The synthesis and characterisation of new macrocyclic complexing agent for use in tumour targeting

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THE SYNTHESIS AND CHARACTERISATION OF NEW MACROCYCLIC COMPLEXING AGENTS FOR USE IN TUMOUR TARGETING

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A Thesis Submitted for

the Degree of Doctor of Philosophy at the University of Durham

July 1993

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DECLARATION

The work herein was carried out at Durham University between October 1988 and August 1991. It has not been submitted for a degree at this or any other University and is the author's own work unless otherwise indicated by reference or footnote.

Publications

Some of the author's research, presented in this thesis, form the basis of the following publications:

1. Synthesis of 1,10-Dithia-4,7,13,16-tetra-azacyclooctadecane, 1-aza-4,7-dithiacyclononane, and N,N'-1,2-Bis(1-aza-4,7dithia-carbonyl) ethane. Structual and Solution Studies of their Silver Complexes. A.S. Craig, R Kataky, R.C. Matthews, D. Parker, G. Ferguson, A. Lough, H. Adams, N. Bailey, H. Schneider, J. Chem. Soc. Perkin Trans., 1523, 2, (1990).

2. Synthesis and Structure of Stable Indium and Gallium Complexes of (R)-1,4,7-Tris(2'-methylcarboxy-methyl)-triazacyclononane. R.C. Matthews, D. Parker, G. Ferguson, B. Kaitner, A. Harrison and L. Royle. Polyhedron 1951, 16, (1991).

3. ^{67}Ga-9N_3 Uptake by Xenografts of Human Melanotic Melanoma in Mice. A. Harrison, C.A. Walker, K.A. Pereira, D. Parker, L. Royle, R.C. Matthews, A.S Craig, Nucl. Med. Comm. 13, 667, (1992).

ACKNOWLEDGEMENTS

I would like to thank David Parker for his guidance and commitment to both the project and myself.

Also I would like to acknowledge the help of the following people: Alice Harrison and Louise Royle for collecting the invaluable biodistribution data and their help in analysing it, Professor George Ferguson for the crystal structure determination, Hermann Schneider for measuring the enthalpies of complexation of the silver complexes, Julia Say, Alan Kenright and Ray Matthews for their help in acquiring the NMR spectra, Mike Jones and Vince McNeilly for running the mass spectra, Mr R Hart and Mr G Haswell for their good humour and glassblowing skills, and to all the other technical staff, and especially the cleaners, in the department.

I acknowledge the financial support of Celltech Ltd.

I would like to thank all the people that have helped me fill my leisure time, and especially Pete for his endless enthusiasm, Jenny for her invaluable support, Penny for her friendship and help in preparing this thesis, Ben for his intellectual stimulus, and not forgetting Paul and his good humoured banter. In addition, a special thank you to Einar and Rod.

Finally, I thank my brother Andrew for his support over the last few years.

ABSTRACT

THE SYNTHESIS AND CHARACTERISATION OF NEW MACROCYCLIC COMPLEXING AGENTS FOR USE IN TUMOUR TARGETING

Macrocyclic complexing agents have been synthesised for the binding of indium(III), gallium(III), yttrium(III), gold(I), silver(I) and rhenium(V) and a comparative study has been made between the macrocyclic complexing agents, $18N_4S_2$, $18N_4S_2Me_4$, $18N_4O_2$ and $18N_4O_2Me_4$ and their ability to bind silver (I).

The gallium(III) and indium(III) complexes of $9N_3C_3Me_3$ and $9N_3C_3Ph_3$ have been investigated extensively both *in vitro* and *in vivo*. The stability of the complexes have been characterised using ^{71}Ga NMR, ^{1}H NMR, U.V. spectral analysis, ligand protonation constants, and complex binding constants. A full X-ray crystallographic structural determination has been obtained for the indium-$9N_3C_3Me_3$ complex. Following confirmation of the excellent binding characteristics of the $9N_3C_3Me_3$ and $9N_3C_3Ph_3$ complexing agents, the *in vivo* kinetic stability of the indium and gallium complexes has been investigated. The $9N_3C_3Me_3$ complexes were exceptionally stable and cleared rapidly (99% within 24 hrs) via the renal excretion pathway. The complexes of the $9N_3C_3Ph_3$ were less stable and also showed a preference for clearance via the kidneys. Further to this, the tumour localising properties in a human melanotic melanoma has been investigated for the ^{67}Ga-$9N_3C_3Ph_3$ complex. The complex has shown a preference for tumour localisation, although a low tumour: blood ratio (1:1) may prohibit its application for tumour targeting.

The stability constants of the silver(I) complexes of $18N_4S_2$, $18N_4S_2Me_4$, $18N_4O_2$ and $18N_4O_2Me_4$ have been measured both in methanolic and aqueous media. The $[Ag\text{-}18N_4S_2Me_4]^+$ complex stability ($\log K_{ML}$= 14.6) is the highest stability constant recorded in methanol. In aqueous media, of the four complexes, the $[Ag\text{-}18N_4S_2]^+$ is the most stable ($\log K_{ML}$=10.4) a reversal of the observed order of complex stability in methanol.

The stability of the new yttrium(III) complexing agent has been ascertained using ^{1}H NMR and HPLC radiometry and proved to be insufficiently stable for *in vivo* use, and was easily displaced by DTPA in a trial experiment.

ABBREVIATIONS

EDTA	Ethylenediamine-N,N,N',N'-tetraacetic acid
DTPA	Diethylenetriamine-N,N,N',N",N"-pentaacetic acid
NTA	Nitrilotriacetic acid
PCA	Polyaminocarboxylate complexing agent
DOTA	1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid
TRITA	1,4,7,10-Tetraazacyclotridecane-1,4,7,10-tetraacetic acid
TETA	1,4,8,11-Tetraazacyclotetradecane-1,4,8,11-tetraacetic acid
DOTA-BMA	4-(N-Benzyl-N-methylcarboxamidomethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid
ODOTRA	1-Oxa-4,7,10-triazacyclododecane-4,7,10-triacetic acid
DTCTA	1,7-Dioxa-4,10,13-triazacyclopentadecane-4,10,13-triacetic acid
$9N_3$	1,4,7-Triazacyclononane
$9N_3C_3$	1,4,7-Triazacyclononane-1,4,7-triacetic acid
$9N_3C_3Me_3$	(R)-1, 4, 7 -Tris (2'-methylcarboxymethyl) - triazacyclononane
$9N_3C_3Ph_3$	-1, 4, 7 -Tris (2'- phenylcarboxymethyl) - triazacyclononane
$9N_3P_3Ph_3$	1, 4, 7 triazacyclononane-1,4,7-triyltris[methylene(phenylphosphinic acid)] -
$9N_3P_3Me_3$	1, 4, 7 triazacyclononane-1,4,7-triyltris[methylene(methylphosphinic acid)] -
$15N_3O_2$	1,4 -Dioxa-7,10,13-tris- (carboxymethyl) - triazacyclopentadecane
RII	Radioimmunoimaging
RIT	Radioimmunotherapy
DNA	Deoxyribonucleic acid
MoAb	Monoclonal antibody
IgG	Immunoglobulin
3,2,3-tet	4,7-Diaza-1,10-dodecanediamine
cyclam	1,4,8,11-Tetraazacyclotetradecane
mal	Maleimide
Me	Methyl
Et	Ethyl
Ac	Acetyl
Ph	Phenyl
Bz	Benzyl
Ts (Tosyl)	4-Toluenesulphonyl
Ar	Aromatic
THF	Tetrahydrofuran
Ln	Lanthanide
MPt	Melting point
dec	Decomposes
IR	Infra-red

UV	Ultraviolet
NMR	Nuclear magnetic resonance
HPLC	High performance liquid chromatography
MS	Mass spectroscopy
CI	Chemical ionisation
DCI	Desorption chemical ionisation
FAB	Fast atom bombardment
TLC	Thin layer chromatography
NBS	N-Bromosuccinimide
PBS	Phosphate buffered saline
en	Ethylenediamine

CONTENTS PAGE

CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

1.1 THE NEED FOR IMPROVED CANCER THERAPY

1.1.1 What Is Cancer?[1,2]

Cancer is a disease of the cell. It normally appears as a growth or *tumour*, a large mass of tissue cells. The tumour is the result of a multi-stage process known as *carcinogenesis*. Carcinogenesis involves, firstly, an initiation step by a *carcinogen,* followed by the second stage, the activation of the damaged cell. The time span between the appearance of the tumour and the initiation step may be many years. Carcinogens can be physical (e.g. U.V. or X-Ray radiation) or chemical (e.g. benzene). What is common to both is the ability to severely damage cellular DNA.

The activation step is less well understood and may be the result of environmental factors (e.g. diet, exposure to sunlight, nicotine and alcohol abuse). However there is some evidence that susceptibility runs in families, suggesting genetic inheritance.

Another possibility is the effect of certain viruses on the immune system which may inhibit the body's natural defence against a mutant cell. Although there is much research and debate over the actual cause of the cell activation, it is clear once the cell is activated a tumour will begin to grow.

The cells of a tumour no longer respond to normal growth mechanisms and may replicate *-their natural function*, at a rate which far exceeds the normal rate, producing a growth and possibly damaging the surrounding tissues. If the tumour remains in a fixed position, it is known as a *benign* tumour. If the tumour fragments and invades other organs, it is a *malignant*

tumour. The process of fragmentation is known as *metastasis*. Metastasis is very difficult to control, and is responsible for the major number of deaths of those people that die from cancer.

1.1.2. The Incidence Of Cancer[3]

Cancer is a very common disease and was responsible for 163,000 deaths in the U.K. in 1990. In the U.K. alone, more than 250,000 people are diagnosed with cancer each year. The incidence of the ten most common forms of cancer are given in figure 1.1 (A). Present statistics show that one in three people will develop cancer during their lifetime. Most (70%) of the people who contract cancer are over 60 years old.

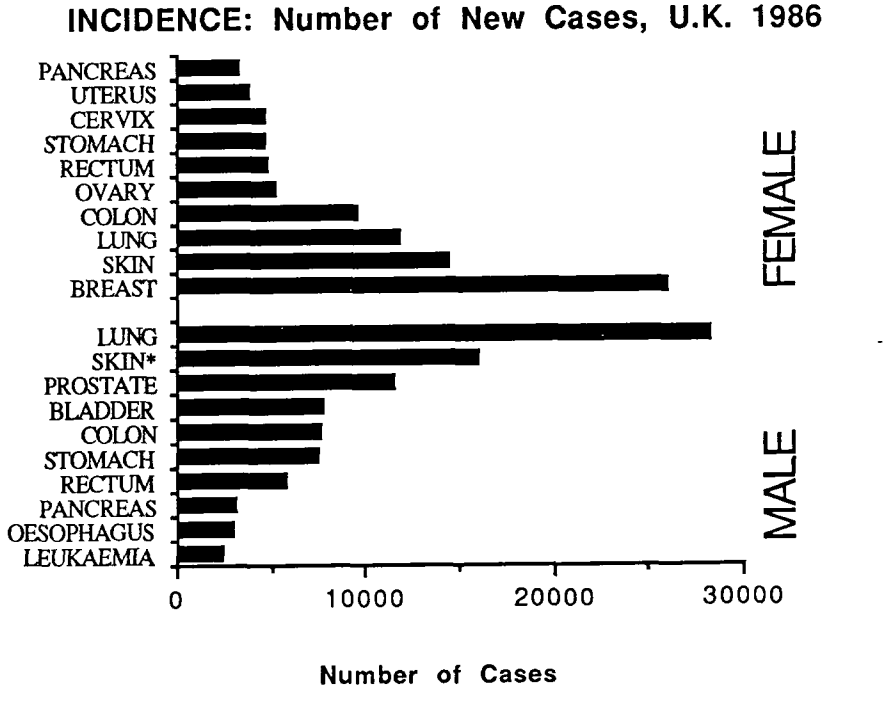


Figure 1.1(A) *The Incidence Of The Ten Most Common Forms Of Cancer In The U.K., 1986.*[2] ** "Skin" does not include malignant melanoma.*

Survival from cancer depends greatly on the stage at which it is diagnosed; the more advanced the disease, the more likely it is that metastasis has occurred and that other tumours are developing away from the primary site. Figure 1.1(B) shows the five year relative survival rates for the ten most common forms of cancer.

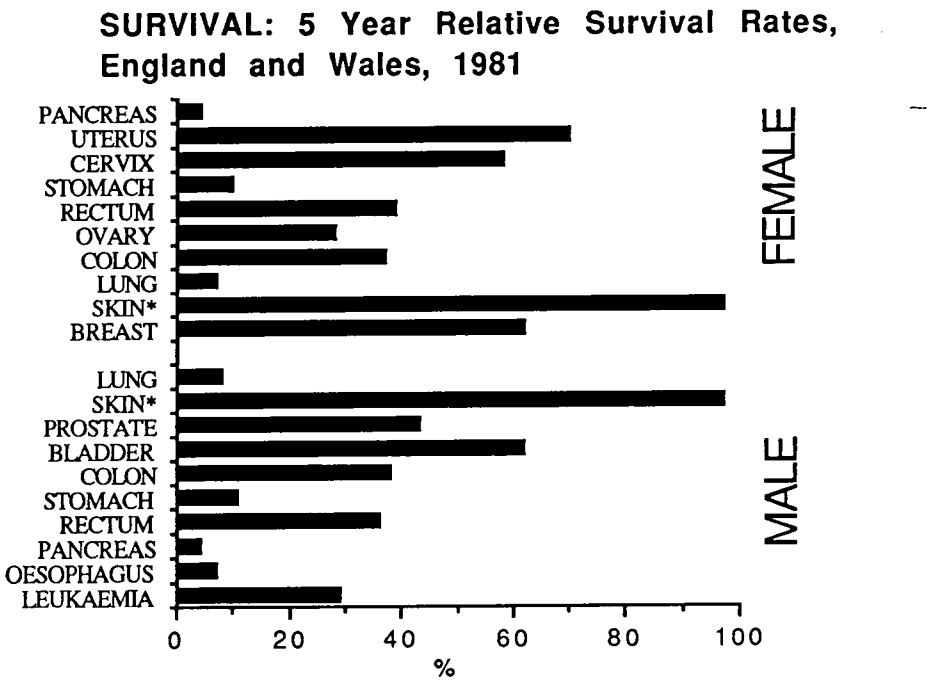


Figure 1.1(B) *The Five Year Survival Rate For Patients With The Ten Most Common Forms Of Cancer. England and Wales, 1981.*[2] **"Skin" does not include malignant melanoma.*

Figure 1.1(C) shows the ten cancers which cause the most deaths in men and women. With men, lung cancer is the major cause of death from cancer (one third), whereas with women, breast cancer is the largest cause of death for women who contract cancer (one fifth).

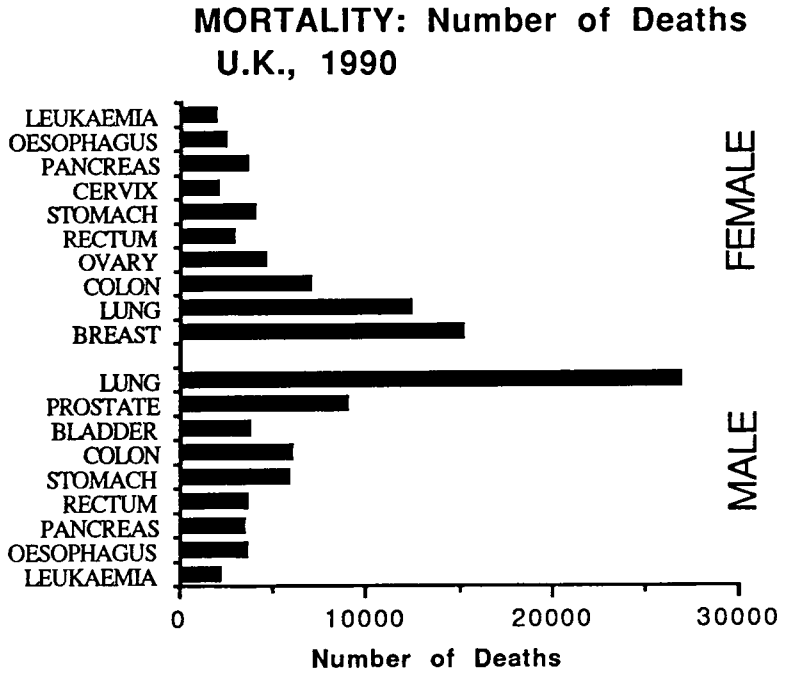


Figure 1.1(A) *The Number Of Deaths From The Ten Most Common Forms Of Cancer In The U.K., 1990.*[2]

1.1.3 The Treatment Of Cancer[1]

Surgery can be used to remove a tumour, and is very effective in the treatment of benign tumours. *Radiotherapy* - the use of X-rays and Gamma-rays, and *chemotherapy* - the use of drugs (e.g. cis-platin and 5-fluorouracil) are used to combat benign tumours *in situ*. These techniques do meet with some success, for example, more than 80% remission is occurring for testicular and ovarian cancers, but this degree of success is limited to the treatment of a small group of cancers. In fact lung, colon, rectum and prostate cancers, are virtually untreatable using chemotherapy. Because chemotherapy involves the destruction of the DNA of tumour cells, a side effect of the technique is the destruction of healthy tissue and possibly vital organs.

The need for new treatment is clear. Those cancers which are currently untreatable, surgery aside, call for new methods for their treatment, and the intrinsic danger of chemotherapy puts the emphasis on finding a new *cell-selective* approach to the treatment of cancer. One such approach is to specifically target the cancer cells using the molecular blood hounds - *monoclonal antibodies.*

1.2 ANTIBODIES

1.2.1 Introduction

Antibodies are proteins known as *immunoglobulins*. They are produced in vertebrates by white blood cells called *B-lymphocytes* in response to invading *pathogens* or other foreign substances. *T-lymphocytes* alert the B-cell to the presence of the foreign substance; the B-cells produce huge quantities of antibodies which recognise and subsequently give rise to their destruction by the white killer cells, or *macrophages*[4]. The mechanism of antigen recognition is achieved by binding to the surface with partial structures (*determinants*) in a highly specific fashion.

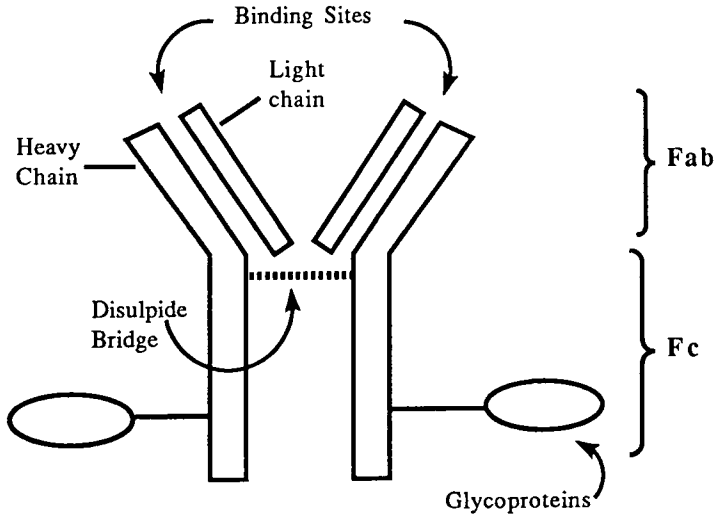


Figure 1.2 *Schematic Diagram Of An Antibody*

Antibodies, in their simplest form, consist of two identical heavy chains and two identical light chains linked together by disulphide bridges in a complex three dimensional structure. The antibody has three regions: of these two are Fab regions (ab stands for antigen binding), consisting of one light chain and part of the heavy chain. Each Fab is a single binding site, thus each molecule is bivalent, giving rise to two identical binding sites. The third

region is the Fc or constant region, consisting of two parts of the heavy chains. The Fc fragment mediates the effector functions by initiating a sequence of immunological reactions which lead to the elimination of the antigen. It is the spatial arrangement of the amino acid residues in the Fab fragments that determine the individual binding specificity of the antibody.

1.2.2 Monoclonal Antibodies And Cancer Therapy

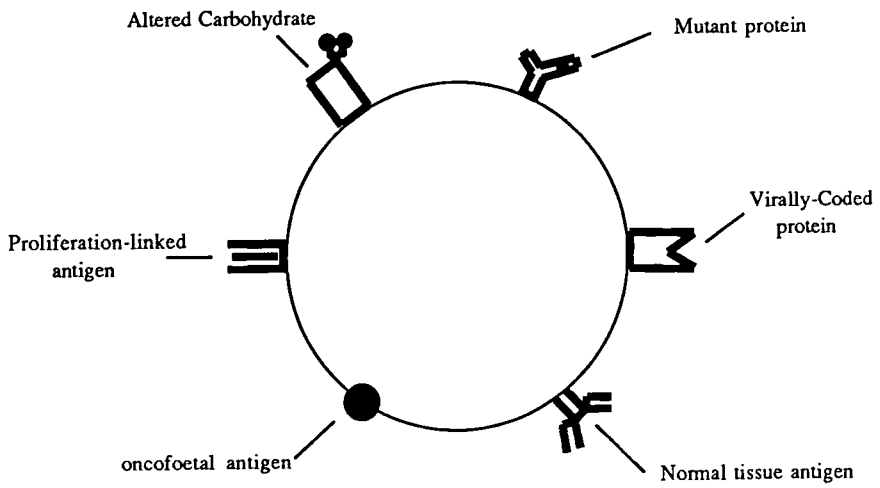


Figure 1.3 *Target Antigens*

A tumour cell expresses glycoproteins and glycolipids (Figure 1.3) on its surface which are not normally expressed. The body's immune system should recognise these as antigenic and the response process described above should take place. Often this is not the case and the mutant cell is allowed to proliferate causing the growth of a tumour. This fact in itself demands to be understood. Why is it that the cell is not recognised and destroyed?

Aside from this apparent malfunction of the immune system, the presence of these glycolipids and glycoproteins can be advantageous. If an antibody, specific to these unique antigens,

could be labelled with an imaging agent (*Radioimmuno-scintigraphy*, RII) or a therapeutic agent (*Radioimmunotherapy*, RIT), this would provide us with a cell selective approach to the treatment of cancer.

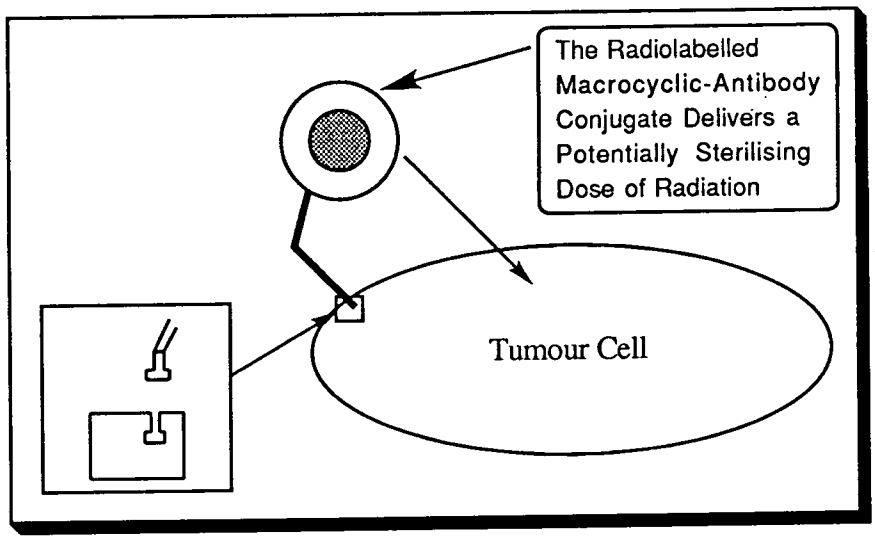


Figure 1.4 *Radioimmunotherapy*

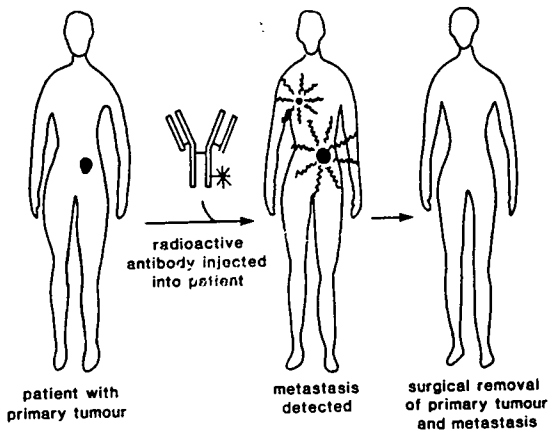


Figure 1.5 *Radioimmunoimaging*[1]

Nearly one hundred years ago, Ehrlich postulated that it may be possible to target therapeutic agents at specific tissues. He suggested that antibodies would be likely agents for the job, but until the 1970's this was an impossible task. At that time, it was impossible to raise a specific antibody for a specific antigen - *a monoclonal antibody*.

In 1975, Kohler and Milstein[7] were responsible for solving this problem. They discovered, that if a plasma-B-cell could be immunised against one antigen and fused to a malignant myeloma cell (*a hybridoma*), the resultant *hybrid* is capable of maintaining the characteristics of both cells, namely, to produce an abundance of antibodies specific to one antigen, (Figure 1.6). Since then, this hybridoma technology has made it possible to produce large quantities of monoclonal antibodies.

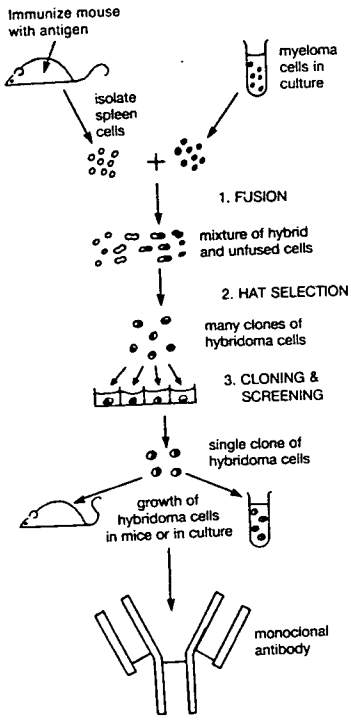


Figure 1.6 *Hybridoma Technology*[1]

1.2.3 Chimaeric Antibodies[8,9] And Antibody Fragments[10]

Kohler and Milstein used mice for their initial work. From the diagram, it is shown that part of the process involves injecting a tumour antigen into a mouse, from which the resultant lymphocytes are taken. The antibodies produced by a mouse are *murine* antibodies and are recognised by the human immune system as foreign or *immunogenic*. If they are used *in vivo* they may cause an immune response that would obliviate the desired process. If a fully human antibody is required, somebody must be inoculated with the tumour antigen. This obviously poses a severe difficulty.

This problem has been partially overcome by employing humanised or *chimaeric* antibodies. These antibodies have the hypervariable region of the mouse for the tumour antigen, but human constant and variable regions. These antibodies should prove less immunogenic than the murine antibodies.

Important advances have also taken place in the production of *antibody fragments* directly from E-coli[10]. An antibody fragment, as the name suggests, is just the binding part of the antibody. Figure 1.7 shows the possible fragments. As the constant region does not take part in the recognition process, it is disposable. Apart from reducing immunogenicity, their smaller size should improve transport time to the tumour site and also allow better access into more hindered sites. These antibody fragments are also cleared more rapidly from the blood and eventually from the body, and should improve the tumour : background ratio.

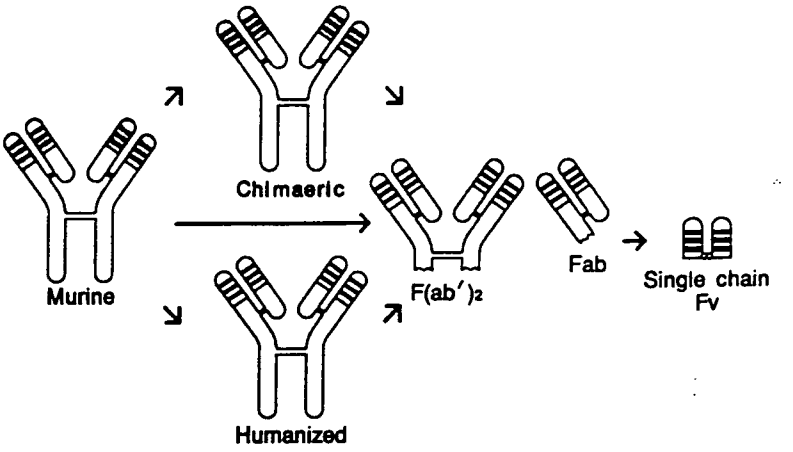


Figure 1.7 *Antibody Fragments of an IgG*

1.3 RADIOIMMUNOTHERAPY AND RADIOIMMUNOIMAGING

1.3.1 Introduction

Tumour targeting employing antibody conjugated radiolabels, allows both therapy and imaging to be achieved within a single treatment protocol[11,12]. The use of monoclonal antibodies provides a method of achieving a high target : non-target ratio. To facilitate the successful use of these techniques a number of factors must be addressed. The method of radiolabelling is of crucial importance (section1.3.3), as is the choice of a suitable radiolabel.

1.3.2 The Choice Of Radionuclide [13,17]

1.3.2.1 Radioimmunoimaging

When imaging is intended, what is required is a high photon density to maximise the resolution of the image produced [13]. For therapeutic use, a radionuclide with high energy is required.

More specifically, for imaging, the minimum possible interaction with the tissue is crucial. Also the nature of the radiation emitted must allow an easy and preferably cheap method of detection. As most in-house detectors are tuned for the detection of technetium 99m (E = 141 keV), a photon energy close to this value would be beneficial. Gamma and positron emitting isotopes of sufficient energy fall into this category. Detection of a single photon emitter can take place using a conventional Anger camera, with a resolution of 1 cm, which can be improved using a tomographic detector. A positron colliding with another electron creates two

colinear photons of 511 keV, and gives improved resolution (3mm) using a positron emission tomography scanner[17].

Common requirements for both imaging and therapy isotopes are as follows:

1) A half-life of between 6 hours and 8 days. The physical process of getting the conjugated antibody (mass = 150000) is relatively slow, $t_{1/2}$ = 24 hours.
2) The production of stable daughter products.
3) Must be cheap and readily available.
4) Must be carrier free (not contaminated with stable isotopes of the element) to achieve maximum dosage for the tumour.
5) The radionuclide must possess the appropriate chemical properties to enable its attachment to the antibody with a specially chosen complexing agent.

A list of possible isotopes is given below.

Radionuclide	$T_{1/2}$	E photon (%)
^{123}I	13.2 hr	159 (83)
^{125}I	60.0 days	27(138)
		35(7)
^{131}I*	8.05 days	364(82)
^{113m}In	1.68 hr	391(66)
^{111}In	2.83 days	171(88)
		247(94)
^{99m}Tc	6.02 hr	141(89)
^{67}Ga	3.25 hr	184(24)
^{82}Rb (PET)	1.4 hr	511
^{64}Cu**(PET)	12.8 hr	511(120)
^{68}Ga (PET)	1.20hr	511(178)

* ^{131}I has an accompanying β^- emission E_{max} 0.188 MeV

** ^{64}Cu has an accompanying β^- emission E_{max} 0.57 MeV

Table 1.1 *List Of Candidates For Tumour Imaging*

Gallium 67 And Indium 111

Despite being more expensive and less easy to detect than technetium 99m, both of these isotopes have a convenient coordination chemistry, and suitable half-lives making them very strong candidates for imaging.

1.3.2.2 Radioimmunotherapy

For radioimmunotherapy, the antibody-radiolabel conjugate needs to be able to deliver a sterilising dose of radiation that is of sufficient energy to cause cleavage of the cellular DNA. This constitutes a dosage of between 600 to 40000 rads. This suggests that

an alpha or beta emitter is the most suitable. There is one more consideration that must be borne in mind -that of the tumour *morphology*[16]. It is impossible for any one nuclide to meet the morphological demands of every tumour. For instance, leukaemia is a single cell cancer, and if a long range emitter was employed, many healthy cells in the local environment would be damaged. In this case it would be best to employ a short range beta or alpha emitter. For large and dense tumours, a long range beta emitter would be best. This would enable the whole tumour to be irradiated, even when antigen distribution is non-uniform, Figure 1.7

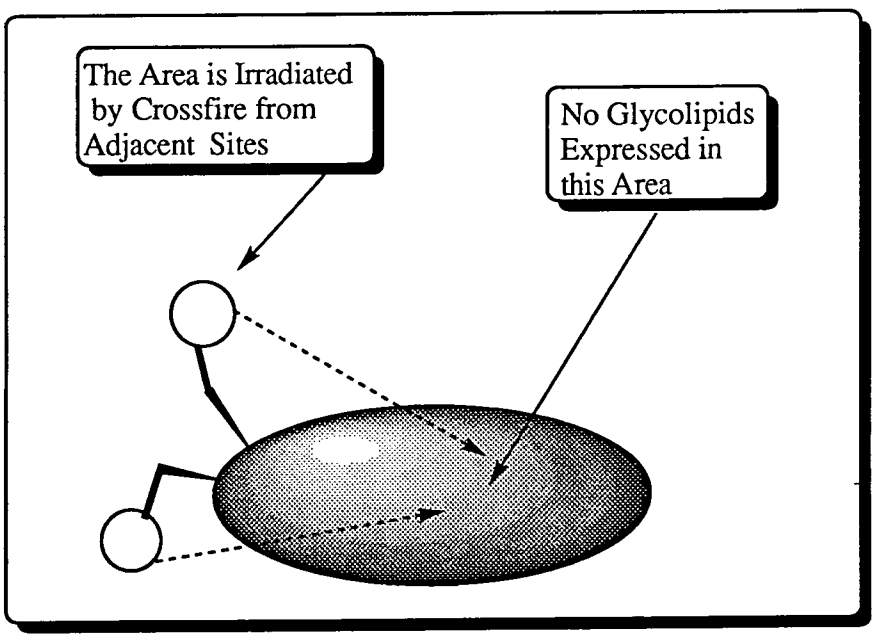


Figure 1.7 *The Remote Part Of The Tumour Where No Tumour Specific Antigens Exist Is Irradiated By The Long Distance Beta Emitter -* ***Crossfire.***

The problem with alpha emitters is that they have a very short range (1 to 2 cell diameters), and therefore are not suitable for most tumours. Also, many alpha emitters produce potentially harmful

daughter products, or possess undesirable chemical properties. Beta emitters that may be suitable are given in table 1.2.

Isotope	Half Life hr	Bmax (Mev%)	Mean Range mm	Gamma (KeV %)
^{67}Cu	62	0.40 (45)	0.2	93 (17)
		0.48 (35)		184 (47)
		0.58 (20)		
^{90}Y	64	2.25 (100)	3.9	----
^{111}Ag	179	1.04 (93)	1.1	342(6)
		0.69 (6)		
^{131}I	193	0.61 (90)	0.4	364(79)
^{161}Tb	166	0.45	0.3	75
		0.57		57 (21)
		0.58		
^{188}Re	17	1.96	3.3	155
		2.12		
^{199}Au	75	0.25 (22)	0.1	158 (76)
		0.3 (72)		

Table 1.2 *Possible Isotopes For Use In RIT*

Rhenium 188

Of these candidates, rhenium 188 is a potential candidate. Unfortunately, the low oxidation state chemistry is rather complex and difficult synthetically, although stable cationic $Re(V)O_2$ complexes with 1,4,8,11- tetrazaundecane have been characterised[18].

Gold 199 And Silver 111

Gold 199 has similar problems to the rhenium 188 isotope associated with its usage, in that its coordination chemistry is complicated by the fact that whilst Au(III) complexes are kinetically inert, they are strongly oxidising and Au(I) complexes - preferring a linear coordination geometry - tend to disproportionate readily. However, silver 111 is much more amenable to complexation. If a more stable complex can be made[18], this isotope could prove to be a very effective therapeutic agent.

Yttrium 90

Yttrium 90 is particularly well suited to therapy. It has a suitable half life ($t_{1/2}$ = 64 hours) and is a pure beta emitter of intermediate energy (2.3 MeV). The daughter products are stable and it is readily available from a generator from its parent strontium 90.

Because yttrium 90 has a range of 3.9mm, it is very suitable for lung, ovarian[19,20], colorectal[21] and breast cancer. However if it becomes dislodged from the antibody, it can build up in the bone marrow causing the potentially lethal myelosuppression. It is crucial that the radiometal is bound irreversibly to the antibody.

1.3.3 Bifunctional Complexing Agents

In order to achieve successful tumour targeting the radiolabel must be irreversibly bound to the antibody. To achieve this a bifunctional complexing agent is required [22,23]. A bifunctional complexing agent consists of an activated moiety that can be conjugated to an antibody, possibly via the NH_2 group of a lysine

residue. This is combined with a complexing agent specifically chosen to bind the radionuclide irreversibly. (Figure 1.8)

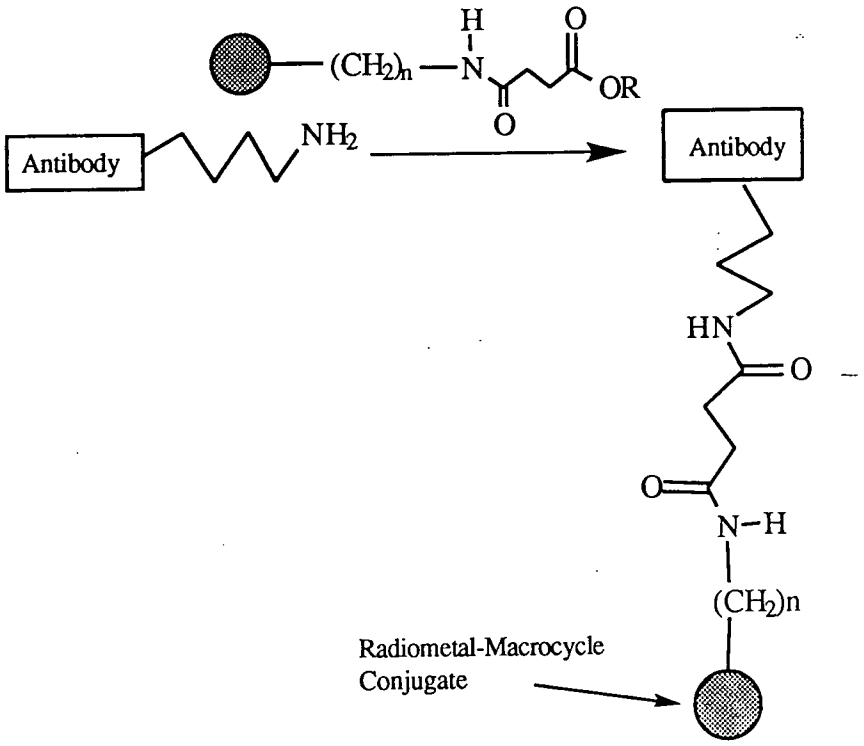


Figure 1.8 *Radiolabelling A Monoclonal Antibody*

The choice of complexing agent is governed by the radiometal ion that will be used as the radiolabel[24]. It is impossible for any one complexing agent to form stable complexes with all of the choice radiometals. Each metal ion will have a distinctive coordination requirement comprising of a specific number and type of donor atoms with a suitable spatial arrangement to achieve coordination saturation. Previous workers concentrated on this one ligand approach employing functionalised EDTA[25,26] or DTPA[27] as the complexing agent for all choice radiolabels. The instability of such complexes *in vivo* predetermined the use of selectively tailored complexing agents. The problems encountered with EDTA and

DTPA, namely acid catalysed decomplexation *in vivo,* may be partially or completely solved by a judicious choice of a macrocyclic complexing agent[28,29].

1.4. MACROCYCLIC COMPLEXING AGENTS.

1.4.1 Introduction

A macrocycle is a polydentate ligand, accommodated by a cyclic array of atoms containing at least three donor atoms; the macrocyclic ring containing at least nine atoms, (Figure 1.10). Macrocyclic complexing agents [29] are a better choice for *in vivo* work for a number of reasons.

1.4.2 Macrocycles - Superior Complexing Agents ?

Macrocycles form complexes with metals that are usually of greater thermodynamic stability than their acyclic counterparts[32] such as EDTA and DTPA.

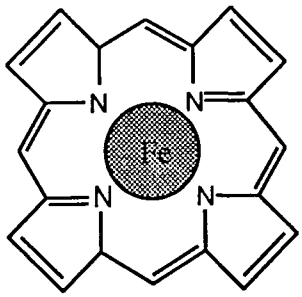


Figure 1.10 *A Macrocyclic Complex*

We should expect an extra increase in the stability constant, due to the formation of another chelate ring - *the chelate effect*. Polydentate chelating agents are always thermodynamically more stable than monodentate ligands. The example below clearly demonstrates this effect:

$$Ni^{2+}(aq) + 6\,NH_3(aq) \longrightarrow Ni(NH_3)_6^{2+} + aq \quad logK=8.6$$

$$Ni^{2+}(aq) + 3\,en(aq) \longrightarrow Ni(en)_3^{2+} + aq \quad logK=18.2$$

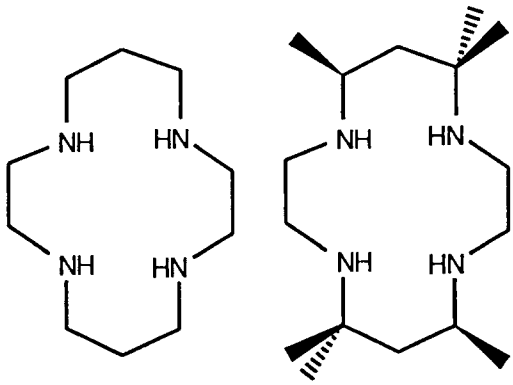
The introduction of three chelate rings produces a large increase in the stability constant (K) and therefore a large decrease in the Gibbs free energy($\Delta G°$).

$$\Delta G° = -RT\ln K = \Delta H° - T\Delta S°$$

The free energy of a reaction is a combination of the enthalpy ($\Delta H°$) and the entropy ($\Delta S°$) of the reaction. The enormous increase in the stability constant is a combination of these parameters. Although the main effect, in this case, is due to the increase in translational entropy of the system when monodentate ligands are replaced by the polydentate ligand.

1.4.3 The Macrocyclic Effect

Macrocyclic complexes are, almost always, kinetically and thermodynamically more stable than their acyclic analogues. The increase in stability found with a macrocyclic ligand far outweighs that which might be expected with the addition of another chelate ring. Cabbiness and Margerum[33] have reported that the copper(II) complex of tet-a was 10,000 times more stable than the copper(II) complex of the acyclic of 2,3,2-tet.



Cyclam **tet-a** **2,3,2-tet**

Complex	Log K
$Cu(2,3,2\text{-tet})^{2+}$	23.9
$Cu(tet\text{-}a)^{2+}$	28

This effect has been primarily ascribed to an increased entropy of complexation.

The Ni(II) complex of cyclam and its acyclic analogue have also been compared by Margerum[33].

Complex	logK	ΔH/kcal mol^{-1}	ΔS/cal$K^{-1}mole^{-1}$
$Ni(2,3,2\text{-tet})^{2+}$	15.8	-13.0	7.4
$Ni(cyclam)^{2+}$	22.2	-19.4	-2.0

In this case the enthalpic factor dominates and the macrocyclic effect results in a stability that is of the order of 10^7 greater than its acyclic analogue.

The extra stability of macrocyclic complexes may be attributed to either favourable enthalpy or favourable entropic contributions. In many cases it is a mixture of both, but often the entropie factor is the main contribution. The conformational change an acyclic

complexing agent must undergo to form the complex, far outweighs that of the already pre-organised macrocycle.

Kinetic Stability is also associated with macrocyclic complexing agents. Most macrocycles show a kinetic inertness and do not readily dissociate, even in acidic conditions. These kinetic properties may also effect the equilibrium constant of such complexes. For instance, it is possible with the open chain series to undergo S_N1 displacements, where the displaced donor can be readily solvated. With the macrocyclic ligand, this is much more difficult because of the rigidity. The cycle does not allow the passage of incoming ligands, or the subsequent solvation and stabilisation of the displaced donor moiety.

1.4.4 Specific Tailoring Of The Macrocycle

Because each macrocycle can be synthesised specifically, containing the required number and type of donor atoms to coordinately saturate the metal ion, this considerably reduces the risk of competitive demetallation *in vivo*. Also, macrocyclic complexes are less sensitive to acid catalysed dissociation[30,31], and therefore tend to be more kinetically stable *in vivo* (the pH in the region of the liver may be as low as 2).

In addition, each macrocycle can be designed to form a neutral or cationic complex, which will not attract protons and other cations encountered in the blood stream, again reducing the chance of the formation of mixed complexes with metals already in relatively high concentration in the body (Ca^{2+} is 1.26 mmol dm^{-3}, Mg^{2+} is 0.8 mmol dm^{-3}, and Zn is 10^{-5} mmol dm^{-3} in human

serum). The overall charge on the complex may also influence its excretion pathway in the body.

1.5 THE SCOPE OF THIS WORK

1.5.1 Introduction

As previously discussed, stable complexes of the chosen radiometals are of primary importance for tumour targeting and *in vivo* work in general. This research aims to synthesise a number of macrocyclic complexing agents and the subsequent complexes, in order to clarify whether the complexing agents are viable candidates for *in vivo* use.

Selected methods of investigation have been employed, including NMR studies, protonation constant measurements, calorimetric determination of binding constants, crystallographic determination of structure, and biodistribution studies.

The remainder of this chapter is concerned with a brief description of the relevant coordination chemistry of the chosen radiolabels, leading to our choice of proposed macrocycle for synthesis.

1.5.2 Indium(III) And Gallium(III) -Coordination Chemistry And Proposed Macrocycles For Synthesis

Indium is a group III metal possessing the electron configuration $(Kr)4d^{10}5s^{2}5p^{1}$. The +3 ion is the common oxidation state. Many examples[34] of neutral octahedral complexes exist with both mono and polydentate ligands.

Indium complexes of EDTA and DTPA have been used in Radioimmunoimaging[35]. These anionic complexes, despite being stable at pH 7, readily protonate at a lower pH and as suggested earlier, are susceptible to competitive demetallation *in vivo*.

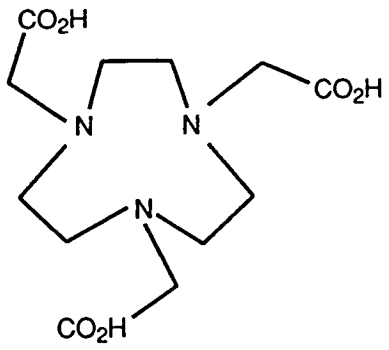
Gallium possesses similar coordination chemistry to indium, the tripositive ion being the most stable. Both the DTPA and EDTA complexes readily protonate at a lower pH.

Both gallium(III) and indium(III) readily hydrolyse in aqueous media. Thus the ligand employed should be tri-basic in order to neutralise the resultant complex. As suggested the complex ought to be hexadentate in order to coordinately saturate the ion.

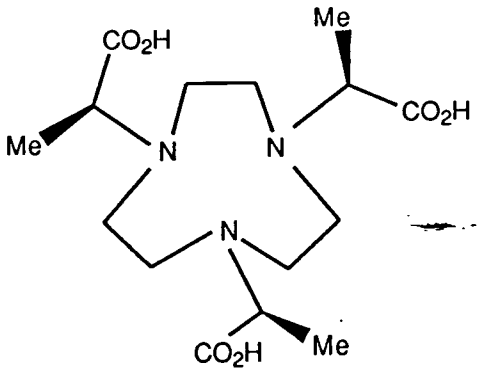
Complexes of indium(III) and gallium(III) with (3) (page 28) have been reported[35], and indium is bound very rapidly under ambient conditions. This suggests that the carboxylate or similar acid donor groups allow fast complexation, a prerequisite for radio isotopic tumour targeting. Also acid donor groups like the carboxylate group or alkylphosphinic acid group[36], will also resist protonation of the complex. The stability of the complexes of $9N_3C_3$ (3) has been studied and it has been shown that the complexes are very stable with respect to acid-catalysed dissociation, therefore being suitable for *in vivo* use.

Bearing this in mind, it was decided to synthesise (4), (5) and (6) (page 28). The addition of the methyl and phenyl groups with (4) and (5) should radically alter the lipophilicity (and the solubility) of the complexes, compared to (3), and should produce some interesting characteristics.

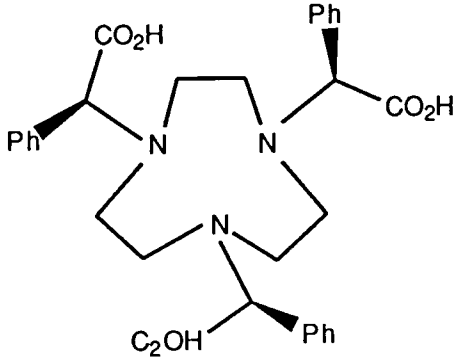
It was hoped in the synthesis of (6) that a successful synthetic route to phosphinic acid - polyazacycles could be found.



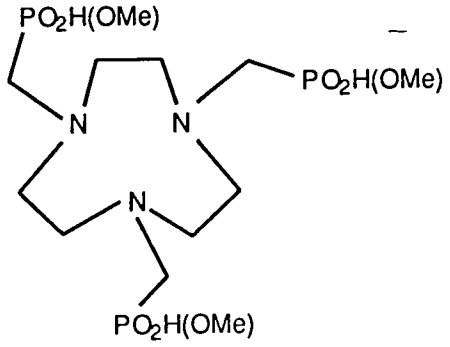
$9N_3C_3$-(3)



$9N_3C_3Me_3$-(4)



$9N_3C_3Ph_3$-(5)

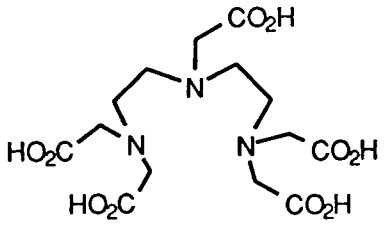


(6)

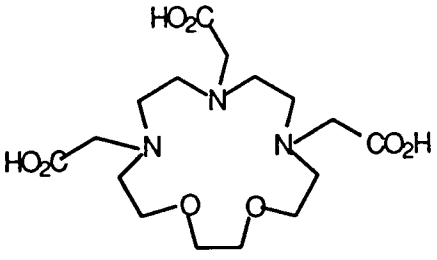
1.5.3 Yttrium Coordination Chemistry And Proposed Macrocycles For Synthesis

Yttrium has the electronic configuration $(Kr)5s^24d^1$. It lies above La in the transition group 3. The values of the atomic and ionic radius lie close to the corresponding values for terbium and dysprosium (the lanthanide contraction). Yttrium resembles the lathanides in its chemistry, forming a very stable tripositive ion. The yttrium(III) ion has low polarisability and tends to form electrostatic bonds with hard donors like O and N. The coordination of the tripositive ion is characterised by octadentate complexes[37].

DTPA is an acyclic complexing agent which forms a thermodynamically stable ($\log K_{ML}$ = 22.1) complex with yttrium(III).



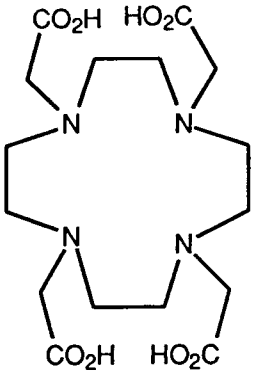
DTPA



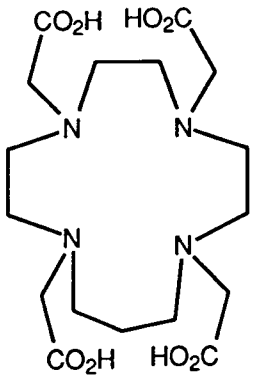
(7)

The good thermodynamic stability is not sufficient for *in vivo* use and the complex undergoes measurable demetallation in the human body[49].

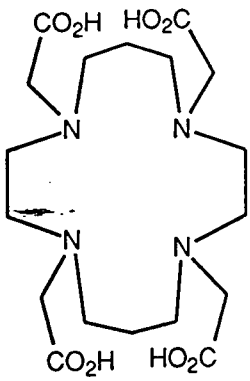
J.P.L. Cox[38] has compared the stabilities of the complexing agents on page 30, concluding that Y(DOTA)$^-$ is the most stable complex. Therefore it was decided in the light of this work to prepare ligand (7), which may be considered as a macrocyclic analogue of DTPA. In Chapter 5 the background chemistry is discussed in more detail.



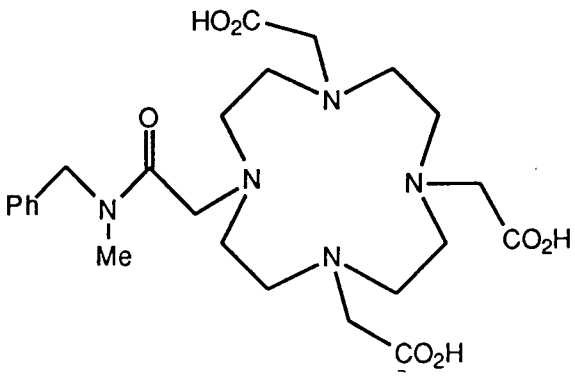
DOTA



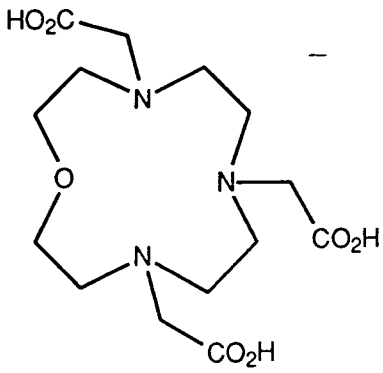
TRITA



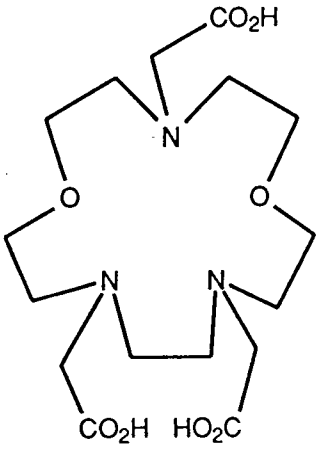
TETA



DOTA-BMA



ODOTRA

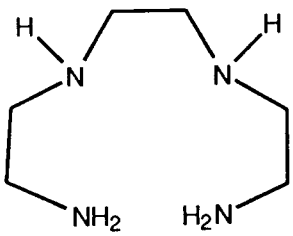


DTCTA

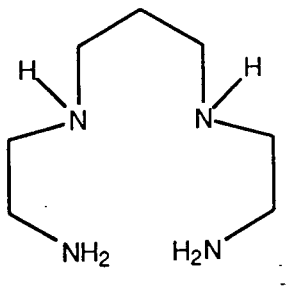
1.5.4 Rhenium And Technetium Coordination Chemistry And Proposed Macrocycle for Synthesis.

The extensive use of the metastable isotope $^{99m}Tc(\gamma = 140$ keV, $t_{1/2} = 6.02$ h) in medicine as a scanning agent for specific vital organs has been documented[46]. In recent years ^{186}Re ($\beta^- = 1.07$ MeV, $t_{1/2} = 90$ h) and ^{188}Re ($\beta^- = 2.12$ MeV, $t_{1/2} = 17$ h) have been suggested as suitable candidates for diagnostic work.[47] As mentioned earlier in this chapter these isotopes may be suitable for RIT if they can be irreversibly bound to a bifunctional complexing agent and subsequently attached to an antibody.

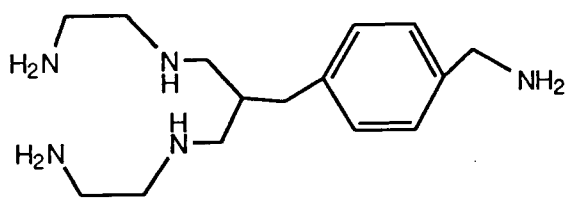
Parker and Roy[48] have made stable complexes with rhenium(V) of the type $[LReO_2]X$, (where X = Cl^-, $CH_3COO_2^-$, PF_6^-) where L is either L_1 or L_2 from below.



L_1



L_2



(9)

It was decided to synthesise an analogue of L_2, functionalising the ligand at the 6 position to allow attachment to an antibody.

Therefore it was proposed to synthesise (9) as a suitable complexing agent for rhenium and technetium.

1.5.5 Gold Coordination Chemistry And Proposed Complexing Agents For Synthesis.

Gold(I) readily disproportionates unless stabilised by strong complexing agents. Gold(III) is more stable but may be unsuitable for *in vivo* use, as it carries a +3 charge that cannot easily be neutralised by the complexing agent. In addition, complexes of gold(III) are strongly oxidising.

