemistry.[62]

Practical advantages can also be gained by using an immobilized species,[76] since in an industrial plant the functionalized polymers can be treated as a heterogeneous system. There have already been industrial techniques developed for the handling of such systems and so no new technology is required. Polymers can easily be used in either batch or continuous flow systems and are generally suitable for both gas and liquid phase reactions. The type of polymer used can be modified to suit any industrial reaction conditions.

The polymer matrix constitutes a special environment for carrying out chemical reactions.[65] For example, it may impose, on molecules diffusing into it, certain defined steric limitations. These limitations may be determined by:-

pore or channel structure;
substituent species on the polymer backbone;
and by the distance between attached species and the polymer backbone.

The polarity of the backbone can also have a pronounced effect on the reaction path of bound species. These parameters can all be varied in order to give a reaction environment which is tailor-made for particular

requirements.

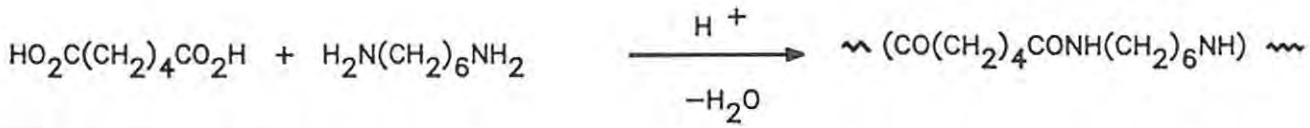
The polymeric carrier restricts translational motion and reactive centres can, in certain circumstances, behave virtually as if they were in solution at infinite dilution. A consequent use of polymers with low loading is that unstable transient species can be stabilized and exploited. Of cause, the opposite can also be achieved, by forcing reactive species into close proximity an effect which can result in fast and selective reactions.[89]

However, as with every new development there are also some disadvantages. The first of these is that the strength of the bond between the reactive species and the polymer backbone must be great enough to withstand the reaction conditions which will be used. If the reactive species is leached off then all the possible advantages will be lost.[63] This remains a serious problem in the case of immobilised metal complex catalysts.[64] Another problem is that reaction rates can be dramatically affected if the diffusion barriers imposed by the polymer are too great. This effect can be minimised by using a polymer with high porosity, but this limits the choice of polymers which can be used.[65]

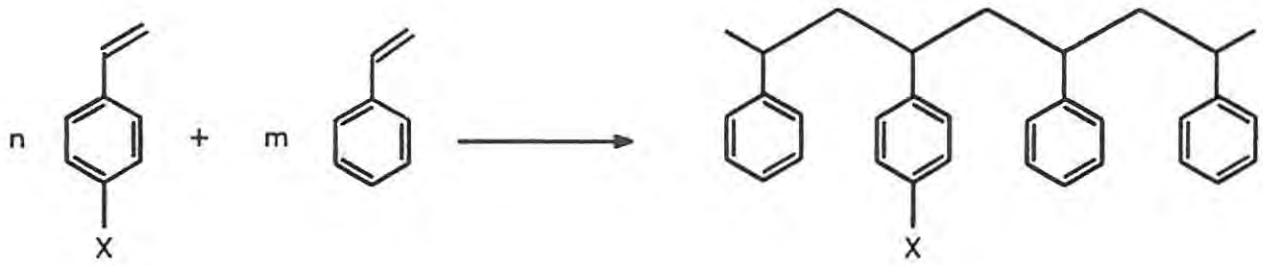
One of the major drawbacks to the functionalized polymer approach is, generally, that these systems have not been

available and when they are available they tend to be very expensive. So decisions have to be made as to whether or not the time and cost in preparing these resins out-weighs the advantages which could be gained. In principle, however, the potential gain in minimizing the dangers involved in handling dangerous chemicals is likely to outweigh the financial costs involved in their use.

1.3.2 Structure and Properties of Functionalized Polymers.



a) Linear Polymer.



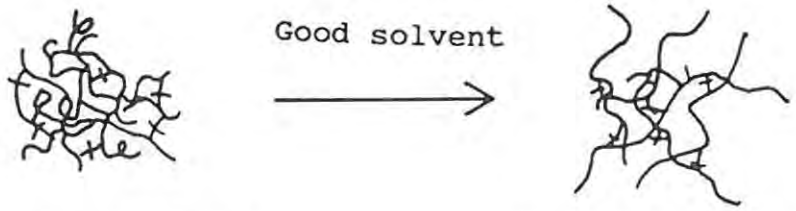
b) Cross–linked Polymer. (for n : m :: 1 : 3)

Scheme 5

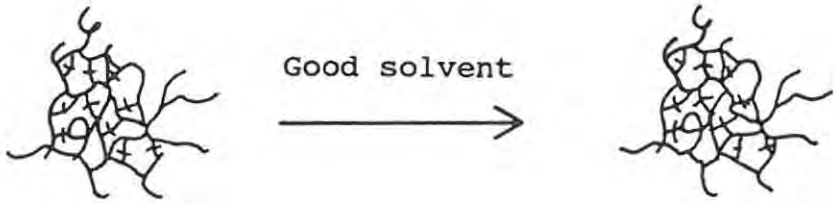
A functionalized polymer can be defined as a synthetic macro-molecule which has functional groups chemically

bound to it.[67] These functional groups can be used as reagents,[69] catalysts,[68] protecting groups[58] etc. The macromolecule can be a linear species capable of forming a molecular solution in a suitable solvent,[70] (see scheme 5a) or alternatively, they can be a cross-linked species (a so-called resin), which though readily solvated by a suitable solvent remains macroscopically insoluble.[60] Most applications have used insoluble polymer resins as the support, these being prepared by suspension co-polymerisation of styrene with divinylbenzene as crosslinker [77] (see scheme 5b). Both laboratory[78] and industrial processes yield spherical particles whose diameter can be closely controlled between 10μm and 1mm depending on the expected use. Methods have been developed whereby the total pore volume, pore size distribution and internal surface area can all be readily controlled[60] using porogens (organic solvents and non-solvents) or by varying the concentrations of the crosslinker.

Lightly cross-linked resins (around 2% cross-linking) which are synthesised in the absence of porogen are called "gel-type" or microporous resins.[79] When dry, these resins are non-porous but on contact with a good solvent a soft gel network is formed with the generation of considerable porosity, depending on the extent of cross-linking (see figure 7). Where the extent of cross-linking is less than 1%, the swollen resins generally



Lightly cross-linked Network expanded



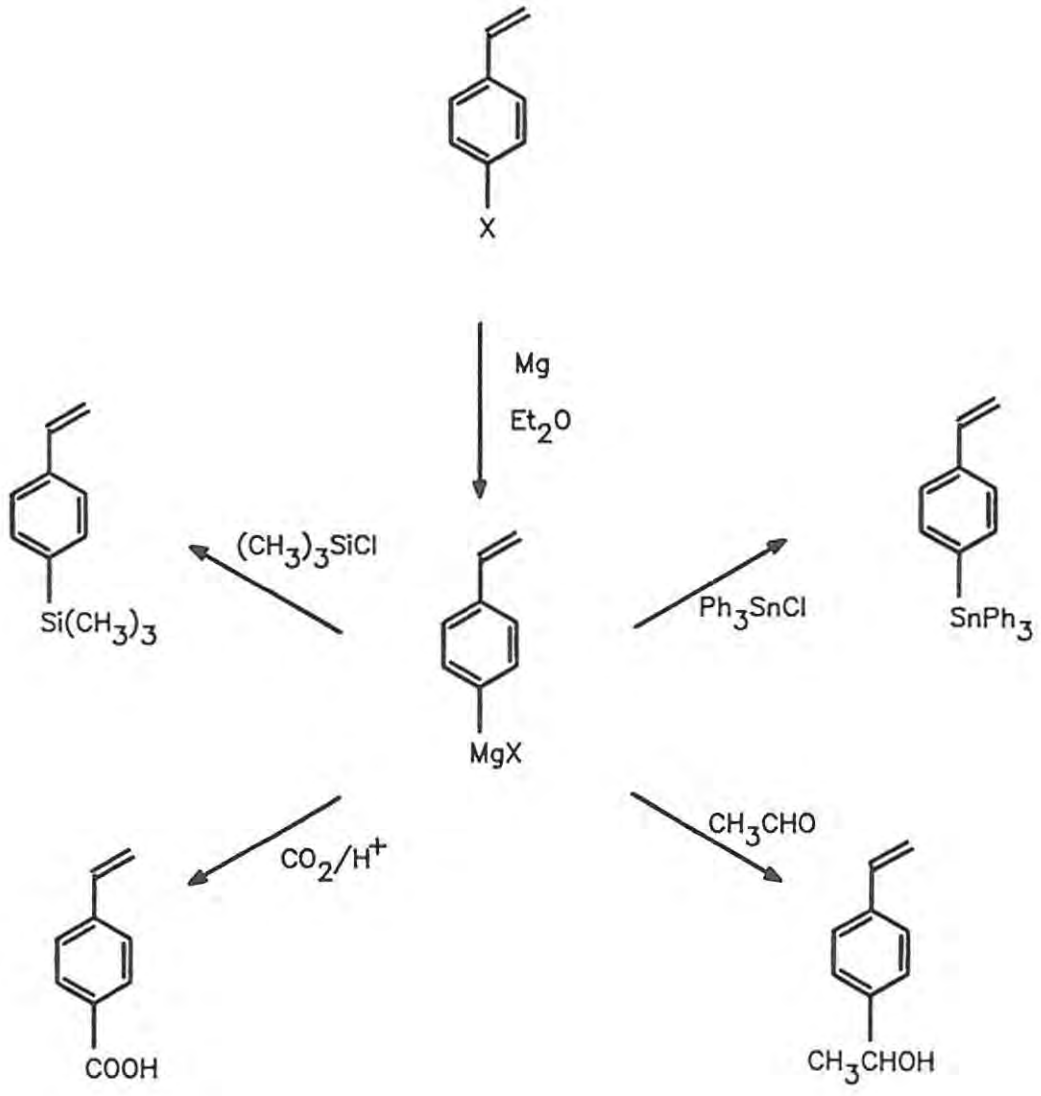
Highly cross-linked Network little changed

Figure 7

have low mechanical stability and are readily fragmented, even with careful handling. By increasing the extent of cross-linking, the mechanical stability also increases but this, in turn, gives rise to acute diffusional limitations which may hinder the rate of reaction.

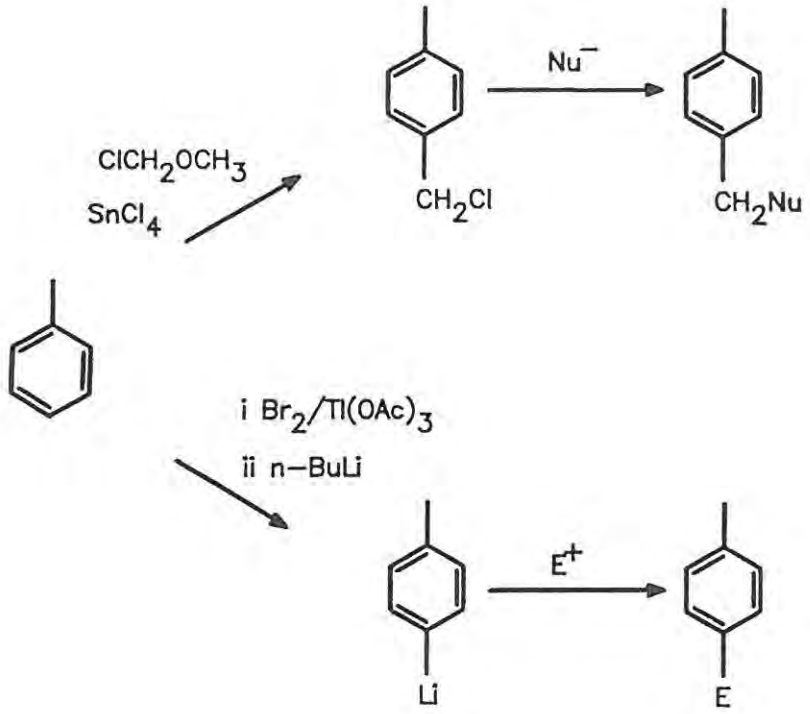
More heavily crosslinked resins are rendered porous by the use of porogens. Where the solvent solvates both monomer and polymer, a fully expanded network is formed with a considerable degree of porosity.[80] Since polymers are porous even in the dry state, the pore network can be accessed by non-solvents and solvents alike. Very small pores with high surface area arise when the porogen is a good solvent for the polymer. The use of a solvent, which is good for the monomer but precipitates the polymer, results in large pores with small surface area.[81] In order to obtain mechanical stability in the

swollen solvated state large quantities (up to 20%) of difunctional co-monomers are used in the preparation. Materials of this type are not readily available from commercial sources.



Scheme 6

Appropriate functional groups leading to the required active species can be introduced in a number of ways. One way is by direct polymerization of monomers which



Scheme 7

already have the required functional groups. Compounds such as Grignard reagents can provide a versatile route to a wide range of monomers (see scheme 6).[83,84] A second way is by modifying the polymer and adding on the required functionality. Examples of this are modification *via* chloromethylation[85] or lithiation[86] (see scheme 7). Usually the task is done by a combination of these two methods, with a bias towards the chemical modification technique. A difficulty with the first method is that considerable manipulation of the co-polymerization procedure may be required to achieve an acceptable yield of the co-polymer and also to ensure a satisfactory physical form.[60] In the second method, commercially available resins of high quality are

normally used and the desired functional groups are introduced using standard organic synthetic techniques.[85,86] While this ensures a product of good physical form, the derivatization procedures must be as free of side reactions as possible.[87] Even so, the polymers prepared in this way rarely have every repeat unit functionalized, and the distribution of groups may not be uniform. Differing synthetic routes may result in differing distributions of functionalized groups, and this is an area which requires further detailed investigation. The usual analytical technique used to follow functionalisation in these systems is infra red absorption spectrometry. This usually gives enough information to calculate the degree of modification and whether the correct modification has occurred. More detailed analyses can be done using nuclear magnetic resonance spectroscopy, but this depends on the nature of the polymer used and the type of modifications involved.[88]

Polystyrene's dominant role as a support structure for functionalized polymers is due to its fulfilling the major criteria for a support.[65] These are that it must be :-

chemically stable but can be functionalized with relative ease;

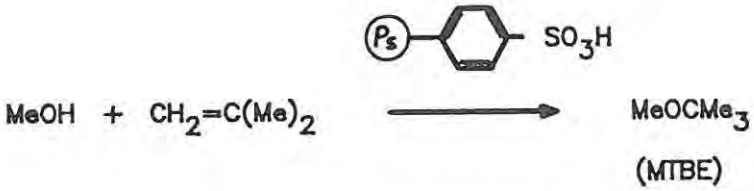
mechanically strong enough to withstand most reaction conditions;

compatible with most organic solvents;

relatively cheap and freely available.

A functional group may be attached to the styrene matrix by various approaches, some of which are illustrated in scheme 2. Nucleophilic displacement on chloromethyl substituents is the most commonly used technique.[66]

1.3.3 Advantages and Disadvantages in Using Functionalized Polymers.



Production of methyl *tert*-butyl ether (MTBE)

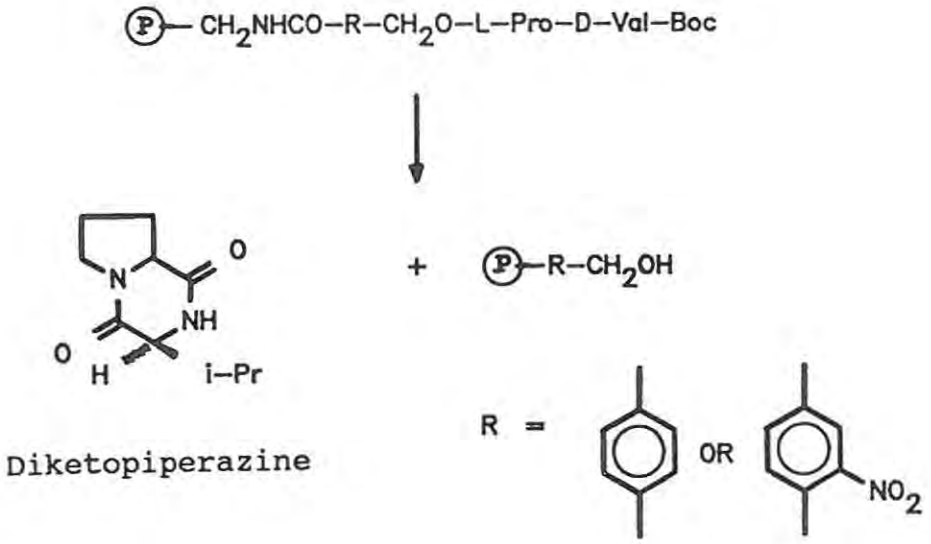
Figure 8

The most obvious advantage in using functionalized polymers is the simplification of product work-up, isolation and separation. In the case of cross-linked polymer resins, simple filtration procedures can be used for isolation and washing, and the need for complex chromatographic techniques can be eliminated. With linear polymers, techniques such as precipitation, sedimentation and ultrafiltration can be employed.[60] Resins also allow for the possibility of automation (in the case of repetitive stepwise reactions) and also the facility of carrying out reactions in flow reactors on a commercial scale. An example of this is the production

of methyl *tert*-butyl ether (MTBE), which is catalysed by a sulphonic acid resin (see figure 8).[71] Supported reagents may also be used in excess to drive reactions to completion, without incurring any penalty in the work-up procedures.

