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SYNTHESES OF NOVEL ACYCLIC AMINO-AMIDO LIGANDS

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by

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ABSTRACT

Towards the labelling of biological macromolecules in contrast media, a synthesis of the novel bifunctional amido-ligands N,N′-bis[2-(N′′,N′′-dimethylamino)ethyl]-4-aminobenzylmalondiamide (**67**) and the 3-aminopropyl derivative (**66**) from appropriately C-functionalized malonates by amidation with N,N-dimethylethylenediamine (**62**) followed by reduction of the respective nitro (**64**) and cyano (**63**) groups is described.

The synthesis of N,N′-bis[2-(N′′,N′′-dimethylamino)ethyl]iminodiacetamide (**73**) from diethyl N-benzyliminodiacetate (**79**) by amidation with (**62**) followed by debenzylation is described. Herein is also reported the unsuccessful attempts to prepare a functionalized pentaamine ligand similar to (**73**) *via* the intermediacy of N,N′-bis[2-(N′′,N′′-dimethylamino)ethyl]-N′′′-(2,2-diethoxy-ethyl)iminodiacetamide (**112**) whose preparation is also detailed. Attempts to this end *via* the Mitsunobu and Steglich coupling of N,N′-bis[2-(N′′,N′′-dimethylamino)ethyl]-N′′′-(2-hydroxyethyl)iminodiacetamide (**100**) with N-*tert*-butyloxycarbonylglycine (**105**) also met with failure. Further failed attempts to secure suitably functionalized intermediates by N-alkylation of diethyl iminodiacetate (**70**) with appropriate electrophiles are described. The successful functionalization of the pentaamine series of ligands by N-alkylation of (**73**) with *p*-nitrobenzoyl chloride (**118**) to give N,N′-bis[2-(N′′,N′′-dimethylamino)ethyl] N′′′-(4-nitrobenzamido)iminodiacetamide (**119**) is presented. The preparation of the non-functionalized novel trioxo heptaamine ligand N,N′,N′′-tris[2-(N′′,N′′-dimethylamino)ethyl]nitrilotriacetamide hydrochloride (**86a**) is also described.

An investigative study towards the assembly of a novel triamine system for encapsulating NMR or isotopic NMR-active metal ions for possible use in diagnostic medicine is reported. The key facet to this end is the reported preparation of N,N′,N′′-tris(2-aminoethyl)propane-1,2,3-tricarboxamide **(89)** by controlled amidation of trimethyl propane 1,2,3-tricarboxylate **(88)** with ethylenediamine.

The syntheses of functionalized and non-functionalized novel tetraamine dioxo and trioxo ligands from glycine, ethyl N-benzylglycinate **(78)**, L-valine, and L-lysine *via* classical peptide synthesis methodology (in part) are described.

ABBREVIATIONS*

APT	attached proton test
Bn	benzyl,$C_6H_5CH_2$-
Boc	*tert*-butyloxycarbonyl,$(CH_3)_3COCO$-
DNA	deoxyribonucleic acid
DOTA	1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid
DTPA	diethylenetriaminepentaacetic acid
EDTA	ethylenediaminetetraacetic acid
MRI	Magnetic Resonance Imaging
NMR	Nuclear Magnetic Resonance
Ph-	benzyl,C_6H_5-
Su	Succinimide
TETA	1,4,8,11-tetraazacyclotetradecane-N,N′,N′′,N′′′-tetraacetic acid

tert	tertiary
δ	chemical shift (ppm)
ν	stretching frequency (cm^{-1})
τ_M	residence time of primary coordination sphere
μ	ionic strength

*Abbreviations not listed above are defined in the text.

CONTENTS

CHAPTER 1

1. MAGNETIC RESONANCE IMAGING (MRI)

1.1 Introduction

Magnetic resonance imaging (MRI) is an investigatory technique used to image soft tissue without interference of bone[1]. A simplistic form of MRI involves the application of a linear magnetic field gradient in order to "spatially encode" nuclei in the subject with different resonant frequencies. The free induction decay (FID) signal following a radio frequency pulse is Fourier transformed to yield a one-dimensional projection of signal amplitude along a particular line through the subject. With the aid of algorithms used in X-ray computer tomography (CT) and other imaging techniques, a series of such projections can be reconstructed into two-dimensional images of NMR intensity.

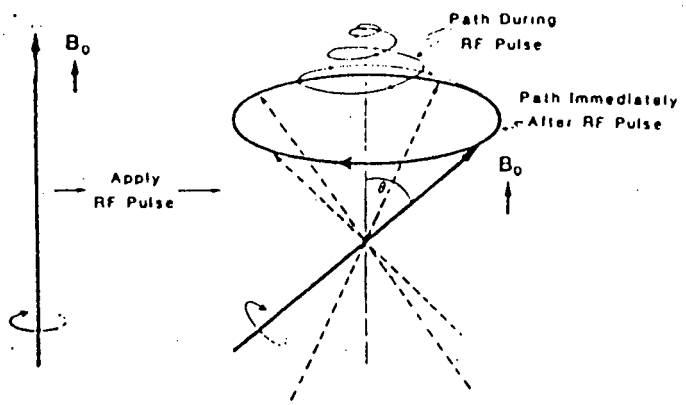


Fig.1 Upon application of a radiofrequency (RF) pulse, the net magnetic moment is perturbed from its equilibrium position, and because of its properties of spin, begins to precess about the static field direction. The angle between the z axis and the magnetization vector continues to increase as long as the pulse remains on. When it is turned off, the vector precesses freely at the final angle, θ and its rotation describes the wall of the cone. The component of magnetization which rotates in the x,y plane (dark area) generates the nuclear signal.

The dependence of ^1H image intensity on tissue relaxation times (which is the basis of image enhancement using paramagnetic agents) is inherent in the basic principles of NMR. Briefly, the net macroscopic magnetization of proton spins, which are aligned parallel with the applied field along the z axis, if perturbed by application of one or more radio frequency pulses. The component of the magnetization along the z axis

"relaxes" back to its equilibrium value with an exponential time constant T_1, the longitudinal (or spin-lattice) relaxation time. (Fig.1).

The time dependence of the magnetization perpendicular to the z axis is characterized similarly by T_2, the transverse (or spin-spin) relaxation time, which measures the time of decay of transverse magnetization to its equilibrium value of zero. In image data acquisition, the pulses are rapidly repeated for each projection.

Depending on the particular *RF* pulse sequence used, the image intensity may be a function of T_1 or T_2 or both, as well as spin density, ρ (the number of spinning nuclei per unit volume). There are numerous factors which determine T_1 and T_2, but a selection of some may be of interest in imaging biological systems. T_1 and T_2 are most sensitive to the degree of molecular motion. In solids and at low temperatures, there is minimal molecular motion and T_1 may be many seconds while T_2 is only microseconds. However, in liquids and at higher temperatures, T_1 and T_2 are almost equal, being about 2 seconds for pure water. (In fact T_2 can never be longer than T_1 and is often substantially shorter). Therefore if the T_1 and T_2 ratio approaches 1, the sample may be assumed to be relatively "liquid-like", and if the ratio is very small, the sample is relatively "solid-like". In NMR imaging, only the signal from "liquid-like" regions is observed; rigidly bound nuclei give essentially zero signal. Thus in proton images, the ^1H nuclei in compact bone are "NMR silent" and usually appear as black on an NMR image.

Variations in T_1 proton relaxation time among different tissues are often related to free water content. Tissues with short T_1 values generally yield greater image intensity than those with longer values since the steady state magnetization along the z axis is greater in the tissue with the fastest relaxation. On the other hand, short T_2 values are always associated with lower signal intensity since this diminishes the net transverse magnetization available for detection.

The greater functional decrease in T_1 dominates the relaxation effects and generates signal enhancement. Employment of "contrast agents" markedly modifies relaxation behaviour by enhancing the signal intensity of the tissue bearing the agent.

1.2 Contrast Agents

Advances in MRI as a clinical diagnostic modality has prompted the need of a new class of pharmaceuticals. These drugs would be administered to patients in order to (1) indicate the status of organ function or blood flow and/or (2) enhance the image contrast between normal and malignant tissue. Since the image intensity in ^1H NMR imaging, largely composed of the NMR signal of water protons, is dependent on nuclear relaxation times, complexes of paramagnetic transition and lanthanide metal ions, which can decrease relaxation times of nearby nuclei *via* dipolar interactions, have received most attention as potential contrast agents. Contrast agents are unique diagnostically in that they are not directly visualized on the NMR image but are detected indirectly by virtue of changes in proton relaxation behaviour.

1.2.1 Historical Background

Bloch first described the use of a paramagnetic salt, ferric nitrate, to enhance the relaxation rates of water protons[2]. The pioneering work of Lautebur in the field of NMR imaging[3] in 1973 was extended to human imaging in 1977[3]. Lautebur, Mendoca and Rudin were the first to show feasibility of paramagnetic tissue discrimination on the basis of differential water proton relaxation times[4]. In their experiments a salt of Mn(II), a cation known to localize in normal myocardial tissue in preference to infarcted regions, was injected to dogs with an occluded coronary artery. The longitudinal proton relaxation rates ($1/T_1$) of tissue samples correlated with Mn(II) concentration and thus normal myocardium could be distinguished from infarcted zone by relaxation behaviour alone. Ferric chloride has been orally administered to humans, by Young *et al.*[5], to enhance the gastrointestinal tract. The diagnostic potential of contrast agents was first demonstrated in patients by Carr *et al.*[6] Gd(III)

diethylenetriamminepentaacetate ($[Gd(DTPA)(H_2O)]^{2-}$) was administered intravenously to patients with cerebral tumours, thus providing enhancement of the lesions in the region of cerebral capillary breakdowns. This is the only agent presently undergoing clinical trials. Recently,[7,8,9] functionalized DTPA and EDTA protein conjugates have been employed to specifically target certain pathological conditions.

1.3 General Requirements for Metal Complexes as NMR Contrast Agents

NMR imaging contrast agents must exhibit biocompatibility as pharmaceuticals in addition to nuclear relaxation probes. Aside from standard pharmaceutical features such as water solubility and shelf stability, the requirements relevant for metal complex-based agents can be classified into three general categories; a review of the literature pertinent to each category follows this section.

1.3.1 Relaxivity

The efficiency with which the complex enhances the proton relaxation rates of water, refered to as relaxivity, must be sufficient to significantly increase the relaxation rates of target tissue. The dosage of the complex at which such alteration of tissue relaxation rates occurs must be non-toxic.

1.3.1.1 Basic Theory of Relaxivity

Relaxation of magnetic resonance signals generally results from the presence of local fluctuating magnetic fields. In pure water, the most important mechanism for production of such a field is the dipole-dipole interaction between neighbouring water protons. Each proton has a magnetic moment, which produces a small magnetic field at a neighbouring proton. As a result of Brownian motion of the water molecules, the magnitude of the field experienced by neighbouring protons fluctuates randomly, producing relaxation. The relaxation times T_1 and T_2 depend not only on the

magnitude of the local fields but also on the time scale of the fluctuations, normally expressed as correlation times. Paramagnetic ions eg. Gd^{3+} and Mn^{2+}, have magnetic dipole moments of the order of 1 000 times that of those protons. Such magnetic moments can produce correspondingly large local fields and can therefore enhance the relaxation rates of water molecules in the vicinity of the ions. The closest protons are those of the water molecules proximal to the ions, and of the water molecules coordinated to the paramagnetic ion in aqueous solution. However, measured relaxation times are those of the bulk water rather than the small fraction bound to the paramagnetic agent. The bulk water is relaxed by the paramagnetic ion because the bound water continually exchanges with free water, distributing the effect of the paramagnetic ion throughout the water. Thus paramagnetism is an underlying property required of metal ions to significantly effect relaxation rates of target tissue.

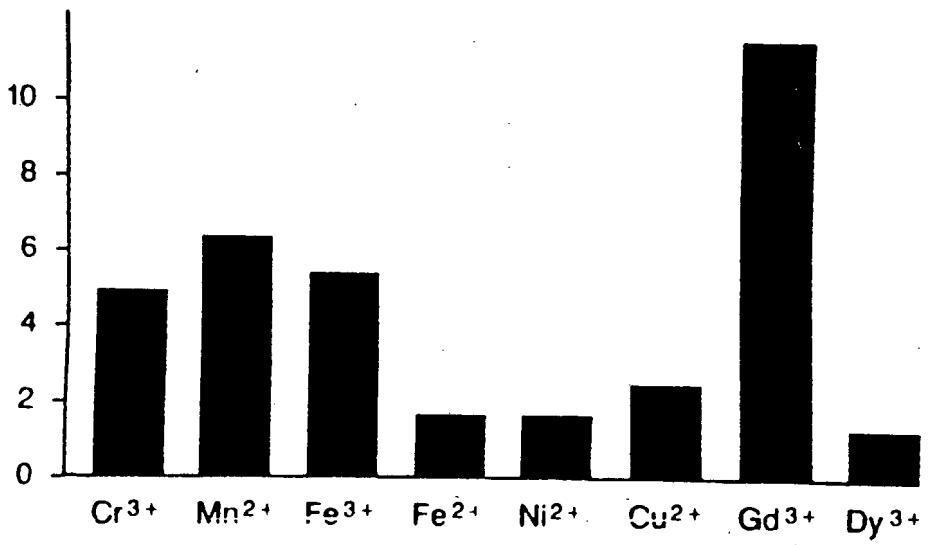


Fig.2 T_1 relaxivity ($mmol^{-1}s^{-1}$) of paramagnetic elements at 20MHz.

Fig.2 shows the relaxivity of various transition and lanthanide series metal ions. From this bar graph, Gd^{3+} and Mn^{2+} emerge as having relatively high relaxivity properties and are expected to enhance the relaxation rates of target tissues.

1.3.1.2 Some of the Properties Contributing to Relaxivity

1.3.1.2.1 Coordination Number, q.

Since contrast agents are not visualized directly in magnetic resonance imaging (MRI), but are detected indirectly *via* their effect on relaxation, the number of coordinated water molecules is important. Gadolinium(III) has between 8-9 coordination sites and its administration would increase the relaxation rate and hence the signal intensity of the tissue concerned. This feature has been observed when gadolinium has been administered[10] as a chloride salt . However, the high toxicity (LD_{50}) of the salt (Fig.3) prevents its use as a contrast agent.

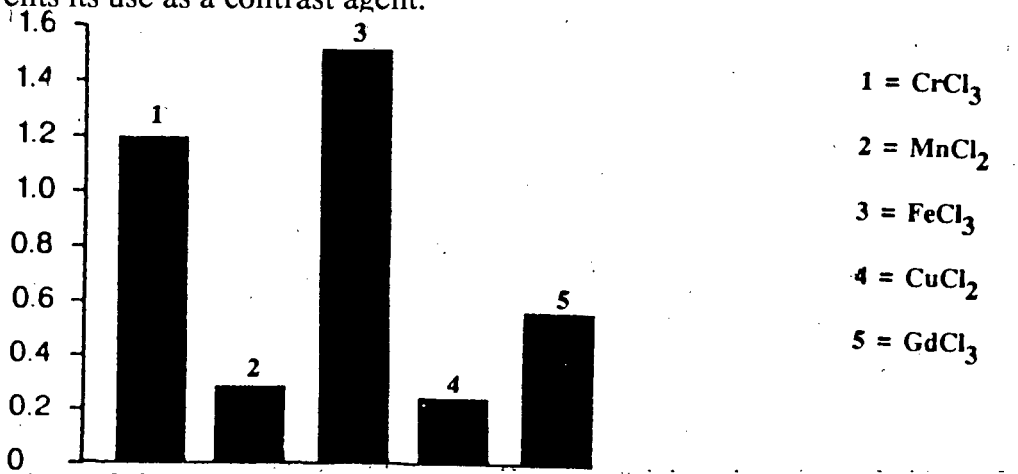


Fig.3 Accute lethal toxicity (LD_{50}) after intravenous administration of metal chlorides in rats (90-110gm). Injection rate was 2mL/min, and the pH of each solution was adjusted to 5 to 7 by NaOH.

In the case of ligated metal ions, the ligand must be designed in such a way as to facilitate maximum exposure of the metal ion centre to the tissue water molecules for an efficient mechanism of relaxivity. There is an associated increase in relaxivity with greater number of coordinated water molecules.

1.3.1.2.2 Rotational Correlation Time, τ_R.

For metal ions with long electron spin relaxation times (T_{1e}), (eg Gd^{3+}, Mn^{2+} and Fe^{3+}) nuclear relaxation is controlled by the rotational correlation time(τ_R). Rotational immobilization of metal complexes in viscous solvents containing

paramagnetic metal ions has been observed to lead to increased relaxation rates[11,12]. Three basic strategies exist to reduce the rotational mobility of metal ion complexes *in vivo*: (1) Distribution of the agent into a tissue or tissue compartment with high microviscosity. (2) Covalent attachment of the complex to a larger molecule such as a protein or antibody prior to injection. (3) Noncovalent binding of the complex in tissue to macromolecules. The first of these ideas have far reaching consequences regarding understanding the relaxivity of metal chelates in tissue. Debye-Stokes predicts that for a spherical molecule of radius a, τ_R, the rotational correlation time, is directly proportional to the viscosity of the medium, η, and the third power of the radius as given by equation (1), where k is the Boltzman constant and T is the absolute temperature. Thus the relaxivity of a complex should be directly proportional to η until τ_R approaches T_{1e} and/or τ_M, the life time of the complex..

$$\tau_R = 4\pi a^3\eta/3kT \qquad \text{..... (1)}$$

1.3.1.2.3 Protein-bound Metal Ions and Chelates

There has been prolific research in the technique of attaching metals ions and chelates to macromolecules. Eisenger, Shulman and Blumberg demonstrated that binding a paramagnetic ion to a macromolecule, in their case, DNA, enhances the water proton relaxation efficiency by lengthening of the rotational correlation time[13]. This phenomenon , which came to be known as proton relaxation enhancement (PRE), has been extended to study hydration and structure of metaloenzymes[14,15,16]. The

effect of linking metal ion chelates on the relaxivity is shown in Table 1.

TABLE 1. Selected Longitudinal Relaxivities (R_1) for Protein-metal Ion Complexes and for Bovine Serum Albumin (BSA) Covalently Labelled with Metal Chelates.

COMPLEX	$R^a_1, M^{-1}s^{-1}$	freq MHz	temp °C	ref
	Gd(III)			
glutamine synthetase	148	22.5	25	b
immunoglobulin	112	20	19	c
BSA	72	24.3	30	17
$(BSA)(GdEDTA)^c_n$	36	20	37	18
EDTA (free)	6.6	20	35	19
$(BSA)(GdDTPA)_n$	19	20	37	18
DTPA (free)	4.1	20	35	19

[a]R_1/metal ion. [b]Eads,C.D., Mulqueen, P., Horrocks, W.D., and Villafranca, J. J. *Biochemistry* **1985**, *24*,1221. [c]Burton, D.R., Forsen, S, Karlstrom, G., *et al. Eur. J. Biochem.*,**1976**, *71*, 519. [d]Reuben, J. *Biochemistry* , **1971**, *15*, 2834. [e]n = 3-10 (average number of chelates/protein molecule).

1.3.2 Specific in Vivo Distribution

Ideally for the complex to be of diagnostic value, it must localize for a period of time in a target tissue or tissue compartment in preference to non-target regions. This forms the basic tenet in any agent-based imaging procedure where detection is a function of its tissue concentration. For MRI, this requirement needs to be qualified: the relaxation rates of the target tissue should be enhanced in preference to other tissues.

However, true targeting is rarely achieved. After administration, the agent equilibrates in several body compartments prior to excretion; preferential distribution of the agent to the desired site is all that can be expected in most circumstances.

The currently investigated contrast agent , [Gd(DTPA)(H_2O)], have appeared to be effective in enhancing relaxivity. They have been used to provide diagnostic information such as the integrity of the blood-brain barrier[20,21,22,23], information which was virtually unattainable without contrast agents. Despite all these apparently favourable features, most of the effective contrast agents suffer the disadvantage of being general in the sense that the localization mechanisms, which include breakdown in the blood-brain barrier[14,16,24] and increased extracellular volume[23], are non-specific.

Recently, paramagnetic metal ions and their chelates have been linked to monoclonal antibodies specific to particular tumour lines. This technique has provided a more direct targeting route. A preliminary account describing the use of Mn-DTPA labelled antimyosin antibody for the detection of infarcted myocardial tissue has appeared[25] as well as employment of synthetic paramagnetic metalloporphyrins to decrease proton relaxation time of tumours.[26,27]

Though the technique of attaching paramagnetic metal ions to monoclonal antibodies was initially greeted with enthusiasm, this method is likely to have far reaching consequences only in radioimaging, where only miniscule concentrations of the label are needed. Relatively high concentrations of paramagnetic agent are required for NMR imaging (roughly 10-100μM), whereas the concentration of antigenic sites in tumours is 0.1μM or less). Despite saturation of such sites with paramagnetically labelled antibody molecules, such conjugates would require 100-1000 chelates per molecule for significant relaxation time differences. Coupled with the obvious deterents associated with toxicity and lower antigenic affinity of these conjugates, this approach, though attractive, is not without problems. Work by Brechbiel *et al.*[7] has reported a mild method for circumventing the problems associated with the affinity of chelates to bind certain available sites on the antibody backbone without loss of immunoactivity.

1.3.3 Stability and Toxicity

The acute and chronic toxicity of an intravenously administered metal complex is related in part to its *in vivo* stability and its tissue clearance behaviour. The transition and lanthanide ions are relatively toxic at doses required for NMR relaxation rate changes (roughly 0.5-5g per patient) and greatly exceeds that of metal ions or complexes used in radioscintigraphy. However, iodine-containing contrast agents are used in computer tomography (CT) and other radiological procedures at much higher doses than NMR agents (*ca* 50-200g per patient). With the development of relatively non-toxic chelates, the contrast-enhanced NMR examination is likely to be safer than similar CT procedures.

Stability and toxicity are therefore treated together to emphasize the historical importance of metal complex stability in determining toxicity in evaluation of NMR agents. The dissociation of a complex generally leads to a higher degree of toxicity stemming from the free metal ion or free chelating ligand. Though the chemist may contribute in the development of safe chelates by synthesis of more stable derivatives and elucidation of dissociation mechanisms under biological conditions, testing and mechanistic understanding of metal complex toxicity requires the expertise of toxicologists and pharmacologists.

1.3.3.1 In Vivo Stability of Metal Complexes

The stability of a metal complex has been treated as a kinetic requirement, not a thermodynamic requirement.[28] The agent must be sufficiently stable to effect the desired contrast. The stability of the metal complex is only required for the duration of the examination, and should be excreted minutes or hours after administration.

Of the metal ions and their chelates studied as contrast agents, the most studied are the trivalent metal ions Gd(III), Mn(III), and Fe(III) because of their long T_{1e} and large magnetic moments (Fig.2). However the long T_{1e}'s and high

relaxivity are unfortunately detrimental to complex stability. The lack of ligand field stabilization energy in complexes of these ions leads, generally, to very labile metal-ligand bonds. Kinetic stability must therefore derive from the structure of an appropriate multidentate ligand. The dissociation kinetics are nevertheless related to the thermodynamics of complexation via the expression of equation 2, where k_a and k_d are the association constant and dissociation constants

$$k_d = k_a/K_{ML} \quad \text{..... (2)}$$

and K_{ML} is the thermodynamic association constant. Thermodynamic considerations are important in identifying the source of instability.

Though a complex may encounter a number of tissue compartments *in vivo*, which may differ with respect to dissociation factors, serum stability is most often evaluated, as has been the practise in radiopharmaceutical applications of metal complexes. A variety of coordinating ligands and proteins as well as metal ions can compete for either the paramagnetic ion or its multidentate ligand, providing a rigorous test for metal complex stability.

Martell discussed in detail the expected stability of Fe(III) complexes in serum from the point of view of designing sequestering ligands for the treatment of iron overload conditions.[29] Moerlein and Welch presented a similar analysis of gallium (III) and indium (III) complexes as radiopharmaceu ticals.[30]

An important thermodynamic sink for trivalent metal ions in serum is their precipitation with commonly occurring anions like hydroxide, phosphate, or carbonate. Table II lists the available solubility product constants (K_{sp}) relevant for contrast agent design. Also shown are calculated values of free metal ion concentration in the presence of both the precipitate and appropriate concentrations of the anion in serum.

TABLE 2. Relevant Solubility Product Constants (K_{sp}) and Calculated Free Concentrations of Gd(III), Fe(III), and Mn(II) under Serum Conditions of Concentration.

Compound	$\log K_{sp}$[a] (25 °C, μ = 0)	free metal ion concn, M[b]
$GdPO_4$	-22.26[c]	4×10^{-5}
$Gd_2(CO_3)_3$	-32.2	5×10^{-10}
$Gd(OH)_3$	-25.6	2×10^{-6}
$FePO_4$	-26.4	3×10^{-19}
$Fe(OH)_3$	-41.5	2×10^{-22}
$MnCO_3$	-9.3	2×10^{-5}
$Mn(OH)_2$	-12.8	2.5

[a]Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum: New York, 1974; Vol. 4. [b]Calculated from K_{sp}'s and protonation constants of anions: pH = 7.4,$[HCO_3^-]$ = 27 mM,$[HPO_4^{2-} + H_2PO_4^-]$ = 2 mM. [c]μ = 0.5.

Both phosphate and carbonate appear to be important for the precipitation of Gd(III), whereas for Fe(III) the formation of hydroxy complexes is favoured. Precipitation of Mn(II) does not appear to pose a problem. The calculation of whether a complex is thermodynamically stable to precipitation of the ion in serum can be approached from the opposite viewpoint, i.e., whether a ligand can solubilize the ion from the precipitate. Martell[29] defined the solubilization constant K_{sol} as the degree of conversion of the free ligand to the metal chelate where T_L is the total concentration of the ligand (equation 3). Low values of K_{sol} reveal an inability of a ligand to solubilize the ion; alternatively it would predict that the complex would be unstable with respect to metal ion precipitation.

$$K_{sol} = [ML]/T_L \quad \text{..... (3)}$$

Very high values of K_{sol} would occur for a thermodynamically stable complex where no precipitate is present. Lauffer[28] mentions the calculation of K_{sol} for complexes of single multidentate ligands (equation 3) and also gives association constants and stability constants in serum with respect to precipitation (K_{sol}) (Table 2) as well as their interpretation. As previously mentioned, $[Gd(DTPA)(H_2O)_n]^{2-}$ is presently undergoing clinical trials. The high stability constant of this complex (log K_{ML} 22.46, Martell *et al.Critical Stability Constants*; Plenum: New York, 1974; Vol. 4) and a correspondingly low solubilization constant (K_{sol} -0.4) are indicative of stability and a tendency to retain Gd(III) metal ion. The higher denticity of DTPA is most likely to also impart kinetic stability. The properties inherent upon any ligand for choice as a metal ion chelator must therefore bear salient features which will secure kinetic and thermodynamic stability. Other contrast agents employing Gd(III) ion as the metal centre, and incorporating ligands with a macrocyclic structure have appeared.[29-31] Ligands like tetraazacyclododecanetetraacetic acid (DOTA) bear this macrocyclic feature. The stability constant of this complex has been estimated from europium (III) by Lincoln *et al.*[32] and another constant by Caheris *et al.*[33] has also been reported, and were in each case found to be higher than $[Gd(DTPA)(H_2O)_n]^{2-}$. $[Gd(DOTA)(H_2O)]^-$ has also been observed to be stable *in vivo*, exhibiting similar biodistribution to $[Gd(DTPA)(H_2O)]^{2-}$.[31,33-36] The low dissociation kinetics of $[Gd(DOTA)(H_2O)]^-$ has been explained in terms of conformational stability and macrocyclic structure. The half life of $[Gd(DOTA)(H_2O)]^-$ is estimated[28] to be over 2000 years at pH 6, however no allowance is made for the presence of labilizing ligands.

Gansow *et al.*[37] perfomed serum stability studies for DOTA, DTPA, and substituted DTPA complexes of Gd(III). The radioactive complexes were incubated at 37°C under a 95% air/5% CO_2 atmosphere to maintain bicarbonate concentration. Loss of Gd(III) from these complexes resulted in radioactive precipitates. Over an observation period of 125h $[Gd(DOTA)(H_2O)]^-$ lost 5% or less radioactivity, whereas $[Gd(DTPA)(H_2O)]^{2-}$ lost 10-20%. Effects of introducing substituents at some defined point on the ligand, e.g. a substituent on the ethylene moeity of EDTA, have been

reported by Meares *et al.*.[38]. They observed a decrease in the dissociation rate of ^{111}In(III) from EDTA. The investigators attributed the apparent increased stability to steric effects, which decrease rates of rearrangement and dissociation. In contrast, substitution at one ethylene of DTPA was not accompanied by an enhancement in stability of the derivatized DTPA compared to the parent ligand. Possibly substitution in DTPA requires to be at both ethylene groups to be effective in the augmentation of kinetic stability.

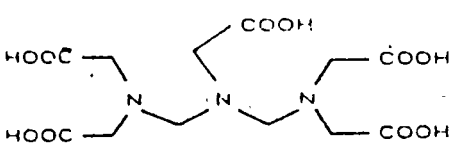
1.3.3.2. Toxicity

Stability of metal complexes and toxicity are interrelated. Toxicity effects arise from: (i) free metal ion, released by partial dissociation, (ii) free ligand, also by dissociation, and (iii) the intact complex. The former two cases pertain more to stability of the metal ion complexes. Another source of toxicity, when considering chelates attached to monoclonal antibodies or other biologically active macromolecules, is the macromolecule itself. Dissociation or metabolic degradation, possesses potential toxicity problems. Also such chelates have a concentration requirement for intravenous administration in the nanomolar region for immunoscintigraphy.[39] This technique poses no serious toxicity problems. Available biological data has pointed to the importance of metal ion dissociation as an important source of toxicity. Both metal ions and free ligands tend to be more toxic than the metal ion complexes. For the former case, this is evident from the LD_{50}'s (interpolated dose at which 50% of animals would die) shown in Fig.3.

1.4 Concluding Remarks.

The introduction given in this chapter serves the purpose of acquainting the reader with some of the theoretical aspects of MRI. In essence, this chapter provides the motivation for the synthesis of the ligands reported in this thesis.

The nature of ligands prepared in this project realized the basic tenents as outlined in section 1.3 (and subsequent subsections). One of the more important features of these ligands is the amide groups which upon coordination lose a proton and may give rise to neutral complexes. These neutral complexes should have a very different biodistribution to the charged $[Gd(DTPA)]^{2-}$ complex and may even pass through the blood brain barrier. In addition, the design of the ligands reported make provision for those relaxation properties in the final metal-ligand system thereby providing a pronounced effect on relaxivity. To exemplify this point, a contrast between the octadentate DTPA (1) ligand and the tetradentate system (84) [this system is selected arbitrarily; most (but not all) of the ligands reported herein have this basic structure] is made.



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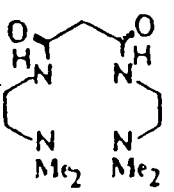


(83)

The planarity of system (84) upon metal-ion coordination allows tissue water access to the metal ion. Such a situation contributes significantly to the mechanism of tissue relaxivity, and subsequently image enhancement of the area being investigated. For the octadentate system (1), only a restricted amount of interaction of tissue water molecules with the metal-ion centre in possible.

Another special feature of the functionalized ligands prepared in this project, is the possibility of attaching them to biological macromolecules. This serves the purpose of (1) attaining specific *in vivo* targeting of certain pathological conditions when attached to monoclonal antibodies specific for a certain tumour line, and (2) reduction of rotational mobility of the metal-ion complex. The latter, for a situation where a paramagnetic metal-ion is employed, results in the enhancement of tissue water protons

via lengthening of the rotational correlation time. These two properties provided the motivation for functionalizing our ligands for attachment to proteins.

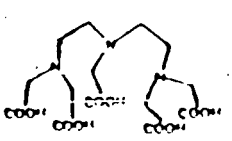
CHAPTER 2

2. REVIEW OF SYNTHESES OF LIGANDS FOR PROTEIN LABELLING.

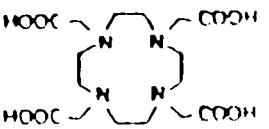
2.1 Introduction

The labelling of biologically important molecules by means of bifunctional chelating agents has become routine since its introduction by Sundberg.[40] The success of this technique relies upon a bifunctional chelating agent which comprises both a powerful metal ion chelating group(s) and a functional group that covalently binds to protein or other biological important molecules without adversely altering their properties. Such ligands have been used as vehicles for carrying radioactive metal ion for attachment to specific monoclonal antibodies in cancer diagnosis.[42a,b]

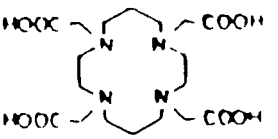
Currently the ligands that are being extensively used for such purposes, at most, incorporate a macrocyclic structure. Ligands such as DTPA (1), DOTA (2), and TETA (3) have been observed to bind metal ions with an overall stability to the complex (ligand + metal ion).[28] However the disadvantage of these ligands is that they are general and exhibit no specificity physiologically.



(1)



(2)

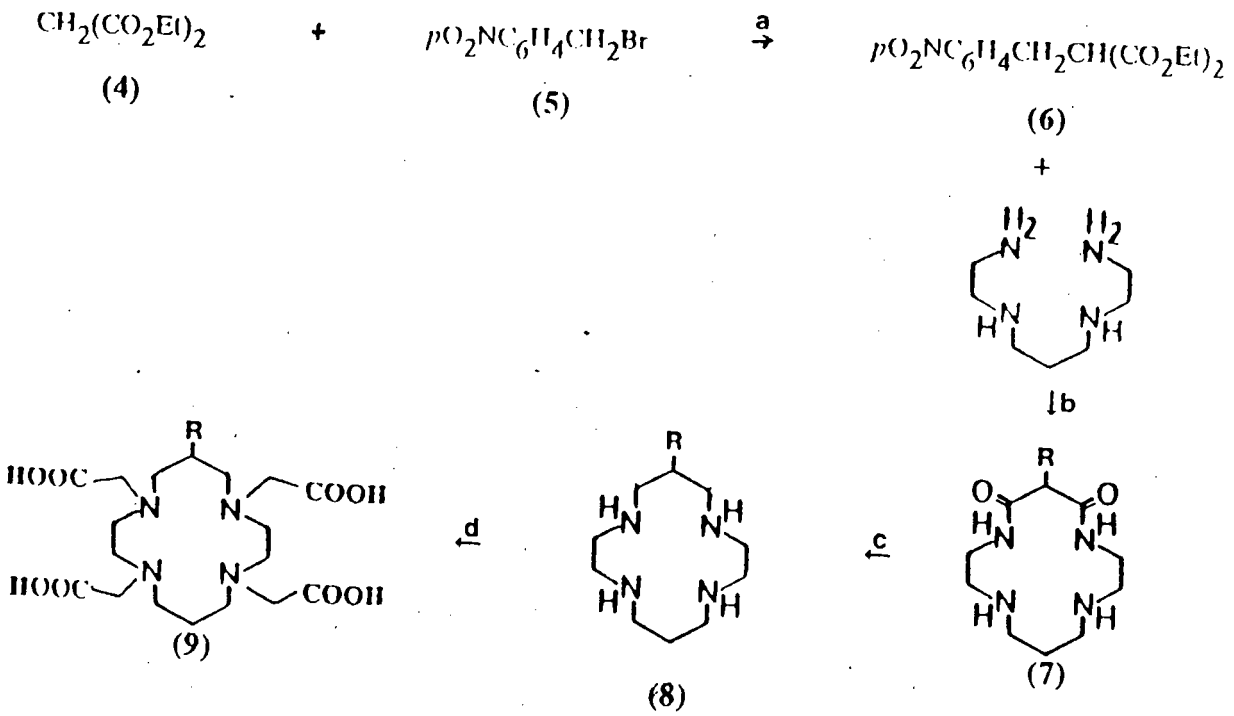


(3)

Fig.4 Non-functionalized octadentate ligands which have been used in contrast media, coordinating gadolinium(III).

2.2 Literature methods for the preparation of functionalized ligands.

Recently functionalized ligands possessing a macrocyclic structure have appeared.[41,42a,b] Meares *et al.*[41] reported a six step synthesis of a functionalized TETA ligand (Scheme 1).

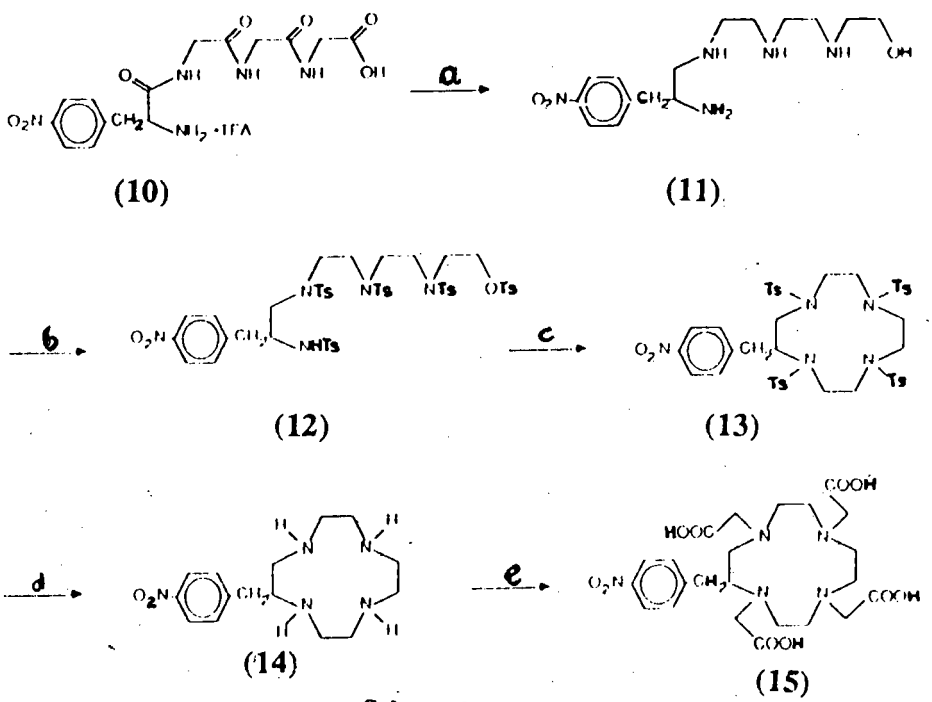


Scheme 1.

The synthesis involved (a) C-alkylation of diethyl malonate (**4**) with *p*-nitrobenzylbromide (**5**) under the standard Michael conditions to generate diethyl *p*-nitrobenzylmalonate (**6**) in a yield of 60%. Subsequent, (b) amidation with the appropriate polyamine 1,3-(2'-aminoethylamino)-propane by a method analogous to that of Tabushi *et al.*[43] gave the cyclic dioxodiamide (**7**) in 16% yield. Subsequent (c) reduction with diborane furnished the cyclic tetraamine (**8**) in a yield of 80%. The next stage involved (d) N-alkylation of the secondary amino groups with bromoacetic acid to

give the tetraacid (**9**) in 13% yield. Recently, this type of alkylation has been employed to prepare similar systems in high yields (77-80%) by the use of caesium carbonate in refluxing ethanol.[42b] Reduction of the *para*-disposed nitro group was achieved with 10% palladised carbon under normal hydrogenation conditions to give the *p*-aminobenzyl-TETA in 90% yield. The ultimate functionalization was either *via* bromoacetylation of the amino group or treatment of the amino group with thiophosgene to generate the isothiocyanate group.

Meares *et al.*[44] recently reported a peptide-directed synthesis of TETA macrocyclic ligand bearing a C-substituted functional group for antibody attachment. Macrocyclic polyamines, the key to macrocyclic bifunctional chelating agents, are synthesized by bimolecular cyclization.[43,45] Competition between polymerization and the desired cyclization is a common problem. Efforts by Meares *et al.*[45b,46] gave unsatisfactory yields. Realizing the difficulties associated with the bimolecular reactions, Meares *et al.*[44] embarked on a peptide-directed synthesis involving an *intra*molecular tosylamide ring closure. For polyazamacrocycles with nitrogens separated by two-carbon chains (e.g. TETA), peptides made from α-amino acids are readily accessible starting materials.[47] Scheme 2 outlines the synthesis of the functionalized TETA macrocycle by the peptide method.



(10) (11) (12) (13) (14) (15)

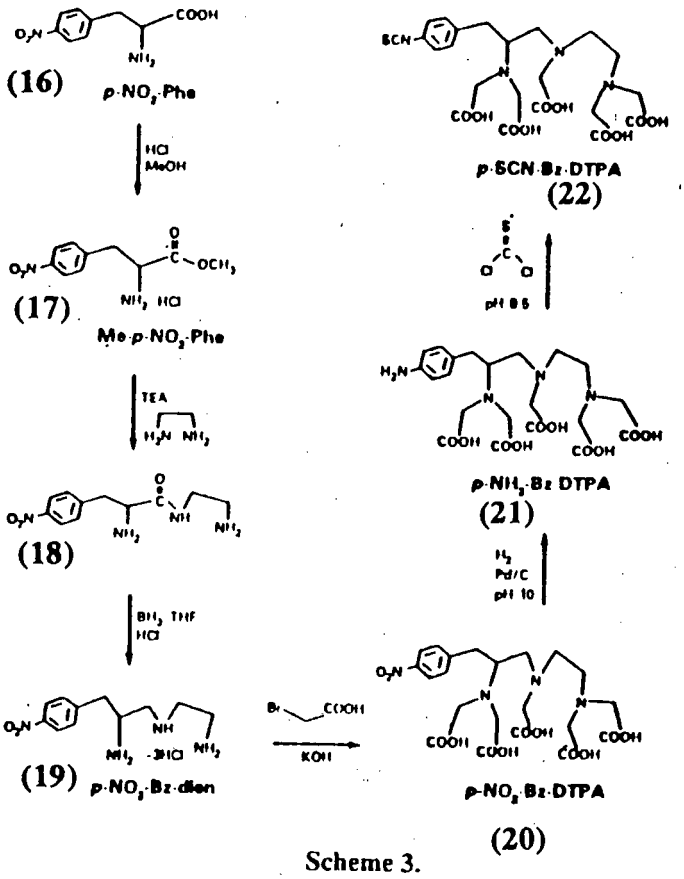
Scheme 2.

Treatment of (**10**) with borane converts peptides to the linear polyamino alcohol (**11**), in which the original peptide backbone has been converted to a C-terminal alcohol, an N-terminal primary amine, and internal secondary amines (Scheme 2, step a).[48] Treatment of (**11**) with *p*-toluenesulphonyl chloride produces a C-terminal tosyl ester, an N-terminal primary amine, an internal secondary amine, and internal tosylamide (**12**) (Scheme 2, step b). Treatment of (**12**) with mild base converts the N-terminal tosylamide to a nucleophile, which displaces the C-terminal tosyl ester and thus forms a macrocyclic ring (**13**) in high yield (79%) (Scheme 2, step c). This *intra*molecular cyclization may be perfomed in very dilute solution, eliminating concern about polymer formation. Subsequent steps involved detosylation of the macrocycle (**13**) which was achieved in 91% yield after chromatography. Treatment of the resulting cyclic tetraamine (**14**) with 5 equivalents of bromoacetic acid at 70°C and at pH 10 for 3h gave the functionalized TETA macrocyle (**15**) in a yield of 58% after chromatography (Scheme 2, step e). The remaining steps involve, as before, reduction of the nitro group of (**15**) and conversion of the resulting amino group into the isothiocyanate group.

The peptide method provides versatility since one can vary side chains on the ring conveniently by selecting appropriate amino acids as building blocks. Loss of enantiomeric purity was found to be unlikely under the reaction conditions involved.[45c] Amino acids such as glycine, β-alanine, and γ-aminobutyric acid are potential sources of 2-,3-, and 4-carbon chains between the nitrogens in the macrocycle.

Another ligand that has been extensively used in the magnetic resonance imaging and diagnostic nuclear medicine is DTPA. The appeal of this ligand is due to the low dissociation rate of the metal ion at physiological pH when chelated by the DTPA (**1**) ligand. This desirable feature has seen its use in nuclear medicine for specific

diagnosis of tumorogenic cells.[7] The requirement for specificity for this ligand has been realized, amongst other workers by Brechbiel and co-workers[7] who reported a six step synthesis (Scheme 3) of a functionalized DTPA ligand.

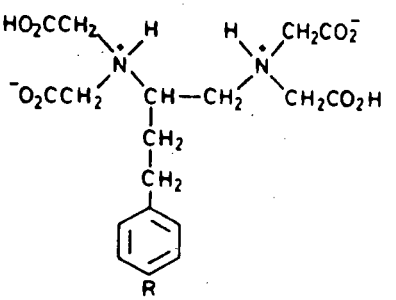


Scheme 3.

Starting from *p*-nitrophenylalanine (**16**), acid catalyzed esterification gave the methyl ester (**17**)[49] in a yield of 88% as a hydrochloride salt. Treatment of the methyl ester (**17**) with an excess of ethylenediamine furnished N-(2-aminoethyl)-*p*-nitrophenylalanine amide (**18**) in 88% yield. The resulting amide was reduced with diborane to afford the triamine trihydrochloride (**19**) in 82% yield. The foregoing triamine (**19**) was reacted with an excess of bromoacetic acid under basic conditions (7*M* KOH), to give, after four purification protocols, 1-(*p*-nitrobenzyl) diethylenetriaminepentaacetic acid (**20**) in 35% yield. Reduction of the nitro group of the pentaacid (**20**) with 10% Pd/C gave the amino pentaacid (**21**) in a yield of 98%.

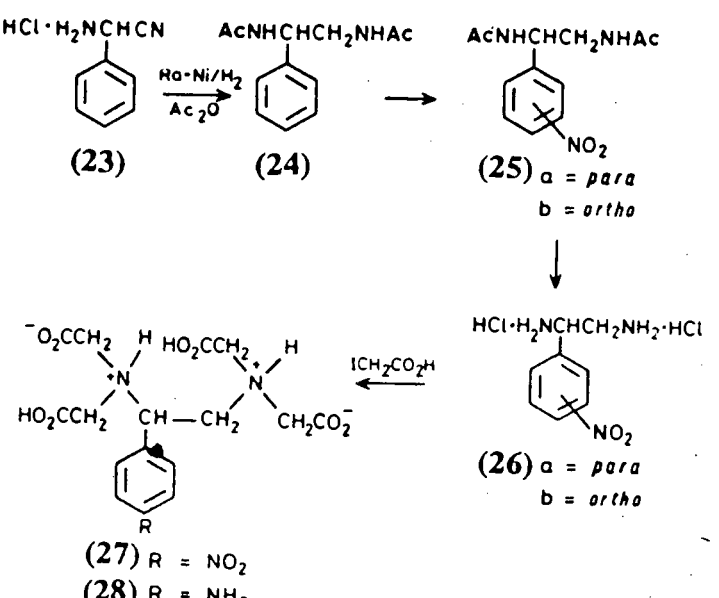
Subsequent treatment of the latter with thiophosgene afforded the isothiocyanate derivative (**22**). The last step therefore sets the stage for protein attachment after metal ion coordination.

Warshawsky *et al.*[8] also reported an improved method for the functionalization of EDTA for attachment to biological macromolecules. Meares *et al.*[40] have prepared 1-(*p*-aminophenylethyl)ethylenediaminetetraacetic acid (**38**) which was



(38) R = NH_2

bound by diazotization to human serum albumin and bovine fibrinogen. The problem of coupling *via* diazotization, as demonstrated by Yeh *et al.*[50], was found to be insufficiently effective in rendering coupling to protein.[51-54] This procedure required the protection of the EDTA function. Meares' route (Scheme 4) was problematic at the nitration (*ortho*:*para*-nitration ratio was 3:2) and alkylation steps.