Gold(I) forms many complexes - linear, trigonal and tetrahedral. Linear complexes are favoured eg, $[Au(PPh_3)_2]^+$. There is a strong tendency for the gold atoms in linear complexes to interact with one another to produce polymeric chains[41], and for this reason it is necessarily difficult to form macrocyclic complexes.

Gold(I) forms a stable complex with ethylenethiourea (Figure 1.11) and an X-ray structure has been determined[41b]. However, the complex is susceptible to nucleophilic attack and is unstable at low and high pH[41c].

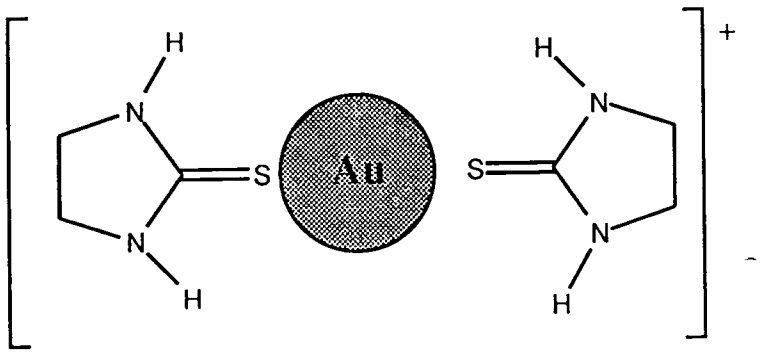


Figure 1.11 *The Gold(I) - Ethylenethiourea Complex*

After carrying out some modelling studies, using information gained from the aforementioned crystal structure, we proposed to synthesise two acyclic complexing agents (56) (figure 1.12) and (57) (figure 1.13). The 'bite' of the two complexing moieties is sufficient to fit the gold(I) ion and the use of a bidentate chelate should provide an advantage (*-the chelate effect*) over monodentate ligands, and may produce a more thermodynamically, as well as more kinetically stable complex.

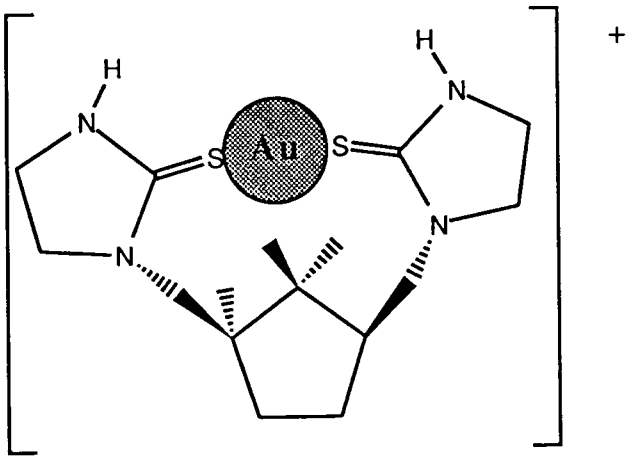


Figure 1.12 *The Proposed Gold(I) Complex Of (56)*

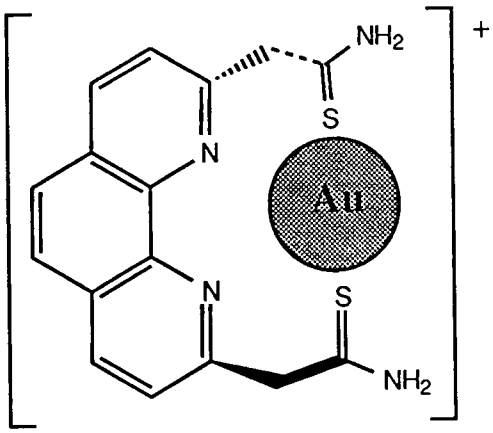


Figure 1.13 *The Proposed Gold(I) Complex Of (57)*

To begin with it was decided to synthesise (12) (figure 1.14), the lipophilic phenyl groups providing us with a monodentate molecule functionalised in such a way as to resemble the bidentate ligands (56) and (57). We hoped, due to the bulky side-arms, ~~th~~at the complexing agents would protect the gold ion from nucleophilic attack providing a more stable complex. Also if (12) was successful as a complexing agent for gold(I) we would synthesise (56) and (57).

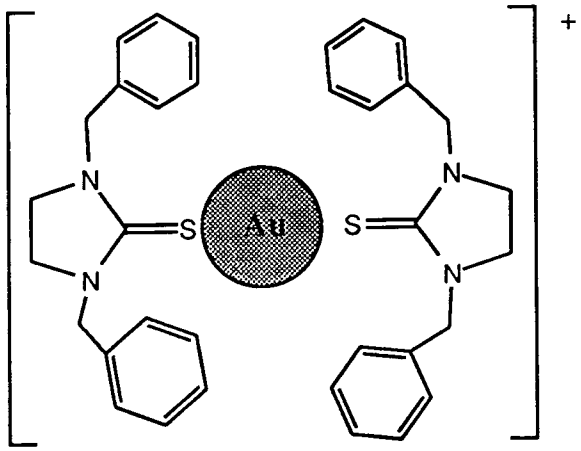
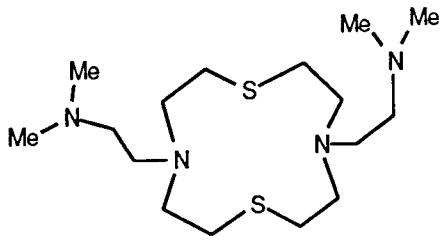


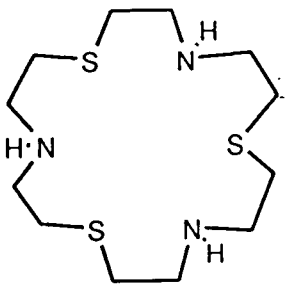
Figure 1.14 *The Proposed Gold(I) Complex Of (12)*

1.5.6 Silver Coordination Chemistry And Proposed Complexing Agents For Synthesis.

Silver prefers the unipositive oxidation state. The Silver(I) ion is large and polarisable, has a d^{10} electron configuration and an ionic radius of 1.15A°. From ligand field considerations, no coordination geometry is preferred, but in practice, linear and tetrahedral coordination are the most abundant. With macrocyclic complexing agents five and six coordinate complexes are common, and seven and eight coordination numbers are also known[42]. Silver(I) shows a preference for soft donor atoms eg. sulphur and nitrogen[43], and prefers the 18-crown cavity size[44]. Even though silver(I) has a greater preference for sulphur over nitrogen, because polysulphur cycles tend to adopt an 'endodentate' conformation[44], a mixed sulphur and nitrogen cycle will be preferred. Taking these points into consideration we proposed to synthesise (13) and (14).

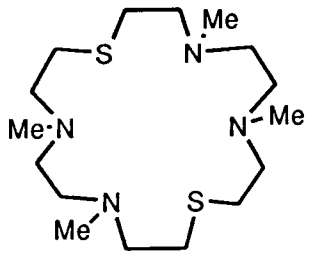


(14)

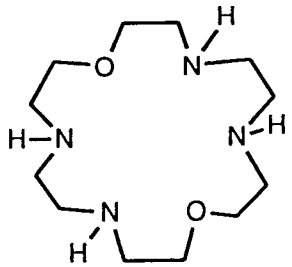


(13)

To further investigate the thermodynamic properties of the silver complexes of (15), (48), (49) and $18N_4S_2$ these macrocycles were synthesised.



(15)



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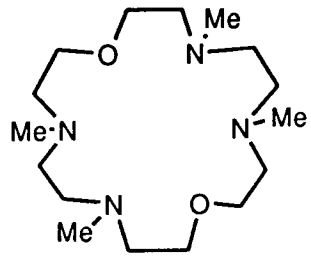

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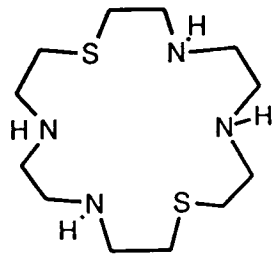

$18N_4S_2$

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CHAPTER TWO

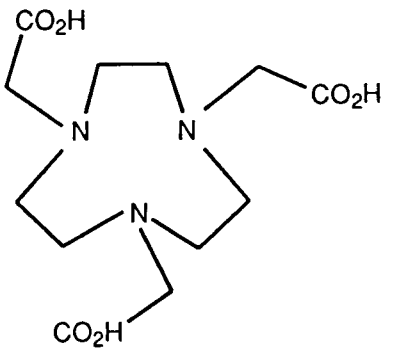
SYNTHESIS AND CHARACTERISATION OF COMPLEXING AGENTS $9N_3C_3Me_3$ AND $9N_3C_3Ph_3$ AND THE ATTEMPTED SYNTHESIS OF A PHOSPHINIC ACID POLYAZACYCLE.

2.0 SYNTHESIS AND CHARACTERISATION OF COMPLEXING AGENTS $9N_3C_3Me_3$ AND $9N_3C_3Ph_3$ AND THE ATTEMPTED SYNTHESIS OF A PHOSPHINIC ACID POLYAZACYCLE.

2.1 THE SYNTHESIS OF THE COMPLEXING AGENTS

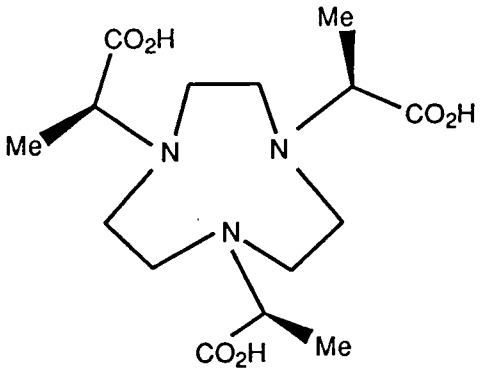
2.1.1 Introduction

The synthesis of $9N_3C_3$ (3) was first reported in 1973[1]. The subsequent formation of neutral stable complexes with Fe(III) and Cr(III) was reported in 1977[2].

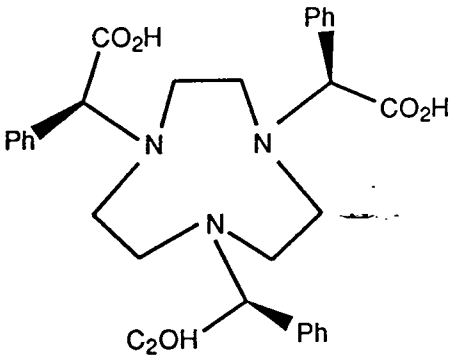


$9N_3C_3$-(3)

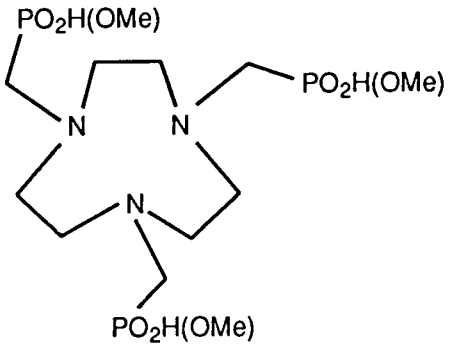
Stable complexes of (3) with indium and gallium have been synthesised in this laboratory[3]. As mentioned in the introduction (4), (5) and (6), are excellent models for the N-functionalised ligands (54) and (55) that may be attached to tumour-localising antibodies and rapidly radiolabelled with indium or gallium isotopes[3].



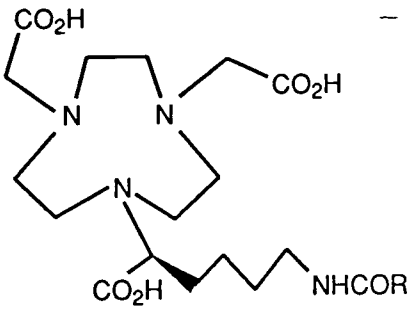
$9N_3C_3Me_3$-(4)



$9N_3C_3Ph_3$-(5)



(6)



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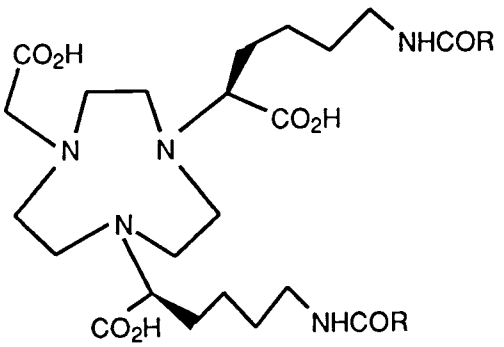
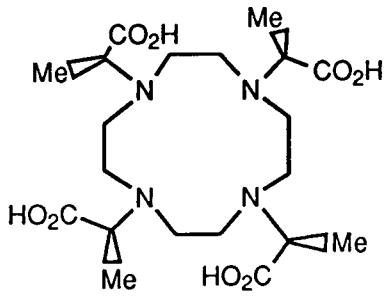


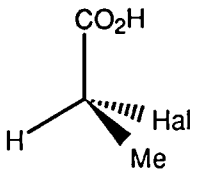
(55)

2.1.2 The Synthesis Of (R)-1, 4, 7 -Tris (2'-Methylcarboxymethyl)-Triazacyclononane(4)

Desreux has reported[4] the synthesis of the supposedly enantiopure tetraazacycle (17).



$12N_4C_4$(17)

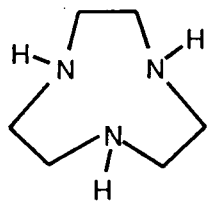


(19a, Hal=Br)

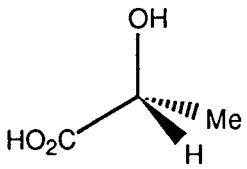
(19b, Hal=Cl)

The synthesis involves the nucleophilic attack of the tetra-amine's nitrogen atoms on the electron deficient carbon of the substituted acid (19a). The configuration of the stereocentre at C-2 is inverted during the concerted S_N2 reaction.

For our synthesis, we used 1,4,7-triazacyclononane (22) with the enantiopure α–chloropropanoic acid (19b).



$9N_3$(22)



(18)

The liberated proton from the amine lowers the pH of the reaction mixture as the reaction proceeds, and is a suitable method of monitoring the reaction. The initial pH of the reaction was adjusted

to pH 10, with lithium hydroxide and the pH was maintained throughout the reaction by adding solid lithium hydroxide. If the pH falls too low, protonation of the nitrogen occurs inhibiting alkylation. If the pH was taken much higher than pH10, the possibility of competitive nucleophilic attack by hydroxide ions is increased (Figure 2.1), resulting in the formation of the unwanted alpha-hydroxy acid (18). Concurrent base catalysed racemisation of the enantiopure alpha-chloro acid is also more likely at elevated pH (Figure 2.2).

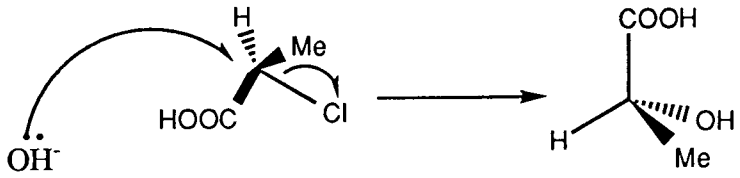


Figure 2.1 *Competitive Nucleophilic Substitution From Hydroxide Ions*

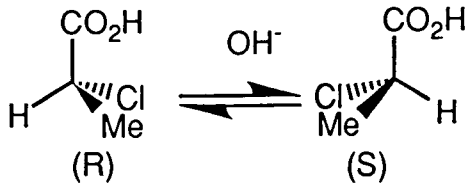
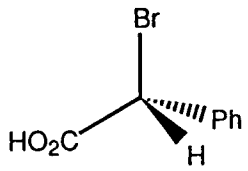


Figure 2.2 *Base Catalysed Racemisation*

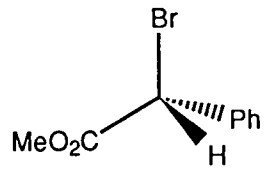
Purification was attempted by recrystallisation at pH 12, 7 and 3. These attempts proved fruitless, and the complexing agent was purified by column chromatography as the tri-ester ($MeOH/H^+$), followed by hydrolysis giving the hydrochloride salt, (HCl(6M) 120°C, 18h).

2.1.3 The Synthesis of 1,4,7-Tris (2'-Phenylcarboxy methyl)-1,4,7-Triazacyclononane (5)

The synthesis of (5) was approached in a similar manner to that of (4). However, the partial insolubility of the phenyl substituted acid (20) in aqueous methanol retarded the progress of the reaction, (followed as a function of the pH of the reaction mixture).



(20)



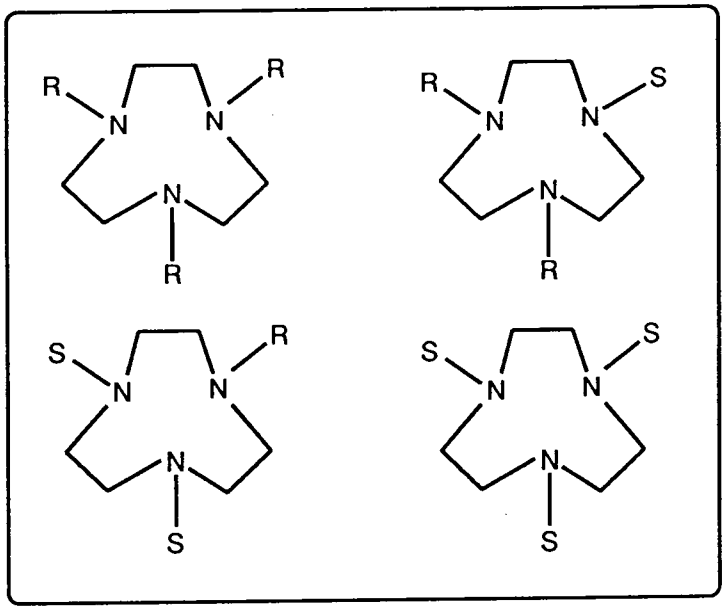
(21)

Therefore the synthesis of the methyl ester (21) of (20) was undertaken and (21) was purified by column chromatography. The reaction of $9N_3$ (22) with the racemic ester in a suitable polar solvent (CH_3CN)[1] was successful, and the compound was purified with column chromatography, followed by acidic hydrolysis.

At this stage, it must be noted that the purified compound was most probably a mixture of the four possible stereoisomers, RRR, SSS, SRS, RSR (i.e. two chiral diastereoisomers).

---

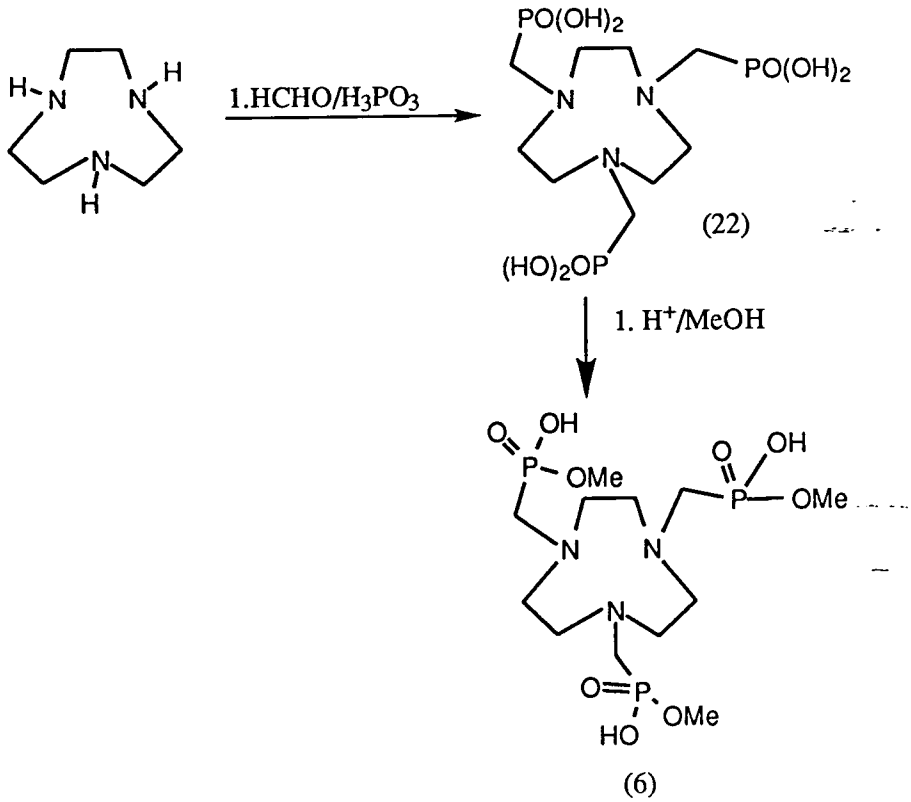
[1] This reaction was tried in a number of solvent/base systems in order to improve the relatively slow rate of alkylation. Acetonitrile and LiOH (pH maintained at 8 - 9) or THF/methanol/ CH_3CN with K_2CO_3were both tried, the latter proved to be the best system and was chosen for further syntheses of this molecule.



The Possible Stereoisomers

2.1.4 The Attempted Synthesis of O,O',O''-Trimethyl-1,4,7-tris(phosphonatomethyl)-triazacyclononane(6)

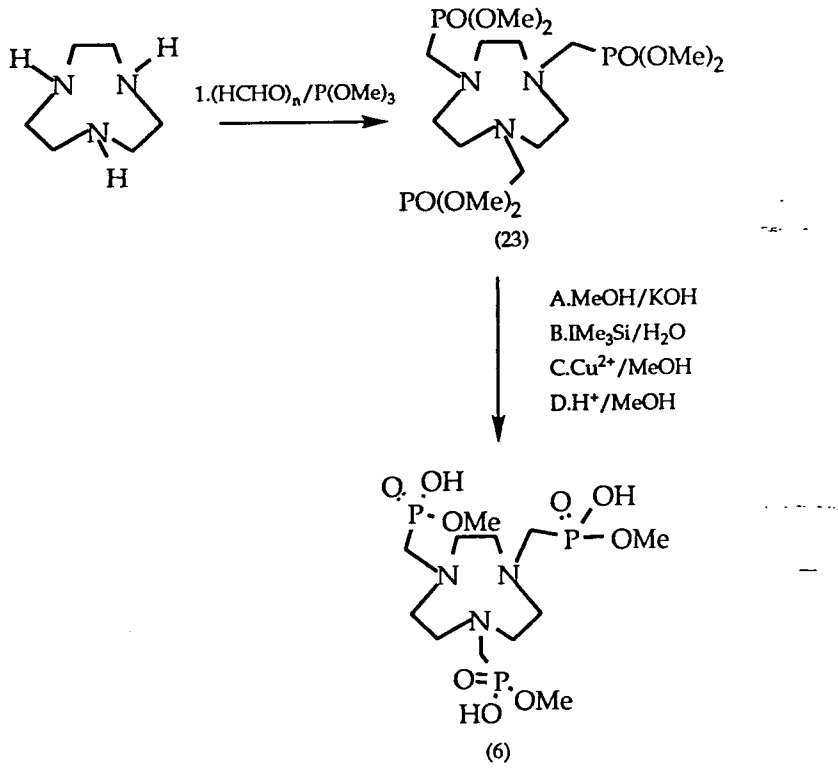
The proposed synthesis of (6) is in Scheme 2.1. The initial reaction of $9N_3$ with formaldehyde and H_3PO_3 to form (22) has been used in this laboratory previously [5] with success.



Scheme 2.1

Following formation of (22) and purification by recrystallisation, the amino phosphinic acid was boiled under reflux in methanol in the presence of sulphuric acid. According to the ^{31}P and ^{1}H NMR and mass spectral analysis, the nitrogen-carbon bond had cleaved under these conditions.

An alternative route was attempted, (Scheme 2.2).



Scheme 2.2

The first step involving the formation of (23) was successful. Selective hydrolysis was attempted by the following four methods:

a) The use of MeOH/KOH was entirely unsuccessful and produced no detectable reaction (NMR and Mass Spectrum).

b) Dealkylation using Me_3SiI, involving alkyl-oxygen cleavage, yielded a large mixture of species as deduced by ^{31}P and ^{1}H NMR.

If this reaction had been selective and therefore successful, in so much as it was mainly the desired product, simple base hydrolysis should have resulted in the target molecule.

c) The use of copper to control the base hydrolysis was suggested by Thomas Kaden and co-workers[6], who achieved selective hydrolysis of diethyl phosphonate moieties attached to tetraazacycles. With or without the copper present, no reaction could be detected even at elevated temperature. This result could be explained as an

effect of the smaller ring size of the 9-membered cycle, and thus the proximity of the copper to the reaction site would be very different than the case with the 12-membered cycle.

d) Finally, acid hydrolysis (H_2SO_4/MeOH) was attempted which produced a large mixture of inseparable products as deduced from ^{1}H and ^{31}P NMR spectra.

2.2 CHARACTERISATION OF $9N_3C_3Me_3$ AND $9N_3C_3Ph_3$

2.2.1 Introduction

This section reports the information acquired about the two new hexadentate ligands $9N_3C_3Me_3$ and $9N_3C_3Ph_3$. The structures of the new complexing agents and of selected complexes (Cu(II), Ca(II), Ga(II), In(III)) were determined using ^{1}H and ^{71}Ga NMR, mass spectral analysis and X-ray crystallographic methods of analysis. Binding constants of $9N_3C_3Me_3$ with Ca(II) and Cu(II) were measured by pH potentiometric titrations, as were the pK_a values of the ligands. Having gained the confidence that the gallium and indium complexes were of similar stability to the parent ligand ($9N_3C_3$), the *in vivo* kinetic stability was investigated through biodistribution studies of the ^{67}Ga-ligand complexes in mice. Further to this, an assessment of the localising properties of the complexes in a xenograft of a human tumour in mice was investigated. The details of that investigation are reported in *chapter 3.*

2.2.2 Synthesis And Structural Analysis Of [In(III).$9N_3C_3Me_3$]

The neutral indium(III) complex was prepared at pH 2 following admixture of equimolar quantities of $9N_3C_3Me_3$ and indium trinitrate solutions. The complex readily crystalised at room temperature, producing clear and colourless crystals. The crystals showed unusual insolubility and would not dissolve in any common laboratory solvent.

X-ray analysis[1] (figure 2.4) revealed that indium is very tightly bound by the oxygen and nitrogen donors, with shorter In-O and In-N bond lengths than any previously reported hexacoordinate indium complex(7). The crystal possesses an orthorhombic space group ($P22_12_1$) and takes the form of a distorted trigonal prism. The angle of distortion (twist angle, figure 2.3) is 20.8°. This value is approximately the same as that found for the ferric complex of the parent triacetate ligand(8)

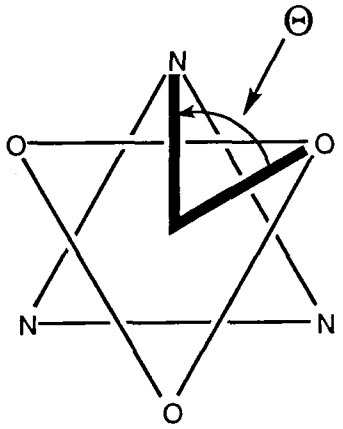


Figure 2.3: *The Twist Angle (Θ) - A measure Of The Distortion From C_3 Symmetry*

[1]The X-ray crystal structure was determined by Professor George Ferguson (Department of Chemistry, University of Guelph, Ontario, Canada).

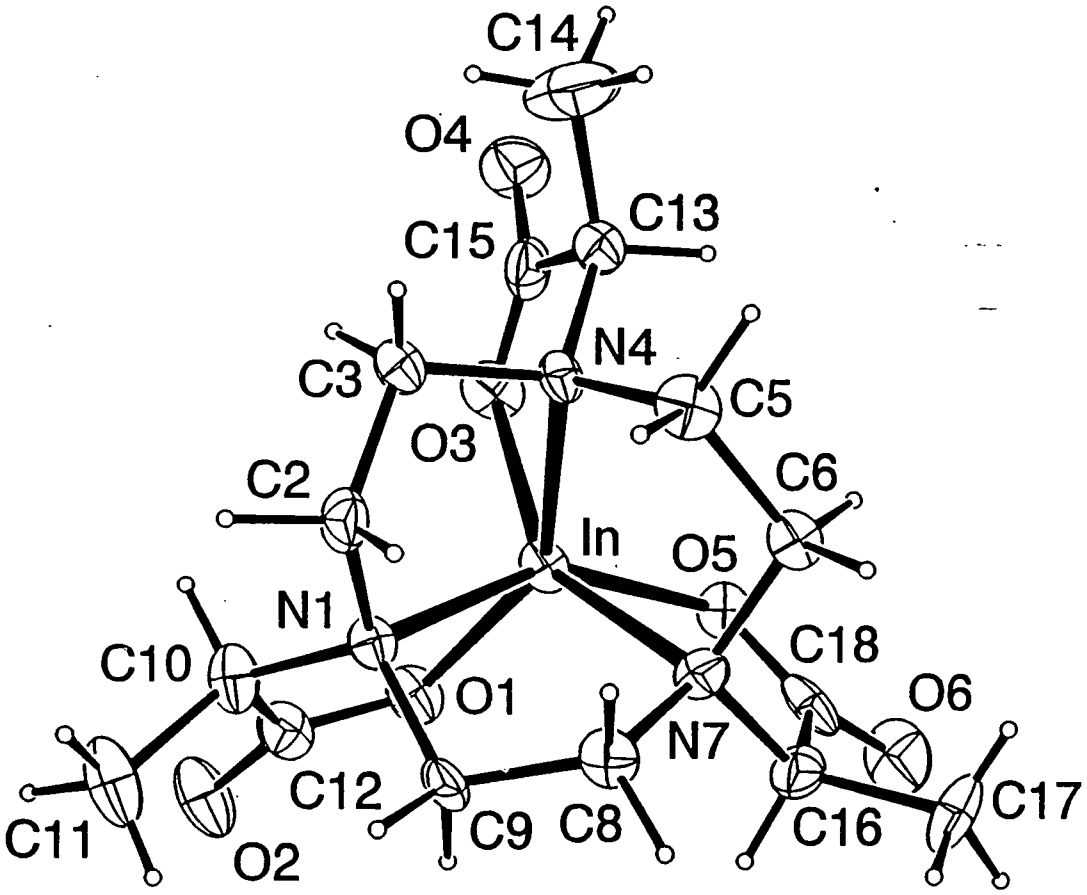


Figure 2.1 *Perspective (ORTEP) View of the Indium -9$N_3C_3Me_3$ Complex.* Selected bond lengths and bond angles: In-N(1) 2.258(4), In-N(4) 2.270(5), In-N(7) 2.254(5), In-O(1) 2.101(4), In-O(3) 2.094(4), In-O(5) 2.094(4)A°; N(1)-In-N(4) 79.2(2), N(1)-In-N(7) 79.7(2), N(4)-In -N(7) 79.3(2), O(1)-In-O(3) 93.6(2), O(1)-In-O(5) 93.9(2), O(3)-In-O(5) 94.1(2), N(4)-In-O(3) 77.0(2), N(7)-In-O(5) 77.0(2), N(1)-In-O(1) 77.7(1).

It is the first structural analysis of a neutral indium(III) complex with hexacoordinate geometry which possesses non-crystallographic C_3 symmetry.

Using a similar method to that described above, an attempt was made to crystalise $9N_3C_3Me_3$ with gallium(III) and copper(II). The admixture of the ligand with gallium trinitrate solution produced an unusual result. After 60 minutes the solution became viscous and opaque. Presumably, this was caused by the insolubility of the complex. The reason why it should show a marked difference to the indium-ligand complex is unclear. The difference in the ion size and thus the polarising strength may render the complex more lipophilic causing it to come out of solution so quickly that it has no time to crystallise uniformly as in the case of the corresponding indium complex.

The copper complex did not crystallise. Changes in the concentration of the solution and in the pH made no difference and the solution did not produce any crystals.

Similar attempts were made with the complexing agent $9N_3C_3Ph_3$ and gallium, indium and copper. With gallium and indium, the complex was very insoluble -similar to the gallium-$9N_3C_3Me_3$ complex, and it did not prove possible to grow any crystals for X-ray analysis. With copper(II), crystals have been grown but they were deliquescent and it was not possible to analyse them using X-ray methods.

2.3 ^{71}Ga NMR INVESTIGATION OF THE STRUCTURE OF THE GALLIUM COMPLEXES OF $9N_3C_3Me_3$ AND $9N_3C_3Ph_3$

Using ^{71}Ga NMR, [$9N_3C_3Me_3$.Ga] can be observed in 6M $HN0_3$ as a singlet at +149 ppm ($\omega_{1/2}$ = 1100Hz). The investigation was problematic due to the aforementioned solubility of the gallium complexes of these ligands. The solution had to be introduced to the NMR probe immediately after mixing, to enable enough time for the measurement to be taken before the complex came out of solution.

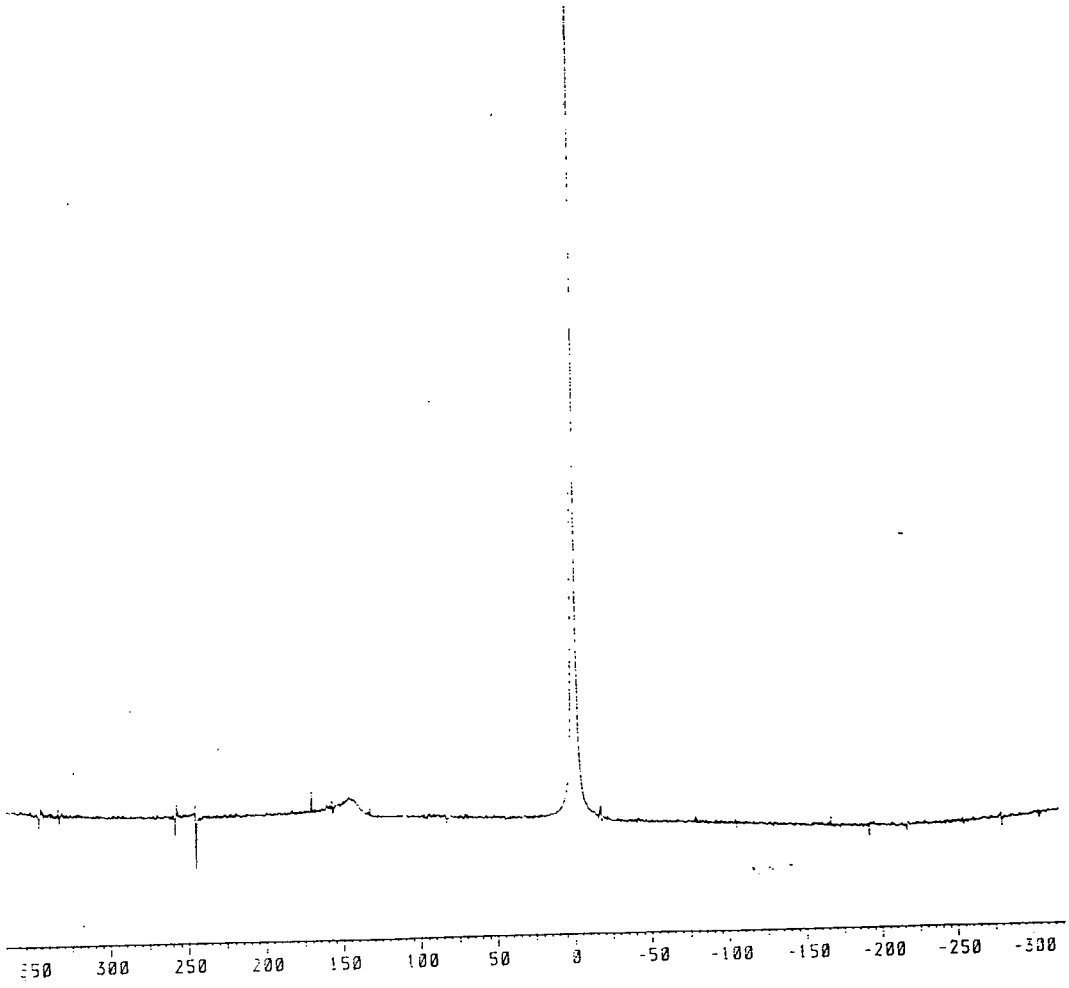


Figure 2.5 ^{71}Ga NMR of the Gallium Complex of $9N_3C_3Me_3$
The reference signal is Gallium trinitrate in 6M HN03

Figure 2.5 shows the ^{71}Ga spectrum, and it can be seen that the signal width of the complex at the baseline is broader than that of the reference complex $Ga(H_2O)_6^{3+}$. The origin of line broadening with quadrupolar nuclei is related to the interaction of the electric field gradient with the quadrupolar moment at the nucleus. Any distortion of the field gradient away from cubic symmetry results in an interaction with the nuclear quadrupole causing an increase in the relaxation times T_1 and T_2. The effect of this is to produce line broadening as illustrated in figure 2.5. The gallium ion in the nitric acid solution is symmetrically surrounded by water molecules producing minimal interaction of the electric field gradient and quadrupole at the nucleus resulting in a very sharp signal peak. The fact that the complex gives such a broad signal would suggest that the symmetry of the complex is distorted from the C_3 symmetry as we might expect from a comparison with other similar complexes.

Complexing Agent	NMR Signal ppm (pH)	$\omega 1/2$ Hz	Complex Symmetry	Twist Angle degrees
$9N_3C_3Me_3$	+149 (0.7)	1100	non C_3	20.5
$9N_3C_3$[9]	+171 (0.7)	210	C_3	0
$9N_3P_3Ph_3$[9]	+132 (0.7)	560	$\approx C_3$	7.9

Table 2.1: *A Comparison Of ^{71}Ga NMR Signals And Complex Symmetry For Related Complexes*

Table 2.1 shows the relationship between line width ($\omega_{1/2}$) and the symmetry of the complex in its crystalline form.

Complexes of $9N_3C_3$ possess crystallographic C_3 symmetry[9] and the $9N_3C_3$-gallium complex produces a sharp signal with similar

line width to $[Ga(H_2O)_6]^{3+}$ ion. Crystallographic analysis of Ga-$9N_3P_3Ph_3$[10] complex shows the complex possesses only approximate crystallographic C_3 symmetry and the ^{71}Ga NMR signal is rather broad ($\omega_{1/2}$ = 560Hz). The $9NC_3Me_3$ complex shows even greater line broadening and this may suggest that the departure from C_3 symmetry in solution is even greater than that of $9N_3P_3Ph_3$. Comparing the twist angle (the angle between the oxygen donor and the nearest nitrogen donor in the ring, (Figure 2.3) of the crystals of each complex (Table 2.1), a similar trend is apparent, suggesting that the solid state conformation is similar to the average conformation in solution.

The insolubility of the gallium complex of $9N_3C_3Ph_3$ prevented any determination of the ^{71}Ga NMR spectrum.

2.4 DETERMINATION OF PROTONATION AND BINDING CONSTANTS OF $9N_3C_3Me_3$

Using pH potentiometric titrations, the protonation and selected binding constants have been obtained for $9N_3C_3Me_3$. The experimental procedure (Section 5.5) employed was similar to that used by Zompa[10] in his investigation of the parent ligand $9N_3C_3$.

The ligand or 1:1 complex of known concentration was titrated against an accurately calibrated alkaline solution and the data analysed using SCOGS to obtain the required equilibrium constants.[1]

Ligand	pK_1	pK_2	pK_3	$logK_{CaL}$	$logK_{CaLH}$	$logK_{CuLH2}$*
$9N_3C_3$ (7a)	11.2	6.10	3.48	10.8	5.75	2.9
$9N_3C_3Me_3$	11.7	5.74	3.16	8.92	5.06	2.77
Difference	0.5	0.36	0.32	0.12	0.69	0.13

Table 2.2 *The pK_a and Stability Constants of $9N_3C_3$ and $9N_3C_3Me_3$*

* From spectraphotometric analysis of the 780 nmand 660 nm bands

The results of our investigation show that complexing agent $9N_3C_3Me_3$ shows similar complexing behaviour to the parent $9N_3C_3$. The steric effect of the methyl group in the alpha position is limited as it is exocyclic. The bond attaching it to the cycle may freely rotate so that the methyl moiety does not interfere with complexation (Figure 2.6 and Figure 2.1).

[1]The analysis of the data was carried out at the University of Durham by Dr Ritu Kataky.

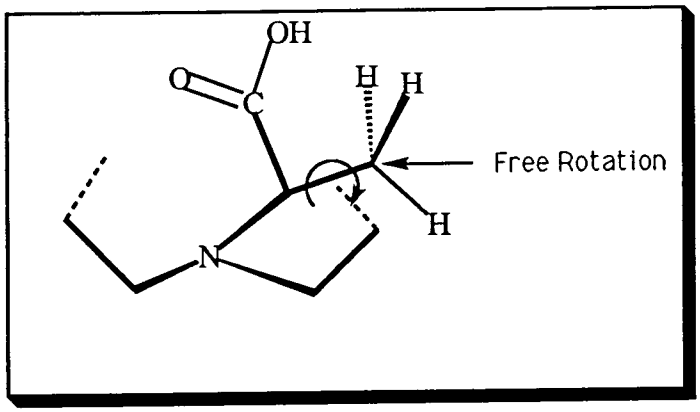


Figure 2.6 *The Alpha-Methyl Group May Rotate Freely*

The electronic effect of the methyl group, may slightly increase the electron density at the carbonyl, although the measurements suggest the effect is negligible.

From this comparison, it is clear that $9N_3C_3Me_3$ possesses similar complexing abilities to the parent $9N_3C_3$ and the effect of the methyl group on the ability to form complexes is minimal, although the change in the solubility of the resultant complexes is marked.

2.5 VISIBLE SPECTRUM ANALYSIS OF $[Cu.9N_3C_3Me_3]^{2+}$ AND $[Cu.9N_3C_3Ph_3]^{2+}$

The copper(II) complexes of $9N_3C_3Me_3$ and $9N_3C_3Ph_3$ are blue. The parent ligand complex $[Cu.9N_3C_3]^{2+}$ forms two distinct species with copper(II) between pH 1.8 and 3.5. Within this pH range, the protonation of the species $[Cu.9N_3C_3]^-$ occurs forming $H[Cu.9N_3C_3]$ [10] (equation 2.1).

$$[Cu.9N_3C_3]^- + H^+ \rightleftharpoons H[Cu.9N_3C_3]$$

Equation 2.1

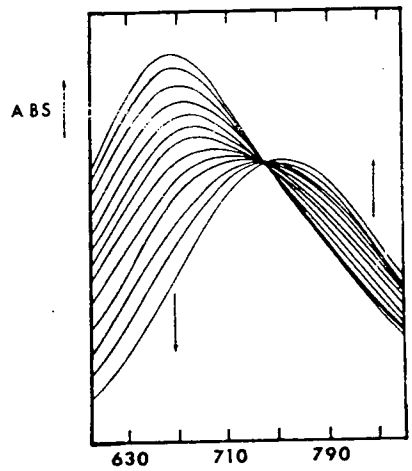


Figure 2.7 *The Visible Spectrum Of The $9N_3C_3$-Copper(II) Complex At Various Values Of pH*[10].

The two species possess different absorption maxima and are distinguishable using visible spectroscopy. Zompa used this quantifiable difference to determine the pK_a value for the equilibrium shown in equation 2.1. Using accurately known concentrations of ligand and copper (II) solutions, the pH was varied between pH 1.8

and pH 3.5 in small accurate increments and the visible spectrum was recorded.

The two distinct peaks at either side of the figure correspond to the two distinct species. Situated between them is the isosbestic point (720 nm), which is related to the equilibrium of the two complexes. Using the information from the visible spectrum and the exact concentrations of the reagents, the pK_a of the protonation can be calculated as follows:

If the extinction coefficients of the species are calculated using the Beer-Lambert equation (1):

$$\log I°/I = A = \varepsilon.l.c \qquad (1)$$

Where the optical density, A, can be measured at λ_{max} for a known concentration, c, to give a value for e at l_{max}. Therefore at an accurately known value of pH the concentration of each species can be calculated, using the Beer-Lambert relationship, by measuring the absorption value at either value of λ_{max} and combining this result with (2).

$$[Cu.L] + [Cu.LH] = [Cu.L]° \qquad (2)$$

Where [Cu.L]° is the initial concentration of copper complex

The pK_a of the protonation (2), can then be calculated from (3);

$$K_{Cu.LH} = \frac{[Cu.L] \cdot [H^+]}{[Cu.LH]} \qquad (3)$$

When I = 1.0M

The pH gives a value of $[H^+]$ (I=1.0), and the concentrations of species can be calculated as described above. Therefore at different values of pH a value for the pK_a can be calculated.

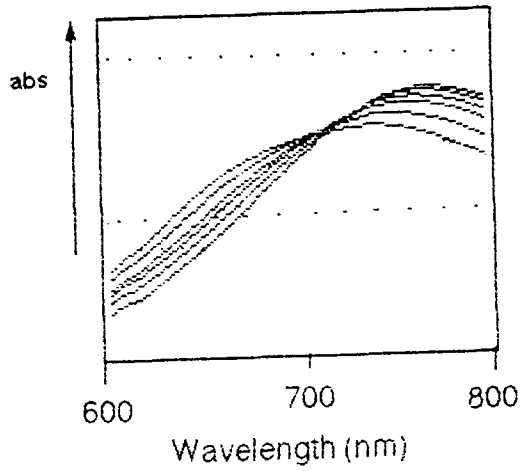


Figure 2.8 *The Visible Spectrum Of The Copper - $9N_3C_3Me_3$ Complex Between pH 2.0 And pH 3.2*

Using a similar but less rigourous method to Zompa's, the experiment was repeated to estimate the pK_a of this protonation for the two new complexing agents.

The visible spectrum, of a known concentration of the Cu-$9N_3C_3Me_3$ complex at a known value of pH(2.0-3.4), was measured, (I=1.0 M, $NaClO_4$). The pH was adjusted in small increments and the spectrum measured again. The visible spectra are shown in figure 2.8 and figure 2.9.

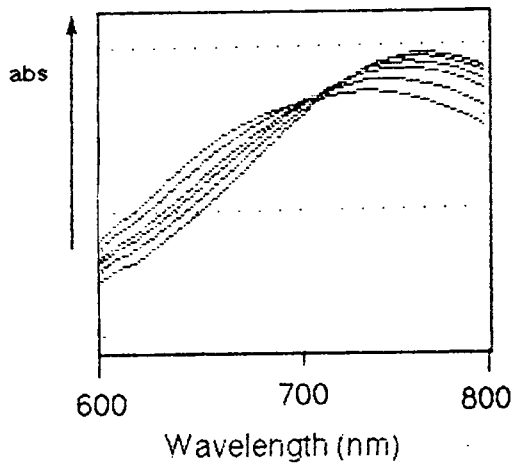


Figure 2.9 *The Visible Spectrum Of The Copper - $9N_3C_3Ph_3$ Complex Between pH 2.0 And pH 3.2*

As with $9N_3C_3$-copper complex , the isosbestic points are clearly visible at 720 nm as are the peaks corresponding to the individual species, within this pH range.

From this information, using the procedure outlined above, we estimated that this pKa for the two new complexing agents is approximately 2.9±0.2 @ 25°C, very similar to that for the $9N_3C_3$-copper complex.

The method Zompa used was extremely thorough, using SQUAD, to analyse his data. Our investigation experiment was much less rigourous, and the value calculated for both values of the pka carries an error of ± 0.2.

2.6 REFERENCES

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CHAPTER THREE

BIODISTRIBUTION STUDIES OF THE GALLIUM AND INDIUM COMPLEXES OF $9N_3C_3Me_3$ AND $9N_3C_3Ph_3$ IN ATHYMIC NU:NU MICE

3.0 BIODISTRIBUTION STUDIES OF THE GALLIUM AND INDIUM COMPLEXES OF $9N_3C_3Me_3$ AND $9N_3C_3Ph_3$ IN ATHYMIC NU:NU MICE

3.1 INTRODUCTION

Interpretation of the U.V spectra, NMR data, crystal analysis and stability constant measurements of the two new complexing agents $9N_3C_3Me_3$ and $9N_3C_3Ph_3$, suggests that both ligands form stable indium(III) and gallium(III) complexes and possess similar coordination chemistry to the parent $9N_3C_3$[4]. As mentioned previously the *in vivo* kinetic stability of the complexes is of the upmost importance to their suitability for use in diagnostic and nuclear medicine (^{111}In, γ, $t_{1/2}$ = 2.83 days; ^{67}Ga, γ, $t_{1/2}$ = 3.25 days; ^{68}Ga, β^+, $t_{1/2}$ = 68min) .

With variable pH (2-8) and a high concentration of cations *in vivo*, it is crucial that the complex remains stable despite the *in vivo* environment. We knew from the results of previous biodistribution[2] investigation that the parent gallium complex, gallium-$9N_3C_3$, possesses high stability *in vivo*, with virtually no decomplexation occurring. A further biodistribution study was undertaken to test and compare the *in vivo* kinetic stability of gallium and indium complexes of $9N_3C_3Me_3$, $9N_3C_3Ph_3$, $9N_3P_3Me_3$* and $9N_3P_3Ph_3$** with that of the parent complex.

Later in this chapter, localising properties of the complexes within a human melanotic melanoma implanted in mice, are reported.

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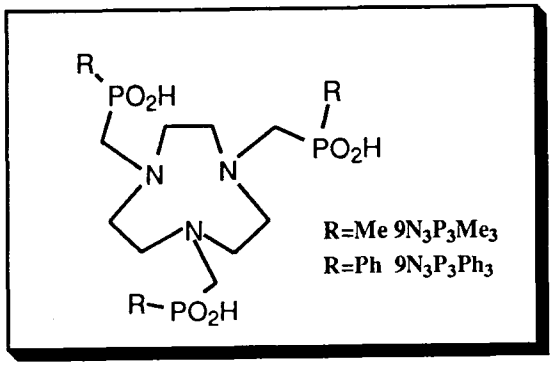
* $9N_3P_3Me_3$ wase synthesised by C. Broan at the University of Durham.

** $9N_3P_3Ph_3$ were synthesised byT. Norman at the University of Durham.

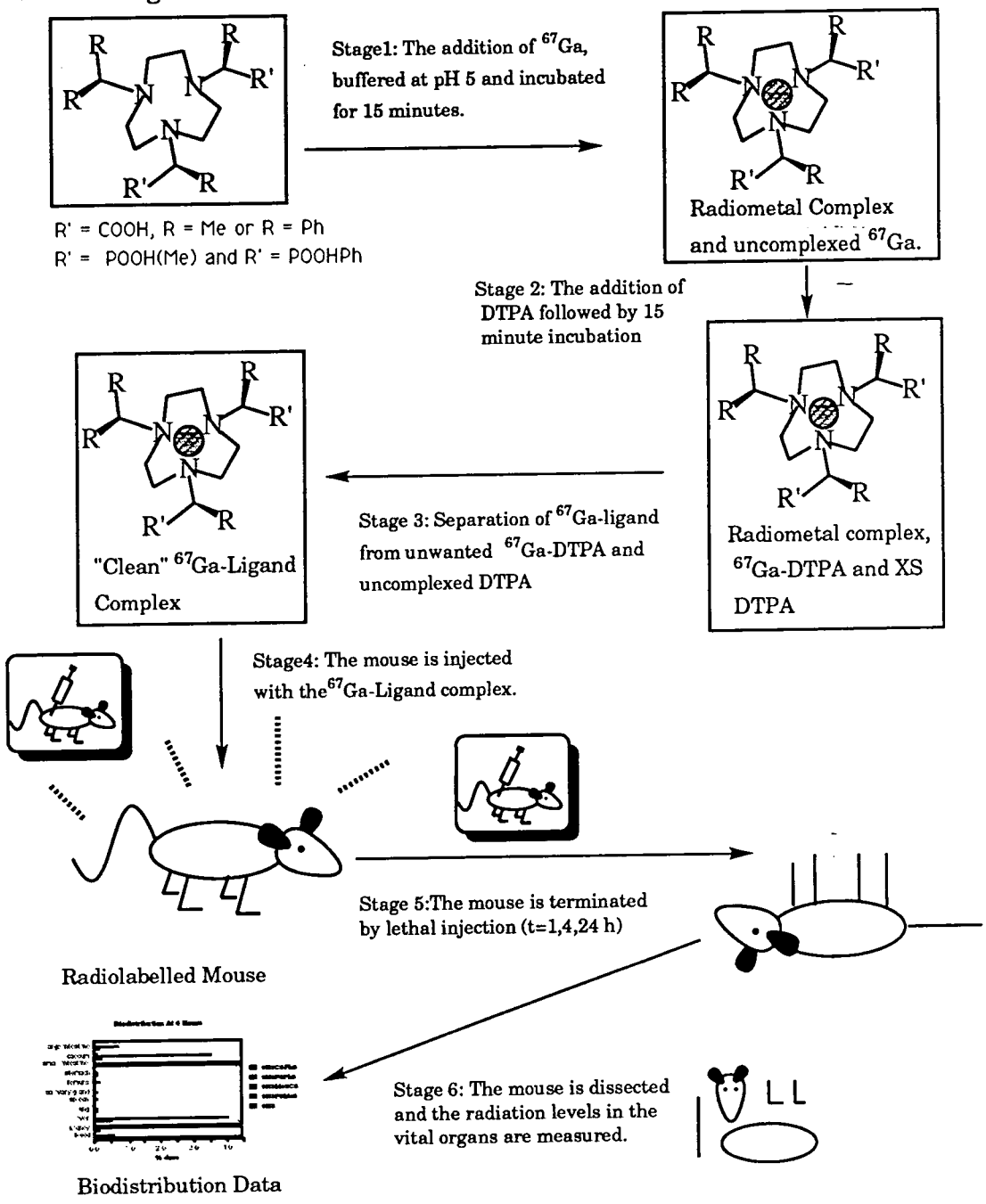
3.1.1 The Phosphinic Acid-9N3 Complexing Agents

All these complexeing agents are derived and synthesised from the cyclic polyamine 1,4,7-triazacyclononane. The physical and chemical properties of $9N_3C_3Me_3$ and $9N_3C_3Ph_3$ have already been discussed in chapter 2. The other two complexing agents, $9N_3P_3Me_3$ and $9N_3P_3Ph_3$ are aminoalkylphosphinic acids and possess distinct chemical differences to the carboxylic acid analogues[5,6]. Compared to a carboxylic acid, the phosphinic analogue is usually more acidic and the complex is more resistant to protonation and acid-mediated decomplexation. In addition, when the oxygen of the phosphinic acid is interacting with a metal, the position of the phosphinic acid is fixed and a new stereogenic centre is produced at the pentavalent phosphorus, allowing the possibility of stereoisomeric complexes. Similarly to the two carboxylate complexing agents they are hexadentate, and possess strong complexing characteristics.



3.2 EXPERIMENTAL METHOD OF INVESTIGATION[1]

Scheme 3.1 shows an overview of the experimental procedures. Details are given in section 5.3.



Scheme 3.1 *Experimental Method*

[1]These experiments were carried out by A. Harrison and L. Royle, MRC, Radiobiology Unit, Didcot, Oxon.