Macromolecules may effectively encapsulate a corrosive species, thus removing it from contact with the surroundings and so rendering it non-corrosive.[72] Attachment to a molecule of large molecular mass may cause the vapour pressure of a noxious species to become virtually zero so minimising the possibility of the noxious species being vaporised.[62] Bonding a toxic species to a macromolecule will also reduce the body's ability to absorb the bound toxic species, thus increasing safety when dealing with hazardous chemicals.[73]

There are also a number of potentially important reactivity changes which may be induced using functionalized polymers.[76] A combination of a high degree of cross-linking, a low level of functionalization, low reaction temperatures and the development of electronic charges near the polymer backbone tends to encourage a situation, which may be regarded as mimicking the solution conditions of "infinite dilution". In these circumstances intermolecular reaction of bound molecules is prevented and such attached molecules can be made



Diketopiperazine

Figure 9 Cylization synthesis of Diketopiperazine.

either to react intramolecularly (i.e. cyclize,[74] see figure 9) or to react selectively with an added soluble reagent. Polymer supported metal complexes with vacant coordination sites may fulfil this description, with the resin inhibiting the normal solution oligomerization processes of such species. Under certain circumstances it is also possible to achieve the opposite state of "high concentration" by heavily loading a flexible polymer matrix with one particular moiety in an attempt to force its reaction with a second polymer-bound species.[89]

As has been mentioned before, there are also a number of major disadvantages. The most important of these is the additional time and cost incurred in the synthesis of the supported reagent or catalyst. This may be offset by

the potential advantages, especially if there are simple methods of regeneration and recycling the species.[90] However, the occurrence of slow reactions and poor yields may be a problem. By changing the support and the reaction conditions these problems can often be overcome, but there are always problems which cannot be readily solved. For example, when the reaction of the species occurs on the polymer backbone, then the final cleavage step may be incomplete and the reaction conditions used in this step may cause degradation of the polymer.[91] The chemical and physical strength of the polymer can also limit the uses, a good example of this being strongly acidic and basic ion-exchange resins, which have a temperature stability limit which has hindered their widespread commercial use. There is also the problem that the capacity of the functionalized polymer is limited and this can be of particular importance in the field of organic syntheses where stoichiometric quantities of supported reagents are required. The presence of the macromolecule hinders characterization of the supported reagents, especially in analytical techniques which require homogeneous solutions. Finally there is always the possibility of side-reactions occurring with the polymer.[88] While a number of classic cross-linking side reactions have been identified,[91] low yields may often be associated with intrapolymeric reactions, many of which have not yet been fully investigated.

1.3.4 Concluding remarks about functionalized polymers.

Despite various disadvantages, there have been several functionalized polymers which have become commercially avalible. The earliest of these were analogues of EDTA (i.e. Dowex A-1, Chelex-100 and Chelex-20.[92]), which are still widely used. Other resins include Duolite GT-73[93], Amborane™-345[94] and Chelite-N.[95] Many other resins which have shown great promise in the laboratory have not been developed further because of the cost and difficulty of production. More research is being done in this field and with more papers being published each year, the future for functionalized polymers seems assured.[96]

1.4 AIMS OF THE PRESENT INVESTIGATION.

The initial aims of the present study were :-

i) The phased development of ligand systems based on amino acids for specific complexation with metal ions.

ii) The development of techniques for the attachment of the ligand systems developed in aim (i) onto a linking group and an initial investigation of the effect of the presence of the linking group on complex formation by the ligand systems.

iii) The development of techniques for linking ligand systems onto a polymer support.

In fact, as will be apparent in the discussion, these aims shifted as the project developed and as new problems came to light.

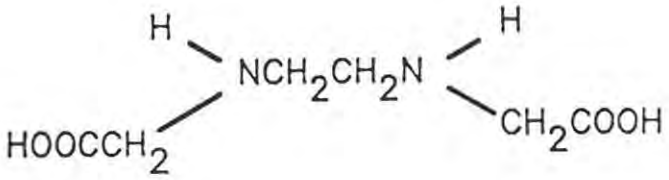
2 DISCUSSION.

The synthetic approach used was broken down into several stages in which the ligands would be developed. In phase I, a range of ligands was synthesised which contained a single amino acid. From the results of complexing studies carried out on this set of ligands, several of the ligands were selected as promising enough to be taken on to Phase II for further development. Phase II involved the development of ligands which contained two amino acids forming systems with chelation properties. Promising compounds from complexation studies on this set of ligands were then used for phase III, which involved development of macrocyclic ligands.

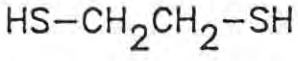
2.1 Phase I: The Synthesis of Single Amino Acid Containing Ligands.

Several readily available amino acid systems were chosen to provide donor centres for the ligands. These included glycine, cysteine, glutamine and others. There were several non-amino acid systems which were also investigated due to their useful complexing ability. These included compounds such as ethylenediamine (ED), ethanedithiol (EDT), dithiothreitol (DTT) and ethylenediaminediacetic acid (EDDA) (see figure 10).

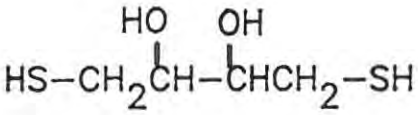
The main objective in phase I was the development of synthetic techniques to permit attachment of amino acids



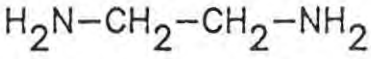
EDDA



Ethanedithiol (EDT)



Dithiothreitol (DTT)

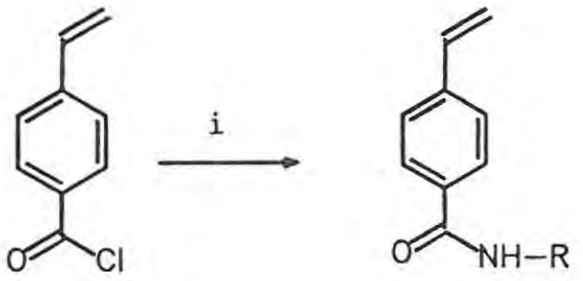


Ethylenediamine (ED)

Figure 10

and other compounds onto a polymeric backbone. It was initially decided to use the monomer, styrene, as a linking group instead of polystyrene, to ease the difficulties that would have been encountered in characterising functional groups on the polymer. The method of attachment (see scheme 8) involved the formation of a peptide bond from the reaction of an amino group of the ligand with the acid chloride group

of the styrene derivative (in the case of ethanedithiol, attachment was through one of the thiol groups).



4

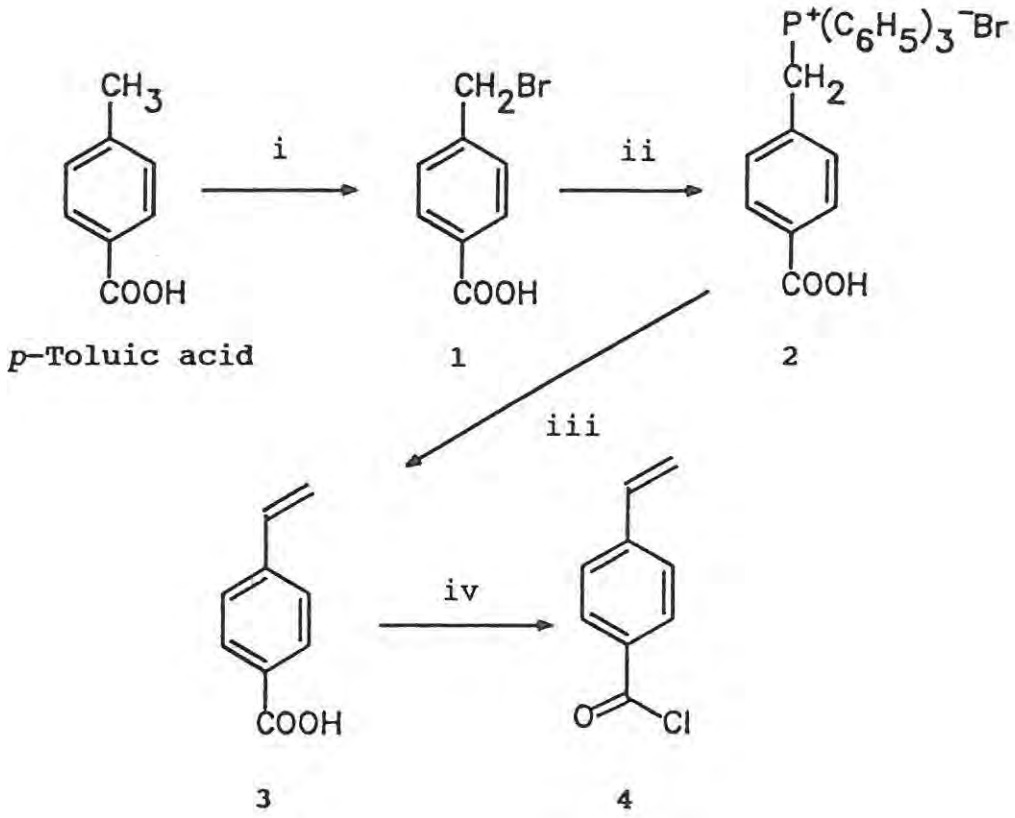
5 R = $CH_2CH_2NH_2$
6 R = CH_2COOH

Reagents

i. H_2N-R

Scheme 8

The acid chloride (4) was synthesised (see scheme 9), using the methods described by Tuleen et al.[100] and Broos et al.,[101] from toluic acid via the benzylic bromide (1) and the triphenylphosphonium salt (2) to form the carboxystyrene (3). The acid chloride (4) was formed using a modification of the methods described by Achinami et al.[103] and Nganie et al.,[104] with the latter method being found to be the most satisfactory affording the product in higher yield (95% as opposed to 54%).



p-Toluic acid 1 2

3 4

Reagents

i.	C_6H_5COBr, NBS.
ii.	$(C_6H_5)_3P$
iii.	HCHO
iv.	$SOCl_2$, 2d.

Scheme 9

2.1.1 Problems In Using Styrene.

The monomer styrene contains a reactive vinyl group which readily undergoes polymerisation reactions. The introduction of ligand systems onto the styrene molecule was accompanied by problems due to the product polymerising. Two attempts were made to link ligand

systems to styrene derivatives, *viz*, ethylenediamine and glycine, to form the compounds *N*-(2-aminoethyl)-4-ethenylbenzamide (5) and *N*-carboxymethyl-4-ethenylbenzamide (6) (see scheme 8). Work-up of these two products proved problematic as they were insoluble in most common solvents, and this made characterisation very difficult. N.m.r. spectra of the two products showed a loss of the vinylic protons consistent with the formation of styrene polymers under the conditions employed. It was considered possible that the polymerisation was catalysed by the liberated HCl and in an attempt to overcome this problem, the reaction was carried out under alkaline conditions. However, work-up again proved to be difficult and the resulting oil could not be characterised fully. Another disadvantage of using the styrene monomer was that the carboxystyrene (3) was not readily available and had to be synthesised in several steps as shown in Scheme 9.

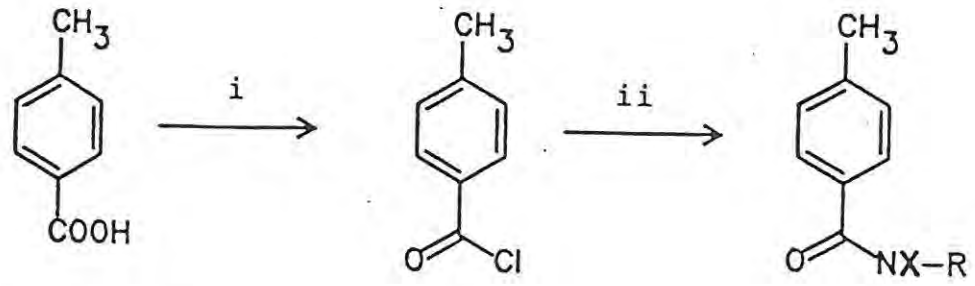
2.1.2 **Design of a "Pseudo-styrene" Monomer.**

In an effort to overcome the difficulties described in section 2.1.1., the vinyl group on the styrene molecule was replaced by a methyl group. By doing this it was hoped that the polymerisation problems would be overcome and that the toluoyl system would act as a suitable, alternative model linking group to styrene. Using this linking group, it was hoped that some information would be gained about the electronic and steric interactions

involved in complex formation. Use of this linking group as a "pseudo-polymer" is expected to permit examination of the limiting aspects of ligand binding without worrying about the effects that the polymer's physical presence would have. The starting product, toluic acid, was cheap and readily available which made it a viable alternative to carboxystyrene (3).

The linkage reactions (see scheme 10)[105] were modifications of those used previously, with the acid chloride being synthesised following the method described by Nganie *et al.*[103] The method for peptide bond formation was the same as the one used in the linkage of ligands to the styrene acid chloride derivative (4). A range of twelve phase I ligand systems (8 - 19) were synthesized using this method (see scheme 10).

One of the problems which was encountered when dealing with amino acids containing highly reactive side chains was the protection and deprotection of these groups to prevent them from taking part in the linkage reaction. An example of this is the linkage of cysteine to the toluoyl acid chloride (see scheme 11). The thiol group of the cysteine molecule was protected using the classic method described by Bodanszky and Bodanszky,[112] where triphenylmethanol is reacted with the thiol group in the presence of boron trifluoride etherate in glacial acetic acid. The addition of such a large protecting



p-Toluic acid 7 8 - 19

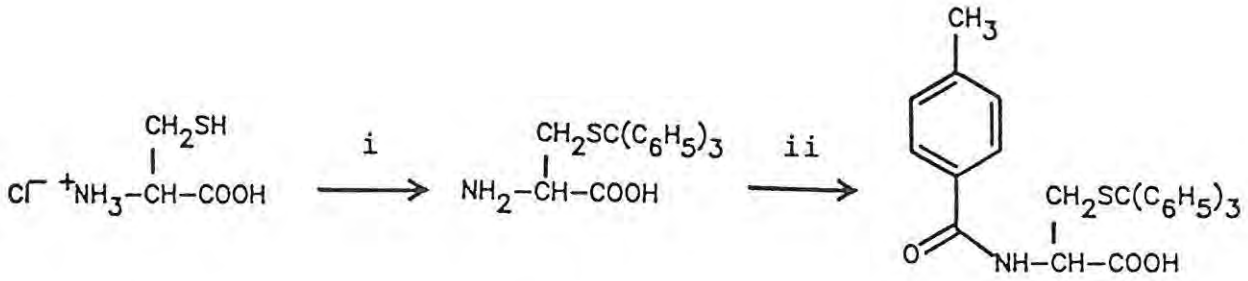
	X	R
8	NH	CH_2COOH
9	NH	$CH_2CONHCH_2COOH$
10	NH	$CH_2CH_2NH_2$
11	NCH_2COOH	$CH_2CH_2NHCH_2COOH$
12	NH	$CH[CH_2SC(C_6H_5)_3]COOH$
13	O	CH_2CH_2OH
14	S	$CH_2CH(OH)CH(OH)CH_2SH$
15	NH	$CH(CH_2OH)COOH$
16	NH	$CH(CHOHCH_3)COOH$
17	NH	$CH(CH_2CONH_2)COOH$
18	NH	$CH(CH_2CH_2CONH_2)COOH$
19	S	CH_2CH_2SH

Reagents

i $SOCl_2$, 2d.
ii HX-R

Scheme 10

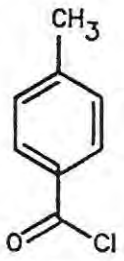
group made manipulation of the compound difficult by significantly reducing solubility of the compound in the usual solvents, as well as making interpretation of the ^1H n.m.r. spectra more difficult due to extensive absorption in the aromatic region. It was also found



19 20 12

Reagents

i $HOC(C_6H_5)_3$

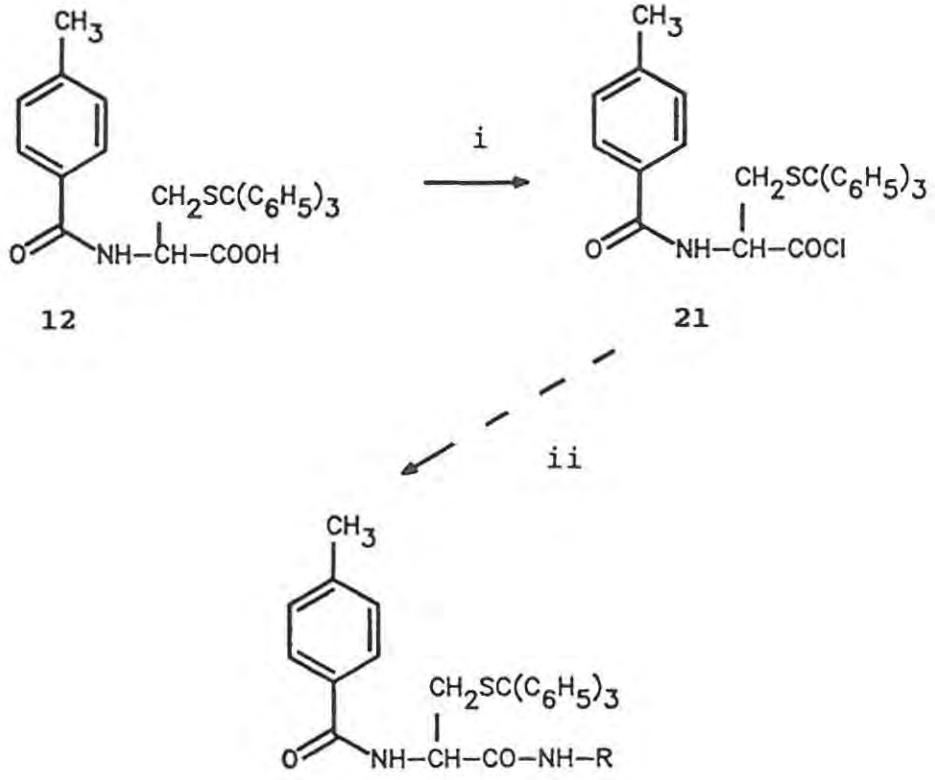
ii 

7

Scheme 11

that the deprotection reaction had a very low yield (13%) and, in some cases, the linkage between the amino acid and the toluoyl group was broken during deprotection, giving toluic acid and cysteine (the starting products).

It was hoped that this approach could be improved to permit addition of further amino acid residues onto the linked amino acid (see scheme 12). This proved difficult as formation of the acid chloride of the linked amino acid (22) proceeded in very low yield. Consequently this



12 21

Reagents

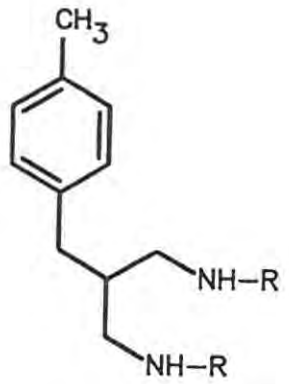
i $SOCl_2$
ii H_2N-R

Scheme 12

approach to dipeptides was not pursued. The glycylglycine derivative (9) was therefore prepared through a more direct approach using the readily available dipeptide glycylglycine and reacting it with toluoyl chloride (7).