(23) **(24)** **(25)** a = *para*, b = *ortho*

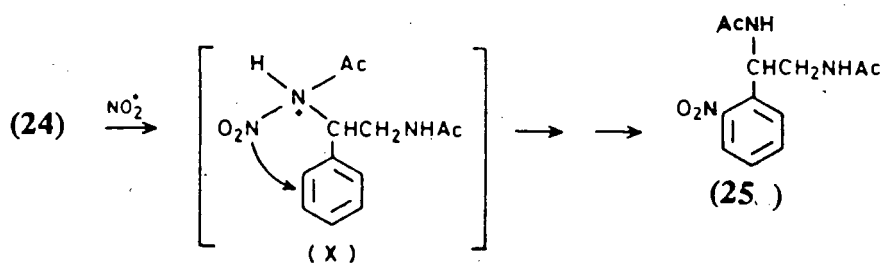
(26) a = *para*, b = *ortho*

(27) R = NO_2

(28) R = NH_2

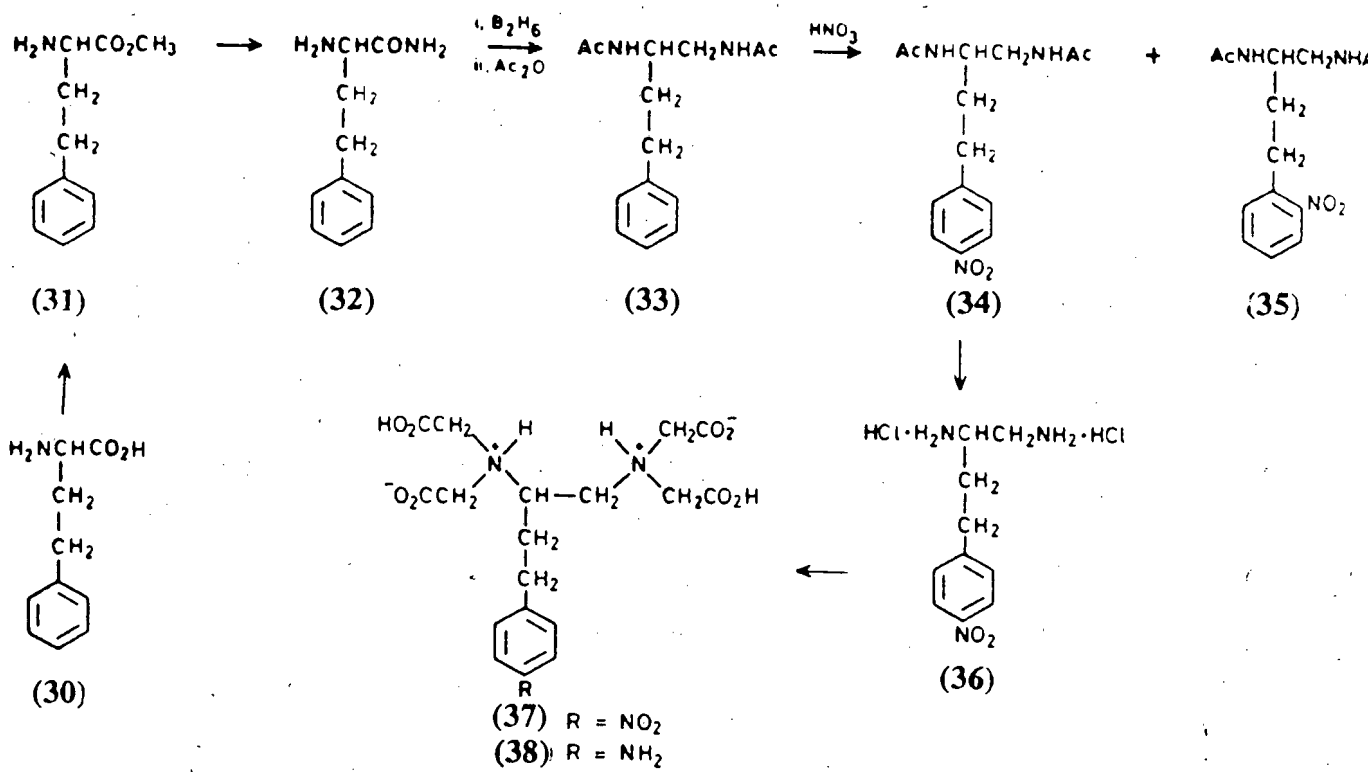
Scheme 4.

Warshawsky *et al.*[8] investigated the temperature parameter in an attempt to suppress *ortho*-nitration. It was found that the α-acetamido-moeity of (24) (Scheme 4) was probably involved in the formation of the *ortho*-isomer through an unstable *N*-nitro-amide intermediate (X) (Scheme 5).



Scheme 5.

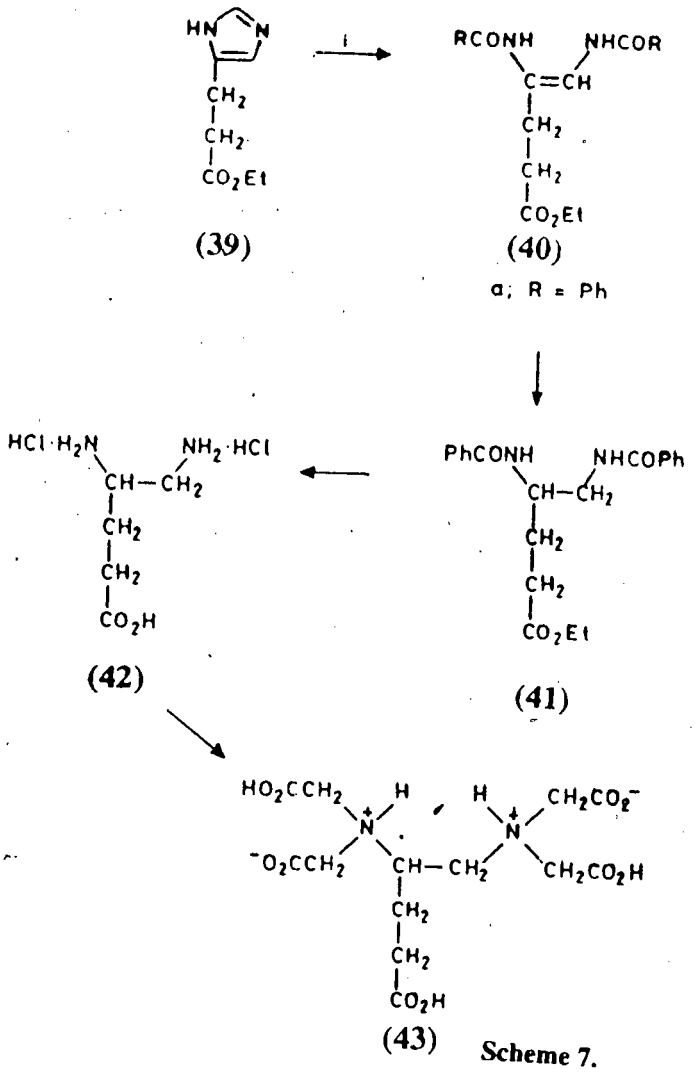
Thus, separation of the acetamido-group by a chain of two or more carbon atoms from the aromatic part of the molecule will diminish the formation of the *ortho*-isomer. This undertaking therefore served as the basis for the modification of the method by Meares.[40] The starting material selected for this purpose was 2-amino-4-phenylbutyric acid (30). Esterification of (30), and subsequent treatment of the pertinent ester (31) with ammonia gave the amide (32) which was reduced with borane to the corresponding amine which was acetylated to give the diacetyldiamide (33) in 75% yield (Scheme 6).



Scheme 6.

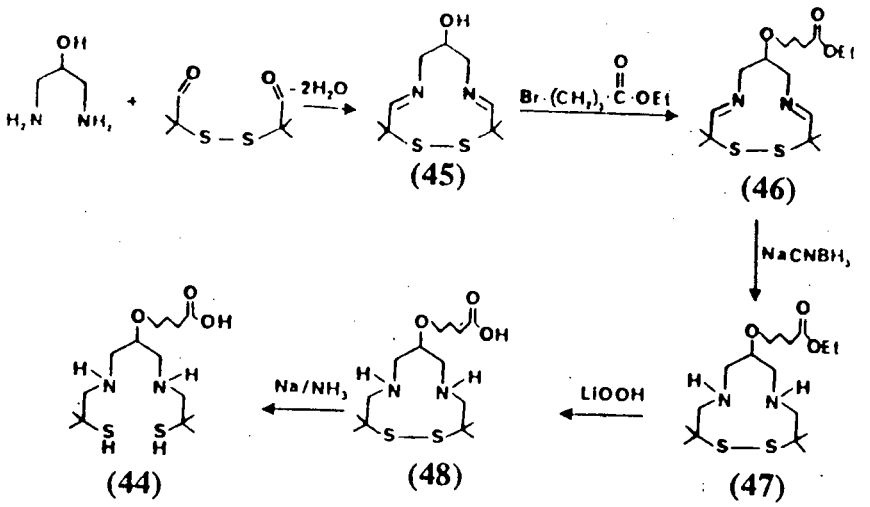
Nitration of the diacetyldiamide (**33**) occurred predominantly at the *para*-position to give (**34**). The alkylation of (**36**) [resulting from the acid hydrolysis of the diacetyldiamide (**33**)] with bromoacetic acid led smoothly to the ethylenediaminetetraacetic acid (**37**) in 71% yield. The nitro compound (**37**) was reduced to the amine (**38**), the bifunctional chelating agent.

Altman *et al.*[9] reported an alternative method of coupling chelates to biological macromolecules. This method incorporated the ethoxycarbonyl as the "spacer-arm" for coupling. Their synthetic strategy was based on the Bamberger[55] ring cleavage dibenzoylation of a suitably substituted imidazole, under the Schotten-Bauman reaction conditions (Scheme 7).



Scheme 7.

The ethyl 3-imidazol-4(5)-yl propanoate (**39**) was treated with benzoyl chloride in ethyl acetate-aqueous sodium hydrogencarbonate to give the unsaturated product (**40a**) in 82% yield. Catalytic reduction of compound (**40**) gave the saturated product (**41**) in 95% yield which was subsequently hydrolyzed to the dihydrochloride (**42**) in 89% yield. The final step involving the introduction of the EDTA function, was achieved by alkylation with bromoacetic acid under basic conditions, giving compound (**43**). On investigating the literature, it appears that acyclic bifunctional ligands are relatively less in use compared with their macrocyclic counterparts. Nonetheless, acyclic ligands have appeared[56,57] and Hnatowich *et al.*[57] recently reported a five step synthesis of an acyclic bifunctional diaminodithiol ligand (**44**) (Scheme 8).

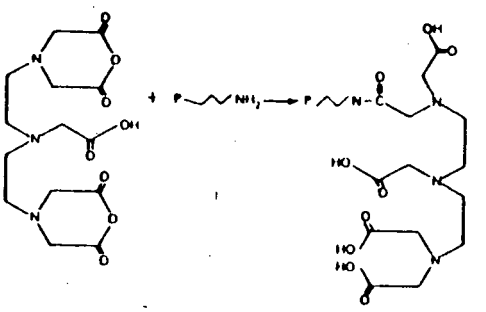


Scheme 8.

The ligand backbone was constructed as described by Billing *et al.*[58,59] through diimine formation by condensation of 1,3-diamino-2-propanol and 2,2'-dithio-bis(2-methylpropanal)[60] in anhydrous benzene to afford the diimine (**45**) in 87% yield. The functionalization was achieved by alkylation of the hydroxyl group according to the method of Jones *et al.*[61] by reacting equimolar amounts of the diimine (**45**) and ethyl 4-bromobutyrate in anhydrous DMF under nitrogen in the presence of potasium carbonate at 65°C for 60 hours, to give the ester (**46**) in 82% yield. Selective reduction of the diimine in (**46**) with sodium cyanoborohydride in acetic acid at 15°C left the disulphide bond intact to give the diamine (**47**) in 89% yield. Hydrolysis of the diamide (**47**) with lithium hydroperoxide (generated *in situ* by reacting lithium hydroxide and hydrogen peroxide at 5°C) as described by Evans[62] gave the acid (**48**) in a yield of 88%. The reduction of the disulphide bond of the acid (**48**) was achieved in a solution of ammonia in the presence of sodium metal at -70°C to give the ligand (**44**) in 62% yield. There was no mention of the mode of coupling to protein. This probably involves formation of an active ester which would react with an amino group on the protein backbone.

2.3 Modes of coupling ligands to protein.

Other modes of covalently attaching liganded metal ion to proteins have been accomplished by acylation with activated carbonyls, aromatic diazonium coupling and bromoacetyl alkylation. These coupling methods have been found to be inefficient.[51-54] The mixed anhydride method has been employed, for instance, in attaching DTPA conjugates to protein e.g. DTPA-isobutyloxycarboxycarbonic anhydride,[63] and DTPA cyclic anhydride (CA-DTPA).[64] The disadvantage of these techniques lies in their susceptibility to hydrolysis *in vivo*. Also the anhydride form of DTPA (**49**) was observed to lose the DTPA structure upon attachment to protein.[54] This phenomenon was confirmed by the observation that indium-DTPA dianhydride system upon coupling to protein, lost indium *in vivo* faster than DTTA which resulted from the loss of DTPA structure (Scheme 9).

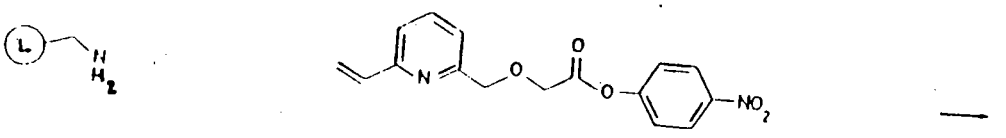


Scheme 9: DTPA-dianhydride (**49**) ---> DTTA (**50**)

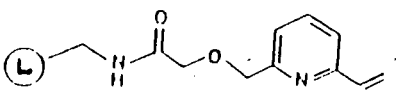
Brechbiel *et al.*,[7] in the light of the inefficiency of other modes of coupling, selected the isothiocyanate group as the mode of choice. This route realized the expediency of the method in terms of easy access of the isothiocyanate group from the amino group by treatment with thiophosgene. This mode of coupling to protein has been used in the

attachment of indium-DTPA conjugates to monoclonal antibodies without loss of the DTPA structure.[7]

An alternative mode of coupling ligands to monoclonal antibodies has recently appeared in the literature.[42a] This method involves the reaction of the amino group on a 'spacer arm' on a particular ligand, with a bifunctional linker molecule (**51**) according to the reaction depicted in Scheme 10.



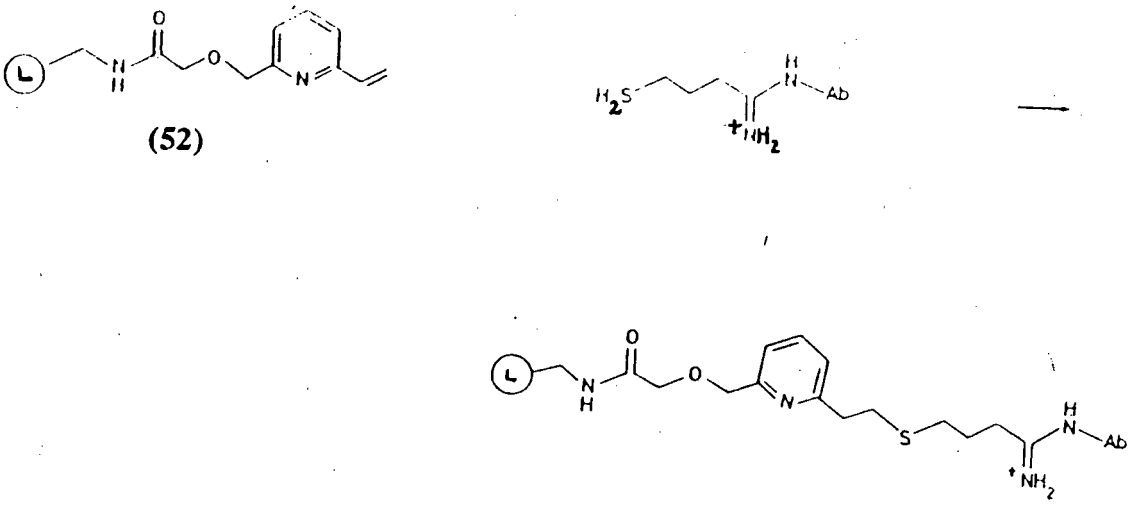
(**51**)



(**52**)

Scheme 10.

The stage prior to coupling to protein involves the modification of the relevant protein or monoclonal antibody by reaction with 2-iminothiolane which will generate thiol groups (typically 3 to 5 per antibody by titration with Ellman's reagent[65]). The resulting derivatized ligand (**52**) will react selectively[42a,b] with the thiol groups on the chemically modified antibody according to Scheme 11.

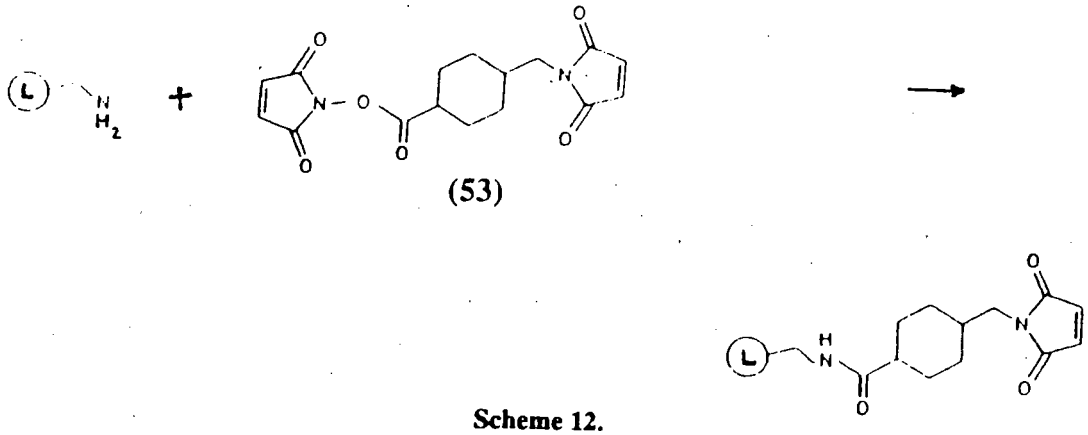


(52)

Scheme 11.

Following coupling between the derivatized ligand (**52**) and antibody, the excess thiol groups are capped by reaction with iodoacetamide. By this method, up to two ligands per antibody are attached without loss of the immunoactivity of the antibody. The isothiocyanate method give an average of 1.4 chelates to the antibody.[7] The former method is undoubtedly superior in terms of attaining a high radioactive yield (particularly important in radiation therapy and diagnosis) than that employed by Brechbiel *et al.*.[7]

Parker *et al.*[42b] also reported an alternative linker molecule for coupling chelates to antibody. This method involves the treatment of the amino group on the 'spacer arm' of the ligand with the N-hydroxysuccinimide ester of N-(4-carboxycyclohexylmethyl)maleimide (**53**)[66] (Scheme 12) as an alternative to (**51**)



(53)

Scheme 12.

In the work reported herein, the mode of protein coupling employs the isothiocyanate group for the reasons already mentioned *vide supra.*

2.4 Objectives of the research.

The project involves the design, synthesis and characterization of novel tetra- and pentaamine ligands directed for protein labelling. The potentiometric stability constants of these ligands will be determined elsewhere.

CHAPTER 3

3. RESULTS AND DISCUSSION

Herein is reported the syntheses of acyclic tetra- and pentaamine ligands from functionalized esters *via* treatment with N,N-dimethylethylenediamine (**62**) to afford the corresponding diamides (and trisamide for those ligands which are not functionalized). As a result, these ligands incorporate strong coordinating amino groups. Moreover, these classes of ligands reported should, as a result of their acyclic structure, be capable of coordinating different metal ions with respect to their ionic size. Compared to their macrocyclic counterparts which impose a restriction on metal ion size, these classes of ligands might therefore be used for most biologically important metal ions which otherwise have different ionic sizes.

The initial step towards functionalization for the class of ligands incorporating the malonyl skeleton (Class I) involves alkylation at α-carbon of diethyl malonate with an appropriate alkylating agent. This is the usual approach for this class of ligands.[41,42a,b] The functional group on the "spacer arm" of the respective ligands is appropriately selected to provide entry to the amino group by chemical modifications compatible with other functional groups on the ligand.

$$R^1CH(CONHCH_2CH_2NMe_2)_2$$

(**63**) $R^1 = NCCH_2CH_2$-

(**64**) $R^1 = pO_2NC_6H_4CH_2$-

CLASS I TYPE

For ligands incorporating the iminodiacyl skeleton (Class II), initial functionalization involves selecting a suitable starting material which can further be elaborated by mild

chemical modifications, to provide a handle for attachment to protein. Of this class of ligands, the preparation of non-functionalized ligands (**73**) and (**86**) is also described.

$R^1N(CH_2CONHCH_2CH_2NMe_2)_2$

(**73**) R^1 = H

(**86**) R^1 = $Me_2NCH_2CH_2NHCOCH_2$-

CLASS II TYPE

Synthetic studies towards the preparation of a functionalized cage complex is also described. To this end, the ligand system based on a tricarboxylic acid was synthesized and the literature methods were adapted to carry out the intended preparation of the tricarboxamide (**89**), which belongs to Class III.

$R^1CH(CH_2CONHCH_2CH_2NH_2)_2$

(**89**) R^1 = $H_2NCH_2CH_2NHCO$-

CLASS III TYPE

The last class of ligands reported is that based on amino acids with the ethylenediamine bridge (Class IV). The synthesis involves selecting an appropriately protected starting amino acid and activation of the carboxyl group by classical peptide synthesis methodology.

$R^1HNCH_2CH_2NHR^2$

R^1 = amino acid residue;

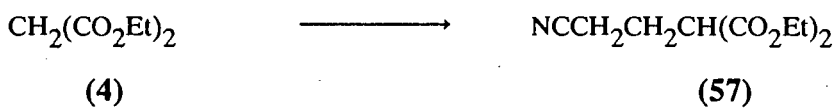
R^2 = either an amino acid residue or an oxalamide

CLASS IV TYPE

Subsequent steps involve the condensation of a suitable diamine (1,2-diaminoethane in this case) and further condensation with an adduct of choice to complete the coordinating function. This class which also embodies non-functionalized ligands, was prepared with the aim of developing a methodology for the functionalized counterparts.

3.1 SYNTHESES OF TETRAAMINE LIGANDS BASED ON FUNCTIONALIZED MALONATES. CLASS I LIGANDS.

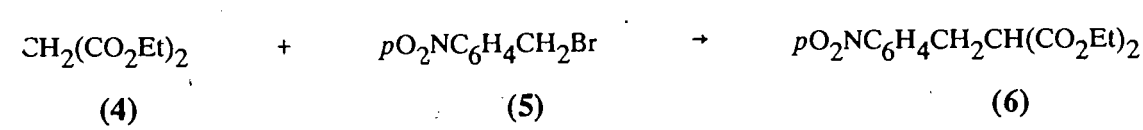
The preparation of this class of ligands required the preparation of suitably functionalized malonic ester derivatives (**6**) and (**57**).



(**4**) (**57**)

Scheme 13.

For the preparation of the cyanoethyl ester (**57**), of the electrophiles selected for the intended C-alkylation of malonic ester (**4**), acrylonitrile provided the best yields (60%), whilst 3-bromoproprionitrile resulted in poor to mediocre yields (32-40%), an observation that has been noted for alkyl bromides.[67] The C-alkylation of malonic ester (**4**) with *p*-nitrobenzylbromide (**5**) as the alkylating agent provided a 44% yield of the *p*-nitrobenzyl ester (**6**) by adapting the literature procedure.[41] The yield obtained for the cyanoethyl ester (**57**) (60%) was consistent with that reported in the literature.[69] In the preparations of both compounds (**6**) and (**57**), the bis-products (**59**) and (**60**), were clearly evident, and contributed to the lowering of yields of (**6**) and (**57**) [this was noticeable for the *p*-nitrobenzyl ester (**6**)].



(**4**) (**5**) (**6**)

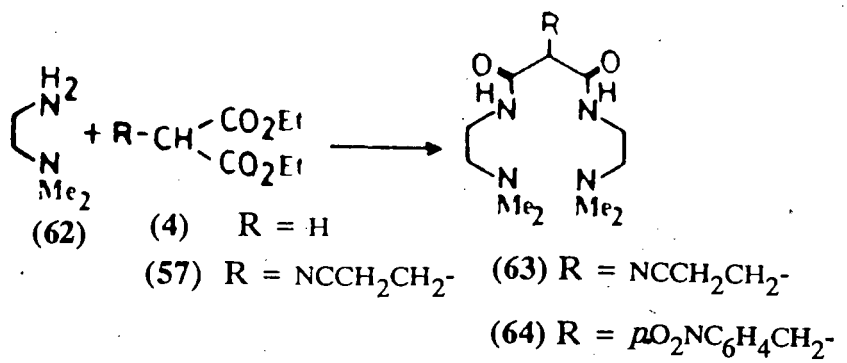
Scheme 13a

$R^1R^2C(CO_2Et)_2$

(59) $R^1 = R^2 = NCCH_2CH_2$-

(60) $R^1 = R^2 = p$-$O_2NC_6H_4CH_2$-

3.2 Synthesis of N,N′-bis[2-(N′′,N′′dimethylamino)ethyl]-2′-cyanoethylmalondiamide (63) and N,N′-bis[2-(N′′N′′-dimethylamino)ethyl] 4-nitrobenzylmalondiamide (64).



(62) (4) R = H

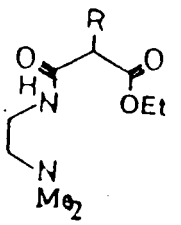
(57) R = $NCCH_2CH_2$- (63) R = $NCCH_2CH_2$-

(64) R = $pO_2NC_6H_4CH_2$-

Scheme 14. EtOH, reflux, 10 days, 70-90%.

The diamides (**63**) and (**64**) were prepared in yields of 90% and 70% respectively, by treatment of the respective cyano (**57**) and *p*-nitrobenzyl (**6**) esters with N,N-dimethylethylenediamine (**62**) in refluxing ethanol for ten days. During the assessment of the conditions for the chain extending amidation reaction for the cyano (**57**) and *p*-nitrobenzyl (**6**) esters, it became evident that the reflux temperature was an important parameter in determining product distribution. This inference emerges from the observation that the major products isolated at relatively low temperatures (room temperature to 60°C), were possibly monoamides, together with little (*ca* 30% in each case) of the desired diamides (**63**) and (**64**). For the amidation of the *p*-nitrobenzyl ester (**6**) at 60°C, the major product (69%) was identified as N-(2-N′,N′-

dimethylaminoethyl)-2-ethoxycarbonyl-3-(4-nitrophenyl)-propanamide (**65**) (Fig.5) on the basis of its ^{1}H-n.m.r.[70b] However at higher temperatures (80-110°C; oil bath), after ten days thin layer chromatography showed total disappearance of the starting esters (**57**) and (**6**) and there was no evidence of monoamides.



(**65**) R = $pO_2NC_6H_4CH_2$-

Fig.5. The structure of the mono amide (65) based on ^{1}H-n.m.r. evidence.

Preliminary evidence in support for amidation *via* the nucleophilic attack at sp^2 hybridized carbon with the concommitant displacement of the ethoxy groups was afforded by infrared spectroscopy of the respective cyano (**63**) and *p*-nitrobenzyl (**64**) diamides, which in each case revealed the disappearance of the bands originally at 1735 cm-^{1} (C=O ester) in (**57**) and (**6**), and the appearance of the bands at 1662 and 1584 cm-^{1}, assigned to carbonyl amide I and II respectively. More evidence was available from the ^{1}H-n.m.r. spectra which revealed for the diamides (**63**) and (**64**) the disappearance of the ethyl ester signal at δ1.20ppm (OCH_2CH_3) and δ4.14ppm (OCH_2CH_3). The cyano compound (**63**) m.p. 122-125°C analyzed satisfactorily for $C_{14}H_{27}N_5O_2$, and the ^{1}H-n.m.r. spectrum revealed the new signals at δ2.1 [$N(CH_3)_2$], 2.4 [$CH_2N(CH_3)_2$], 3.3 (*J* 5.8Hz, CH_2NHCO). The triplet centered at δ3.49ppm in the

^{1}H-n.m.r. of the starting ester (**57**) shifted upfield to δ2.8ppm, which is consistent with the introduction of the relatively shielding amide function.

Table 3. Characteristic ^{1}H-n.m.r. signals of N,N-dimethylaminoethyl moeity.

a c b d
-CONHCH$_2$CH$_2$NMe$_2$

Proton	δ(ppm)
a	6.0-8.2
b	2.45-2.50
c	4.0-4.2
d	2.2-2.3

The *p*-nitrodiamide (**64**) m.p. 144-146°C, which analyzed satisfactorily for $C_{18}H_{29}N_5O_4$, revealed the same features for the N,N-dimethylaminoethylenediamine moeity as the diamide (**63**) (See Table 3). For the diamide (**64**), the doublet and triplet at δ3.28ppm and δ3.63ppm respectively in the ^{1}H-n.m.r. of the ester (**6**), resonated as a complex signal at δ3.35ppm which incorporated the methylene protons contiguous to the amide function (usually at δ3.9 -4.2ppm). This observation was confirmed by an integration of seven protons.

Mass spectrometry revealed molecular ions m/z 297 for the cyanodiamide (**63**) and m/z 379 for the *p*-nitrobenzyldiamide (**64**) , thus confirming their formulas.

3.2.1 A 2D-n.m.r. study of (63). Assignment of ^{1}H- and ^{13}C- n.m.r of (63) by COSY and HETCOR.

In the ^{1}H-n.m.r. spectrum of the diamide (**63**) (Fig.6), the intriguing multiplets at δ3.09ppm and δ3.5ppm (denoted by an asterisk in Fig.6) were since confirmed to belong

to the CH_2NHCO portion of the N,N-dimethylethylenediamine moeity. This assignment was established by a COSY experiment (see the appendix for the spectrum) which showed the connectivity with the H-2 protons at δ2.4ppm on C-2 (the numbering is as delineated in the diagram on Fig.6).

$NCCH_2CH_2CH(CONHCH_2CH_2NMe_2)_2$

(63)

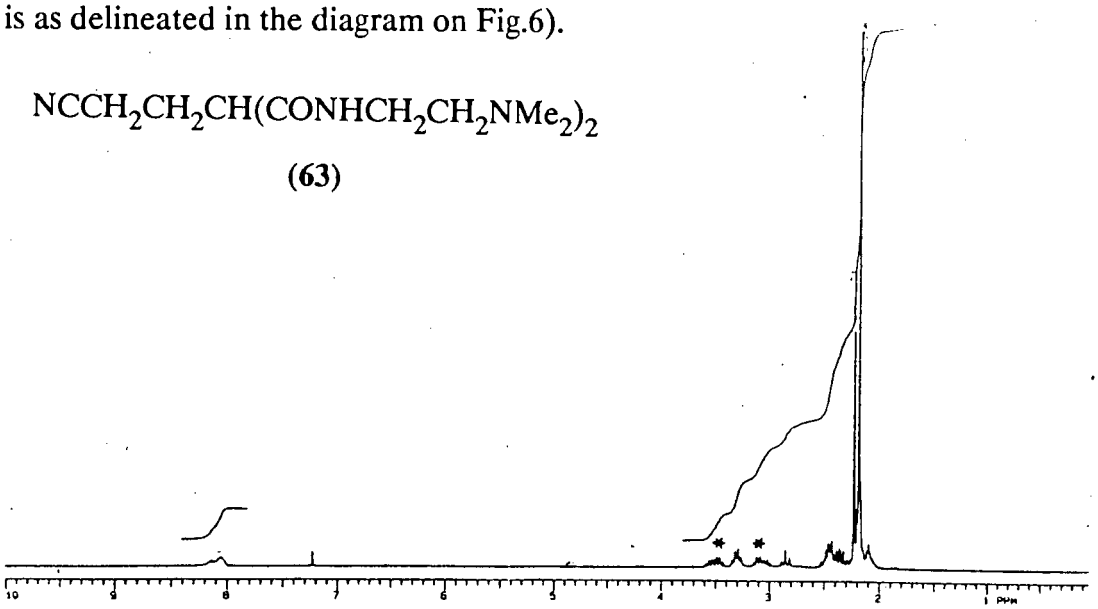


Fig.6 ^{1}H-n.m.r. spectrum (200MHz) of the cyanodiamide (63) recorded at 25°C.

Further evidence for compound (**63**) was provided by the ^{13}C-n.m.r. spectrum (Fig.7) which revealed the expected six line signals within the aliphatic region δ20-60ppm. The ^{13}C-n.m.r. assignments were confirmed by a HETCOR experiment (see the appendix for the spectrum) and are shown in Table 4.

Table 4. ^{13}C-n.m.r. spectral assignments of the diamide (63).

δ_c (ppm)	Carbon assignment (C-n)[a]
29.22 33.90	CH_2CH_2CN (C-5 and C-6)
36.85 37.07	CH_2NHCO (C-3)
45.19 45.42	$N(CH_3)_2$ (C-1)
52.64	OCCHCO (C-4)
57.79 57.92	$CH_2N(CH_3)_2$ (C-2)
170.46[b]	NHCO (C-8)
172.05[b]	CN (C-7)

[a]C-n notation is used arbitrarily to denote the carbon resonances being assigned and follows the same numbering as in Fig.7 diagram. This numbering is also consistent with the H-n notation denoting the various protons in Fig.6 diagram. [b]The assignment of these signals were not HETCOR-assisted.

$NCCH_2CH_2CH(CONHCH_2CH_2NMe_2)_2$

(63)

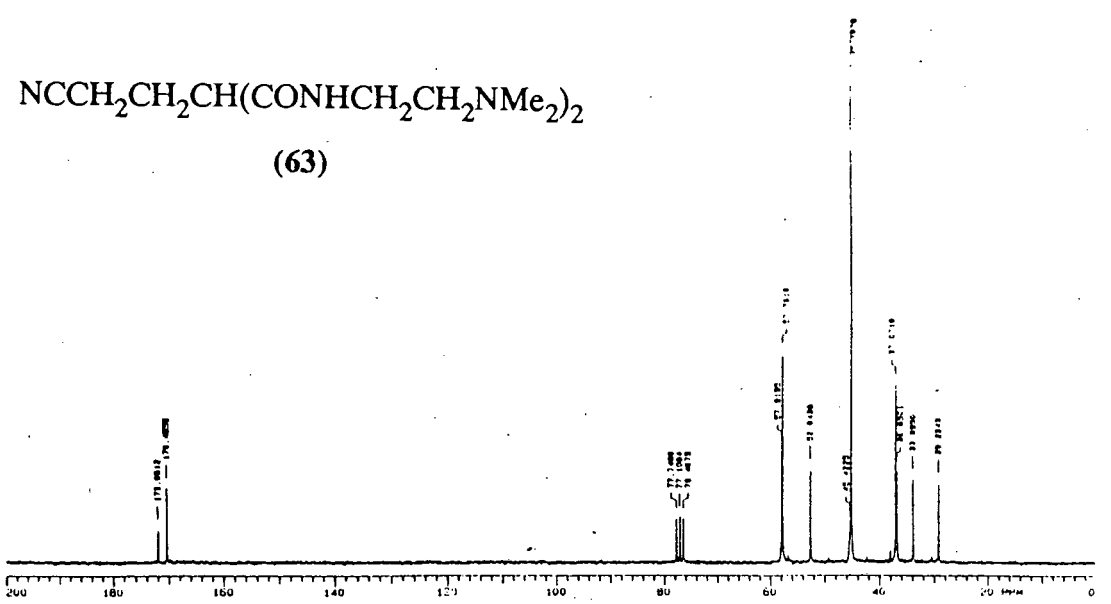
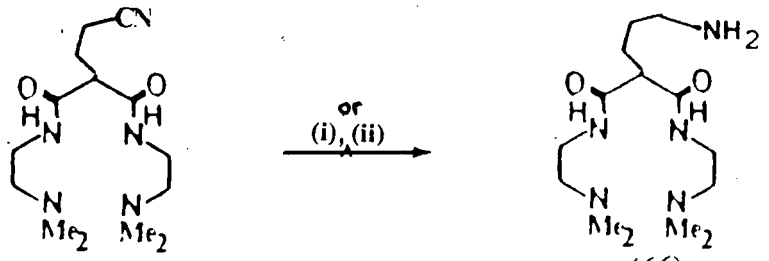


Fig.7 ^{13}C-n.m.r. spectrum (50.3MHz) of the diamide (63) recorded at 25°C.

3.3 Reduction of the cyanodiamide (63) and p-nitrobenzyl- diamide (64) to the respective amino diamides (66) and (67).3.3.1 Attempts to prepare the aminopropyldiamide (66).



(63) (66)

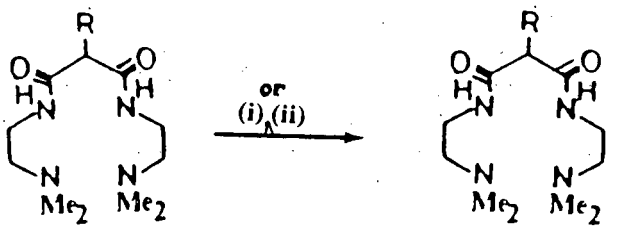
Scheme 15.(i) Ra-Ni (W-2), EtOH-NH_3, H_2 (10bars), 80-90°C; (ii) PtO_2, MeOH-HCl, H_2 (6bars), r.t.

Initially, the reduction of the cyanodiamide (**63**) to the aminopropyl diamide (**66**) was effected with Raney nickel (W-2 type) in ethanol/ammonia at 10 bars pressure at a temperature between 80-90°C. The resulting residue following work up furnished a product whose analysis was at variance with that for the reduction product (**66**). Attempts to characterize the isolated product were not undertaken; the preferred and simpler route for the preparation of the aminodiamide (**66**) employed platinum oxide (PtO_2) as the catalyst at 6 bars of hydrogen pressure in acidic methanol for 24 hours. This mild procedure furnished the product as a syrup in a yield of 80% (crude).

Evidence in support for the partially successful functional group transformation was available from the ^{13}C-n.m.r. spectrum of the product (**66**) which revealed *ca* 45% disappearance of the signal at δ172ppm (*C*N). The foregoing feature indicated seemingly successful reduction of the cyano group. Prolonged hydrogenation failed to improve the efficacy of the reduction. Infrared spectroscopy data could not be used

diagnostically to account for the C≡N → CH_2NH_2 transformation since the band due to C≡N is reportedly weak[70a] and was not observed in the infrared spectrum of the cyanodiamide (**63**). In spite of this, however, infrared spectroscopy revealed the characteristic pair of N-H (1°amine) bands in the region 3200-3000 cm^{-1} though unusually weak. Owing to the disappointing foregoing results, the *p*-nitrobenzyldiamide (**64**) was selected for ensuing studies because of the relative readiness with which the nitro group was reduced (*vide infra*).

3.3.2 Preparation of the *p*-aminobenzylmalondiamide (67).



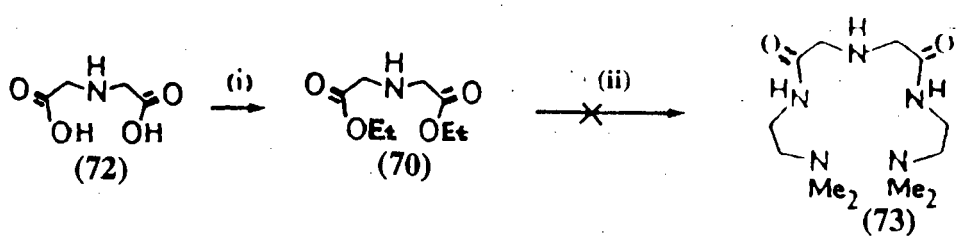
(**64**) R = $pO_2NC_6H_4CH_2$- (**67**) R = $pH_2NC_6H_4CH_2$-

Scheme 16. 10% Pd-C, ammonium formate, r.t., 16h, 60%;(ii) 10% Pd-C, H_2, 16h, 98%.

The *p*-aminobenzyldiamide (**67**) was initially prepared in 60% yield by reduction of the *p*-nitrobenzyldiamide (**64**) using the Pd-C/ammonium formate system at room temperature.[71] The ^1H- n.m.r. spectrum of the reaction mixture showed the reaction to have gone to *ca* 95% completion. [The intensities of the downfield signal due to the aromatic protons in the the *p*-nitrophenyl ring were, compared with those of the corresponding protons which shifted upfield due to the introduction of an electron donating amino group in (**67**)]. Improved yields (98%) were achieved by hydrogenating

products.The ^{1}H-n.m.r. spectrum of the partially purified reaction mixture [free of the starting bromide (**69**)] revealed the presence of the ethoxy group (triplet and quartet systems at δ1.25ppm and δ4.14ppm), as well as a series of D_2O exchangeable protons [NH of either of the products (**70**) or (**71**)].

Exhaustive purification of the isolated material failed to afford the iminodiester (**70**) in sufficiently pure form. The infrared spectrum of the partially purified product revealed v(C=O) amide I and II bands at 1650 and 1583 cm-^{1} respectively, as well as a band at 1740 cm-^{1}, assigned to v(C=O) ester. These features cannot be treated as serendipitous as they pointed strongly to N-acetylation product as well as the N-alkylation product. The relative proportions of the N-acetylation and N-akylation products by ^{1}H-n.m.r. spectroscopy was not undertaken.

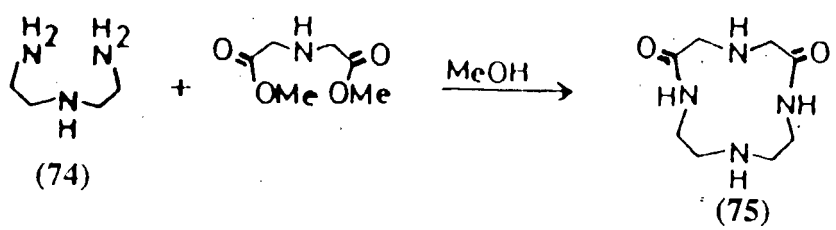


Scheme 18. (i) EtOH, HCl, reflux, 16h, 88%; (ii) $H_2NCH_2CH_2NMe_2$, EtOH, reflux, 3 days.

A simpler route towards the preparation of the diethyl iminodiacetate ester (**70**) was accessible from the commercially available iminodiacetic acid (**72**) [Though the procedure of Scheme 16 (leading ultimately to (**73**) for preparing (**70**) seemed appropriate as the diacid (**72**) was not readily available, the major deterrent was the

undesirable side reactions]. Acid catalyzed esterification of the iminodiacetic acid (**72**) provided the ester (**70**) in 88% yield (Scheme 17). Amidation of the ester (**70**) with the diamine (**62**) was expected to furnish the diamide (**73**). However this reaction resulted in an intractable material which failed to give the iminodiamide (**73**). One reason may be due to the nucleophilicity of the secondary amine on the intermediate ester (**70**) potentially competing with the N,N-dimethylaminoethylenediamine (**62**) in the attack at the ester carbonyl.

Kimura[72] outlined a synthetic scheme for the macrocyclic ligand (**75**) by treating the methyl ester equivalent of (**70**) (unprotected at the secondary amine) with (**74**) in methanol, though no details of the yields and experimental conditions were given (Scheme 19):



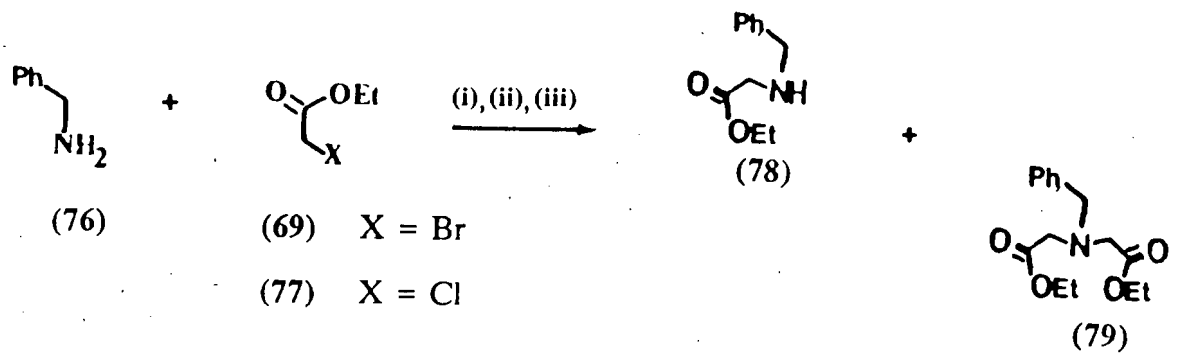
(74) (75)

Scheme 19.

In view of the problem encountered in preparing the diamide (**73**) directly from the diethyl ester (**70**), it was decided to employ the protected diethyl iminodiacetate (**79**). Treatment of the ester (**70**) with benzylbromide in triethylamine with temperatures ranging from room temperature to 60°C, gave the diethyl-N-benzyliminodiacetate (**79**) in very low yield (5%) with substantial recovery of unreacted ester (**70**). The poor yield associated with this procedure in preparing the protected ester (**79**) excluded it as the

route of choice. An alternative procedure for achieving the desired protection was sought and section 3.4.1.2 highlights such endeavours.

3.4.1.2 Synthesis of diethyl N-benzyliminodiacetate (79)[73].



Scheme 20. (i) Et_3N, 60°C, 16h, 72% [from (**77**)] or 81% [from (**69**)]; (ii) (**78**), Et_3N, reflux, 8h, 48%; (iii) NaH (1.1eq), DMF, -30°C → r.t., 58%.

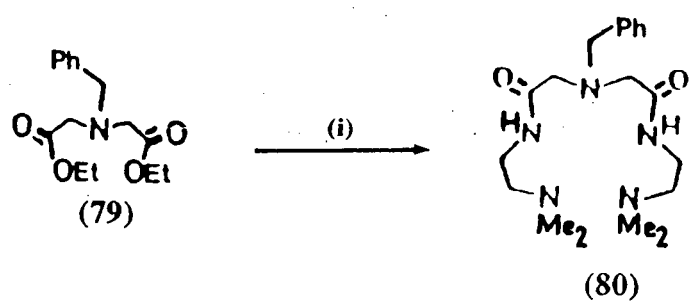
An alternative route in the synthesis of the N-benzyl ester (**79**) is shown in Scheme 20. The N-benzyl product (**78**) was obtained by refluxing benzylamine (**76**) with ethyl chloro acetate (**77**) in the presence of triethylamine, and gave predominantly the mono N-alkylated ester (**78**) in 72% yield as well as the protected ester (**79**) in 20% yield. [The mono N-alkylated ester (**78**) was obtained in 81% yield when diethyl ether was the solvent together with 1% of (**79**)]. Treatment of the mono ester (**78**) with sodium hydride in DMF and ethyl bromoacetate (**69**) at -30°C raised the yield of the protected ester (**79**) to 58%.

A more direct route for preparing (**79**) from benzylamine (**76**) was sought. Mono-N-alkylation was observed to proceed efficiently at room temperature in relatively short reaction times (4-6 hours) with triethylamine as solvent, and even less when diethyl ether was the solvent (2.5 hours) compared to the formation of the diester (**79**).

However, prolonged reaction times (2-3 days, at room temperature) using a large excess of alkylating agent [(**69**) or (**77**)], raised the yield of the protected diester (**79**) to 48%. The mono-N-alkylation product (**78**) was converted to the diester (**79**) in 48% yield by treatment with ethyl bromoacetate (**69**) in refluxing triethylamine.

In executing a more direct route to the diester (**79**), based on the above-mentioned observations, the molar ratio of benzylamine to alkylating agent was altered from 1:1 (monoalkylation conditions) to 1:4, and the effect of temperature on product distribution was investigated. As anticipated, a reversal in product distribution was observed and the desired N-dialkylated product (**79**) was isolated in 65% yield together with the monoester (**78**) (11%). The latter was readily converted to (**79**) as previously described (*vide supra*). The diester (**79**) prepared by this route exhibited similar thin layer chromatographic behaviour as the same product prepared by an independent route.[73] The ^{1}H-n.m.r. spectrum of the ester (**79**) showed the expected triplet and quartet system at δ1.20ppm and δ4.14ppm respectively, the five phenyl ring multiplet signal at δ7.3ppm, the benzyl methylene proton signal at δ3.49ppm, as well as those at δ3.65ppm (NCH_2CO), all of which were consistent with structure (**79**).

