NOVEL LIPIDIC-AMINO ACID BASED DRUG AND PEPTIDE DELIVERY SYSTEM

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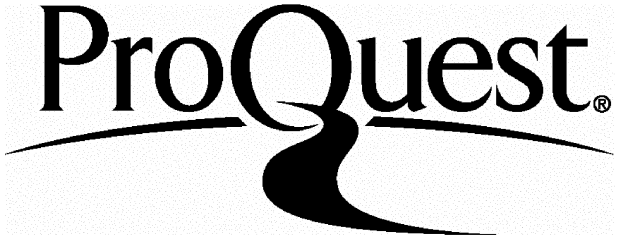

CONTENTS

ABSTRACT

The lipidic amino acids are synthetic α-amino acids with long alkyl side chains. The lipidic amino acids and their homo oligomers represent compounds combining the structural properties of proteins and peptides and the physical characteristics of lipids and membranes.

The lipidic amino acids were synthesized from the appropriate alkyl bromide and diethyl-acetamido malonate. Resolution was achieved enzymatically using Acylase I or chemically by forming diastereomers of the amino acids with an optically pure α-pinene derivative.

The aim of the project was the synthesis of the lipidic amino acids and peptides and their conjugation with natural peptides including elastase inhibitors and enkephalins. The lipidic amino acids and their oligomers were envisaged to increase the membrane permeability of peptides. In addition, it is envisaged that the long alkyl side chains of the lipidic amino acids will physically protect against the metabolic breakdown of the enzyme labile elastase and enkephalin peptides.

The use of the lipidic amino acids as a polymeric drug delivery system was investigated. Random polymerisation of homo- and hetero-trimers by the active ester method yielded as series of products to be tested for drug encapsulation capacity and metabolic stability. Low solubility of these products limited their use in drug encapsulation and stability studies.

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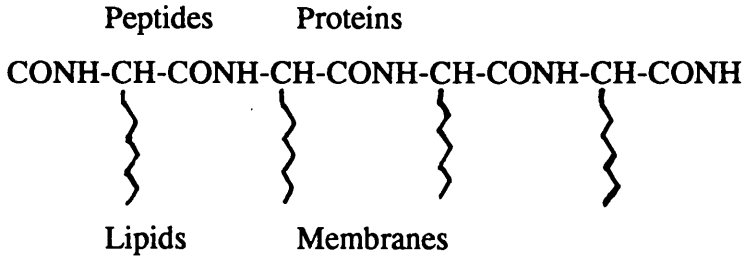
This thesis is dedicated to the memory of my father, whose support was always unfailing.

TO MY LATE FATHER

1. INTRODUCTION

The lipidic amino acids are synthetic α-amino acids with long alkyl side chains, they and their homo-oligomers represent a class of compounds which combine the structural features of lipids with those of amino acids and peptides. The lipidic amino acids can be condensed in the same way as natural amino acids to give oligomers and polymers. Interest has been shown in the amino acids and their derivatives as lubricants[1], cosmetics[2], polishes[3] and surface improvers for ceramics[4]. In addition, the lipidic amino acids and oligomers can be used as detergents, water resistant (waterproof) and biocompatible films and coatings[5]. Our particular interest is to use them as a drug delivery system.

The lipidic amino acids have dual character, the long chain resembling a fatty acid side chain and the polar head resembling amino acids. Condensation of the lipidic amino acids to the lipidic peptides, which are chimeric in nature, combines the physical, chemical and biological properties of peptides and proteins, with those of lipids and membranes (Fig. 1)



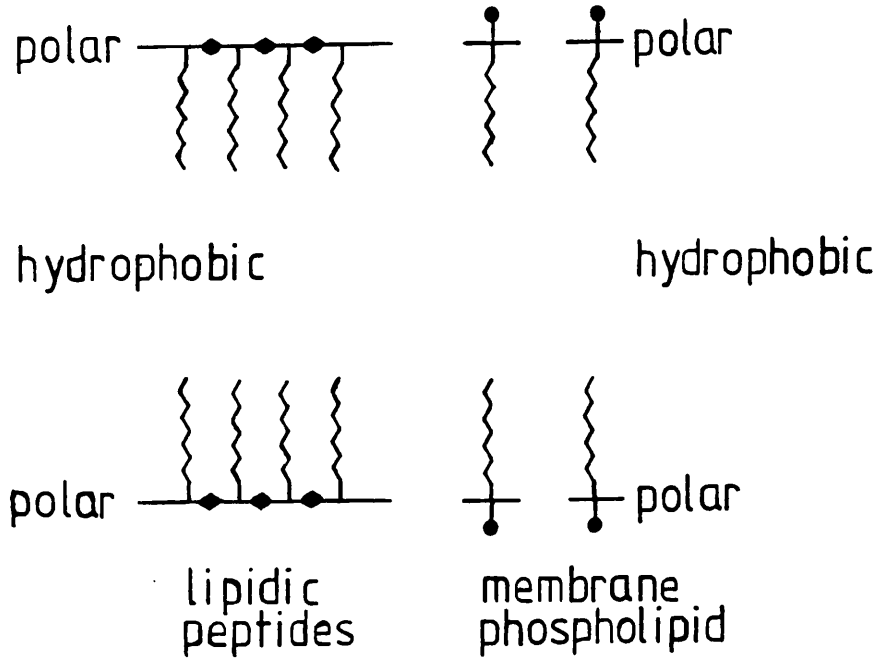
(Fig. 1)

The polymers formed by condensation of the lipidic amino acids are anticipated to be biocompatible, biodegradable, non toxic and nonimmunogenic[6]. This, and the chimeric nature of these peptides and polymers, confer on them the potential as a drug delivery system.

The lipidic peptides can be homopeptides or heteropeptides containing other amino acids. The hydrophobicity of the lipidic peptides is controlled by the length of the alkyl side chain or the number of amino acid residues in the peptide. The inclusion of other amino acids with charged groups would alter hydrophobicity. A combination of these would produce lipidic peptides with differing properties.

During the polymer synthesis carried out in this work, the alkyl chain length remained the same, but natural amino acids were included in the peptide chain to confer different properties.

The lipidic peptides resemble the phospholipid bilayer of a cell membrane. (Fig. 2).



(Fig. 2)

This similarly can be explored as a means of drug delivery. Poorly-absorbed compounds can be comigrated or enveloped within the peptide. The lipidic nature of the conjugate will assist the passage of the active compound across biological barriers thus aiding absorption. The lipidic peptides can also confer stability to biologically labile compounds by protecting them from enzyme degradation.

1.1. Drug Delivery

The ideal of Paul Ehrlich's 'magic bullet' has long been sought in the treatment of disease. The targeting of a drug to the required site of action and the maintenance of a therapeutically effective dose for a prolonged period have long been strived for. In addition, the reduction of drug in the vicinity of non-target cells is another major aim. To attain this ideal, several methods have previously been investigated. This thesis examines the efficient delivery of medicinal substances, specifically the use of the lipidic amino acids.

1.2 Established methods enhancing drug absorption

To improve absorption, a poorly absorbed drug can be modified physically or chemically.

1.2.1 Physical methods

The carrier requirements of different therapeutic agents vary widely, therefore no single carrier system can be used for all drugs.[7] There is a general requirement for drug carriers to possess:

(i) The carrier-agent conjugate must retain the agents activity unchanged unless the conjugate can be degraded at the site of action giving the active agent.

(ii) The carrier must be non-toxic

(iii) The carrier must be non-immunogenic and non-antigenic and not alter the antigenicity of the drug.

(iv) The carrier must be biodegradable.

(v) The carrier must retain their characteristics following conjugation i.e. antibodies must be able to complex with the specific antigen and the clearance rate of macromolecules must not be adversely affected.

1.2.1.1 Macromolecules

Macromolecular drug carriers previously used include antibodies[9] and other proteins[10,11]. The production of sufficient numbers of antibodies and the non-specific nature of immunoglobulins[12] including their alteration on the presentation of drugs have limited their use. Conversely the glycoproteins with the presence of sugar groups and their selectivity in the binding of receptors, have given potential targeting ability, for example, polylactose-LDL binds to hepatic parenchymal cells whilst asialoglycoproteins (galactose) binds hepatic parencymal sinusoidal cells.

1.2.1.2 Cellular carriers

Erythrocytes, neutrophils and platelets are used as cellular drug carriers. Erythrocytes due to their availability and the extensive knowledge of their production and metabolism are widely used. Erythrocytes can be used as enzyme carriers which

they naturally contain[12] to treat patients who are enzyme deficient, or as drug carriers[13] to deliver drugs to the liver or macrophages which intercept old erythroctes. Cell entrapped desferroxamine injected into patients with metal overload chelates the metal more quickly than the drug alone.

Neutrophils localise in inflammatory lesions therefore neutrophils loaded with Inoxine allow imaging of lesions whilst platelets have been used successfully in infant idiopathic thrombocytopenia.

1.2.1.3 Liposomes

Liposomes were first observed in the 1960's by Bangham and his collaborators. It was found that phospholipids dispersed in water formed multilayered vesicles. Each layer being a bimolecular lipid membrane with the layers enclosing internal aqueous compartments[14,15,16]. Liposomes resemble cellular membranes and are made from phospholipids but sterols, glycolipids, organic bases or acids, even membrane proteins or artificial polymers, have been used.

Liposomes now consist of one or more concentric bilayers with the aqueous compartments allowing water soluble substances to be entrapped. The potential use of liposomes as drug carriers was first made in 1972[17] by Gregoriadis and co-workers. Liposomes can be administered by various routes eg. topically, parenterally, by inhalation and orally.

Topically liposomes are now used in cosmetic creams to enable greater penetration and longer action of the active ingredients.

Liposomes injected subcutaneously, interperitoneally and intramuscularly are drained away via the lymphatics therefore they can be used to delivery drugs to the lymph nodes and to detect metastatic spread in lymph nodes by encapsulating lymphoscintigraphic material. Liposomes administered intravenously are largely taken up by phagocyte cells in the reticuloendothelial system such as the liver and spleen in both animals[18,19] and humans[20,21] thus limiting their use as general drug carriers. The major interest in liposomes is their potential of targeting active compounds to the reticuloendothelial for treating parasitic, fungal (eg leishmaniasis, schistosomiasis and malaria) and bacterial macrophage-related diseases[22], viral diseases[23], and cancer metastasis[24].

Liposomes are used in immunology as carriers of antigens and adjuvants[25] and so have found use in autoimmune diseases where they decrease the number of circulatory autoantibodies thus controlling and improving the disease. Most recently a lipophile azidothymidine (AZT) analogue has been incorporated into liposomes for the treatment of human acquired immunodeficiency syndrome (AIDS). The liposomal compounds are active in vitro and may be important for the treatment of the macrophage-related pool of the viral cause of the disease[26].

Orally administered liposomes are readily degraded in the gastro intestinal tract[27]. Some liposomes such as those composed of distearyl phosphatidyl choline/cholesterol are resistant to degradation but fail to cross the intestinal tissue[28]. Liposomes may not only be ineffective in increasing oral absorption, but have been shown to decrease the absorption of well absorbed substances such as glucose[29]. Liposome formulations for intranasal and inhalation uses[30], have shown promise in the delivery of therapeutic compounds.

1.2.1.4 Micelles

Micelles are clusters of surfactant molecules which form in solution above a critical concentration (CMC), when the interface is saturated with surfactant[31]. Entrapment of the drug molecules within micellar structure has been attempted with limited success. Incorporation of heparin and poorly absorbed antibiotics within a mixed bile salt/fatty acid micelle have resulted in improved oral absorption[32]. Micelles are physically unstable in the biological environment thus limiting their use.

1.2.1.5 Solubility Enhancers

The transmembrane passage of compounds is, according to the pH-partition hypothesis dependent of their lipophilicity, with hydrophobic compounds being more readily transported than hydrophillic ones. As hydrophobicity increases however, there is a decrease in aqueous solubility and in extremely hydrophobic compounds poor solubility may be the rate limiting step in drug absorption. Solubility enhancers improve the rate and extent of absorbtion of compounds with poor solubility. The best of these being the cyclodextrins[33].

1.2.1.6 Absorption Enhancers

Absorption enhancers have been used to increase the absorption of some poorly-absorbed compounds by increasing cell membrane permeability. Several absorption enhancers have been investigated, they include sodium salicylate[34], glycerides[35], surfactants[36], fatty acid emulsions[37], and 1-dodecylazacycloheptanone[38]. A combination of oleic acid and 1-dodecylazacycloheptanone enhance absorption by being incorporated into the cell membrane[39], this mechanism may be shared by other enhancers. Nishihata[40] et al showed that the improved rectal absorption seen for some ß-lactam antibiotics with chelating agents involves calcium ion chelation. The use of absorption enhancers is limited due to their potential irreversible effects on membranes and the problem of the absorption enhancers reaching the correct concentration at the required site.

1.2.2 Chemical Methods

Drug design is a balance between chemical modifications for potency and those allowing delivery and absorption. One method of avoiding the complexity of this problem is the use of bioreversible chemical modifications, prodrugs, to improve absorption.

1.2.2.1 Prodrugs

Extensive studies have been made in the use of prodrugs[41] to improve the absorption of poorly absorbed molecules. The type of prodrug linkage used is dependent on the functional group of the drug molecule requiring modification.

The conversion of prodrug to drug molecule can occur by several reactions, the most common being enzyme hydrolysis. Besides enzyme activation of prodrugs, physiological pH 7.4 can be used in triggering release of active drug. Here the prodrugs are labile at pH 7.4 but stable at lower pH 3.4.

A summary of bioreversible modifications and linkages commonly used follows.

1.2.2.1.1 Esters

Esters are the most popular of the prodrug linking groups. Their popularity

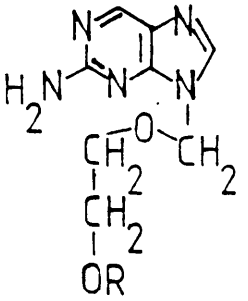
is primarily due to the presence of esterases found in blood. liver and other organs or tissues which break down the ester bond. The ester linkage allows the attachment to a wide variety of groups such as carboxyl, hydroxyl or thiol groups thus introducing almost any required degree of hydrophilicity or lipophilicity. Several examples of ester derivates of drugs are found in literature.

Hydrophobic esters of aspirin[42], indomethacin[43] and other non-steroidal inflammatories have been made. The esters were made to reduce the gastro-intestinal irritation found with these compounds, but maintain oral absorption.

Hydrophobic esterification of benzodiazepines such as lorazepam and oxazepam[44,45] allows them to cross the blood-brain barrier.

GABA (γ-aminobutyric acid) is a naturally occurring inhibitory neurotransmitter which has potential as an antiepileptic agent. GABA does not however, cross the blood-brain barrier after systemic administration. Esterification of GABA increases lipophilicity enabling measurable amounts of GABA within the central nervous system[46,47].

Alanyl (1b) and glycyl (1c) esters of the antiviral acyclovir (a) increase hydrophilicity enabling acyclovir to be given by injection[48].



1 R

a) H

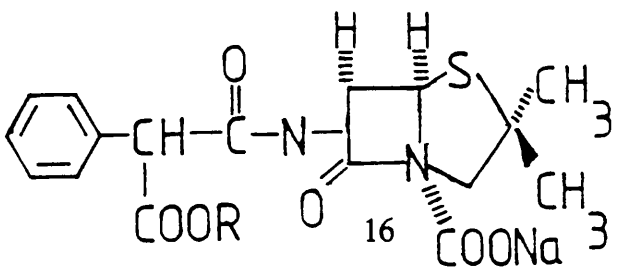
b) HCl H_2N CH (CH_3) CO

c) HCl H_2N CH_2 CO

1

A similar increase in hydrophilicity is achieved for metronidazole with the use of amino acids[49,50].

Simple hydrophobic (2b) alkyl and aryl esters of carbenicillin (2a) are orally absorbed and hydrolysed in vivo to the parent antibiotic (2a).



2 R

a) H

b) Me, Et, benzyl

16

1.2.2.1.2 Acyloxyalkyl esters

In other penicillins the simple alkyl and aryl esters are not sufficiently labile in vivo to release the free penicillin acid. This problem was overcome by Jansen and Russel in 1965[51], by the use of special double esters (acyloxy alkyl esters). It was found that acyloxymethyl ester of benzyl-penicillin was rapidly hydrolysed to the free penicillin acid. The first step in the hydrolysis of the acyloxyalkyl esters is the enzymatic cleavage of the terminal ester with the formation of an unstable hydroxymethyl ester which is unstable and rapidly dissociates to the penicillin acid and formaldehyde (Scheme 1),

$$\text{Drug-COO-CH}_2\text{ OCOR} \xrightarrow{\text{esterase}} \text{Drug-COO CH}_2\text{OH} + \text{RCOOH}$$

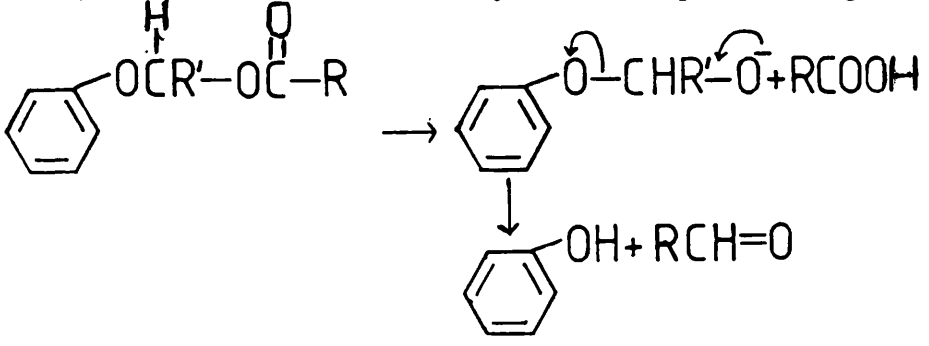
$$\downarrow \text{ fast}$$

$$\text{Drug-COOH} + \text{CH}_2\text{O}$$

(Scheme 1)

The difference in enzymatic stability between the alkyl or aryl esters and the acyloxyalkyl esters of ß-lactam antibiotics is due to steric hindrance of the penicillin carboxyl group, thus a terminal acyloxyalkylester is less hindered.

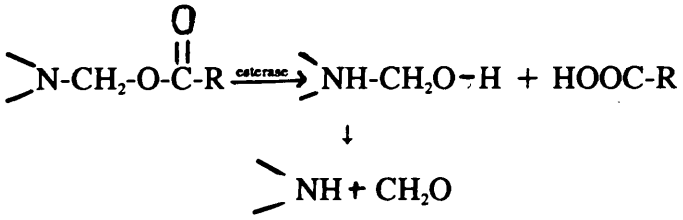
Several double ester prodrug forms of ampicillin are now on the market, pivaloyloxymethyl[52] ester (pivampicillin), the phthalidyl ester[53,54] (talampicillin) and the ethoxycarbonyloxyethyl ester[55] (bacampicillin). The use of the double ester prodrugs has been extended to cover other drugs, such as indomethacin and other non-steroidal anti-inflammatory drugs[56], cromoglycic acid[57] and methyldopa[58]. The double esters have been extended to the use of phenolic drugs such as oestradiol phenylephrine and the catecholamines[59,60]. The resulting ethers are hydrolysed again via a sequential reaction (Scheme 2) to yield the free phenolic drug.



(Scheme 2)

The enzymic hydrolysis of the phenolic esters yields an unstable hemiacetal which then breaks down to the parent phenol.

The use of the acyloxyalkyl prodrug group has been extended to nitrogen containing compounds giving N-acyloxyalkyl derivatives. The breakdown of these prodrugs is again a sequential process with the enzymic removal of the acyl group and then the unstable intermediate breaking down (scheme 3)[61].



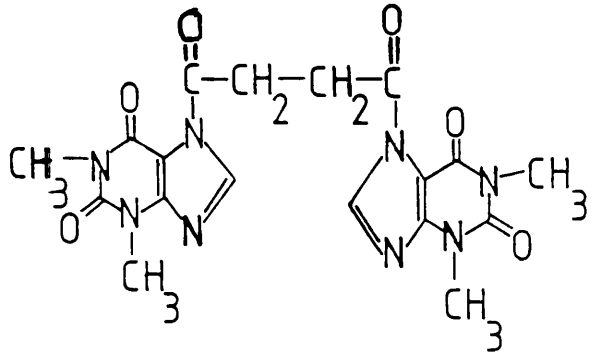
(Scheme 3)

The N-acyloxycarbonyl derivative has been used in GABA, resulting in some crossing of the blood-brain barrier[62]. Its use in phenytoin[63] improves oral absorption, whilst in theophylline[64], 6-mercaptopurine[65] and 5-fluorouacil[66] the prodrug allows for delivery through the skin.

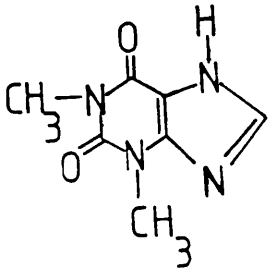
1.2.2.1.3 Amides

N-acylation of amines to give the amide is used rarely as the amides produced are relatively stable in vivo although some are susceptible to enzyme cleavage whilst others are chemically labile.

N-acyl derivatives of theophylline and allopurinol have been made. The theophylline prodrug 7,7-Succinyldithiophillene[67] (6) is used for chemically controlled release of theophylline (7).

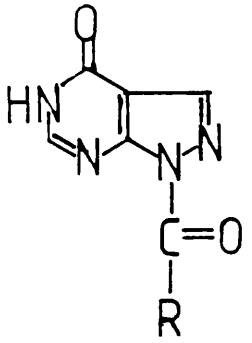


(6)



(7)

Prodrugs of allopurinol (8), another common drug, have been made to allow rectal and parenteral administration[68].



(8)

The use of γ-glutamyl derivatives has shown that there is active uptake and metabolism in the kidney, where there is a concentration of enzymes capable of cleaving the γ-glutamyl derivatives. This has allowed the production of kidney specific prodrugs which have a desired action on the kidney and urinary tract, eg sulphamethoxazole[69].

1.2.2.1.4 Schiff Bases

Schiff bases (imines) are potentially useful prodrugs[70,71], although mainly at present used for amines, their use could be extended to aldehyde or ketonic groups. The use of Schiff bases is limited due to their instability in aqueous solution, but in use they greatly reduce the basic character of amine, thus increasing lipophilicity and therefore the ability of drugs to cross membranes.

Prodrugs other than those already mentioned also exist. Ring opened derivatives act as prodrugs for cyclic compounds as in the barbiturates[72]. Cyclic prodrug derivatives are found in drugs having more than one functional group an example of this are the pyrrolides and Δ-pyrrolines of GABA[73].

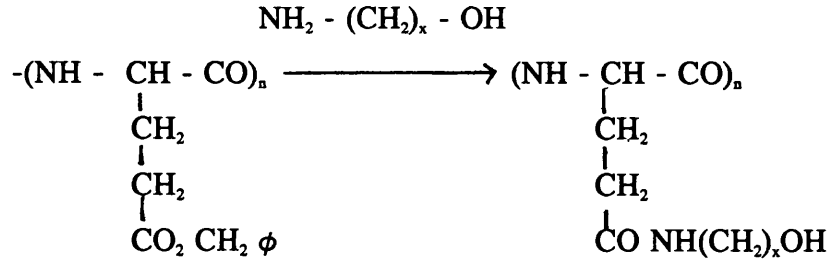
Phosphate group prodrugs exist for purine and pyrimidine nucleosides with phospholid derivative of 1ßD arabinofuranosylacytosine having greater anti-leukaemic activity[74].

1.2.2.1.5 Polymeric Prodrugs, Drug Carriers and Controlled Release Matrices

The use of macromolecules as drug delivery systems both as conjugates or as polymer capsules have been investigated. Many polymer capsules made are attempts

at controlled release, these systems often require the release of the drug from the polymer system and thus also act as prodrugs. Polylactic acid was one of the early biodegradable polymers used as an implant to deliver narcotic agents[75,76] and in 1973[77] as an implantation film as a carrier for d-norgestrel, a contraceptive agent. Due to the success of polylactic acid, other hydroxy acids have been investigated, in 1967[78] polylactic and polyglycolic acid co-polymers were used as drug delivery systems for fertilizers and insecticides as well as drugs. Polymeric systems can be biologically inert e.g. poly[N-(2-hydroxypropyl) methylacrylamide[79] or have biological activity e.g. poly(-α-lysine)[80] which is cytotoxic.

α-Amino acids have been investigated as polymeric drug carriers. In poly α-amino acid systems various drugs can be encapsulated in a polymer shell,[81] intermixed with polymer or covalently bound to the polymer backbone[82]. The α-amino acids with greatest potential as drug carriers are, L-glutamic, L-aspartic and co-polymers of these with L-leucine or L-valine. Drugs can be covalently bound to the polymer backbones by attachment to the carboxyl group of L-glutamic and L-aspartic acids. Drug attachment to polymers is generally via a spacer group, carried out by reacting an α-ω-hydroxyalkylamine with a poly (α-amino acid) e.g. poly(γ-benzyl-L-glutamate). (Scheme 4). The length of the spacer group can be varied thus affecting the lipophilicity of the polymer.



Scheme 4

As well as the carbonate ester links, other groups have been used as polymer links including esters[83], acid labile amides[84], disulphides[85] and succinate diesters[86].

The α-amino acid polymers and co-polymers have varying properties of hydrophilicity and hydrophobicity depending on the α-amino acids used and the length and type of space group. These variations allow controlled released properties to be introduced into the peptide drug carriers.

The work undertaken in this project involved the synthesis and characterisation of α-amino acid polymers and co-polymers to be used for drug encapsulation and linking. Also, the introduction of controlled release properties by varying polymer structure. In addition to polymer delivery systems, hydrophobic derivertisation has been shown to increase membrane permeability thus increasing the uptake of poorly absorbed compounds. The lipophilic α-amino acids (lipidic acids) have been used in subsequent chapters to increase uptake of short chain peptides.

2. 1 History of Enkephalins

Opium has been known for centuries and morphine the opium derivative for 200 years. The presence of endogenous opioid peptides was discovered in the last 10-15 years when electrical stimulation of the periaqueductal gray region in rat brains, produced naloxone reversible analgesia[27,86,89] (naloxone is an opiate antagonist).

After the discovery of naloxone reversibility, the active factor was purified and simultaneous attempts were made to identify specific opiate binding site in the brain.

Stereospecific binding sites within the CNS were reported by three research groups[90-92]. The presence of an opiate receptor and naloxone reversibility indicated the existance of an endogenous opioid moiety.

In 1975, several groups demonstrated that pituitary and brain extracts contained compounds with opiate like activity[93-95]. Hughes and Kosterlitz[96] subsequently identified two pentapeptides: methionine enkephalin and leucine enkephalin, these pentapeptides differ only in their C-terminal amino acids. The existence of these two very similar opioid peptides has fuelled a great deal of research.

The opioid peptides are divided into different categories[97].

2.1.1 Endogenous Peptides

1. The pentapeptides [Met]- (9) and [Leu]-enkephalin (10).
2. Pentapeptides arising from the biosynthesis of enkephalin precursors. These include those arising from adrenal pro-enkephalins: [Met]-enkephalin-Arg-Gly-Leu (11), Peptide E (12), [Met]-enkephalin-Arg-Phe (13), dynorphin (14), α

and β neoendorphin (15), and PH-8P (16).

3. β-Endorphin (17), (endorphins are peptides that arise from β-lipotropin), and the related α, γ and δ endorphins.

2.1.2 Exogenous Peptides

1. Pronase resistant peptides e.g. β-casomorphin 5 and 7 (18). These are derived from amino acids 60-66 of β-casein.
2. Dermorphin (19) and derived peptides. (This heptapeptide was isolated from frog skin).
3. This group includes peptides with opiate like properties not caused by direct receptor interaction e.g. kyotorphin (20), which appears to act by releasing enkephalin.

Tyr-Gly-Gly-Phe-Met Tyr-Gly-Gly-Phe-Leu

[Met]-enkephalin (9) [Leu]-enkephalin (10)

Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-Gly-Arg-Pro-Glu-Trp-
Trp-Met-Asp-Tyr-Gln-Lys-Arg-Tyr-Gly-Gly-Phe-Leu

Peptide E (12)

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln

Dynorphin (14)

Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro

β-Neoendorphin (15)

α-Neoendorphin (as above with Lys at end).

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile

PH-8P (16)

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-
Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu

β-Endorphin (17)

(α-Endorphin aa 1-16)

(γ-Endorphin aa 1-17)

(δ-Endorphin aa 1-27)

Tyr-Pro-Phe-Pro-Gly-Pro-Ile (18)

β-Casomomorphin-5 (aa 1-5)

β-Casomorphin-7

Tyr-DAla-Phe-Gly-Tyr-Pro-Ser-NH_2

Dermorphin (19)

Tyr-Arg

Kyotorphin (20)

The endogenous peptides arise from three types of biogenetic precursors, these are pro-opiomelanocortin (POMC), proenkephalin and prodynorphin.

The activity of exogenous opioid peptides is comparable to [Met]-enkephalin. The dermorphins, which have a D-Ala substituted for Gly^2 are 40-times more potent than [Met]-enkephalin in isolated guinea pig ileum (GPI) and 1000-times more potent in the rat hot plate test by intracerebroventricular injection[98].

2.2 Structure Activity Relationships

The structure activity study of a large number of derivatives gives the following indications[99]:

1. The N-terminal tetrapeptide is the minimum sequence required for opiate activity.
2. The three amino acid residues of the N-terminal are the most important for activity.
3. Substitution of Gly^4 retains activity.
4. O-Methylation of Tyr^1 retains activity[100], while O-sulphation[101] destroys it.
5. Substitution of Tyr^5 and/or modification of the C-terminal are readily tolerated.

The various endogenous opioids have different selectivities for the μ, δ and κ opiate receptors. Many of the synthetic analogues and derived peptides also have receptor subtype specificity and this enables the biological properties of the receptor subtypes to be investigated.

2.2.1 Opioid Peptide Precursors

The opioid peptides, like other CNS peptides, are synthesised as large inactive polypeptides. In these polypeptide structure sequences several small active molecules are found, which are released by enzymic splitting. Three genes code for the opioid polypeptide precursors, giving three precursors pro-opiomelanocortin (which contains a wide variety of biologically active peptide sequences), proenkephalin and prodynorphin.

2.2.2 Enkephalin Selectivities for the μ and δ Opiate Receptors

Various opiate receptor subtypes are thought to exist and are called μ, κ, σ and δ receptors. The existance of these various receptor subtypes allows for the design of compounds targetted for each receptor, thus giving a specific biological result.

The μ, κ and δ receptor subtypes are important for analgesia. The extended length enkephalins, dynorphins and endorphins occupy the κ receptor , while the pentapeptides and smaller enkephalins affect the μ and δ receptors. Potency tests for enkephalins refer to GPI (guinea pig ileum) which is rich in μ and MVD (mouse vas deferens) which is rich in δ receptors.

2.2.3 The Effect of Chain Length and C-Terminal Substitution on μ and δ Receptor

[Leu]-enkephalin has a greater δ receptor specificity than the [Met]-enkephalin and substitution of Gly^2 with $D\text{-}Ala^2$ retains this relationship. Incorporation of lipophilic D-amino acids at position 2 further increases selectivity due to the presence of an auxiliary binding site[102,103].

Esterification[104-106] or amidation[107,108] of the C-terminal carboxylic acid gives an increased μ specificity, as does the loss of Gly^3, giving the tetrapeptide[109,110] amides analogous to the dermorphins and casomorphins.

In tripeptides, where the C-terminal is substituted by a phenylpropylamide μ selectivity predominates due to the phenylpropylamide substituting for the phenylalanine side chain[102]. Deletion of Gly^2 and Gly^3 results in loss of tripeptide (Tyr-Phe-Met-NH_2) activity[99], indicating the distance between the aromatic rings of

Tyr and Phe is important.

The Tyr-D-Ala dipeptide with a phenylpropylamide or phenylethylamide ring substituted at the C-terminus retains potent μ selectivity[109].

2.2.4 The Effect of Phe^4 on μ and δ Receptor Selectivities

Phe^4 has a dramatic effect on μ:δ selectivity. Tetrapeptide (21) is μ selective as is its decarboxylated analogue (22). Changing the phenylethyl group of (22) to aliphatic hydrophobic side chains as in (23) results in increased μ receptor specificity and decreased δ receptor recognition. μ selectivity is further enhanced by subtituting D-Ala^2 with D-Met^2 in (24), due to better binding at the lipophilic auxiliary site[110].

Tyr-D-Ala-Gly-Phe

(21)

Tyr-D-Ala-Gly-NH-$(CH_2)_2$-C_6H_5

(22)

Tyr-D-Ala-Gly-NH-$CH(CH_3)CH_2CH(CH_3)_2$

(23)

Tyr-D-Met-Gly-NH-CH-$(CH_3)CH_2CH(CH_3)_2$

(24)

Summmary of requirements for μ receptor specificity:

(a) The size and hydrophobic content of these peptides enables them to fit the conformational space occupied by the morphine alkaloids.

(b) The peptides exist in a highly folded conformation, as shown by the morphinomimetic activity of conformationally constrained analogues incorporating α-aminoisobutyric acid[112].

(c) The folded conformation is the lowest energy form of peptide conformation.

(d) There is external orientation of the lateral side chains of any D-amino acid at position 2, but not for the L-enantiomers. This allows additional bonding interaction at a lipophilic binding site on the receptor.

(e) There is an energetically viable overlap between the tyrosine and the phenethyl chain of morphine. This occurs for δ specific peptides.

(f) There is similar spatial orientation of the C-6-α-hydroxyl group of

morphine and the critically important amide carbonyl group of Gly[3][111,113-115].

Requirements for μ opiate receptor binding site specificity[116]:

(a) Decreasing the number of amino acids in the enkephalin chain

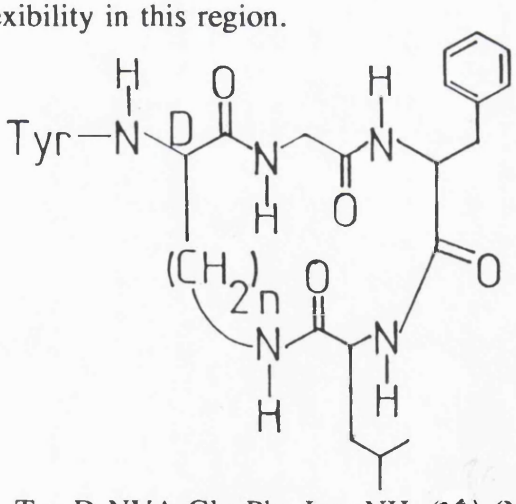
(b) Increasing lipophilicity at the C-terminal by esterification, amidation and decarboxylation.

(c) Replacement of the aromatic Phe[4] residue with a lipophilic aliphatic side chain.

(d) Introduction of a hydrophobic D-amino acid at position 2 in the enkephalin chain.

(e) The use of hydrophobic amino acid as the fifth residue to occupy an additional receptor binding site.

The preparation of cyclic enkephalins gives μ selective compounds, because cyclisation reduces the conformational degrees of freedom. Cyclisation involves the production of a cyclic lactam between basic D-amino acids in position 2 and the C-terminal carboxylic acid[117]. Tyr[1] is exocyclic, thus the cyclic enkephalins maintain the necessary flexibility in this region.



n=2 (25)
n=1 (26)
n=3 (27)
n=4 (28)

Tyr-D-NVA-Glu-Phe-Leu-NH_2 (28) (NVA = norvaline)

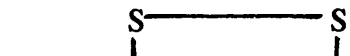
Compound (25) contains a 14-membered lactam ring, incorporates D-2,4-diaminobutyric acid as the bridging ligand, and is 17 times more potent than the [Leu]-enkephalin in the GPI assay and 7 times less potent in the MVD test.

Comparison of (25) with the open chain analogue (28) indicates that μ selectivity is a direct result of conformational restriction[118].

Variation of conformational restriction can be brought about by changing the number of methylene groups in the basic amino acid. Analogues (26), (27) and (28) show μ receptor specificity, with increased μ, as the size of the lactam ring increases (i.e. the number of methylene groups increases).

The most potent analogue (27) is 51 times as potent as [Leu]-enkephalin in the GPI assay and 12 times less potent in the MVD. The cyclic enkephalins have the same configurational requirements in amino acids 1-5 as the linear enkephalins[119].

Substitution of cysteine at position 2 and 5 of the enkephalin chain with cyclisation through disulphide bonds gives active compounds.



(29)

Compound (29) is a strong analgesic causing respiratory depression and it is 100 times more potent than morphine in competing for its receptor[120]. The use of [D-Cys^2, D-Cys^5] and [D-Cys^2, Cys^5]-enkephalin shows the cyclic disulphides are non-selective towards the μ and δ receptors[121,122]. The use of penicillamine (β,β-dimethylcysteine), which restrains conformation like aminoisobutyric acid and forms disulphide bridges produces δ specific peptide.

The DADLE (30) [Leu]-enkephalin analogue is δ selective, here Gly^2 is replaced with D-Ala^2, and Leu^5 is replaced by its D-Leu enantiomer.

Tyr-D-Ala-Gly-Phe-D-Leu (30) DADLE

DADLE (30) has a three fold preference for the δ receptor. Lipophilic D-amino acids at position 2 enhance μ selectivity, introduction of hydrophilic groups in this position should enhance δ selectivity. Decreasing the enkephalin chain length favours μ selectivity thus increasing it slightly may enhance δ affinity.

Replacement of Gly^2 with hydrophilic Ser^2 and the introduction of Thr^6 in [Leu]-enkephalin produced (31) (DSLET), which is 10 times more potent at the δ receptor than DADLE (30)[123].

Tyr-Y-Gly-Phe-Leu-Thr Y=Ser (31)

Y=Thr (32)

Replacement of Gly^2 with Thr^2 gives deltaenkephalin (DTLET) (32) which has greater δ specificity than (31)[124].

Comparing compounds 30, 31 and 32 shows the effect of hydrophilicity and lipophilicity of the second amino acid in enkephalin.

In summary δ opiate receptor specificity is enhanced by:

(1) The presence of an aromatic ring at the equivalent position of phenylalanine at position 4.

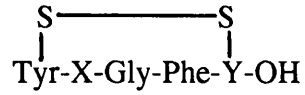
(2) The presence of a hydrophilic C-terminus that allows the Phe^4 aromatic ring to be fitted into its δ binding site i.e. the amino acids following Phe^4 play a key role in determining δ specificity.

(3) The presence of a hydrophilic side chain on the second amino acid such as Ser or Thr strongly enhances δ specificity.

(4) An extended rather than folded energetically available conformation of the peptide.

Extended peptide conformations are thought to be important for δ selectivity although cyclic δ specific peptides have been produced.

Cyclic [D-Cys^2, D- or L-Cys^5]-enkephalins show slight μ selectivity, but replacement of cysteine with penicillamine (Pen, β,β dimethylcysteine) produces the most δ specific peptides known.

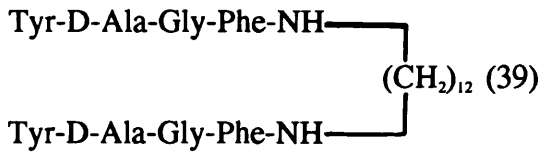


X	Y
D-Pen	D-Cys (33)
"	Cys (34)
"	D-Pen (35)

"	Pen	(36)
D-Cys	D-Pen	(37)
D-Cys	Pen	(38)

The replacement of D-Cys^2 with D-Pen^2 (33) gives a δ selective peptide, while replacement of Cys in positions 2 and 5 gives even greater selectivity. Compound (35), having D-Pen^2 and D-Pen^5, is the most selective ligand[125]. The δ receptor selectivity of the penicillamine compounds is due to additional conformational rigidity in these cyclic analogues conferred by the gem-dimethyl groups of penicillamine.

Rigidity has been confered to the enkephalins by dimerisation via a spacer group of varying length. The tetrapeptide dimer (39), with a 12-methylene group spacer unit has optimum δ selectivity[125,126].



(39)

This tetrapeptide (39) has 4 times the δ selectivity of (31). Dimer selectivity is thought to be due to the cross-linking of receptors by the two linked individual enkephalin terminals[126].

2.2.5 MINIMUM ENKEPHALIN CHAIN LENGTH NECESSARY FOR ANALGESIA

[Met]- and [Leu]-enkephalin show no analgesic activity because they are rapidly inactivated by peptidases, but substitution of Gly^2 by D-Ala^2 in the enkephalinamides (40) and (41), gives analgesic activity[128,129].

Tyr-D-Ala-Gly-Phe-Met-NH_2 (40)

Tyr-D-Ala-Gly-Phe-Leu-NH_2 (41)

The number of units in the peptide sequence required for analgesia has been studied by synthesising deletion peptides.

Tyr-D-Ala-Phe-Met-NH_2 (42)

Tyr-D-Ala-Phe-Leu-NH_2 (43)

Tyr-D-Met(O)-Gly-N-Methylphenylethylamide (44)

Tyr-D-Ala-3-Phenylpropylamide (45)

Tyr-D-Met(O)-3-Phenylpropylamide (46)

Tetrapeptides (42) and (43)[130], have analgesic activity as does tripeptide (44) and dipeptides (45) and (46)[132-135]. In peptides (44)-(46) the arylalkylamide groups at the C-terminus seem to be needed for analgesic activity. The aromatic ring in the amide substituent, replacing the aromatic ring in phenylalanine in [Met]- and [Leu]-enkephalins. It appears that the minimum chain length required for enkephalin based analgesia is a dipeptide.

2.3 STRUCTURE ACTIVITY RELATIONSHIPS IN THE ENKEPHALINS

2.3 1.The Enkephalin N-Terminus

Modification of the tyrosine amino group by acylation, alkylation, deamination and extension by the addition of other amino acids, or its removal by replacing tyrosine with 4-hydroxyphenylpropionic acid results in loss of biological activity in both [Leu]- and [Met]-enkephalin[136].

N-methylation of Tyr^1 results in little change of potency in the GPI assay in [Leu]- and [Met]-enkephalin. Synthesis of a series of N-alkyl [Met]-enkephalins from ethyl to octyl, shows a decrease in potency and receptor affinity[137]. N,N-dimethylation of the [Leu]-enkephalin N-terminal leads to decreased potency, while blocking this group with an acetyl or butoxycarbonyl group leads to no biological activity. A mono or unsubstituted N-terminal amino group is required for opioid activity.

It is thought that a region of the opiate receptor prefers cationic species in the Tyr^1 area this region being restricted to 3.3Å[138], thus strongly basic guanadino analogues have been produced. Compound (47) containing $D\text{-}Met^2$ in a tetrapeptide gives increased potency, but no receptor specificity.

$$NH_2\text{-}C(=NH)\text{-Tyr-D-Met-Gly-Phe-}NH_2 \quad (47)$$

The guanadino derivative (47) is 26 times more potent than the amino analogue (48) and 4.4 times more potent than morphine[139].

Tyr-D-Met-Gly-Phe-NH_2 (48)

The addition of a variety of lipo- and hydrophilic amino acids[140], acidic and basic[141], at the N-terminal lowers potency but does not change selectivity. Increasing amino acid chain length decreases potency rapidly as the chain length is extended[142,143]. A series of [Met]-enkephalin analogues β-Ala, GABA and 5-aminopentanoic acid lack activity and have minimal receptor affinity[141].

It appears most N-terminal substitution decreases potency, N-methylation may enhance biological activity but generally little change is tolerated at this position of he enkephalin molecule.

2.3.2 Tyrosine[1] Structure-Activity Relationships

Tyrosine requirements are very stringent. Deletion of Tyr[1] in both [Leu] and [Met] enkephalin results in loss of activity, as does introduction of the Tyr enantiomer D Tyr, receptor affinity is also lost[144]. Substitution of the tyrosine hydroxy group by p amino or DOPA (3,4 dehydroxyphenylamine) results in loss of agonist activity.

The replacement of tyrosine by p-OH-Phe Gly or homo (2-amino-4-(4-hydroxyphenyl)-butyric acid) tyrosine and other groups such as Gly, Ala, D-Ala and His, also results in activity loss.

Many rigid analogues have been synthesised to constrain tyrosine in a fixed conformation, but these are inactive. It appears that any change in the tyrosine molecule results in a decrease in biological activity[145-147].

2.3.3 Glycine[2] Structure Activity Relationships.

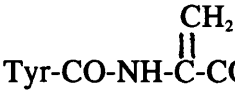
The second enkephalin residue is glycine. Here the requirements are less stringent and changes can be made. Gly[2] is important in determining μ or δ receptor specificity, the selectivity being determined by hydrophillic or lipophilic side chains of the amino acids.

Many changes have been made at the second residue in both [Leu] and [Met] enkephalins[103-106]. The introduction of D-amino acids result in increased biological activity. Replacement of Gly by D-Ala results in in increased biological activity, and the more lipophilic amino acids D-Leu[2] and D-Ala[2] show an increase in potency and greater receptor selectivity[108,148].

The introduction of hydrophillic D-Ser[2] increases potency, but not all D-amino

acid substitutions result in potency. The natural amino acid, D-Phe^2, Pro^2, D-Pro^2, D-Trp^2 and D-Val^2 are less active. The inclusion of non α-amino acids ß-alanine^2 and ϵ amino hexanoic acid[149] leads to loss of biological activity. The preferred substitution of Gly^2 for analgesia is D-Ala^2 or D-Met^2.

To confer rigidity to the molecules the dehydroamino acids have been produced. The opiate receptor affinity of Δ-Ala^2 (49) has been studied and has shown δ receptor selectivity[150]. The replacement of Gly^2 with sarcosine (Sar) (50), N-methylglycine, results in 1% or less of the biological activity of [Met] enkephalin.



Tyr-CO-NH-C-COGly Phe Leu (49)

(D-Ala^2-[Leu]enkephalin)

Tyr-Sar-Gly-Phe-Leu (50)

The N-substitution of Gly^2 with acetic acid (51) or ß-hydroxyethyl (52) residues results in compound (51) having no action and (52) having 0.43 times the activity of [Met] enkephalin at the δ receptor, but only 0.06 times the activity at the μ receptor.

Tyr-(R)Gly-Gly-Phe-Leu

R = CH_2CO_2H (51)

R = CH_2CH_2OH (52)

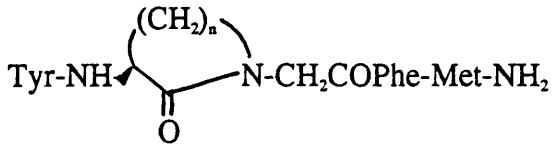
Tyr-(Gly-Gly-Phe-Leu)$_2$ (53)

Tyr $CONHCH_2$-CS-Gly-Phe-Leu (54)

The hydroxy ethyl side-chain in (52) may be mimicking the selective effects of Ser^2 and Thr^2. The dimeric form of the [Leu] enkephalin having a common Tyr^1 has been synthesized and shown δ selectivity but weak potency(53). The Gly^2 carbonyl group has been replaced with a thiocarbonyl, the thioamide (54) has 2.6 times the μ receptor activity and 9.1 times the δ receptor activity of [Leu] enkephalin[151].

Various rigid analogues have been produced by incorporating rigid amino acids in place of Gly^2 or by ring formation between Tyr^1 and Gly^2. Methylene

bridging of the α-carbon and of Gly² results in a series of bonded analogues (48-50)

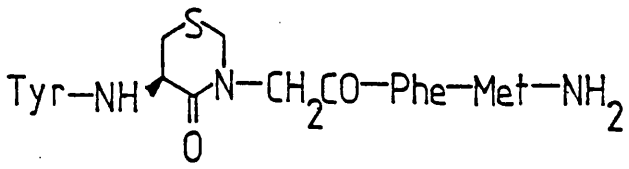


55 (n = 2)

56 (n = 3)

57 (n = 4)

Compound (56) has 0.1 times the potency of [Met] enkephalin, the others less than 0.01 times the potency. The thiazolidinone derivative (58), an analogue of a cyclic methionine, has only 3% the activity of [Met] enkephalin. The amide nitrogens of Gly² and Gly³ of [Leu] enkephalin have been bonded by an ethylene group (59) resulting in a biologically inactive molecule[152]. Similar results were observed from the [Met] enkephalin derivatives. Formation of an imidazolidinone (60) between Tyr¹ and Gly² nitrogen results in a drastic reduction of biological activity[151,152] as does the diketopiperazine (61), partly due to its blockade of the Tyr¹ amino group. The rigid analogues of Gly² have not been successful.



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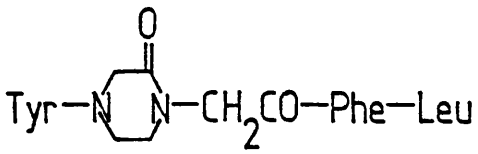

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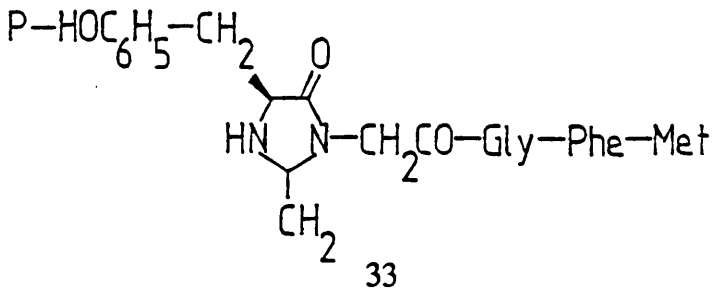

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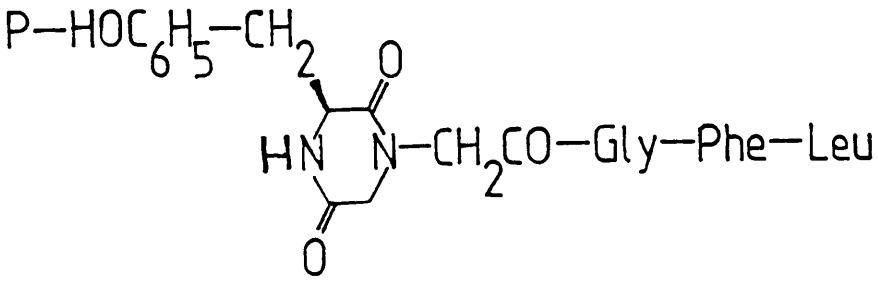

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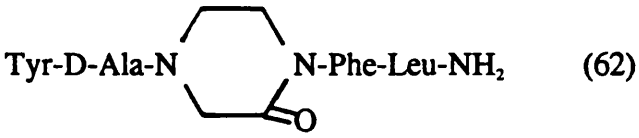
2.3.4 Glycine[3] Structure-Activity Relationships

Gly[3] is an important part of the molecule, deletion results in loss of μ receptor activity to 0.2% of that of [Met] enkephalin[155]. Introduction of other natural amino acids decreases activity as does the incorporation of D-Ala[3], with μ and δ receptor activity being 2% or less. The replacement of Gly[3] by Sar[3] and dehydroalanine[156] with both the D- and L-isomers was ineffective as was the ß-alanine derivative and Aib[3]. Backbone methylation, the replacement of Gly[3] with Sar[3] in [Leu] enkephalin, reduces δ activity to only 0.3% of [Met]. Enkephalin with a thioamide retains μ equivalence to [Leu] enkephalin but δ activity is reduced by 85%. A rigid analogue (62) where the Gly[2] and Gly[3] amide nitrogens are linked by an ethylene bridge is inactive[157].



(62)

Structure-activity relationships for Gly[3] indicate that all modifications, except for the thioamide group, reduce biological activity.

2.3.5 Phenylalamine[4] Structure Activity Relationships

Structure activity relationship plus receptor type selectivities have been determined for Phe[4]. The replacement of Phe[4] by D-Phe[4], results in loss of biological activity.

Replacement of Phe[4] with other amino acid losses both μ and δ activity; the exception is Trp[158].

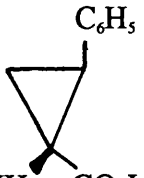
The amino acids replacing Phe[4] do not have a large lipophilic side chain that

is required to replace the aromatic ring of Phe in μ selective ligands. Substitution of Phe^4 by Δ^2-Phe produces analogues (63) and (64), which possess high affinity for the opiate receptor, with (63) having higher δ selectivity and these dehydro analogues possess in vivo analgesic properties[159].

Tyr-Gly-Gly-Δ^2-Phe-Leu (63)

Tyr-D-Ala-Gly-Δ^2-Phe-Leu (64)

The cyclopropyl derivative (65) is a conformationally restricted Phe^4 analogue, with both enantiomers being less active than the parent at both the μ and δ receptor[160].



Tyr-D-Ala-NH CO-Leu (65)

Substitution of the aromatic ring of Phe^4 has been investigated. The p NO_2 group on Phe^4 produces analogues with increased μ and δ receptor activity.

Tyr-D-Ala-Gly-p-NO_2 Phe - X

X = Leu-NH_2 (66)

= D Leu (67)

= Met (68)

Compound (66) is becoming more μ active, compound (67) more δ active and compound (68) is indiscriminate[161,162].

Meta and para substitutes of Phe^4 on the analgesic peptide, metkephamid (69) have been studied. In meta-substituion an electronegative halogen appears to give the best δ potency and analgesia. Other electronegative groups such as trifluoromethyl and electron donating groups are less efficient. A similar pattern to the meta substituents is obtained by the para substitutes, with electron withdrawing groups giving the most potent peptides. Fluorine and nitro para groups having similar δ potencies, but fluorine being 8 times more potent as an analgesic[163].

The D-p-chloro derivative is less active than the L-p chloro derivative. The electron-donating and acidic p-hydroxy group when Phe^4 is replaced with Tyr^4 is less active than D-p-chloro in the δ assay and has weak analgesic properties.

Tyr-D-Ala-Gly-Phe(Me)Met-NH_2

(69)

Loss of the Phe^4 aromatic ring does not affect activity. Reduction of the aromatic ring to the hexahydro derivative produces analgesic enkephalins[164,165] Incorporation of Phe(6H)^4 in the [D-Met^2] enkephamides gives peptides that are more μ potent and less δ potent.

Backbone alkylation of Phe^4 in the tetrapeptide Tyr-D-Ala-Gly-Phe($\overset{R}{\underset{|}{}}$)-$NH_2$ has been studied. δ potency peaks at the N-ethyl and is 90 times more potent than the unsubstituted parent[166].

R = C_2H_5 (70)

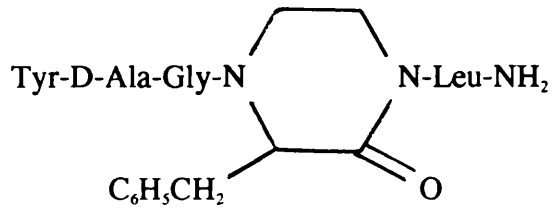
R = $CH_2CH{=}CH_2$ (71)

R = CH_2-c-C_3H_5 (72)

R = $CH_2CH{=}C(CH_3)_2$ (73)

δ activity is also shown by the opiate antagonist, mixed agonist-antagonist substituents (71-73). Analgesic efficacy is achieved by alkylation of the Phe^4 amide nitrogen the N-ethyl analogue being 20 times more potent than the parent which is 150 times more potent than the N-methyl. Analgesic potency decreases with larger lipophilic substituents and shorter hydrophillic derivatives.

The thioamide group at Phe^4 produces conformationally restrained enkephalin analogues. The [Leu] enkephalin derivative containing the thioamide in place of the amide group of Phe^4 is equivalent to [Leu] enkephalin at the μ and δ receptor. Joining the Phe^4 amide nitrogen and Leu^5 amide by a 2-carbon bridge produced a rigid analogue (74). The diasteriomers were separated and neither bound the opiate receptor and both had only weak analgesic properties.



(74)

In summary, many changes can be made at Phe^4 without loss of activity. The Phe aromatic ring can be reduced or substituted. The amide nitrogen can also be alkylated and Phe^4 replaced with other aromatic amino acids such as Trp^4.

2.3.6 Methionine^5/Leucine^5 Structure Activity Relationships.

Structural and conformational changes at Met^5/Leu^5 positions are well tolerated. Removal of the terminal amino acid retains μ and δ activity. D-Ala^2 results in more potent tetrapeptides and D-Leu^5 gives decreased μ activity but increased δ activity. Replacement of L-Met with D-Met gives a peptide similar to [D-Leu] enkephalin where μ activity is reduced and δ activity unchanged. Oxidation of methionine sulphide to sulphoxide and sulphone decreased both μ of activity. The D-Ala^2 sulphoxides are more potent than the parent Met derivative[167].

Decarboxylation of [Met] and [Leu]-enkephalin gives the tetrapeptides (75) and (76).

Tyr-Gly-Gly-Phe-R

R = $NH(CH_2)_2SCH_3$ (75)

R = $NH(CH_2)_2CH(CH_3)_2$ (76)

Replacement of Met^5/Leu^5 with hydrophilic groups such as hydroxyalkyl amides or aminoalkylamides (77,78) leads to enhancement of μ effects and a decrease in δ activity[168,169].

Try-D-Ala-Gly-MePhe-R

R = $NH(CH_2)_2N(CH_3)_2$ (77)

R = $NH(CH_2)NO(CH_3)_2$ (78)

Compounds (77 and 78) are potent analgesics. Compound (79) has a similar, but stronger effect than (77) and (78) due to its lipophilic group.

Try-D-Ala Gly Phe-N-$(CH_2)_2CH(CH_3)_2$ (79)

$CH_2C_6H_5$

Compound (79) has 9 times the GPI potency of [Met] enkephalin and only 0.5 times the potency in the MVD assay.

Reduction of [Met] and [Leu] enkephalin to the alcohols (80) and (81) retains receptor and GPI activity.

Try-Gly-Gly-Phe-R

R = Met-ol (80)

R = Leu-ol (81)

Try-D-Ala-Gly Phe-Met-ol (82)

The [D-Ala^2, Met^2-ol] derivative (82) and its sulphoxide derivative possess strong opiate receptor affinity and are potent parenteral analgesics. The reduction of the terminal amino acid to the corresponding alcohol produces biologically potent enkephalins.

Replacement of Met^5/Leu^5 with other naturally occurring amino acids results in reduced biological activity with the exception of isoleucine and norleucine. Biological activity was also lost in the substitution of 6-aminohexanoic acid (83) at position 5, but the [D-Met^2] analogue of (83) was very potent equivalent to 0.75 of that of parenteral morphine.

Tyr-Gly-Gly-Phe-NH$(CH_2)_6CO_2H$ (83)

Replacement of the carbonyl group of amino acids by phosphonic acid and incorporation of these as the fifth amino acid produces some analgesic activity (84 and 85)[170].

Tyr-Gly-Gly-Phe-R

R = $(NH\text{-}CH(CH_2CH(CH_3)_2PO_3H_2)$ (84)

R = $(NH\text{-}CH(CH_2CH_2SCH_3)PO_3H_2$ (85)

The chloromethyl ketone derivative (86) of (30) (DADLE) binds irreversibly to the high affinity opiate binding site producing a long-lasting dose-dependent analgesia[176,172].

Tyr-D-Ala Gly Phe-NH-CH-$COCH_2Cl$

$CH_2CH(CH_3)_2$ (86)

Incorporation of dehydro Leu^5 (87 and 88) leads to retardation of enzymatic hydrolysis of the amide bond.

Try-Gly-Gly-Phe-Δ^{Z}-Leu (87)

Tyr-D-Ala-Gly-Phe-Δ^{Z}-Leu (88)

Both (87) and (88) have μ and δ receptor affinity equivalent to [Leu] enkephalin with (87) having no analgesic activity and (88) 2% of morphine[173].

N-backbone methylation of [Leu]-enkephalin and the D-Ala^2 analogue does not alter in vitro potency or selectivity. The increased resistance to enzymatic hydrolysis produced by N-methylation increases analgesic potency of enkephalin analgesics. Production of rigid analogues with the Met^5 or Leu^5 amides in a six-membered ring produced a [Leu] enkephalin analogue 4.5 times as potent as morphine in analgesia, while the [Met] enkephalin analogue has greater opiate receptor affinity but equivalent analgesia to morphine.

2.3.7 The Enkephalin C-Terminus

The effect of C-terminal amidation on biological activity is variable. A series of N-alkylamides of [D-Ala^2, Met^5] enkephalin showed GPI potency with the most potent being ethyl and n-propyl, both had analgesic effect 2.1 times that of morphine[174]. Increasing the chain length to six amino acids by addition of Thr^6 and Ser^6 yields δ-specific peptides.

2.4 Enkephalin Based Opioid Antagonists

Introduction of cyclopropylmethyl or allyl substituents to the N-terminus in the case of monosubstitution leads to agonists with sharply reduced biological activity or partial agonists[175,176]. The N,N-diallyl compound (89) and the isosteric analogue (91) with the Gly^3-Phe^4 band replaced by CH_2S are pure antagonists with δ opiate receptor selectivity[177,178]. Antagonists (89) and (90) possess a 10-30 fold greater selectivity in antagonising [Leu] enkephalin compared with morphine, in MVD preparations. Structure activity investigations mainly of the second and fifth enkephalin residues has produced congeners with lower affinity and selectivity[179]. The structure activity relationship data developed for agonist enkephalins is not transferable to the antagonist series. Replacement of the two glycine residues in (89) with Aib residues (90) produces a highly selective δ receptor antagonist. Compound (91) is equivalent to naloxone at the δ receptor but has no μ and k receptor activity below 5 μm

concentration[180]. A series of antagonists, are based on the secondary amides of a tetrapeptide. One of these peptide (92) is a pure agonist in the MVD with a 10-fold selectivity for the μ receptor. The Phe[4] methyl group and the secondary amide are vital antagonist properties.

N,N-(Allyl)$_2$Tyr-Gly-Gly-Phe-Leu (89)

N,N-(Allyl)$_2$Tyr-Gly-NH(CH$_2$)$_2$S-CH(CH$_2$C$_6$H$_5$)CO-Leu (90)

N,N-(Allyl)$_2$Tyr-Aib-Aib-Phe-Leu (91)

Tyr-D-Ala-Gly-MePhe-N(CH$_3$)(CH$_2$)$_3$C$_6$H$_5$ (92)

2.5 Clinically Investigated Enkephalin Analgesics

Three peptides have been clinically tested, metkephamid (93)[181], DAMME (94)[182] and [D-Met^2,Pro^5] enkephalinamide (95)[183]. These peptides contain modifications to retard enzymatic hydrolysis eg. D-amino acids, backbone methylation or reduction of a carboxyl group to the alcohol.

Tyr-D-Ala-Gly-Phe-Me-Met-NH$_2$ (93)

Try-D-Ala-Gly-Me-Phe-Met (O)-ol (94)

Tyr-D-Met-Gly-Phe-Pro-NH$_2$ (95)

Metkaphamid (93) is 1.7 times more δ selective than [Met] enkephalin in the MVD assay and 100 times more potent than morphine by ICV administration[184]. Compound (94) reported by Sandoz in 1977[185], is a potent analgesic, by ICV administration it is 1000 times more potent than morphine. However its analgesic effects orally in humans were less than that of morphine.

[Met^2,Pro^5] enkephalinamide (95) reported in 1977[186-187] is 70 times more potent than morphine as an analgesic by ICV administration.

2.6.1 The Chemical Anatomy of the Enkephalins

The enkephalins (9) and (10) are peptide opiate based analgesics. Due to their peptide character they are susceptible to proteolytic enzymes as well as metabolic, transport, tissue distribution and CNS penetration problems inherent in the rigid opiates. The biological activity of the enkephalins shows the following trends:

1) The first two residues with a lipophilic amide side chain is the minimum chain length for analgesia.
2) An unsubstituted or monosubstituted amino group in tyrosine is necessary as is the phenolic hydroxyl. Other changes result in reduced activity.
3) D-amino acids at position two increase activity relative to Gly^2 whilst L-amino acids decrease it.
4) Little change is tolerated at Gly^3.
5) Phe^4 can be radically changed with the aromatic group being reduced or substituted by electron withdrawing groups. Phe^4 can be substituted by Trp but not most other amino acids.
6) Position 5 can be radically changed. Met^5 oxidation to the sulphoxide (not the sulphone) gives enhanced analgesic properties. Leu^5/Met^5 can be decarboxylated or replaced by natural and unnatural lipophilic amino acids. The carboxyl group can be reduced to the alcohol resulting in retained or enhanced analgesic potency. Formation of the amides results in enhanced stability.
7) Potent analgesics are produced by amide backbone substitution at Phe^4 and Met^5/Leu^5 but introduction at Gly^2 or Gly^3 decreases activity.

Functional groups influencing μ and δ opiate receptor specificities.

2.6.2 μ receptor specificity.

1) Decreasing the number of residues in the peptide.
2) Replacement of Gly^2 with lipophilic D-amino acids.
3) Replacement of Phe^4 by a large lipophilic chain.
4) Lipophilic amino acids at position^5
5) A folded conformation.

2.6.3 δ receptor specificity.

1) Phe^4 aromatic ring.
2) A hydrophilic C-terminus.
3) Hydrophilic D-Amino acid at Gly^2.
4) An extended conformation.

2.7 BIOLOGICAL EFFECTS OF OPIOIDS

The existance of opiate receptors, found in the mid 1970's, led to a search for naturally occurring opiate molecules. This search led to the discovery of [Met] and [Leu] enkephalin and their precursors.

The biological effects of opiates in humans include sedation, miosis, euphoria (in the presence of discomfort) dysphoria (in the absence of discomfort), nausea and emesis, analgesia, antitussive, respiratory depression, inhibition of gastrointestinal transit, urinary retention, tolerance, and physical dependence.

2.7.1 Multiplicity of Opiate Receptors

Using behaviourial, physiological or pharmacological tests, three distinct opiate receptors were identified the μ, k and σ receptors. The μ receptor is associated with bradycardia, miosis, respiratory deceleration, indifference and analgesia. The σ receptor is associated with tachycardia, mydriasis, respiratory acceleration and delirium. The k receptor is associated with miosis, analgesia, sedation and little change in pulse or respitory rate.

The μ receptor binds morphine, its prototypic ligand and is the predominant receptor in guinea pig ileum. [Leu] and [Met]-enkephalin bind at the δ receptor , the peptide binding receptor found in mouse vas deferens[188] and benzmorphines bind at the k receptor which predominates in the rabbit vas deferens[189]. Not all peptides are δ specific with [Tyr-D-Ala-Gly-Me Phe-Met(O)-ol] and [Tyr-D-Ala-Gly-MePhe-Gly-ol] binding the μ receptor with greater affinity than the δ receptor, as does morphiceptin [Tyr-Pro-Phe-Pro-NH2] and some of its analogues.

D-Ala^2-D-Leu^5 (DADLE) (31) is the prototype δ receptor agonist[190] but is only three times more δ selective than μ, but several other enkephalin analogues are more μ-selective. DSL ET[Tyr-D-Ser-Gly-Phe-Leu-Thr][191,192] and DTLET[Tyr-D-Thr-Gly-Phe-Leu-Thr] are 8 and 23 times more δ-selective respectively.