2.2 Phase II: The Synthesis of Bis-(amino acid) Ligands.

In phase II, three different approaches were tried in order to afford a dual chain system consisting of two amino acid molecules separated by approximately three



Basic back-bone for attachment of two amino acid groups [R = CH(R')COOH]

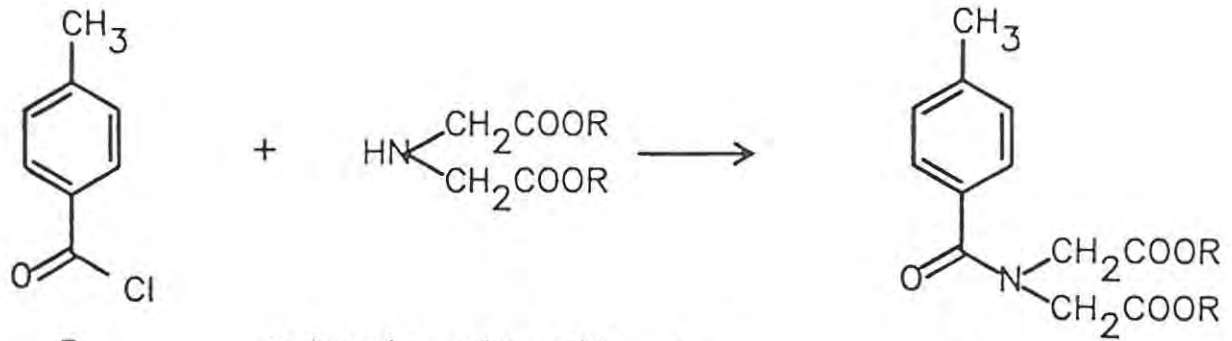
Figure 11

other atoms as shown in figure 11.

2.2.1 Route 1: Iminodiacetic acid approach.

In route 1, use was made of the two carboxylic acid groups present in the iminodiacetic acid molecule. It was hoped that the central nitrogen atom would provide an easy means of attachment for the iminodiacetic acid molecule to the acid chloride (7) (see scheme 13).

However, application of the linkage reactions developed in Phase I [in which the amino group of the ligand was reacted with an acid chloride functionality on the



7 Iminodiacetic acid 32 33 R = H R = CH_2CH_3 R = CH_3 23 24 25

Scheme 13

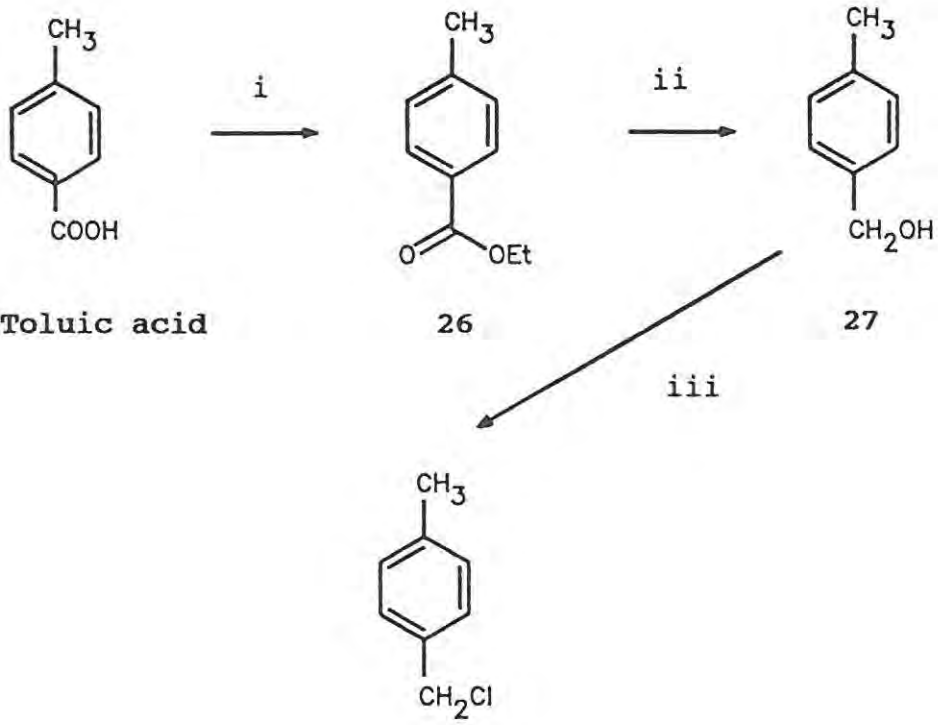
linking group (toluoyl chloride (7))] resulted in recovery of the starting materials with no product being formed. This was possibly due to hydrolysis of toluoyl chloride (7) by water present in the solvent and it was decided to repeat the reaction in an organic medium to overcome this problem. However, a major drawback to this approach was that iminodiacetic acid is only sparingly soluble in most organic solvents, resulting in little or no product being formed. To increase the solubility of the iminodiacetic acid system it was decided to synthesise the diethyl (32) or dimethyl (33) esters (see figure 12). These esterification reactions[114] afforded

24 25

Diethyl and dimethyl esters of iminodiacetic acid.

Figure 12

the diethyl and dimethyl esters in very low yield (21% and 15% respectively). Coupling of these two esters with the toluoyl chloride (7) was effected in basic medium (NaH/THF) under N_2. However, both of these coupling reactions proceeded in very low yield (12% and 25% for the diethyl and dimethyl esters respectively). The repeated isolation of the starting material, *p*-toluic



p-Toluic acid **26** **27**

28

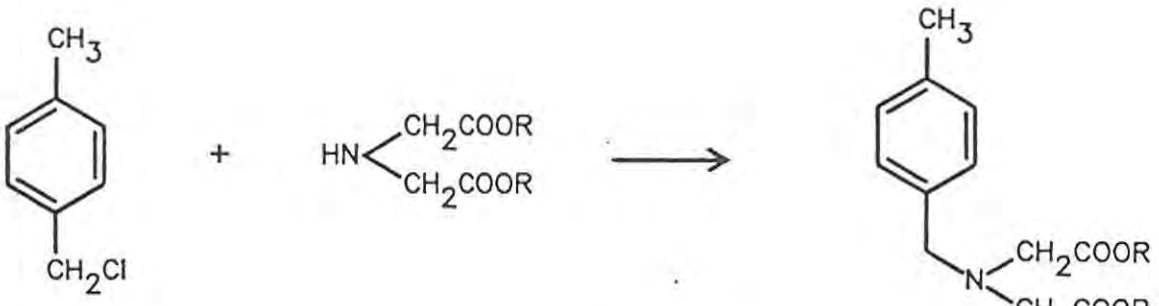
Reagents

i	EtOH/H⁺
ii	LAH
iii	$SOCl_2$

Scheme 14

acid, was attributed to hydrolysis of the acid chloride, toluoyl chloride (7). Another linking group was then investigated to overcome these problems. The new linking group to be investigated was α-chloro-*p*-xylene (28). This was synthesised from the readily available *p*-toluic acid (see scheme 14). *p*-Toluic acid was esterified to form the ethyl ester (26) which was, in turn, reduced to the alcohol (27), which was easily chlorinated using thionyl chloride to give the benzylic chloride (28). This chloride (28) is obtainable commercially but, due to problems in having this compound transported by air, it had to be synthesised.

The coupling reaction between α-chloro-*p*-xylene (28) and



28 Iminodiacetic acid R = H 29
32 R = CH_2CH_3 30
33 R = CH_3 31

Scheme 15

iminodiacetic acid (see scheme 15) was effected in organic medium (Et_2O), in the presence of a strong base

(NaH), following a similar pattern to the malonic ester synthesis (see section 2.2.2). It was hoped that the strong base would remove the proton on the nitrogen atom, thus allowing for electrophilic attack of this anion on the halide (28). However, poor solubility and the presence of two acidic protons on the two carboxylic acid functionalities of the iminodiacetic acid resulted in large quantities of base having to be used and, also, in relatively poor yields being obtained (32%)

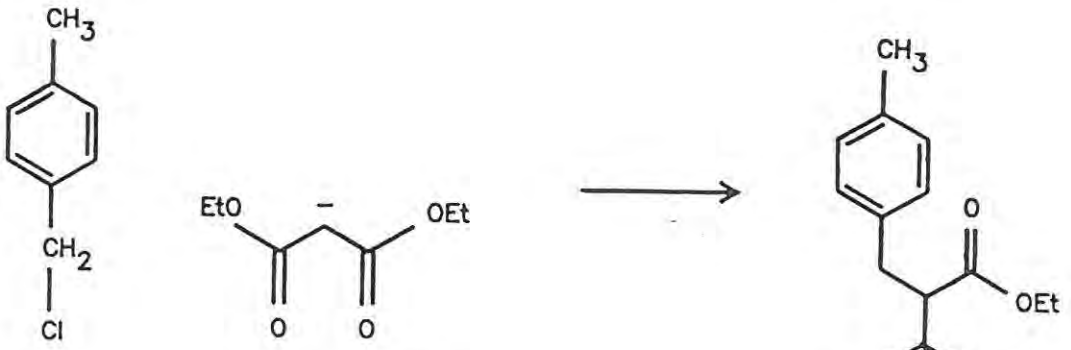
The approach was therefore modified and reactions were carried out using the disodium salt of iminodiacetic acid as well as both the diethyl and dimethyl esters of iminodiacetic acid (see Scheme 15). All these reactions afforded their respective products in very poor yields (15%, 10% and 12% respectively). Since the linking reaction would be an important step in a multi-step synthesis, it was decided to look at further, alternative approaches.

2.2.2 **Routes 2 and 3: Malonic ester synthesis approach.**

Routes 2 and 3 followed a similar theme, in that both involved the malonic ester synthesis[97] approach to link a bifunctional group (malonic ester) onto the pseudo-styrene (7). The malonic ester could then be modified in two different ways to allow for linkage of this system to two amino acid molecules.

The synthesis of the α-substituted malonic ester compound, which was common to both approaches, was carried out from the available starting materials α-chloro-*p*-xylene (28) and diethyl malonate.

The malonic ester synthesis involves the abstraction of an α-hydrogen from the malonic ester, using a strong base (sodium ethoxide, generated *in situ* by dissolving sodium metal in dry ethanol), and subsequent

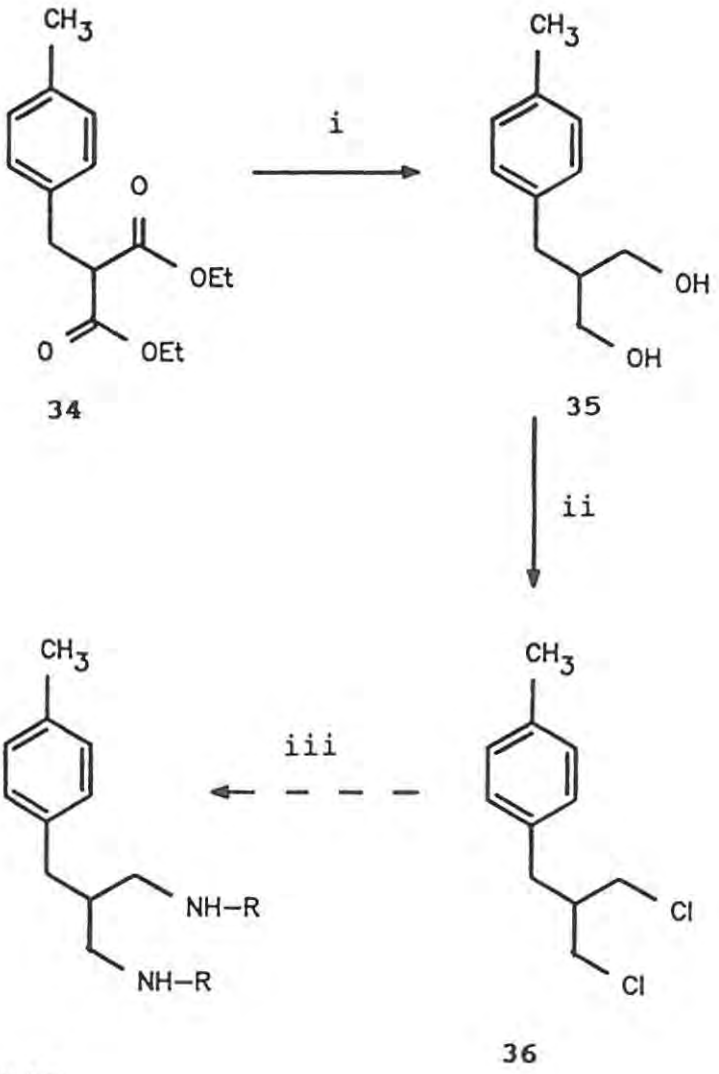


28 Malonic ester carbanion 34

Scheme 16

nucleophilic attack of this carbanion on the benzylic halide (see scheme 16). Care was taken to prevent the second α-hydrogen from being abstracted by the base, by limiting the amount of base present. The substituted malonic ester (34) could then be treated in two different ways.

Route 2, outlined in Scheme 17, involved reduction of the ester functionalities, in compound (34), to form the corresponding dialcohol (35). This dialcohol (35) could



34 35 36

Reagents

i	LAH
ii	$SOCl_2$
iii	$R-NH_2$

Scheme 17

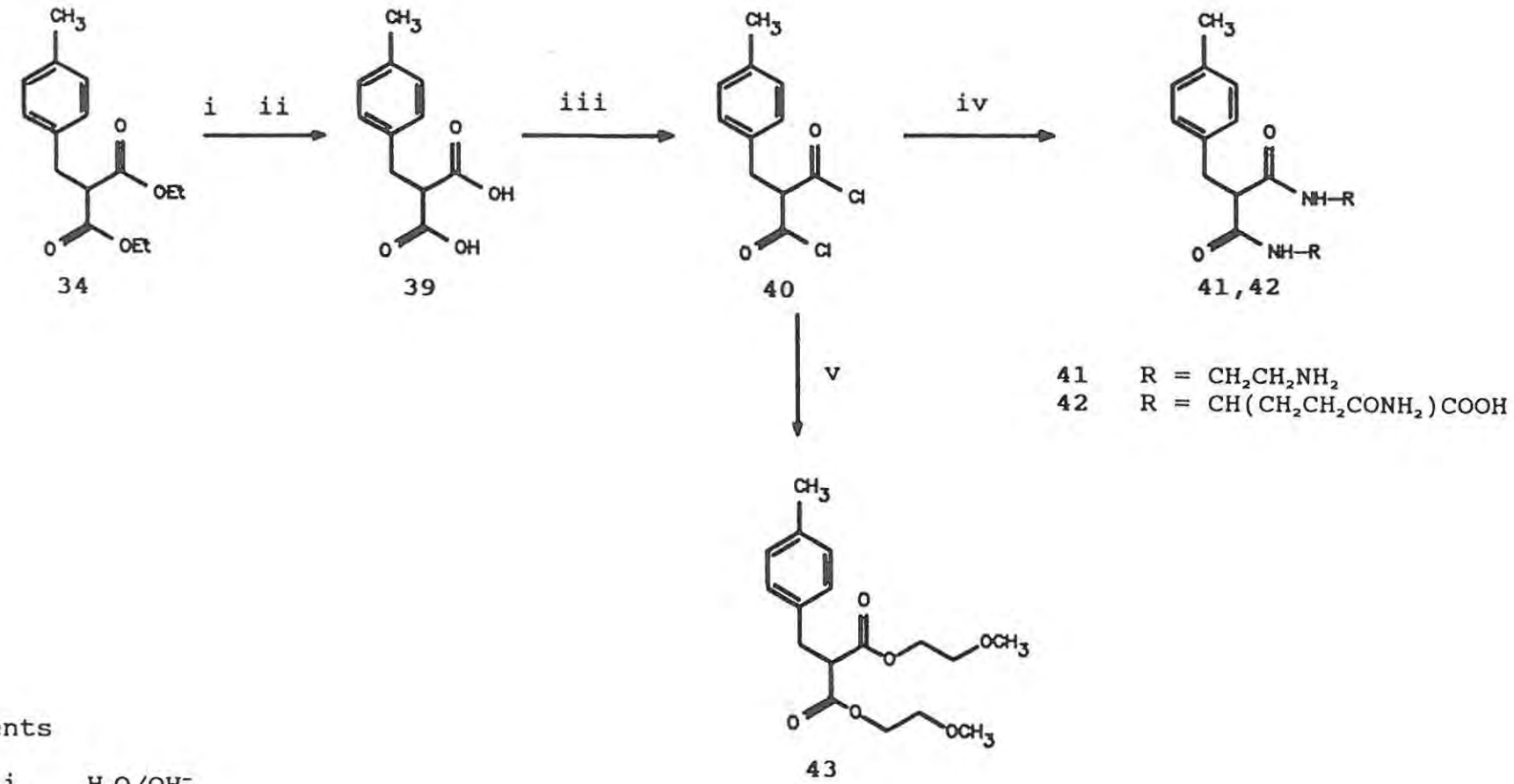
then be chlorinated to the dichloride (36) to give two

sites for amino acid attachment.

Route 3 was the synthetic route found to be most suitable. Due to the risk of decarboxylation of malonic acid systems under acidic conditions, hydrolysis of the ester (**34**) was achieved by alkaline hydrolysis using aqueous base (KOH) (see scheme 18). The substituted diacid (**39**) could then be treated with thionyl chloride to afford the diacid chloride (**40**) which could now undergo peptide linkage. Two compounds were synthesised by this method, *viz.*, the ethylenediamine derivative (**41**) and the *L*-glutamine derivative (**42**). Another compound, having a similar structure, was synthesised from the acid chloride (**40**) and methoxyethanol (see scheme 18). The bis-(methoxyethyl) compound (**43**) was of interest due to its similarity to crown ether systems.

2.3 Phase III. The synthesis of cyclic ligand systems.

Cyclic ligand systems have a higher degree of rigidity and are more preorganised than open chain chelate ligand systems. The advantages of cyclic systems over chelate systems were discussed in section 1.1.7. Thus, in phase III, two known cyclic ligand systems were synthesized in a direct, logical extension of their respective phase II analogues, *viz.* compounds (**45**) and (**47**).