3.4.1.3 Amidation of diethyl N-benzyliminodiacetate (79) with the diamine (62).



(79) (80)

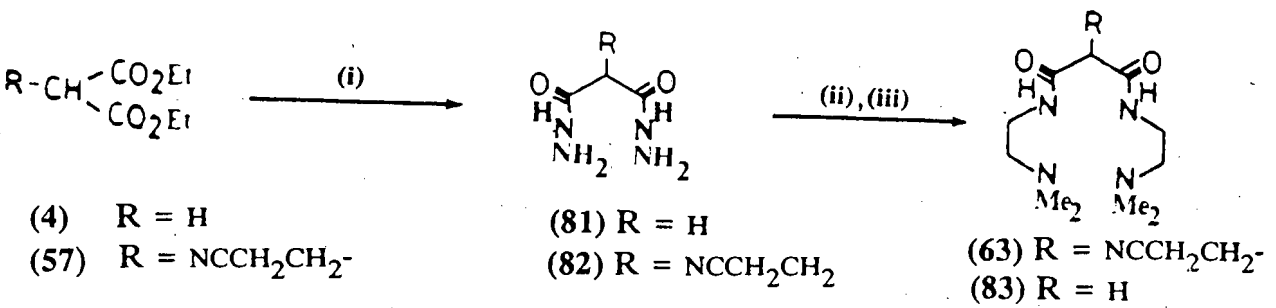
Scheme 21. (i) (**62**), EtOH, reflux, 3 days, 85%.

Treatment of the protected ester (**79**) with excess of the diamine (**62**) in ethanol under reflux for a duration of three days, gave the N-protected bisamide (**80**) as an orange-tan syrup in 84% yield after chromatography, which analyzed for $C_{19}H_{33}N_5O_2$. The ^{1}H-n.m.r. of the orange syrupy diamide (**80**) revealed the same features observed for the diamides (**63**) and (**64**) with respect to the N,N-dimethylaminoethylenediamino moeity. Of note was the respective shift of the singlets at δ3.49ppm and δ3.65ppm as observed in the ^{1}H-n.m.r. of the ester (**79**), to δ3.19ppm and δ3.91ppm in the ^{1}H-n.m.r. of the diamide (**80**). The former is significant in showing the shielding effect of the amide function upon the methylene protons *alpha* to the amide carbonyl. The infrared spectrum of the diamide (**80**) also revealed *inter alia* the amide carbonyl stretching frequencies of type I and II at 1653 and 1532 cm-^{1} respectively. Finally mass spectroscopy established the molecular formula for the diamide (**80**) by a peak at m/z 364 (M^+ + H).

An interesting observation regarding the amidation of the N-protected diester (**79**) was that the reaction was essentially complete in three days (t.l.c control), which was relatively rapid compared to the duration for the same reaction with malonic ester derivatives (**57**) and (**6**). The reactivity of the diethyl ester (**79**) towards amidation was comparable to that of the more reactive electrophile diethyl oxalate (**101**). A possible explanation may be attributable to the tertiary nitrogen in the ester (**79**) exerting a significant electron withdrawing inductive effect at the ester carbonyl. This phenomenon would enhance the electrophilicity of this center and as such the nucleophilic attack of the diamine (**62**) with the concomitant displacement of the ethoxy group should occur readily. The malonic ester derivatives lack this feature and the relatively longer reaction periods for efficient amidation is evidence of their relatively diminished electrophilicity. Enhancement of the cyano ester (**57**) towards amidation

was investigated with the hope of circumventing the unreasonably lengthy reaction periods under mild conditions (section 3.4.1.4).

3.4.1.4 Conversion of the diethyl esters (4) and (57) to their respective dihydrazides (81) and (82). Enhancement of reactivity towards amidation *via in situ* generation of the diazides of (4) and (57).

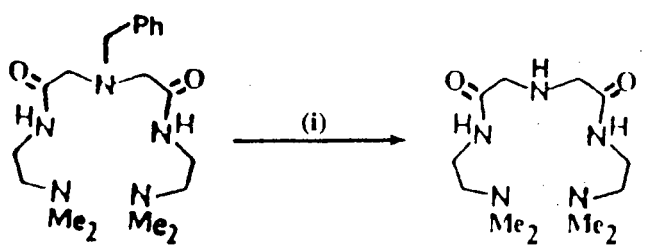


Scheme 22. (i) $H_2NNH_2.H_2O$, MeOH, r.t., 99%; (ii) Bu^tONO,7*M* HCl, or $NaNO_2$, 5*M* HCl, THF(aq), -15°C; (iii) **(62)**, THF,-30°C → r.t., 24h, 70-79%.

The bishydrazides **(81)**[74] and **(82)** were both prepared, in pure form by the literature method[75] in yields of 99%. Subsequently these hydrazides **(81)** and **(82)** were treated with either *tert*-butyl nitrite (Bu^tONO)[76] or nitrous acid (generated *in situ* i.e. HCl + $NaNO_2$)[75] in aqueous tetrahydrofuran at -15°C for 3 hours. Reaction with 2.1 equivalents of the diamine **(62)** at temperatures from -30°C to ambient furnished the respective diamides **(83)** and **(63)** in 70-79% yields. The products prepared by this method were identical spectroscopically with the products prepared *via* amidation of the malonic esters **(4)** and **(57)**. The dihydrazide of the nitro ester **(6)** was not prepared because this material was no longer available. It was envisaged that the dihydrazide, and subsequently the diazide derived from **(6)** would exhibit similar behaviour towards amidation.

With such an undertaking, an alternative method towards improving the rapidity of bisamidation of the malonic ester derivatives (one day *versus* ten days) was desirable. Acyl chlorides as alternative intermediates to esters have been used in the syntheses of some ligand structures for sequestering metal ions. Attempts in this direction, in particular the hydrolysis step giving rise to the free dicarboxylic acid (as required prior to conversion to the acyl chloride) proved inefficient in terms of retrieving reasonable yields of the dicarboxylic acid (generally yields were below 45%). This observation was probably due to the observed poor extraction of the pertinent diacid into organic solvents employed (dichloromethane, chloroform, diethyl ether, and ethyl acetate). The diazide route was preferred because of the high yields of the intermediate dihydrazides (>98%), and reasonable purity. Because of the explosive nature of the diazide, this route is only suitable for small scale preparations; nevertheless it does enhance the reactivity of the malonic ester intermediates.

3.4.1.5 Debenzylation of the N-benzyliminodiamide (80).



(80) (73)

Scheme 23. (i) PtO_2, H_2, EtOAc-MeOH (7:3), r.t., 24h, 98%.

The imino diamide (**73**) was obtained in 98% yield by debenzylation of the N-benzyl diamide (**80**) by hydrogenation over platinum oxide (PtO_2) in ethanol-chloroform (90:10). Preliminary evidence for the successful debenzylation was from the infrared spectrum of the deprotected diamide (**73**) which revealed the absence of the bands at 1600 and 1515 cm^{-1}, assigned to the phenyl ring. The infrared spectrum of the bisamide (**73**) also showed the absence of the monosubstituted phenyl ring bands originally observed at 774 and 744 cm^{-1} in the infrared spectrum of the N-benzyl diamide (**80**). The ^{1}H-n.m.r. spectrum of (**73**) (Fig.8) substantiated these findings with the observed absence of the aromatic five proton complex at δ7.50ppm as well as the benzyl methylene proton singlet observed at δ3.65ppm in the ^{1}H-n.m.r. spectrum of (**80**). The new D_2O exchangeable signal at δ3.47ppm was in conformity with the unmasking of the secondary amine.

$HN(CH_2CONHCH_2CH_2NMe_2)_2$

(73)

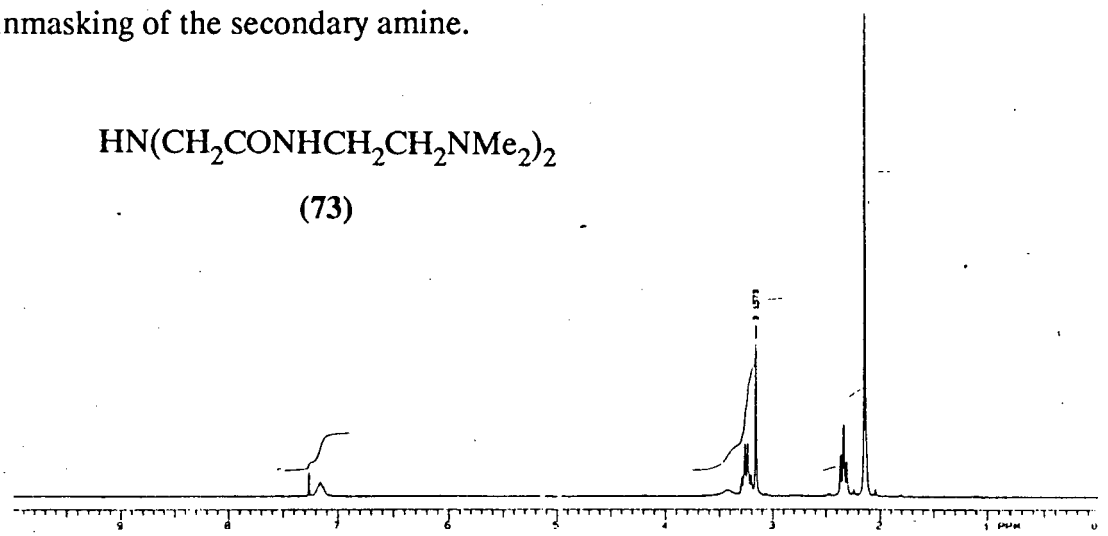


Fig.8. ^{1}H-n.m.r. spectrum (200MHz) of the iminodiamide (73) recorded at 25°C.

The ^{13}C-n.m.r. spectrum (Fig.9) of the iminodiamide (**73**) also showed the expected four carbon resonances in the range δ30-60ppm [The ^{13}C-n.m.r. assignments of the iminodiamide (**73**) are given in Table 5].

Table 5. ^{13}C-n.m.r. assignments of the iminodiamide (73).

δ_c (ppm)	Carbon assigned	(C-n)[a]
36.35	CH_2NHCO	(C-3)
45.10	NCH_3	(C-1)
52.56	$CH_2N(CH_3)_2$	(C-2)
58.25	NCH_2CO	(C-4)
170.94	CONH (C-5)	

[a]C-n notation is used arbitrarily to denote a particular carbon being assigned and follows from the numbering as shown on the diagram of (73) (Fig.9).

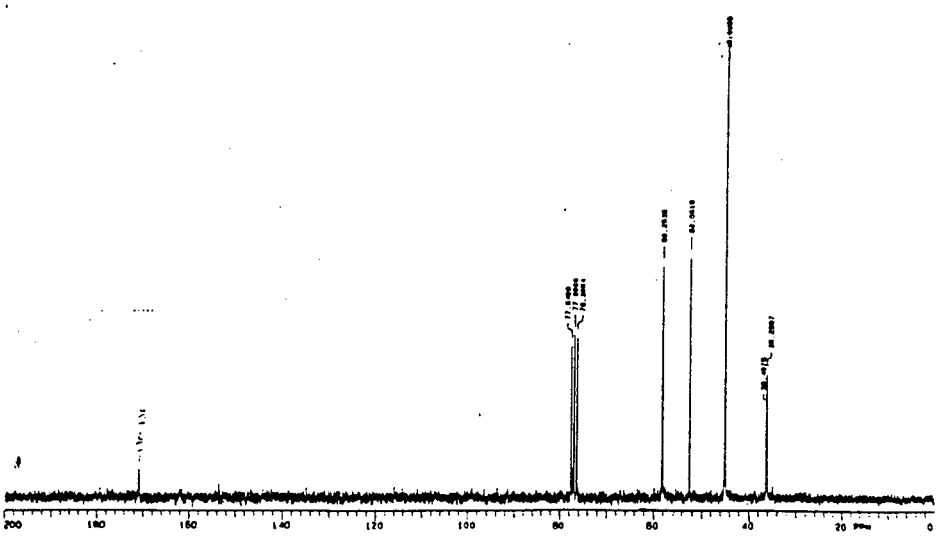
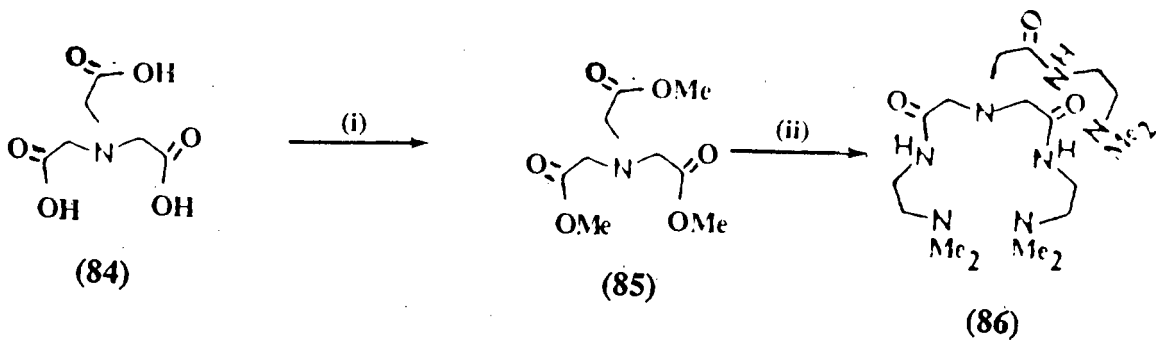


Fig.9. ^{13}C-n.m.r. spectrum (50.3MHz) of the iminodiamide (73) recorded at 25°C.

The mass spectrum of the debenzylation product also revealed the highest peak at m/z 273 in agreement with the molecular ion of the bisamide (73).

3.4.1.6 A synthesis of the nitrilotriacetamide (86).



Scheme.24. (i) MeOH, HCl, reflux, 16h, 83%; (ii) (**62**), reflux, 3 days, 66%.

The rationale for the synthesis of the trisamide (**86**) followed the sequence: acid (**84**) → ester (**85**) → trisamide (**86**). The triester (**85**) was prepared in 83% yield from the commercially available nitrilotriacetic acid (**84**) by conventional acid catalyzed esterification procedure. The infrared spectrum of the esterification product revealed a band centered at 1742 cm-^{1} and was assigned to ν(C=O ester). The ^{1}H-n.m.r. spectrum of the ester (**85**) revealed the singlets at δ3.53 (NCH_2CO) and δ3.58ppm (OCH_3). The ^{13}C-n.m.r. spectrum of the ester (**85**) also showed the expected three carbon resonances at δ51.41ppm (OCH_3), δ54.68ppm (NCH_2CO), and δ170.88ppm (*C*O). The mass spectrum of the triester confirmed the structure by showing the peak at m/z 233 in agreement with the molecular peak for the triester (**85**).

The triester (**85**) and the diamine (**62**) were reacted under the usual amidation conditions to give, after column chromatography, the trisamide (**86**) $C_{18}H_{39}N_7O_3$, as an orange syrup in 66% yield. Spectroscopic evidence was consistent in all respects with that expected for the trisamide (**86**). Both the ^{1}H- and ^{13}C-n.m.r. spectra of the trisamide (**86**) and its trihydrochloride (**86a**) were investigated. The signals at δ2.21ppm [N(CH_3)$_2$], δ2.45ppm [CH_2N(CH_3)$_2$], δ3.21ppm (NCH_2CO), and δ3.41ppm

(CH_2NHCO) in the ^{1}H-n.m.r. of the trisamide, shifted downfield to δ2.93ppm, δ3.31ppm, δ3.95ppm, and δ3.65ppm respectively in the ^{1}H-n.m.r. of the trihydrochloride **(86a)**. These observed shifts are in agreement with the protonation of the relatively basic tertiary nitrogen centres, which results in a deshielding effect on protons attached to them. The downfield shift of the order Δδ0.87ppm was the most pronounced of all the observed chemical shift differences. The feeble basicity of the amide nitrogen was notable for the observed change in chemical shift of the signal at δ3.38ppm in **(86)** to δ3.64ppm in **(86a)** for (CH_2NHCO) was the smallest compared with that of the protons contiguous to non-amide nitrogens.

$N(CH_2CONHCH_2CH_2NMe_2)_3$

(86)

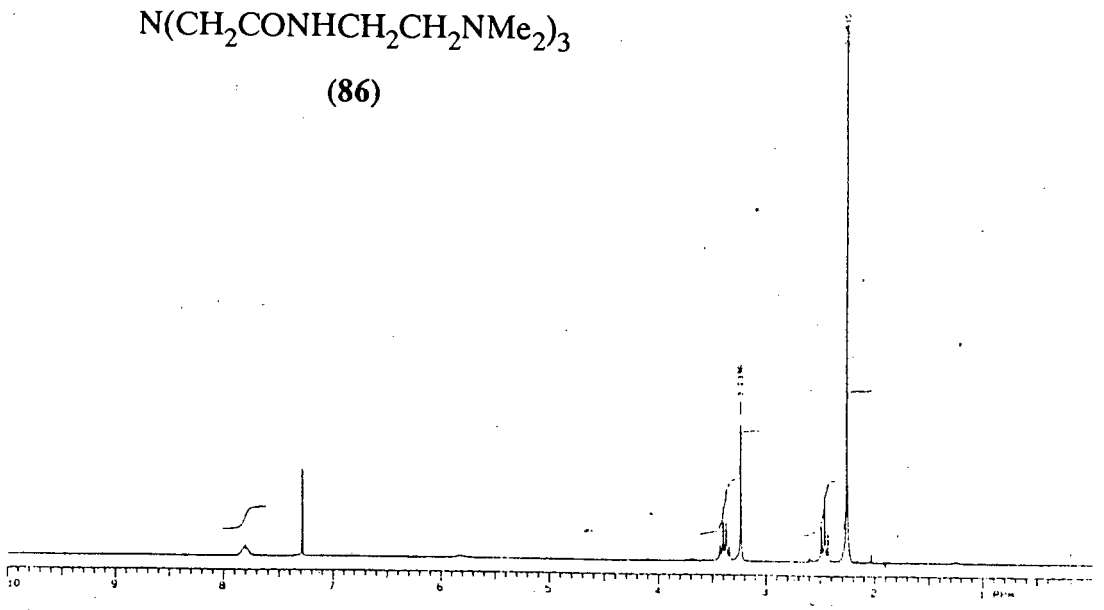


Fig. 10. ^{1}H-n.m.r. spectrum (200MHz) of the trisamide (86) recorded at 25°C.

$N(CH_2CONHCH_2CH_2NMe_2)_3.3HCl$

(86a)

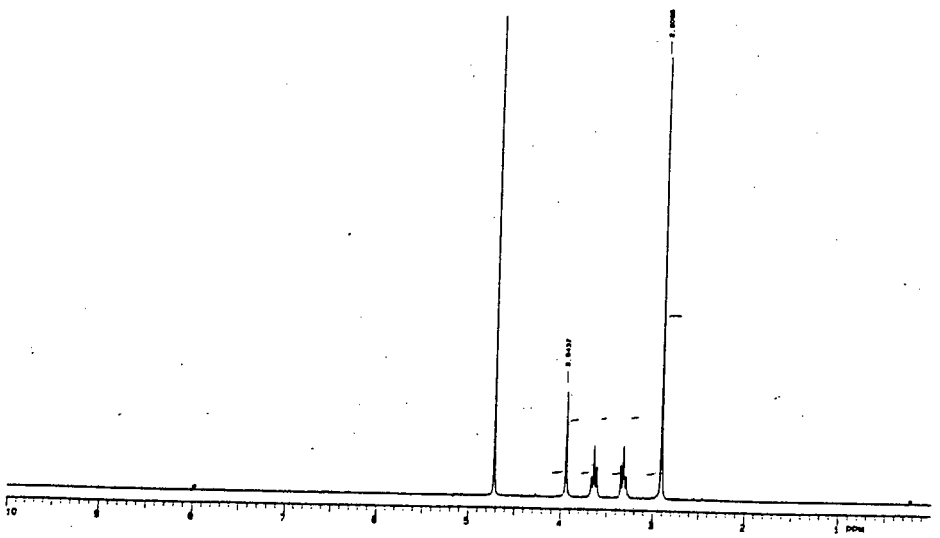


Fig.11. ^{1}H-n.m.r. spectrum of the trihydrochloride (86a) recorded at 25°C.

The ^{13}C-spectra of the trisamide (**86**) and (**86a**) were assigned by comparison of the ^{13}C-n.m.r spectrum with the model oxalodiamide (**150**) (see appendix, Fig.4a). Amidation of the triester (**85**) shifted the signal observed at δ54.68ppm in the spectrum of (**85**) to δ59.20ppm in the trisamide (**86**), which in turn shifted to δ57.70ppm in the trihydrochloride (**86a**) and was assigned to NCH_2CO. This assignment was corroborated by a comparison with the HETCOR assignment of the same carbon in the hydroxydiamide (**100**) (see 3.4.2.3). The signal at δ45.16ppm in the spectrum of compound (**86**) which shifted to δ43.84ppm in the hydrochloride (**86a**) and was assigned to $N(CH_3)_2$ [The assignment of this signal was confirmed by the ^{13}C (APT) of (**150**)]. The new signal at δ36.72ppm in the spectrum of the trisamide (**86**), shifted to δ35.34ppm in the spectrum of the hydrochloride (**86a**) and was assigned to CH_2NHCO, by comparison with the HETCOR assisted assignment of the same carbon of the diamide (**63**), and ^{13}C (APT) of (**150**). The assignment of the signal at δ58.15ppm to $CH_2N(CH_2)_3$ followed with all the other carbon resonances having been assigned and is consistent with the resonance of this carbon as established by the APT of (**150**).

$N(CH_2CONHCH_2CH_2NMe_2)_3$

(86)

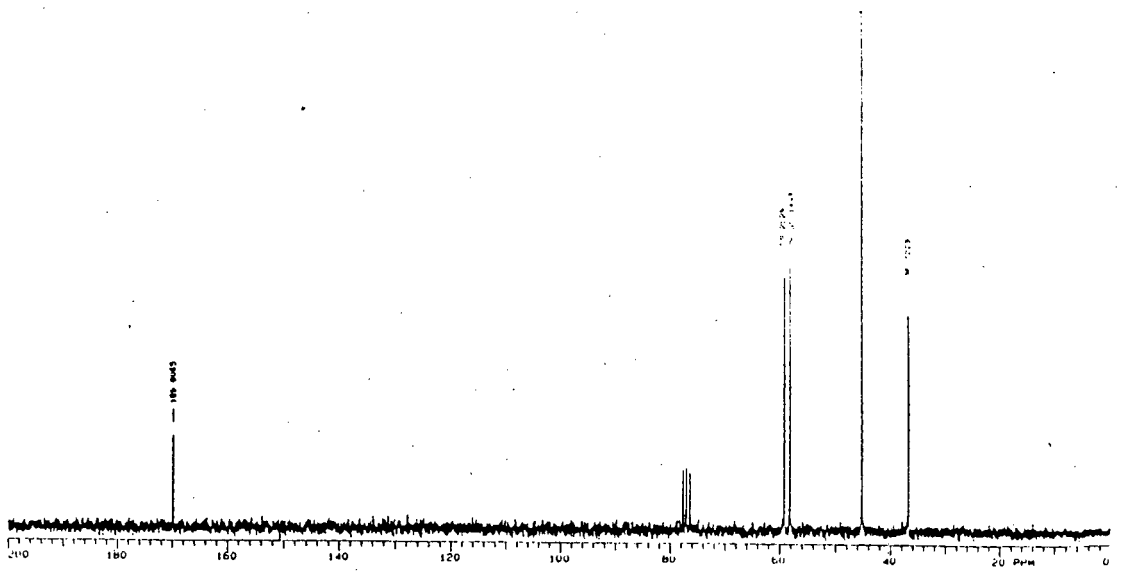


Fig.12. ^{13}C-n.m.r. spectrum (50.3MHz) of the trisamide (86) recorded at 25°C.

$N(CH_2CONHCH_2CH_2NMe_2)_3.3HCl$

(86a)

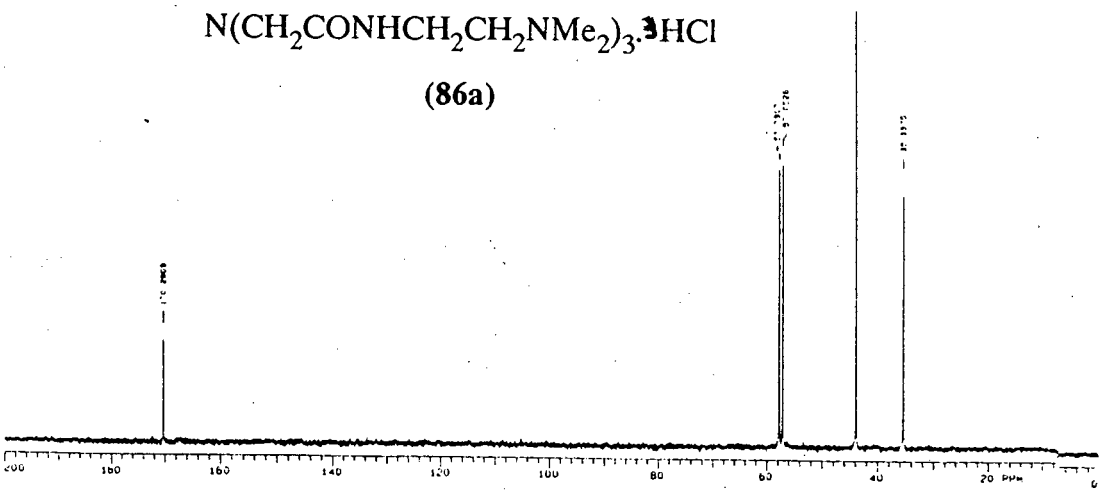


Fig.13. ^{13}C-n.m.r. spectrum (50.3MHz) of the trihydrochloride (86a) recorded at 25°C.

3.4.2 Functionalized pentaamine Ligands. Investigation of Synthetically Feasible Routes for the Functionalization of the Diamide Bearing the Iminodiamide (73) Skeleton.

3.4.2.1 Considerations.

It was of interest to incorporate a facility in the structural backbone of the iminodiamide (73) which would enable its attachment to biological macromolecules. Nitration of the benzyl diamide (80) and subsequent reduction to unmask the amino group is impracticable for it would lead to debenzylation and ultimately to (73). In view of the obvious disadvantage, alternative routes were considered which would incorporate a desirable feature for forthcoming chemical elaboration which would ultimately converge to a functionality best disposed for covalent attachment to biological macromolecules.

$$HN(CH_2CONHCH_2CH_2NMe_2)_2$$

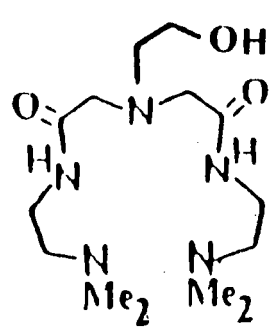
(73)

A short communication by Olah *et al.*[91] described a direct and highly efficient electrophilic aromatic amination *via* trimethylsilylazide/triflic acid reagent. The yields reported in some mono substituted phenyl compounds were generally high (73-96%). However the isomer distribution was in the order *ortho* > *para* > *meta*. The latter, in the context of this work is disadvantageous; the interest is in having a *para* substituted amino group because the steric factor critically determines the efficiency of coupling to biological macromolecules. For the *para* case this condition is satisfied (the coordinating function of (80) is far removed from the macromolecule), and is significant in proceeding from the *ortho* to the *meta* isomer. Moreover the Lewis acid catalyst

(aluminium trichloride has previously been used [92]) trifluoromethanesulphonic acid employed at elevated temperatures (50-70°C) for this type of amination is not compatible with the diamide function of compound **(80)**. As such, exploitation of this methodology towards functionalizing the N-benzyl iminodiamide **(80)** was not practically investigated based on the abovementioned arguments.

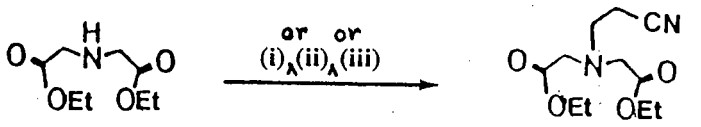
Diethyl iminodiacetate **(70)** was initially chosen as the starting compound for the purposes of introducing the desired functionalization. Alkylation of the secondary amine in the ester **(70)** with an alkylating agent propitious for further mild chemical transformation en route to the ultimate functional group appeared the best approach. The various routes towards achieving these goals are discussed in the next section 3.4.2.2 .

An alternative approach for obtaining a suitable intermediate which appeared somewhat more attractive than the previous one based on **(70)**, realized the versatility inherent in employing the intermediate in the form of the hydroxyethyliminodiacetic acid **(97)**. Thus, by employing facile chemoselective transformations on the acid **(97)**, which would result in the retention of the 2-hydroxyl group, it should be feasible to prepare the substituted iminodiamide **(100)**. With the hydroxyl group readily accessible, various transformations leading to the desired features best suited for coupling to biological macromolecules *via* the isothiocyanate are feasible. Section 3.4.2.4 highlights the abovementioned endeavours.



(100)

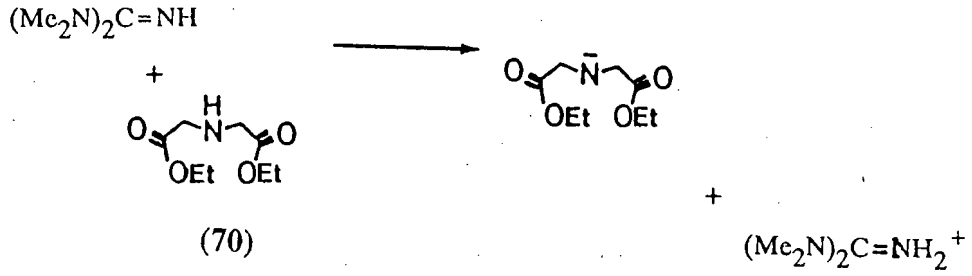
3.4.2..2 Attempted N-alkylation of diethyl iminodiacetate (70).



(70) (96)

Scheme 25. (i) $BrCH_2CH_2CN$, $KHCO_3$, CH_3CN, reflux; (ii) $CH_2{=}CNCN$, $KHCO_3$, CH_3CN, reflux; (iii) $CH_2{=}CHCN$, TMG, CCl_4, r.t..

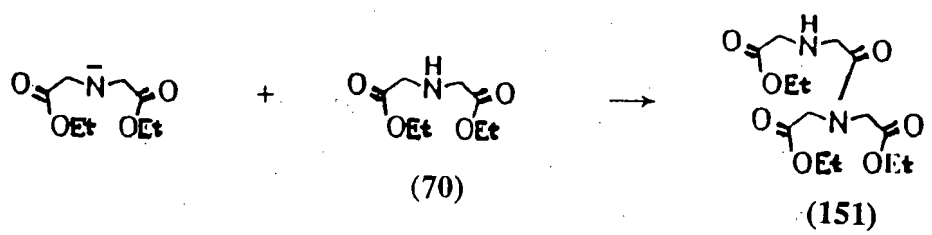
N-cyanoethylation at the amino group of the ester (**70**) was attempted using either 3-bromopropionitrile (**54**) or acrylonitrile (**56**) as the alkylating agents in the presence of potassium bicarbonate in refluxing acetonitrile, and with acrylonitrile (**56**) in the presence of tetramethylguanidine in carbon tetrachloride. None of the three routes investigated furnished the desired diethyl N-(2-cyanoethyl)iminodiacetate (**96**), though the mass spectrum of the product formed by the third procedure (Scheme 25) revealed a peak at m/z 242 (4%) corresponding to M^+ for (**96**). In attempts to purify the isolated product, however, column chromatography was found ineffective, and vacuum distillation resulted in decomposition. It was considered that a strong organic base like tetramethylguanidine would abstract the proton from the -NH- residue in (**70**) more readily.(Scheme 26).



Scheme 26.

This reaction was not investigated further owing to the difficulty in obtaining a pure product in sufficient quantity.

The difficulties associated with effecting N-alkylation of the ester (**70**) by the various routes attempted were discouraging. Though cyanoethylation of amines is a well documented procedure[93] effected at room temperature for some amines, secondary amines usually require the application of heat to effect cyanoethylation. For our purposes, elevated temperatures in cyanoethylating (**70**) can result in undesirable side reactions by the secondary amine attacking the carbonyl carbon of another molecule of (**70**) to give the amide (**151**) (Scheme 27).

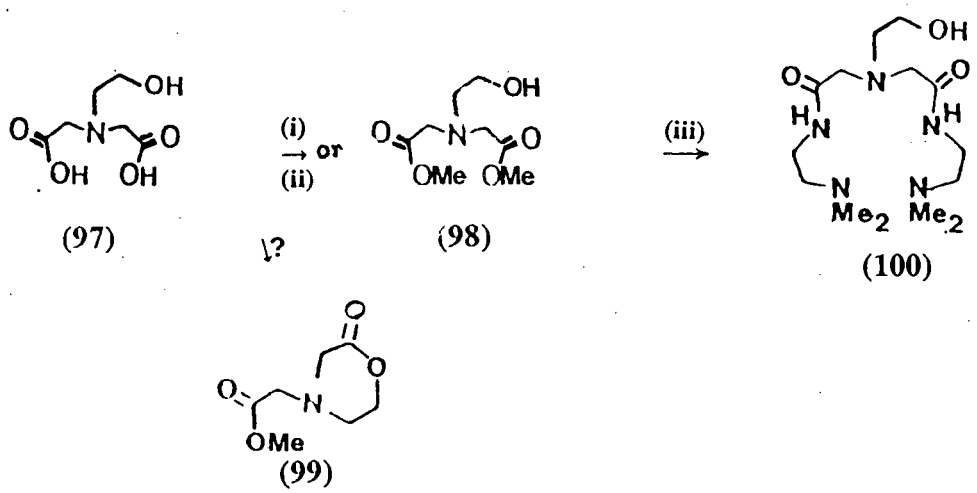


Scheme 27. Same conditions as for Scheme 25, (iii).

As a last resort in the attempted functionalization of the ester (**70**), it was treated with 3-bromopropionitrile (**54**) in the presence of 4-(dimethylamino)pyridine (DMAP) in dichloromethane. After 60h, the reaction mixture gave a product whose mass spectrum revealed a weak peak at m/z 242, corresponding to the molecular ion for the desired ester (**96**). However, the ^{1}H-n.m.r. spectrum of the isolated product did not account for the successful formation of (**96**) but was similar in all respects to the spectrum of the starting ester (**70**), contaminated with residual 4-(dimethylamino)pyridine. Perhaps mass spectroscopy revealed only a trace of the desired product (**96**) which was otherwise not detectable by thin layer chromatography.

Further attempts to achieve the functionalization of (**70**) by the abovementioned methods were abandoned.

3.4.2.3 A synthesis of the hydroxyethyliminodiacetamide (100).



Scheme 28. (i) MeOH, HCl, reflux, 16h, 72%; (ii) CH_2N_2, MeOH, 0°C, 2.5h, 96%; (iii) (**62**), MeOH, reflux, 3 days, 75%.

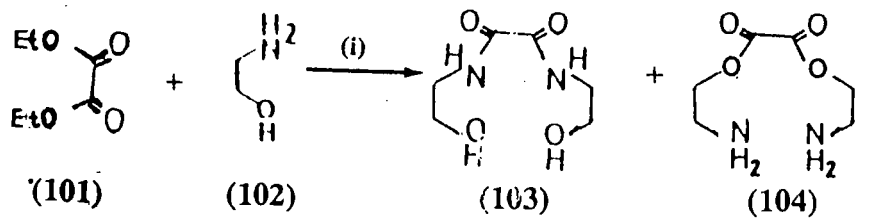
The synthesis of the N-(2-hydroxyethyl) diamide (**100**) followed the sequence: (**97**) → (**98**) → (**100**). The N-(2-hydroxyethyl) dimethyl ester (**98**) was prepared from the acid (**97**) *via* the previously described esterification procedure in a yield of 72%, or in more improved yields (98%) with ethereal diazomethane. Thin layer chromatographic analysis of the ester (**98**) prepared by acid catalyzed esterification revealed an apparently homogeneous product, whilst the ^{1}H-n.m.r. revealed fine splitting for the methoxy signals at δ3.45ppm and δ3.65ppm. Moreover the integration for the methylene protons adjacent to the 2-hydroxyl group at δ2.82ppm was for more than two protons. These features were thought to be a contaminant arising from a side product of the acid catalyzed esterification of (**97**). The possibility of contaminants arising from a possible byproduct was investigated in an attempt to explain the anomalous features in the ^{1}H-n.m.r. spectrum of the hydroxy ester (**98**).

The possibility of lactonization leading to structure (**99**) was ruled out based on spectroscopic evidence.

The infrared spectrum of the isolated product (**98**) revealed only a band at 1742 cm-^{1} which was assigned to the ester (C=O) absorption. Exhaustive purification by repeated column chromatography of the apparently impure ester (**98**), failed to change the observed inconsistent integration for the methylene protons signal centered at δ2.8ppm in the ^{1}H-n.m.r. spectrum of the ester (**98**). The infrared spectral bands at 3465 and 1410 cm-^{1} for the ester (**98**) are attributed to the O-H stretching and bending respectively. The mass spectrum also revealed a peak at m/z 205 which satisfactorily accounted for the molecular ion of (**98**). On close examination of the mass spectrum of (**98**), a peak centred at m/z 173, which could suggest the lactone (**99**), is more likely to arise from the loss of methanol from the molecular ion, m/z 205. In the light of the foregoing, the effect of heat during the acid catalyzed esterification of (**97**) may lead to

some lactonization. To verify this possibility, a milder technique for the preparation of the methyl ester (**98**) was embarked upon. Thus treating the diacid (**97**) with excess ethereal diazomethane at low temperature furnished the diester (**98**) in 96% yield. The ^{1}H-n.m.r. and mass spectra of this diester (**98**) were identical with those obtained for the acid catalyzed preparation of the ester (**98**), thus excluding the lactonization possibility.

At this point it seemed prudent to investigate the participation of the 2-hydroxyl group of the ester (**98**) during amidation with the diamine (**62**). A model study was conducted which employed diethyl oxalate (**101**) as the ester equivalent of the dimethyl ester (**98**) (Scheme 29), on the basis of its comparable reactivity towards amidation as esters bearing the iminodiacetyl moeity.



Scheme 29. (i) EtOH, reflux, r.t. → 95°C, 5h → 4days, 99%; (ii) (**62**), EtOH, r.t., 3 days, 98%.

The nucleophile which was selected for the purposes of this model study was 2-aminoethanol (**102**) where the hydroxyl and the amino groups thereof would mimic the 2-hydroxyl portion of the ester (**98**) and the free amino group in (**62**) respectively. Examination of the isolated product with the intent of establishing the reaction pattern in terms of product distribution was conducted. Of the physical techniques employed for analysis, infrared spectroscopy appeared to be the most informative. Since the possible product(s) are isomeric, mass spectroscopy should differentiate between the structures (**103**) and (**104**). The infrared spectrum of the isolated product (which was chromatographically homogeneous) revealed relevant absorption bands at 1638 and 1538 cm^{-1} which were assigned to the respective C=O amide I and II stretching frequencies. The isolated product was thus assigned structure (**103**) as no bands in the region 1730-1735 cm^{-1} (C=O ester) were observed which would have been in support of (**104**). The ^{1}H-n.m.r. spectrum confirmed the structure by showing the multiplets at δ3.8ppm (CH_2NHCO) and δ4.65ppm (CH_2OH) as well as the broad proton signals at δ4.2ppm (O*H*) and δ7.7ppm (N*H*CO). The ^{13}C-n.m.r. spectrum also revealed signals *inter alia* at δ40.51 (CH_2NHCO), δ67.67 (CH_2OH) and δ160.48ppm (NHCO). Further evidence was forthcoming from the mass spectrum where the highest peak at m/z 176 confirmed the molecular ion for (**103**). The symmetrical fragmentation of the diamide (**103**) gave a fragment ion at m/z 88. The peak at m/z 158, due to the loss of H_2O, was particularly convincing in excluding structure (**104**) in favour of (**103**).

In the light of the foregoing evidence, protection of the 2-hydroxyl group of (**98**) was deemed not necessary and therefore amidation was expected to proceed as expected without the participation of this group. Moreover, this model study established the feeble nucleophilicity of the hydroxyl group, and as such excluded any possibilities of formation of the lactone (**99**). The peak at m/z 173 in the mass spectrum of the ester (**98**) can be assigned to a loss of methanol under electron impact conditions on the

strength of this model. The possibility of dehydration due to the reflux temperatures employed for the acid catalyzed esterification was considered. The mass spectrum of the ester **(98)** revealed a peak at m/z 187, which results from the loss of H_2O from the molecular ion m/z 205. Whilst this was the case, ^{1}H-n.m.r. did not account for any alkenyl system by showing the vinyl triplet and doublet system at *ca* δ4.0-5.0ppm. Moreover infrared spectroscopy did not show any evidence of an alkenyl product as no bands at 3030 (=C-H) or 1500-1600cm-^{1} (C=C) were observed. Therefore, based on the evidence of ^{1}H-n.m.r. and infrared spectroscopy the peak at m/z 187 in the mass spectrum of **(98)** resulted from dehydration under electron impact conditions. The anomalous features in the ^{1}H-n.m.r. spectrum of the intermediate ester **(98)** were possibly due to the contaminants inherent in the starting acid **(97)**.

Having established the non-participating role of the hydroxyl prepared by acid catalyzed esterification of the corresponding acid **(97)**, the diester **(98)** was reacted with excess diamine **(62)** under the usual amidation conditions (Scheme 28). Thin layer chromatography of the reaction mixture revealed two spots. The more mobile spot (R_F 0.56-0.89) was not identified; the less mobile spot (R_F 0.20-0.44) was found to be the desired hydroxy diamide **(100)** (67%) on the basis of its ^{1}H-n.m.r. (Fig.14).

$HOCH_2CH_2N(CH_2CONHCH_2CH_2NMe_2)_2$

(100)

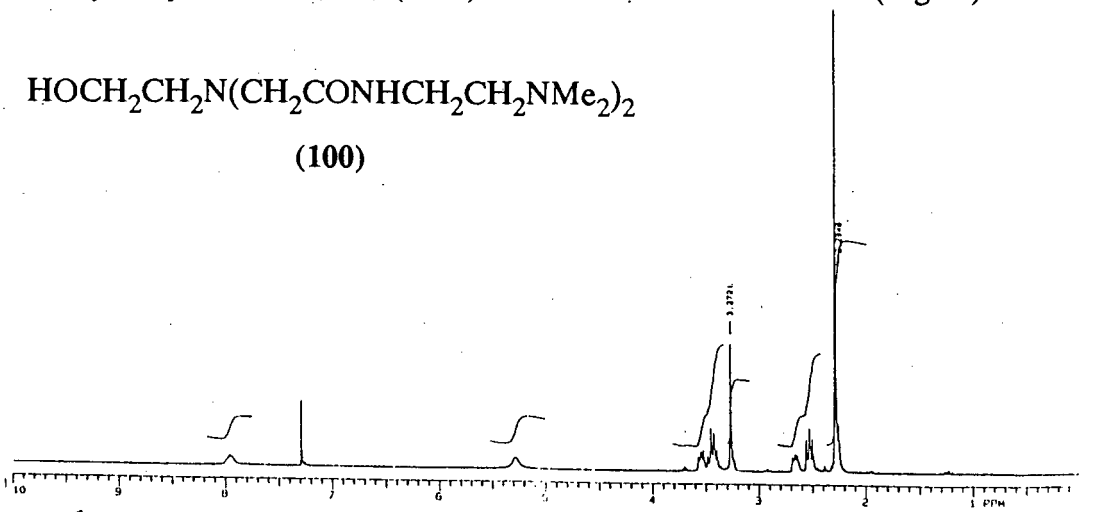


Fig.14. ^{1}H-n.m.r. spectrum (200MHz) of (100) recorded at 25°C.

The ^{13}C-n.m.r. spectrum (Fig.15) of the diamide (**100**) showed the expected six line carbon resonnaces in the region δ35-65ppm. The signals due to the amide moiety of (**100**) were assigned by comparison with the ^{13}C-n.m.r. assignments of (**150**) (see Appendix 4a). The carbon signals of the N-(2-hydroxyethyl) moeity of (**100**) were assigned by heteronuclear correlation spectroscopy [HETCOR (see appendix) Table 6].

$(Me_2NCH_2CH_2NHCO\text{-})_2$

(150)

Table 6. ^{13}C-n.m.r. assignments of (100) by a HETCOR experiment.

δ_c (ppm)	Carbon assignment
36.24	CH_2NHCO
45.01	NCH_3
58.35	$CH_2N(CH_3)_2$
58.53	CH_2OH
59.55	CH_2N
60.60	NCH_2CO
169.90	$NHCO$

[b]The C-n notation is used arbitrarily to denote the carbon of assigned in (**100**) (Fig 15).

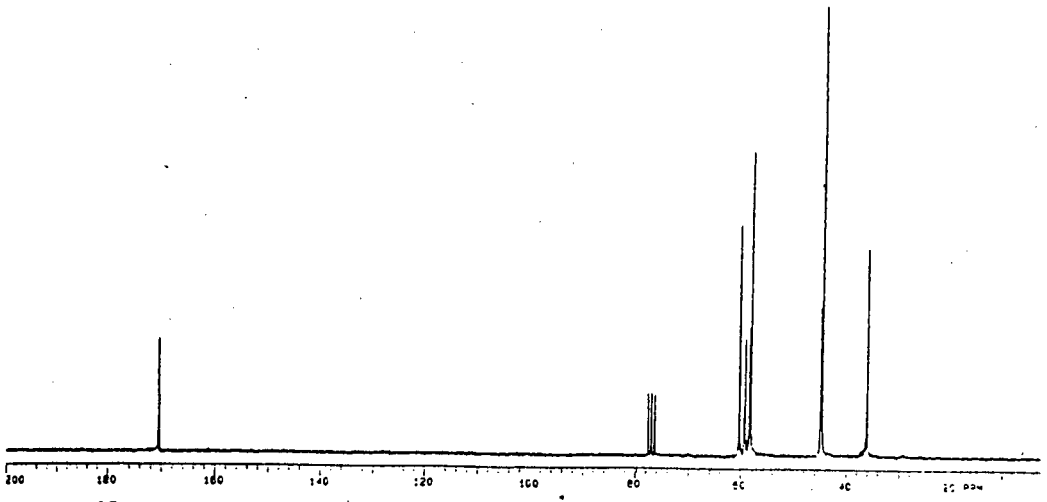
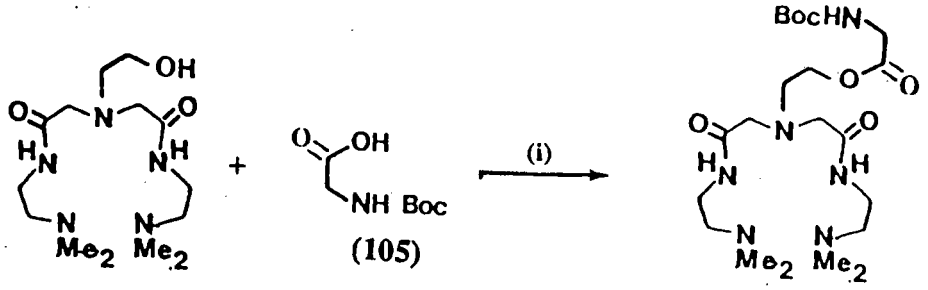


Fig.15. ^{13}**C-n.m.r. spectrum (50.3MHz) of (100) recorded at 25°C.**

The mass spectrum of the diamide **(100)** confirmed the structure by showing the peak at m/z 317, which accords with the molecular ion. The peak at m/z 299 was assigned to the loss of H_2O from the molecular ion and was strong evidence for the retention of the 2-hydroxyl group upon esterification of the diacid **(97)** (by acid catalysis) and during amidation, and thus served to substantiate further the validity of an earlier model (Scheme 29).