2.7.2 Analgesia

Several behaviourial tests are used to evaluate the efficacy of opioids as analgesics. These include writhing[193], formalin[194], tail flicks[195], hot plate[196] and flinch jump tests[197] performed using rodents, the skin twitch response performed using

dogs[198] and the discrete trial shock titration procedure used in primates[199,200].

The μ receptor was initially attributed to the analgesic activity of opioids, due to its distribution in the brain and because regions involved in the processing of nociceptive information were enriched in μ sites[201,202]. Some k agonists have shown analgesic activity, although this effect could not be dissociated with their additional μ receptor activity. The selective k agonist U-50488[203] produces analgesia, this and the analgesic activity of k agonists which have μ receptor antagonists, slow K activation also produces analgesia.

K receptors are found in the spinal cord, a CNS site involved in the processing of nociceptive information[204]. The δ agonist analgesic activity particularly after ICV or intrathelical administration, and the existence of δ receptors in regions of the CNS involved in processing or nociceptive information suggest that analgesic opioid activity cannot be attributed to one opiate receptor subtype.

2.7.3 Respiratory Depression

Respiration is monitored using several techniques including measurement of respiratory rate, tidal volume, minute volume (the product of respiratory rate and tidal volume), the arterial tension of CO_2 and 0_2, integrated phrenic nerve activity and responsitivity to CO_2. Using these techniques opioids have been shown to alter respiratory rate, rhythmicity, pattern and minute volume[205,206]. Morphine has been found to affect frequency and tidal volume control mechanisms of the respiratory centre independently[207] and to depress the peripheral hypoxic drive to respiration[208]. Response to CO_2 is also decreased by opioids, by shifting the CO_2 stimulus respiratory response curve. The decrease in total respiration is attributed to failure of the respiratory centre to respond to CO_2. The opioid peptides [Met] enkephalin, ß-endorphin and D-Ala^2-D-Leu^5 enkephalin also depress respiration[209-211]. Studies indicate that the respiratory depressant and analgesic effects of opioids are mediated by different opiate receptors. The respiratory depressant effects of opioids cannot be attributed to activation of any one opiate receptor subtype, but both μ and δ receptors are involved[212].

2.7.4 Gastrointestinal Motitility

Gastrointestinal motility (GI) is decreased by opioids due to increased tone of portions of the stomach, small intestine and large intestine and a decrease in the number of propulsive contractions of the small and large intestines[213]. Both peripheral and central sites mediate gastrointestinal mobility reduction[214,215]. Ward and Takemori[216] suggest that the centrally mediated effect of opioids on GI mobility is mediated by μ receptors, whereas the peripherially mediated GI effects are mediated by μ and k receptors. Porreca and Burks[217] also conclude that centrally mediated actions are due to μ receptors while the opioid effects on GI exerted at the level of the spinal cord are mediated by δ and μ receptors.

2.7.5 Dependence

Drug dependence is defined as 'a state, psychic and sometimes physical, resulting from the interaction between a living organism and a drug, characterised by behavioural and other responses that always include a compulsion to take the drug on a continuous or periodic basis in order to experience its pshychic effects, and sometimes to avoid the discomfort of its absence'[218].

Several animal models of physical and psychic drug dependence have been developed. *In vivo* dependence studies indicate that μ and k- selective opioids have different dependence profiles. k receptor agonists do not suppress abstinence symptoms in morphine dependent animals, and the abstinence syndromes exhibited by k and μ agonists are different. In general no physiological significance can be assigned to one opiate receptor subtype, but each different subtype appears to mediate the pharmacological effects of opioids to a different extent[219].

3. Elastase Inhibitors

The lipidic amino acid drug delivery system involves the use of lipidic amino acid oligomers to carry drugs, amino acids or pepitdes. It has been designed with the twin objectives of enhancing membrane permeability due to increased lipophilicity and enhancing the stability of the parent compound[220]. Compounds attached to the lipidic amino acid drug delivery system are stabilised by decreasing the rate of biodegradation due to protection of attached moieties by the long hydrophobic chains

of the lipidic amino acids[221]. The lipidic amino acid delivery system has been applied to the synthesis of elastase inhibitors.

Elastases are proteolytic enzymes which have an affinity for elastin, found in elastic fibres, and they have the ability to degrade virtually all components of connective tissue[222]. Interest in the elastase enzymes has arisen due to their possible involvement in connective tissue disorders and inflammatory diseases. The disorders that are thought to involve elastases are pancreatitis[223], emphysema[224-227], artherosclerosis[228], adult respiratory distress syndrome[229], gingivitis, glomerular nephritis[230], rheumatoid and osteo arthritis[229,230]. There may also be some involvement of elastase in cancer metastasis[231,235].

The physiological function of elastases is to act as a defence mechanism, in which their proteolytic activity destroys foreign materials such as microorganisms, inflammatory mediators or necrotic tissue following phagocytosis by macrophages.

The body is protected from elastase proteolysis by the existence of protease inhibitors[232]. The disorders caused by elastases occur when elastase release is uncontrolled. The normal lung, during infection and inflammation, is protected from elastase proteolysis by the protease inhibitor α1-antitrypsin (now called α1-proteinase inhibitor), which is the major serum inhibitor of leucocyte proteases. In some individuals, the mechanism protecting against elastase proteolysis is non-operative, because they have an α1- proteinase deficiency due to genetic or other causes. The lack of protection against the elastase enzyme leads to emphysema[233] or respiratory distress syndrome[230,234].

Elastase may also indirectly accelerate the progress of the disorders associated with it in two ways. Elastases can cleave complement component C'5 to C'5a, complement being a group of proteins mediating the combination of antigen and antibody and so they are important in the immune response[235]. C'5a is a potent chemoattractant for neutrophils, and produces further inflammation. Elastase may contribute to the pathology of rheumatoid arthritis by stimulating the synthesis of rheumatoid factor thus progressing the disease further[236].

Other inhibitors of leucocyte mediated proteolysis include acid stable inhibitors from mucous secretions[237,238], from seminal plasma[239] and from salivary glands[240,241]. The inhibitor α2- macroglobulin is found in synovial fluid and is thought to be the

elastase inhibitor that prevents proteolysis of cartilage and hence protects against arthritis[242].

The elastase inhibitors α1-proteinase and α2-macroglobulin are circulating inhibitors and control elastin turnover in conjunction with leucocyte and tissue elastase.

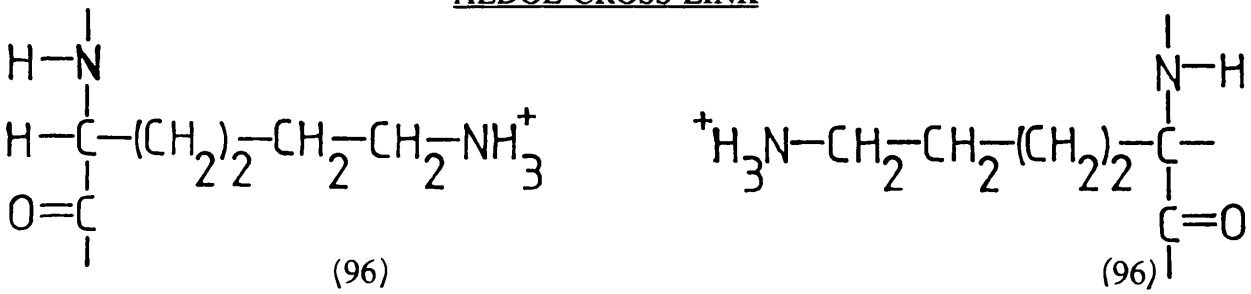
3.1 Elastin Structure[243]

Elastin is found in elastic fibres and is the substrate of the elastases. One of the properies of elastic fibres is that they can stretch to several times their own length and then rapidly return to their original size. Large amounts of elastin are found in the walls of blood vessels and in ligaments, with smaller amounts found in skin, tendon and loose connective tissue.

The amino acid composition of elastin is distinctive. One-third of the amino acid residues in elastin are glycine, there is also a high proportion of proline but few polar amino acids. Elastin is rich in nonpolar aliphatic residues such as alanine, valine, leucine and isoleucine.

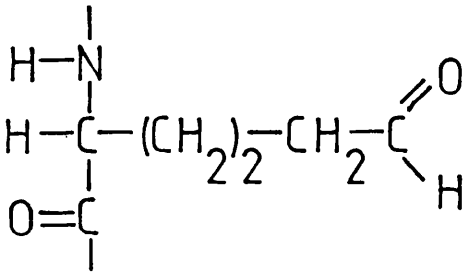
Mature elastin is cross-linked, making it insoluble and difficult to analyse. To overcome elastin insolubility, studies have been done on the soluble precursor of elastin, proelastin. The sequence of proelastin have shown alanine and lysine rich regions, where aldol cross-linking occurs (Fig.3).

ALDOL CROSS LINK

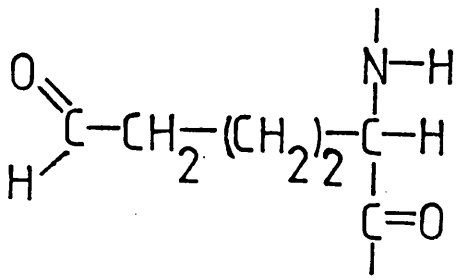


(96) (96)

Lysine Residues



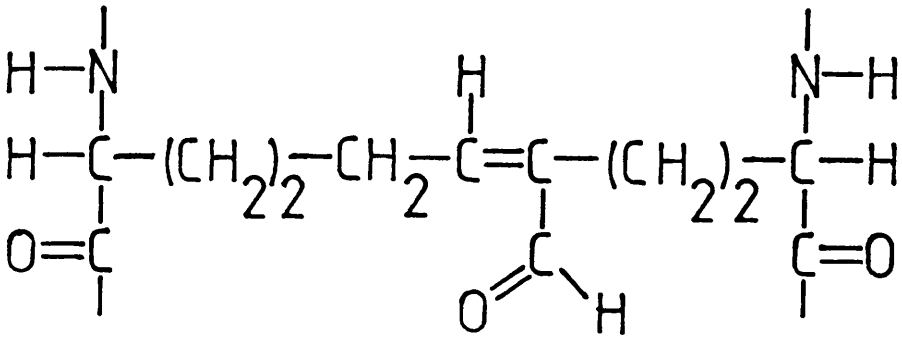
(97)



(97)

Aldehyde Derivatives of Lysine

(ALLYSINE)



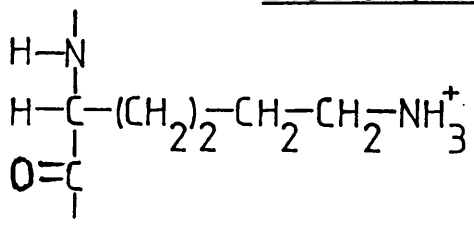
(98)

Aldol Cross Link

Fig.3

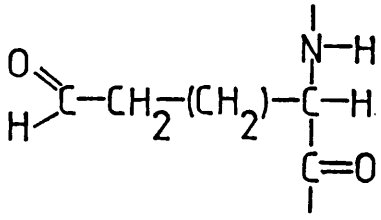
The lysine residues (96) of elastin are also involved in cross-linking other than of the aldol type, these are lysinonorleucine (Fig.4) and desmosine (Fig.5). Lysinenorleucine (99) is derived from an aldehyde derivative of lysine (97) and a lysine residue (96) reacting via a Shiff base (100).

LYSINONORLEUCINE CROSS LINK



(96)

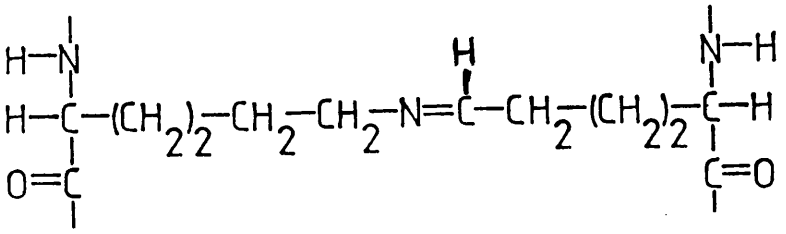
Lysine Residue



(97)

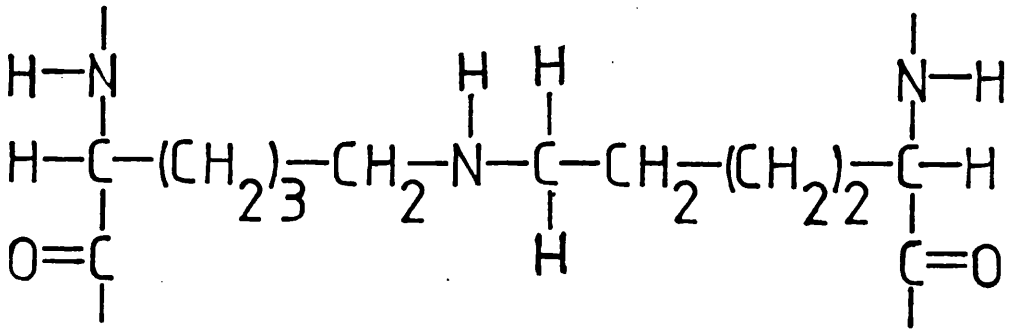
Aldehyde Derivative

of Lysine



(100)

Schiff Base



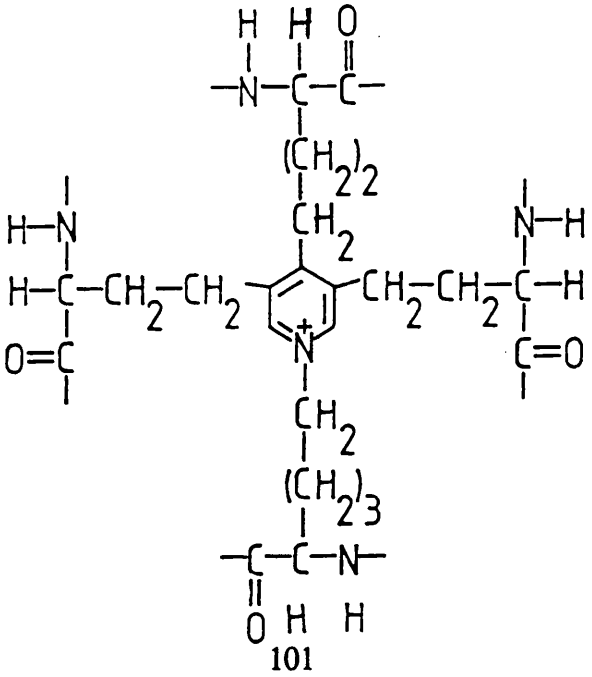
(99)

Lysinonorleucine

Fig. 4

Desmosine (VI) is derived from four lysine side chains.

DESMOSINE



101

Fig.5

The elastin cross-links are thought to be important in giving the elastic fibres the ability to stretch and return to their original size. The regions between the cross-links are rich in valine, glycine and proline residues.

3.2 Elastase Regulation[228]

Elastase is regulated by inhibitors which prevent proteolysis of normal healthy tissue. It is when the regulatory processes are prevented that elastase is thought to be involved in diseases.

There are three ways in which elastase can escape regulation:

3.2.1 Enzyme release in concentrations sufficient to overwhelm the available inhibitor.

This may occur for example when intraphagosomal digestion and even extracellular digestion occur in the vicinity of large numbers of polymorphonuclear leucocytes or in the microspaces between these and elastase susceptible substrates (eg basement membranes and cartilage surfaces.).

3.2.2. Release of enzyme in close proximity to its substrates which then successfully

compete with the α1-proteinase inhibitor (α1Pi) for binding elastase.

3.2.3 Enzymes are released into sites where local α1Pi molecules have been inactivated by oxidation. Oxidation of the inhibitor may be important in accounting for elastase activity at the foci of inflammation, or in the lung. This occurs because the stimuli releasing the elastase also release a variety of highly reactive reduced species of oxygen (superoxide anion + H_2O_2 + OH^-). These activated oxygen species can themselves damage tissues but they also inactivate α1Pi and this inactivated species reacts with elastase at 0.05% the rate of normal elastase inhibitor.

3.3 Elastase Mediated Tissue Injury in Pulmonary Disease[228]

Elastase mediated injury to the airways has been implicated as a pathogenic factor in cystic fibrosis. Polymorphonuclear (PMN) elastase has been found in patients with cystic fibrosis. Patients with cystic fibrosis suffer with lung infection and when infected with Psuedomonas aeruginosa, the α1Pi is destroyed by the bacterial metalloelastase. The bacterial enzyme destroying α1Pi and PMN appear to act synergistically in the airways of patients with cystic fibrosis, causing further deterioration of lung tissue[244].

Increased levels of active PMN elastase are found in patients with adult respiratory distress syndrome, here inactivated α1Pi is also found[245,246]. Activated leucocytes and macrophages in the lungs of patients with adult respiratory syndrome oxidise α1Pi, resulting in the inhibitor being inactive against elastase.

Emphysema is a third pulmonary disease in which PMN, monocyte and macrophage elastases have been implicated as causing parenchymal lung injury, this involves the walls of the repiratory bronchioles. Cigarette smoking is a major risk factor in this disease and this can be due to several reasons[247]:-

(i) recruitment of phagocytes to the lung
(ii) release of elastases in the interstitium and airspaces
(iii) funtional activity of α1Pi in lung secretion

3.4 Elastase Mediated Vascular Tissue Injury[248,249]

Elastase is released from neutrophils phagocytosing foreign bodies or immune complexes. Deposition of immune complexes in arteries, which occurs in immune complex diseases, results in digestion and disruption of the elastic lamina, that is the arterial wall is damaged.

Elastase can induce the release of vasoactive amines from mastcells and platelets directly, or from mastcells indirectly by the generation of C'5a from C'5. Clot promoting activity has been reported and neutrophil mediated inflammatory reactants are accompanied by fibrin formation.

3.5 Elastase Mediated Nephrotoxic Nephritis[248]

In nephrotoxic nephritis antiglomerular basement membrane antibody and complement bind along the basement membrane. Neutrophils accumulate and adhere to the immune reactants, thus bringing them in close contact with the basement membrane. Elastase is released from the adherent neutrophils, to remove the foreign bodies, but also causes proteolysis of the basement membranes, resulting in non-slective proteinuria and severe necrotising glomerulonephritis.

3.6 Properties of Elastases[250]

Elastases exist in the digestive tract and pancreas but are also found in neutrophils, monocytes, macrophages, platelets, fibroblasts and smooth muscle cells.

3.6.1. Digestive Tract Elastase[243]

The human pancreatic elastase enzyme is similar to trypsin and chymotrypsin in its action and structure. It is secreted by the pancreas as a zymogen and activated by cleavage of a single peptide bond. The new amino terminus created by zymogen cleavage turns inward and interacts electrostatically with the carboxylate of aspartate 194.

Forty per cent of the amino acid sequences of elastase, trypsin and chymotrypsin are identical and the similarity is greater in the amino acid residues located in the interior of the enzymes.

Elastase, as with trypsin and chymotrypsin, is inhibited by fluorophosphates

and has an active-site serine residue. The sequence around the active site serine is the same in all three enzymes, ie Gly-Asp-Ser-Gly-Gly-Pro.

The tertiary structure of elastase is very similar to trypsin and chymotrypsin and each has an oxyanion hole which is involved in the enzyme action.

The catalytic mechanisms of all these enzymes are also very similar. They are effective catalysts, because they bind the transition state of the reaction. The oxyanion hole and the charge relay network are the essential structural elements of each enzyme in promoting the formation of the transient tetrahedral intermediates.

3.6.2. Neutrophil Elastase[250]

Human neutrophil leucocyte elastase is a serine proteinase (ie a serine residue is present in position 195). The primary amino acid sequence contributes a nucleophilic hydroxyl group to attack carbonyl carbons of target peptide bonds. Valine or alanine residues are preferred in the P1 position of the target protein. The enzyme is a single chain polypeptide with a basic isoelectric point. It has several isoenzyme forms, is active at neutral pH, has a molecular weight of 33,000 daltons and comprises 18-20% carbohydrate. It is synthesised primarily in promyelocytes and stored in the cytoplasmic azurophil granules of maturing neutrophils. Neutrophil elastase is discharged into tissues when the lung cell encounters objects to be phagocytised, or after cell death.

Physiological turnover of connective tissue macromolecules may be a function of the elastases secreted by histocytes (tissue macrophages), smooth muscle cells and fibroblasts. Hydrolysis of matrix macromolecules by neutrophil elastase is a pathological process, in which a wide variety of structurally important proteins and glycoproteins can be attacked by the enzyme. Neutrophil elastase attacks elastin but also cleaves core proteins of proteoglycan molecules in the connective tissue ground substance. Damage to the proteoglycan molecules interferes with water entrapment by proteoglycan complexes, and in the case of joint cartilage spoils mechanical function.

Neutrophil elastase acts on collagen by transecting across its helical portion. Collagen is a major supporting component of lung connective tissue, blood vessels and the gut. The structural integrity of epithelial and endothelial basement

membranes, is also maintained by collagen. Fibronectin, a major cell adhesion molecule critical to the organisation of many tissues, is another structural macromolecule susceptible to attack by the neutrophil enzyme.

As well as connective tissue components, many plasma proteins are also hydrolysed by neutrophil elastase. This category of substrates includes immunoglobulins, clotting factors (including fibrinogen) and complement proteins. Formed blood elements can also be affected by the enzyme; these include lymphocyte activation and platelet aggregation.

3.6.3. Monocyte Elastase[250]

Human monocytes contain elastase which although different from the neutrophil enzyme in amount and subcellular location is antigenically and biochemically related. Monocyte elastase is found on the cell surface (ie plasma membrane), therefore although the concentration is much less than in neutrophils its exposed location results in considerable damage being done as monocytes move through connective tissues. Monocyte elastase attacks elastin, fibronectin and also degrades serum amyloid A (which is important in inflammatory states).

3.6.4. Macrophage Elastase [250]

Macrophage elastase is not stored in the cell or localised to plasma membrane but is secreted into the extracellular medium, and is thought to be a metallo-enzyme. Macrophage elastase is resistant to inhibition by α1Pi and may actually digest this antiprotease. The macrophages are thought to produce an inhibitor of their own elastases.

Degradation of connective tissue matrix molecules by macrophages appears to involve the co-operative interplay of several enzymes primarily elastase and plasminogen activator. Plasminogen activator is not only a secretory product of the cell but is also present in macrophage plasma membranes. The extensive pericellular matrix-dissolution charactristics of macrophages acting in close physical contact with matrix substrates, is thought to be due to plasminogen activator. By converting plasminogen to plasmin in inflammatory exudative fluids, macrophage plasminogen activator can indirectly facilitate elastinolysis. Plasminogen activator aids elastinolysis

as plasmin is effective at stripping away proteoglycans and fibrin, thus unmasking target collagen and elastin fibres. Plasmin may also activate latent macrophage elastase. Thus matrix degradation by macrophages is characterised by (1) co-operativity between surface bound and secreted enzymes; and (2) delivery of enzymes directly onto insoluble matrix components avoiding soluble phase protease inhibitors.

Macrophages bind and internalise neutrophil elastase, because they have a specific membrane receptor, in addition to other neutrophil glyoprotein enzymes. Macrophages subsequently release the neutrophil enzyme in an active form; or a process accelerated by macrophage cell injury. Macrophages serve as a vector for the harboured neutrophil protease, carrying it to tissue sites that it ordinarily might not reach and bringing it into closer proximity with its target in the extracellular matrix. Macrophage delivered neutrophil elastase may also escape the soluble protease inhibitors by being internalised.

Macrophages are designed as lung defence cells, therefore their presence at sites of injury is not proof of their role in the injury process. Macrophages synthesise small amounts of elastase inhibitors including α1Pi and α2-macroglobulin (inhibitor of neutrophil elastase) as well as an inhibitor to their own metallo-enzyme.

3.6.5. Elastase of Platelets Fibroblasts and Smooth Muscle[250]

Elastolytic enzymes are also found in fibroblasts, smooth muscle, and platelets. Aortic smooth muscle cell elastase may participate in elastin turnover in normal arterial wall and in the pathologic elastolysis of artherosclerosis.

Human skin fibroblasts also contain an elastase as do platelets. Fibroblast elastase is similar to that of neutrophils while platelet elastase is immunologically different.

3.7 Possible Role of Polymorphonuclear Neutrophil (PMN) Elastase in Joint Disease[251]

The first step in degradation of joint cartilage matrix proteoglycan occurs when bacteria, viruses, microcrystalline deposits (eg monosodium urate) or antigen-antibody complexes appear in the synovial spaces. These materials may be present as a particulate suspension throughout the synovial fluid or as an adsorbed layer in the

articular surfaces, or both. The second step involves the liberation of intrinsic diffusable factors (eg certain bacteria) or the activation of the complement system (e.g.by antigen-antibody complexes) to form a concentration gradient of PMN chemoattractants. This leads to a chemotactic response and emigration of large numbers of PMN's to the joint space.

In the third step, PMN leucocytes entering the joint fluids engulf the suspended particles or attempt to engulf particles adsorbed onto cartilage surfaces and so release lysosomal enzymes into the synovial fluid or directly onto the articular cartilage surfaces.

Once the lysosomal enzymes have been released from the leucocytes in the joint spaces, endogenous antiproteases present in synovial fluid [252], may act to protect susceptible substrate molecules from proteolysis. Under certain conditions in the joint the formation of inhibitory complexes between leucocyte proteases and synovial antiproteases may be prevented and the enzyme may remain active. Enzyme activity is retained when the enzyme is released in sufficient amounts to saturate antiproteases present. This occurs in septic arthritis or gout. The concentration of free antiproteases may also be lowered by complexing with bacterial proteases in septic joints as occurs in lung infections[253]. In rheumatoid arthritis a different mechanism may interfere with efficient removal of proteases that leak out of neutrophils. Release of enzymes from neutrophils in direct contact with connective tissue, results in an excess of protease to inhibitor at the tissue surface, this does not reflect the situation elsewhere in the joint space. The concentration of free inhibitors in the bulk phase of synovial fluid does not always accurately reflect their local concentration in the immediate vicinity of the target tissue. Local imbalance of enzyme and inhibitor, added to the less efficient penetration of α1Pi into the cartilage as compared to leucocyte neutral proteases, results in matrix damage by PMN proteases in inflammed joints being further increased.

3.8 Polymorphonuclear Proteases and Carcinogenesis[236]

PMN proteases degrade interstitial components of connective tissues and may also alter the surface properties of living cells in inflammed tissues. Alteration of cellular surface properties alter the growth behaviour of the cell, this may be

significant in carcinogenesis.

The growth stimulation induced by brief treatment with proteolytic enzymes is transient and normal growth control reappears soon after the cells are able to restore their original surface structure. Chronic or intermittent irritation of tissues may lead to repeated exposure of cells to leucocyte proteases with a consequent long term effect upon cellular growth. Hyperplasia of epithelial linings observed in response to chronic irritation may be based partly on changes in cellular growth due to protease exposure. The hyperplastic response of carcinogens appears to be associated with their tumour promoting activity.

Elastases produced from many different sites causes a number of diseases. The production of a synthetic inhibitor to the elastase enzymes would be beneficial in the treatment of elastase promoted diseases. The aim of the research done was to produce an elastase inhibitor.

3.9 Synthetic Inhibitors Of Human Elastase[254]

Elastases are naturally inhibited by an excess of α1Pi, disease states arise when the enzyme-inhibitor balance is disturbed. Enzyme-inhibitor imbalance can be due to several reasons, the genetic deficiency of α1Pi, the oxidation of α1Pi, or the production of excess enzyme. The current treatment for enzyme inhibitor imbalance is:

(1) administration of excess natural inhibitor (α1Pi);

(2) other naturally occuring high molecular weight protease inhibitors eg Eglin C; and

(3) low molecular weight synthetic inhibitors.

(1) and (2) are now produced by genetic engineering methods.

The design of potent synthetic elastase inhibitors involves the understanding of the catalytic triad, consisting of aspartic acid, histidine and serine which facilitate the formation of the enzyme-inhibitor tetrahedral adduct (Fig.6a).

Synthetic inhibitors are generally designed to interfere with the formation of the enzyme-inhibitor adduct and can bind to the enzyme in a reversible (covalent or non-covalent) or irreversible way.

The synthetic inhibitors fall into two structural types:- the peptidic or heterocyclic.

3.10 Peptidic Reversible Inhibitors of Elastase

Four classes of potent reversible peptidic elastase inhibitors have been reported : the peptidyl boronic acids (102)[255], trifluoromethyl ketones (103)[256], aldehydes (104,105,106)[257,258]; and α-keto esters (107)[259] (table 1).

Trifluoromethyl ketones (103), aldehydes (104,105,106) and α-keto esters (107) contain an electrophilic carbonyl and were designed to form reversible covalent adducts with the enzyme. The trifluoromethyl ketones are the most potent synthetic elastase inhibitors. The fluorinated ketone carbonyl facilitates the enzyme catalysed addition of the active site serine to the ketone carbonyl to form a stable hemi-ketal adduct.

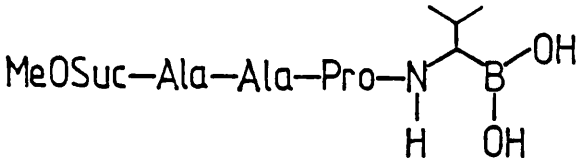
The aldehydes (104,105,106) and α-keto esters (107) are believed to inhibit elastase in a similar way. The aldehydes are potent selective inhibitors of human neutrophil elastase. The α-keto esters span the active site of human neutrophil elastase and are more potent than the corresponding α-keto acids.

PEPTIDIC INHIBITORS OF ELASTASE

Reversible

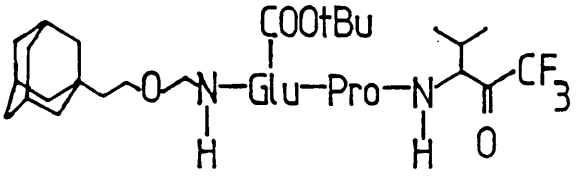
Table 1

Boronic acids



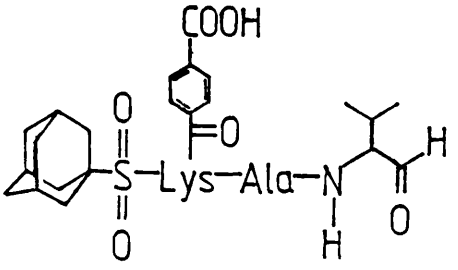
(102)

Trifluoromethyl ketone

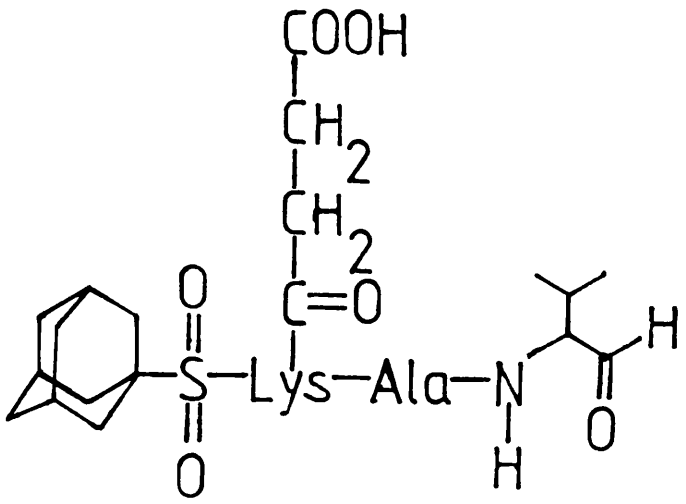


(103)

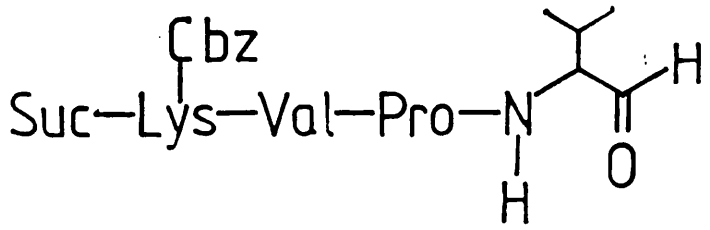
Aldehydes



(104)

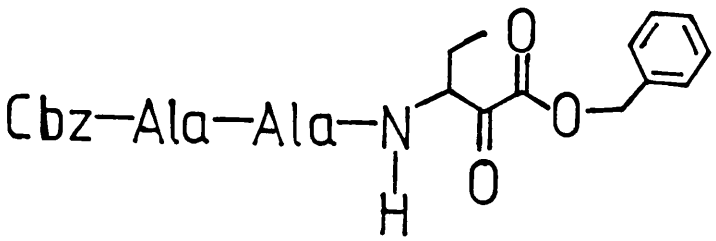


(105)



(106)

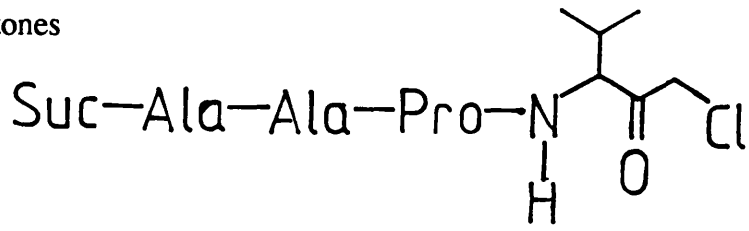
α-Keto esters



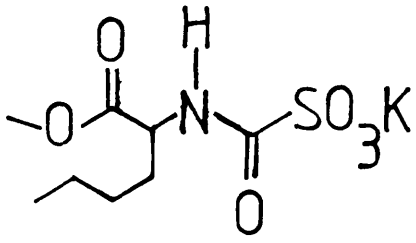
(107)

Irreversible

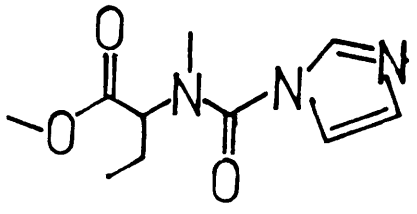
Chloromethyl ketones (108)



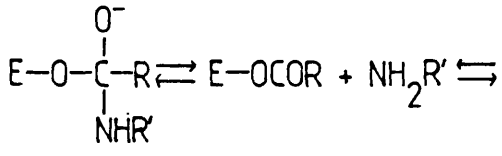
Sulphonate salts (109)



Latent Isocyanates (110)



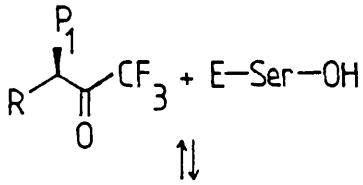
a) $E{-}OH + RCONHR' \rightleftarrows E{-}OH{\cdot}RCONHR' \rightleftarrows$

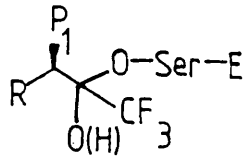


$\rightleftarrows E{-}OCOR + NH_2R' \rightleftarrows$

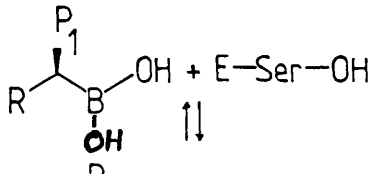
$E{-}OH{\cdot}RCO_2H \rightleftarrows E{-}OH + RCO_2H$

b)





c)



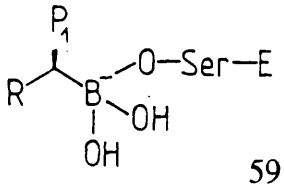


Fig.6 (a) Typical reaction sequence for serine protease and inhibitor with formation of tetrahedral adduct.

(b) Fluorinated ketone inhibitor reacts with active serine site to form a stable hemiketal adduct.

(c) Formation of the tetrahedral covalent adduct of serine active site with the boronic acid inhibitor.

The use of boronic acids as transtion state analogue inhibitors of serine proteases was introduced by Koehler and Lienhard in 1971[260]. The boronic acid inhibitor is bound as a tetrahedral covalent adduct with the active site serine hydroxyl (Fig.6b).

The peptidic inhibitors contain an amino acid sequence based on synthetic substrates of elastase. Selective substrates have been designed where the structures mimic the natural sequences that may be present in elastin[261]. The peptidic inhibitors have the amino acids alanine, proline and valine, because elastin is rich in these.

3.11 Irreversible Elastase Inhibitors

3.11.1 Peptidic Irreversible Inhibitors of Elastase

Irreversible peptidic inhibitors include the peptidyl chloromethyl ketones (108)[262], the amino acid sulphonate salts (109)[263] and the amino acid derived latent isocyanates (110)[264]. The chloromethyl ketones inhibit serine proteases, initially by forming an enzyme-inhibitor tetrahedral adduct between the inhibitors reactive carbonyl and the active site serine hydroxyl. Formation of the enzyme-inhibitor adduct is followed by irreversible alkylation of the active histidine site[265]. The sulphonates irreversibly inactivate elastase with the release of a bisulphate ion.

3.11.2 Heterocyclic Irreversible Inhibitors of Elastase

A new series of cephalosporins (111) are a novel class of heterocyclic inhibitors of elastase (Table 2). Cephalosporins substituented in the 7α position are potent elastase inhibitors[266]. The sulphones are the most active members of the series, which quickly and irreversibly inactivate the enzyme. The mechanism of action of the cephalosporin sulphone elastase inhibitors involves the formation of a reversible complex, followed by the acylation of an active site residue.

Other irreversible heterocyclic elastase inhibitors include (isocoumarin-)3-alkoxy-7-amino-4-chloroisocoumarin (112)[267]. The inhibitor 7-amino-4-chloro-isocoumarin was designed to acylate the enzyme with the release of a quinone imine methide which could then irreversibly inactivate the enzyme by alkylation of an active site nucleophile. Many ynenol lactones (113,114)[268] are substrates for elastase while the ynenol lactones with an unsubstituted acetylene terminus are inhibitors of elastase.

3.12 Heterocyclic Reversible Inhibitors of Elastase

The isocoumarin, 3,4-dichloroisocoumarin (118)[269], the substituted 2-pyrones (117)[270,271] and the benzoxazinones (115,116)[272-274] are all mechanism based reversible inhibitors of elastase.

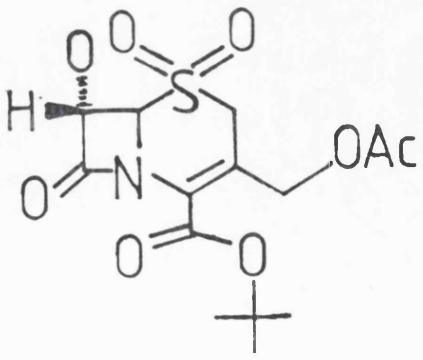
The 3,4-dichloroisocoumarins (118) contain a masked acid chloride capable of acylating an active site nucleophile. The 2-pyrones (117) are modelled on the natural elastase inhibitor elasnin, obtained from bacteria. Such inhibitors are not found in the body, here the elastase enzymes are themselves broken down enzymically. The potency of the 2-pyrones depends on the nature and position of alkyl chains on the ring. The benzoxazinones (115,116) are thought to acylate the enzyme, which can either undergo ring closure to re-form intact benzoxazinone or hydrolysis to liberate an N-acylanthranilic acid.

HETEROCYCLIC INHIBITORS OF ELASTASE

Irreversible

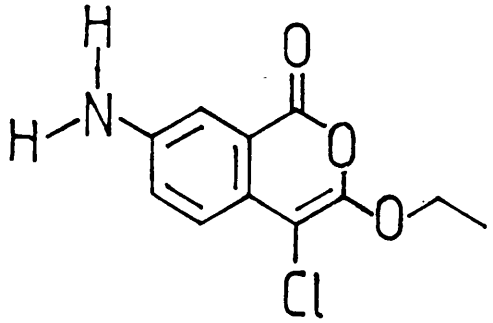
Table 2

Cephalosporins



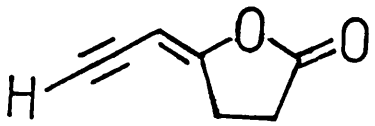
(111)

Chloroisocoumarins

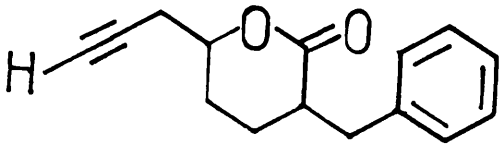


(112)

Ynenol lactones



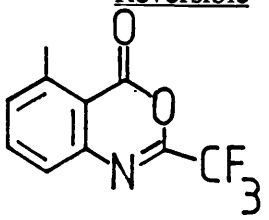
(113)



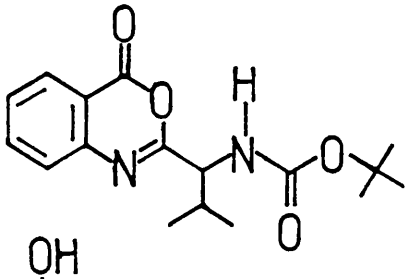
(114)

Reversible

Benzoxazinones

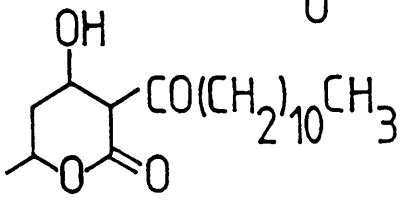


(115)

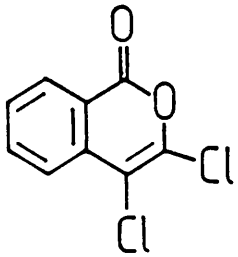


(116)

2-Pyrones



(117)



Chloroisocoumarins

(118)

3.13 Pharmacology of Elastase Inhibitors

The pharmacological profile of the synthetic elastase inihibtors is tested in animal models with induced emphysema.

The chloromethylketones are effective when administered intrathecally, parentally and orally, although the renal toxicity observed by administration of these compounds renders them unsafe for clinical use.

The peptide boronic acids administered intrathecally or intraperitonially appeared to be effective and showed no overt toxicity.

The peptide aldehyde inhibitors are active when given intrathecally or intraperitonially. Compounds 104 and 105 (Table 1) appear to have no overt toxicity while compound 106 (Table 1) is a very potent tight binding reversible inhibitor. The instability of compound 106 in vivo means it is not a desirable compound for clinical use.

3.14 Elastase Inhibitors Synthesised in this Work

Due to the toxicity of the inhibitors already existing, or the lack of activity by oral administration, the synthesis of reversible peptidic elastase inhibitors was undertaken. The inhibitors synthesised possessed an elastin mimicing amino acid sequence Ala-Ala-Pro-Val, with lipidic groups attached to it. The lipidic groups enable the inhibitors to attach to cellular membranes, so protecting the native elastin from being degraded. The lipidic amino acid conjugated inhibitors were designed to be potent orally active inhibitors lacking toxicity.

The major work was the synthesis of the elastase inhibitors where tetrapeptide (119) was conjugated to the lipidic amino acid and its oligomers. A lipophilic moiety is required[275,276], and using lipidic amino acids enabled control of lipophilicity by altering the amino acid chain length or the size of the oligomer produced.

Tetraaminodecanoic acid was the lipidic amino acid used in this work . The monomer, dimer and trimer being conjugated to Ala-Ala-Pro-Val(119).

The oleoyl, myristoyl and p-methoxycinnamoyl-5-amino-hexanoyl derivatives of Ala-Ala-Pro-Val were synthesised for comparative biological studies with the lipidic amino acid derivatives.

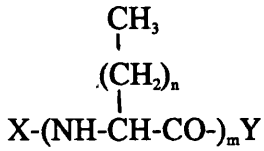
The potency of the compounds synthesised as human leucocyte elastase

inhibitors was determined. Succinyl trialanine para-nitroanilide was used as the enzyme substrate. The assay was carried out in the presence of lipidic amino acid inhibitor concentrations of 0-50μg/ml. The relative potencies of the monomer, dimer and trimer could be determined.

4. DISCUSSION

The lipidic amino acids are known and were first synthesized by Albertson in 1946[277]. The lipidic amino acids II, XXXIX and XXXX were synthesized by the Albertson method.

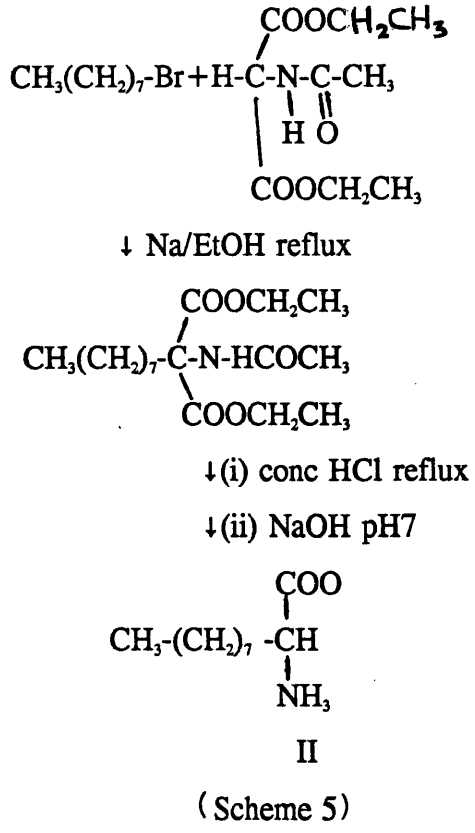
4.1 Lipidic amino acids and oligomers synthesized.



	X	Y	n	m
II,XXXIX, XXXX	H	OH	7,11,13	1
XXXXI,XXXXII,XXXXIII	BOC	OH	7,11,13	1
XXXXXIV, XXXXV, XXXXVI	H	OCH_3	7,11,13	1
XXXXVIII, XXXXVII, XXXXIX	BOC	OCH_3	7,11,13	2
L, LI, LII	BOC	OH	7,11,13	2
LIII, LIV, LV	BOC	OCH_3	7,11,13	3

Aminodecanoic acid, (the ten carbon amino acid) was synthesized by refluxing 1-bromooctane with diethylacetamidomalonate in the presence of sodium ethoxide (Scheme 5).

The strongly basic conditions due to sodium ethoxide allows dissociation of the proton at position 2 of the diethylacetamidomalonate and thus the nucleophilic attack at this position by the 1-bromooctane giving the diethyl-octylacetamido malonate. Hydrolysis of this intermediate with HCl and subsequent neutralisation with NaOH yields the required amino acid. The same procedure was used for XXXIX and XXXX, the bromoalkanes used here being 1-bromododecane and 2-bromotetradecane respectively.



(Scheme 5)

The amino acids produced by this method are racemic. Work involving polymer synthesis required the resolution of the racemic mixture into the two enantiomers, whilst the elastase and opiate derivatives were synthesized using the racemic mixture.

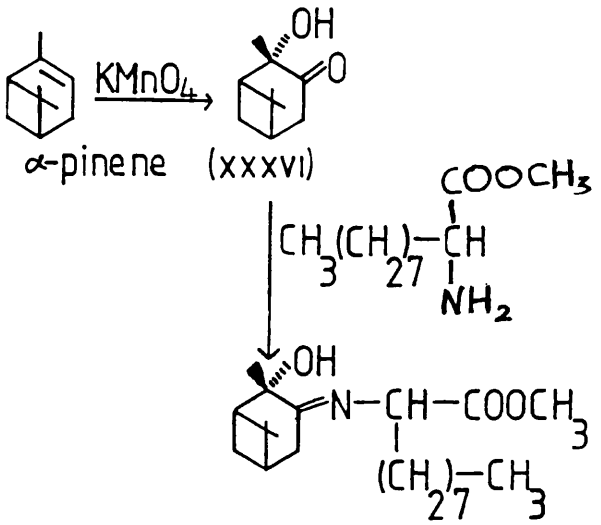
Amino Acid Resolution

Two methods of resolution were employed during the work, firstly enzymic and secondly chemical.

Enzymic resolution of aminodecanoic acid was reported by Birnbaum et al., 1953[278] and was employed during this work. The enzyme acylase I, which specifically hydrolyses the N-chloroacetyl derivative of the dl-amino acid was used to yield the l-amino acid. The d-amino acid N-chloroacetyl derivative remained in solution and was liberated by acid hydrolysis. Chemical resolution was effected using a chiral agent. Ogun et al[279] had used a chiral α-pinene derivative (+)-(1R,2R,5R)-2-hydroxypinan-3-one in the stereoselective alhylation of its Schiff bases derived from glycine and alanine tertiary esters. The racemic lipidic amino acid and the chiral ketol were condensed, with the condensation product being resolved.

The methyl ester of aminodecanoic acid was refluxed in benzene or toluene with the chiral ketol XXXVI (obtained by $KMnO_4$ oxidation of α-pinene), with a catalytic amount of borontrifluoride dietherate (Scheme 6).

The reaction was monitored by tlc and continued until the ketol was no longer detected. The schiffs base (XXXVII) produced two diasteroisomers which were separated by silica gel chromatography and the aminodecanoic acid methyl ester (VI) was obtained by hydrolysis of the Schiffs base with hydroxylamine hydrochloride.



(Scheme 6)

The absolute configuration of the chiral carbon of each enantiomer was determined by circular dichroism (CD) spectroscopy. A comparison of the CD spectra of the enzymatically resolved and chemically resolved enantiomers was made. (Fig. 7 l-isomer enzymic resolution. Fig. 9 d-isomer enzymic resolution. Fig. 8 l-isomer chemical resolution. Fig. 10 d-isomer chemical resolution).

It was found that the enantiomer with the positive CD spectrum was the l-isomer and the enantiomer with the negative spectrum was the d-isomer.

Figures 7 and 8

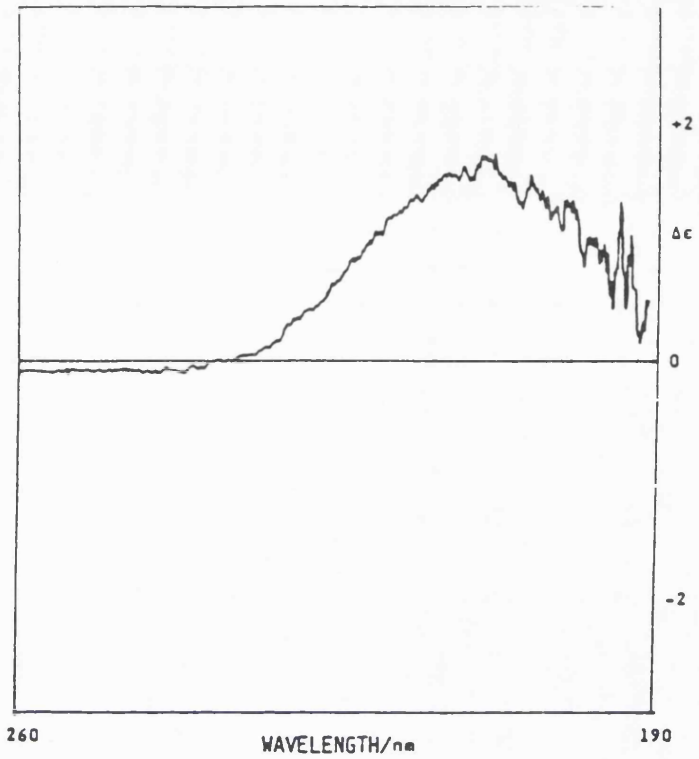


Fig. 7 Circular Dichroism Spectrum of L-C_{10}OMeHCl synthesised from enzymatically resolved L-C_{10}amino acid

(MeOH, conc.g/ml 0.00104, path length 0.05cm)

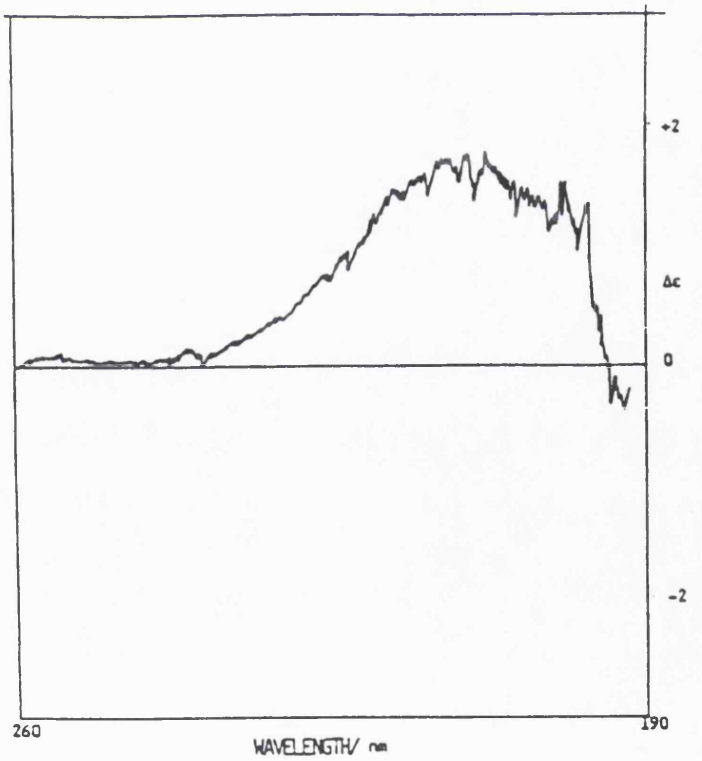


Fig. 8 Circular Dichroism Spectrum of L-C_{10}OMe.HCl resolved with (1S,2S,5S)(-)-2-hydroxypinan-3-one

(MeOH, conc. 0.00104g/ml, path length 0.05cm)

Figures 9 and 10

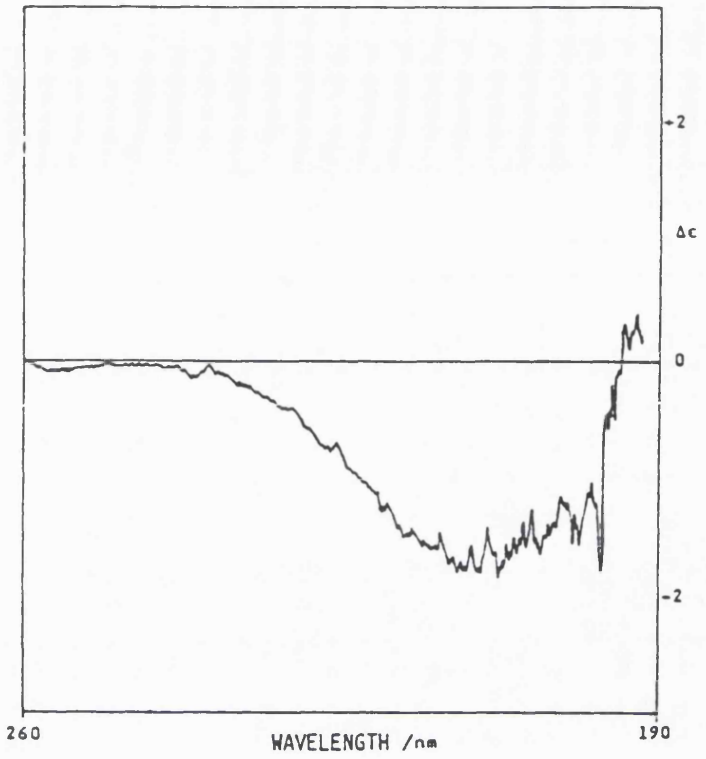


Fig. 9 Circular Dichroism Spectrum of DC_{10}OMeHCl synthesised from enzymatically resolved DC_{10} amino acid

(MeOH, conc.g/ml 0.00108, path length 0.05cm)

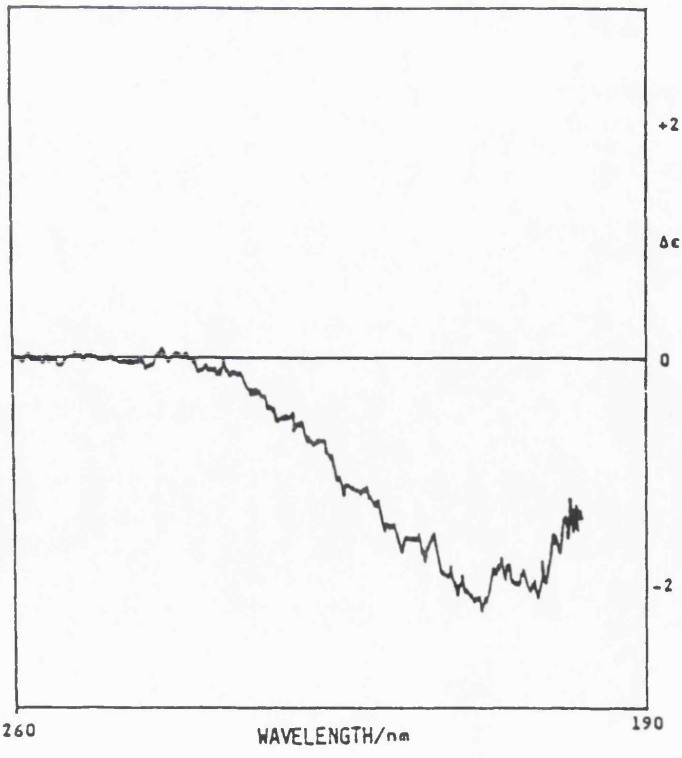


Fig. 10 Circular Dichroism Spectrum of DC_{10}OMe.HCl resolved with (1S,2S,5S)(-)-2-hydroxypinan-3-one

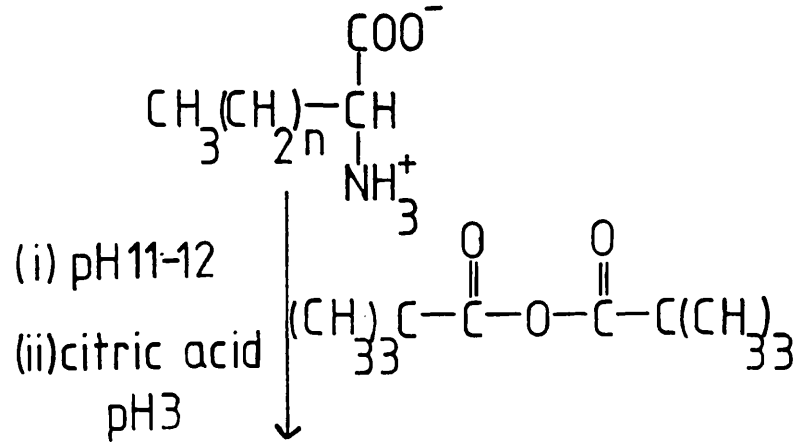
(MeOH, conc.g/ml 0.00112g/ml, path length 0.05cm)

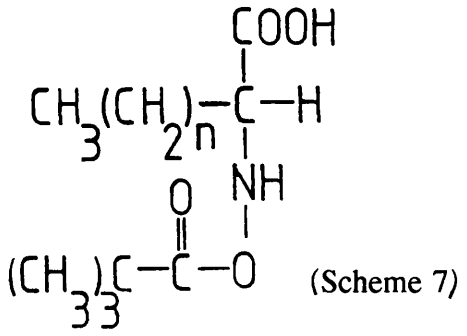
In peptide synthesis the amino and carboxyl functions must be protected to prevent unrequired side ractions.

4.3 Protection of the Amino Group

Many protecting groups are available for the amino function (Bodansky)[280]. The group used in this work was the tertiarybutoxy carbonyl group (BOC). This was chosen for its ease of formation, removability and the solubility of the products in organic solvents.

The BOC-protected amino acids were obtained by reacting the symmetrical anhydride ditertiary butyl dicarbonate ($(Boc)_2O$) with the lipidic amino acids (Scheme 7).

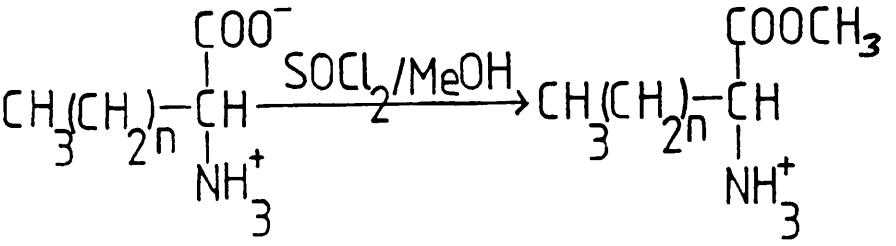




(Scheme 7)

4.4 Protection of the Carboxyl Function

The most widely used protecting group of the carboxylic acid function is the ester function of various types[280]. The methyl esters are used in the protection of the lipidic amino acids for this work. The methyl ester hydrochloride was obtained by reacting the amino acid with thionyl chloride in methanol (Scheme 8).



(Scheme 8)

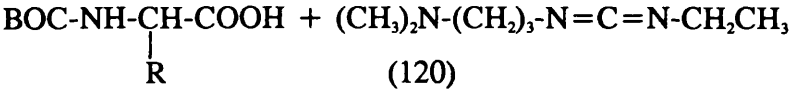
4.5 Synthesis of Peptides

Peptide synthesis was carried out via the mixed anhydride method.

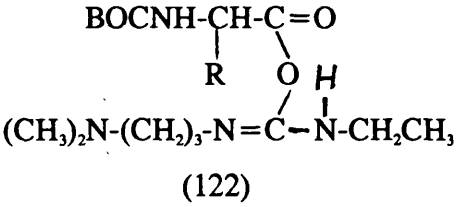
4.5.1 Synthesis of Lipidic Amino Acid Homo-oligomers

The lipidic amino acids homo-oligomers were synthesised by coupling the appropriate amino and carbonyl protected compounds. The aminodecanoic acid dimer (LVIII) was synthesised by the coupling of XXXXI and XXXXIV using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDAC) as a coupling agent and 1-hydroxybenzotriazole (HOBt) to direct the pathway of the reaction.

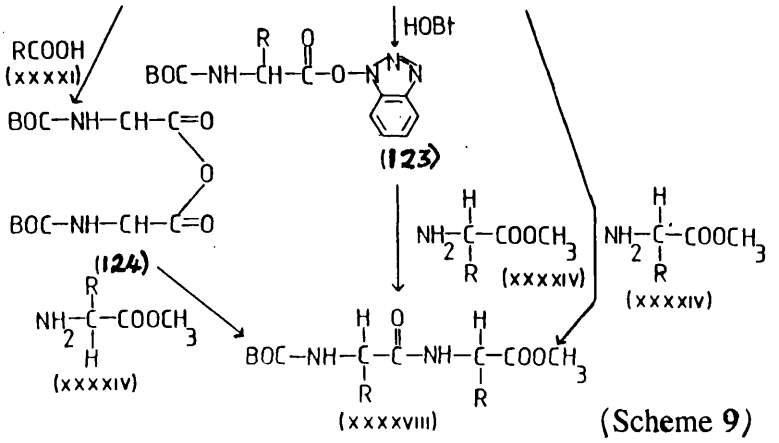
Initially BOC-aminodecanoic acid (XXXXI) is added to the coupling agent EDAC (120) with tertiary amine, HOBt (121) and then carboxyl protected aminodecanoic acid (XXXXIV) added (Scheme 9).



(120)



(122)



(Scheme 9)

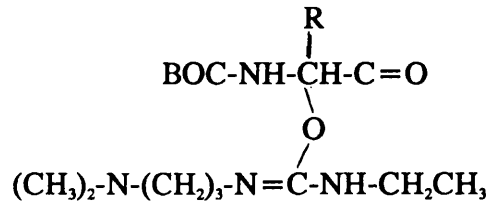
The acylurea (122) is an activated carboxyl compound which can undergo several reactions:

(1) A BOC protected amino compound (XXXXI) can attack the reactive intermediate (122) to form a symmetric anhydride (124) which then reacts with a carbonyl protected amino acid (XXXXIV) to form a peptide.

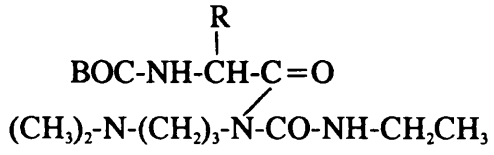
(2) Reaction of the active intermediate (122) with the carbonyl protected amino acid (XXXXIV) gives the required dipeptide (XXXXVIII).

(3) Reaction of the active intermediate (122) with HOBt forming an active ester (123) which reacts by nucleophilic attack with the methyl ester (XXXXIV) giving the protected dipeptide.

(4) O-N migration of the active intermediate (122) forms the N-acylurea (124), the inclusion of HOBt in the reaction is primarily to prevent this pathway (Scheme 10) which results in loss of starting material and difficulty in separation of the required product.



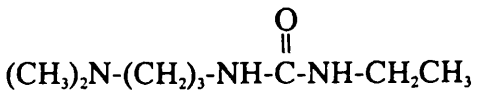
(122)



(124)

(Scheme 10)

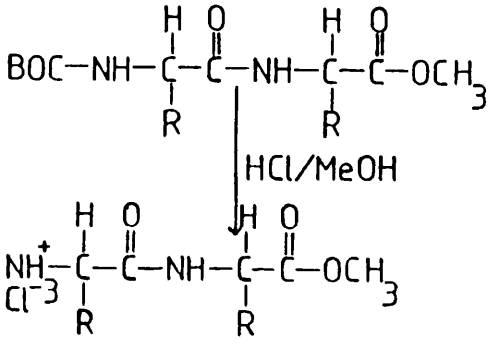
A by-product of the coupling reaction is urea (125). EDAC urea is water soluble and easily removed from the reaction mixture by washing with aqueous solution, this is why EDAC was used as the coupling agent for the lipidic amino acids.



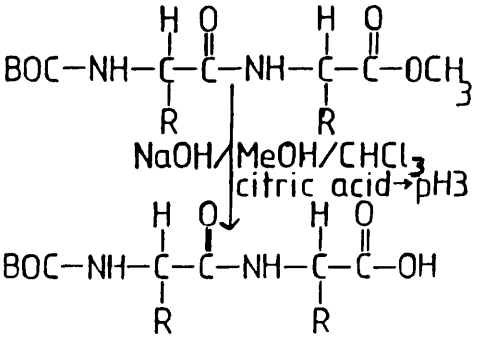
(125)

The above method of coupling was used in the synthesis of all the dimers XXXXVII and XXXXIX and also in the synthesis of the lipidic amino acid trimers LIII, LIV and LV.

To synthesize the lipidic amino acid trimers or any peptides produced in this work, removal of the tertiary butoxyl carbonyl and ester protecting groups must be achieved. The Boc group is acid labile and is removed by anhydrous trifluoroacetic acid or anhydrous hydrogen chloride in methanol, the latter being mainly the method used (Scheme 11). Removal of the methyl ester group was by saponification with NaOH in methanol the reaction being followed by tlc (Scheme 11a).



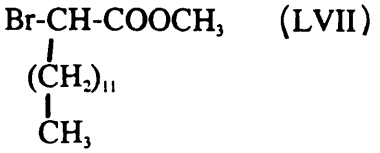
(Scheme 11)



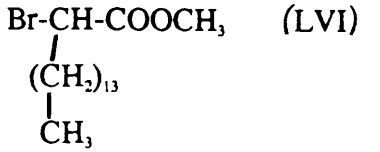
(Scheme 11a)

4.5.2 Synthesis of bromo-alkanes and bromoalkane lipidic amino acid derivatives

Bromoalkane methyl ester (LVI, LVII) were synthesized via method [A] and the coupling of the bromoalkanes to lipidic amino acids was carried out via method [D].