3.3 BIODISTRIBUTION DATA FOR THE GALLIUM COMPLEXES IN NON-TUMOUR BEARING MICE.

3.3.1 The Data

Gallium One Hour	Citrate	9N3	9N3P3-Me3	9N3C3-Me3	9N3P3-Ph3	9N3C3-Ph3
Tissue	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose
blood	11.55 39.13	0.15 0.53	0.09 0.31	0.11 0.41	0.12 0.40	0.15 0.55
kidney	3.92 2.04	4.57 3.28	3.18 1.89	8.37 5.20	3.49 1.97	5.78 4.55
liver	2.61 4.89	0.65 1.18	0.14 0.26	2.10 4.01	0.65 1.32	0.41 0.87
lung	5.20 0.98	0.42 0.08	0.24 0.05	0.17 0.03	0.14 0.03	0.14 0.030
spleen	2.26 0.27	0.11 0.02	0.07 0.01	0.10 0.01	0.08 0.01	0.07 0.011
salivary glands	2.83 0.62	0.08 0.03	0.07 0.02	0.08 0.02	0.05 0.01	
urine	19.4	449.8	257.4 0.28	268.8	119.00	146.3
brain		0.017 0.008			0.010 0.0047	0.009 0.0045
femur *2	3.16 0.56	0.07 0.01	0.07 0.01	0.07 0.01	0.05 0.10	0.07 0.014
gall bladder		0.034			0.185	0.471
muscle skel.	1.18	0.06	0.08	0.06	0.05	0.06
stomache	0.80	0.05	0.03	0.03	0.13 0.07	0.07 0.05
sm. intestine	4.92	0.61	0.36	0.79	15.7 31.87	25.2 57.39
caecum	1.03	0.06	0.08	0.11	4.6 3.45	0.07 0.06
la. intestine	1.65	0.08	0.04	0.10	1.04 0.70	0.06 0.05
no. of mice	4	5	7	4	4	3
mean wt g.	34.0	36.7	35.2	37.2	33.5	36.9

Table 3.1 *The Biodistribution at 1 Hour*

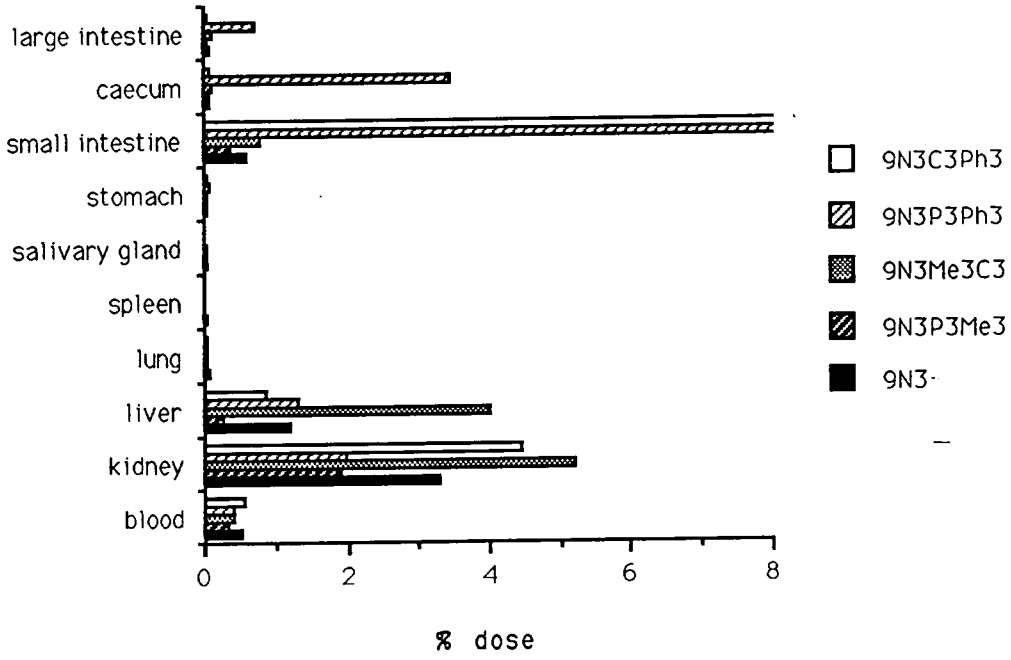
Gallium 4 Hours	Citrate	9N3	9N3P3-Me3	9N3C3-Me3	9N3P3-Ph3	9N3C3-Ph3
Tissue	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose
blood	8.26 30.37	0.005 0.021	0.005 0.018	0.023 0.081	0.058 0.02	0.121 0.420
kidney	4.63 2.95	1.11 0.76	1.86 1.21	3.74 2.39	0.75 0.47	0.551 0.293
liver	3.09 6.44	0.29 0.73	0.084 0.17	1.34 2.8	0.3 0.55	0.216 0.367
lung	3.62 0.97	0.05 0.01	0.05 0.011	0.06 0.013	0.05 0.01	
spleen	3.17 0.47	0.04 0.006	0.033 0.005	0.07 0.008	0.036 0.004	0.055 0.006
salivary glands	2.32 0.62		0.03 0.008	0.05 0.012	0.032 0.008	
urine	7.49	16.5	15	18 2	32	10 0.7
brain		0.009 0.004			0.005 0.002	0.007 0.003
femur *2	3.39 0.67	0.019 0.003	0.022 0.004	0.036 0.006	0.035 0.007	0.082 0.014
gall bladder		0.001		0.05	1.35 0.018	0.100
muscle	0.92	0.016 0.02	0.015		0.029 0.006	
stomach	0.90	0.02	0.069	0.014	0.05 0.033	0.09
small intestine	7.44	0.17	0.21	0.36	0.37 0.74	1.35
caecum	. 2.79	0.28	0.19	0.52	13.3 11.4	19.46
large intestine	2.24	0.30	0.12	0.51	16.7 15.3	21.19
no. of mice	3	4	3	4	3	4
mean weight g.	36.2	41.5	38.3	34.7	34.7	34.9

Table 3.2 *The Biodistribution at 4 Hours*

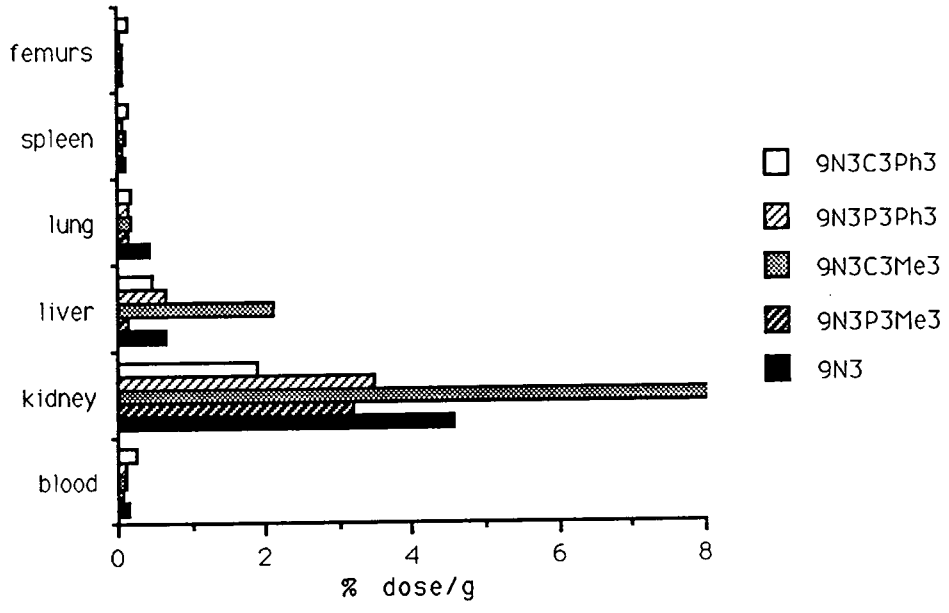
24 Hours	Citrate	9N3	9N3P3-Me3	9N3Me3-C3	9N3C3-Ph3	9N3P3-Ph3
Tissue	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose
blood	1.66 6.23	0.002 0.007	0.001 0.004	0.0245 0.089	0.122 0.347	0.0195 0.0701
kidney	3.46 2.10	0.021 0.012	0.359 0.208	0.107 0.062	0.296 0.153	0.121 0.0716
liver	3.39 7.18	0.124 0.235	0.0385	0.124 0.251	0.342 0.602	0.161 0.32
lung	1.81 0.42	0.037 0.009	0.0157 0.0031	0.059 0.01	0.180 0.048	0.027 0.0057
spleen	2.53 0.33	0.028 0.004	0.0226 0.003	0.0527 0.007	0.169 0.017	0.028 0.0035
salivary glands	2.32 0.60		0.025 0.0059			0.024 0.0056
urine	3.35	0.22	0.186 0.22	0.22		0.064
brain				0.0067 0.0029	0.006 0.003	0.017 0.0008
femur *2	4.41 0.82	0.015 0.002	0.0164 0.003	0.0978 0.0166	0.278	0.0437 0.0074
gall bladder					0.001	0.09 0.0007
muscle	0.43	0.005	0.0078	0.0193	0.026	0.0062
stomach	1.21	0.006	0.1036	0.036	0.084	0.017 0.012
small intestine	6.59	0.03	0.0603	0.14	0.322	0.045 0.1
caecum	2.13	0.03	0.0234	0.21	0.279	0.083 0.081
large intestine	2.87	0.03	0.0234	0.16	0.162	0.072 0.068
no. of mice	3	3	4	5	4	3
mean weight g.	37.9	37.5	34.8	36.1	30.6	35.8

Table 3.3 *The Biodistribution at 24 Hours*

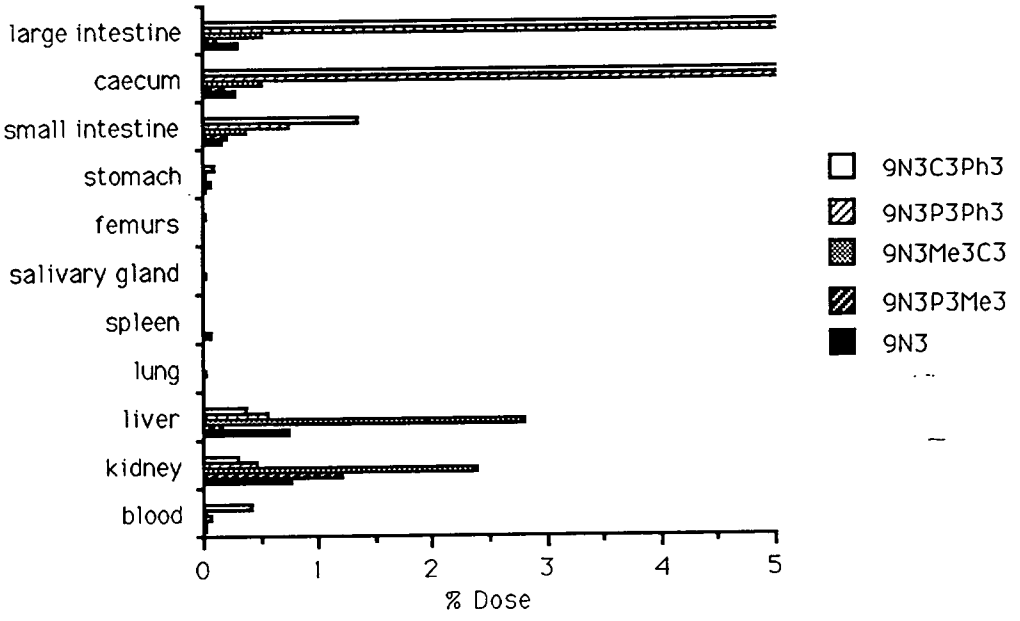
GRAPH 1a: Biodistribution (% Dose) of the Gallium Complexes at 1 hour



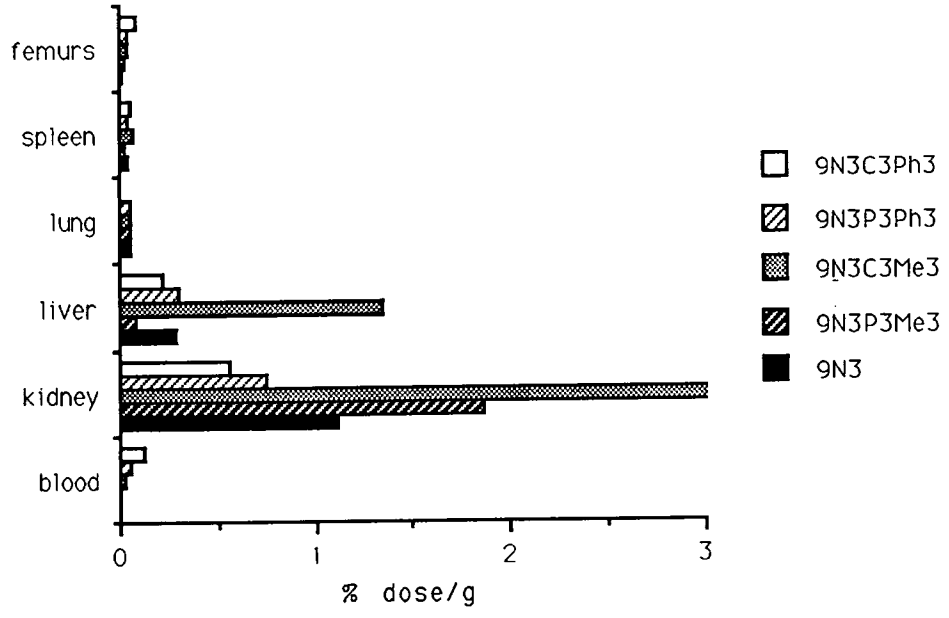
GRAPH 1b: Biodistribution (% dose/g) of the Gallium Complexes at 1 hour



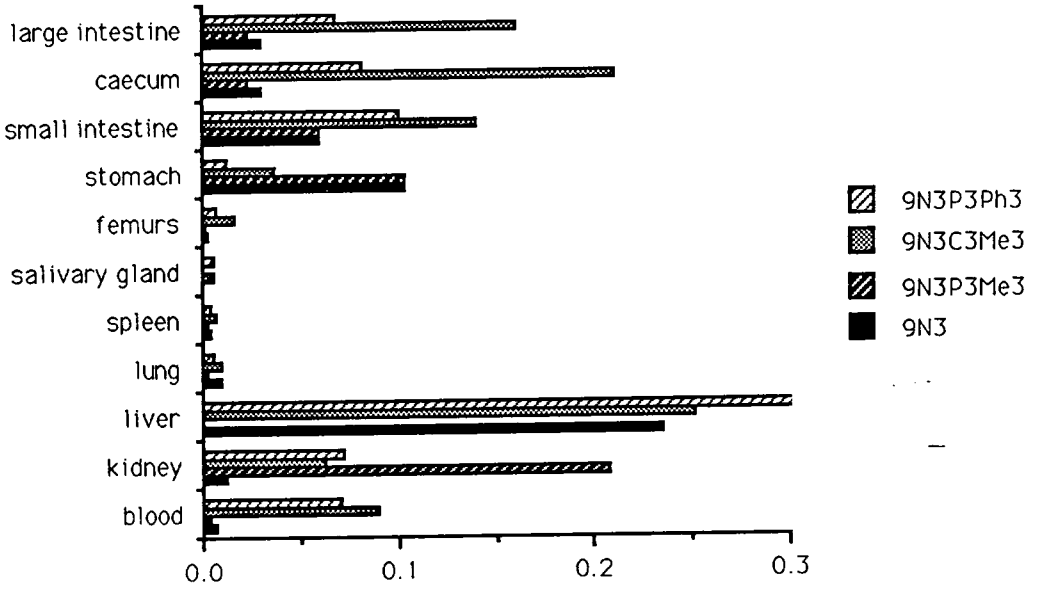
GRAPH 2a: Biodistrition (%dose) of the Gallium Complexes At Four Hours



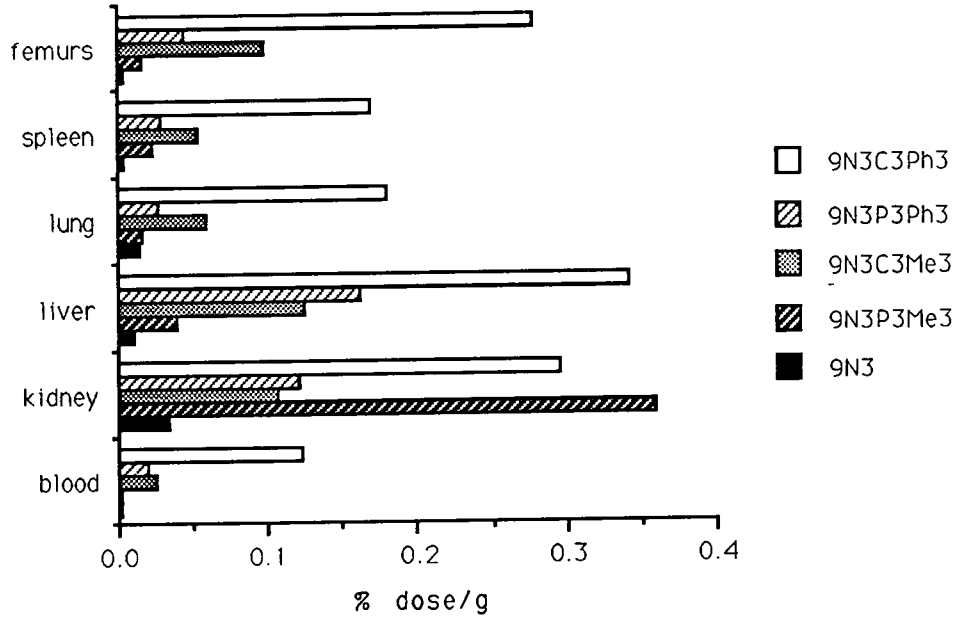
GRAPH 2b: Biodistribution (%dose/g) of the Gallium Complexes at Four Hours



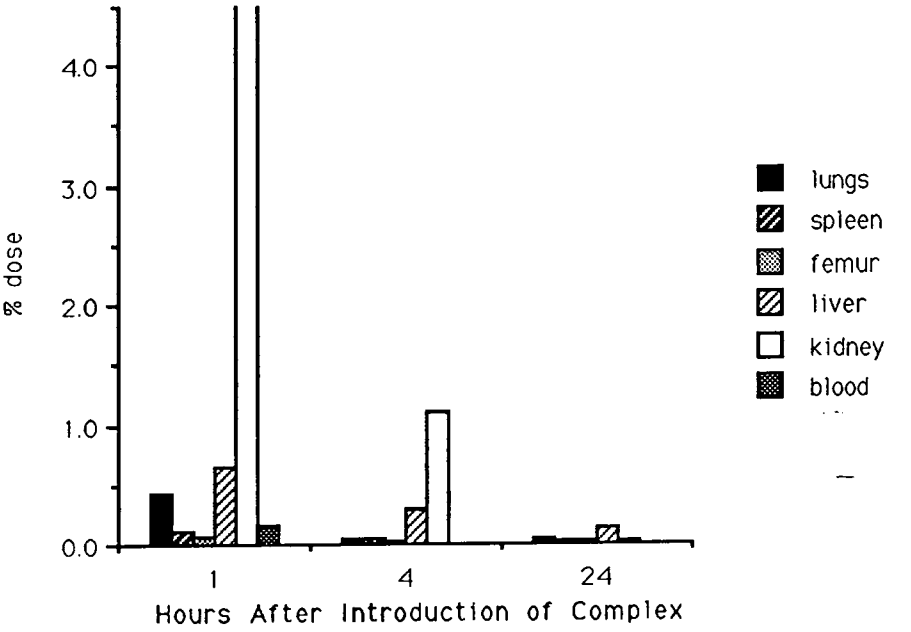
GRAPH 3a: Biodistribution (%dose) of the Gallium Complexes At 24 Hours



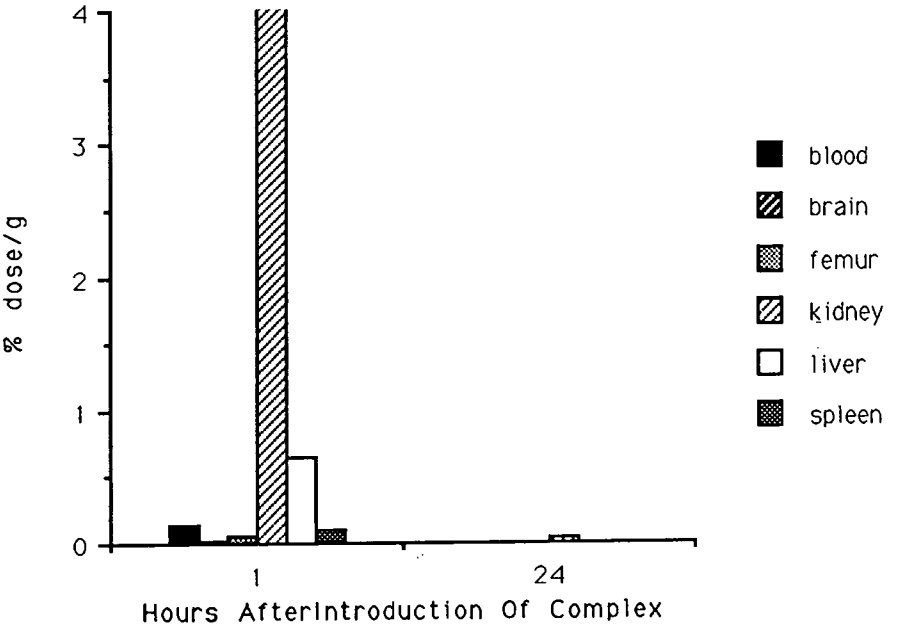
GRAPH 3b: Biodistribution (%dose/g) of the Gallium Complexes At 24 Hours



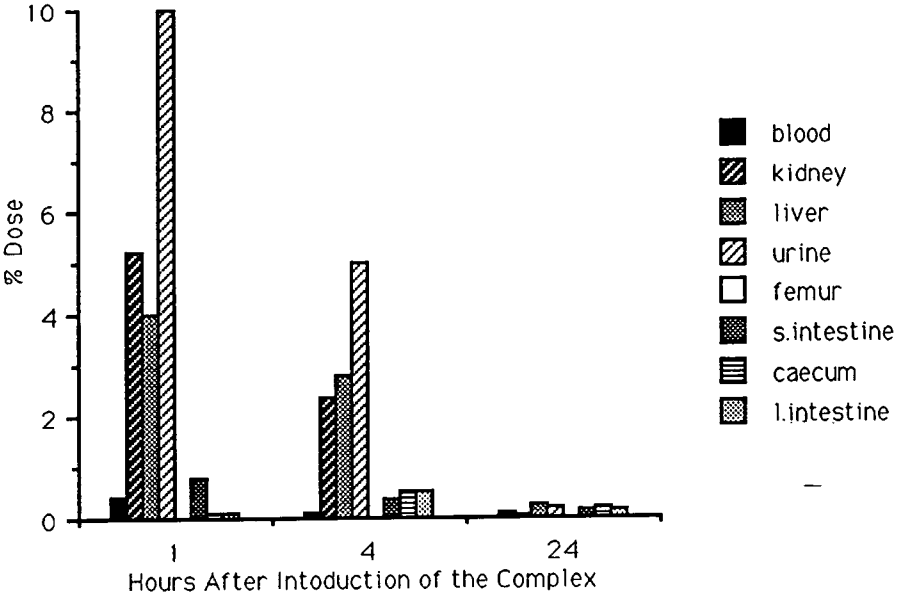
GRAPH 4a: Biodistribution of Gallium-9N3



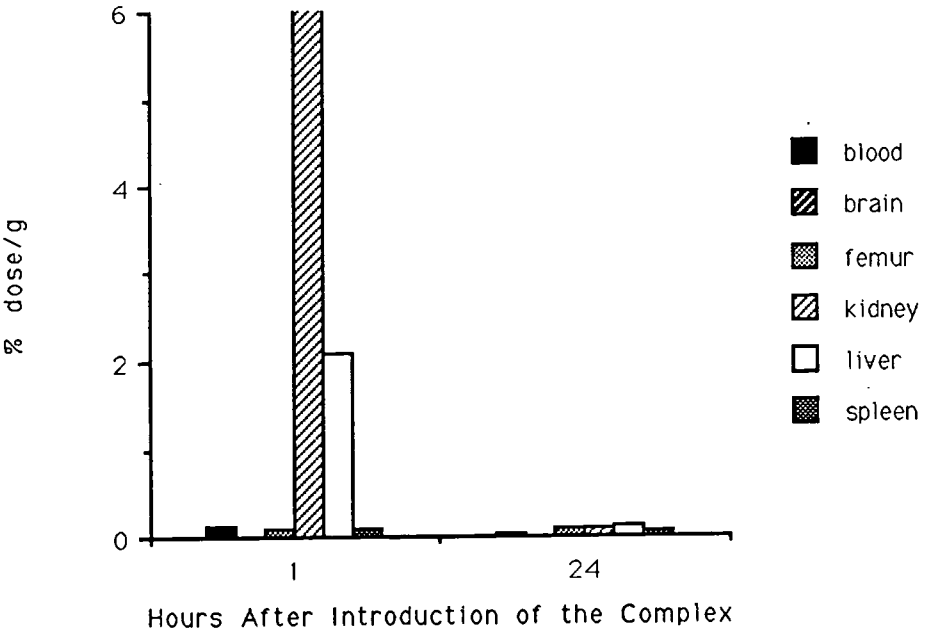
GRAPH 4b: Biodistribution (%dose/g) of Gallium-9N3



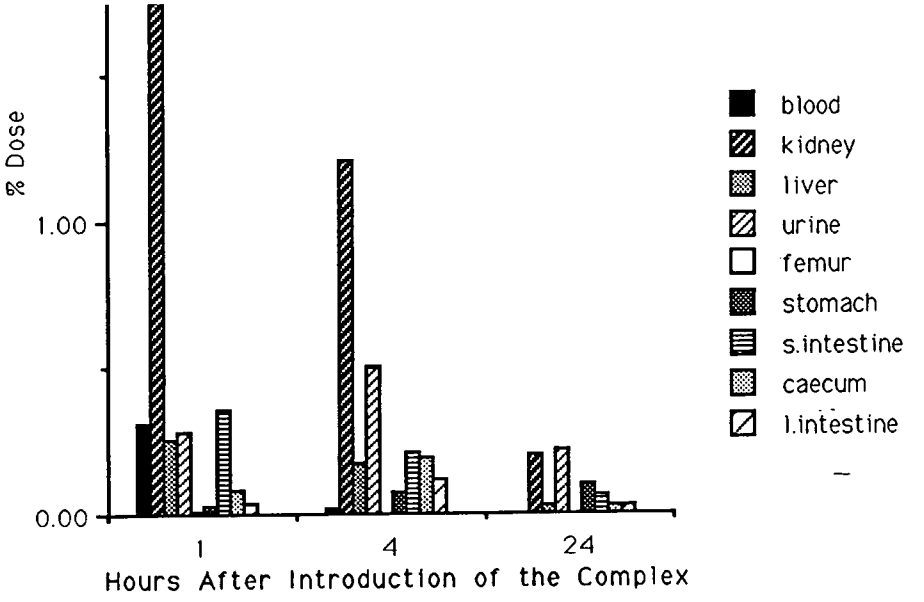
GRAPH 5a: Biodistribution of Ga-9N3C3Me3



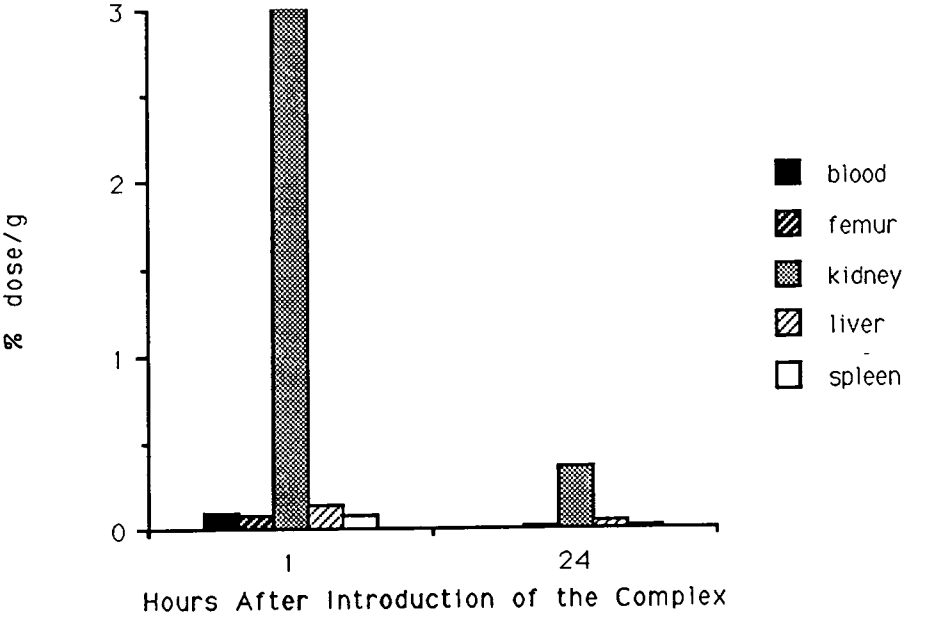
GRAPH 5b: Biodistribution of Ga-9N3C3Me3



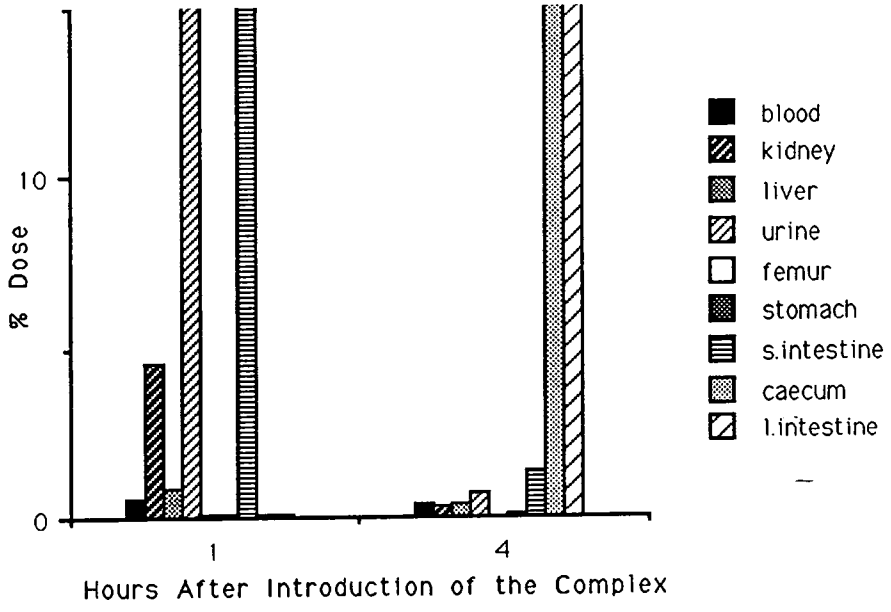
GRAPH 6a: Biodistribution (% dose) of Ga-9N3P3Me3



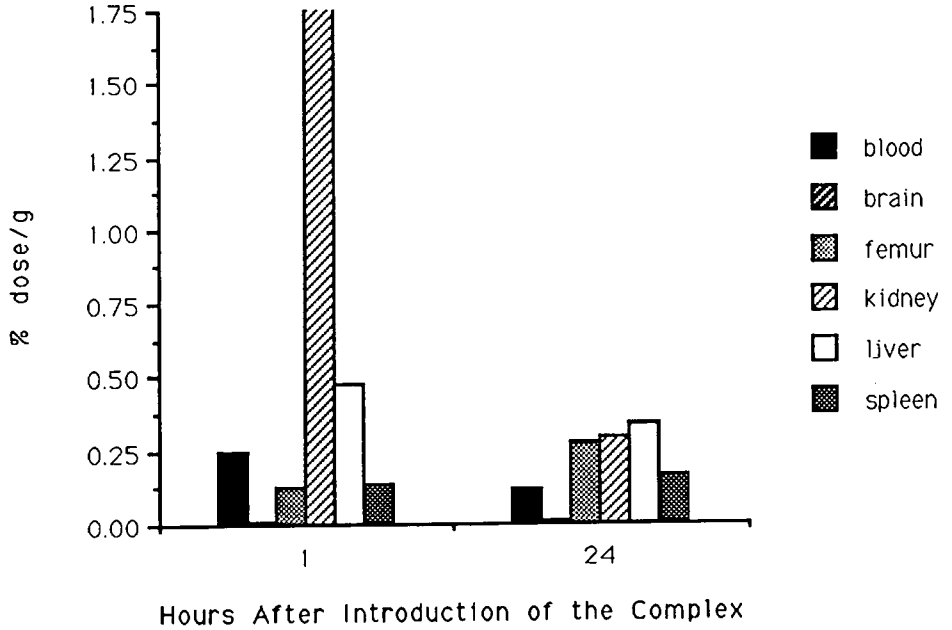
GRAPH 6b: Biodistribution (% dose/g) of Ga-9N3P3Me3



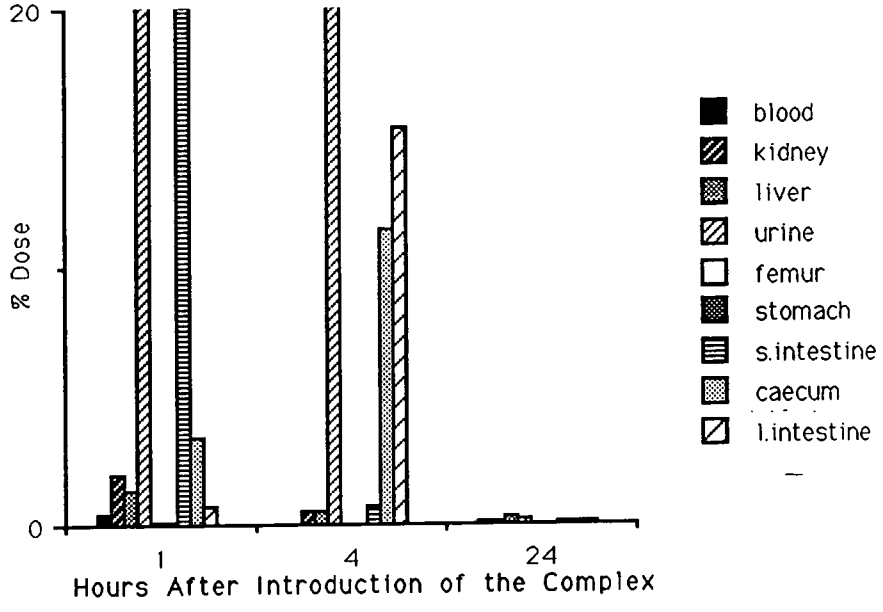
GRAPH :7a Biodistribution (%dose) of Gallium-9N3C3Ph3



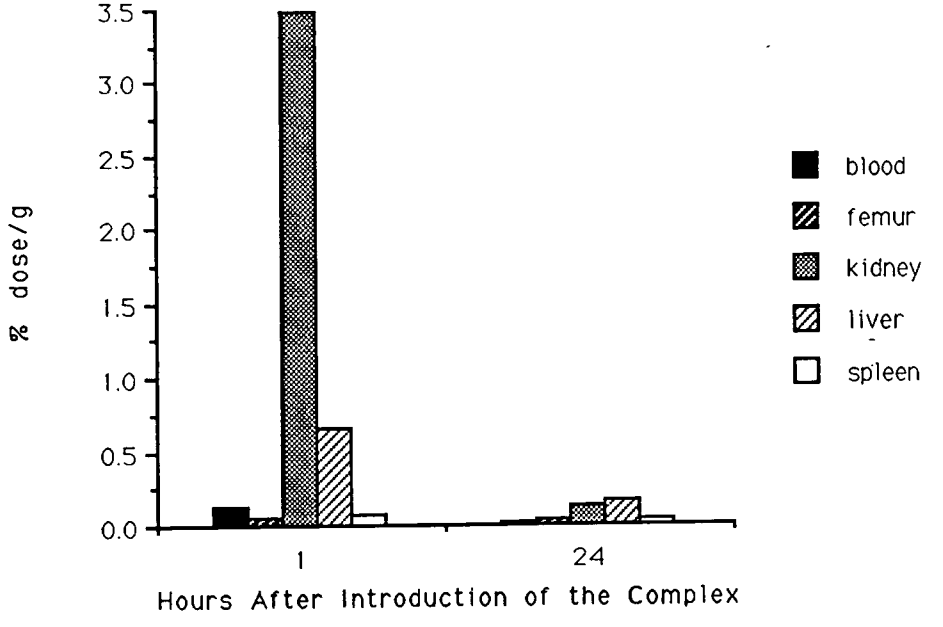
GRAPH 7b: Biodistribution (%dose/g) of Gallium-9N3C3Ph3



GRAPH 8a: Biodistribution (% dose)
of Ga-9N3P3Ph3



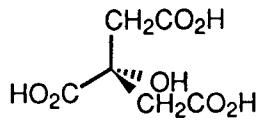
GRAPH 8b: Biodistribution (% dose/g)
of Ga-9N3PePh3



3.3.2 The Stability Of The Gallium Complexes *In Vivo*

During the preparation of the the complexes (Section 5.3), the uncomplexed ^{67}Ga is removed from the sample using a ten-fold excess of DTPA. All the complexes remained intact in the presence of the DTPA. This confirmed our previous experimental measurements and also demonstrates the high kinetic stability of all the complexes.

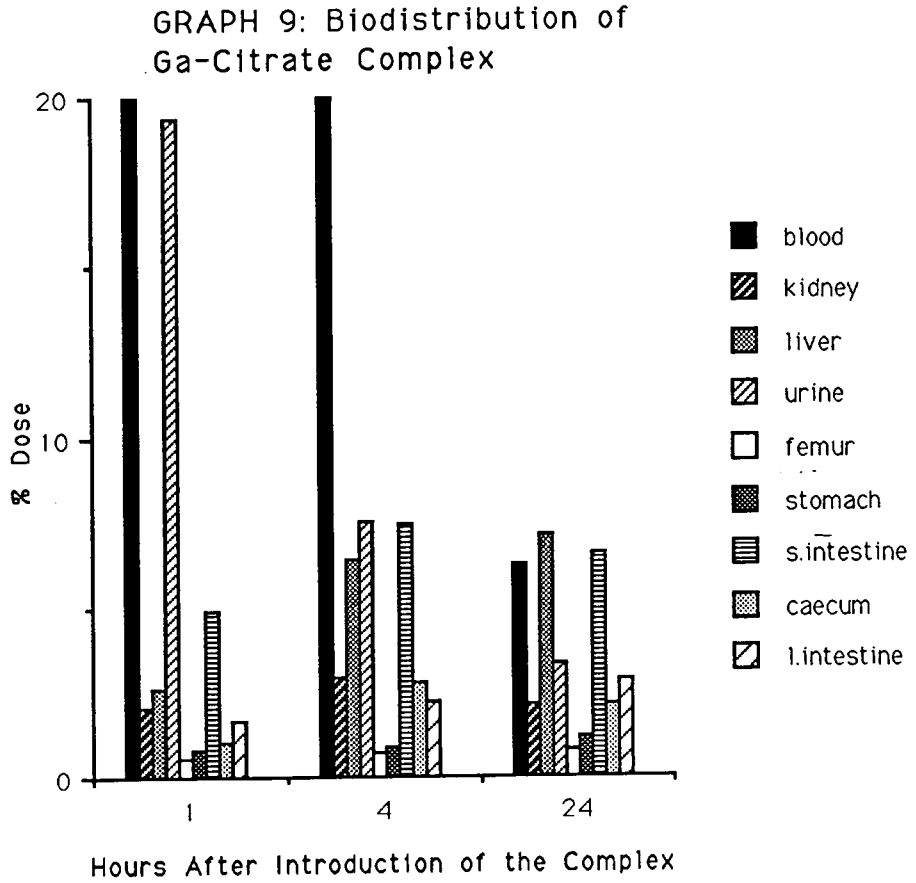
The stability of the complexes *in vivo* can be ascertained visually when comparing the graphical data for the gallium-citrate complex (graph 9) with that of the gallium-$9N_3C_3$ complex.



CITRATE

The biodistribution of the weak gallium-citrate complex was undertaken to compare the results against and provide a bench mark for assessing the stability of the complexes. The citrate complex readily dissociates *in vivo*. Therefore much of the gallium-67 localises in the body (liver and skeleton) and clears more slowly from other tissues than a stable complex.

The new complexing agents contrast well with the citrate complex and have almost completely cleared after 24 hours. This indicates that the complexes remain intact *in vivo*.



3.3.3 The Fate Of The ^{67}Ga-Complexes

The parent $9N_3C_3$ complex (graph 1 and 4) is cleared mainly through the renal *pathway* (kidneys). The activity of the complex in the kidneys is 4.57, 1.11, and 0.021 % dose g^{-1} at 1, 4, and 24 hours respectively. The measured activity that remains at 24 hours is very small indicating the complex has mostly been excreted, presumably intact.

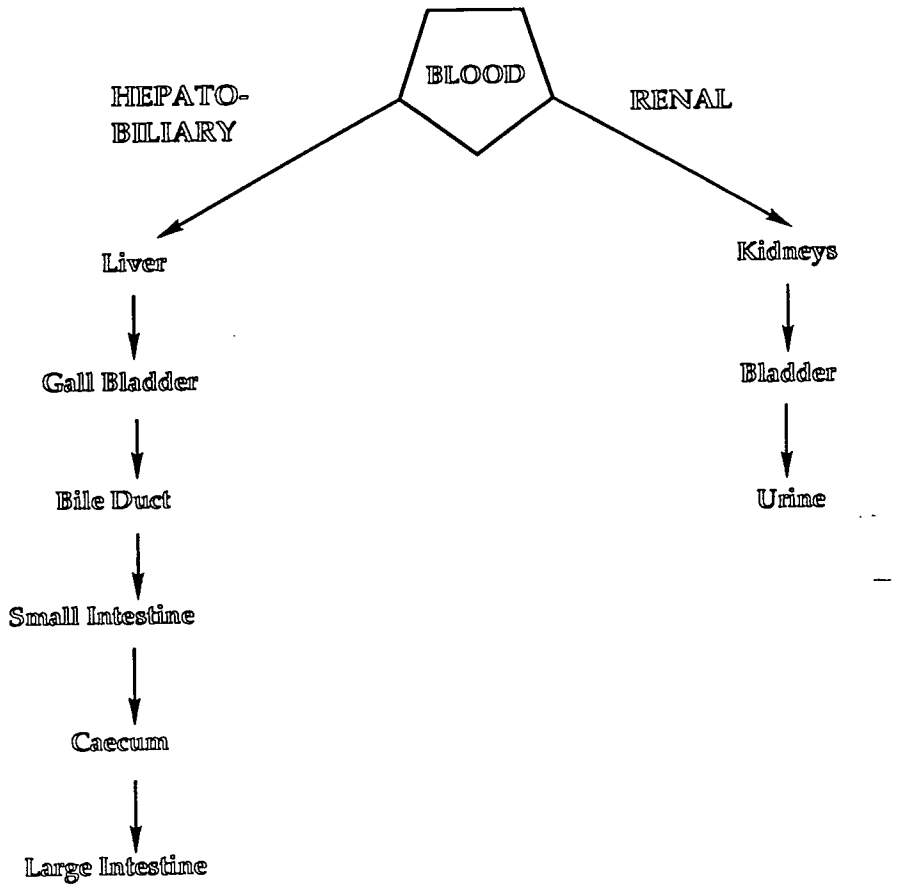


Figure 3.1 *Renal And Hepato-Biliary Excretion Pathways*

The ^{67}Ga-$9N_3P_3Me_3$ complex is excreted via the kidney and the biodistribution is very similar to the parent complex, ^{67}Ga-$9N_3C_3$. After 24 hours most of the activity has cleared.

The ^{67}Ga-$9N_3C_3Me_3$ also shows a substantial concentration (8.37 % dose g^{-1}) in the kidney at one hour. In addition, graph 1b indicates that, after one hour, there is a substantial amount of ^{67}Ga-$9N_3C_3Me_3$ in the liver. On closer inspection of graph 2a, there is no indication of the complex in the small and large intestine. Figure 3.1 describes the two main excretion pathways. It is slightly surprising that the high concentration of the complex in the liver is not mirrored with a proportionally high concentration in the small and large intestine - the *hepato-biliary* excretion pathway. The explanation for this

anomaly may be that the complex is unstable *in vivo*. Graphs 5a and 5b show that in femur, kidney, liver and blood there is a little activity ($\approx$ 0.25 % dose g^{-1}). Comparing this to the activity of the other complexes at 24 hours (graph 3a and 3b), the concentration of the ^{67}Ga-$9N_3C_3Me_3$ is less than that of the other complexes. Therefore it is unlikely that decomplexation is the cause of this phenomenon. The presence of the ^{67}Ga-$9N_3C_3Me_3$ complex in the liver in such a relatively high concentration cannot be explained at present.

After one hour, the ^{67}Ga-$9N_3C_3Ph_3$ and ^{67}Ga-$9N_3P_3Ph_3$ complexes show a comparatively lower concentration in the kidney than the other complexes. In addition, high concentrations in the small and large intestines (graph 1a and 8a) have been observed. This indicates that a proportion of the complex is passing through the liver and bile duct and into the intestines to be excreted in the caecum. This preference for the *hepato-biliary* route may be related to the lipophilicity of these complexes. The addition of phenyl groups to the complex, does alter the solubility characteristics of their complexes, and may be expected to influence the *in vivo* pharmacokinetics of these complexes as well.

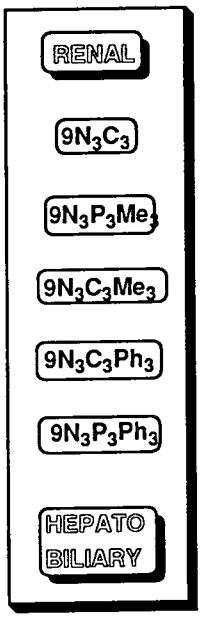
The clearance of all the complexes is fast, after 24 hours less than 1 % of each complex remains in the body.

3.3.4 Conclusions

The experiment was designed to determine the eligibility of the gallium complexes of the new complexing agents for use *in vivo*. All the complexes demonstrated excellent *in vivo* stability, and all of them cleared quickly from the body, with little evidence of dissociation.

Secondly, the modification to the lipophilicity of the complexes introduced by the presence of the methyl and phenyl groups affects the way the complexes are processed by the body. The more lipophilic complexes tend to excrete via the hepato-biliary route, whereas the parent and $9N_3P_3Me_3$ are mainly excreted through the kidneys.

The following list shows the order which is observed.



The application of the new $^{67}Ga(\gamma)$ complexes for *in vivo* imaging of the liver, bile duct and small intestine is feasible as the concentration in these organs, although small, compared to the background may be within the lower limits that allow successful imaging. Also the use of ^{68}Ga for positron emission tomography (PET) or stable gallium for *in vivo* NMR detection may also be feasible.

3.4 BIODISTRIBUTION OF THE INDIUM COMPLEXES IN ATHYMIC NU:NU MICE

3.4.1 Introduction

In chapter 1 (1.3.2), it was noted that ^{111}In is a good imaging isotope. This gamma emitter has a half-life of 2.83 days and is a potential candidate for single photon emission computed tomography (SPECT).

Although indium(III) has a larger ionic radius than the gallium ion, indium forms strong complexes with the $9N_3C_3$ family of complexing agents.(4)

	Gallium(III)	Indium(III)
Ionic Radius A° (hexacoordinate)	0.76	0.94

A second set of biodistribution experiments, similar to the gallium work, was made using indium 111 as the radiometal.

Once again the aim of the investigation was to determine the stability of the indium complexes *in vivo* and observe if the complexes displayed a preference for a particular excretion pathway, i.e. whether the more lipophilic indium complexes were excreted via the hepato-biliary route.

3.4.2 Experimental Method Of Investigation

The experimental details were analogous to those used in the gallium work.

3.4.3 The Data

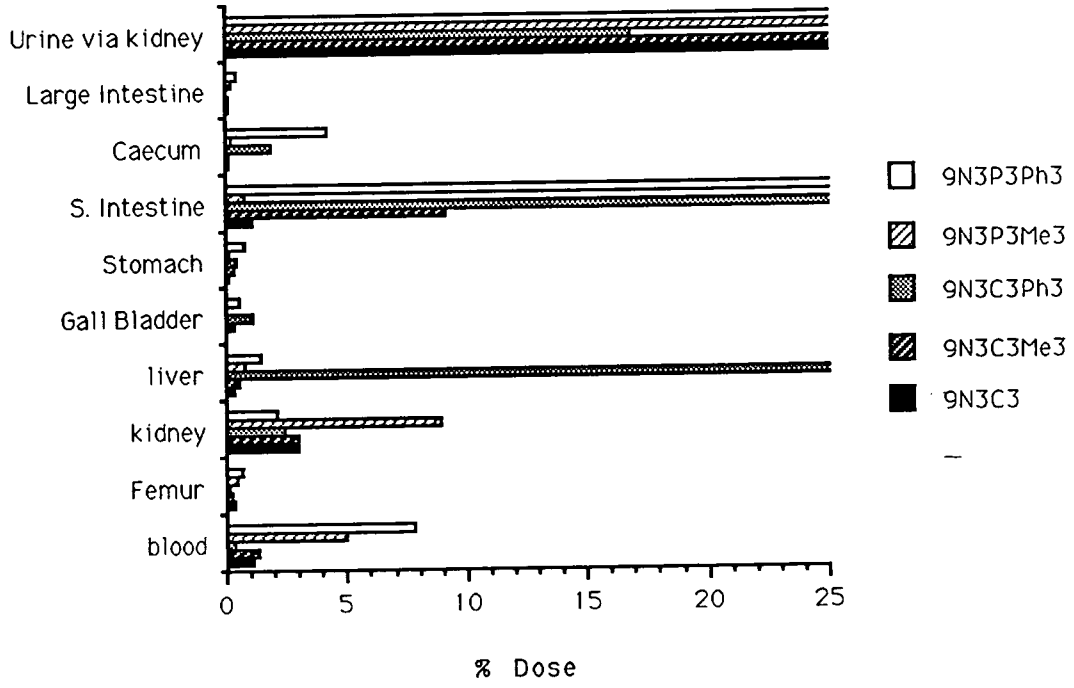
In (1 Hour)	9N3	9N3P3-Me3	9N3C3-Me3	9N3P3-Ph3	9N3C3-Ph3
Tissue	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose
Blood	0.28 1.08	1.38 4.95	0.38 1.37	2.06 7.79	0.12 0.38
Brain	0.024 0.010	0.049 0.02	0.028 0.013	0.055 0.026	0.011 0.005
Femur	0.34	0.45	0.21	0.64	0.134
Kidney	4.55 2.99	13.81 8.91	4.81 2.96	3.53 2.04	4.2 2.38
Liver	0.25 0.28	0.39 0.79	0.29 0.58	0.68 1.40	23.3 40.50
Lung	0.33 0.07	0.74 0.13	0.4 0.08	1.03 0.20	0.20 0.03
Muscle	0.12	0.30	0.13	0.33	0.04
Skeleton	1.28	1.62	0.73	2.41	0.42
Spleen	0.12 0.02	0.35 0.05	0.14 0.02	0.42 0.05	0.14 0.02
Gall Bladder	0.02	0.01	0.28	0.56	1.15
Stomach	0.08	0.11	0.33	0.8	0.45
S.Intestine	1.08	0.78	9.18	30.21	29.8
Caecum	0.09	0.17	0.14	4.18	1.87
L.Intestine	0.09	0.19	0.12	0.45	0.03
Urine Via Kidney	79.77	69.77	70.59	27.85	16.6
Total Gut	1.35	1.25	9.77	35.64	32.16
no. of mice	9	4	4	8	4
mean weight	38g	34.9	35.2	37.2	31.9

Table 3.4 *The Biodistribution Of The Indium Complexes At One Hour*

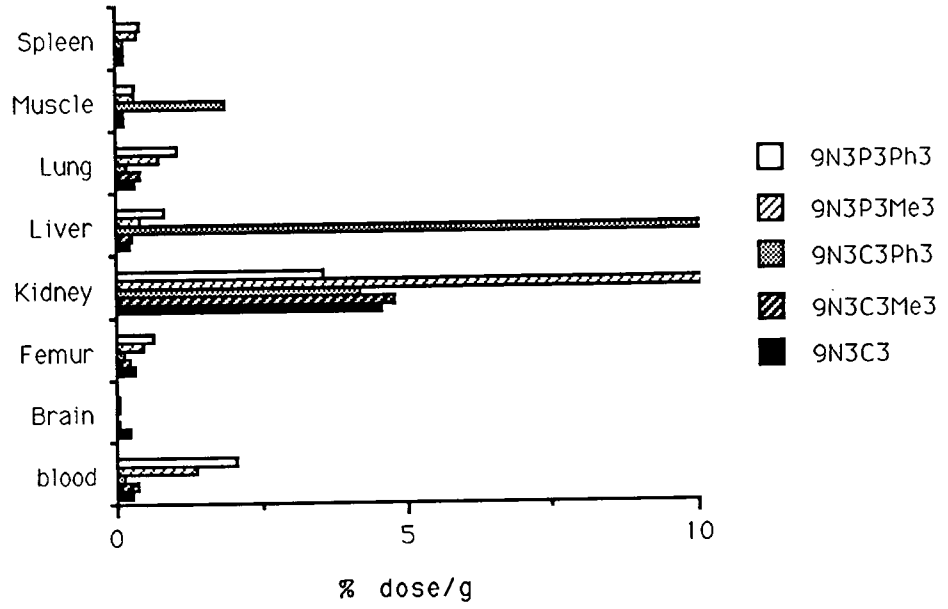
In (24 Hours)	9N3	9N3P3-Me3	9N3C3-Me3	9N3P3-Ph3	9N3C3-Ph3
Tissue	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose	%dose/g %dose
Blood	0.002 0.009	0.31 0.99	0.002 0.007	0.25 0.93	0.034 0.105
Brain	0.001 0.0006	0.03 0.013	0.0008 0.0003	0.03 0.013	0.004 0.002
Femur	0.05	0.67	0.019	0.80	0.10
Kidney	0.19 0.13	2.78 1.74	0.17 0.10	2.89 1.81	1.02 0.55
Liver	0.05 0.10	1.09 1.84	0.04 0.07	0.84 1.73	14.72 24.28
Lung	0.02 0.004	0.56 0.09	0.010 0.002	0.43 0.08	0.073 0.012
Muscle	0.004	0.21	0.002	0.19	0.024
Skeleton	0.17	2.13	0.065	1.18	0.31
Spleen	0.02 0.002	0.44 0.05	0.012 0.001	0.47 0.06	0.124 0.01
Gall Bladder	0.0004	0.02	0.0005	0.007	0.13
Stomach	0.008	0.11	0.004	0.12	0.16
S.Intestine	0.05	2,.19	0.04	1.11	0.67
Caecum	0.11	0.31	0.14	0.4	11.37
L.Intestine	0.05	0.41	0.05	0.34	0.74
Total Gut	0.21	3.01	0.23	1.97	2.94
no. of mice	4	4	4	7	4
mean weight g.	38.3	32.0	34.1	-	31.0

Table 3.5 *The Biodistribution Of The Indium Complexes At 24 Hours*

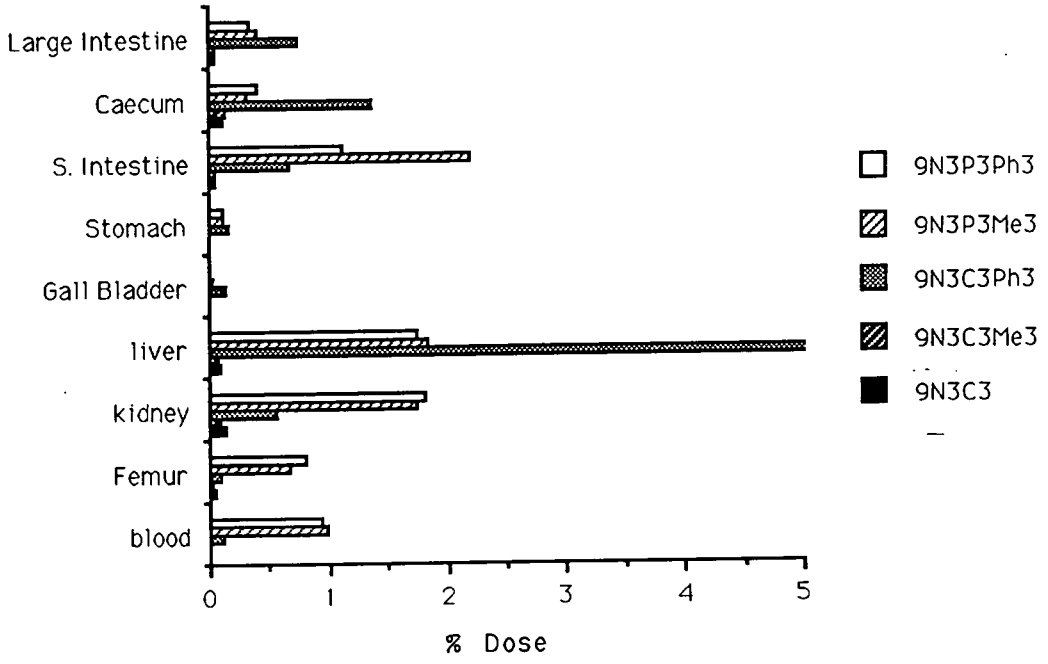
GRAPH 10a: Biodistribution of (% dose)the Indium Complexes 1 hour after introduction