3.4.2.4 Attempted esterification of N-*tert*-butyloxycarbonyl-glycine (105) with the hydroxy diamide (100).

3.4.2.4.1 Steglich's esterification method.[94]



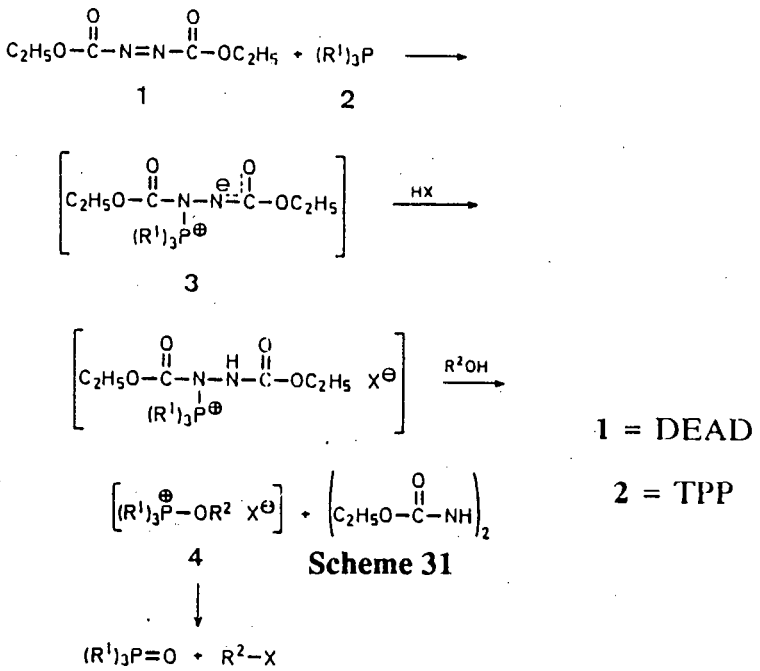
(100) **(105)** **(106)**

Scheme 30. (i) DCC, DMAP, CH_2Cl_2, r.t. → 80°C.

The coupling of a hydroxyl group and a carboxylic entity effected by dicyclohexylcarbodiimide (DCC) in the presence of catalytic amount of 4-(dimethylamino)pyridine (DMAP) to furnish an ester has been exploited in the syntheses of biologically active molecules,[95] though the yields varied between 20-45%. Recently, a similar methodology has been applied to the synthesis of (+)-colletodiol $C_{14}H_{20}O_6$[95] in good yield. The same methodology was therefore adapted to prepare the ester **(106)**, but the attempts to this end met with failure. Prolonged reaction times (up to five days), failed to yield the ester **(106)**. An alternative method which parallels this form of coupling was next investigated (section 3.4.2.6)

3.4.2.4.2 Mitsunobu esterification.[96]

In view of the failure to prepare the ester **(106)** by the abovementioned route, an alternative route was sought. The Mitsunobu coupling has been effectively and extensively utilized in, amongst others, the esterification of carboxylic acids.[96,97,98] The Mitsunobu esterification involves *inter*molecular dehydration reaction occurring between alcohols and acidic components on treatment with diethyl azodicarboxylate (DEAD) and triphenylphosphine (TPP), where DEAD acts as a dehydrating agent. Therefore the condensation of alcohols with acids mediated by DEAD/TPP system involves, in general, initial activation of DEAD as shown in Scheme 31.



1 = DEAD

2 = TPP

Scheme 31

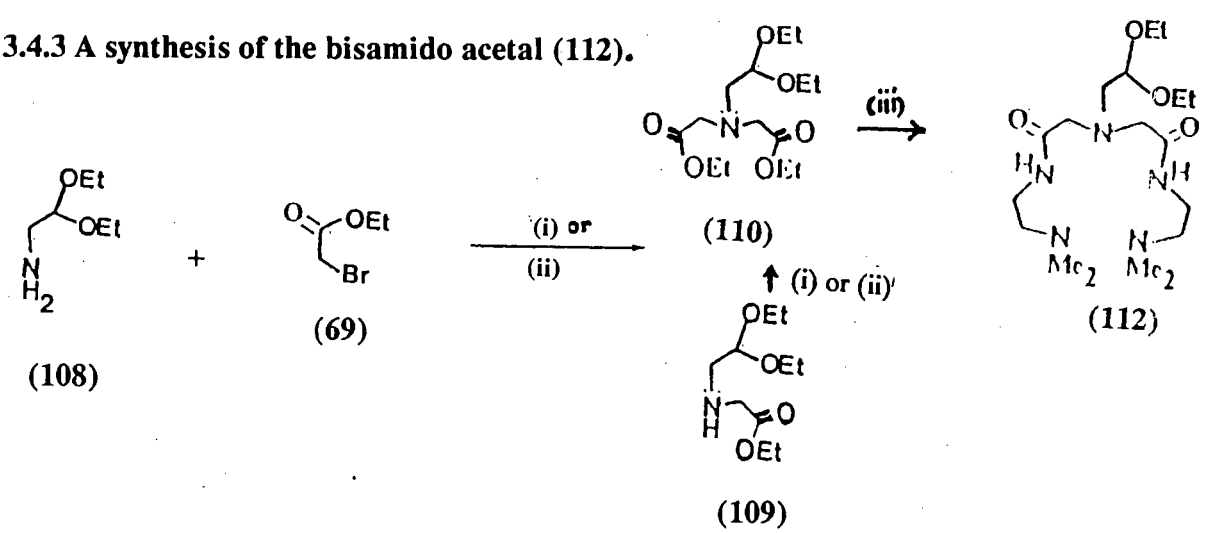
The advantage of this route as an alternative for the condensation of (**100**) and (**105**) lies in the mild and neutral conditions, as well as functional group selectivity exhibited in this condensation. Thus the compounds (**100**) and (**105**) were allowed to react in the presence of DEAD and TPP. Thin layer chromatography analysis of the reaction mixture revealed starting materials (**100**) and (**105**) after 16 hours. Prolonged reaction times at room temperature for up to 3 days did not effect the reaction to any appreciable extent as judged by thin layer chromatography. A new spot with R_F 0.15 was isolated as an intracticable gum which was found to contain, in part, triphenylphosphine (TPP). Attempts to purify this residue chromatogaphically resulted in a product which gave unacceptable analyses for the expected ester (**106**).

The possibilities of the diethyl azodicarboxylate having polymerized or decomposed cannot be the reason for the sluggish nature of the reaction for it was purified prior to use and shown to be pure by ^{1}H-n.m.r. and gas chromatography-mass spectroscopy. It therefore appeared that probably the Boc-glycine (**105**), is not sufficiently acidic to effect protonation as in Scheme 31 thereby retarding the reaction.

In selecting the Boc-glycine (**105**) as the acidic component, it was hoped that, since it incorporates a protected amino group, a successful condensation would be followed by mild acid hydrolysis to provide the key intermediate with the free amino group. Subsequent treatment of this amino group with thiophosgene was to complete the task of functionalizing the pentaamine "dioxo" ligands prior to protein labeling.

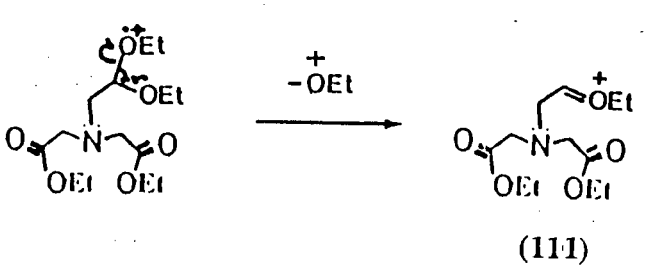
It was therefore decided to use a relatively more acidic component e.g.*p*-nitrobenzoic acid (**107**) which was thought capable of functionalizing (**100**).[The *para* disposed nitro group would also stabilize the carboxylate anion, as protonation is required in the first step (Scheme 31), and as such, ionization will occur more readily compared with (**105**)]. However, the low solubility of *p*-nitrobenzoic acid (**107**) in organic solvents prevented the test of this postulate since self-condensation reactions have been known to occur for sparingly soluble acid components.[96] At this stage, condensation of (**100**) with any suitable acid component by either the Steglich or Mitsunobu methods was not investigated further.

3.4.3 A synthesis of the bisamido acetal (112).



Scheme 32. (i) Et_3N, reflux, 2.5 days, (**110**) (45%), (**109**) (14%); (ii) CH_2Cl_2, K_2CO_3, 2.5 days, (**110**) 95%, (**62**) EtOH, reflux, 3 days, 89%.

The synthesis of the diamide (**112**) is outlined in Scheme 32. The synthesis of (**112**) was undertaken as a seemingly feasible route for the purposes of gaining entry to a suitably functionalized pentaamine "dioxo" ligand. The N-dialkylation of the aminoacetal (**108**) with ethyl bromoacetate (**69**) in triethylamine gave in 45% yield key intermediate ester (**110**) together with the mono N-alkylated product (**109**) in 14% yield [This product (**109**) was readily converted to the diester (**110**) by methods analogous to those described for (**78**), section 3.4.1.2]. Superior yields (98%) and a purer product were obtained when the N-dialkylation reaction was conducted in the presence of anhydrous potassium carbonate. The infrared spectrum of the diester (**110**) revealed the ester carbonyl stretching frequency at 1742 cm-^{1}. ^{1}H-n.m.r. spectroscopy also revealed signals due to the diester (**110**), with the triplet at δ1.2ppm [partially obscured by the acetal methyl proton signal (OCH_2CH_3) at δ1.25ppm], the quartet system at δ4.14ppm (OCH_2CH_3, ester), as well as the methylene proton signal at δ3.4ppm (NCH_2CO). All these features were consistent with the diethyl N,N-diacetate moeity of (**110**). The highest peak in the mass spectrum at m/z 305 (obtained below 100°C) corresponded with the ester (**110**). [At higher temperatures, the molecular ion was not clearly apparent; instead, the highest peak occurred at m/z 260 [M^+(**110**)-45], due to the loss of one of the four ethoxy groups. The fragmentation of the acetal function is reported to occur more readily.[99] On this basis, it is proposed to assign the loss of m/z 45 to structure (**111**) (Fig.16):



(111)

Fig.16. Proposed mechanism resulting in the loss of 45 a.m.u.

The peak at m/z 232 resulted from the fragmentation of acetal

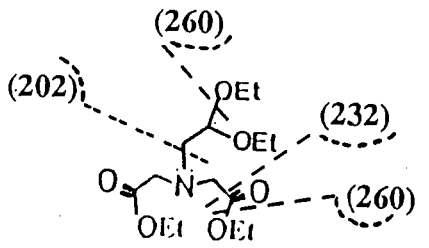
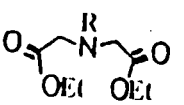


Fig.17. Fragmentation in the mass spectrum of (110).

diester (**110**) by loss of $\cdot CO_2Et$, for which there was a fragment ion at m/z 73.This mode of fragmentation, also seen in the mass spectrum of (**79**), appears to be common in the esters bearing the iminodiacetyl skeleton with the exception being the hydroxy ester (**98**). The peak at m/z 202 corresponds with the loss of the fragment $[C_5H_{11}O_2]^{\cdot}$, as confirmed by a complementary peak at m/z 103. Another commonly observed fragmentation for esters incorporating the iminodiacetyl skeleton (Fig. 18 illustrates these types) as



(**70**) R = H

(**79**) R = $PhCH_2$-

(**110**) R = $(EtO)_2CHCH_2$-

Fig.18. A representation of the esters incorporating the iminodiacyl moeity.

in (**110**), is the loss of $[C_6H_{13}O_2]$ (also seen as a complementary fragment ion at m/z 117) to give the fragment ion at m/z 188. This was also observed for compound (79), (R = $PhCH_2$), by loss of m/z 91 to give the same ion at m/z 188.

Following the successful preparation of the ester (**110**) in high yield, the next step involved the bisamidation of the diester (**110**) with the diamine (**62**) under the previously described conditions (Scheme 32) to give the acetaldiamide (**112**) in a yield of 89%, as an orange-yellow syrup. Infrared spectroscopy revealed the amide carbonyl I and II bands at 1651 and 1584 cm^{-1} respectively, as well as C-H stretches (NCH_3, and NCH_2), at 2830-2782 cm^{-1}. The ^{1}H-n.m.r. spectrum of compound (**112**) (Fig.19) revealed the usual N-methyl proton singlet at δ2.25ppm, the methylene proton signal contiguous to the N-methyl nitrogen centred at δ2.50ppm, which appeared as a deformed triplet (*J ca.* 6Hz), and the multiplet at δ3.41ppm. These features were consistent with the chain extending bisamidation by the diamine (**62**).

$(EtO)_2CHCH_2N(CH_2CONHCH_2CH_2NMe_2)_2$

(112)

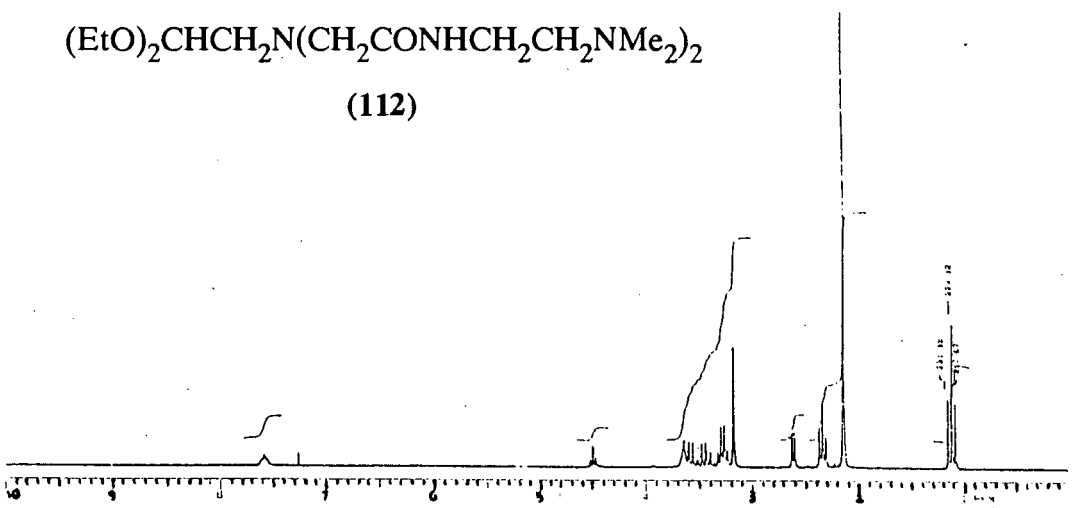


Fig.19. ^{1}H-n.m.r. spectrum (200MHz) of (112) recorded at 25°C.

The ^{13}C-n.m.r. spectrum (Fig.20) of the diamide (**112**) revealed the expected eight carbon resonances in the region δ15-115ppm. A HETCOR spectrum of compound

(**100**) (appendix) and a fully assigned ^{13}C-n.m.r. spectrum of the aminoacetal (**108**) were used to assign the ^{13}C-n.m.r. signals of the diamide (**112**).

Table 7. ^{13}C-n.m.r. assignments of the diamide (112) by comparison of the HETCORS of compounds (100) and (108).[a]

δ_c (ppm)	Carbon assignment
15.16	$OCH_2\mathit{C}H_3$
36.51	$\mathit{C}H_2NHCO$
45.04	NCH_3
57.64	$CH\mathit{C}H_2N$
58.24	$\mathit{C}H_2N(CH_3)_2$
59.80	NCH_2CO
62.58	OCH_2CH_3
101.28	$OC\mathit{H}O$
170.36	$NH\mathit{C}O$

[a]See appendix, Fig.3a.

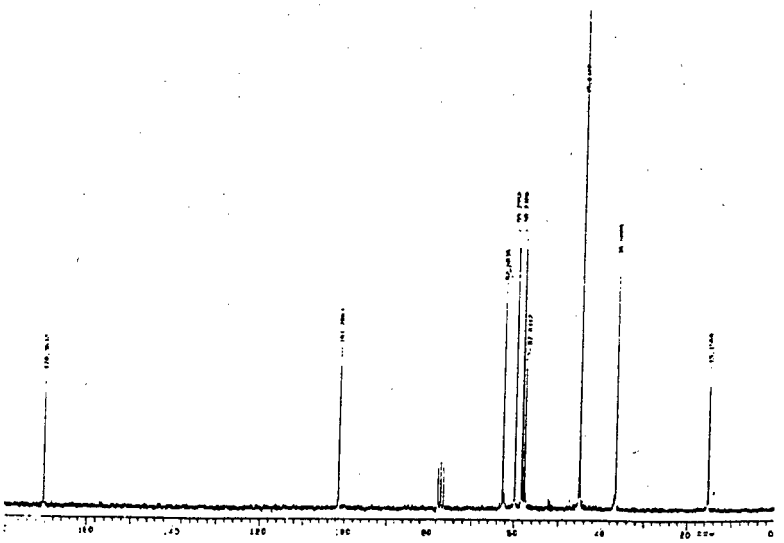
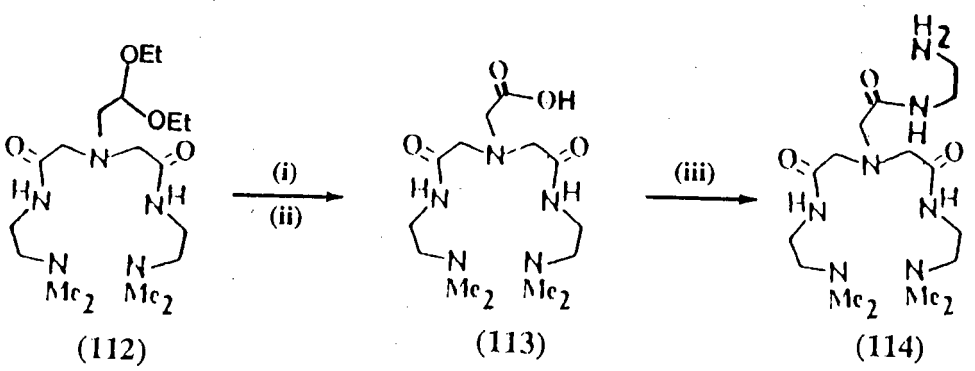


Fig.20. ^{13}C-n.m.r. spectrum (50.3MHz) of the bisamide (112) recorded at 25°C.

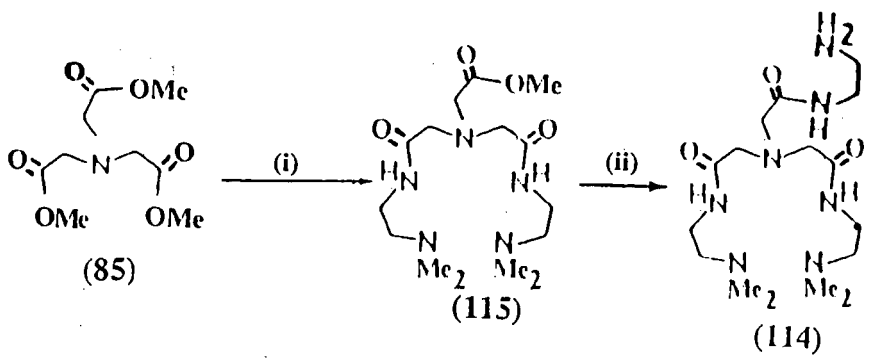
More evidence for the diamide (**112**) was adduced from the mass spectrum which revealed the highest mass at m/z 389 for the molecular ion. A detailed mass spectral assignment of the diamide (**112**) is dealt with in section 3.6.3.4.

With the successful preparation of the diamide (**112**), it was hoped that acid hydrolysis of the acetal, followed by oxidation to the carboxylic acid (**113**) would provide an alternative coupling procedure *via* either a "mixed anhydride" method,[100] or DCC-mediated coupling of (**113**) with ethylenediamine to give (**114**) (Scheme 33) as had been considered.



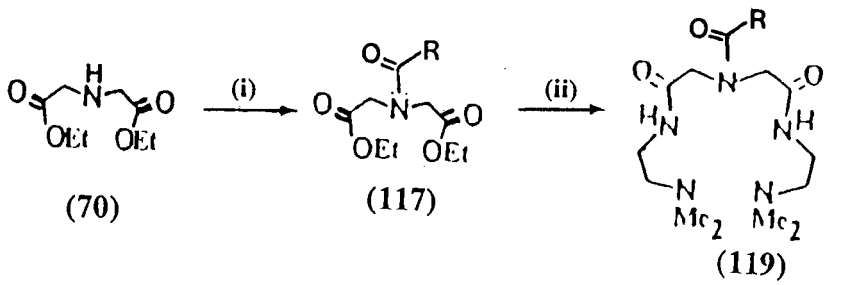
Scheme 33. (i) H^+, THF, -30°C; (ii) $KMnO_4$; (iii) DCC, $H_2NCH_2CH_2NH_2$.

The rationale employed during the early efforts towards the preparation of (**114**) involved, first, the bisamidation of one equivalent of the triester (**85**) with two equivalents of (**62**) to give the ester diamide (**115**). The ester diamide (**115**) was to be reacted with ethylenediamine to provide the ester diamide (**114**) (Scheme 34):



Scheme 34. (i) (**62**) 2.0eq, MeOH, reflux, 4-15 days; (ii) ethylenediamine, MeOH, 60°C.

Though the procedure of Scheme 34 is relatively shorter by comparison, the inefficiency of this reaction in terms of duration (15 days) to ensure bisamidation, prompted the

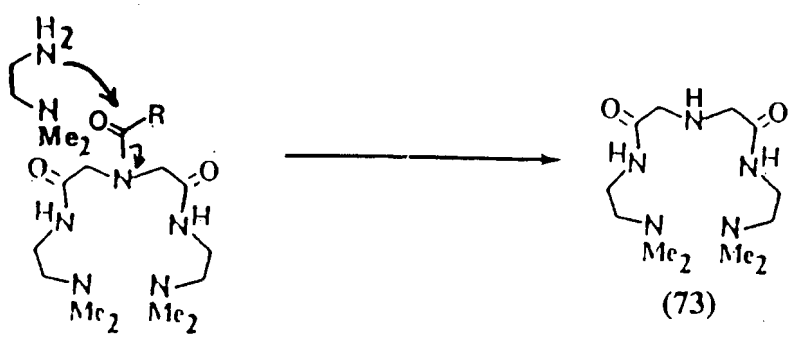


R = p-$O_2NC_6H_4$-

Scheme 36. (i) p-$O_2NC_6H_4COOH$ (**107**), DCC, CH_2Cl_2, r.t., 24h, 80% or p-$O_2NC_6H_4COCl$ (**118**), $MgSO_4$-K_2CO_3, r.t., 24h, 87%; (ii) (**62**), EtOH, reflux, 3 days.

The p-nitrobenzamido ester (**117**) was prepared by treating the diethyl iminodiacetate (**70**) with p-nitrobenzoic acid, *via* DCC coupling for 24h in a yield of 80%, as yellow crystalline flakes, $C_{15}H_{18}N_2O_7$, m.p. 69-70°C. A better yield of 87%, was obtained when the ester (**70**) was reacted with an equimolar amount of freshly-prepared p-nitrobenzoyl chloride (**118**) in dichloromethane in the presence of excess K_2CO_3. The infrared spectrum of the ester (**117**) revealed the =C-H aryl stretching frequency at 3293 cm^{-1}, the ester carbonyl at 1742 cm^{-1}, the tertiary amide carbonyl at 1641 cm^{-1}, and the symmetric and unsymmetric stretching bands for the conjugated NO_2 group at 1553 and 1350 cm^{-1} respectively. The 1,4-disubstitution band at 840 cm^{-1} was also accounted for in the infrared spectrum of the ester (**117**). ^{1}H-n.m.r. spectroscopy also confirmed the successful coupling by showing the disubstituted benzene ring proton doublets at δ7.61ppm (2- and 6-H, J 8.9Hz), and δ8.10ppm (3- and 5-H, J 8.9Hz). Mass spectroscopy further corroborated the structure (**117**) by showing the highest peak at m/z 338 for the molecular ion. Apart from the characteristic fragmentation resulting in peaks at m/z 292 (loss of one ethoxy fragment, and m/z 265 (loss of CH_2CO_2Et), the peak at m/z 188 was readily assigned to the loss of the p-nitrobenzoyl fragment, thus

provided strong evidence for the ester (**117**) [The complementary fragment ion arising from this event was also accounted for by the peak at m/z 150]. With the successful and efficient preparation of the functionalized ester (**117**), the next stage involved the bisamidation with the diamine (**62**) in refluxing ethanol for three days. Preliminary evidence for the successful bisamidation was afforded by the infrared spectrum of the isolated product which revealed the amide carbonyl I and II stretches at 1660 and 1584 cm^{-1} respectively. The infrared spectrum of the isolated product did not show the aryl =C-H stretching at 3400-3200 cm^{-1}, NO_2 symmetric and asymmetric stretches respectively at 1550 and 1350 cm^{-1}, *nor* the 1,4-disubstitution band at 840 cm^{-1}. Thus far these features accounted for the unexpected loss of the *p*-nitrobenzoyl moiety. ^{1}H-n.m.r. spectroscopy also confirmed these findings as the doublets originally at δ8.10ppm (3- and 5-H) and δ7.61ppm (2- and 6-H) in the ^{1}H-n.m.r. spectrum of compound (**117**) were not forthcoming in the same region in the ^{1}H-n.m.r. spectrum of the isolated product. To reconcile these observations, a possible event which might have led to the apparent loss of the *p*-nitrobenzoyl moeity is that proposed in Scheme 37.



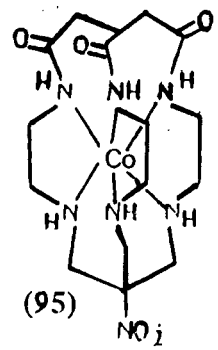
(**117**) (**73**)

Scheme 37. A proposed event leading to the loss of the p-nitrobenzoyl moeity.

The *p*-nitro group in the intermediate ester (**117**) is exerting an electron withdrawing mesomeric effect at the *ortho* and *para* positions. This will inductively enhance the electrophilicity of the tertiary amide carbonyl, and as such another electrophilic centre is established in competition with the ester carbonyl center. Given the prolonged (3-3.5 days) refluxing conditions for amidation reactions, transamidation (resulting in the loss of *p*-nitrobenzoyl moeity) is likely to have been a significant event competing with amidation at the ester carbonyl carbon. The ^{1}H-n.m.r. of the isolated product also appeared to be similar to that of the iminodiacetamide (**73**) also in terms of R_F value.

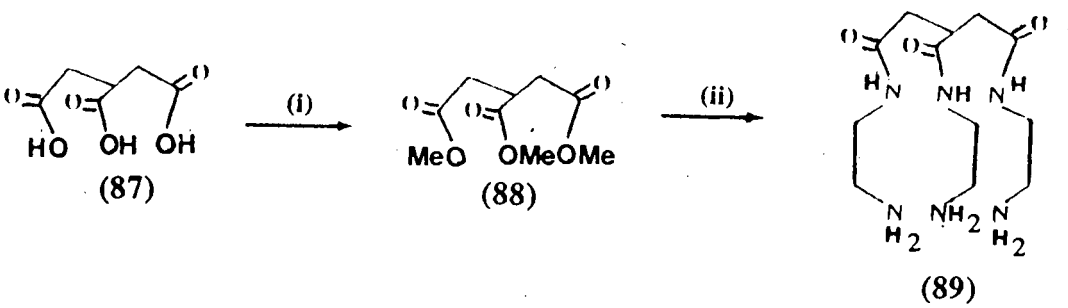
As a result of this undesirable side reaction, an alternative route was sought which would incorporate the same *p*-nitrobenzoyl moeity. The debenzylation product (**73**) of Scheme 23 was a viable key intermediate for the alternative preparation of (**119**). Thus N-alkylation of (**73**) with *p*-nitrobenzoyl chloride (**118**) in chloroform resulted in a high yield (89%) of the diamide (**119**). This was isolated as the dihydrochloride, a hygroscopic cream-white powder. The analysis of the dihydrochloride, $C_{19}H_{30}N_6O_5.2HCl$ conformed with that expected for **(119).2HCl.**

3.5 TOWARDS THE SYNTHESIS OF A FUNCTIONALIZED CAGE COMPLEX. SYNTHESIS OF THE TRICARBOXAMIDE (89). CLASS III LIGANDS.



(95)

TARGET COMPOUND

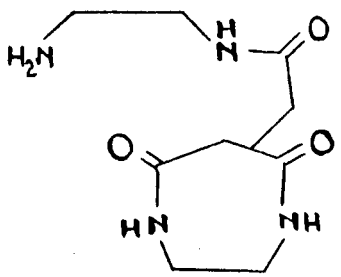


Scheme 38. (i) CH_2N_2, 0°C, 10min., 99%; (ii) $H_2NCH_2CH_2NH_2$ (xs), < 0°C, 3 days, 99%.

The rationale followed in the preparation was similar to that described for the trisamide (**86**): i.e. acid → ester → trisamide. The trimethyl ester (**88**) was prepared in 99% yield by treating the 1,2,3-propane tricarboxylic acid (**87**) with an excess of ethereal diazomethane. The infrared spectrum of the resulting ester (**88**) showed the expected ν(C=O) band at 1735 cm-^{1}. The ^{1}H-n.m.r. spectrum of the triester (**88**) revealed a multiplet at δ2.63ppm, assigned to CHC*H*$_2$CO, a quartet at δ3.19ppm (*J* 6.7Hz, C*H*CH$_2$) and the methoxy signals at δ3.61ppm and δ3.65ppm. ^{13}C-n.m.r. spectroscopy also revealed the expected five carbon resonances at δ34.99ppm (*C*H$_2$CH), δ37.26ppm (*C*H$_2$CH), d51.21ppm (CH$_2$*C*HCH$_2$) δ171.70ppm (CH$_2$*C*O), and δ173.51ppm (CH*C*O). Subsequent reaction of the trimethyl ester (**88**) with an excess of ethylenediamine at low temperature (-5°C to room temperature) gave the tricarboxamide (**89**) (99%), as a semi-crystalline, slightly hygroscopic gum which was pure by ^{1}H-n.m.r. spectroscopy and elemental analysis. The ^{1}H-n.m.r. of the tricarboxamide (**89**) revealed a multiplet at δ2.53ppm (CHC*H*$_2$CO) which appeared to be a multiplet in the ^{1}H-n.m.r. spectrum of the triester (**88**). The multiplet at δ2.64ppm, upon expansion appeared to be a pair of partially superimposed unsymmetrical triplets and satisfactorily integrated for six methylene protons

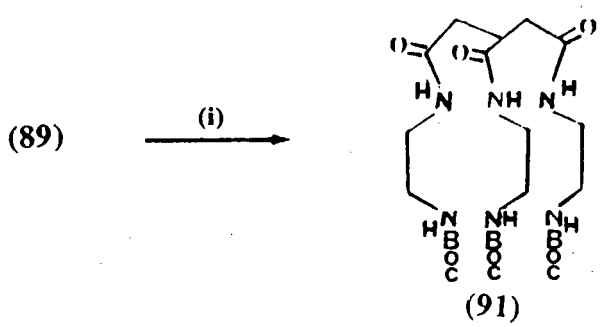
[$CH_2CH_2NH_2$, 4H belonging to the C-1 and C-3 portion of the 2-aminoethylcarboxamide moeity of **(89)** and 2H belonging to the C-2 portion of the 2-aminoethylcarboxamide moeity)]. The multiplet at δ3.05ppm was found to incorporate the methine proton signal (CH_2CHCO) [originally at δ3.19ppm in the spectrum of **(88)**], and the unsymmetrical triplet at δ3.17ppm (*J ca.* 6.3Hz) was assigned to the methylene protons adjacent to the amide function of the C-2 portion ($CHCONHCH_2CH_2$). The ^{13}C-n.m.r. spectrum of the tricarboxamide **(89)** displayed the expected eight carbon resonance which included the carbon resonances for the carbon atoms attached to the amino groups at δ40.87ppm (for the C-2 chain), and at δ42.03ppm (C-1 and C-3 chains). The spectrum of compound **(89)** also showed *inter alia*, the signals for the carbon atoms adjacent to the amide function (those on the N,N′-ethylenediamine moeity, CH_2NHCO) at δ39.49ppm (for C-2 chain) and δ38.45ppm (for the C-1 and C-3 chains). The signals at δ34.99ppm ($CHCH_2CO$) and δ38.45ppm ($CHCH_2CO$) in the ^{13}C-n.m.r. spectrum of the triester **(88)** were partially superimposed in the ^{13}C-n.m.r. spectrum of the tricarboxamide **(89)** and appeared at δ40.23ppm and δ40.28ppm respectively. The downfield shift of these signals highlights the deshielding effect of the amide function on these carbons. Furthermore, the expected two carbonyl carbon signals were observed at δ173.82ppm (CH_2CO) and δ176.23ppm ($CHCO$) in the spectrum of **(89)** [These signals were formerly at δ171.70ppm and δ173.51ppm in the spectrum of the triester **(88)**].

These results thus far excluded any possibility of *intra*molecular nucleophilic attack having occurred which would have resulted in structure **(90)**. This was found when the addition of the trimethyl ester **(88)** to ethylenediamine was conducted at room temperature. Therefore by adding the ester **(88)** slowly at low temperature to a concentrated solution of ethylenediamine, the formation of the cyclic compound **(90)** and any other undesirable side reactions was avoided.



(90)

To further establish that all the three primary amino groups in the tricarboxamide (**89**) were free, a small amount of (**89**) was treated with di-*tert*-butyloxydicarbonate in methanol (Scheme 39). The ^{1}H-n.m.r. spectrum of the trisBOC derivative (**91**), m.p.179-181°C, revealed the *tert*-butyloxy signals at δ1.45ppm and δ1.52ppm in a ratio of 2:1, and as such confirmed that the three amino groups in (**89**) are indeed free. Elemental analysis accorded with the formula $C_{27}H_{50}N_6O_9$.



(91)

Scheme 39. (i) $(Boc)_2CO$, MeOH, r.t., 16h, 75%.

The mass spectrum of the tricarboxamide (**89**) showed it to be labile as no molecular ion, expected at m/z 302 was observed. The highest fragment ion at m/z 256 (2%) was assigned to the loss of the fragment $[CH_2CH_2NH_2]^{\cdot}$ from the molecular ion. Attempts

to record the mass spectrum of **(89)** at lower temperatures failed to show any molecular ion.

3.5.1 Encapsulation of the tricarboxamide (89).

Significant tumour uptake of the radioactively-labelled cobalt cage of type **(92)**, referred to by its trivial name diNOsar" (for dinitrosarcophagine) has been observed in this laboratory.[77]

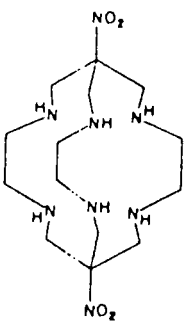
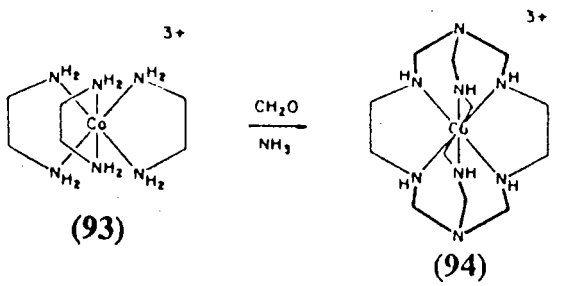


Fig.21. Structure of the "diNOsar (92).

Prior to reporting on the attempts at synthesizing the equivalent of the "diNOsar" cage, a brief review of the processes involved will be described, to put our efforts into perspective.

Cyclic multidentate chelating agents are the most potent yet often the most selective of metal coordinating agents and Sargeson *et al.*[78-83] have described the synthesis of macropolycyclic ligands especially suited for binding transition metals. The first-developed approach led to the octaazamacrocycle **(94)** (trivial name = "sepulchrate",[78-81]) which seemed accessible to several obvious changes, one of which was to vary the central atoms of the ligand "caps" (shown by heavy lines, Fig.22).

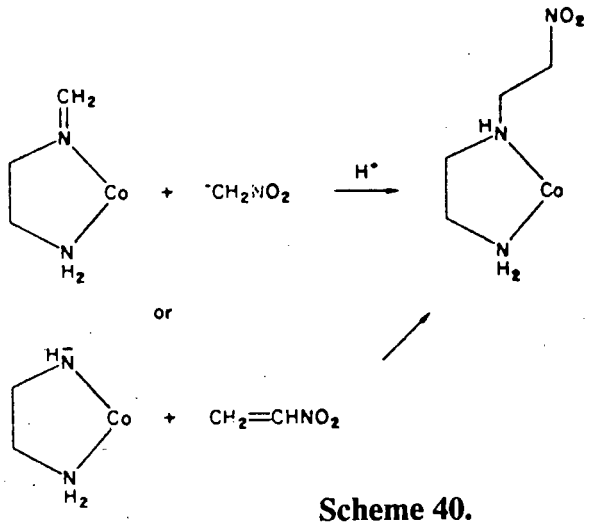


(93) (94)

Fig.22. Represantation of the first-developed procedure (94).

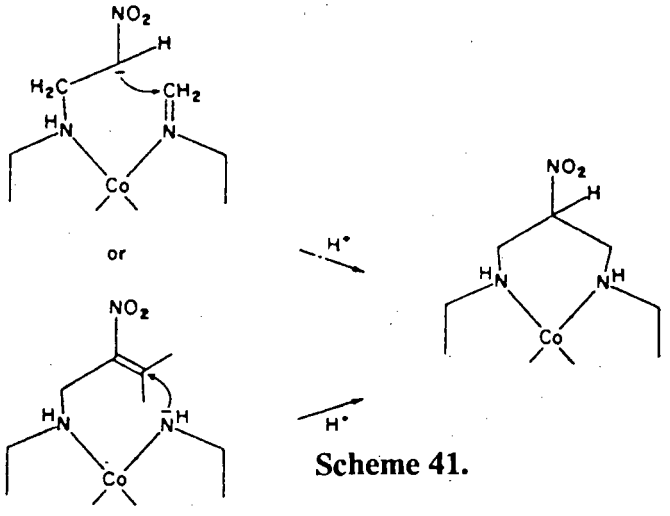
In (**94**) the nitrogen atoms are derived from ammonia formally acting as a tribasic acid under preparative conditions. In principle, any other tribasic acid can be expected to provide a substitute for ammonia. Sargeson *et al* have described a synthetic procedure for Co(diNOsar)$^{3+}$ (**92**), from (**93**)[84], *via* trisubstitution at the reactive carbon of nitromethane instead of that at the nucleophilic nitrogen of ammonia which results in the "cap" (dark lines) of (**94**).

The synthesis of this type of "cage" complex has been developed on the understanding of macrocycle formation reactions involving Co(III) amine complexes and formaldehyde in aqueous solution.[85] Sargeson[84] proposed a mechanism for the encapsulation of (**93**) to (**92**) using the nitromethane-formaldehyde system. Thus in the first step free nitromethane anion might add to a coordinated methyleneimine or free nitroethylene arising from base catalyzed condensation between CH_3NO_2 and HCHO or might be attacked by a protonated coordinate amine (Scheme 40).



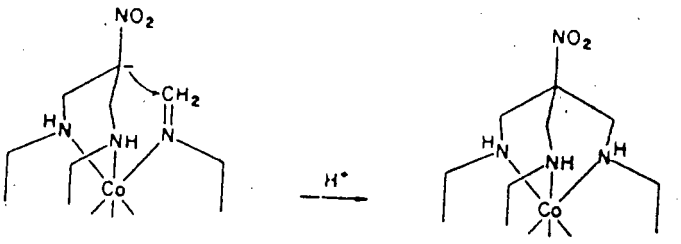
Scheme 40.

The second step would necessarily be *intra*molecular, with two possibilities (Scheme 41). The third reaction step leading to completion of the "cap" must involve carbanion addition to a coordinated imine since activated olefin can no longer be



Scheme 41.

at the now tertiary (and ultimately bridgehead) carbon (Scheme 41).



Scheme 42.

For simplicity, postulating the formation of $Co(diNOsar)^{3+}$ through a series of carbanion additions to coordinated imines was preferred, though evidence to exclude more complex processes is not conclusive. *Inter-* and *intra*molecular additions of coordinated deprotected amines to unsaturated carbon are well known.[86-89] [and must be the first step in formation of a coordinated imine from $Co(en)_3^{3+}$, (where en = ethylenediamine) and formaldehyde]. However other processes involving carbanion and other nuleophilic additions to coordinated amines are also well known.[86,89]

The interest in undertaking the same "capping" procedure on the tricarboxamide (**89**) realized the implications of introducing a group (NO_2) for subsequent functionalization. Thus the nitro group from the "capping" procedure could be reduced to the amino group, and ultimately the isothiocyanate. The highly charged nature of the cage complex used in our laboratory resulted in rapid excretion, and as such it was of interest to investigate a modified system. Bearing these factors in mind, the synthetic

feasibility of (**95**) was investigated with the view of establishing reaction conditions and yields of the subsequent steps using the inexpensive $CoCl_2.6H_2O$.

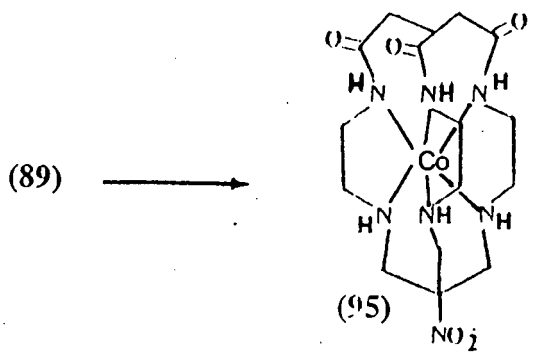


Fig.23

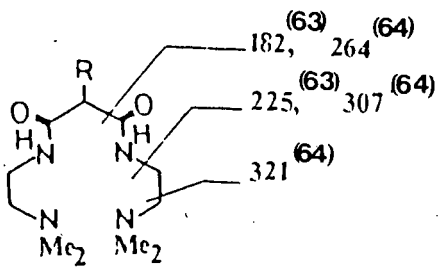
The attempted "capping" was peformed according to a variation of the procedure shown in Fig. 22, but following the method of White and Lawrence *et al.*[90]. Disappointingly, such a procedure met with failure; the isolated product proved extremely hygroscopic and prone to decomposition [as evident from the colour change (darkening) at room temperature]. Although an alternative method for effecting the desired "capping" is well documented,[84] it was not extended to the tricarboxamide (**89**) owing to the failure of the methodology of Fig.23. The method described by Sargeson *et al.*[84] has been succesfully applied in our laboratory, and by analogy, there appeared to be scope for the tricarboxamide (**89**). Further investigations in this direction were not undertaken beyond the preparation of the tricarboxamide (**89**), and could be a subject of further investigation in an independent programme. The reaction conditions for the preparation of the crucial trisamidation product (**89**) have been established as well as the efficacy (99% yield and high purity) and reproducibility of the trisamidation protocol.

3.6 MASS SPECTROMETRY FRAGMENTATION PATTERNS OF THE VARIOUS LIGANDS ("DIOXO-LIGANDS"). CLASS I AND II LIGANDS.

The mass spectral fragmentation patterns of the various ligands synthesized is discussed in this section, which highlights some characteristic fragmentation patterns of these type of ligands.

3.6.1 Ligands incorporating the derivatized malonyl skeleton. Class I.

The cyanodiamide (**63**), and the *p*-nitrobenzyldiamide (**64**), both showed similar fragmentation about the N,N-dimethylenediamine moeity. The peak at m/z 225 in the mass spectrum of (**63**) was assigned to the loss of $[CH_2CH_2NMe_2]$ and the loss of this fragment was also observed in the mass spectrum of (**64**) by the peak at m/z 307. A second cleavage by the loss of the fragment ion $[Me_2NCH_2CH_2CONH]^+$, m/z 115, was evident by the complementary peaks at m/z 182 and m/z 264 in the mass spectra of the respective diamides (**63**) and (**64**). Though the mass spectra of the diamides (**63**) and (**64**) did reveal peaks at m/z 58, attributable to $[Me_2NCH_2]^+$, the corresponding fragment ion at m/z 239 was not observed in the mass spectrum of (**63**) whereas there was a peak at m/z 321 in the mass spectrum of (**64**) which was evidence for the loss of m/z 58 fragment.



(**63**) R = $NCCH_2CH_2$-

(**64**) R = *p*-$O_2NC_6H_4CH_2$-

Fig.24. Common fragmentation pattern of ligands (63) and (64).

3.6.2 Ligands incorporating the iminodiacyl skeleton. Class II.

This class is comprised of the N-protected bisamide (**80**), the iminodiamide (**73**), N-(2-hydroxethyl)bisamide (**100**), and the trisamide (**86**). As was observed for the Class I bisamides, the peak at m/z 58 was evident in all the mass spectra of the Class II bisamides and the trisamide (**86**), corresponding with the loss of the fragment $Me_2NHCH_2^{\bullet}$. However the peaks resulting from the loss of this fragment were only seen in the mass spectra of compounds (**73**), (**80**), and (**86**) and not in that of compound (**100**). Thus the mass spectra of compounds (**73**), (**80**), and (**86**) respectively revealed the fragment ions at m/z 293, m/z 116, and m/z 344, each of which is consistent with the loss of $[Me_2NCH_2]$, i.e. 58 mass units.

The mass spectrum of compound (**100**), m/z 317 did not show the peak at m/z 259 nor did it reveal a peak at m/z 245, the former being consistent with the previously observed trends, whilst the latter would result from the loss of the fragment $[Me_2NCH_2CH_2]$. However, the peaks resulting from the loss of 72 a.m.u were observed in the mass spectra of compounds (**73**), (**80**), and (**86**). The mass spectra of compound (**80**), (**86**), and (**100**) also showed the fragment ion, $[CONHCH_2CH_2NMe_2]^+$, m/z 115. The complementary peaks resulting from such an event were accounted for at m/z 248 for compound (**80**), m/z 286 for compound (**86**), and m/z 202 for compound (**100**).

3.6.3 Fragmentation patterns unique to the respective ligands.

For clarity, the unique fragmentation patterns giving rise to the different fragments are discussed separately from those which appear to be resulting from the loss of common fragments as discussed *vide supra*.

3.6.3.1 Fragmentation pattern for (80).

$C_6H_5CH_2N(CH_2CONHCH_2CH_2NMe_2)_2$

(80)

Table 8. Fragmentations in the mass spectrum of compound (80).

m/z	Relative intensity (%)	Assignment
364	3	M^+
306	5	$M^+ - Me_2NCH_2\cdot$
293	15	$M^+ + H - Me_2NCH_2CH_2\cdot$
273	4	$M^+ - PhCH_2$
248	23	$M^+ - Me_2NCH_2CH_2NHCO\cdot$
130	20	$H + Me_2NCH_2CH_2NHCOCH_2^+\cdot$
91	100	$PhCH_2^+$

Apart from the peaks at m/z 306 and m/z 293 [M^+-72+H], of note was the clean fragmentation of the benzyl fragment $[PhCH_2]^+$, observed at m/z 91, which resulted in a peak at m/z 273 $[N(CH_2CONHCH_2CH_2NMe_2)_2]^+$. The peak at m/z 234 resulted from the cleavage of $[CH_2CONHCH_2CH_2NMe_2]^+$, m/z 130 [accounted for in the mass spectrum of compound **(80)**] from the molecular ion, m/z 364.