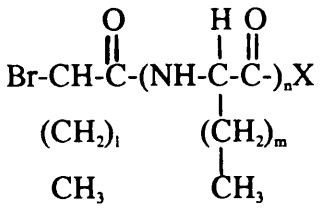


(LVII)



(LVI)

4.5.3.1 Summary of bromoalkane lipidic amino acid derivatives



	l	m	n	x
LVIII	13	11	1	OCH_3
LIX	13	11	1	OH
LX	13	11	2	OCH_3

4.5.3.2 Synthesis of Hetero-oligomers

Phenylalanine, lysine and glycine were natural amino acid used in the synthesis of potential drug carrying polymers.

Glycine was chosen as due to its simplicity no racemisation can occur during the polymerisation process[281].

Lysine was chosen due to the ability to attach drug molecules to the chain amino group thus enabling the carrying of molecules containing acidic and alcoholic groups.

The final choice of natural amino acid was phenylalanine. This was chosen due to its hydrophobic nature and also the existence of enzymes that break peptide bonds at this amino acid.

The hetero-oligomers and polymers were produced to give modified solubility characteristics compared to the homo-oligomers and possibilities for drug conjugation.

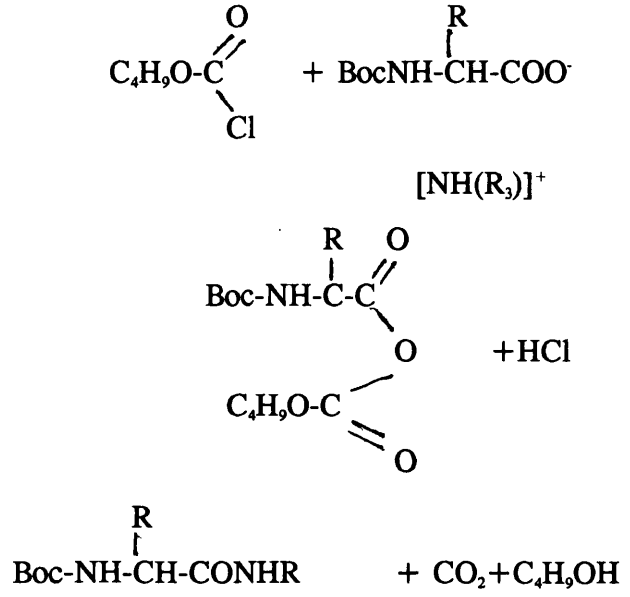
Synthesis of the hetero-oligomers was undertaken stepwise as for the homo-oligomers, with a tripeptide being synthesized, and undergoing polymerisation. Initially the fully proteced dipeptides were produced which were deprotected and then the tripeptide synthesized. Different coupling methods were used however, in the synthesis of the hetero-peptides.

4.6 Coupling with the Use of Isobutyl Chloroformate

Coupling using isobutyl chloroformate was undertaken for various hetero-di

and tripeptides.

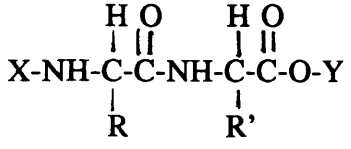
Isobutycholorformate coupling occurs via the mixed anhydride method of peptide synthesis. The active intermediate is the carbonic acid half ester which decompose to give the corresponding alcohol (here it is butanol) and carbon dioxide. (Scheme 12)



Scheme 12

Alcohol and carbon dioxide are the by products of the reaction (Scheme 12). Therefore no side products are formed. The tertiary base used during synthesis varied availability, the following being used: N-methylmorpholine (NMM), trithylamine (TEA) or DIPEA.

4.7 List of dipeptides synthesised with method of synthesis.

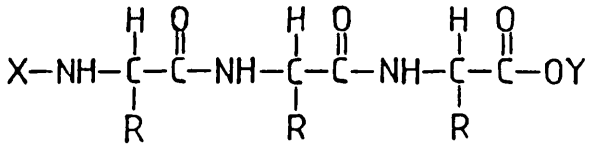


X	R	R'	Y	Method of synthesis
	$CH_2C_6H_5$	H	CH_3	DCC
"		$(CH_2)_7CH_3$	CH_3	Isobutychloroformate

"	$(CH_2)_7CH_3$	$CH_2C_6H_5$	CH_3	Isobutychloroformate
$(CH_3)_3CO\text{-}C(=O)$	$(CH_2)_4NHC(=O)\text{-}OCH_2C_6H_5$	H	C_2H_5	Isobutychloroformate
"	$(CH_2)_7CH_3$	H	CH_3	Isobutychloroformate
"	$(CH_2)_4NHC(=O)\text{-}OCH_2C_6H_5$	$(CH_2)_7CH_3$	H	Saponification
H	$CH_2C_6H_5$	H	CH_3	HCl gas

Fully protected dipeptides were synthesized and deprotected in order to allow the synthesis of the required tripeptide which were to undergo polymerisation.

4.8 Tripeptides synthesized



X	R	R_1	R_2	Y	method
$(CH_3)_3COC(=O)$	$(CH_2)_4NHCOCH_2C_6H_5$	$(CH_2)_{27}CH_3$	$(CH_2)_{27}CH_3$	CH_3	IBCF
"	$(CH_2)_{27}CH_3$	$(CH_2)_4NHCOCH_2C_6H_5$	H	CH_3	IBCF
"	"	$CH_2C_6H_5$	"	"	DCC
"	"	$(CH_2)_4NHCOCH_2C_6H_5$	"	H	saponify
"	"	$CH_2C_6H_5$	"	"	"

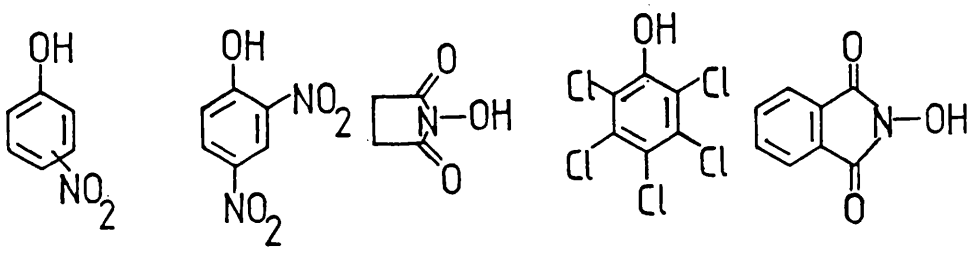
The hetero tripeptides produced were deprotected at the carbonyl terminus to allow the addition of an active ester, the group required for polymerisation.

Polymerisation was carried out by two widely used methods. The first being the active ester method and the second the Leuch's anhydride method.

4.9 Polymerisation methods

4.9.1 Polymerisation by the Active Ester Method

Several active esters can be used for polymerisation[280]. Active esters increase the rate of activity by allowing successive reactions to occur. The ability to allow successive reactions is due to the electrophilic nature of the carbonyl carbon brought about by the electron withdrawing effect of the esters. Many of the esters used for polymerisation are aryl esters, with the nitropherol esters[282-284] being widely used, particularly 2,4-dinitrophenol (126). Other esters which have been used include N-hydroxysuccinamide (127), pentachloro-phenyl (128) and N-hydroxy-phtalimide (129).

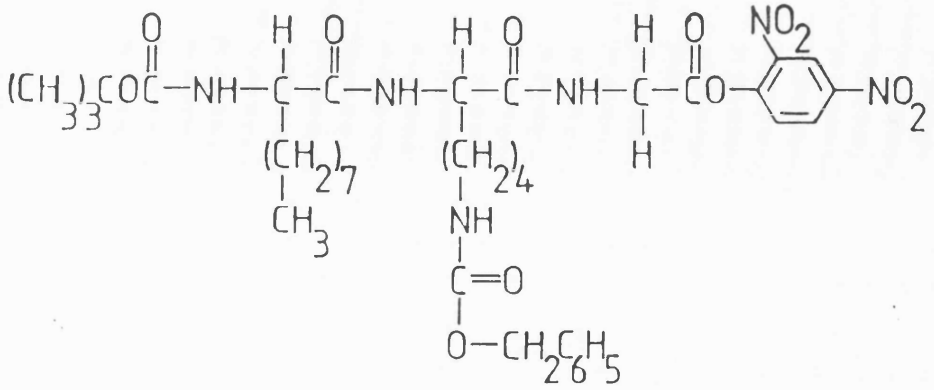


(126) (127) (128) (129)

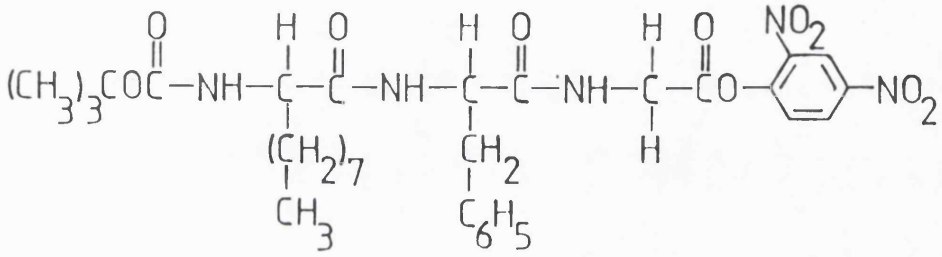
Terminal glycine has often been used in polymerisation to prevent any racemisation. Bloom et al[285] used nitrophenol esters in the synthesis of their oligopeptides and sequential polypeptides, not always using glycine as the terminal amino acid. In their work they reported molecular weights in the region of 6,000 to 15,000 depending on the initial tripeptide and they reported little or no racemisation.

De Tar et al[284] also using nitrophenol active esters, polymerised tripeptides to give sequential polypeptides with an average molecular weight of 12,000.

The 2,4-dinitrophenol group was attached to the deprotected tripeptides via EDAC coupling. Two 2,4-dinitrophenol active ester tripeptides were produced.



(XXV)



(XXVIII)

Separation of these compounds created difficulty due to breakdown on silica gel during chromatography.

The protected active ester tripeptides were deprotected at the amino terminus by trifluoroacetic acid hydrolysis. Polymerisation of tripeptide XXX was initially followed by NMR, with DMSO as solvent with NMM as the basic initiator. The reaction was allowed to proceed for 18 hours with spectra taken at regular intervals showing major changes in the amino and α-carbon regions of the spectra.

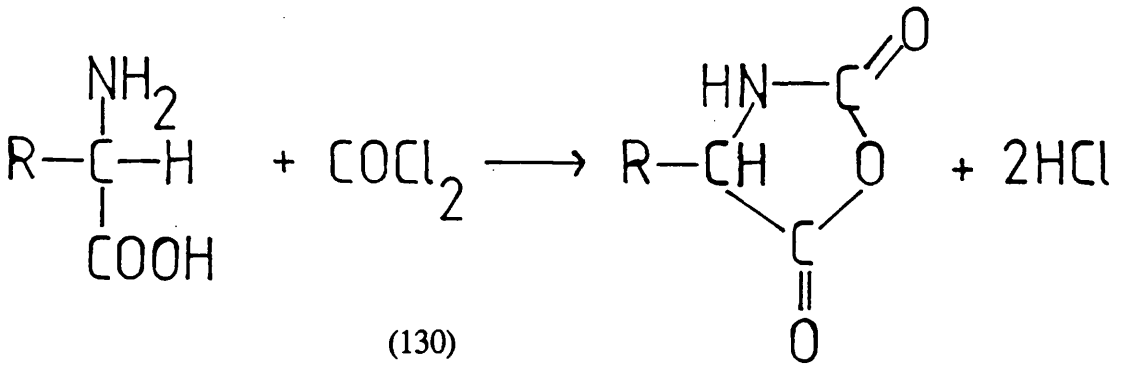
Polymerisation of active ester XXV was carried out with TEA as the basic initiator, the reaction was allowed to proceed for 27 hours.

In both polymerisations a gel was obtained, which on adding methanol, gave a fine white solid which was collected by filtration. This solid was then insoluble or gave rise to gel formation on the addition of solvents.

4.9.2 Leuchs Anhydride method of Polymerisation

Polymerisation via the Leuchs anhydride method requires the synthesis of a Leuchs anhydride, by the phosphogenation of the unprotected amino acid. This reaction

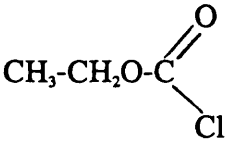
is normally undertaken by phosgene gas (130) (Scheme 13) but due to its toxicity other reagents were sought to give the required product.



(130)

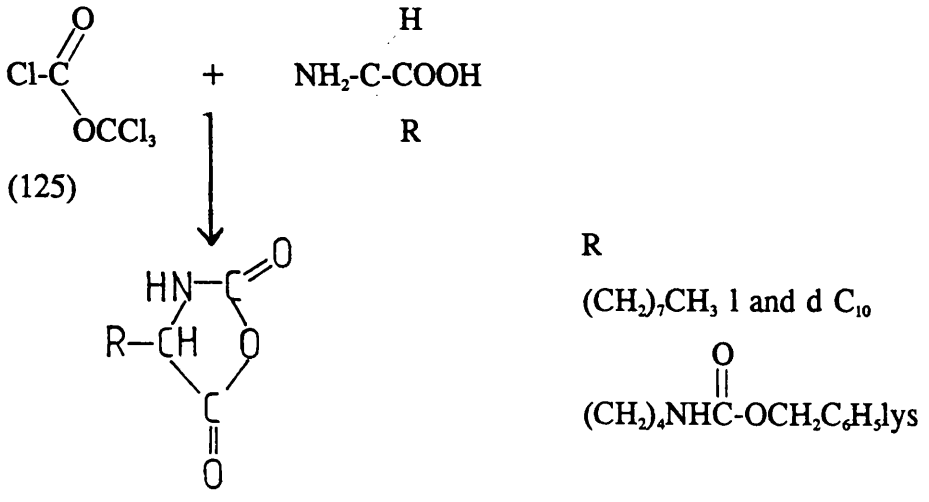
(Scheme 13)

Ethyl chloroformate (131) was used in an attempt to obtain the Leuchs anhydride but NMR and melting points indicated the required product had not been obtained.



(131)

Trichloromethyl chloroformate (diphosgene) (132), then a new product, was used via the method of Parrod and Spach[286] to obtain the Leuchs anhydrides of d-aminodecanoic acid, l-aminodecanoic acid and Nϵ-carbobenzoxylysine (Scheme 14)



(Scheme 14)

Leuchs anhydrides have been synthesized for all naturally occurring amino acids[287] and their use in polymerisation and peptide synthesis has been widely reported[288-290]. The use of Leuchs anhydrides in polymerisation and copolymerisation has been widely used and these were the methods employed during this work. A dilute solution of the Leuchs anhydride was used approximating 5% and the reaction in benzene was allowed to proceed for 24 hours.

The N-carboxy anhydride method of peptide synthesis is often the simplest method for the preparation of free peptides as the reaction proceeds almost quantitatively at optimum conditions with no intermediates needing to be islolated and purified. It was because of the ability for perpetual reaction with no side products, that made this a method of choice for some of the polymerisation work.

Polymerisation via the Leuchs anhydride method gave four polymers:

(i) d-aminodecanoic acid polymer XXXV

(ii) l-aminodecanoic acid polymer XXXII

(iii) an l-aminodecanoic acid-lysine 1:1 polymer XXXIII

(iv) l-aminodecanoic acid-lysine 10:1 polymer XXXIV

The addition of benzene allowed filtration of the K_2CO_3 present in the reaction. Addition of methanol gave a white precipitate which in drying became insoluble.

4.10 Solid Phase Peptide Synthesis

Manual and automated solid phase peptide synthesis were used to increase the rate of peptide production.

4.10.1 Manual Solid Phase Peptide Synthesis

VII was attached to chloromethylated Merrifield resin by refluxing in EtOH for 48hrs. The level of binding to the resin was found and the quantity of the reactants needed was calculated. Coupling to the resin was carried out in a stepwise manner. Following each coupling, the N-terminal protecting group was deprotected, and the next amino acid added. To ensure coupling had occurred at each step a small amount of resin was removed from the reaction by transesterification (using MeOH and

TEA). At the second coupling tlc indicated two spots, complete deprotection following the first coupling had not occurred, therefore the deprotection medium was changed from TFA in DCM to HCl in DEM and finally HCl in dioxane. Tlc again indicated incomplete deprotection therefore manual synthesis was abandoned and automated solid phase peptide synthesis was undertaken.

4.10.2 Automated Solid Phase Peptide Synthesis

Glycine was bound to chloromethylated resin as the N-α-tertiarybutoxycarbonyl calcium salt, glycine being chosen to avoid racemisation on cleavage The amount of amino acid bound was evaluated by hydrolysis of a small amount of resin, the reactants subsequently used being relative to the bound glycine, 2.5 equivalents being used to ensure complete reactivation. Firstly, VII and then N-α-tertiarybutoxycarbonyl-N-ϵ-carbobenzoxylysine were coupled to glycine with the amount of free amino groups calculated at each step using the method of V.K. Sarin et al[291] ., to ensure complete coupling at each step.

The tripeptide resin-glycine-aminodecanyl-N-α-butoxycarbonyl-N-ϵ-carbobenzoxylysine once formed, was cleaved from the resin by hydrazinolysis, but crystallisation and purification of the tripeptide could not be achieved. Solid phase peptide synthesis was abandoned in favour of solution phase synthesis.

4.11 Molecular Weight Determination

The molecular weight of the polymers synthesised was to be determined. There was great difficulty in the dissolution of the polymers, but with the use of solvent mixtures in conjunction with small quantities of polymer dissolution was achieved. Various methods of molecular weight determination were used.

4.11.1 Vapour Pressure Osmometry

Vapour pressure osmometry was initially the method of choice. This technique measures resistance changes by the use of a wheatstone bridge. The osmometer consists of two cells. One filled with solvent and the second with solutions of varying concentration. The different rates of evaporation from the two cells gives a resistance measurement and this is used to calculate the molecular

weight.

The vapour pressure osmometer is first allowed to saturate with benzene vapour over 48 hours and then standardised with benzil. Once standardised, XXXIII was dissolved in C_6H_6 giving a gel from which no evaporation appeared to occur and no resistance changes measured. Disruption of the gel structure with a second solvent could not be carried out, as this would affect vapour pressure due to differences in evaporation rates of the different solvents. This method was abandoned.

4.11.2 Viscometry

In viscometry, the time taken for the solvent and solutions of varying concentration to pass between two points in a viscometer are recorded. These are related to the molecular weight by the intrinsic viscosity which is found during experimentation.

$[\eta] = \eta_2/c$ as $C \rightarrow O$ $\eta_r = (\eta_o - 1)$

η = viscosity of solution

η_o = viscosity of solvent

XXXIII was dissolved in C_6H_6 giving a gel, the flow times of various solutions were recorded (Table 5).

Fig. 14 η_r/c against c was plotted giving an exponential curve. Extrapolation of the graph to c=o could not be made, and a different formula was used to calculate the molecular weight from the results.

$$\eta_r/c_2 = t\text{-}to/toC_2 + (1/\varrho_o - \acute{v}_2)t/to$$

to = time of flow of solvent

t = time of flow of solution

C_2 = concentration of solution

ϱo = density of solvent

$\acute{v}_2$ = partial molar volume

Partial molar volume is the volume taken by the molecule. As no value was

available for the partial molar volume a standard was chosen from molecules having similar constituents.

Fig. 15 of η/c versus c was plotted again giving an exponential curve which did not allow extrapolation to zero. The results of the viscometry molecular weight determination indicated that the solutions possessed complex behaviour particularly at higher concentrations.

Viscometry measurements with more dilute solutions could not be made due to inaccuracies in measuring solutions of very similar concentration. The behaviour of the polymer in solution could be explained by aggregation of the polymer molecules. Aggregation would account for the formation of gel in solution and the exponential curve produced at higher concentrations.

4.11.3 Electrophoresis

Gel electrophoresis is used to determine the molecular weight of proteins. The polymers produced in this work are protein like therefore gel electrophoresis was used to determine the molecular weight of the polymers. L-aminodecanoic acid polymers were placed on a 10% SDS gel. No movement of the polymer from the point of injection at the baseline occurred, therefore no information was obtained about the molecular weight.

4.11.4 Light Scattering

Light scattering was the main method use of molecular weight determination. To ensure correct technique and user error, the molecular weight of dextrans was determined.

Light scattering is dependent on measuring the amount of scatter caused by placing the solution cell in the path of a laser beam. The amount of light scattered is measured and related to the size of the molecular solution, allowing molecular weight to be determined. L-aminodecanoic acid polymer (XXXII) was dissolved in DMSO giving a viscous solution, which could not be filtered. To avoid contamination and maintain a particle free system great care was taken.

Readings were taken at varying angles of scatter from 60° to 120°. This was done in order to get an idea of molecular shape as well as size. The refractive index

of the solution was calculated by experiment as it was required for the calculation of molecular weight.

$n = n_o + (dn/dc)c$ n = refractive index of solution

n_o = refractive index of solvent

c = concentration of solvent

$\Delta n = K\Delta d$ K = constant 0.9675×10^{-3}

d = experimental readings

From the experimental results, calculations gave n values which were plotted against concentration (Fig.17). The slope of the graph giving dn/dc, this being used to calculate molecular weight.

The equation for molecular weight determination is:

$$\text{Intercept} = 1/M/\frac{4\pi^2 n_o^2 (dn/dc)2}{\lambda^4 Lb}$$

λ = wavelength of light used in light scattering

b = constant 3.2198×10^{-3}

$L = 6.023 \times 10^{23}$

$n_o = 1.478$

$dn/dc = 6.22816 \times 10^{-4}$

The calculated readings and refractive index were used to give a Zimm plot which was done by computer. Zimm plots take into account readings at all angles of scatter, giving results for both molecular weight and shape. The Zimm plot gave eroneous results, the molecular weight being calcuated as 9.2991×10^{-3}.

The results were re-examined and the perspex block readings (which was used as a standard) were found to have drifted with time, therefore the Zimm plot was recalculated using the average reading for the standard. The molecular weight calculated = 1.206×10^{-2}.

With the eroneous results obtained, simple molecular weight calculations were carried out using only a 90° angle.

C/S_{90} against C was plotted (Fig.16), the intercept = 0.812

$$0.812 = 1/M/0.1127672$$

$$1/M = 0.095$$

$$M = 10.92$$

This molecular weight was again eroneous. The method was tried again with a different polymer.

L-aminodecanoic acid-lysine 1:1 polymer was dissolved in benzyne and trifluoroethanol added to prevent aggregation. The experimental details were as in the l-aminodecanoic acid polymer, with readings taken at 90° angle and the refractive index being found, allowing the molecular weight to be calculated.

Intercept of $C/S_{90} = 6.6 \times 10^{-3}$

$dn/dc = 0.403$

$1/M = 260.27$

$M = 7.93 \times 10^{-4}$

Due to eroneous results this method was again abandoned without result.

4.12 Rayleigh Interference Optics and Schlieren Optics

All the methods attempted had failed to give satisfactory molecular weight results. The polymers were sent for more complex experimentation, external to the department. The l-aminodecanoic acid polymer was solubilised in toluene + 5% TFE ($_N$1mg/ml) and examined by Rayleigh Interference optics using a Model E and Schlieren optics using an MSE Centriscan.

The Rayleigh Interference optics showed virtually no deviation of the fringes in a 12,, d.s. cell (even at speeds up to 50,000rpm)

In Schlieren optics initially very little movement was seen but with a more sensitive phase plate system a result was obtained. At 40,000rpm no boundary resolved, but the Schlieren trace showed negative deflection both near to the base and near to the meniscus. After 1 hour the trace became time invariant with a small negative deflection (Fig.22).

This result is consistent with the sample having a density very close to the

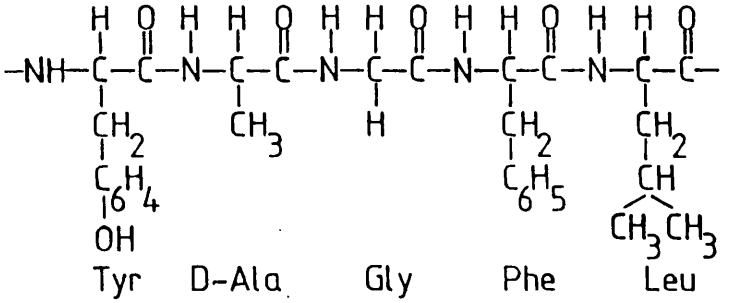
solvent probably a little higher. Under these conditions estimation of molecular weight is impossible, as the ratio d(dc/dr) by $(1-\upsilon\varrho_o)$ required for Schlieren work is non-evaluable when both numerator and denominator are close to zero. The equilibrium state appeared to be rapidly reached, hence the translational diffusion coefficient must be quite small. The polymer molecular mass thus being around 20000.

The closeness of the mass density of solute and solvent and $(1-\upsilon\varrho_o)$ to zero also explains why no deviation was obtained with the Rayleigh fringes and erroneous results in the light scattering.

In conclusion, the synthesis of the polymers was straightforward while the molecular weight determination and characterisation proved to be impossible.

5. Enkephalins

Enkephalins were first discovered in 1975, the objectives of the enkephalin project are the solution phase synthesis of enkephalin derivatives, having increased lipophilicity, increased ability to cross the blood brain barrier and increased stability to enzymic breakdown. The conjugation of lipidic amino acids and their oligomers to the Leu enkephalin sequence (10) enhances the ability to cross biomembranes (eg. gut, blood-brain barrier).



(10)

In addition to conjugation of the lipidic amino acids to the carbonyl terminus, inclusion of the lipidic amino acids in the pentapeptide sequence was also carried out.

The Leu-enkephalin sequence first identified by Hughes and Kosterlitz[76] is selective for the δ receptor as compared to the Met-enkephalin (9). Inclusion of D-Ala as opposed to the naturally occurring Gly in position 2 increases activity and the compounds containing D-Ala were synthesized[108,148].

The enkephalins are important because they mimic the opiate analgesics and

are, due to their small size and hydrophobic content, able to fit the conformational space occupied by the morphine alkaloids[96]. The ability to use enkephalins in analgesia or other disorders and avoid dependence, respitory or gastric effects has long been investigated.

The rationale for conjugation of the lipidic amino acid and its oligomers is its ability to increase lipophilicity without toxicity or bioincompatibility. The oligomers also enable the lipophilicity to be altered by an increase or decrease in the chain length of the amino acid or the length of the oligomers.

5.1 Chemistry of the Enkephalins

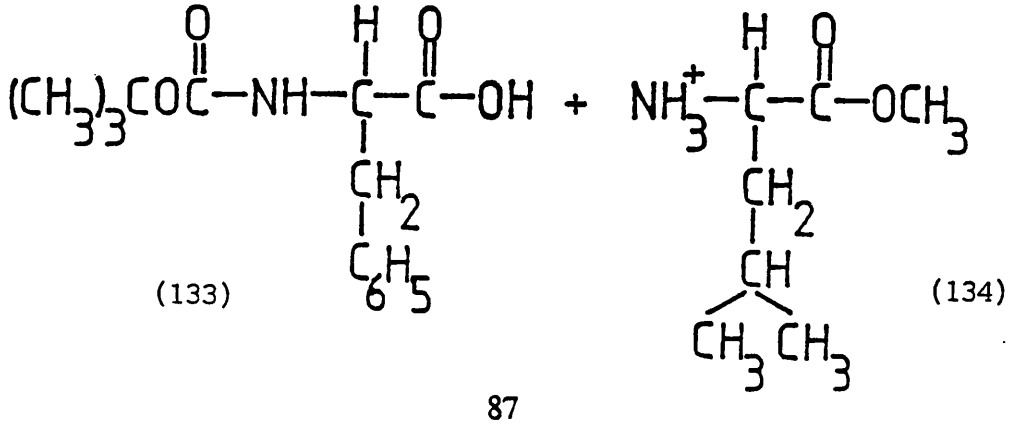
The enkephalins were made by using solution phase peptide synthesis in a stepwise method.

The synthesis of the enkephalins was done in four separate ways depending on the inhibitor being produced. In all cases the protecting groups used were the N-α-tertiarybutoxy carbonyl group (BOC) and methyl esters (Me).

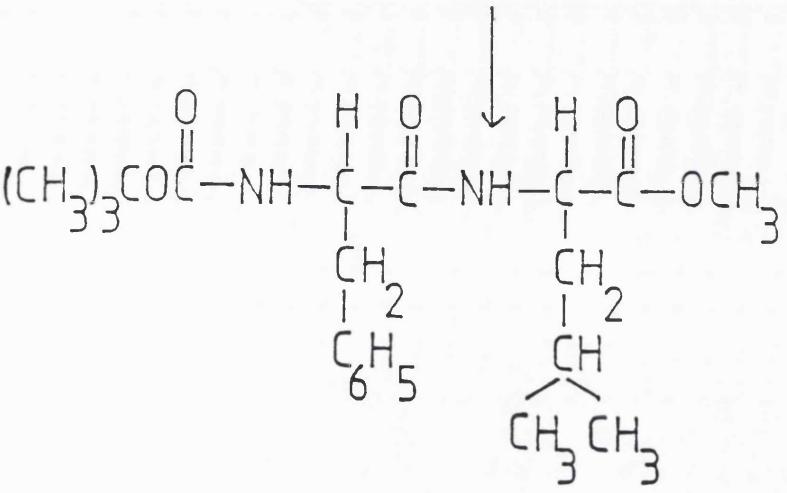
Initially the compounds having the lipidic amino acids substituted with the pentapeptide sequence were produced. Substitutions were made at position 2, 4 and 5 of the pentapeptide sequence (10) because altering positions 1 and 3 has been found by other workers to decrease activity. Introduction of lipidic amino acids within the sequence (10) increases lipophilicity thus increasing μ receptor specificity.

5.2 Enkephalin synthesis

First, N-α-tertiarybutoxycarbonyl phenylalanine (133) (Boc Phe) and leucine methyl ester hydrochloride (Leu O Me HCl) (134) were coupled using dicyclohexylcarbodiimide (DCC) giving the dipeptide Boc Phe Leu O Me (LXIV)(Scheme 15).



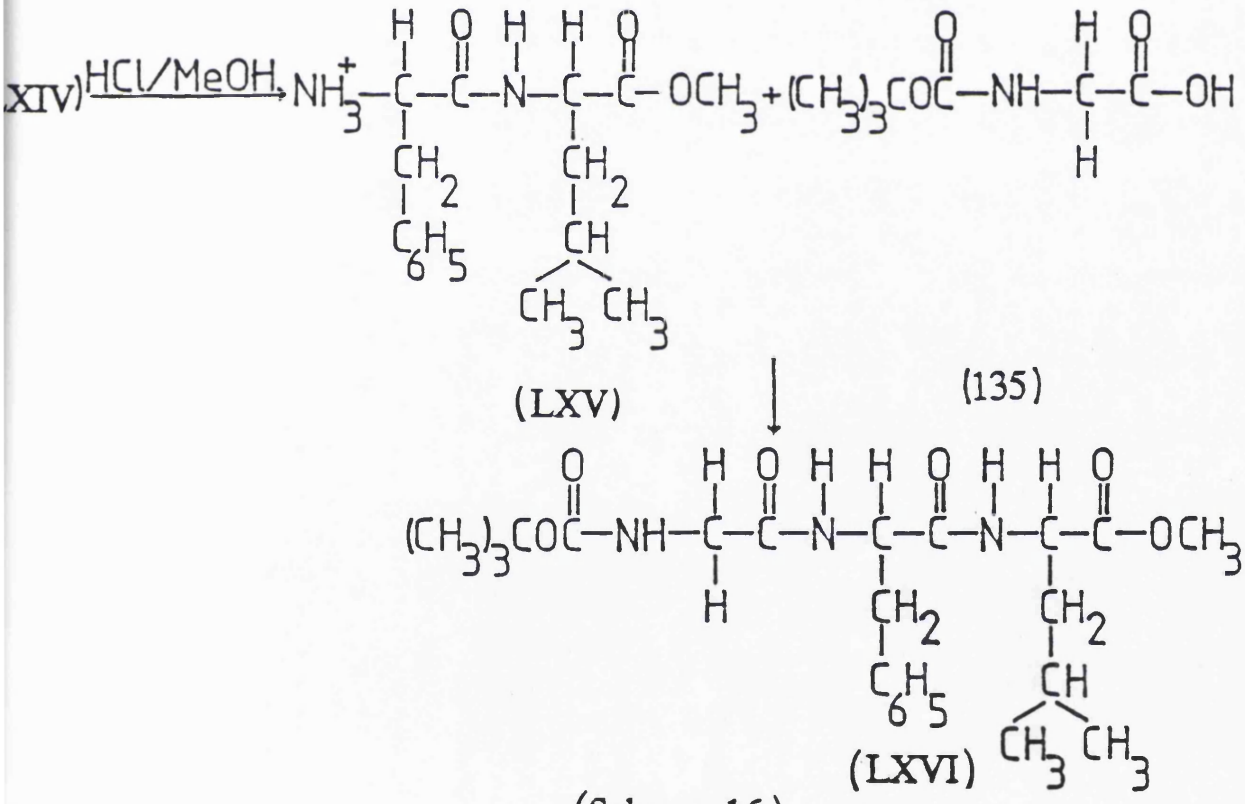
(133) (134)



(LXIV)

(Scheme 15)

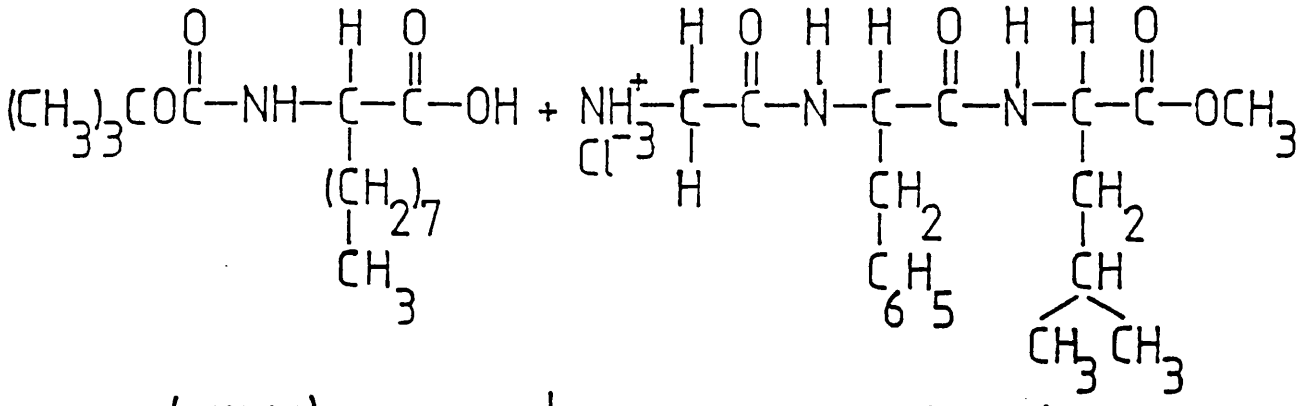
Following acidic deprotection of the amino group giving (LXV) addition of Boc Gly (135) gave Boc Gly Phe Leu O Me (LXVI) (Scheme 16).



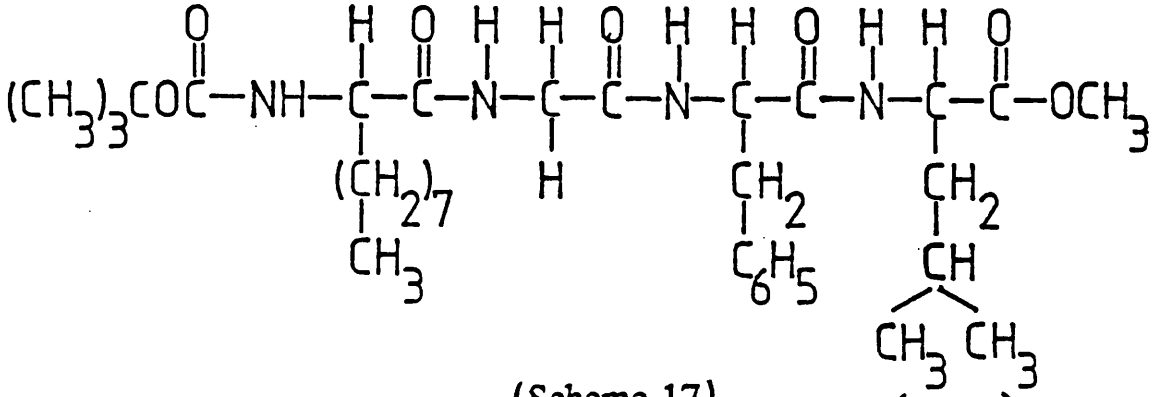
(Scheme 16)

N-terminal deprotection of tripeptide (LXVI) yielded (LXVII) which was used in the synthesis of the Leu-enkephalin (10) and a substitution peptide having 2-aminodecanoic (C_{10}) acid in position 2 LXXVIII. Boc C_{10} (XXXXI) was coupled to

(LXVII) in EDAC to give (LXIX) (Scheme 17).

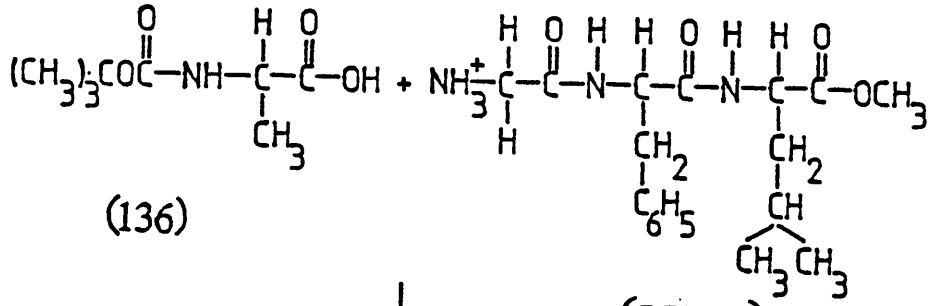


(XXXXI) (LXVII)

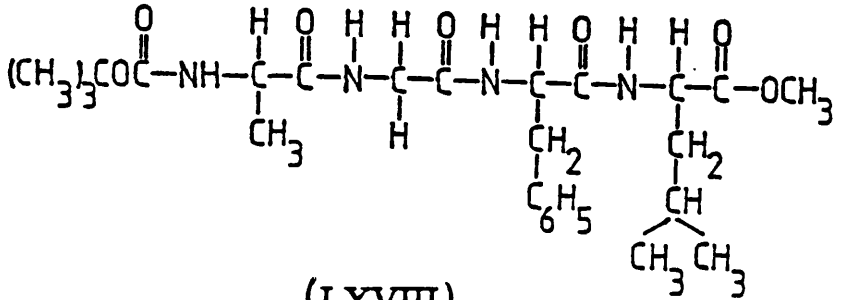


(Scheme 17) (LXIX)

Boc-D-alanine (136) (Boc-D-Ala) and (LXVII) were coupled to give the tetrapeptide (LXVIII) (Scheme 18).



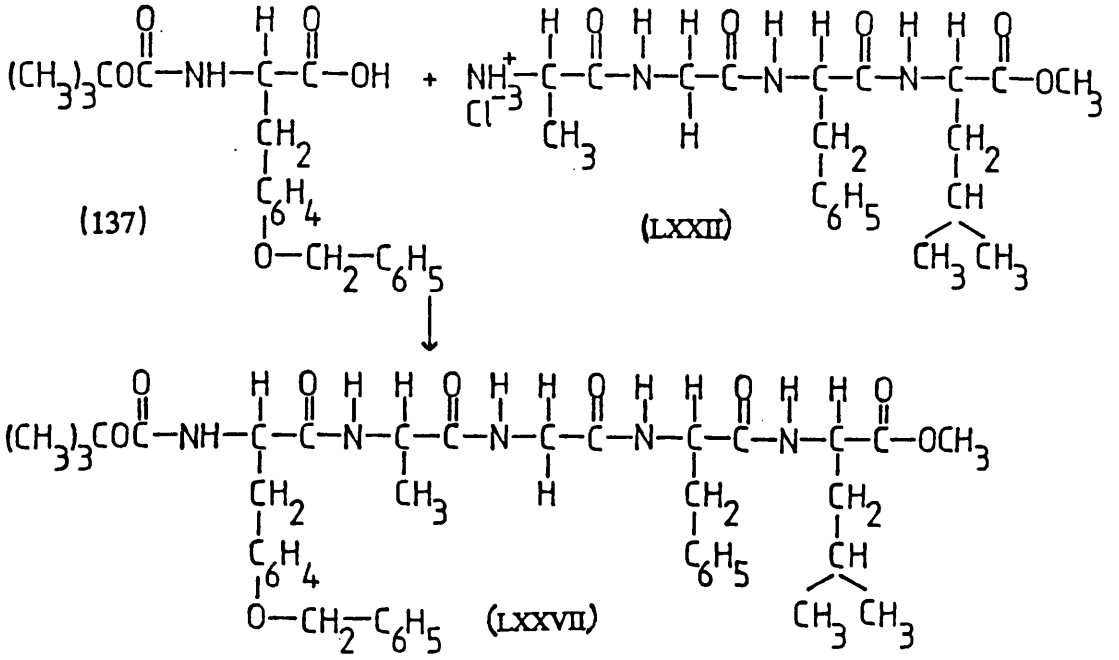
(136) (LXVII)



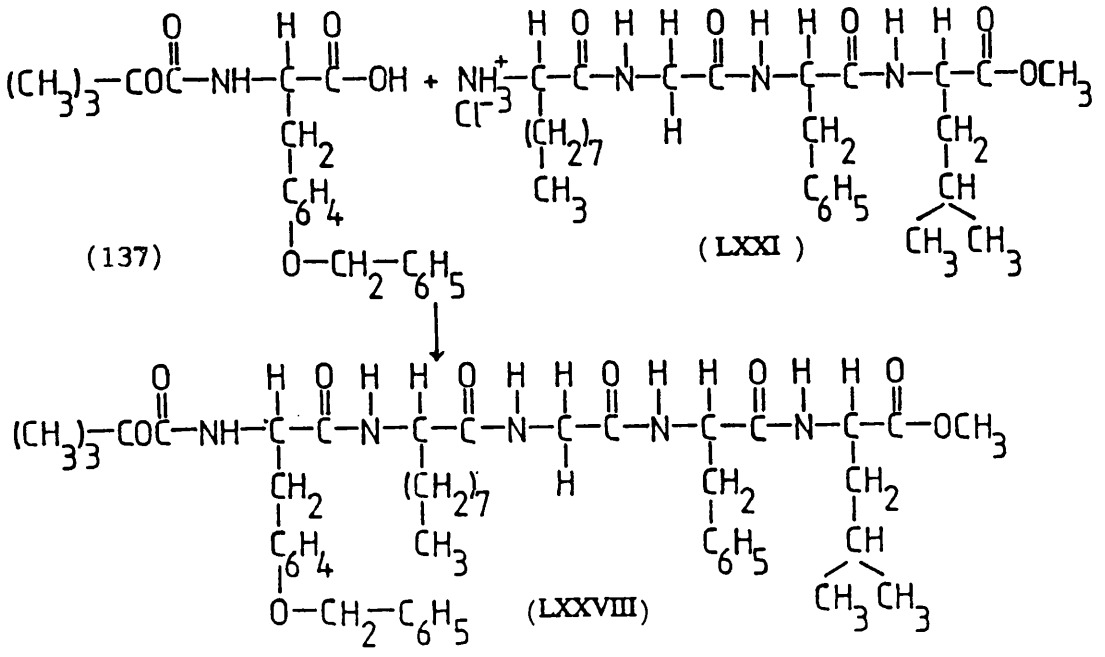
(LXVIII)

(Scheme 18)

Peptides (LXIX) and (LXVIII) were N-terminus deprotected (LXXI and LXXII) and coupled with Boc-Tyrosine (benzyl) hydroxy (137) (Boc Tyr [Bzl OH]) giving the enkephalin pentapeptide (LXXVII) (Scheme 19) and the enkephalin substituted with C_{10} in position 2 (Boc Tyr (Bzl)OH)-L-C_{10} Gly Phe Leu Me) (LXXVIII). (Scheme 20)



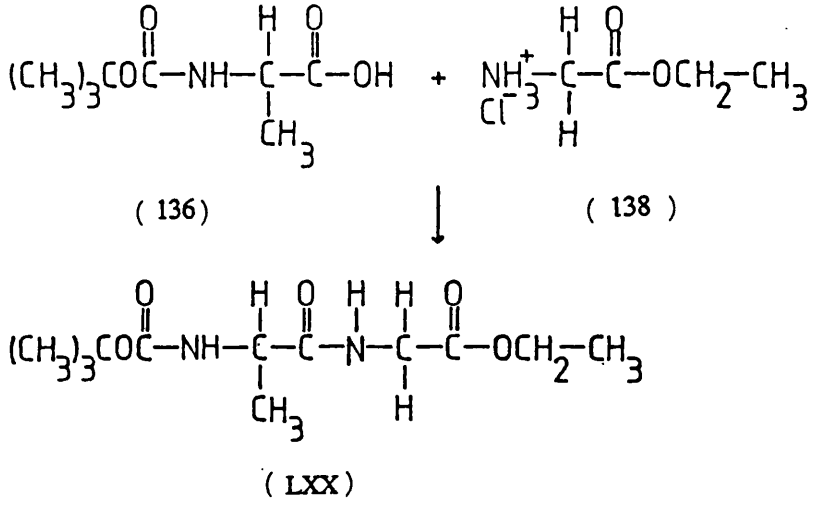
(Scheme 19)



(Scheme 20)

To obtain 2-aminotetradecanoic acid (C_{14}) substitution in position 4 the synthesis was via a different route.

Boc Ala (136) and glycine ethyl ester hydrochloride (Gly O Et HCl) (138) were coupled giving dipeptide (LXX) (Scheme 21).

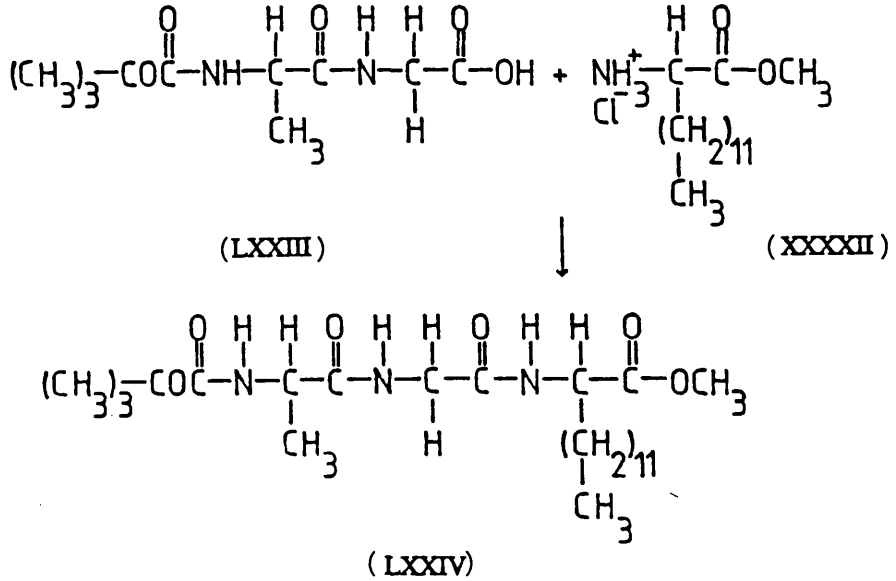


(136) (138)

(LXX)

(Scheme 21)

Deprotection of (LXX) at the C-terminus gave (LXXIII) which was coupled to 2-aminotetradecanoic acid methyl ester hydrochloride (XXXXII) giving (LXXIV) (Boc D Ala Gly C_{14} O Me) (Scheme 22).



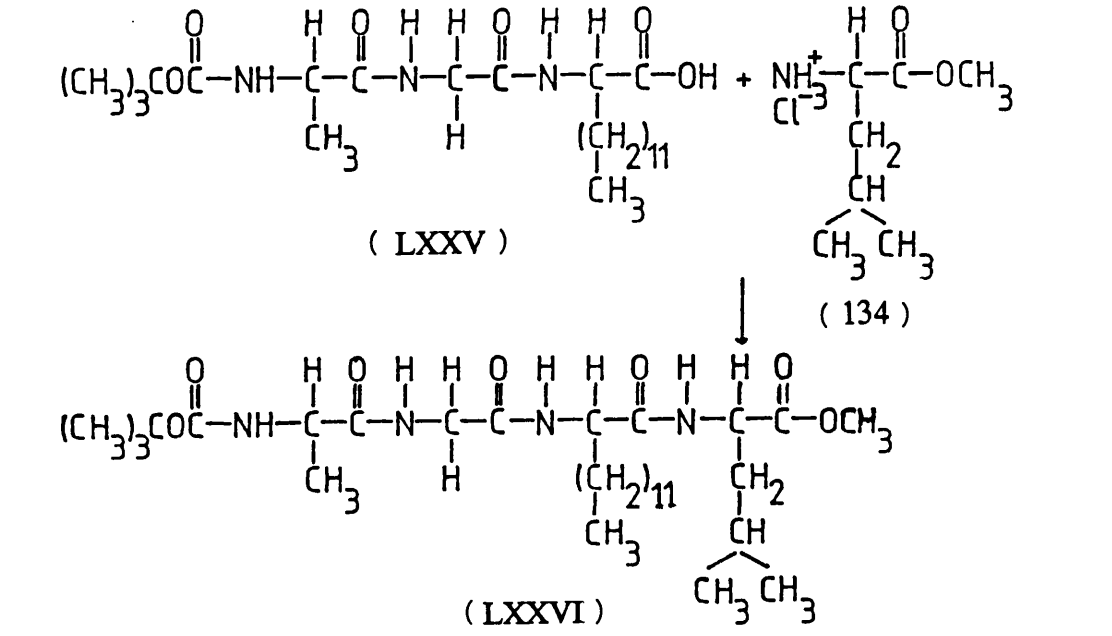
(LXXIII) (XXXXII)

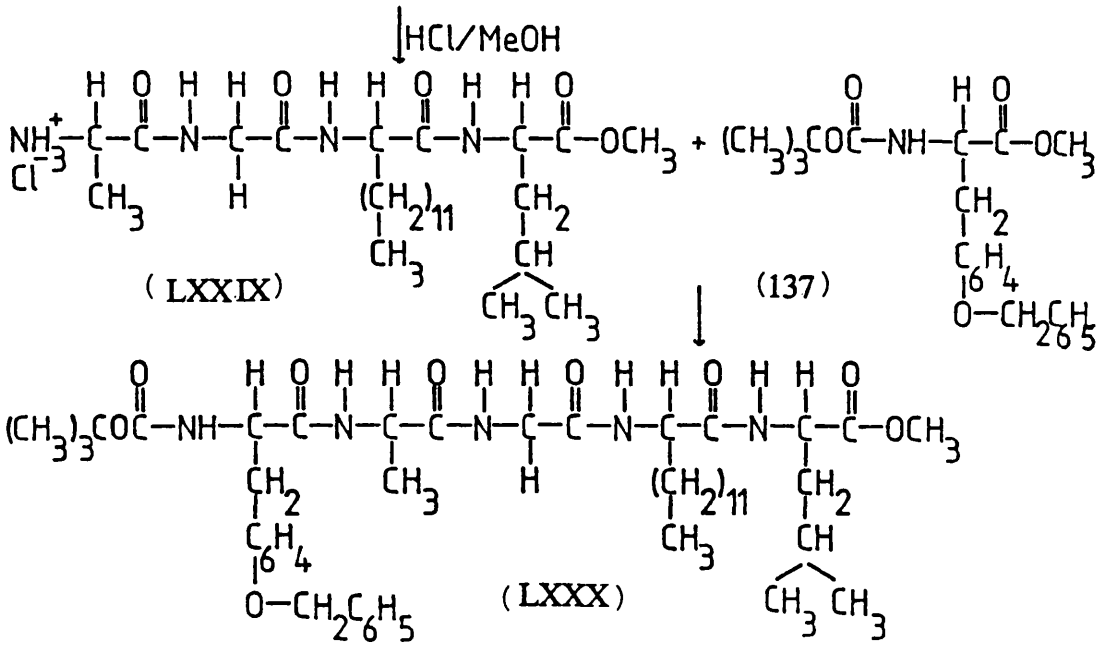
(LXXIV)

(Scheme 22)

Peptide (LXXIV) was deprotected at the C-terminus giving (LXXV) and

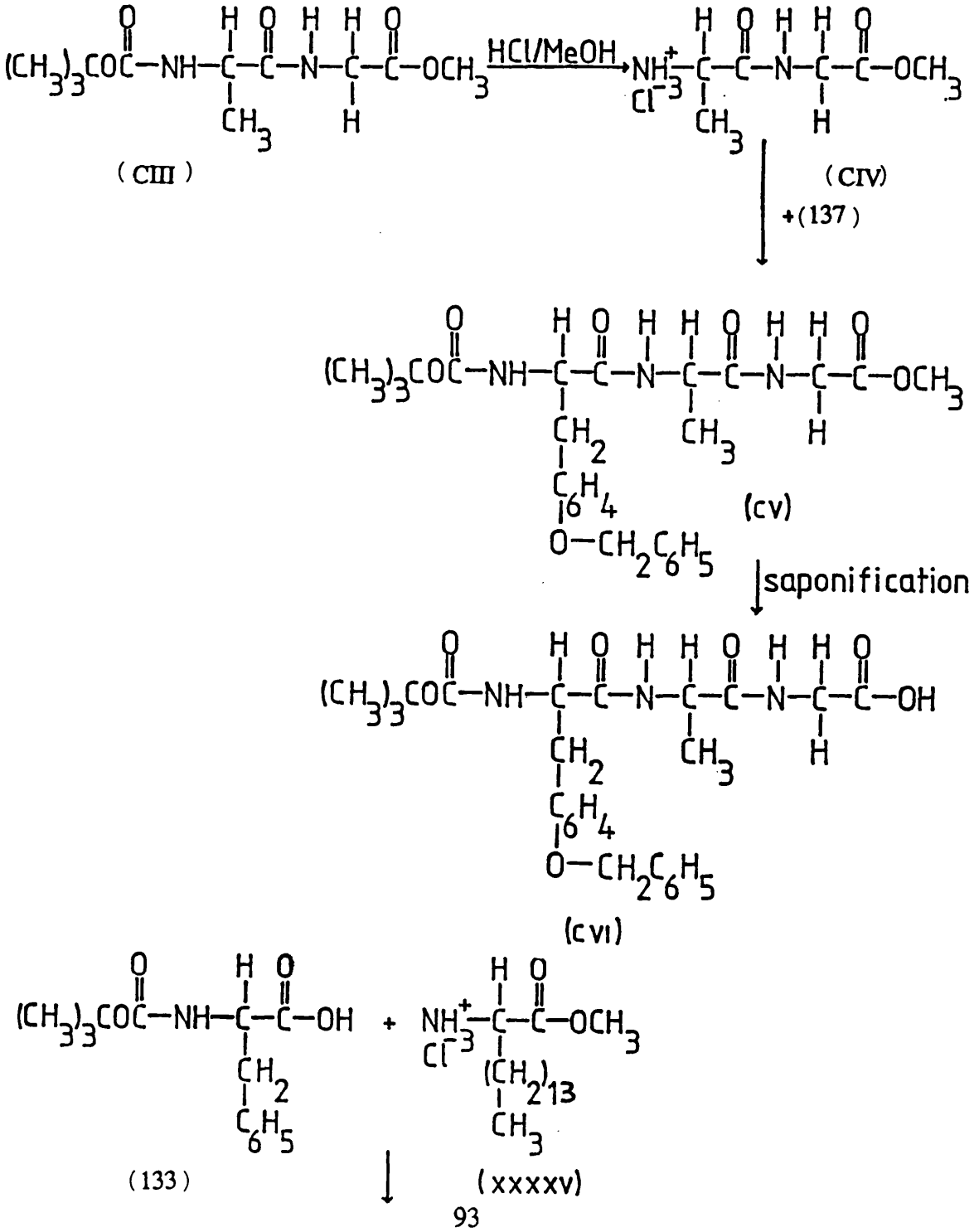
coupled to Leu OMe HCl (134) giving (LXXVI) which was deprotected at the amino group (LXXIX) and coupled with Boc Tyr (Bzl) OH (137) giving (LXXX) the position 4 substituted enkephalin (Scheme 23).

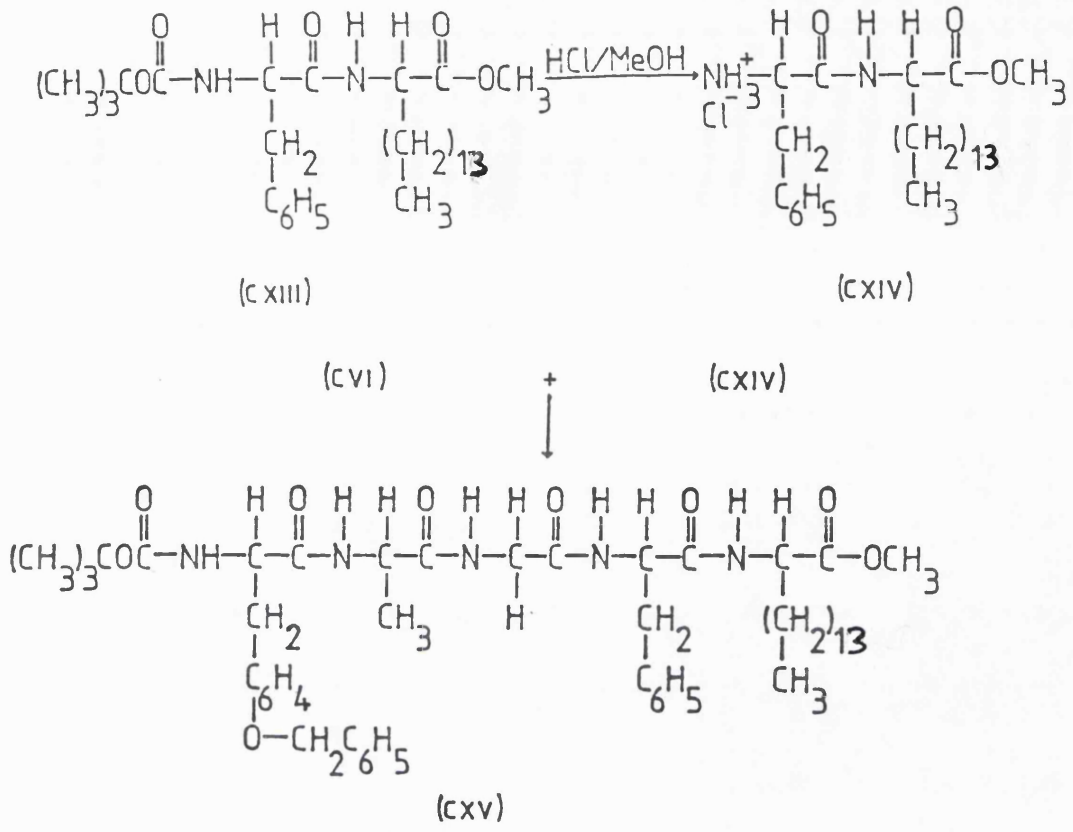




(Scheme 23)

2-Aminohexadecanoic acid (C_{16}) position 5 substituted enkephalin was produced by the N-terminal deprotection of (CIII) giving (CIV) which was coupled to (137) giving the tripeptide (CV). Boc Phe (133) was coupled to XXXXV giving dipeptide CXIII. C-terminal deprotection of (CV) giving (CVI) and N-terminal deprotection of (CXIII)' giving (CXIV) followed by coupling gave the position 5 substituted enkephalin (CXV). (Scheme 24).



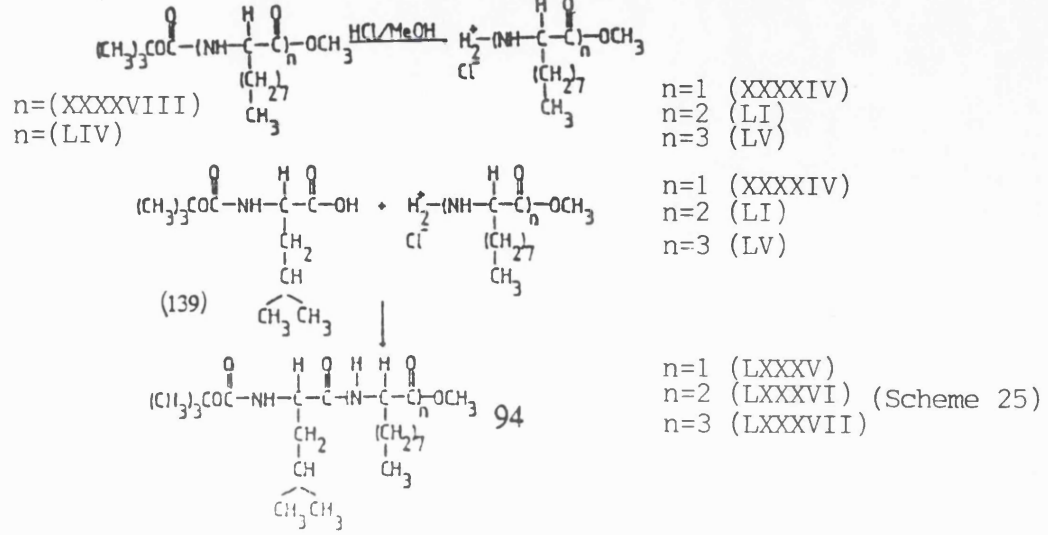


(Scheme 24)

5.3 AMIDE LINKED CONJUGATES

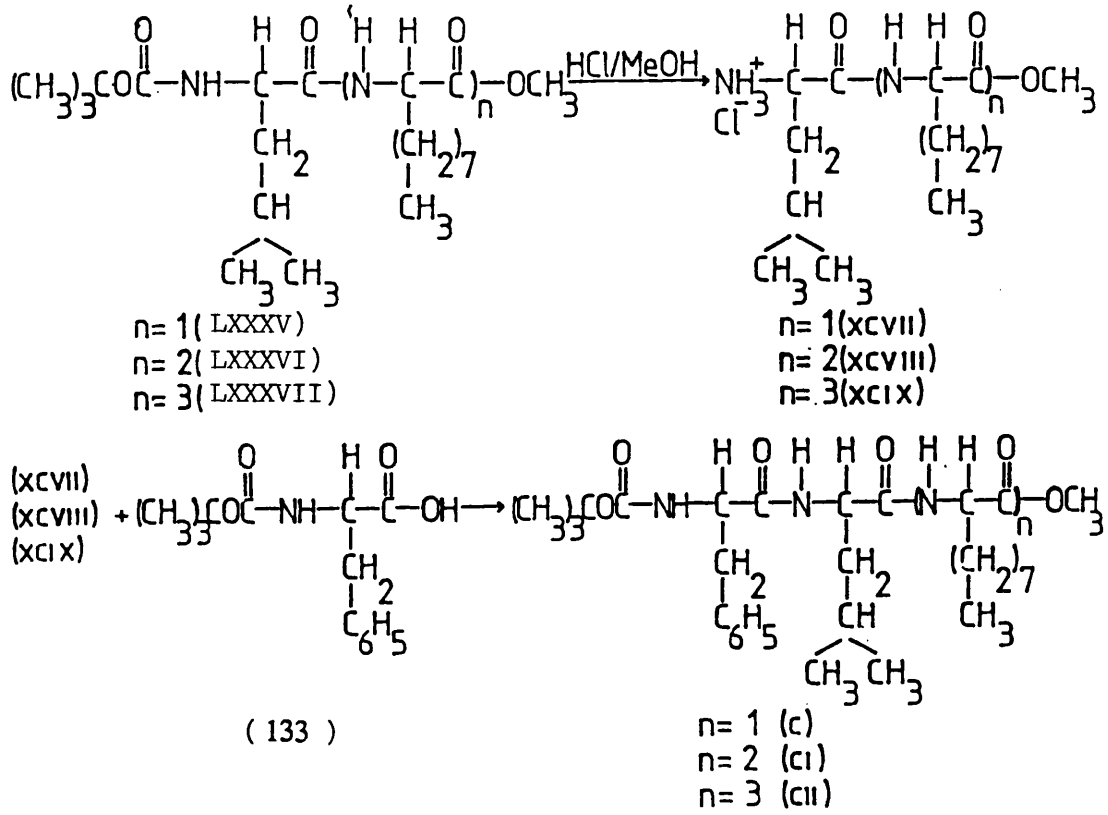
Conjugation of 2-aminodecanoic acid (C_{10}) and its oligomers to the enkephalin sequence (10), via an amide linkage, was the second group of compounds produced, where lipidic amino acids were coupled, EDAC was used.

Aminodecanoic acid methyl ester hydrochloride XXXXIV and its dimer (Boc C_{10} C_{10} OMe) (XXXXVIII) and trimer (Boc C_{10} C_{10} C_{10} OMe) (LIV) were used for conjugation. XXXXIV was coupled with Boc Leu OH (139) giving (Boc Leu C_{10} OMe) (LXXXXV). (XXXXVIII) and (LIV) were N-terminally deprotected giving (LI) and (LV) and these were coupled with (139) giving (Boc Leu C_{10} C_{10} OMe) (LXXXVI) and (Boc Leu C_{10} C_{10} C_{10} OMe) (LXXXVII)(Scheme 25).