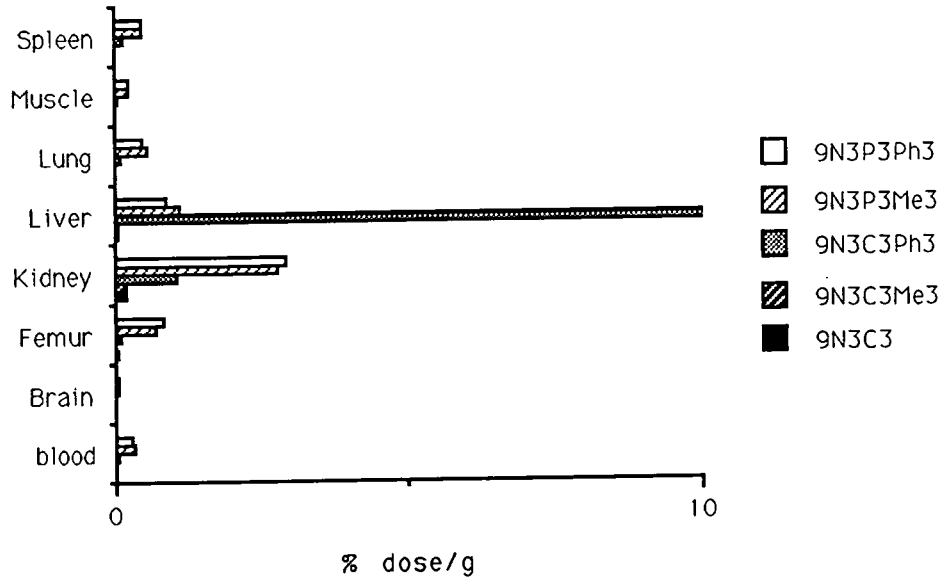
GRAPH 10b: Biodistribution (% dose/g) of the Indium Complexes 1 hour after introduction



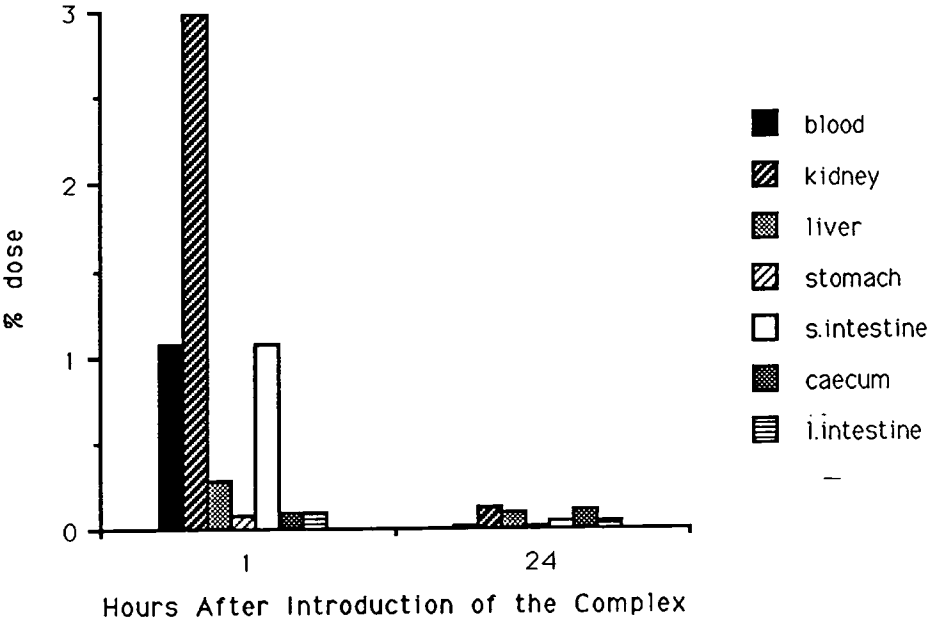
GRAPH 11a: The Biodistribution (% dose) of the Indium complexes 24 hr after their introduction



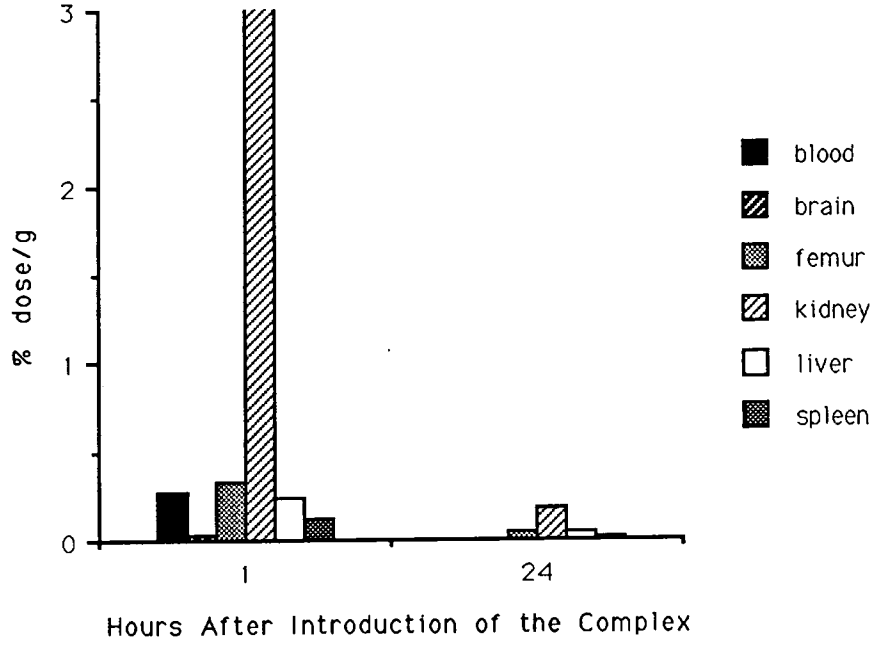
GRAPH 11b: The Biodistribution (% dose/g)of the Indium Complexes 24 hr after their introduction



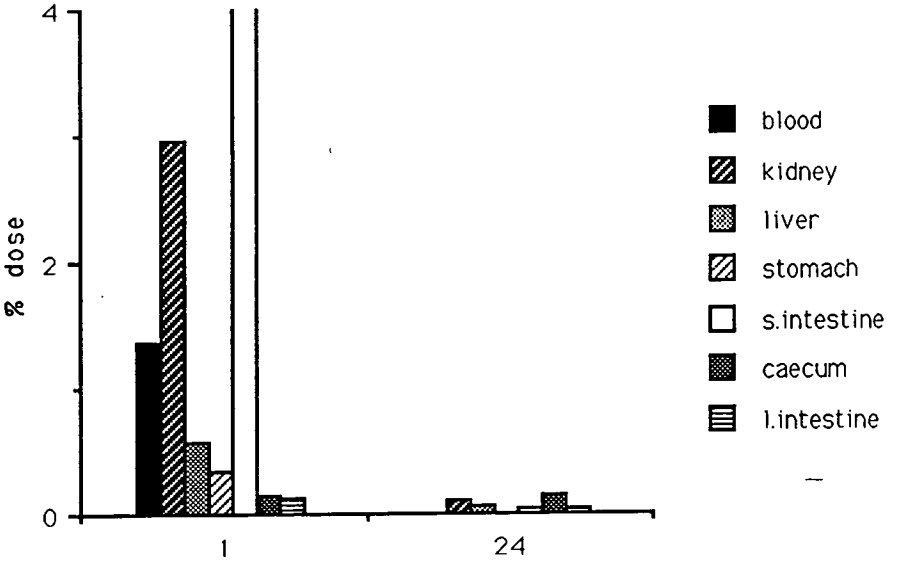
GRAPH 12a: Biodistribution (% dose)
of Indium-9N3C3



GRAPH 12b: Biodistribution (% dose/g)
of Indium-9N3C3

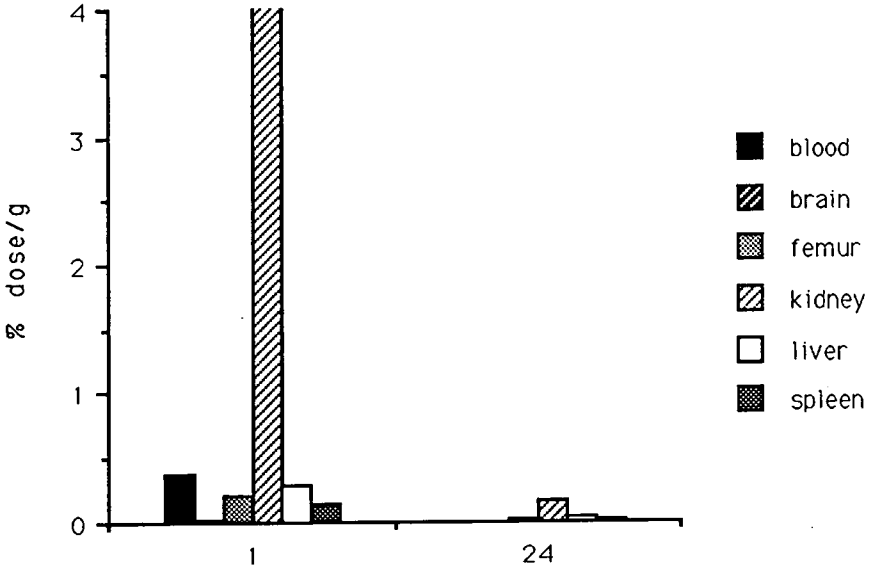


GRAPH 13a: Biodistribution (% dose)
of Indium-9N3C3Me3



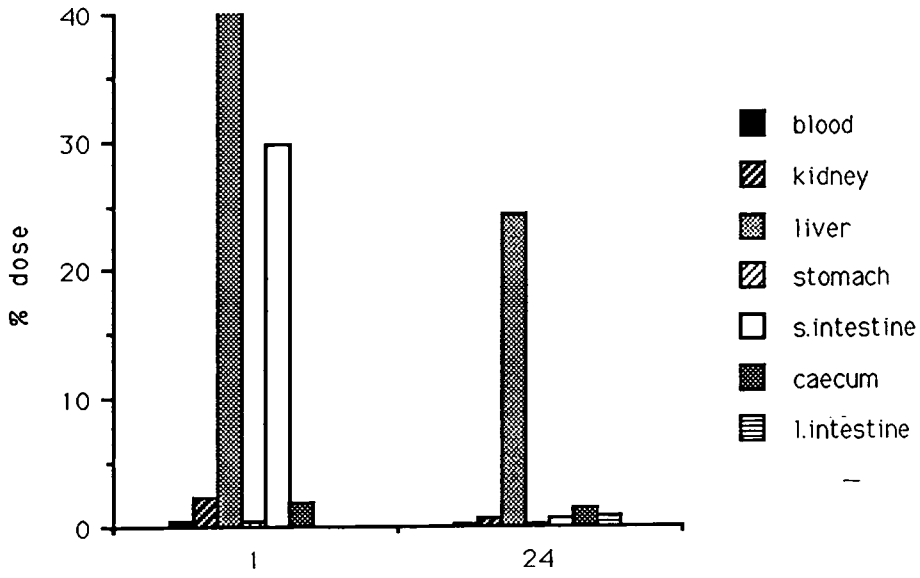
Hours After Introduction of the Complex

GRAPH 13b: Biodistribution (% dose/g)
of Indium-9N3C3Me3



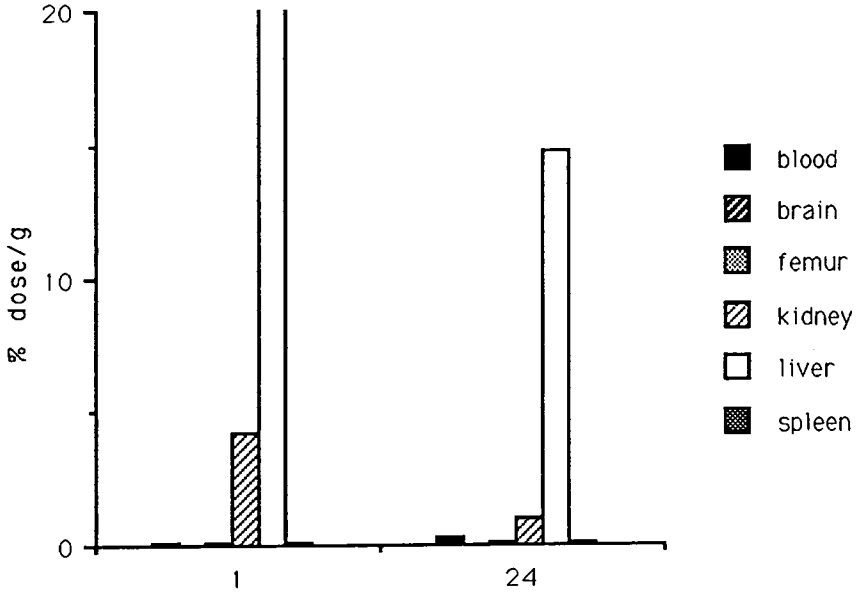
Hours After Introduction of the Complex

GRAPH 14a: Biodistribution (% dose) of Indium-9N3C3Ph3



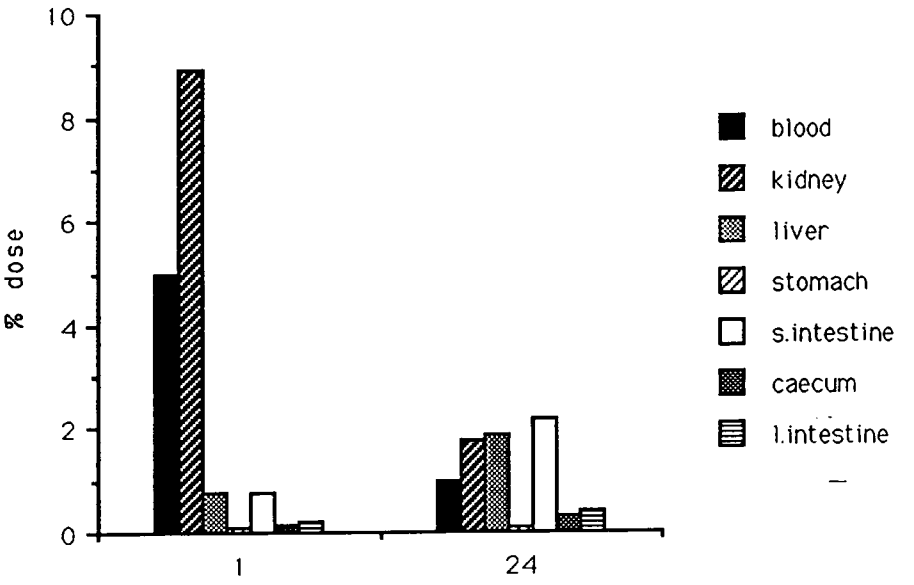
Hours After Introduction of the Complex

GRAPH 14b: Biodistribution (% dose/g) of Indium-9N3C3Ph3



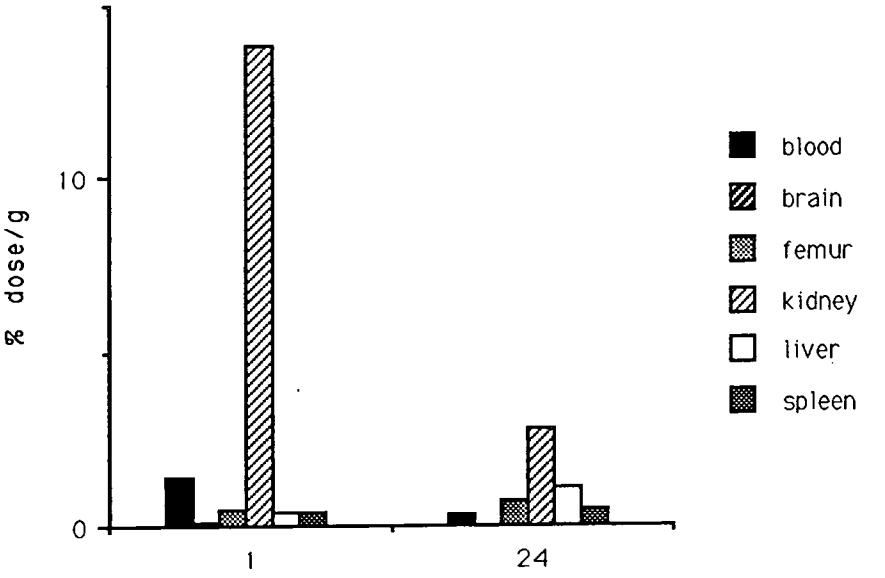
Hours After Introduction of the Complex

GRAPH 15a: Biodistribution (% dose)
of Indium-9N3P3Me3



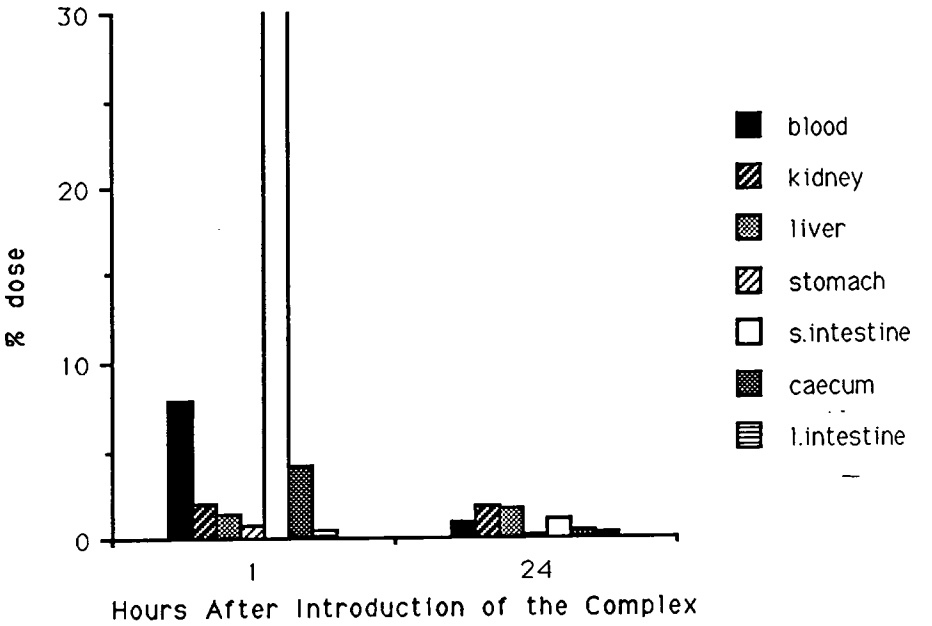
Hours After Introduction of the Complex

GRAPH 15b: Biodistribution (% dose/g)
of Indium-9N3P3Me3

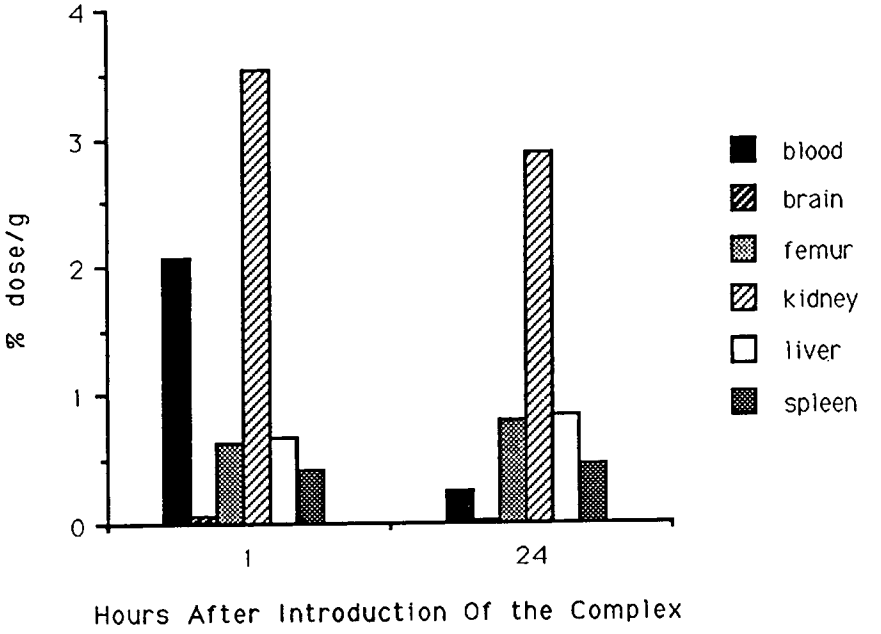


Hours After the Introduction of the Complex

GRAPH 16a: Biodistribution (% dose)
of Indium-9N3P3Ph3



GRAPH 16b: Biodistribution (% dose/g)
of Indium-9N3P3Ph3



3.4.4 The Stability Of The ^{111}In-Complexes In Vivo

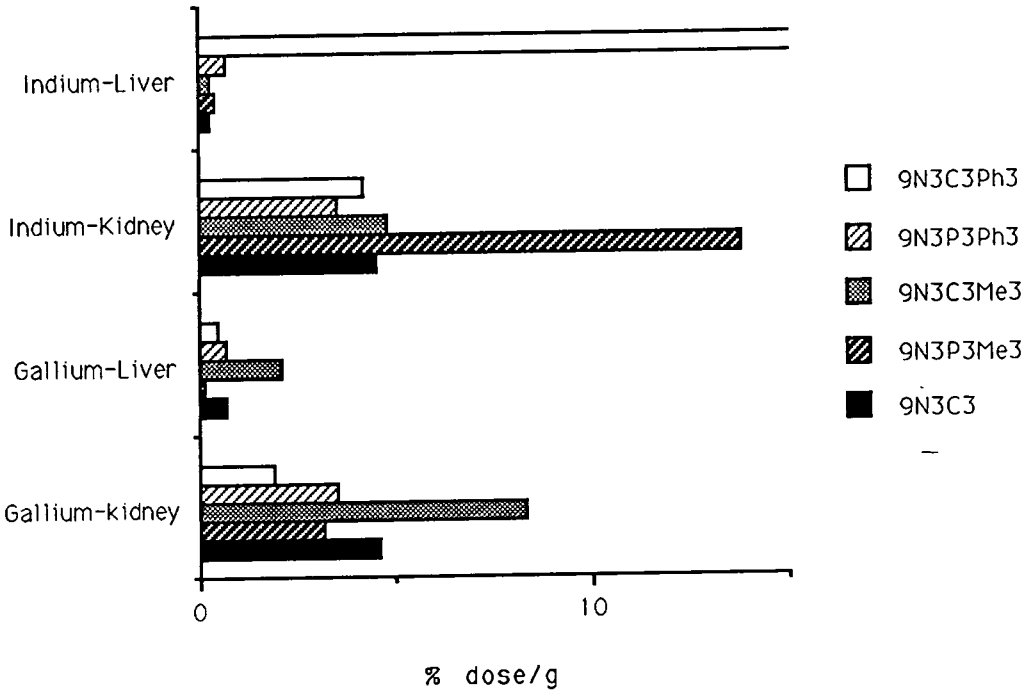
Comparing graphs 11a and 11b with graphs 3a and 3b (the biodistribution of the gallium and indium complexes at 24 hours), the amount of radioactivity still present with some of the indium complexes is greater by almost a factor of 10 in certain organs (kidney, liver, femur) and this effect is most notable with the $9N_3P_3Ph_3$ indium complex.

This suggests that, in general, the indium complexes are less stable *in vivo* than the analogous gallium complexes. An explanation for this may lie in the size of the ion. The larger indium(III) ion may be too large to allow the macrocycle to bind properly. It is possible that the indium ion is less well bound by the 'tighter bite' of the $9N_3$ moiety. The position of the pendant acid groups will be affected and they may not be able to bind cooperatively at their optimum In-O distances.

3.4.5 The Fate Of The Indium Complexes.

Graph 17 compares the biodistribution data at one hour to see if the indium complexes are excreted in a similar manner to those of gallium.

GRAPH 17: Biodistribution in the Liver and Kidney after 1 Hour



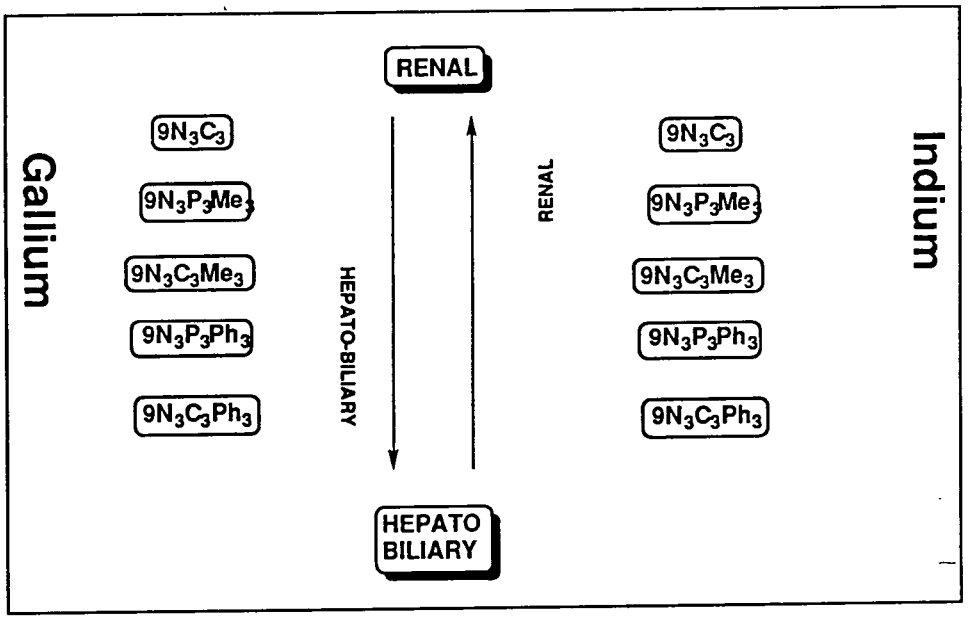
The parent indium-$9N_3C_3$ complex is excreted via the renal pathway (Graphs 10 and 12).

The ^{111}In-$9N_3C_3Me_3$ and ^{111}In-$9N_3P_3Me_3$ complexes also show renal excretion, with very little activity detected in the liver, (Graphs 10, 13 and 14). The unexplained presence of a large concentration of the ^{67}Ga-$9N_3C_3Me_3$ complex in the liver does not occur with the indium analogue. However graph 1a shows, after one hour, a relatively large concentration in the intestines indicating that a proportion of the complex is passing through the liver, and excreted via the hepato-biliary pathway.

The ^{111}In-$9N_3C_3Ph_3$ and ^{111}In-$9N_3P_3Ph_3$ complexes display a preference for the hepato-biliary pathway (Graphs 1, 14 and 15). After one hour, graph 10a shows that both complexes were present in the caecum. This gives an indication of the rate at which the complexes are cleared via the hepato-biliary route (< 1 hour).

3.4.6 Conclusions

The experiment was designed to determine the eligibility of the indium(III) complexes for their use *in vivo*.. The complexes demonstrated less adequate *in vivo* stability than the analogous gallium complexes and there is evidence for dissociation with activity present in the liver, in particular, after 24 hours. The In-$9N_3C_3Ph_3$ complex appears to be the most unstable, the high concentration of indium in the liver after 24 hours is probably due to the free indium being scavenged by transferrin and deposited in the liver. The modification to the lipophilicity of the complexes introduced by the presence of the methyl and phenyl groups affects the way the complexes are processed by the body. With the gallium complexes, the more lipophilic complexes (phenyl) tend to excrete via the hepato-biliary route, whereas the parent, $9N_3P_3Me_3$ and $9N_3C_3Me_3$ are mainly excreted through the kidneys. The indium complexes show a similar order of preference which is shown below. The only difference is that the ^{111}In-$9N_3C_3Me_3$ shows a slight preference for the hepato-biliary route, although it is excreted mostly via the renal pathway. The excretion preferences for the complexes are summarised below.



3.4.7 A Second Investigation Of The Biodistribution Of The ^{111}In-9N3C3Ph3 Complex

Graphs 14a and 14b, indicated that the ^{111}In-9N3C3Ph3 complex was unstable *in vivo*. A second sample of the 9N3P3Ph3 was submitted for biodistribution to clarify this.

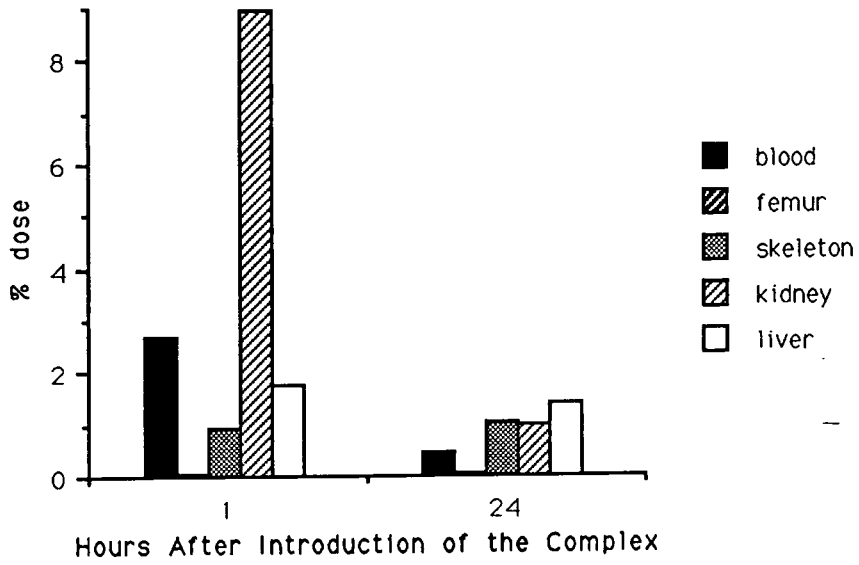
3.4.8 The Data

The experimental details are the same as those used in the previous experiments. The data is presented below (Table 3.6):

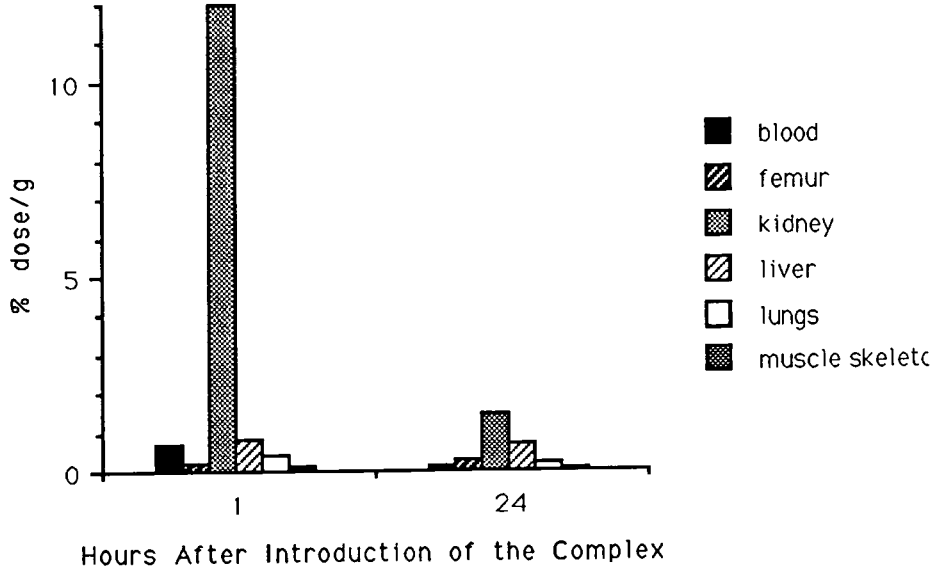
	$9N_3C_3Ph_3$ One Hour	$9N_3C_3Ph_3$ 24 Hours
Tissue	**%dose/g %dose**	**%dose/g %dose**
Blood	0.694 2.70	0.115 0.478
Brain	0.036 0.017	
Femur	0.234 0.038	0.247 0.039
Skeleton	0.915	1.03
Heart	0.219 0.034	
Kidneys	12.0 8.935	1.48 0.96
Liver	0.821 1.77	0.664 1.4
Lungs	0.446 0.095	0.177 0.036
Spleen	0.166 0.019	0.17 0.016
Gall bladder	0.100	
Stomach	0.095	0.038
S. Intestine	8.86	0.437
Caecum/L.Int-estin	1.638	0.425
Urine	66.3	0.08

Table 3. 6 *The Biodistribution Of The ^{111}In-$9N_3C_3Ph_3$ Complex*

GRAPH 18a: Biodistribution (% dose) of the Indium-9N3C3Ph3 complex (second investigation)



GRAPH 18b: The Biodistribution (% dose/g) of the Indium-9N3C3Ph3 complex (second investigation)



3.4.9 Discussion

The new results indicate that the complex was excreted quickly and remained stable. The excretion pathway is similar to the analogous gallium complex and travels via the liver (Hepato-biliary) with some going via the renal pathway. This is substantially different to the results of the previous experiment, where we concluded that the complex was unstable.

This difference may be explained by the influence of the ligand's stereochemistry. The sample, used in the second experiment, was from the first batch of elutants, containing the triester, taken off the alumina column. The previous sample had been from the main fraction of elutants. If the diastereoisomers have different polarities, then it is possible that each sample may have contained a larger amount of either diastereomeric pairs, e.g. SSS and RRR or SRS and RSR. If that was the case, the results indicate that the isomer present in the first experiments forms a less stable complex with indium, than the predominant isomer used in the second experiment.

Figure 3.3 represents the possible solid state conformation of the two diastereomers. The drawing is based on the conformation of the $9N_3C_3Me_3$ ligand when complexed with indium (Section 2.3). Phenyl groups have been added in place of the methyl groups.

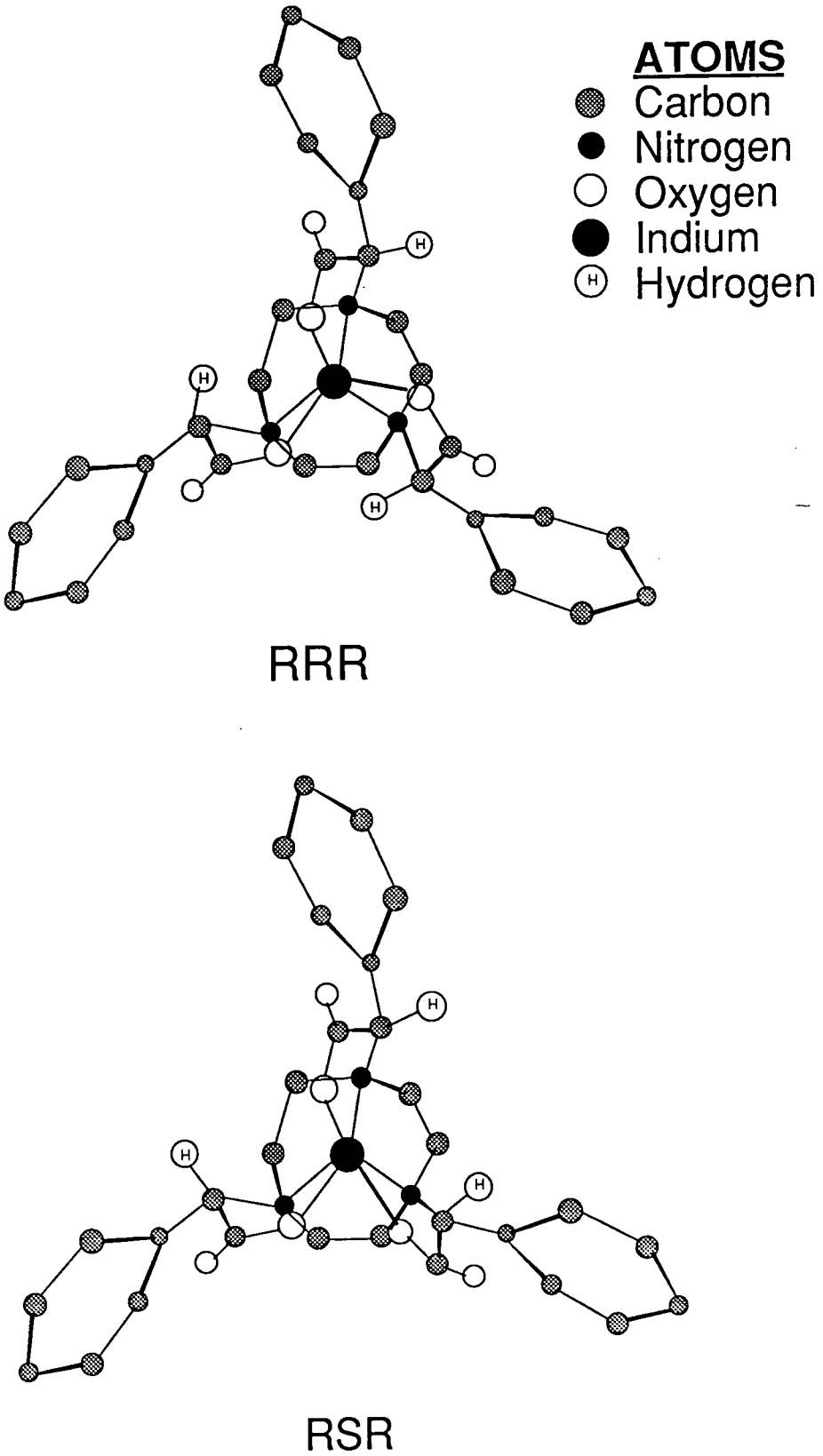


Figure 3.3 *Schematic Representation of the Complex's Solid State Conformation*

With the RSR (and SRS) isomer, the acid group attached to the carbon with the S configuration is twisted in the opposite direction to the other carboxylate groups (Figure 3.3) The effect of this may influence the ability of the macrocycle to adopt a conformation that provides optimum donor-atom-In bond lengths. Therefore it may be possible that one of the diastereomeric pairs is less stable than the other.

To summarise, it seems that each experiment was carried out using a mixture of the $9N_3C_3Ph_3$ isomers which contained more of one set of diastereomers than the other. Those diastereomers predominant in the second experiment form a much more stable complex than those isomers predominant in the first experiment.

To further investigate this phenomena would necessitate the separation of the isomers, identification of the configuration of each one, and a further biodistribution study.

3.5 GALLIUM-67 COMPLEX UPTAKE BY XENOGRAFTS OF HUMAN MELANOTIC MELANOMA IN MICE[1]

3.5.1 Introduction

This section reports on the biodistribution of the gallium 67 complexes 9N3, $9N_3P_3Me_3$, $9N_3C_3Ph_3$, DOTA and citrate in mice that had previously grafted with a human melanotic melanoma (HX118).

The incentive for this study arose from the results of a investigation into the biodistribution of the gallium-9N3 complex in tumour bearing mice.

The summarised results are as follows;

^{67}Ga-9N3 complex cleared faster from all tissues (and the blood) than from the tumour.
The concentration ratio *xenograft : blood* was ≈ 20:1, 4 hours after introduction of the complex.
After 4 hours only 4.5 % of physical decay has occurred ensuring near maximum signal : noise ratio.

This led to a suspicion that the complex was specifically binding to melanin and may provide a useful means of detecting melanotic melanoma metasteses using SPECT.

[1]The data collection was carried out by A. Harrison and L . Royle, MRC, Radiobiology Unit, Didcot

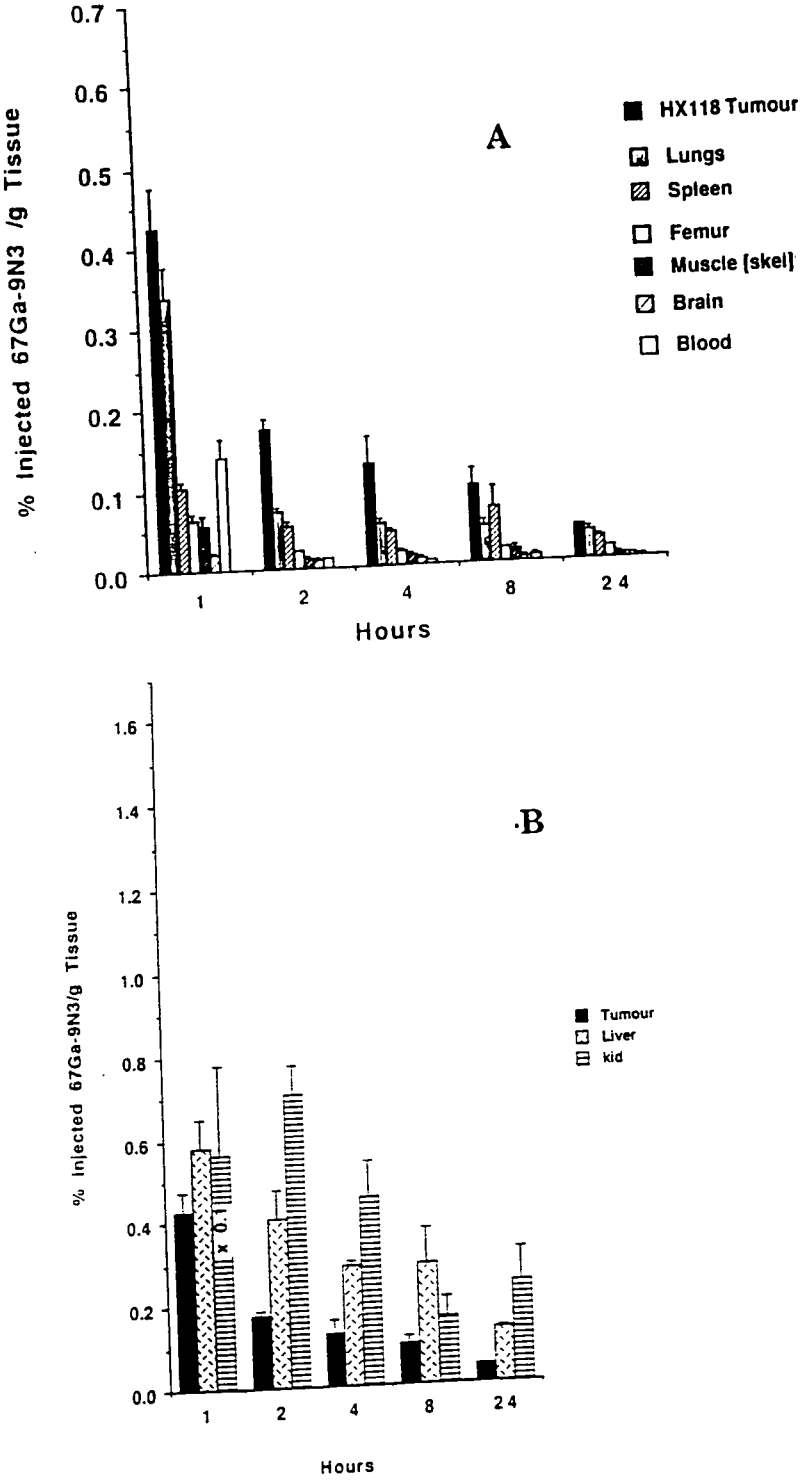
3.5.2 Experimental Method

The experimental method used was similar to that given in chapter 5. The major difference is that the mice had been previously injected subcutaneously in the flank with HX118 cells. After 6 to 8 weeks, the tumour volume is large enough (0.05ml) for their use in these experiments. At this time the mice are injected with 10-25 μCi of the radiometal complex. A fatal injection of sodium pentabarbitone was administered to kill the mice at 1, 2, 4, 8 and 24 hours.

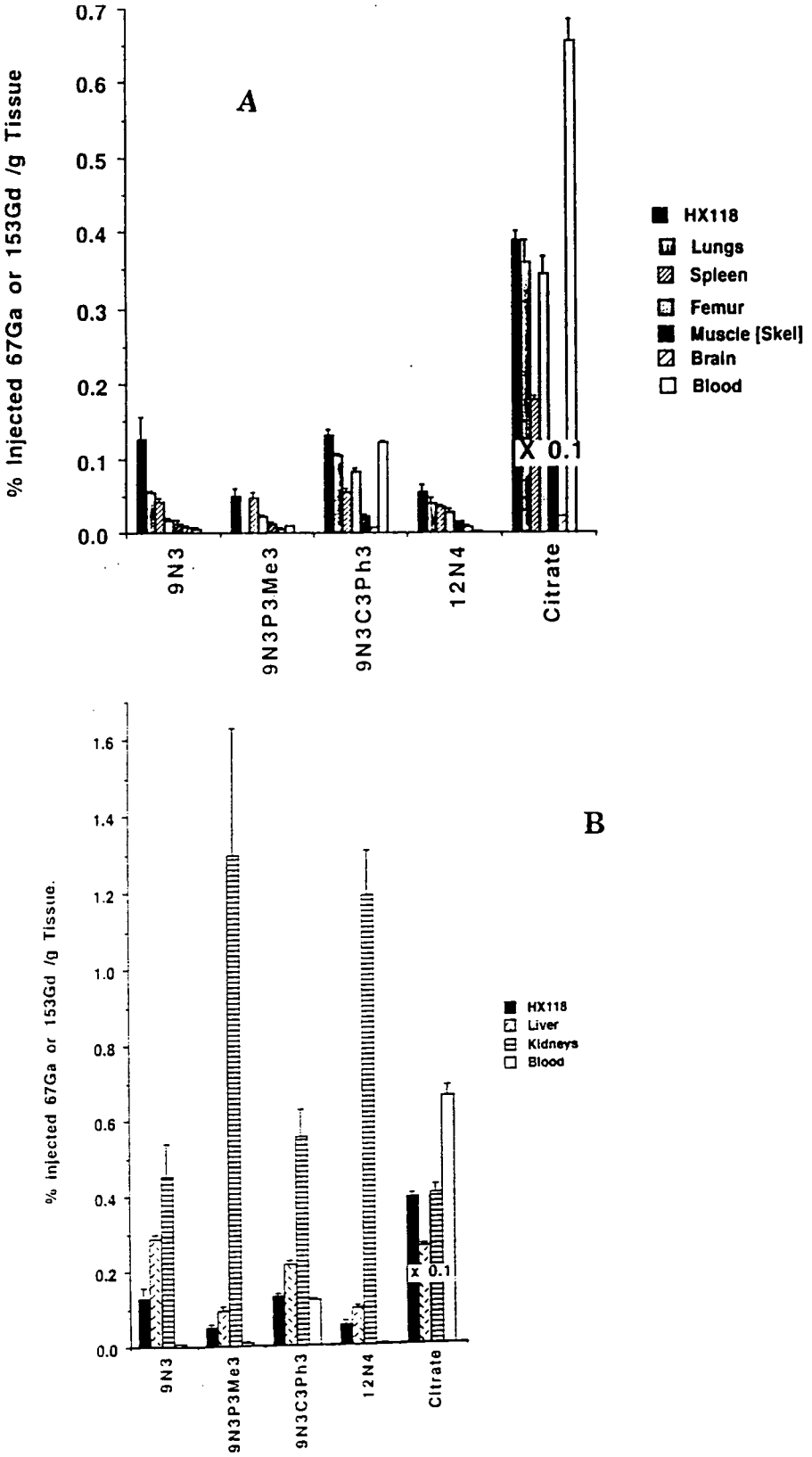
Also ^{153}Gd-DOTA was investigated as part of the experiment. Because the ^{153}Gd-DOTA complex is anionic and remains extracellular, it was hoped to to compare its biodistribution with those of the other (charge neutral) complexes, that can diffuse into the cells.

3.5.3 The Biodistribution of the ^{67}Gallium Complexes in Tumour-Bearing Mice

The results of the investigation are shown in graphs 19-20.



Graph 19 (A+B): Concentration of 67Ga-9N3 in blood, HX118 tumour and seven other tissues between 1 and 24 h after injection.



Graph 20 (A+B): *Concentration Of ^{67}Ga And Of ^{153}Gd In HX118 Tumour And Other Tissues 4 h After Injection.*

The data from the graphs is summarised below;

^{67}Gallium-9N$_3$C$_3$

At all times the the radioactivity produced by the complex, as compared to all the other organs, was greatest in the tumour (approximately twice), except for the liver and the kidneys, where the activity was 2 and 3 times that of the tumour.

The complex was excreted through the kidneys (RENAL).

The Other Complexes

The gallium-citrate complex demonstrates what occurs when a weak complex is introduced *in vivo* and dissociates, clearing slowly from all the organs and especially slowly from the liver, lungs and femur. At 4 hours the concentration of the gallium in the citrate treated mice was very high, and the amount ^{67}Ga in the blood is 5.8%g^{-1} -this value is higher than in any other tissue and the HX118 tumour.

The lipophilic complexes ^{67}Ga-9N$_3$C$_3$Ph$_3$ and ^{67}Ga-9N$_3$P$_3$Ph$_3$ cleared from the blood comparatively more slowly than Ga-9N$_3$C$_3$ and the concentration in the tumour at 4 hours was ≈ 0.13% g^{-1}. Both of the complexes cleared quickly and we may conclude that they remained intact *in vivo*.

The ^{153}Gd-DOTA complex cleared faster than Ga-9N3 in most tissues. This resulted in much lower concentrations in these tissues.

3.5.4 The Tumour : Blood Ratios At 4 Hours

The tumour : blood ratios are given in table 3.6. It is essential to have a high value for this ratio if the tumour is to be detected successfully (SPECT, PET).

Of all the complexes tested only the $9N_3C_3$ complex may meet the required ratio for successful imaging (tumour: blood ≈ 21).

	Injected	*Activity*	*% g^{-1}*	
^{67}Ga-Complex	Blood	Tumour	Tumour: Blood	Clearence
$9N_3C_3$	0.005	0.127	21.5	Renal
$9N_3P_3Me_3$	0.008	0.05	5	Renal
$9N_3C_3Ph_3$	0.121	0.13	1.1	H.-Biliary
DOTA*	0.002	0.055	22	Renal
Citrate	5.57	3.88	0.64	---

* ^{153}Gd

Table 3.6 *Concentrations of Radioactivity in Tumour and Blood at 4 h.*

The ^{67}Ga-$9N_3C_3$ and ^{67}Ga-$9N_3C_3$Ph3 complexes, which were eliminated via the renal pathway, had the highest tumour:blood ratios. The $9N_3C_3Ph_3$ complex is excreted via the hepato biliary route. As mentioned earlier in this chapter, the rate of clearance via the liver is very much fast than renal excretion with these complexes. This may partially explain the very low tumour:blood ratio for this complex.

3.5.5 Conclusions

The results from this investigation confirm the previous results[4] that the concentration of ^{67}Ga-$9N_3C_3$ complex is greater in the HX118 xenograft than in the blood at 4 hours. The mechanism by

which the gallium-$9N_3C_3$ complex localises in the melanin is not understood, but is likely to be related to the slower rate of clearance of the complex from the tumour cells. Also this behaviour is not specific to the melanotic melanoma[1]. Despite this, the tumour localising abilities of the complex in human melanotic melanoma grafted in mice are adequate for *in vivo* imaging.

3.6 REFERENCES

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7. Private communication, A. Harrison.

CHAPTER FOUR

SYNTHESIS AND INVESTIGATION OF THE COMPLEXING AGENTS 18N4S2, 18N4S2Me4, 18N4O2, 18N4O2Me4, N,N-DIBENZYLETHYLENETHIOUREA (12), 15N3O2C3 (7) AND LIGAND (9) AND THEIR SILVER(I), GOLD(I), YTTRIUM(III), AND RHENIUM(V) COMPLEXES.

4.0 SYNTHESIS AND INVESTIGATION OF THE COMPLEXING AGENTS $18N_4S_2$, $18N_4S_2Me_4$, $18N_4O_2$, $18N_4O_2Me_4$, N,N-DIBENZYLETHYLENETHIO-UREA (12), $15N_3O_2C_3$ (7) AND LIGAND (9) AND THEIR SILVER(I), GOLD(I), YTTRIUM(III), AND RHENIUM(V) COMPLEXES.

4.1 INTRODUCTION

This chapter is split into four sections. Each section describes the synthesis or attempted synthesis of the macrocycles described in section 1.5. Where the synthesis was successful, the results, outlined below, of the complexation experiments are reported.

The first section reports the comparison of the stability of the silver(I) complexes of $18N_4S_2$, $18N_4S_2Me_4$, $18N_4O_2$, and $18N_4O_2Me_4$. This investigation involves the use of calorimetrically determined entropies of complexation and pH - metric determination of protonation constants of the ligands and binding constants of the complexes.

The second section describes the complexation of gold(I) by the new complexing agent (12).

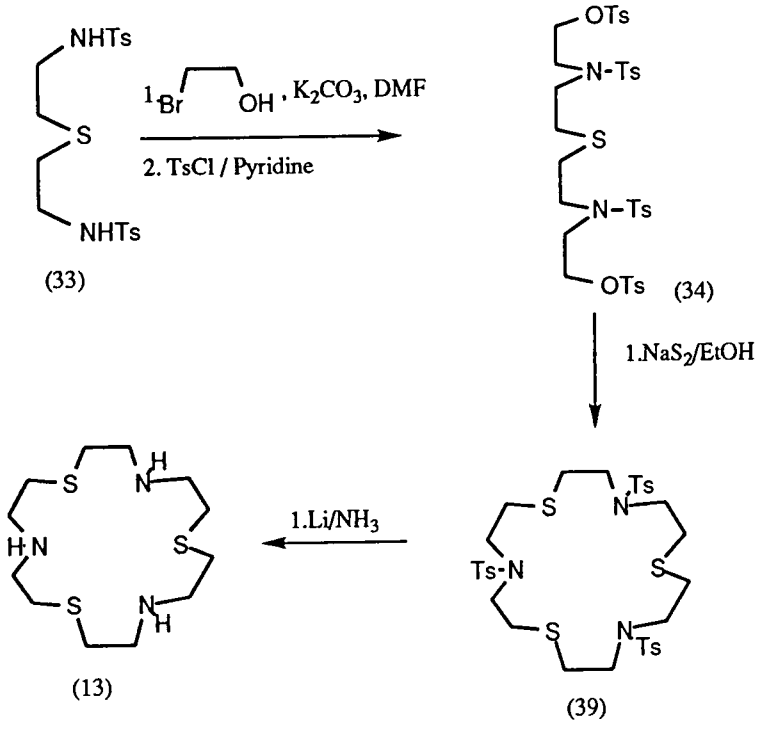
The third section reports the characterisation of the yttrium(III) complex of $15N_3O_2C_3$ (7). ^1H NMR and HPLC radiometric methods were used to determine its possible use for *in vivo* applications.

The final section of the chapter reports attempted the synthesis of the rhenium complex of (9).

4.2 THE SYNTHESIS OF THE SILVER(I) COMPLEXING AGENTS $18N_4S_2Me_4$, $18N_4O_2$, AND $18N_4O_2Me_4$, THE ATTEMPTED SYNTHESIS OF LIGAND (13) AND (14) AND THE COMPARISON OF THE STABILITY OF THE SILVER (I) COMPLEXES OF $18N_4S_2$, $18N_4S_2Me_4$, $18N_4O_2$, AND $18N_4O_2Me_4$

4.2.1 The Attempted Synthesis Of 1,7,13-Trithia-4,10,16-Triazacyclooctadecane (13)

Aza-thia macrocycles are difficult to prepare by standard macrocyclisation methods, but may be made from either tosylamide/thiol[10] or amine/acid chloride[11] precursors.

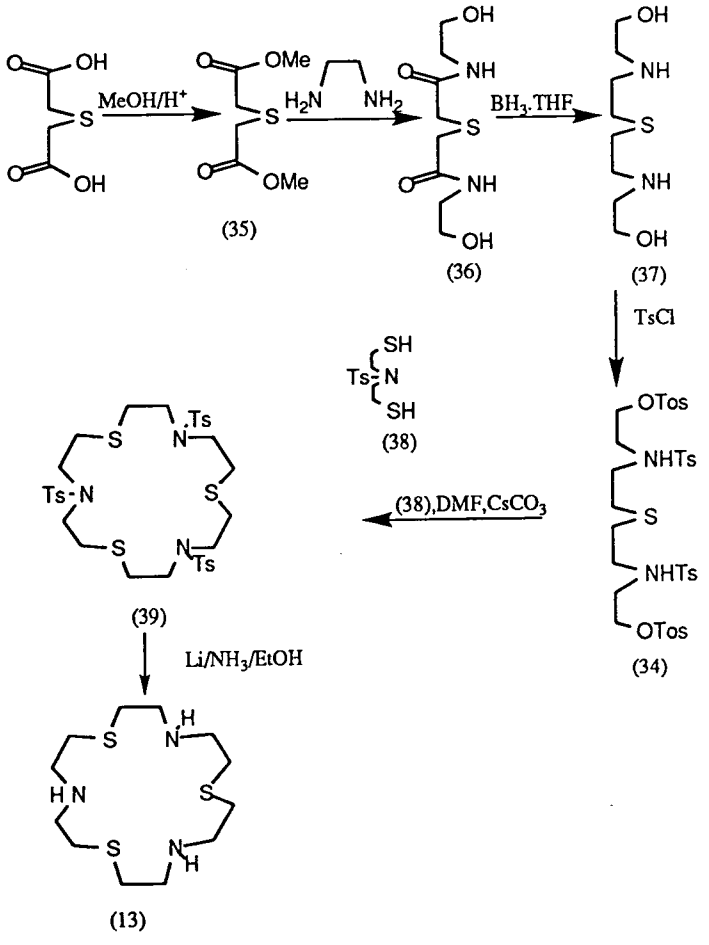


Scheme 4.1

The original synthetic proposal, Scheme 4.1, resulted in a mixture of tosylated material which proved intractable, and it was decided to attempt the longer synthesis outlined in scheme 4.2.