3.6.3.2 Fragmentation pattern for (86).

$N(CH_2CONHCH_2CH_2NMe_2)_3$

(86)

Table 9. Fragmentations in the mass spectrum of compound (86).

m/z	Relative intensity (%)	Assignments
402	6	$M^+ + H$
344	3	$M^+ + H - Me_2NCH_2$
331	28	$M^+ + 2H - Me_2NCH_2CH_2$
286	>0.5	$M^+ - Me_2NCH_2CH_2NHCO$
272	18	$M^+ - Me_2NCH_2CH_2NHCOCH_2$
130	31	$H + Me_2NCH_2CH_2NHCOCH_2^+$

The fragmentation mode for the trisamide (**86**) does not significantly differ from that of compound (**80**), as both show fragment ions corresponding with the respective loss of Me_2NHCH_2, $Me_2NHCH_2CH_2$, and $Me_2NHCH_2CH_2NHCO$ (See tables 8 and 9). Of particular note was the fragment at m/z 272, which was observed to be 1 a.m.u from compound (**73**), as well as the fragment resulting from the loss of m/z 91 in the mass spectrum of compound (**80**). A similar structure was envisioned and the following mechanism was proposed to explain how the structure corresponding to m/z 272 may be formed:

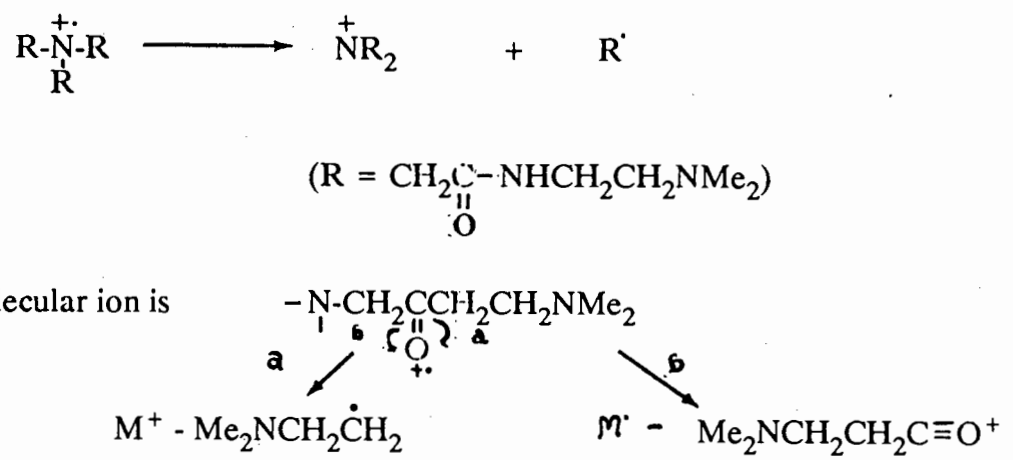


Fig.25. Proposed mechanism which results in the fragment with m/z 272.

The fragment ion at m/z 130 was indeed observed in the mass spectrum of compound **(86)**. The tendency for the mechanism depicted in Fig.25 is inherent in the proposed structure for fragment ion m/z 272.

3.6.3.3 Fragmentation pattern for (100).

$HOCH_2CH_2N(CH_2CONHCH_2CH_2NMe_2)_2$

(100)

Table 10. Fragmentations in the mass spectrum of compound (100).

m/z	Relative intensity (%)	Assignment
317	2	M^+
299	1	$M^+ - H_2O$
230	5	$M^+ - Me_2NCH_2CH_2NH\text{-}H$
188	8	$M^+ - Me_2NCH_2CH_2NHCOCH_2$
130	30	$H + Me_2NCH_2CH_2NHCOCH_2^{+\cdot}$

The peak at m/z 188 in the mass spectrum of compound **(100)** resulted from the loss of the fragment $Me_2NCH_2CH_2NHCOCH_2^{\cdot}$. The peak at m/z 299 is the result of dehydration of the molecular ion. The loss of the fragment $[Me_2NCH_2CH_2NH]$ from the molecular ion which was also observed in the mass spectrum of compound **(100)**, would account for the peak at m/z 229. The following mechanism (Fig.26) is proposed to show how the fragment ion at m/z 229 results, possibly forming the morpholinone ion.

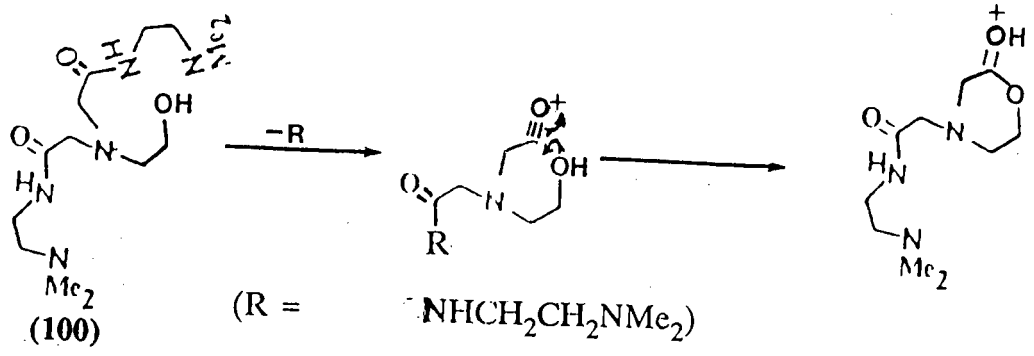


Fig.26 Proposed mechanism leading to the fragment with m/z 229.

3.6.3.4 Fragmentation pattern for (112).

$(EtO)_2CHCH_2N(CH_2CONHCH_2CH_2NMe_2)_2$

(112)

The mass spectrum of compound **(112)** revealed the expected acetal ethoxy fragmentation by showing the peak at m/z 344. The peak at m/z 103 [also observed for the ester **(110)**], complemented the peak at m/z 286.

Table 11. Fragmentations in the mass spectrum of compound (112).

m/z	Relative intensity (%)	Assignment
389	19	M^+
344	26	$M^+ - EtO\cdot$
331	0.5	$M^+ - Me_2NCH_2\cdot$
317	23	$M^+ - Me_2NCH_2CH_2\cdot$
286	44	$M^+ - (EtO)_2CH$
272	24	$M^+ - (EtO)_2CHCH_2\cdot$
260	21	$M^+ - Me_2NCH_2CH_2NHCOCH_2\cdot$
103	87	$(EtO)_2CH^+\cdot$

The aforeassigned peaks are unique to the diamide of structure **(112)**, and provide with other characteristic peaks at m/z 272, m/z 130, and m/z 73 further evidence in support for **(112)**. The peak at m/z 317 was complemented by a peak at m/z 73 (100%), and the peak at m/z 272 had a weak complementary peak at 117.

The ethyl ester monoamide (**124**), m.p. 39-40°C was formed as yellow elongated triangles in 89% yield, together with 4% of the bisamide (**128**) as white fine needles, m.p. 206-210°C. The infrared spectrum of the monoamide (**124**) revealed the expected ester and amide bands at 1735, 1662, and 1584 cm^{-1} respectively, all of which were consistent with the intended monoamidation of diethyl oxalate (**101**) with benzylamine (**76**). ^{1}H-n.m.r. spectroscopy revealed for monoester (**124**), the triplet at δ1.35 ppm (*J* 7.2Hz, OCH_2CH_3) and quartet at δ4.3 ppm (*J* 7.2Hz, OCH_2CH_3) for the ethoxy ester moiety. Furthermore, the spectrum displayed the coupling between the benzyl methylene protons ($PhCH_2$) and the amide nitrogen proton (CONH) by the two proton doublet at δ4.49ppm (*J* 6.1Hz, $PhCH_2NHCO$), which collapsed into a singlet upon D_2O exchange. Mass spectroscopy further corroborated the structure by revealing the molecular ion at m/z 207 in agreement with the molecular formula for compound (**124**).

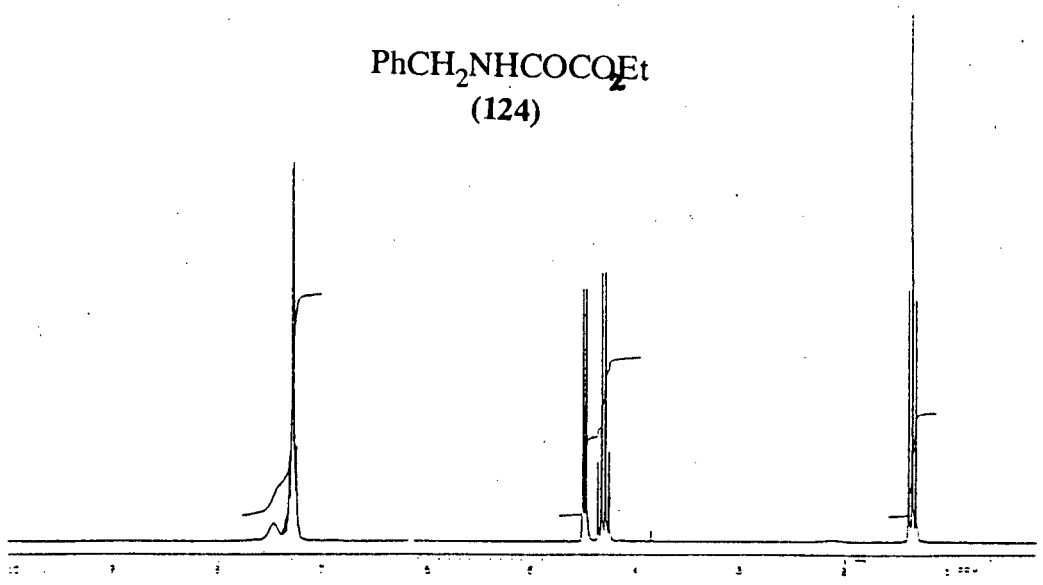
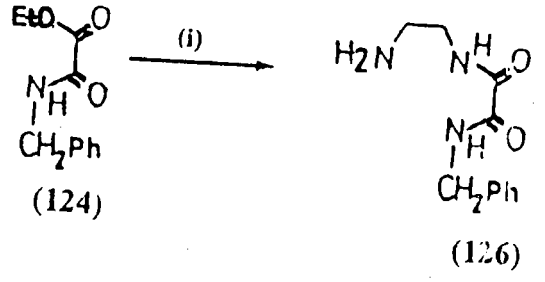


Fig.27. ^{1}H-n.m.r. spectrum (200MHz) of the ester monoamide (124) recorded at 25°C.

Having successfully and efficiently obtained compound (**124**),

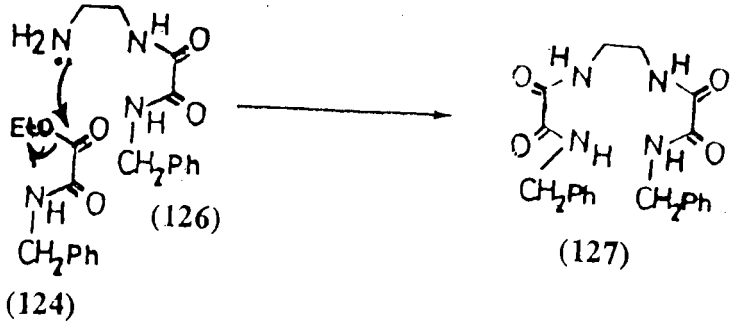
the next sequence involved the amidation of the foregoing ester (**124**) with neat ethylenediamine to provide the requisite diamide (**126**).



(124) (126)

Scheme 46. (i) MeOH-CH_2Cl_2 (1:1), $H_2NCH_2CH_2NH_2$, r.t., 18h, 50%.

The intended amidation of the ester (**124**) followed conditions reminiscent to those described for the monoamidation of diethyl oxalate (**101**) with benzylamine (**76**) (Scheme 45). The amidation procedure thus provided the pale yellow diamide (**126**), m.p. 134-136°C in a mediocre yield (50%), together with the white flakes of the tetraamide (**127**) $C_{20}H_{22}N_4O_4$, m.p. >200°C, in *ca* 50% yield.



(124) (126) (127)

Scheme 47. Intermolecular attack leading to the tetraamide (127).

The tetraamide resulted from the *inter*molecular bisamidation (Scheme 47). Characterization and confirmation of the structure of the tetraamide (**127**) was

revealed the doublet at δ4.14ppm (*J* 5.6Hz, HNCH_2CO), as well as that at δ4.67ppm (*J* 6.4Hz, PhCH_2NHCO).

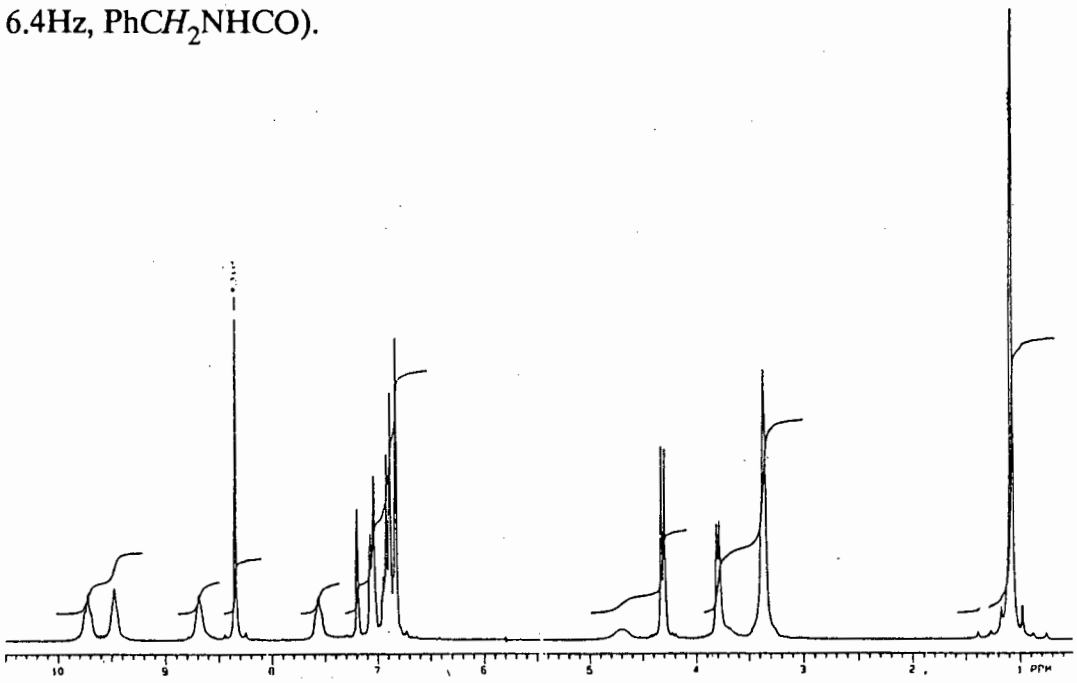
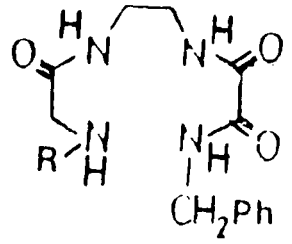


Fig.28. ^{1}H-n.m.r. spectrum (200MHz) of the trisamide (129) recorded at 25°C.

^{13}C-n.m.r. spectroscopy revealed the expected phenyl ring carbon atoms and the four carbonyl carbon resonances (Table 12).

Table 12. ^{13}C-n.m.r. partial assignments of selected characteristic signals for to the trisamide (129).

δ (ppm)	Assignments
127.49, 127.94, 128.90, 139.30	phenyl ring signals (*Ph*CH_2)
156.89	*C*O^{t}Bu
161.30, 161.60	*C*OCONHBn
170.95	*C*ONH



R = BOC-

(129)

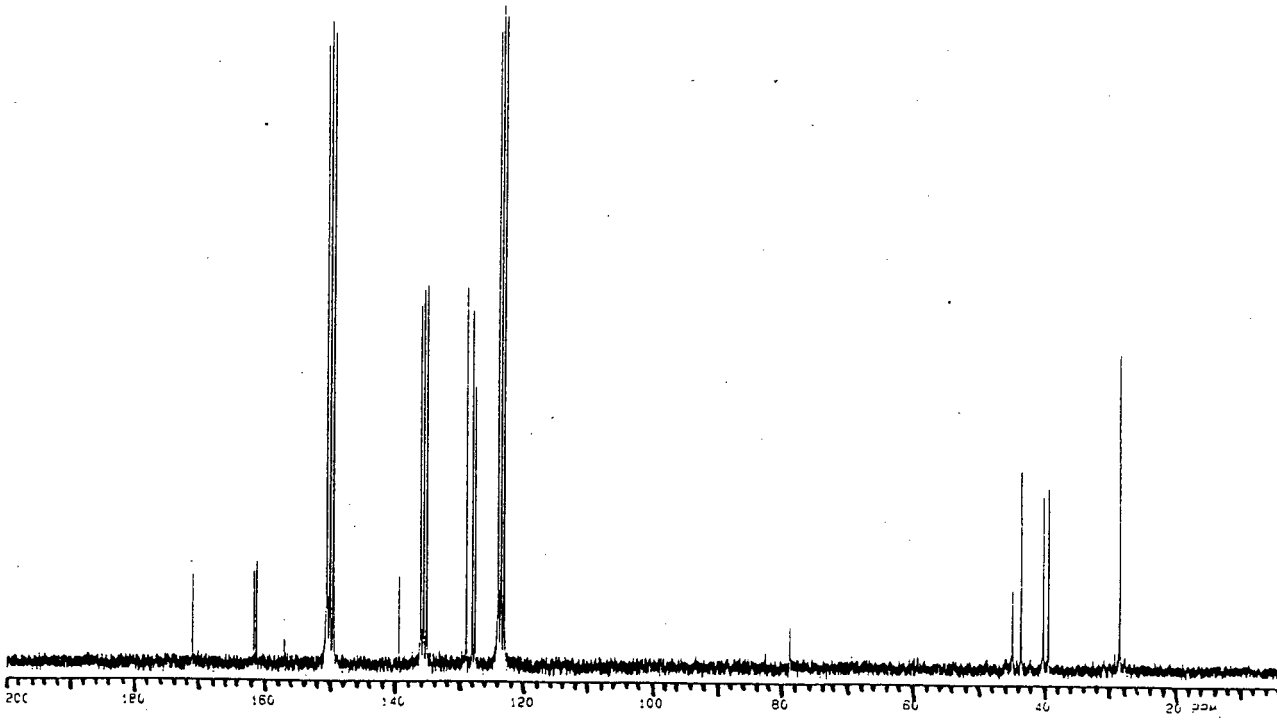
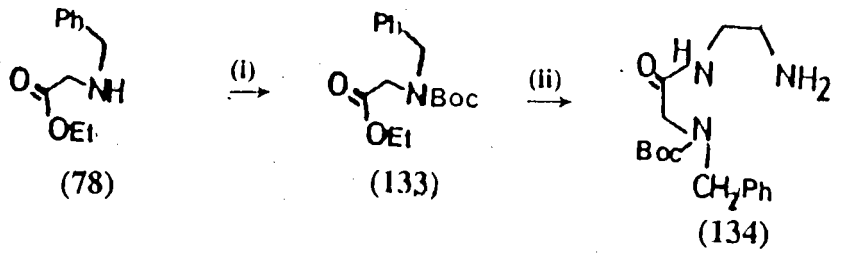


Fig.29. ^{13}C-n.m.r. spectrum (50.3MHz) of the trisamide (129) recorded at 25°C.

Removal of the N-Boc-protecting group in **(129)** would furnish **(130)**. It was decided not to proceed to this step; the usually hygroscopic nature of the hydrochloride salts [as would be the case upon hydrochloric acid treatment of **(129)**

to **(130)**] leads to difficult handling for the purposes of stability constant determinations. Fresh preparation of the trisamide **(130)** from **(129)** is therefore recommended.

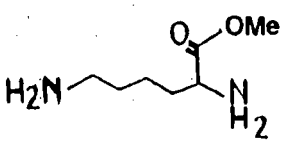
Having prepared the progenitor of compound **(130)**, i.e **(129)**, the preparation of the analogue of the former i.e. **(130)** was pursued. The construction of the trioxo ligand **(130)** was based on the utility of ethyl N-benzyl glycinate **(78)** and differs from the approach to compound **(129)** with regards to the retention of the benzyl group at the α-amino position (substitution effects about the coordinating function have been found to impart slow dissociation kinetics of metal ions[38], as well as playing a role in determining lipophilicity or lipophobicity behaviour *in vivo*; the decision therefore to retain the benzyl groups was motivated by these previously noted observations). Ethyl N-benzyl glycinate **(78)** which was prepared in good yield (72-81%) (Scheme 20) was used for the purposes of preparing compound **(133)**, the key intermediate to compound **(134)**. The tendency of the monoamidation of the "active ester" **(121)** to proceed to the diamide **(122)** had been observed earlier (*vide supra*). This methodology was expected to be more expedient compared to that invoking path (b) (Scheme 44) [**(124)** → **(126)**, 50%], but required the control of the monoamidation process. To validate an earlier intuition [i.e. that stemming from the observation that failure to provide the monoamide **(125)** from the "active ester" **(121)** was due to the highly reactive nature of compound **(121)**; thus employing the ethyl or methyl ester form was expected to provide for the intended control of monoamidation as it is of relatively diminished reactivity compared to the "active ester" form], the ethyl ester **(78)** was selected to this end as it was readily available. As a precautionary measure, ethyl N-benzyl glycinate **(78)** was protected at the α-amino position by treatment with di-*tert*- butyl dicarbonate [$(Boc)_2O$] at room temperature to provide the bisN-protected ethyl glycinate **(133)** (99.4%) (Scheme 48).



(78) (133) (134)

Scheme 48. (i) Boc_2O, $CHCl_3$, r.t., 2.5h, 99.4%; (ii) 0°C , $H_2NCH_2CH_2NH_2$, → r.t., 19h, 84%.

Parker *et al.*[42b] in their intended monoamidation of lysine methyl ester (**149**), proceded without protecting the N^{ϵ}- and N^{α}-amino groups and the monoamidation process proceded without incident (92%); Brechbiel *et al.*[7] also employed the same methodology without the protection of the N^{α}-amino group (Scheme 3, Ch2)].



(149)

The ^{1}H-n.m.r. spectrum of the bis N-protected ester (**133**) revealed the disappearance of the broad proton singlet originally observed at δ1.98ppm (N*H*) in

the ^{1}H-n.m.r. spectrum of compound (**78**); the new nine proton singlet at δ0.96ppm [$C(CH_3)_3$] provided proof for the successful *tert*butyloxycarbonylation of the secondary amine of ester (**78**).

$PhCH_2N(Boc)CH_2CO_2Et$

(133)

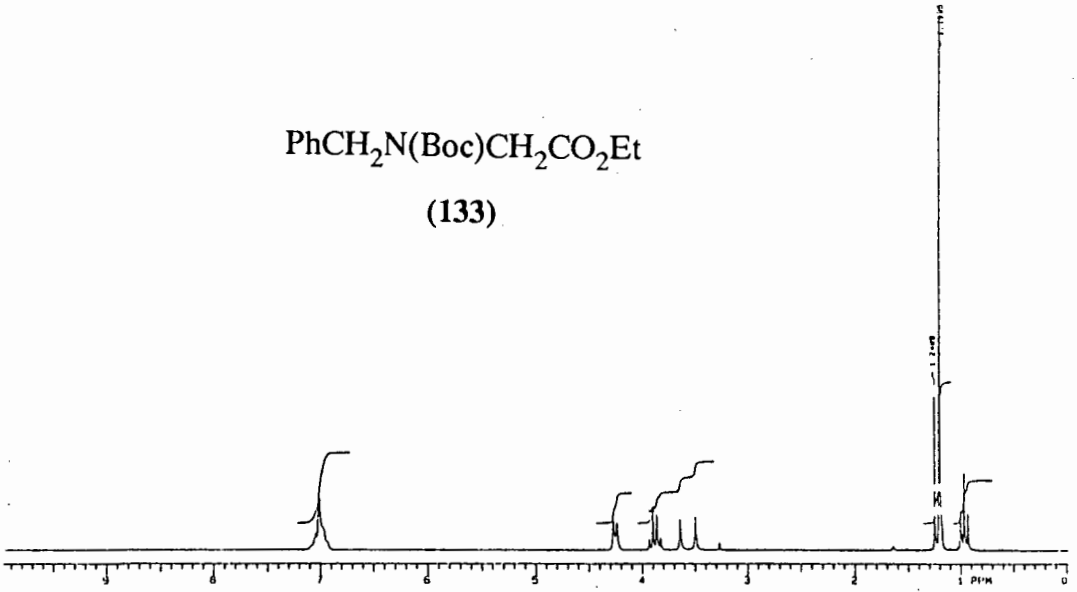


Fig.30. ^{1}H-n.m.r. spectrum (200MHz) of the bisN-protected glycine ethyl ester (133) recorded at 25°C.

Following the efficient preparation of the requisite bisN-protected ester (**133**), the next transformation required the monoamidation of the ester (**133**) to furnish the intermediate adduct (**134**). Controlled introduction of the ethyl bisN-protected glycinate (**133**) to a solution of ethylenediamine (which also served as solvent) gave the monoamide (**134**) in good yield (84%). Preliminary evidence was adduced from thin layer chromatographic analysis which revealed a ninhydrin-active spot (single spot, R_F 0.70-0.90) (after exhaustive high vacuum treatment to eliminate any adventitious amine]. The infrared spectrum of compound (**134**) revealed the disappearance of the ester carbonyl stretching frequency band observed at 1751cm^{-1} in the infrared spectrum of the original ester (**133**). Regrettably the diagnostic primary amine doublet pair was obscured by the urethane and amide N-H stretches, and the single broad band observed at 3293 cm^{-1} was assumed to incorporate

amide, urethane and primary amine N-H absorptions. The band at 1669 cm-^{1} was assigned to the amide I carbonyl stretching and that at 1546 cm-^{1} to be due to a combination of amide and urethane II stretching. The ^{1}H-n.m.r. spectrum of the monoamide (**134**) confirmed the successful monoamidation event by revealing the signals at δ2.25ppm (CH_2NH_2), δ2.65ppm (CH_2NH_2), and δ3.15ppm (CH_2N Boc), all of which were in conformity with the newly introduced 1,2-diaminoethane moeity.

$PhCH_2N(Boc)CH_2CONHCH_2CH_2NH_2$

(134)

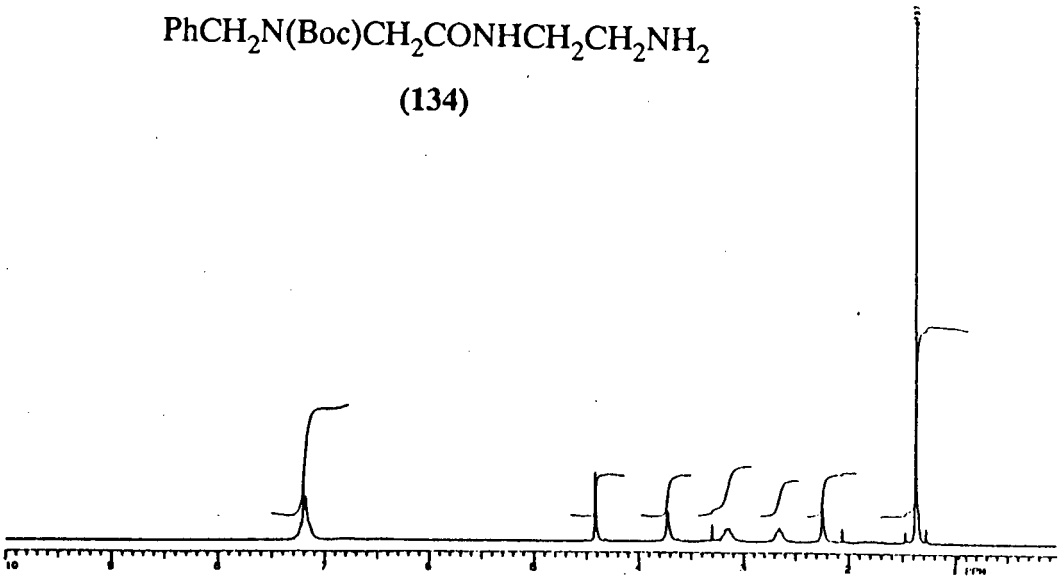
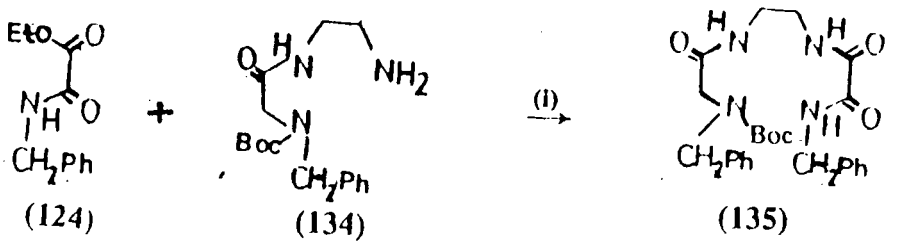


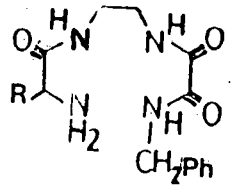
Fig.31. ^{1}H-n.m.r. spectrum of the monoamide (134) recorded at 25°C.

With the monoamide (**134**) in hand, the *inter*molecular nucleophilic attack by the free amino group of compound (**134**) on the ester (**124**) furnished the condensation product (**135**), m.p. 199-201°C, in 89% yield as a white powder.



Scheme 49. (i) **(124)**, $CHCl_3$, 16h, r.t., 89%.

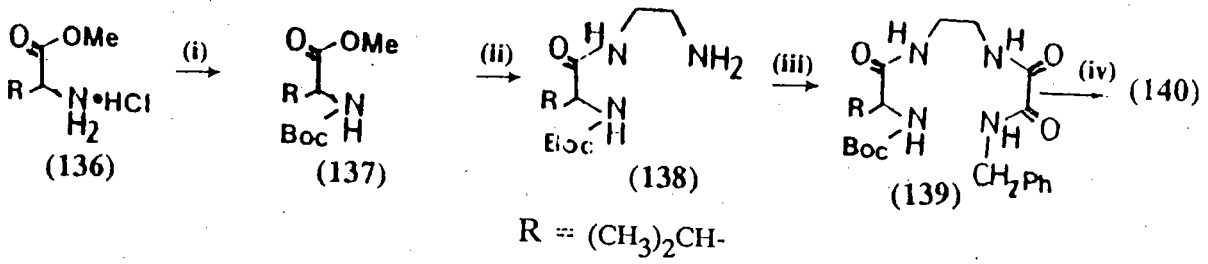
Attempts to obtain an analytically pure sample by recrystallization however failed to provide acceptable combustion analysis data. The ^{1}H-n.m.r. of the product was not recorded; the sample did not exhibit good solubility in most of the solvents commonly used [as was the case with compound **(127)**], a factor which affected the resolution and resulted in a complecated spectrum. Of the possible contaminants, residual ethylenediamine [from the preparation of the intermediate monoamide **(134)**] was likely to have further reacted with the ester monoamide **(124)** giving rise to products like **(126)** and **(127)**. Mass spectroscopy data accounted for such structures with peaks at m/z 221 [**(126)**] and m/z 382 [**(127)**] and thus substantiated the notion of ethylenediamine-mediated side reactions. As a precautionary measure, it appeared well advised to conduct a prolonged high-vacuum treatment of the monoamide **(134)** to ensure complete removal of ethylenediamine. On an analytical scale this was possible as the analytical sample of **(134)** gave satisfactory confirmatory data. Further investigation into the process of Scheme 49 was not undertaken.



R = $(CH_3)_2CH$-

(140)

The synthetic concept of Scheme 48 was extended to another analogue of compound **(129)** (substituted at the α-carbon by the isopropyl group) i.e. **(140)** and as such demonstrated the general applicability of this methodology.



Scheme 50. (i) Boc-ON, 1,4-dioxane, r.t., 2.5h, 92%; (ii) MeOH, $H_2NCH_2CH_2NH_2$, r.t., 18h, 88%; (iii) **(124)**, MeOH-$CHCl_3$, r.t., 1h, 81%; (iv) MeOH, H^+, r.t., 10min., 98%.

For the preparation of compound **(140)**, L-valine methyl ester **(136)** was first converted to the N-Boc compound **(137)** in excellent yield (92%). This was achieved by *tert*-butyloxycarbonylating the ester hydrochloride **(136)** with 2-*tert*-butyloxycarbonyl-2-phenyloxyiminonitrile (Boc-ON) in 1,4-dioxane . The infrared spectrum revealed the urethane carbonyl bands at 1663 and 1525 cm^{-1} (C=O amide I and II respectively). The ^{1}H-n.m.r. spectrum confirmed the successful protection

by displaying the signal at δ1.35ppm [C(CH_3)$_3$]. Monoamidation of the protected methyl ester (**137**) as previously described, provided the monoamide (**138**) as a yellow amorphous solid in 88% yield. The infrared spectrum provided preliminary evidence for the monoamide by revealing the absence of the ester carbonyl stretching (1742 cm-^{1}), and the presence of the expected bands at 1663 and 1584 cm-^{1} (C=O amide I and urethane and amide II respectively). Further evidence for compound (**138**) was adduced from the ^{1}H-n.m.r. spectrum which confirmed the 1,2-diaminoethane moeity with the signals at δ2.30ppm (CH_2NH_2), δ2.75ppm (*J* 5.8Hz, CH_2NH_2), and δ3.25ppm (*J* 5.8Hz, CH_2NHCO).

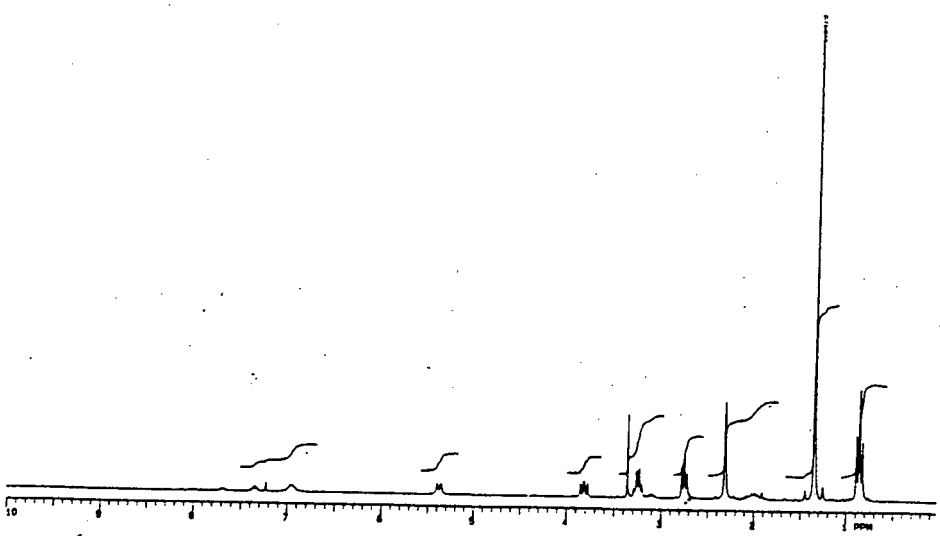


Fig.32. ^{1}H-n.m.r. spectrum (200MHz) of compound (138) recorded at 25°C.

Following the efficient preparation of the monoamide (**138**), the remaining sequence was condensation with the monoamide ester (**124**), a procedure which provided the intermediate compound (**139**) in good yield (81%) [Scheme 50, (iii)]. Preliminary evidence for the successful coupling of compounds (**124**) and (**138**) was provided by the infrared spectrum of compound (**139**) which revealed new bands for the phenyl ring at 3065 cm-^{1} (=C-H aryl) and 697 cm-^{1} (monosubstitution).

Furthermore, the ^{1}H-n.m.r. spectrum of the trisamide (**139**) displayed new signals at δ4.69ppm (PhC*H*$_2$NHCO) and δ7.25ppm (*Ph*) which were further evidence for the coupling event. More evidence for the trisamide (**139**) was adduced from the mass spectrum which revealed m/z at 420 in conformity with the molecular formula $C_{21}H_{32}N_4O_5$ for compound (**139**). The final step in the synthesis involved the removal of the Boc-protecting group *via* hydrogen chloride treatment of compound (**139**) in methanol to provide the final product (**140**) (Scheme 50, third step). ^{1}H-n.m.r. provided evidence for the successful deprotection by displaying the disappearance of the nine proton signal originally at δ1.46ppm [C(C*H*$_3$)$_3$] in the spectrum of the trisamide (**139**).

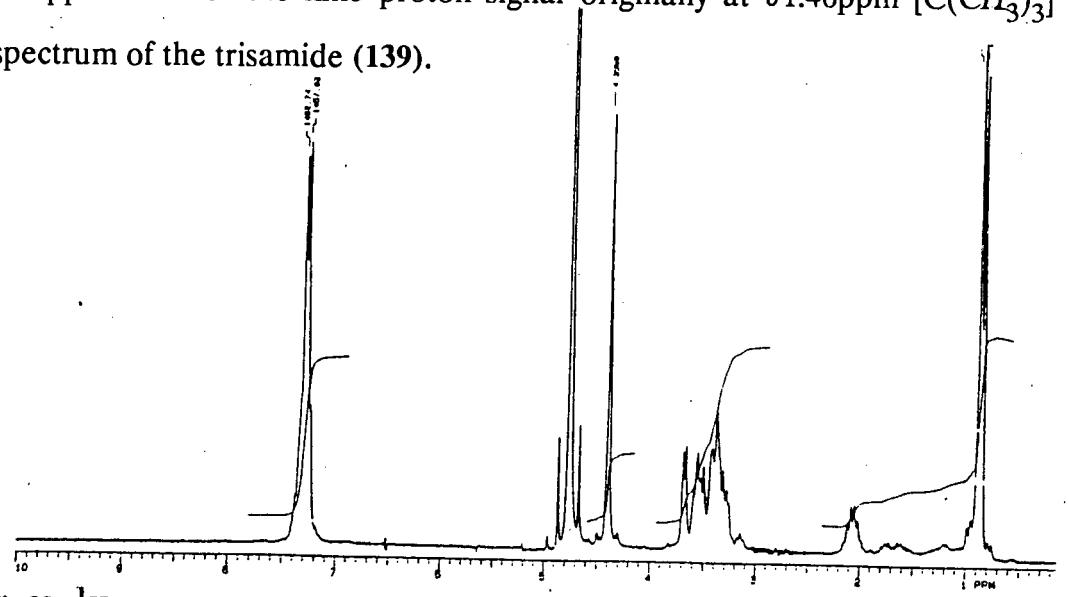
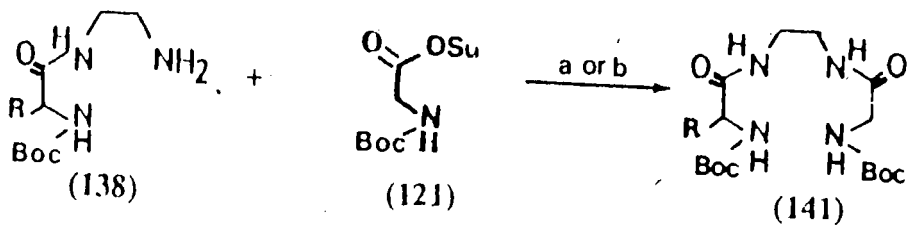


Fig.33. ^{1}H-n.m.r spetrum (200MHz) of the trisamide (140) recorded at 25°C.

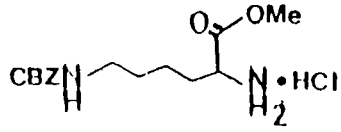
It was shown earlier that monoamidation of esters (CO_2R, R = Et- or Me-) can be controlled more readily compared to the monoamidation of "active esters", and bisamidation is a relatively insignificant event [as seen in the sequence (**121**) → (**125**) yielding mainly (**123**) whereas (**133**) → (**134**), and (**137**) → (**138**) proceeded readily without the detection of bisamidation products]. To demonstrate the generality of the monoamidation methodology based on the "conventional esters"[107], the preparation of the bisamide (**141**) was undertaken (Scheme 51).



$R = (CH_3)_2CH-$

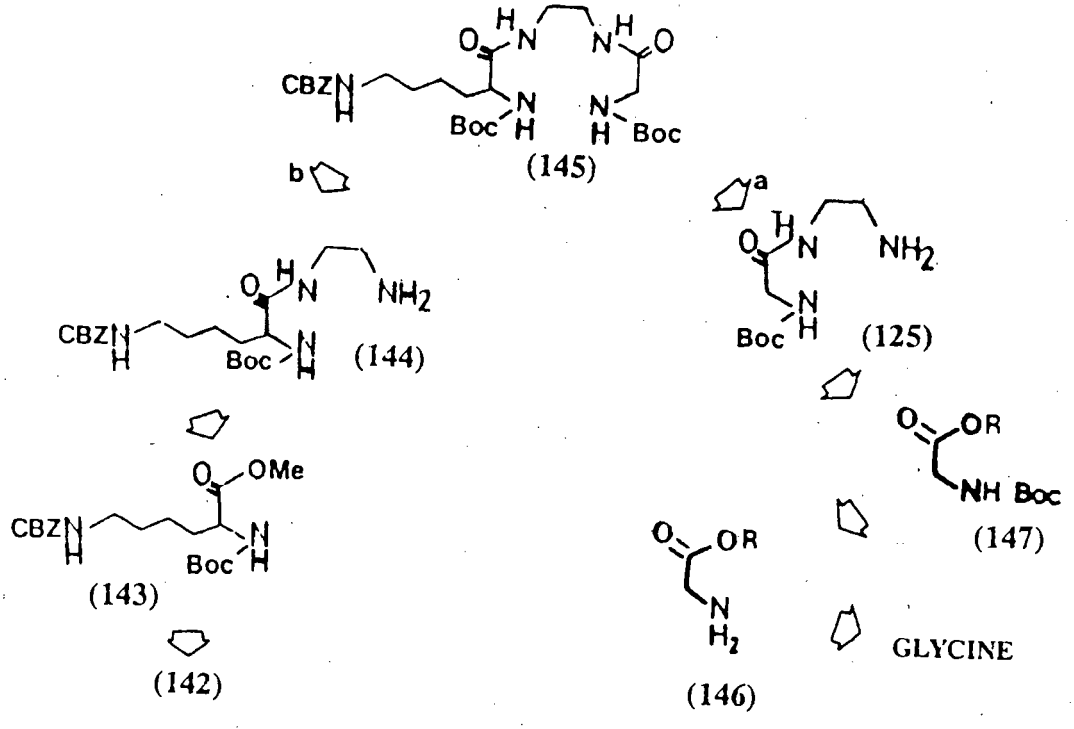
Scheme 51. (a)1,4-dioxane-$CHCl_3$ (99:1), r.t., 0.75h, 62%;(b)DCC, 1,4-dioxane, r.t., 10h, 98.6%.

Two routes leading to compound (**141**) were investigated. The route involving path (a) required the preparation of the "active ester" (**121**) whilst that of path (b) employed a direct coupling between compounds (**105**) and (**138**). The coupling process of path (a) provided the bisamide (**141**) in 78.5% yield whereas that of path (b) delivered compound (**141**) in 98.6% yield. The superior method of path (b) was the route of choice. Further evidence for the bisamide (**141**) was supported by mass spectroscopy. The mass spectrum displayed no peak at m/z 416 (M^+), but revealed the highest peak at m/z 286, corresponding with M^+-$C_6H_{12}NO_2$·, i.e. loss of $(CH_3)_3COCONHCH_2$· fragment. Combustion analysis of the hygroscopic bisamide (**141**) was satisfactory for the formula $C_{19}H_{36}N_4O_6 \cdot \frac{1}{2} H_2O$.



(142)

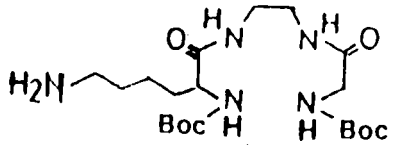
The choice of compound (142) realized the incorporation of the N^{ϵ}-amino group (this will serve as a "spacer arm" with which attachment to biological macromolecules could be effected), and which, following a series of chemoselective transformations, can be unmasked in preparation for thiophosgenation. The retrosynthetic analysis of (145) presented in Scheme 52 shows the conceptual approaches considered for the construction of the functionalized L-amino acid derived dioxo ligand(s).



Scheme 52. Retrosynthetic analysis of compound (145) which shows feasible assembly pathways.

Cleavage of the amide bond from the (1S)-5(aminopentyl)-1-(carboxyacyl) portion [path (a)] leads to (N-*tert*-butyloxycarbonylamino)-glycyl-1,2-diaminoethane (125). [Monoamidation of compound (147) with ethylenediamine also provides (125)].

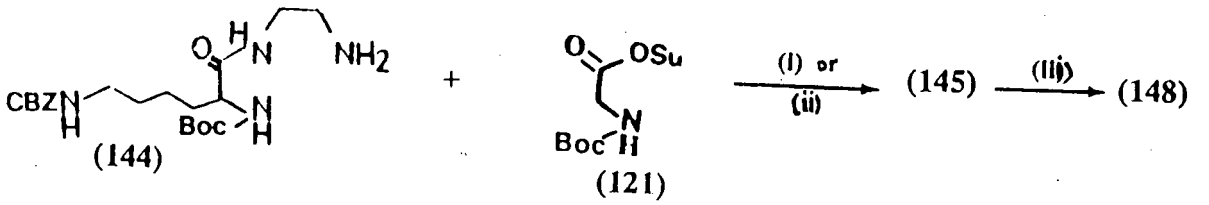
Access to compound (147) requires the initial esterification of glycine to provide the ester (146) (R = Me- or Et-), followed by *tert*butyloxycarbonylation of the N^{α}-amino group of the ester (146) to provide compound (147). The coupling of compounds (121) and (144) would furnish compound (145) the precursor to compound (148) by selective deprotection of the N^{ϵ}-amino group.



(148)

The five step sequence of path (a), would also provide a feasible pathway directed towards the assembly of compound (148). The meagre availability of glycine at the time of the study prompted the option for path (b). This situation could be circumvented by ethereal diazomethane treatment of the readily available N-*tert*butyloxycarbonylglycine (105) or esterification under Mitsunobu conditions.[96] Attempts to this end were not undertaken (the transformations towards achieving the delineated concepts of path (a) still remained involved).

Based on the involved nature of the requirements of path (a), the synthetic thrust was directed towards developing the logistics of path (b). The protection of the N^{α}-amino group was the first step of the sequence (142) → (143) → (144) → (145). The protection event proceeded efficiently (99-100%) by *tert*-butyloxycarbonylation using either di-*tert*-butyl carbonate or 2-*tert*-butyloxycarbonyl-2-phenyloximinonitrile.



Scheme 53. (i) 1,4-dioxane, r.t., 19h, 77% or (ii) DCC, (**105**), 1,4-dioxane, 96%; (iii) 10%Pd/C, MeOH-EtOAc (95:5), 36h, 98%.

The first route to be investigated was path (i) (Scheme 53), which required the condensation of the monoamide (**144**) with the "active ester" (**121**). The activation of carbonyl centres *via* the *O*-succinimide esters ("active esters") for mediating condensation reactions with nucleophilic species is a well known concept particularly in peptide chemistry.[103] The intended coupling process leading to compound (**145**) *via* path (a) was achieved in good yield (77%). Though the transformation (**144**) → (**145**) [path (a)] was plausibly efficient, it required the preparation of the "active ester" (**121**) (Scheme 43), which in turn is only formed in moderate yields (54%). In an attempt to avoid the activation process of compound (**105**) *via* the formation of the "active ester" (**121**) (Scheme 43) and with the view of optimizing the overall sequence (**142**) → (**143**) → (**144**) → (**145**), a direct coupling of compounds (**105**) and (**144**) was sought. The key facet of any successful approach stemming from compound (**105**) must rest on its intrinsic ability to undergo condensation with the monoamide without the intermediacy of compound (**121**). A dicyclohexylcarbodiimide-mediated coupling of compounds (**105**) and (**144**) [path (ii)] provided for this task satisfactorily and resulted in a 96% yield of the pale yellow slightly hygroscopic compound (**145**), $C_{28}H_{45}N_5O_8$, as confirmed by combustion analysis. The route of path (ii) required no chromatographic purification and delivered the product (**145**) in practically pure form. Evidence for the successsful coupling by both pathways (a) and (b) was afforded by ^{1}H-n.m.r. spectrum which revealed, for the bisamide (**145**), the disappearance of the proton

signal at δ1.75ppm (CH_2NH_2) [in the ^{1}H-n.m.r. of the monoamide (**144**)]. The complex signal at δ2.85-3.89ppm is assigned to the methylene protons adjacent to the primary amino group. This feature highlighted the functional group transformation and was consistent with the previously observed trends for Class I and II ligands.