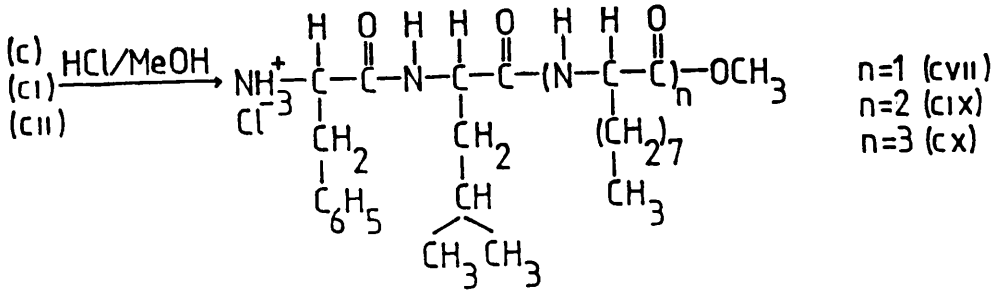
(Scheme 25)

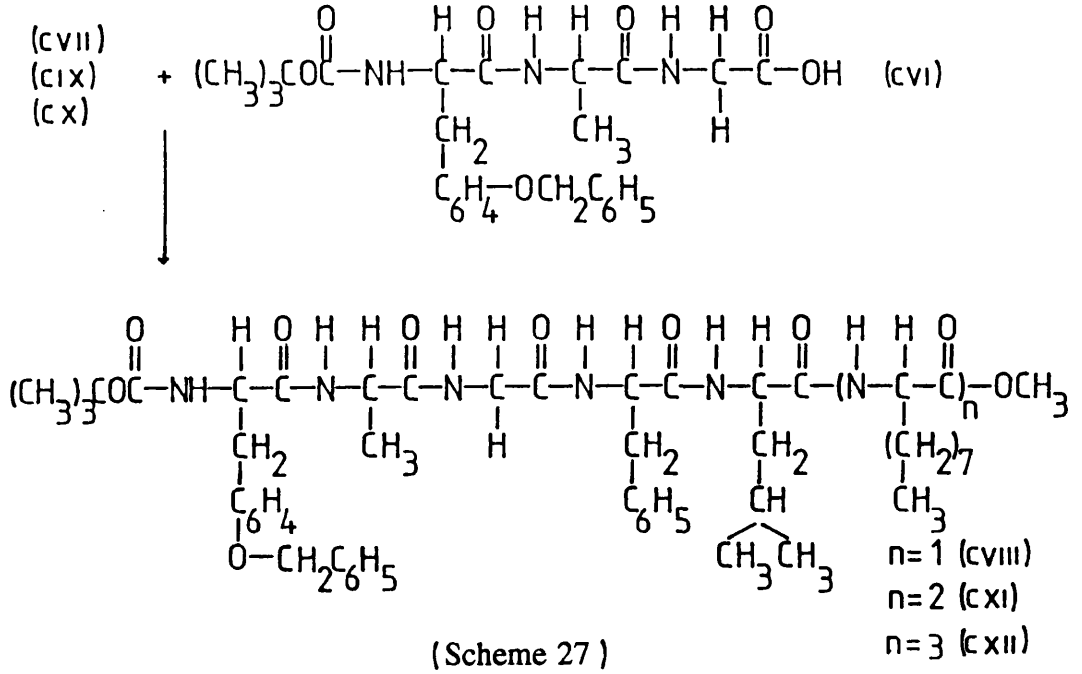
Compounds (LXXXV), (LXXXVI) and (LXXXVII) were N-terminally deprotected giving (XCVII), (XCVIII) and (XCIX) which were reacted with (133) giving (Boc Phe Leu C_{10} OMe) (C),(Boc Phe Leu C_{10} C_{10} OMe) (CI) and (Boc Phe Leu C_{10} C_{10} C_{10} OMe) (CII) (Scheme 26).



(Scheme 26)

The amino groups of (C), (CI) and (CII) were deprotected giving (CVII), (CIX) and (CX) these synthons were coupled to (CVI) giving the amide conjugated enkephalin derivatives (Boc Tyr (Bzl)OH D Ala Gly Phe Leu C_{10} O Me) (CVIII) (Boc Tyr (Bzl) OH D Ala Gly Phe Leu C_{10} C_{10} O Me) (CXI) and (Boc Tyr (Bzl) OH D Ala Gly Phe Leu C_{10} C_{10} O Me) (CXII). (Scheme 27)



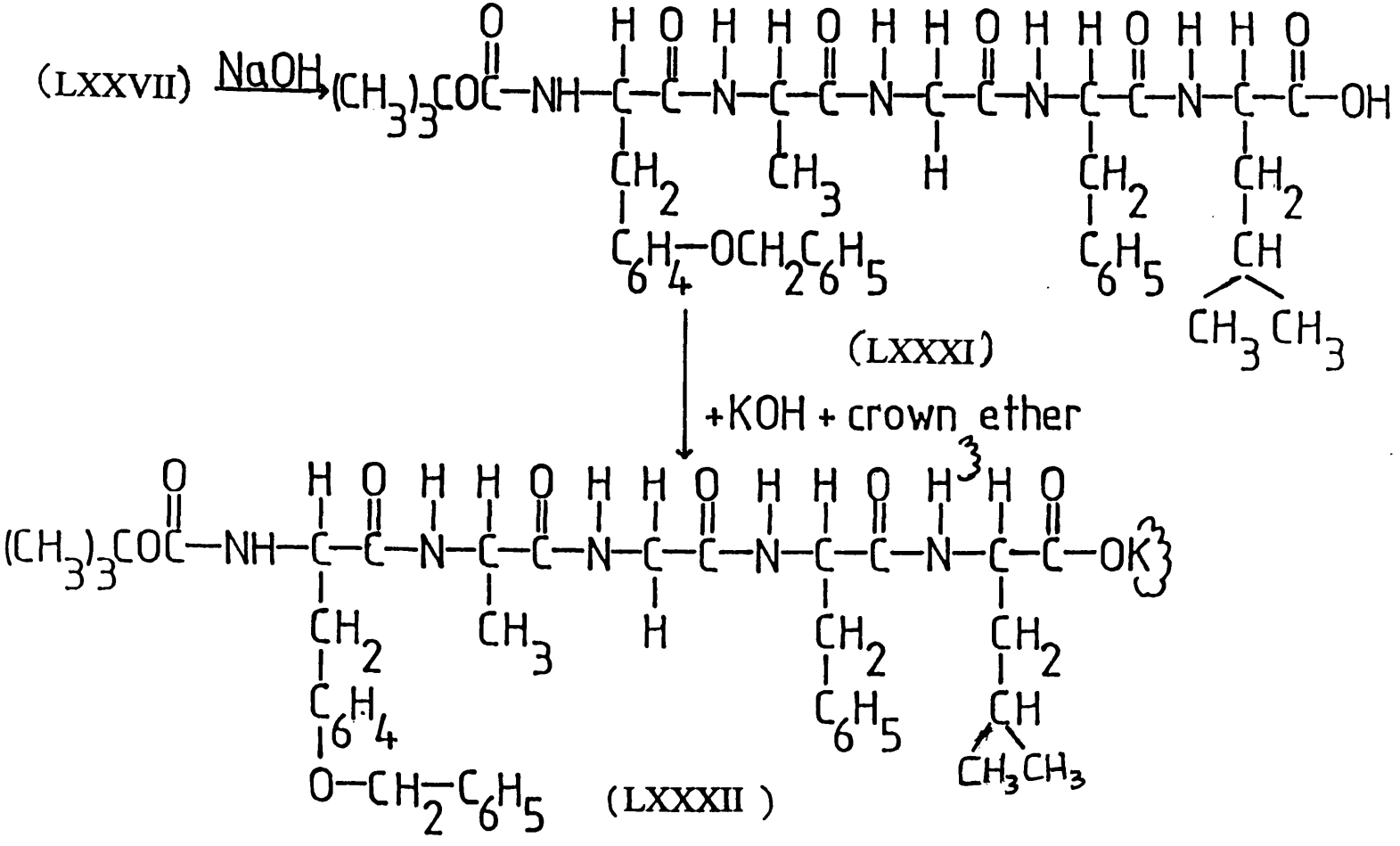


(Scheme 27)

5.4 ESTER LINKED CONJUGATES

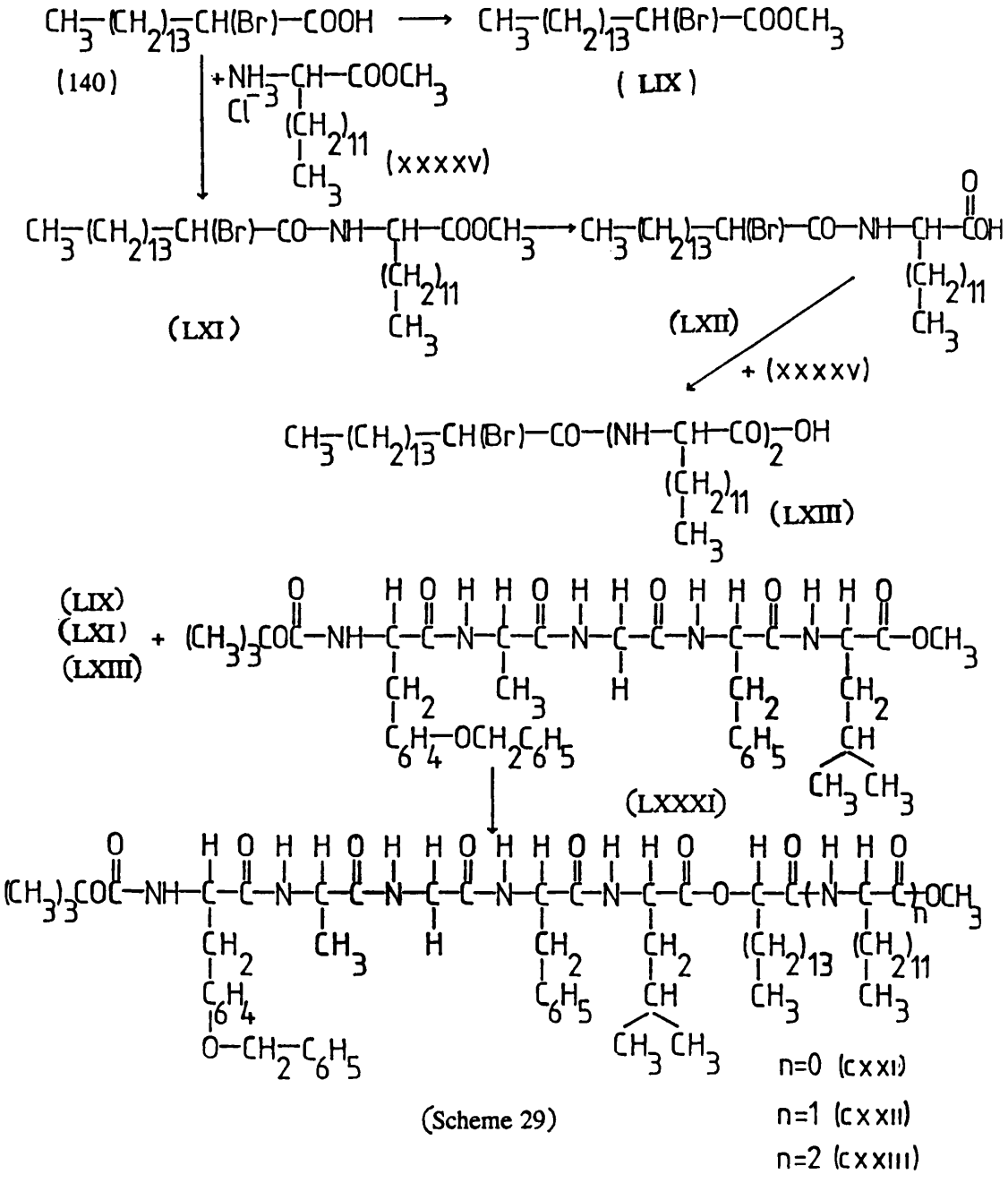
The third group of enkephalin derivatives produced were the ester linked lipidic amino acid derivatives. The ester link is important as a pro-drug linkage which can be readily broken by esterases in vivo. Esters also increase enkephalin activity.

The synthesis was first tackled by deprotecting the carbonyl of (LXXVII) giving (LXXXI) and producing a potassium, 18 crown-6-ether salt. (LXXXII) (Scheme 28). This enables coupling to be done without using harsh conditions but merely by adding and removing from the coupling group a nucleophile; the crown ether acts as a catalyst.



(Scheme 28)

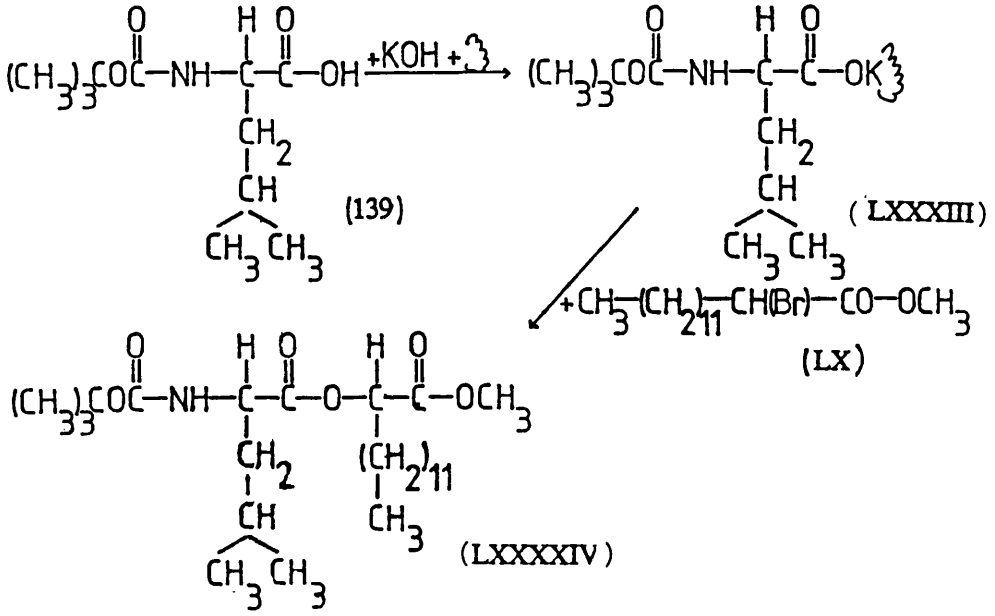
The production of an ester linkage was achieved by the coupling (LXXXI) with bromo derivatives of the lipidic amino acids. 2-bromohexadecanoic acid (Br C_{16}) (140) was carbonyl protected (LIX) but also coupled with 2-aminotetradecanoic acid methyl ester hydrochloride (XXXXV) giving (LXI) (Br-C_{16}-C_{14} OMe). LXI was carbonyl deprotected giving LXII and coupled to (XXXXV) giving (LXIII) (Br C_{16} C_{14} C_{14} OMe). Compounds (LIX), (LXI) and (LXIII) were then coupled with (LXXXI) giving (CXXI) (Boc Tyr (Bzl)OH D Ala Gly Phe Leu O C_{16} OMe), (CXXII) (Boc Tyr (Bzl)OH D Ala Gly Phe Leu O C_{16} C_{14} OMe) and (CXXIII) (Boc Tyr (Bzl)OH D Ala Gly Phe Leu $C_{6\ 14}$ C_{14} OMe) (Scheme 29).

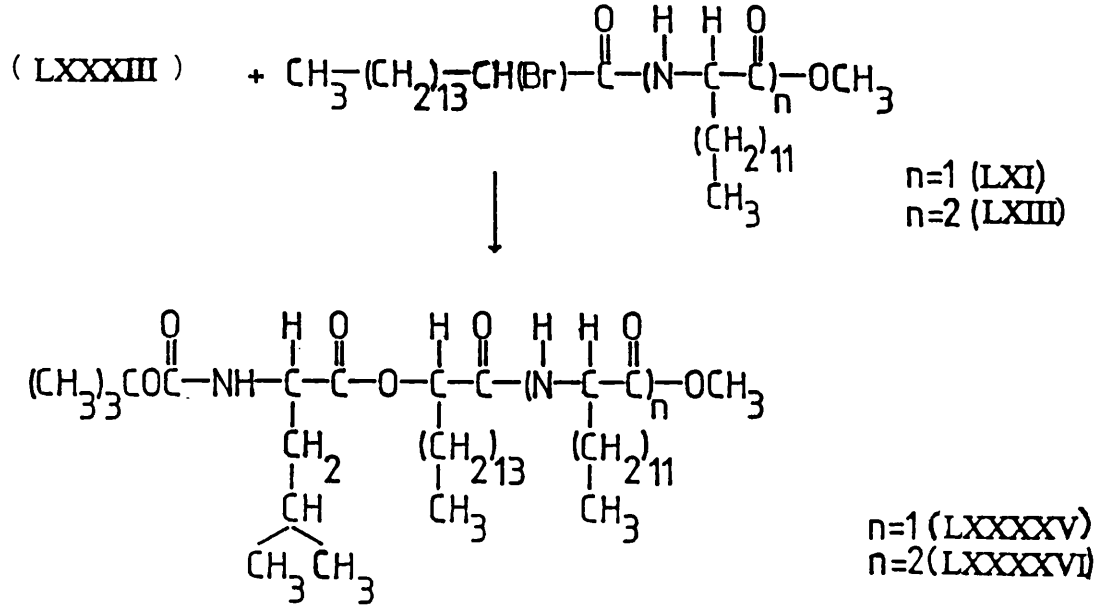


(Scheme 29)

NMR and MS spectra of (CXXI) (CXXII) and (CXXIII) did not indicate the presence of the required compounds, therefore the synthesis was revised.

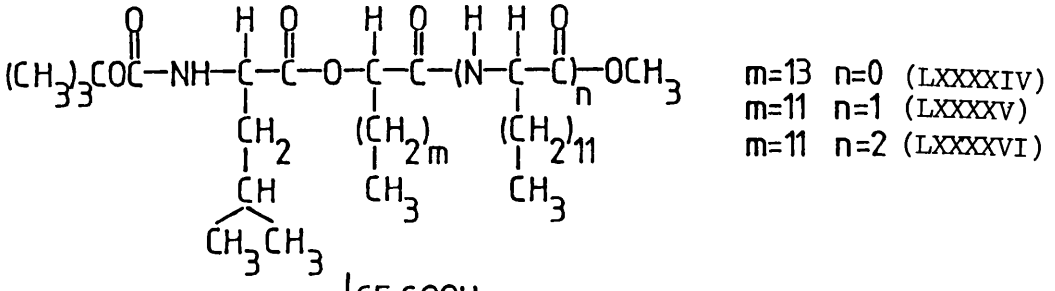
The potassium 18-crown-6-ether salt of (Boc Leu OH) (139) was made giving LXXXIII (Boc Leu OK) and this was coupled with (LX), (LXI) and (LXIII) giving (Boc Leu O C_{14} O Me) (LXXXXIV), (Boc Leu O C_{16} C_{14} O Me) LXXXXV and (Boc Leu O C_{16} C_{14} C_{14} O Me) (LXXXXVI) (Scheme 30).

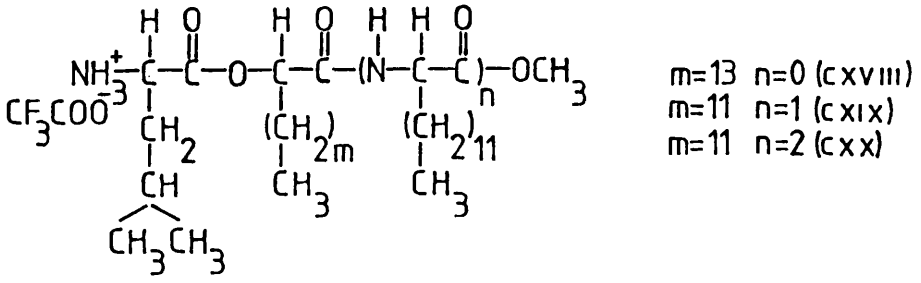


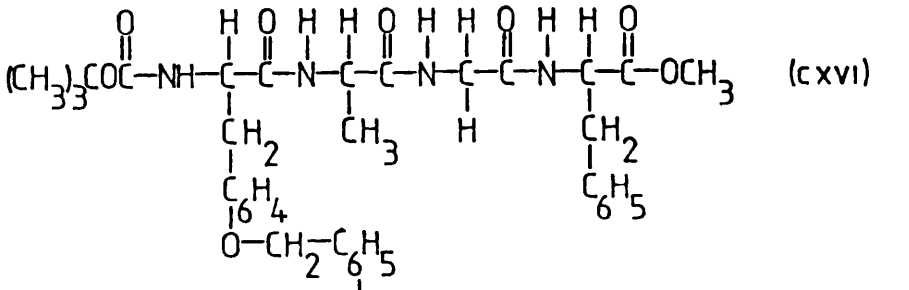


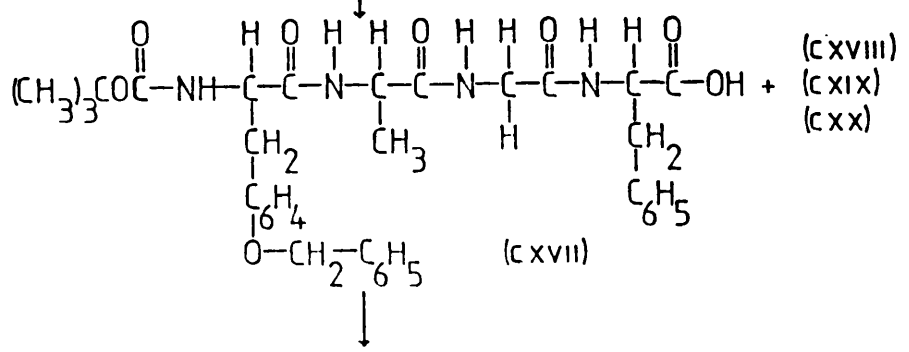
(scheme 30)

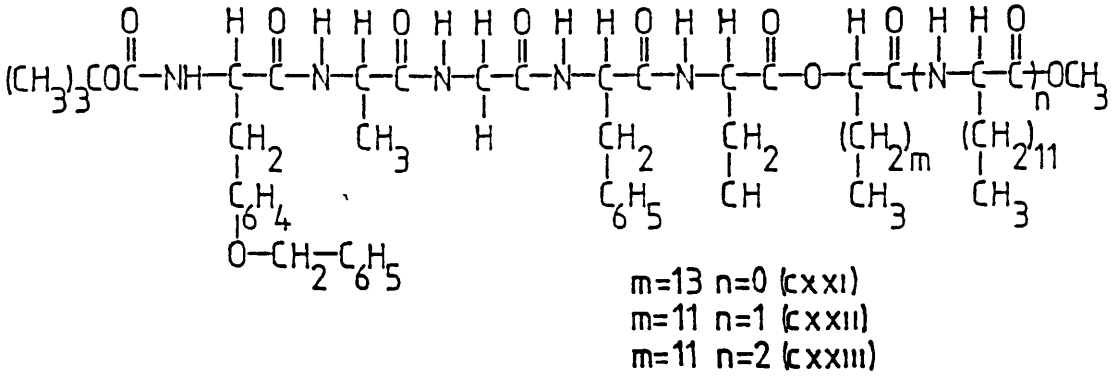
The amino groups of (LXXXXIV), (LXXXXV) and (LXXXXVI) were deprotected, trifluoroacetic acid being used instead of hydrochloric acid due to the milder conditions, giving (CXVIII), (CXIX) and (CXX). Compounds (CXVIII), (CXIX) and (CXX) were coupled with the synthon (Boc Tyr Bzl (OH) D Ala Gly Phe OH) CXVII produced by coupling (CVI) with phenylalanine methyl ester hydrochloride (Phe OMe HCl) (128) giving (Boc Tyr (Bzl) OH D Ala Gly Phe OMe) (CXVI). Carbonyl group deprotection of (CXVI) gave (CXVII) which coupled to (CXVIII), (CXIX) and (CXX) gave the ester linked enkephalin conjugates (CXXI), (CXXII) and (CXXIII) (Scheme 31).











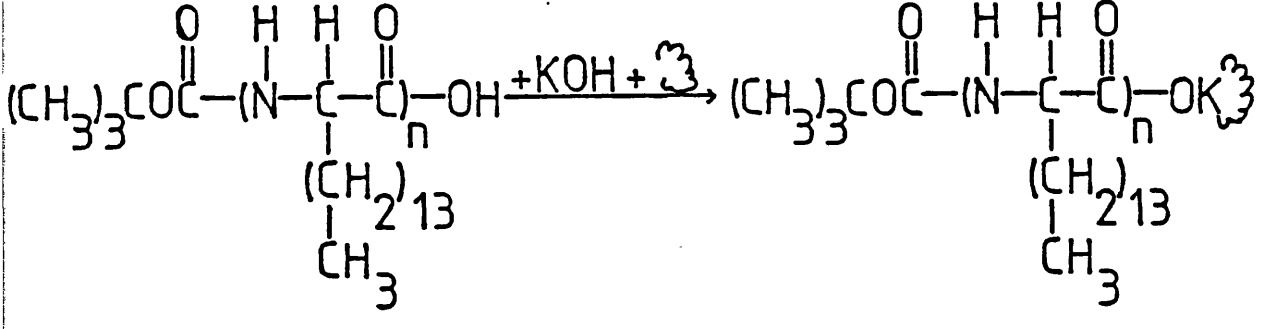
m=13 n=0 (CXXI)
m=11 n=1 (CXXII)
m=11 n=2 (CXXIII)

(Scheme 31)

5.5 DI-ESTER LINKED ENKEPHALIN CONJUGATES

The di-ester linked enkephalin lipidic amino acid conjugates were the final compounds in the series to be synthesised. The di-ester link is widely used as a pro-drug linkage 2-aminohexadecanoic acid (C_{16})and its oligomers were used in the di-ester linkage with enkephalins.

XXXXIII, LIII, and LVIII were reacted with KOH and 18-crown-6-ether giving the potassium 18-crown-6 salts (Boc C_{16} OK) (LXXXVIII) (Boc C_{16} C_{16} OK) and (LXXXIX) and (Boc C_{16} C_{16} C_{16} OK) LXXXX (Scheme 32).



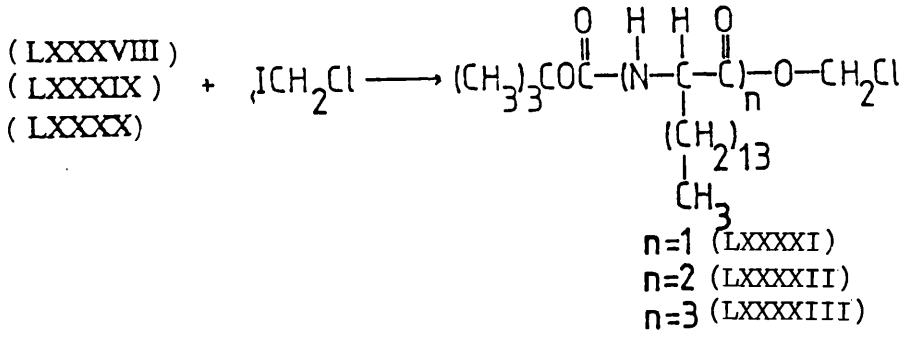
n =1(XXXXIII)
n =2(LIII)
n =3(LVIII)

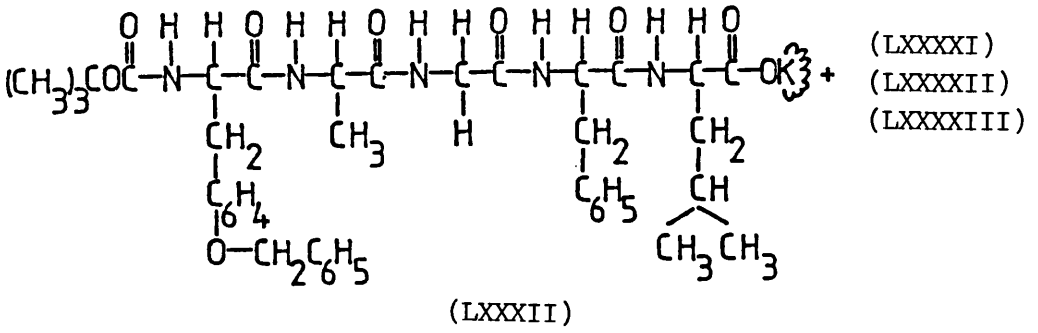
n =1(LXXXVIII)
n =2(LXXXIX)
n =3(LXXXX)

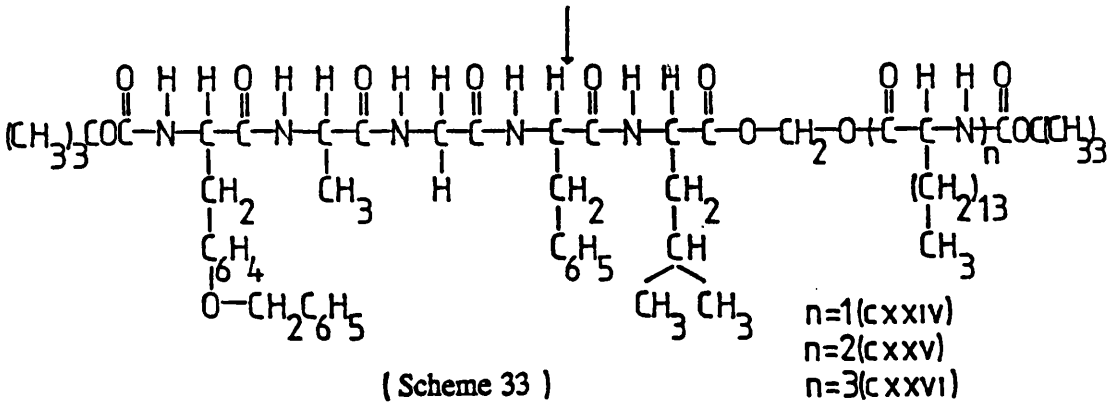
(Scheme 32)

Compounds (LXXXVIII), (LXXXIX) and (LXXXX) were then reacted with chloroiodomethane (ICH_2 Cl) (142) giving (Boc C_{16}-OCH_2 Cl) LXXXXI. (Boc C_{16} C_{16}-OCH_2 Cl) (LXXXXII) and (Boc C_{16} C_{16} C_{16} O CH_2 Cl) (LXXXXIII), these were then reacted with (LXXXII) giving the required enkephalin di-ester linked conjugates (Boc Tyr (Bzl) OH D Ala Gly Phe Leu OCH_2 O C_{16} Boc) (CXXIV) (Boc Tyr (Bzl) OH D Ala Gly Phe Leu O CH_2 O C_{16} C_{16} Boc) CXXV and (Boc Tyr (Bzl) OH D Ala

Gly Phe Leu OCH_2 O C_{16} C_{16} C_{16} Boc) CXXVI. (Scheme 33)







(Scheme 33)

The ability of the enkephalins to cross the blood-brain barrier is very limited as is their oral use, due to enzymic breakdown and their lack of lipophilicity. The work carried out is an attempt to address these problems.

5.6 SUBSTITUTION OF LIPIDIC AMINO ACIDS WITHIN THE ENKEPHALIN MOLECULE

The positions within the enkephalin molecule replaced are 2,4 and 5. The tyrosine molecule in position 1 does not allow for any rearrangement as its removal or change of configuration result in loss of activity[136-153]. Position 2 is less critical in enkephalin activity. Altering Gly^2 to D-amino acids increases activity and both the μ and δ receptor whilst hydrophobic amino acids increase μ activity[108-148]. The introduction of 2-aminodecanoic acid in this position increases lipophilicity thus should increase μ selectivity. The 2-aminodecanoic acid used is racemic therefore it should also increase activity due to the presence of the D-isomer.

Position 3 was not altered as Gly^3 is a critical section of the enkephalin molecule which does not allow manipulation[155-156].

Phe^4 has been previously altered by other groups but except for Trp other substitutions result in decreased activity[158]. The amino acids which have been used have not had large lipophilic side chains, necessary to replace the aromatic ring. In this work Phe^4 has been replaced by 2-aminotetradecanoic acid which due to its long chain should replace the aromatic ring and so maintain or enhance activity.

The final position altered was Leu^5. Here structural and conformational changes have given active compounds. The inclusion of 2-aminohexadecanoic acid should therefore not remove activity but the inclusion of a hydrophobic group at position 5 should enhance μ selectivity.

The work in this area was done to examine how the inclusion of the lipidic amino acids would affect activity. The increase in hydrophobicity may in addition increase the ability of these substituted enkephalins to cross biomembranes, and confer on them stability by reducing enzymic breakdown.

5.7 AMIDE LINKED ENKEPHALIN LIPIDIC AMINO ACID CONJUGATES

Enkephalins are more active when the terminal carbonyl is reduced to the amide or ester, the increased activity of the amides is usually due to enhanced stability[174,94].

One clinically investigated enkephalin derivative with an amide on the terminal carbonyl is metkephamid which is over 100 times more potent than morphine[184].

The use of amide linkages as pro-drugs is limited because amides are relatively stable *in vivo*. There are certain amides that are sufficiently chemically labile to act as pro-drugs and amides formed with amino acids are generally susceptible to enzymatic cleavage *in vivo.*

The rationale for the use of the lipidic amino acid as amide linked conjugates is the increased stability and activity conferred by the amide group on enkephalins. The lipidic amino acid conjugate should therefore increase stability due to its ability to protect the enkephalin from enzymic breakdown and increase its lipophilicity, enabling the enkephalins to cross biomembranes and thus the blood-brain barrier.

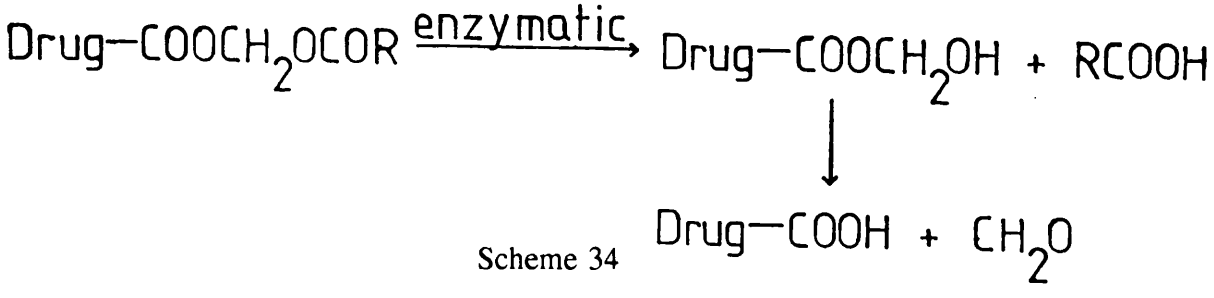
5.8 ESTER LINKED ENKEPHALIN LIPIDIC AMINO ACID CONJUGATES

The ester group is a widely used pro-drug for carbonyl and hydroxyl functions because it is easily hydrolysed *in vivo.* Ester hydrolysis *in vivo* is achieved by esterase found in the blood, liver and other organs and tissues.

Esters as well as being labile groups increase the lipophilicity of the parent compound. This increases absorption through biomembranes and in the case of enkephalins should increase the ability to cross the blood-brain barrier[44,45].

The reasons for ester conjugation of the lipid amino acids to the enkephalins were that reduction of the terminal carboxyl group increases activity (as in the case of the amides) and the possible increase in ability to cross the blood-brain barrier[174].

The di-ester linkage was used because of the stability of some esters to hydrolysis thus making them unsuitable pro-drugs as is true in some penicillin esters. To overcome this problem Jansen and Russell showed that double esters are rapidly hydrolysed in the blood and tissues[51]. Here, the first step in the hydrolysis of the di-ester is enzymatic cleavage of the terminal ester bond forming a highly unstable hydroxymethyl ester which rapidly dissociates to the parent drug and formaldehyde (Scheme 34).



Scheme 34

The increased rate of di-ester cleavage may be due to lack of steric hindrance of the terminal ester as compared to the drug ester.

5.9 CONCLUSION

The derivatisation of the enkephalin molecule is done to increase stability, activity and hydrophobicity.

The inclusion of the lipidic amino acids on the enkephalin molecule either by substitution or by conjugation increases hydrophobicity. This increase should allow greater crossing of biomembranes and the blood-brain barrier.

It was hoped that the correct substitution position would increase activity and alter enkephalin receptor selectivity from δ to μ.

Amide and ester or di-ester linkages were used in the enkephalin molecule for several reasons. Reduction of the terminal carbonyl to amides and ester is known to increase enkephalin activity. In addition, esters and amides act as pro-drugs which deliver the drug effectively and selectively to the active site, the subsequent removal of these groups then leaves the free parent molecule. The ester and amide groups were therefore used to take the enkephalin molecule to the required active site and then release the enkephalin by spontaneous or enzymatic transformation. The pro-drug linkages to the enkephalin molecule should therefore enhance efficacy and reduce the toxicity and unwanted side effects, by controlling their absorption.

6. Elastase Inhibitors

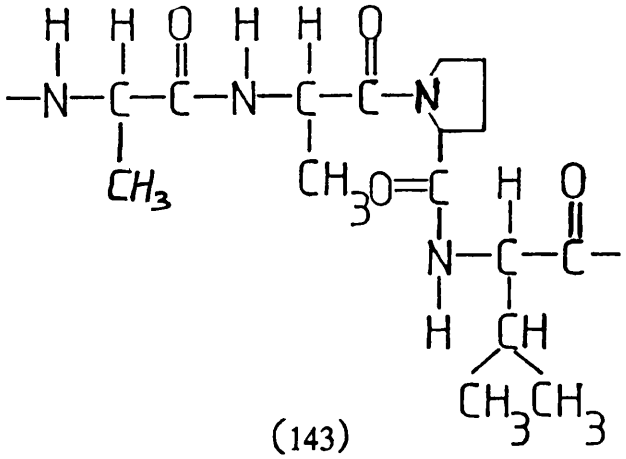
Elastases are proteolytic enzymes which degrade elastin found in elastic fibres and are involved in connective tissue disorders and inflammatory diseases.

The objective of the work described is the solution phase synthesis of elastase inhibitors with increased lipophilicity. The conjugation of lipidic amino acids and their oligomers to the tetrapeptide sequence (143) increases lipophilicity and enhances their ability to cross biomembranes (eg gut, blood brain barrier) and also the ability to attach to the cell membrane. The elastase inhibitors produced could prevent proteolysis and the subsequent damage caused to cartilage and elastic fibres.

One of the substrates of the elastases is elastin. Structurally, elastin has a

repeating amino acid sequence, the amino acids present being mainly nonpolar with aliphatic side chains. The elastase inhibitors synthesised have a common tetrapeptide sequence, which partially mimics the amino acid sequences found in elastin. The recognition of the elastase inhibitor sequence will allow the inhibitor to bind the elastase enzyme preventing the elastin from breaking.

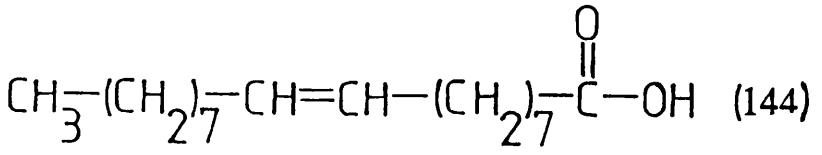
The tetrapeptide sequence synthesised for inclusion in the elastase inhibitors is Ala-Ala-Pro-Val (143). 143 has been synthesized by Hornebeck et al[292] and found to inhibit the action of human leucocyte and pancreatic (porcine) elastase.



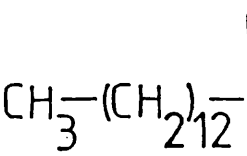
(143)

The tetrapeptide sequence Ala-Ala-Pro-Val (143) alone is not a potent inhibitor but becomes so when attached to a lipophilic moiety. The importance of the lipophilic group attached to the elastin mimicing peptide sequence is the ability to anchor the inhibitor to the cell membrane, so protecting elastin.

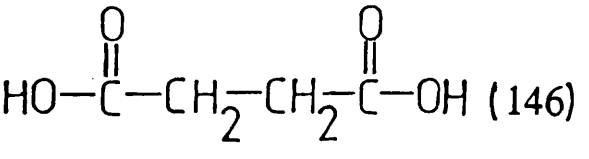
The lipophilic groups which have previously been used in the elastase inhibitors include oleic acid (144), myristic acid (145), succinic acid (146) and methoxycinnamoyl-5-amino-hexanoate (147). The lipidic amino acids and their oligomers were attached via carbodiimide coupling to the peptide sequence (143) to act as the lipophilic moiety. The hydrophobicity of the lipidic oligomers can be controlled by increasing or decreasing the number of amino acids constituting the hydrophobic group or by altering the length of the amino acid alkyl chain.



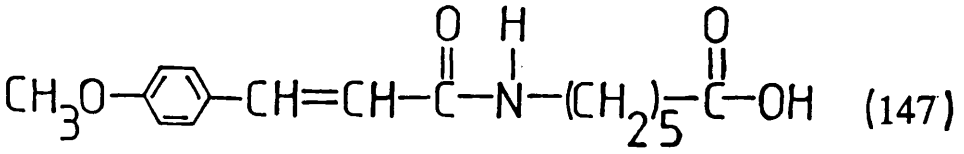
(144)



(145)



(146)



(147)

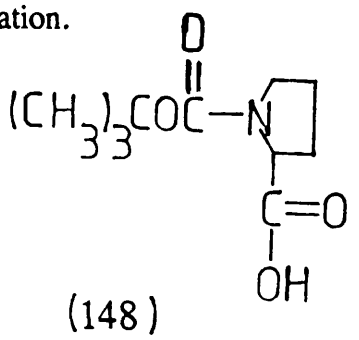
The rationale for the synthesis of elastase inhibitors containing lipidic amino acid oligomers was first, the ability to control their hydrophobicity and secondly to standardise the structure of the hydrophobic group.

To date all the lipidic amino acids and their oligomers have passed all biocompatibility and toxicological tests, it is thus hoped that toxicity or antigenicity problems will not occur.

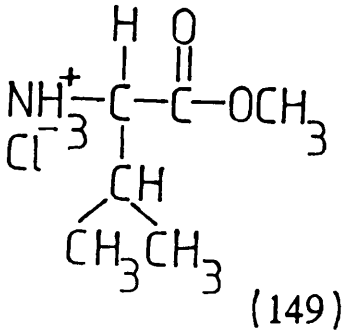
6. 1 Chemistry of the Elastase Inhibitors

The synthesis of the elastase inhibitors was carried out using solution phase peptide synthesis, in a stepwise method.

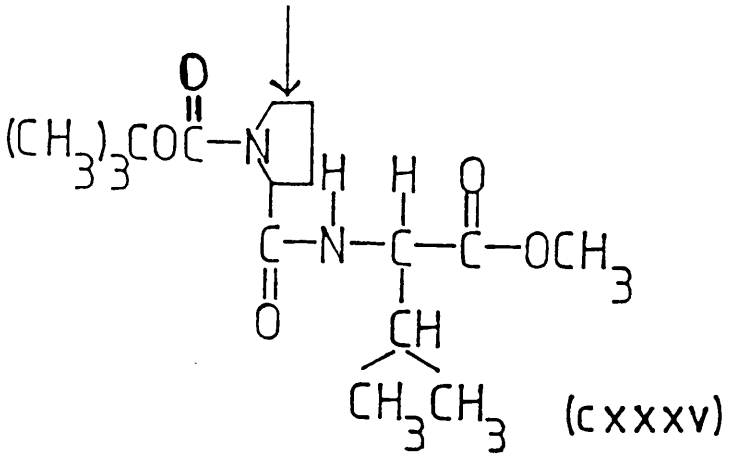
N-α-tertiarybutoxycarbonyl proline (BocPro) (148) and valine methyl ester hydrochloride (Val OMe HCl) (149) were coupled using dicyclohexylcarbodiimide (DCC) giving the dipeptide Boc-Pro-Val OMe (CXXXV) (Scheme 35). The Boc group was used to protect the terminal amino group of dipeptide (CXXXV) and a methyl ester was used to protect the terminal carboxyl group. Boc and methyl ester protecting groups were chosen due to their ease of synthesis and removal, the Boc group can be removed by acid hydrolysis and the methyl ester by alkali saponification.



(148)



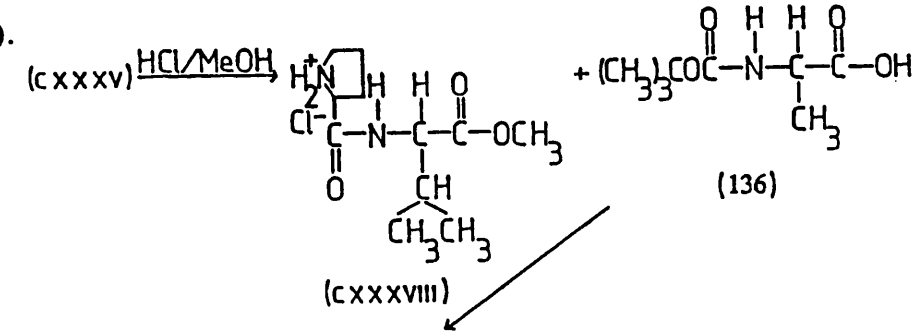
(149)



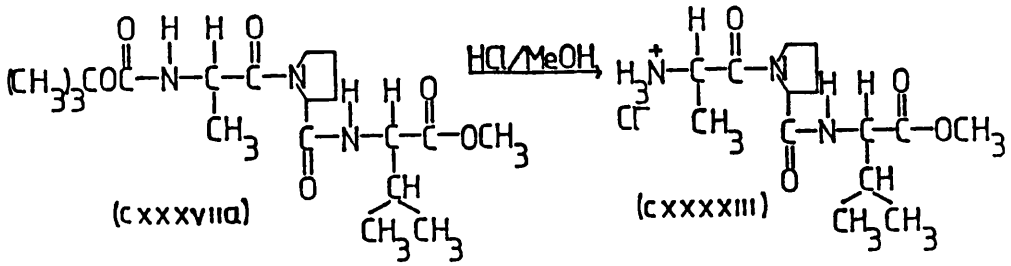
(CXXXV)

Scheme 35

The synthesis of the elastase inhibitor was continued with the deprotection of the terminal amino group of (CXXXV) and the addition of BocAla (136) giving BocAla-Pro-Val-OMe (CXXXVIIa). Tripeptide (CXXXVIIa) was deprotected at the terminal amino group to yield amine (CXXXXIII), with (CXXXXIII) acting as the core group to which a series of peptides and peptide derivatives were added (scheme 36).



(CXXXV) (136) (CXXXVIII)

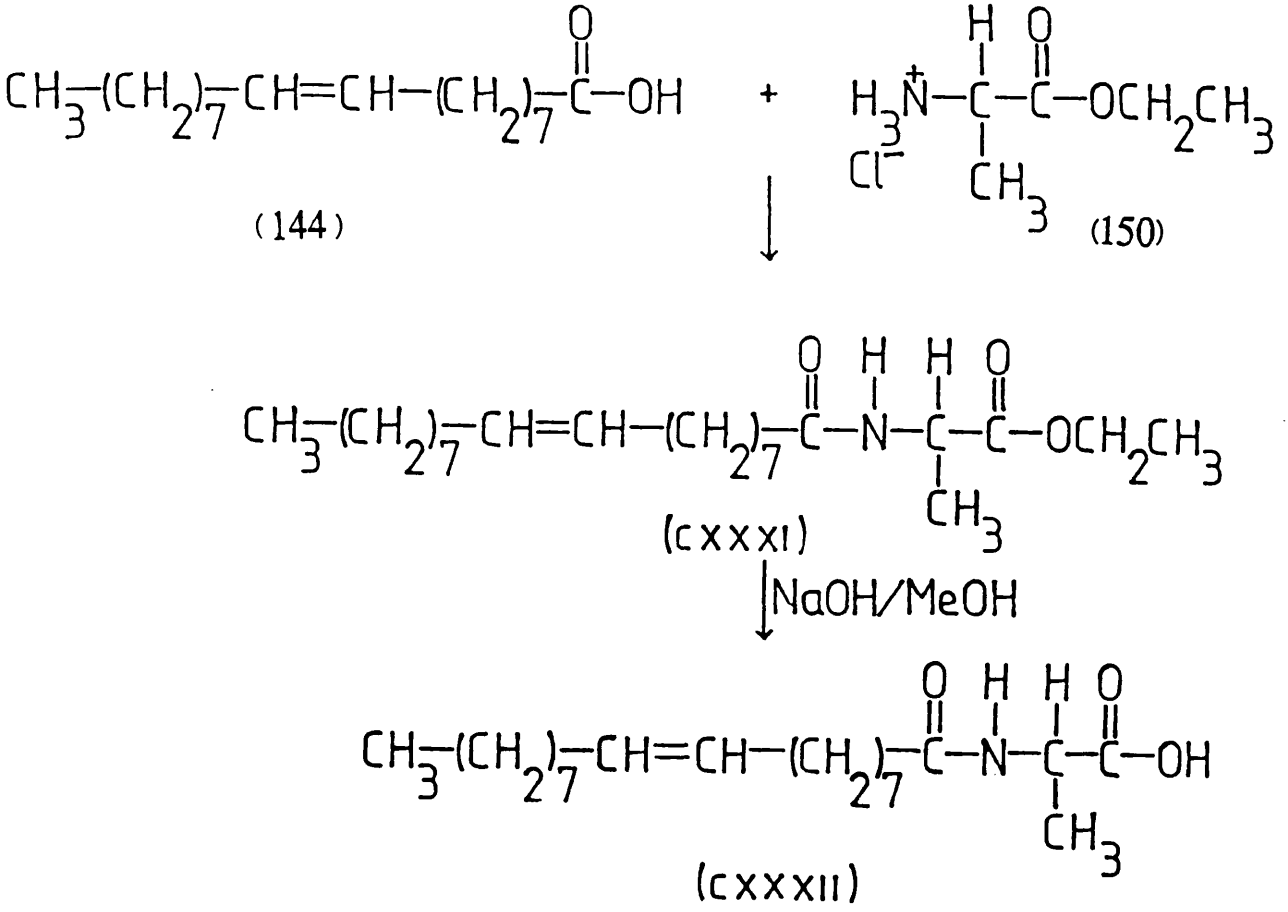


(CXXXVIIa) (CXXXXIII)

Scheme 36

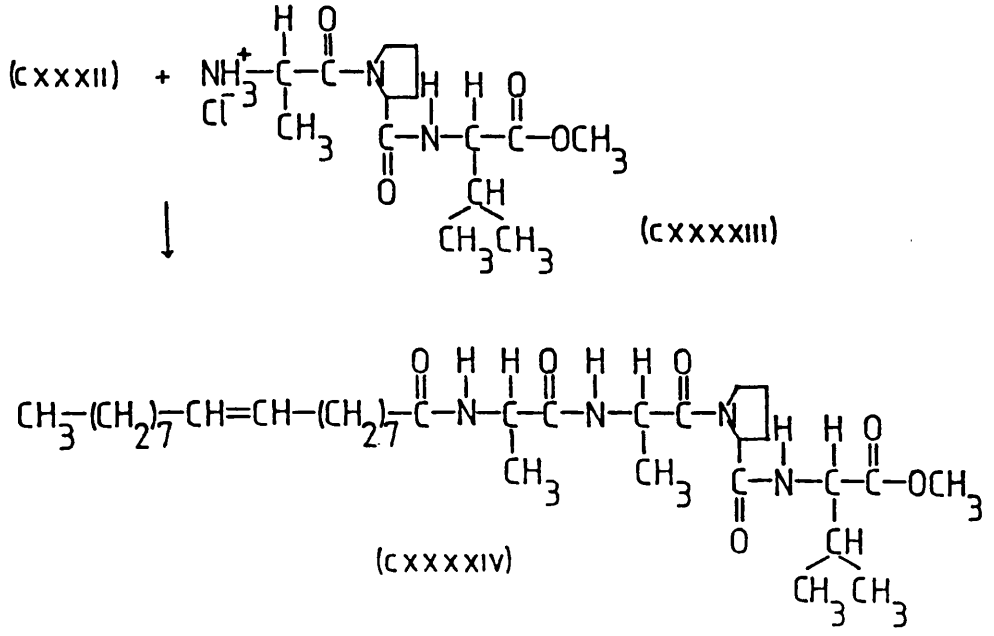
The second alanine in the elastase mimicing sequence (143) was included in the lipophilic synthon, prior to its combination with amine (CXXXXIII). The synthor method was used to aid stability and solubility.

Oleic acid (144) and alanine ethyl ester hydrochloride (150) were coupled with DCC to give oleoyl-alanine ethyl ester (CXXXI), which was subsequently deprotected at the carbonyl terminus to give the free acid (CXXXII) (scheme 37).



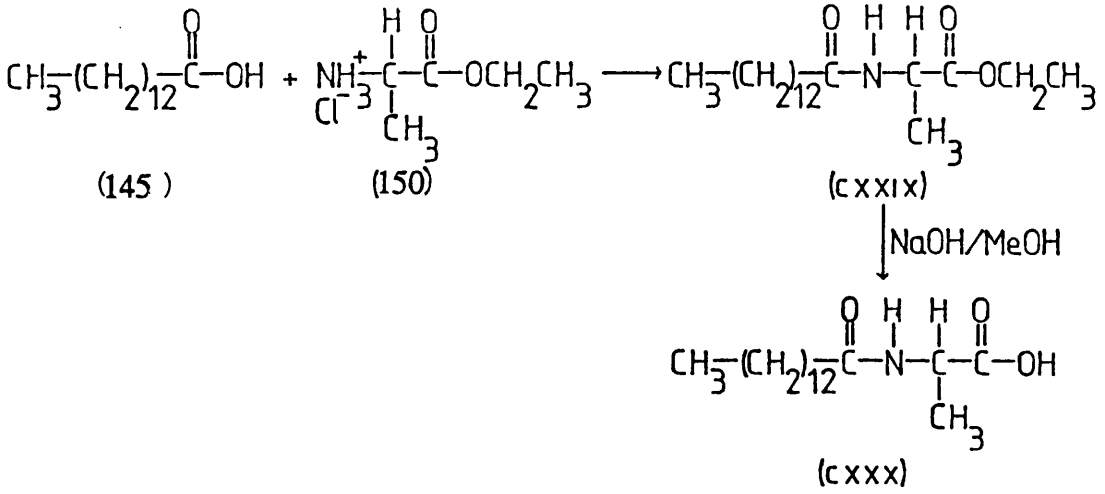
(Scheme 37)

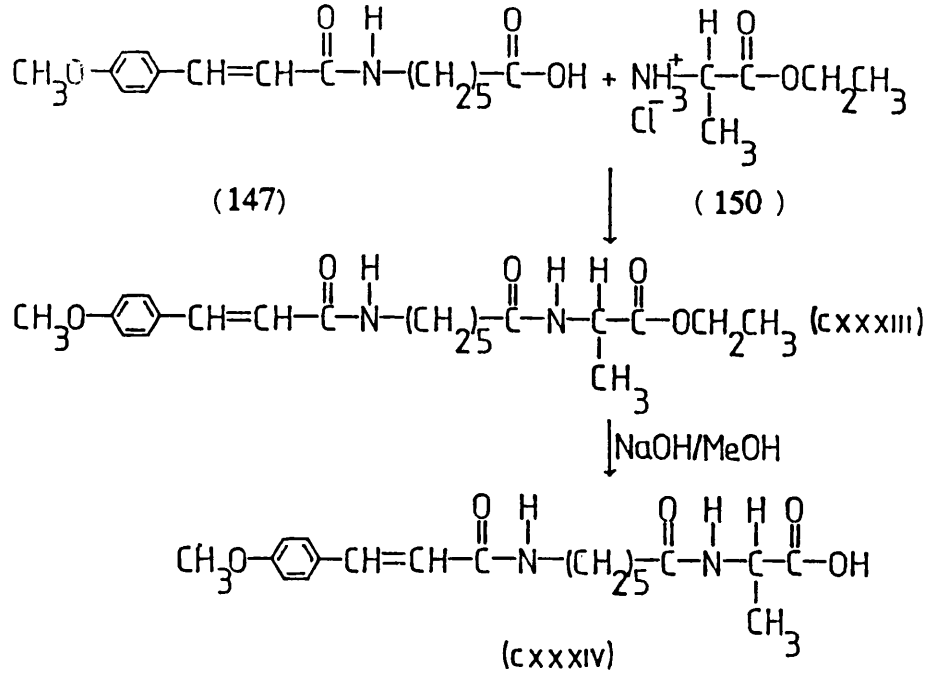
The coupling of (CXXXXIII) and (CXXXII) via the DCC method gave the elastase inhibitor Oleoyl-Ala-Ala-Pro-ValOMe (CXXXXIV) (scheme 35).



(Scheme 38)

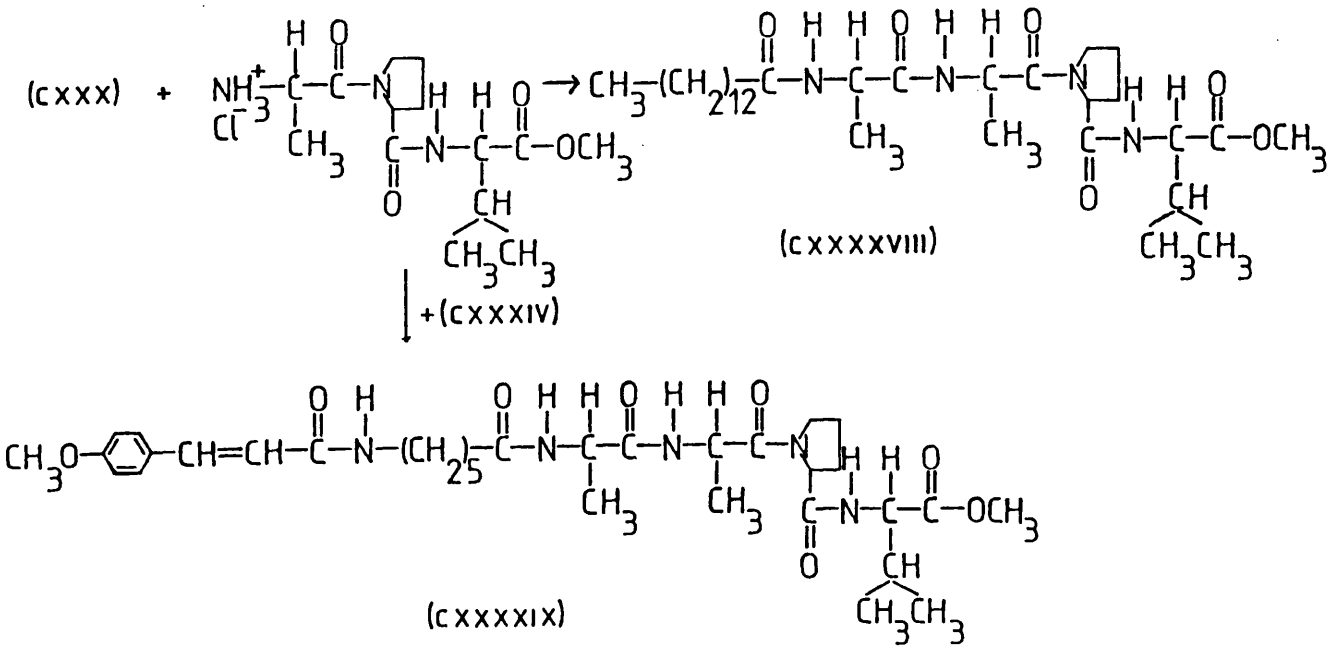
Alanine ethyl ester hydrochloride (150) was coupled with myristic acid (145) and p-methoxycinnamoyl-5-amino hexanoate (147) giving myristoyl-alanine ethyl ester (CXXIX) and methoxycinnamoyl-alanine ethyl ester (CXXXIII) respectively (scheme 39).





(Scheme 39)

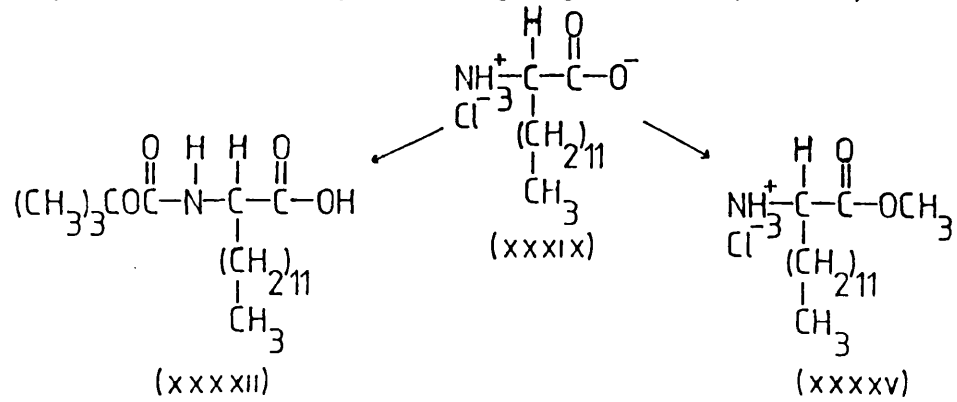
Ester cleavage of compounds Myr-Ala-OEt (CXXIX) and p-Moxy-Ala-OEt (CXXXIII) gave the free acids Myr-AlaOH (CXXX) and p-Moxy-AlaOH (CXXXIV) which when coupled to tripeptide (CXXXXIII) produced the elastase inhibitors myristoyl-Ala-Ala-Pro-Val-OMe (CXXXXVIII) and p-methoxycinnamoyl-5-aminohexanoyl-Ala-Ala-Pro-Val-OMe (CXXXXIX) (scheme 40).



(Scheme 40)

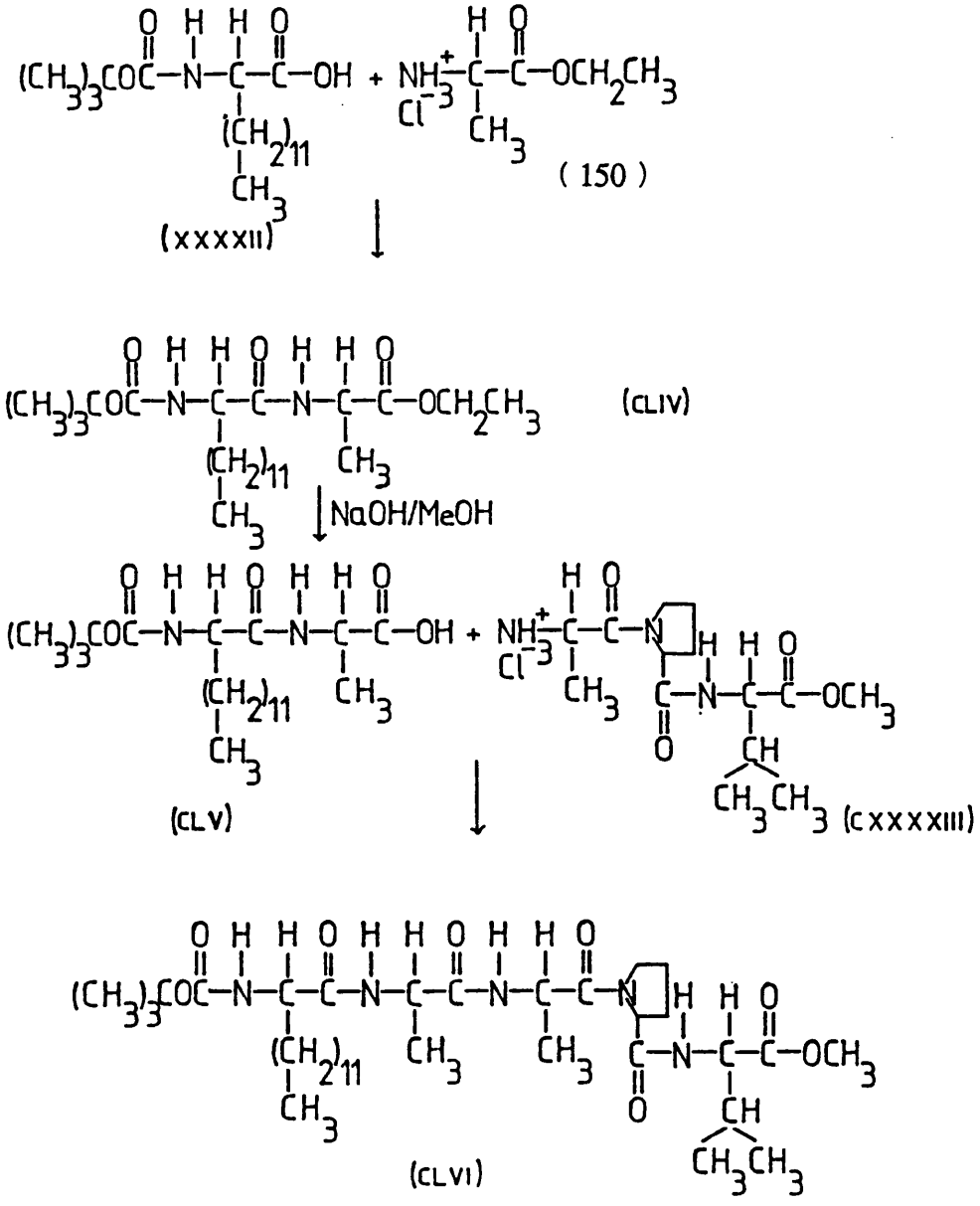
Inhibitors were then produced with lipidic amino acids as the lipophilic moiety.

The lipidic amino acid aminotetradecanoic acid (XXXIX), was used in the synthesis of the elastase inhibitors. The acid (XXXIX) was protected as the Boc derivative at the terminal amino group giving Boc-C14OH (XXXXII) and as the methyl ester at the carboxyl terminus giving C14-OMe (XXXXV).



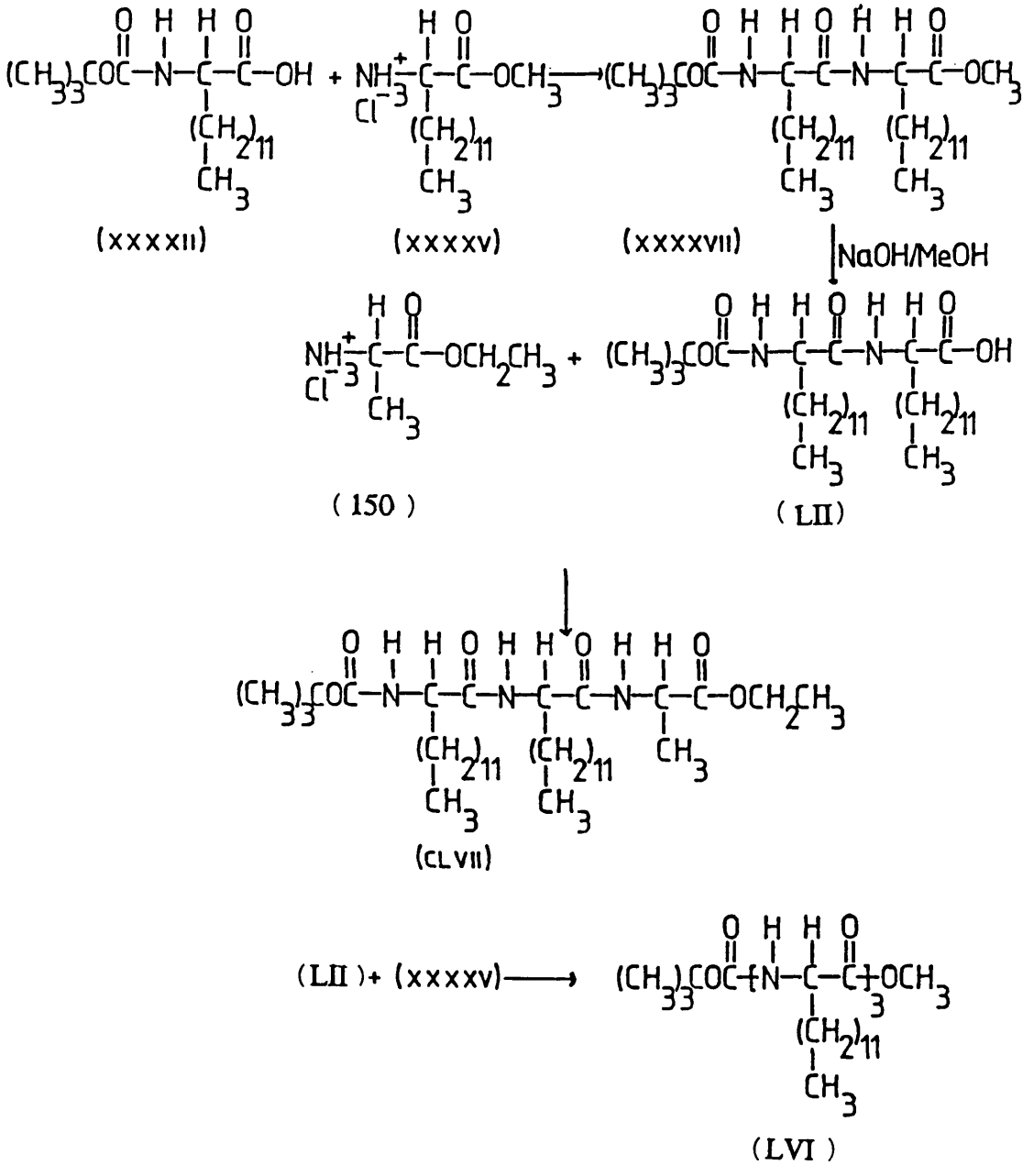
BocC14OH (XXXXII) and Ala-OEt HCl (150) were coupled via literature method giving the protected dipeptide (CLIV). To facilitate purification, 1-ethyl-3(3-dimethyl)-1-aminopropyl carbodiimide (EDAC) was used in coupling involving lipidic amino acids because the urea by-product formed from this carbodiimide is water soluble.