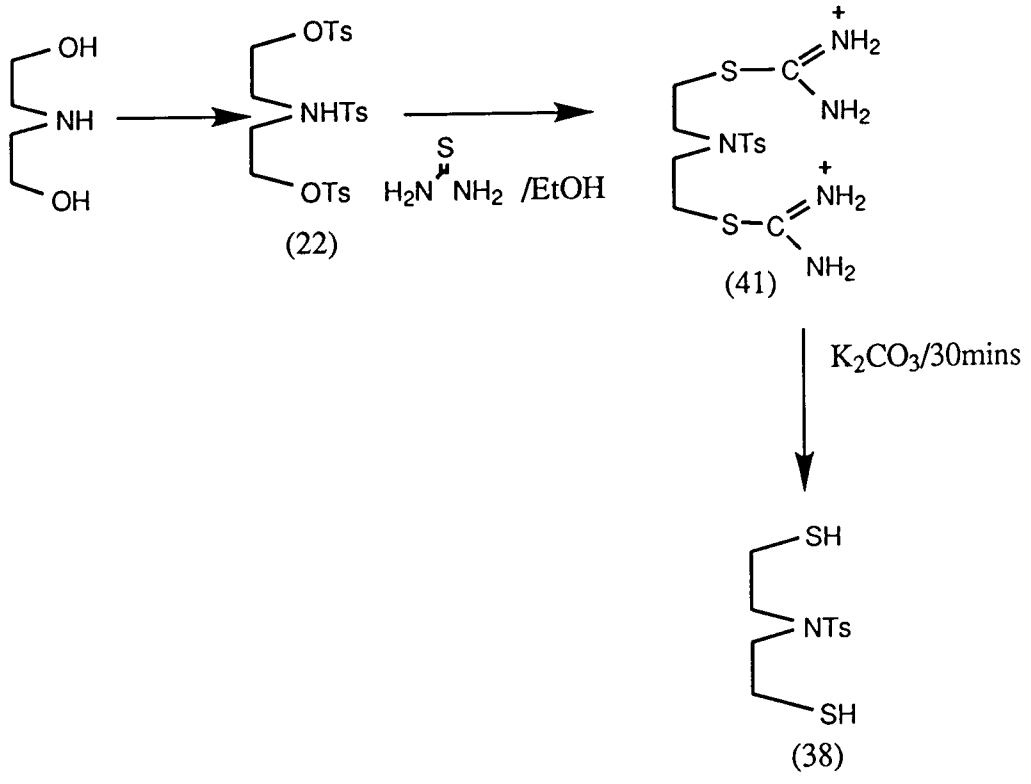
The esterification of thioglycolic acid with methanol yielded compound (35). Aminolysis (24 hour reflux in ethanolamine) resulted in a 43% yield of the diamide (36). Successful reduction was carried out with Borane-THF.

After trying a variety of solvents and temperature variations, tetratosylation was achieved with the highest yield using a mixed solvent system of acetonitrile and chloroform, with the initial reaction temperature at -15°C. The reaction could be monitored by TLC. After 4 hours, the reaction was allowed to warm up to room temperature, and was filtered after a further four hours. Column chromatography on alumina purified the reaction mixture.



Scheme 4.2

Synthesis of the dithiol (38) was carried out using a standard procedure, (Scheme 4.3)[12] However, this method resulted in a mixture of two compounds, presumably the thiol and the related disulphide, which could not be separated by chromatography. To remedy this problem, the reflux time of the intermediate salt (41), was reduced to thirty minutes. This resulted in the formation of the dithiol, free from the disulphide.



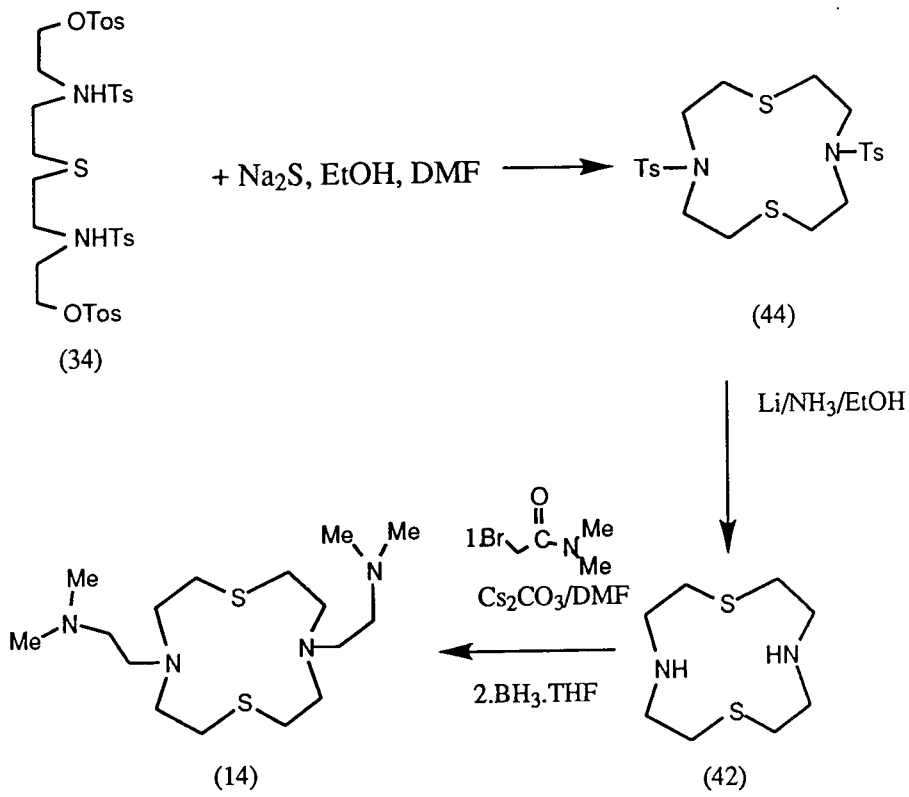
Scheme 4.3

The cyclisation step was attempted in DMF using caesium carbonate as the base. The reaction was followed by TLC, and after seven days, and elevation of temperature, there was no reaction. If more laboratory time had been available, different conditions could have been tried to facilitate the formation of the cyclic molecule (39).

4.2.2 THE ATTEMPTED SYNTHESIS 1,7-BIS(DIMETHYLAMINOETHYL)-4,10-DITHIA-1,7-DIAZACYCLOOCTADECANE (14)

4.2.2.1 ROUTE 1

The reaction of (34) with sodium sulphide was attempted, Scheme 4.4. The insolubility of sodium sulphide was a concern and a mixture of DMF and ethanol seemed to provide slight solubility and a finer suspension. The heterogenous reaction mixture was heated to 80 °C for four days but no chemical change was observed.

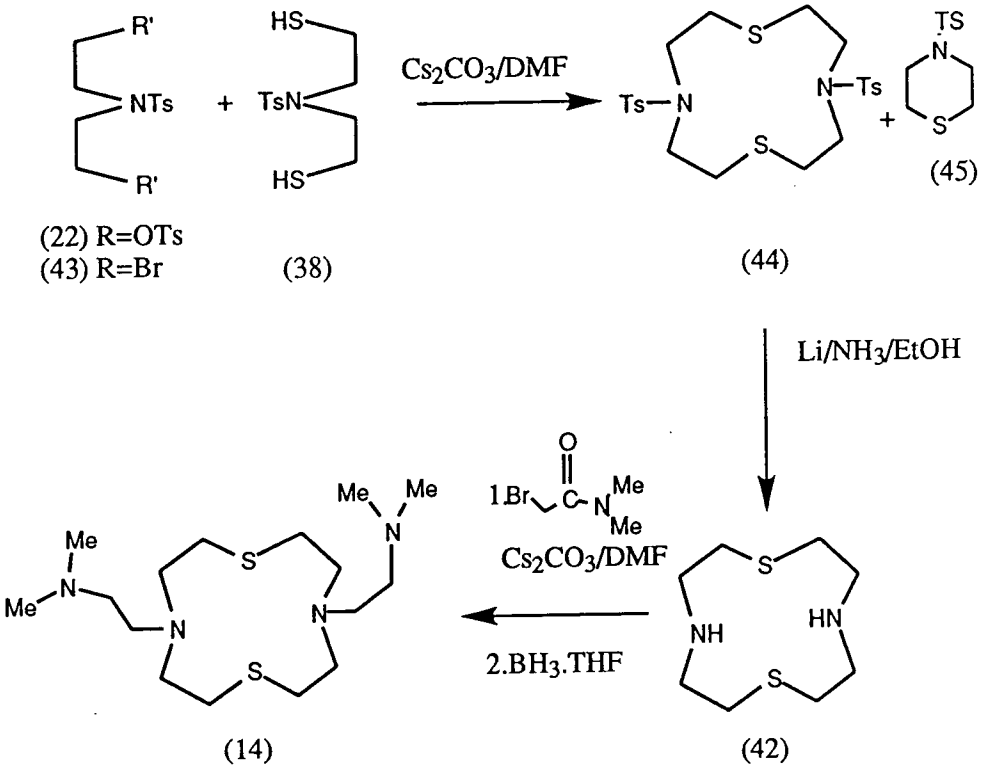


Scheme 4.4

4.2.2.2 ROUTE 2

Reaction of (22) with the dithiol (38), Scheme 4.5, produced no detectable change. Whereas reaction of (43) with the dithiol (38),

produced a mixture of products with (45) as the main component of the mixture (NMR and mass spectral analysis). The nature of the reaction pathway that facilitated the formation of this product (and why the reaction conditions favoured (45) is not clear). The deprotonation of the thiol and subsequent nucleophilic attack at the opposite carbon is the obvious reaction pathway. Displacement of a leaving group must have occurred, although the nature of the leaving group is unclear.



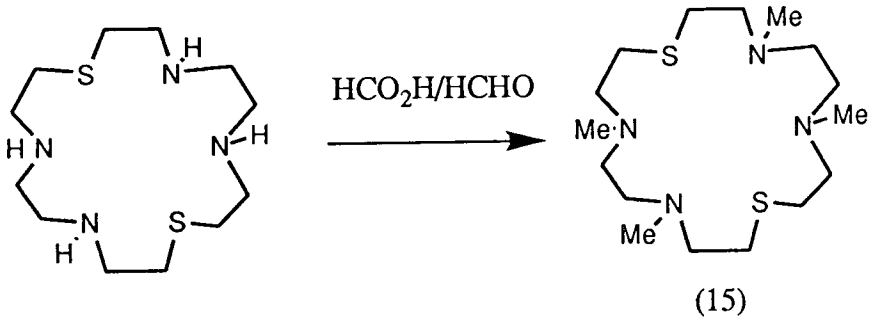
Scheme 4.5

4.2.2.3 Conclusions

Both of these attempts proved inconclusive. However, other similar cyclisation reactions[11,12] have proved successful. With more laboratory time, and a variation of the reaction conditions, both of these routes may be successful in producing an alternative route to the thiol/acid chloride method of producing $12N_2S_2$

4.2.3 SYNTHESIS OF N,N',N'',N''' - TETRAMETHYL-1, 10-DITHIA-4,7,13,16 - TETRAAZA - CYCLOOCTADECANE (15)

To further investigate the thermodynamic properties of (15)[12], the tetramethylated $18N_4S_2$ complexing agent was synthesised (Scheme 4.6). Tetramethylation was carried out using the Eschweiler-Clarke methylation procedure. Equimolar quantities of acetic acid and formaldehyde (37%) was added with $18N_4S_2$ and heated to 100°C for twenty-four hours. Purification of the tetra-amine was achieved by a recrystallisation from hexane.

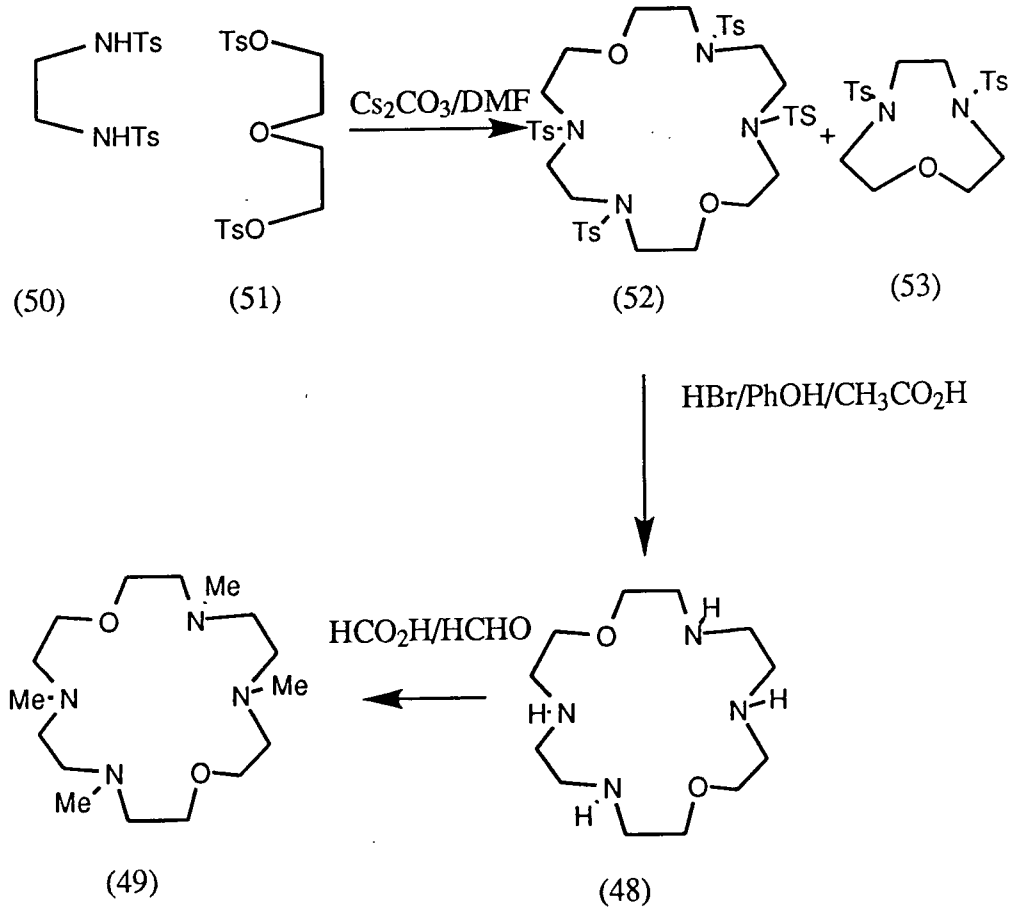


(15)

Scheme 4.6

4.2.4 SYNTHESIS OF N, N', N'', N''' - TETRAMETHYL -1,10-DIOXA -4, 7, 13, 16 - TETRAAZACYCLOOCTADECANE (49)[12]

In order to make comparative thermodynamic studies with the tetramethylated $18N_4S_2$ (15) and its silver(I) complex, it was necessary to synthesise the $18N_4O_2$ macrocycle (48)[12], and its tetramethyl derivitives (49) (Scheme 4.7).



Scheme 4.7

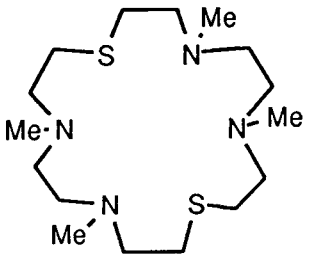
The cyclisation (DMF, caesium carbonate) of (50) and (51) gave two main products, the 9-membered (53), and the 18-membered (52) rings in a ratio of 2:1 respectively. Detosylation was effected using

HBr/acetic acid/phenol, followed by recrystallisation from hexane to give the $18N_4O_2$ as a colourless solid in 28% yield. Tetramethylation was carried out using the Eschwieler-Clarke methodology as with the $18N_4S_2$.

4.2.5 COMPARISON OF THE SILVER(I) COMPLEXES OF $18N_4S_2$, $18N_4S_2Me_4$, $18N_4O_2$, AND $18N_4O_2Me_4$.

4.2.5.1 Introduction

This section describes the results of the thermodynamic and pH - metric investigation of the silver(I) complexes of (14), (48), (49) and $18N_4S_2$.



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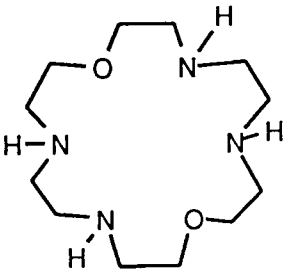

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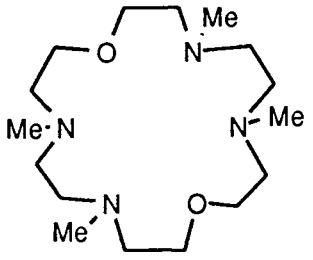

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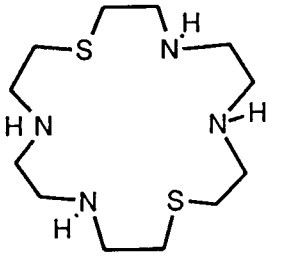
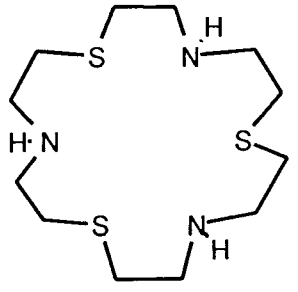


$18N_4S_2$

The failed synthesies of (13) and (14) prevented any complexation studies with these molecules.



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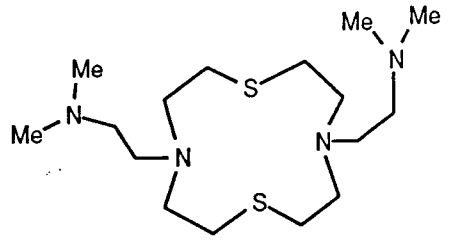

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4.2.5.2 Complex Stability As Determined By A Thermodynamic Study Of Complexation[1]

The entropies of complexation for the silver complexes of $18N_4S_2$, $18N_4S_2Me_4$, $18N_4O_2$ and $18N_4O_2Me_4$ were calculated from calorifically determined enthalpies of complexation.

Ligand	log K_s@/ dm^3 mol^{-1}	-ΔH/ kJ mol^{-1}	TΔS/ kJ mol^{-1}
N_4S_2	14.1	77.0	+ 3.3
$N_4S_2Me_4$	14.6	102.1	- 18.7
N_4O_2	11.2	59.5	+ 4.4
$N_4O_2Me_4$	13.4	84.3	- 7.8
N_2O_4*	10.0	51.4	+ 5.7
N_2S_4*	13.7	83.2	- 5.0
S_2O_4*	10.3	64.0	- 5.3
O_6**	4.6	38.3	- 12.1

* Data from ref. 3. ** Data from ref.4.
@ Errors in log K are typically (± 0.1) Or less, and for ΔH ± 0.3 kJ mol-1 or less

Table 4.1 *Stability Constants, Entropies And Enthalpies Of Complexation*

4.2.5.3 Discussion

The data in table 4.1 indicates that the silver (I) complex of $18N_4S_2Me_4$ possesses the highest stability constant of all the 1:1 complexes with these ligands. It is 10 times greater than the oxygen analogue and slightly more stable than the unfunctionalised parent macrocycle, ($18N_4S_2$).

The extra stability as a result of tetra methylation (as also seen with the $18N_4O_2$ complex) can be explained in terms of an increase in the enthalpy of complexation (table 4.1, third column). This suggests

[1]Carried out by H. Schneider, Max-Planck Insitut fur Biophysikalische Chemie, D-3400, Gottingen, FRG.

that a tertiary nitrogen is a superior σ–donor for silver(I) in methanol solution.

Closer scrutiny of the table also shows that the enthalpic advantage afforded by the tertiary amine is offset by the decrease in the entropy of complexation for these methylated macrocycles. A difference of 22 kJ mol^{-1} and 12.2 kJ mol^{-1} between the $18N_4S_2$, $18N_4O_2$ complexes and the methylated analogues may be understood as a result of two factors:

Firstly, the presence of the methyl groups causes a strained transition state leading to the complexation, which forces the macrocycle into an unfavourable conformation before complexation is attained. The obvious reason for this is that the methyl groups are interacting unfavourably with their neighbours.

Secondly, the desolvation of the ligand is an important contributory factor in the ΔS term. The secondary amines of (14) will form stong hydrogen bonds with the solvent whereas the methylated macrocycle cannot, and futhermore solvation is inhibited by the presence of the bulky methyl groups. Undoubtedly the higher (unfavourable) enthalpy of ligand desolvation for the secondary amine cycles compared to the tertiary amine analogues is also contributing to the observed ΔH differences.

4.2.5.4 Complex Stability As Determined By A Study Of The Protonation Constants Of The Ligands And The Binding Constants Of The Silver Complexes[1].

The protonation and binding constants of the silver (I) complexes of $18N_4S_2$ and $18N_4S_2Me$ in aqueous solution were measured (298K, 0.05 mol dm^{-3} NMe_4NO_3) (Table 4.2). The method employed was to use pH-metric titrations in the absence and presence of Ag^+.

Ligand	pK_1	pK_2	pK_3	pK_4	log K_{AgL}	log K_{AgLH}	log K_{AgLH2}	log K_{AgLH3}
N_4S_2	9.26	8.45	5.81	4.88	10.4 (7.91)	9.05 (5.40)	6.00 (3.94)	4.13
N_4S_2-Me_4	8.82	8.35	4.13	3.71	9.47 (7.41)	8.06 (4.60)	4.31	-
$N4O_2$*	9.67	8.85	6.61	3.21	-	-	-	-

Table 4.2 *Protonation for the ligands and binding constants for the silver(I) complexes. (298K, H_2O, I =0.1 NMe_4NO_3). Values in parenthese refer to protonation constants of the complexes (±0.1). * Ref 5.*

Two experimental points worthy of note are that firstly, the protonation constants were obtained using SCOGS and formation constants by SUPERQUAD and also all the pH-Metric data was analysed by SCOGS and SUPERQUAD

[1]Data analysed by R. Kataky, Unversity of Durham , Durham.

4.2.5.6 Discussion

The protonation constants for $18N_4S_2$ and $18N_4S_2Me_4$ are similar to those for the $18N_4O_2$ which have been reported previously[5].

The effect of the sulphur atoms has little effect on the basicity of the nitrogen atoms. The methylated molecule shows a slightly lower pK_a value which is accounted for by less effective solvation of the conjugate acid.

However, the binding constants for the 1:1 complexes in water are of the order of 10^4 times lower than those recorded in methanol (Table 4.3). The explanation for this lies in the better solvation of the cation and of the ligand provided by the water molecules.

Complex	Log K dm3 mol-1 (methanol)	Log K dm3 mol-1 (water)	Δlog K
N_4S_2	14.1	10.4	3.7
$N_4S_2Me_4$	14.6	9.47	5.13

Table 4.3 *A Comparison Of The Silver(I) Complex's Binding Constants In Water And Methanol*

Also the order of the magnitude of the binding constants for $18N_4S_2$ and $18N_4S_2Me_4$ is reversed in water. The explanation for this is in accord with the previous discussion: an enhanced negative entropy of complexation, whose main contributory factor is the ligand desolvation. In the case when water is the solvent, the non-methylated ligand is more highly solvated by water, and the increase

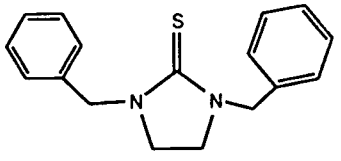
in entropy when these molecules are released (complexation), outweighs the loss of enthalpic energy needed to remove them.

4.3 SYNTHESIS AND CHARACTERISATION OF THE COMPLEX OF GOLD(I) WITH THE NEW COMPLEXING AGENT N,N-DIBENZYLETHYLENE - THIOUREA(12) AND THE ATTEMPTED SYNTHESIES OF LIGAND (56) AND LIGAND (57)

4.3.1 The Synthesis Of The Complexing Agents For Gold(I).

4.3.1.1 Introduction

It was decided to synthesise (12), in the hope that the addition of the lipophilic phenyl groups would provide us with a monodentate molecule functionalised in such a way as to resemble the bidentate ligands (56) and (57), (Section 1.5.5). The synthesis of (12) was successful and two methods were employed to attempt the formation of the gold(I) complex. The failed syntheses of (56) and (57) prevented any complexation studies with these molecules.



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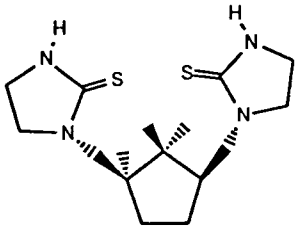

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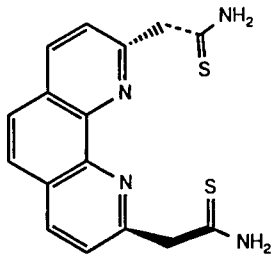
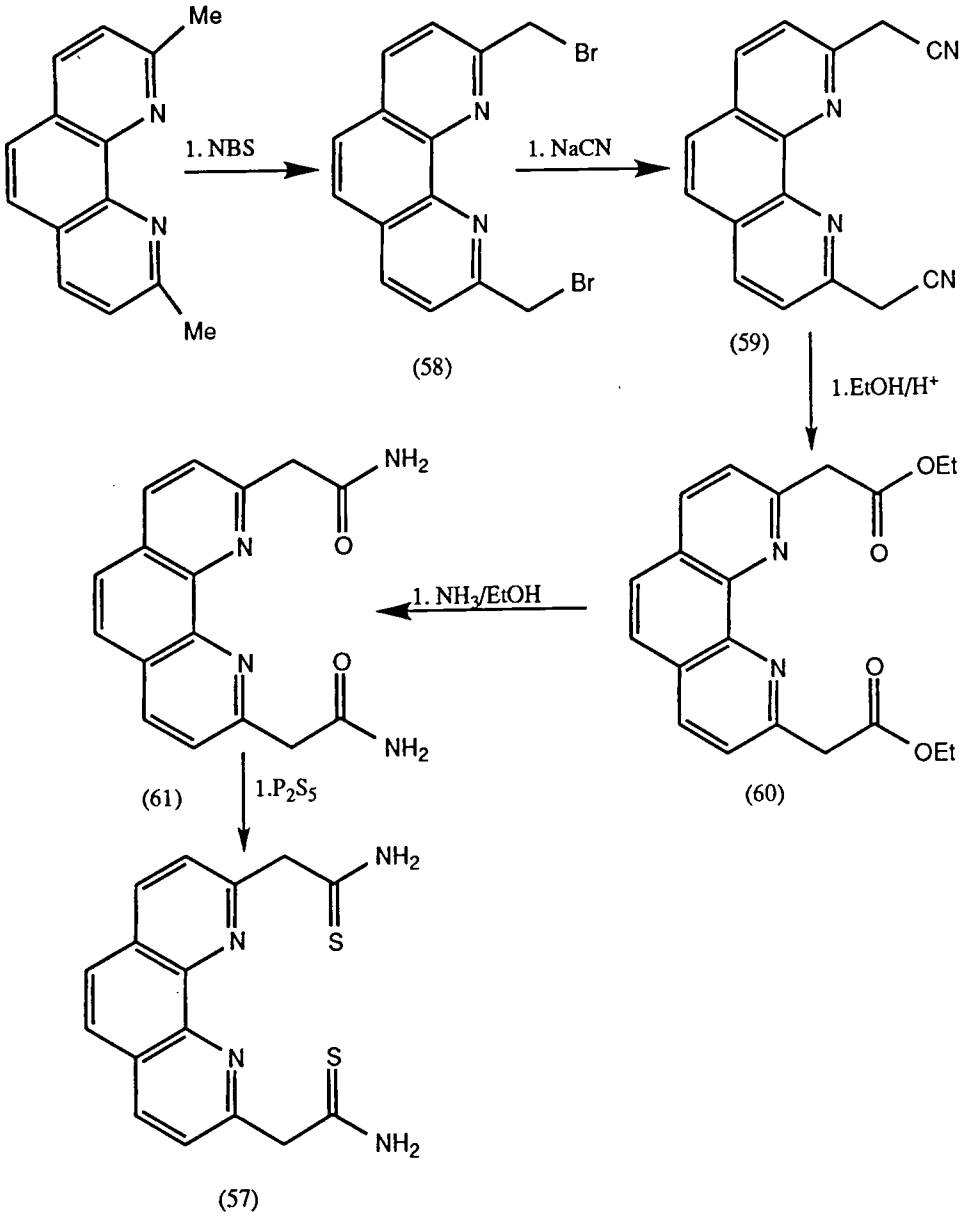


(57)

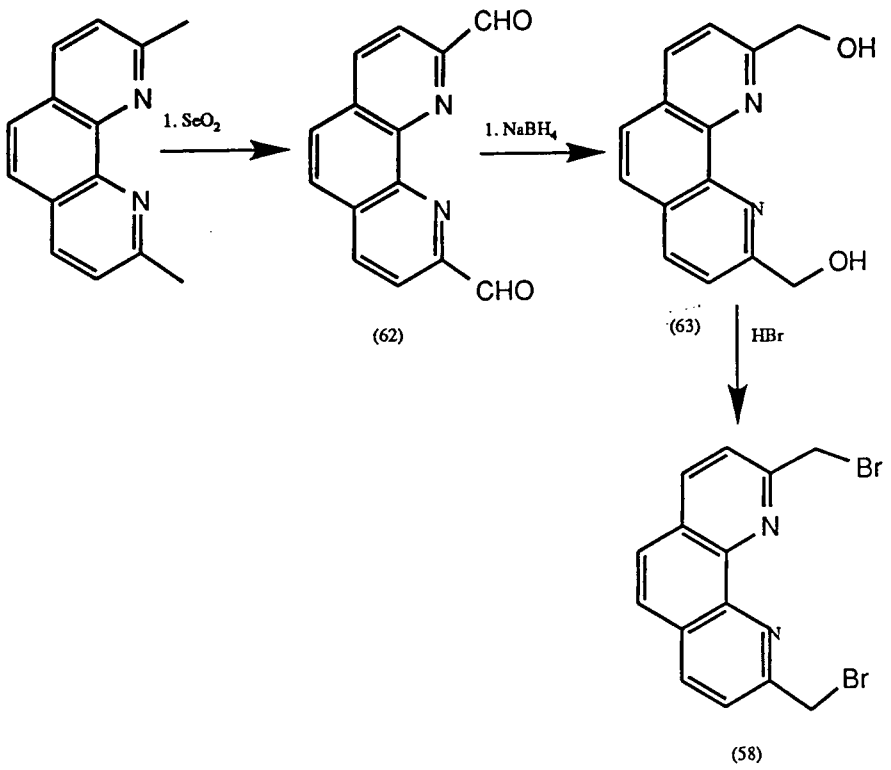
4.3.2 The Attempted Synthesis of (57)

The synthetic route outlined in scheme 4.8 begins with the selective mono-bromination of 2,9-dimethyl-1,10-phenanthroline using NBS. This was unsuccessful and the reaction produced mixed products of the di- and tri-brominated species, despite varying the reaction time and concentration of the reactants.



Scheme 4.8

Another approach, to synthesis (58), was attempted[15] (Scheme 4.9).



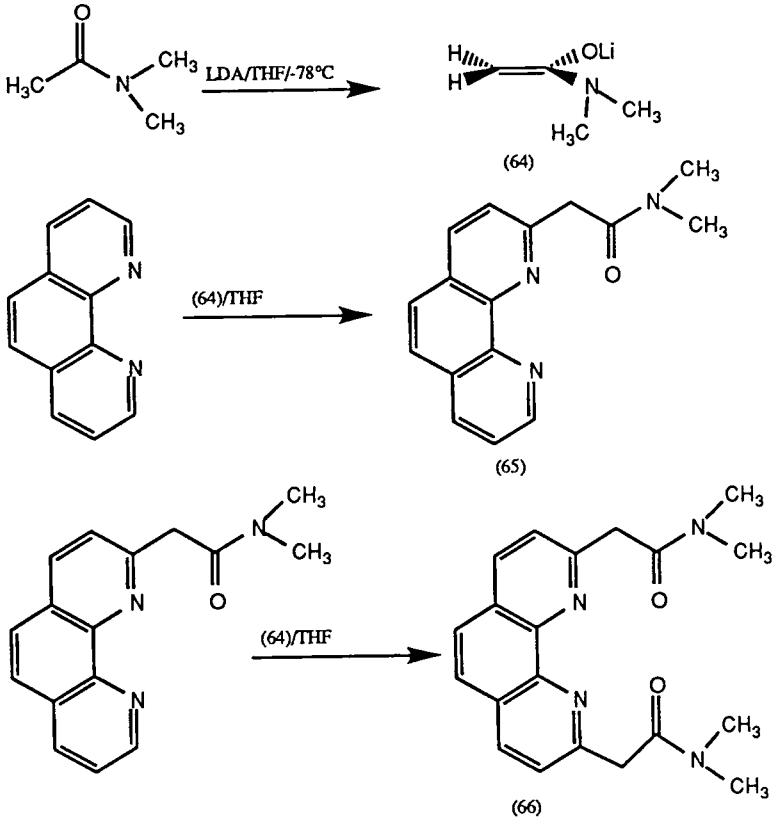
Scheme 4.9

The difficulty in this scheme was the use of borohydride to reduce the carbonyl to the alcohol. The reaction was very unreliable and the yield always unpredictable and small. Work up conditions were optimised to give the maximum and cleanest yield, but the product was always contaminated by an unknown species. We presumed that the boron from the reducing agent was binding to the phenanthroline ring and this was the cause in yield fluctuation and contamination of the product. Bromination was successful, and cyanation (Scheme 4.8) was attempted using sodium cyanide. The

micro-analysis confirmed that the mono nitrile was the major isolated product. The synthesis was not completed due to more emphasis being placed on the synthesis and data collection for the $9N_3$ complexing agents.

Synthesis of ligand (57) has also been attempted using a different strategy involving the use of lithium amide (Scheme 4.10).

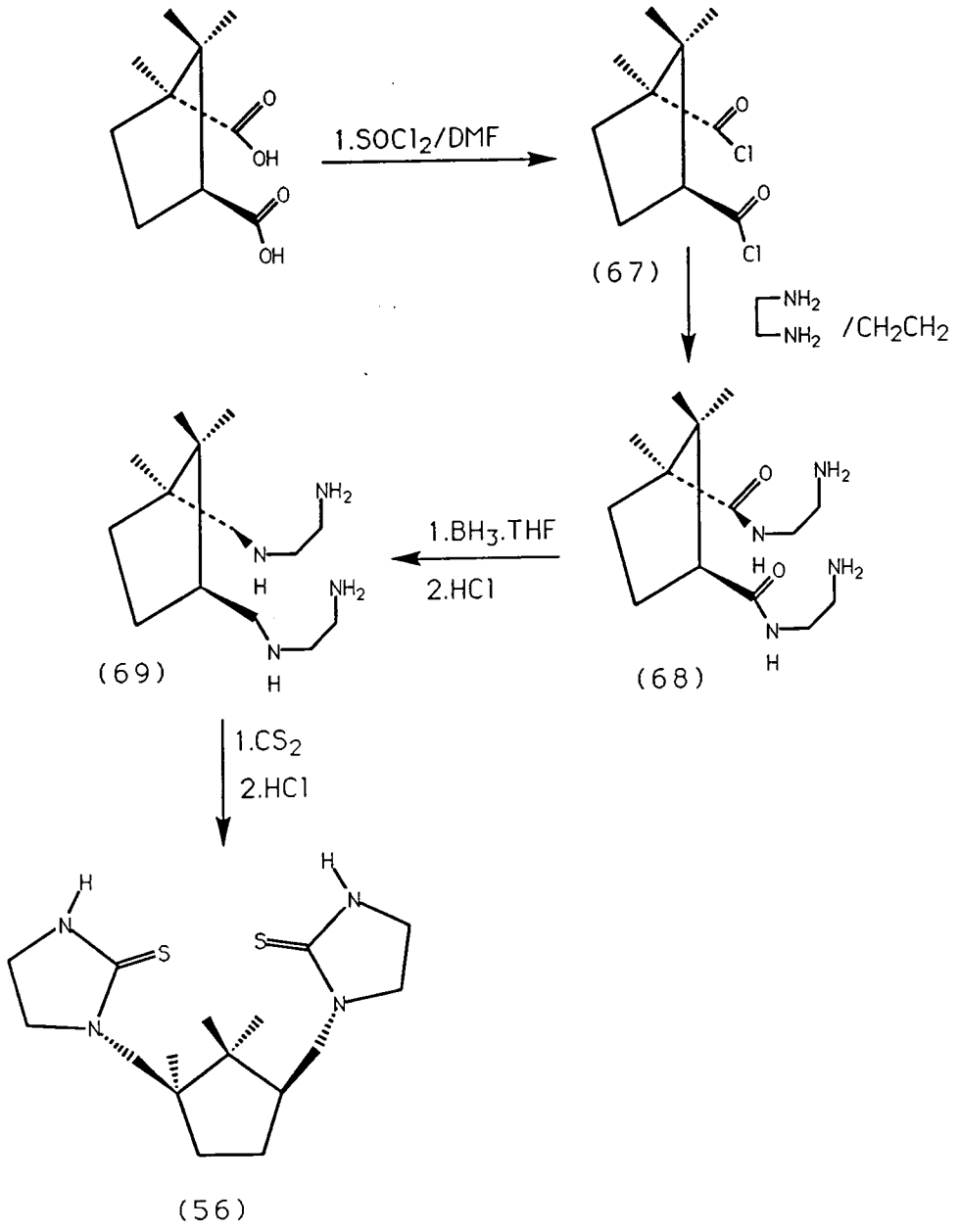
The monoalkylated phenanthroline (65) was detected in a low yield, but the subsequent di-alkylation was unsuccessful.



Scheme 4.10

4.3.3 The Attempted Synthesis Of Ligand (56)

Our initial strategy was to synthesise the diamide (68), and reduce this to give the tetra-amine (69), followed by cyclisation to give the desired ligand (56), (Scheme 4.11).



Scheme 4.11

Unfortunately, the formation of the amide via the diacid chloride, was unsuccessful. There was ^{1}H and I.R. evidence that the

monoalkylated species had formed but no indication of the formation of the dialkylated molecule. We were conscious of the possible intramolecular reaction forming camphoric anhydride (Figure 4.1). An I.R of the reaction mixture showed the distinctive stretch at 1770 cm^{-1}.

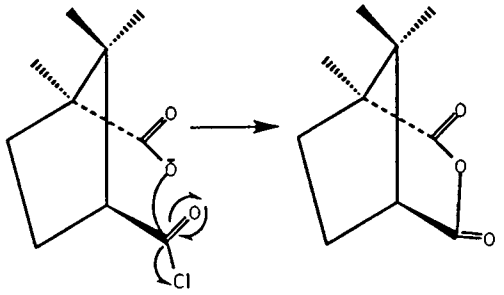
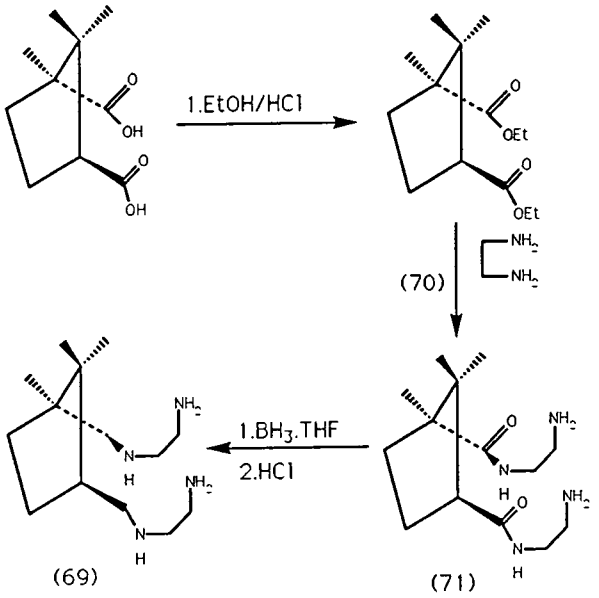


Figure 4.1 *The formation of Camphoric Anhydride.*

We proposed a different synthetic pathway, firstly forming the ester of the camphoric acid to inhibit anhydride formation (Scheme 4.12).

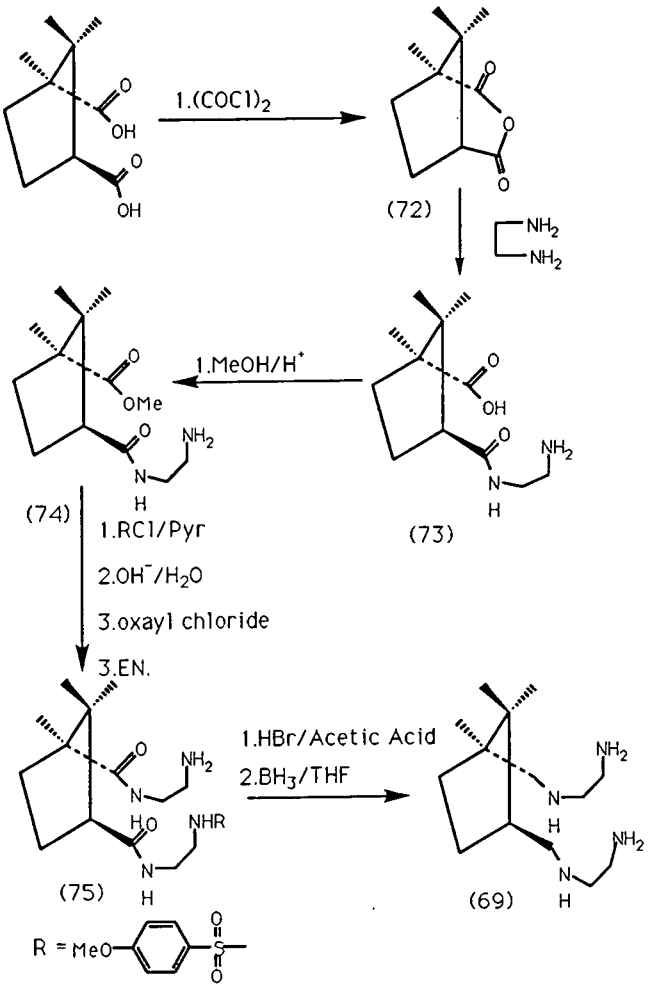


Scheme 4.12

This esterification was successful (50% yield) but the conversion of (70) to the diamide took 14 days and the reaction mixture still

contained starting material. The reduction was problematic also, as the diamide did not dissolve in THF. The reduction took 3 weeks at reflux and resulted in a complex mixture which proved intractable.

Formation of the diacid chloride was attempted again, this time using oxalyl chloride; the infra red spectrum (I.R. 1750, 1810 cm^{-1}) of the product suggests that the anhydride had formed. A new synthetic sequence was scheme 4.13.



Scheme 4.13

The reaction of the anhydride (72) with ethylenediamine may give (73), which may be protected by forming the methyl ester (74). The reaction of (21) with methoxybenzenesulphonyl chloride, followed

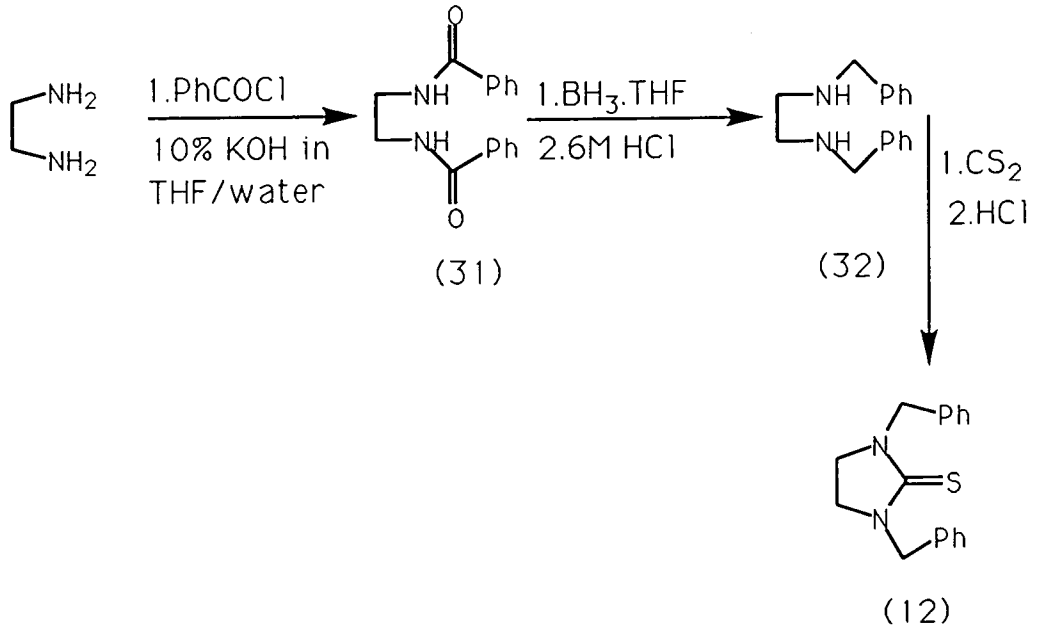
by saponification and treatment with oxalyl chloride and ethylene diamine would give (75). Deprotection of the amine followed by a reduction may gives us (69), which should be readily cyclised, using the CS_2 reaction, to give ligand (56).

This synthetic scheme was not carried out as the synthesis of the polyaza and polyoxaaza ligands took precedence.

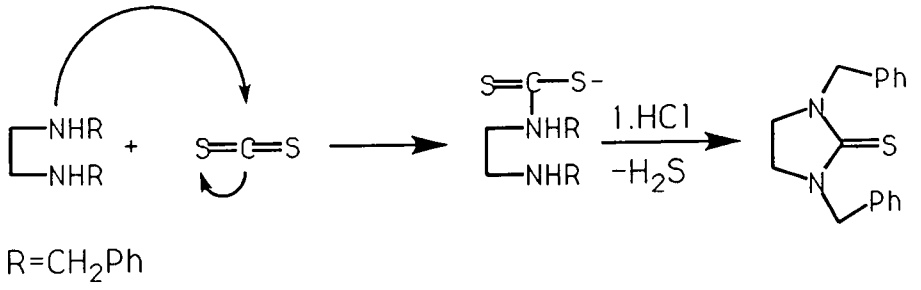
4.3.3 THE SYNTHESIS OF N,N-DIBENZYLETHYLENETHIOUREA (12)

N-alkylation of thiourea, a more direct route to (12), was not attempted as it was assumed that S-alkylation would occur preferentially.

N-benzoylation of ethylenediamine, followed by subsequent reduction with BH_3.THF yielded (32) in a modest yield. The cyclisation step[16] (Scheme 4.15) involves nucleophilic attack on the electron-deficient carbon centre of the carbon disulphide, followed by protonation and a second nucleophilic attack resulting in the formation of (12) (Scheme 4.14).



Scheme 4.14

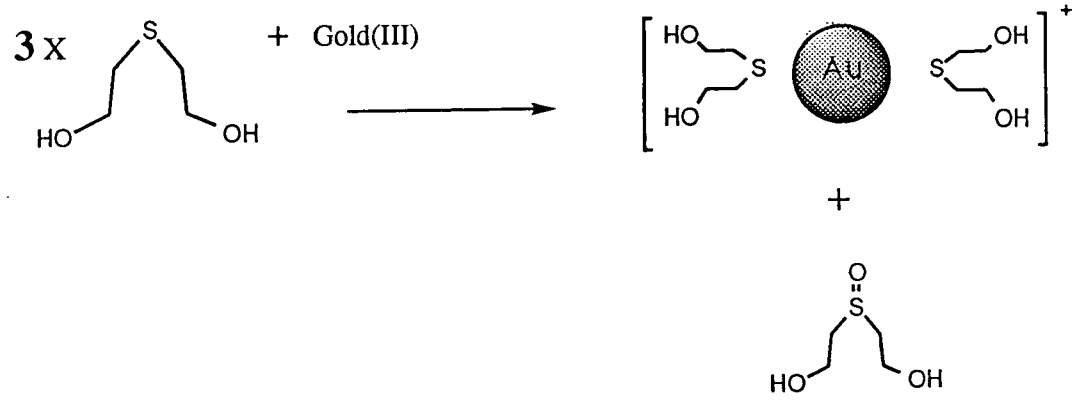


Scheme 4.15

4.3.4 Complexation Of Gold(I) By N,N-Dibenzylethylenethiourea (12)

Parker and Roy[17] have shown that thiodiglycol is a suitable reducing agent for gold(III). The propensity for thiodiglycol to reduce gold(III) is coupled with its ability to form a stable gold(I) complex in water. The complex is strong enough to stop the disproportionation of the gold(I) ion, but is easily displaced by a stronger donor. It was for these reasons that thiodiglycol was chosen as the reducing agent which permitted an attempt to form the complex of (12) with the gold(I) ion stabilised and reduced in one step. Evidence for the reduction and stability of the gold ion is given by ^1H NMR and is also indicated by the lack of appearance of a gold mirror.

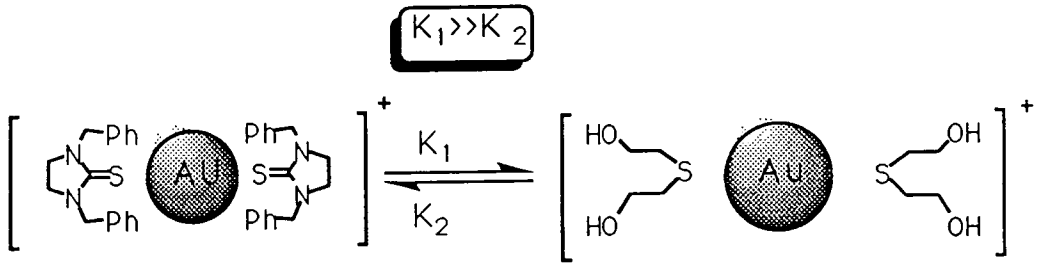
The experimental procedure (Scheme 4.16) involved mixing of the gold(III) precursor with three equivalents of thiodiglycol. Gentle warming of the solution is all that is necessary to form the gold(I) precursor.



Scheme 4.16

After the formation of the intermediate, one equivalent of (12) was added. At this stage no reaction could be observed (precipitation, colour change, etc) and to facilitate the precipitation of the complex a large anion was added (BPh_4^-, PF_6^-). After the addition of these

anions, no precipitation was observed. ^{1}NMR showed that the complex in solution was the gold complex of thiodiglycol. This suggested that the thoiodiglycol was in some way preventing (12) from binding. The possible reasons for this may have been that (12) was thermodynamically a weaker ligand and thiodiglycol binds in preference (Equation 4.1).



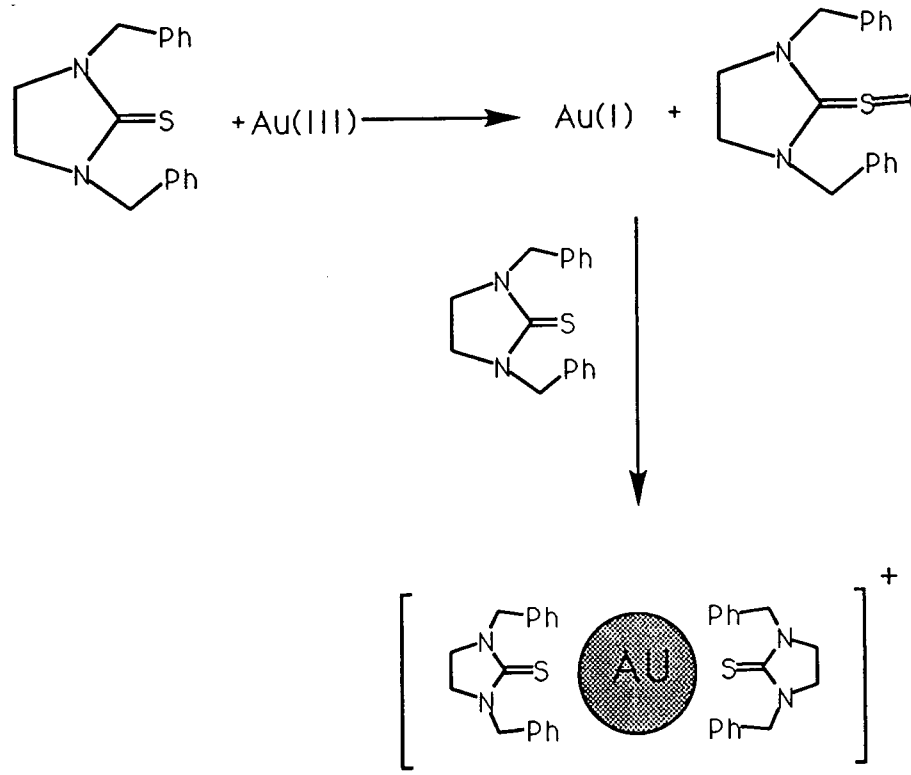
Equation 4.1

Another possible reason may be that the presence of the bulky benzyl moiety could be causing a steric hindrance and creating an energy maximum in the kinetic profile which drastically slows down the rate of complexation.

After incubating the solution at 40°C for 24 hours no precipitate was observed.

4.3.5 Use Of N,N-Dibenzylethylenethiourea (12) To Reduce Gold(III) To Gold(I).

Another attempt was made to form the complex using (12) itself to reduce the gold(III) *in situ* (Scheme 4.17).



Scheme 4.17

After warming the coloured solution of gold(III) with (12) the solution became colourless[1] ^1H NMR showed the characteristic signal shift for (12) indicating that the change in colour of the solution was due to the reduction of the gold(III). The addition of the larger anion, tetraphenylborate caused the precipitation of a pale yellow solid. Microanalysis, NMR and FAB mass spectral analysis indicated that the complex had formed, although it was contaminated

[1]Prolonged or excessive heating resulted in disproportionation, suggesting that the complex was unstable.

with a coprecipitate, probably a salt of teraphenylborate. ^{1}H NMR indicated that the previously equivalent protons of the ligand had become nonequivalent upon complexation.

4.4 SYNTHESIS OF $15N_3O_2C_3$ (7)AND CHARACTERISATION OF THE YTTRIUM COMPLEX

4.4.1 Introduction

As mentioned briefly in chapter 1, a substantial amount of research has taken place in the use of polyaza and polyazaoxa macrocycles to complex yttrium(III)[6]. The work of Cox and Parker is briefly summarized below and gives a formative overview of the precedent set by their extensive research.

4.4.1.1 Stability Constants Of Tetraazatetracarboxylic Acid Macrocyclic Complexes of Yttrium(III)

Cox reports the stability constants of five known yttrium complexes given in table 4.4

Complex	DOTA[a]	TRITA[a]	TETA[a]	DTPA[8]	EDTA[8]
YL	24.9	19.6	16.3	22.1[b]	18.1[b]

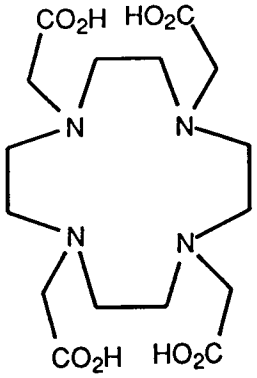
Table 4.4 *Stability Constants (Log K) of the Yttrium Complex.*

a) T = 298 ± 0.1K I = 0.1M [$(CH_3)_4NNO_3$].