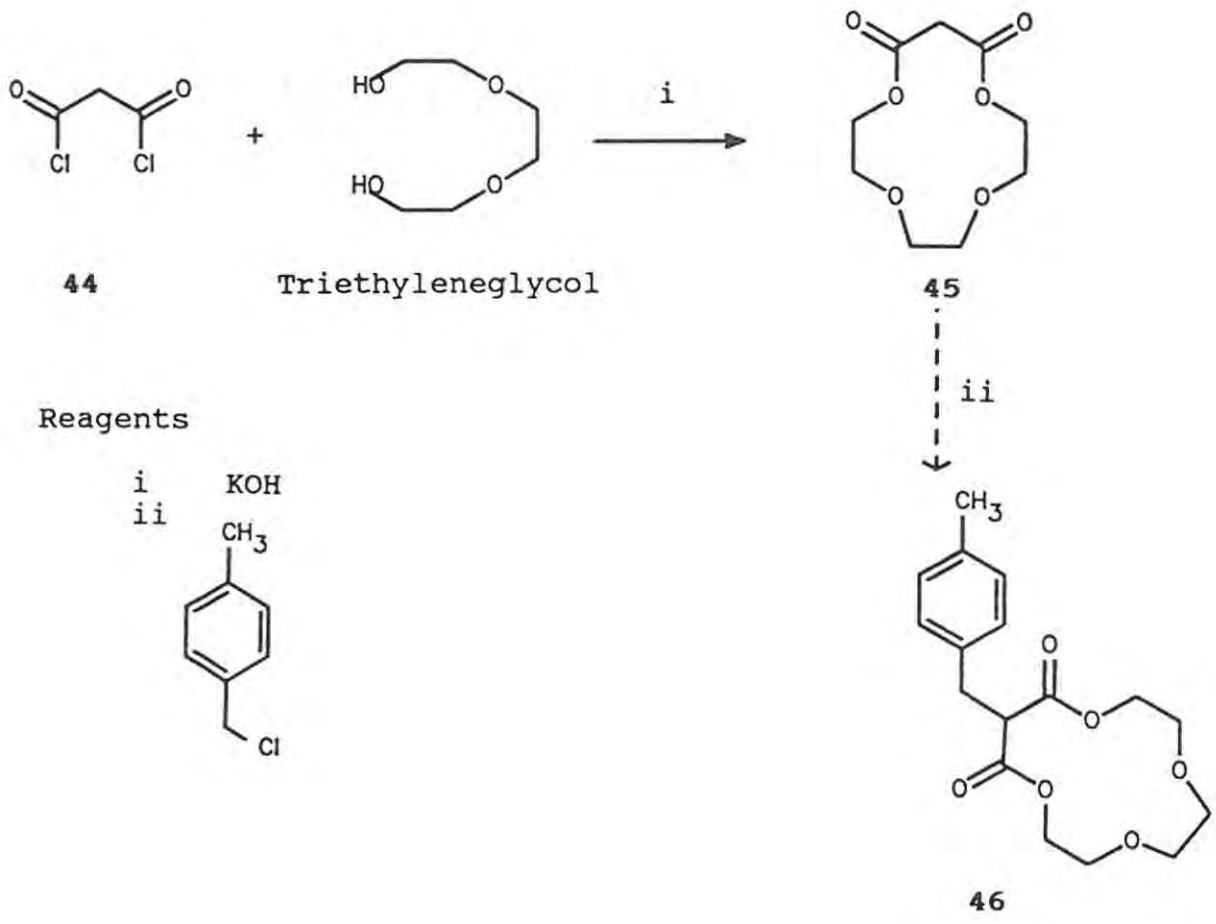
Reagents

i	H_2O/OH^-
ii	H^+
iii	$SOCl_2$
iv	H_2N-R
v	$HOCH_2CH_2OCH_3$

Scheme 18

2.3.1 Synthesis of a crown-ether ligand system.

The crown-ether ligand system (45) was prepared by the alcoholysis reaction of triethyleneglycol (heated with



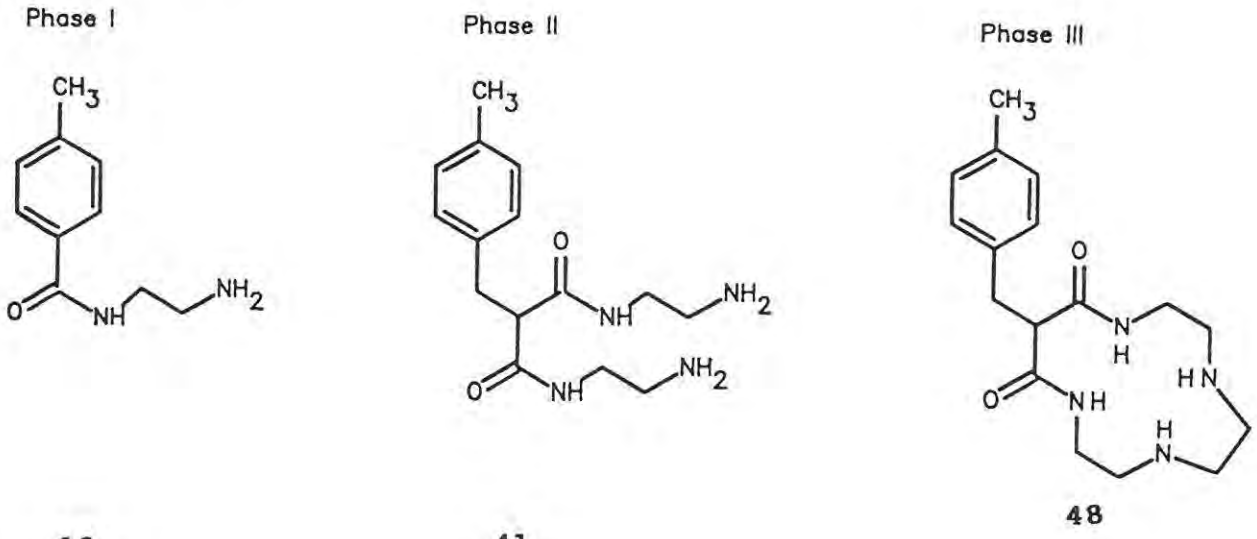
Scheme 19

KOH over a flame) and malonyl dichloride (added drop-wise) as shown in scheme 19, giving a fair yield (32%). This compound (45) was to be reacted further to give similar structures to those synthesised in phase I (13)

and phase II (41), by α-alkylation with α-chloro-*p*-xylene as shown in scheme 19. However, time constraints did not allow for this latter reaction to be investigated and this development should be explored in a future project.

2.3.2 Synthesis of a cyclic tetraaza ligand.

As indicated in figure 13, the tetraaza ligand system (48) is a direct extension of the toluoyl ethylenediamine (10) ligand system developed in phase I and the bis-(ethylenediamine) (41) ligand system developed in phase II.



10 41 48

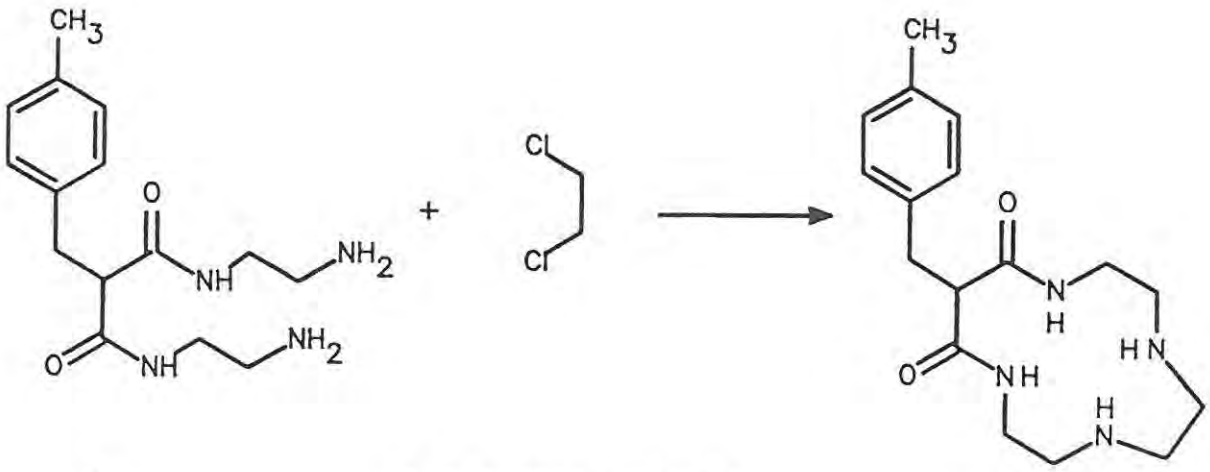
Ethylenediamine analogues of phase I, II and III ligands.

Figure 13

The complexation characteristics of the ligand 1,4,7,10-

tetraazacyclotridecane-11,13-dione (**47**) have been extensively studied.[98] In previous investigations, most workers have attempted to attach this ligand to a polymer or some other ligand system via one of the ring nitrogen atoms. However, studies have shown that this approach drastically reduces the coordinating abilities of the ligand system.[98] An extensive literature search revealed only a few cases where one of the carbon atoms was used in a linking reaction of any sort.[99] Our proposed route, for attaching this ligand system through the carbon onto the linking group, α-chloro-*p*-xylene (**28**), was to have followed one of three possible routes.

The first route starts with the previously synthesised phase II ligand system, (**41**), and requires closing of a

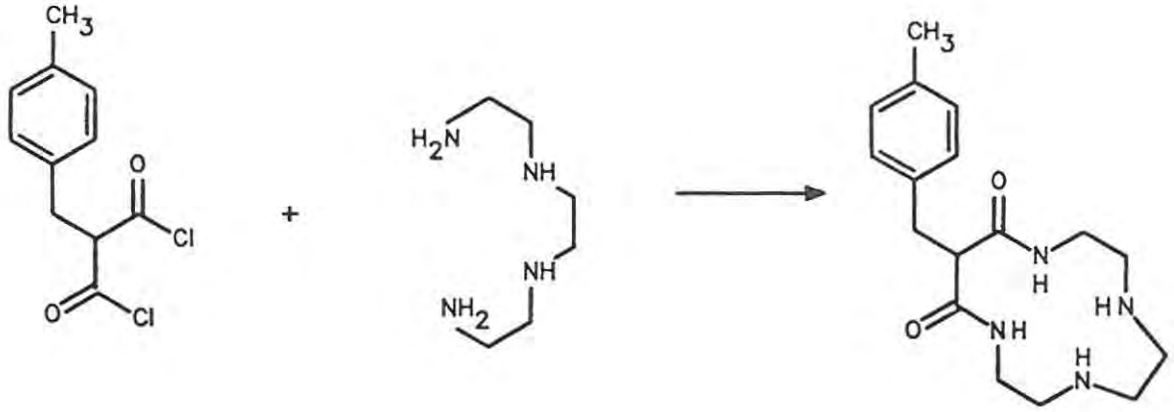


41 1,2-Dichloroethane **48**

Scheme 20

ring containing the two ethylenediamine arms. A possible method of achieving this would be to use nucleophilic substitution by the terminal primary amino groups of each "arm" on a dihalide compound such as 1,2-dichloroethane (see scheme 20). Although this reaction looks simple enough on paper, it has several disadvantages; these being possible side reactions such as the reaction of each amino group with separate dichloride molecules and the difficulty in dissolving the diamine (**41**) in common solvents. At this point it should also be noted that one of the aims of the project was to attach the ligand system onto a polymer and, in view of the difficulties in using polymers in synthetic reactions (as described in section 1.3) we wished to reduce the number of reactions which would have to be carried out on the polymer itself. In scheme 20 the polymeric equivalent to the starting material (**41**) would have involved four reactions to synthesise. In order to reduce the number of steps to synthesise a starting material, we examined other possible synthetic routes.

The second possible route involves nucleophilic acyl substitution of the terminal amino groups in triethylenetetramine on the acid chloride (**40**) formed in phase II of this project (see scheme 21). This approach has the advantage that there are fewer steps required in the synthesis of the acid chloride (**40**), but it has the disadvantages of possible side reactions and the

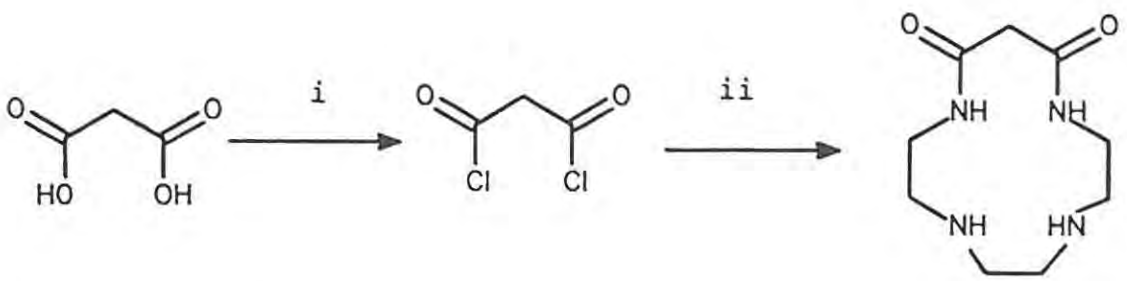


40 Triethylenetetramine **48**

Scheme 21

difficulties associated with manipulation of the acid chloride (**40**) (which would have to be kept dry and which is difficult to purify). These difficulties prompted us to investigate an alternative approach.

The third route involves the initial formation of the cyclic ligand system with subsequent addition of this ligand system onto the linking group. The synthesis of the cyclic ligand system, 1,4,7,10-tetraazacyclotridecane-11,13-dione (**47**), has been reported previously and we followed the method of Buttafava *et al.*[120] which involves reaction of malonyl dichloride (**44**) and triethylenetetramine (see scheme 22). Malonyl dichloride was initially synthesised from the reaction of thionyl chloride and malonic acid over 3 days at 50°C giving a yellow liquid of malonyl



Malonic acid **44** **47**

Reagents

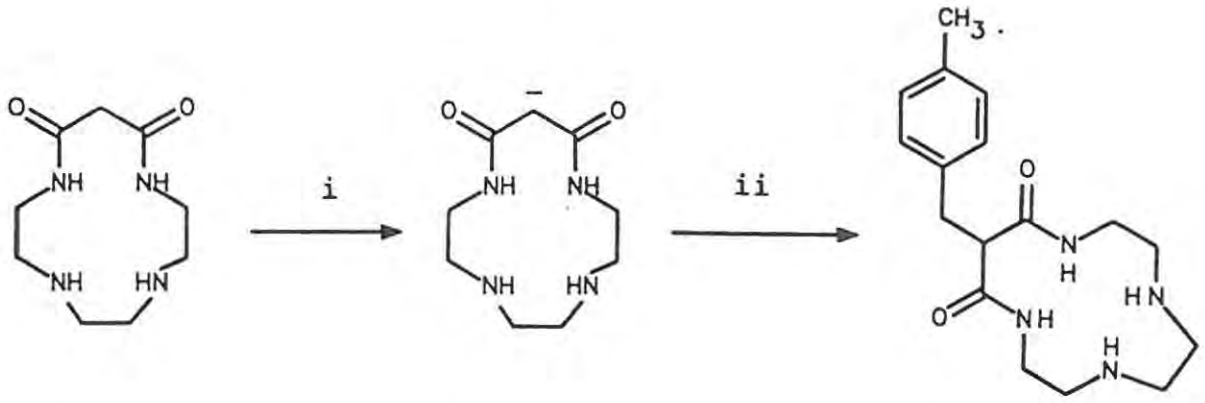
i $SOCl_2$, 50°C, 3d.
ii Triethylenetetramine

Scheme 22

dichloride (**44**) in fair yield (16%) (see scheme 22). Purification of the malonyl dichloride (**44**) proved to be difficult and so in later syntheses we used commercially obtained malonyl dichloride.

The cyclization reaction proceeded well in ethanol but the final product proved difficult to separate and low yields were obtained when using flash chromatography. In an attempt to overcome this problem the copper complex of the cyclic ligand system was formed and this proved to be insoluble in ethanol and so precipitated out. The product was then recovered by washing the complex in brine, and good yields (75%) were obtained.

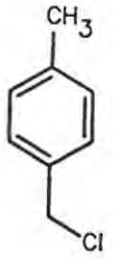
The plan was to then attach this ligand system onto the



47 Carbanion 48

Reagents

i $Na^+ {}^-OEt$

ii 

Scheme 23

linking group, α-chloro-*p*-xylene (28), adopting a modification of the malonic ester synthesis approach used in phase II (see scheme 23). However, the presence of the protons on the amine and amide groups within the ring may be expected to be a complicating factor, although the pKa values for the α-methylene protons and the amino and amido protons show that the α-methylene protons are more acidic in nature and it is resonable to expect them to react more readily than the other protons. However, the difference in the pKa values is

not very great and so there will be competition in the reaction to abstract a proton and this will reduce yields and the chance of the reaction being successful. We attempted this reaction using one equivalent of sodium ethoxide and the reaction yielded starting material. Unfortunately time constraints meant that this reaction could not be investigated further at this time and this project was brought to a close at this point.

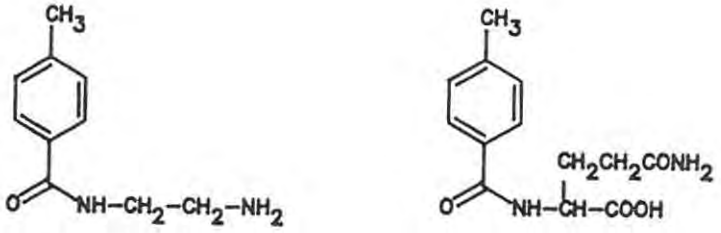
2.4 Coordination studies of phase I, II and II ligand systems.

After a number of ligands had been developed in each phase, they were subjected to a series of tests to assess their complexing potential. Compounds which were found to have complexing potential were taken onto the next stage and developed further. It should be noted that the complexation studies undertaken in this project have been exploratory rather than definitive and this area will be investigated in depth in subsequent studies.