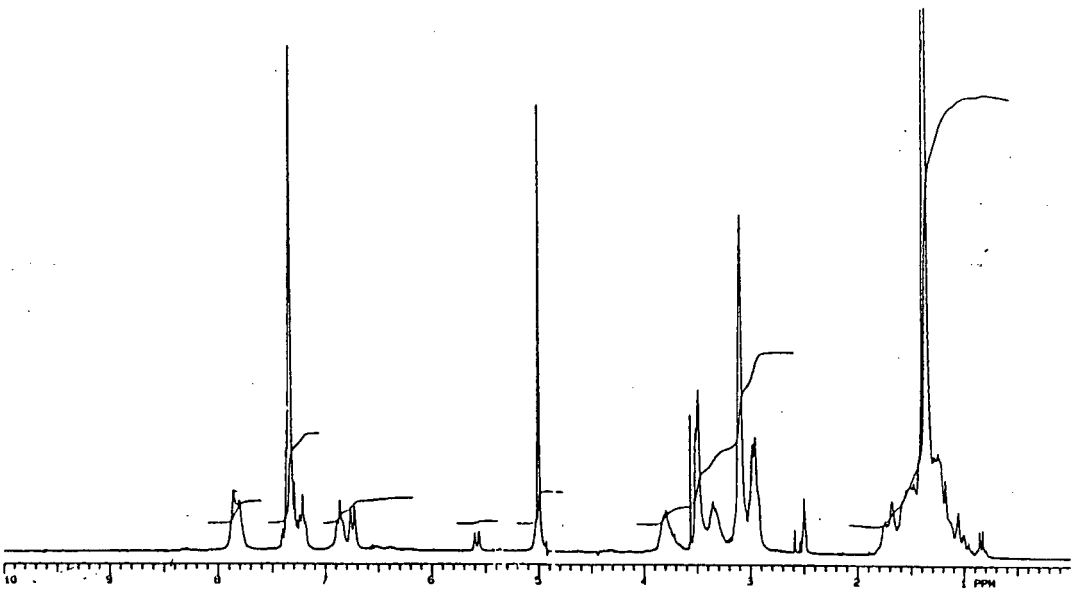


Fig.36. ^{1}H-n.m.r. spectrum (200MHz) of the bisamide (145) recorded at 25°C.

Having prepared the the bisamide (**145**), the transformation preceding thiophosgenation involved deprotection at the ϵ-amino group. This process was achieved in 98% yield by hydrogenolysis [Scheme 53, (iii)] to give the pale yellow hygroscopic powder of the aminopentyl compound (**148**) whose combustion analysis was consistent with the molecular formula $C_{20}H_{39}N_5O_6.H_2O$. The infrared spectrum revealed the absence of the bands at 3065 cm-^{1} (=C-H), and 697 cm-^{1} (monosubstitution) which served as preliminary evidence for the successful deprotection event. Furthermore, the ^{1}H-n.m.r. spectrum of the amino diamide (**148**) confirmed the successful deprotection by revealing the absence of the benzyl methylene protons (PhCH_2) [originally at δ5.05ppm in the ^{1}H-n.m.r. spectrum of the bisamide (**145**)], and the five proton signal (*Ph*) [originally at δ7.32ppm in the ^{1}H-

n.m.r. spectrum of the bisamide (**145**)]. The mass spectrum corroborated the structure by revealing m/z 446 in accordance with the molecular formula for compound (**148**).

$H_2N(CH_2)_4CH(NHBoc)CONHCH_2CH_2NHCOCH_2NHBoc$

(148)

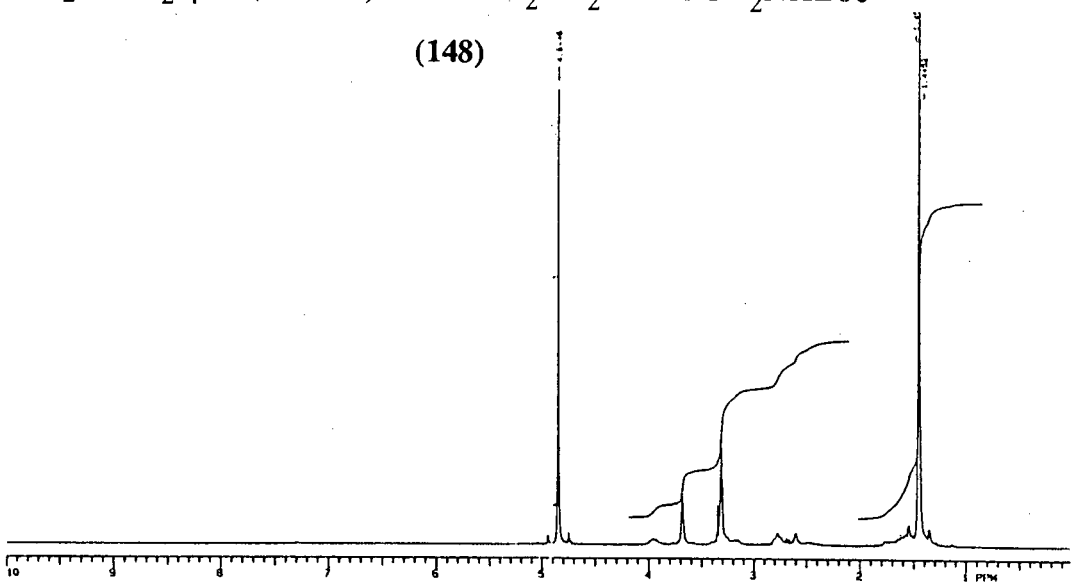


Fig. 37. ^{1}H-n.m.r spectrum (200MHz) of the aminobutyl diamide (148) recorded at 25° C.

3.8. Mass Spectroscopy of Selected Ligands Derived from Amino Acids.

The fragmentation patterns of Class **IV** ligands differ remarkably from those of observed for Class I-II systems. In this class of ligands, there exists some notable trends which serve the purpose of providing diagnostic information.

In this section, an attempt is made to identify common mass spectral patterns among the ligands as well as those features unique to the individual class. The mass spectral fragmentation of the "dioxo" series [compounds (**141**) and (**148**)] and the "trioxo" series [compounds (**129**) and (**139**)] is discussed in this section.

3.8.1 Common fragmentation patterns of the "dioxo" ligands (141) and (148).

Both compounds **(141)** and **(148)** incorporate the Boc-group in their structures and by this token, the facile fragmentation of $C_4H_9^{+\cdot}$ is accountable by the peak at m/z 57 in the mass spectra of these compounds. The peak at m/z 116 which corresponds to the fragment ion $C_5H_{10}NO_2^{+}$ was observed in both mass spectra of compounds **(141)** and **(148)**. Surprisingly, in both cases, no fragment ion corresponding to this event was present. The *tert*-butyloxy fragment ion $C_4H_9O^{+\cdot}$, denoted by a peak at m/z 73, common for both compounds **(141)** and **(148)** though not very strong, is accompanied by a stronger peak at m/z 74 ($C_4H_9OH^{+}$) in each case.

It must be stated though, that for compound **(148)**, the event resulting from the loss of the fragment ion with 73 a.m.u. can also arise from the aminobutyl residue as $[H_3N(CH_2)_4]^{+\cdot}$. The foregoing event was confirmed by a weak complementary peak at m/z 374.

3.8.2 Fragmentation unique to compounds (141) and (148).

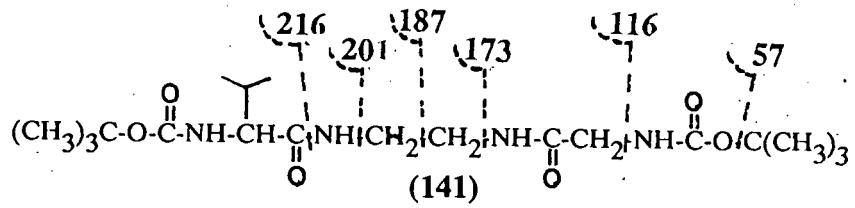


Fig.38. A diagramatic representation of the fragmentation patterns in the mass spectrum of compound (141).

$$Me_3COCONHCHCONHCH_2CH_2NHCOCH_2NHCOOCMe_3$$
$$(CH_2)_4NH_2$$

(148)

Table 13. Fragmentation in the mass spectrum of compound (148).

m/z	Relative intensity (%)	Assignment
446	3	$M^+ + H$
373	1	$M^+ + H - C_4H_{11}N\cdot$ or $-C_4H_{11}N$
326	4	$M^+ + 2H - (CH_3)_3COCO\cdot$
316	2.5	$M^+ + H - (CH_3)_3COCONHCH_2$
297	10	$M^+ - 2H - 2(CH_3)_3CO$
259	< 3	$C_{12}H_{25}N_3O_3^{+\cdot}$ or $[C_{12}H_{24}N_3O_3+H]^{+\cdot}$
245	3	$[C_{10}H_{18}N_3O_4 + H]^+$ or $[C_{11}H_{23}N_3O_3+H$
230	3	$[C_{11}H_{21}N_2O_3+ H]^{+\cdot}$
201	13	$[C_{10}H_{21}N_2O_2]^{+\cdot}$

The fragmentation patterns unique to compound **(148)** are adumbrated in Table 13. The fragmentation leading to the ion at m/z 346 was accounted for in the spectrum of compound **(148)** by the peak at m/z 101. The fragmentation resulting in peaks at m/z 245, 316, and 229 respectively were all accounted for by the complementary peaks at m/z 201, 130, and 217 respectively. Of note was the observed fragmentation about the 1,2-diaminoethane moeity which resulted in the peak at m/z 259. The foregoing event was confirmed by the presence of the complementary peak at m/z 187 (weak), but a stronger peak occurred at m/z 188. Interestingly a similare mode of fragmentation had been observed for compound **(141)**.

For the Class III dioxo ligands, the fragmentation patterns shown in Fig.38 and Table 13 provide evidence for the respective structures of compounds **(141)** and **(148)**.

3.8.3 Common fragmentation patterns of the trioxo ligands (129) and (139).

$BocNHCH_2CONHCH_2CH_2NHCOCONHCH_2Ph$

(129)

$BocNHCH(CHMe_2)CONHCH_2CH_2NHCOCONHCH_2Ph$

(139)

As for the dioxo systems, their trioxo counterparts also exhibit some diagnostic fragment ions common for both compounds **(129)** and **(139)**. Since both ligands incorporate the Boc-group, the expected fragment ions at m/z 57 ($C_4H_9^{+\cdot}$), m/z 73 ($C_4H_9O^{+\cdot}$), and m/z 116 [$(CH_3)_3COCONH^{+\cdot}$] were observed in the mass spectra of both compounds **(129)** and **(139)**. This feature had been similarly observed for the dioxo systems, and based on this observation, these peaks are therefore diagnostic of the tetraamine dioxo and trioxo ligands derived from amino acids incorporating the Boc-group.

Other common features in the fragmention pattern which are unique to compounds **(129)** and **(139)** are the fragment ions denoted by peaks at m/z 91 ($C_6H_5CH_2^+$) and m/z 106 ($C_6H_5CH_2NH^{+\cdot}$). These features are consistent with the N-benzyloxalyl moeity of compounds **(129)** and **(139)**.

The fragmentation about the 1,2-diaminoethane moeity was also accounted for in the spectra of compounds **(129)** and **(139)**. This feature had been previously observed for the dioxo ligands. For compound **(129)**, the event resulting in the ion with a peak at m/z 230 ($M^+ - C_6H_4CH_2NHCOCONHCH_2 + H$), though weak, was accompanied by the complementary fragment ion at m/z 191. Compound **(139)**

also exhibited similar behaviour and the peak at m/z 191 was also accompanied by the complementary fragment ion at m/z 188 (M^+ - 191 + H).

For compounds **(129)** and **(139)**, the mass spectral fragmentation modes are very similar, giving rise to similar complementary fragment ions as well as the ions corresponding to the respective fragmentations.

3.8.4 Fragmentation patterns unique to compounds (129) and (139).

For compound **(129)**, the fragmentation mode discussed in the preceding section remains to be the sole observable feature characteristic of this compound. A notable deviation from this norm is the peak at m/z 279 which corresponds to M^+ - 99, 2 a.m.u. less than the fragment ion $(CH_3)_3COCO^{+\cdot}$ at m/z 101. The loss of 99 a.m.u. from the molecular ion results in a fragment ion with a peak at m/z 277. This peak is weak in the spectrum of compound **(129)**; a stronger peak at m/z 279 is assigned to the fragment ion (M^+ + 2H - C_5H_9O).

Table 14. Selected fragmentations in the mass spectrum of compound (139)

m/z	Relative intensity %	Assignment
260	15	$M^+ - C_9H_8NO_2$
249	3	$M^+ - C_9H_{19}NO_2$
212*	53	$M^+ - C_5H_{10}NO_2 \cdot C_7H_7$

The fragmentation denoted with an asterisk (*) is an intriguing one. Simultaneous fragmentation of $C_5H_{10}NO_2$ and C_7H_7 from the molecular ion is an unusual event. It was reasoned that the fragment ion at m/z 212 could have occurred possibly *via* a sequential cleavage of the $C_5H_{10}NO_2$ and C_7H_7 fragments. Thus the ion at m/z 212 would have resulted from a secondary fragmentation which follows from either the

($M^+ - C_5H_{10}NO_2\cdot$) cleavage followed by the loss of $C_7H_7\cdot$, or *vice versa*.

CHAPTER 4

4. Conclusion.

The synthesis of functionalized bisamides (**63**) and (**64**) from C-alkylated malonic esters (**57**) and (**6**) *via* bisamidation with the diamine (**62**) was successfully and efficiently achieved. Prolonged reaction periods at elevated temperatures were necessary for the efficient condensation between esters (**57**) and (**6**) with the diamine (**62**) which was required in a large excess. The reduction of the *p*-nitro group of compound (**64**) provided the amino derivative (**67**) for thiophosgenation prior to attachment to protein. The observed sluggish (*ca* 45%) reduction of the cyano compound (**63**) to the corresponding aminopropyl derivative (**66**) prompted the development of the sequence (**64**) → (**67**) for use in subsequent studies. Therefore by employing well precedented synthetic principles, the development of the novel tetraamine dioxo ligands belonging to the Class I category has been successfully realized and applied.

The synthesis of functionalized pentaamine dioxo ligands (Class II) has been successfully achieved (80%). The preparation of a transiently protected intermediate (**79**) improved the sequence (**79**) → (**80**) → (**73**), without the participation of the secondary group of the ester (**70**) in the sequence (**70**) → (**73**). The efficacy with which the intermediate (**79**) was prepared demonstrated the temperature parameter and duration of the reaction in delivering the ester (**79**). The ultimate functionalization of the pentaamine ligands of type (**73**) with compounds (**100**) and (**112**) as key intermediates met with failure. The successful preparation of the key functionalized ester (**117**) [an alternative following unsuccessful attempts in grafting N-alkylation of compound (**70**) with a range of suitable electrophiles] failed to provide compound (**119**). To this end, the debenzylation product (**73**) was committed and provided the desired intermediate

(119) efficiently. The synthetic concept of this strategy is general and open to various substituents. The preparation of compound **(119)** highlighted the successful functionalization of the pentaamine ligand **(73)**.

Furthermore, the ligand **(86)**, belonging to a Class II ligand system was efficiently prepared *via* the transformations **(84)** → **(85)** → **(86)**. This procedure provided a non-functionalized ligand system.

The preparation of the tricarboxamide **(89)** was successfully achieved in good yield (99%) from the triester **(88)** *via* trisamidation under controlled conditions. The employment of the esters of type RCO_2R'(R = alkyl, R′ = Et- or Me-) as intermediates to the respective ligands was based on the versatility of this process; this has been established in the work reported in this thesis (*vide infra*). The only prolonged amidation procedure was in the preparation of compounds **(63)** and **(64)** from esters **(57)** and **(6)** respectively; the unreasonably lengthy duration for such a transformation was successfully circumvented by employing the intermediacy of the diazide forms of esters **(57)** and **(6)**.

Though the intended "capping" of the tricarboxamide **(89)** was unsuccessful, there remains scope for the improvement of this process. The key assembly of compound **(89)** was not committed further for "capping" purposes, but served to demonstrate the control of the trisamidation reaction profile (of esters of type RCO_2R') leading substantially to the initially elusive tricarboxamide **(89)** [Incidence of *intra*molecular cyclization leading to compound **(90)** had been a significant event].

Finally, the development of functionalized and non-functionalized tetraamine dioxo and trioxo ligands (Class III) from amino acids have been successfully accomplished.

Commencing the synthesis with esters of type RCO_2R^1, followed by monoamidation with the appropriate diamine system grafted the key intermediates towards the assembly of dioxo and trioxo systems without incident. This methodology was found to be general and high yielding. Furthermore the previously noted versatility of esters of type RCO_2R^1 was affirmed by the successful, almost exclusive formation of the monoamides **(134)**, **(138)**, and **(144)** without the tendency towards bisamidation [as established by the observed bisamidation event leading to compound **(122)**] in the intended monoamidation of the "active ester" **(121)**. The requisite intermediates necessary for the coupling process with the respective monoamides was efficiently achieved by invoking the amidation sequence based on RCO_2R'-type esters [**(76)** + **(101)** → **(124)**]. The coupling of the carboxylic intermediate **(105)** with the respective monoamides **(138)** and **(144)** provided the corresponding intermediates **(141)**, and **(145)** in relatively superior yields whereas the coupling which employed the "active ester" **(121)** resulted in moderate to good yields of the intermediates **(141)** and **(145)**. Consequently the more efficient route was developed and thus established the route of choice for the assembly of systems similar (or akin) to **(141)** and **(145)**.

For the functionalized system **(145)**, the succesful demonstration of selective protecting group deprotection was to conveniently eventuate in the selective thiophosgenation at the N^{ϵ} amino group of compound **(148)**. The deprotection at the N^{α}-amino group was to be the post-thiophosgenation event. The logistics undertaken in this rationale, though not tested for practical feasibility, provided a reasonable account for the intended functionalization.

The major objectives of this project have been achieved with reasonable success, with a few areas warranting further investigation. The sequence **(4)** → **(57)** and **(6)**,

whilst providing material for proceeding into subsequent steps, *albeit* in low yields can now be avoided since the appropriately C-alkylkated malonic ester derivatives (**57**) and (**6**) are commercially available.

The unexpectedly sluggish reduction of the cyano compound (**63**) and the functionalization of the pentaamine ligands with the hydroxy diamide (**100**) as the intermediate to this end represent the only processes which met with failure.

This is the present state of the project.

Future Plans.

(1) Potentiometric investigation of the metal ion coordinating ability of Class I, II, and III ligands should provide some indication about the system exhibiting the most stability. Deprotection of the α-amino groups in compounds **(122)**, and **(141)** must precede such studies.

(2) For the functionalized ligands, notably compounds **(66)**, **(67)**, and **(148)**, thiophosgenation of the amino groups of these compounds should provide the corresponding isothiocyanates. For compound **(148)**, the ultimate step must be the deprotection of the α-amino groups to unmask the coordinating function.

(3) The investigation into the binding of the isothiocyanates from (2) to proteins is to be undertaken.

CHAPTER5

EXPERIMENTAL

Melting points were measured on a Fisher-Johns apparatus and are uncorrected. Infrared spectra were obtained as nujol mulls between NaCl plates unless otherwise stated, on a Perkin Elmer 985 infrared spectrophotometer. Unless otherwise stated, ^{1}H-n.m.r. spectra were recorded on a Bruker WH-90, 90MHz spectrometer for solutions in [^{2}H]-chloroform, the internal reference being tetramethylsilane (Me_4Si). The ^{13}C -n.m.r. spectra were recorded on the Varian VXR-200 at 50.3MHz and the solvent used is the same as that in which the ^{1}H-n.m.r. was recorded unless otherwise specified. The symbols *s*, *d*, *t*, *q*, *dq* and *m* refer to signal multiplicities singlet, doublet, triplet, doublet of quartet and unresolved multiplet, respectively. The mass spectra were recorded on a VG-MICROMASS 16F mass spectrometer at 70eV, the ion source temperature between 180-220°C. Elemental analyses were obtained on a Hireaus CHN-RAPID analyzer.

All solvents were freshly distilled prior to use. Ethanol and methanol were distilled first from calcium oxide (CaO) then from Mg/I_2. N,N-Dimethylethylenediamine (Aldrich), pyridine, and triethylamine (Merck) were each distilled first from anhydrous potassium hydroxide then from calcium hydride, and dichloromethane was distilled from calcium hydride and/or phosphorus pentoxide before use. Nitrilotriacetic acid (Aldrich) was used as received. Moisture-sensitive reactions were conducted in flame-dried glassware under dry nitrogen atmosphere and all other reactions were fitted with a guard tube containing silica gel or calcium chloride ($CaCl_2$). Dried organic extracts were filtered prior to evaporation under reduced pressure below 50°C. All reactions were monitored by t.l.c on aluminium plates coated with silica gel 60 F_{254} and the spots were visualized under ultra violet light or by exposing the plates to iodine (I_2) vapour. Column chromatography refers to dry-packed columns using silica gel as adsorbent (70-

230mesh, Merck). All solvents used for column chromatography were of reagent grade and were distilled before use. The ratios of solvent mixtures used for column chromatography refer to volume by volume (v/v) unless otherwise stated. Light petroleum refers to the fraction of b.p. 60-80°C and ether to diethyl ether.

Diethyl 2-cyanoethylmalonate (57). - Method (a). To a solution of freshly distilled diethyl malonate **(4)** (16g, 100mmole) and freshly cut sodium metal (1.15g, 50mmole) in absolute ethanol (125mL) was added dropwise 3-bromopropionitrile **(54)** (6.7g, 50mmole) over 0.25h followed by heating under reflux for 8h. The excess ethanol was evaporated under reduced pressure and the reaction mixture was poured into ice-water and the whole was extracted with ether (4x50mL). The combined extracts were washed with deionized water (3x50mL) and dried (Na_2SO_4). The filtered ethereal extract was evaporated and the resulting residue was fractionated *in vacuo* to afford unreacted diethyl malonate (3.25g) at 44°C /0.6mm as forerun. The ^1H- n.m.r. of the second fraction revealed traces of diethyl malonate. Refractionation of this fraction *in vacuo* gave the desired **cyano ester (57)** (1.25g, 12%) as a colourless liquid b.p. 120°C /1.0mm (Lit.[68] 104-110°C/0.6mm) (Found: C, 56.5; H,7.0; N,6.8. $C_{10}H_{15}NO_4$ requires C, 56.3; H, 7.0; N, 6.6%). The spectral data and t.l.c behaviour of this fraction was in agreement with the same compound arrived at by method (c) below.

Method (b).

3-Iodopropionitrile (55). - Bromopropionitrile **(54)** (5g, 37,3mmole) was stirred with potassium iodide (9.1g) in methylethylketone (MEK) and the resulting yellow solution was heated under reflux for 19h. The mixture was cooled and filtered and the yellow precipitate was washed with small volumes of methylethylketone. The washings were combined and evaporated under diminished pressure to give a pale yellow residue

which was dissolved in benzene (25mL) and shaken with 10% sodium thiosulphate (20mL). The organic layer was further washed with deionized water (2x25mL), and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave a residue which upon fractionation *in vacuo* afforded the **title compound (55)** (6g, 89%) b.p. 65-70°C/5.0mm. (Found:C, 20.1; H, 2.4; N, 7.9. C_3H_4NI requires C, 19.9; H, 2.2; N, 7.7%);ν_{max} (KBr, film) 552, and 498 (C-I) cm^{-1}; ^1H n.m.r: δ2.98 (2H, m, CH_2CH_2CN), and 3.06 (2H, m, CH_2CH_2I); m/z (rel. int.%) 181 (100%, M^+), 141 (9), 127 (31), and 54 (90).

To a well stirred solution of sodium (120mg, 5.1mmole) in dry absolute ethanol (15mL) and diethylmalonate **(4)** (1.60g, 10mmole) was added dropwise 3-iodopropionitrile **(55)** (920mg, 5.1mmole) over 10 minutes. The mixture was stirred rapidly at room temperature for 4h, then heated under gentle reflux for a further 5h. The reaction mixture was allowed to cool to ambient temperature and left to stir for 16h. Work up as in method (a) above gave unreacted diethylmalonate (800mg) b.p. 45°C/0.6mm and fractions which, though homogeneous on t.l.c, gave unacceptable analyses for the ester **(57)**. This reaction was not investigated further and the prefered route to the cyanoester **(57)** was by method (c) below.

Method (c). To an ice-cooled stirred solution of freshly cut sodium (540mg, 23mmole) in dry ethanol (triply distilled from Mg/I_2, 100mL) and freshly redistilled diethylmalonate **(4)** (69g, 432mmole), was added slowly acrylonitrile **(56)** (20g, 377mmole) over 1.5h such that the temperature remained below 35°C. The reaction mixture was allowed to stir for a further 0.6h under continuous ice-cooling of the flask. Work up was as described in method (a) above. The following fractions were obtained: (i) unreacted **diethyl malonate (4)** (13.76g) b.p. 44°C /0.65mm; (ii) the desired **ester (57)** (20.23g, 60%), b.p. 104-105°C /0.6mm, 115-120°C /0.9mm, [Lit.[68,69], as in method

(a)] (Found: C, 56.1; H, 7.1; N, 6.6. $C_{10}H_{15}NO_4$ requires C, 56.1; H, 7.0; N, 6.6%); ν_{max} (neat) 2243 (C≡N), and 1735 (C=O) cm-^{1}; ^{1}H-n.m.r: δ1.20 (6H, t, *J* 7.2Hz, OCH_2CH_3), 2.1-2.7 (4H, m,CH_2CH_2CN), 3.49 (1H, t, *J* 7.8Hz, OCC*H*CO), and 4.20 (4H, q, *J* 7.2Hz, OCH_2CH_3); m/z (rel. int.%) 213 (100, M^+), 168 (64), 69 (60), and 55 (100); and (iii) a dark yellow oil which crystallized on cooling to give **diethyl bis(2-cyanoethyl)malonate (59)** (14.20g, 14% based on original acrylonitrile), as white needles. m.p. 60-61°C (ethanol-water) (Lit.[68] 61.5°C) (Found: C, 58.9; H, 7.0; N, 10.5. $C_{13}H_{18}N_2O_4$ requires C, 58.6; H, 6.8; N, 10.6%); ^{1}H n.m.r: δ1.28 (6H, t, *J* 7.2Hz, OCH_2CH_3), 2.1-2.6 (8H, m, CH_2CH_2CN), and 4.20 (4H, q, *J* 7.2Hz, CH_2CH_3); m/z (rel. int.%) 266 (100, M^+), 221 (20), and 108 (100).

N,N'-Bis[2-(N''N''-dimethylamino)ethyl] 2-cyanoethylmalondiamide **(63).** - Diethyl 2-cyanoethylmalonate **(57)** (2.00g, 9.4mmole) and N,N-dimethylethylenediamine **(62)** (3.30g,38mmole) were dissolved in 96% ethanol (20mL) and heated under reflux on an oil bath (95°C) with vigorous stirring. The reaction was monitored by t.l.c [eluant: ethyl acetate-methanol, 7:3] and shown to be complete after 10 days. The excess ethanol was evaporated under reduced pressure and the resulting orange-yellow amorphous residue was triturated with ether brought about immediate crystallization of the *product* **(63)** (2.50g, 90%) as pale yellow flakes m.p. 122-127°C (light petroleum-ethyl acetate-ether) (Found: C, 56.8; H, 9.3; N, 23.7. $C_{14}H_{27}N_5O_2$ requires C, 56.6; H, 9.1; N, 23.6%); ν_{max} 3287 (N-H amide) , 1662, and 1584 (C=O amide) cm-^{1}; ^{1}H n.m.r. (200MHz) δ2.0-2.05 [4H, m, obscured by $N(CH_3)_2$, CH_2CH_2CN], 2.1 [12H, s, $N(CH_3)_2$], 2.35 [4H, m, $CH_2N(CH_3)_2$], 2.8 (1H, deformed t, *caJ* 7.5Hz, OCC*H*CO), 3.27 (4H, m, CH_2NHCO), and 8.05br (2H, s, CON*H*, D_2O exchangeable); ^{13}C-n.m.r. δ29, 34, 37, 45, 58, 170.5, and 172; m/z (rel. int.%) 297 (8, M^+), 228 (13), 210 (7), 174 (18), 88 (24), 71 (91) and 69 (46).

N,N'-Bis[2-(N'',N''-dimethylamino)ethyl 2-aminopropylmalondi amide **(66).** - **Method (a).** The cyano diamide **(63)** (220mg, 0.97mmole) was dissolved in ethanol (10mL) followed by the addition of Raney nickel (W-2 type) (*ca* 200mg in 5mL ethanol) in a single portion. Ammonia (15mL) was added and the reaction vessel, fitted with a magnetic stirrer, was placed in an autoclave where the temperature was maintained between 80-90°C under hydrogen at 10 bars. After 6h, the catalyst was removed by filtration through a Celite 535 bed in a sintered glass funnel. The ethanol and residual ammonia were evaporated off under reduced pressure to afford a product whose analysis was at variance with that for the desired title diamide **(66)**. The prefered method to the amino diamide **(66)** was by method (b) below.

Method (b). The cyano diamide **(63)** (400mg) was dissolved in dry methanol (25mL) and the resulting solution was cooled to 5°C (ice-salt bath) followed by saturation with dry hydrogen chloride gas for 0.5h. The reaction flask was then charged with platinum oxide (PtO_2) and the whole was hydrogenated at atmospheric pressure until the uptake of hydrogen ceased (24h). The reaction mixture was filtered through a celite 535 bed on a fine fritt funnel which was washed well with dry methanol (15mL). Slow evaporation of the filtrate at 45°C under high vacuum gave the *title compound* **(66)** (630mg, 96%), as a hygroscopic trihydrochloride salt. ^{13}C-n.m.r. revealed that the reaction had gone *ca* 45% to completion.

Diethyl *p*-nitrobenzylmalonate (6). - To a well stirred solution of diethylmalonate (3.2g, 20mmole) and sodium (320mg, 13.9mmole) in dry absolute ethanol (40mL) was added a hotethanolic solution of *p*-nitrobenzyl bromide (3g, 13.9mmole). The reaction mixture was heated under reflux for 18h. Ice (50g) was added and the resulting precipitate of **diethyl bis-(p-nitrobenzyl)malonate (60)** was filtered and washed with cold ethanol (20mL) and oven dried (70°C) (3.6g, 60%), m.p. 166-167°C (ethanol)

(Found: C, 58.2; H, 5.1; N, 6.5. $C_{21}H_{22}N_2O_6$ requires C, 58.6; H, 5.1; N, 6.5%); ν_{max} 1735 (C=O), 1600 (C=C), 1552 and 1355 (NO_2 conjugated) cm-^{1}; ^{1}H- n.m.r. δ1.12 (6H, t, *J* 7.2Hz, OCH_2CH_3), 3.27 (4H, s, $PhCH_2$),4.08 (4H, q, *J* 7.2Hz, OCH_2CH_3), 7.29 (4H, d, *J* 8.1Hz, 2- and 6-H) and 8.10 (2H, d, *J* 8.7Hz, 3- and 5-H); m/z (rel. int.%) 430 (75%,M^+), 416 (7), 316 (12), 267 (15), 252 (8), and 57 (100). The filtrate was evaporated to dryness and exhaustively extracted with ether (4x50mL). The combined ether extracts were dried (Na_2SO_4), evaporated to dryness and the resulting oil was chromatographed on a silica gel column [eluant: petroleum ether-ethyl acetate, 9:1-8:2] to yield the starting material, *p*-nitrobenzyl bromide (**5**) (90mg, 3%), and the **title compound** (**6**) (1.8g, 44%) as pale yellow powdery crystals. m.p. 59.5-60°C (ethanol-water) (Lit.[41], 58-60°C) (Found: C,56.8; H, 5.75; N, 4.65. $C_{14}H_{17}NO_4$ requires C, 56.9; H, 5.8; N, 4.7%); ν_{max} 1736 (C=O), 1605 (C=C), 1552 and 1350 (NO_2 conjugated) cm-^{1}; ^{1}H-n.m.r (200MHz) δ1.26 (6H,t, *J* 7.14Hz , OCH_2CH_3), 3.29 (2H, d, *J* 7.75Hz, $PhCH_2CH$), 3.65 (1H, t, *J* 7.75Hz, $OCCHCO$), 4.15 (4H, q, *J* 7.14Hz, OCH_2CH_3), 7.35 (2H, d, *J* 8.7Hz, 2- and 6-H) and 8.10 (2H, d, *J* 8.7Hz, 3- and 5-H); m/z (rel. int. %) 295 (70%,M^+), 250 (60), 204 (57), 194 (82), and 176 (74).

N,N'-Bis[2-(N' ',N' '-dimethylamino)ethyl]p-nitrobenzymalondiamide (**64**). - A slurry of diethyl *p*-nitrobenzylmalonate (**6**) (1.0g, 3.39mmol) in 96% ethanol (8mL) was warmed on an oil bath (60°C) until dissolved. The triply-distilled N,N-dimethylethylenediamine (**62**) was added in three portions at 24h intervals over 3 days (Total: 12g, 136mmole). The reaction mixture was vigorously stirred for 0.25h followed by heating under reflux on an oil bath (100-110°C) with continuous stirring. The reaction mixture was worked up as before. Crystallization of the amorphous residue was promoted by the addition of small volumes of ether (2x5mL, total) to give the *title diamide* (**64**) as orange-yellow flakes (900mg, 70%) m.p. 144-145°C (Petroleum ether-ether) (Found: C, 56.5; H, 7.7; N, 18.3. $C_{18}H_{29}N_5O_4$ requires C, 56.9 ;H, 7.7 ;N, 18.5%); ν_{max} 3315 (N-H amide), 1664,

1553 (C=0 amide I and II), 1602, 1513 (C=C), 1533, 1346 (NO_2 conjugated), 835 and 749 (1,4-disubstitution) cm-^{1}; ^{1}H-n.m.r. (200MHz) δ2.13 [12H, s, N(CH_3)$_2$], 2.2-2.45 [4H, m, CH_2N(CH_3)$_2$], 3.1-3.35 (7H, m, CH_2CH_2NHCO, PhCH_2 and OCC*H*CO), 6.9br (2H, s, CON*H*), 7.35 (2H, d, *J* 8.85Hz, 2- and 6-H) and 8.10 (2H, d, *J* 8.85Hz, 3- and 5-H); m/z (rel. int.%) 379 (6%, M^+), 350 (17), 307 (13), 264 (3), 219 (4), 115 (18), and 58 (100).

N,N'-Bis[2-(N'',N''-dimethylamino)ethyl]-2-(4-aminobenzyl) malondiamide (**67**). - The foregoing *p*-nitrobenzyl diamide (**64**) (230mg, 0.61mmole) in ethanol was charged with 10% Pd-C (70mg) and was hydrogenated at atmospheric pressure until no further hydrogen uptake was observed (16h). The catalyst was filtered off through a Celite 535 bed in a small sintered funnel. Removal of the ethanol under diminished pressure afforded the *amino diamide* (**67**) (210mg, 98%) as an orange hygroscopic powder. (The elemental analysis for this product was unsatisfactory owing to its hydroscopic nature.); ν_{max} 3300, 3321 (N-H), 1664, 1553 (C=O amide I and II), 1600, 1515(C=C), 835 and 748 cm-^{1} (1,4-disubstitution); ^{1}H-n.m.r. (200MHz) δ2.13 [12H, s, N(CH_3)$_2$], 2.2-2.5 [4H, m, CH_2N(CH_3)$_2$], 3.1-3.4 (7H, m, CH_2NHCO, PhCH_2CH, OCC*H*CO), 6.9br (1H, s, N*H*CO, D_2O exchangeable), and 7.43 (4H, m, 2-H, 3-H, 4-H, 5-H, and 6-H); m/z (rel. int.%) 349 (10%, M^+), 279 (15), 234 (10), 146 (11), and 106 (35).

N-Benzylglycin ethyl ester (78). - Method (a). To a vigorously stirred solution of benzylamine (**76**) (2g, 19mmole) in dry triethylamine (15mL), was added ethyl chloroacetate (**77**) (2.29g, 19mmole) in one portion. The reaction mixture was stirred at room temperature for 0.5h during which a precipitate of triethylamine hydrochloride formed. The reaction mixture was warmed on an oil bath (60°C) and left stirring for 20h. Triethylamine was evaporated under reduced pressure and the resulting residue

was poured into a saturated solution of potassium hydrogen carbonate (20mL). The whole mixture was extracted with ethyl acetate (3x100mL) and the extracts were combined, washed with deionized water and dried (Na_2SO_4). Evaporation of the dried organic extracts afforded **ethyl N-benzylglycinate (78)**, pure by ^{1}H-n.m.r. (2.65g, 72%) as a yellow liquid, b.p. 125-130°C/7.0mm (Lit.[73] 139-140°C/11mm) (Found: C, 68.6; H, 7.5; N, 7.3. $C_{11}H_{15}NO_2$ requires C, 68.4; H, 7.8; N,7.3%). The ^{1}H-n.m.r. and behaviour on t.l.c [eluant:benzene-ethanol, 5:1] were the same as that for the compound prepared by method (b) below.

Method (b). Benzylamine (**76**) (10g, 9.35mmole) in dry ether (60mL) was cooled to 0°C and ethyl bromoaacetate (**69**) (7.44g, 44.6mmole) was added during 0.17h. After 0.75h the reaction mixture was filtered to remove the precipitate and the ethereal phase was evaporated to give a yellow oily residue which was taken up in carbon tetrachloride (CCl_4). The whole was washed well with deionized water (4x100mL), dried (Na_2SO_4) and evaporated under diminished pressure giving an orange oil. Column chromatography on silica gel [eluant: benzene-ethanol, 95:5] of the oil furnished first **diethyl N-benzyliminodiacetate (79)** [100mg, 1% based on ethyl bromoacetate (**69**)] (Found: C, 64.4; H, 7.8; N, 5.1. $C_{15}H_{21}NO_4$ requires C, 64.5; H, 7.5; N, 5.0%). This compound is best prepared by method (b) below. [T.l.c behaviour and ^{1}H-n.m.r. of this product were in agreement with the product of method (b)], and **N-benzylglycine ethyl ester (78)** (7g, 81%) as a dark yellow liquid b.p. 130-135°C/8.5mm [Lit.[73], same as in method (a)] (Found:C, 68.3; H, 7.8; N, 7.3. $C_{11}H_{15}NO_2$ requires C, 68.4; H, 7.8; N, 7.3%); ν_{max} (film) 3524, 3338 (imine N-H), 1735 (C=O ester), 1584w (N-H bending), 1515 (C=C), 737 and 697 (monosubstitution) cm-^{1}; ^{1}H-n.m.r (200MHz) δ1.20 (3H, t, *J* 7.14Hz, OCH_2CH_3), 1.98 (1H, s, CH_2NHCH_2, D_2O exchangeable), 3.39 (2H, s, $NHCH_2CO$), 3.62 (2H, s, $PhCH_2$),

4,14 (2H, q, *J* 7.14Hz, OCH_2CH_3) and 7.30 (5H, m, *Ph*); m/z (rel. int. %) 193 (11%, M^+), 120 (17), 106 (17) and 91 (100).

Diethyl N-benzyliminodiacetate (79). - Method (a). To a well stirred solution of benzylbromide (200mg, 12.6mmole) in diethyl ether (40mL), was added, during 0.4h an ethereal solution of diethyl iminodiacetate (**70**) (109mg, 0.58mmole). The cloudy reaction mixture was warmed to 50°C (oil bath), for 2.5h. The precipitate that formed was filtered, and the solvent was removed under reduced pressure. Column chromatography of the resulting residue, eluting with benzene-ethanol, 99:1-95:5 provided the excess benzylbromide, followed by the **title compound** (**79**) (80mg, 5%); subsequent fractions furnished the ester (**70**) (189mg, 80%). The title compound is best prepared by method (b) below.

Method (b). To a stirred solution of benzylamine (**76**) (4g, 37.4mmole) in triethylamine (70mL) was added, during 0.5h, ethyl bromoacetate (13.7g, 82.0mmole) which resulted in the immediate formation of a white precipitate. The reaction mixture was heated under reflux for 18h and worked up as described in the synthesis of the monoester (**78**) above, except that the aqueous phase was saturated with sodium chloride and the whole was exhaustively extracted with ethyl acetate (7x100mL). The combined extracts were washed with deionized water (8x100mL) and dried ($MgSO_4$). Evaporation of the dried organic phase left a dark orange residue which upon column chromatography [eluant: benzene-ethanol, 95:5] afforded first the **title compound** (**79**) (6.8g, 65% based on benzylamine) [R_F 0.72 (benzene-ethanol, 5:1: Lit.[73], 0.74] (Found: C, 64.2; H, 7.4; N, 5.0. $C_{15}H_{21}NO_4$ requires C, 64.5; H, 7.5; N, 5.0%); ν_{max} (film) 1750 (C=O ester), 1515 (C=C), 737 and 697 (monosubstitution) cm^{-1}; ^{1}H-n.m.r. (200MHz) δ1.26 (6H, t, *J* 7.1Hz, OCH_2CH_3), 3.49 (4H, s, $OCCH_2N$), 3.65 (2H, s, $PhCH_2N$), 4.14 (4H, q, *J* 7.1Hz, OCH_2CH_3) and 7.30 (5H, m, *Ph*); m/z (rel.

int.%) 279 (72%, M^+),206(62), 192 (10), 188 (10) and 91 (100). The second product was **ethyl N-benzylglycinate (78)** (800mg, 11% based on benzylamine) which exhibited the same t.l.c behaviour and had the same ^{1}H-n.m.r. as the product prepared by methods (a) and (b) above.

Method (c). N-benzylglycine ethyl ester **(78)** (300mg, 1.55mmole) in triethylamine (15mL) was stirred with ethyl bromoacetate **(69)** (260mg, 1.56mmole) for 0.5h. The resulting turbid reaction mixture was heated under reflux for 6h and left at 60°C for 16h. The reaction mixture was poured into water previously saturated with potassium hydrogencarbonate. The whole mixture was extracted with ethyl acetate (3x50mL) after saturating with sodium chloride, and was extracted with ethyl acetate-chloroform (1:1, v/v, 2x50mL). The combined extracts were washed with deionized water (4x100mL), dried ($MgSO_4$) and concentrated under reduced pressure. The resulting residue was column chromatographed [eluant: benzene-ethanol, 99:1-95:5] to yield the **diethyl ester (79)** [(200mg, 46% based on N-benzylglycine ethyl ester **(78)**] (the foregoing fraction revealed the same t.l.c behaviour and ^{1}H-n.m.r. as the title compound prepared by method (b) above) and the starting material **(78)** (95mg, 32%).

Method (d). To a well-stirred solution of ethyl bromoacetate **(69)** (71mg, 0.43mmole) and N-benzylglycine ethyl ester **(78)** (112mg, 0.58mmole) in dry dimethylformamide (DMF) (10mL) at -30°C, was added slowly a mixture of sodium hydride (50% suspension in oil, 14.4mg, 0.58mmole) in dry dimethylformamide (4mL). The reaction mixture was warmed to room temperature, then to 60°C on an oil bath. Water (5mL) was added and the reaction mixture was diluted with chloroform (20mL). The organic phase was separated, dried (Na_2SO_4) and evaporated to dryness to give an orange-brown oil. Preparative layer chromatography [eluant as in method (b) above] afforded the **diethyl ester (78)** [70mg, 58% based on ethyl bromoacetate **(69)** together with

starting material (**78**) (40mg, 38%). The ^{1}H-n.m.r. of the former fraction was the same as for the chief product from method (c) above.

N,N'-Bis[2-(N'',N''-dimethylamino)ethyl]-N'''-2-benzyliminodiacetamide (**80**). - To a well-stirred solution of the diethyl ester (**79**) (650mg, 2.33mmole) in 96% ethanol (15mL) was added in one portion N,N-dimethylethylenediamine (**62**) (3g, 34mmole). The reaction mixture was heated under reflux for 30h. T.l.c [chloroform-methanol, 5:1] showed the absence of the starting ester and revealed a product spot at R_F 0.15-0.20 [eluant: methanol-chloroform-triethylamine, 20:10:1]. The reaction was allowed to stir under reflux for a further 16h. The excess ethanol and amine were removed *in vacuo* leaving a dark orange-brown viscous oil which was column chromatographed [eluant: methanol-chloroform-ammonia (aq), 60:40:10] to afford the *title diamide* (**80**) (770mg, 87%) as an orange syrup. (Found: C, 62.8; H, 8.7;N, 18.9. $C_{19}H_{33}N_5O_2$ requires C, 62.8; H, 9.1; N, 19.3%); ν_{max} ($CHCl_3$) 3310, 3060 (amide N-H), 1653, 1532 (C=O amide), 1603 (C=C), 774 and 744 (monosubstitution) cm-^{1}; ^{1}H-n.m.r. (200MHz) δ2.25 [12H, s, N(CH_3)$_2$], 2.45 [4H, t, *J* 5.8Hz, CH_2N(CH_3)$_2$], 3.17 (4H, s, NCH_2CO), 3.35 (4H, m, CH_2NHCO), 3.65 (2H, s, PhCH_2N), 7.29 (5H, m, *Ph*) and 7.5br (2H, s, CON*H*, D_2O exchangeable); ^{13}C-n.m.r: δ 36.35, 45.13, 58.42, 58.58, 59.58, 127.64, 128.49, 129.15, 136.92, and 170.10; m/z (rel. int. %) 364 (4%, M^+ + H), 363 (3), 273 (4), 248 (23), 234 (21), 134 (26), 113 (2) and 91 (100).

Synthesis of the dihydrazides of (4) and (57). General procedure. - To a stirred solution of the ester (10mmole) in absolute ethanol (20mL) at room temperature in a flask covered with aluminium foil was added hydrazine hydrate (25mmole). The reaction mixture was allowed to stir at room temperature for 24h. The excess ethanol and hydrazine hydrate was removed *in vacuo* (40°C, bath) [CAUTION: Dihydrazides

are light sensitive; (**82**) is heat sensitive]. Residual hydrazine hydrate was removed by drying in a dessicator containing concentrated sulphuric acid.

Malonic dihydrazide (81). - This was obtained as a white precipitate; purified by recrystallization from petroleum ether-ethyl acetate (8:2), then methanol-chloroform (99:1) to give a sample with a melting point 154-154.5°C (Lit.[74], 154-154.5 °C) (Found: C, 27.5; H, 6.0; N, 42.4. $C_3H_8N_4O_2$ requires C, 27.3; H, 6.1; N, 42.4%); ν_{max} 3296-3200 (N-H amine and amide), 1664 (C=O, amide I), and 1533 (C=O, amide II) cm^{-1}; ^{1}H-n.m.r. (D_2O): δ2.82 (2H, s, OCC*H*$_2$CO).

2-cyanoethylmalonic dihydrazide (82). - This was obtained as a reddish semi-crystalline material:^{1}H-n.m.r. δ (D_2O) 2.89 (1H, deformed t, *J ca* 6.8Hz, OCC*H*CO), and 2.1-2.7 (4H, m, C*H*$_2$C*H*$_2$CN). The product was both heat and light sensitive. Mass spectrometry resulted in decomposition, and no molecular ion corresponding to m/z 185 was observed. Infrared (nujol) showed similar features as for (**81**) above. The ν(C≡N) stretching frequency was not observed for (**82**).

Preparation of the Malondiamide (83) and cyano diamide (63) via the diazide derived from (81) and (82). General Procedure. - A suspension of the appropriate dihydrazide (10mmole) in tetrahydrofuran (10mL) was cooled to -30°C (acetone-dry ice). Hydrogen chloride in tetrahydrofuran (5*M*; 5mL) was added, followed by *tert*-butylnitrite or sodium nitrite (21mmole). The mixture was stirred for 0.5h at -15°C until a clear solution was obtained. After careful evaporation of the solvent (40°C/0.2mm), the residue was extracted with chloroform (2 x 15mL) precooled to -10°C, then washed with cold water, 5% aqueous sodium hydrogencarbonate, cold water, then dried ($MgSO_4$). Into the dried chloroform extract of the diazide, N,N-dimethylaminoethylenediamine (**62**) (2.1eq) was added at -5°C (ice-salt cooling) and

the whole was stirred for 24h whilst warming to room temperature. Careful evaporation of the solvent furnished the diamides (**83**) and (**63**), having the same spectroscopic properties with the diamides prepared by the amidation of esters (**4**) and (**57**).