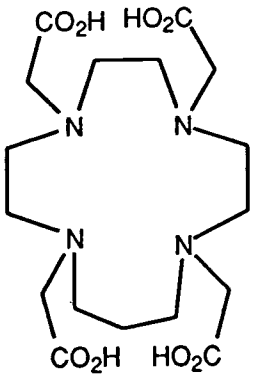
b) T = 298K I = 0.1M [KNO_3]

The order of magnitude of the stability constants measurements are:

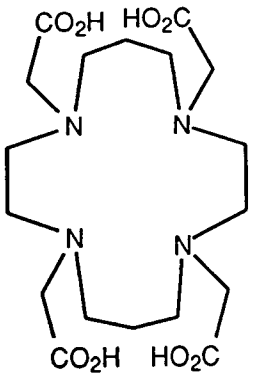
$$Y(DOTA)^- > Y(DTPA) > Y(TRITA)^- > Y(EDTA)^{2-} > Y(TETA)^-$$



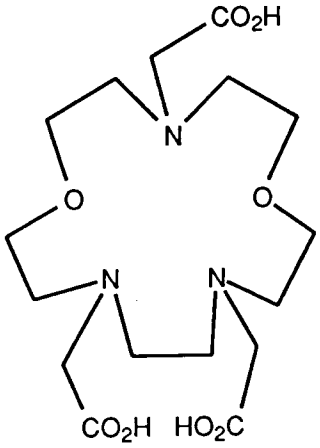
DOTA



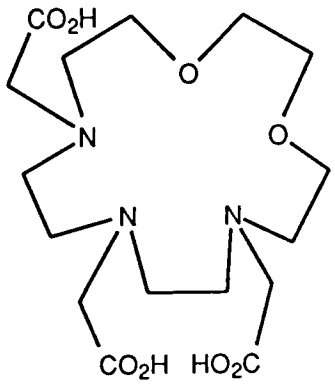
TRITA



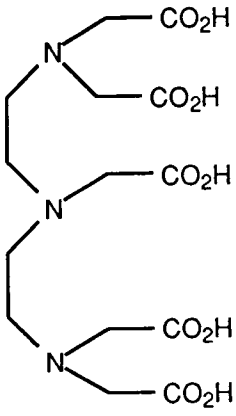
TETA



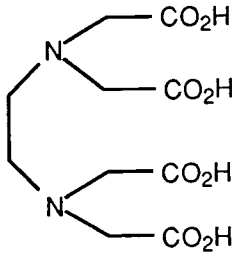
DTCTA



$15N_3O_2C_3$ (7)



DTPA



EDTA

In a summary of this work, Cox discussed the possible reasons for this trend. He suggested the following explanations:

> The propylenediamine groups of the six-membered chelate rings are more sterically crowded, due to unfavourable eclipsing interactions, than the smaller five-membered chelate rings.
>
> The macrocyclic conformation - that is the position adopted by the macrocycle after complexation - is dependent on the size of the chelate rings. The conformation adopted by $Y(DOTA)^-$ is the antiprismatic quadrangular [3,3,3,3] conformation. This is known to be the most stable conformation for octadentate yttrium complexes, as the repulsion energy between donors is minimised. The other possibility is a dodecahedral conformation, which is a less stable structure due to unfavourable steric interactions.

4.4.1.2 Measurement Of The Rate Of Uptake Of Yttrium(III) By The Macrocyclic Complexes

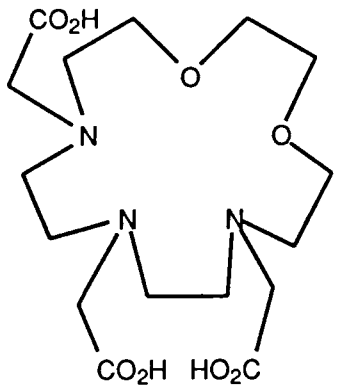
If the eventual use of the yttrium complexes is the specific targeting of radionucleides *in vivo*, the rate of uptake by the complexing agent is a critical factor in a successful application. This becomes obvious when taking into consideration the half-life of the yttrium nuclide and the time taken for the radio-conjugate to localise at the tumour site. (^{90}Y: β^- (2.3 MeV), $t_{1/2}$ = 64 hours)

Using ^1H NMR, Cox[1] was able to estimate the rate of uptake of the yttrium complexes of DOTA, TRITA, ODOTRA, and DTCTA. His results show that all the complexes are fully formed within ten minutes.

Further to this, the rate of forward binding was assessed by HPLC radiometry. He concludes that the approximate order for the extent of yttrium uptake is:

DOTA > DTCTA > ODOTRA ≈ TRITA >> TETA

4.4.1.3 $15N_3O_2C_3$ - A New Macrocyclic Complexing Agent For Yttrium(III).



$15N_3O_2C_3$ (7)

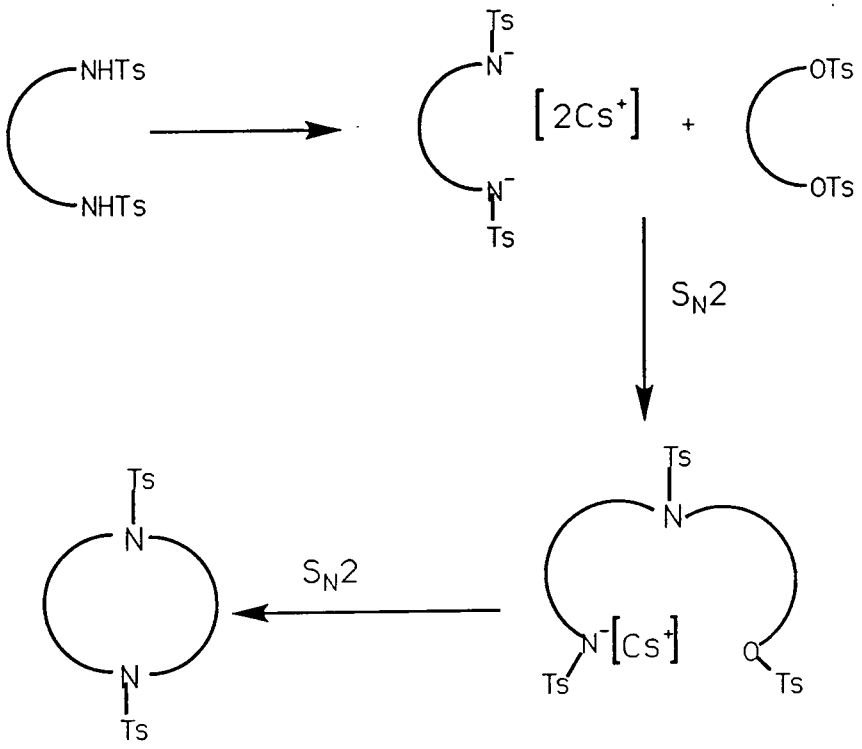
The yttrium complex of DTCTA which was synthesised by Cox[6], proved to be a poorer complexing agent than DOTA. The presence of the five-membered chelates and the overall neutrality of the complex appeared to be offset by the presence of the harder oxygen donors and possibly a more strained conformation.

It was decided to prepare $15N_3O_2C_3$, which could be considered as a macrocyclic analogue of DTPA, with three contiguous nitrogen donors. The macrocycle was synthesised and characterised by FAB MS, ^1H NMR and HPLC radiometry.

4.4.2 SYNTHESIS OF 7,10,13-TRIS(CARBOXY METHYL)-1,4 -DIOXA-7,10,13-TRIAZACYCLOPENTA -DECANE (7)

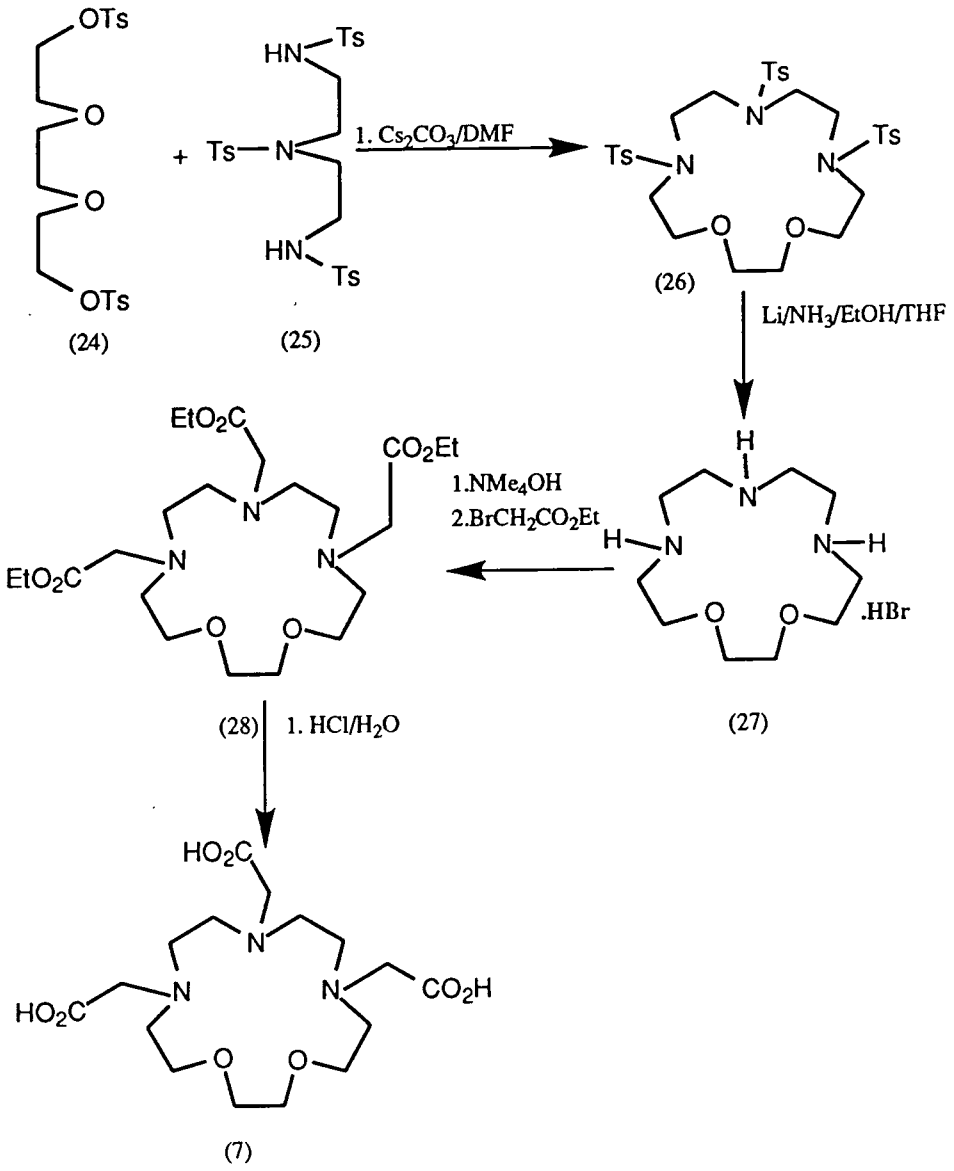
The crucial step in this synthesis (Scheme 4.18) is the cyclisation step (Scheme 4.19), and a judicious choice of base is required[7c]. It is thought that because of the size of the caesium ion, the use of caesium carbonate is advantageous. The reaction proceeds via the di-

caesium salt followed by two nucleophilic displacements of the tosyl groups .



Scheme 4.18

An excess of base is required, as the bicarbonate ion is not sufficiently basic to deprotonate the tosyl-amide at a reasonable rate. It has been suggested that the ion-pairing ability of caesium carbonate in DMF (caesium is a large ion and is poorly solvated by such dipolar solvents) causes contact pairs to be formed rather than purely solvated ions. The effect of this is to bring caesium into close proximity to the counter-ions and to promote intramolecular reactions as opposed to oligomer-forming intermolecular reactions. In addition, due to the large size of the caesium ion, it enables the S_N2 reaction to occur on the surface of the ion, with a prearranged position, creating a favourable entropy change for the reaction.



Scheme 4.19

Detosylation of (26) was at first attempted with hydrobromic acid, acetic acid, and phenol. The isolated yield of this reaction was less than 5%. The use of liquid ammonia and lithium metal was much more successful, giving yields of up to 80%. The final stages of this synthesis involved alkylation followed by hydrolysis of (28), to yield the target molecule which was isolated as the hydrochloride salt.

4.4.3 Estimation Of The Rate Of Yttrium Uptake Using ^1H NMR

Using a similar experimental method as Cox[6] a series of ^1H NMR experiments were made to make a semi-quantitive assessment of the forward rate of yttrium uptake by $15N_3O_2C_3$.

An ^1H NMR spectra was recorded of a 0.028 $mol^{-1}dm^{-3}$ solution of the complex at pD 5 as a comparison for the ^1H NMR of the yttrium complex.

Next the solution of the macrocycle was mixed with one equivalent of yttrium nitrate (in D_2O) and immediately placed within the NMR probe and the spectra recorded. After a further 10, 50, 110 and 170 minutes the spectrum was measured again. After three hours, ten more equivalents of yttrium were added and the ^1H NMR measured after 30 minutes. Figure 4.2 shows some of the spectra.

The spectrum of the complex is complicated and precludes any detailed analysis. The presence of the yttrium metal causes the various signals to spread out. The complex pattern suggests that previously equivalent methylene protons were diastereotopic and once the ligand is bound to the yttrium ion the position of these protons becomes fixed and they are no-longer symmetrically equivalent. Also the sharpness of the spectral peaks indicate that the ring is rigid.

After ten minutes there was no significant change in the ^1H NMR spectrum. Neither was there any change when further yttrium(III) was added. Therefore it was concluded that the complex must have formed within 10 minutes.

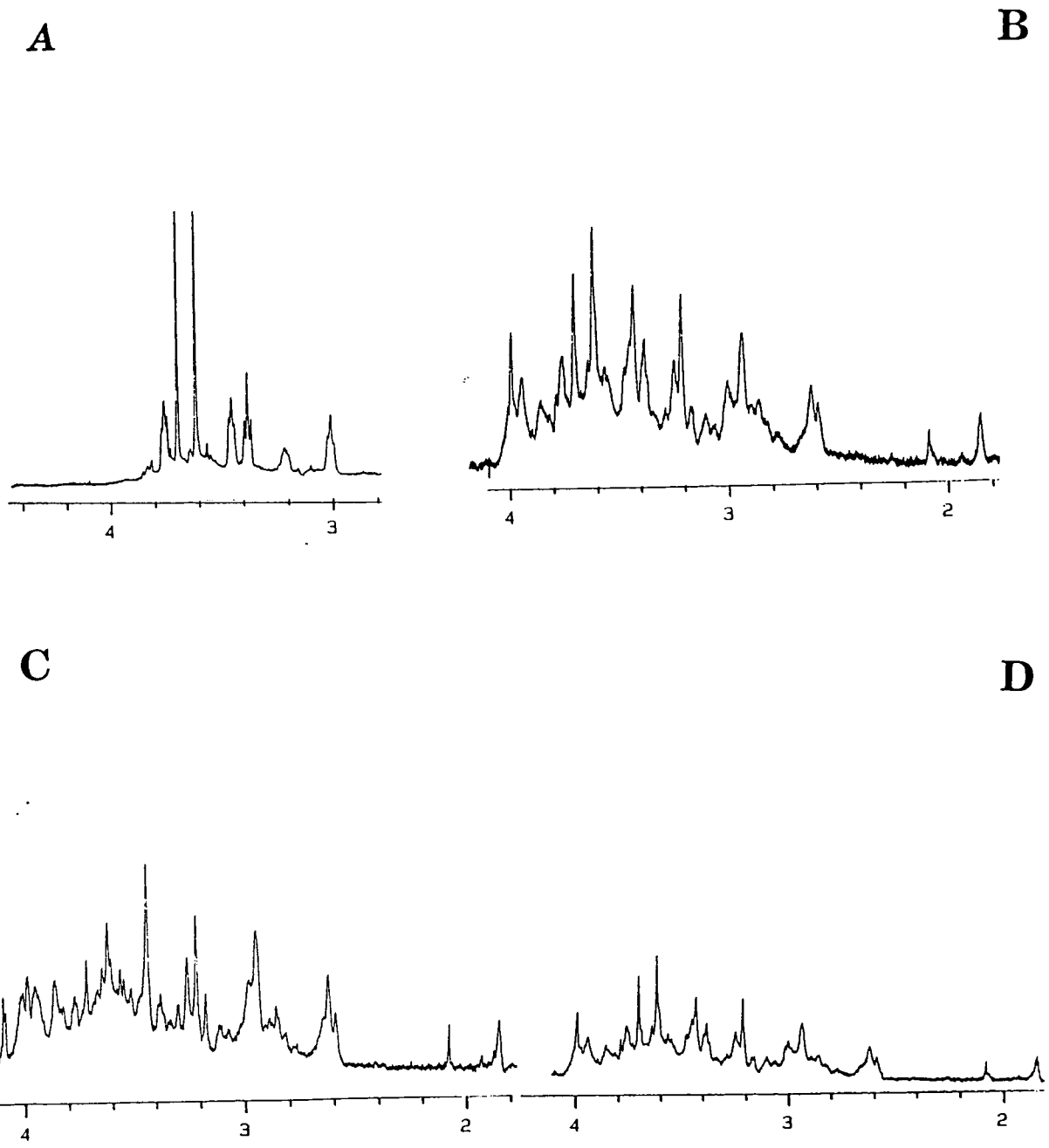


Figure 4.2 *^1H NMR Spectra (400MHz, D_2O, pD 5, 298K) of; A) $15N_3O_2C_3$ B) $Y(15N_3O_2C_3)$ after 10 min. C) $Y(15N_3O_2C_3)$ after 3 hours. D) $Y(15N_3O_2C_3)$ after 3.5h and with 11 equivs. of Y^{3+}.*

4.4.4 An Assessment Of The Kinetic And Thermodynamic Strength Of $^{153}Gd(15N_3O_2C_3)$ Using HPLC Radiometry[1] .

Using ^{153}Gd a semi-quantitive assessment was made of the kinetic and thermodynamic stability of the new complexing agent. Because ^{153}Gd is physically and chemically very similar to yttrium it was chosen as a suitable radioisotope to test the new macrocycle.

Gd-$15N_3O_2C_3$ was prepared by incubating 2 mmols of $15N_3O_2C_3$ in 200 nM ammonium acetate solution (pH 6.5) with trace amounts of ^{153}Gd at 37°C for between 0.5 and 5 hours.

The ^{153}Gd-$15N_3O_2C_3$ was then incubated with DTPA;

a) Ten-fold excess of DTPA for 5 mins..

b) Hundred-fold excess of DTPA for 5 mins..

c) One-fold excess of DTPA for 5 mins..

d) Ten-fold excess of DTPA for 30 mins..

a) 1. The gadolinium and $15N_3O_2C_3$ were incubated together for 30 minutes before the addition of the DTPA.

a) 2. The gadolinium and $15N_3O_2C_3$ were incubated together for 4 hours before the addition of the DTPA.

The results of these experiments are given in table 4.6.

---

[1] Experiments performed by A. Harrison and L. Royle, MRC, Radiobiology Laboratory, Didcot, Oxon.

Retention Time (mins)	a) 1.	a) 2.	b)	c)	d)	Species
[DPTA]	10 x	10 x	100 x	1 x	10 x *	Detected
4.4	6.04	6.49	5.70		4.73	Gd-15$N_3O_2C_3$
4.9	4.35	4.60		36.64		Gd-15$N_3O_2C_3$
6.8		0.08				Intermediates
7.5	1.35	0.89		10.91		Intermediates
9.9			35.85			Gd-DTPA
10.2			58.29			Gd-DTPA
10.9	88.26	87.95				Gd-DTPA
11.3				52.45	95.27	Gd-DTPA
14.2			0.16			Gd-DTPA

Table 4.6 *The Retention Times and Peak Areas. * 30 Minutes incubation period. The letters a) to d) refer to the preparation conditions described on the previous page.*

4.4.5 Discussion

The HPLC results indicate that the DTPA had stripped the gadolinium from the Gd-15$N_3O_2C_3$ complex with ease. When 10 and 100 equivalents of DTPA are present, approximately 90% of the gadolinium is complexed by the DTPA. When only one equivalent of DTPA present 36% of the Gd-15$N_3O_2C_3$ complex remains intact.

The difference in incubation period a) 1. (30min) and a) 2. (4 h) made no significant difference to the results, which confirmed the results the of ^1H NMR experiment i.e that the complex must have formed formed quickly (< 30 min).

DTPA forms a very thermodynamically stable complex with yttrium(III) (log K_{ML} = 22.1)[8]. The successful challenge by DTPA on the Gd-15$N_3O_2C_3$ complex indicates substantial kinetic instability in the Gd-15$N_3O_2C_3$ complex.

4.4.6 Conclusions

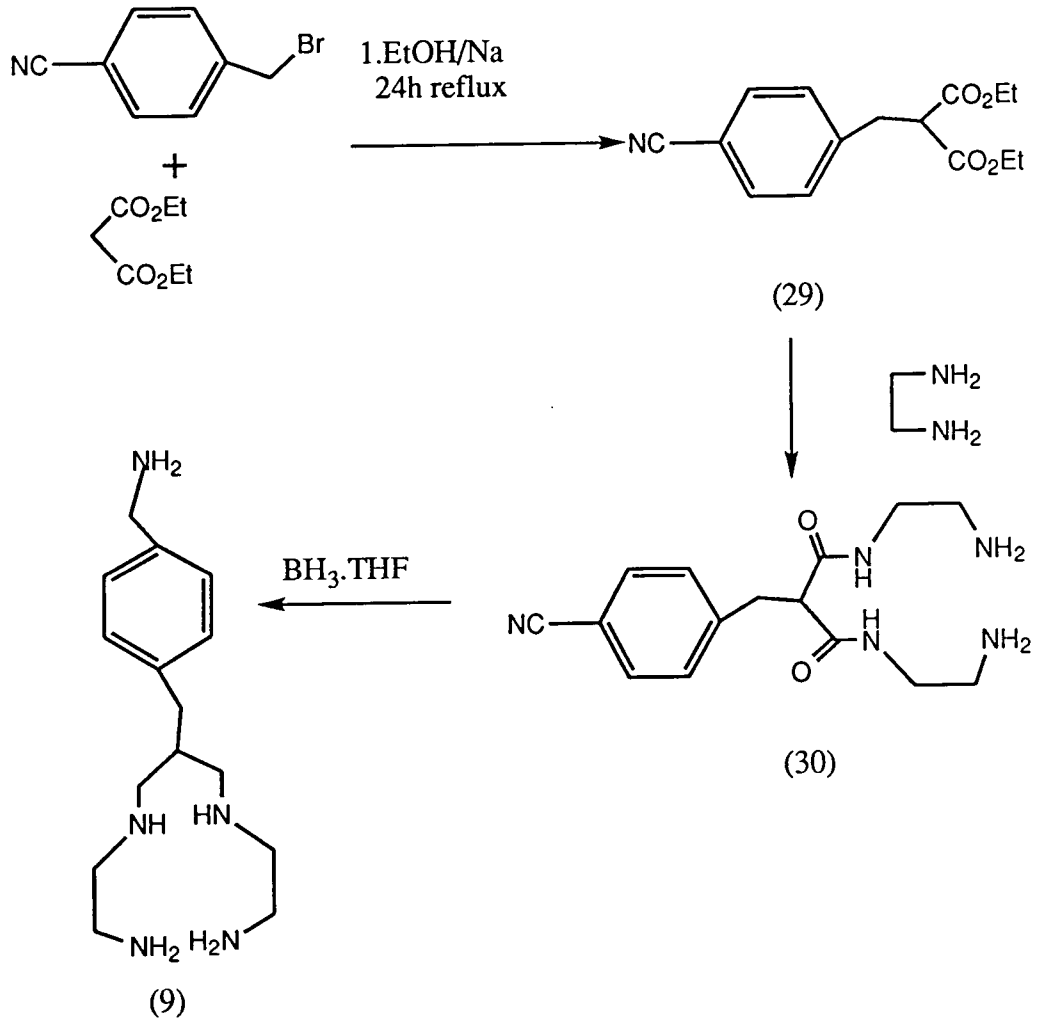
The ^{1}H NMR experiment gave evidence to suggest that the Yttrium-$15N_3O_2C_3$ 1:1 complex is formed quickly (< 10 min) at pH 5. This was confirmed (for Gd) by HPLC radiometry.

The kinetic instability of the complex has been demonstrated using a DTPA challenge and the complex is therefore deemed unsuitable for *in vivo* use.

4.5 THE SYNTHESIS OF LIGAND (9) AND THE ATTEMPTED SYNTHESIS OF THE RHENIUM COMPLEX OF (9).

4.5.1 Synthesis Of (9) 1,9-Diamino-(5-p-aminomethylbenzyl) -3,7-diazanonane(9)

The first step in this reaction scheme (Scheme 4.20) had been previously carried out in this laboratory[13]. The same procedure was followed. The formation of the di-amide was achieved by reaction of the diester (48h, 60°C) using dried ethylene diamine as the solvent and reactant.



Scheme 4.20

The following reduction reduces both the amide groups and the nitrile in a single step. BH_3.THF was the reducing agent used, and completion of the reaction was deduced by monitoring the infra-red spectrum of quenched samples of the reaction mixture. However, the work up and extraction of the amine (9) proved not to be straightforward. The work-up begins with the cooling of the reaction mixture followed by the quenching of the excess borane by adding methanol dropwise. Next, the solution was refluxed in HCl (6M) for 2 hours, and the water removed under reduced pressure.

This procedure is followed with a methanol wash to convert any of the boron species to the volatile $B(OMe)_3$, which can be removed, alongside the methanol, under reduced pressure. In most cases, the residue is dissolved in a sodium hydroxide solution bringing the pH to 14, thereby freeing the amine. Extraction is achieved using dichloromethane.

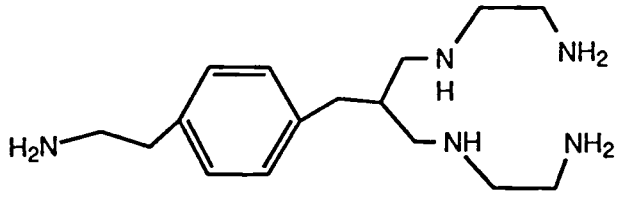
At this point, the mixture containing (9) was a white emulsion which would not separate. With the other half of the reaction products, the same procedure was repeated. After washing with methanol, instead of basifying the solution to pH 14, a different method of extraction was used. The approximate quantity of sodium hydroxide needed to neutralise the salt was dissolved in the minimum quantity of water. This was added to the solid residue. The mixture was warmed and the pH monitored and maintained (addition of sodium hydroxide), above pH 7. When all the residue had dissolved, the water was removed under reduced pressure. The neutralised amine residue was dissolved in hot dichloromethane, filtered, dried over a drying agent and the solvent removed under reduced pressure,

It is important to note that the solvent used to extract the compound was dichloromethane as opposed to chloroform, the reason being that at pH > 7, there is a possibility of the chloroform losing H-Cl, and forming the highly reactive dichlorocarbene, which may have reacted with the amine and contaminated the product. Recrystallisation from chloroform /methanol gave the pure product.

The next stage in the synthesis was to functionalise the pendant arm, so it may be attached to an antibody. Selective acylation (PhCOCl) of the primary amine was attempted using a Pipes buffer (pH 6.4) to selectively protonate the ring amine groups to allow for selective acylation. Unfortunately the pentaamine (9) would not remain in solution with the pH stabilised below pH 7. Dioxan was added to aid the solubility of the compound but was unsuccessful. Selective acylation was not achieved using this strategy.

4.5.2 THE ATTEMPTED SYNTHESIS OF THE RHENIUM COMPLEX OF (9)

This section reports the attempted synthesis of the rhenium complex of (9).



(9)

The synthetic method that was used was similar to the method employed by Parker and Roy[9].

The complexing agent was dissolved in chloroform and the rhenium was added as trans-$ReOCl_3(PPh_3)_2$. The mixture was stirred at room temperature and a small quantity of a yellow/green solid precipitated and was collected by filtration.

Micro-analysis of this compound showed it to contain less than 5% rhenium, suggesting a 1:1 complex had not been formed. ^1H NMR showed no indication of complexation.

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CHAPTER FIVE

EXPERIMENTAL PROCEDURES

5.0 EXPERIMENTAL PROCEDURES

5.1 INTRODUCTION

Throughout this work, temperatures are quoted in °C, and pressures in mbar. The alumina used in chromatography was Merck alumina, activity II-III, 70-230 mesh and unless stated always pre-soaked in ethyl acetate to deactivate it.

Proton and carbon-13 NMR spectra were recorded on a Varian 400, Varian Gemini 200, or a Bruker AC250 spectrometer. Proton and carbon chemical shifts are quoted in ppm to higher frequency of TMS. Coupling constants are given in Hz.

Infrared spectra were recorded on a Perkin-Elmer 577 spectrometer, as a thin film for liquid samples or a Nujol mull for solids, between NaCl plates. Mass spectra were recorded on a VG 7070E mass spectrometer, using CI, DCI, FAB, or EI ionisation modes as stated.

All solvents were dried using the normal laboratory procedures and all reactions were carried out under a dried inert gas (Nitrogen or Argon) unless the solvent used is water. "Water" refers to deionised water throughout. DMF and BH_3.THF refer to Aldrich 'Sure-Seal' reagents.

5.2 THE SYNTHESIS OF THE COMPLEXING AGENTS

5.2.1 (R) - 1,4,7-TRIS(2'-METHYLCARBOXYMETHYL)-TRIAZACYCLONONANE(4)

(R) - 1,4,7-Tris(2'-Methylcarboxymethyl)-Triazacyclononane (4)

To a mixture of triazacyclononane (1.5g, 11.7mmol) and (S)-(-)-2 chloropropionic acid (4.44g, 40.95mmol) in water (50 cm^3) was added lithium hydroxide until the pH reached 10. The mixture was vigorously stirred at 45 °C and the pH periodically monitored and maintained with the addition of more lithium hydroxide. During the reaction, samples were removed and examined using ^1H NMR to ascertain the amount of acid which had hydrolysed and, if necessary, up to 3 equivalents of acid were added. After 10 days, the reaction was complete (TLC, NMR). The pH was adjusted to 2 (HCl) and the mixture filtered and the water evaporated under reduced pressure. The addition and evaporation of methanol to the residue (x3) ensured that it was free from water. Esterification was achieved by refluxing the residue in methanol (20 cm^3) with 1 cm^3 of sulphuric acid present. After 24 hours the mixture was cooled, neutralised with dry sodium bicarbonate, filtered and the solvent removed under reduced pressure. Purification was achieved on an alumina column using methanol (0-2%) / dichloromethane followed by acid hydrolysis (6M HCl, 60°C, 4hr) to give a white crystalline solid. (2.3g, 61%) m.p. 265°C

Ester:

NMR: δ_H ($CDCl_3$) 3.6 (9H,s,0C<u>H</u>$_3$), 3.5 (3H,quartet,C<u>H</u>(Me)-C0$_2$Me), 2.7 (12H, m, N-C<u>H</u>$_2$-C<u>H</u>$_2$-N), 1.25 (9H, d, C<u>H</u>$_3$); δ_C ($CDCl_3$) 173.86 (carbonyl), 60.95 (OMe), 52.99 (β-C), 50.1 (N-CH_2-CH_2-N), 15.7 (-CH_3); IR (Nujol) 2900 (CH_3), 1730 (CO); MS: m/z (DCI,NH_3) 404 (M^+ + 17)100% , 389 (M^+ + 2) 22%.

Acid:

Analysis found: C, 45.20; H, 7.51; N, 10.40; $C_{15}H_{27}N_3O_6.HCl.H_2O$ requires C, 45.06; H, 7.56; N, 10.51 %. NMR: δ_H (D_2O) 4.0 (3H, quartet, N-C<u>H</u>(Me)-COOH), 3.3 (12H, m,(N-C<u>H</u>$_2$-C<u>H</u>$_2$-N), 1,4 (9H,d -C<u>H</u>$_3$); δ_C (D_2O) 175.0 (carbonyl), 60.55 (β–C), 46.41 (N-CH_2-CH_2-N), 10.51 (-CH_3); IR (Nujol) 1740 (carbonyl);MS: m/z (DCI, NH_3) 362 (M^++17) 100% .

5.2.2 1,4,7-TRIS(2'-PHENYLCARBOXYMETHYL)-1,4,7-TRIAZACYCLONONANE (5)

(2)-Bromophenylmethylpropanoate (21)

To a mixture of (2)-bromo-phenylacetic acid (5g, 0.23mol) in methanol (50 cm^3) was added sulphuric acid (1 cm^3) and the vigorously stirred mixture was brought to reflux for 24 hours. The cool acidic mixture was neutralised with excess sodium bicarbonate and following filtration, the solvent was removed under reduced pressure. Purification was achieved on an alumina column eluted with CH_2Cl_2/hexane (1:100) and produced a clear oily residue after

the elutants had been removed. (4.3g, 81%). b.p.112°C, 3mmHg [lit[7] 113°, 3mmHg]; NMR: δ_H ($CDCl_3$) 7.4 (5H, s, phenyl), 4.6 (1H, m, CH(Ph)-C), 3.71 (3H, s, OMe); δ_C ($CDCl_3$): 171.1 (carbonyl), 136.1, 128.1, 127.9, 126.1 (aromatic), 70.2 (β-carbon); MS: m/z (DCI) 230 (M^++1) 64%.

1,4,7-Tris(2'-phenylcarboxymethyl)-1,4,7-triazacyclononane (5)

To a vigorously stirred solution of 1,4,7-triazacyclononane (0.200g, 15.6mmol) with potassium carbonate (0.72g, 52mmol) in acetonitrile (10 cm^3) was added the ester (21) (1.18g, 51.5 mmol). The temperature of the mixture was adjusted to 60°C and maintained for 7 days. The cooled mixture was filtered and the solvents removed under reduced pressure. Purification by an alumina column eluted with (0-2%) methanol in dichloromethane resulted in a clear oily residue. After checking the purity of the ester using carbon and proton NMR, the compound was hydrolysed (6M HCl, 60°C, 24hr), to yeild a colourless solid, (3.8g, 48%).

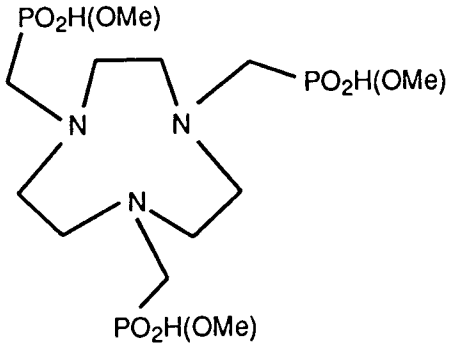
Ester

NMR: δ_H ($CDCl_3$): 7.35 (15H, s, Phenyl), 4.37(3H,quartet, N-C<u>H</u>(Ph)-CO), 3.60 (9H, s, OMe), 2.75, 2.99 (12H, d, AA'BB', J=13.1, CH_2-N); δ_C ($CDCl_3$): 173.68 (carbonyl), 135.9, 128.5, 126.8, 125.3 (aromatic), 71.49 (β-C), 52.8, 54.8 (N-CH_2-CH_2-N); MS: m/z (DCI) 574 (M^++1) 100%.

Acid

Analysis found: C, 61.0; H, 6.11; N, 6.9; $C_{30}H_{33}N_3O_6.HCl.H_2O$ requires C, 61.48; H, 6.19; N, 7.17 %. NMR: δ_H (CD_3OD): 7.44 (15H, s, Phenyl), 3.8 (3H,quartet,N-C<u>H</u>(Ph)-C), 3.1 (12H,m ,N-C<u>H</u>(Ph)-C); δ_C (CD_3OD): 173.71 (carbonyl), 137.9, 129.5, 128.8, 128.3 (aromatic), 72.51 (b-C), 51.9, 53.9 (N-CH_2-CH_2-N);MS: m/z (DCI, NH_3) 532 (M^++1) 100%.

5.2.3. THE ATTEMPTED SYNTHESIS OF O,O',O''-TRIMETHYL-1,4,7-TRIS(PHOSPHONATOMETHYL)-TRIAZACYCLONONANE(6)



(6)

1,4,7-Tris(phosphonoxy methyl)-triazacyclononane(22)

To a stirred solution of 1,4,7-triazacyclononane (0.19g, 1.5mmol) and phosphorous acid (0.374g, 4.6mmol) in 6M hydrochloric acid (3 cm^3) at 120°C was added aqueous formaldehyde (37%, 0.38g,

4.7mmol) dropwise over 2.5 hours. After evaporation the solid was redissolved in the minimum quantity of water (0.5 cm^3) and 3 cm^3 of acetone added. The solution was decanted off; the resulting oil was dissolved in the minimum quantity of water and acetone added until the solution became turbid. One drop of water was added and the mixture was warmed until clear. The cooled solution was filtered and the white crystals collected (0.23g, 47%). NMR: δ_H (D_2O) 3.55 (12H, s, N-CH_2-CH_2-N), 3.31 (16H, d(J_{PH}=11.9 Hz), N-CH_2-P); δ_P (D_2O) 11.65 (s). [lit[6]].

O,O',O''-Trimethyl-1,4,7-tris(phosphonatomethyl)-triazacyclononane(6)

A stirred solution of (22) (0.1g, 0.2mmol) with 1 cm^3 of sulphuric acid and methanol, was heated under reflux for 3 days. ^1H and ^{13}C NMR and mass spectral analysis indicated that the sample had degraded.

Hexamethyl-1,4,7-Tris(phosphonatomethyl)-1,4,7-triazacyclononane(23)

To a stirred solution of 1,4,7-triazacyclononane (0.2g, 1.6mmol) and paraformaldehyde (0.24g, 5.22mmol) was added trimethylphosphite (0.58g) and the mixture was brought to reflux for 24 hours. After evaporation of the solvent the oil was dissolved in dichloromethane, decanted off and evaporated to yield a brown oil . The oil was chromatographed on alumina, eluting with 1% methanol / dichloromethane (Rf 0.8) to yield a colourless oil (39%). Found 495.38649, $C_{27}H_{36}N_3O_9P_3$ requires495.38538; NMR: δ_H ($CDCl_3$) 3.8,

3.75 (9H,d,OMe), 3.05, 3.01 (6H, d, CH_2-P), 2.95 (12H, s, N-CH_2). δ_P ($CDCl_3$) 31.68 (s); m/z (DCI, NH_3) 512 (M^++ 17).

5.2.4 1,4 -DIOXA-7,10,13-TRIS (CARBOXYMETHYL) - TRIAZACYCLOPENTADECANE(7)

1,8-Bis(p-toluenesulphonato)-3,6-dioxa-octane (24)

To a solution of triethyleneglycol (5g, 26mmol) in pyridine was added p-toluenesulphonylchloride (15g, 26mmol). Following dissolution of all the solid, the flask was cooled to -18 °C for 48 hours. The solution was poured onto ice and filtered, washed with hydrochloric acid (1M, 2x50cm^3) and water (2x50cm^3). The solid was dissolved in dichloromethane, dried with potassium carbonate and was evaporated to yield an oil which was recrystallised from methanol (11.9g, 70%).m.p. 80-83°C [lit[7] 80-82°C]; NMR: δ_H ($CDCl_3$) 7.34, 7.77 (8H, AA'BB', J= 8, OTs), 4.0 (4H, m, CH_2-OTs), 3.6 (4H, m, O-CH_2), 3.4 (6H, s, OCH_2), 2.43(6H, s, Me); MS: m/z (DCI) 476 (M+17).

1,4-Dioxa-7,10,13-tris(p-toluenesulphonato)-7,10,13-triazacyclopentadecane(26)

To a solution of the ditosylate (24) (3.22g, 5.7mmol) and caesium carbonate (3.88g, 11.4mmol) in DMF (250 cm^3) was added a solution of the tritosylamide (25) (2.6g, 5.7mmol) in DMF (100 cm^3) over 2.5 hours. The mixture was heated to 60°C for 24 hours. The DMF was

removed under vacuum (150°C, 0.1 mmHg) and the residue dissolved in hot chloroform and filtered while still hot. Following evaporation the solid was recrystallised from hot toluene (70%, 2.7g). Analysis found: C, 54.70; H, 5.93; N, 6.3; $C_{31}H_{41}N_3O_8S_3$ requires C,54.77; H, 6.10; N, 6.18 %. NMR: δ_H ($CDCl_3$) 7.83 7.32 (4H, d, AA'BB',.(J=8.1), aromatic H), 7.72, 7.32 (8H, d, AA'BB'(J=8.1), aromatic H), 3.58 (4H, t, O-C$\underline{H}_2$-CH_2-N), 3.50 (4H, s, CH_2-O), 3.39 (4H, m, N-CH_2), 3.29 (8H, m, N-CH_2), 2.44 (3H, s, Me) 2.43 (6H, s, CH_3).; δ_C ($CDCl_3$) 142.3, 142.1, 141.8,137.9,137.6, 126.9 (aromatic), 68.2 67.3 (O-CH_2), 43.5, 45.6, 42.4 (N-CH_2), 23.1, 22.4 (CH_3) MS: m/z (DCI) 682 (M^++3) 100%, 680(M^++1) 32%.

1,4,-Dioxa-7,10,13-triazacyclopentadecane(27)

To a solution of the tosylamide (26) (0.94g, 1.3mmol) in THF (24 cm^3) was added 5 cm^3 of ethanol and the mixture was cooled to -78°C under an atmoshere of argon gas. Ammonia was condensed into the mixture using a cold finger until about 50 cm^3 had been added. To this was added lithium metal(0.5g). The solution became intense blue and was left for 2 hours with the temperature maintained. After allowing the temperature to gradually rise and the ammonia to bubble off through a trap, water was added (20 cm^3) and the solvents were removed under reduced presure. The residue was dissolved in 6M HCl (50 cm^3) and washed with ether (3x50cm^3). The water was removed and the residue redissolved in 6M sodium hydroxide solution (25 cm^3), washed with dichloromethane (3x50cm^3), and dried with anhydrous magnesium sulphate. After filtration the solvents were removed under reduced pressure and the residue

dissolved in chloroform; dried again using anhydrous magnesium sulphate, filtered and the solvent was evaporated under reduced presssure. The solid residue was recrystallised from dichlorometane/cyclohexane to give a white crystalline solid. (0.28g, 85%). Found (M^++1) 218.1832 $C_{10}H_{24}N_3O_2$ requires 218.1869. NMR: δ_H ($CDCl_3$): 3.61(4H, m, O-CH_2), 3.59 (4H, s, O-CH_2), 2.74 (12H, mult, N-CH_2). ^{13}C NMR δ_C ($CDCl_3$): 69.3 (O-C), 69.2 (O-C), 48.5, 48.4, 47.3 (N-C); MS m/z (DCI) 219 (M^++2).

7,10,13-Tris(ethoxycarbonylmethyl)-1,4-dioxa-7,10,13-triazacyclopentadecane (28)

To a stirred solution of (27) (150mg, 0.68mmol) and caesium carbonate (760mg, 2.04mmol) was added ethylbromoacetate (0.28 cm^3, 2.3mmol.) The stirred mixture was heated to 60°C and left for 24 hr. The alkylation was monitored by TLC. After cooling, the mixture was filtered and the solvent removed under reduced pressure. The oily residue was purified by column chromatography (alumina) using 0-2% methanol in dichloromethane as the elutant, giving a clear oil. (134mg, 44%), Analysis found: 476.5763 (M^++1) $C_{22}H_{42}N_3O_8$ requires 476.5891. NMR δ_H ($CDCl_3$): 4.15 (6H, m, O-$C\underline{H}_2$-CH_3), 3.58 (8H, mult.,O-CH_2), 3.44 (4H, s.,CH_2-N), 3.39 (2H, s, CH_2-N), 2.85 (12H, m, CH_2-N) 1.27 (9H, m, O-CH_2-$C\underline{H}_3$).; δ_C ($CDCl_3$): 170.53, 170.49 (carbonyl), 69.87, 69.79, (N-CH_2-CO), 59.49, 59.44, 59.33, 55.41 (O-CH_2), 45.1, 46.9, 48.3 (CH_2-N), 13.36, 13.35 (CH_3); MS: m/z (DCI) 476 (M^++1) 90%).

1,4 -Dioxa-7,10,13-tris- (carboxymethyl) - triazacyclopentadecane(7)

Hydrolysis of the triester (28) was carried out in 6M HCl (60°C, 4hr). The solvents were removed under reduced pressure to yield the hydrochloride salt, a glassy solid. NMR δ_H (D_2O): 4.07 (4H, s, N-CH_2-C=O or CH_2O), 3.82 (6H, s, N-CH_2-C=O or CH_2), 3.41-3.63 (16H, m, NCH_2, OCH_2); δ_C (D_2O): 170.5, 165.4 (carbonyl), 67.1,66.9 (CH_2-O), 65.3 (CH_2-C=O) , 47.2, 49.3,52.1, 52.3 (CH_2-N). MS (FAB) 392 (M^+ + 1).

5.2.5 SYNTHESIS OF 1,9-DIAMINO-5-(P-AMINOMETHYLBENZYL) 3,7-DIAZANONANE)(9)

Diethyl-p-cyanobenzylmalonate(29)

To a solution of sodium ethoxide in ethanol (1 mol dm^{-3}, 125 cm^3) was added dropwise at room temperature diethylmalonate (20g, 125mmol) in ethanol (50 cm^3). The mixture was stirred for thirty minutes. α-Bromo-p-toluonitrile (12g, 61mmol) was added in DMF (60 cm^3) dropwise, and the mixture refluxed for twenty-four hours. After filtration, and the addition of water, an oil formed and the water was decanted off. The oil was dissolved in ether, dried over potassium carbonate, filtered, and the solvent removed under reduced pressure. The product was purified by fractional distillation at 0.01mm/Hg at 50°C. (9g, 51%). TLC R_f 0.25 [lit. 0.26[2]] (20% EtOAc/petrol); NMR: δ_H ($CDCl_3$): 7.61, 7.32 (4H, d,J=8, aromatic),

4.2 (4H, m, OCH_2), 3.4 (1H, t,C<u>H</u>) 3.27 (2H, m, CH_2-phenyl), 1.2 (6H, t, CH_3); δ_C 172.3 (CO), 141.2, 139.8, 132.6, 131.1 (aromatic), 123.5(CN), 63.4 (O-CH_2), 41.4, 39.2 (CH_2-Ph), 21.3(CH_3); I.R.(thinfilm): 3010 (CH), 2980(CH), 2230(CN); MS: m/z 276 (M^++1) 100%.

1,9-Diamino-5-(p-cyanobenzyl)-4,6-dioxa-3,7-diazanonane(30)

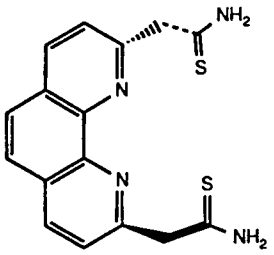
To a neat solution of ethylenediamine (100 cm^3) was added (29) (2.5g, 0.11mmol). The mixture was heated to 60°C for 48h. After the solution cooled, the ethylenediamine was removed at reduced pressure leaving a solid yellow residue. NMR: δ_H(MeOD) 7.72, 7.41 (4H, d, J=8, aromatic) 3.48(7H, m, CH_2, CH, N-CH_2), 2.9 (4H, m, N-CH_2); δ_C (MeOD) 171.8 (C=O), 146.2 , 133.7, 131.5, 120.18, (aromatic), 111.8 (CN), 46.9, 45.1(CH_2-N), 43.5(C-CO), 37.4 (C-aromatic); MS m/z (DCI, NH_3) 320 (M^++17) 100%.

1,9-Diamino-5-(p-aminomethylbenzyl)-3,7,diazanonane(9)

To a solution of BH_3.THF (52mmol) was added the diamide (30) (1g, 3.4mmol), and the mixture was heated to reflux for 72h. After cooling, the mixture was quenched by the dropwise addition of methanol. After the borane was deemed to have been quenched, the methanol was removed under reduced pressure, and the residue refluxed in 6M HCl (250 cm^3) for 3.5 hours. After the solution had cooled the water was removed under reduced pressure and the residue washed with methanol to convert any of the remaining boron

species to the volatile $B(OMe)_3$. After the methanol had been removed (rotary evaporator), the estimated quantity of NaOH required to neutralise the hydrochloride salt was dissolved in the minimum quantity of water. This basic solution was added to the solid residue and warmed until all the solid had dissolved, maintaining the pH at > 7. The water was removed under reduced pressure and the dichloromethane (100 cm^3) added to the solid residue. This mixture was heated to reflux until the solid had dissolved. The cool solution was dried over anhydrous potassium carbonate, filtered and the solvent removed under reduced pressure, to yield a clear oily residue. The compound was purified by recrystallisation from chloroform/methanol to yield a glassy solid, (45%, 0.46g). Analysis found: C, 64.38; H, 9.47; N, 25.14; $C_{15}H_{29}N_5$ requires C, 64.48; H, 9.46; N, 25.06%; NMR: δ_H ($CDCl_3$) 7.87, 7.46 (4H, d, AA'BB', J=8.1, aromatic) 3.4 (4H, m, CH_2-phenyl), 3.13-2.51 (12H, m, N-CH_2) 1.3 (1H, m, CH) ; δ_C ($CDCl_3$) 140.71, 138.67, 128.74, 126.759, (aromatic), 52.71, 52.36, 51.06, 45.84, 41.2 (CH_2-N, CH_2-phenyl), 21.2 (CH); MS: m/z (DCI, NH_3) 296 (M^++17) 33%.

5.2.6 THE ATTEMPTED SYNTHESIS OF (57)



1,10-Phenanthroline-2,9-dicarbaldehyde (62)

To a stirred solution of selenium dioxide (7.5g, 68mmol) in aqueous dioxan (96% w/v) was added 2,9-dimethyl-1,10-phenanthroline (3.0g, 14.9mmol) and the mixture was heated to reflux for two hours. The red solution was filtered hot through celite and cooled. The orange precipitate was collected by filtration (3.8g, 76%). m.p. 231-232. [lit.(1) 231°C] ν_{max} 2900 (br), 1700 (CHO, s). NMR δ_H (DMSO) 10.2 (CHO), 8.81 (2H, AB, aromatic), 8.43 (2H, AB, aromatic), 8.25 (2H, s, aromatic) ; MS: m/e (DCI) 237 (M+1) 100%.

2,9-Bis(hydroxymethyl)1,10-phenanthroline (63)

To a stirred solution of (62) (1g, 4.4mmol) in 50 cm^3 of ethanol, sodium borohydride (0.45g, 12mmol) was added slowly to prevent overheating. The mixture was refluxed overnight. After evaporation the residue was redissolved in the minimum volume of water, filtered hot and allowed to cool. The orange crystals were collected by filtration (10%). m.p. 196-199° [litt.(1) 197-199°] NMR: δ_H (DMSO)

8.94,(2H, AB, Ar), 8.37 (2H,AB, Ar), 5.31 (2H, s, Ar) 4.9(4H,s,CH_2). I.R. ν_{max} 3600-3100, 1630, 1510, 1170, 850 cm^{-1}; MS: m/z (DCI) 241 (M^++1), 100%.

2,9-Bis(bromomethyl)1,10-phenanthroline hydrobromide. (58)

A stirred solution of (63) (1.35g, 5.8mmol) in 48% (w/v) hydrogen bromide solution (60 cm^3) was heated to reflux over-night. The solution was cooled and solid potassium hydroxide added until precipitation was complete (2.5g, 95%). Analysis found; C, 44.2; H, 3.5; N, 7.3; $C_{14}H_{12}N_2Br_2$ requires C, 43.2; H, 3.15; N, 7.3; m.p. 111-112°C [lit.(1) 110-111]; NMR: δ_H (DMSO) 8.63, 8.04 (2H, AB, J=11.2, Ar), 8.12 (2H, s, Ar), 4.93 (4H, s, CH_2). MS: m/z (DCI) 359 (M+1).

2-cyanomethyl-9-bromo-1,10-phenanthroline(59)

To a stirred solution of (13) (0.18g, 0.35mmol) in 20 cm^3 of ethanol was added potassium cyanide (0.056g, 0.87mmol) and the mixture heated to reflux overnight. The cooled solution was poured onto aqueous sodium carbonate (20 cm^3, 5%) and extracted with dichloromethane (4x20cm^3), dried with potassium carbonate and evaporated to yield a brown oil (0.06g, 70%).; Analysis found; C, 56.79; H, 4.05; N, 9.27; $C_{16}H_{10}N_3Br$ requires C,57.7; H, 3.3; N, 13.46 NMR: δ_H (DMSO) 7.8, 8.5, 7.95 (6H, mult., Ar), 4.9 (4H, s, CH_2-Br); 4.1 (2H, s, CH_2-CN); δ_C (DMSO) 150.1, 145.7, 136.5, 125.6, 129.0, 110 (aromatic) 36.7 (CH_2) 34.7 (CH_2): I.R:. (thinfilm) 2950, 2220 (CN); MS m/z 312, (M^++1)100%.

2,9-BIS(N,N-DIMETHYLACETEMIDO)1,10-PHENANTHROLINE (15)

2-(N,N-dimethyleneacetamido)-1,10-phenanthroline (65)

1) *Preparation of LDA*

To a solution of diisopropylamine (6.2 cm^3, 44mmol) in 10 cm^3 of THF was added butyllithium (17 cm^3, 2.55M, 44mmol) and the solution stirred for twenty minutes at -78°C.

2) *Preparation of Lithiate (64)*

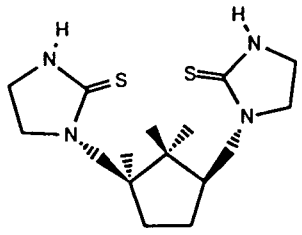
To a solution of LDA (44mmol) was added N,N-dimethyl acetmide (4 cm^3, 44mmol) and the solution stirred for sixty minutes at room temperature.

To a suspension of phenanthroline (1g, 5.1 mmols) in benzene (20 cm^3) was added (64) (44mmols) and the mixture was stirred overnight at room temperature. Water was added (10 cm^3) at 0°C and the aqueous layer separated and extracted with dichloromethane (4x20cm^3). After combining organic washings, 20g of manganese dioxide was added. The reaction was followed by TLC. When it was complete 20g of magnesium sulphate was added and the filtered solution evaporated to yield a dark red oil. δ_H ($CDCl_3$) 8.7 (2H,m , Ar), 7.7 (2H, m, Ar),, 7.1 (2H, m, Ar), 3.6 (4H, s, CH2-C=O), 2.63 (3H, s, CH3), 2.54(3H, s, CH3). MS: m/z (DCI) 266 (M+1).

2,9-Bis(N,N-dimethylacetamide)-1,10-phenanthroline (66)

Procedures 1), 2) and 3) were repeated substituting the mono-alkylated phenanthroline (65) for phenantholine in procedure 3). No further reaction could be detected.

5.2.7 THE ATTEMPTED SYNTHESIS OF LIGAND (56)



Diethyl camphorate(70)

To a solution of camphoric acid (20g, 100mmol) in ethanol (500 cm^3) was added sulphuric acid (2 cm^3) and the mixture brought to reflux for 48 hours. Sodium carbonate solution (5%) was added to pH 7 and the mixture evaporated. Water (100 cm^3) and ether (100 cm^3) were added forming an inseparable viscous solid. After evaporation to give a clear oil, ether was added (100 cm^3), the solution washed with water (3x20cm^3), dried over magnesium sulphate and evaporated to leave a clear oil (10g, 50%). NMR: $\delta_H(CDCl_3)$ 4.12 (3H, m, CH_2), 4.15 (3H, m, CH_2) 2.3 (1H, m, CH), 1.85 (2H, m, CH_2), 1,63 (2H, m, CH_2) 1.21-0.97 (15H, m, CH_3); I.R. 2900 (CH,br), 1720 (MeOC=O) cm-1. M/e (DCI) 257 (m+1, 100%).

2,5-Bis-(N-2-aminoethylcarbamoyl)-1,1,2-trimethylcyclopentane(71)

A solution of (70) (10g, 50mmol) in dioxan (10 cm^3) was added dropwise over one hour to 50 cm^3 of ethylenediamine. It was heated under reflux for 2 weeks. The reaction was followed by its I.R spectrum. The solvents were removed under vacuum (20°C, 0.3mmHg) and a brown gum resulted, which was used directly without purification. I.R. 1650 (C=O, sh); MS: m/z (CI) 285 (M^++1) 80%.

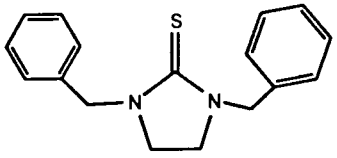
2,5-Bis(4'-amino-2'aza-butyl)-1,1,2-trimethylcyclopentane(69)

Borane-THF (150 cm^3, 131mmols) was added to (71) (3.7g, 13mmols) and the mixture brought to reflux for three weeks. The reaction was followed by the change in its I.R. spectrum. After addition of methanol (50cm^{-3}) followed by evaporation and reflux with 6M hydrochloric acid (100 cm^3) for two hours, the mixture was brought to pH 14 with solid potassium hydroxide and extracted with dichloromethane (5x20cm^3). The carbon-13 spectrum of the resulting oil contained a myriad of signals suggesting the product had degraded.