2.4.1 Complexation Studies of Phase I Ligands.

The study of the ligand system's ability to complex was carried out using a grid matrix approach. Ten ligands, (8, 9, 10, 11, 14, 15, 16, 17, 18 and 19), which had been purified, were used in the study. Samples of each ligand, dissolved in ethanol or a mixture of ethanol and a small quantity of DMSO, were treated with ethanolic solutions of several different metal salts, [$FeCl_3$, $Co(NO_3)_2$, $Cu(NO_3)_2$ and $CdCl_3$]. The colours of the mixtures were noted and compared to standards of the metal salts dissolved in the same solvent systems. These tests were done on each of the ten phase I ligand systems.

Two of these ligands (10 and 18) (see Figure 14) showed dramatic colour changes indicating complex formation.



phase I ligands, showing complexation potential.

Figure 14

Due to interfering solvent effects, other methods of analysis such as ultra-violet spectroscopy proved to be ineffective in showing if complex formation was taking place and which atoms were being used in complex formation. It was decided to develop these two ligands further, in phase II, on the grounds that they had shown strong colour changes which indicated that some complex formation had taken place.

2.4.2 Complexation Studies of Phase II Ligands.

This study took the same form as for the phase I ligands. Both the bis-(ethylenediamine) (41) and the bis-(glutamine) (42) compound showed colour changes with both cobalt (II) and copper (II) salts. However, the bis-(glutamine) compound (42) was more difficult to work with as it was very insoluble, even in DMSO.

2.4.3 Complexation studies of phase III ligands

This study took the same form as the previous

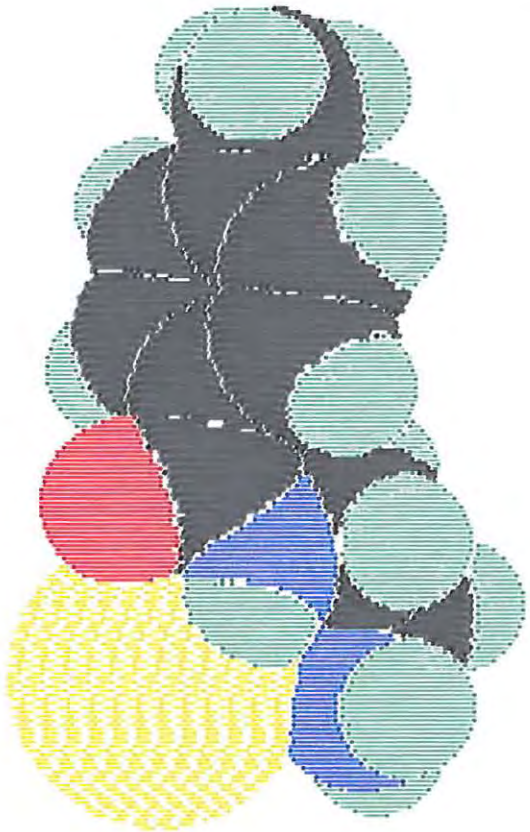
complexation studies in phase I and II. Only the tetraaza ligand system (**47**) was used in the study. A copper (II) complex precipitated out of the ethanolic solution on addition of the copper (II) salt and this was collected by filtration. Time constraints once again curtailed any in-depth investigation into the properties of this complex.

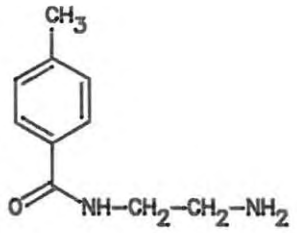
2.5 MOLECULAR MODELLING.

Molecular modelling by computer simulation was done to give an initial idea as to the conformations these ligand systems would take when complexing with a metal ion. The ethylenediamine systems in phases I to III have been modelled, and their space filled diagrams are shown in figures 15, 16 and 17.

Use was made of Tripos Associates' Alchemy II and the cation was initially bound to the ligand system, to get the basic conformation, and then the bonds were broken and hydrogens added to the ligand. The system was then subjected to an energy minimization routine which iterated until the energy gradient fell below 0.01. The program was modified to include the Cu^{2+} cation.

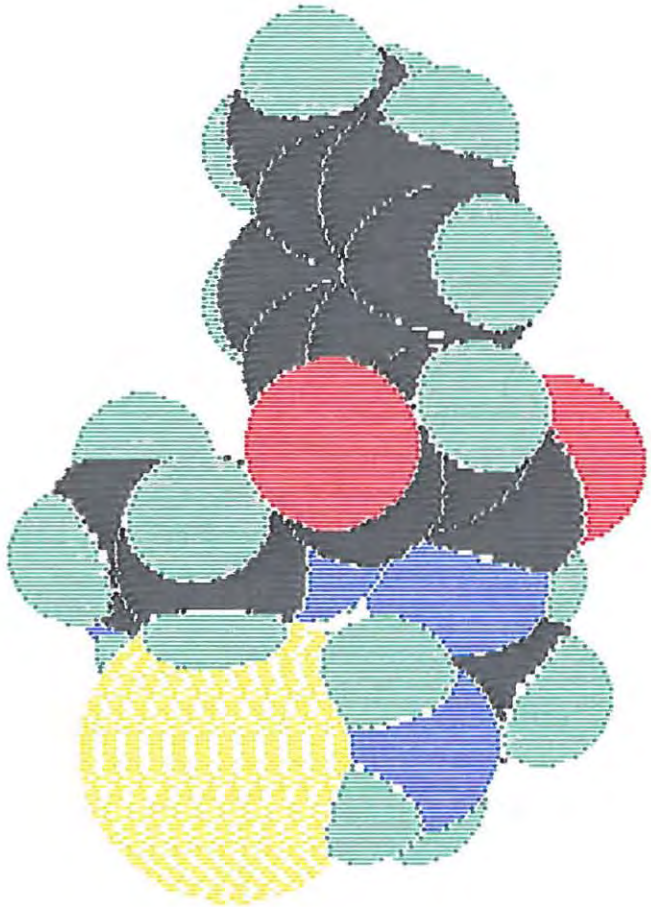
Detailed conclusions about chelation potential based solely on this molecular modelling are premature. Further investigation will include the integrated application of molecular modelling, X-ray crystallography and spectroscopy to establish conformational preferences.

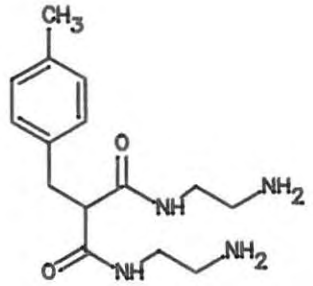




(10)

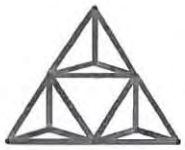
Figure 15





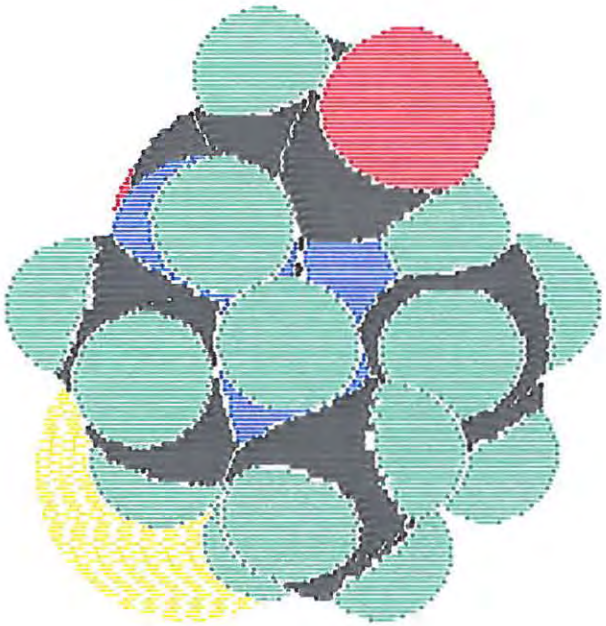
(41)

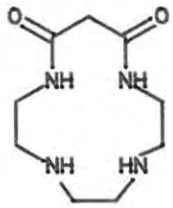
Figure 16



Alchemy II
TRIPOS Associates
St. Louis. Mo.

PHASE II





(47)

Figure 17

2.6 CONCLUSIONS.

In this project a range of ligand systems was successfully synthesized using a phased approach. Synthetic techniques were developed for the linkage of these ligand systems onto pseudo-polymer linking groups and these techniques will be developed further to include linking of the ligand systems onto various polymer supports. The research has provided a necessary survey of developments in the fields of ligand design and biomimetic ligand systems, and in the area of functionalized polymers. The project was an initial investigation for the research group into this area and it has provided a sound basis on which future research projects can be launched.

Future investigations may develop the linking of cyclic ligand systems onto polymeric support reagents and a more comprehensive investigation into the complexation potential of these compounds. Such investigations could include detailed conformational and X-ray crystallographic analyses.

3 EXPERIMENTAL.

3.1 GENERAL.

All melting points were determined on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra were recorded on a Perkin Elmer 180 grating spectrophotometer using KBr discs unless otherwise stated. U.v. spectra were recorded on a Beckman 5240 spectrophotometer using ethanolic solutions, unless otherwise stated. 60 MHz ^{1}H n.m.r. spectra were recorded on a Perkin Elmer R12a n.m.r. instrument using tetramethylsilane as an internal standard and $CDCl_3$ as the solvent unless otherwise stated. 400 MHz ^{1}H and ^{13}C n.m.r. spectra were recorded on a Bruker 400 AMX spectrometer, using tetramethylsilane as an internal standard and $CDCl_3$ as the solvent unless otherwise stated. Low resolution mass spectra were run on a Hewlett Packard 5988A gas chromatograph linked to a Hewlett Packard 598A mass spectrometer. High resolution mass spectra were run on a KRATOS MS80 RF.

Molecular modelling and molecular mechanics were performed on a CW16 AT computer with an EGA card using *Tripos Associates' Alchemy II*.

T.l.c. analysis was performed on MERCK Silica gel $60F_{254}$ pre-coated plastic plates and flash chromatography was carried out with MERCK Silica gel 60 [particle size

0.040-0.063mm (230-400 mesh ASTM)].

Due to difficulties encountered in the purification of some samples and the resulting inaccuracy with combustion elemental analysis, mass spectrometry was used for elemental analysis.

3.2 SYNTHETIC PROCEDURES.

α-Bromo-p-toluic acid (1). – Benzoyl chloride (0.211g, 1.5mmol) was added cautiously to a well stirred mixture of *p*-toluic acid (2.771g, 20mmol) and *N*-bromosuccinimide (3.662g, 20mmol) in CCl_4 (25ml). The resulting mixture was boiled under reflux for 1h., then cooled and filtered. The residue was washed with pentane (3 x 10ml) and H_2O (50ml) to afford the crude product (4.375g, 73%), which was recrystallised from hot acetone to give pure α-bromo-*p*-toluic acid (1), m.p. 225°C (lit.,[100] 229°C); υ_{max}/cm^{-1} 3000 - 2650 br (COOH) and 1680 (CO); δ_H (60MHz) 2.51 (2H,s,$BrCH_2$) and 7.20 (4H,dd,ArH).

p-Carboxybenzyltriphenylphosphonium bromide (2).[101] – To a mixture of dry acetone (260ml) and α-bromo-*p*-toluic acid (1) (8.610g, 0.04mol) was added triphenylphosphine (10.511g, 0.04mol) in dry acetone (40ml) and the mixture was boiled under reflux for 1 h. The resulting precipitate was filtered off and washed with Et_2O to yield, as a white powder, *p*-carboxybenzyltriphenylphosphonium bromide (2) (7.730g, 40%). This product, which was not purified further, was used to prepare *p*-carboxystyrene (3).

p-Carboxystyrene (3).[101] – A solution of NaOH (6.0g) in H_2O (30ml) was added drop-wise over 15 min. to a well stirred suspension of *p*-carboxybenzyltriphenylphosphonium bromide (2) (9.409g, 0.02 mol) in

37% aq. formaldehyde (80ml) and H_2O (40ml). During stirring for 1 h., the starting material dissolved and a new precipitate formed. The solid was filtered off and washed with H_2O (3 x 30ml). Acidification of the filtrate with 5**M**-HCl precipitated the product which was collected at the pump (1.928g, 68%). The crude product was recrystallised from 70% aq. EtOH to give white plates of *p*-carboxystyrene (**3**), m.p. 143°C (lit.,[102] 144°C), υ_{max}/cm^{-1} 2800br (COOH), 1680 (CO), 1610 and 1560; δ_H (60MHz, DMSO-d_6/$CDCl_3$) 5.62 (2H,m,CH_2), 6.90 (1H,m,CH), 7.83 (4H,dd,ArH) and 10.12 (1H,s,COOH).

p-*Ethenylbenzoyl chloride* (**4**).[103] — To a well stirred solution of *p*-carboxystyrene (**3**) (1.003g, 7mmol) in dry $CHCl_3$ (20ml), thionyl chloride (3.0ml, 41mmol) was added drop-wise and the resulting mixture was heated at 55°C for 1 h., protecting with a drying tube. The $CHCl_3$ was distilled off, and additional $CHCl_3$ (30ml) was then added and distilled off leaving the crude product, which was distilled under vacuum to afford pure ethenylbenzoyl chloride (**4**) (0.585g, 54%), b.p. 61°C/18mmHg (lit.[103], 66°C/20mmHg); υ_{max}/cm^{-1} (thin film) 2980br (CH) and 1775 (CO); δ_H (60MHz) 3.24 (2H,m,CH_2), 7.02 (1H,m,CH) and 7.43 (4H,dd,ArH).

Alternative method.[104]

A mixture of *p*-carboxystyrene (**3**) (1.257g, 9mmol) and thionyl chloride (1.5ml, 15mmol) was stirred at room temperature for 2 d. Excess thionyl chloride was

distilled off under vacuum leaving the crude *p*-ethenylbenzoyl chloride (4) (1.452g, 95%) which was purified by distillation under vacuum.

N-*(2-aminoethyl)-4-ethenylbenzamide*[105] (5). – *p*-Ethenylbenzoyl chloride (4) (0.521g, 3mmol) in dry Et_2O (2ml) was added drop-wise to a well stirred mixture of ethylenediamine (0.5ml, 7mmol) in dry Et_2O (10ml) in a flask fitted with drying tube. The resulting precipitate was filtered off and washed successively with 10% aq. NaOH and satd. aq. NaCl solution. The resulting product proved to be insoluble in most common solvents and was presumed to have undergone acid catalysed polymerisation; this method was not investigated further.

Alternative Method.

To a well stirred, cooled (ice-bath) solution of ethylenediamine (0.5ml, 7mmol) in 10% NaOH (7ml), *p*-ethenylbenzoyl chloride (4) (1.264g, 6mmol) was added drop-wise over 1 h. The resulting mixture was stirred at this temperature for 1 h. and then neutralized with dil. aq. H_2SO_4 to afford a viscous oil. Repeated attempts to purify this product proved unsuccessful; this approach was also not investigated further.

N-*carboxymethyl-4-ethenylbenzamide* (6). – *p*-Ethenylbenzoyl chloride (4) (0.521g, 3mmol) in dry Et_2O (2ml) was added drop-wise to a well stirred mixture of

glycine (0.511g, 7mmol) in dry Et_2O (10ml) in a flask fitted with drying tube. The resulting precipitate was filtered off and washed successively with 10% aq. NaOH and satd. aq. NaCl solutions. The resulting product proved to be insoluble in most common solvents and was presumed to have undergone an acid catalysed polymerisation; this method was not investigated further.

Alternative Method.

To a well stirred cooled (ice-bath) solution of glycine (0.501g, 7mmol) in 10% NaOH aq. (7ml) was added *p*-ethenylbenzoyl chloride (4) (1.112g, 6mmol) drop-wise over 1 h.. The resulting mixture was stirred at this temperature for a further 1 h.. Neutralisation with dil. aq. H_2SO_4 afforded an oil. Repeated attempts to purify the product proved unsuccessful; this approach was also not investigated further.

p-*Toluoyl chloride*[103] (7). — A solution of thionyl chloride (12.0ml,165mmol) in dry $CHCl_3$ (20ml) was added drop-wise over 15 min., to a hot (55°C) solution of *p*-toluic acid (2.010g,15mmol) in dry $CHCl_3$ (50ml). The resulting mixture was stirred at 55°C for 1 h. The $CHCl_3$ and excess thionyl chloride were removed by distillation, and the remaining green liquid was distilled under vacuum to afford a clear solution of *p*-toluoyl chloride (7) (0.843g, 46%); b.p. 110°C/20mmHg (lit.[106], 225-227°C); υ_{max}/cm^{-1} (thin film) 3030 (CH) and

1735 (CO); δ_H (60MHz) 2.21 (3H,s,CH_3) and 7.53 (4H,dd,ArH).

Alternative Method.[104]

A solution of *p*-toluic acid (5.015g, 37 mmol) and thionyl chloride (4.735g, 40mmol) was stirred in an apparatus fitted with a drying tube for 48 h. at room temperature. Excess thionyl chloride was distilled off under vacuum leaving the crude *p*-toluoyl chloride (4.580g, 88%).

N-*carboxymethyl-4-methylbenzamide* (**8**). – *p*-Toluoyl chloride (**7**) (7.543g, 49mmol) was added drop-wise over 1 h. to a cooled (ice-bath) solution of glycine (3.410g, 45mmol) in 10% aq. NaOH (50ml). The resulting mixture was stirred at 0°C for a further hour. The mixture was then neutralised with dil. H_2SO_4 and the resulting white precipitate filtered off, washed with H_2O and then dried. The crude product was recrystallized from EtOH-H_2O to give N-*carboxymethyl-4-methylbenzamide* (**8**) (5.890g, 67%), m.p. 131°C; υ_{max}/cm^{-1} 3350 (NH), 2950br (CH) and 1750 (CO); δ_H (60MHz, DMSO-d_6/$CDCl_3$) 2.41 (3H,s,CH_3), 2.62 (1H,s,NH), 6.11 (2H,d,CH_2), 7.51 (4H,dd,ArH) and 9.98 (1H,s,COOH).