Following carbonyl deprotection of (CLIV), BocC14OAla OH (CLV) was coupled with Ala-Pro-Val OMe (CXXXXIII), giving the elastase inhibitor Boc-C14-Ala-Ala-Pro-Val OMe (CLVI) (scheme 41).



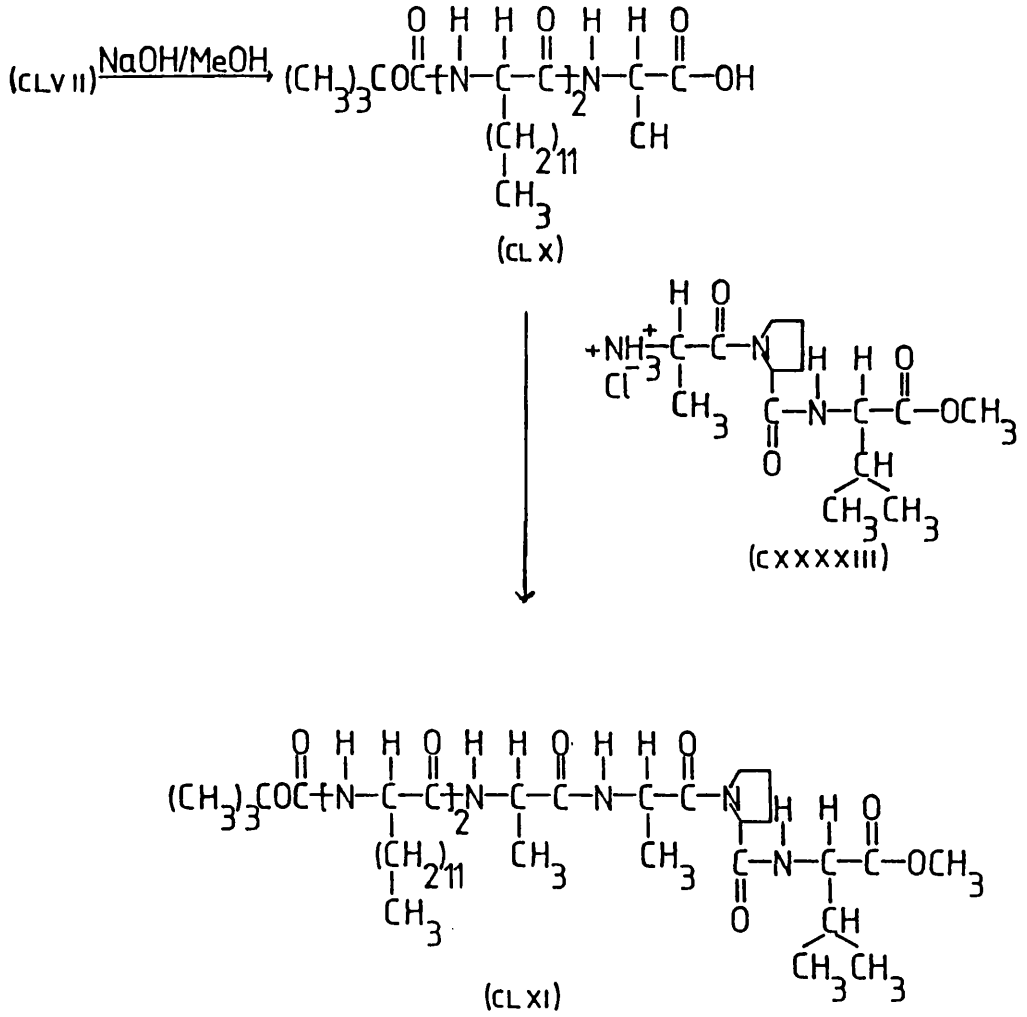
(Scheme 41)

The dipeptide produced by the coupling of BocC14OH (XXXXII) and C14 OMe HCl (XXXXV) Boc-C14-C14OMe (XXXXVII), was deprotected at the carbonyl terminus giving the free acid (LII). The coupling of BocC14-C14OH (LII) with C14OMe HCl (XXXXV) and Ala OEt HCl (150) gave the lipidic tripeptide BocC14-C14-C14 OMe (LVI) and tripeptide BocC14-C14-Ala OEt (CLVII) (scheme 42).



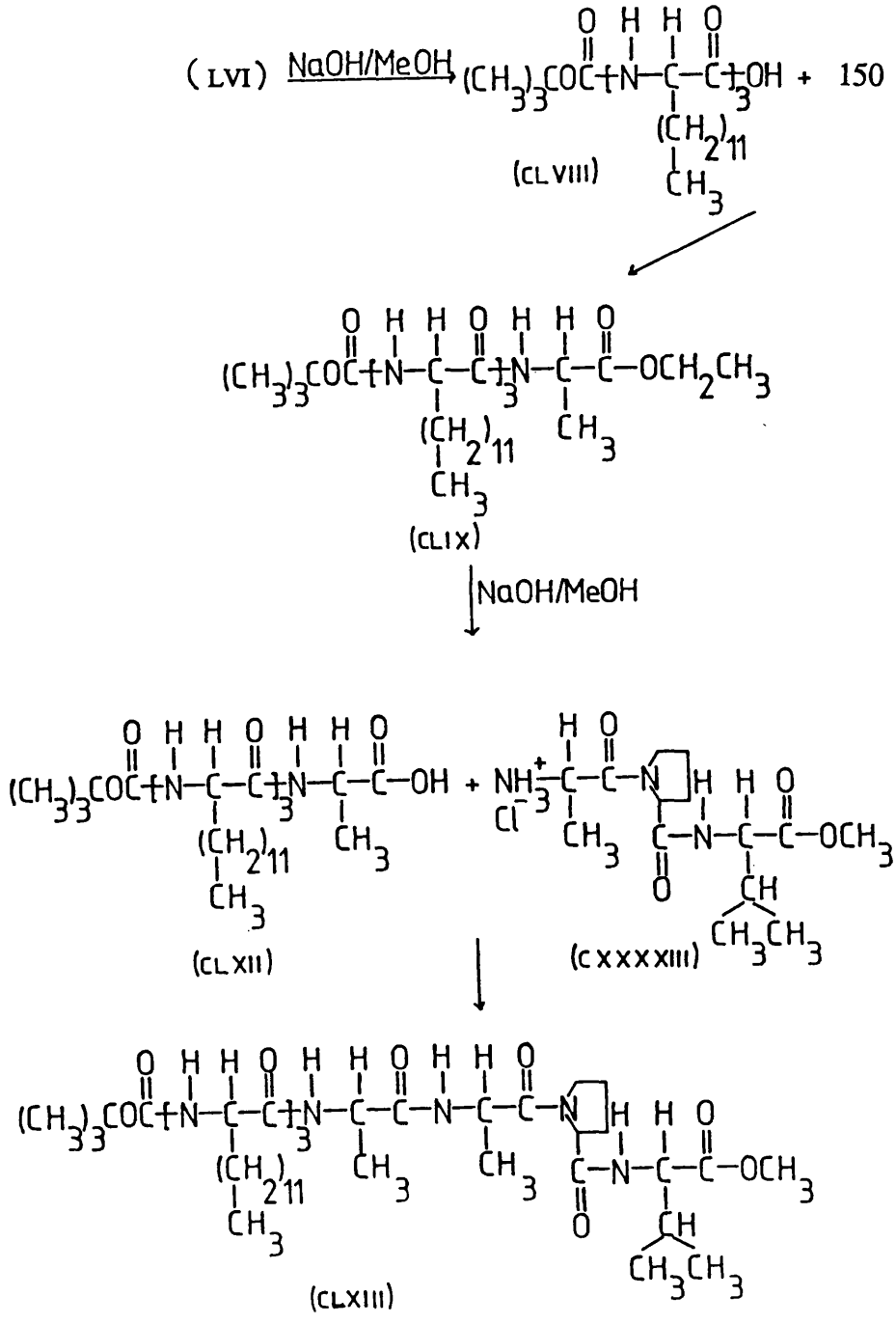
(Scheme 42)

Deprotection of the terminal carbonyl group of BocC14-C14-C14 OMe (LVI) and BocC14-C14-AlaOEt (CLVII) gave the free acids Boc-C14-C14-AlaOH (CLX) and Boc-C14-C14-C14OH (CLVIII). Compound (CLX) was coupled with Ala-Pro-ValOMe (CXXXXIII), giving the elastase inhibitor Boc-C14-C14-Ala-Ala-Pro-ValOMe (CLXI), which has the elastin mimicing peptide (143) conjugated to the novel lipidic amino acid dipeptide (scheme 43).



(Scheme 43)

Boc-C14-C14-C14-AlaOEt (CLIX) was synthesised by coupling Boc-C14-C14-C14OH (CLVIII) and (150), the tetrapeptide produced was deprotected at the carbonyl terminus to give Boc-C14-C14-C14-AlaOH (CLXII). The additon of (CLXII) and tripeptide Ala-Pro-ValOMe (CXXXXIII) gave the final elastase inhibitor Boc-C14-C14-C14-Ala-Ala-Pro-ValOMe (CLXIII) (scheme 44).



(Scheme 44)

Throughout the synthesis of the elastase inhibitors, purification and separation of the required peptides was done by using silica flash column chromatography or silica preparative TLC.

The oleic (144), myristic (145) and methoxycinnamoyl-5-amino-hexanoate (147) conjugated elastase inhibitors were used in activity comparisons between these and the novel lipidic amino acid elastase inhibitors.

6.2 Synthesis of Elastase Inhibitors to Study Structure Activity Relationships

The lipidic amino acid conjugated elastase inhibitors have been synthesised using racemic lipidic amino acids. Initially, the racemic compounds were produced as elastase inhibition had to be shown by these novel products before the preferred stereochemistry of the lipophilic group was determined.

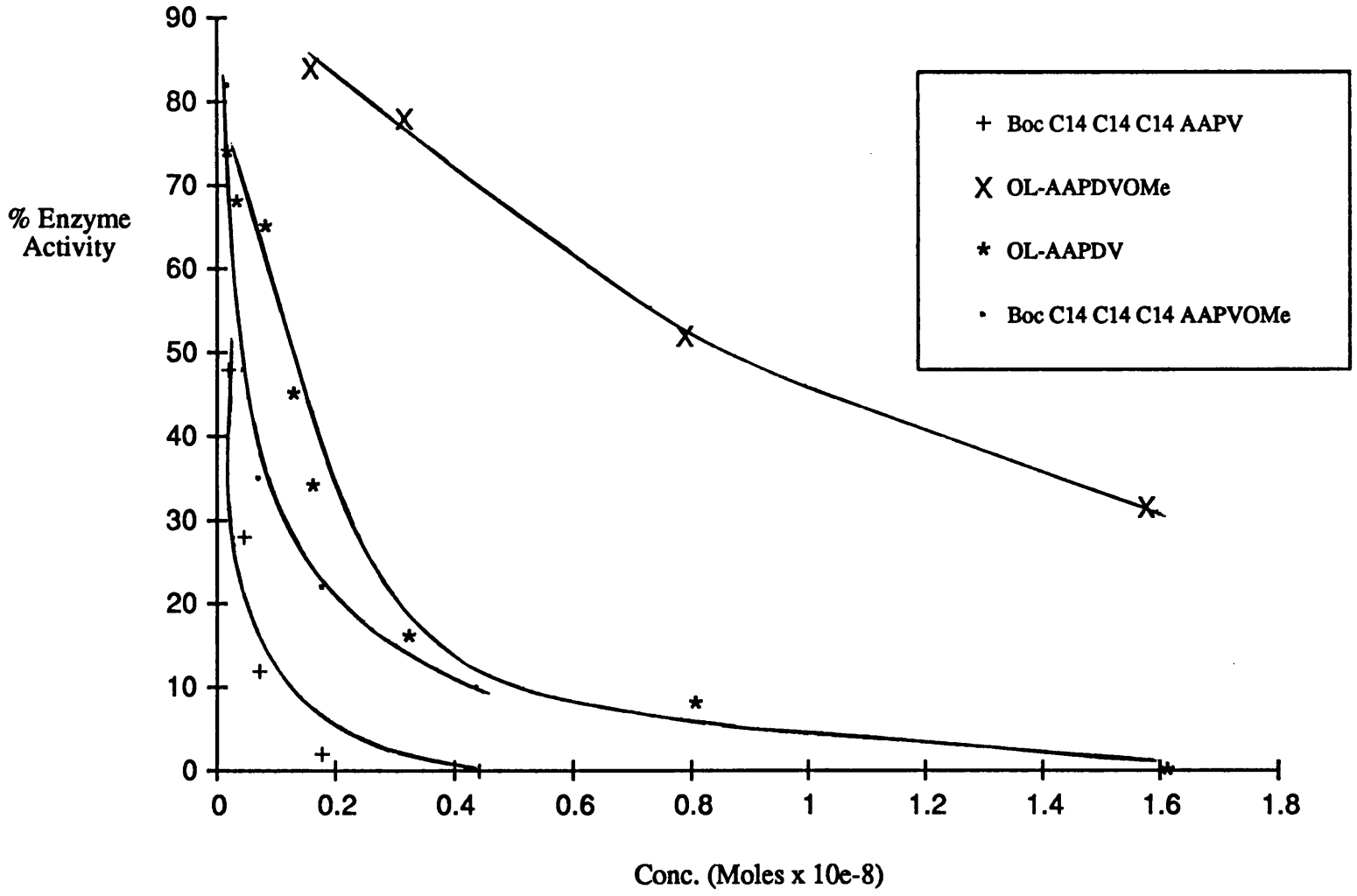
The effect of steroechemistry on elastase inhibitor activity was studied by changing L-Val to D-Val in the tetrapeptide sequence Ala-Ala-Pro-Val (143). Tripeptide (CXXXXII), containing the D-Val isomer, was produced in exactly the same way as that containing L-Val, and coupled to Oleoyl-AlaOH (CXXXII) as before giving two oleic conjugated products, Oleoyl-Ala-Ala-Pro-L-ValOMe (CXXXXIC) and Oleoyl-Ala-Ala-Pro-D-ValOMe (CXXXXVI).

The activity of elastase inhibitors having free acids at the carbonyl terminus was compared, to those protected by methyl ester, (Fig. 23). The effect of a free acid carbonyl compared to a protected carbonyl on the potency of the lipidic amino acid inhibitors is marked, with the inhibitory capacity of the methyl esters being approximately 30% that of the free acids.

2D COESY NMR spectra were used to analyse the lipidic amino acid conjugate elastase inhibitors, containing lipidic amino acid monomer, dimer and trimer. 2D COESY spectra indicate through-bond connectivities, thus enabling the assignment of α-H and NH protons and the characterisation of the compounds.

The purity of all the elastase inhibitors synthesised and used in biological testing was examined using analytical HPLC, with the eluted fragments collected and identified by mass spectrometry. The lipidic amino acid used in the synthesis of the elastase inhibitors was racemic and the diasterioisomers produced were separated by HPLC. Mass spectrometry was used to prove the identity of the diasterioisomers.

Fig. 23 Graph of % Enzyme Activity against Conc. Comparing the Effect of Carboxyl Terminus Protection



6.3 Biology of the Elastase Inhibitors

The compounds produced in this work were tested for biological activity. The method used was that Hornebeck et al. Here the inhibitor and substrate are first incubated and the enzyme added. The amount of substrate unaffected by the enzyme is then measured and the inhibition quoted as a percentage. The results quoted compare the compounds having oleic acid as the lipidic moiety with those having the lipidic amino acid monomer, dimer and trimer.

6. 4 Biological Results

The oleic acid derivative (CXLIV) was the most potent inhibitor therefore the results quoted use this compound for comparison with the lipidic amino acid derivatives.

The activity of human leucocyte elastase (elastin products) (2μg/ml) on succinyl trialanine para-nitroanilide (0.78μg/ml) as substrate was determined in the presence of the lipidic amino acid derivatives in concentrations of 0-50μg/ml.

According to the results all the compounds tested are inhibitors of human leucocyte elastase to differing degrees.

Table 48

Table of Concentration against % Enzyme Activity in the Presence of Inhibitors

conc.	% Enzyme Activity in the Presence of Inhibitors					
μg/ml	Boc C14 AAPV	Boc 2C14 AAPV	Boc 3C14 AAPV	OL-AAPV	OL-AAPDV	OL-AAPDV Me Ester
10	4				0	31.5
5	22	0	0		8,(20)	52
2	46	8	2	1	16,(18)	78
1	60			8	34	84
0.8		12	12	18	45	
0.5		34	28	22	65	
0.2		56	48	54	68	
0.1					74	

For direct comparitive measurement of inhibition to be made the concentration in molarity was used.

RMM of BocC14AAPV = 681

RMM of BocC14C14AAPV=906

RMM of Boc C14C14C14AAPV=1131

RMM of Gl-AAPV=620

RMM of Ol-AAPDV=620

RMM of Ol-AAPDVOMe=634

Table 49

Table of Concentration Conversion from μg/ml to Molarity

Conc.in μg/ml	[x10-8] Boc C14	[x10-8] Boc 2C14	[x10-8] Boc 3C14	[x10-8] Ol-AAPV	[x10-8] Ol-AAPDV	[x10-8] Ol-AAPDVMe
10	1.4684				1.6129	1.5773
5	0.7432	0.5519	0.4421		0.8065	0.7886
2	0.2937	0.2208	0.1768	0.3226	0.3226	0.3155
1	0.1468			0.1613	0.1613	0.1577
0.8		0.0883	0.0707	0.1290	0.1290	
0.5		0.0552	0.0442	0.0806	0.0806	
0.2		0.0221	0.0177	0.0323	0.0823	
0.1					0.0161	

Table 50

Table of % Enzyme Activity in the Presence of Inhibitors Against Concentration in Molarity

% Act. Boc C14	Conc.x10^{-8} Boc C14	% Act. Boc2C14	Conc.x10^{-8} Boc2C14	% Act. Boc3C14	Conc.x10^{-8} Boc3C14	% Act. Ol-AAPV	Concx10^{-8} Ol-AAPV
4	1.4684	0	0.5519.	0	0.4421	1	0.3226
22	0.7342	8	0.2208	2	0.1768	8	0.1613
46	0.2937	12	0.0883	12	0.0707	18	0.129
60	0.1468	34	0.0552	28	0.0442	22	0.0806
		56	0.0221	48	0.0177	54	0.0326

Percentage activation against concentration was plotted giving Fig. 24. This gave a comparison of the lipidic amino acid inhibitors against the oleoyl linked inhibitory sequence.

Fig. 24 Graph of % Enzyme Activity against Inhibitor Conc. comparing OL-AAPV with the Lipidic Amino Acid Inhibitors

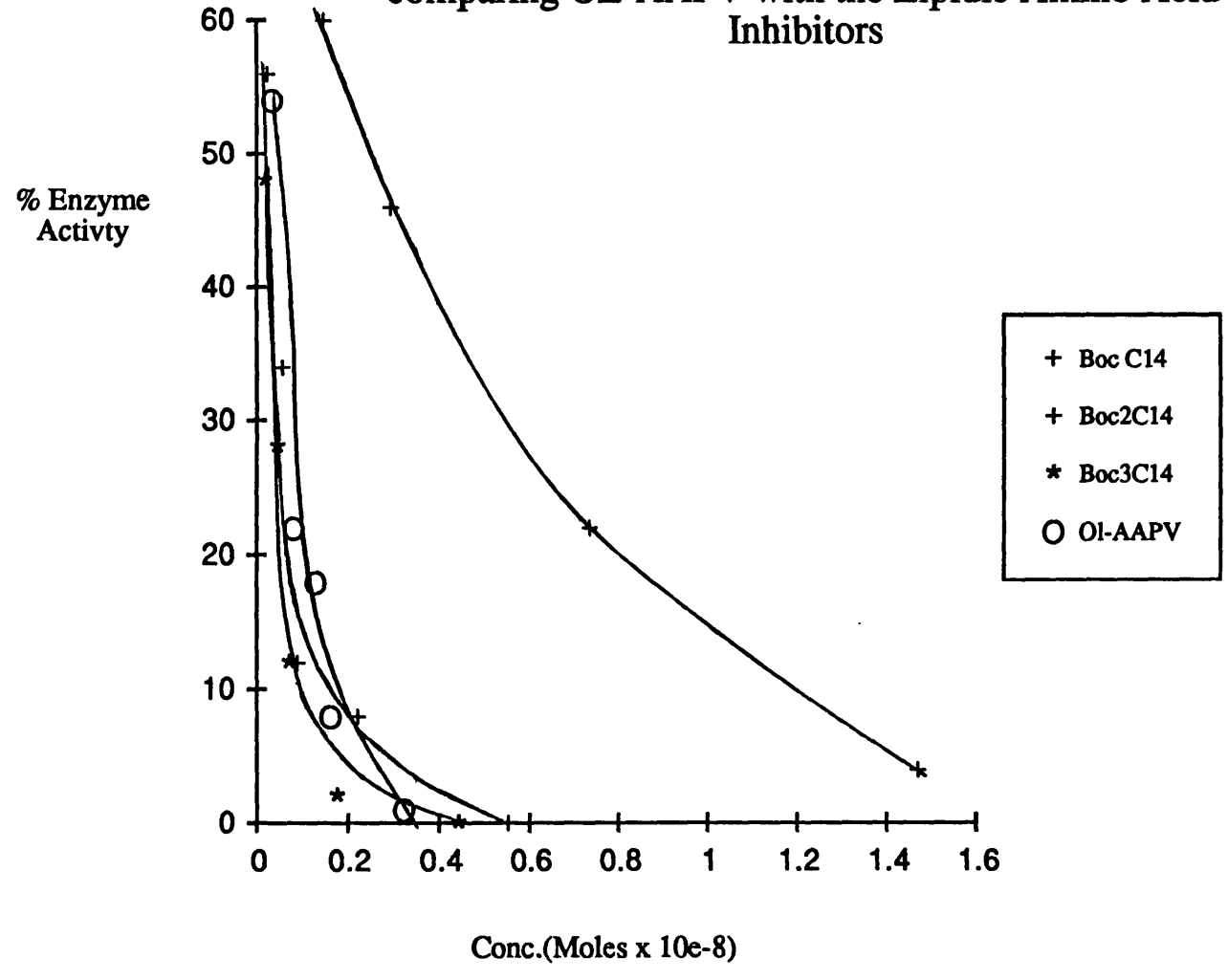


Table 51

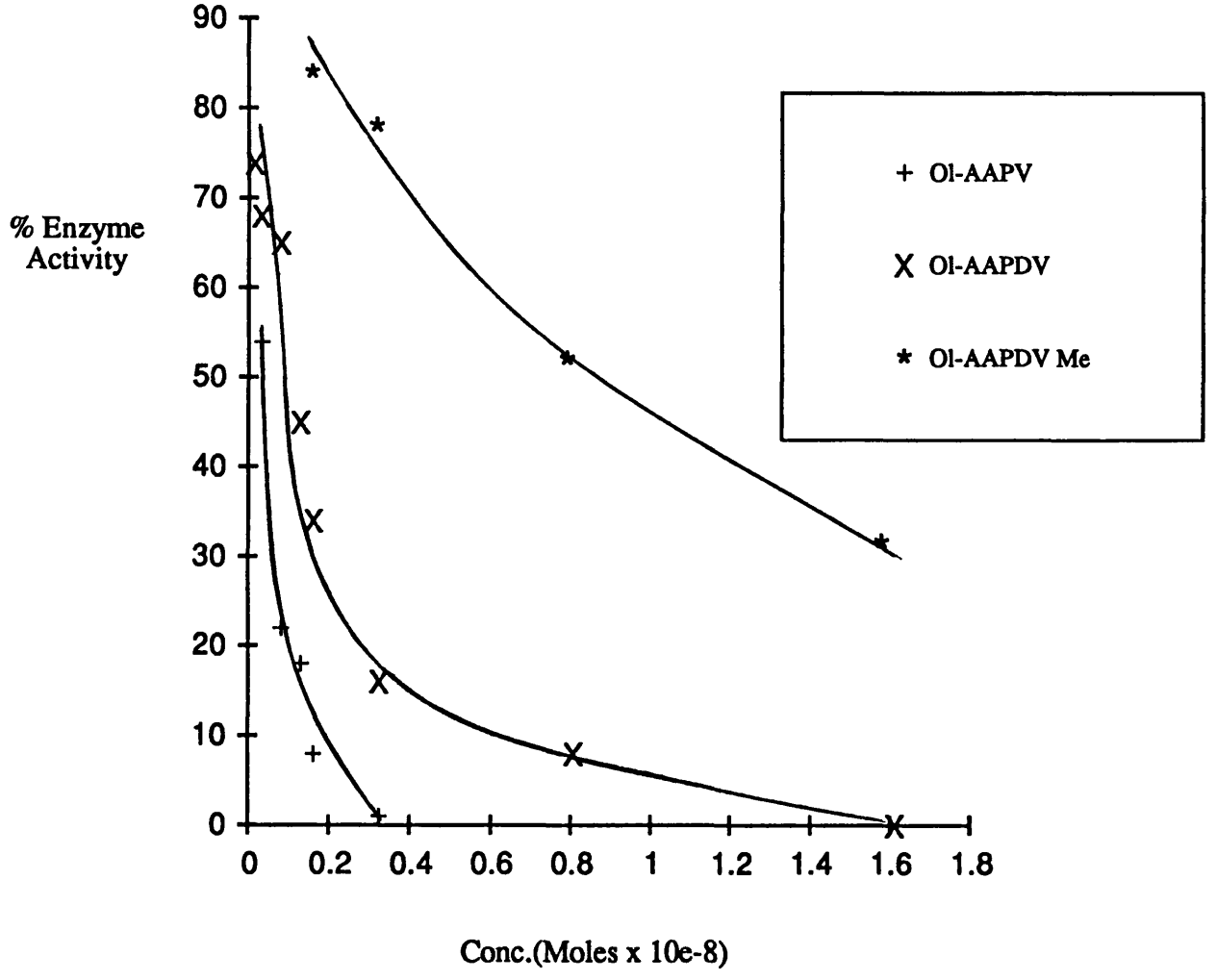
Table of % Enzyme Activity in the Presence of Inhibitor Against Concentration

% Activity Ol-AAPV	Conc.x10^{-8} Ol-AAPV	% Activity Ol-AAPDV	Conc.x10^{-8} Ol-AAPDV	% Activity Ol-AAPDV Me ester	Conc.x10^{-8} Ol-AAPDV Me ester
1	0.3226	0	1.6129	31.5	1.5773
8	0.1613	8,(20)	0.8065	52	0.7886
18	0.1290	16,(18)	0.3226	78	0.3155
22	0.0806	34	0.1613	84	0.1577
54	0.0323	45	0.1290		
		65	0.0806		
		68	0.0323		
		74	0.0161		

The results tabulated above are represented graphically (Fig. 25).

To compare the inhibitory effect of the replacement of L valine by D valine and also the inclusion of methyl ester at the terminal carboxyl end.

Fig. 25 Graph of % Enzyme Activity comparing the inhibitory effect of L- and D- isomers and Terminal End Protection



From the pharmacological results it can be seen that inhibition of the elastase enzyme increases from the monomer through the dimer and trimer (Figs.23,24). The elastase inhibitors conjugated with the lipidic amino acid dimer and trimer, as the lipidic moieties are more potent than the oleic acid derivative which was used as the standard (Fig. 24).

If the concentration of the derivatives giving 50% inhibition of elastase activity (IC_{50}) is evaluated from the dose response curves, the results are as follows:-

Inhibitor	IC_{50}
Oleoyl-AAPV	4.66×10^{-7}
Monomer	3.05×10^{-6}
Dimer	3.60×10^{-7}
Trimer	1.50×10^{-7}

The IC_{50} values of the tested compounds increase with lipophilicity. The effect of stereochemistry of the elastin mimicing tetrapeptide on elastase inhibition was also studied in vitro. When the stereochemistry of part of the oleic derivative was changed from L-Val to D-Val, the potency of the inhibitor decreased (Fig. 25).

The effect of free acid on potency was also studied. The presence of protecting groups at the carboxyl terminus decreases potency (Fig. 23).

In addition to their activity as elastase inhibitors the lipidic amino acid derivatives were tested as inhibitors of plasmin and urokinase.

The activity of plasmin was assayed calorimetrically using tosyl- Gly-Pro-Arg-p-nitranilide acetate as substrate in pH 8.4 buffer. The activity of urokinase was assayed with benzoyl B Ala-Gly-Arg-p-nitranilide as substrate. The compound having the monomer as the lipidic moiety inhibited 88% of plasmin activity at a concentration of 10μg/ml, with the inhibitory capacity decreasing as concentration increased up to 50μg/ml. The monomeric peptide derivative inhibited urokinase activity by 46% at a concentration of 50μg/ml.

The compound having the lipidic amino acid trimer as the hydrophobic moiety reduced plasmin activity by 36% and 44% at 10μg and 50μg/ml respectively. The

inhibition of urokinase by the trimeric derivative was 22% at 50μg/ml.

In contrast to HLE, the inhibitory effect of the lipidic amino acid derivatives on plasmin and urokinase decreases as the number of lipidic amino acid residues ie from monomer through to trimer.

Due to the inhibition of elastase by the lipidic amino acid trimer deriverative of Ala-Ala-Pro-Val further experiments were carried out on rabbit skin.

6. 5 *In vivo* studies of the Elastase Inhibitory Activity of Boc-C_{14}-C_{14}-C_{14}-Ala-Ala-Pro-Val in Rabbit skin.

The inhibitor and HLE were injected intradermally in the rabbit skin. The elastic fibres were visualised on microscopic sections and their surface was quantified by morphometry. The results were compared with those obtained without injection of the inhibitor or of the enzyme.

Hairs were removed from the back of a male white Bouscat rabbit weighing 1500g and the following solutions were injected intradermally at six different sites.

1) 0.2 ml of phosphate buffered salve pH 7.4 (PBS).
2) 500μg of inhibitor in a 1:1 mixture of EtOH and PBS.
3) 7.6μM HLE in 0.2ml PBS.
4) 250μg of inhibitor in 0.1ml of a 1:1 mixture of EtOH-PBS, followed in 15 minutes by 7.6μM of HLE in 0.1ml of PBS.
5) 7.6μM of HLE in 0.1ml PBS followed in 15 minutes by 250μg of inhibitor in a 1:1 mixture of EtOH-PBS.
6) 7.6μM HLE and 250μg of inhibitor were injected together in a mixture of PBS and 50μl of EtOH.

Skin biopsies were taken 3 hours after the injections from the regions 1-6. After fixing, dehydration and embedding in paraffin in several sections of 5-6μm thick, visualisation of the elastic fibres were achieved by staining with Orcein of Unna (see Fig. 26-30). Morphometric analysis was performed on the stained sections by the method of Lantesoul and Lay[293], using a computer programme developed by the Ecole de Mines of Fontenebleau. The area and the volume fractions of 5 distinct fields were evaluated. (Table 52).

Table 52 Table of in vivo elastase inhibitory activity of Boc $C_{14}C_{14}C_{14}$ Ala-Ala-Pro-Val

		1	2	3	4	5	$A_{omg}=EA_o/5$	$V_{omg}=A_o \times 0.25$
1	A A_o-A $(256)^2$	2849 0.0434 (4.3%)	3542 0.05404 (5.404%)	4793 0.0737 (7.31%)	3277 0.05 (5%)	3401 0.0518 (5.18%)	0.05446sd 5.44 ± 0.9	0.0136 1.36%
2	A A_o	4670 0.0712 6 (7.12)	3335 0.0508 (5.08%)	3289 0.0501 (5.0%)	4763 0.0726 (7.26%)	5658 0.0863 (8.6%)	0.0662 6.62% ± 127	0.0165 1.65%
3	A A_o	1051 0.0157 (5.6%)	2042 0.0366 (3.66%)	1862 0.0284% (2.84%)	1220 0.0186 (1.86%)	769 0.0117 (1.17%)	0.0222 2.2% ± 0.9	0.0148 1.48%
4	A A_o	3685 0.0788 (7.88%)	2453 0.0374 (3.74%)	1472 0.0224 (2.24%)	6003 0.0915 (9.15%)	5918 0.0903 (9.03%)	0.0595 5.95% ± 2.7	0.0148 1.48%
5	A A_o	5166 0.0788 (7.88%)	5734 0.0874 (8.74%)	5054 0.0771 (7.7%)	3496 0.0533 (5.33%)	3675 0.0566 (5.6%)	0.0705 7.05% ± 1.3	0.0176 1.76%
6	A A_o	6309 0.0962 (9.6%)	5821 0.0888 (8.88%)	3603 0.0549 (5.49%)	3625 0.0553 (5.53%)	6097 0.0930 (9.30%)	0.0776 7.76% ± 1.8	0.0194 1.94%

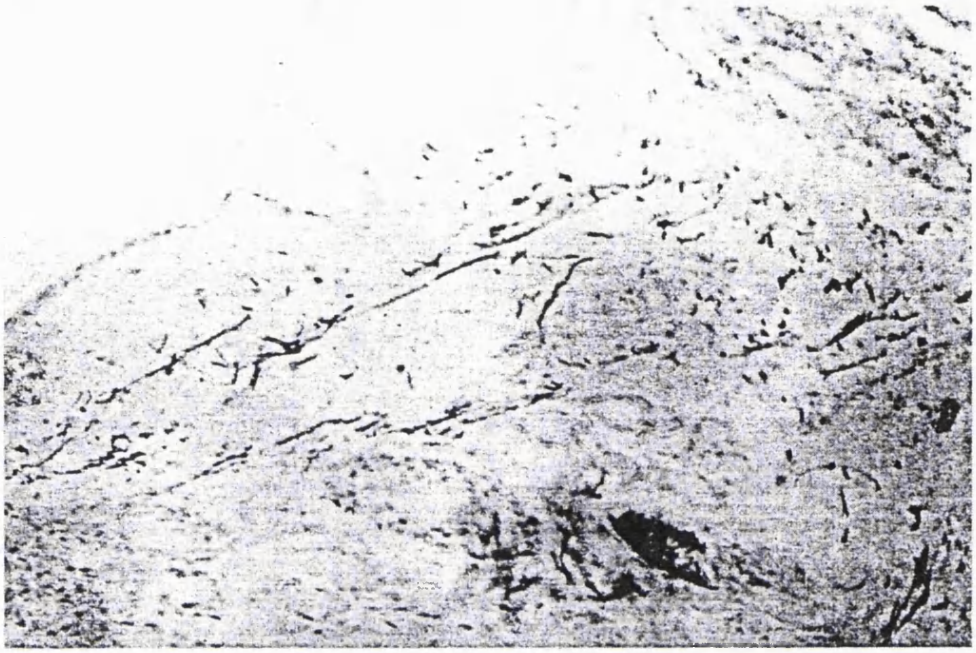
A = area of field

A_o = area of the elastic fibres related to the field

V = volume fraction of elastic fibres

No. 1 Standard

Fig. 26



No. 3

Fig. 27

No. 4

Fig. 28

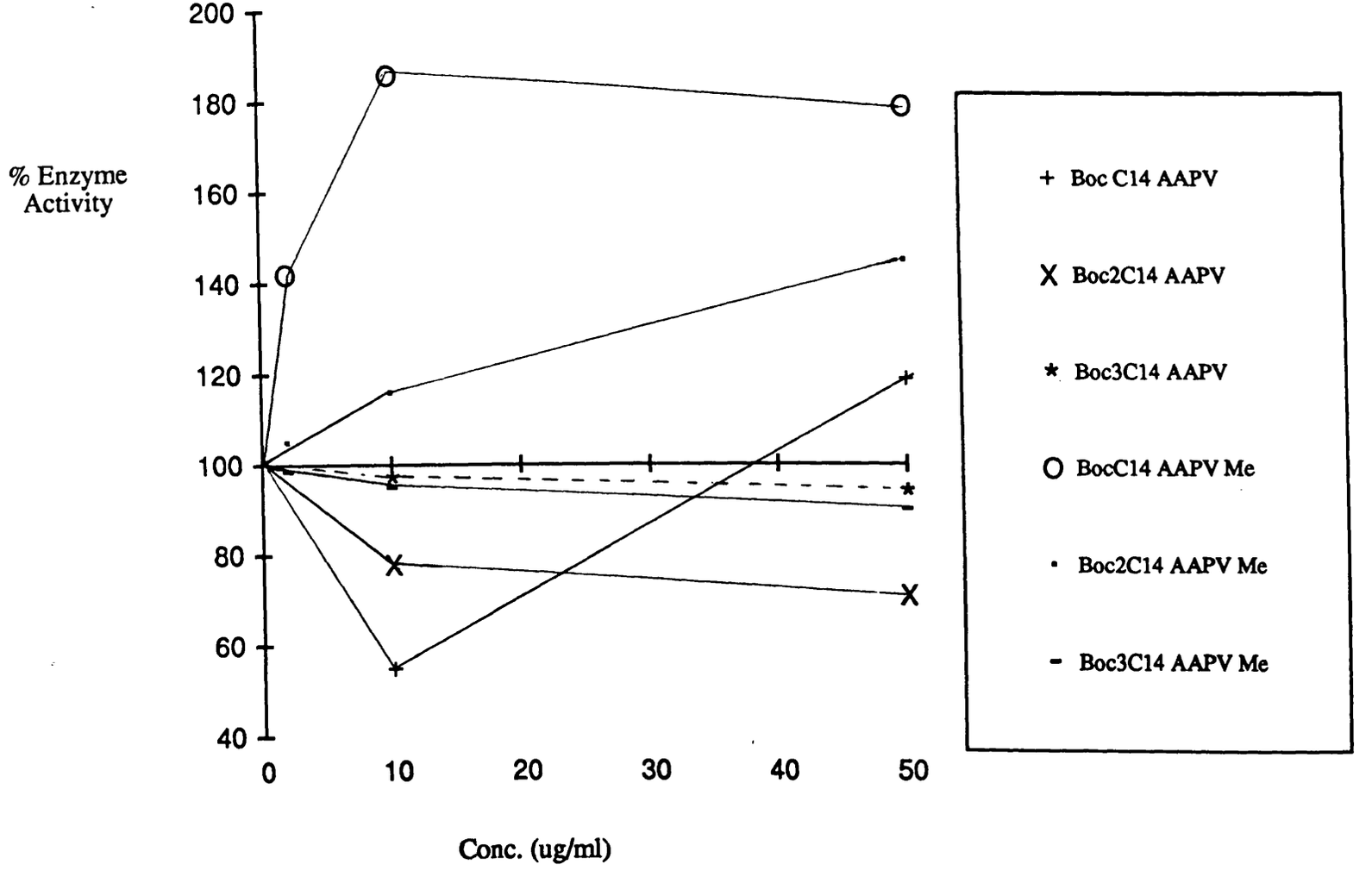
No. 5

Fig. 29

No. 6

Fig. 30

Fig. 31 Graph of Porcine Pancreatic Elastase Activity against Inhibitor Conc.



The effect of the lipidic amino acid monomer, dimer and trimer derivatives of Ala-Ala-Pro-Val were examined as the free carboxyl acid or the protected methyl ester. These inhibitors were examined against HLE, porcine pancreatic elastase (PPE), trypsin and plasmin.

Table 53 Inhibitory effect of the lipidic amino acid derivatives

Enzyme	Boc C_{14} AAPV	Boc$C_{14}C_{14}$ AAPV	Boc$C_{14}C_{14}$ C_{14} AAPV	Boc C_{14} AAPV OMe	Boc$C_{14}C_{14}$ AAPV OMe	Boc$C_{14}C_{14}$ C_{14} AAPV OMe
PPE 2.5μg/ml)	10μg/ml 55% 50μg/ml 119%	78% 71%	97% 94%	2μg/ml 142% 10μg/ml 186% 50μg/ml 179%	105% 116% 145%	98.5% 95% 90%
Plasmin 12mU/ml	10μg/ml 37 50μg/ml 51	0% 0%	0% 0%	4% 62%	106% 95%	10% 38%
Trypsin 2.5μg/ml	10μg/ml 107% 50μg/ml 172%	38% 86%	303% 634%	132% 115%	118% 87%	68% 92%
HLE				10μg/ml 0% 50μmg/ml 0%	0% 0%	0% 0%

Graphs of % enzyme activity against concentration of inhibitor were plotted, Fig. 31 related to PPE, Fig. 32 relates to plasmin and Fig. 33 to trypsin.

Fig. 32 Graph of Plasmin Activity against Inhibitor Conc.

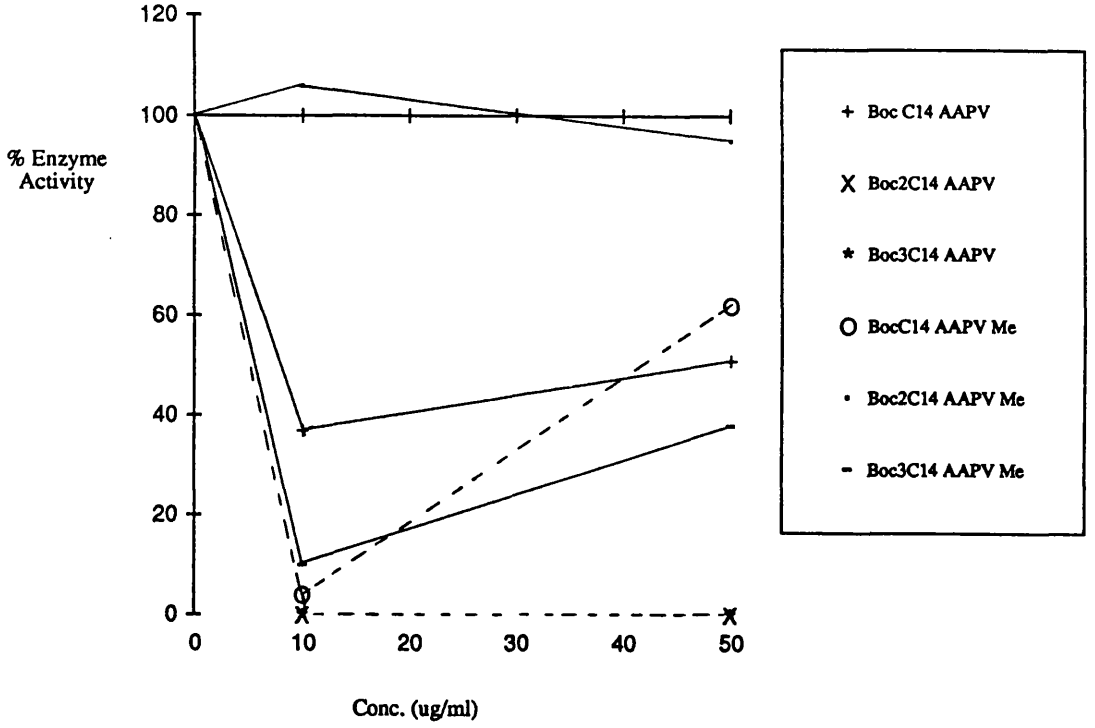
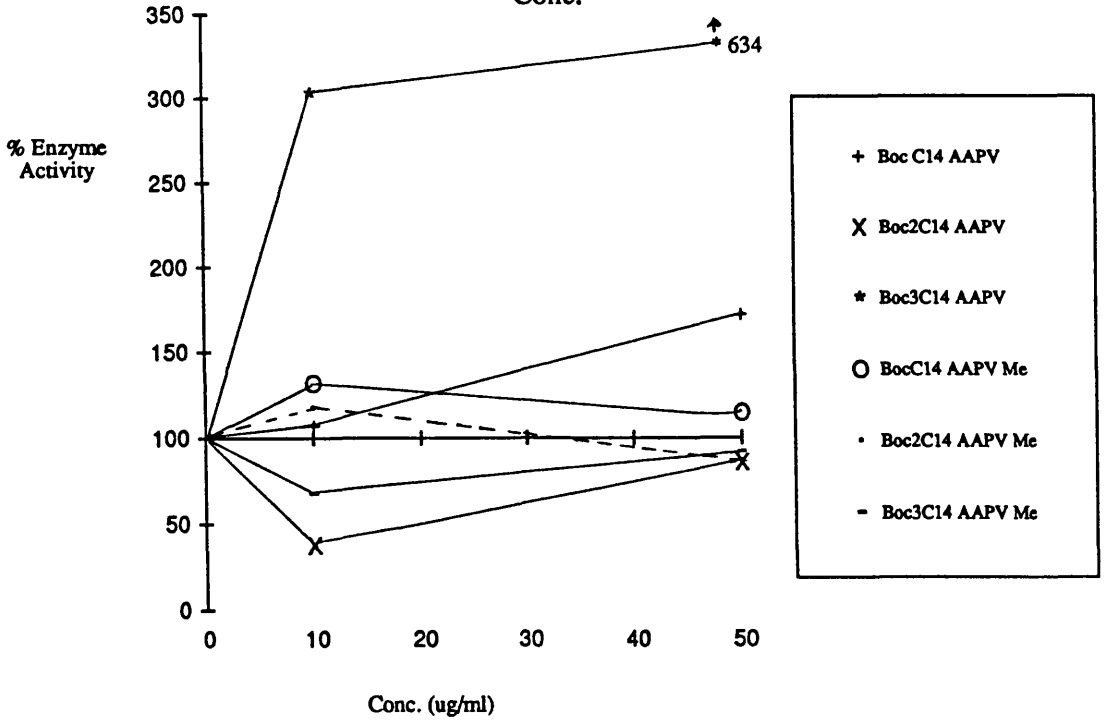


Fig. 33 Graph of % Trypsin Activity against Inhibitor Conc.



Conclusion

The lipidic amino acid derivatives (CLVI),(CLXI) and (CLXIII) are good inhibitors of Cathepsin B and weak inhibitors of other serine proteases, thus exhibiting elastoprotective activity. The elastase inhibitor conjugated with the lipophilic amino acid trimer was a more potent inhibitor than the dimer, which was itself more potent than the monomer. The most active compound was determined by that requiring the lowest concentration to prevent elastase action.

The study of the in vivo elastase inhibitory activity can be summarised as below.

Table 54

	A_o Average %	Standard Deriv.
(1) PBS	5.44	+0.9
(2) Inhibitor	6.62	+1.3
(3) HLE	2.20	+0.9
(4) Inhibitor 15 min + elastase	5.95	+2.7
(5) Elastase 15 min + inhibitor	7.05	+1.3
(6) Elastase + inhibitor (Simultaneously)	7.76	+1.3

The detailed results indicate that there is no significant difference between the control injection area (1) and the sites where the inhibitor was injected with or without the enzyme (2,4,5,6). The injection site of the uninhibited elastase (3), exhibited a threefold decrease in the area and volume fraction of the elastic fibres related to the control and to the enzyme treated regions ($p > 0.0001$). It can be concluded that under the experimental conditions used application of the inhibitor before or after the administration of the elastase protects the elastic fibres in the skin. The use of radiolabelled fatty acid substituted Ala-Ala-Pro-Val derivatives was found to easily penetrate animal skin. Thus the C_{14} trimeric derivative of Ala-Ala-Pro-Val may exert elastoprotecting effect in the skin when incorporated in cosmetic preparations as creams or lotions.

The effect of C_{14} monomer, dimer and trimer derivatives of Ala-Ala-Pro-Val, as the free acid and the methyl ester protected carboxyl were studied for their inhibitory effect on PPE, plasmin and trypsin.

In PPE the methyl ester of the monomer and dimer enhances the activity,

whilst the trimer decreases enzymic activity. The free acid decreases PPE activity in all cases, whilst at higher concentrations the monomer appears to enhance activity.

The activity of trypsin is decreased by the methyl ester trimer, whilst the monomer and dimer initially increases activity, but at high concentration trypsin activity is decreased. The free acid activity of the monomer, dimer and trimer Ala-Ala-Pro-Val vary in their action against trypsin. The monomer appears to increase trypsin activity at higher concentrations whilst the dimer decreases activity with concentration. The trimer results are totally erroneous with activity increasing up to 600 fold. This behaviour is thought to be due to micelle formation with increasing activity at higher concentrations.

In the case of plasmin, the enzyme activity decreases free acid experiments whilst the methyl ester again shows a decrease in enzyme activity but the dimer has a greater activity than the monomer.

In conclusion the enzyme activity appears to decrease with monomer through to trimer as a general trend. There is a problem however, which could be due to the formation of micelles which these compounds readily form.

MOLECULAR WEIGHT DETERMINATION

Several different techniques were used to determine the molecular weight of the polymers produced. The techniques used included gel filtration, high pressure lipid chromatography, light scattering, viscometry, vapour pressure osmometry, Rayleigh Interference Optics and Schlieren optics.

1. VAPOUR PRESSURE OSMOMETRY

The chamber of the vapour pressure osmometer was saturated with benzene over 48 hours and then the instrument was standardised with benzil before the molecular weight determination of the aminodecanoic acid-lysine copolymer (XXXIV).

Benzil (0.1526g) was dissolved in benzene (10 mls) and readings obtained on the wheatstone bridge were taken at various concentrations

Table 3

Conc.(g/l)	ΔR 1	ΔR 2	ΔR 3	ΔR 4	Avg . ΔR	ΔR/C
1.526	1.03	1.03	1.03		1.03	0.675
3.052	1.94	1.92	1.93	1.96	1.9375	0.6348
6.104	3.85	3.80	3.79		3.8144	0.6247
9.156	5.57	5.57	5.60		5.58	0.6094
15.26	0.56	8.60	8.51		8.5567	0.5607

Fig. 11

Extrapolation of the graph (Fig.11) to concentration=0 gives an intercept=0.6725227. A second experiment was run for standardisation.
Benzil (0.1500g) was dissolved in benzene (10 mls).

Fig. 11 Graph of △R/C against Conc. of Aminodecanoic Acid - Lysine Copolymer 1:1

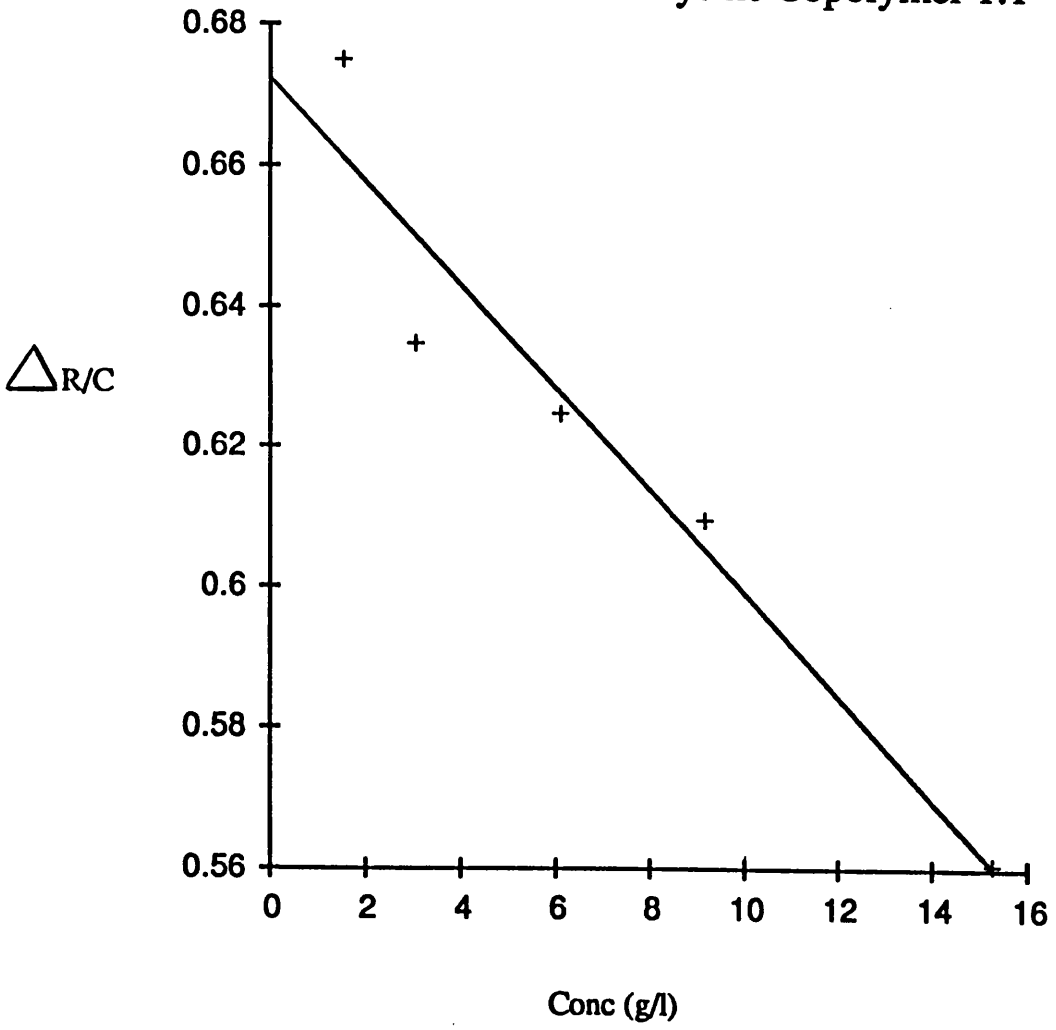


Table 4

Conc.(g/l)	ΔR 1	ΔR 2	ΔR 3	ΔR 4	Avge.ΔR	ΔR/C
1.5	0.96	0.96	0.96		0.96	0.64
3.0	1.89	1.90	1.90		1.8967	0.6322
6.0	3.68	3.71	3.67		3.7578	0.6144
9.0	5.71	5.57	5.63		5.6367	0.6263
15.0	8.97	9.07	9.01		9.0167	0.60111

Fig. 12

From Fig.12 point 4 was incorrect and therefore omitted to give Fig.13.

Fig. 13

When extrapolated to conc=0, the intercept (ΔR/C)=0.6393396

Following successful standardisation, determination of the molecular weight XXXIII was attempted, however even at dilute solutions the polymer gave gel like solutions thus no reading could be obtained on the vapour pressure osmometer.

2. VISCOMETRY

XXXIV(0.0534g) was dissolved in benzene (10mls,) dissolution was very difficult and ultrasonication was used to aid dissolution, the solutions obtained having a gel like appearance. The solution was placed in the viscometer and the time taken for the meniscus to travel between two points was taken.

Table 5

Solution (g/100ml.)	Time (sec)					
Benzene	196.13	197.15	195.60	196.95	194.70	197.24
0.1068	No movement of the meniscus was observed					
0.01068	205.88	212.94	212.68	211.31	212.13	
0.0236	218.36	216.22	216.12	216.30		
0.032088	217.50	218.02	218.33	219.60	219.16	219.73
0.0534	226.00	226.36	226.22			

Fig. 12 Graph of△R/C against Aminodecanoic Acid - Lysine Copolymer 10:1

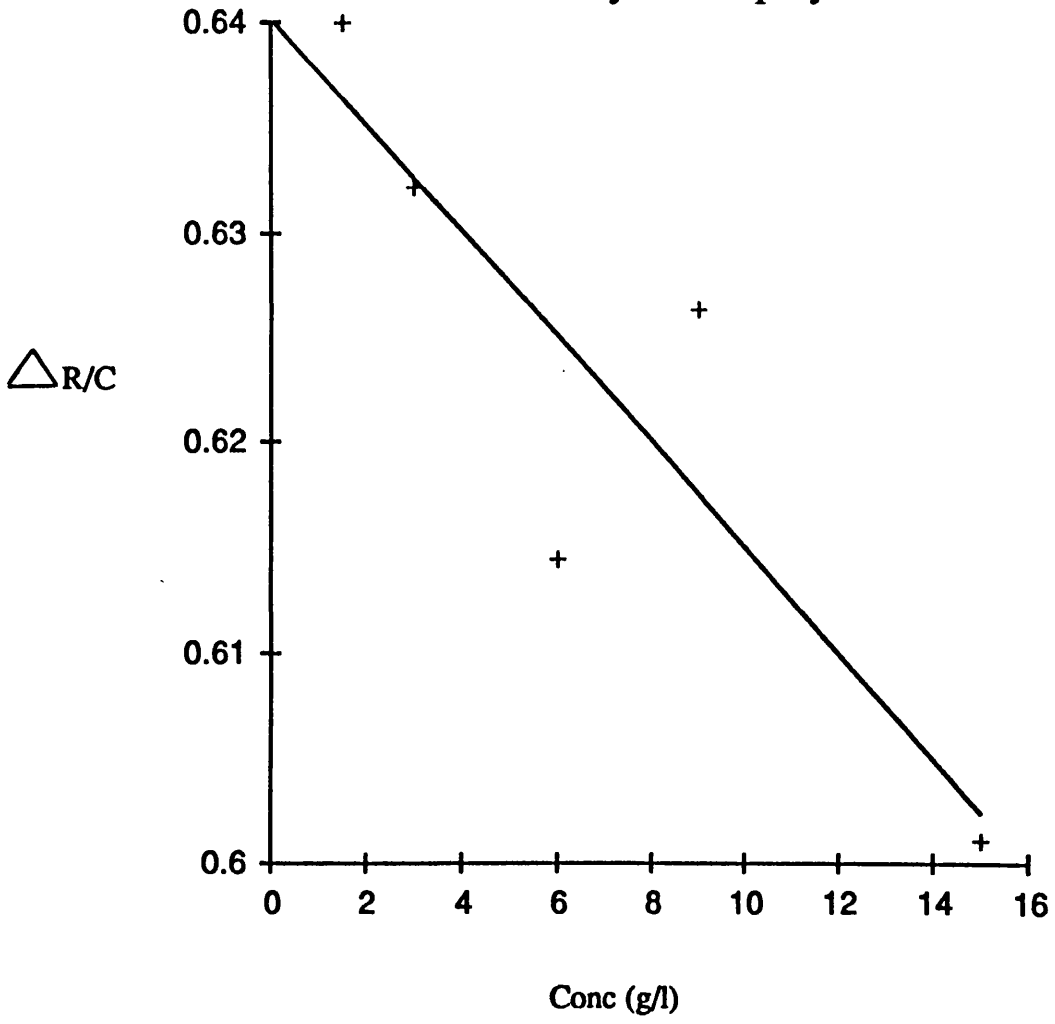


Fig. 13 Graph of △R/C against Conc. of Aminodecanoic Acid - Lysine Copolymer 10:1 (corrected)

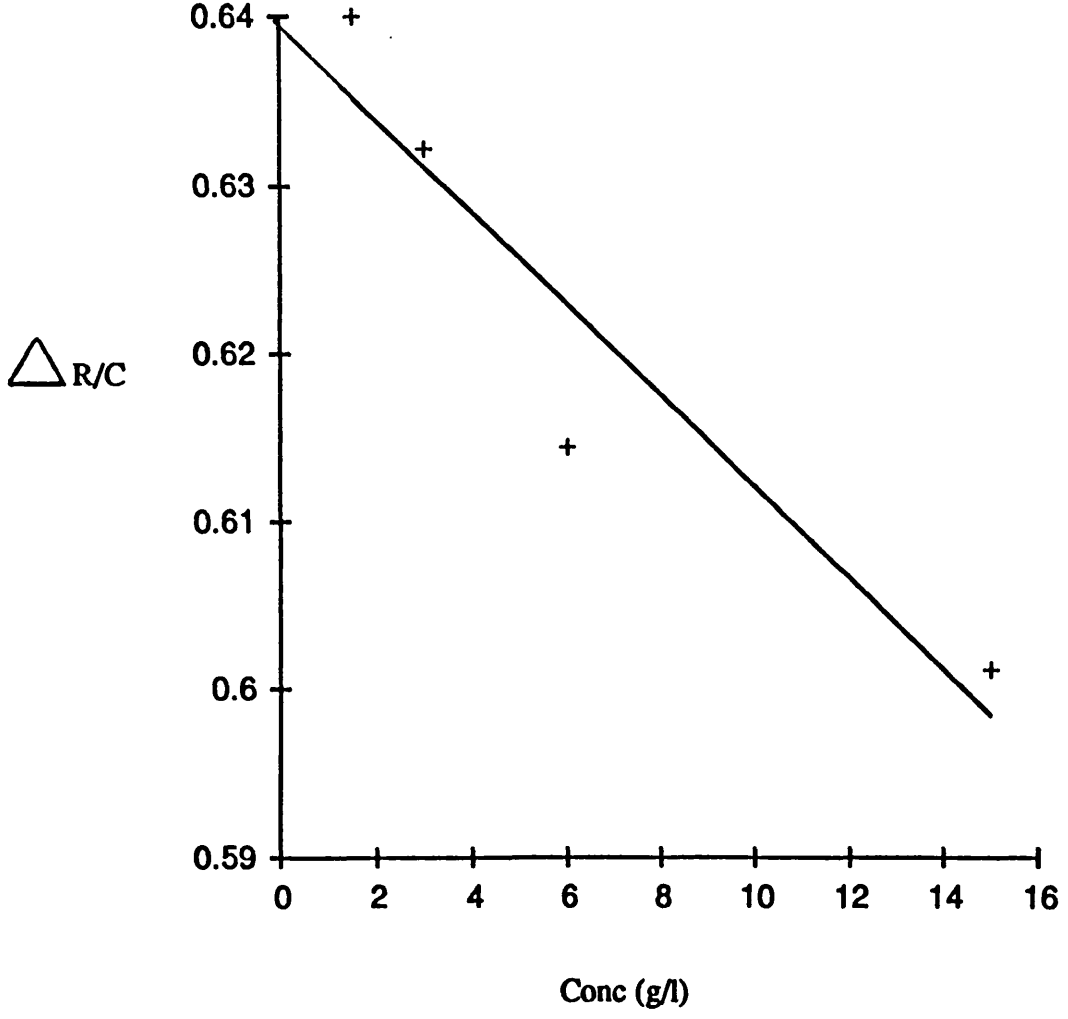


Table 6

Conc. (g/100ml.)	Average time (sec.)	ήs	ήs/c
Benzene	197.113		
0.01068	212.5833	0.07848	7.3487
0.02136	216.2133	0.0969	4.5365
0.03204	219.50	0.11357	3.5446
0.0534	226.1933	0.1475	2.7628

To calculate the intrinsic viscosity [ή] s/c was plotted against c. Fig. 14

As C→0 ήs/c = [ή]

ή = viscosity of solution

ήo = viscosity of solvent

ήs - (ήM/ 0-1)

No extrapolation to C = O could be made as the graph obtained is exponential, the same results were used with a different formula.

$$ή/C_2 = t\text{-}t_o/t_oC_2 + \{1/\rho\text{-}\upsilon_2\}t/t_o$$

Table 7

Conc. (g/1000mls)	$t\text{-}t_o/t_o$	$t\text{-}t_o/t_oC_2$
0.1068	0.0784844	0.7348727
0.2136	0.0969	0.45365
0.3204	0.11357	0.3544771
0.534	0.14753	0.2762755

Table 8

Conc.(g/l)	$1/\rho_o\text{-}\upsilon_2)$	t/t_o	$(1/\rho_o\text{-}\upsilon_2)t/t_o$	$+t\text{-}t_o/t_oC_2$
0.1068	-0.0333	1.0785	-0.03595	0.6989227
0.2136	-0.0333	1.0969	-0.03656	0.41709
0.3204	-0.333	1.11357	-0.037119	0.317358
0.534	-0.333	1.14753	-0.038251	0.2380245

ρ_o = density ofbenzene υ= partial molar volume (assumed to be = 0.7)

s/C_2 was plotted against C_2. Fig. 15

Extrapolation to C_2 = O (Fig. 15) cannot be made as an exponential graph as obtained. Therefore this method of molecular weight determination was abandoned.