Camphoric anhydride(72)

To a suspension of camphoric acid (1g, 5mmols) in dichloromethane (20 cm^3) was added oxalyl chloride (2 cm^3) and one drop of DMF. The mixture was stirred at room temperature until the suspension became clear. Solvents were removed under vacuum (0.1mmHg), dichloromethane added (5 cm^3) and removed under vacuum (x3). Dichloromethane (10 cm^3) was added and to this solution was added ethylenediamine (1 cm^3) in dichloromethane (10 cm^3). After evaporation, a white gummy solid resulted. I.R. 1650 (RHN-C=O,HO-C=O, br) cm^{-1}. MS: m/z (DCI) 243 (M+1).[lit [4]]

5.2.8 N,N-DIBENZYLETHYLENETHIOUREA(12)



N,N-Dibenzoylethylenediamine(31)

To a stirred solution of aqueous KOH in THF (100 cm^3, 10% KOH w/v, 50% THF) and ethylenediamine (5g, 28mmol) at 0°C was added benzoyl chloride (11.9g, 85mmol) over 1 hour. The mixture was stirred at room temperature for 24 hours. The white solid was filtered off, washed with methanol and dried under vacuum. (4.5g, 60%). m.p. 75°C; I.R.; ν_{max} (thin film) 3280 (NH, Br), 1625 (NC=O) cm^{-1}; MS: m/z (CI) 269 (M+1).

N,N-Dibenzylethylenediamine (32)

BH_3.THF (1M, 75 cm^3, 75mmol) was added to dry (31) (2g, 7.5 mmol) and the mixture was stirred at reflux for 24 hours. Methanol was added dropwise to quench the excess borane until the effervescence had ceased. After evaporation of the solvent, 6M HCl (200 cm^3) was added to the oil and the mixture left to boil under reflux for three hours. After evaporation, 50 cm^3 of distilled water was added along with solid KOH and the solution brought to pH 14. Extraction with dichloromethane (3x20cm^3) followed by drying with potasium carbonate and evaporation yielded a clear oil. (1.67g, 90%). b.p 196°C. 4mmHg [lit[7] 195°C]; NMR: δ_H ($CDCl_3$) 1.4 (2H, s, N-H), 2.5 (4H, s, CH_2-Ph), 3.6 (4H, s, CH_2-N), 7.1 (10H, s, Ph); δ_C ($CDCl_3$) 141.2, 138.6, 132.1, 129.3 (aromatic), 65.1 (N-CH_2), 54.1(N-CH_2-CH_2-N); I.R: ν_{max} 3000-3500 (NH,Br), 1200 cm^{-1}; MS: m/z (CI) 241 (M+1).

N,N-Dibenzylethylenethiourea(12)

To a stirred solution of (32) (1.81g, 8.6 mmol) in aqueous ethanol (150 cm^3, 1:1) at O°C was added carbon disulphide (0.65g, 8.6mmol) in dioxan (100 cm^3) over one hour. After the mixture was heated at 100°C for three hours, conc. HCl (10 cm^3) was added and the mixture was heated under reflux for 12 hours. After evaporation the solid was redissolved in chloroform (50 cm^3) and filtered whilst hot. The cooled solution was filtered and the crystals washed with water and dried under vacuum. (1.6g, 70%). m.p. 81°C. Analysis found: C, 71.91; H, 6.42; N, 9.53; $C_{17}H_{18}N_2O$ requires C, 72.3; H, 6.42; N, 9.92; NMR: δ_H ($CDCl_3$) 3.38 (4H, s, CH_2-Ph), 4.89 (4H, s, CH_2-N), 7.35

(10H, s, Ph); δ_C ($CDCl_3$), 182.8 (C=S), 136.3, 128.5, 128.1, 127.5 (aromatic), 51.6, 45.3 (CH_2-N) MS: m/z (CI) 283 (M+1), 100%.

5.2.9 ATTEMPTED SYNTHESIS OF 1,7,13-TRITHIA-4,10,16-TRIAZACYCLOOCTADECANE(13)

1,10-Bis(p-toluenesulphanato)-3,8-N,N-bis(p-toluenesulphanotoamide) -5-thia-decane(34)

To a solution of (33) (2.3mmol, 1g) in 50 cm^3 DMF was added 2-bromoethanol (5mmol, 0.36 cm^3). The mixture was heated to 60°C for 5 days. This resulted in a mixture of tosylated material which proved intractable.

Dimethylthiodiglycolate(35)

The esterification of thiodiglycolic acid was achieved by refluxing the acid (0.32mol, 48g) in 100 cm^3 of methanol and 5 cm^3 of acetyl chloride for 48h. Any excess acid was neutralised with potassium carbonate. The pure ester was extracted into dichloromethane and was isolated as a colourless oil. (0.31mol, 95%) b.p 137°C, 11mmHg [lit[4] 135°,11mmHg]; NMR: $\delta_H(CDCl_3)$ 3.2(6H,s,OMe), 2.97(4H,s,CH_2); δ_C ($CDCl_3$) 169.6(C=O), 51.8(OMe), 32.8(CH2); IR (thin film), 1730 (carbonyl); MS m/z 179 (M^++1).

3,8-(N,N'-2'-hydroxy ethyl)-thiodiglycolamide(36)

The diester(35) (32mmol) was added to 100 cm^3 of ethanolamine. The mixture was refluxed overnight. The solvent was removed under reduced pressure and the pale yellow solid recrystallised from ethanol/toluene. (31g, 43%) m.p 245°C; Analysis found C, 40.77; H, 6.92; N, 11.74; $C_8H_{16}N_2O_4S$ requires C, 40.69; H, 6.82; N, 11.85; NMR: δ_H (CD_3OD) 4.59 (4H, s, S-CH_2), 3.31 (4H, t, J=5.7, CH_2-OH, 2.99 (4H, m, CH_2-N), 2.22 (2H, t, OH); δ_C (CD_3OD) 172.0 (carbonyl), 61.4 (CH_2-OH), 43.2 (CH_2-N), 36.5 (CH_2-S); IR (thin film) 3100-3500 (OH), 1650 (carbonyl); MS m/z (EI) 237 (M^++1), 219,176.

3,9-Diaza-6-thia-undecan-1,11-diol(37)

To a solution of BH_3.THF (8 equivalents) was added (36) (15g , 0.06mol) and the solution was heated under reflux for 4 days. The cool solution was quenched by the dropwise addition of methanol. Excess methanol was added and removed under reduced pressure. The residue was washed with ether. The residue was refluxed in 6M HCl (30cm^3) for 2 hours. The water was removed under reduced pressure and the residue washed with ether (3x20cm^3). The residue was dissolved in a solution of NaOH (25cm^3, 0.1mol dm^{-3}) and the amine extracted with dichloromethane (3x20cm^3). The solution was dried over K_2CO_3, filtered and the solvents removed to give a grey oil (5g, 33%). An alternative method of extracting the amine from the basic solution was the use of a anion exchange column. NMR: δ_H ($CDCl_3$) 3.4 (4H,t,O-C<u>H</u>2), 2.7 (4H,t,O-CH_2-C<u>H</u>$_2$-N), 2.5 (4H,t,S-CH_2-

$C\underline{H}_2$-N) 2.3 (S-CH_2); δ_C ($CDCl_3$) 62.7 (CH_2O), 51.6 (CH_2-N), 48.9 (CH_2-N), 31.1 (CH_2-S); MS m/z 209 (M^++1).

3,9-N,N'-Bis(p-toluenesulphanato)-1,11-bis(p-toluenesulhonyl)-3,9-Diaza-6-thia-undecan-1,11-diol(34)

To a solution of (37) (100mgs, 0.026mmol) in acetonitrile/chloroform ($3cm^3$) (50:50) and Et_3N (0.16g, 1.58mmol) at -15°C was added tosylchloride (0.3g, 1.5mmol). The temperature was maintained at -15°C and the reaction vigorously stirred for 4h. The reaction mixture was allowed to warm up to room temperature and was filtered after a further 4hours. The solvents were removed under reduced pressure and the residue purified on an alumina column eluted with 0.75% methanol in dichloromethane to yield a colourless oil. NMR: δ_H ($CDCl_3$) 7.49 ,7.46 (4H,AA'BB',aromatic H), 7.65, 7.34 (4H,AA'BB',aromatic H), 4.15 (4H,t,J=5.4,CH_2-O), 3.41 (4H,t,J=5.4,O-CH_2-$C\underline{H}_2$-N), 3.25 (4H,t,J=8.4,CH_2-N), 2.69 (6H,t,J=8.4,CH_2-S) 2.45, 2.41 (CH_3); δ_C 145.2, 143.9 ,135.91, 132.16, 130.06, 129.97, 129.91, 130.6, 127.98, 127.26 (aromatic), 68.9 (CH_2-O), 50.14, 48.28(CH_2-N), 30.41 (CH_2-S), 21.66, 21.51 (CH_3-aromatic); MS m/z 826 (M^++1).

N-(p-toluene-sulphonyl)-3-aza-1,5-bis(p-toluenesulphonato)pentane (22)

To a solution of toluene-p-sulphonyl chloride (107.8g, 0.565mol) in pyridine ($100cm^3$) was added a solution of bis-(2-hydroxyethyl)amine (16.5g, 0.157mol) in pyridine ($90cm^3$) dropwise

over a period of 30 min. The mixture was left at - 18°C for 48h. After pouring the mixture onto ice (400g), with stirring until the ice melted, the mixture was left at room temperature for 2h to give a yellow-brown solid which was collected by filtration and twice recrystallised from ethanol-toluene (5:1) to give a yellow solid (75.0g, 84%); m.p. 96-97°C [lit[7] 96-98°]; (Found: C, 52.5; H, 4.92; N, 1.81. $C_{25}H_{29}NO_8S_3$ requires C, 52.9; H, 5.11; N, 2.11); NMR: δ_H ($CDCl_3$) 7.75 (2H, d, J=8.1, part of AA'BB' system, aromatic H), 7.35 (2H, d, J=8.1, part of aromatic AA'BB' system, aromatic H), 7.28 (4H,d, J=8.1, part of AA'BB' system, aromatic), 4.11 (4 H, t, J=5.9, CH_2 -O), 3.37 (4H, t, J=5.9, CH_2-N), 2.43 (3 H, s, CH_3), and 2.35 (6H, s, CH_3). MS: m/z (DCI, NH_3) 585 (M^++ 18), 568 (M^++1).

N-(Tolyl-p-sulphonyl)- 3-azapentane-1,5-dithiol, (38)

Thio-urea (22.8g, 0.299mol) was added to a solution of compound, (22), (75.0g, 0.136mol) in dry ethanol (500cm^3) and the solution was heated under reflux, under a nitrogen atmosphere for 30h. The solvent was evaporated under reduced pressure and the residue taken up in saturated sodium bicarbonate solution (250cm^3) and heated under reflux for 30 minutes. The cooled solution was adjusted to pH 7 with hydrochloric acid (6 mol dm^{-3}). The aqueous layer was extracted with dichloromethane (3x250cm^3), dried (anhydrous $MgSO_4$), filtered and the solute chromatographed on "flash" silica gel eluting with dichloromethane:methanol, (199:1). Evaporation of the elutes gave a clear oil which crystallised over a period of 24h (32.7g, 83%); R_F 0.5 [silica gel: CH_2-Cl_2-MeOH (99:1)]; Analysis found (M^++1) 292.0490. $C_{11}H_{17}NO_2S_3$ requires 292.0496;

NMR: δ_H ($CDCl_3$) 7.70 (2H, d, J=8, part of AA'BB' system, aromatic H), 7.33 (2H, d, J=8.1, part of AA'BB' system, aromatic H), 3.28 (4H,t, J=7.5, N-CH_2), 2.74 (4H, m, S-CH_2), 2.43 (3H, s, CH_3), and 1.44 (2H, t, J=8.5, SH); δ_C ($CDCl_3$) 21.4 (CH_3), 23.8 (CH_2-S), 52.6 (CH_2-N), and 126.7, 126.9, 129.7, 135.9, 143.6 (aromatic C). MS: m/z (DCI, NH_3) 292 (M^++1).

1,7,13-Trithia-4,10,16-N,N,N-(p-toluenesulphonyl)-4,10,16-Triazacyclooctadecane(39)

To a solution of (34) (2g, 2.43mmol) and $CsCO_3$ (2g, 6mmol) in DMF (80 cm^3) was added (38) (0.78g, 2.1 equivalents). The solution was heated to 55°C with vigorous stirring for 2 weeks. After the removal of the DMF under reduced pressure, no signs of reaction could be discerned (TLC, ^1H NMR).

5.2.10 ATTEMPTED SYNTHESIS OF 1,7-BIS(DIMETHYLANIMOETHYL)-4,10-DITHIA-1,7-DIAZACYCLODODECANE(14)

Route 1

To a solution of Na_2S (0.04g, 0.026mmol) in ethanol (10 cm^3) was added (34) (220mg, 0.267mmol), and $CsCO_3$ (0.09g, 2 equivalents).

The mixture was refluxed at 40°C for 4 days with no detectable reaction taking place (TLC, ^1H NMR).

Route 2

N-(p-toluene-sulphonyl)-3-aza-1,5-bis(p-toluenesulphonato)pentane (22)

See above in section 5.2.9.

N-(p-toluene-sulphonyl)-3-aza-1,5-dibromopentane (43)

To a solution of (22) (2g, 3.52mmols) in DMF was added potassium bromide and the solution stirred at 60°C for 48 hours. The DMF was removed under reduced pressure and the residue dissolved in dichloromethane and washed with water (3 x 50cm^3). The organic solution was dried with potassium carbonate, filtered and the solvent evaporated to yield a pale yellow solid. Analysis found C, 34.23; H, 3.97; N, 3.71% $C_{11}H_{15}NSBr_2$ requires C, 34.30; H, 3.93; N, 3.63%; NMR: $\delta_H(CDCl_3)$ 7.7, (2H,d,J=8, part of AA'BB', aromatic), 7.39 (2H,d,J=8,part of AA'BB, aromatic H), 2.43 (3H, s, CH_3), 2.21 (8H, s, $C\underline{H}_2$-$C\underline{H}_2$). δ_C ($CDCl_3$) 141.6,141.1. 136.7,136.2 (aromatic), 71.3 (CH_2-N-tosyl), 21.5 (CH_2-Br). MS: m/z 386 (M^++1).

1,4-Dithia-7,10-N,N-Bis-(p-toluenesulponyl)-7,10-diazacyclodecane(44)

Using reagent (22)

To a solution of (22) (2.0g, 3.4mmol) and caesium carbonate (2.2 equivalents) in DMF (200 cm^3) was added a solution of (38) (1.0g, 3.25mmol) in DMF (100 cm^3) with vigorous stirring. The mixture was stirred at room temperature for two hours and monitored by TLC. The temperature was raised to 80°C after 3 days. TLC showed no change in the starting reagents.

Using reagent (43)

To a solution of (43) (1.1g, 2.65mmol) and caesium carbonate (2.2 equivalents) in DMF (200 cm^3) was added a solution of (38) (0.8g, 2.7mmol in DMF (100 cm^3) with vigorous stirring. The mixture was stirred at 65°C for 4 days. The solvent was evaporared under reduced pressure and the residue dissolved in dichloromethane and washed with sodium hydroxide solution and dried over potassium carbonate, filtered and the solvent evaporated. The residue was dissolved in toluene, filtered and the solvent evaporated. The product was purified using an alumina column eluted with (1:1) dichloromethane:toluene. The oil was recrystallised from a toluene/hexane mixture (9:1).(0.3g, 22%).Analysis found; C, 51.24; H, 5.93, N, 5.44; $C_{11}H_{15}NO_2S_2$ requires C, 51.34; H, 5.87; N, 5.44; The analysis confirms that the major isolated product is molecule (45): NMR: $\delta_H(CDCl_3)$ 7.72 (2H, d, ,J=8.1, part of AA'BB' system, aromatic

H), 7.31 (2H, d, J=8.1, part of AA'BB' system, aromatic H), 3.65 (4H,t,CH_2-N), 3.01(4H,t, CH_2-S). δ_C ($CDCl_3$) 142.5, 135.7, 128.8, 125.9 (aromatic), 50.5 (CH_2-N), 38.9(CH_2-S); MS: m/z (DCI) 258 (M^++1).

5.2.11 N,N',N'',N''' - TETRAMETHYL-1,10-DITHIA-4,7,13,16 - TETRAAZACYCLOOCTADECANE (15)

N,N',N'',N''' - Tetramethyl-1,10-dithia-4,7,13,16-tetra-aza-cyclo-octadecane, (15).

1,10-Dithia-4,7,13,16-tetra-azacyclo-octadecane (80.0mg, 0.274mmol) was heated at 95°C with formaldehyde (0.24cm^3, 37% solution) and formic acid (0.32cm^3) for 20h. To the cooled solution was added hydrochloric acid (1.0mol dm^{-3}, 5.0cm^3) and the solution evaporated under reduced pressure to give a pale brown residue. After dissolving in water (3cm^3) the pH was adjusted to 14 with KOH solution and the solution extracted with dichloromethane (5 x 5cm^3), dried (anhydrous K_2CO_3) and the solvent removed under reduced pressure to give a colourless residue. Recrystallisation from hexane gave a white solid (52.0mg, 55%); m.p. 43-44°C; (Found: C, 54.8; H, 10.8; N, 15.5. $C_{16}N_{36}N_4S_2.1/2H_2O$ requires C, 54.6; H, 10.4; N, 15.9); NMR: δ_H ($CDCl_3$) 2.64 (16H, s, CH_2-N, CH_2-S), 2.51 (8H, s, CH_2-N) and 2.27 (12H, s, CH_3); δ_C ($CDCl_3$) 29.2 (CH_3), 43.1 (CH_2-S), and 55.2, 57.7 (CH_2-N). MS: m/z (DCI, NH_3) 350 (M^++2), 351 (M^++3), 335, 323, 262, 161.

5.2.12 N,N',N'',N'''-Tetramethyl-1,10-dioxa-4,7,13,16-tetra-aza-cyclo-octadecane (49)

N,N',N'',N'''-Tetrakis(tosyl-p-sulphony)-1,10-dioxa-4,7,13,-16-tetra-azacyclo-octadecane, (52), and N,N'-bis (tolyl-p-sulphonyl)-1-oxa-4,7-diazacyclononane, (53).

Caesium carbonate (8.26g, 25.4mmol) was added to a solution of 3-oxa-1,5-bis (tolyl-p-sulphonyloxy) pentane (5.00g, 12,1mmol) in anhydrous DMF (50cm^3) under a nitrogen atmosphere. A solution of N'N''-bis (tolyl-p-sulphonyl) ethane-1,2-diamaine (4.44g, 12.1mmol) in anhydrous DMF (50cm^3) was added dropwise over a period of 4h with vigorous stirring. The reaction mixture was stirred at room temperature for 12h and heated to 60°C for 4h. The solvent was removed under reduced pressure, the residue taken up in dichloromethane (100cm^3) and washed with distilled water (2 x 100cm^3). The organic layer was dried (anhydrous $MgSO_4$), filtered and the solvent removed under reduced pressure to give a pale yellow solid. The mixture was taken up in hot toluene (40cm^3) and the 18-membered ring compound was collected as a white solid by filtration (warmed filtration apparatus) (1.06g, 20%). The nine-membered ring compound was obtained from the cooled filtrate as a crystalline solid, collected by filtration and dried *in vacuo* (10^{-2}mmHg) (2.17g, 41%).

***Compound* (53)**- M.p. 160 - 161°C; (Found: C, 54.8; H, 6.02; N, 6.34. $C_{20}H_{26}N_2O_5S_2$ requires C, 54.8; H, 5.94; N, 6.39;) NMR: δ_H ($CDCl_3$) 7.70 (4H, d , J= 8.1, part of AA' BB', aromatic H), 7.32 (4H, d,

J=8.2, part of AA'BB' system, aromatic H), 3.90 (4H, t, J=4.3 Hz, CH_2-O), 3.47 (4H, s, N-CH_2-CH_2-N), 3.26 (4H, t, J=4.3, CH_2-N), and 2.43 (6H, s, CH_3). MS: m/z (CI, NH_3) 440 (M^++2), 439 (M^++1) and 283.

***Compound* (52)** - M.p. 242-244°C; Found: C, 55.0; H, 6.13; N, 6.12. $C_{40}H_{52}N_4O_{10}S_4$ requires C, 54.8; H, 5.94; N, 6.39); δ_H ($CDCl_3$) 7.71 (8H, d, J=8.1, part of AA' BB', aromatic H), 7.32 (8H, d, J=8.1, part of AA' BB', aromatic H), 3.54 (8H, t, J=4.9, CH_2O), 3.32 (8H, s, N-CH_2-CH_2-N), 3.22 (8H, t, J=4.8, CH_2-N), and 2.44 (12H, s, CH_3). MS: m/z (CI, NH_3) 880 (M^++ 2) and 722.

10-Dioxa-4,7,13,16-tetra-azacyclo-octadecane (48)

A solution of hydrogen bromide in acetic acid (45%, 100cm^3) and phenol (5.0g, 53mmol) was added to compound (52) (1.25g, 1.43mmol) and the solution heated under reflux for 6 days. Diethyl ether (40cm^3) was added to the cooled reaction mixture and a fine white precipitate collected by filtration. This was taken up in distilled water (40cm^3), basified with aqueous KOH (30%) and extracted with dichloromethane (4x40cm^3). The organic layer was dried (anhydrous K_2CO_3), filtered and the solvent removed under reduced pressure. The residue was recrystallised from dichloromethane-hexane to give a colourless crystalline solid (90mg, 25%); m.p. 58 - 60°C; NMR: δ_H ($CDCl_3$) 3.59 (8H, t, J=4.9, CH_2-O), 2.80 (8H, t, J=4.0, CH_2-N), 2.78 (8H, s, N-CH_2-CH_2-N), and 2.07 (4H, br, s, NH); δ_C ($CDCl_3$) 70.0 (CH_2-O) and 49.2 (CH_2N); MS: m/z (DCI, NH_3) 262 (M^++1) and 204, 131.

N,N',N'',N'''-Tetramethyl-1,10-dioxa-4,7,13,16-tetra-aza-cyclo-octadecane (49)

1,10-Dioxa-4,7,13,16-tetra-azacyclo-octadecane (60mg, 23mmol) was heated at 95°C with formaldehyde (37%, 0.20cm^3) and formic acid (0.27cm^3) for 20h. Hydrochloric acid (1.0mol dm^{-3}, 5.0cm^3) was added to the cooled solution and the solvent removed under reduced pressure. The residue was redissolved in water (3.0cm^3) and adjusted to pH 14 with potassium hydroxide. After extraction with dichloromethane (5 x 5cm^3), the organic layer was dried (anhydrous K_2CO_3), filtered and the solvent removed under reduced pressure. The residue was treated with hexane (3 x 5cm^3), the extracts combined and the solvent removed under reduced pressure to give a clear oil which crystallised on cooling (5°C) (59mg, 80%); m.p. = 23 - 25°C; NMR: δ_H($CDCl_3$) 3.55 (8H,t, J= 5.5, CH_2-O), 2.63 (8H, t, J=5.5, CH_2-N), 2.59 (8H, s, NCH_2-CH_2-N), and 2.28 (12H, s, CH_3).

5.2.13 Indium(III) Complex of (R) - 1,4,7-Tris(2'-Methylcarboxymethyl)-Triazacyclononane (4)

A solution of indium trinitrate (30.4mgs, 1x10^{-4}mols) in 0.001 mol dm^{-3} nitric acid (1 cm^3) was added to a mixture of (R) - 1,4,7-Tris(2-Methylcarboxymethyl)-triazacyclononane (4) (34.9mgs, 1x10^{-4}mols) in 0.001 mol dm^{-3} nitric acid (1 cm^3). The solution was gently warmed and allowed to cool to room temperature. After 24 hours an opaque crystalline solid precipitated. The mother liquors were decanted off, and the residue washed with acetone (2 cm^3 x 3) and the solvent allowed to evaporate. The crystals were insoluble in all

common laboratory solvents. Analysis found: C, 38.08; H, 5.38; N, 8.82; $C_{15}H_{24}O_6N_3In.0.9H_2O$ requires C, 38.12; H, 5.4; N, 8.88 ; MS: m/z (FAB) 458 (M^++1) 3%.

5.2.14 SYNTHESIS OF GOLD(I) COMPLEX OF N,N-DIBENZYLETHYLENETHIOUREA

Using N,N-Dibenzylethylenethiourea as the Reducing Agent.

To a solution of the ligand (12) (0.3g, 1.05mmol) in chloroform (5 cm^3) was added potasium tetrachloroaurate (0.13g, 0.35mmol). The mixture was gently warmed and the solution became colurless. After cooling, tetramethylammonium tetraphenylborate(0.35mmol) was added and a pale yellow solid precipitated, the solution was filtered and the solid dried *in vacuo.* (32%, 0.12g) Analysis found C, 59.4; H, 4.98; N, 6.3 $C_{58}H_{56}N_4S_2Au$ requires C, 64.4; H, 5.2; N, 5.1%;Analysis for Au gave 12.3%, complex requires 18.25 % NMR ^1H (DMSO) 7.13, 7.12, 7.10, 7.03 (5H, s, aromatic), 4.28, 4.23 (4H, AB,J=20Hz, N-CH_2), 4.15, 3.95, (4H,t,N-CH_2); δ_C(DMSO) 23.2 (C=S), 135.6, 134.4, 126.4, 125.3, 125.2, 124.3, 121.5 (aromatic), 56.24, 50.81, 45.50, 44.86, 44.21 (N-CH_2); MS m/z (FAB) 762(M^++1) 33%.

5.2.15 ATTEMPTED SYNTHESIS OF THE RHENIUM(V) COMPLEX OF 1,9-Diamino-(5-p-AMINOMETHYL-BENZYL) 3,7-DIAZANONANE)(9)

To a solution of the ligand (9) (0.5 mmol) in chloroform (20 cm^3) was added trans $ReOCl_3(PPh_3)_2$ (0.21g, 0.4mmol). The solution was stirred at room temperature for 15 minutes. A pale green precipitate was collected and washed with chloroform (3x3cm^3) and ether (3x3cm^3) and dried over P_2O_5 in vacuo.(0.006g, 5%) Analysis for rhenium gave Re (4.9%) 1:1 complex requires 34%.

5.3 THE EXPERIMENTAL METHODS USED IN THE BIODISTRIBUTION STUDIES*

5.3.1 Preparation of the ^{67}Gallium and ^{111}Indium Complexes

The complexes were prepared as follows**

1. A 250 µl solution of 2nM of the macrocycle is added to 100-200 µCi ^{67}Ga and 0.2 M ammonium acetate at pH 5.

2. The solution is incubated at 37°C for 30 minutes before the addition of a ten-fold excess of DPTA for 5 minutes to quench any unreacted gallium 67.

3. The mixture is purified by HPLC on an AX300 anion exchange column with 0.2% ammonium acetate pH 6.8, 10% acetonotrile as the running buffer at 1ml/minute.

4. The peak corresponding to the ^{67}Ga-complex, as measured by a radiometer, was collected and left at 37°C overnight to allow the acetonitrile to evaporate.

5. This solution is then diluted with PBS in preparation for the injection.

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* These experiments were carried out by A. Harrison and L. Royle, MRC, Radiobiology Unit, Didcot, Oxon.

** Quantities may differ with respect to each complex

5.3.2 The Experimental Details of the Biodistribution in Athymic NU:NU Mice*

The experimental details, as represented in scheme 3.1 are as follows.

1. The subcutaneous injection of congenitally athymic nu:nu mice (8-10 weeks) with a suspension of human melantoic melanoma (HX118). After 6 weeks the tumour volume is checked (minimum 0.5 ml) before proceeding.**

2. The mice were injected with 10-25 μCi of the radiometal complex.

3. At 1, 4 and 24 hours*** the mice were terminated with a lethal injection of sodium pentabarbitone.

4. Samples of blood and urine were removed and analysed.

5. Other tissues, as specified in the results tables, were analysed for radioactivity.

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* These experiments were carried out by A. Harrison and L. Royle, MRC, Radiobiology Unit, Didcot, Oxon.

** This only applies to the experiments where the mice had been induced with a tumour (4.4.1)

*** This may vary between experiments

5.4 ^{1}H NMR DETERMINATION OF THE RATE OF YTTRIUM UPTAKE BY (7)

Spectra were recorded on a Varian 400 spectrometer at 293K. The pD was kept constant at 5.0 by a d_3-sodium acetate (0.14 M)-d_4-acetic acid (0.06 M) buffer in D_2O). Ligand and metal ion concentrations were both 0.028 M.

Yttrium (III) was added to the ligand in solution as $Y(NO_3)_3.6D_2O$, and the D_2O salt obtained by adding D_2O to $Y(NO_3)_3.6H_2O$, evaporating and repeating this process twice.

Reagents

$Y(NO_3)_2.6H_2O$ was obtained from Alfa; and used as recieved.

5.5 STABILITY CONSTANT AND PROTONATION CONSTANT MEASUREMENTS FOR THE $9N_3C_3Me_3$ COMPLEXING AGENT[1]

Potentiometric titrations were carried out at 298 ± 0.1K. The molarities of tetramethylammonium hydroxide (Sigma) titrant solutions were corrected for carbonate contamination by previous titration against dilute hydrochloric acid (0.1 M, BDH). Ionic strength was kept constant with tetramethylammonium nitrate (0.1 M, Sigma).

The titration system consisted of a double-walled glass cell thermostatted at 298(±0.1)K containing the ligand solution coupled to a Mettler DV401 automatic burette containing the titrant. The pH was monitored with a Corning 001854 combination microelectrode. Titrations were controlled by a BBC microcomputer which also handled data storage. Data were transferred to an MTS mainframe computer by the program KERMIT and analysed using the program SUPERQUAD.

5.6 X-ray Crystal Structure Determination[3]

The X-ray crystal structure was determined by Professor George Ferguson (Department of Chemistry, University of Guelph, Ontario, Canada). The cell and intensity data were collected with an Enraf-Nonius CAD-4 diffractometer using graphite monochromated Mo-K^{α} radiation. All calculations were carried out on a PDP11-73 computer system using the SDP-Plus system of programs and data therein. The structures were solved by the heavy-atom method. Hydrogen atoms (visible in difference maps) were allowed for, and refinement was by full-matrix least squares calculations with all non-H atoms allowed ansiotropic motion.

Additional material available from the Cambridge Crystallographic Data Centre comprises H-atom co-ordinates, thermal parameters and remianing bond length and angles.

Cell data, experimental details, positional and thermal parameters and molecular dimensions for each structure are given in the Appendix.

5.7 DETERMINATION OF THE BINDING CONSTANTS FOR THE SILVER(I) COMPLEXES

5.7.1 pH - Metric titrations - Water.

(i) Apparatus

The titration cell was a double-walled glass vessel (capacity 5 cm^3) which was maintained at 25°C, using a Techne Tempette Junior TE-8J. Titration solutions were stirred using a magnetic stirrer and kept under an atmosphere of nitrogen. Titrations were performed using an automatic titrator (Mettler DL20, 1 cm^3 capacity) and burette functions (volume increments and equilibration time) were controlled by a BBC microprocessor. The pH was measured using a Corning 001854 combination microelectrode which was calibrated using buffer solutions at pH 4.008 ($CO_2H.C_6H_4CO_2K$, 0.05mol dm^{-3}) and pH 6.865 (KH_2PO_4, 0.025mol dm^{-3}-Na_2HPO_4, 0.025mol dm^{-3}). Data was stored on the BBC microprocessor and transferred to the MTS mainframe using KERMIT and subsequently analysed by two non-linear least-squares programs SCOGS and SUPERQUAD.

(ii) Acid-dissociation constants

Stock solutions of the ligand (0.002mol dm^{-3}) in Milli-Q water (25.0 cm^3) with nitric acid (1mol equiv. per amine nitrogen of the ligand) and tetramethylammonium nitrate (I = 0.10mol dm^{-3}) were prepared. In each titration 3.5 cm^3 of the stock ligand solution was titrated with tetramethylammonium hydroxide (0.109mol dm^{-3}), the

exact molarity of which was determined by titration against hydrochloride acid, 0.100mol dm^{-3}.

Methanol. Metal binding constants.* Stock solutions were prepared as above with the addition of one equivalent of silver nitrate. Titrations were performed as beofre.

Using the assumption that only 1:1 complex formation was occurring we have equations (1) and (2).

$$C°_{Ag} = [Ag^+] + [AgL^+] \qquad (1)$$

$$C°_{L} = [L] + [AgL^+] \qquad (2)$$

Here $C°_{Ag}$ was the initial concentration of silver ion and $C°_{L}$ was the overall concentration of ligand in solution (free or complexed).

Thus:

$$K_s = \frac{C°_{Ag} - [Ag^+]}{[Ag^+]\,(C°_{L} - [AgL^+])} = \frac{C°_{Ag} - [Ag^+]}{[Ag^+](C°_{L} - C°_{Ag} + [Ag^+])} \qquad (3)$$

Varying the initial silver ion concentration from 5 x 10^{-4} to 5 x 10^{-4} to 5 x 10^{-3} mol dm^3 did not affect the calculated K_s values. The enthalpies of complexation (ΔH) were measured by standard calorimetric methods using a Tronac 450 microcalorimeter.

A solution of silver nitrate (1.0mmol, 20 cm^3) in methanol was titrated with a solution of the ligand in methanol (0.02mol dm^3). The ionic strength was kept constant at I = 0.05 mol dm^3 by addition of tetramethyl ammonium percholate. The concentration of free silver

* These analyses by Dr H.J. Buschmann, Krefeld, Germany.

ion was measured using a silver ion selective electrode (Metrohm EA282) with a second silver electrode as a reference electrode. The emf observed could be used directly to determine the free silver ion concentration, according to the Nernst equation, which simplifies to equation (4),

$$E = E_0 + A\ln[Ag^+] \qquad (4)$$

where $[Ag^+]$ is the concentration of free silver ion and A is a constant which may be determined using appropriate calibration solutions. The stability constant K_s which refers to the reaction, equation (5)

$$Ag^+ + L = AgL^+ \qquad (5)$$

is defined by equation (6)

$$K_S = \frac{[AgL^+]}{[Ag^+]\,[L]} \qquad (6)$$

K_s is the concentration stability constant, assuming that the activity coefficients of the three species are equal to unity.

5.8 REFERENCES

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APPENDIX

Colloquia And Conferences

UNIVERSITY OF DURHAM

Board of Studies in Chemistry

COLLOQUIA, LECTURES AND SEMINARS GIVEN BY INVITED SPEAKERS

A list of all the research collquia, lectures and seminars arranged by the Department of Chemistry during the period of the author's residence as a postgraduate student is presented: "*" indicating the author's attendance.

1st October to 31st July 1991

6th October 1988	Schmutzler, Professor R. (Technicsche Uniersitat Braunschweig) Fluorophosphines Revisited - New Contributions to an Old Theme *
18th October 1988	Bollen, Mr. F. (Durham Chemistry Teachers'Centre) Lecture about the use if SATIS in the classroom
18th October 1988	Dingwall, Dr. J. (Ciba Geigy) Phosporus-containing Amino Acids: Biologically Active Natural and Unnatural Products*
18th October 1988	Dingwall, Dr. C.J. Ludman (University of Durham) The Energetics of Explosives *
21st October 1988	von Rague Schleyer, Professor P. (Universitaty Erlangen Nurnberg) The Fruitful Interplay Between Calculational and Experimental Chemistry
27th October 1988	Rees, Professor C.W. (Imperial College, London) Some Very Heterocyclic Compounds *
9th November 1988	Singh, Dr. G. (Teesside Polytechnic) Towards Third Generation Anti-Leukaemics*
10th November 1988	Cadogan, Professor J. I. G. (British Petroleum) From Pure Science to Profit

16th November 1988	McLauchlan, Dr. K.A. (University of Oxford) The Effect of Magnetic Fields on Chemical Reactions
24th November 1988	Baldwin, Dr. R. R. and Walker, Dr R.W. (University of Hull) Combustion: Some Burning Problems *
1st December 1988	Snaith, Dr. R. (University of Cambridge) Eyptian Mummies: What, Where, Why and How?
7th December 1988	Hardgrove, Dr. G. (St. Olaf College , USA) Polymers in the Physical Chemistry Laboratory
9th December 1988	Jager, Dr. C. (Friedrich-Schiller University GDR) NMR Investigations of Fast Ion Conductors of the NASICON Type
14th December 1988	Mortimer, Dr. C. (Durham Chemistry Teachers' Centre) The Hindeberg Disaster - An Excuse for Some Experiments
26th January 1989	Jennings, Professor R. R (University of Warwick) Chemistry of the Masses*
1st February 1989	Walters, Mr. D. and Cressey, T. (Durham University Teachers' Centre) GCSE Chemistry 1988: A Coroner's Report
2nd February 1989	Hall, Professor L. D. (Addenbrooke's Hospital, Cambridge) NMR - A Window to the Human Body*
9th February 1989	Baldwin, Professor J.E. (University of Oxford) Recent Advances in Living Metathesis*
15th February 1989	Butler, Dr. A. R. (University of St. Andrews) Cancer in Linxiam: The Chemical Dimensions*

16th Febraury 1989	Aylett, Professor B.J. (Queen Mary College, London) Silicon-Based Chips: The Chemist's Contribution
22nd February 1989	MacDoughall, Dr. G. (University of Edinburgh) Vibrational Spectroscopy of Model Catalytic Systems
23rd February 1989	Johnson, Dr. B.F.G.(University of Cambridge) The Binary Carbonyls
1st March 1989	Errington, Dr. R. J. (University of Newcastle-Upon-Tyne) Polymetalate Assembly in Organic Solvents
9th March 1989	Marko, Dr. I. (University of Sheffield) Catalytic Asymmetric Osmylation of Olefins
14th March 1989	Revell, Mr. P. (Durham Chemistry Teachers' Centre) Implementing Broad and Balanced Science
15th March 1989	Aveyard, Dr. R. (University of Hull) Surfactants at your Surface
20th April 1989	Casey, Dr. M. (University of Salford) Sulphoxides in Stereoselective Synthesis*
27th April 1989	Crich, Dr. D. (University College London) Some Novel Uses of Free Radicals in Organic Synthesis)
3rd May 1989	Ashman, Mr. A. (Durham Chemistry Teachers' Centre) The Chemical Aspects of the National Curriculum
3rd May 1989	Page, Dr. P.C.B. (University of Liverpool) Stereocontrol of Organic Reactions Using 1,3- dithiane - 1 - oxides *
10th May 1989	Wells, Professor P. B. (University of Hull) Catalyst Characteristics of Activity
11th May 1989	Frey, Dr. J. (University of Southampton)

	Spectroscopy of the Reaction Path: Photodissociation Raman Spectra of NOCl
16th May 1989	Stibr, Dr. R. (Czechoslovak Academy of Sciences) Recent Developments in the Chemistry of Intermediate-Sited Carboranes
17th May 1989	Moody, Dr. C.J. (Imperial College London) Reactive Intermediates on Heterocyclic Synthesis *
23rd May 1989	Paetzold, Professor P. (Aachen) Iminoboranes XB=NR: Inorganic Acetylenes?
14th June 1989	Jones, Dr. M.E. (Durham Chemistry Teachers' Centre) Discussion Session on the National Curriculum
15th June 1989	Pola, Professor J. (Czechoslovak Academy of Sciences) Carbon Dioxide Laser Induced Chemical Reactions - New Pathways in Gas-Phase Chemistry)
28th June 1989	Jones.Dr. M.E. (Durham Chemistry Teachers' Centre) GCSE and A Level Chemistry 1989
11th July 1989	Nicholls, Dr. D. (Durham Chemistry Teachers' Centre) Demo: Liquid Air *

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COLLOQUIA, LECTURES AND SEMINARS GIVEN BY INVITED SPEAKERS

1st October 1989 to 31st July 1990

17th October 1989	Palmer, Dr. F . (University of Nottingham) Thunder and Lightning*
25th October 1989	Floriani, Prof. C. (University of Lausanne) Molecular Aggregates - A Bridge Between Homogeneous and Heterogeneous Systems
1st November 1989	Badyal, Dr. J. P. S. (University of Durham) Breakthroughs in Heterogeneous Catalysis
9th November 1989	Greenwood, Prof. N. N. (University of Leeds) Novel Cluster Geometries in Metalloborane Chemistry*
10 November 1989	Bercaw, Prof. J. E. (California Institute of Technology) Synthetic and Mechanistic Approaches to Ziegler - Natta Polymerization of Olefins
13 November 1989	Becher, Dr. J. (University of Odense) Synthesis of New Macrocyclic Systems Using Heterocyclic Building Blocks*
16 November 1989	Parker, Dr. D. (University of Durham) Macrocycles, Drugs and Rock 'n' Roll*
29 November 1989	Cole-Hamilton, Prof. D. J. (University of St. Andrews) New Polymers from Homogeneous Catalysis
30 November 1989	Hughes, Dr. M. N. (King's College, London) A Bug's Eye View of the Periodic Table

4th December 1989	Graham, Dr. D. (B. P. Research Centre) How Proteins Adsorb to Interfaces
6th Decenber 1989	Powell, Dr. R. L. (ICI) The Development of C.F.C. Replacement
7th December 1989	Butler, Dr. A. (University of St. Andrews) The Discovery of Penicillin : Facts and Fancies*
13 December 1989	Klinowski, Dr. J. (University of Cambridge) Solid-State NMR Studies of Zeolite Catalysts
15th December 1989	Huisgen, Prof. R. (Universität München) Recent Mechanistic Studies of [2 + 2] Additions
24 January 1990	Perutz, Dr. R. N. (University of York) Plotting the Course of C-H Activations with Organometallics*
31 January 1990	Dyer, Dr. U. (Glaxo) Synthesis and Conformation of C-Glycosides
1st February 1990	Holloway, Prof. J. H. (University of Leicester) Noble Gas Chemistry
7th February 1990	Thompson, Dr. D. P. (University of Newcastle upon Tyne) The Role of Nitrogen in Extending Silicate Crystal Chemistry
8th February 1990	Lancaster, Rev. R. (Kimbolton Fireworks) Fireworks - Principles and Practice*
12 February 1990	Lunazzi, Prof. L. (University of Bologna) Application of Dynamic NMR to the Study of Conformational Enantiomerism*
14th February 1990	Sutton, Prof. D. (Simon Fraser University, Vancouver) Synthesis and Applications of Dinitrogen and Compounds of Rhenium and Iridium
15 February 1990	Crombie, Prof. L. (University of Nottingham)

	The Chemistry of Cannabis and Khat
21st February 1990	Bleasdale, Dr. C. (University of Newcastle upon Tyne) The Mode of Action of some Anti - Tumour Agents*
22nd February 1990	Clark, Prof. D.T. (ICI Wilton) Spatially Resolved Chemistry (using Nature's Paradigm in the Advanced Materials Arena)
28th February 1990	Thomas, Dr. R. K. (University of Oxford) Neutron Reflectometry from Surfaces
1st March 1990	Stoddart, Dr. J. F. (University of Sheffield) Molecular Lego
8th March 1990	Cheetham, Dr. A. K. (University of Oxford) Chemistry of Zeolite Cages
21st March 1990	Powis, Dr. I. (University of Nottingham) Spinning Off in a Huff : Photodissociation of Methyl Iodide
23 March 1990	Bowman, Prof. J. M. (Emory University) Fitting Experiment with Theory in Ar-OH
9th July 1990	German, Prof. L. S. (Soviet Academy of Sciences) New Syntheses in Fluoroaliphatic Chemistry : Recent Advances in the Chemistry of Fluorinated Oxiranes
9th July 1990	Platanov, Prof. V.E. (Soviet Academy of Sciences, Novosibirsk Polyfluoroindanes : Synthesis and Transformation
9th July 1990	Rozhkov, Prof. I. N. (Soviet Academy of Sciences, Moscow) Reactivity of Perfluoroalkyl Bromides

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COLLOQUIA, LECTURES AND SEMINARS GIVEN BY INVITED SPEAKERS

1st August 1990 to 31st July 1991

11th October 1990	Macdonald, Dr. W.A. (ICI Wilton) Materials for the Space Age*
24th October 1990	Bochmann, Dr. M. (University of East Anglia) Synthesis, Reactions and Catalytic Activity of Cationic Titanium Alkyls
26th October 1990	Soulen, Prof. R. (South Western University, Texas) Preparation and Reactions of Bicycloalkenes
31st October 1990	Jackson, Dr. R.F.W. (University of Newcastle upon Tyne) New Synthetic Methods : a-Amino Acids and Small Rings *
1st November 1990	Logan, Dr. N. (University of Nottingham) Rocket Propellants
6th November 1990	Kocovsky, Dr. P. (University of Uppsala) Stereo-Controlled Reactions Mediated by Transition and Non-Transition Metals *
7th November 1990	Gerrard, Dr. D. (British Petroleum) Raman Spectroscopy for Industrial Analysis
8th November 1990	Scott, Dr. S.K. (University of Leeds) Clocks, Oscillations and Chaos*
14th November 1990	Bell, Prof. T. (SUNY, Stoney Brook, USA) Functional Molecular Architecture and Molecular Recognition
21st November 1990	Pritchard, Prof. J. (Queen Mary & Westfield College) Copper Surfaces and Catalysts*

28th November 1990	Whitaker, Dr. B.J. (University of Leeds) Two-Dimensional Velocity Imaging of State-Selected Reaction Products
29 November 1990	Crout, Prof. D. (University of Warwick) Enzymes in Organic Synthesis
5th December 1990	Pringle, Dr. P.G. (University of Bristol) Metal Complexes with Functionalised Phosphines*
13th December 1990	Cowley, Prof. A.H. (University of Texas) New Organometallic Routes to Electronic Materials
15th January 1991	Alder, Dr. B.J. (Lawrence Livermore Labs., California) Hydrogen in all its Glory
17th January 1991	Sarre, Dr. P. (University of Nottingham) Comet Chemistry
24th January 1991	Sadler, Dr. P.J. (Birkbeck College London) Design of Inorganic Drugs : Precious Metals, Hypertension & HIV
30th January 1991	Sinn, Prof. E. (University of Hull) Coupling of Little Electrons in Big Molecules : Implications for the Active Sites of Metalloproteins and other Macromolecules
31st January 1991	Lacey, Dr. D. (University of Hull) Liquid Crystals
6th February 1991	Bushby, Dr. R. (University of Leeds) Biradicals and Organic Magnets
14th February 1991	Petty, Dr. M.C. (Durham University) Molecular Electronics
20th February 1991	Shaw, Prof. B.L. (University of Leeds) Syntheses with Coordinated, Unsaturated Phosphine Ligands*
28th February 1991	Brown, Dr. J. (University of Oxford) Can Chemistry Provide Catalysts Superior to Enzymes?

6th March 1991	Dobson, Dr. C.M. (University of Oxford) NMR Studies of Dynamics in Molecular Crystals
7th March 1991	Markam, Dr. J. (ICI Pharmaceuticals) DNA Fingerprinting
24th April 1991	Schrock, Prof. R.R. (M.I.T.) Metal-Ligand Multiple Bonds and Metathesis Initiators
25th April 19921	Hudlicky, Prof. T. (Virginia Polytechnic Institute) Biocatalysis and Symmetry Based Approaches to the Efficient Synthesis of Complex Natural Products
20th June 1991	Brookhart, Prof. M.S. (University of North Carolina) Olefin Polymerizations, Oligomerizations and Dimerizations Using Electrophilic Late Transition Metal Catalysts*
29th July 1991	Brimble, Dr. M.A. (Massey University, New Zealand) Synthetic Studies Towards the Antibiotic Griseusin-A*

RESEARCH CONFERENCES

1. U.K. Macrocycle Group, Annual Meeting, University of Durham, 19 April, 1989.

2 R.S.C. Perkin Division, N.E. Regional Meeting, University of York, 16 December, 1989.

3 RSC Graduate Symosium, University of Durham, 12 April 1989

4 R.S.C Dalton and Industrial Division, Inorganics In Human Health, UCL, 1989.

CRYSTAL DATA

Space Group Cell Dimentions Orthorhombic $P22_12_1$

a 6.6785(21) b 15.551(3) c 17.361(3)

Volume 1803.1(7)A**3

Empirical Formula : $C_{15}H_{25.80}InN_3O_{6.90}$

$C_{15}H_{25.80}InN_3O_{6.90}$ or $C_{15}H_{24}InN_3O_6.0.9H_2O$

Cell dimensions obtained from 25 reflections with 2 theta angle in the range 15.00-32.00 degrees.

Crystal dimensions: 0.29 x 0.22 x 0.08 mm

FW = 473.41 Z = 4 F(000) = 964

Dcalc 1.744Mg.m^{-3}, mu = 1.33mm^{-1},

lambda = 0.70930°A, 2Theta(max) = 53.8°

The internsity data was collected on a Nonius diffractometer, using the theta/2theta scan mode.

the h,k,l ranges are: 0 8, 0 18, 0 22.

No. of reflections measured	2267
No. of unique reflections	2266
No. of reflections with Inet > 5sigma(Inet)	1986

Absorbtion corrections were made.

The minimum and maximum transmission factors are 0.758165 and 0.896510.

The last least squares cycle was calculated with 51 atoms, 245 parameters and 1973 out of 1973 reflections.

Weights based on counting statistics were used.

The residuals are as follows:

For significant reflections, RF 0.029, RW 0.025, GoF 2.28

For all reflections,

Where RF = sum(F_0-F_c)/sum(F_0),

RW = sqrt [sum(w(F_0-F_c)**2)/(No. of reflns - No. of params.)]

GoF = sqrt [sum(w(F_0-F_c)**2)/(No. of reflins - No. of params.)]

The maximum shift/sigma ratio was 0.200.

In the last D-map, the deepest hole was -0.44e/A**3,

and the highest peak 0.380e/A**3

Table . Calculated hydrogen coordinates (C-H 0.95 Å).

H2A	.4344	.3543	.3587
H2B	.5244	.2934	.2970
H3A	.4379	.4142	.2326
H3B	.2291	.4168	.2716
H5A	.4195	.3100	.0921
H5B	.5192	.2827	.1690
H6A	.4442	.1588	.1081
H6B	.2295	.1903	.0890
H8A	.4480	.0777	.2608
H8B	.5271	.1695	.2436
H9A	.4586	.1667	.3706
H9B	.2491	.1267	.3562
H10	.1151	.3339	.4058
H11C	.1757	.2720	.5237
H11A	.2946	.1991	.4833
H11B	.3739	.2928	.4810
H13	.1000	.3585	.0868
H14C	.1361	.5053	.0770
H14A	.2537	.5061	.1540
H14B	.3434	.4607	.0823
H16	.1302	.0376	.2285
H17C	.1717	-.0443	.1201
H17A	.2780	.0325	.0805
H17B	.3748	-.0111	.1517

DEPOSITION DATA

Table . Anisotropic thermal parameters U_{ij} ($\times 10^2$). 90-39

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
In	1.971(15)	4.418(20)	2.425(16)	.147(22)	.151(16)	.041(22)
N1	2.4 (2)	3.7 (3)	2.4 (2)	-.1 (3)	.3 (2)	.1 (3)
C2	3.1 (4)	4.3 (4)	3.5 (4)	-.8 (3)	-.1 (3)	-1.1 (3)
C3	4.4 (4)	3.7 (3)	3.3 (4)	.0 (3)	.8 (4)	.1 (3)
N4	2.8 (3)	3.1 (3)	2.7 (3)	-.1 (3)	.3 (3)	-.3 (2)
C5	2.8 (3)	5.1 (4)	2.7 (3)	-.1 (3)	.8 (3)	.2 (3)
C6	3.1 (4)	4.2 (4)	2.0 (3)	.5 (3)	.3 (3)	-.1 (3)
N7	2.7 (3)	2.9 (3)	2.6 (3)	.0 (3)	-.1 (3)	.2 (2)
C8	2.6 (3)	4.0 (3)	4.1 (4)	.7 (2)	-.4 (3)	-.1 (3)
C9	2.8 (4)	4.4 (4)	2.9 (4)	-.6 (3)	-.5 (3)	.3 (3)
C10	3.2 (3)	6.0 (5)	2.5 (3)	.5 (3)	.2 (3)	-1.1 (3)
C11	5.6 (4)	11.9 (7)	3.0 (3)	-.2 (7)	-.4 (3)	-1.7 (5)
C12	3.8 (3)	5.3 (5)	3.4 (3)	1.2 (3)	1.2 (3)	.3 (3)
C13	4.5 (4)	4.0 (4)	3.1 (4)	.1 (3)	-.6 (3)	.1 (3)
C14	7.6 (6)	5.7 (5)	6.6 (6)	.2 (5)	1.1 (6)	2.8 (5)
C15	4.2 (5)	4.5 (4)	3.4 (4)	1.2 (4)	-.4 (4)	-.8 (3)
C16	4.7 (4)	3.3 (4)	2.9 (3)	-.7 (3)	.3 (3)	.2 (3)
C17	7.9 (6)	4.0 (4)	5.7 (5)	.5 (5)	-.5 (5)	-2.0 (4)
C18	5.1 (5)	5.7 (5)	2.4 (4)	-2.4 (4)	-.1 (4)	.8 (3)
O1	2.8 (2)	6.8 (3)	2.8 (2)	-.5 (2)	.5 (2)	.3 (2)
O2	5.7 (3)	11.0 (5)	2.9 (2)	-.6 (3)	1.7 (2)	-.1 (3)
O3	3.2 (2)	5.3 (3)	4.5 (3)	1.7 (2)	.4 (2)	.4 (2)
O4	6.5 (4)	5.9 (4)	5.3 (4)	2.7 (3)	.1 (3)	1.1 (3)
O5	2.6 (3)	5.3 (3)	3.7 (3)	-.4 (2)	-.2 (2)	-.4 (2)
O6	6.9 (5)	6.8 (4)	6.8 (4)	-3.0 (3)	-1.1 (4)	-.2 (3)
OW1	7.1 (7)	17.9 (1.9)	7.9 (1.1)	4.6 (9)	-2.3 (9)	-4.0 (1.5)
OW2	17.2 (2.1)	32.2 (9.1)	7.9 (1.4)	-7.9 (3.8)	-.3 (3)	4.6 (4.9)

.....