N-*Toluoylglycylglycine* (**9**). – Toluoyl chloride (**7**) (7.012g, 45mmol) was added drop-wise to a well-stirred, pre-cooled (0°C, ice-bath) solution of glycylglycine (5.940g, 45mmol) in 10% aq. NaOH (50ml). The resulting

mixture was left stirring at this temperature for 1 h. Acidification with dil. H_2SO_4 afforded a white precipitate which was collected by filtration and washed with H_2O (3 x 25ml) to afford the crude product (5.431g, 47%), which was recrystallized from EtOH to give N-*toluoylglycylglycine* (9), m.p. 229°C; (Found: M^+ 250.096. $C_{12}H_{14}N_2O_4$ requires *M* 250.095); υ_{max}/cm^{-1} 3350 (NH), 2910br (CH) and 1730 (CO); δ_H (400MHz DMSO-d_6/$CDCl_3$) 1.9 (3H,s,CH_3), 3.1 (2H,d,CH_2CONH), 3.3 (2H,d,CH_2COOH) and 6.5 - 7.2 (4H,dd,ArH); δ_C (400MHz DMSO-d_6/$CDCl_3$) 19.7 (CH_3), 29.2 (NHCH_2CONH), 41.4 (CH_2COOH), 126.0 - 139.8 (ArC), 141.4 (C_6H_4CO), 165.5 (CH_2CONH) and 181.2 (COOH).

N-*(2-aminoethyl)-4-methylbenzamide*[6] (**10**). — To a well-stirred, cooled (ice-bath) mixture of excess ethylenediamine (3ml) in dry Et_2O (20ml), *p*-toluoyl chloride (7) (4.621g,30mmol) in dry Et_2O (10ml) was added drop-wise over 15 min. The resulting mixture was stirred at room temperature for 1 h. The resulting precipitate was filtered off and washed successively with aq. 10% NaOH and satd. aq. NaCl to afford the crude product (4.817g, 91%), which was purified by recrystallization from EtOH to give crystals of N-*(2-aminoethyl)-4-methylbenzamide* (**10**), m.p. 238°C; (Found: M^+ 161.084. $C_{10}H_{11}NO$ (M^+ - NH_3) requires 161.084); υ_{max}/cm^{-1} 3310 (NH), 2905br (CH) and 1720 (CO); δ_H (400MHz) 2.23 (3H,s,CH_3), 2.59 (2H,s,CH_2NH_2) 3.65 (2H,s,NHCH_2) and 7.24 - 7.75 (4H,dd,ArH); δ_C (400MHz) 20.2 (CH_3), 33.7 (CH_2NH_2), 54.8

($NHCH_2$), 126.2 - 140.2 (ArC) and 166.7 (CONH).

N-Toluoylethylenediamine diacetic acid (11). – Using the same experimental procedure as for the synthesis of *N-carboxymethyl-4-methylbenzamide* (8), toluoyl chloride (7) (2.015g, 13mmol) was added to ethylenediamine diacetic acid (2.107g, 15mmol) in 10% aq. NaOH (20ml). Work-up resulted in *N-toluoylethylenediamine diacetic acid* (11) (1.858g, 49%), m.p. 207-210°C; (Found: M^+ 294.137. $C_{14}H_{18}N_2O_5$ requires M 294.121. This result was taken at very low intensity and so is not accurate.); υ_{max}/cm^{-1} 2950br (COOH), 1740, 1610 (CO) and 1525; δ_H (60MHz) 2.40 (3H,s,CH_3), 3.75 (4H,s,NCH_2), 4.12 (4H,s,CH_2COOH), 7.30 (4H,s,ArH) and 8.13 (2H,s,COOH).

N-Toluoyl-S-triphenylmethyl-L-cysteine) (12). – Toluoyl chloride (7) (1.545g, 10mmol) was added dropwise to a cold (0°C, ice-bath) solution of *S*-triphenylmethyl-*L*-cysteine (21) (3.645g, 10mmol) in 10% aq. NaOH (20ml) and stirred for 30 mins. The resulting mixture was acidified with 5*M* HCl and extracted into EtOAc (3 x 50ml). The combined extracts were washed with satd. aq. NaCl and dried (anhydr. $MgSO_4$). Evaporation of the solvent afforded a pale yellow, amorphous solid (2.023g, 32%) which was recrystallized from Et_2O to give *N-Toluoyl-S-triphenylmethyl-L-cysteine)* (12), m.p. 231°C; υ_{max}/cm^{-1} 3040 - 2600 (COOH) and 1710 (CO); δ_H (60MHz, DMSO-d_6/$CDCl_3$) 1.32 (1H,t,CH), 2.15 (2H,d,CH_2),

2.41 (3H,s,CH_3), 2.89 (1H,d,NH) and 7.56 (18H,m,ArH).

S-(2,3-dihydroxy-4-mercaptobutyl) toluene-4-thiocarboxylate (**14**). – Toluoyl chloride (**7**) (2.012g, 15mmol) in dry Et_2O (20ml) was added drop-wise to a solution of 2,3,-dihydroxy-1,4-dimercaptobutane (2.213g, 15mmol) in dry Et_2O (20ml). The mixture was boiled under reflux for 4 h. and the cooled solution washed with 10% aq. NaOH and the Et_2O layer was dried (anhydr. $MgSO_4$). Evaporation of the solvent afforded an amorphous, white solid (2.330g, 61%) which was recrystallized from EtOH to give *S-(2,3-dihydroxy-4-mercaptobutyl) toluene-4-thiocarboxylate* (**14**), m.p. 263°C; υ_{max}/cm^{-1} 3310 (OH), 3100 (CH) and 1710 (CO); δ_H (60MHz, DMSO-d_6/$CDCl_3$) 1.51 (2H,m,CH), 2.40 (3H,s,CH_3), 3.41 (4H,m,CH_2), 7.65 (4H,dd,ArH) and 10.71 (2H,s,OH).

N-(1-carboxy-2-hydroxyethyl)-4-methylbenzamide (**15**). – The experimental procedure for the synthesis of *N*-carboxymethyl-4-methylbenzamide (**8**) was followed, using toluoyl chloride (**7**) (3.024g, 20mmol), serine (2.059g, 20mmol) and 10% aq. NaOH (30ml). The work-up afforded an amorphous, white solid (3.214g, 72%) which was recrystallized from EtOH to give *N-(1-carboxy-2-hydroxyethyl)-4-methylbenzamide* (**15**), m.p. 191°C; (Found: M^+ 223.086. $C_{11}H_{13}NO_4$ requires: M 223.085); υ_{max}/cm^{-1} 3350 (OH), 2950br (CH) and 1710 (CO); δ_H (60MHz) 2.40 (3H,s,CH_3), 3.91 (2H,d,CH_2), 4.62 (1H,m,CH), 7.61

(4H,dd,ArH) and 8.23 (2H,s,OH).

N-*toluoylasparagine* (16). – The experimental procedure for the synthesis of *N*-carboxymethyl-4-methylbenzamide (8) was followed, using toluoyl chloride (7) (2.384g, 15mmol), *L*-asparagine (2.004g, 15mmol) and 10% aq. NaOH (30ml). The work-up afforded a white precipitate (2.579g, 68%) which was recrystallized from EtOH to give N-*toluoylasparagine* (16), m.p. 131°C; (Found: M^+ 237.095. $C_{12}H_{15}NO_4$ requires: M 237.100); υ_{max}/cm^{-1} 3320 (OH), 2950br (CH) and 1690 (CO); δ_H (60MHz, DMSO-d_6/$CDCl_3$) 2.41 (3H,s,CH_3), 2.80 (2H,m,CH_2), 3.51 (1H,m,CH, 6.50 (1H,s,NH_2) and 7.56 (4H,dd,ArH).

N^{α}-*toluoylthreonine* (17). – The experimental procedure for the synthesis of *N*-carboxymethyl-4-methylbenzamide (8) was followed, using toluoyl chloride (7) (3.021g, 20mmol), *L*-threonine (2.024g, 17mmol) and 10% aq. NaOH (30ml). The work-up afforded an oily white precipitate (2.899g, 72%), which was recrystallized from EtOH/H_2O to give N^{α}-*toluoylthreonine* (17), m.p. 142°C; (m/z Found 250.0579. $C_{12}H_{14}N_2O_4$ requires: 250.0554); υ_{max}/cm^{-1} 3400 (OH), 2950br (CH) and 1680 (CO); δ_H (400MHz, DMSO-d_6/$CDCl_3$) 2.22 (3H,s,CH_3), 2.23 (2H,s,CH_2), 2.56 (1H,s,C*H*), 3.61 (1H,s br,COOH) and 7.03 - 7.75 (4H,dd,ArH); δ_C (400MHz, DMSO-d_6) 21.1 (CH_3), 40.4 (CH_2), 47.9 (CH), 126.7 - 129.2 (ArC), 141.5 (CONH), 142.7 ($CONH_2$) and 168.0 (COOH).

N^{α}-*Toluoylglutamine* (18). – The experimental procedure for the synthesis of *N*-carboxymethyl-4-methylbenzamide (8) was followed, using toluoyl chloride (7) (4.363g, 28mmol), *L*-glutamine (4.098g, 28mmol) and 10% aq. NaOH (20ml). The work-up afforded a white precipitate (5.766g, 78%) which was recrystallized from EtOH to give N^{α}-*toluoylglutamine* (18), m.p. 151°C; υ_{max}/cm^{-1} 3400 (COOH), 3190 and 1610 (CO); δ_H (400MHz, DMSO-d_6/$CDCl_3$) 2.29 (2H,s,$CHCH_2CH_2$), 2.30 (3H,s,CH_3), 7.12 (2H,dd,CH_2CONH_2), 7.54 (1H,s,CH) and 7.65 - 7.85 (4H,DD,ArH); δ_C (400MHz, DMSO-d_6/$CDCl_3$) 21.0 (CH_3), 21.2 ($CHCH_2CH_2$), 21.4 (CH_2CONH_2), 127.3 - 129.6 (C_6H_4), 142.1 (ArCO), 143.0 ($CONH_2$) and 168.5 (COOH) (Peak due to NHCHCOOH calculated to appear at 42.0, possibly hidden under the DMSO peaks).

S-*(2-mercaptoethyl) toluene-4-thiocarboxylate* (19). – A solution of toluoyl chloride (7) (4.997g, 32mmol) in dry Et_2O (20ml) was added drop-wise to a solution of ethanedithiol (3.048g, 32mmol) in pyridine (2.6ml, 32mmol) and dry Et_2O (10ml) in a flask fitted with aq. $KMnO_4$ scrubbers. The mixture was stirred at r.t. overnight and the resulting precipitate was dissolved in EtOAc (3 x 30ml) and washed with 10% aq. NaOH (3 x 15ml) and 5*M*-HCl, and dried (anhydr. $MgSO_4$) Evaporation of the solvent left yellow crystals (3.528g, 52%) which were recrystallized from EtOH to give S-*(2-mercaptoethyl) toluene-4-thiocarboxylate* (19), m.p. 219°C; υ_{max}/cm^{-1} 1660

(CO); δ_H (400MHz) 2.04 (3H,s,CH_3), 2.62 (2H,t,CH_2SH), 3.02 (2H,t,CH_2CH_2), 3.67 (1H,br s,SH) and 6.88 - 7.48 (4H,dd,ArH); δ_C (400MHz) 20.8 (CH_3), 30.0 (CH_2SH), 37.1 (SCH_2), 126.4 - 143.6 (ArC) and 189.6 (CO).

L-*cysteine hydrochloride* (**20**).[110] — To a suspension of L-cysteine (13.001g, 110mmol) in H_2O (30ml) was added conc. HCl (15ml) and the mixture stirred for 30 mins. The H_2O was evaporated off to give a solid residue (11.659g, 70%) which was recrystallized from MeOH-Et_2O to give *L*-cysteine hydrochloride (**20**), m.p. 174°C (lit.[111],175-178°C); δ_H (60MHz, D_2O) 3.21 (2H,d,CH_2) and 4.50 (1H,t,CH).

S-*triphenylmethyl*-L-*cysteine* (**21**). — A mixture of *L*-cysteine hydrochloride (**20**) (10.016g, 63mmol) and glacial acetic acid (70ml) was warmed (steam-bath) to 60°C. Triphenylmethanol (16.553g, 63mmol) was added drop-wise and the mixture was again heated to 60°C. Boron trifluoride etherate (9ml) was added and the mixture heated to 80°C for 30 mins. The cooled mixture was left stirring for 45 mins. before being transferred to a large beaker where EtOH (95ml), H_2O (32ml) and anhydr. sodium acetate (19.017g, 230mmol) were added, forming a cloudy white precipitate. The addition of H_2O (250ml) formed a gum which was collected by freezing the gum solid (ice-bath) and filtering. The gum was washed successively with H_2O, acetone and Et_2O affording a white

crystalline solid (11.348g, 50%) which was recrystallized from DMF-H_2O to give *S*-triphenyl-*L*-cysteine (**21**), m.p. 182°C (lit.[112], 183.5°C); υ_{max}/cm^{-1} 3000 - 2600 br (COOH) and 1720 (CO); δ_H (60MHz, DMSO-d_6) 3.17 (2H,d,CH_2), 4.50 (1H,t,CH), 4.90 (2H,br s,NH_2) and 7.51 (15H,s,ArH).

N,N'-*bis(carboxymethyl)-4-methylbenzamide* (**23**). – *p*-Toluoyl chloride (**7**) (3.038g,20mmol) was added drop-wise to a cooled (ice-bath) solution of *N*-carboxymethylglycine (2.024g,15mmol) in 5M aq. NaOH (25ml). The mixture was stirred at 0°C for 1 h. and at room temperature for 1 h. Acidification with 5M aq. HCl caused precipitation which was collected by filtration to afford a white solid (2.249g,56%) which was identified as the starting materials *N*-carboxymethylglycine and p-toluic acid.

N,N'-*bis(carboxymethyl)-4-methylbenzamide, diethyl ester* (**24**) – Iminodiacetic acid, diethyl ester (**32**) (1.341g,7mmol) was added to a well stirred suspension of NaH (0.338g,14mmol) in dry Et_2O (20ml) and the resulting mixture was boiled under reflux for 2 h. Toluoyl chloride (**7**) (1.546g,10mmol) was added drop-wise to the cooled mixture and then the resulting mixture was boiled under reflux for a further 7 h. The excess NaH was neutralised by the cautious drop-wise addition of H_2O to

the ice-cold mixture and then by acidification with dil. aq. H_2SO_4. The mixture was then extracted with CH_2Cl_2 and the extracts dried (anhydr. $MgSO_4$). Evaporation of the solvent afforded a brown oil (0.261g,12%) which was purified on a flash column ($CHCl_3$-EtOAc 10-1). This afforded the starting ester (32) and some p-toluic acid. Repeated attempts to increase the yield proved fruitless and this approach was abandoned.

N,N'-bis(carboxymethyl)-4-methylbenzamide, dimethyl ester (25) – Using the same experimental procedure as for the synthesis of *N,N'-bis*(carboxymethyl)-4-methylbenzamide, diethyl ester (24), iminodiacetic acid dimethyl ester (33) (0.565g,5mmol) was added to NaH (0.249g,10mmol) in dry Et_2O (20ml). Toluoyl chloride (7) (1.613g,10mmol) was added and the mixture boiled under reflux for 5 h. Work up afforded a brown oil (0.289g,25%) which yielded only starting materials. This approach was not investigated further.

Ethyl 4-methylbenzoate (26). – Conc. H_2SO_4 (5ml) was added drop-wise to a well stirred solution of *p*-toluic acid (13.994g, 103mmol) in dry EtOH (50ml).The resulting mixture was boiled under reflux for 8 h., after which the excess EtOH was distilled off. The aqueous layer was neutralized with aq. $NaHCO_3$ and then extracted with Et_2O (3 x 25ml). The combined extracts were dried (anhydr. $MgSO_4$) and the solvent was evaporated off to afford, a

sweet smelling, orange oil (14.829g, 88%), which was distilled to give ethyl 4-methylbenzoate (26), b.p. 118°C/20mmHg (lit.[107], 122°C/22mmHg); υ_{max}/cm^{-1} (thin film) 2975m (CH) and 1735s (CO); δ_H (60MHz) 0.95 (3H,t,CH_3CH_2), 1.91 (3H,s,$CH_3C_6H_5$), 3.94 (2H,q,CH_3CH_2) and 7.37 (4H,dd,ArH).

α-Hydroxy-p-xylene (27). — Ethyl 4-methylbenzoate (26) (13.059g, 0.08mol) in THF (20ml) was added drop-wise to a stirred suspension of LAH (1.920g, 50mmol) in dry THF (30ml) under dry N_2. The resulting mixture was boiled under reflux for 4 h., after which the excess LAH was quenched by the cautious, sequential addition of H_2O (1ml), 10% aq. NaOH (1ml) and H_2O (3ml). The white crystalline material was filtered off and the filtrate was added to H_2O (20ml) and extracted with Et_2O (3 x 25ml). The combined ether extracts were washed with satd. aq. NaCl and dried (anhydr. $MgSO_4$). Evaporation of the Et_2O afforded α-hydroxy-*p*-xylene (27) as a white solid (7.834g, 81%), m.p. 59-61°C (lit.[108], 61-62°C); υ_{max}/cm^{-1} 3400br (OH) and 2960br (CH); δ_H (60MHz) 2.00 (1H,s,OH), 2.30 (3H,s,CH_3), 4.58 (2H,s,CH_2) and 7.22 (4H,s,ArH).

α-Chloro-p-xylene (28). — Thionyl chloride (4.871g, 41mmol) was added drop-wise to a stirred pre-cooled (ice-bath) solution of α-hydroxy-*p*-xylene (27) (5.019g, 41mmol) in a flask fitted with a drying tube. The

resulting solution was stirred at room temperature for 2d. The excess thionyl chloride was removed under vacuum and the residual, crude product (5.030g, 92%) was distilled to give α-hydroxy-*p*-xylene, b.p. 110°C/20mmHg (lit.[109], 200°C), υ_{max}/cm^{-1} (thin film) 3200br (OH), 2960 (CH) and 1520; δ_H (60MHz) 2.39 (3H,s,CH_3), 6.49 (2H,s,CH_2Cl) and 7.21 (4H,s,ArH).