N,N'-Bis[2-(N'',N''-dimethylamino)ethyl] iminodiacetamide (**73**). - **Method (a).** Diethyl iminodiacete (**70**) (210mg, 1.11mmole), and N,N-dimethylaminoethylenediamine (**62**) (391mg, 4.44mmole), in absolute ethanol (20mL) were heated under reflux for 3 days. After the usual work up, a pale yellow intractable gum was isolated but the elemental analysis was unsatisfactory for the desired *title compound* (**73**). Further investigation into this method of preparing the diamide (**73**) was abandonned in preference of method (b) below.

Method (b). To the N-benzyl diamide (**80**) (670mg, 1.84mmole) dissolved in absolute ethanol (15mL) was added platinum oxide (PtO_2) (50mg). The heterogeneous mixture was hydrogenated at atmospheric pressure until no further hydrogen uptake was observed (24h). The reaction mixture was worked up in the usual way and afforded the product as an orange oil which was column chromatographed [eluant: methanol-chloroform-ammonia (aq), 9:5:5] to provide the *title compound* (**73**) (490mg, 98%) R_F 0.2-0.6 (eluant as for chromatography) (Found: C, 54.0; H, 9.9; N, 25.6. $C_{12}H_{27}N_5O_2$ requires C, 53.7; H, 10.1; N, 25.8%); ν_{max} 3307, 2966 (N-H), 2681, 2469, 2336 (C-H), and 1662, 1533 cm-^{1}(C=O amide); ^{1}H-n.m.r. (200MHz) δ2.13 [12H, s, N(CH_3)$_2$], 2.33 [4H, t, *J* 6.1Hz, CH_2N(CH_3)$_2$], 3.15 (4H, s, NCH_2CO), 3.25 (4H, m, CH_2NHCO), 3.4br. (1H, s, CH_2N*H*CH_2,. D_2O exchangeable), and 7.15br. (2H, s, N*H*CO, D_2O exchangeable); ^{13}C n.m.r: δ36.35, 45.10, 52.56, 58.25, and 170.94; m/z (rel. int.%) 273 (3%, M^+), 215 (2), 201 (3), 203 (37), 158 (10), 144 (3), 129 (9), 115 (9), 72 (54), and 58 (100).

Trimethyl nitrilotriacetate **(85).** - Nitrilotriacetic acid **(84)** (3.5g, 18.3mmole) in dry methanol (200mL) containing 10 drops of concentrated sulphuric acid was heated with dry benzene (150mL) for 19h using a Dean Stark trap. The reaction mixture was cooled to ambient temperature. The solvents were removed under reduced pressure, chloroform (100mL) was added to the resulting residue and the whole mixture was washed with a saturated aqueous solution of sodium hydrogencarbonate (5x50mL), and finally with deionized water (5x50mL) then dried ($MgSO_4$). Evaporation of the dried extracts gave an oil which was column chromatographed (short column) [eluant: chloroform-methanol, 95:5] furnishing the desired *title ester* **(85)** [2.52g, 59% or 69% based on recovered starting free acid **(84)**. Prolonged reflux (2-3days) resulted in 83% of **(85)**] R_F 0.75 (chloroform-methanol, 95:5) (Found: C, 46.0; H, 6.3; N, 6.0. $C_9H_{15}NO_6$ requires C, 46.4; H, 6.5; N, 6.0%); ν_{max} (film) 2863-2760 (C-H), and 1740 cm-^{1} (C=O); ^{1}H-n.m.r (200MHz) δ3.53 (6H, s, NCH_2CO), and 3.58 (9H, s, OCH_3); ^{13}C-n.m.r.: δ51.41, 54.68, and 170.88; m/z (Rel. Int.%) 233 (14%, M^+), 201 (3), and 174 (100).

N,N',N''-tris[2-(N''',N'''-dimethylamino)ethyl] nitrilotriacetamide **(86)** - To a well stirred solution of trimethyl nitrilotriacetate **(85)** (1.59g, 6.82mmole) in methanol (15mL), was added N,N-dimethylethylenediamine **(62)** (5.41g, 61.4mmole). The whole was heated under reflux until complete disappearance of the triester had resulted (t.l.c control, 3.5 days). The reaction mixture was allowed to reflux for a further 2 days. The excess methanol and amine were removed under reduced pressure and the resulting residue was dissolved in ethyl acetate (5mL) and adsorbed onto a silica gel (35-70mesh) prior to application onto silica gel column. Elution with methanol-chloroform-ammonia (aq) (7:3:1) furnished the pure *title compound* **(86)** (1.81g, 66%), as an orange viscous oil, R_F 0.25-0.35 (methanol-chloroform-ammonia(aq), 60:30:10)

(Found: C, 54.0; H, 9.3; N, 24.1. $C_{18}H_{39}N_7O_3$ requires C, 53.8; H, 9.8; N, 24.4%); ν_{max} (film) 3287, 3011 (N-H amide), 2973-2777 (C-H), and 1656, 1549 (C=O amide) cm^{-1}; ^{1}H-n.m.r. (200MHz) δ2.21 [18H, s, $N(CH_3)_2$], 2.45 (6H, t, *J* 5.74Hz, sharpens on D_2O exchange, CH_2CH_2N), 3.15 (6H, s, NCH_2CO), 4.14 (6H, m, CH_2CH_2NHCO), and 8.10br (3H, s, NHCO, D_2O exchangeable); m/z (rel. int.%) 402 (6%, M^+ + H), 344 (3), 331 (28), 272 (18), 130 (31), and 58 (100).

N,N'N''-tris[2-(N''',N'''-dimethylamino)ethyl]- nitrilo-triacetamide trihydrochloride (**86a**). - The foregoing trisamide (**86**) (1.02g, 2.53mmole) was dissolved in dry methanol followed by saturation of the solution with dry hydrochloric acid gas for 0.5h with ice-cooling of the reaction flask. The methanol was removed *in vacuo* (water bath, 45°C) to give the hygroscopic salt (1.25g, 96%) ^{1}H-n.m.r. (200MHz, D_2O, pH 1.5) δ2.92 [18H, s, $N(CH_3)_3$], 3.32 (6H, deformed t, CH_2CH_2N), 3.63 (6H, t, *J* 6.1Hz, CH_2CH_2NHCO), and 3.92 (6H, s, NCH_2CO); ^{13}C-n.m.r.: δ35.34, 43.84, 57.05, 57.73, and 170.30.

Dimethyl N-(2-hydroxyethyl) iminodiacetate (**98**). - **Method (a).** To a stirred mixture of N-(2-hydroxyethyl) iminodiacetic acid (**97**) (2.4g, 13.2mmole) in methanol (150mL) and benzene (175mL), was added 10 drops of concentrated hydrochloric acid. The mixture was heated at reflux with a Dean-Stark trap for 9h. The reaction mixture was cooled, a saturated aqueous solution of sodium hydrogencarbonate (50mL) was added and the bulk of the organic solvents were removed under reduced pressure. The resulting aqueous phase was saturated with sodium chloride and extracted exhaustively with ethyl acetate (8x50mL). The combined extracts were washed with deionized water (5x100mL), and dried ($MgSO_4$). Evaporation of the dried extracts furnished the *title ester* (**98**) (2g, 72%). An analytical sample was obtained by column chromatography, eluting with petroleum ether-ethyl acetate (7:3). (Found: C, 46.2; H, 7.2; N, 7.2.

$C_8H_{15}NO_5$ requires C, 46.8; H, 7.4; N, 6.8%); ν_{max} (film) 3462br (OH), 2953, 2851 (C-H), 1742 (C=O), and 1410 (OH bend) cm-^{1}; ^{1}H-n.m.r. (200MHz) δ2.80 (2H, skewed t, CH_2CH_2N), 3.35 (4H, s, NCH_2CO), 3.45 and 3.65 (6H,three each, split s, OCH_3), 4.33 (2H, m, NCH_2CH_2OH), and 5.3br (1H, s, *OH*); m/z (rel. int.%) 205 (4%, M^+), 188 (21), 174 (40), 146 (44), and 114 (97).

Method (b). A magnetically stirred slurry of N-(2-hydroxyethyl) iminodiacetic acid (**97**) (3.07g, 17.33mmole) in methanol (150mL) at 0°C was treated with an excess of ethereal diazomethane (*ca* 1.5 xmmole of acid) freshly prepared from N-nitrosomethyl urea[106], until the yellow colour persisted. The excess diazomethane was removed by slow warming to room temperature of the reaction mixture. Evaporation of the solvents left a dense yellow oil which was passed through a short column of silica gel [eluant: chloroform-methanol, 95:5] to give the pure *dimethyl N-(2-hydroxyethyl) iminodiacetate* (**98**) (3.40g, 96%), identical in terms of its ^{1}H-n.m.r., infrared, and mass spectrum with the product prepared by method (a) above.

N,N'-Bis-(2-hydroxyethyl) oxalodiamide (**103**). - To a stirred solution of diethyl oxalate (**101**) (2.22g, 15.2mmole) in absolute ethanol (15mL), was added, under nitrogen, 2-hydroxyethylamine (**102**) (2.05g, 33.6mmole) in absolute ethanol (5mL). The reaction mixture was stirred at room temperature for 10min, and warmed to 60°C for 0.5h which caused the precipitation of the product (**103**). The reaction mixture was cooled to ambient temperature, followed by the evaporation of the excess ethanol to give the *title compound* (**103**) (2.65g, 99%), as a white gleaming powder. m.p. 100°C (darkens), 165.5-166.5°C (acetone-methanol) (Found: C, 41.3; H, 6.8; N, 15.7. $C_6H_{12}N_2O_4$ requires C, 40.9; H, 6.9; N, 15.9%); ν_{max} 3287 (OH), 1647, 1538 (C=O amide), and 1295 (O-H bend) cm-^{1}; ^{1}H-n.m.r. (200MHz, CF_3CO_2D) δ3.8 (4H, m, CH_2NHCO), 4.2 (2H, s, *OH*, D_2O exchangeable), 4.65 (4H, m, CH_2OH), and 7.7br. (2H, s, N*H*CO); ^{13}C-

n.m.r: δ40.51, 67.67, and 160.48; m/z (rel. int.%) 176 (95%, M^+), 158 (60), and 58 (100).

N,N'-Bis-[2-(N'',N''-dimethylamino)ethyl] N'''-(2-hydroxyethyl) iminodiacetamide **(100).** - To a solution of dimethyl-N-(2-hydroxyethyl) iminodiacetate **(98)** (1.23g, 6mmole) in ethanol (10mL) was added N,N-dimethylethylenediamine **(62)** (2.12g, 24mmole) as a fast drip. The reaction mixture was well stirred for 0.5h at room temperature and heated under reflux for 3 days with continued stirring. The ethanol and the amine were removed *in vacuo* and the resulting residue was chromatographed on silica gel [eluant: methanol-chloroform-ammonia (aq), 40:30:1] to furnish the desired *title diamide* **(100)** (1.27g, 66.8%) as an orange syrup, R_F 0.20-0.44 (eluant as for chromatography) (Found: C, 52.7; H, 10.1; N, 21.9. $C_{14}H_{31}N_5O_3$ requires C, 52.97; H, 9.8; N, 22.1%); ν_{max} (CCl_4) 3309 (OH), 2826, 2782 (C-H), and 1651, 1543 (C=O amide I and II) cm^{-1}; ^{1}H-n.m.r. (200MHz) δ2.25 [12H, s, $N(CH_3)_2$], 2.35 [4H, deformed t, $CH_2N(CH_3)_2$], 2.51 (2H, skewed triplet, sharpens to a well defined triplet on D_2O exchange, *J ca* 5.3Hz, CH_2CH_2OH), 3.25 (4H, s, NCH_2CO), 3.43 (4H, m, CH_2CH_2NHCO), 3.55 (2H, m, sharpens to a well defined triplet on D_2O exchange *J* 5.25Hz Hz, CH_2CH_2OH), 3.9br (1H, s, OH, D_2O exchangeable), and 5.3br (2H, s, NHCO, D_2O exchangeable); ^{13}C-n.m.r: δ35.47, 44.34, 57.88, 58.68, 59.67, 60.82, and 172.10; m/z (rel. int.%) 317 (2%, M^+), 299 (1), 287 (2), 247 (4), 229 (5), and 188 (8).

Diethyl N-(2,2-diethoxyethyl) iminodiacetate **(110).** - **Method (a).** The 2,2-diethoxyaminoethane **(108)** (2g, 15mmole) in dry triethylamine (10mL) was added to a stirred solution of dry triethylamine (150mL) under nitrogen, followed by the addition of ethyl bromoacetate **(69)** (10g, 60mmole) in one portion under a nitrogen stream. The reaction mixture was stirred rapidly for 10 min with immediate precipitation of the

triethylamine hydrobromide. The reaction mixture was flushed with nitrogen for 0.5h then heated under reflux for 48h, and stirred at room tempeature for 44h. Absolute ethanol (100mL) was added to the reaction mixture which was again flushed with nitrogen for 0.5h then heated under reflux for another 12h. The solvents were removed under reduced pressure and a saturated aqueous solution of sodium hydrogen carbonate (50mL) was added and the whole mixture was extracted exhaustively with ethyl acetate-chloroform (1:1, v/v) (5x50mL). The combined extracts were washed well with deionized water (5x50mL) and dried ($MgSO_4$). Evaporation of the dried filtered extracts gave an orange-brown residue (3.75g) which was column chromatographed [eluant: chloroform-methanol, 95:5] to give, first the *title compound* (**110**) (2.08g, 45%) (Found: C, 54.9; H, 9.1; N, 4.9. $C_{14}H_{27}NO_6$ requires C, 55.1; H, 8.9; N, 4.6%); ν_{max} (film) 2973-2238 (C-H), and 1742 (C=O) cm-^{1}; ^{1}H-n.m.r. (200MHz) δ *ca* 1.2 (6H, t, *J* 7.20Hz, OCH_2CH_3), *ca* 1.25 [6H, t, *J* 7.15Hz, $CH(OCH_2CH_3)$, 2.71 [2H, d, *J* 5.52Hz,$NCH_2CH(OCH_2CH_3)$], 3.4 (4H, s, NCH_2CO), 3.5-3.8 [4H, m, $CH(OCH_2CH_3)$], 4.15 (4H, q, *J* 7.20Hz, OCH_2CH_3), and 4.6 [1H, t, *J* 7.15Hz, $CH(OCH_2CH_3)$]; m/z (rel. int.%) 305 (15%, M^+), 232 (23), 202 (30), 186 (23), 116 (12), and 103 (100); and (b) *Ethyl N-(2,2-diethoxyethyl) aminoglycinate* (**109**) (450mg, 14%), as an orange oil. R_F 0.56 (eluant as for chromatography) (Found: C, 55.1; H, 9.6; N, 6.6. $C_{10}H_{21}NO_4$ requires C, 54,8; H, 9.7; N, 6.4%); ν_{max} 3146, 3007 (NH), and 1740 (C=O) cm-^{1}; ^{1}H-n.m.r. (200MHz) δ *ca*1.2 (3H, t, *J* 7.20Hz, OCH_2CH_3), *ca* 1.3 [6H, t, *J* 7.15Hz, $CH(OCH_2CH_3)$], 2.0br (1H, s, N*H*, D_2O exchangeable), 2.71 (2H, d, *J* 5.52Hz, NCH_2CH), 3.42 (2H, s, NCH_2CO), 3.5-3.8 [4H, m, $CH(OCH_2CH_3)$], 4.15 (2H, q, *J* 7.20Hz, OCH_2CH_3), and 4.6 [1H, t, $CH(OCH_2CH_3)$]; m/z (rel. int.%) 219 (20%, M^+) 149 (3), 146 (9), and 103 (103).

Method (b). To a stirred heterogeneous mixture of the 2,2-diethoxy aminoethane (**108**) (2g, 15mmole), and anhydrous sodium carbonate (30g) in dry dichloromethane

(150mL) was rapidly added ethyl bromoacetate (**69**) (10g, 60mmole) dropwise. The reaction flask was fitted with a reflux condenser, flushed with nitrogen (20 min), and heated under a positive pressure of nitrogen for 60h. The reaction mixture was filtered of the salts and excess sodium carbonate, concentrated *in vacuo* to *ca* 10mL and loaded on to a silica gel column. Elution with chloroform-methanol (95:5) furnished the analytically pure sample of the *ester* (**110**) (3g, 98%) identical both in t.l.c behaviour and spectroscopically with the product (**110**) prepared by method (a) above. There was no evidence of the monoalkylation *product* (**109**) as judged by t.l.c.

N,N'-Bis[2-(N'',N''-dimethylamino)ethyl]-N'''-(2,2-diethoxyethyl)iminodiacetamide (**112**). - Diethyl N-(2,2-diethoxyethyl)iminodiacetate (**110**) (300mg, 0.982mmole), was dissolved in absolute ethanol (8mL) with magnetic stirring under nitrogen, followed by the addition of N,N-dimethylaminoethylenediamine (**62**) (520mg, 5.89mmole) in one portion. The reaction was stirred at room temperature for 0.5h, followed by heating under reflux for 3 days. The reaction mixture was fitted with a vacuum take-off adaptor and attached to a vacuum pump with liquid-nitrogen trap in line. Removal of the solvent and other volatiles left an orange-yellow oil. Column chromatography [eluant: methanol-chloroform-ammonia (aq), 95:5:5] afforded the analytical sample of the *title compound* (**112**) (340mg, 89%), as an orange-yellow syrup, R_F 0.35-0.40 (flame shaped;same eluant as for chromatography) (Found: C, 55.7; H, 9.7; N, 18.2. $C_{18}H_{39}N_5O_4$ requires C, 55.5; H, 10.1; N, 17.9%); ν_{max} 2830, 2782 (C-H), 1651 (C=O, amide I), and 1584 (C=O, amide II)cm^{-1}; ^{1}H-n.m.r. (200MHz) δ1.22 (6H, t, *J* 7.2Hz, OCH_2CH_3), 2.25 [12H, s, $N(CH_3)_2$], 2.50 [4H, deformed t, *J* 6.1Hz, sharpens upon D_2O exchange, $CH_2N(CH_3)_2$], 2.72 (1H, d, *J* 5.2Hz, CH_2CH), 3.17 (4H, s, NCH_2CO), 3.41 (4H, m, CH_2NHCO), 3.5-3.8 (4H, m, OCH_2CH_3), 4.5 (1H, t, *J* 5.2Hz, NCH_2CH), and 7.8br. (2H, s, NHCO, D_2O exchangeable); ^{13}C-n.m.r: δ15.16, 36.51, 45.04, 57.64, 58.24, 59.80, 62.58, 101.28, and 170.36; m/z (Rel. Int.%) 389 (19%, M^+), 344 (26), 331

(<0.5%), 319 (23), 286 (44), 272 (24), 260 (21), 130 (55), 103 (87), 72 (100), and 58 (100).

Diethyl iminodiacetate (70). - Method (a). To the well stirred heterogeneous mixture of ethyl glycinate (free base) **(68)** (100mg, 0.971mmole) and solid sodium hydrogen carbonate (900mg) in dry dichloromethane (20mL), was added ethyl bromoacetate **(69)** (162mg, 0.971mmole). The reaction mixture was stirred at room temperature for 48h, then warmed to 60°C for 16h, after which it was filtered, washed with water (2x20mL) and dried (Na_2SO_4). Evaporation of the dried organic phase furnished a gum. Column chromatography of this residue failed to furnish a product with acceptable analyses for the **ester (70)**. ^{1}H-n.m.r. and infrared spectrometry showed this residue to be a mixture of N-acylation and N-acetylation products. Method (b) below was the prefered route to the ester **(70)** was by method (b) below.

Method (b). To a slurry of iminodiacetic acid (Aldrich) **(72)** (2g, 15mmole) in ethanol (250mL) and benzene (200mL) was added 10 drops of concentrated hydrochloric acid. The stirred mixture was heated to reflux for 16h using a Dean-Stark trap. The cooled mixture was evaporated under diminished pressure to low volume (*ca* 5mL), followed by the addition of a saturated aqueous solution of sodium hydrogencarbonate (30mL). The resulting aqueous phase was extracted exhaustively with chloroform (8x50mL) and the combined organic extracts were dried ($MgSO_4$) and evaporated to give an orange oil which was filtered through a pad of silica gel [eluant: chloroform-methanol, 99:1] to give the **ester (70)** (2.5g, 88%). Column chromatography [eluant: chloroform-methanol, 95:5] gave the pure **ester (70)**. R_F 0.33-0.68 (eluant as for chromatography) (Found: C, 50.7; H, 7.7; N,7.3. $C_8H_{15}NO_4$ requires C, 50.8 ; H, 8.0; N, 7.3%); ν_{max} 3306 (N-H), 2936-2783 (C-H),and 1742 (C=O ester) cm-^{1}; ^{1}H-n.m.r. (200MHz) δ1.20 (6H, t, *J* 7.15Hz, OCH_2CH_3), 2.15br (1H, s, CH_2NHCH_2, D_2O exchangeable), 3.41 (4H, s,

NCH_2CO), and 4.15 (4H, q, *J* 7.15Hz, OCH_2CH_3); m/z (rel. int.%) 189 (6%, M^+), 143 (3), and 116 (100).

Attempts to prepare diethyl N-(2-cyanoethyl) iminodiacetate (96) by N-alkylation of diethyl iminodiacetate (70).

Method (a). To a stirred solution of diethyl iminodiacetate (**70**) (500mg, 2.65mmole) in acetonitrile (15mL) under nitrogen at room temperature was added potassium carbonate (460mg, 3.33mmole), followed by acrylonitrile (960mg, 18mmole) in acetonitrile (5mL). The reaction mixture was stirred at room temperature for 0.5h then heated under reflux for 24h. The dark-brown mixture was cooled, filtered, and concentrated to low volume under reduced pressure. The resulting residue was taken up in carbon tetrachloride (50mL), which was washed exhaustively with deionized water (5x50mL), and dried ($MgSO_4$). Column chromatography [eluant: chloroform-methanol, 95:5] afforded a dark-brown mobile oil, the elemental analysis of which was unsatisfactory for the title compound (**96**).

Method (b). To a well-stirred solution of the iminodiacetic ethyl ester (**70**) (530mg, 2.80mmole) in carbon tetrachloride (8mL) was added tetramethylguanidine (TMG) (322mg, 2.80mmole) in carbon tetrachloride (5mL), immediately followed by a rapid dropwise addition of acrylonitrile (9g, 0.17mole). The reaction mixture turned from orange to dark orange-brown after 18h. T.l.c indicated absence of starting material and the reaction was therefore quenched with 5*M* HCl, and diluted with carbon tetrachloride (45mL). The organic phase was exhaustively washed with deionized water (6x30mL) and saturated aqueous sodium hydrogencarbonate (3x30mL) then dried ($MgSO_4$). Evaporation of the dried organic phase gave a dark-brown oil which was column chromatographed to give a dark orange-brown liquid. Attempts to vacuum

distil a small amount of this fraction resulted in decomposition. Gas chromatography-mass spectroscopy of the isolated residue revealed m/z 242 [M^+ **(96)**, *ca* 4%]. This however was not the highest molecular ion. Purification of the resultant product proved cumbersome and therefore this reaction was not explored further.

Method (c). Diethyl iminodiacetate **(70)** (300mg, 1.59mmole) in dichloromethane (15mL) was treated with N-(2-cyanoethyl)-4-(dimethylamino)pyridinium bromide salt (372mg, 1.63mmole) at room temperature for 60h. T.l.c indicated a new spot with R_F0.47-0.71 (chloroform-methanol, 95:5). The reaction mixture was diluted with dichloromethane (40mL), and the whole mixture was sequentially washed with 1*M* aqueous hydrochloric acid (2x 30mL), saturated aqueous sodium hydrogencarbonate (2x30mL), and water (2x30mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated to give a deep yellow oil of comparable R_F as the starting ester **(70)** and was confirmed by ^{1}H-n.m.r. to be mainly starting material (>90%).

Diethyl N-(p-nitrobenzamido)iminodiacetate **(117).** - **Method (a).** To a stirring mixture of diethyl iminodiacetate **(70)** (420mg, 2.22mmole) and *p*-nitrobenzoic acid **(107)** (370mg, 2.22mmole) in dry dichloromethane (25mL), was added, under nitrogen, at 0°C (ice-bath) N,N-dicyclohexylcarbodiimide (DCC) (460mg, 2.22mmole). The reaction mixture was stirred for 3h after which the reaction warmed to room temperature. After 21h, the resultant precipitate of dicyclohexylurea (DCU) was filtered and washed with small volumes of dichloromethane (2x 10mL). The filtrate was diluted with more dichloromethane (30mL), successively washed with 1*M* hydrochloric acid (2x 30mL), saturated sodium hydrogencarbonate (2x30mL), water (3 x30mL), and finally dried ($MgSO_4$). Evaporation of the dried organic phase gave the *title compound* **(117)** (600mg, 80%), as a yellow syrup which crystallized to yellow plates *in vacuo* R_F 0.73 (chloroform-methanol, 95:5), m.p. 69-70°C (ether-ethyl acetate) as pale yellow

crystals (Found: C, 53.2; H, 5.1; N, 8.6. $C_{15}H_{18}N_2O_7$ requires C, 53.3; H, 5.3; N, 8.3%); ν_{max} 3293 (C-H), 1739 (C=O, ester), 1641 (C=O, 3°amide), 1457, 1374 (NO_2, conjugated), and 857 (1,4-disubstitution) cm^{-1}; ^1H- n.m.r. (200MHz) δ1.2 (6H, t, *J* 7.2Hz, OCH_2CH_3), 4.0 (4H, s, NCH_2CO), 4.14 (4H, q, *J* 7.2Hz, OCH_2CH_3), 7.56 (2H, d, *J* 8.9Hz, 2- and 6-H), and 8.25 (2H, d, *J* 8.9Hz, 3- and 5-H); m/z (rel. int.%) 338 (8%, M^+), 292 (11), 265 (17), 188 (37), and 150 (100).

***p*-Nitrobenzoyl chloride (118).**[104] - To a vigorously stirred mixture of *p*-nitrobenzoic acid (**107**) (1.77g, 10.6mmole) in dry dichloromethane (30mL), were added, under argon atmosphere at room temperature, oxalyl chloride (2.7g, 21.3mmole) and dimethylformamide (1 drop). The reaction mixture was stirred until the *p*-nitrobenzoic acid was fully dissolved (0.5-0.75h), after which it was stirred for a further 0.5h. The solvent was removed under vacuum and the residue was redissolved in dichloromethane (30mL), and evaporated to dryness to give the crude title compound (**118**) (1.89g, 96%),as a deep yellow powder. This material was used in the subsequent step without further purification.

Method (b). To a well-stirred solution of diethyl iminodiacetate (**70**) (610mg, 3.23mmole) in dry dichloromethane (50mL) at room temperature, anhydrous potassium carbonate (5g) and anhydrous magnesium sulphate (10g) were added. The reaction flask was flushed with nitrogen whilst the freshly prepared acyl chloride (**118**) (600mg, 3.23mmole) was added in small portions (slight foaming occurred). The reaction mixture was stirred under nitrogen for 1h after which t.l.c (chloroform-methanol, 95:5) revealed the absence of the starting ester (**70**). The mixture was filtered of the excess potassium carbonate, and anhydrous magnesium sulphate. The filtrate was concentrated *in vacuo* to give the *product* (**117**) (950mg, 87%) having the same spectroscopic properties as the product prepared by method (a) above.

N,N'-Bis[2-(N'',N''-dimethylamino)ethyl] N'''-(4-nitrobenzamido)iminodiacetamide **(119). Method (a).-** Diethyl N-(*p*-nitrobenzamido)iminodiacetate **(117)** (700mg, 2.07mmole), and N,N-dimethylaminoethylenediamine **(62)** (730mg,8.28mmole) were heated to reflux (90-110°C; oil bath) under nitrogen for 3days. The reaction was worked up in the usual way to give the crude title compound **(119)**. Column chromatography [eluant: methanol-chloroform-ammonia (aq), 95:5:5] furnished a product with R_F 0.19 (eluant as for chromatography). Though chromatographically homogeneous, the ^{1}H-n.m.r. spectrum of the product revealed an absence of the *p*-nitrobenzamido moeity and was identified as the hydrolysis product similar to **(73)**: δ2.25 [12H, s, N(CH_3)$_2$], 2.33 [4H, t, *J* 5.9Hz, CH_2N(CH_3)$_2$], 3.30 (4H, m, CH_2NHCO), 4.1br. (1H, s, N*H*, D_2O exchangeable), and 6.0br. (2H, s, N*H*CO, D_2O exchangeable). This product was not further characterized and the preferred route to **(119)** was by method (b) below.

Method (b). To a stirred solution of the imino diamide **(73)** (1.5g, 5.49mmole) in chloroform-dichloromethane (5:95, v/v) (75mL) under nitrogen at 0°C (ice-bath), freshly prepared *p*-nitrobenzoyl chloride **(118)** (1.02g, 5.49mmole) was added, in small portions, over 0.25h. The reaction mixture whilst warming to room temperature changed from an initially deep yellow colour to a dark-orange colour and over 18h was accompanied by the precipitation of the product. Filtration of the reaction mixture furnished the crude material as a slightly brown powder which was recrystallized in chloroform to give the pure *compound* **(119)** as a creamy-white hygroscopic dihydrochloride **(119a)** (2.2g, 81%) (Found: C, 46.1; H, 6.5; H, 16.95. $C_{18}H_{32}Cl_2N_6O_4$ requires C, 46.1; H, 6.5; N, 16.96%); ν_{max} (free base, film) 3291 (N-H, amide), 3031 (=C-H), 1660 [C=O (amide I),and C=O (3° amide], 1584 (C=O, amide II), 1550, 1350 (NO_2, conjugated), 1495 (aryl), and 745 (1,4 disubstitution) cm-^{1}; ^{1}H-n.m.r.

(free base, 200MHz) δ2.26 [12H, s, $N(CH_3)_2$], 2.48 [4H, m, $CH_2N(CH_3)_2$], 3.89 (4H, m, CH_2NHCO), 3.92, 4.10 (2H each, s, NCH_2CO), 7.2br. (1H, s, NHCO, D_2O exchangeable), 7.72 (2H, d, *J* 8.9Hz, 2- and 6-H), 8.21 (2H, d, *J* 8.9Hz, 3- and 5-H), and 8.46br. (1H, s, NHCO, D_2O exchangeable) ; m/z (rel. int.%) (free base) 433 (M^+, 0.3%).

Trimethyl 1,2,3-propanetricarboxylate (**88**). - The stirred mixture of 1,2,3-propane tricarboxylic acid (**87**) (1.43g, 8.12mmole) in dry ether (10mL) at -5°C (ice-salt bath), was treated with excess of ethereal diazomethane until the yellow colour persisted (0.5h). The reaction mixture was allowed to warm slowly to room temperature (to remove excess diazomethane). Careful evaporation of the solvent (45°C/2.0mm) furnished the pure *title ester* (**88**) (1.76g, 99%), as a pale orange mobile oil. R_F 0.80 [methanol -chloroform -ammonia (aq), 7:3:1] (Found: C, 49.4; H, 6.8. $C_9H_{14}O_6$ requires C, 49.6; H, 6.5%); ν_{max} 1735 cm^{-1} (C=O ester) ; ^{1}H-n.m.r. (200MHz) δ2.63 (4H, m, $CHCH_2CO$), 3.19 (1H, q, *J* 6.74Hz, CH_2CHCH_2), 3.61 (6H, s, $CH_2CO_2CH_3$), and 3.65 (3H, s, $CHCO_2CH_3$); ^{13}C-n.m.r: δ34.99, 37.26, 51.21, 171.70, and 173.51; m/z (rel. int.%) 219 (0.93%, M^+ + H), 187 (98), 159 (12), and 127 (100).

N,N′,N′′- tris(2-aminoethyl)propane-1,2,3- tricarboxamide (**89**). - To a rapidly stirred methanolic solution of N,N-ethylenediamine (8.04g, 44.7mmole) at -5°C (ice-salt) was added slowly over 0.5h, a methanolic solution of the foregoing ester (**88**) (1.39g, 6.38mmole; *ca* 0.3*M*). The reaction mixture was stirred at -5°C for 2.5h, and allowed to reach ambient temperature followed by continued vigorous stirring over 3 days. The starting ester (**88**) was completely reacted [t.l.c (methanol-chloroform-ammonia (aq), 7:3:1] after the 3 day period, and the methanol and excess amine were removed *in vacuo* (60°C/2.0mm) to afford the pure *title compound* (**89**) (1.91g, 99%), as a pale yellow semi-crystalline gum which upon storage in vacuo at 5°C, yielded a white

crystalline material, R_F 0.08-0.18 (methanol-chloroform-ammonia (aq), 7:3:1) (Found: C, 47.5; H, 8.4; N, 27.5. $C_{12}H_{26}N_6O_3$ requires C, 47.7; H, 8.7; N, 27.8%); ν_{max} 3340-3150 (N-H), 1662, and 1584 (C=O amide) cm-^{1}; ^{1}H-n.m.r. (CD_3OD, 200MHz): ^{1}H-n.m.r. intractable, multiplet at δ3.2-3.4 obscurred by solvent signal; δ_H (D_2O) 2.53 (4H, m, $CHCH_2CO$), *ca* 2.63 and 2.64 (6H, m, $CH_2CH_2NH_2$, 4 and 2 each), 3.05 (3H, m, CH_2CHCO and $CHCONHCH_2CH_2$), and 3.17 (4H, unresolved t, *J* 6.32Hz, $CH_2CONHCH_2CH_2$); ^{13}C-n.m.r: δ38.45, 39.49, 40.23, 40.28, 40.82, 42.03, 173.82, and 176.23; m/z (rel. int.%) 258 (M^+-$CH_2CH_2NH_2$, 2%), 243 (3), 215 (1), 213 (100), 200 (44), and 155 (42).

***tert*-Butyloxycarbonylation of the foregoing tricarboxamide (89).** - The tricarboxamide **(89)** (120mg, 0.397mmole) in methanol-dichloromethane (95:5) was treated with di-*tert*-butyloxydicarbonate (330mg, 1.512mmole). After 24h, removal of the solvents from the mixture under reduced pressure gave, following trituration with dichloromethane (3x5mL) (to remove excess Di-tert-butyloxydicarbonate), and filtration, the *tri-N-(tert-butyloxycarbonyl) derivative* **(91)** (72.4mg, 30%). Cooling of the filtrate at -5°C furnished additional product (Total: 108mg, 75%) m.p. 159°C (darkens), 175-180°C (decomposition) (methanol-dichloromethane) (Found: C, 53.5 ;H, 8.3; N, 13.6. $C_{27}H_{50}N_6O_9$ requires C, 53.8; H, 8.4 ;N, 13.9%).

Attempted "capping" of the tricarboxamide (89).[90] To the vigorously stirring solution of the tricarboxamide **(89)** (230mg, 0.762mmole) in methanol (20mL), was added cobaltous chloride ($CoCl_2$-$6H_2O$) (180mg, 0.762mmole) after which purple powdery crystals were deposited. Nitromethane (2g) in methanol (5mL) was added to the well stirred solution followed by aqueous formaldehyde (40%, 2g) which resulted in the purple precipitate dissolving and a colour change through dark orange-red to orange. Finally triethylamine (2g) was added to the reaction mixture and the mixture was

heated at *ca* 60°C (oil bath) for 2h. The excess methanol was evaporated under reduced pressure and yielded an intracticable hygroscopic residue whose analyses were at variance with that for the desired compound. The capping procedure was not pursued further.

N-*tert*-Butyloxycarbonylglycine (105)[105] - To a solution of glycine (8g, 10.7mmole), triethylamine (1.59g, 15.7mmole) in 50% aqueous acetone (45mL) was added 2-*tert*-butyloxycarbonyloxyimino-2-phenylacetonitrile (Boc-ON) (2.91g, 11.7mmole) with stirring at room temperature. After stirring for 2.5h, the acetone was removed under reduced pressure, and ethyl acetate-dichloromethane (1:0.5, v/v) (40mL) was added to the aqueous phase. The organic phase was separated, and the aqueous phase was acidified with 1*M* citric acid, and extracted with ethyl acetate (2x20mL) and the combined extracts and organic washings were dried ($MgSO_4$). The dried organic phase was evaporated to dryness to give the **title compound (105)** (17.5g, 88%), as gleaming white crystals; m.p. 86-87°C (petroleum ether-ethyl acetate) (Lit.[105], 86.5-87.5°C, 86-88°C) (Found: C, 48.1; H, 7.5; N, 8.03. $C_7H_{13}NO_4$ requires C, 47.99 ;H, 7.48; N, 8.0%); ν_{max} 3303 (O-H), 3323 (N-H), 1680 (C=O, carboxyl), 1738, 1757 (C=O, amide), and 1585 (C=O, amide/urethane) cm^{-1}; ^{1}H-n.m.r: δ1.45 [9H, s, $C(CH_3)_3$], 3.30 (2H, s, NCH_2CO), 8.1br. (1H, s, N*H*CO), and 10.1br. (1H, s, O*H*); m/z (rel. int.%) 160 [3%, $M^+ - CH_3$], 130 (9), 120 (81), 102 (5), and 57 (100).

Attempts to synthesize *N,N'-bis-[2-(N'',N''-dimethylamino)ethyl] N'''[ethyl 2'-(N''''-tert-butyloxycarbonylglycinate]iminodiacetamide* **(106).**

(a) Steglich esterification method. - The N-(2-hydroxyethyl) diamide **(100)** (240mg, 0.76mmole) was added to a vigorously stirred solution of N-*tert*-butyloxycarbonyl glycine **(105)** (133mg, 0.76mmole), with dicyclohexylcarbodiimide (DCC) (156mg, 0.76mmole),

and 4-(dimethylamino)pyridine (DMAP) (96mg, 0.76mmole) in ethanol-free dichloromethane (30mL) under nitrogen. The reaction mixture was heated under reflux for 2h, then was kept at 45°C for 19h. The solvent was removed under reduced pressure and the resulting residue was triturated with ethyl acetate (3mL), and dichloromethane (10mL) after which the mixture was filtered through a celite pad. The resulting filtrate was concentrated and was found by t.l.c. to consist of largely starting hydroxy diamide (**100**).

(a) Mitsunobu coupling. - To a well stirred solution of N-*tert*-butyloxycarbonylglycine (**105**) (608mg, 3.47mmole) under nitrogen at room temperature, were added diethylazodicarboxylate (DEAD) (604mg, 3.74mmole) in dry dichloromethane (40mL), a solution of the hydroxy diamide (**100**) (880mg, 2.77mmole) in dichloromethane (8mL) and triphenylphosphine (910mg, 3.74mmole). The reaction mixture was stirred under nitrogen at room temperature for 24h then was concentrated to low volume (*ca* 5mL) under reduced pressure. Ethyl acetate (5mL) and hexane were added to the residue until turbidity persisted. The mixture was stored at 5°C for 48h, and the resulting precipitate was filtered off. The filtrate was concentrated and applied onto a silica gel column. Elution with chloroform (100%) (400mL), then methanol-chloroform-ammonia (aq) (7:3:1) furnished a chromatographically homogeneous product, R_F 0.30-0.62 (methanol, 100%), 0.61-0.92 [methanol-chloroform-ammonia (aq), 7:3:1] which was found, by ^{1}H-n.m.r., to be a mixture of starting reagents triphenylphosphine, and N-*tert*-butyloxycarbonylglycine (**105**). Subsequent fractions from the column furnished an intractable gum whose elemental analysis was unsatisfactory for compound (**106**).

N,N'-Bis[2-(N'',N''-dimethylamino)ethyl] N'''-(methoxycarbonylmethyl) iminodiacetamide (**115**). - To a well- stirred solution of the

trimethyl nitrilotriacetate ester (**85**) (500mg, 2.15mmole) in methanol (20mL), was added the diamine (**62**) (378mg, 4.29mmole) in one portion. The reaction mixture was heated under reflux for 15 days. T.l.c analysis [eluant: methanol-chloroform-ammonia (aq), 9:5:5] of the reaction mixture revealed signifcant amounts of the starting ester (**85**), trisamide (**86**) as well as a new spot (R_F 0.35-0.40) corresponding with (**115**) as determined by comparison with the compounds (**85**) and (**86**). Gas chromatography coupled to mass spectrometry of the reaction mixture revealed peaks at m/z 233 (**85**), 345 (**115**), and 402 (**86**). Further investigations into the aspects of this reaction were abandonned based on the disadvantageous profile of the reaction. The reaction mixture was enriched with more of the diamine (**62**) to prepare more of the trisamide (**86**).

N-(*tert*-butyloxycarbonyl)glycine succinimide ester (121).[103] - N,N-dicyclohexylcarbodiimide (DCC) (10.75g, 52.2mmole) was added to a cooled solution (ice-bath) solution of N-(*tert*-butyloxycarbonyl)glycine (**105**) (9.14g, 52.2mmole) and N-hydroxysuccinimide (**120**) (6.0g, 52.2mmole) in 1,4-dioxane (200mL). The reaction mixture was allowed to warm to room temperature with continued stirring for 8h, followed by cooling in the refrigerator for 16h. The formed dicylohexylurea (DCU) was filtered and washed well with 1,4-dioxane (2x50mL). The combined washings and filtrate were concentrated under reduced pressure to yield a colourless oil which immediately crystallized. The compound was triturated with petroleum ether (2x5mL) and filtered to obtain 10.14g (71.6%), m.p. 164-166°C. The analytical sample had m.p. 168-172°C (propan-2-ol) (Lit.[103], 168-170°C), R_F 0.23 (petroleum ether-ethyl acetate, 6:4) (Found: C, 48.7; H, 5.7; N, 10.3. $C_{11}H_{16}N_2O_6$ requires C, 48.5; H, 5.9; N, 10.3%); ν_{max} 3297 (N-H urethane), 1786 (C=O imide), 1735 (C=O ester), 1675 (C=O amide I), 1528 (C=O amide II), and 1374 [C-H, $C(CH_3)_3$] cm^{-1}; ^{1}H-n.m.r: δ1.35 [9H, s, $C(CH_3)_3$], 2.75 (2H, s, NCH_2CO), 4.21 (4H, m, $OCCH_2$), and 5.0br. (1H, s, N*H*COO,

D_2O exchangeable); m/z (rel. int.%) 257 (1%, M^+-CH_3), 199 (11), 174 (5), 171 (9), and 57 (100).

N,N′-Bis[N-(*tert*-butyloxycarbonyl)-glycyl]-1,2-diaminoethane (122). - To a well stirred solution of the succinimide ester of N-*tert*-butyloxycarbonylglycine (**121**) (2.06g, 7.57mmole) in dry dimethoxyethane (DME) (45mL) at 0°C (ice-bath), was added, dropwise, ethylenediamine (230mg, 3.78mmole) in dimethoxyethane (5mL). The reaction was allowed to warm to room temperature during 24h. The white precipitate that formed was filtered by suction to yield the **title compound** (**122**) (1.11g, 78.5%), m.p. 176-179°C (chloroform-propan-2-ol) (Found: C, 51.5; H, 8.2; N, 15.2. $C_{16}H_{30}N_4O_6$ requires C, 51.3; H, 8.1; N, 14.96%); ν_{max} 3270 (N-H amide and urethane), 1668 (C=O amide I and urethane), 1530 (C=O amide II and urethane), and 1364 (C-H, $C(CH_3)_3$] cm^{-1}. This compound was not further analyzed beyond combustion analysis and infrared spectroscopy.

Methyl N^{α}-Boc-N^{ϵ}-(carbobenzyloxy)-L-lysinate (143). - Method (a). A slurry of (2S)-Nϵ-Benzyloxycarbonyl lysine methyl ester hydrochloride (**142**) (1.40g, 4.23mmole) in dry dichloromethane-chloroform (7:3, v/v) (50mL) was treated with triethylamine (440mg, 4.23mmole), followed by the addition of di-*tert*butyloxycarbonyl dicarbonate (982mg, 4.50mmole) under nitrogen at room temperature. The reaction was judged to be complete after 1.5h (t.l.c control, eluant petroleum ether-ethyl acetate, 6:4), and the reaction mixture was successively extracted with saturated aqueous sodium hydrogencarbonate (2x20mL), and deionized water (2x50mL). The combined aqueous extracts were back extracted with chloroform (2x20mL), and the combined organic phases were dried ($MgSO_4$). Evaporation of the dried organic phase furnished a yellow oil which was chromatographed on a silica gel column [eluant: petroleum ether-ethyl acetate, 6:4] to provide the pure **title compound** (**143**) (1.65g, 99%), as a viscous pale

yellow oil, R_F 0.76 (eluant as for chromatography) (Found: C, 60.5; H, 7.6; N, 6.7. $C_{20}H_{30}N_2O_6$ requires C, 60.9; H, 7.7; N, 7.1%); ν_{max} (film) 3346 (N-H urethane), 3065 (=C-H aryl), 2933, 2864 (C-H, CH_3), 1705 [C=O, urethane and ester (masked)], 1522 (C=O amide II), 1450 (aryl), 1365 [C-H, $C(CH_3)_3$], and 753 (monosubstitution) cm^{-1}; ^{1}H-n.m.r. (200MHz) δ1.41 [9H, s, $C(CH_3)_3$], 1.53 [6H, m, $NHCH_2(CH_2)_3$], 3.15 [2H, m, $NHCH_2(CH_2)_3$], 3.70 (3H, s, OCH_3), 4.3br. and 4.95br. (each 1H, s, NHCOO), 5.07 (3H, m, chiral CH, obscurred by $PhCH_2$ singlet), and 7.33 (5H, s, *Ph*); m/z (rel. int.%) 395 (0.9%, M^+ + H), 364 (1), 338 (4), 336 (< 1), 231 (8), 215 (< 1), 189 (1), 59 (33), and 57 (100).

Method (b). (2S)-N^{ϵ}-Benzyloxycarbonyl-2-lysine methyl ester hydrochloride (**142**) (3.33g, 9.98mmole) in 1,4-dioxane (50mL) was treated with triethylamine (1.21g, 11mmole), followed by the addition of the BOC-ON reagent (3.12g, 12.7mmole). T.l.c (eluant as for chromatography, method (a) above) revealed the reaction to be complete (2h). The triethylamine hydrochloride salt was filtered off and the filtrate was concentrated under reduced pressure to low volume (*ca* 5mL) and the resulting residue was loaded on to a silica gel column. Elution with petroleum ether-ethyl acetate, 99:1-85:5 provided the pure compound (**143**) (3.9g, 100%), identical in all respects (T.l.c, ^{1}H-n.m.r., and IR) with the product prepared by method (a) above.

N-*tert*Butyloxycarbonyl valine methyl ester (137). - To a stirring slurry of valine methyl ester hydrochloride (**136**) (3.34g, 19.9mmole) in dry 1,4-dioxane (75mL), at room temperature, was added triethylamine (2.14g, 21.1mmole) which resulted in the immediate precipitation of triethylamine hydrochloride salt. The heterogeneous mixture was stirred for 10min. followed by the addition of the BOC-ON reagent (4.90g, 19.9mmole) in one portion. The reaction was stirred at room temperature for 16h after which the salt was filtered and the filtrate was concentrated under reduced pressure.

The resulting orange residue was chromatographed on a silica gel column [eluant: petroleum ether-ethyl acetate, 99:1] to give the pure **title compound (137)** (4.20, 92%), as an orange-yellow oil, R_F 0.56 (petroleum ether-ethyl acetate, 6:4) (Found: C, 57.0; H, 8.7; N, 6.2. $C_{11}H_{21}NO_4$ requires C, 57.2; H, 9.1; N, 6.1%); 3345 (N-H urethane), 2975 (C-H, CH_3), 1742 (C=O ester), 1712 (C=O urethane), 1498 (C=O amide II), and 1389,1366 [C-H, $C(CH_3)_3$] cm-^{1}; ^{1}H-n.m.r (200MHz) δ0.82 [6H, m, $CH(CH_3)_2$], 1.34 [9H, s, $C(CH_3)_3$], 2.0 [1H, m, $CH(CH_3)_2$], 3.63 (3H, s, OCH_3), 4.10 (1H, m, asymmetric *CH*), and 5.0 (1H, unresolved d, N*H*COO); ^{13}C-n.m.r: δ17.48, 18.80, 28.14, 31.14, 51.79, 58.43, 79.51, 84.92, 155.50, and 172.71.