Anisotropic temperature factors are of the form:

$$\exp[-2\Pi^2(h^2U_{11}a^{*2} + k^2U_{22}b^{*2} + l^2U_{33}c^{*2} + 2hkU_{12}a^*b^* + 2hlU_{13}a^*c^* + 2klU_{23}b^*c^*)].$$

DEPOSITION DATA

Torsion angles for $C_{15}H_{24}InN_3O_6 \times 0.9H_2O$. 90-39

N4	In	N1	C2	7.6(| 3)	N4	In	N1	C9	-111.9(| 4)
N4	In	N1	C10	130.4(| 4)	N7	In	N1	C2	88.4(| 3)
N7	In	N1	C9	-31.1(| 3)	N7	In	N1	C10	-148.7(| 4)
O1	In	N1	C2	-149.6(| 4)	O1	In	N1	C9	90.9(| 3)
O1	In	N1	C10	-26.8(| 3)	O3	In	N1	C2	-61.9(| 3)
O3	In	N1	C9	178.6(| 4)	O3	In	N1	C10	60.9(| 3)
O5	In	N1	C2	132.7(| 4)	O5	In	N1	C9	13.3(| 3)
O5	In	N1	C10	-104.4(| 3)	N1	In	N4	C3	-30.5(| 3)
N1	In	N4	C5	89.7(| 3)	N1	In	N4	C13	-148.5(| 4)
N7	In	N4	C3	-111.9(| 4)	N7	In	N4	C5	8.3(| 3)
N7	In	N4	C13	130.1(| 4)	O1	In	N4	C3	15.1(| 3)
O1	In	N4	C5	135.3(| 4)	O1	In	N4	C13	-102.9(| 4)
O3	In	N4	C3	90.8(| 4)	O3	In	N4	C5	-149.0(| 4)
O3	In	N4	C13	-27.1(| 3)	O5	In	N4	C3	178.9(| 4)
O5	In	N4	C5	-61.0(| 3)	O5	In	N4	C13	60.9(| 3)
N1	In	N7	C6	-112.3(| 4)	N1	In	N7	C8	7.5(| 3)
N1	In	N7	C16	129.5(| 4)	N4	In	N7	C6	-31.0(| 3)
N4	In	N7	C8	88.9(| 4)	N4	In	N7	C16	-149.2(| 4)
O1	In	N7	C6	177.6(| 4)	O1	In	N7	C8	-62.5(| 3)
O1	In	N7	C16	59.5(| 3)	O3	In	N7	C6	13.2(| 3)
O3	In	N7	C8	133.1(| 4)	O3	In	N7	C16	-105.0(| 4)
O5	In	N7	C6	90.2(| 4)	O5	In	N7	C8	-149.9(| 4)
O5	In	N7	C16	-28.0(| 3)	N1	In	O1	C12	17.0(| 3)
N4	In	O1	C12	-29.0(| 3)	N7	In	O1	C12	88.2(| 4)
O3	In	O1	C12	-100.1(| 4)	O5	In	O1	C12	165.5(| 4)
N1	In	O3	C15	89.7(| 4)	N4	In	O3	C15	18.7(| 4)
N7	In	O3	C15	-26.0(| 4)	O1	In	O3	C15	167.8(| 5)
O5	In	O3	C15	-98.1(| 4)	N1	In	O5	C18	-22.8(| 4)
N4	In	O5	C18	92.7(| 4)	N7	In	O5	C18	22.1(| 4)
O1	In	O5	C18	-95.9(| 4)	O3	In	O5	C18	170.2(| 5)
In	N1	C2	C3	17.3(| 3)	C9	N1	C2	C3	131.0(| 6)
C10	N1	C2	C3	-100.6(| 5)	In	N1	C9	C8	51.9(| 3)
C2	N1	C9	C8	-66.0(| 5)	C10	N1	C9	C8	164.8(| 7)
In	N1	C10	C11	162.5(| 5)	In	N1	C10	C12	33.1(| 3)
C2	N1	C10	C11	-77.1(| 5)	C2	N1	C10	C12	153.5(| 6)
C9	N1	C10	C11	51.3(| 4)	C9	N1	C10	C12	-78.1(| 5)
N1	C2	C3	N4	-48.2(| 4)	C2	C3	N4	In	51.0(| 3)
C2	C3	N4	C5	-67.9(| 5)	C2	C3	N4	C13	165.0(| 7)
In	N4	C5	C6	16.7(| 3)	C3	N4	C5	C6	131.1(| 6)
C13	N4	C5	C6	-101.8(| 6)	In	N4	C13	C14	160.6(| 6)
In	N4	C13	C15	32.3(| 3)	C3	N4	C13	C14	48.8(| 5)
C3	N4	C13	C15	-79.6(| 5)	C5	N4	C13	C14	-78.5(| 6)
C5	N4	C13	C15	153.1(| 7)	N4	C5	C6	N7	-48.2(| 4)
C5	C6	N7	In	51.8(| 3)	C5	C6	N7	C8	-65.0(| 5)
C5	C6	N7	C16	164.7(| 7)	In	N7	C8	C9	18.4(| 3)
C6	N7	C8	C9	131.7(| 6)	C16	N7	C8	C9	-98.5(| 6)
In	N7	C16	C17	161.6(| 6)	In	N7	C16	C18	31.2(| 3)
C6	N7	C16	C17	50.4(| 5)	C6	N7	C16	C18	-80.1(| 5)
C8	N7	C16	C17	-79.6(| 6)	C8	N7	C16	C18	149.9(| 7)
N7	C8	C9	N1	-49.8(| 4)	N1	C10	C12	O1	-22.9(| 3)
N1	C10	C12	O2	159.2(| 7)	C11	C10	C12	O1	-152.7(| 7)
C11	C10	C12	O2	29.4(| 4)	C10	C12	O1	In	-2.4(| 2)
O2	C12	O1	In	175.4(| 6)	N4	C13	C15	O3	-20.3(| 3)
N4	C13	C15	O4	162.0(| 9)	C14	C13	C15	O3	-150.5(| 9)
C14	C13	C15	O4	31.8(| 5)	C13	C15	O3	In	-5.1(| 3)
O4	C15	O3	In	172.4(| 7)	N7	C16	C18	O5	-16.6(| 3)
N7	C16	C18	O6	165.1(| 9)	C17	C16	C18	O5	-147.2(| 9)
C17	C16	C18	O6	34.5(| 5)	C16	C18	O5	In	-10.2(| 3)
O6	C18	O5	In	167.9(| 7)						

Columns are 10Fo, 10Fc, 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	0,	0, 1		6	406	411	3	6	1073	1124	5	2	177	206	5
2	3054	3191	15	7	202	196	4	7	337	327	4	3	103	99	8
4	272	291	3	8	926	919	5	8	962	955	5	4	244	258	5
6	112	101	6	10	730	728	4	9	419	427	4	6	307	317	5
8	1565	1603	7	11	171	163	7	10	932	924	4	7	255	253	5
10	1262	1229	6	12	738	728	4	11	180	182	7	8	474	474	4
12	1095	1087	5	14	273	267	6	12	805	806	4	9	263	269	6
14	786	787	5	16	439	436	6	13	155	153	9	10	439	453	5
16	371	364	6	18	547	550	6	14	466	474	5	11	153	165	8
18	381	388	6	20	433	441	7	15	163	180	9	12	316	303	6
20	191	179	10		0,	4, 1		18	255	265	8	13	204	203	7
22	172	143	11	0	1971	1983	5	20	202	205	10	14	202	199	8
	0,	1, 1		1	511	506	2		0,	7, 1		15	170	161	9
2	412	407	2	2	145	184	5	1	128	144	5	16	299	291	7
3	77	81	5	4	659	689	3	2	473	454	3	17	138	141	12
4	1208	1192	5	5	95	93	6	4	271	263	4	18	247	255	9
6	244	233	3	6	1044	1054	5	5	174	171	5		0,	10, 1	
8	853	849	4	7	188	198	4	6	427	443	4	0	1011	998	5
10	675	678	4	8	1439	1412	7	7	353	356	4	1	252	259	5
11	102	109	10	9	591	575	4	8	268	271	5	2	942	970	5
12	762	770	4	10	821	813	4	9	288	287	5	3	467	468	4
13	151	162	8	11	295	303	5	10	314	316	5	4	925	942	5
14	164	149	8	12	623	636	4	12	529	530	5	5	587	572	4
16	441	432	6	13	164	179	9	13	192	207	8	6	670	653	4
18	597	606	6	14	494	477	5	14	394	397	6	7	265	259	5
20	456	463	7	16	217	224	8	15	178	193	9	8	380	373	5
22	343	344	8	18	302	291	7	16	377	376	6	9	153	114	8
	0,	2, 1		20	174	175	11	17	205	225	9	10	315	318	6
0	2689	2736	13		0,	5, 1		18	321	321	7	12	478	480	5
1	215	215	2	3	111	113	5	19	153	143	12	13	221	192	7
2	1755	1780	4	4	177	189	4	20	314	298	8	14	352	355	6
4	1824	1840	5	5	178	156	4		0,	8, 1		15	210	218	8
5	149	143	5	6	273	243	4	0	1183	1177	6	16	204	204	8
6	1097	1102	6	7	158	151	5	1	288	308	4	17	146	125	12
7	183	170	4	8	354	357	4	2	1002	1018	5	18	149	159	13
8	635	645	3	9	428	432	4	3	350	350	4		0,	11, 1	
9	185	184	5	10	580	578	4	4	1078	1097	6	1	95	94	11
10	945	949	4	11	187	172	6	5	491	500	4	2	88	58	12
11	196	211	6	12	739	737	4	6	1087	1144	6	4	321	331	5
12	927	952	5	14	348	363	6	7	233	222	5	5	180	197	7
13	159	176	8	16	492	482	6	8	1010	1009	5	6	545	562	4
14	772	764	5	17	216	246	8	9	216	235	6	7	218	215	7
16	193	191	8	18	454	452	6	10	678	685	4	8	292	281	6
18	363	360	6	19	202	207	9	11	150	156	9	12	185	178	8
20	200	189	9	20	401	395	7	12	501	507	5	14	284	281	7
22	139	145	14		0,	6, 1		13	160	162	9	15	159	167	11
	0,	3, 1		0	737	782	4	14	340	360	6	16	288	291	8
1	258	248	2	1	548	544	3	16	264	270	7	17	171	162	10
2	1545	1495	5	2	753	789	4	18	258	261	8	18	203	207	10
3	129	142	5	3	311	309	3	20	206	190	10		0,	12, 1	
4	90	85	7	4	1357	1356	6		0,	9, 1		0	698	686	4
5	73	63	8	5	418	424	3	1	172	173	5	1	559	538	4

Columns are 10Fo 10Fc 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	0, 12, 1			13	175	190	11		1, 1, 1			15	134	151	11
2	702	696	4	14	132	137	14	1	1082	1083	4	16	474	484	6
3	457	455	5		0, 16, 1			2	356	344	2	18	483	492	6
4	462	476	5	0	176	165	8	3	260	248	3	20	339	349	7
5	311	306	5	1	123	113	11	4	1099	1088	5		1, 4, 1		
6	239	223	6	2	267	263	7	6	772	762	4	0	1672	1701	5
7	173	151	8	3	236	229	7	7	280	274	4	1	387	385	3
8	187	172	7	4	345	330	6	8	457	453	3	2	1466	1448	6
9	198	176	7	5	303	284	7	10	461	475	4	3	238	254	3
10	318	305	6	6	305	285	7	12	636	630	4	4	1478	1485	6
11	249	261	7	7	247	246	8	13	237	213	6	5	120	119	6
12	389	391	6	8	250	254	8	14	541	545	5	6	1644	1632	7
13	298	293	7	9	201	187	9	15	204	214	8	7	171	185	5
14	283	284	7	10	206	213	9	16	587	598	5	8	1149	1170	6
15	217	231	9	11	171	173	10	17	296	303	7	9	199	197	6
16	156	168	12	12	155	154	12	18	562	562	6	10	879	900	4
17	154	143	12		0, 17, 1			20	361	348	7	11	335	333	5
	0, 13, 1			8	136	131	12		1, 2, 1			12	741	734	4
1	108	80	11	11	111	114	16	0	1547	1533	4	13	205	213	8
2	160	167	8		0, 18, 1			1	160	139	4	14	659	665	5
3	167	166	8	0	160	158	11	2	1137	1160	5	15	202	216	8
8	143	145	10	1	229	205	9	4	1963	1966	6	16	379	374	6
9	111	62	11	2	184	182	10	5	82	77	8	18	359	359	7
10	251	245	7	3	176	158	10	6	1590	1604	7	20	264	249	8
11	124	114	12	4	188	194	10	7	309	306	4		1, 5, 1		
12	210	200	8	5	178	170	10	8	1059	1053	5	0	397	382	3
13	174	153	9	6	162	181	11	9	83	89	10	1	106	99	6
14	225	215	9	7	215	214	9	10	757	768	4	2	173	172	4
15	142	124	13	8	173	169	11	11	351	351	5	3	239	225	3
16	223	247	10	9	171	173	12	12	867	867	4	4	192	198	4
	0, 14, 1				1, 0, 1			13	177	178	8	5	381	370	3
0	415	414	5	0	2482	2523	12	14	622	621	5	6	372	366	3
1	312	311	6	1	102	57	4	16	285	277	6	7	143	141	6
2	356	359	6	2	1624	1620	5	18	291	284	7	8	530	518	4
3	316	295	6	3	187	198	4	20	236	234	9	9	202	177	6
4	271	266	6	4	778	791	4	21	114	126	16	10	545	546	4
5	310	302	6	5	468	459	3		1, 3, 1			11	176	196	8
6	300	282	6	6	1097	1133	6	0	410	390	2	12	477	481	5
7	289	290	6	7	370	380	3	1	312	308	3	13	243	247	7
8	339	327	6	8	891	899	4	2	951	940	5	14	402	391	5
9	259	264	7	9	112	136	8	3	86	90	7	16	441	431	6
10	292	284	7	10	934	961	5	4	246	243	3	17	156	146	10
11	221	227	8	11	124	136	9	5	310	287	3	18	424	430	6
12	244	264	8	12	806	802	4	6	832	827	4	19	129	153	14
13	171	168	10	13	363	366	5	7	144	125	5	20	318	319	8
14	205	212	10	14	597	602	5	8	773	764	4		1, 6, 1		
	0, 15, 1			15	122	134	13	9	199	206	5	0	1679	1672	6
7	142	151	10	16	179	157	8	10	577	565	4	1	860	845	4
8	140	145	11	18	246	225	7	11	188	179	7	2	1275	1271	6
9	153	138	10	19	191	212	9	12	537	533	5	3	323	315	3
10	164	173	10	20	197	183	9	13	222	231	7	4	914	981	5
11	172	150	10	21	119	147	16	14	355	373	6	5	272	266	4

Columns are 10Fo 10Fc 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	1, 6, 1			18	245	229	8	11	177	179	8	11	171	169	10
6	876	896	4	19	145	127	12	12	271	261	7	12	200	192	9
7	183	185	6		1, 9, 1			13	183	179	9	13	104	118	16
8	896	904	4	0	289	293	4	14	319	310	7	14	141	142	13
9	318	321	5	1	202	208	5	15	124	120	12	15	137	116	13
10	714	733	4	2	147	170	6	16	296	285	8		1, 15, 1		
11	265	252	6	3	181	189	6	17	218	200	9	0	128	151	10
12	691	699	4	4	162	191	7	18	211	212	11	1	127	147	11
13	229	228	7	5	207	200	6		1, 12, 1			6	93	97	15
14	520	539	5	6	194	192	6	0	612	610	4	8	140	118	10
15	138	144	11	7	221	220	6	1	375	380	5	9	168	141	9
16	328	331	6	8	345	345	5	2	591	616	4	10	157	163	11
17	101	101	15	9	268	283	6	3	233	232	7	11	184	178	10
18	285	275	7	10	500	503	5	4	555	547	5	13	157	158	12
20	198	193	10	11	231	251	7	5	192	189	7		1, 16, 1		
	1, 7, 1			12	426	419	5	6	495	479	5	0	172	155	9
0	433	430	3	13	242	259	7	7	250	249	7	1	255	272	7
1	320	315	3	14	323	315	6	8	452	438	5	2	245	235	7
3	246	242	4	15	111	69	13	9	329	332	6	3	301	310	7
4	189	177	5	16	335	333	7	10	401	406	6	4	281	287	7
5	119	109	7	17	128	120	13	11	225	220	7	5	314	314	7
6	230	258	5	18	328	312	8	12	355	358	6	6	257	234	7
7	279	294	5	19	164	145	12	13	165	163	10	7	254	249	7
8	316	331	5		1, 10, 1			14	236	235	8	8	212	195	8
9	470	472	4	0	773	763	4	15	127	143	14	9	168	168	11
10	484	466	5	1	423	432	4	16	205	189	10	10	171	168	10
11	321	310	6	2	795	773	4		1, 13, 1			12	123	122	15
12	523	518	5	3	337	336	5	0	96	101	13		1, 17, 1		
13	176	172	8	4	734	737	4	1	148	140	9	5	102	86	14
14	389	403	6	5	387	394	5	2	260	285	6	10	113	104	15
16	440	431	6	6	717	726	4	3	190	191	8		1, 18, 1		
17	111	99	13	7	472	481	5	4	224	230	7	0	179	165	10
18	377	363	7	8	541	547	5	5	109	112	11	1	244	246	8
19	130	123	13	9	344	353	5	10	146	134	10	2	182	180	10
20	298	288	8	10	407	398	5	11	169	147	9	3	218	219	9
	1, 8, 1			11	246	245	7	12	214	195	8	4	151	158	12
0	1081	1095	5	12	338	332	6	13	146	166	11	5	214	187	9
1	617	620	3	13	174	172	9	14	219	219	9	7	170	165	11
2	910	921	5	14	329	328	7	15	142	133	13	8	142	128	12
3	406	408	4	16	260	263	8	16	208	200	10		2, 0, 1		
4	774	784	4	18	204	204	10		1, 14, 1			0	73	102	8
5	398	414	4		1, 11, 1			0	372	364	5	1	156	148	5
6	890	867	4	1	230	230	6	1	330	326	6	2	1343	1349	6
7	317	318	5	2	285	310	5	2	384	382	5	3	555	536	3
8	749	738	4	3	192	167	7	3	294	294	6	4	1633	1643	7
9	394	406	5	4	337	344	5	4	350	347	6	5	576	542	3
10	520	515	5	5	135	138	9	5	265	275	6	6	1735	1708	7
11	309	309	6	6	244	260	6	6	378	373	6	7	148	136	6
12	426	424	5	7	275	270	6	7	316	323	6	8	1002	1027	5
13	183	167	8	8	143	160	9	8	382	395	6	10	926	913	4
14	365	375	6	9	124	141	11	9	283	293	7	12	801	791	4
16	330	355	7	10	187	176	8	10	292	313	7	14	449	449	5

Columns are 10Fo 10Fc 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	2,	0, 1		3	744	718	4	14	468	478	5	4	822	822	4
15	241	257	7	4	561	540	3	15	262	266	7	5	368	376	4
16	325	322	7	5	598	568	3	16	406	415	6	6	852	850	4
17	165	177	10	6	622	627	3	17	160	157	11	7	306	299	5
18	287	262	7	7	612	590	3	18	415	416	7	8	648	631	4
19	177	179	10	8	283	289	5	20	324	325	8	9	292	283	6
20	257	249	8	9	214	220	6		2,	6, 1		10	520	506	5
21	131	131	15	10	573	580	4	0	1519	1500	7	11	290	306	6
	2,	1, 1		11	355	365	5	1	337	342	3	12	460	459	6
0	181	181	4	12	478	482	5	2	1383	1386	6	13	200	203	8
1	851	811	4	13	260	239	6	3	254	263	4	14	359	362	6
2	234	245	4	14	498	514	5	4	746	745	4	16	299	297	7
3	372	373	3	15	156	151	10	5	194	191	5	17	155	126	11
4	519	509	3	16	457	464	6	6	848	860	4	18	219	202	9
5	738	716	4	17	140	142	11	7	233	235	5	19	152	129	12
6	311	323	4	18	445	433	6	8	785	777	4		2,	9, 1	
7	772	748	4	20	340	344	8	9	253	251	6	1	156	164	6
8	183	159	6	21	136	141	14	10	590	595	4	2	202	226	6
9	126	110	8		2,	4, 1		11	273	259	6	3	105	126	10
10	536	540	4	0	896	910	5	12	446	447	5	4	146	151	7
12	645	642	4	1	132	134	5	13	142	141	10	5	138	129	8
13	108	128	12	2	1554	1553	6	14	410	407	6	6	254	263	6
14	720	740	5	3	395	383	3	16	352	360	6	7	180	176	7
15	214	204	8	4	1560	1542	7	18	279	268	8	8	219	212	7
16	493	511	6	5	673	659	3	19	175	169	11	9	196	212	7
17	185	189	9	6	1314	1270	7	20	168	192	13	10	327	322	6
18	374	364	7	7	348	347	4		2,	7, 1		11	254	254	7
20	271	270	9	8	681	660	4	1	607	597	3	12	326	320	6
	2,	2, 1		10	604	603	4	2	697	685	3	13	181	175	9
0	1484	1478	6	11	90	92	14	3	114	95	7	14	328	334	7
1	286	290	3	12	605	593	5	4	509	502	4	15	149	141	11
2	1390	1434	6	13	246	242	7	5	198	188	5	16	310	316	7
3	413	407	3	14	490	492	5	6	265	253	5	17	130	152	14
4	954	901	5	15	294	321	7	7	224	220	6	18	329	318	8
5	370	360	3	16	418	422	6	8	158	179	8		2,	10, 1	
6	1675	1573	7	17	160	136	9	9	281	279	5	0	431	440	4
7	337	325	4	18	368	361	7	10	384	382	5	1	389	397	5
8	1302	1299	6	20	241	237	9	11	237	229	7	2	541	557	4
9	231	231	5		2,	5, 1		12	332	335	6	3	310	309	5
10	800	788	4	0	458	451	3	13	114	108	12	4	655	650	4
11	293	294	5	1	786	766	4	14	365	378	6	5	359	352	5
12	544	548	5	2	321	313	3	15	118	116	13	6	821	806	4
13	191	196	8	3	900	885	4	16	419	416	6	7	364	376	5
14	466	474	5	4	716	705	4	17	142	158	12	8	671	683	5
15	290	286	7	5	595	575	3	18	392	375	7	9	266	274	6
16	453	462	6	6	780	767	4	19	122	102	15	10	471	481	5
18	343	332	7	7	325	310	4	20	280	269	8	11	247	246	7
20	255	228	8	8	289	296	5		2,	8, 1		12	287	301	7
	2,	3, 1		10	338	352	5	0	745	746	4	13	259	251	7
0	491	479	3	11	308	302	5	1	308	313	4	14	306	292	7
1	510	501	3	12	351	358	6	2	869	877	4	15	161	148	10
2	483	480	3	13	287	311	6	3	336	335	4	16	258	255	9

Columns are 10Fo 10Fc 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	2, 10, 1			0	291	279	6	5	188	181	11	8	1032	1038	5
18	185	180	11	1	327	326	6	6	147	131	13	9	223	232	7
	2, 11, 1			2	346	347	6	7	140	151	15	10	720	731	4
0	83	86	14	3	242	238	7		3, 0, 1			11	265	266	6
1	251	228	6	4	402	396	6	0	359	400	3	12	489	477	5
2	194	192	7	5	224	228	8	2	1031	1024	5	13	345	345	6
3	149	127	9	6	338	333	6	3	313	294	4	14	378	380	6
5	149	144	9	7	230	224	7	4	1424	1456	7	15	271	284	7
7	167	167	9	8	235	227	8	5	168	159	6	16	467	490	6
8	228	223	7	9	172	193	10	6	1193	1207	6	18	269	272	8
9	266	265	6	10	177	173	10	7	121	105	9	20	121	126	15
10	332	333	6	11	151	168	11	8	996	999	5		3, 3, 1		
11	274	288	7	12	140	128	12	9	158	172	8	0	120	82	6
12	290	288	7	13	166	168	11	10	704	711	4	1	487	498	3
13	178	203	9	14	129	148	15	11	368	378	5	2	640	633	3
14	309	307	7		2, 15, 1			12	643	643	5	3	565	565	3
15	181	171	10	1	171	182	9	13	329	338	6	4	450	456	3
16	272	263	8	2	105	100	13	14	408	406	6	5	432	417	4
17	193	186	11	3	124	138	11	15	204	205	9	6	288	302	5
	2, 12, 1			4	156	165	10	16	466	489	6	7	451	470	4
0	474	448	5	5	119	106	11	18	291	288	8	8	324	325	5
1	351	342	5	6	126	150	12	19	173	159	11	9	369	369	5
2	509	489	5	7	118	98	12	20	222	210	10	10	536	544	5
3	271	266	6	8	184	171	9		3, 1, 1			11	386	389	5
4	457	459	5	9	178	165	9	0	221	222	4	12	438	426	5
5	293	303	6	10	145	153	12	1	266	278	4	13	124	139	12
6	524	543	5	11	131	142	14	2	216	223	4	14	406	424	6
7	332	338	6	12	101	95	17	3	537	554	3	15	210	207	8
8	469	474	5		2, 16, 1			4	265	260	4	16	422	415	6
9	281	289	6	0	239	244	8	5	655	653	4	18	356	372	7
10	388	378	6	1	283	308	7	6	294	306	5	19	185	185	11
11	228	226	7	2	258	252	7	7	868	852	4	20	359	368	8
12	272	264	7	3	265	264	7	8	369	352	5		3, 4, 1		
13	177	181	10	4	265	264	7	9	506	511	4	0	874	896	4
14	226	201	8	5	220	215	8	10	563	562	5	1	382	377	3
16	152	144	12	6	227	232	8	11	397	379	5	2	1121	1135	6
	2, 13, 1			7	166	167	10	12	451	442	5	3	543	533	3
1	89	95	14	8	157	149	11	14	415	426	6	4	1085	1097	6
3	182	169	8	9	138	120	11	15	275	268	7	5	623	621	4
4	115	139	12	10	115	103	14	16	360	366	7	6	859	858	4
5	261	278	6	11	126	114	15	17	235	251	8	7	380	389	5
6	163	178	9	12	128	106	14	18	347	328	7	8	695	688	4
7	248	250	7		2, 17, 1			19	166	176	11	10	511	528	5
8	163	163	9	2	94	104	15	20	402	390	7	12	481	473	5
9	205	220	8	4	121	123	13		3, 2, 1			13	282	289	7
10	149	140	10	7	104	131	17	0	1227	1220	6	14	323	316	7
11	204	177	8		2, 18, 1			1	480	477	3	15	336	334	6
12	245	230	7	0	187	172	10	2	1316	1317	6	16	320	335	7
13	144	143	12	1	232	221	8	3	107	113	7	17	152	166	11
14	247	246	9	2	211	186	9	4	698	717	4	18	173	173	11
15	157	138	11	3	205	208	10	6	987	1008	5	19	129	136	15
	2, 14, 1			4	186	176	10	7	307	290	5		3, 5, 1		

Columns are 10Fo 10Fc 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	3, 5, 1			11	226	222	7	4	559	554	5	5	280	290	7
0	238	223	4	12	335	316	6	5	256	241	6	6	173	175	9
1	524	508	3	13	190	166	8	6	459	452	5	7	213	238	8
2	202	200	5	14	398	404	6	7	235	234	7	8	225	233	8
3	654	639	3	15	293	290	7	8	410	419	6	9	169	167	9
4	383	379	4	16	375	365	7	10	389	406	6	10	203	214	9
5	419	413	4	17	200	210	10	11	178	190	9	11	166	165	10
6	533	513	4	18	265	244	9	12	351	361	6	12	190	174	10
7	262	254	5		3, 8, 1			13	244	236	8	14	180	142	11
8	336	342	5	0	762	768	4	14	269	286	8		3, 14, 1		
9	201	178	7	1	220	218	6	15	203	199	10	0	366	362	6
10	340	318	5	2	844	850	4	16	165	153	12	1	307	305	6
11	243	247	7	3	214	223	6	17	157	156	13	2	345	349	6
12	436	429	5	4	826	818	4		3, 11, 1			3	290	290	7
14	498	503	6	5	287	289	5	0	120	120	10	4	368	363	6
15	228	239	8	6	654	653	4	1	151	139	9	5	257	270	7
16	431	416	6	7	380	372	5	2	175	180	8	6	282	273	7
17	214	220	9	8	491	489	5	3	190	201	8	7	220	211	8
18	276	266	9	9	110	107	12	4	193	195	7	8	228	210	8
19	160	171	12	10	499	496	5	5	358	360	6	9	184	176	9
	3, 6, 1			11	159	141	9	6	168	169	9	10	201	192	9
0	1022	1031	5	12	488	503	6	7	329	327	6	11	248	243	8
1	436	427	4	13	222	220	8	8	289	293	6	12	224	211	9
2	1031	1047	5	14	394	408	6	9	194	188	8	13	205	205	10
3	522	518	4	15	245	241	8	10	317	334	7		3, 15, 1		
4	859	878	4	16	265	256	8	11	216	204	8	0	117	111	13
5	433	433	4	17	182	173	11	12	250	256	8	1	203	211	9
6	807	816	4	18	174	179	12	13	222	214	8	2	108	99	13
7	434	445	4		3, 9, 1			14	169	167	11	3	121	115	12
8	718	710	4	1	257	260	6	15	196	207	11	7	115	133	13
9	223	234	7	2	246	268	6	16	167	160	11	8	151	134	11
10	582	581	5	3	393	385	5		3, 12, 1			10	129	145	14
11	196	216	8	4	312	309	5	0	479	492	5	11	135	108	13
12	476	478	5	5	387	374	5	1	289	288	6	12	138	127	14
13	160	160	10	6	328	323	5	2	382	380	5		3, 16, 1		
14	332	323	6	7	305	319	6	3	374	375	6	0	339	338	6
15	183	167	9	8	278	271	6	4	320	308	6	1	200	218	9
16	251	241	8	9	343	357	6	5	362	375	6	2	251	245	7
17	225	231	9	10	185	164	8	6	297	302	6	3	165	176	10
18	166	168	12	11	349	351	6	7	339	355	6	4	190	175	9
19	225	244	10	12	230	215	8	8	323	338	6	5	188	177	9
	3, 7, 1			13	206	200	9	9	235	208	7	6	227	221	9
1	344	356	4	14	271	266	8	10	311	313	7	7	176	164	10
2	316	325	5	15	227	229	9	11	196	183	9	8	235	250	9
3	404	403	4	16	275	260	8	12	251	237	8	9	114	140	17
4	347	341	5	17	178	183	11	13	180	183	10	10	188	172	11
5	440	457	4	18	247	242	10	14	187	182	10		3, 17, 1		
6	260	257	6		3, 10, 1			15	144	153	14	2	132	120	12
7	498	499	4	0	503	488	5		3, 13, 1			3	145	142	12
8	229	219	6	1	295	308	5	2	154	178	9	4	106	125	16
9	234	241	7	2	491	484	5	3	191	210	8	5	153	132	12
10	270	256	6	3	293	283	5	4	174	157	8		3, 18, 1		

Columns are 10Fo 10Fc 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	3, 18, 1			10	486	480	5	2	188	182	7	16	253	259	9
1	142	141	13	12	497	502	6	3	476	471	4	17	191	196	11
2	131	129	13	13	242	243	8	5	478	479	4		4, 8, 1		
3	149	118	12	14	384	374	7	6	125	119	10	0	703	720	4
	4, 0, 1			15	237	244	8	7	407	408	5	1	298	305	6
0	353	333	4	16	363	354	7	8	391	405	5	2	593	588	4
1	99	87	9	17	233	257	10	9	490	485	5	3	157	156	8
2	810	821	4	18	166	189	13	10	464	461	5	4	479	470	5
3	199	217	6	19	201	212	11	11	326	328	6	5	150	145	9
4	1041	1039	5		4, 3, 1			12	423	410	6	6	289	293	6
6	874	892	5	0	189	201	6	13	268	263	7	7	308	314	6
7	310	308	6	1	631	634	4	14	380	376	6	8	369	365	6
8	554	557	5	2	246	248	5	15	266	277	8	9	250	227	7
9	483	486	5	3	638	649	4	16	323	313	7	10	371	380	6
10	381	380	6	4	264	265	5	17	201	201	10	11	261	264	7
11	261	267	7	5	500	512	4	18	237	213	10	12	370	365	6
12	351	353	6	6	361	363	5		4, 6, 1			13	286	288	7
13	186	199	9	7	269	272	6	0	785	786	4	14	283	294	8
14	347	348	7	8	336	332	6	1	99	83	11	15	246	250	9
16	356	366	7	9	499	501	5	2	753	747	4	16	172	184	12
17	239	237	9	10	307	316	6	3	227	238	6	17	137	139	15
18	235	224	9	11	425	437	6	4	712	723	4		4, 9, 1		
19	278	273	9	12	323	331	7	5	278	273	6	0	141	132	9
	4, 1, 1			13	321	335	7	6	556	548	5	1	527	535	5
1	688	704	4	14	377	372	6	7	359	359	5	2	126	111	10
2	160	163	7	15	247	250	8	8	573	583	5	3	445	449	5
3	661	659	4	16	369	375	7	9	262	256	7	4	157	173	9
4	195	168	6	18	285	267	9	10	584	572	5	5	279	278	6
5	556	565	4		4, 4, 1			11	219	217	8	6	173	174	9
6	451	438	5	0	1024	1024	5	12	500	482	6	7	192	198	8
7	377	392	5	1	124	134	9	13	215	211	8	8	280	298	7
8	416	425	5	2	1007	1006	5	14	269	276	8	9	271	274	7
9	577	596	5	3	246	255	5	15	200	202	9	10	271	263	7
10	248	265	7	4	796	786	4	16	143	139	14	11	283	288	7
11	361	378	6	5	290	284	5	17	157	165	13	12	210	194	8
12	209	214	8	6	650	656	4		4, 7, 1			13	198	185	9
13	250	238	7	7	326	336	5	0	241	255	6	14	138	132	12
14	344	363	7	8	690	684	4	1	302	290	5	15	139	148	14
15	224	243	9	9	178	179	8	2	251	255	6	16	171	186	12
16	373	371	7	10	650	655	5	3	552	559	4		4, 10, 1		
17	166	156	11	11	128	135	12	4	255	246	6	0	612	637	5
18	262	253	9	12	539	525	5	5	595	598	5	1	364	370	6
	4, 2, 1			13	255	250	8	6	276	268	6	2	464	484	5
0	785	785	4	14	383	355	6	7	370	355	5	3	260	281	6
2	918	925	5	15	218	216	9	8	427	426	5	4	327	330	6
3	196	175	6	16	246	249	9	9	251	247	7	5	182	179	8
4	1068	1056	5	17	202	198	10	10	379	386	6	6	257	261	7
5	254	257	5	18	115	103	17	11	263	258	7	7	213	206	8
6	674	692	4	19	176	177	12	12	323	332	7	8	355	352	6
7	447	442	5		4, 5, 1			13	281	295	7	9	183	173	9
8	601	580	5	0	447	456	4	14	258	259	8	10	351	338	6
9	297	311	6	1	436	455	4	15	285	292	8	11	246	240	8

Columns are 10Fo 10Fc 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	4, 10, 1			3	232	222	8	6	159	147	10	4	465	469	5
12	251	264	8	4	274	254	7	7	179	164	9	5	300	319	6
13	225	218	9	5	222	231	8	8	252	269	7	6	425	421	5
14	197	215	11	6	215	219	9	9	458	453	6	7	210	211	7
15	211	189	9	7	207	222	9	10	299	309	7	8	507	505	5
16	189	166	11	8	201	189	10	11	363	351	6	9	360	356	6
	4, 11, 1			9	132	140	14	12	280	300	8	10	474	485	6
1	236	244	7	10	194	185	10	13	363	355	7	11	282	271	7
3	317	323	6	11	128	147	15	14	357	338	7	12	311	298	7
4	169	163	9		4, 15, 1			15	230	232	9	13	249	227	8
5	325	340	6	0	169	170	10	16	235	220	9	14	222	215	9
6	129	142	12	1	181	192	9	17	141	149	14	15	137	154	14
7	217	229	8	2	168	166	9		5, 2, 1			16	223	210	10
8	222	207	8	3	116	120	13	0	597	598	4	17	190	190	11
9	179	169	9	4	158	147	11	2	719	732	4		5, 5, 1		
10	235	232	8	5	130	124	13	3	186	183	7	0	155	154	8
11	195	200	9	6	102	117	17	4	714	698	4	1	371	371	5
12	189	190	10	7	171	186	11	5	258	254	6	2	241	246	6
13	143	162	13	8	121	116	15	6	573	568	5	3	393	392	5
	4, 12, 1			9	173	190	11	7	474	473	5	4	207	197	7
0	486	486	5		4, 16, 1			8	430	418	6	5	466	463	5
1	239	243	7	0	213	230	9	9	241	246	7	6	110	117	13
2	397	373	6	1	178	157	10	10	464	463	6	7	484	488	5
3	212	223	8	2	161	157	11	12	320	332	7	8	196	211	8
4	327	325	6	3	146	144	12	13	275	274	7	9	609	621	5
5	266	269	7	5	148	178	13	14	272	275	8	10	305	303	7
6	311	300	6	6	130	106	13	15	261	250	9	11	362	372	6
7	310	311	7	7	195	205	10	16	268	250	8	12	261	277	8
8	346	342	6		4, 17, 1			17	198	200	12	13	286	274	7
9	179	170	9	2	106	113	17		5, 3, 1			14	254	261	8
10	273	262	7		5, 0, 1			0	120	130	10	15	207	221	11
11	121	105	13	0	693	695	4	1	680	685	4	16	228	223	10
12	197	191	10	2	680	696	4	2	228	231	6	17	148	145	14
14	184	182	11	3	245	268	7	3	608	620	4		5, 6, 1		
	4, 13, 1			4	721	710	4	4	256	247	6	0	402	394	5
0	109	102	13	5	115	125	13	5	419	430	5	1	183	191	8
1	224	235	8	6	603	612	5	6	174	177	8	2	489	483	5
2	148	151	10	7	448	453	6	7	227	210	7	3	325	338	6
3	211	207	8	8	537	541	5	8	183	180	8	4	507	523	5
4	166	180	10	9	358	382	6	9	573	578	5	5	241	257	7
5	205	204	8	10	439	429	6	10	290	276	7	6	516	525	5
6	160	154	10	12	311	286	7	11	457	471	6	7	274	280	7
7	217	225	8	13	221	210	8	12	317	333	7	8	479	469	5
8	199	201	9	14	261	249	8	13	380	385	7	9	341	342	6
9	243	242	8	15	217	209	9	14	366	371	7	10	360	364	6
10	196	200	10	16	293	279	8	15	231	229	9	11	230	236	8
11	197	219	10	17	225	210	10	16	258	254	9	12	235	235	8
12	168	150	12		5, 1, 1			17	148	150	14	13	188	205	10
	4, 14, 1			1	863	878	4		5, 4, 1			14	195	189	10
0	314	305	7	2	106	98	12	0	740	747	4	15	150	152	13
1	208	202	8	3	776	773	4	2	622	629	4	16	180	187	12
2	264	261	7	5	545	532	5	3	130	129	10		5, 7, 1		

Columns are 10Fo 10Fc 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	5, 7, 1			1	284	297	7	9	151	172	13	5	207	215	8
0	120	130	11	2	402	393	6	10	156	136	12	6	443	444	6
1	336	322	6	3	319	334	6		5, 14, 1			7	230	240	8
3	460	466	5	4	301	296	7	0	180	197	11	8	386	384	6
4	194	201	8	5	264	272	7	1	136	117	12	9	228	221	8
5	437	446	5	6	295	291	7	2	201	195	9	10	348	343	7
6	233	243	7	7	226	211	8	4	192	181	10	11	229	218	9
7	308	326	6	8	338	341	7	5	153	140	12	12	229	218	9
8	263	256	7	9	191	186	9	6	157	154	12	13	278	277	9
9	398	409	6	10	296	292	7		5, 15, 1			14	207	183	10
10	221	218	8	11	174	178	11	0	108	95	14	15	225	226	11
11	283	300	8	12	203	206	10	3	116	127	16		6, 3, 1		
12	184	164	9	13	164	167	12	4	153	119	12	0	140	145	10
13	222	237	9	14	174	160	12	5	143	136	14	1	645	626	5
14	200	184	10		5, 11, 1			6	127	141	16	2	121	111	11
15	202	200	10	0	229	243	8		5, 16, 1			3	560	548	5
16	202	206	11	1	362	365	6	0	124	120	14	4	158	155	9
	5, 8, 1			2	193	191	9		6, 0, 1			5	401	394	6
0	384	380	5	3	363	386	6	0	404	416	5	6	126	127	12
1	242	254	7	4	176	164	9	2	431	425	5	7	406	398	6
2	444	440	5	5	318	316	7	4	460	440	6	8	162	148	11
3	252	244	6	6	153	153	10	6	494	499	6	9	507	511	6
4	452	449	5	7	250	247	8	7	289	283	7	10	181	174	10
5	145	149	11	8	165	169	11	8	429	436	6	11	378	372	7
6	425	419	6	9	229	228	9	9	272	275	8	12	193	204	10
7	181	184	9	10	179	177	11	10	397	384	7	13	233	235	9
8	400	406	6	11	191	180	10	11	192	184	10	14	240	250	10
9	225	235	8	12	181	169	11	12	201	194	10	15	230	237	10
10	334	343	7	13	164	168	13	13	230	242	10		6, 4, 1		
11	232	225	8		5, 12, 1			14	195	184	11	0	449	446	5
12	255	251	8	0	233	212	8	15	279	262	9	1	205	205	8
13	182	177	11	1	192	213	9		6, 1, 1			2	464	455	5
14	190	184	11	2	281	271	7	1	686	683	5	4	426	403	6
15	141	155	15	3	230	229	8	3	640	647	5	6	389	376	6
	5, 9, 1			4	311	321	7	4	105	107	14	7	191	186	9
0	335	343	6	5	221	217	8	5	452	456	6	8	290	276	7
1	572	588	5	6	283	274	7	6	157	136	10	9	290	287	7
2	267	270	7	7	188	180	9	7	358	351	6	10	286	271	7
3	369	376	6	8	207	172	9	8	250	259	8	11	267	235	8
4	186	171	8	9	124	147	14	9	477	478	6	12	206	191	10
5	234	235	7	10	134	128	14	10	274	282	8	13	215	212	10
6	135	127	11	11	165	153	11	11	413	406	7	14	222	198	10
7	284	282	7		5, 13, 1			12	215	203	9	15	216	202	11
8	185	171	9	0	153	126	10	13	290	307	9		6, 5, 1		
9	307	322	7	1	257	260	8	14	218	225	10	0	158	177	9
10	190	202	10	2	104	122	15	15	249	239	10	1	373	367	6
11	216	203	9	3	173	184	10		6, 2, 1			2	157	170	9
12	179	176	10	4	112	116	14	0	429	424	5	3	472	479	5
13	203	190	10	5	170	152	10	1	93	122	15	4	192	184	8
14	230	197	9	6	176	168	10	2	422	419	5	5	507	520	5
	5, 10, 1			7	193	188	10	3	148	168	10	6	138	133	11
0	460	470	6	8	180	176	11	4	442	453	5	7	490	483	6

Columns are 10Fo 10Fc 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	6, 5, 1				6, 9, 1				7, 0, 1			6	179	171	10
8	112	110	15	0	213	218	8	0	472	491	6	7	198	193	10
9	449	439	6	1	382	364	6	2	387	386	6	8	207	201	10
10	151	136	12	2	184	186	9	4	350	342	7	9	182	180	11
11	269	257	8	3	386	392	6	5	149	139	12	10	235	237	11
12	180	166	11	4	183	188	10	6	227	231	9	11	163	154	14
13	206	193	11	5	357	353	6	7	182	184	11		7, 5, 1		
14	204	191	10	6	159	162	10	8	208	208	10	0	229	253	8
	6, 6, 1			7	343	331	7	9	176	173	12	1	475	465	6
0	293	286	6	8	142	144	13	10	205	203	11	2	179	198	10
1	312	315	6	9	281	283	8	11	135	167	17	3	545	551	6
2	380	380	6	10	158	141	12	12	177	175	13	5	477	477	6
3	201	196	8	11	235	219	9		7, 1, 1			7	367	363	7
4	462	470	6	12	231	214	10	1	398	387	6	8	181	185	12
5	138	136	11		6, 10, 1			3	507	499	6	9	246	238	9
6	431	437	6	0	250	233	7	5	503	510	6	10	176	161	12
7	211	207	8	2	257	246	7	6	129	124	14	11	213	222	11
8	265	249	7	4	225	204	8	7	546	540	6		7, 6, 1		
9	255	258	8	5	140	164	12	8	203	182	9	0	184	195	10
10	142	138	12	6	205	201	10	9	443	430	7	2	205	194	9
11	168	173	12	7	165	169	11	10	145	134	14	3	146	172	12
12	171	144	11	8	179	190	11	11	351	361	9	4	228	220	8
13	210	209	10	9	193	200	11		7, 2, 1			5	199	193	9
14	226	220	11	10	188	194	11	0	415	419	6	6	210	214	9
	6, 7, 1			11	161	139	12	1	98	113	15	7	156	149	12
0	144	139	10		6, 11, 1			2	332	321	7	8	225	192	9
1	385	367	6	0	160	156	10	3	107	122	15	9	158	144	12
2	147	139	10	1	262	271	8	4	292	281	7	10	177	167	12
3	478	483	6	2	154	155	11	5	124	101	12		7, 7, 1		
4	185	186	9	3	300	305	7	6	222	204	8	0	204	196	9
5	441	438	6	4	162	150	12	8	203	213	10	1	447	437	6
6	209	223	9	5	284	268	8	9	187	186	11	2	187	171	9
7	387	394	6	6	153	149	12	10	249	259	10	3	459	449	7
8	181	177	9	7	250	241	9	11	224	228	10	4	134	127	13
9	364	373	7	9	236	223	9	12	228	215	10	5	379	380	7
11	268	263	8		6, 12, 1				7, 3, 1			7	341	345	8
12	144	131	14	0	144	104	12	0	155	153	10	8	210	213	10
13	190	176	11	2	173	171	11	1	461	466	6	9	273	274	9
	6, 8, 1			3	115	133	16	2	109	86	13		7, 8, 1		
0	229	209	8	4	212	216	9	3	490	493	6	0	262	256	8
1	165	196	10	5	139	126	13	5	479	465	6	2	221	216	9
2	306	312	7	6	238	225	9	7	472	482	6	3	169	170	11
3	230	242	8	7	104	88	17	8	132	145	14	4	190	192	10
4	377	367	6	8	199	177	11	9	374	364	7	5	172	165	11
5	192	208	9		6, 13, 1			11	251	269	10	6	183	198	11
6	336	339	7	1	224	221	9	12	162	172	15	7	115	121	17
7	159	148	10	3	207	211	10		7, 4, 1			8	239	221	9
8	239	246	8	4	161	151	8	0	291	287	7		7, 9, 1		
9	194	201	10	5	199	184	10	2	238	226	8	1	275	235	8
10	201	204	10		6, 14, 1			3	107	119	15	2	107	128	16
11	141	143	13	0	144	152	14	4	238	220	8	3	342	348	7
12	217	177	10	1	167	146	11	5	203	198	9	4	127	135	15

Columns are 10Fo 10Fc 10Sig, * for Insignificant

l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig	l	kFo	Fc	Sig
	7, 9, 1				8, 0, 1			0	271	280	8	4	188	172	11
5	336	349	8	0	313	332	8	2	214	216	10	5	132	146	15
6	147	139	13	2	238	235	9	4	158	148	12	6	147	165	14
7	325	304	8	4	146	141	14		8, 3, 1				8, 5, 1		
	7, 10, 1				8, 1, 1			1	430	428	7	1	472	462	7
0	205	210	10	1	456	438	7	3	406	403	7	3	359	376	8
2	226	223	9	3	426	437	7	4	176	172	12	4	155	137	12
3	124	124	15	4	124	113	15	5	409	407	7	5	307	302	9
4	190	203	11	5	425	432	8	6	169	167	13		8, 6, 1		
	7, 11, 1			6	133	136	15		8, 4, 1			0	106	121	17
1	206	197	10	7	410	416	8	0	173	168	11	2	127	110	15
2	118	116	16		8, 2, 1			2	149	148	13	3	131	151	16

Table . Final Atomic Coordinates ($x10^4$, $x10^5$ for In) and B_{iso} ($Å^2$) with e.s.d.'s in parantheses.

	x	y	z	B_{iso}	occ.
In	.02357(4)	.24234(2)	.24128(2)	2.32(1)	
N1	.2560 (6)	.2538 (3)	.3352 (2)	2.2 (2)	
C2	.4055 (10)	.3208 (4)	.3143 (4)	2.9 (3)	
C3	.3293 (9)	.3803 (3)	.2506 (4)	3.0 (3)	
N4	.2424 (9)	.3326 (3)	.1844 (3)	2.3 (2)	
C5	.3979 (10)	.2829 (4)	.1404 (3)	2.8 (3)	
C6	.3327 (10)	.1905 (4)	.1267 (3)	2.4 (3)	
N7	.2561 (8)	.1476 (3)	.1977 (3)	2.1 (2)	
C8	.4130 (8)	.1368 (3)	.2581 (4)	2.8 (3)	
C9	.3460 (10)	.1660 (4)	.3372 (3)	2.6 (3)	
C10	.1413 (8)	.2738 (4)	.4072 (3)	3.1 (3)	
C11	.2571 (10)	.2580 (6)	.4806 (3)	5.4 (4)	
C12	-.0663 (9)	.2297 (4)	.4061 (3)	3.3 (3)	
C13	.1216 (10)	.3898 (4)	.1330 (4)	3.1 (3)	
C14	.2232 (14)	.4733 (5)	.1094 (5)	5.2 (4)	
C15	-.0894 (12)	.4056 (5)	.1677 (4)	3.2 (3)	
C16	.1428 (10)	.0666 (4)	.1806 (3)	2.9 (3)	
C17	.2519 (13)	.0053 (5)	.1284 (5)	4.6 (4)	
C18	-.0712 (13)	.0859 (5)	.1550 (4)	3.5 (3)	
O1	-.1403 (6)	.2093 (3)	.3403 (2)	3.2 (2)	
O2	-.1518 (7)	.2189 (3)	.4671 (2)	5.1 (3)	
O3	-.1515 (6)	.3510 (3)	.2182 (2)	3.4 (2)	
O4	-.1889 (9)	.4658 (3)	.1449 (3)	4.6 (3)	
O5	-.1428 (7)	.1617 (3)	.1689 (2)	3.1 (2)	
O6	-.1628 (9)	.0279 (4)	.1246 (4)	5.4 (3)	
OW1	.6084 (21)	.0378 (9)	-.0069 (10)	8.7(1.0)	0.5
OW2	.6840 (31)	.4704 (46)	.0005 (48)	15.1(3.9)	0.4

B_{iso} is the mean of the principal axes of the thermal ellipsoid.

Table . Bond lengths (Å).

In-N(1)	2.258(4)	C(10)-C(11)	1.512(8)
In-N(4)	2.254(5)	C(10)-C(12)	1.547(8)
In-N(7)	2.270(5)	C(12)-O(1)	1.286(7)
In-O(1)	2.101(4)	C(12)-O(2)	1.215(7)
In-O(3)	2.094(4)	C(13)-C(14)	1.522(11)
In-O(5)	2.094(4)	C(13)-C(15)	1.552(10)
N(1)-C(2)	1.489(8)	C(15)-O(3)	1.288(9)
N(1)-C(9)	1.491(8)	C(15)-O(4)	1.214(9)
N(1)-C(10)	1.498(7)	C(16)-C(17)	1.503(11)
C(2)-C(3)	1.528(9)	C(16)-C(18)	1.526(11)
C(3)-N(4)	1.486(8)	C(18)-O(5)	1.296(10)
N(4)-C(5)	1.504(8)	C(18)-O(6)	1.211(9)
N(4)-C(13)	1.496(8)	O(4)...OW(2)(i)	2.65(8)
C(5)-C(6)	1.520(9)	O(4)...OW(2)(ii)	2.84(6)
C(6)-N(7)	1.491(8)	O(6)...OW(1)(i)	2.752(17)
N(7)-C(8)	1.492(8)	O(6)...OW(1)(iii)	2.749(17)
N(7)-C(16)	1.499(8)	OW(1)-OW(1)(iv)	1.20(3)
C(8)-C(9)	1.514(10)	OW(2)-OW(2)(v)	0.92(14)

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Symmetry codes:

(i)	x-1, y, z	(iv)	x, -y, -z
(ii)	x-1, -y+1, -z	(v)	x, -y+1, -z
(iii)	x-1, -y, -z		

Table . Bond angles (°).

N(1)-In-N(4)	79.7(2)	C(5)-C(6)-N(7)	113.0(5)
N(1)-In-N(7)	79.7(2)	In-N(7)-C(6)	102.7(3)
N(1)-In-O(1)	77.7(1)	In-N(7)-C(8)	108.6(4)
N(1)-In-O(3)	117.3(2)	In-N(7)-C(16)	105.4(4)
N(1)-In-O(5)	147.7(2)	C(6)-N(7)-C(8)	113.0(5)
N(4)-In-N(7)	79.3(2)	C(6)-N(7)-C(16)	112.7(5)
N(4)-In-O(1)	148.0(2)	C(8)-N(7)-C(16)	113.5(4)
N(4)-In-O(3)	77.0(2)	N(7)-C(8)-C(9)	113.4(5)
N(4)-In-O(5)	117.0(2)	N(1)-C(9)-C(8)	111.9(5)
N(7)-In-O(1)	118.1(2)	N(1)-C(10)-C(11)	114.1(5)
N(7)-In-O(3)	147.3(2)	N(1)-C(10)-C(12)	110.9(4)
N(7)-In-O(5)	77.0(2)	C(11)-C(10)-C(12)	113.3(5)
O(1)-In-O(3)	93.6(2)	C(10)-C(12)-O(1)	117.7(5)
O(1)-In-O(5)	93.9(2)	C(10)-C(12)-O(2)	118.2(5)
O(3)-In-O(5)	94.1(2)	O(1)-C(12)-O(2)	124.1(6)
In-N(1)-C(2)	109.9(3)	N(4)-C(13)-C(14)	115.4(6)
In-N(1)-C(9)	102.8(3)	N(4)-C(13)-C(15)	110.6(5)
In-N(1)-C(10)	105.5(3)	C(14)-C(13)-C(15)	112.0(6)
C(2)-N(1)-C(9)	112.1(4)	C(13)-C(15)-O(3)	116.9(6)
C(2)-N(1)-C(10)	113.6(5)	C(13)-C(15)-O(4)	119.4(7)
C(9)-N(1)-C(10)	112.2(4)	O(3)-C(15)-O(4)	123.6(7)
N(1)-C(2)-C(3)	112.1(5)	N(7)-C(16)-C(17)	114.1(6)
C(2)-C(3)-N(4)	112.8(4)	N(7)-C(16)-C(18)	111.4(5)
In-N(4)-C(3)	103.0(4)	C(17)-C(16)-C(18)	113.8(6)
In-N(4)-C(5)	110.5(3)	C(16)-C(18)-O(5)	118.0(6)
In-N(4)-C(13)	106.4(4)	C(16)-C(18)-O(6)	117.0(7)
C(3)-N(4)-C(5)	112.3(5)	O(5)-C(18)-O(6)	125.0(8)
C(3)-N(4)-C(13)	112.0(4)	In-O(1)-C(12)	117.8(4)
C(5)-N(4)-C(13)	112.0(5)	In-O(3)-C(15)	118.8(4)
N(4)-C(5)-C(6)	111.6(5)	In-O(5)-C(18)	117.5(5)

Table . Bond angles (°).

N(1)-In-N(4)	79.72(18)	C(5)-C(6)-N(7)	113.0(5)
N(1)-In-N(7)	79.74(18)	In-N(7)-C(6)	102.7(3)
N(1)-In-O(1)	77.70(15)	In-N(7)-C(8)	108.6(4)
N(1)-In-O(3)	117.31(18)	In-N(7)-C(16)	105.4(4)
N(1)-In-O(5)	147.70(18)	C(6)-N(7)-C(8)	113.0(5)
N(4)-In-N(7)	79.31(18)	C(6)-N(7)-C(16)	112.7(5)
N(4)-In-O(1)	148.03(17)	C(8)-N(7)-C(16)	113.5(4)
N(4)-In-O(3)	77.03(18)	N(7)-C(8)-C(9)	113.4(5)
N(4)-In-O(5)	116.98(18)	N(1)-C(9)-C(8)	111.9(5)
N(7)-In-O(1)	118.05(17)	N(1)-C(10)-C(11)	114.1(5)
N(7)-In-O(3)	147.31(17)	N(1)-C(10)-C(12)	110.9(4)
N(7)-In-O(5)	76.95(19)	C(11)-C(10)-C(12)	113.3(5)
O(1)-In-O(3)	93.64(17)	C(10)-C(12)-O(1)	117.7(5)
O(1)-In-O(5)	93.90(17)	C(10)-C(12)-O(2)	118.2(5)
O(3)-In-O(5)	94.11(18)	O(1)-C(12)-O(2)	124.1(6)
In-N(1)-C(2)	109.9(3)	N(4)-C(13)-C(14)	115.4(6)
In-N(1)-C(9)	102.8(3)	N(4)-C(13)-C(15)	110.6(5)
In-N(1)-C(10)	105.5(3)	C(14)-C(13)-C(15)	112.0(6)
C(2)-N(1)-C(9)	112.1(4)	C(13)-C(15)-O(3)	116.9(6)
C(2)-N(1)-C(10)	113.6(5)	C(13)-C(15)-O(4)	119.4(7)
C(9)-N(1)-C(10)	112.2(4)	O(3)-C(15)-O(4)	123.6(7)
N(1)-C(2)-C(3)	112.1(5)	N(7)-C(16)-C(17)	114.1(6)
C(2)-C(3)-N(4)	112.8(4)	N(7)-C(16)-C(18)	111.4(5)
In-N(4)-C(3)	103.0(4)	C(17)-C(16)-C(18)	113.8(6)
In-N(4)-C(5)	110.5(3)	C(16)-C(18)-O(5)	118.0(6)
In-N(4)-C(13)	106.4(4)	C(16)-C(18)-O(6)	117.0(7)
C(3)-N(4)-C(5)	112.3(5)	O(5)-C(18)-O(6)	125.0(8)
C(3)-N(4)-C(13)	112.0(4)	In-O(1)-C(12)	117.8(4)
C(5)-N(4)-C(13)	112.0(5)	In-O(3)-C(15)	118.8(4)
N(4)-C(5)-C(6)	111.6(5)	In-O(5)-C(18)	117.5(5)