N,N'-bis*(carboxymethyl)-4-methylbenzylamine* (**29**). — *N*-carboxymethylglycine (8.134g,61mmol) was added to a well-stirred suspension of NaH (2.965g,124mmol) in dry Et_2O (20ml) and the mixture was boiled under reflux for 5 h. α-Chloro-*p*-xylene (**28**) (6.003g,43mmol) was added to the cooled mixture and then boiled under reflux for 7 h. The excess NaH was neutralised by the cautious drop-wise addition of H_2O to the ice-cooled mixture and then acidified with dil. aq. H_2SO_4. The mixture was then extracted with CH_2Cl_2 and the extracts were dried over anhydr. $MgSO_4$. Evaporation of the solvent afforded a brown oil (3.214g,32%) which was purified on a flash column (using $CHCl_3$-EtAc 10-1) to give, as a white solid, N,N'-bis*(carboxymethyl)-4-methylbenzylamine* (**29**), m.p. 213°C; υ_{max}/cm^{-1} 3350 (NH), 3200 (COOH) and 1710 (CO); δ_H (60MHz, D_2O) 1.21 (4H,m,CH_2COOH), 2.20 (3H,s,CH_3), 4.53 (2H,m,$C_6H_4CH_2$) 7.34 (4H,s,ArH) and 9.15 (2H,s,COOH).

Alternative Method.

N-carboxymethylglycine (3.991g,30mmol) and NaOH (1.003g,25mmol) were dissolved in MeOH (20ml) and H_2O

(20ml) and heated to 60°C. α-Chloro-*p*-xylene (28) (2.0026g,30mmol) was divided into two portions and the first was added drop-wise to the hot solution. A further quantity of NaOH (1.025g,25mmol) was added followed by the rest of the α-chloro-*p*-xylene. The mixture was boiled under reflux for 3 h. and then the MeOH was distilled off and the remaining aqueous layer was acidified to pH 2.5 and left over night in the cold room and the precipitate of N,N'-bis*(carboxymethyl)-4-methylbenzylamine* (29) (1.045g,15%) was collected.

N,N'-bis*(carboxymethyl)-4-methylbenzylamine, diethyl ester* (30). – Using the same experimental procedure as for the synthesis of *N,N'-bis*(carboxymethyl)-4-methylbenzylamine (29), *N*-carboxymethylglycine diethyl ester (32) (0.951g,5mmol) was added to NaH (0.217g,10mmol) in dry Et_2O (20ml) and the mixture was boiled under reflux for 5 h. α-Chloro-p-xylene (28) (0.984g,7mmol) was added and the mixture boiled under reflux for a further 7 h. Work-up resulted in a brown oil (0.147g,10%) which on further purification afforded starting materials as the major components.

N,N'-bis*(carboxymethyl)-4-methylbenzylamine, dimethyl ester* (31). – Using the same experimental procedure as for the synthesis of *N,N'-bis*(carboxymethyl)-4-methylbenzylamine (29), *N*-carboxymethylglycine dimethyl ester (33) (0.817g,6mmol) was added to NaH

(0.321g,12mmol) in dry Et_2O (20ml) and the mixture was boiled under reflux for 5 h. α-Chloro-p-xylene (28) (0.843g,6mmol) was added and the mixture boiled under reflux for a further 7 h. Work-up resulted in a brown oil (0.191g,12%) which on further purification afforded starting materials as the major components.

Diethyl iminodiacetate (32). — HCl gas[113] was bubbled through a boiling, well-stirred mixture of *N*-carboxymethylglycine (1.032g,8mmol) and EtOH (50ml) for 1 h. The solution formed was then boiled under reflux for a further 3 h. The solution was neutralised with $CaCO_3$ and extracted with CCl_4. The extracts were dried (anhydr. $MgSO_4$) and evaporation of the solvent afforded a brown oil (3.175g,21%) which was distilled to give a brown oil of diethyl iminodiacetate (32), b.p. 147°C/15mmHg (lit.[114], 133°C/11mmHg); υ_{max}/cm^{-1} (thin film) 3025, 1765 and 1610 (CO); δ_H 1.75 (6H,t,CH_3), 3.54 (4H,s,$HNCH_2$), 3.98 (4H,q,CH_3CH_2) and 6.31 (1H,br s,HN).

Dimethyl iminodiacetate (33). — The experimental procedure for the synthesis of diethyl iminodiacetate (32) was followed using carboxymethylglycine (0.982g,7mmol) in MeOH (50ml). The work-up afforded a brown oil (0.178g,15%) which was distilled under vacuum to give a brown oil of dimethyl iminodiacetate (33), b.p. 102°C/15mmHg (lit.[114], 126/33mmHg); υ_{max}/cm^{-1} (thin film) 1690 (CO); δ_H (60MHz, D_2O) 3.25 (4H,s,CH_2), 3.65

(6H,s,CH_3) and 5.64 (1H,br s,HN).

Diethyl 2-[(4-methylphenyl)methyl]-1,3-propanedioate (**34**). – Diethyl malonate (16.0ml, 71mmol) in dry EtOH (10ml) was added drop-wise to a well-stirred suspension of sodium ethoxide in EtOH [generated by the addition of sodium metal (5.011g, mmol) to dry EtOH (50ml) under N_2]. The resulting mixture was boiled under reflux for 2 h. α-Chloro-*p*-xylene (**28**) (11.061g, 79mmol) was then added drop-wise to the hot mixture, which was then boiled under reflux for a further 1 h. to form a bright yellow precipitate. The cooled reaction mixture was diluted with H_2O (50ml) and extracted with Et_2O (3 x 50ml). The combined extracts were washed with satd. aq. NaCl and dried (anhydr. $MgSO_4$). Evaporation of the solvent afforded the crude product (10.044g, 48%) which was distilled under vacuum to give diethyl 2-[(4-methylphenyl)methyl]-1,3-propanedioate (**34**), b.p. 155°C/15mmHg (lit.,[115] 178-180°C/20mmHg); υ_{max}/cm^{-1} (thin film) 2980, 1740 (CO) and 1520; δ_H (400MHz) 1.17 (6H,s,CH_3CH_2), 2.23 (3H,s,$CH_3C_6H_4$), 3.10 (2H,s,$C_6H_4CH_2$), 4.00 (4H,s,CH_3CH_2) and 7.05 (4H,s,ArH).

2-[(4-Methylphenyl)methyl]-1,3-propanediol (**35**). – Diethyl 2-[(4-methylphenyl)methyl]-1,3-propanedioate (**34**) (1.054g, mmol) was added drop-wise to a well stirred suspension of lithium aluminium hydride (LAH) (0.166g,) in dry tetrahydrofuran (THF) (20ml). The

resulting mixture was boiled under reflux for 12 h. and the excess LAH was quenched by the cautious, sequential addition of H_2O (1ml), 10% aq. NaOH (1ml) and H_2O (3ml). The white crystalline material was filtered off and the filtrate extracted with Et_2O (3x25ml). The combined extracts were washed with satd. aq. NaCl and dried (anhydr. $MgSO_4$). The solvent was evaporated off and the crude product (0.631g, 88%) was recrystallized from EtOH to give white crystals of *2-[(4-methylphenyl)methyl]-1,3-propanediol* (35), m.p. 72°C; υ_{max}/cm^{-1} 3450br (OH), 3030 (CH) and 1540; δ_H (400MHz) 2.30 (3H,s,CH_3), 2.32 (2H,d,$C_6H_4CH_2$), 2.81 (1H,br s,OH), 3.70 (1H,m,CH), 4.55 (4H,d,CH_2OH) and 7.16 (4H,m,ArH); δ_C (400MHz, $CDCl_3$) 20.9 (CH_3), 33.8 ($C_6H_4CH_2$), 43.8 (CH), 65.2 (CH_2OH) and 128.8 - 130.4 (ArC).

2-[(4-Methylphenyl)methyl]-1,3-dichloropropane (36). — Thionyl chloride (1.678g,14mmol) was added drop-wise to a well stirred, ice cold solution of 2-[(4-methylphenyl)methyl]-1,3-propanediol (35) (1.271g,7mmol) in Et_2O (20ml). The resulting solution was stirred at 0°C for 1h. after which the solvent was evaporated off affording a dark green solution which was used without further purification.

2-[(4-Methylphenyl)methyl]-1,3-propanedioic acid (39).[116] — Diethyl 2-[(4-methylphenyl)methyl]-1,3-propanedioate (34) (11.035g, 42mmol) was added drop-wise to a stirred

solution of KOH (6.123g, 109mmol) in H_2O (6ml) and EtOH (20ml) and the solution was then boiled under reflux for 6 h. The excess EtOH was distilled off under vacuum and the remaining aqueous solution was acidified to pH 4 with dil. H_2SO_4. The solution was then extracted with Et_2O and the combined extracts were washed with satd. aq. NaCl and dried (anhydr. $MgSO_4$). The solvent was then evaporated off to give a white crystalline solid which was recrystallised from EtOH to afford 2-[(4-methylphenyl)methyl]-1,3-propanedioic acid (**39**) (3.582g, 41%), m.p. 153°C (lit.[117],154-155°C); υ_{max}/cm^{-1} 3050 - 2650 br (COOH) and 1725 (CO); δ_H (60MHz, DMSO-d_6/$CDCl_3$) 1.25 (1H,t,*J* 6.0,C*H*), 2.31 (3H,s,CH_3), 3.42 (2H,m,CH_2) and 7.37 (4H,s,ArH).

2-[(4-Methylphenyl)methyl]malonyl dichloride (**40**).[118] – Thionyl chloride (5ml, excess) was added drop-wise to a well stirred solution of 2-[(4-methylphenyl)methyl]-1,3-propanedioic acid (**39**) (2.013g, 10mmol) in dry EtOH (20ml) in a flask fitted with a drying tube. The resulting solution was maintained at 45 - 50°C for 3 d.. The excess thionyl chloride was then removed by distillation under vacuum to afford the crude product, *2-[(4-methylphenyl)methyl]malonyl dichloride* (**40**) (1.523g, 64%)[b.p. 201°C/1mmHg; υ_{max}/cm^{-1} (thin film) 3020br (CH), 1785br (CO), 1600 and 1520; δ_H (60MHz) 2.38 (3H,s,CH_3), 3.30 (2H,d,*J* 8.2, CH_2), 4.45 (1H,t,*J* 7.9,CH_2) and 7.15 (4H,s,ArH)] which was used without further

purification.

N,N'-bis*(2-aminoethyl)-2-[(4-methylphenyl)methyl]-1,3-propanediamide* (**41**). – Ethylenediamine (0.969g,9.5mmol) was added drop-wise to a well stirred solution of [(4-methylphenyl)methyl]malonyl dichloride (**40**) (1.006g,4mmol) in dry Et_2O (10ml). A thick yellow precipitate formed and the mixture was partitioned between EtOAc (3x50ml) and H_2O (50ml). The organic layer was washed with satd. aq. NaCl and dried (anhydr. $MgSO_4$). Evaporation of the solvent afforded a yellow solid (0.693g,59%) which was recrystallized from EtOAc to give N,N'-bis*(2-aminoethyl)-2-[(4-methylphenyl)methyl]-1,3-propanediamide* (**41**), m.p. 330°C dec; δ_c (400MHz, DMSO-d_6) 20.8 (CH_3), 36.8 ($CONHCH_2$), 54.8 (CH[CONH]), 95.6 ($C_6H_4CH_2$), 128.5 - 136.3 (ArC) and 168.9 (CO).

N,N'-Bis-*(glutamyl)-2-[(4-methylphenyl)methyl]-1,3-propanediamide* (**42**). – *L*-glutamine (3.013g,20mmol) was added to a well stirred solution of [(4-methylphenyl)methyl]malonyl dichloride (**40**) (2.123g,86mmol) in dry THF (20ml). The resulting mixture was boiled under reflux for 2h. Evaporation of the solvent afforded a brown oil which was purified on a flash column (ethyl acetate:hexane :: 1:3). Three fractions were obtained but none of these three proved to be the desired product. Repeated attempts to obtain the desired product failed and so this experiment was

abandoned.

Di(2-methoxyethyl) 2-[(4-methylphenyl)methyl]-1,3-propanedioate (**43**). – To a well stirred solution of 2-[(4-methylphenyl)-methyl]-1,3-propanedioic acid (**39**) (1.033g, 5mmol) in methoxyethanol (20ml, excess), conc. H_2SO_4 was added drop-wise. The solution was boiled under reflux for 8h and then extracted with Et_2O (3 x 25ml). The extracts were washed with satd. aq. NaCl and dried (anhydr. $MgSO_4$). Evaporation of the solvent afforded the crude product (1.103g, 68%), which was purified by distillation to give di*(2-methoxyethyl) 2-[(4-methylphenyl)methyl]-1,3-propanedioic acid* (**43**), b.p. 164°C/1mmHg, (Found: M^+ 324.189. $C_{17}H_{24}O_4$ requires M 324.178); υ_{max}/cm^{-1} (thin film) 2925br (CH) and 1735 (CO); δ_H (400MHz) 2.28 (3H,s,$CH_3C_6H_4$), 3.19 (2H,d,*J* 7.8,$C_6H_4CH_2$), 3.31 (6H,s,CH_3O), 3.50 (4H,q,*J* 5.1,CH_3OCH_2), 3.73 (1H,t,*J* 7.9,$CHCH_2$), 4.23 (4H,t,*J* 4.8,$COOCH_2$) and 7.06 (4H,m,ArH).

Malonyl dichloride (**44**). – Malonic acid (10.094g,97mmol) and thionyl chloride (21ml,289mmol) were heated at between 45 and 50°C under N_2 for 3 d. The resulting solution was then heated for a further 6 h. at 60°C. Evaporation of the excess thionyl chloride afforded a dark green liquid with a large quantity of charred material suspended in it. Purification by careful vacuum distillation afforded a yellow liquid of

malonyl dichloride (**44**) (2.179g,16%), b.p. 43°C/15mmHg (Lit.,[119] 60°C/20mmHg); δ_H (60MHz) 4.32 (2H,s,CH_2).

1,4,7,10-Tetraoxacyclotridecane-11-13-dione[122] (**45**). – A suspension of triethyleneglycol (5.357g,30mmol) and KOH (4.095g,60mmol) was heated over a flame, boiling for 1 min., to afford a brown oil which was spread over the sides of the flask while it solidified during cooling. Malonyl dichloride (4.233g,30mmol) was added drop-wise to the solid and the reaction was kept under control by the careful addition of the malonyl dichloride as well as cooling when needed in an ice-bath. The resulting mixture was then heated for 3 mins. over a flame, and then CH_2Cl_2 (30ml) was added and mixed well. The mixture was then extracted with water (3 x 30ml) and the organic extracts were collected, dried over anhydr, $MgSO_4$ and the solvent was then evaporated off affording a brown solid (2.231g,32%). Recrystallization of this solid from $CHCl_3$ and hexane afforded 1,4,7,10-tetraoxacyclotridecane-11-13-dione (**45**), m.p. 50°C (Lit.,[122] 51.5-52.5°C); υ_{max}/cm^{-1} 1710; δ_H (60MHz) 3.40 (2H,s,$COCH_2CO$), 3.64 (4H,s,OCH_2CH_2O), 3.75 (4H,t,$CO_2CH_2CH_2$) and 4.35 (4H,t,CO_2CH_2).

1,4,7,10-Tetraazacyclotridecane-11,13-dione[120] (**47**). – A solution of diethyl malonate (4.004g,25mmol) and triethylenetetramine (3.656g,25mmol) in EtOH (20ml) was boiled under reflux for 3 d. Evaporation of the solvent

afforded a brown oil which was purified on a flash column (ethyl acetate : hexane 1:10) to afford 1,4,7,10-tetraazacyclotridecane-11,13-dione (**47**) (1.125g,21%), m.p. 186°C (Lit.,[121] 188-189°C); δ_H (60MHz) 2.61 (4H,dd,NHC*H*$_2$C*H*$_2$NH), 2.82 (8H,dd,CONHC*H*$_2$C*H*$_2$NH) and 3.14 (2H,s,COCH$_2$CO).

Alternative Method.

Triethylenetetramine (3.688g,25mmol) was added drop-wise to a well stirred, ice cold solution of malonyl dichloride (3.513g,25mmol) and pyridine (4.025g,51mmol) in THF (20ml). The resulting mixture was boiled under reflux for 1 h. The resulting solid which was formed was filtered off and dried. Recrystallization from EtOH afforded 1,4,7,10-tetraazacyclodecane-11,13-dione (**47**) (1.932g,36%).

4. REFERENCES.

1. a) P.M. May and R.A. Bullman, *Prog. Med. Chem.*, 1983, **20**, 266; b) R.A. Bullman, *Struct. Bonding*, 1987, **67**, 91; c) D. Bryce-Smith, *Chem. Soc. Rev.*, 1986, **15**, 93.
2. A. Albert, *Selective Toxicity*, Chapman and Hall, London, 1973, p. 20.
3. A.E. Martell, R.J. Motekaitis and M.J. Welch, *J. Chem. Soc., Chem. Commun.*, 1990, 1748.
4. a) R.B. Lauffer, *Chem. Rev.*, 1987, **87**, 901; b) E. Deutsch, K. Libson, S. Jurisson and L.F. Lindoy, *Prog. Inorg. Chem.*, 1983, **30**, 75.
5. R.D. Hancock and A.E. Martell, *Chem. Rev.*, 1989, **89**, 1875.
6. S.K. Sahni and J. Reedijk, *Coord. Chem. Rev.*, 1984, **1**, 59.
7. B.R. Green and R.D. Handcock, *J. S. Afr. Inst. Min. Metall.*, 1982, **82**, 303.
8. M.M. Crutchfield, *J. Am. Oil Chem. Soc.*, 1978, **55**, 58.
9. S. Chaberek and A.E. Martell, *Organic Sequestering Agents*, Wiley and Sons, New York, 1959, p 32.
10. K.B. Nielson and D.R. Winge, *J. Biol. Chem.*, 1983, **258**, 13065.
11. R.G. Pearson, *J. Am. Chem. Soc.*, 1963, **85**, 3533.
12. G. Schwarzenbach, *Adv. Inorg. Chem. Radiochem.*, 1961, **3**, 257.

13. R.D. Hancock, *Pure Appl. Chem.*, 1986, **58**, 1445.

14. H. Sigel and R.B. Martin, *Chem. Rev.*, 1982, **82**, 385.

15. First reported by G. Wiedeman, *Justus Liebigs Ann. Chem.*, 1848, **68**, 323; and brought into prominence by H. Schiff, *Ber. Dtsch. Chem. Ges.*, 1896, **29**, 298.