Ethyl N-benzyloxamate **(124).** - To a well stirred solution of freshly distilled diethyl oxalate **(101)** (12.41g, 84.9mmole) in dichloromethane (50mL) at 0°C (ice-bath), was added under nitrogen, the freshly distilled solution of benzylamine **(76)** (4.55g, 40.6mmole) in dichloromethane (30mL) over 0.8h. The reaction mixture was allowed to warm gradually to room temperature during 2h. T.l.c followed by spraying with ninhydrin and charring (180°C) revealed the complete reaction of benzylamine **(76)**. Evaporation of the solvent and other volatiles left a fine white powdery material admixed with the yellow oil. Petroleum ether-ether (60:40, v/v) was added to the foregoing residue and filtered to give *Bis(N-benzyl)oxalodiamide* **(128)** (450mg, 4%), as a gleaming powder m.p. 206-210°C (decomposition) (Found: C, 72.0; H, 6.3; N, 10.4. $C_{16}H_{20}N_2O_2$ requires C, 71.6; H, 6.0; N, 10.4%); ν_{max} 3327 (N-H amide), 3031 (=C-H aryl), 1676 (C=O amide I), 1527 (C=O amide II), 1494 (aryl), and 742 (monosubstitution) cm-^{1}; ^{1}H-n.m.r (200MHz) δ4.49 (4H, d, *J* 6.1Hz, collapses to a s upon D_2O exchange, $PhCH_2NHCO$), 7.31 (10H, m, *Ph*), and 7.35br. (2H, s, N*H*CO, D_2O exchangeable); m/z (rel. int.%) 268 (24%, M^+), 177 (100), 134 (4), 106 (55), and 91 (100). The filtrate was concentrated under reduced pressure and the resulting residue was chromatographed on a silica gel column. Elution with petroleum ether-

ethyl acetate (99:1) provided the unreacted diethyl oxalate (**102**) (4.85g); the *title compound* (**124**) was then eluted with ethyl acetate-methanol (70:30) to give an orange oil which crystallized to elongated yellow triangles (7.48g, 89%), R_F 0.08 (petroleum ether-ethyl acetate, 9:1), 0.72 (ethyl acetate-methanol, 7:3), m.p. 39-40°C (petroleum ether-ethyl acetate, 9:1) (Found: C, 63.5; H, 6.3; N, 7.2. $C_{11}H_{13}NO_3$ requires C, 63.8; H, 6.3; N, 6.8%); ν_{max} 3270 (N-H amide), 3051 (=C-H aryl), 1740 (C=O ester), 1675 (C=O amide I), 1526 (C=O amide II), 1495 (aryl), and 740 (monosubstitution) cm-^{1}; ^{1}H-n.m.r. (200MHz) δ1.35 (3H, t, *J* 7.2Hz, OCH_2CH_3), 4.31 (2H, q, *J* 7.2Hz, OCH_2CH_3), 4.49 (2H, d, *J* 6.1Hz, collapses to a singlet upon D_2O exchange, $PhCH_2NHCO$), 7.31 (10H, m, *Ph*), and 7.5br. (1H, s, N*H*CO, D_2O exchangeable); ^{13}C-n.m.r: δ13.87, 43.81, 63.10, 127.80, 127.87, 128.72, 136.71, 156.40, and 160.55; m/z (rel. int.%) 207 (100, M^+), 178 (31), 134 (4), 133 (24), and 91 (100).

N-benzyl N'-(2-aminoethyl)oxalodiamide (**126**). - To a rapidly stirred solution of ethylenediamine (640mg, 10.6mmole) in dichloromethane (45mL) at 0°C (ice-bath) was added a solution of N-benzyl-2-ethoxycarbonyloxalamide (**124**) (1.50g, 7.25mmole) in dichloromethane (10mL). The reaction mixture was allowed to warm to room temperature during 18h. The white precipitate that formed was filtered, taken up in methanol, boiled briefly (10min.), and filtered hot to give *N,N'-bis(benzyl)oxalyl]-1,2-diaminoethane* (**127**) (320mg, 48%), m.p. > 200°C. (Found: C, 63.0; H, 5.9; N, 14.7. $C_{20}H_{22}N_4O_4$ requires C, 62.8; H, 5.8; N, 14.65%); ν_{max} 3324 (N-H amide), 3064 (=C-H, aryl), 1662 (C=O amide I), 1580 (C=O amide II), 1494 (aryl), and 750 (monosubstitution) cm-^{1}; [^{1}H-n.m.r. not recorded because the product did not exhibit solubility in most organic solvents]. The filtrate was concentrated under reduced pressure to provide the *title compound* (**126**) (800mg, 49.9%) as a pale yellow powder, m.p. 134-136°C. (Found: C, 59.5; H, 7.2; N, 18.6. $C_{11}H_{15}N_3O_2$ requires C, 59.7; H, 6.8; N, 18.99%); ν_{max} 3200-3120 (N-H amine and amide), 3065 (=C-H aryl), 1663 (C=O

amide I), 1580 (C=O amide II), 1495 (aryl), and 752 (monosubstitution) cm-^{1}; ^{1}H-n.m.r. (200MHz, pH 1.5, D_2O) δ3.21 (2H, unresolved t, *J* 5.9Hz, CH_2NH_2), 3.62 (2H, unresolved t, *J* 5.9Hz, CH_2NHCO), 4.47 (2H, s, $PhCH_2$), and 7.35 (5H, m, *Ph*); ^{13}C-n.m.r: δ37.70, 39.60, 43.91, 128.48, 129.63, 137.91, 161.30, and 162.61; m/z (rel. int.%) 221 (0.5%, M^+), 192 (33), 130 (< 0.5), 106 (19), 91 (100), 43 (69), and 30 (100).

N'[N''-(N'''-tert-butyloxycarbonylglycyl)-2-aminoethyl]-N-benzyloxalodiamide (**129**). - To a stirred hot solution of N-benzyl oxalodiamide (**126**) (100mg,0.37mmole) in methanol (10mL), was added, dropwise a solution of the succinimide ester (**121**) (90mg, 0.37mmole) in chloroform (5mL). The reaction mixture was stirred for 5h, at room temperature and kept at -5°C (refrigerator) for 16h. The precipitate that formed was collected by suction; the filtrate was treated with petroleum ether-ether (99:1) to induce further precipitation. The combined precipitated residues provided the *title compound* (**129**) (100mg, 71.95%), m.p. 175-176°C (methanol) (Found: C, 57.0; H, 7.25; N, 14.6. $C_{18}H_{26}N_4O_5$ requires C, 57.1; H, 6.9; N, 14.8%); ν_{max} 3230 (N-H amide and urethane), 3066 (=C-H aryl), 2987, 2875 (C-H, CH_3), 1662 (C=O amide I), 1580 (C=O amide and urethane II), 1495 (aryl), 1364 [C-H, $C(CH_3)_3$], and 698 (monosubstitution) cm-^{1}; ^{1}H-n.m.r. (200MHz, C_5D_5N) δ1.44 [9H, s, $C(CH_3)_3$], 3.72 (4H, s, CH_2NHCO), 4.14 (2H, d, *J* 5.6Hz, NCH_2CO), 4.67 (2H, d, *J* 6.4Hz, collapses to a s upon D_2O exchange, $PhCH_2NHCO$), *ca* 7.2 (5H, m, *Ph*), 7.9br. (1H, unresolved t, N*H*COO, D_2O exchangeable), 9.1br. (1H, s, N*H*CO, D_2O exchangeable), 9.8br. (1H, s, N*H*CO, D_2O exchangeable), and 10.1 (1H, unresolved t, N*H*CO, D_2O exchangeable); ^{13}C-n.m.r: δ28.43, 39.33, 40.13, 43.46, 44.76, 78.73, 127.94, 128.90, 139.30, 156.89, 161.20, 161.61, and 170.95.

Ethyl N-Benzyl-N-(tert-butyloxycarbonyl)glycinate (**133**). - To a vigorously stirred solution of ethyl N-benzylglycinate (**78**) (3.17g, 19.2mmole) in dry chloroform (70mL), was

added, dropwise, a solution di-*tert*-butyl dicarbonate (Boc_2O) (4.19g, 19.2mmole) in dry chloroform (5mL). T.l.c (benzene-ethanol, 95:5) indicated the reaction to be complete after 2h, and the reaction was left to stir at room temperature for a further 14h. Removal of the solvent under reduced pressure provided the pure *title compound* (**133**) (5.6g, 99.4%) as an orange-yellow oil [An analytical sample was obtained by column chromatography (petroleum ether-ethyl acetate, 6:4], R_F 0.41-0.58 (flame-shaped spot) (Found: C, 65.6; H, 7.9; N, 4.8. $C_{16}H_{23}NO_4$ requires C, 65.5; H, 7.9; N, 4.8%); ν_{max} (film) 3070 (=C-H aryl), 2979, 2935 (C-H, CH_3), 1751 (C=O ester), 1699 (C=O urethane), 1493 (C=O amide and urethane II), 1394, 1368 [C-H, $C(CH_3)_3$], and 722 (monosubstitution) cm-^{1}; ^{1}H-n.m.r. (200MHz) δ0.96 (3H, t, *J* 7.2Hz, OCH_2CH_3), 1.20 [9H, s, $C(CH_3)_3$], 3.49, 3.65 (1H each, s, NCH_2CO), 3.84 (2H, q, *J* 7.2Hz, OCH_2CH_3), and 4.14 (2H, d, *J* 7.3Hz, $PhCH_2NHCO$); ^{13}C-n.m.r: δ14.07, 27.28, 28.16, 47.55 (d), 51.18 (d), 54.13, 60.80, 80.36, 127.35, 128.01, 128.34, 137.39 (d), 155.57 (d), and 169.79.

N-(2-Aminoethyl) N'-(tert-butyloxycarbonyl)-N'-(benzyl)glycinamide (**134**). - To a vigorously stirred solution of freshly distilled ethylenediamine (20mL) was added, during 0.25h a solution of N-diprotected glycine ethyl ester (**133**) (1.60g, 5.45mmole) in methanol (8mL). The reaction was complete after 1h as judged by t.l.c (petroleum ether-ethyl acetate, 6:4). The excess ethylenediamine and methanol were removed *in vacuo* leaving an orange-brown residue which was chromatographed on a silica gel column eluting first with ethyl acetate. Elution with methanol-chloroform-ammonia (aq), 30:75:5-50.45.5 furnished the *title compound* (**134**) (1.45g, 86.6%) as a bright yellow oil, R_F 0.70-0.90 (methanol-chloroform-ammonia (aq), 30:75:5) (Found: C, 61.0; H, 8.2; N, 13.2. $C_{16}H_{25}N_3O_3.\frac{1}{2}H_2O$ requires C, 60.7; H, 8.1; N, 13.2%); ν_{max} (film) 3293 (N-H amide and urethane), 3063 (=C-H aryl), 2974, 2870 (C-H, CH_3), 1668 (C=O amide I), 1548 (C=O amide II and urethane), 1493 (aryl), 1364 [C-H, $C(CH_3)_3$], and 720 (monosubstitution) cm-^{1}; ^{1}H-n.m.r. (200MHz) δ1.37 [9H, s, $C(CH_3)_3$], 2.21 (2H, s,

CH_2NH_2, D_2O exchangeable), 2.65 (2H, unresolved t, CH_2NH_2), 3.14 (2H, m, CH_2NHCO), 3.72 (2H, s, NCH_2CO), 4.40 (2H, s, $PhCH_2N$), 6.8br. (1H, s, NHCO, D_2O exchangeable), and 7.15 (5H, m, *Ph*); ^{13}C-n.m.r: δ28.28, 41.01, 41.54, 50.66, 51.94, 80.98, 127.77, 128.65, 137.37, 156.00 (weak), and 169.69.

N'[N''-(N'''-benzyl-N'''-(tert-butyloxycarbonyl)glycyl)-2-aminoethyl]-N-benzyloxalodiamide **(135).** To a well stirred solution of the monoamide **(134)** (500mg, 1.63mmole) in chloroform (10mL) was added a solution of the ester monoamide **(124)** (337mg, 1.63mmole) during 0.25h. The reaction mixture turned cloudy and after 3.5h, a slightly tan precipitate deposited. More chloroform was added (25mL) and the reaction was stirred at room temperature for a further 1.5h. The reaction mixture was chilled for 3h at -5°C and the resulting precipitate was filtered, washed with small amounts of chilled chloroform. A sample of this material was recrystallized in methanol-chloroform to give a cream-white powder with a m.p. 199-201°C, yield: 481mg (89%). Elemental analyses of this product were unsatisfactory for the title compound **(135)** despite repeated recrystallization in various solvent mixtures. The ^{1}H-n.m.r. spectrum was also not recorded for the product showed poor to no solubility in most conventionally employed solvents.

N-(2-Aminoethyl)-N$^{\epsilon}$-(benzyloxycarbonyl)-N$^{\alpha}$-(tert-butyl-oxycarbonyl)lysinamide **(144).** - The ester **(143)** (1.50g, 3.80mmole) was taken up in methanol (8mL) and added dropwise to a vigorously stirred solution of ethylenediamine (20mL) at room temperature. The reaction was judged to be complete by t.l.c (petroleum ether-ethyl acetate, 7:3) and the reaction was worked up as before to give a bright yellow residue which upon chromatography on a silica gel column provided the pure *title compound* **(144)** (1.60g, 89%) as a bright yellow oil, R_F 0.47 (methanol-chloroform-ammonia (aq), 85:15:5) (Found: C, 58.1; H, 8.0; N, 12.7. $C_{21}H_{34}N_4O_5.CH_3OH$ requires C, 58.1; H, 8.4;

N, 12.3%); ν_{max} (film) 3320 (N-H, 2°amide and 1° amine), 3065 (=C-H aryl), 2933, 2867 (C-H, N-CH_2/CH_3), 1669 (C=O amide I), 1526 (C=O amide and urethane II), 1450 (aryl), 1389, 1364 [C-H, $C(CH_3)_3$], and 697 (monosubstitution) cm^{-1}; ^{1}H-n.m.r. (200MHz) δ1.40 [9H, s, $C(CH_3)_3$], 1.50 [6H, m, $NHCH_2(CH_2)_3$], 1.75br. [3H, s, CH_2NH_2 and CH_3OH (occluded), D_2O exchangeable], 2.75 (2H, t, *J* 5.9Hz, CH_2NH_2), 3.15 [2H, m, $NHCH_2(CH_2)_3$], 3.25 (2H, m, CH_2NHCO), 4.0br. (1H, s, NHCOO, D_2O exchangeable)*, 5.05 (2H, s, $PhCH_2$), 6.2br. (1H, s, NHCOO, D_2O exchangeable)*, 5.42 (1H, m, CH_2CH asymmetric), 6.82br. (1H, s, NHCO, D_2O exchangeable), and 7.32 (5H, s, *Ph*). (*Interchangeable)

N-(2-Aminoethyl)-N$^{\alpha}$-(tert-butyloxycarbonyl)valinamide **(138).** - The title compound was prepared [from L-valine methyl ester hydrochloride **(136)**] in an analogous manner as previously described for compounds **(134)** and **(144)** above. Column chromatography on silica gel eluting first with ethyl acetate (100%) removed any residual impurities; the product was eluted with methanol-chloroform-ammonia (aq), 50:15:5 to provide the *title compound* **(138)** (2.63g, 85%) as an orange-yellow syrup which crystallized into a pale orange-yellow amorphous powder upon trituration with petroleum ether-ether and storage at -5°C (refrigerator) for 40h. (Found: C, 55.5; H, 9.9; N, 16.2. $C_{12}H_{25}N_3O_3$ requires C, 55.6; H, 9.7; N, 16.2%); ν_{max} (CCl_4) 3223 (N-H amide, amine, and urethane), 2976, 2870 (C-H, CH_3), 1668 (C=O amide I), 1583 (C=O amide and urethane II), and 1365 [C-H, $C(CH_3)_3$] cm^{-1}; ^{1}H-n.m.r. (200MHz) δ0.85 [6H, d, *J* 6.5Hz, $CH(CH_3)_2$], 1.35 [9H, s, $C(CH_3)_3$], 2.0 [1H, m, $CH(CH_3)_2$], 2.30 (2H, s, CH_2NH_2, D_2O exchangeable), 2.75 (2H, t, *J* 5.8Hz, CH_2NH_2), 3.25 (2H, m, CH_2NHCO), 3.81 (1H, m, *CH*), 5.49 (1H, d, *J* 8.5Hz, NHCOO, D_2O exchangeable), and 6.85br. (1H, s, NHCO, D_2O exchangeable).

N-(N''-Boc-valyl)-N'-(N'''-benzyloxamoyl)-1,2-diaminoethane (**139**). - To a rapidly stirred solution of the valinamide (**138**) (270mg, 0.88mmole) in methanol-chloroform (99:1, v/v) at room temperature, was added ethyl N-benzyloxamate (**124**) (220mg, 0.88mmole) in small portions during 0.25h. The reaction was stirred for a further 1h. The solvents were evaporated under reduced pressure to provide a pale yellow powdery residue which was taken up in chloroform (30mL) and boiled briefly (*ca* 5 min.). The undissolved material was filtered, oven-dried (80°C) to provide the pure *title compound* (**139**) (240mg, 65%) [Cooling of the filtrate at 5°C (refrigerator) provided an additional 60mg of product]. Total yield was 300mg (81%), as white flakes, m.p. 211-213°C. (Found: C, 59.7; H, 7.5; N, 13.3. $C_{21}H_{32}N_4O_5$ requires C, 60.0; H, 7.7; N, 13.3%); ν_{max} 3236 (N-H amide and urethane), 3065, 2938, 2839 (C-H, CH_3), 1667 (C=O amide I), 1530 (C=O amide and urethane II), 1389, 1365 [C-H, $C(CH_3)_3$], and 697 cm^{-1}; ^{1}H-n.m.r. (200MHz, C_5D_5N) δ1.04 [6H, d, *J* 6.8Hz, $CH(CH_3)_2$], 1.46 [9H, s, $C(CH_3)_3$], 2.35 [1H, m, $CH(CH_3)_2$], 3.85 (4H, m, CH_2NHCO), 4.50 (1H, m,, *CH*), 4.69 (2H, d, *J* 6.4Hz, collapses to a singlet upon D_2O exchange, $PhCH_2NHCO$), 7.25 (5H, m, *Ph*), 7.8 (1H, d, *J* 8.8Hz, N*H*COO, D_2O exchangeable), 9.17 (1H, unresolved t, N*H*CO, D_2O exchangeable), 9.74 (1H, unresolved t, N*H*CO, D_2O exchangeable), and 10.13 (1H, t, N*H*CO, D_2O exchangeable); ^{13}C-n.m.r: δ18.39, 19.78, 28.48, 31.76, 39.19, 40.16, 43.46, 60.87, 78.57, 127.57, 127.94, 128.88, 139.32, 156.79, 161.19, 161.58, and 173.11; m/z (rel. int.%) 420 (3%, M^+), 347 (26), 304 (0.6), 260 (14), 248 (2), 212 (55), 204 (8), 177 (22), 116 (50), 107 (72), 91 (100), and 72 (100).

N-valyl-N'-(N''-benzyloxamoyl)-1,2-diaminoethane hydrochloride (**140**). - A slurry of compound (**139**) (190mg, 0.45mmole) in methanol-tetrahydrofuran (90:10, v/v) (70mL) was warmed (60°C; oil-bath) until dissolved, followed by cooling to 0°C (ice-bath). Dry hydrogenchloride gas was bubbled through the cooled solution for 10min. The solvents were removed under reduced pressure (<50°C; water-bath) to provide the

title compound (**140**) (160mg, 99%) as an off-white hygroscopic powder. (Found: C, 52.5; H, 7.2; N, 15.5. $C_{16}H_{25}ClN_4O_3.\frac{1}{2}H_2O$ requires C, 52.5; H, 7.0; N, 15.3%); ν_{max} (KBr) 3200-3100 (N-H amide and amine), 3065 (=C-H aryl), 1668 (C=O amide I), 1580 (C=O amide II), 1495 (aryl), and 697 (monosubstitution) cm^{-1}; ^{1}H-n.m.r. (200MHz, D_2O) δ0.85 [6H, m, $CH(CH_3)_2$], 1.70 [1H, m, $CH(CH_3)_2$], 3.1-3.75 (5H, m, CH_2NHCO, and CH), 4.40 (2H, s, $PhCH_2$), and 7.3 (5H, m, *Ph*); ^{13}C-n.m.r: δ17.54, 18.23, 30.47, 38.91, 39.48, 43.84, 59.42, 128.14, 128.43, 129.56, 137.89, 161.39, 161.89, and 170.22.

N-(N''-Boc-valyl)-N'-(N'''-Boc-glycyl)-1,2-diaminoethane (**141**). - **Method (a).** To a well stirred yellow solution of the N-protected valinamide (**138**) (350mg, 1.35mmole) in 1,4-dioxane-$CHCl_3$ (99:1, v/v) (20mL) at room temperature, was added N-(*tert*-butyloxycarbonyl)glycine hydroxysuccinimide ester (**121**) (370mg, 1.35mmole) in small portions. The yellow solution turned a cloudy pale yellow after the final addition of compound (**121**) but soon cleared into a bright orange-yellow colour. After 0.75h, the solvents were removed under reduced pressure to give an orange syrup which was triturated with ether (2x20mL). Evaporation of the ether under reduced pressure gave a yellow powder. The foregoing powder was dissolved in chloroform (20mL), and ether was added dropwise which promoted the precipitation of the product. The whole mixture was chilled at 5°C (refrigerator) for 14h. Filtration by suction, followed by drying *in vacuo* (45°C/0.5mm, 16h) provided the pure *title compound* (**141**) (440mg, 78.5%), identical in all respects (^{1}H-n.m.r., ^{13}C-n.m.r., and IR) with the compound prepared by method (b) below.

Method (b). To a well stirred solution of the valinamide (**138**) (360mg, 1.39mmole) and N-(*tert*-butyloxycarbonyl)glycine (**105**) (240mg, 1.39mmole) in 1,4-dioxane (40mL) at 0°C was added dicyclohexylcarbodiimide (DCC) (290mg, 1.39mmole). The reaction

was allowed to briefly warm to ambient temperature followed by cooling at 5°C (refrigerator) overnight (10h). Filtration of the dicyclohexylurea (DCU), and concentration of the filtrate under reduced pressure furnished an orange-yellow oil which was triturated with petroleum ether-ether (2x5mL) and the triturant was removed under reduced pressure to finally give the *diamide* (**141**) (570mg, 98.6%) as a yellow hygroscopic powder. (Found: C, 53.7; H, 8.5; N, 13.1. $C_{19}H_{36}N_4O_6.\frac{1}{2}H_2O$ requires C, 53.6; H, 8.6; N, 13.2%); ν_{max} 3230 (N-H amide and urethane), 2937, 2836 (C-H, CH_3), 1665 (C=O amide I), 1529 (C=O amide and urethane II), and 1365 [C-H,$C(CH_3)_3$] cm^{-1} ; ^{1}H-n.m.r. (200MHz) δ0.91 [6H, m, $CH(CH_3)_2$], 1.42 [18H, s, $C(CH_3)_3$], 2.1 [1H, m, $CH(CH_3)_2$], 3.39 (5H, m, CH_2NHCO, and CH), 3.77 (2H, d, *J* 5.9Hz, $HNCH_2CO$), 3.84 (1H, dd, *J* 6.4Hz, and 2.04Hz, NHCOO), 5.22 (1H, d, *J* 8.5Hz, NHCOO, D_2O exchangeable), 5.45 (1H, unresolved t, NHCO, D_2O exchangeable), and 6.85br. (1H, s, NHCO, D_2O exchangeable); ^{13}C-n.m.r: δ17.84, 19.29, 25.46, 28.32, 30.63, 39.36, 44.25, 60.21, 77.20, 80.14, 156.10, 170.69, and 172.85;m/z (rel.int.%) 287 (M^+-$C_6H_{12}NO_2$+H, 2%), 268 (13), 212 (13), 199 (4), 186 (11), 172 (11), 116 (46), 72 (100), and 57 (77).

N-(N^{α}-Boc-N^{ϵ}-benzyloxycarbonyl-L-lysyl)-N'-(N''-Boc-glycyl)-1,2-diaminoethane (**145**). - **Method (a).** The title compound was prepared by adaptation of the method as described for compound (**141**) above. Thus the lysinamide (**142**) (1.13g, 2.67mmole) in 1,4-dioxane (5mL) at room temperature was added, during 0.25h a solution of the N-(*tert*-butyloxycarbonyl)glycine hydroxysuccinimide ester (**121**) (730mg, 2.67mmole) in 1,4-dioxane (3mL). The reaction was judged to have gone appreciably into completion (*ca* 95% by t.l.c) after 19h. The solvent was removed under reduced pressure and the resulting residue was chromatographed on a silica gel column [eluant: methanol-chloroform-ammonia (aq), 80:5:5 - 95:5:5] to provide the *title compound* (**145**) [1.19g, 77% or 86% based on recovered starting material (**144**) (140mg, 10.6%], as an orange syrup which crystallized to a pale yellow hygroscopic powder. This product was

identical in all respect (^{1}H-n.m.r., and IR) with the product prepared by method (b) below.

Method (b). To a well stirred solution of the lysinamide (**144**) (370mg,0.88mmole) and N-(*tert*-butyloxycarbonyl) glycine (**105**) (150mg, 0.88mmole) in 1,4-dioxane (25mL) at 0°C (ice-bath) was added N,N-dicyclohexylcarbodiimide (DCC) (180mg, 0.88mmole). The reaction was allowed to warm slowly to ambient temperature during 6h, and subsequently cooled at 5°C (refrigerator) for 16h. The precipitate of dicyclohexylurea (DCU) was filtered off and the filtrate was concentrated under reduced pressure to give an orange syrupy residue which was triturated with petroleum ether-ether. Storage of the foregoing mixture at 5°C (refrigerator) for 24-30h afforded the *diamide* (**145**) (490mg, 96%) as hygroscopic pale yellow powder, R_F 0.42 (methanol-chloroform-ammonia (aq), 30:75:5) (Found: C, 58.3; H, 8.0; N, 11.8. $C_{28}H_{45}N_5O_8$ requires C, 58.0; H, 7.8; N, 12.1%); ν_{max} (film before crystallization) 3325 (N-H amide and urethane), 3070 (=C-H aryl), 2973, 2829 (C-H, CH_3), 1669br. (C=O amide I), 1526 (C=O amide and urethane II), 1450 (aryl), 1389, 1365 [C-H, $C(CH_3)_3$], and 697 (monosubstitution) cm-^{1}; ^{1}H-n.m.r. [200MHz, $(CD_3)_2SO$]: δ1.37 [18H, s, $C(CH_3)_3$], 1.52 [6H, m, $NHCH(CH_2)_3$], 2.85-3.89 [9H, m, 3 x $NHCH_2$, $NHCH_2CO$, and CH], 4.99 (2H, s, $PhCH_2$), 5.59 (1H, d, NHCOO, D_2O exchangeable), 6.74 (1H, d, NHCOO, D_2O exchangeable), 6.83br. (1H, unresolved t, NHCO, D_2O exchangeable), 7.34 (5H, s, Ph), and 7.81 (1H, unresolved d, NHCOO, D_2O exchangeable).

N-(N^{α}-Boc-L-lysyl)-N'-(N''-Boc-glycyl)-1,2-diaminoethane (**148**). - Compound (**145**) (1.03g, 1.78mmole) in methanol-ethyl acetate (95:5, v/v) (45mL), and 10% Pd/C (500mg) was hydrogenolyzed at atmospheric pressure until no further hydrogen uptake was observed (36h). The heterogeneous mixture was worked up in the usual way to

provide the pure *title compound* (**148**) (770mg, 97%), as a pale yellow hygroscopic powder. (Found: C, 52.0; H, 8.5; N, 14.9. $C_{20}H_{39}N_5O_6.H_2O$ requires C, 51.8; H, 8.9; N, 15.1%); ν_{max} 3323 (N-H amide and urethane), 2936, 2829 (C-H, CH_3), 1669br. (C=O amide I), 1525 (C=O amide and urethane II), 1389, and 1364 [C-H, $C(CH_3)_3$] cm^{-1}; ^{1}H-n.m.r. (200MHz, CD_3OD) δ1.45, 1.46 [18H, s, $C(CH_3)_3$], 1.52 [8H, m, $H_2NCH_2(CH_2)_3$ and CH_2NH_2], 2.5-2.8 (4H, m, NHCOO and $COCH_2NHCO$), 3.1-3.4 (6H, m, CH_2NHCO and CH_2NH_2), 3.69 (2H, s, NHCO), and 3.95 (2H, m, CH); m/z (rel. int.%) 446 (3%, $M^+ + H$), 373 (1), 326 (4), 295 (10), 259 (<3), 245 (3), 214 (13), and 57 (80).

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107 The phrase "conventional esters" in this context is used to delineate between the "active esters" of type **(121)** and the ethyl or methyl esters (RCO_2R', R' = Et- or Me-)

APPENDIX

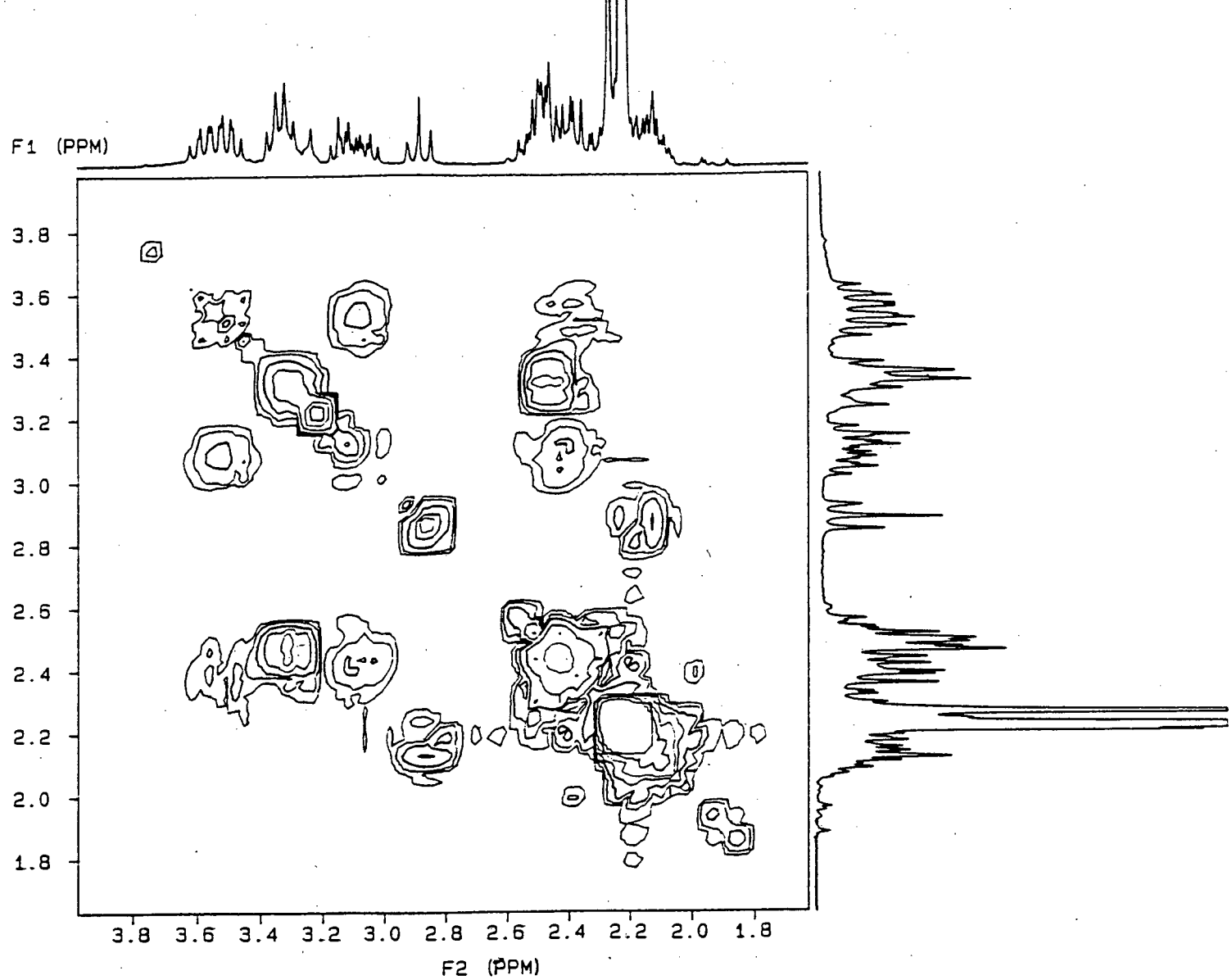

Fig.1a. COSY spectrum of the cyanodiamide (63).

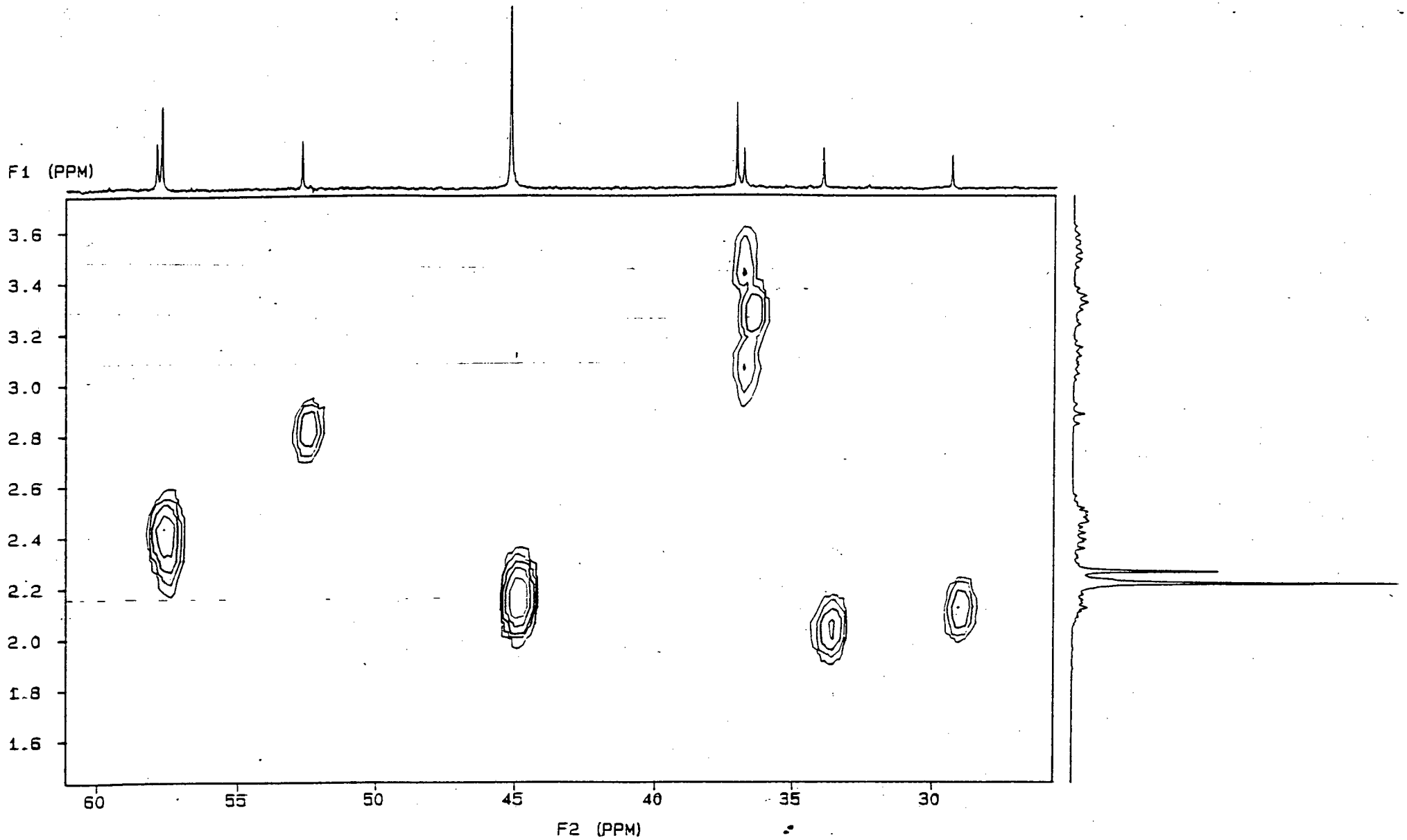

Fig.2a. HETCOR spectrum of the cyanodiamide (63)

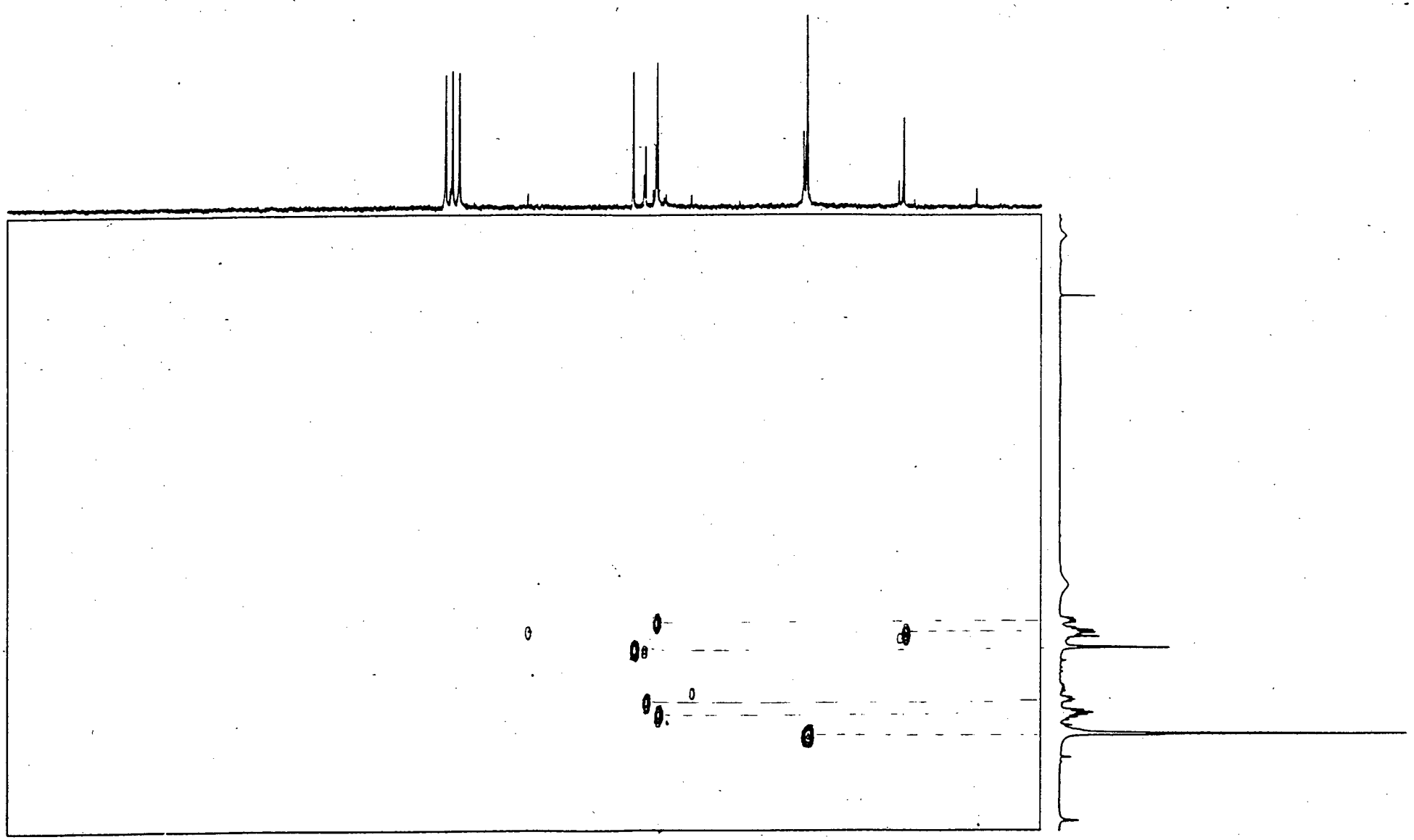

Fig.3a. HETCOR spectrum of the hydroxydiamide (100)

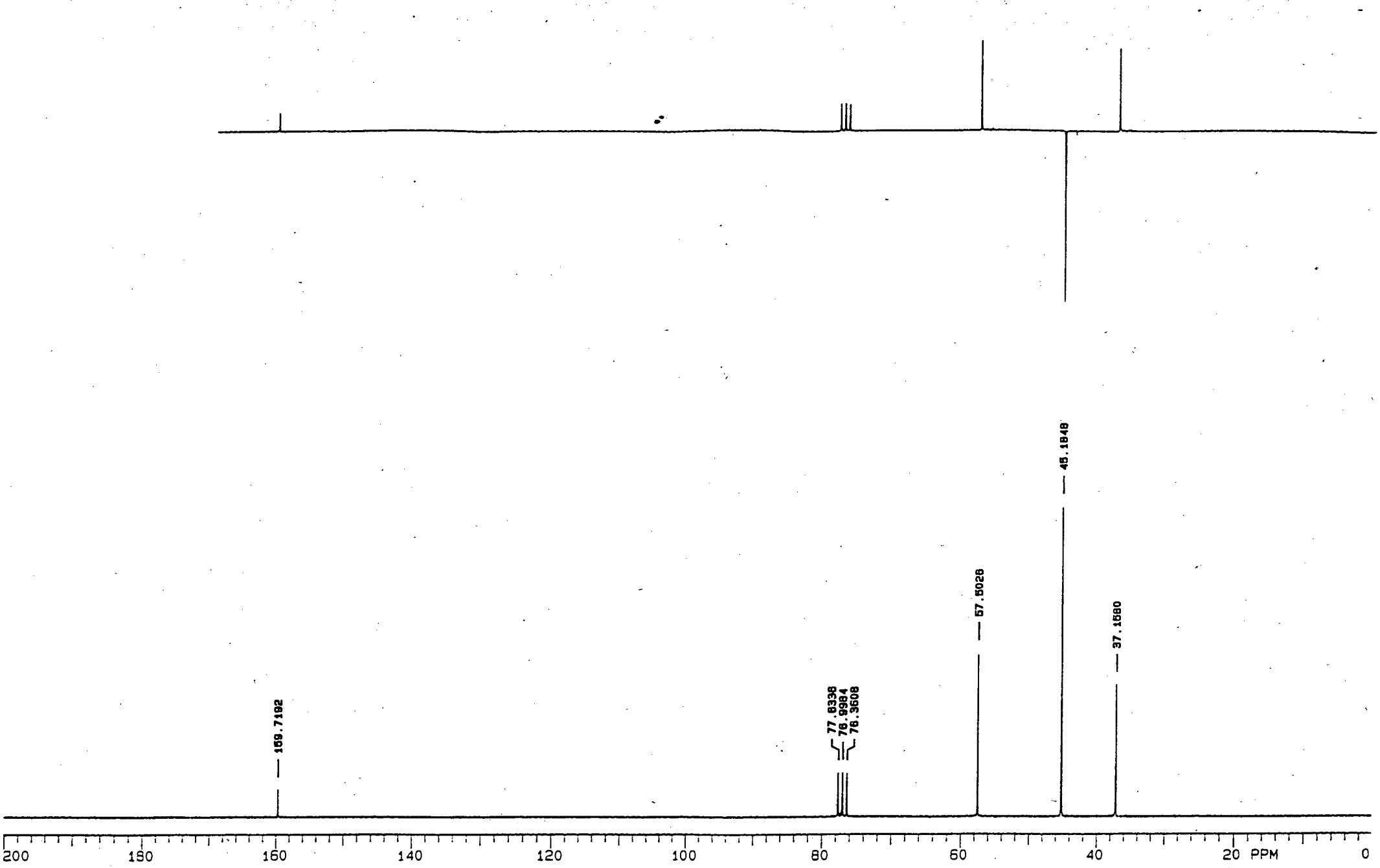

Fig.4a. ^{1}H-n.m.r. spectrum of the oxalodiamide (150)

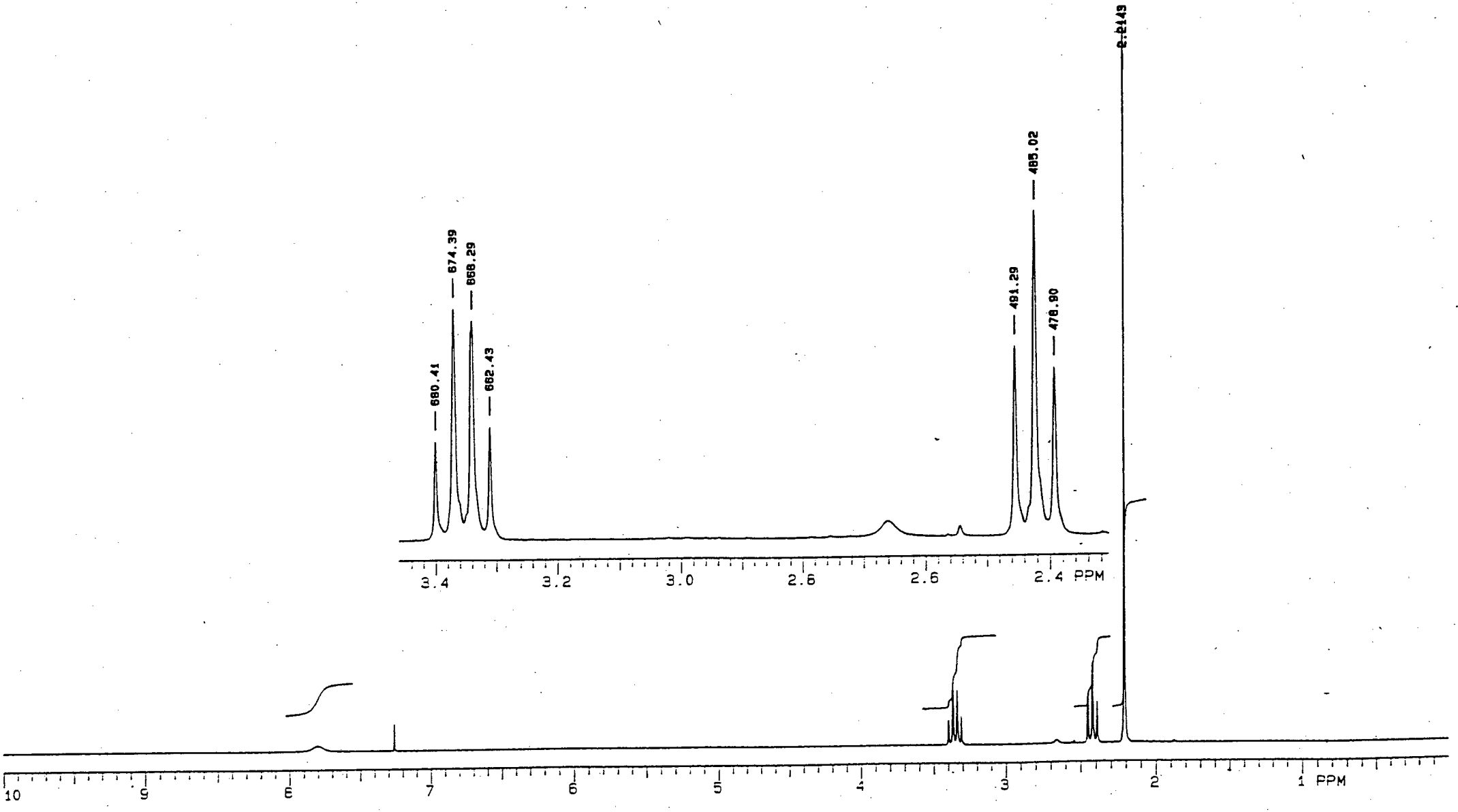

Fig.5a. APT spectrum of the oxalodiamde (150)