Fig. 14 Graph of ηS/C against Conc. of Aminodecanoic Acid - Lysine Copolymer 1:1

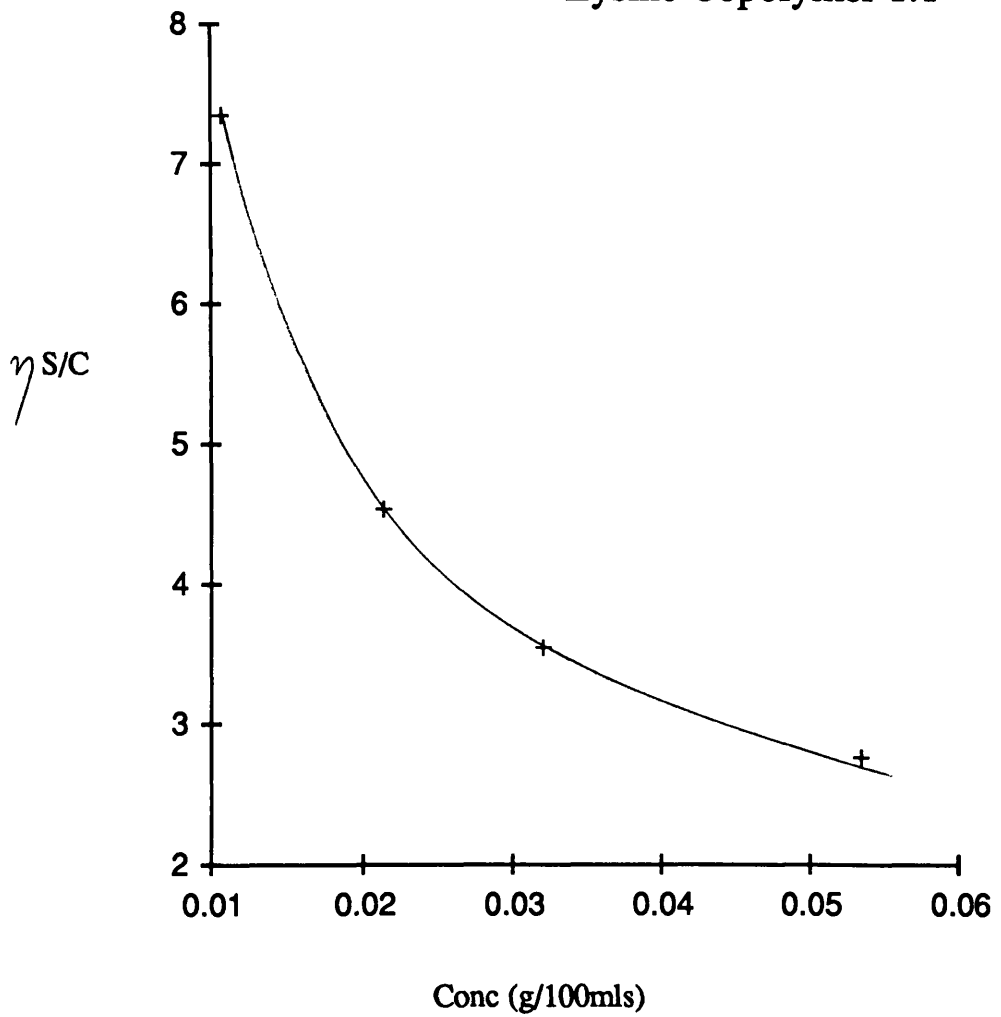
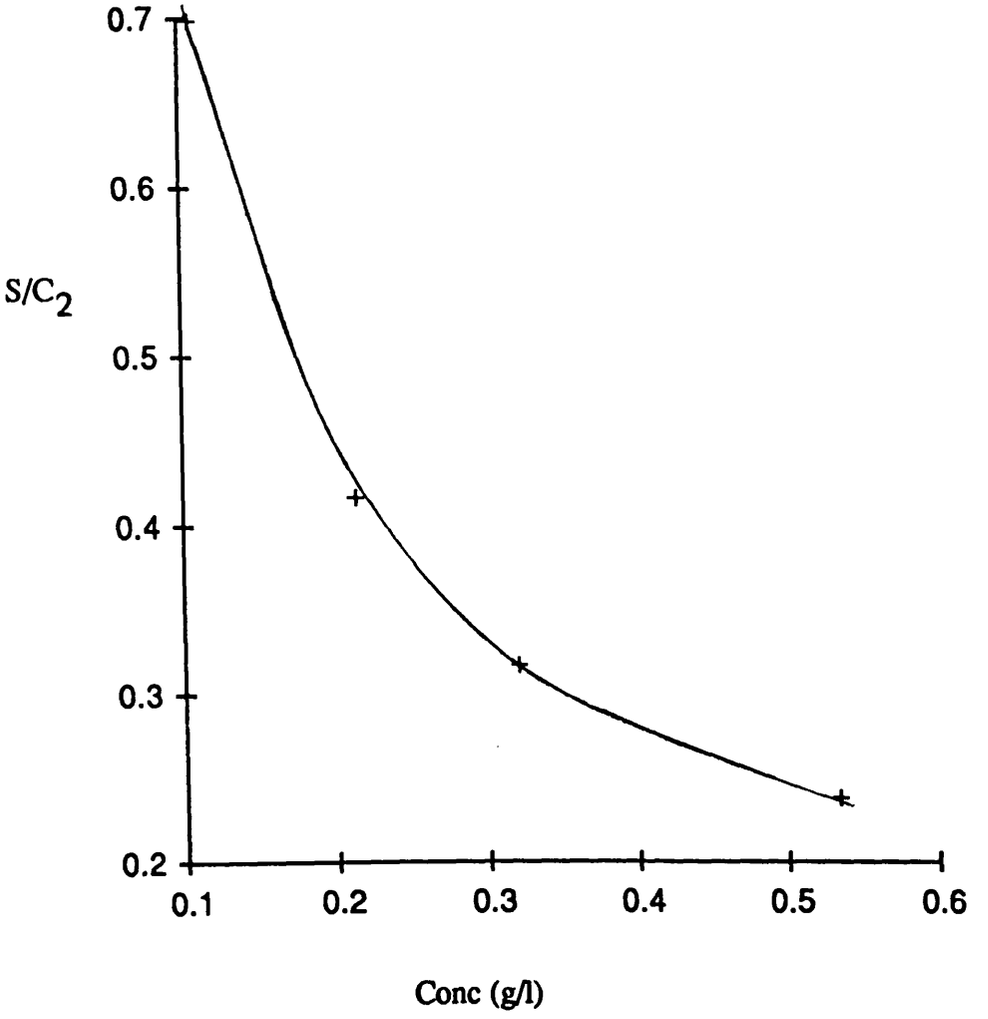


Fig. 15 Graph of S/C_2 against C for Aminodecanoic Acid - Lysine Copolymer 1:1



3. LIGHT SCATTERING

Molecular Weight Determination of l-aminodecanoic acid Polymer XXXIII

Light scattering measurements were taken through varying angles to that molecular shape as well as molecular weight could be determined.

XXXII (0.20995g) was dissolved in dimethylsulphoxide (DMSO). (100 mls). (PB is Perspex Block used as standard)

Table 9

Readings

60° Angle

Conc.(g/100ml)	P.B.1.	P.B.2	1	2	3	4	P.B.3	P.B.4
DMSO	4.91	4.91	3.06	3.05	3.02		5.11	5.13
0.20995	4.74	4.73	4.40	4.48	4.50	4.43	4.73	4.73
0.16796	4.03	4.04	4.20	4.24	4.37	4.18	4.02	4.00
0.104795	5.31	5.28	3.85	3.75	3.80		5.30	5.29
0.014199	4.85	4.87	3.18	3.18	3.20		4.97	4.97

Table 10

70° Angle

Conc.(g/100ml)	P.B.1	P.B.2	1	2	3	4	P.B.3	P.B.4
DMSO	5.13	5.10	2.12	2.29	2.21	2.22	5.11	5.10
0.20995	4.74	4.73	3.71	3.84	3.77	3.70	4.75	4.74
0.16796	3.99	3.99	2.37	2.33	2.34		3.92	3.90
0.104795	5.29	5.27	2.35	2.34	2.42		5.21	5.22
0.04199	4.96	4.97	2.17	2.21	2.22		4.99	4.99

Table 11

80° Angle

Conc.(g/100ml)	P.B.1	P.B.2	1	2	3	4	P.B.3	P.B.4
DMSO	5.09	5.10	2.04	2.01	2.01		4.92	4.91
0.20995	4.72	4.73	3.59	3.58	3.56		4.79	4.76
0.16796	3.90	3.89	2.25	2.28	2.25		4.03	4.02
0.104975	5.21	5.22	2.34	2.28	2.30		5.11	4.88
0.04199	4.99	4.99	2.08	2.06	2.03		4.89	4.88

Table 12

90° Angle

Conc.(g/100ml)	P.B.1	P.B.2	1	2	3	4	P.B.3	P.B.4
DMSO	4.89	4.89	1.83	1.78	1.86		4.93	4.92
0.20995	4.75	4.75	3.49	3.51	3.51		4.81	4.80
0.16796	4.02	4.02	2.16	2.17	2.18		3.97	4.01
0.104975	5.13	5.12	2.27	2.32	2.29		5.24	5.23
0.04199	4.90	4.91	2.01	2.01	2.05		4.79	4.78

Table 13

100° Angle

Conc.(g/100ml)	P.B.1	P.B.2	1	2	3	4	P.B.3	P.B.4
DMSO	4.91	4.91	1.66	1.65	1.64		4.89	4.86
0.20995	4.80	4.79	3.77	3.79	3.77		4.72	4.71
0.16796	3.99	4.01	2.16	2.22	2.25		4.03	4.05
0.104975	5.22	5.23	2.30	2.20	2.39		5.33	5.33
0.04199	4.76	4.78	1.91	1.91	1.92		4.78	4.77

Table 14

110° Angle

Conc.(g/100ml)	P.B.1	P.B.2	1	2	3	4	P.B.3	P.B.4
DMSO	4.83	4.83	1.75	1.72	1.74		4.72	4.73
0.20995	4.70	4.69	4.02	4.10	4.16	4.13	4.73	4.74
0.16796	4.03	4.04	2.34	2.31	2.39		3.98	3.97
0.104975	5.32	5.33	2.43	2.41	2.50		5.39	5.41
0.04199	4.81	4.78	2.10	2.05	2.12		4.82	4.81

Table 15

120° Angle

Conc.(g/100ml)	P.B.1.	P.B.2	1	2	3	4	P.B.3	P.B.4
DMSO	4.72	4.69	2.09	2.07	2.06		4.75	4.76
0.20955	4.72	4.73	4.77	4.64	4.55	4.64	4.69	4.69
0.16996	3.96	3.97	2.53	2.59	2.57		3.99	4.00
0.104975	5.40	5.41	3.35	3.37	3.36		5.39	5.41
0.04199	4.81	4.81	2.10	2.76	2.76		4.71	4.69

$I = \frac{\text{A.V. reading Polymer}}{\text{AV. P.B.}}$ $S = I_{polymer} - I_{DMSO}$

Table 16

60° Angle

Conc. (g/100ml)	Av.P.B. before	Av. Reading	Av.P.B after	Av.P.B.	I	S
DMSO	4.91	3.0433	5.12	5.015	0.6068	
0.20995	4.735	4.4525	4.73	4.7325	0.9408	0.334
0.16796	4.035	4.2067	4.01	4.0325	1.0458	0.439
0.104975	5.295	5.295	3.80	5.295	0.7177	0.1109
0.04199	4.86	3.1867	4.97	4.915	0.6484	0.0416

Table 17

70° Angle

Conc. (g/100ml)	Av.P.B. before	Av.Reading	Av.P.B. after	Av.P.B.	I	S
DMSO	5.115	2.21	5.105	5.100	0.4325	
0.20995	4.36	3.755	4.745	4.74	0.7922	0.3597
0.16796	3.99	2.3467	3.91	3.95	0.5941	0.1616
0.104975	5.28	2.37	5.215	5.2475	0.4516	0.0191
0.04199	4.965	2.20	4.99	4.9775	0.4420	0.0095

Table 18

80° Angle

Conc. (g/100ml)	Av.P.B. before	Av. Reading	Av.P.B. after	Av.P.B.	I	S
DMSO	5.095	2.02	4.915	5.005	0.4036	
0.20995	4.725	3.5767	4.775	4.75	0.7530	0.3494
0.16796	3.895	2.26	4.025	3.96	0.5707	0.1671
0.104975	5.215	2.3067	5.11	5.1625	0.4468	0.0432
0.04199	4.99	2.0567	4.885	4.9375	0.4165	0.0129

Table 19

90° Angle

Conc. (g/100ml)	Av.P.B. before	Av.Reading	Av.P.B. after	P.B.	I	S
DMSO	4.89	1.8233	4.925	4.9075	0.3715	
0.20995	4.75	3.5033	4.805	4.7775	0.7333	0.3618
0.16796	4.025	2.17	3.99	4.005	0.5418	0.1703
0.14975	5.125	2.2933	5.235	5.18	0.4427	0.0712
0.04199	4.905	2.0367	4.795	4.845	0.4204	0.0489

Table 20

100° Angle

Conc. (g/100ml)	Av.P.B. before	A.v. Reading	Av.P.B. after	P.B.	I	S
DMSO	4.91	1.65	4.875	4.8925	0.3373	
0.20995	4.795	4.78767	4.715	4.755	0.7943	0.457
0.16796	4.00	2.21	4.04	4.02	0.5498	0.2125
0.104975	5.225	2.3633	5.33	5.2775	0.4478	0.1105
0.04199	4.77	1.9133	4.775	4.7725	0.4009	0.0636

Table 21

110° Angle

Conc. (g/100ml)	Av.P.B. before	Av.Reading	Av.P.B. after	Av.P.B.	I	S
DMSO	4.83	1.7367	4.725	4.7775	0.3635	
0.20095	4.695	4.1025	4.735	4.715	0.8701	0.5066
0.16796	5.035	2.3467	4.735	4.385	0.5352	0.1717
0.104975	5.325	2.4467	5.40	5.3625	0.4563	0.0928
0.04199	4.975	2.09	4.815	4.805	0.4350	0.0715

Table 22

120° Angle

Conc. (g/100ml)	Av.P.B. before	Av.Reading	Av.P.B. after	Av.P.B.	I	S
DMSO	4.795	2.0733	4.755	4.73	0.4383	
0.20995	4.725	4.65	4.69	4.7075	0.6440	0.5495
0.16796	3.965	2.5533	3.995	3.98	0.6440	0.2057
0.104975	5.405	3.36	5.40	5.4025	0.6219	0.1836
0.04199	4.81	2.74	4.70	4.755	0.5762	0.1379

The results were used to give a Zimm plot to derermine molecular weight and shape. A plot of c/S_{∞} against c was made to give an indication of molecular weight alone. (Fig. 16).

Table 23

Conc.(g/100 ml)	c/S_{∞}
0.20995	0.5803
0.16796	0.9863
0.104975	1.4744
0.04199	0.8587

From Fig. 16 the intercept was found to be 0.812. To calculated the molecular weight, the refractive index of XXXII was found. The refractive index was measured using a green laser.

REFRACTIVE INDEX OF THE 1-AMINODECANOIC ACID POLYMER

Table 24

Conc.(g/100ml)	d1						Av.d1
DMSO	4.790	4.770	4.725	4.750	4.742	4.794	4.76383
0.04199	6.50	6.509	6.516	6.513	6.502	6.498	6.50633
0.104975	4.623	4.558	4.554	4.580	4.573	4.558	4.57433
0.16796	4.379	4.389	4.379	4.359	4.379	4.210	4.22783

Fig. 16 Graph of C/S_{90} against Conc. of Aminodecanoic Acid polymer

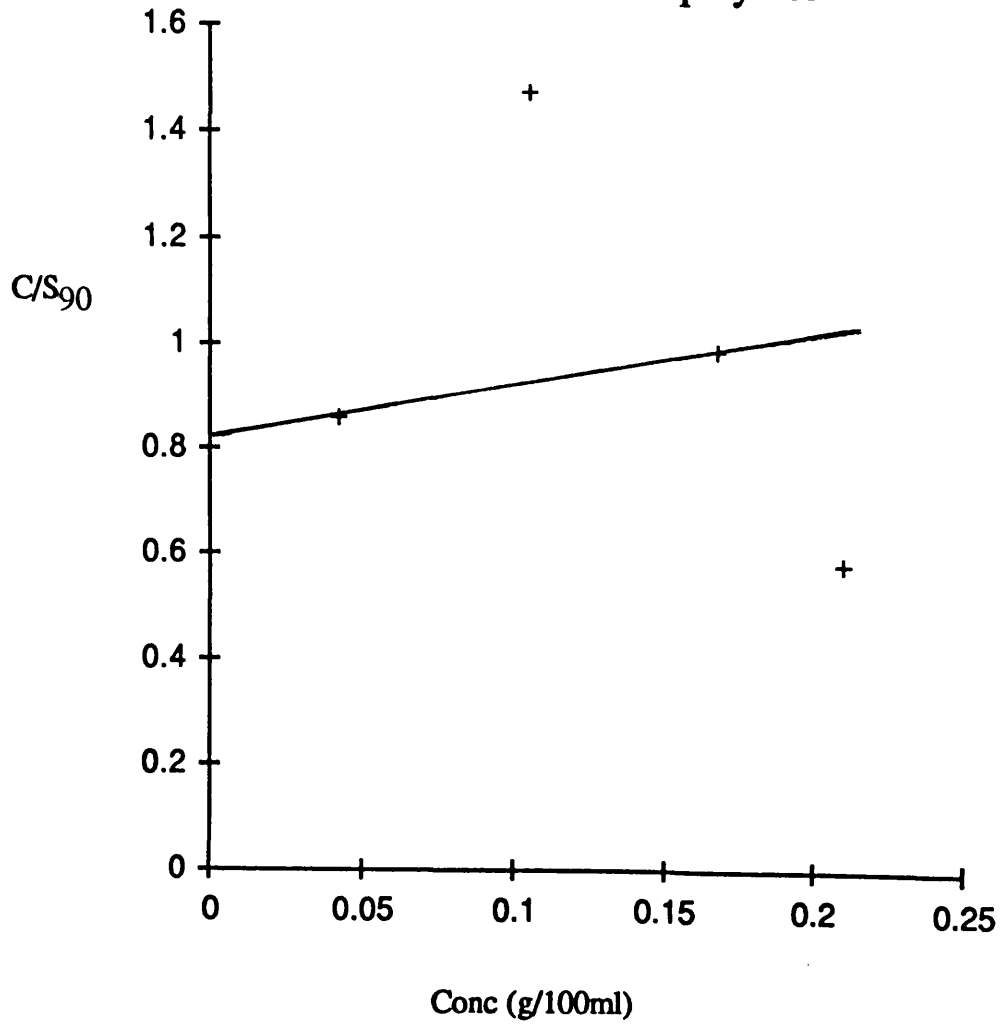


Table 25

Conc. (g/100ml)	d2						Av.d2
DMSO	4.606	4.608	4.619	4.610	4.602	4.604	4.6085
0.04199	2.935	2.899	2.949	2.958	2.950	2.961	2.942
0.104975	4.763	4.799	4.798	4.771	4.789	4.788	4.77967
0.16976	4.962	4.986	4.961	4.968	4.992	4.995	4.77976

Table 26

Conc. (g/100ml)	d2-d1	Δd $d_{polymer}-d_{solvent}$	Δn
DMSO	-0.15533		
0.04199	-3.56433	-3.4092	$-3.2904*10^{-3}$
0.104975	0.20534	0.36067	$3.4895*10^{-4}$
0.16797	0.60683	0.76216	$7.3739*10^{-4}$
0.20095	0.88167	1.037	$1.0033*10^{-3}$

Δn against concentration (C) was plotted. (Fig. 17).

giving $dn/dc=6.22816 \times 10^{-4}$

is the equation used in refractive index measurements

$n=n_o+(dn/dc)c$

$\Delta n=K\Delta d$

n_o = refractive index of solvent

K = constant$=0.9675*10^{-3}$ (100ml/g)

S obtained from light scattering and dn/dc from the refractive index measurements were used in a computer programme to give a Zimm plot, which would give information about molecular weight and shape.

The results obtained from the Zimm plots were:

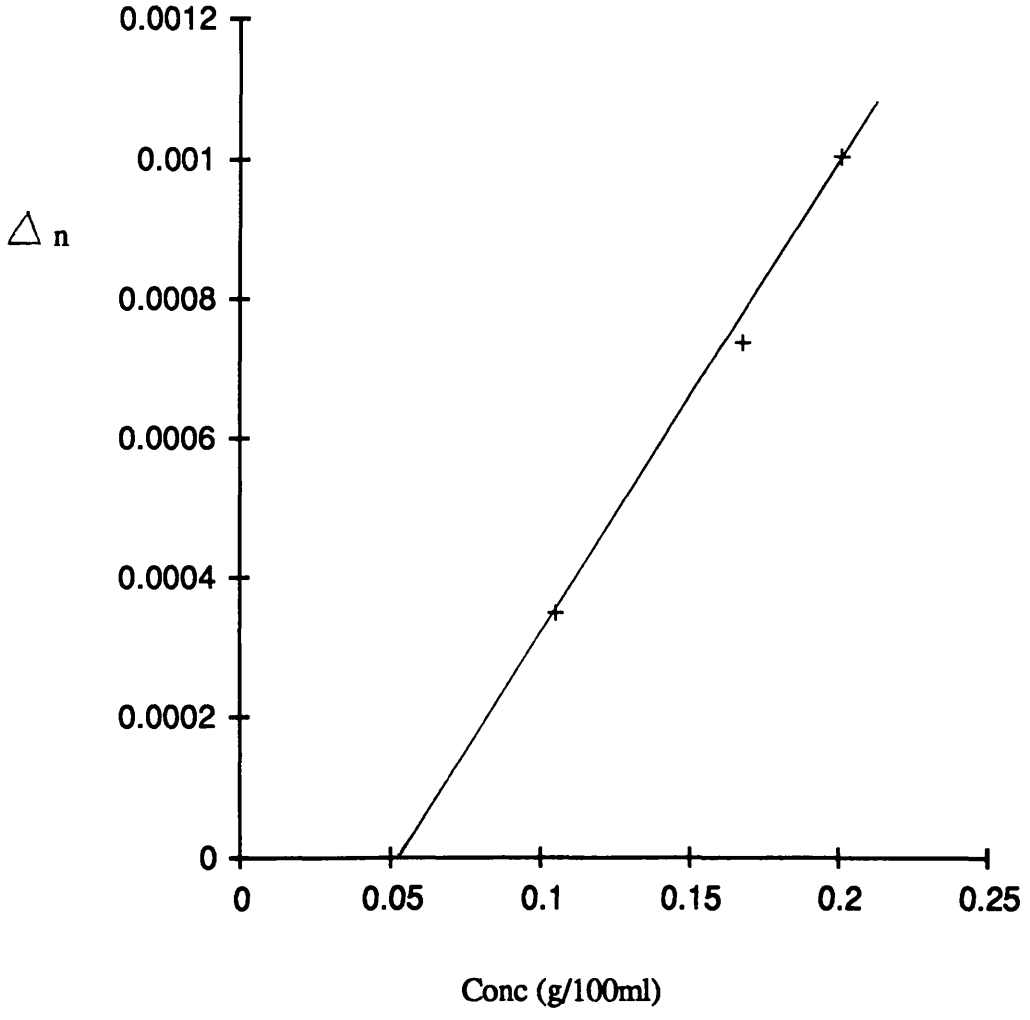
Weight average molecular weight = 9.2291×10^{-3}

Mean square radius = -0.5593×10^{-4}

Second virial coefficient = 0.1634×10^{-2}

Third virial coefficient = 0.1634×10^{-2}

Fig. 17 Graph of △n against Conc. of Aminodecanoic Acid Polymer



The results obtained gave no indication to the size of the polymer, therefore the readings obtained were re-examined. The perspex block measurements changed over the varying concentrations in a time dependent manner. This was corrected for by taking the averave of P.B. readings and the calculations were made.

Table 27

Angle	60°	70°	80°	90°	100°	110°	120°
PB Av.	4.796	4.805	4.770	4.773	4.7431	4.809	4.715

Table 28

60° Angle

Conc.(g/100ml)	I	S	c/S
DMSO	0.6345		
0.20995	0.9284	0.2939	0.7144
0.16796	0.8771	0.2426	0.6923
0.104975	0.7923	0.1578	0.6652
0.04199	0.6644	0.0299	1.4043

Table 29

70° Angle

Conc. (g/100ml)	I	S	c/S
DMSO	0.4600		
0.20995	0.7815	0.3215	0.6530
0.16796	0.4884	0.0284	5.9141
0.104975	0.4932	0.0332	3.1619
0.04199	0.4579	-0.0021	-19.9952

Table 30

80° Angle

Conc.(g/100ml)	I	S	c/S
DMSO	0.4235		
0.20995	0.7498	0.3263	0.6434
0.16976	0.4738	0.0503	3.3392
0.104975	0.4836	0.0601	1.7467
0.04199	0.4312	0.0077	0.9331

Table 31

90° Angle

Conc. (g/100ml)	I	S	C/S_∞
DMSO	0.3844		
0.20995	0.7386	0.3452	0.5927
0.16976	0.4575	0.0731	2.2977
0.104975	0.4836	0.0991	1.0593
0.04199	0.4294	0.045	1.0717

Table 32

100° Angle

Conc. (g/100ml)	I	S	c/S
DMSO	0.3479		
0.20995	0.7963	0.4484	0.4682
0.16976	0.4659	0.118	1.4234
0.104975	0.4983	0.1504	0.6980
0.04199	0.4034	0.0555	0.7566

Table 33

110° Angle

Conc. (g/100ml)	I	S	c/S
DMSO	0.3611		
0.20995	0.8531	0.4920	0.4267
0.16976	0.4880	0.1269	0.7555
0.104975	0.5088	0.1477	0.7107
0.04199	0.4346	0.0735	0.5713

Table 34

120° Angle

Conc.(g/100ml)	I	S	c/S
DMSO	0.4394		
0.20995	0.9856	0.5462	0.3844
0.16976	0.5433	0.1039	1.6166
0.104975	0.7122	0.2728	0.3848
0.04199	0.5808	0.1414	0.2970

A correction factor to account for the drift in time of the perspex block readings was found and applied to all further calculations.

Table 35

60° Angle

Conc. (g/100ml)	Av. reading	I	S	c/S
DMSO	2.9104	0.6068		
0.20995	4.5122	0.9408	0.3340	0.6285
0.16796	5.0156	1.0458	0.4390	0.3826
0.104975	3.4419	0.7177	0.1109	0.9469
0.04199	3.1095	0.6484	0.0416	1.0094

Table 36

70° Angle

Conc.(g/100ml)	Av. reading	I	S	c/S
DMSO	2.0781	0.4325		
0.20995	3.8065	0.7922	0.3597	0.5837
0.16796	2.8547	0.5941	0.1616	1.0393
0.104975	2.1701	0.4516	0.0191	5.4835
0.04199	2.1238	0.4420	0.0095	4.42521

Table 37

80° Angle

Conc. (g/100ml)	Av. reading	I	S	c/S
DMSO	1.9252	0.4036		
0.20995	3.5198	0.7530	0.3494	0.6009
0.16796	2.7223	0.5707	0.1671	1.0051
0.104975	2.1313	0.4468	0.0432	2.4289
0.04199	1.9869	0.4165	0.0129	3.2433

Table 38

90° Angle

Conc. (g/100ml)	Av. reading	I	S	c/S
DMSO	1.7622	0.4036		
0.20995	3.4780	0.7333	0.3618	0.5803
0.16796	2.5699	0.5418	0.1703	0.9861
0.104975	2.0998	0.4427	0.0712	1.4739
0.04199	1.9938	0.4204	0.0489	0.8592

Table 39

100° Angle

Conc. (g/100ml)	Av. reading	I	S	c/S
DMSO	1.5996	0.3373		
0.20995	3.7672	0.7943	0.4570	0.4595
0.16796	2.6075	0.5498	0.2125	0.7906
0.104975	2.2140	0.4478	0.1105	0.9499
0.04199	1.9015	0.4009	0.0636	0.6602

Table 40

110° Angle

Conc. (g/100ml)	Av. reading	I	S	c/S
DMSO	1.7482	0.3635		
0.20995	4.1843	0.8701	0.5066	0.4144
0.16796	2.5736	0.5352	0.1717	0.9784
0.104975	2.1942	0.4563	0.0928	1.1317
0.04199	2.0917	0.4350	0.0715	0.5876

Table 41

120° Angle

Conc. (g/100ml)	Av. reading	I	S	c/S
DMSO	2.0667	0.4383		
0.20995	4.6574	0.9787	0.5495	0.3821
0.16796	3.0367	0.6440	0.2057	0.8163
0.104975	2.9324	0.6219	0.1836	0.5717
0.04199	2.7170	0.5762	0.1379	0.3044

A Zimm plot was recalculated, molecular weight = 1.206×10^{-2} this being an eroneous result. C/S_{90} versus C was plotted for the results corrected for the perspex block (Fig. 18), and those corrected for both the perspex block and the readings (Fig. 19). C/S_{90} versus C intercept = 0.812, this and the refractive index was used to calculated molecular weight.

Fig. 18 Graph of C/S_{90} against Conc. of L-Aminodecanoic Acid Polymer

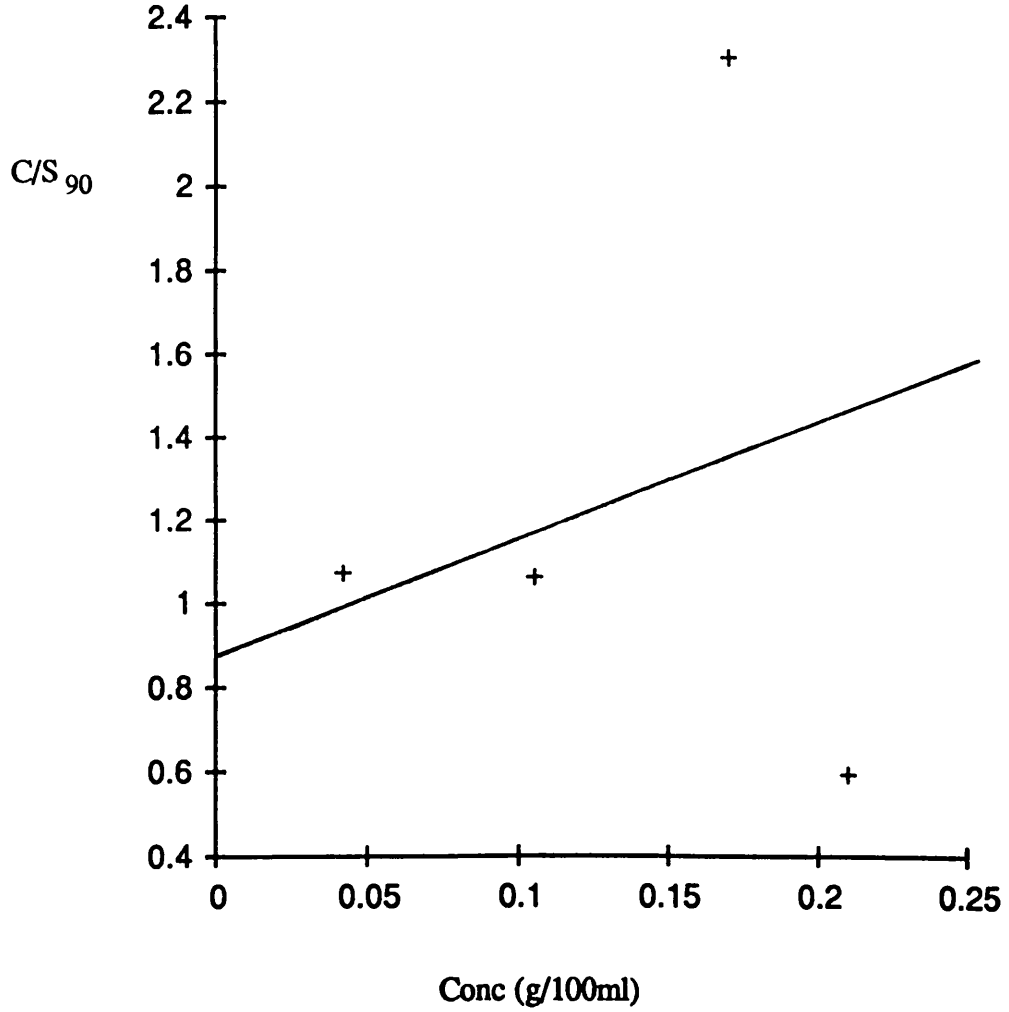
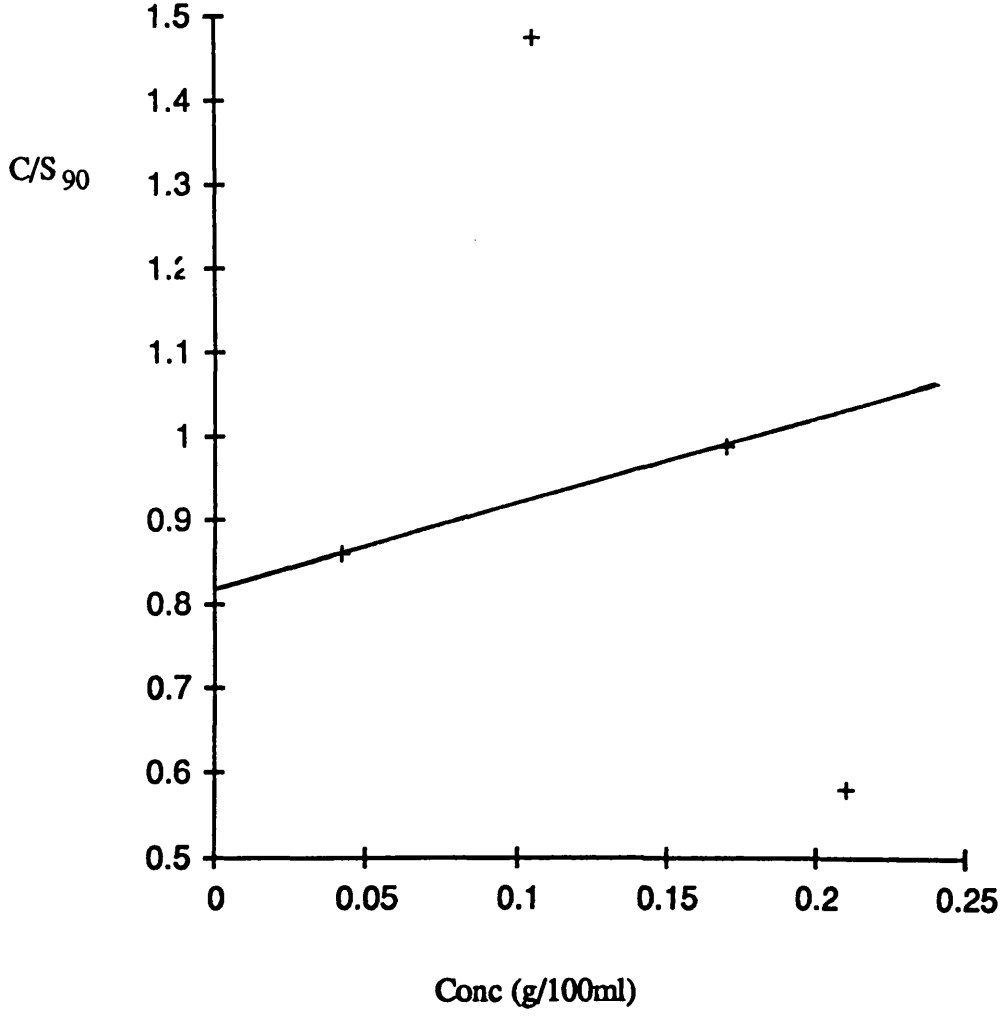


Fig. 19 Graph of C/S_{90} against Conc. of L-Aminodecanoic Acid Polymer (corrected)



Intercept = $I/M/\frac{4M^{2n}2}{2^4 2b}(dn/dc)^2$

λ = wavelength of light used in light scattering

b = constant 3.2189×10^{-3}

L = 6.028×10^{-3} Avogadro constant

no = 1.478

dn/dc = 6.22816×10^{-4}

8.012 = I/M/0.1127627

1/M = 0.0915

M = 10.92

The determination was abandoned due to eroneous results.

MOLECULAR WEIGHT DETERMINATION OF AMINODECANOIC ACID-LYSINE CO-POLYMER BY LIGHT SCATTERING

Aminodecanoic acid-lysine 1:1 co-polymer (XXXIII) (0.0206g) was dissolved in benzene (9.9mls) and the volume made up to 10ml with trifluoroethanol (TFE). In this experiment readings were only taken at a 90° angle and a secondary standard (C_6H_6 and TFE at the same dilution as in the polymer solutions) was used.

Table 42

Solution	Backgrd.		Block		2° stand	
(g/100ml)	before		before		before	
SOLVENT	0.23	0.23	5.78	5.77		
0.01015	0.24	0.24	5.75	5.79	3.09	3.10
0.0203	0.26	0.24	5.74	5.73	3.01	3.02
0.05075	0.25	0.25	5.74	5.76	3.10	3.10

Solution	Readings			2° Stand		Block		Backgrnd.	
(g/100ml)				after		after		after	
SOLVENT	3.21	3.08	3.13			5.79	5.79	0.24	0.23
0.01015	7.18	7.22	7.12	3.11	3.08	5.75	5.72	0.25	0.24
0.0203	8.19	8.21	8.26	3.09	3.08	5.75	5.75	0.26	0.25
0.05075	9.16	9.14	9.20	3.06	3.04	5.75	5.72	0.26	0.25

Table 43

Solution	Av. Back.	Av. Block.	Av.2° Stand.	Av. Read.	Av.2° Stand.	Av. Block	Av. Backg.
SOLVENT	0.23	5.775		3.14		5.79	0.235
0.01015	0.24	5.77	3.095	7.1733	3.095	5.735	0.245
0.0203	0.25	5.735	3.015	8.22	3.085	5.75	0.255
0.05075	0.25	5.755	3.10	9.1667	3.05	5.735	0.255

Table 44

SOLUTION	Av. Backgrd.	Av. Perspex block	Av.2° stand.	Av. Read.
SOLVENT	0.2325	5.7825	3.14	3.14
0.01015	0.2425	5.7525	3.095	7.1733
0.0203	0.2525	5.7425	3.05	8.22
0.05075	0.2525	5.745	3.075	9.1667

Table 45

SOLUTION	I_{90}	S_{90}	c/S_{90}
SOLVENT	0.5299		
0.01015	1.2778	0.73398	0.01383
0.0203	1.4513	0.9274	0.02189
0.05075	1.622971	1.0091	0.04617

C/S_{90} against C was plotted (Fig. 20).

Refractive Index calculation of polymer (XXXIII) were made using a blue laser.

The Refractive Index of the polymer was found using a blue laser.

Fig. 20 Graph of C/S_{90} against Conc. of Aminodecanoic Acid - Lysine Copolymer 1:1

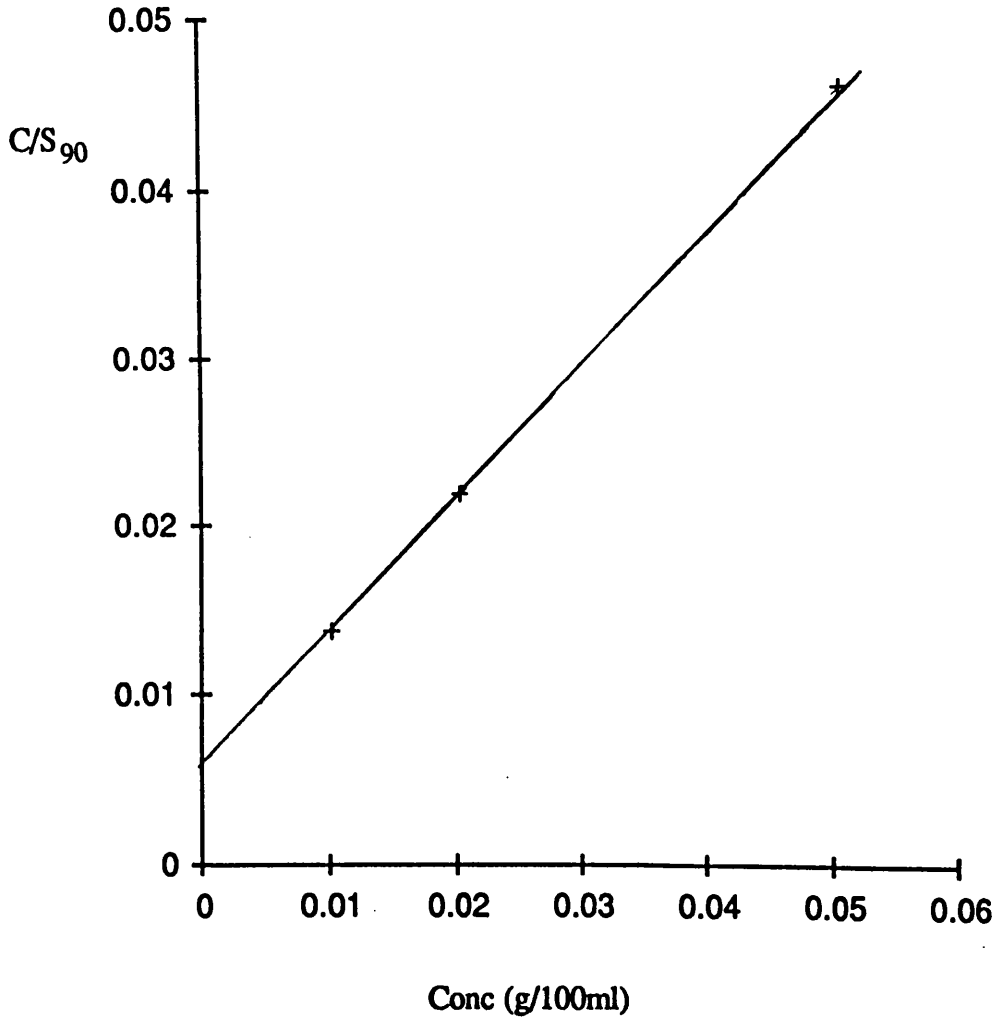


Table 46

SOLUTION (g/100ml) Benzene/TFE	d1					d2			
(9.9ml.0.1m)	4.655	4.675	4.624			4.45	4.40	4.447	
0.00714	3.449	3.445	3.478	3.435	3.425	5.715	5.90	5.621	5.64
0.0051	4.592	4.613	4.621	4.610	4.630	4.545	4.50	4.471	4.46
0.0102	1.865	1.872	1.86	1.85	1.849	7.078	7.018	7.025	7.02
0.0204	0.545	0.528	0.555	0.56	0.550	8.214	8.239	8.220	8.20

Table 47

SOLUTION (g/100ml)	Av. d1	Av.d2	d2-d1	Δd	Δn
SOLVENT	4.6513	4.4323	-0.219		
0.00714	3.4464	5.6904	2.244	2.463	2.38295×10^{-3}
0.0051	4.6132	4.4948	-0.1184	0.1006	9.73305×10^{-5}
0.0102	1.8592	7.0352	5.176	5.395	5.21966×10^{-3}
0.0204	0.5476	8.2164	7.6688	7.8878	7.63145×10^{-3}

Δn versus C was plotted Fig. 21.

dn/dc = 0.402945 was used to calculate molecular weight. C/S_{∞} Intercept (From Fig. 20) = 6.6×10^{-3}

$$6.6 \times 10^{-3} = 1/M \frac{4M^2 n_o^2 (dn/dc)^2}{2^4 Lb}$$

$$1 = 1260.27\ M = 7.93 \times 10^{-4}$$

This molecular weight is erroneous, therefore light scattering was abandoned.

4. Rayleigh Interference Optics and Schlieren Optics

The aminodecanoic acid polymer was studied by Rayleigh Interference Optics using a Model E. The polymer was solubilised in toluene and 5% TFE with ultrasonication in a 12mm d.s. cell, with virtually no deviation of the fringes being obtained even at speeds of 50,000 rpm. XXXII was examined with Schlieren optics

Fig. 21 Graph of △n against Conc. of Aminodecanoic Acid - Lysine Copolymer 1:1

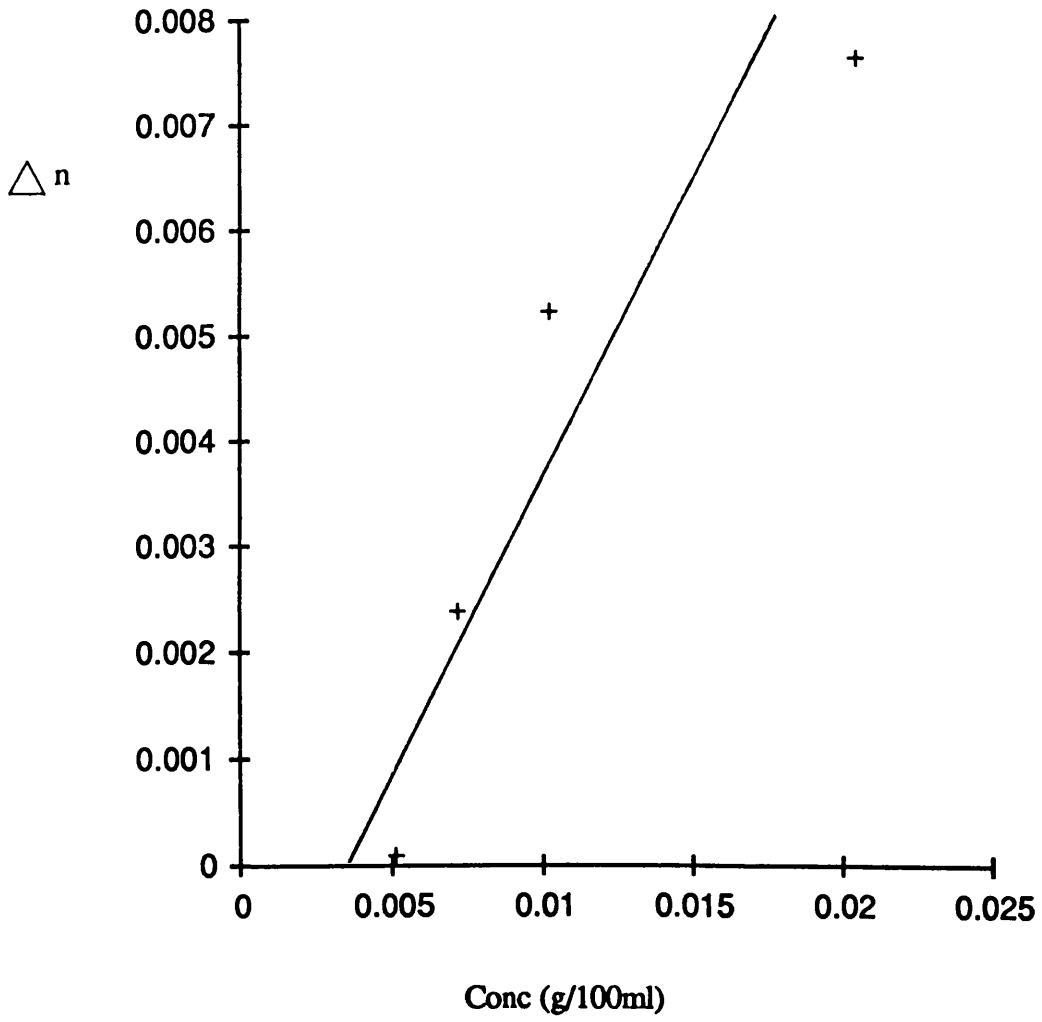
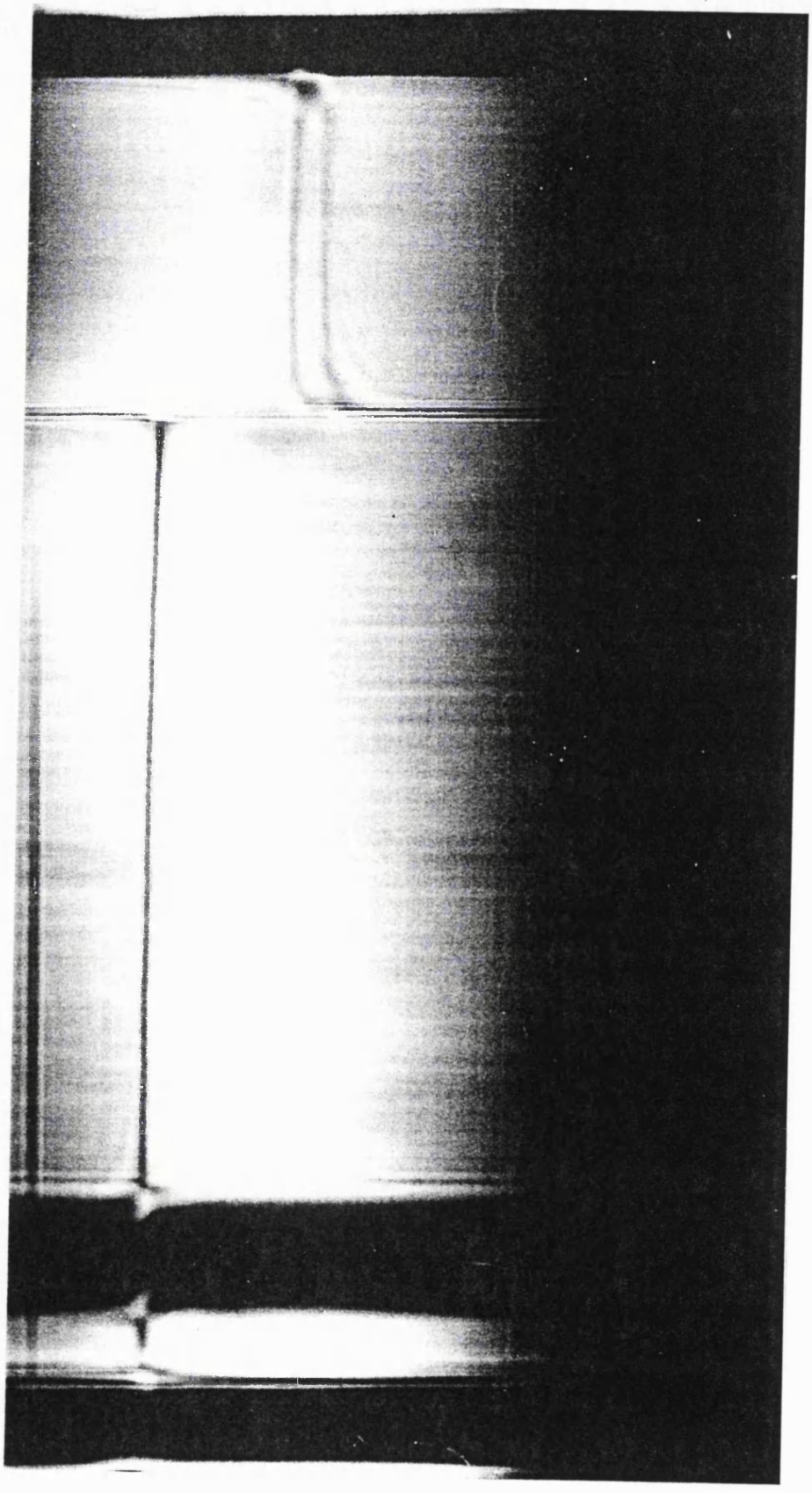


Figure 22



using an MSE Centriscan again very little movement was seen, whist using the MkII MSE Analytical 40,000 rmp no boundary resolved, but the Schlieren trace showed negative deflection both near to the base and near to the meniscus. After one hour the trace became time invariant, with a constant but small negative deflection. Fig. 22.

Again this gave no satisfactory results, so the determination of the molecular weight of the polymers synthesised was abandoned.

GENERAL EXPERIMENTAL DETAILS

INFRA RED SPECTRA

Infra-red spectra were recorded with a Perkin Elmer 84 Spectrophotometer.

NUCLEAR MAGNETIC RESONANCE (N.M.R) SPECTRA

N.M.R. spectra were recorded on a Bruker WP80 SY 80Mhz Spectrometer with tetramethylsilane as internal standard 8 = 0 , a Varian XL300 and Bruker AM500 at 300 and 500 MHz respectively. The following abbreviations are used for the description of signals: s = singlet, d = doublet, t = triplet, q = quartet and m = an unresolved multiplet.

MASS SPECTRA

Mass spectra were ran on a VG Analytical ZAB SE instrument using fast atom bombardment.

MELTING POINTS

Melting points were determined using an Electrothermal melting point apparatus and are uncorrected.

AMINO ACIDS

Amino acids used were of l-configuration unless otherwise stated.

MOLECULAR WEIGHT DETERMINATION

Rayleigh Interference Optics carried out by Model E Schlieren optics determined by MSE Centriscan Light Scattering was carried out on a Brice-Phoenix

CHROMATOGRAPHY

The silica gel used for analytical layer chromatography (t.l.c.) was Kieselgel PF_{254} applied as a 0.5m layer.

Plates used for preparative layer chromatography were 20 x 20 x 1mm thick using Kieselgel $PF_{254,366}$.

Silica for column and flash column chromatography was Kieselgel G.

Microanalyses were carried out by Mr W Baldeo at the School of Pharmacy, University of London.

T.L.C. Systems:

System A	10% vv/ Methanol 90% chlorocorm
System B	30% v/v Chlorform 70% ethylacetate
System C	5% v/v methanol 95% chloroform
System D	70% Ethyl Acetate 30% chloroform
System E	40% Ethyl Acetate 60% chloroform
System F	30% Hexane 70% ethyl acetate
System G	30% Hexane 70% diethyl ether
System H	10% Ethyl acetate 40% chloroform 50% petroleum ether
System I	30% Methanol 70% chloroform
System J	50% Ethyl acetate 50% chloroform

EXPERIMENTAL

LIPIDIC AMINO ACIDS

DIETHYLOCTYLACETAMIDO MALONATE (I)

Sodium metal (7.46g, 0.32M) was slowly added to freshly dried ethanol (155mls) to produce sodium ethoxide to which diethylacetamido malonic ester (66.80g, 0.36M) was added.

On dissolution of the malonic ester, 1-bromooctane (65.52g, 0.34M) was added to the mixture and this was refluxed for 12 hrs. The reaction was followed by TLC. Solvent mixture A.

The crude reaction mixture was poured into an ice-water mixture (250 mls) to give diethyloctylacetamido malonate as a white needle shaped crystalline product which was dried in vacuo (96.33g, 95.4%) m.p. 48°C (lit. m.p. 52°C) from ether

υ max 3440, 3300, 2930, 2860, 1745, 1645, 1510, 1440, 1365, 1260, 1225, 1190, 1015

NMR ($CDCl_3$) 0.87 (3H,t.CH_3 alkyl. 6.8Hz); 1.20 (18H,m,CH_2 x 6 alkyl,CH_3 x 2 ethyl); 2.01 (3H, s,OCH_3); 2.30 (2H, m,$C_\beta H_2$ alkyl); 4.20 (4H, 2 x CH_2 ethyl); 6.76 (1H, s,NH)

FOUND: C,62.18; H,9.51; N,4.43%

CALCULATED FOR: $C_{17}H_{31}NO_5$ C,62.01; H,9.42; N,4.26%

MS, M/Z (%) $C_{17}H31NO_5$ (329) 329 (1.3), 256 (63.3), 214 (100), 210 (45.5), 171 (10.4), 141 (14.3), 43 (29.9).

dl-α-2-AMINODECANOIC ACID (II)

I (18.95g, 0.06M) was refluxed in concentrated hydrochloric acid (HCl) (58mls) for 7 hrs. On cooling to room temperature the aminodecanoic acid hydrochloride salt was dissolved in temperature, the aminodecanoic acid hydrochloride salt was dissolved in a 1:1 ethanol-water mixture, and the free acid was precipitated with ammonium hydroxide solution (25%), at pH7 to 8. The amino acid was collected by vacuo, and washed with water, ethanol, and ether (10.43g, 93%)

m.p. 259°C (dec.) (lit. m.p.260°dec).

υ max 3000 (broad), 2920, 2840, 2115, 1580, 1405, 1315, 1185, 850, 660.

FOUND C,64.29; H,11.57; N,7.36%

CALCULATED FOR: $C_{10}H_{21}NO_2$ C,64.17; H,11.76; N.7.49%

MS M\Z (%) 188 (100%), (M^+ + H) 172 (5.52%), 142 (68.66%), 67 (8.28%.

CHLOROACETAMIDO-α-2-AMINODECANOIC ACID (III)

II (4.66g, 0.024M), was suspended in ethyl acetate(EtOAc) (100mls) and freshly distilled chloroacetyl chloride (4.33g, 0.038M) was added. The suspension was refluxed for 2hrs until dissolution had occurred and any remaining solid was removed by filtration. The filtrate was evaporated to give a yellowish oil, which readily solidified giving creamy white crystals (6.32g, 100%). From pet. ether 40-60 m.p. 84-85°C (5.66g, 89.5%) a white crystalline solid was obtained (lit. m.p. 92°C).

υ max. 3370, 2910, 2610, 2560, 1720, 1625, 1530, 1400, 1240, 1220, 1150, 1000, 900, 780, 650.

NMR ($CDCl_3$) 0.8 (3H, t. CH_3 alkyl) 63Hz; 1.3 (12H, s.CH_2 x 6 alkyl); 1.9 (2H, m.$C_\beta H_2$ alkyl); 4.1 (2H, s.CH_2Cl); 4.7 (1H, s.$C_\alpha H$); 7.05(1H, d.NH) 7Hz; 8.2 (1H, s.COOH)

FOUND: C,54.70; H,8.46; N,5.45%

CALCULATED FOR: $C_{12}H_{22}NO_3Cl$ C,54.65; H,8.35; N,5.31%

MS m/z (%) $C_{12}H_{22}NO_3Cl$ (265 or 263) 266 (5.6%) (M^++1) 264 (16.7%) (M^++1), 228 (10.4%), 220 (33.3%), 218 (100%), 186 (14.6%), 184 (29.2%), 153 (16.7%), 151 (47.9%), 135 (14.6%), 133 (43.8%)

1-α-2-AMINODECANOIC ACID (IV)

II (2.15g, 8.16 x 10^{-3}M) was dissolved in distilled water (400 mls) (a little heat was required for this). The pH was adjusted to 7.2 by the addition of 2N lithium hydroxide (LiOH), the solution was incubated at 38°C for ½hr for equilibration. Acylase I enzyme (0.300g), was added to the solution and the mixture was incubated

for 48hrs.

After the reaction had proceeded for 24hrs the pH of the solution which had dropped was readjusted to 7.2 and a further amount of Acylase I (0.100g) was added, and the incubation continued for a further 24hrs. The l-aminodecanoic acid produced was collected by filtration and washed firstly with water, then ethanol and finally ether. This was then dried (0.68g, 89%) m.p. 259°C (dec) (lit. m.p. 260°C) $[\alpha]=+30.7°$ in glacial acetic acid (lit. $[\alpha]=+31°$).

υ max 3000, 2920, 2840, 2115, 1580, 1510, 1405, 1315,
1185, 850, 660 as for all aminodecanoic acid.

FOUND: C, 64.13; H,11.30; N,7.48%.

CALCULATED FOR: $C_{10}H_{21}NO_2$ C,64.16; H,11.11; N,7.67%

MS m/z (%) $C_{10}H_{21}NO_2$ (187) 188 (M^++1) (100%), 172 (4.83%),142 (40.83%), 67 (4.14%)

<u>d-α-2-AMINODECANOIC ACID</u> (V)

Following the removal of IV the pH of the filtrate was reduced to 2 with concentrated hydrochloric acid (conc. HCl) to give a white precipitate which was then collected by filtration. The solid obtained was dissolved in acetone, and any insoluble material removed by filtration.

The solvent was evaporated to yield a white solid (1.2g) which was then refluxed with HCl (3N, 36mls) for 2 hrs. On cooling the solution was neutralized with ammonium hydroxide soltuion to give d-aminodecanoic acid (0.71g, 93%) m.p. 259°C (dec). $[\alpha]=-30.2°$ (literature value=31°) in glacial acetic acid.

υ max 3000 (broad), 2920, 2840, 2115, 1580, 1405, 1315,
1185, 850, 660 as for all aminodecanoic acid.

FOUND: C,64.13; H, 11.30; N, 7.48%

CALCULATED FOR: $C_{10}H_{21}NO_2$ C,63.92; H,11.32; N,7.45%

MS m/z (%) $C_{10}H_{21}NO_2$ (187) 188 (100%) (M^++1), 172 (2.76%), 142 (35.86%), 67 (2.07%)

1-α-2-AMINODECANOIC ACID METHYL ESTER HYDROCHLORIDE SALT (VI) [Method A]

Thionyl chloride ($SOCl_2$) (1.7g, 0.041M) was slowly added to ice-cooled methanol (170 mls). To the methanolic solution IV (1.7g, 9.09 x 10^{-3}M) was added, and the reaction was refluxed for 8 hrs., the end point being determined by T.L.C. (system A). The solvent was evaporated yielding a white solid m.p. 127°C from acetone (1.57g, 85.9%).

υ max ($CHCl_3$ film) 3350 (broad), 2960, 2930, 2860, 1750, 1600, 1510, 1470, 1440.

NMR ($CDCl_3$) 0.87 (3H, t. CH_3 alkyl, 6.5Hz); 1.22 (10H,s. CH_2 alkyl); 1.43 (1H, m. $C_\gamma H$ alkyl); 1.53 (1H, m. $C_\gamma H$ alkyl); 2.03 (2H, q.$C_\beta H_2$, 7.8Hz); 3.8 (3H, s. CH_3 methyl ester); 4.05 (1H, t. $C^\alpha H$,m 7.8Hz); 8.83 (3H, s. NH_3^+)

FOUND: C,55.30; H,10.01; N,5.77%

CALCULATED FOR: $C_{11}H_{24}NO_2Cl$ C,55.57; H,10.17; N.5.89%

MS m/z (%) $C_{11}H_{24}NO_2Cl$ 202 (1.57%) (M^++1-HCl), 142 (100%), 88 (5.9%), 83 (3.94%), 74 (7.09%). 69 (7.9%), 56 (12.6%).

TERTIARY-BUTOXYCARBONYL-1-α-2-AMINODECANOIC ACID (VII)

SOLUTION A- To a 1:1 mixture of a tert-butanol : water mixture (40mls) with the pH previously adjusted to 11-12 with sodium hydroxide (NaOH) solution (5N) IV (2g, 0.0107M) was added and the pH again readjusted to 11-12.

SOLUTION B- Ditertiarybutyloxycarbonyl (3.5g, 0.017M) was dissolved in tert-butanol. (4mls).

Solution B was added to solution A at room temperture wth the pH being continually monitored and maintained between 11-12 with NaOH (5N). Once the solution had cleared and there was no rapid pH drop, the soltuion was stirred for a further 30 mins. at room temperature. Water (H_2O) (20mls) was added to the reaction mixture and the pH adjusted to 4 with solid citric acid, the product being extracted with EtOAc.

The solvent was then dried over anhydrous sodium sulphate ($Na2SO_4$) and evaporated to yield a clear oil (3.03g, 99.34%).

υ max 3450, 3310, 2950, 2870, 1815, 1630, 1500, 1480, 1380.

NMR ($CDCl_3$) 0.87 (3H, t. CH_3 alkyl, 5.92Hz); 1.3 (10H,s. CH_2 alkyl); 1.45 (9H, s. $(CH_3)_3$ BOC); 1.66 (2H, m. $C_\gamma H_2$ alkyl); 1.84 (2H, m. $C_\beta H_2$ alkyl); 4.30 (1H, m. $C_\alpha H$); 4.97 (1H, d. NH, 7.89Hz); 6.10 (1H, s. COOH)

MS m/z (%) 473 (23), 472 (M+2Na-H) + (86), 450 (M+NH) (33), 393 (15), 373 (22), 372 (100), 370 (23), 282 (23), 282 (14), 176 (23), 119 (10), 57 (26).

GENERAL METHODS OF PEPTIDE SYNTHESIS

1 Mixed Anhydride Method of Peptide Synthesis [B]

Freshly distilled base (1 equivalent) was added to the amino group protected amino acid or peptide (1 equivalent) dissolved in chloroform or dichloromethane. This solution was stirred at -10°C (ice-salt bath) for 10 minutes to equilibrate and then freshly distilled isobutyl chloroformate (IBCF) (1.1 equivalents) was added. To this the carboxyl group protected amino acid or peptide (1 equivalent) plus base (1 equivalent) in solvent was added and the reaction mixture was then stirred overnight.

When the reaction was complete as determined by TLC the misture was extracted:

(i) 1N HCl

(ii) 0.1N NaOH solution or saturated sodium bicarbonate ($NaHCO_3$)

(iii) brine

The extraction was carried out several times to remove any colouration, the solvent was dried over anhydrous Na_2SO_4 and evaporated to yield the required peptide.

The base can be N-methyl morpholine (NMM), triethylamine (TEA) or diisopropylethylamine (DIPEA).

2. DICYCLOHEXYLCARBODIIMIDE IN PEPTIDE SYNTHESIS (DCC) [C]

The amino group protected amino acid or peptide (1 equivalent) was dissolved in chlorform ($CHCl_3$) or dichloromethane (DCM) then DCC (1.1 equivalent) was added. To this solution, the salt of the amino acid or peptide (1 equivalent) to be coupled plus base (1 equivalent) were added, and this was allowed to stir at room temperature for 5-6 hours, when the reaction had gone to completion as determined by TLC, any dicyclohexyl urea (DCU) produced during the reaction was removed by filtration and the reaction mixture extracted:-

(i) 1N HCl

(ii) 0.1N NaOH solution or saturated $NaHCO_3$

(iii) brine

The solvent was then dried over anhydrous sodium sulphate and removed to yield the required peptide.

Although the urea produced during the reaction can be removed by filtration, due to its partial solunbility in the solvents used for coupling there is always some of this product left as an impurity. In order to overcome this DCC can be replaced with a water soluble carbodiimide such as N-ethyl-N'-(3 - dimethylaminopropyl)carbodiimide, (EDAC) this procudes a water soluble urea which can be removed readily by extraction.

PHENYLALANINE METHYL ESTER HYDROCHLORIDE SALT (VIII)

$SOCl_2$ (2.21g, 0.186M), in MeOH (200mls), and phenylalanine (Phe) (2g, 0.012M) were reacted as in [A] giving a white solid. m.p.= 157-159° (literature value 158-162°) from EtOAc (1.84g, 84.8%).

υ max 3045, 2930, 2885, 1740, 1545, 1370, 750.

NMR ($CDCl_3$) 3.30 (2H, m. CH_2 phenyl group); 3.65 (3H, s. CH_3 methyl ester); 4.30 (1H, m. $C^{\alpha}H$); 7.30 (5H, s. C_6H_5 phenyl group); 8.60 (3H, s. NH_3^+ broad)

FOUND C,55.27; H,6.45; N,6.51%

CALCULATED FOR $C_{10}H_{14}NO_2Cl$ C,55.69; H,6.54; N6, 49%

MS m\z (%) M^+ 180 (100%) 164, (3.33%), 131 2.56%), 120 (39.5%), 103 (3.75%), 88 (4.58%), 77 (1.25%)

N-α-TERTIARYBUTOXYCARBONYLPHENYLALANYLGLYCINE METHYL ESTER (IX)

Glycine methyl ester hydrochloride salt (1.00g, 7.97 mM) (DIPEA) (1.03g, 7.97mM) t-BOCphenylalanine (Boc Phe) (2.14g, 8.08mM) and DCC (1.81g, 8.77mM) in DCM were reacted via method C. The reaction was monitored by TLC (system B) yield = 2.67g (99.7%). Purification was carried out using silica gel chromatography giving a white solid yield = 2.14g (79.9%) mp 93-95°C.

υ max 3390. 3330, 2990, 2935, 1750, 1685, 1530, 1370,
1280, 1225, 1210, 1155, 950, 750.

NMR ($CDCl_3$) 1.40 (9H, s. (CH_3)$_3$ Boc); 3.08 (2H, m. CH_2 Phe);
3.73 (3H, s. CH_3 methyl ester); 4.00 (2H, oct. CH_2 Gly, 17.6Hz, 6.6Hz);
4.40 (1H, m. $C_\alpha H$ Phe); 4.97 (1H, m. NH Phe); 6.4 (1H, s. NH Gly); 7.26 (5H, m. C_6H_5 Phe)

FOUND C,60.68; H,7.21; N,8.59%

CALCULATED FOR $C_{17}H_{24}N_2O_5$ C,60.70; H7.19; N,8.33%

MS m/z (%) $C_{17}H_{24}N_2O_6$ (352) 337 (15.17%), 281 (46.21%), 265 (2.76%), 249 (2.76%), 237 (77.93%), 218 (2.07%), 190 (4.14%), 164 (10.34%), 146 (7.59%), 132 (2.45%) 120 (100%), 104 (2.76%), 90 (5.52%)

M^+ expected = 336

N-α-TERTIARY-BUTOXYCARBONYL-N-ϵ-CARBOBENZOXYLYSYL-α-2-AMINODECANOIC ACID METHYL ESTER (X)

NMM (1.08g, 10.70mM), t-BOC-N-ϵ-carbobenzoxylysine (tBoc-N-ϵ-CBz Lys) (1.57g, 4.13mM), IBCF (0.81g, 5.93mM) and VI (1.00g, 4.98mM) in $CHCl_3$ (25mls) were reacted via method [B] the reaction being followed by TLC (system A)

Yield = 1.87g (80.6%) mp = 72-74°C

υ max 3340, 3300, 3080, 3040, 2920, 2860, 1740, 1690,

1680, 1665, 1540, 1520, 1450, 1370, 1320, 1275, 1265, 1240, 1170, 1140, 1050, 1010, 750, 710, 700.