16. S. Scheiner and W.N. Lipscomb, *J. Am. Chem. Soc.*, 1977, **99**, 3466.

17. a) M.S.B. Munson, *J. Am. Chem. Soc.*, 1965, **87**, 2332; b) R.W. Taft, J.F. Wolf, J.L. Beauchamp, G. Scorrano and E.M. Arnett, *J. Am. Chem. Soc.*, 1978, **100**, 1240; c) J.S. Uppal and R.H. Staley, *J. Am. Chem. Soc.*, 1982, **104**, 125; d) M.M. Kappes and R.H. Staley, *J. Am. Chem. Soc.*, 1982, **104**, 1813 and 1819.

18. G. Nord, *Comments Inorg. Chem.*, 1985, **4**, 193.

19. D.J. Cram, T. Kaneda, R.C. Helgeson, S.B. Brown, C.B. Knobler, E. Maverick and K.N. Trueblood, *J. Am. Chem. Soc.*, 1985, **105**, 3645.

20. a) R.D. Hancock, *J. Chem. Soc., Dalton Trans.*, 1980, 416; b) R.D. Hancock and F. Marsicano, *Inorg. Chem.*, 1978, **17**, 560; c) R.D. Hancock and F. Marsicano, *Inorg. Chem.*, 1980, **19**, 2709.

21. R.M. Izatt, J.S. Bradshaw, S.A. Nielsen, J.D. Lamb, J.J. Christensen and D. Sen, *Chem. Rev.*, 1985, **85**, 271.

22. a) K.R. Adam, K.P. Dancey, B.A. Harrison, A.J.

Leong, L.F. Lindoy, M. McPartlin and P.A. Tasker, *J. Chem. Soc., Chem. Commun.*, 1983, 1351; b) K.P. Adam, A.J. Leong, L.F. Lindoy, H.C. Lip, B.W. Skelton and A.H. White, *J. Am. Chem. Soc.*, 1983, **105**, 4645.

23. N. Okatu, K. Toyoda, Y. Moriguchi and K. Ueno, *Bull. Chem. Soc. Jpn.*, 1967, **40**, 2326.

24. G.T Morgan and H.D.K. Drew, *J. Chem. Soc.*, 1926, **117**, 1456.

25. G. Scharzenbach, *Helv. Chim. Acta.*, 1952, **35**, 2344.

26. A.W. Adamson, *J. Am. Chem. Soc.*, 1954, **76**, 1578.

27. a) R.D. Hancock and F. Marsicano, *J. Chem. Soc., Dalton Trans.*, 1976, 1096; b) W.R. Harris, *J. Coord. Chem.*, 1983, **13**, 16.

28 R.D. Hancock, G.J. McDougall and F. Marsicano, *Inorg. Chem.*, 1979, **18**, 2847.

29. L. Gunnar Sillén and A.E. Martell, *Stability Constants of Metal-Ion Complexes*, The Chemical Society, London, 1964, p. 12.

30. a) N.F. Curtis and D.F. Cook, *J. Chem. Soc., Chem. Commun.*, 1967, 962; b) D.C. Olson and J. Vasilevskis, *Inorg. Chem.*, 1969, **8**, 1611; c) D.P. Rillema, J.F. Endicott and E. Papacostantinous, *Inorg. Chem.*, 1971, **22**, 1021.

31. a) D.C. Olson and J. Vasilevskis, *Inorg. Chem.*, 1973, **10**, 463; b) L.D. Diaddavio, W.R. Robinson and D.W. Margerum, *Inorg. Chem.*, 1983, **22**, 1021.

32. R.L. Deming, A.L. Allred, A.E. Dahl, A.W. Merlinger

and M.O. Kostner, *J. Am. Chem. Soc.*, 1976, **98**, 4132.

33. a) D.K. Cabbiness and D.W. Margerum, *J. Am. Chem. Soc.*, 1969, **91**, 6540; b) D. Hinz and D.W. Margerum, *Inorg. Chem.*, 1974, **13**, 2941.

34. R.D. Hancock and G.J. McDougall, *J. Am. Chem. Soc.*, 1980, **102**, 6551.

35. J.M. Lehn, *Acc. Chem. Res.*, 1978, **11**, 49.

36. A.M. Sargeson, *Pure Appl. Chem.*, 1984, **56**, 1603.

37. K.P. Wainwright and A. Ramasubbu, *J. Chem. Soc., Chem. Commun.*, 1982, 277.

38. R.D. Hancock and A.E. Martell, *Comments Inorg. Chem.*, 1988, **6**, 237.

39. J. Cornforth, *Proc. Roy. Soc. London*, 1978, **203**, 101.

40. I.M. Klotz, G.P. Royer and I.S. Scarpa, *Proc. Nat. Acad. Sci. USA*, 1971, **68**, 263.

41. a) M.A. Hierl, E.P. Gamsen and I.M. Klotz, *J. Am. Chem. Soc.*, 1979, **101**, 6020; b) E.J. Delancy, L.E. Wood and I.M. Klotz, *J. Am. Chem. Soc.*, 1982, **104**, 799.

42. D.J. Cram, *Science*, 1983, **219**, 1177.

43. D.J. Cram and H.E. Katz, *J. Am. Chem. Soc.*, 1983, **105**, 135.

44 a) M. Margoshes and B.L. Vallee, *J. Am. Chem. Soc.*, 1957, **79**, 4813; b) P. Pulido, J.H.R. Kagi and B.L. Vallee, *Biochemistry*, 1966, 5, 1768.

45 a) J.H.R. Kagi, S.R. Himmelhoch, P.D. Whanger, J.L.

Bethume and B.L. Vallee, *J. Biol. Chem.*, 1974, **249**, 3537; b) R.H.O. Buhler and J.H.R. Kagi, *Febs Lett.*, 1974, **39**, 229.

46 a) M. Margoshes and B.L. Vallee, *J. Biol. Chem.*, 1960, **235**, 3460; b) J.H.R. Kagi and B.L. Vallee, *J. Biol. Chem.*, 1961, **236**, 2435.

47 a) M. Webb and L. Magus, *Chem. Biol. Interact.*, 1976, **14**, 357; b) G.F. Nordberg, *Environ. Physiol. Biochem.*, 1972, **2**, 7.

48 M.M. Kissling and J.R.H. Kagi, *Febs Lett.*, 1977, **82**, 247.

49 Y. Kojima, C. Berger, B.L. Vallee and J.R.H. Kagi, *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 3413.

50 I-Y. Huang, A. Yoshida, H. Tsunoo and H. Nakajima, *J. Biol. Chem.*, 1977, **252**, 8217.

51 A. Yoshida, B.E. Kaplan and M. Kimura, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 486.

52 A.C. Legon and D.J. Millen, *Chem. Soc. Revs.*, 1987, **16**, 467.

53 R.C. Hider, *Struct. Bond.*, 1984, **38**, 25.

54 H. Stunzi, *Aust. J. Chem.*, 1979, **32**, 21.

55. E. Simon, *Ann.*, 1839, **31**, 265.

56. H. Staudinger, *Ber.*, 1920, **53**, 1073.

57. Ion exchange resins as acid and base catalysts are not dealt with in detail here as there are many good reviews available, i.e. M.J. Astle, *Ion Exchangers In Organic and Biochemistry*, eds. C. Calman and T.R.E. Kressman, Interscience, New York,

1957, chapter 36, p. 658 and F. Helfferich, *Ion Exchange*, McGraw-Hill, New York, 1962, chapter 2.

58. R.B. Merrifield, *J. Am. Chem. Soc.*, 1963, **85**, 2149.

59. a) P.D. Verweij, W.L. Driessen, J. Reedijk, B. Rowatt and D.C. Sherrington, *Reactive Polymers*, 1990, **13**, 83; b) D.C. Neckers, *Nouveau Journal De Chimie*, 1982, **6**, 645; c) C. Kantipuly, S. Katragadda, A. Chow and H.D. Gesser, *Talanta*, 1990, **37**, 491; d) J.H. Hpdgkin, *Chemistry and Industry*, 1979, 152; e) S.D. Alexandratos, *Separation and Purification Methods*, 1988, **17**, 67; f) K. Petrak, *Reactive Polymers*, 1989, **10**, 231.

60. P. Hodge and D.C. Sherrington, *Polymer-supported Reactions in Organic Synthesis*, Wiley-Interscience, Chichester, 1980, chapter 1.

61. A. Akelah, *Reactive Polymers*, 1988, **8**, 273.

62. D.C. Sherrington, *Chemistry and Industry*, 1991, **1**, 15.

63. A. Patchornik and M.A. Kraus, *Pure Appl. Chem.*, 1975, **43**, 503.

64. W.T. Ford (Ed), *Polymeric Reagents and Catalysts*, Washington D.C., J. Am. Chem. Soc., 1986, chapter 1.

65. W.H. Daly, *Makromol. Chem.*, 1979, **Suppl. 2**, 3 - 25.

66. J.M.J. Frechet and M.J. Farrell, *Functionalization of Crosslinked Polystyrene Resins By Chemical Modifications: A Review*, in *Chemistry and Properties of Crosslinked Polymers*, S.S. Labona,

ed., Academic Press, New York, 1977, p. 231.

67. A. Akelah and D.C. Sherrington, *Chem. Rev.*, 1981, **81**, 557.

68. R.H. Grubbs, L.C. Kroll and E.M. Sweet, *J. Makromol. Sci. Chem.*, 1973, **A 7**, 1047.

69. P. Hodge, *Synthesis and Separations Using Functional Polymers*, Wiley, Chichester, 1988, p. 23.

70. D.H. Solomon, ed., *Kinetics and Mechanism of Polymerization, Vol 3, Step-growth Polymerization*, Dekker, New York, 1972, chapter 1.

71. H. Widdecke, *Syntheses and Separations Using Functional Polymers*, Wiley, Chichester, 1988, Chapter 4.

72. M. Tomoi and W.T. Ford, *Syntheses and Separations Using Functional Polymers*, Wiley, Chichester, 1988, Chapter 5.

73. a) R.A. Bulman, O. Vanderborght and S. Van Puymbroeck, *Health Phys.*, 1983, **44**, 428; b) F. Bakir, S.F. Damlusi, T.W. Clarkson, J.C. Smith and R.A. Doherty, *Science*, 1973, **181**, 230.

74. a) Y.H. Chang and W.T. Ford, *J. Org. Chem.*, 1981, **46**, 3756; b) Y. Nambu, T. Endo and M. Okawara, *J. Polym. Sci., Polym. Chem. Edn.*, 1980, **18**, 2793.

75. E. Girault, R. Eritja and E. Pedroso, *Tetrahedron Lett.*, 1981, 3779.

76. N.K. Mathur, C.P. Narang and R.E. Williams, eds., *Polymers as Aids in Organic Synthesis*, Academic

Press, New York, 1980, chapter 2.

77. R. Arshady, G.W. Kenner and A. Ledwith, *J. Polymer Sci., Chem.*, 1974, **12**, 2017.

78. a) D.C. Sherrington, D.J. Craig, J. Dalgleish, G. Domin, J. Taylor and G.V. Meehan, *Europ. Polymer J.*, 1977, **13**, 73; b) J.A. Greig and D.C. Sherrington, *Polymer*, 1978, **19**, 163.

79. J.A. Greig, W.M. MacKenzie and D.C. Sherrington, *Polymer*, 1977, **18**, 1291.

80. a) W.L. Sederel and G.J. De Jong, *J. Appl. Polymer Sci.*, 1973, **17**, 2835; b) J.R. Millar, D.G. Smith, W.E. Marr and T.R.E. Kressman, *J. Chem. Soc.*, 1963, 218; c) J.C. Moore, *J. Polymer Sci. A.*, 1964, **2**, 835.

81. a) K.A. Kun and R. Kunin, *J. Polymer Sci. A1*, 1968, **7**, 2689; b) J.R. Millar, D.G. Smith and T.R.E. Kressman, *J. Chem. Soc.*, 1965, 304.

82. R. Rabinowitz and R. Marcus, *J. Org. Chem.*, 1961, **25**, 4157.

83. A.N. Senear, J. Wirth and R. Neville, *J. Org. Chem.*, 1960, **25**, 807.

84. J.R. Leebrick and H.E. Ramsden, *J. Org. Chem.*, 1958, **23**, 935.

85. a) K.W. Pepper, H.M. Paisley and M.A. Young, *J. Chem. Soc.*, 1953, 4097; b) R.S. Feinberg and R.B. Merrifield, *Tetrahedron*, 1974, **30**, 3209.

86. a) R.H. Grubbs and S.C.H. Su, *J. Organometallic Chem.*, 1976, **122**, 151; b) M.J. Farrall and J.M.J.

Frechet, *J. Org. Chem.*, 1976, **41**, 3877.

87. J.M.J Frechet and M.J. Farrall, *The Chemistry and Properties of Cross-linked Polymers*, S.S. Labana, ed., Academic Press, New York, 1977, p. 59.

88. A.Guyot, *Reactive Polymers*, 1989, **10**, 113.

89. J.I. Crowley and H. Rapoport, *Acc. Chem. Res.*, 1976, **9**, 135.

90. a) J. Yoshida, R. Nakai and N. Kawabata, *J. Org. Chem.*, 1980, **45**, 5269; b) J. Yoshida, J. Hashimoto and N. Kawabata, *J. Org. Chem.*, 1982, **47**, 3575.

91. a) K.W. Pepper, H.M. Paisley and M.A. Young, *J. Chem. Soc.*, 1953, 4097; b) G.D. Jones, *Styrene*, R.H. Boundy and R.F. Bayer, eds., Reinhold, New York, 1952, Chapter 14, p. 674.

92. a) R. Boniforti, R. Ferraroli, P. Frigieri, D. Heltai and G. Queirazza, *Anal. Chim. Acta.*, 1984, **162**, 33; b) W.G. Mitchell and M.M. Jones, *J. Inorg. Nucl. Chem.*, 1978, **40**, 199; c) T. Yoshioka, *Bull. Chem. Soc. Japan*, 1985, **58**, 2618; d) E. Oikawa and K. Ohtomo, *React. Polym. Ion. Exch., Sorbents*, 1988, **8**, 17.

93. M.D. Rosenzweig, *Chem. Eng.*, 1975, **82**, 60.

94. G.V. Myasoedova, I.I. Antol'skaya and S.B. Savvin, *Talanta*, 1985, **32**, 1105.

95. K.H. Lieser, M. Erboy and B. Thybusch, *Angew. Makromol. Chem.*, 1987, **152**, 167.

96. C. Kantipuly, S. Katragadda, A. Chow and H.D. Gesser, *Talanta*, 1990, **37**, 491.

97. J. March, *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, Wiley-Interscience, 3rd Ed., New York, 1984.

98. F.P. Bossu and D.W. Margerum, *J. Am. Chem. Soc.*, 1976, **98**, 4003.

99. E. Kimura, T. Koike, R. Machida, R. Nagai and M. Kodama, *Inorg. Chem.*, 1984, **23**, 4181.

100. D.L. Tuleen and B.A. Hess, *J. Chem. Educ.*, 1971, **48**, 476-477.

101. R. Broos, D. Tavernier and M. Anteunis, *J. Chem. Educ.*, 1978, **55**, 813.

102. C.S. Marvel and C.G. Overberger, *J. Am. Chem. Soc.*,1945, **67**, 2250.

103. K. Achinami, N. Abe, Y. Ishizuka, Jap P 160,365/1979 (CA 92:146910t)

104. A. Nganie, D. Derouet, A. Fernandez and C.P. Pinazzi, *Eur. Polym. J.*, 1986, **22(6)**, 431-438.

105. M. Bodanszky and A. Bodanszky, *The Practice of Peptide Synthesis*, Springer-Verlag, Berlin, 1984, p. 89.

106. *Beilsteins Handbuch Der Organischen Chemie*, eds. B. Prager, P. Jacobson, P. Schmidt and D. Stern, Julius Springer, Berlin, 1926, vol. 9, p. 486.

107. *Dictionary of Organic Compounds*, Eyre and Spottiswoode Ltd., London, 4th ed., vol. 5, p. 3074.

108. *Dictionary of Organic Compounds*, Eyre and Spottiswoode Ltd., London, 4th ed., vol. 3, p. 1826.

109. *Dictionary of Organic Compounds*, Eyre and Spottiswoode Ltd., London, 4th ed., vol. 2, p. 695.

110. Following the method given in *Vogel's Textbook of Practical Organic Chemistry*, eds., B.S. Furniss, A.J. Hannaford, V. Rogers, P.W.G. Smith and A.R. Tatchell, Longman, London and New York, 1978, 4th ed, p. 252.

111. *Beilsteins Handbuch Der Organischen Chemie*, eds. B. Prager and P. Jacobson, Julius Springer, Berlin, 1922, vol. 4, p. 506.

112. M. Bodanszky and A. Bodanszky, *The Practice of Peptide Synthesis*, Springer-Verlag, Berlin, 1984, p. 83.

113. *Vogel's Textbook of Practical Organic Chemistry*, eds., B.S. Furniss, A.J. Hannaford, V. Rogers, P.W.G. Smith and A.R. Tatchell, Longman, London and New York, 1978, 4th ed, p. 296.

114. *Beilsteins Handbuch Der Organischen Chemie*, eds. B. Prager and P. Jacobson, Julius Springer, Berlin, 1922, vol. 4, p.366.

115. *Beilsteins Handbuch Der Organischen Chemie*, ed. F. Richter, Springer, Berlin, 1946, 2nd Supplementary series, vol. 9, p. 631.

116. B.S. Furniss, A.J. Hannaford, V. Rogers, P.W.G. Smith and A.R. Tatchell, *Vogel's Textbook of Practical Organic Chemistry*, Longman, London and New York, 1978, 4th ed, p. 493.

117. S. Scheler and F. Endermann, S.A. Patent, ZA

6707085/1968.

118. C. Raha, *Org. Synth.*, 1953, 33, 20.

119. R. Chittaraja, *Org. Synth.*, 1953, 33, 20.

120. A. Buttafava, L. Fabbrizzi, A. Perotti, A. Poggi and B. Seghi, *Inorg. Chem.*, 1984, 23, 3917.

121. E. Kimura, T. Koike, R. Machida, R. Nagai and M. Kodama, *Inorg. Chem.*, 1984, 23, 4181.

122. J.S. Bradshaw, C.T. Bishop, S.F. Nielsen, R.E. Asay, D.R.K. Masihdas, E.D. Flanders, L.D. Hansen, R.M. Izatt and J.J. Christensen, *J. Chem. Soc., Perkins 1*, 1976, 2505.