NMR ($CDCl_3$) 0.87 (3H, t. CH_3 alkyl, 6.34Hz); 1.2 (12H,m.6 x CH_2 alkyl C_{10}); 1.45 (9H, s. $(CH_3)_3$ Boc); 1.53 (1H, m. $C_\beta H$ C_{10}); 1.66 (1H, m.$C_\beta H$ C_{10}) 1.81 (4H, m. 2 x CH_2 lys); 3.2 (2H, m. $C_\beta H_2$ lys); 3.67 (3H, s. CH_3 methyl ester); 4.05 (1H, m. $C_\alpha H$ C_{10}); 4. 15 (2H,m,$C_\epsilon H_2$ys; 4.6 (1H, m. C_α H lys); 4.95 (1H, s. NH lys); 5.08 (2H, s. CH_2 benzyl group); 5.10 (1H, s. NH lys ϵ); 6.50 (1H, d. NH C_{10}, 4.5Hz); 7.3 (5H, m. Phenyl group)

FOUND C,63.88; H,8.79; N,7.44

CALCULATED FOR $C_{30}H_{49}N_3O_7$ C,63.90; H,8.77; N,7.46

MS m/z (%) $C_{30}H_{49}N_30_7$ (56) 564 (16.1) M+1) 550 10.3), 534 (8.9), 464 (82.4), 455 (11.9), 429 (15.5), 357 (8.3), 33.1 (7.9), 324 (15.1), 295 (12.8), 282 (11.7), 253 (10.3), 170 (17.9), 154 (9.1), 91 (17.1).

N-α-TERTIARYBUTOXYCARBONYL-N-ϵ-CARBOBENZOXYLYSYL GLYCINE ETHYL ESTER (XI)

XI is as method B. BOC CBz lys 6.47mM), NMM (1.30g, 2.94mM), IBCF (0.97g, 7.11mM) and glycine ethyl ester hydrochloride (0.90g, 6.47mM) were reacted. Following extraction and chromatographic purification (system C) yield = 2.47g (82.1%) mp 85-87°C.

υ max 3380, 3325, 2990, 2940, 1750, 1685, 1655, 1280, 770

NMR ($CDCl_3$) 1.26 (3H, t. CH_3 ethyl ester, 6.67Hz); 1.44 (9H, s.$(CH_3)_3$ Boc); 1.6 (4H, m. 2 x CH_2 lys); 3.20 (2H, q. $C_\beta H_2$ lys, 5Hz);4.06 (1H, m. $C_\alpha H$ gly); 4.12 (5H, m. CH_2 ethyl , $C_\epsilon H_2$ lys and gly $C_\alpha H$);4.20 (1H, m. $C_\alpha H$ lys); 4.93 (1H, s. broad lys NH urethane); 5.14 (3H, s. CH_2 benzyl group NH, lys ϵ); 6.59 (1H, s. broad NH, gly); 7.36 (5H, m. phenyl group)

FOUND C,52.21; H,7.35; N,8.97

CALCULATED FOR $C_{23}H_{35}N_3O_7$ C,59,34; H,7.58; N,9.03; 0.24.06

MS m/z (%) $C_{23}H_{35}N_3O_7$ (465), 466 (19.7) (M^{+1}), 453 (7.1), 367 (45.3), 353 (8.0), 246 (20.5), 220 (10.3), 206 (8.4), 177 (6.7),161 (7.1), 91 (11.9).

N-α-TERTIARYBUTOXYCARBONYL-α-2-AMINODECANOYL-GLYCINE METHYL ESTER (XII)

VII (1.00g, 3.48mM) NMM (0.70g, 6.94mM), IBCF (0.52g, 3.81 mM) and glycine methyl ester hydrochloride (0.48, 3.48 mM) were reacted. Following extraction and evaporation yield = 1.10g (88.3%) mp 71-73°C.

υ max 3385, 3335, 2995, 1750, 1690, 1655, 1370, 1270, 1160, 1100, 710

NMR ($CDCl_3$) 0.98 (3H, t. CH_3 alkyl chain C_{10}); 1.36 (12H,s.6 x CH_2 C_{10}); 1.40 (9H, s. $(CH_3)_3$ Boc); 1.90 (2H, m. C_β H_2 C_{10}); 3.71 (3H, s. CH_3 Me esters); 4.00 (2H, m. CH glycine); 4.18 (1H, m. $C_\alpha H$ C_{10}); 5.00 (1H, s. NH C_{10}); 6.35 (1H, s. NH gly)

FOUND C,58.82; H,9.73; N,7.98

CALCULATED FOR $C_{17}H_{34}N_2O_6$ C,58.93; H,9.89; N,8.09; 0,23.09

MS m/z (%) $C_{17}H_{34}N_2O_6$ 362, 347 (23.9), 333 (8.1), 247 (44.7), 234 (7.9), 218 (15.2), 204 (9.3), 191 (8.3), 163 (7.4),147 (11.5), 129 (18.8), 115 (13.5), 92 (9.8), 57 (17.6)

DEPROTECTION OF PEPTIDES

1. Ester Hydrolysis [D]

Removal of the ester groups used to protect the peptides was carried out by alkali hydrolysis . Three equivalents of alcoholic potassium hydroxide (KOH) solution (1:1 ethanol-water) were used as compared to one equivalent of peptide. The solution was stirred at room temperture for approximately 15 minutes with the reaction being followed by TLC. When hydrolysis had been completed the pH of the reactants was reduced to 3 using 1N HCl and the solution was extracted with EtOAc. The EtOAc solution was then dried over Na_2SO_4 and evaporated in vacuo, to give the required compound.

2. Tertiarybutyloxycarbonyl Group Removal [E]

(i) To each gram of the peptide to be deprotected a mixture (1:1) of trifluoroacetic acid (TFA) (freshly distilled) and $CHCl_3$ (30mls) was added. The reactants were stirred at room temperature for 1.5hrs and the reaction was followed by TLC. Once the peptide had been deprotected the solvent was evaporated in vacuo and the removal of TFA was effected by the use of ether, leaving the trifluoroacetate salt.

(ii) Deprotection of the peptides was also effected by dissolving in dry DCM (freshly distilled) with dry HCl gas being bubbled through the soltuion, for 30 minutes, the reaction again being monitored by TLC. (The gas was produced by the addition of sulphuric acid to ammonium sulphate). When the reaction had reached completion the solvent was evaporated in vacuo leaving the hydrochloride salt.

N-α-TERTIARYBUTOXYCARBONYL-N-ε-CARBOBENZOXYLYSYL-2-α-AMINODECANOIC ACID (XIII)

(X) (0.5g, 8.88mM) was deprotected by alkali hydrolysis method [D] (KOH, 1M, 0.9mls). The reaction being followed by TLC (system C). Following pH adjustment and extraction yield = 0.42g (86.2). NMR indicated the loss of the methyl group.

N-α-TERTIARYBUTOXYCARBONYL-N-ε-CARBOBENZOXYLYSYL-GLYCINE (XIV)

(XI) (0.75g, 1.61mM) was deprotected using alcoholic method [D] KOH (1M, 5mls) and followed by TLC (system C). Yield = 0.6g (84.8%). Characterisation by IR indicated the loss of the ester carbonyl and the appearance of the acid peak.

N-ε-CARBOBENZOXYLYSYL-GLYCINE ETHYL ESTER TRIFLUOROACETATE SALT (XV)

(XI) (1.00g, 2.2mM) was deprotected via the TFA method [E]. Yield =

1.05g (100%) TLC (system A) indicated the disappearance of starting material. IR showed the loss of ester carbonyl and the appearance of the acid carbonyl.

PHENYLALANYL-GLYCINE METHYL ESTER HYDROCHLORIDE (XVI)

IX (1.98g, 5.89mM) in DCM (20mls) was deprotected with HCl gas (method [E]ii) the reaction was followed by TLC (system D). Yield following evaporation = 1.60g (100%). IR indicated the disappearance of the urethane carbonyl.

N-α-TERTIARYBUTOXYCARBONYL-N-ϵCARBOBENZOXYLYSYL-2-α-AMINODECANOYL-GLYCINE METHYL ESTER (XVII)

XIII (0.08g, 0.182mM), NMM (0.036g, 0.36mM) ICBF (0.023g, 0.20mM) and glycine methyl ester (0.98g, 0.20mM) in $CHCl_3$ (1.5ml) were reacted as in method B. TLC (system A and C) indicated a small impurity. Purification was carried out by preparative TLC (system C). Yield = 0.013g (83.3%) mp 101°C
υ max 3340, 3285, 3090, 2920, 1760, 1695, 1655, 1530. 1265

NMR ($CDCl_3$) 0.98 (3H, t. CH_3 C_{10} alkyl chain); 1.24 (16Hs 6 x. CH_2 C_{10} 2x CH_2 lys); 1.40 (9H, s. $(CH_3)_3$ Boc); 1.68 (2H, m. $C_\beta H_2$ lys); 1.87 (2H, m. $C_\beta H_2$ C_{10}); 3.19 (2H, m. $C_\epsilon H_2$ lys); 3.68 (3H, s.CH_3 Me ester); 4.04 (3H, m. CH_2 gly, $C_\alpha H$ C_{10}); 4.50 (1H, m. $C_\alpha H$ lys); 5.03 (2H, m. NH_αlys NH ϵ lys);5.09 (2H, s. CH_2 benzyl); 6.50 (1H, d. NH C_{10}); 6.80 (1H, s. NH gly);7.32 (5H, m. C_6H_5 benzyl group)

MS m/z (%) $C_{32}H_{52}N_4O_8$ (620), 621 (M+1) (20.1), 607 (5.3), 521 (34.7), 486 (12.5),471 (15.8), 432 (5.3), 324 (7.1), 297 (15.2), 232 (8.6), 135 (7.9), 112 (4.2), 91(89.7), 82 (6.8), 56 (16.4).

N-α-TERTIARYBUTOXYCARBONYL-N-ϵ-CARBOBENZOXYLYSYL-α-2-AMINODECANOYL-α-2-AMINODECANOIC ACID METHYL ESTER (XVIII)

XIII (0.42g, 7.64mM), NMM (0.16g, 1.58mM) IBCF (0.11g, 0.81mM) and VI (0.16g, 0.67mM) in $CHCl_3$ (7mls) were reacted via method [B]. Yield = 0.45g (96.6%) mp = 105-108°C.

υ max (thin film) 3340, 3300, 3080, 3040, 2920, 2860, 1740, 1690, 1680, 1665, 1450, 1320, 1265, 1170, 1140, 1010, 750, 770, 700.

NMR ($CDCl_3$) 0.98 (6H, t. CH_3 2 x C_{10}); 1.36 (24H,s. 2 x CH_2 alkyl chain C_{10}); 1.41 (9H, s. $(CH_3)_3$ Boc); 1.65 (4H, m.2 x CH_2 lys); 1.90 (4H, m.2 x $C_\beta H_2$ C_{10}); 3.18 (2H, q. $C_\beta H_2$ lys); 3.70 (3H, s. CH_3ester); 4.07 (2H, m. $C_\alpha H$ C_{10}); 4.59 (1H, m. $C_\alpha H$ lys); 4.92 (1H, broad s, NH lys); 5.08 (2H, s. CH_2 benzyl group); 5.11 (1H, s. NH ϵ lys); 6.48 (1H, d. NH C_{10}); 6.52 (1H, d.NH C_{10})7.31 (5H, m. C_6H_5)

FOUND C,65.53; H,9.36; N,7.65; 0.17.47

CALCULATED FOR $C_{40}H_{68}N_4O_8$ C6,65.16; H,9.18; N,7.38

MS m/z M^+ $C_{40}H_{68}N_4O_8$ (7.32), 733 (22.08) (MH), 633 (46.67), 619 (8.54), 599 (31.67), 564 (28.75), 550 (13.33), 506 (9.58),499 (12.92), 480 (9.17), 464 (10.97), 450 (5.00), 430 (2.71), 371 (1.88), 356 (27.1), 330 (3.23), 202 (16.46), 174 (7.92), 142 (58.75), 128 (9.17), 91 (100), 82 (37.5), 57 (37.5).

N-α-TERTIARYBUTOXYCARBONYL-2-AMINODECANOYL-N-ϵ-CARBOBENZOXYLYSL-GLYCINE METHYL ESTER (XIX)

VII (0.6g, 2.23mM), NMM (0.44g, 4.36mM), IBCF (0.33g, 2.42mM), and XV (0.85g,2.08mM) were reacted in $CHCl_3$ (15mls). Following extraction and evaporation and TLC (system C) yield = 0.95g (73.5%) mp = 100°C.

υ max 3340, 3290. 3090, 3060, 1760, 1695, 1650, 1530, 1265

NMR ($CDCl_3$) 0.98 (3H, t. CH_3 C_{10} , 6.92Hz); 1.20 (16H,s.6 x CH_2 C_{10} 2 x CH_2 lys); 1.40 (9H, s. $(CH_3)_3$ Boc); 1.70 (2H, m. $C_\beta H_2$ lys);

1.85 (2H, m. $C_\beta H_2C_{10}$); 3.19 (2H, m. $C_\epsilon H_2$ lys); 3.68 (3H, s., CH_3 methyl ester); 4.03 (3H, m. CH_2 gly and $C_\alpha H$ C_{10}); 4.46 (1H, m. C_α H lys) 5.02 (2H, d. NH C_{10} and NH ϵ lys); 5.09 (2H, s. CH_2 benzyl group); 6.71 (1H, d. NH α lys); 6.86 (1H, s. NH gly); 7.35 (5H, m. C_6H_5 benzyl group)

FOUND C,61.93; H,8.27; N,9.16

CALCULATED FOR $C_{32}H_{52}N_4O_8$ C,61.94; H,8.39; N,9.03

MS m/z (%) $C_2H_{52}N_40_8$ (620) 535 (1.88), 521 (30.94), 432 (2.29), 413 (2.29), 387 (3.13), 352 (4.38), 324 (1.25), 244 (9.17), 232 (20.63), 218 (7.08), 199 (8.33), 188 (3.33), 174 (11.25), 154 (1.88), 142 (100), 129 (8.33), 112 (3.33), 91 (91.67), 82 (7.5), 69 (11.88), 56 (18.33).

<u>N-α-TERTIARYBUTOXYCARBONYL-α-2-AMINODECANOYL-PHENYLALANYL-GLYCINE METHYL ESTER</u> (XX)

(VII) (0.68g, 2.37mM), EDAC (0.53g, 2.60mM), DIPEA (0.30g, 2.33mM), (XVI) (0.64g, 2.35mM) in DMF (14mls) reacted. Following filtration, extraction and evaporation, yield = 1.06g (89.5%) mp 101°-104°C.

υ max 3420, 3310, 3105, 2960, 2910, 1740, 1680, 1660, 1645, 1540, 1520, 1380, 760, 700.

NMR ($CDCl_3$) 0.87 (3H, t. CH_3 alkyl C_{10}, 6.3Hz); 1.22 (12H,s.6 x CH_2 C_{10}); 1.39 (9H, s. $(CH_3)_3$ Boc); 1.65 (2H, m. $C_\beta H_2$ C_{10}); 3.15 (2H, d. CH_2 Phe, 6.3Hz); 3.72 (3H, s. CH_3 methyl ester); 3.88 (1H, m. $C_\alpha H$ C_{10}); 3.98 (2H, t CH_2 Gly, 5.6Hz, 19Hz); 4.79 (2H, t. NH C_{10}, $C_\alpha H$ Phe); 6.48 (1H, d. NH Phe, 6.3Hz); 6.77 (1H, s. NH Gly); 7.27 (5H, m. C_6H_5 Phe)

FOUND C,63.80; H,8.35; N,8.23.

CALCULATED FOR $C_{27}H_{43}N_3O_6$ C,64.12; H,8.58; N.8.31

Fab m/e M^+ 506 (17.5), 476 (1.66), 450 (17.5), 406 (10.83), 361 (11.66), 337 (50), 317 (5.83), 281 (5.85), 237 (32.10), 142, (27.10), 120 (100), 98 (2.5), 83 (2.09), 69 (3.33), 55 (4.59).

N-α-TERTIARYBUTOXYCARBONYL-α-2-AMINODECANOYL-N-ϵ-CARBOBENZOXYLYSYL-GLYCINE (XXI)

XIX (0.51g, 0.823mM) was deprotected in alcoholic KOH solution (2.5ml, 1M) as in method [D], the reaction being followed by TLC (system A). On completion the reaction mixture was adjusted to 3 and then extracted with EtOAc. Yield = 0.5g (100%) IR indicated the loss of the ester carbonyl.

N-α-TERTIARYBUTOXYCARBONYL-α-2-AMINODECANOYL-PHENYLALANYL-GLYCINE (XXII)

Deprotection of XX (0.75g, 1.49mM) with 1N alcoholic KOH (4.59mls as in method [D]) yielded a clear oil 0.72g (98.7%). IR indicated the loss of the ester carbonyl and the appearance of carboxylic acid peak.

N-α-TERTIARYBUTOXYCARBONYL-α-2-AMINODECANOYL-N-ϵ-CARBOBENZOXYLYSYL-GLYCINE p-NITROPHENOL ESTER (XXIII)

p-Nitrophenol (0.07g, 0.504mM), XXI (0.195g, 0.32mM), and EDAC (0.05g, 0.323mM) in DCM (2mls) were reacted. Following extraction and evaporation yield = 0.09g (39%) from EtOAc-petrol 60-80 mp 141°C.
υ max 3430, 3410, 3030, 2930, 2870, 1715, 1680, 1650, 1640, 1590, 1550, 1370, 1145, 835

NMR ($CDCl_3$) 0.98 (3H, t. CH_3 alkyl C_{10}); 1.22 (12H,s.6x CH_2 C_{10}); 1.45 (9H, s.$(CH_3)_3$ Boc); 1.55 (2H, m. $C_\beta H_2$ lys); 1.62 (2H, m. $C_\beta H_2$ C_{10}); 1.82 (4H, m.2 x CH_2 lys); 3.20 (2H, m. $C_\epsilon H_2$ lys); 3.90 (1H, m. $C_\alpha H$ C_{10}); 4.02 (1H, m. C_α H lys); 4.32 (2H, m. CH_2 gly); 4.99 (1H, s. NH C_{10});5.10 (2H, s. CH_2 benzyl group); 5.12 (1H, s. NH lys ϵ); 6.50 (1H, d. NH lys); 6.72 (1H, s. NH gly); 6.92 (2H, d. AB system para $NO_2\phi$); 7.30 (5H, m. C_6H_5 lys); 7.43 (2H, d. AB system para $NO_2\phi$)

MS m/z (%) $C_{37}H_{53}N_5O_{10}$ (727) 728 (MH)(6.1), 628 (13.2), 606 (25.9) 601 (7.5), 593 (6.9), 589 (8.8), 521 (11.5), 441 (6.1), 333 (7.5), 288 (5.3), 195 (6.9), 139 (7.4), 123 (38.4), 91 (100), 56 (76.3).

N-α-TERTIARYBUTOXYCARBONYL-α-2-AMINODECANOYL-PHENYLALANYL-GLYCINE-p-NITROPHENOL ESTER (XXIV)

XXII (0.94g, 1.91mM) and p-nitrophenol (0.32g, 2.29mM) in DCM (19mls) were stirred at 0°C and DCC (0.43g, 2.09mM) added. The reaction was stirred for 2.5hrs and followed by TLC (system D), following filtration and evaporation yield = 1.12g (95.8%) mp = 132°C.

υ max 3420, 3410, 3025, 2490, 2865, 1710, 1680, 1645, 1590, 1555, 1370, 1140, 820.

NMR ($CDCl_3$) 0.88 (3H, t. CH_3 C_{10}); 1.22 (12H,s.6x CH_2 C_{10}); 1.37 (9H, s.$(CH_3)_3$ Boc); 1.65 (2H, m. C_8H_2 C_{10}); 3.15 (2H, d. CH_2 Phe); 3.90 (1H, m. $C_\alpha H$ C_{10}); 4.00 (2H, m. CH_2 gly); 4.80 (2H, t. NH C_{10} C_α H Phe); 6.47 (1H, d. NH Phe); 6.70 (1H, s. NH Gly); 6.93 (2H, d. AB system para $NO_2\phi$); 7.25 (5M, m. C_6H_5 Phe); 7.42 (2H, d. AB system para $NO_2\phi$);

MS m/z (%) $C_{32}H_{44}N_4O_8$ (612) 634 $(M+Na)^+$ (10.6), 613 (8.7) $(M+1)^+$. 513 (5.3), 476 (15.9), 418 (6.6), 377 (11.8), 316 (5.7), 272 (8.8), 91 (100), 56 (35.9).

α-2-AMINODECANOYL-N-ε-CARBOBENZOXYLYSYL-GLYCINE-p-NITROPHENOL ESTER TRIFLUOROACETATE (XXV)

XXIII (0.07g, 0.096mM) was deprotected using TFA in $CHCl_3$ (2ml) method [Ei], TLC (system A) being used to follow the reaction. Following evaporation yield = 0.06g (93.9%). IR indicated the loss of the urethane carbonyl.

α-2-AMINODECANOYL-PHENYLALANYL-GLYCINE-p-NITROPHENOL ESTER HYDROCHLORIDE (XXVI)

LXXIV (0.26g, 0.414mM) in DCM (30mls) was deprotected with HCl gas, (method [Eii]). the deprotection followed by TLC (system D) yield = 0.23g (95%). IR indicated the loss of the urethane carbonyl and the appearance of NH stretch.

α-2-AMINODECANOYL-N-ε-CARBOBENZOXYLYSYL-GLYCINE POLYMER (XXVII)

The polymerisation reaction of XXV (0.06g, 0.096mM) was carreid out in an NMR tube to observe the reaction. XXV in DMSO (0.5ml) and NMM (0.09g, 0.91mM) (to neutralise the TFA) were placed in an NMR tube and the reaction followed at various times (0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 60, 90, 120, 240 and 360 mins). Spectral differences could be seen mostly in the α and NH regions. The specturm becoming broad as polymerisation proceeded.

α-2-AMINODECANOYL-PHENYLALANYL-GLYCINE POLYMER (XXVIII)

TEA (0.0424g, 0.419mM) was added to XXVI (0.23g, 0.419mM) in DMSO (0.4ml) (a 60% solution). The reaction was stirred for 27hrs and the polymer precipitated by the addition of $CHCl_3$ (5mls) and MeOH (50mls) with stirring for 2hrs. The polymer was filtered and then resuspended at -10°C in MeOH (15mls) and ether (45mls). The polymer was then filtered and resuspended in ether (25mls) and then filtered and resuspended in water (10mls). Following collection and drying of the polymer yield = 0.184g. Spectra could not be obtained as due to insolubility no NMR was obtained and no breakdown could be observed on MS.

SYNTHESIS INVOLVING THE PRODUCTION OF HOMOPOLYMERS AND POLYMERISATION VIA AMINO ACID ANHYDRIDES

N-CARBOXY-α-l-2-AMINODECANOIC ACID ANHYDRIDE (XXIX)

1. Synthesis of the carboxy anhydride was initially tried using ethyl chloroformate (ECF).

To IV (0.g, 1.07mM) in dioxane (1ml), NMM (0.08g, 0.792mM) and ECF (0.13g, 1.18mM) were added the reaction was stirred at -10°C. After 30 mins the reaction mixture solidified. The solid was collected and found to be IV, evaporation of the eluant gave an oil which was solidified from $CHCl_3$ petroleum ether. NMR indicated the required product had not been obtained, therefore the synthesis was abandoned.

The reaction was again attempted IV (0.1g, 0.535mM) NMM (0.04g, 0.396mM) and ECF (0.7g, 0.59 mM) in dioxane (0.5ml) were refluxed overnight. Examination of the products showed the required product had not been obtained and the use of ethyl chloroformate was abandoned.

Disphogene (0.125g, 0.628mM) in dry tetrahydroflurane (THF) (5mls) was maintained at 60°C for 6hrs. The temperature was reduced to 40°C and IV (0.2g, 1.07mM) was added, the reaction being stirred until IV had completely dissolved and TLC (system E) was used to observe the reaction.

Evaporation of the THF yielded a white oil which was soluble in $CHCl_3$. The remaining fine white ppt was removed. Evaporation of $CHCl_3$ and the addition of ether (2.5mls) and hexane (5mls) and the placement of the mixture in the freezer (-14°C) gave white crystals 0.15g (66%). TLC (system A) indicated the presence of an impurity therefore the product was recrystallised from ether hexane and then toluene (0.08g, 33%) mp 85°C. TLC (system E) indicated an impurity. NMR however indicated the product was pure and the impurity was caused by the breakdown of the product on the silica plates.

υ max 3420, 1830, 1765

NMR ($CDCl_3$) 0.85 (3H,t,CH_3 C_{10} alkyl chain); 1.30 (12H,m,CH_2, C_{10} alkyl chain);

1.90 (2H,m,C_8H_2, C_{10}); 4.30 (1H,m,C $_\alpha$H C_{10}); 5.80 (1H,s, NH, C_{10});

FOUND C,61.87; H,8.95; N,6.61

CALCULATED FOR $C_{11}H_{19}NO_3$ C,61.95; H,8.98; N,6.57; 0,22.50%

MS m/z (%) $C_{11}H_{19}NO_3$ 213 (32.4), 168 (15.7), 156 (4.2), 143 (6.6), 129 (99.6), 101 (21.3), 96 (5.1), 82 (10.3), 69 (5.7), 56 (3.3), 43 (8.9), 32 (4.5).

N-CARBOXY-α-d-2-AMINODECANOIC ACID ANHYDRIDE (XXX)

Diphosgene (0.53g, 2.67MM) and V (1.0g, 5.35mM) in THF (25mls) were reacted as in XXIX. Yield = 0.78g (69%). Following recrystallisation yield = 0.53g (47%) mp = 86°C.

υ max 3420, 1830, 1765

NMR ($CDCl_3$) 0.86 (3H,t,CH_3 C_{10} alkyl chain); 1.30 (12H,m,CH_2 CH_{10} alkyl chain);1.85 (2H,m, $C_\beta H_2$ C_{10}); 4.33 (1H,m, $C_\alpha H$ C_{10}); 5.60 (1H,m,NH C_{10})

FOUND C,61.89; H,8.93; N,6.80%

CALCULATED FOR $C_{11}H_{19}NO_3$ C,61.95; H,8.98; N,6.57; 0.22.50%

MS m/z (%) $C_{11}H_{19}NO_3$ 213 (30.7), 168 (12.3), 156 (5.2), 143 (5.8), 124 (87.8), 101 (17.9), 82 (9.9), 69 (6.1),43 (6.1).

N-CARBOXY-α-N-ϵ-CARBOBENZOXY-LYSINE ANHYDRIDE (XXXI)

N-ϵ-Carbobenzoxy-lysine previosuly synthesized was recrystallised (methanol/water) and used for anhydride synthesis.

υ max 3345, 3060, 3040, 2950, 2865, 1690, 1615, 1580, 1540, 1530, 1510, 1420, 1320, 1270, 745, 695.

Diphosgene (0.283g, 1.55mM) and N-ϵ-carbobenzoxy-lysine (0.40g, 1.50mM) in THF (10mls) were reacted as for XXIX. Yield = 0.298g (63%) following recrystallisation yield = 0.208 (43.8%) mp = 93°C.

υ max 3410, 3315, 3040, 3010, 2970, 1850, 1760, 1710, 1550, 1100, 770, 700.

NMR ($CDCl_3$) 1.49 (2H, m. CH_2 lys); 1.83 (2H, m. $C_\gamma H_2$ lys); 1.95 (2H, m. $C_\beta H_2$ lys); 3.25 (2H, m.$C_\epsilon H_2$ lys); 4.37 (1H, m. $C_\alpha H$ lys); 4.90 (1H, s. NH ϵ lys); 5.11 (2H, s. CH_2 benzyl); 6.79 (1H, s. NH broad); 7.35 (5H, m. C_6H_5 benzyl)

FOUND C,58.99; H,5.84; N,8.98

CALCULATED FOR $C_{15}H_{18}N_2O_5$ C,58.80; H,5.93; N,9.15

MS m/z (%) $C_{15}H_{18}N_2O_5$ (306) 307 (12.8), 281 (5.4), 263 (15.7), 200 (100), 173 (33.3) 150 (27.1), 146 (7.2), 91 (8.6).

POLYMERISATION OF CARBOXY ANHYDRIDES VIA HOMO AND CO-POLYMERISATION

1-α-2-AMINODECANOIC ACID POLYMER (XXXII)

XXIX (0.05g, 0.23mM) and K_2CO_3 (0.010mg) in dried benzene (1ml) (giving a 5% solution) were stirred at room temperature for 24hrs. Carbon dioxide was released during the reaction and a viscous oil was produced. The oil was insoluble in ether and $CHCl_3$ with MeOH causing precipitation. The K_2CO_3 was removed by the addition of benzene (C_6H_6) and the decanting of the C_6H_6 solution. Evaporation gave a thin film yield = 0.04g.

υmax 3380, 2950, 2920, 2850, 1720, 1650, 1540,

NMR ($CDCl_3$) 1.00 (m, C_{10} alkyl region); 1.50 (m, C_{10} alkyl region);
7. 30 (m, NH C_{10}); 7. 50 (S, NH,C_{10})

Identification of the polymer was attempted. The NMR is unclear with complex regions in the alkyl and NH positions and Mass spectrometry could not pick up a definate M^+ or breakdown pattern. Several solvent mixtures were used to separate the polymer, but this was not achieved.

ENZYME DIGESTION OF 1- α-2-AMINODECANOIC ACID POLYMER

The enzymic breakdown of the polymer was investigated with thermolysine as the enzyme. XXXII (300 μl of a 0.01% dispersion in $CHCl_3$ was added to Tris buffer (100mM Tris, 2mM calcium) and thermolysine (100 μg/ml) at pH 7.5-8.0 at room temperature. The pH was readjusted to 8 and thermolysine (3.5μg) added. The solution was stirred at 50°C and samples taken at hourly intervals for ninhydrin testing. No increase in amine groups was observed even after overnight incubation. This was probably due to dissolution difficulties.

l-α-2-AMINODECANOIC ACID AND LYSINE POLYMER 1:1 (XXXIII)

XXIX (0.010g, 0.46mM) and XXXI (0.14g, 0.465mM) were added to C_6H_6 (4.8mls) to give a 5% solution. K_2CO_3 (50mg) was added and the reaction

mixture was stirred at room temperature for 24hrs. A dense white oil was produced with C_6H_6 being added to allow the removal of the K_2CO_3 and MeOH added to precipitate the polymer. Removal of the solvent gave a white solid yield = 0. 1521g NMR could not be obtained due to the insolubility of the polymer.

l-α-2-AMINODECANOIC ACID AND 1-N-ϵ-CARBOBENZOXYLYSINE POLYMER 10:1 (XXXIV)

XXIX (0.10g, 0.46mM) and XXXI (0.014g, 0.0458mM0 in C_6H_6 (2.3mls) with K_2CO_3 (20mg) were reacted as in XXXIII. Yield = 0.0727g. NMR and mass spectra could not be obtained for the polymer.

dl-α-2-AMINODECANOIC ACID POLYMER (XXXV)

XXX (0.045g, 0.207mM) and K_2CO_3 (0.010mg) in C_6H_6 (0.9ml) were reacted as in XXXI. Yield = 0.038g. NMR and mass spectra were not obtained.

PINENE KETOL 6 (-)-(1S,2S,5S)-2-HYDROXYPINAN-3-ONE (XXXVI)

Ref: Carbon & Pierce 1971. (+) α-Pinene (100g, 0.734M) in 90% aqueous acetone (889g) was placed in an ice salt bath and allowed to equilibrate. Powdered potassium permanganate ($KMnO_4$) (200g, 1.265) was added to the reaction mixture over a 10hr period (10 x 20g) then the reaction mixture was vigorously stirred for 24hrs at -10°C, the reaction being monitored by TLC (system F).

Following filtration, to remove the reduced $KMnO_4$, the filtrate was evaporated leaving 250mls and the aqueous solution was extracted with ether (5 x 150mls). The ethereal solution was extracted with water (3 x 30mls) and $NaHCO_3$ solution (2 x 30mls) dried over anhydrous Na_2SO_4 and evaporated giving a pale yellow oil (49.6g, 40.2%). Two fractions of oil were collected by distillation under vacuum.

(i) 84-88°C at 1.25mm (32.13g)

(ii)77-79°C at 0.65mm (10.78g)

The fractions were crystallised from pentane but on collection the solid readily melted with TLC (system F) indicating some impurity. Purification was carried out using silica gel chromatography (system F) Yield = 38.54g mp = 32°C (lit. mp = 32°C)

[α]-36.8 in $CHCl_3$ lit [α] 38.0 Ogusi et al., 1978.

υmax (im solution in CCl_4) 3610, 3500, 1710

NMR ($CDCl_3$) 0.88 (3H,s, CH_3 C^1);36 (3H,s, CH_3 on bridge); 1.37 (3H,s,CH_3 on bridge); 1.67 (1H,m, C^4H, $11H_2$); 2.13 (3H,m,OH C^3H_2); 2.43 (1H,m, C^2H); 2.59 (2H,m, C^5H_2).

Accurate mass

m/e 169.122 9858

FOUND: C,71.02; H,9.62; O,20.36

CALCULATED FOR: $C_{10}H_{16}O_2$ C71.39; H,9.58; O,9.02.

dl-α-2-AMINODECANOIC ACID AND KETOL 6 OXIME (XXXVII)

dl-α-2-aminodecanoic acid methyl ester hydrochloride (II) (3.00g, 0.013M) and NMM (1.3g, 0.013M) in dry C_6H_6 (300mls) were stirred for 30mins in a nitrogen atmosphere and XXXVI (1.69g, 0.011M) added followed by a catalytic amount of boron trifluoride (300mg). The reaction mixture was refluxed at 85°C for 8hrs, the reaction being monitored by TLC (system G). When the ketol had reacted the reaction mixture was filtered and the filtrate evaporated giving a pink oil. Yield = 2.67g (72.7%) The oil was purified by silica gel chromatography (system F) giving the diasterioisomers as colourless oils Rf 0.54 (0.96g, 72%) Rf 0.32 (0.90g, 67.4%)

The spectral data for the two compounds are identical.

IR (K Br) υ max 3340, 2980, 2930, 2850, 1745, 1650, 1460, 1430, 1200, 730, 720.

NMR ($CDCl_3$) 0.9 (3H,t,CH_3); 1.2 (12H,m,$(CH_2)_6$); 1.3 (3H,s,CH_3); 1.5 (3H,s,CH_3); 1.6-2.7 (9H,m,pinane skeleton); 3.7 (3H,s,OCH_3); 4.1 (1H,m,CH)

HYDROLYSIS OF OXIME XXXVII GIVING IVand V

Hydrolysis of XXXVII was achieved with 0.5N hydroxylamine hydrochloride soluiton (30% v/v water in ethanol).

XXXVII top spot (0.96g, 2.83mM) in EtOH (5mls) and hydroxylamine hydrochloride (0.30g, 4.23mM, 8.6mls) XXXVII bottom spot (0.90g, 2.65mM) in EtOH (5mls) and hydrosylamine hydrochloride (0.28g, 3.98mM, 8.1mls).

Both fractions were stirred at room temperature for 12 hours and monitored by TLC (system G). A further fraction of hydroxylamine hydrochloride was added to each and the hydrolysis allowed to proceed to completion. The ethanolic solution was extracted with $CHCl_3$ (15mls) and the $CHCl_3$ solution then extracted with 1NHCl (5mls), $NaHCO_3$ solution (5mls) and water (5mls) Purification of both hydrolysed fractions was by silica gel chromatorgaphy (system A). After evaporation the resulting oil was taken up in EtOAc and HCl gas passed through the solution. The solvent was then evaporated , titration of the oil with ether giving a white solid. Top spot 0.43g, Bottom spot 0.41g. Comparison of the CD spectra for these compounds and those obtained by enzymic hydrolysis allowed the nature of the diasteriomers to be assigned.

Top spot Rf 0.54 m.p. 132-133.5°C

υ max 3150, 2960, 2850, 2600, 2010, 1745, 1610, 1475, 1465, 1460, 1235, 760.

NMR ($CDCl_3$) 0.80 (3H,t,CH_3); 1.26 (12H,m, $(CH_2)_6C_{10}$); 1.50 (2H,m,C_8H_2); 3.70 (3H,s,OCH_3); 4.10 (1H,m,$C_\alpha H$); 8.8 (3H,br,s. NH_3).

Circular Dichroism spectrum Fig. 7

Δ_ϵ = + 1.60 (λ_{210} C (1.04 mg/ml)

A positive CD spectrum indicates l configuration.

Elemental analysis:

CALCULATED FOR $C_{11}H_{24}NO_2Cl$ MW = 237.27

CALCULATED C,55.57; H,10.17; N,5.89

FOUND C,55.49; H,10.22; N,5.95

Bottom spot R_f 0.32

mp 130-132°C

IR (υ max) 3150, 2960, 2920, 2850, 2600, 2010, 1745, (C = 0s t ester), 1610, 1475, 1465, 1460, 1235, 760.

NMR ($CDCl_3$) 0.8 (3H,t, CH_3; 1.26 (12H,m, $(CH_2)_6$); 1.5 (2H,m, $C_\beta H_2$); 3.8 (3H,s, OCH_3); 4.1 (1H,m, $C_\alpha H$); 8.9 , (3H, brs NH_3).

Circular Dichroism spectrum Fig. 9

$\Delta\epsilon$ = -1.68 (λ_{210}), C(1.12mg/ml)

Therefore assigned d configuration .

Elemental analysis $C_{11}H_{24}NO_2Cl$ MW 237.77

CALCULATED FOR C,55.57; H,10.17; N,5.89

FOUND C,55.33; H,10.13; N,5.87

SOLID PHASE PEPTIDE SYNTHESIS

Solid phase peptide synthesis both manual and automated, was used to produce core tripeptides for polymerisation.

Manual solid phase peptide synthesis

Chloromethylated Merrifield resin (10g, 0.75m Eq/gm) VII(1.0g, 3.69mM) and TEA (0.34g, 3.35mM) in dry EtOH (20mls) were refluxed for 48hrs. The resin was filtered and dried, with the extent of binding determined before further coupling was undertaken.

The extent of binding was determined by the removal and accurate weighing of resin (200mg) which was then placed on pyridine (2mls) and heated at 100°C for 1hr. 50% acetic acid (20mls) concentrated nitric acid (5mls) and silver nitrate solution (2mls) were added and this was titrated against sodium thiocyanate solution with ferric ammonium sulphate as indicator. 4.81mEq of chloride were found not to have been replaced with 2.69 mEq replaced (i.e. 2.69 mM, 0.77g VII on renin).

The following reagents were used in the synthesis procedure:-

Dioxane (removal of the peroxides was achieved by passing the dioxane through an alumina column)

$CHCl_3$ (distilled)

DCM (distilled)

TEA (distilled)

TFA

The reaction scheme is as follows (for 10g of resin)

Table 54

Step	Reagent	Volume (ml)	Time (mins)
1	DCM x 3	50	5
2	Abs. EtOH x 3	50	3
3	DCM x 3	50	3
4	TFA (10mls in 50 mls (DCM)	30	
5	DCM x 3	40	5
6	Boc AA in DCM	40	5
7	DCC in DCM	20	overnight

Each coupling reaction involved the use of 2.5 equiv. of VII and 2.5 equiv. of DCC.

First coupling

VII (1.93g, 6.73mM) in DCM (40mls) was placed in the reaction vessel and EDAC (1.29g, 6.70mM) added, the reaction was shaken overnight. The reaction mixture was aspirated and the resin washed as in Table 54.

The extent of coupling was determined by transesterification of the peptide from the resin. Her resin (200mg accurately determined) was removed from the reaction vessels and MeOH (4mls) and TEA (0.5ml) added with stirring for 18hrs.

The resin was then washed with MeOH and the solvent evaporated giving a white solid examined by TLC (system H) giving one spot.

Second Coupling

VII (1.94g, 6.76mM) in DCM (80mls) and EDAC (1.,29g, 6.70mM) were treated as in the first coupling stage. Following transesterification and TLC (system H), two spots were obtained indicating that incomplete deprotection of the peptide was occuring with TFA in DCM, therefore this procedure was abandoned for automated solid phase peptide synthesis.

Automated Solid Phase Peptide Synthesis

TERTIARY BUTOXYCARBONYLGLYCINE CAESIUM SALT (XXXVIII)

Tertiarybutoxycarbonyl glycine (3.00g, 0.017mM) in EtOH (30mls) and water (10mls) was pH adjusted to 7 with 2M caesium carbonate solution. The solvent was evaporated and the compound lyophilised giving a white solid (5.2g, 99.6%) at 50°C for 12hrs. The resin was then filtered and washed with (i) DMF, (ii) DMF:water 1:1 (400mls) (iii) DMF (200mls) (iv) MEOH (400mls) and DCM (400mls) and the dried (23.95g) with the extent of binding calculated. 0.743 mEq g of chloride had been replaced in the resin (99.1%) therefore the resin was used in the automated synthesiser.

COUPLING USING THE SOLID PHASE PEPTIDE SYNTHESISER

5g of resin was used (ie 3.72 mEq of glycine) with 2.5 equivalents of reactants amino acid and dicyclohexylcarbodiimide.

N-α-tertiarybutoxycarbonyl-N-ϵ-carbobenzoxylysine (3.35g, 9.3mM) in DCM (12mls), VII (2.669g, 9.3mM) in DCM (20mls), DCC (4.793g, 0.0186M) in DCM (80mls) and DIPEA (10% v/v in DCM) were placed in the synthesiser and the standard coupling followed as in Table 55.

TABLE 55

REAGENTS	REPEAT	MIX	MLS
Methylene Chloride	4	1	55
TFA	1	5	55
TFA	1	25	55
Wash		20	
Methylene Chloride	3	1	55
Diisopropylethylamine	2	2	55
Methylene Chloride	3	1	55
Dimethyl Formamide	2	1	55
Amino Acid		1 (12 or 20)	
Dicyclohexylcarbodiimide		3	36.66
Dimethylformamide	2	1	55
Ethanol	3	1	55

Once synthesised the peptide was cleaved from the resin, by hydrozinolysis.

30 equivalents of hydrazine (3.58g, 0.11M) in DMF (25mls) were added to the resin and shaken for 50hrs, the resin then washed with DCM :(5 x 20mls). Evaporation of the solvent yield 1.79g (77.9%) followed by TLC (system A,I and J) indicated the presence of one ninhydrin positive spot. Crystallisation could not be achieved and TLC (system B) indicated two spots and the breakdown of the peptide again the synthesis was abandoned.

dl-α-2- AMINOTETRADECANOIC ACID (XXXIX)

Sodium metal (2.12g) was added slowly to absolute EtOH (70ml).Diethylacetamidomalonate malonic ester (20.84g, 0.096M) and 1-bromododecane .(28.26g, 0.1130M), were reacted as in I. Yield = 55.25g Hydrolysis with conc. H_2SO_4 (100ml) gave dl-α-2-aminotetradecanoic acid. Yield =, 23.25g (99.7%).

dl-α-2-AMINOHEXADECANOIC ACID (XXXX)

The method used was as for II and XXXIX

Na (2.07g)diethylacetamido malonic ester (20.05g, 0.0923M), and bromotetradecane (29.86g, 0.1077M). Yield = 23.12g (92.43%).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINODECANOIC ACID (XXXXI)

II (2.5g, 0.0134M) and ditertiary butoxycarbonyl (4.4g, 0.0128M) were reacted as in VII. Yield = 3.51g. (95.0)mg 64-66°C.

NMR $CDCl_3$) 0.88 (3H,t,CH_3); 1.27 (12H,m,6 x CH_2); 1.45 (9H,s,$(CH_3)_3$ Boc); 1.76 (2H,m,$C_\beta H_2$); 4.29 (1H,m,$C_\alpha H$); 4.95 (1H,s,NH).

MS m/z (%) $C_{15}H_{29}NO_4$ (287) 333 (17), 332 $(M^+ + 2Na\ H)^+$,(100), 311 (18), 310 $[M + Na]^+$ (99), 254 (30), 232 (58), 188 (12), 186 (14), 142 (17), 57 (41).

FOUND: C, 62.87; H,10.14; N,4.59.

CALCULATED FOR: $C_{15}H_{29}NO_4$ (287.3) C,62.68; H,10.17; N,4.87).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANOIC ACID (XXXXII)

XXXIX (10.5g, 0.0413M) and ditertiarybutyldicarbonate (13.06g, 0.06M) were reacted as in VII. Yield = 7.52g (55.0%).

Recrystallization from acetonitrile (ACN) Yield = 6.7g (49%) mp 62-64°C.

NMR ($CDCl_3$) 0.89 (3H,s,CH_3); 1.21 (20H,m,10 x CH_2); 1.43 (9H,s,$(CH_3)_3$ Boc); 1.67 (2H,m,$C_\beta H_2$); 4.27 (1H,m,$C_\alpha H$); 4.95 (1H,s,NH)

MS m/z (%) $C_{19}H_{37}NO_4$ M^+ (343) 389(21), 388 (M + 2Na+H) (100),367 (12), 366 (M+Na) (60), 310 (18), 288 (57), 57 (30)

FOUND: C,66.51; H,10.59; N,4.11

CALCULATED FOR: $C_{19}H_{37}NO_4$ (343.4) C,66.43; H,10.86; N,4.08.

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOHEXADECANOIC ACID (XXXXIII)

XXXX (10g, 0.037M), suspended in t-BuOH H_20 2:3 (200ml) and di-tertiarybutyldicarbonate (12.11g, 0.0555M) were reacted as for VII. Yield = 8.80g (66.25%) mp. 79-80°C.

NMR ($CDCl_3$) 0.85 (3H,t,CH_3); 1.23 (24H,m, 12 x CH_2); 1.42 (9H,s,$(CH_3)_3$ Boc); 1.74 (2H,m,$C_\beta H_2$); 4.16 (1H,m,$C_\alpha H$); 5.00 (1H,m,NH);

MS m/e (%) $C_{21}H_{41}NO_4M+$ (371), 394 (M+Na) (100), 373 (11), 372 (M+H), 294 (70), 57 (23).
FOUND: C,67.69; H,11.11; N,3.85.
CALCULATED FOR: $C_{21}H_{41}NO_4$ (371.55) C,67.88; H,11.12; N,3.77.

dl-α-2-AMINODECANOIC ACID METHYL ESTER HYDROCHLORIDE (XXXXIV)

II (2.27g, 1.21 x 10^{-2}M), $SOCl_2$ (2.28g, 0.055M) and MeOH (225mls) were reacted as in method [A]. Yield=2.01g (70%) mp 95-97°C.

NMR ($CDCl_3$) 0.9 (3H,t,CH_3); 1.2 (12H,m,$(CH_2)_6$); 1.35 (2H,s,$C_\beta H_2$); 3.8 (3H,s,CH_3 Me ester); 3.9 (1H,m,$C_\alpha H$); 8.8 (3H,bs,NH_3).

MS m/z (%) $C_{11}H_{24}NO_2Cl$ (237) 203 (13), 202 $[M+H]^+$ 100, 142 (26)
FOUND: C,55.83; H,10.45; N,5.75.
CALCULATED FOR: $C_{11}H_{24}NO_2Cl$ (237.77) C,55.57; H,10.17; N,5.89.

dl-α-2-AMINOTETRADECANOIC ACID METHYLESTER HYDROCHLORIDE (XXXXV)

XXXIX (5.0g, 0.0205M),MeOH (100ml) and $SOCl_2$ (13.5g, 8.5ml, O.1144M) were reacted by method [A] as in VI. Yield = 6.04g (100%) mp 103-104°C..

NMR ($CDCl_3$) 0.9 (3H,t,CH_3); 1.25 (20H,m,10 x CH_2); 1.35 (2H,s,$C_\beta H_2$);
3.70 (3H,s.CH_3 Me ester); 3.90 (1H,m,$C_\alpha H$); 9.10 (3H, broad s, NH_3);

MS m/e M^+ (%) $C_{15}H_{32}NO_2Cl$ (293,295) 259 (16), 258 (M+H) (100) 198 (22)
FOUND: C,61.27; H,10.87; N,4.59.
CALCULATED FOR: $C_{15}N_{32}NO_2Cl$ C,61.30; H,10.98; N,4.77.

dl-α-2-AMINOHEXDECANOIC ACID METHYL ESTER HYDROCHLORIDE (XXXXVI)

XXXX (5.0g, 0.0185M), MeOH (100ml) and $SOCl_2$ (12.12g, 7.63ml, 0.1027M) were reacted by method [A] as in VI. Yield = 3.20g (53.8%). mp 105-106°C.

NMR($CDCl_3$) 0.86 (3H,t,CH_3); 1.26 (24H,m,12 x CH_2); 1.45 (2H,m,$C_\beta H_2$);
3.82 (3H,s,CH_3 Me ester); 4.03 (1H,t,$C_\alpha H$); 8.84 (3H,broad s, NH_3).

MS m/e M^+ (%) $C_{17}H_{36}NO_2Cl$ (321,323) 287 (19) 286 (M+H) (100), 226 (35)
FOUND: C, 61.41; H,11.21; N,4.38;
CALCULATED FOR: $C_{17}H_{36}NO_2Cl$ (321.92) C,63.42; H,11.27; N,4.35

N-α-TERTIARYBUTOXYCARBONYL-dl-α-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID METHYL ESTER (XXXXVII)

XXXXV (2.22g, 7.5639mM) was suspended in DCM (100ml) and TEA (1.53g, 0.0151M) added, the reaction mixture was then stirred at 0°C.

HOBt (1.02g, 7.5483mM), XXXXII (2.5g, 7.5529mM) and 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide (EDAC) (1.45g, 7.5639mM) were added and the reaction allowed to stir overnight.

The reaction mixture was filtered and evaporated, EtOAc was then added, any solid deposited was filtered and the organic layer extracted with 10% citric acid, $NaHCO_3$ and brine. The EtOAc solution was dried over $MgSO_4$. Yield = 3.67g (85.1%)

NMR ($CDCl_3$) 0.88 (6H,t, 2 x CH_3); 1.27 (40H,m, 20 x CH_2); 1.44 (9H,s, $(CH_3)_3$ Boc); 1.77 (4H,m, 2 x $C_\beta H_2$); 3.73 (3H,s,OCH_3 Me ester); 4.04 (1H,s,$C_\alpha H$); 4.58 (1H,s,$C_\alpha H$); 4.93 (1H,s,Boc NH); 6.49 (1H,s,NH).

MS m/e (%) M^+ $C_{34}H_{66}N_2O_5$ (582), 605 (M+Na) (100), 553 (17), 505 (79), 198 (21), 119 (19), 77 (13), 57 (29)

FOUND: C,69.91; H,11.38; N,4.59.

CALCULATED FOR: $C_{34}H_{66}N_2O_5$ (582) C,70.06; H,11.41; N,4.81

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINODECANOYL-2-AMINODECANOIC ACID METHYL ESTER (XXXXVIII)

XXXXI (2.5g, 9.1mM) XXX (2.16g, 9.09mM), HOBt (1.23g, 9.102mM), TEA (1.84g,0.0182M) and EDAC (1.86g, 9.703mM). Yield = 3.96g (94.9%). Compound was purified by flash chromatography.

NMR ($CDCl_3$) 0.85 (6H,t, 2 x CH_3); 1.23 (24H,s, 12 x CH_2); 1.43 (9H,s,$(CH_3)_3$ Boc);1.58,1.80 (4H,broad m, 2 x $C_\beta H_2$); 3.73 (3H,s, OCH_3 Me ester); 4.08 (1H,m, $C_\alpha H$); 4.57 (1H,m, CαH); 5.00 (1H,m,Boc NH); 6.67-6.57 (1H,m, NH)

MS m/e M^+ (%) $C_{26}H_{50}N_2O_5$ (470), 5151 (M+2Na+H) (3) 493 (M+Na) (100), 441 (15), 393 (45), 335 (1), 293 (2), 280 (1), 142 (4), 119 (5), 88 (1), 77 (2), 57 (3).

FOUND: C,66.31; H,10.55; N,5.81

CALCULATED FOR: $C_{26}H_{50}N_2O_5$ (470.68) C,66.34; H,10.71; N,5.95.

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOIC ACID METHYL ESTER (XXXXIX)

Synthesis was as for the tBoc C_{14}-C_{14} OMe. (XXXXVII) (XXXXIII) (2.5g, 6.96mM), XXXXVI (2.24g, 6.967mM), HOBt (0.94g, 6956mM), TEA (1.40g, 0.0138M) and EDAC (1.33g, 6.938mM). Yield = 270g (61.9%).

NMR ($CDCl_3$) 0.87(6H,t,2 x CH_3C_{16}), 1.24(48H,s,CH_2C_{16}), 1.43(9H,s,$(CH_3)_3$ Boc), 1.74(4H,m,2x$C_\beta H_2$), 3.73(3H,s,OCH_3Me ester), 4.06(1H,s,$C_\alpha H$), 4.58(1H,s,$C_\alpha H$), 4.96(1H,s,Boc NH), 6.50(1H,broad s, NH).

MS m/e M^+(%) $C_{38}H_{74}O_5$ (638), 661(M^++Na) (100), 639(M^++H)(27), 561(54), 504(11), 481(13), 230(45), 119(38), 77(19).

N-α-TERTIARYBUTOXYCARBONYL-dl-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID (L)

XXXXVIII (1.5g, 3.28mM) dissolved in MeOH (5ml) and DCM (10ml) and 1N NaOH (7.22ml) added in the same way as for XXI. Yield = 1.45g (99.3%).

NMR ($CDCl_3$) 0.85 (6H,t,2 x CH_3C_{10}); 1.23 (24H,s,12 x CH_2C_{10}); 1.43 (9H,s. $(CH_3)_3$ Boc); 1.87, 1.70, 1.58 (4H,m,2 x $C_\beta H_2C_{10}$); 4.55 (2H,m,2 x $C_\alpha H$ C_{10}); 5.45 (1H,m,Boc NH); 7.05 (1H,m,NH); 8.50 (1H,m, COOH).

MS m/e M^+ (%) $C_{25}H_{48}N_2O_5$ (456) 501 (M+2Na+H) (36) 479 (M+Na) (17), 449 (6), 401 (100), 379 (22) 301 (6), 232 (6), 142 (23), 57 (22).

FOUND: C,65.68; H,10.41; N,6.01

CALCULATED FOR: $C_{25}H_{48}N_2O_5$ C,65,75; H,10.59; N,6.14.

dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER HYDROCHLORIDE (LI)

XXXXVIII (0.83g, 1.24mM) was dissolved in MeOH (15ml) and DCM (5ml) and HCl bubbled through as in method [E]. Yield = 0.50g (100%)

NMR ($CDCl_3$) 0.89 (6H,t,2x CH_3C_{10}); 1.29 (24H,m, 12 x CH_2C_{10}); 1.90 (4H,m, 2 x $C_\beta H_2C_{10}$); 3.82 (3H,s,OCH_3 Me ester); 4.31 (2H,m,2 x $C_\alpha H$ C_{10}); 7.90 (1H,m,CONH)

MS m/z M^+(%) $C_{21}H_{43}N_2O_3Cl$ (406, 408) 371 $(M+H-HCl)^+$ (23), 357 (26), 234

(3), 202 (9), 142 (100), 56 (10).

FOUND: C,61,81; H,10,55; N,6,70.

CALCULATED FOR: $C_{21}H_{43}N_2O_3Cl$ (407.3) C,61.96; H,10.65; N,6.88.

N-α-TERTIARYBUTOXCARBONYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID (LII)

XXXXVII (1.5g, 2.63mM) was dissolved in MeoH (6.4ml) and 1N NaOH (5.8ml) added. On the addition of NaOH a precipitate was formed resulting in slow hydrolysis therefore DCM (30ml) was added to give clear solution and the hydrolysis was allowed to proceed overnight. The solvent was evaporated and EtoAc added, LII was insoluble and therefore extracted with ether. Yield = 1.30g (88.7%).

NMR ($CDCl_3$) 0.88 (6H,t, 2 x CH_3); 1.23 (40H,s, 20 x CH_2); 1.43 (9H,s, $(CH_3)_3$ Boc);1.87-1.53 (4H,m,2 x $C_\beta H_2$); 4.55 (2H,m, 2 x $C_\alpha H_2$); 5.40 (1H,m,Boc NH); 7.05 (1H,m,NH)

MS m/e M^+ (%) $C_{33}H_{64}N_2O_5$ (568), 613 (M+2Na+H) (31), 591 (M+Na) (7), 561 (39), 535 (43), 513 (100) 388 (21), 288 (45), 119 (20), 57 (42).

FOUND: C,69.65; H,11.30; N,4.95.

CALCULATED FOR: $C_{33}H_{64}N_2O_5$ (568.86) C,69.67; H.11.34;N.4.92.

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOIC ACID (LIII)

XXXXIX (1.10g, 1.75mM) was dissolved in DCM (25ml) and MeOH (10ml) IN NaOH (3.6ml) was added and the reaction allowed to stir overnight, as for LII. Yield = 0.87g (80.9%).

NMR ($CDCl_3$) 0.87(6H,t,2xCH_3), 1.23(48H,s,24xCH_2), 1.43(9H,s$(CH_3)_3$Boc), 1.73(4H,m,2x$C_\beta H_2$),4.45(2H,m,2xC_αH), 5.30(1H,m,BocNH), 7.05(1H,m,NH).

MS m/e M^+ (%) $C_{37}H_{72}N_2O_5$ (524), 669(M+2Na+H)(26), 647(M+Na)(19), 617(31), 5679(100), 545(20), 445(46), 374(19), 119(22), 57(32).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER (LIV)

The synthesis of the 2-aminodecanoic acid trimer followed the same pattern of synthesis as the dimers.

XXXXIV (0.77g, 3.242mM) was dissolved in DCM (30ml) and TEA (0.66g, 6.522mM), HOBt (0.55g, 3.256mM). L (1.45g, 3.258mM) and EDAC (0.62g, 3.23mM) added. Yield = 1.70g (82.1%).

NMR $CDCl_3$ 0.85 (9H,t,3 x CH_3); 1.25 (36H,s,18 x CH_2 C_{10}); 1.43 (9H,s,$(CH_3)_3$ Boc);1.75 (6H,m,3 x $C_\beta H_2$ C_{10}); 3.71 (3H,s, OCH_3 Me ester); 4.51,4.39,4.02(3H,m,3 $C_\alpha HC_{10}$); 4.96 (1H,m,Boc NH); 6.68, 6.56 (2H,m, 2 x NH)

MS m/e M^+ (%) $C_{36}H_{69}N_2O_6$ (639), 664 (40), 663 (M+Na) (100), 562 (27), 202 (30), 142 (92), 57 (13).

FOUND: C,67.56; H,10.55; N,6.81.

CALCULATED FOR: $C_{36}H_{69}N_3O_6$ C,67.56; H,10.87; N,6.57.

dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER HYDROCHLORIDE (LV)

LIV (0.63g. 0.986mM) was dissolved in MeOH (15ml), DCM (5ml) and HCl passed through as in method E. Yield = 0,57g (100%).

NMR ($CDCl_3$) 0.85 (9H,m, 3 x CH_3 C_{10}); 1.24 (36H,m, 18 x CH_2 C_{10}); 2.41 (6H,m, 3 x $C_\beta H_2$ C_{10}); 3.75 (3H,m, OCH_3); 4,38 (3H,m, 3 x $C_\alpha H$ C_{10}); 8.14 (2H,m, 2 x CONH).

MS m/z M^+ (%) $C_{31}H_{60}N_3O_4Cl$ (575.5), 540 $(M^+H\text{-}HCl)^+$(10), 371(8), 339(9),

311(8), 202(30), 142(100).

FOUND: C,68.58; H,10.91; N,7.61.
CALCULATED FOR: $C_{31}H_{60}N_3O_4Cl$ (574.32) C,69.10; H,11.22; N,7.80

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID METHYLESTER (LVI)

$C_{14}O$ MeHCl (0.685.g, 2.334mM) was dissolved in DCM (23ml) then TEA (0.472g, 466mM). HOBt (0.315g, 2.331mM) Boc $C_{14}C_{14}OH$ (1.30g, 2.334mM) and EDAC (0.447g, 2.332mM) were added as in XXXXVII. Yield = 1.62g (87.1%).

NMR ($CDCl_3$) 0.87 (9H,t, 3 x CH_3 C_{14}); 1.25 (60H,m, 30 x CH_2 C_{14}); 1.44 (9H, s, $(CH_3)_3$ Boc); 1.75 (6H,m, 3 x $C_\beta H_2$ C_{10}); 3.71 (3H,m, OCH_3 Me ester); 4.51, 4.39, 4.02 (3H,m, 3 x $CH_\alpha H$ C_{14}); 4.94 (1H,s, Boc NH); 6.72, 6.56 (2H,m, 2 x NH)

MS m/e M^+ (%) $C_{48}H_{93}N_3O_6$ (807), 832 (55), 831 (M^+ Na) (100) 731(14), 346(13), 258(12), 198(48), 57(10).

FOUND: C,70.98; H,11.11; N,5.22.
CALCULATED FOR: $C_{48}H_{93}N_6O_6$ (807.25) C,71.32; H,11.60; N,5.32.

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOIC ACID METHYL ESTER

(LVII)

XXXXVI (0.45g, 1.3997mM) was dissolved in DCM (20ml) and LIII (0.85, 1.387mM) TEA (0.28g, 2.767mM) HOBt (0.19g, 1.406mM) and EDAC (0.27g, 1.408mM) were added. Yield = 1.15g (92.2%).

NMR ($CDCl_3$)0.87(9H,t,3xCH_3 C_{16}), 1.25(72H,s,36xCH_2 C_{16}),
1.44(9H,s,Boc$(CH_3)_3$), 1.74(6H,m, 3x$C_\beta H_2$ C_{16}), 3.71(3H,s,OCH_3Me ester),
4.02,438,4,50(3H,m,3x$C_\alpha H$ C_{16}), 4.93(1H,m,BocNH),
6.56,6,72(2H,m,2xNH).

MS m/e M^+(%) $C_{54},H_{105}N_3O_6$ (891), 915(43), 914$(M+Na)^+$(100), 814(17), 402(26), 304(10), 254(33), 57(15).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOHEXDECANOYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOIC ACID (LVIII)

LVII (1.32g, 1.48mM) was dissolved in DCM (2.5ml) and MeOH (15ml) IN NaOH (4.5ml) was added and the reaction proceeded as in method [D]. Yield = 1.03 (79.3%)

NMR ($CDCl_3$) 0.86(9H,t,3xCH_3 C_{16}), 1.23(72H,s,36xCH_2 C_{16}),
1.43(9H,s,$(CH_3)_3$)Boc), 1.72(6H,m,3x$C_\beta H_2$ C_{16}),
4.19,4.30,4.38(3H,m,3x$C_\alpha H$ C_{16}), 4.35(1H,m,BocNH), 7.35(2H,m,2xNH).
MS m/e M^+(%) $C_{53}H_{103}N_3O_6$(877), 901(14), 900$(M+Na)^+$(32), 844(11), 800(14), 676(27), 578(16), 434(25), 254(100), 57(39).

2-BROMOHEXADECANOIC ACID METHYL ESTER (LIX)

$SOCl_2$ (1.77g, 16.2ml) was added dropwise to cooled MeOH (100ml) and 2 bromohexanoic acid (5.0g, 0.015M) was added and the mixture refluxed overnight. Evaporation of the solvent gave an oil. Yield = 3.70g (70.6%).

NMR ($CDCl_3$) 0.89 (3H,t,CH_3 alkyl chain); 1.39 (22H,s,CH_2 alkyl chain);
1.45 (2H,broad m,$C_\gamma H_2$); 2.00 (2H,m,$C_\beta H_2$); 3.77 (3H,broad m,CH_3 Me ester); 4.26 (1H,m,C_2H)

MS m/e M^+ (%) $C_{17}H_{33}O_2Br$ (349,351) 352 (M^++1) (478), 350 (M+1) (49.2), 306 (100), 270 (53.1), 198 (7.9), 154 (11.2), 137 (29.5), 95 (14.9), 81 (16.1), 69

(27.3), 55 (38.9).

2-BROMOTETRADECANOIC ACID METHYL ESTER (LX)

2-bromohexadecanoic acid (5.00g, 0.0163M) and $SOCl_2$ (15.1ml) in MeOH (100ml) reacted as in LVII. Yield = 3.81g (72.9%).

NMR ($CDCl_3$) 0.98 (3H,t,CH_3 alkyl chain); 1.27 (18H,s,9 x CH_2 alkyl chain); 1.44 (2H,m,$C_\gamma H_2$ alkyl chain); 2.00 (2H,m,$C_\beta H_2$ alkyl chain); 3.77 (3H,d,CH_3 methyl ester); 4.24 (1H,m,C_2H);

MS m/e M^+ (%) $C_{15}H_{29}O_2Br$ (320,322) 323 (50.9) (M^++1), 321 (57.1) (M^++1), 277 (100), 241 (47.6), 199 (5.9), 154 (10.0), 137 (30.0) 109 (10.0), 95 (15.3), 87 (44.7), 81 (17.1), 69 (27.1), 55 (40.6).

2-BROMOHEXADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID METHYL ESTER (LXI)

XXXXV (1.00g, 3.407mM) was dissolved in DCM (20ml) and EtOAc (15ml), 2-bromohexadecanoic acid (1.14g, 3.257mM), TEA (0.34g, 3.360mM) and EDAC (0.65g, 3.391mM) were added as in method [C]. Yield = 1.34g, (71.7%).

NMR ($CDCl_3$) 0.88 (6H,t,2 x CH_3 C_{16} C_{14}); 1.30 (40H,s,20 x CH_2 alkyl C_{16} C_{14}) 1.43 (2H,m,$C_\gamma H_2$ C_{16}); 1.71 (2H,m,$C_\gamma H_2$ C_{14}); 1.88 (2H,m,$C_\beta H_2$ C_{16}); 2.06 (2H,m,$C_\beta H_2$ C_{14}); 3.76 (3H,s,CH_3 Me ester); 4.32 (1H,m,$C_\alpha H$ C_{14}); 4.55 (1H,m,C_2H C_{16}); 6.77 (1H,t,NH)

MS m/e M^+ (%) $C_{31}H_{60}NO_3Br$ (573,575) 599 (52.4) (M^++Na) 597 (56.8) (M^++Na), 577 (21.8), 575 (24.1), 517 (22.4),495 (9.4), 411 (5.9), 344 (7.9), 332 (8.8), 258 (20.3), 198 (100), 176 (36.5), 154 (18.2), 137 (18.8), 95 (14.4), 81 (15.0), 69 (24.7), 55 (36.2).

2-BROMOHEXADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID (LXII)

LXI (0.6g, 1.04mM) was dissolved in MeOH (15ml) and DCM (15ml) and IN NaOH (2.3ml) added as in method [D].

Yield = 0.57g (97.9%)

NMR ($CDCl_3$) 0.89 (6H,t,CH_3 x 2 C_{16} C_{14}); 1.30 (40H,s,20 x CH_2 C_{16} and C_{14}); 1.48 (4H,m,$C_\gamma H_2$ C_{14}, $C_\gamma H_2$ C_{16}); 1.73 (2H,m,$C_\beta H_2$ C_{14}); 2.06 (2H,m,$C_\beta H_2$ C_{16}); 4.35 (1H,m,$C_\alpha H$ C_{14}); 4.56 (1H,m,$C_\alpha H$ C_{16}); 6.77 (1H,m,NH C_{14})

MS m/e M^+ (%) $C_{30}H_{58}NO_3Br$ (559,561), 606 (M^++2Na) (23.2), 604 (M^++2Na) (28.2), 584 (M^++Na) (40.0), 582 (45.9), 562 (M^++H) (4.7), 560 (6.8), 516 (27.6), 436 (9.7), 332 (10.4), 252 (7.6), 198 (100), 176 (20.9), 137 (13.5), 81 (16.5), 69 (26.2), 55 (45.3).

2-BROMOHEXADECANOYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID METHYL ESTER (LXIII)

LXII (0.57g, 1.02mM), XXXXV (0.30g, 1.02mM) HOBt (0.14g, 1.036mM) in DCM (20ml) TEA (0.103g, 1.018mM) and EDAC (0.195g, 1.017mM) were reacted as in XXXXVII. Yield = 0.57 (71.2%).

NMR ($CDCl_3$) 0.87 (9H,t,3 x CH_3 C_{16} 2 x C_{14}); 1.29 (58H,29 x CH_2 alkyl C_{16}, C_{14}); 1.43 (2H,m,C_γH2 C_{16}); 1.72 (4H,m,2 x $C_\gamma H_2$ C_{14}); 1.89 (2H,m,$C_\beta H_2$ C_{16}); 2.06 2H,m,$C_\beta H_2$ C_{14}); 2.08 (2H,m,$C_\beta H_2$ C_{14}); 3.76 (3H,s,CH_3 Me ester); 4.30 (1H,m,$C_\alpha H$ C_{16}); 4.51 (1H,m,$C_\alpha H$ C_{14}); 4.56 (1H,m,$C_\alpha H$ C_{14});6.78 (1H,m,NH C_{16}-C_{14}); 6.87 (1H,m,NH C_{14}-C_{14})

MS m/e M^+ (%) $C_{45}H_{87}N_2O_4Br$ (798,800) 822 (43.7), 820 (41.2), 801 (22.3), 803 (26.1), 757 (19.7), 632 (8.9), 523 (36.7), 451 (42.6), 334 (15.8), 332 (17.9), 258 (11.2), 198 (100), 176 (40.6), 154 (25.3), 137 (11.7), 95 (13.8), 81 (13.9), 69 (22.4), 55 (35.3).

ENKEPHALINS

EXPERIMENTS

N-α-TERTIARYBUTOXYCARBONYL-PHENLALANYL-LEUCINE METHYL ESTER (LXIV)

Method F

In a 250 ml round bottom flask equipped with magnetic stirrer and drying tube, Leucine methyl ester hydrochloride (Leu O Me HCl) (5.0g, 27.5 mM), N-α-tertiarybutoxycarbonyl phenylalanine (Boc Phe) (7.3g, 27.3 mM) and HOBt (3.72g, 27.3 mM) were dissovled in EtOAc (50 ml) and DCM (100 ml). TEA (2.78g, 27.3 mM) was added and the reaction mixture cooled to -10°C in an ice/salt bath. DCC was added to the reaction, which was allowed to stir overnight. The precipitated DCU was filtered and the solvent evaporated in vacuum. EtOAc (20 ml) was added to the residue any precipitate filtered and the filtrate washed with 10% citric acid (5 ml), $NaHCO_3$ (sat) (5 ml) and brine (5 ml). The organic layer was dried over (anh Na_2SO_4 and evaporated. Yield = 10.50g (97.4%). White crystalline solid mpt = 96-98°C

NMR ($CDCl_3$) 0.93 (6H, t,2xCH_3 Leu); 1.43 (9H, s, $(CH_3)_3$ Boc); 1.53 (3H, m,$C_\beta H_2$ Leu, $C_\gamma H$ Leu);3.07 (2H, d,$C_\beta H_2$ Phe); 3.69 (3H, s,CH_3 Me ester); 4.34 (1H, m,$C_\alpha H$ Leu); 4.53 (1H, m,$C_\alpha H$ Phe); 4.99 (1H, broad s, Boc NH, Phe); 6.26 (1H, d,NH Leu); 7.26 (5H,m,C_6H_5 Phe).

MS m/e M^+(%) C_{21} H_{32} N_2 O_5 (392), 437(M^++2Na)(2.9), 415(M^+ + Na)(100), 399 (1.8), 363(4.4), 337(2.5), 315(38.2), 293(4.4), 223(2.9), 164(1.3), 146(2.1), 120 (5.3), 86(3.8), 57(6.8).

PHENYLALANYL-LEUCINE METHYL ESTER HYDROCHLORIDE (LXV)

Method G.

LXIV (2.79g, 7.57 mM) was dissolved in MeOH (60 ml) and 30% HCl in MeOH (6 ml) added. The reaction mixture was refluxed for 20 mins. and the solvent evaporated. Yield = 2.40g (96.4%) Oil.

NMR ($CDCl_3$) 0.85 (6H,t,2xCH_3 Leu); 1.62 (3H,d,$C_\beta H_2$ Leu; $C_\gamma H$ Leu) 3.43 (2H,m,$C_\beta H_2$ Phe); 3.65 (3H,s,CH_3Me ester); 4.36 (1H,q,$C_\alpha H$ Leu); 4.60 (1H,broad s,$C_\alpha H$ Phe); 7.34 (5H,m,C_6H_5 Phe); 7.63 (1H,d,NH Leu); 8.29 (3H,broad s, NH_3^+ Phe)

MS me/ M^+ (%) $C_{16}H_{25}N_3O_3Cl$ (328, 330), 315(M^+-HCl+Na)(2.9), 293(M^+-HCl) (100), 279(2.6), 233(2.4), 225(1.8), 217(1.2), 198(2.4), 146(13.6), 133(14.7), 120 (47.3), 103(2.4), 91(2.9), 86(6.9), 77(1.8), 55(1.2).

N-α-TERTIARYBUTOXYCARBOXY-GLYCYL-PHENYLALANYL-LEUCINE METHYL ESTER (LXVI)

Boc Gly (1.29g, 7.28 mM), LXV (2.40g, 7.28 mM), HOBt (13.4g, 7.28 mM), TEA (0.7g, 7.28 mM) and DCC (1.50g, 7.28 mM) in Et0Ac (20 ml) and DCM (25 ml) were reacted as in Method F. Yield = 2.46g (77.5%) mpt = 100 - 102°C.

NMR ($CDCl_3$) 0.88 (6H,d,d 2xCH_3 Leu); 1.45 (9H,s,$(CH_3)_3$ Boc); 1.54 (3H,m,$C_\beta H_2$ Leu, CγH Leu); 3.08 (2H,m,$C_\beta H_2$ Phe); 3.68 (3H,s,CH_3Me ester); 3.76 (2H,m,CH_2 Gly); 4.52 (1H,m,$C_\alpha H$ Leu); 4.66 (1H,q,C_α Phe) 5.08 (1H, broad s, NH Gly); 6.31 (1H,d,NH Leu); 6.89 (1H,d, NH Phe); 7.27 (5H,m,C_6H_5 Phe)

MS m/e M^+ (%) C_{23} H_{35} N_3 O_6 (449), 494(M^+ + 2Na)(6.8), 472(M^+ + Na)(100), 420(8.0), 372(56.6), 299(5.0), 199(5.3), 173(8.1), 133(11.1), 57(60.0).

GLYCYL-PHENYLALANYL-LEUCINE METHYL ESTER HYDROCHLORIDE (LXVII)

LXVI (2.0g, 4.45 mM), was dissolved in MeOH (40 ml) and 30% HCl in MeOH (4 ml) added, as in method G. Yield = 1.70g (99%).

NMR($CDCl_3$) 0.86(6H,d,2xCH_3Leu); 1.58(1H,m,C_γHLeu); 1.76(2H,m,$C_\beta H_2$Leu); 3.07(2H,broads,$C_\beta H_2$Phe); 3.61(3H,s,CH_3Me ester); 3.82(1H,broads,CH Gly);

3.99(1H,broad, CH Gly); 4.39(1H, broad s, $C_\alpha H$ Leu); 4.84(1H,broads,CαHPhe); 7.19(5H,broad s, C_6H_5 Phe); 7.50(1H,broad s,NH Leu); 7.88(3H,broad s,NH_2 Gly NH Phe); 8.54(1H, broad s, NH Gly).

MS m/e M^+ (%)$C_{18}H_{28}N_3O_4$ Cl (385/387), 721(2 x M^+-HCl + Na)(5.4), 699(2x M^+-HCl)(7.5), 638(8.6), 499(13.2), 431(98), 372(2xM^+-HCl)(100), 350(M^+-HCl)(622), 293(21.8), 225(9.4), 205(26.7), 177(42.2), 146(64.7), 120(79.5), 86(38.2), 73(8.4), 57(7.4).

N-α-TERTIARYBUTOXYCARBONYL-D-ALANYL-GLYCYL-PHENYLALANYL LEUCINE METHYL ESTER (LXVIII)

LXVII (1.5g, 3.89 mM) Boc D Ala (0.74g, 3.89 mM), HOBt (o.53g, 3.89 mM) TEA (0.39g, 3.89 mM) and DCC (0.81g, 3.89 mM) were dissolved in EtOAc (10 ml) and DCM (10 ml) as in Method F. Yield = 1.83g (90.4%).

NMR ($CDCl_3$) 0.90 (6H,d.d. 2xCH_3 Leu); 1.37 (3H,d,CH_3 Ala); 1.46 (9H,s, $(CH_3)_3$ Boc); 1.59 (3H,m,$C_\beta H_2$ Leu, $C_\gamma H$ Leu); 3.11 (2H,m,CH_2 Phe); 3.69 (3H,s,OCH_3 Me ester); 3.89 (2H,m,C_α Gly); 4.12 (1H,t,$C_\alpha H$ D Ala);4.50 (1H,m,$C_\alpha H$ Leu); 4.68 (1H,q, $C_\alpha H$ Phe); 4.99 (1H,d, NH D Ala); 6.50 (1H,m, NH Leu); 67.2 (1H,t,NH Gly); 6.84 (1H,m,NH Phe);7.26 (5H,m,C_6H_5 Phe)

MS m/e M^+ (%) $C_{26}H_{40}N_4O_7$ (520), 543(M^++Na)(5.7), 521(M^+H)(10.6), 499(6.5), 431(26.5), 421(5.6), 320(26.2), 293(27.1.), 276(9.4), 225(8.8), 146(35.9), 133 (18.8), 120(100), 86(19.4), 57(18.5).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINODECANOYL-GLYCYL PHENYLALANYL-LEUCINE METHYL ESTER (LXIX)

LXV (0.65g, 1.69 mM), II (0.46g, 1.69 mM), HOBt (0.23g, 1.69 mM), TEA (0.17g, 1.69 mM), and DCC (0.35g, 1.69 mM) were dissolved in EtOAc (10 ml) and DCM (10 ml). The reaction was carried out as Method F. Yield = 1.00g (92.4%).

NMR ($CDCl_3$) 0.90 (9H,m,CH_3 C_{10}, 2 x CH_3 Leu); 1.29 (12H,s,CH_2 x 6 C_{10});

1.46 (9H,s,$(CH_3)_3$ Boc); 1.74 (5H,m,$C_\beta H$,C_{10},$C_\beta H_2$ Leu, $C_\gamma H$ Leu), 3.05 (2H,m,CH_2 Phe); 3.66 (3H,s,CH_3 Me ester); 3.92 (1H,m,$C_\alpha H$ C_{10}); 4.13 (1H,m,CH Gly); 4.24 (1H,mH, Gly); 4.53 (1H,m,C_α H Leu) 4.82 (1H,m,$C_\alpha H$ Phe); 5.37 (1H,m, NH C_{10}); 6.98 (1H,d,NH Gly); 7.21 (5H,m,C_6H_5 Phe); 7.72 (1H,d,NH Leu); 7.86 (1H,d,NH Phe)

MS m/e (%) C_{33} H_{54} N_4 O_7 (618) 633(M^+ + 2Na)(4.4), 642(M^+ + Na)(100), 584(2.9), 541(20.6), 468(2.4), 441(2.0), 324(2.6), 176(3.5), 120(5.9, 57(3.8).

N-α-TERTIARYBUTOXYCARBONYL-D-ALANYL-GLYCINE ETHYL ESTER (LXX)

tBoc D Ala (1.5g, 7.93 mM), Gly O Et HCl (1.11g, 7.93 mM) HOBt (1.07g, 7.93 mM), TEA (0.80g, 7.93 mM) and DCC (1.64g, 7.93 mM) were reacted as in Method F. Yield = 1.90g (87.4%).

NMR ($CDCl_3$) 1.29 (3H,t,CH_3 Et ester); 1.38 (3H,d,CH_3 Ala); 1.45 (9H,s,$(CH_3)_3$ Boc); 4.02 (2H,d,CH_2 Gly); 4.21 (3HH ,q,CH_2 Et ester, $C_\alpha H$ Ala); 4.95 (1H,broad d,NH Ala); 6.58 (1H, broad s, NH Gly);

MS m/e (%) $C_{12}H_{22}N_2O_5$ (274) 319(M^+ + 2Na)(1.5), 297(M^+ + Na)(100), 275(M^+ + 1)(3.2), 219(18.2), 197(12.1), 175(11.2), 199(1.8), 104(9.4) 88(5.9), 57(60.6).

dl-α-2-AMINODECANOYL-GLYCYL-PHENYLALANYL-LEUCINE METHYL ESTER HYDROCHLORIDE (LXXI)

LXIX (0.75g, 1.17 mM) was dissolved in MeOH (15 ml) and 30% HCl in MeOH (1.5 ml) was added, as in Method G. Yield = 0.67g (100%).

NMR ($CDCL_3$) 0.8 (9H, m.2xCH_3 Leu, CH_3 C_{10}); 1.21 (12H, s,CH_2x6,C_{10}); 1.37 (2H, m,$C_\beta H_2$, C_{10}); 1.55 (3H,m,$C_\beta H_2$ Leu C_γ H Leu) 3.13 (2H, broad d, CH_2 Phe); 3.69 (3H,d, CH_3 Me ester); 4.04 (2H,m,$C_\alpha HC_{10}CH$ Gly); 4.33 (1H, broad s, CH Gly); 4.57 (2H,m,$C_\alpha H$ Leu $C_\alpha H$ Phe); 7.17 (5H,s,C_6H_5

Phe); 7.43 (1H, broad s, NH Gly); 8.06 (3H,broad m, C_{10}); 8.46 (1H, broad s, NH Leu); 8.57 (1H, broad s, NH Phe).

MS m/e M^+ (%) $C_{28}H_{47}N_4O_5$ Cl (554/556) 579(M^+ + H)(23.5), 475(11.2), 430 (5.7), 324(6.8), 176(5.3), 120(7.4), 91(15.9), 57(6.3).

D-ALANYL-GLYCYL-PHENYLALANYL-LEUCINE METHYL ESTER HYDROCHLORIDE (LXXII)

LXVIII (0.75g, 1.44 mM), was dissolved in MeOH (15 ml) and 30% HCl in MeOH (1.5 ml) added, as in Method G. Yield = 0.60g, (91.1%).

NMR ($CDCl_3$) 0.89 (6H,t,2xCH_3 Leu); 1.37 (3H,t,CH_3 D Ala); 1.50 (1H,m,C_γ H Leu); 1.61 (2H,m,$C_\beta H_2$ Leu); 3.26 (2H,m,CH_2 Phe); 3.54 (3H,s,CH_3 Me ester); 3.70 (2H,m,CH_2 Gly); 4.15 (1H, broad s, C_αH Ala); 4.32 (1H,broad s, C_αH Leu); 4.43 (1H, broad s, C_αH Phe); 7.16 (5H, broad m, C_6H_5 Phe); 7.50 (3H,broad s,NH_3^+ D Ala); 7.73 (1H,broad t,NH Gly); 8.05 (2H,m,NH Phe, NH Leu).

MS m/e M^+ (%) $C_{21}H_{33}N_4O_5$ Cl (456/458) 443(M^+-HCl+Na)(6.3), 420(M^+-HCl) (100), 293(12.9), 264(15.7), 225(9.1), 146(37.4), 120(67.8), 91(45.9), 57(20.2).

N-α-TERTIARYBUTOXYCARBONYL-ALANYL-GLYCINE (LXXIII)

LXX (0.88g, 3.21 mM) was dissolved in MeOH (30 ml) and NaOH (7.06 ml) added. The reaction was stirred and followed by TLC, as in method D.

NMR ($CDCl_3$) 1.40 (3H,d,CH_3 Ala); 1.47 (9H,s,$(CH_3)_3$ Boc); 4.06 (1H, broad s, C_αH Gly); 4.08 (1H, broad s, C_α Gly); 4.30 (1H, broad s, C_αH Ala); 5.07 (1H, broad s, NH Ala); 6.86 (1H, broad t, NH Gly).

MS m/e M^+(%) $C_{10}H_{18}N_2O_5$ (246), 291 (M^++2Na)(66.6), 269(M^++Na)(100), 247 (M^+H)(2.6), 191(26.0), 160(19.1), 154(16.8), 136(12.4), 107(4.4), 57(9.6).

N-α-TERTIARYBUTOXYCARBONYL-D-ALANYL-GLYCYL-dl-α-2-AMINOTETRADECANOIC ACID METHYL ESTER (LXXIV)

XXXXV (0.82g, 2.80 mM) was dissolved in DCM (10 ml) TEA (0.28g, 2.80 mM) HOBt (0.38g, 2.80mM), LXXIII (0.69g, 2.90mM) in Et0Ac (10 ml) and EDAC (0.54g, 2.80 mM) were added as in method F.

NMR($CDCl_3$) 0.83 (3H,t,CH_3 C_{14}); 1.29 (2OH,s, 10 xCH_2 C_{14}); 1.37 (3H,d.CH_3D Ala);1.43 (9H,s,$(CH_3)_3$ Boc); 1.74 (2H,m,C_β H_2,C_{14}); 3.72 (3H,d,CH_3Me ester); 3.85 (½H,d, CH Gly); 3.91 (½H,d, CH Gly); 4.03 (½H,d, CH Gly); 4.10 (½H,d, CH Gly); 4.14 (1H,m,C_αH C_{14}); 4.53 (1H,m,C_α H Ala); 4.98 (1H,t, NH Ala); 6.66 (1H,m, N H C_{14}); 6.80 (1H, q, N H gly).

MS m/e M^+ (%) $C_{25}H_{47}N_3O_6$ (485), 530(M^+ + 2Na)(2.2), 508(M^+ + Na)(100), 486(M+H)(2.1), 430(11.2), 408(17.9), 315(6.8), 258(20.0), 198(29.7), 88(4.7), 57 (15.6).

N-α-TERTIARYBUTOXYCARBONYL-D-ALANYL-GLYCYL-dl-α-2-AMINOTETRADECANOIC ACID (LXXV)

LXXIV (1.07g, 2.21 mM) was dissolved in MeOH (28ml) and lN NaOH (4.85 mM), added as for method D. Yield = 0.85g (81.8%).

NMR ($CDCl_3$) 0.88 (3H,t,CH_3 C_{14}); 1.26 (20H,s, 10 x CH_2 C_{14}); 1.36 (3H,d,CH_3 D Ala); .45 (9H, s,$(CH_3)_3$ Boc); 1.72 (1H, m, C_β H C_{14}); 1.89 (1H,m, C_βH C_{14}); 3.89 (1H,d, C_αH Gly); 4.11 (1H,d, C_α H Gly); 4.22 (1H,m,C_αH C_{14}); 4.46 (1H,m, C_αH D Ala); 5.16 (½H, broad s, NH D Ala); 5.27 (½H, broad s, NH D Ala); 7.06 ((1H,d, NH Gly); 7.33 (1H, broad s, NH C_{14}).

MS m/e M^+ (%) $C_{24}H_{45}N_3O_6$ (471), 494 (M^+ + Na)(6.5), 472(M^+ + 1)(2.1), 416 (14.4), 372(12.1), 301(30.9), 244(46.5), 225(12.6), 198(100), 173(9.4), 133(19.7), 88(9.4), 57(38.2).

N-α-TERTIARYBUTOXY-D-ALANYL-GLYCYL-dl-α-2-AMINOTETRADECANOYL-LEUCINE METHYL ESTER (LXXVI)

LXXV (0.85g, 1.81 mM), leucine methyl ester hydrochloride (0.33g, 1.8mM), TEA (0.18g, 1.81 mM), HOBt (0.24g, 1.81 mM) and EDAC (0.35g, 1.81 mM) were reacted as in method F. Yield = 0.90g (83.4%).

NMR ($CDCl_3$) 0.91 (9H,m, 2x CH_3 Leu,CH_3 C_{14}); 1.26 (20H,s, 10 x CH_2 C_{14}); 1.35 (3H,d,d, CH_3 Ala); 1.45 (9H,s,$(CH_3)_3$ Boc); 1.66 (4H,m, $C_\gamma H$ Leu, $C_\beta H_2$ Leu, $C_\beta H$ C_{14}); 1.89 (1H,m, $C_\beta H$ C_{14}); 3.70 (3H,d, CH_3 Me ester); 4.98 (2H,m, CH_2 Gly); 4.19 (1H,m, C_α H C_{14}); 4.46 (1H,m,C_α H Ala)4.56 (1H,m, C_α H Leu); 5.11 (1H,d, NH D Ala); 6.83 (2H,m,NH C_{14} NH Leu);7.02 (1H,t,NH Gly).

MS m/e M^+ (%) $C_{31}H_{58}N_4O_7$ (598), 643(M^+ + 2Na)(7.4), 621(M^+ + Na)(100), 565 (6.2), 521(50.9), 450(5.3), 348(7.4), 279(3.2), 198(31.2), 146(6.8), 86(5.1), 57 (8.8).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY)-D-ALANYL-GLYCYL-PHENYLALANYL-LEUCINE-METHYL ESTER (LXXVII)

LXXII (0.60g, 1.31 mM), Boc Tyr (Bzl)OH (0.49g, 1.31 mM), TEA (0.13g, 1.31 mM) HOBt (0.18g, 1.31 mM) and DCC (0.27g, 1.31 mM), were reacted as in Method C. Yield = 0.60g (60.5%) Purity was determined by TLC (system J) and purified by prep TLC.

NMR ($CDCl_3$) 0.87 (6H,m, 2x CH_3 Leu); 1.22 (3H,d, CH_3 Ala); 1.40 (9H,s,$(CH_3)_3$ Boc); 1.58 (1H, m, C_γ H Leu); 1.81 (2H,m,$C_\beta H_2$ Leu); 3.00 (4H,broad m, CH_2 Phe,CH_2 Tyr); 3.67 (3H,s, CH_3 Me ester); 3.90 (2H,m, CH_2 Gly);4.30 (2H,m, C_αH Ala, C_αH Leu); 4.56 (1H,m, C_α H Phe); 4.85 (1H,m, C_α H Tyr); 5.02 (2H,s, CH_2 Benzyl); 5.62 (1H,d, NH Tyr); 6.62 (1H, broad s, NH Leu); 6.71 (1H,t, NH Gly); 6.92 (2H,d, C_6H_4AB Tyr); 6.81 (1H,d, NH D Ala); 7.00 (1H,NH Phe); 7.14 (2H,d, C_6H_4(AB) Tyr); 7.22 (5H,m, C_6H_5 Phe); 7.40 (5H,m,C_6H_5 Benzyl)

MS m/e M^+ $C_{42}H_{55}N_5O_8$ (757), 781(M^+ + Na)(14.7), 578(9.4), 431(70.3), 410 (38.5), 310(100), 292(22.6), 272(33.8) 253(17.2), 226(19.5), 197(19.4), 147(20.9), 91(66.5), 57(28.2).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL-(BENZYL-HYDROXY)-dl-α-2-AMINODECANOYL-GLYCYL-PHENYLALANYL-LEUCINE METHYL ESTER (LXXVIII)

LXXI (0.67g, 1.42 mM), Boc Tyr (Bzl)OH (0.63g, 1.42 mM), HOBt(0.19g, 1.42 mM), TEA (0.14g, 1.42 mM) and EDAC (0.27g, 1.42 mM) were reacted as for Method F. Purification was by prep TLC (system J) Yield = 0.65g (52.4%).

NMR ($CDCl_3$ 0.87 (9H,m,CH_3 C_{10} CH_3 x 2 Leu); 1.30 (12H,m,6xCH_2 C_{10})1.41 (9H,d, $(CH_3)_3$ Boc); 1.55 (1H, m,C_γH Leu_{10}); 1.71 (1H,m,C_βH C_{10}); 1.94 (3H,m,C_βH C_{10} $C_\beta H_2$ Leu); 2.78 (2H,m,$C_\beta H_2$ Phe); 3.06 (2H,m,$C_\beta H_2$ Tyr); 3.68 (3H,d,CH_3 Me ester); 3.83 (1H,m, C_αH C_{10}); 4.26 (2H,q,CH_2 Gly); 4.37 (1H,m,C_αH Leu); 4.50 (1H,m,C_αH Phe); 4.75 (1H,m,C_αH Tyr); 5.03 (2H,d,CH_2 Benzyl); 5.63 (1H,d,NH Tyr); 6.78 (2H,m,NH Leu, NH C_{10}); 6.90 (2H,d,d, C_6H_4 AB Tyr); 7.10 (2H,dd, C_6H_4 AB Tyr);7.22 (2H,m,C_6H_5 Phe); 7.22 (10H,m,C_6H_5 Benzyl, NH Gly, NH Phe, 3H of C_6H_5 Phe).

MS m/e M^+ (%) $C_{42}H_{53}N_5O_9$ (773) 781(14.4), 578(9.7), 449(9.1), 431(70.3), 410 (38.8), 372(6.2), 310(100), 292(22.6), 272(33.5), 253(17.1), 226(19.4), 197(18.8), 147(20.6), 91(66.8), 57(27.6).

D-ALANYL-GLYCYL-dl-α-2-AMINOTETRADECANOYL-LEUCINE METHYL ESTER HYDROCHLORIDE (LXXIX)

LXXVI (0.84g, 1.40 mM) was dissolved in MeOH (17 ml) and 30% HCl in MeOH (1.7 ml) added as for Method G. Yield = 0.69g (98.6%).

NMR ($CDCl_3$) 0.93 (9H,t,2xCH_3 Leu, CH_3 C_{14}); 1.30 (20H,s, 10 x CH_2 C_{14}); 1.38 (3H,d,d CH_3 D Ala); 1.59 (3H,m, C_γ H Leu, C_βH Leu, C_β H C_{14}); 1.75 (2H,m,C_βH Leu, $C_\beta HC_{14}$); 3.69 (3H,s, CH_3 Me ester); 4.10 (1H, broad s, C_α

H C_{14}); 4.43 (3H,broad s, C_α H Ala CH_2 Gly); 4.65 (1H, broad s, C_α H Leu); 7.99 (3H, broad s, NH_3^+ D Ala); 8.34 (1H,broad s, NH C_{14}); 8.50 (1H,broad s, NH Leu); 8.65 (1H, broad s, NH Gly).

MS m/e M^+ (%) $C_{26}H_{57}N_4O_5Cl$ (534/536), 521(M^+-HCl+Na)(100), 507(36.3), 499 (M^++1)(8.23), 450(5.6), 419(6.5), 407(2.1), 348(5.7), 255(5.0), 198(78.2), 146 (12.9), 86(13.5), 55(11.5).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL-(BENZYL HYDROXY)-D-ALANYL-GLYCYL-dl-α-2-AMINOTETRADECANOYL-LEUCINE METHYL ESTER (LXXX)

LXXIX (0.74g, 1.38 mM), tBOC Tyr (Bzl)OH (0.51g, 1.38 mM), HOBT (0.19g, 1.38 mM) TEA (0.14g, 1.38 mM) and EDAC (0.27g, 1.38 mm) were reacted as for Method F. Purity was observed by TLC (systems J and H). Yield = 0.92g (78.1%).

NMR ($CDCl_3$) 0.89 (9H,m,CH_3 C_{14}, 2 x CH_3 Leu); 1.19 (20H,s, 10 xCH_2 C_{14});1.38 (9H,d,$(CH_3)_3$ Boc); 1.64 (2H,m,C_γH Leu, C_βH C_{14});1.82 (3H,m,C_βH C_{14}, $C_\beta H_2$ Leu); 3.10 (2H,m,$C_\beta H_2$ Tyr); 3.68 (3H,d,CH_3 Me ester); 3.92 (1H,d, CH Gly); 4.13 (1H,d, CH Gly);4.40 (1H,m,C_αH C_{14}); 4.64 (3H,m,C_α H Tyr, C_α Leu, C_α H Ala);4.99 (2H,s,CH_2 Benzyl); 5.56 (½H,d,NH Tyr); 5.80 (½H,d,NH Tyr);6.88 (2H,m,C_6H_4 AB Tyr); 7.10 (2H,m,C_6H_4 AB Tyr); 7.39 (5H,m,C_6H_5Benzyl); 7.60 (2H,broad s, NH C_{10}, Leu); 7.74 (½H,m, NH Gly);7.84 (1H,m,½NH Gly, ½ NH Ala); 8.05 (½H,broad s, NH Ala);

Ms m/e M^+ (%) $C_{47}H_{73}N_5O_9$ (851) 874(M^+ + Na)(100), 817(7.6), 646(5.3), 577 (16.5), 547(6.5), 522(11.8), 476(5.3), 435(9.4), 316(7.4), 279(5.9), 226(15.9), 198(38.5), 173(5.3), 146(9.4), 91(38.2), 57(23.5).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY)-D-ALANYL-GLYCYL-PHENYLALANYL-LEUCINE (LXXXI)

(LXXVII) (1.12g, 1.48 mM) was dissolved in MeOH (10 ml) and 1N NaOH

(3.25 ml) added to the reaction and proceeded as for Method D. Yield = 0.31g (28.2%).

NMR ($CDCl_3$ + MeOH) 0.88 (6H,m,2 x CH_3 Leu); 1.13 (3H,d, CH_3 Ala); 1.43 (9H,s(CH_3)$_3$ Boc); 1.64 (1H,m,$C_\gamma H$ Leu); 1 .93 (2H,m,$C_\beta H_2$ Leu);3.12 (4H,broad m,$C_\beta H_2$ Phe, $C_\beta H_2$ Tyr); 4.02 (2H,broad s,CH_2 Gly);4.38 (2H,m,$C_\alpha H$ Ala, $C_\alpha H$ Leu); 4.63 (1H,m,$C_\alpha H$ Phe). 4.90 (1H,m,$C_\alpha H$ Tyr) 5.03 (2H,s,CH_2 Benzyl); 5.74 (1H,broad s,NH Tyr); 6.88 (1H, broad s, NH D Ala); 6.92 (1H, NHGly); 6.95 (2H,d,C_6H_4 AB Tyr);7.12 (1H,broad s, NH Phe); 7.18 (2H,d,C_6H_4 AB Tyr); 7.26 (6H,m,C_6H_5 Phe, NH Leu); 7.43 (5H,m,C_6H_5 Benzyl).

MS m/e M^+ (%) $C_{41}H_{53}N_5O_9$ (759) 805(M+ 2 Na)$^+$(3.5), 782(M + Na)$^+$(3.5), 704 (3.8), 682(5.9), 529(100), 486(10.9), 432(100), 394(8.9), 329(15.9), 304(12.1), 226 (61.8), 176(77.6), 154(20.3), 120(16.5), 91(41.3).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYLHYDROXY)-D-ALANYL-GLYCYL-PHENYLALANYL-LEUCINE POTASSIUM CROWN ETHER (LXXXII)

Method H

LXXXI (0.300g, 0.40 mM) was dissolved in EtOH:H_20 1:1 (2 ml) KOH (0.0216g, 0.40 mM) and 18-crown-6-ether (0.1067g, 0.40 mM) were added and the reaction stirred for 2 hours. The EtOH was removed and the water removed by lyophilisation. Yield = 0.42g (100%).

NMR ($CDCl_3$) 0.87 (6H,m,2 x CH_3 Leu); 1.12 (3H,d,CH_3 D Ala); 1.42(9H,s,(CH_3)$_3$ Boc); 1.66 (1H,m,$C_\gamma H_2$ Leu); 1.95 (2H,m,$C_\beta H_2$ Leu); 3.02 (2H,broad m, $C_\beta H_2$ Phe); 3.13 (2H,m,$C_\beta H_2$ Tyr); 3.63 (24H,s,CH_2 19 crown 6 ether); 4.04 (2H,m,CH_2 Gly); 4.44 (2H,m,$C_\alpha H$ D Ala $C_\alpha H$ Phe) 4.86 (1H,m,$C_\alpha H$ Tyr); 4.93 (1H,m,$C_\alpha H$ Leu); 5.02 (2H,s,CH_2 Benzyl); 5.68 (1H,broad s, NH Tyr); 6.79 (1H, broad s, D Ala); 6.86(1H,t,NH Gly); 6.91 (2H,d,C_6H_4 AB Tyr); 7.02 (lH,broad s,NH Phe); 7.20 (2H,d,C_6H_4AB Tyr); 7.24 (6H,m,C_6H_5 Phe,

NH Leu); 7.40 (5H,m,C_6H_5 Benzyl).

MS m/e M^+ (%) $C_{53}H_{76}N_5O_{15}K$ (1061) 782(M^++Na^+ H)(7.3), 668(11.5), 594 (15.9), 517(8.2), 303(100), 268(18.3).

N-α-TERTIARYBUTOXYCARBONYL-LEUCINE POTASSIUM SALT 18-CROWN-6-ETHER (LXXXIII)

Boc Leu OH (5.00g, 20,1 mM), KOH (1.12g, 20.1 mM) and 18-crown-6-ether (5.31g, 20.1 mM) were dissolved in 1:1 EtOH:H_20 (50 ml) as per method H. Yield = 10.35g, (98.4%).

NMR ($CDCl_3$) 0.87 (6H,m,2 x CH_3 Leu); 1.34 (9H,s,$(CH_3)_3$ Boc); 1.66 (3H,m, $C_\beta H_2$ Leu, $C_\gamma H$ Leu); 3.54 (24H,s,CH_2 Crown ether); 4.04 (lH,m,$C_\alpha H$ Leu) 5.55 (lH,d,NH Leu)

MS m/e M^+ (%) $C_{23}H_{44}N\ O_{10}$ K(535.32) 529(1.8), 461(1.8), 395(2.9), 303(100), 287(60.3), 272(10.3), 242(6.5), 176(32.4), 119(9.1), 55(12.9)

N-α-TERTIARYBUTOXYCARBONYL-D-ALANYL-GLYCYL-PHENYLALANINE METHYL ESTER (LXXXIV)

LXXIII (4.5g, 18.3 mM), Phe OME HCl (3.94g, 18.3 mM), TEA (1.85g, 18.3 mM), HOBt (2.47g, 18.3 mM) and DCC (3.77g, 18.3 mM) were reacted as in Method F. Yield = 6.44g (86.5%). The compound was purified by flash chromatography (system J) Yield = 5.20g (69.8%).

NMR ($CDCl_3$) 1.30 (3H,d, CH_3 Ala); 1.41 (9H,s, $(CH_3)_3$ Boc); 3.07 (2H, m, CH_2 Phe); 3.62 (3H,s, CH_3 Me ester); 3.86 (2H,m, CH_2 Gly); 4.14 (1H,t, $C_\alpha H$ Ala); 4.78 (1H,q,$C_\alpha H$ Phe); 5.24 (1H,d, NH Ala); 6.98 (1H,d, NH Gly);7.10 (3H,m. 2H of C_6H_5 NH Phe); 7.27 (3H,m,3H of C_6H_5 Phe)

MS m/e M^+ (%) $C_{20}H_{29}N_3O_6$ (407)431 (M^+ + NH + H)(100), 408(M^+ + H) (35.0), 308(100), 292(25.3), 272(23.5), 253(18.8), 226(22.1), 197(18.8), 147(24.4),

91(94.7), 57(42.6).

N-α-TERTIARYBUTOXYCARBONYL-LEUCYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER (LXXXV)

Boc Leu OH (0.56g, 2.24 mM), XXXXIV (0.50g, 2.24 mM), TEA (0.23g, 2.24 mM) HOBt (0.30g, 2.24 mM) and EDAC (0.43g, 2.24 mM) were reacted in DCM (15 ml) as per method F. Yield = 0.83g (85.6%).

NMR ($CDCl_3$) 0.88 (3H,t,CH_3 C_{10}); 0.95 (6H,m, 2x CH_3 Leu); 1.29 (12H,s, CH_2 x 6 C_{10}); 1.46 (9H,s, $(CH_3)_3$ Boc); 1.70 (2H,m,$C_\beta H$, C_{10},$C_\gamma H$ Leu); 1.83 (3H,m,$C_\beta H$ C_{10} $C_\beta H_2$ Leu); 3.72 (3H,s,CH_3 Me ester); 4.11 (1H,m,$C_\alpha H$ C_{10}); 4.56 (1H,m,C_α H Leu); 4.84 (1H, broad s, NH Leu); 6.50 (½H,d, NH C_{10}); 6.61 (½,d, NH C_{10}).

MS me/ M^+ (%) $C_{22}H_{42}N_2O_5$ (414) 437(M^++Na)(100), 415(M^++H)(5.0), 359 (17.1), 337(40.9), 315(35.3), 202(20.3), 142(41.8), 130(12.6), 86(37.1), 57(35.0).

N-α-TERTIARYBUTOXYCARBONYL-LEUCYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER (LXXXVI)

Boc Leu OH (0.276g, 1.11 mM), LI (0.45g, 1.11 mM), TEA (0.11g, 1.11 mM) HOBt (0.15g, 1.11 mM) and EDAC (0.21g, 1.11 mM) were reacted as in Method F. Yield = 0.57 g (88.3%).

NMR ($CDCL_3$) 0.88 (6H,t,CH_3 2 x C_{10}); 0.97 (6H,m, 2 x CH_3 Leu); 1.31 (24H,s, 12xCH_2 C_{10}); 1.47 (9H,s, $(CH_3)_3$ Boc); 1.67 (3H,m,2x$C_\beta H$, C_{10}, $C_\gamma H$, Leu);1.83 (4H,m,2x$C_\beta H$, C_{10}, $C_\beta H_2$ Leu); 3.73 (3H,t,CH_3 Me ester); 4.09 (1H, broad s, C_α H C_{10}); 4.31 (1H, broad s, C_α H C_{10}); 4.53 (1H, broad s, $C_\alpha H$ Leu); 4.84 (1H, broad s, NH Leu); 6.51 (2H,m, NH 2x C_{10}).

MS m/e M^+m (%) $C_{32}H_{61}N_3O_6$ (583), 607(M^++H+Na)(100), 507(3.8), 435(7.1), 371(3.8), 202(14.7), 142(38.5), 86(6.8), 57(10.3).

N-α-TERTIARYBUTOXYCARBONYL-LEUCYL-dl-2-AMINODECANOYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER (LXXXVII)

Boc Leu OH (0.08g, 0.313 mM), LV (0.18g, 0.313 mM), TEA (0.03g, 0.313 mM), HOBt (0.04g, 0.313 mM) and soluble DCC (0.06g, 0.313 mM) were reacted as in Method F in EtOAc (10 ml), and DCM (5 ml). Yield = 0.15g (63.8%).

NMR ($CDCl_3$) 0.86 (9H,t, CH_3 C_{10} x 3); 0.97 (6H,m,CH_3 x 2 Leu); 1.27 (36H,s,CH_2 x 18 C_{10}); 1.45 (9H,s,$(CH_3)_3$ Boc); 1.67 (4H,m,3xC_βH C_{10}, C_γ H Leu); 1.82 (5H,m, 3 x C_βH, C_{10}, 2 x C_βH, Leu); 3.71 (3H,d,OCH_3 Me ester); 4.03 (1H,m, C_α H C_{10}); 4.32 (1H,m,C_αH C_{10}); 4.33 (1H,m,C_αH C_{10}); 4.53 (1H,m, C_αH Leu); 4.88 (1H, broad s, NH Leu); 6.53 (3H, m, NH_3 x 3 C_{10}).

MS m/e M^+ (%) $C_{42}H_{80}N_4O_7$ (752), 798(M^++2 Na)(1.5), 776(M^++H+Na)(64.7), 676(17.1), 663(33.8), 563(19.7), 446(3.8), 371(11.8), 327(5.6), 283(3.2), 202 (17.9), 142(100), 86(5.6), 57(15.3).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOHEXADECANOIC ACID POTASSIUM SALT 18 CROWN 6 ETHER (LXXXVIII)

XXXXIII (0.208g, 0.560 mM) was dissolved in EtOH:H_2O 1:1 (5 ml) and potassium hydroxide (0.031g, 0.5606 mM) and 18 crown 6 ether (0.148g, 0.5606 mM) were added, as in method H. Yield = 0.377g (100%).

NMR ($CDCl_3$) 0.89 (3H,t,CH_3C_{16}); 1.33 (24H,s,CH_2 x 12 C_{16}); 1.43 (9H,s,$(CH_3)_3$ Boc); 1.87 (2H,m, $C_\beta H_2$ C_{16}); 3.52 (24H,s, crown ether);4.21 (1H,broad s,C_α H C_{16}); 5.02 (1H,broad d,NH C_{16}).

MS m/e M^+ (%) $C_{33}H_{64}NO_{10}K$ (673) 370(10.9), 356(11.2), 303(100), 299(7.5), 226(12.7), 133(42.1), 90(27.3), 57(67.2).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOIC ACID POTASSIUM SALT 18 CROWN 6 ETHER (LXXXIX)

LIII (0.1919g, 0.307 mM) was dissolved in EtOH:H_20 1:1 (10 ml), KOH (0.0172, 0.307 mM), and 18 crown 6 ether (0.0812g, 0.307 mM) were added, and reacted as for method H. Yield = 0.285g (100%).

NMR ($CDCl_3$) 0.88 (6H,t,2 x CH_3 C_{16}); 1.32 (48H,s,CH_2 x 2 x 24 C_{16}); 1.43 (9H,s,$(CH_3)_3$ Boc); 1.85 (4H,broad m, 2 x $C_\beta H_2$ C_{16}); 3.52 (24H,s, crown ether); 4.12 (1H,broad s, $C_\alpha H$ C_{16}); 4.45 (1H, broad s, $C_\alpha H$ C_{16}); 5.00 (lH, broad m, NH Boc C_{16}); 6.72 (1H, broad d, NH C_{16}).

MS m/e M^+ (%) $C_{48}H_{95}N_2O_{11}K$ (926) 623(11.8), 608(9.1), 594(8.7), 538(6.4), 510(8.5), 405(8.0), 303(100), 264(16.3), 226(17.9), 133(21.5), 92(6.1), 57(25.3).

N-α-TERTIARYBUTOXYLCARBONYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOIC ACID POTASSIUM SALT 18 CROWN 6 ETHER (XC)

LVII (0.3g, 0.242mM) was dissolved in EtOH:H_20 1:1, KOH (0.0135g, 0.242mM) added and the reactin stirred at room temperature for 2hrs. The EtOH was evaporated and the water removed by lyophilisation. Yield = 0.38 (100%).

NMR ($CDCl_3$) 0.87 (9H,t,3 x CH_3 C_{16}); 1.35 (72H,s,CH_2 x 36 C_{16}); 1.42 (9H,s, $(CH_3)_3$ Boc); 1.88 (6H,broad m, 3 x $C_\beta H_2$ C_{16}); 3.53 (24H,s, crown ether); 4.10 (1H,broad s, $C_\alpha H$ C_{16}); 4.30 (1H,broad s, $C_\alpha H$ C_{16}); 4.58 (1H,broad s, $C_\alpha H$ C_{16}); 4.97 (1H,broad m, NH Boc C_{16}); 6.68 (1H,broad d, NH C_{16}); 6.76 (1H,broad d, NH C_{16}).

MS m/e M^+ (%) $C_{97}H_{126}N_3O_{12}K$ (1563) 1260(12.1), 1245(8.5), 1216(9.3), 1160 (6.2), 1146(10.9), 1116(6.4), 908(7.1), 656(8.4), 641(5.6), 625(11.1), 405(7.3), 303 (100), 264(17.6).

N-α-TERTIARYBUTOXYLCARBONYL-dl-α-2-AMINOHEXADECANOYL-O-METHYLENE CHLORIDE (XCI)

Method K

XXXXIII (0.39g, 0.5975 mM) in DMF (10 ml) and chloroiodomethane ($ClCH_2I$) (2.0442g, 0.84 ml) were stirred at room temperature and the reaction followed by tlc (system L). After 48 hours the reaction was complete, EtOAc (10 ml) was added and the solution washed with brine (5 ml), 3% sodium bicarbonate (5 ml) and brine (5 ml). The EtOAc solution was dried over $NaSO_4$ (anh) and evaporated. Yield = 0.22g (91.3%)

NMR ($CDCl_3$) 0.86 (3H,t,CH_3 C_{16}); 1.26 (24H,s,12 x CH_2 C_{16}); 1.48 (9H,m,$(CH_3)_3$ Boc); 1.70 (2H,broad s, $C_\beta H_2$ C_{16}); 4.17 (1H,m,$C_\alpha H$ C_{16}); 4.45 (1H,broad s,OCH_2Cl); 4.69 (1H,broad s, OCH_2Cl); 5.71 (1H,m,NH C_{16})

MS m/e M^+ (%) $C_{22}H_{42}NO_4Cl$ (419/421) 391(1.2), 320(5.9), 300(12.9), 270(21.2), 226(56.2), 133(5.0), 95(6.2), 81(6.8), 68(10.2), 56(100).

N-α-TERTIARYBUTOXYCARBONYL-dl-α2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOYL-O-METHYLENE CHLORIDE (XCII)

LIII (0.34g, 0.367 mM) and $ClCH_2I$ (1.29g, 0.5 ml) in DMF (10 ml) and reacted as in method K. Yield = 0.16g (64.5%).

NMR ($CDCL_3$) 0.90 (6H,t, CH_3 2 x C_{16}); 1.30 (48H,s, CH_2, 2 x 24 C_{16}); 1.49 (9H,s, $(CH_3)_3$ Boc); 1.57 (2H, broad m, 2x $C_\beta H$ x C_{16}); 1.85 (2H,m,2 x $C_\beta H$ C_{16}); 4.09 (1H,broad s,$C_\alpha H$ C_{16}); 4.31 (1H,broad,$C_\alpha H$ C_{16}); 4.60 (2H,m,OCH_2Cl); 4.94 (1H,broad s, NH Boc C_{16}); 5.63 (½H,d,NH C_{16}); 5.88 (½H,d,NH C_{16}).

MS m/e M^+ (%) $C_{38}H_{73}N_2O_5Cl$ (672/674) 617(2.6), 573(8.5), 320(5.29), 270(11.2), 226(100), 68(6.8), 56(27.6).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOYL-O-METHYLENE CHLORIDE (XCIII)

LVIII (0.38g, 0.242mM) in DMF (10ml) was stirred with $ClCH_2I$ (0.85g, 3.17mM) as in method K. Yield = 0.178g (56.3%).

NMR ($CDCl_3$) 0.88 (9H,t,CH_3 C_{16} x 3); 1.29 (72H,s,CH_2 3 x 24 C_{16}); 1.43 (9H,d,$(CH_3)_3$ Boc); 1.80 (6H,m,3 x $C_\beta H_2$ C_{16}); 4.10 (2H,broad s,OCH_2 Cl); 4.29 (1H,broad s,$C_\alpha H$ Boc C_{16}); 4.45 (1H,broad s, $C_\alpha H$ C_{16}); 4.56 (1H,broad s,$C_\alpha H$ C_{16} -OCH_2 Cl); 4.87 (1H,broad s, NH Boc C_{16}); 5.64 (1H,d,NH C_{16}); 5.83 (1H,d,NH C_{16})

MS m/e M^+ (%) $C_{54}H_{104}N_3O_6Cl$ (925,927) 950(M^++Na)(6.8), 927(M^+)(3.5), 925 (2.9), 695(7.1), 617(18.8), 573(52.6), 525(14.7), 505(11.2), 477(13.5), 420(8.2), 391(9.4), 316(40.0), 270(100).

N-α-TERTIARYBUTOXYCARBONYL-LEUCYL-O-TETRADECANOIC ACID METHYL ESTER (XCIV)

LXXXIII (2.5 g, 4.69 mM) was dissolved in DMF (20 ml) and LX (1.50g, 4.67 mM) was added. The reaction proceeded as in method K. Yield = 1.56g (71.0%).

NMR ($CDCl_3$) 0.87 (3H,t,CH_3 C_{14}); 0.98 (6H,t,2x CH_3 Leu); 1.34 (2OH,s,CH_2 x10 C14); 1.47 (9H,s,$(CH_3)_3$ Boc); 1.87 (2H,m,$C_\gamma H$ Leu, $C_\beta H$ C_{14}); 2.00 (3H,m, $C_\beta H_2$ Leu, $C_\beta H$ C_{14}); 3.78 (3H,d,CH_3 Me ester); 4.29(1H,m, $C_\alpha H$ Leu); 4.39 (1H,broad s,$C_\alpha H$ C_{14}); 4.88 (½,m,NH Leu)5.02 (½H,m,NH Leu).

MS m/e M^+ (%) $C_{26}H_{49}N_2O_6$ (471), 470(M^+-1)(1.76), 372(100), 277(15.6), 241 (7.1), 199(5.9), 154(6.2), 130(36.5), 86(56.8), 68(15.6), 56(68.5).

N-α-TERTIARYBUTOXYCARBONYL LEUCYL-O-HEXADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID METHYL ESTER (XCV)

LXXXIII (0.2g, 0.375mM) and LIX methyl ester (0.244g, 0.415mM) in DMF (10ml) were reacted as method K. Yield = 0.33g (97.6%).

NMR ($CDCl_3$) 0.91 (6H,t,CH_3 C_{16}, CH_3 C_{14}); 0.98 (6H,m,2 x CH_3 Leu);1.32 (44H,s,CH_2 x 10 C_{14}, CH_2 x 12 C_{16}); 1.59 (9H,s,$(CH_3)_3$ Boc);1.71 (3H,m,$C_\beta H$ C_{14}, $C_\beta H$ C_{16}, $C_\gamma H$ Leu); 1.87 (4H,m,$C_\beta H$ C_{14}, $C_\beta H$ C_{16}, $C_\beta H_2$ Leu); 3.73 (3H,m,CH_3 Me ester); 4.32 (1H,broad s, $C_\alpha H$ Leu);4.54 (2H,broad s, $C_\alpha H$ C_{16}, $C_\alpha H$ C_{14}); 4.89 (1H,broad s, NH Leu);5.18 (1H,broad s, NH C_{14}).

MS me/ M^+ (%) $C_{42}H_{80}N_2O_7$ (724) 723 (M^+-1)(2.6), 660(9.4), 625(35.9), 512 (12.6), 494(5.3), 258(38.8), 198(100), 158(7.4), 130(51.2), 112(6.2), 86(91,2), 68(21.5), 56(74,7).

N-α-TERTIARYBUTOXYCARBONYL-LEUCYL-O-HEXADECANOYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID METHYL ESTER (XCVI)

LXXXIII (0.135g, 0.2533mM) and LXIII (0.20g, 0.2946mM) were reacted as in method K. Yield = 0.2373 (86.0%)

NMR $CDCl_3$) 0.90 (9H,t,CH_3 C_{16}, 2 x CH_3 C_{14}); 0.98 (6H,m,2 x CH_3 Leu); 1.30 (64H,s,CH_2 x 12 C_{16}, CH_2 x 10 C_{14}, CH_2 x 10 C_{14}); 1.59 (9H,s,$(CH_3)_3$ Boc); 1.70 (4H,m,$C_\beta H$ C_{14} $C_\beta H$ C_{14}, $C_\beta H$ C_{16} $C_\gamma H$ Leu); 1.87 (5H,m,$C_\beta H$ C_{16}, CH C_{14}, $C_\beta H$ C_{14}, $C_\beta H_2$ Leu); 3.72 (3H,m,CH_3 Me ester); 4.32 (1H,broad s, $C_\alpha H$ Leu); 4.52 (3H,broad m,$C_\alpha H$ C_{16}, 2 x $C_\alpha H$ C_{14});4.89 (1H,broad s, NH Leu); 5.17 (2H,broad s, NH C_{14} x 2);

MS m/e M^+ (%) $C_{56}H_{107}N_3O_8$ 949) 972(M^++Na)(1.76), 949(M^+)(1.86), 894(2.4), 625(9.7), 512(2.9), 466(3.2), 258(30.3), 198(100), 158(5.0), 130(30.3), 86(36.2), 56(28.5).

LEUCYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER HYDROCHLORIDE (XCVII)

LXXXV (0.51g, 1.23mM), was dissolved in MeOH (10.2ml), 30% HCl in MeOH (1.02m, 1.02ml) as in method G. Yield = 0.43g (100%),

NMR ($CDCl_3$) 0.87 (3H,t.CH_3 C_{10}); 0.98 (6H,m,2 x CH_3 Leu); 1.31 (12H,s,CH_2 x 6 C_{10}); 1.85 (5H,broad m, $C_\gamma H$ Leu, $C_\beta H_2$ C_{10}, $C_\beta H$ Leu); 3.73(3H,d,CH_3 Me ester); 4.11 (½H,m,$C_\alpha H$ C_{10}); 4.26 (½H,m,$C_\alpha H$ C_{10}); 4.45 (1H,m,$C_\alpha H$ Leu); 7.77 (½H,m,NH C_{10}); 8.01 (½H,m,NH C_{10}); 8.16 (1H,broad s, NH Leu); 8.34 (1H,broad s, NH Leu); 8.59 (1H,broad s, NH Leu)

MS m/e M^+ (%) $C_{17}N_{35}N_2O_3Cl$ (350/352) 337(M^+-HCl+Na)(3.5), 315(M^+-HCl+H)(100), 301(15.8), 202(18.5), 142(14.7), 86(65.3).

LEUCYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER HYDROCHLORIDE (XCVIII)

LXXXVI (0.27g, 0.463mM) was dissolved in MeOH (54ml) and 30% IN HCl in MeOH (0.54ml) added as in method G. Yield = 0.24g (100%).

NMR ($CDCl_3$) 0.85 (6H,t,CH_3 2 x C_{10}); 0.98 (6H,m,2 x CH_3 Leu); 1.28 (24H,s,12 x CH_2 C_{10}); 1.76 (7H,m, $C_\gamma H$ Leu, 2 x $C_\beta H_2$ C_{10}, $C_\beta H_2$ Leu);3.74 (3H,t,CH_3 Me ester); 4.14 (1H,broad s,$C_\alpha H$ C_{10}); 4.48 (2H,broad m,$C_\alpha H$ C_{10}, $C_\alpha H$ Leu); 8.24 (2H,broad s, NH 2 x C_{10}); 8.45 (2H,broad s, NH Leu x 2); 8.61 (1H,broad s, NH Leu)

MS m/e M^+ (%) $C_{27}H_{54}N_3O_4Cl$ (519/521) 506(M^+-HCl+Na)(5.1), 483(M^+-HCl) (100), 469(5.2), 439(5.4), 271(11.5), 158(6.7), 142(8.3), 86(12.4)

LEUCYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER HYDROCHLORIDE (XCIX)

LXXXVII (0.10g, 0.133mM) was dissolved in MeOH (2ml) and 30% HCl in MeOH (0.2ml) added as in method G. Yield = 0.09g (100%).

NMR ($CDCl_3$) 0.86 (9H,t,CH_3 C_{10} x 3); 0.98 (6H,d,2 x CH_3 Leu); 1.30 (36H,s,CH_2 C_{10} x 3); 1.72 (9H,m.3 x $C_\beta H_2$ C_{10}, $C_\beta H_2$ Leu $C_\gamma H$ Leu); 3.70 (3H,m,CH_3 Me ester); 4.12 (1H,broad s,$C_\alpha H$ C_{10}); 4.45 (3H,broad m, 2 x $C_\alpha H$ x C_{10}, $C_\alpha H$ Leu); 7.54 (1½H,d,NH C_{10}); 7.87 (1½H,d,NH C_{10}); 8.18 (1H,broad s, NH Leu); 8.45 (1H,broad s, NH Leu); 8.64 (1H,broad s, NH Leu)

MS m/e M+ (%) $C_{37}H_{73}N_4O_5Cl$ (688/690) 675(M^+-HCl+Na)(6.9), 643(M^+-HCl+H)(100) 639(9.8), 609(36.8), 545(12.3), 503(7.7), 461(11.4), 404(6.9), 374(15.6), 345(7.6), 277(8.4), 741(9.8), 88(30.3), 57(5.3).

<u>N-α-TERTIARYBUTOXYCARBONYL-PHENYLALANYL-LEUCYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER</u> (C)

Boc Phe (0.317g, 1.198mM), XCVII (0.4199g, 1.198mM), HOBT (0.1619g, 1.198mM), TEA (0.1212g, 1.198mM), EDAC (0.2297g, 1.198mM) were reacted as in method F. Yield = 0.4821 (71.7%).

NMR ($CDCl_3$) 0.88 (9H,t,CH_3 C_{10}, 2 x CH_3 Leu); 1.24 (12H,s,CH_2 C_{10}); 1.45 (9H,s, $(CH_3)_3$ Boc); 1.59 (2H,m,$C_\beta H$ C_{10} $C_\gamma H$ Leu); 1.77 (3H,m,$C_\beta HC_{10}$, $C_\beta H_2$ Leu); 3.06 (2H,d,$C_\beta H_2$ Phe); 3.64 (3H,d,CH_3 Me ester); 4.27 (1H,m,$C_\alpha H$ C_{10}); 4.42 (1H,m,$C_\alpha H$ Leu); 4.50 (1H,m,$C_\alpha H$ Phe); 4.82 (1H,broad s,NH Phe);6.25 (½H,d,NH C_{10}); 6.34 (½H,d,NH C_{10}); 6.42 (½H,d,NH Leu); 6.54 (½H,d, NH Leu); 7.26 (5H,m,C_6H_5 Phe)

MS m/e M^+ (%) $C_{31}H_{51}N_3O_6$ (561) 583(M^++Na)(36.4), 562(M^++H)(100), 547 (11.2), 462(6.3), 370(7.6), 336(10.7), 183(15.8), 91(33.4), 57(64.1).

<u>N-α-TERTIARYBUTOXYCARBONYL-PHENYLALANYL-LEUCYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER</u> (CI)

Boc Phe (0.1173g, 0.4426mM), XCVIII (0.23g, 0.4425mM), HOBT (0.0598, 0.4426mM), TEA (0.0448g, 0.,4426mM) and EDAC (0.0849g, 0.4426mM) were reacted as method F. Purification by flash column chromatography was undertaken. Yield = 0.276g (85.3%)

NMR ($CDCl_3$) 0.88 (6H,t,CH_3 2 x C_{10}); 0.94(6H,t,2 x CH_3 Leu); 1.30(24H,s,12 x CH_2 C_{10}); 1.45(9H,s,$(CH_3)_3$ Boc); 1.65 (3H,m,2 x $C_\beta H$ C_{10}, $C_\gamma H$ Leu); 1.85 (4H,m,2$C_\beta H$ C_{10},$C_\beta H_2$Leu); 3.11(2H,d,$C_\beta H_2$ Phe); 3.71 (3H,s,CH_3 Me ester); 4.07 1H,m,$C_\alpha H$ C_{10});4.30(1H,m,$C_\alpha H$ C_{10}); 4.35(1H,m,$C_\alpha H$ Leu); 4.52(1H,m,$C_\alpha H$ Phe); 5.00(1H,broad s, NH Phe); 6.17(1H,broad s,NH Leu); 6.52(2H,m,NH 2 x C_{10}); 7.26 (5H,m,C_6H_5 Phe)

MS m/e M^+ (%) $C_{41}H_{70}N_4O_7$ (730) 753 (M^++Na)(100), 653(13.6), 631(7.1), 463(12.3), 316(8.7), 316(8.7), 285(11.2), 117(5.6), 91(15.1), 86(9.4), 57(7.6).

N-α-TERTIARYBUTOXYCARBONYL-PHENYLALANYL-LEUCYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANYL-2-AMINODECANOIC ACID METHYL ESTER (CII)

Boc Phe (0.0327g, 0.1234mM), XCIX (0.085g, 0.1234mM), HOBT (0.0167g, 0.1234mM), TEA (0.0125g, 0.1234mM) and EDAC (0.0237g, 0.1234mM) were reacted as for method F. Following work up and purification by preparative TCL yield = 0.091g (82.0%).

NMR ($CDCl_3$) 0.87 (9H,t,CH_3 3 x C_{10}); 0.94 (6H,m,2 x CH_3 Leu); 1.27 (36H,s,CH_2 x 18 C_{10}); 1.43 (9H,s(,$CH_3)_3$ Boc); 1.63 (4H,m,3 x $C_\beta H$ C_{10}, $C_\gamma H$ Leu); 1.78 (5H,m,3 x $C_\beta H$ C_{10}, $C_\beta H_2$ Leu); 3.10 (2H,d,$C_\beta H_2$ Phe); 3.70 (3H,s,CH_3 Me ester); 4.02 (1H,m,$C_\alpha H$ C_{10}); 4.35 (3H,broad m,2 x $C_\alpha H$ C_{10}, $C_\alpha H$ Leu);4.52 (1H,m,$C_\alpha H$ Phe); 4.98 (1H,broad s,NH Phe); 6.24 (1H,broad s, NH Leu); 6.50 (3H,m,NH 3 x C_{10}); 7.28 (5H,m,C_6H_5 Phe)

MS m/e M^+ (%) $C_{51}H_{89}N_5O_8$ (889) 912(M^++Na)(100), 890(M^++H)(63.7), 876 (31.8), 776(17.5), 630(9.7), 599(13.8), 569(18.1), 487(8.7), 317(14.2), 164(11.3), 91(5.9), 86(11.4), 55(15.3).

N-α-TERTIARYBUTOXYCARBONYL-D-ALANYL-GLYCINE METHYL ESTER (CIII)

Boc D Ala (1.97g, 10.4mM), glycine methyl ester hydrochloride (1.308g,

10.4mM), HOBT (1.4085g, 10.4mM), DCC (2.15g, 10.4mM) and TEA (1.05g, 10.4mM), in EtOAc (35ml) and DCM (15ml) were reacted as in method C. The reaction was followed by tlc (system J) and the compound purified by silica gel column. Yield = 2.67g (97%).

NMR $CDCl_3$) 1.38 (3H,d,CH_3 Ala); 1.45 (9H,s, $(CH_3)_3$ Boc); 3.76 (3H,s,CH_3 Me ester); 4.05 (2H,d,CH_2 Gly); 4.22 (1H,broad s, $C_\alpha H$ D Ala); 4.95 (1H,ds,NH D Ala); 6.62 (1H,s,NH Gly).

MS m/e M^+ (%) $C_{11}H_{20}N_2O_5$ (260) 283(M^++Na)(100), 269(2.5), 261(M^++1)(47.7) 219(17.6), 183(12.1), 161(11.2), 88(7.1), 57(60.1).

D-ALANYL-GLYCINE METHYL ESTER HYDROCHLORIDE (CIV)

CIII (2.57g, 9.88mM) was refluxed for 20 mins. in MeOH (51.4ml) with 30% HCl in MeOH (51.4ml), as in method G. Yield = 1.93g (99.4%).

NMR ($CDCl_3$) 1.45 (3H,d,CH_3 Ala); 3.99 (2H,d,CH_2 Gly); 4.51 (1H,broad s,$C_\alpha H$ Ala); 7.58 (1H,s,NH Gly); 8.26 (3H,broad s, NH_3^+ Ala);

MS m/e M^+ (%) $C_6H_{13}N_2O_3Cl$ (196/198) 183(M^+-HCl-Na)(6.6), 160(M^+-HCl)(100), 147(6.9), 103(10.1), 76(8.2), 55(15.7).

N-α-TERTIARYBUTOXYCARBONYL TYROSINYL (BENZYL HYDROXY)-D-ALANYL-GLYCINE METHYL ESTER (CV)

Boc Tyr (Bzl OH) (3.4g 9.80mM), CIV (180g, 9.16mM), TEA (0.9269g, 1.16mM), HOBT (1.2378g, 9.16mM), EDAC (1.756g, 9.16mM) in EtOAc (50ml) and DCM (50ml) were reacted as per method F. Yield = 4.50g (95.7%)

NMR ($CDCl_3$) 1.25 (3H,d,CH_3 D Ala); 1.42 (9H,s,$(CH_3)_3$ Boc); 3.00 (2H,m,$C_\beta H_2$ Tyr); 3.72 (3H,s,CH_3 Me ester); 3.92 (1H,d,d,CH Gly); 4.06 (1H,d,d,CH Gly); 4.19 (1H,m,$C_\alpha H$ Ala); 4.46 (1H,m,$C_\alpha H$ Tyr); 4.96 (1H,broad m,NH Tyr); 5.04 (2H,s,CH_2 C_6H_5); 6.74 (1H,t,NH Ala); 6.91 (2H,d,C_6H_4AB Tyr),

6.79 (1H,d,NH Gly); 7.03 (2H,d,C_6H_4 AB Tyr); 7.40 (5H,m, C_6H_5 Benzyl).

MS m/e M^+ (%) $C_{27}H_{35}N_3O_7$ (513) 558(M^++2Na)(4.2), 536(M^++Na)(100), 436 (12:2), 408(22.8), 226(4.9), 197(5.5), 154(5.2), 136(4.7), 91(19.2), 57(9.9).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY)-D-ALANYL-GLYCINE (CVI)

CV (1.02g, 1.987mM) in MeOH (20ml) was deprotected using IN NaOH (4.37ml) as in method D the reaction was followed by tlc. Yield = 0.633g (63.8%).

NMR ($CDCl_3$) 1.42 (3H,d,CH_3 D Ala); 1.48 (9H,s,$(CH_3)_3$ Boc); 3.11 (2H,m,$C_\beta H_2$ Tyr); 4.12 (2H,m,$C_\alpha H$ Gly); 4.46 (1H,m,$C_\alpha H$ Ala); 4.87 (1H,m,$C_\alpha H$ Tyr); 5.06 (2H,s,CH_2 Bz Tyr); 5.63 (1H,d,NH Tyr); 6.39 (1H,m,NH Gly);6.83 (1H,d,NH Ala); 6.93 (2H,d,C_6H_4 AB Tyr); 7.11 (2H,d,C_6H_4 AB Tyr);7.42 (5H,m,C_6H_5 Benzyl).

MS m/e M^+ (%) $C_{26}H_{33}N_3O_7$ (499)499(17.1), 31(100), 315(16.5), 272(13.8) 253(25.3), 239(9.1), 226(17.6), 197(28.2), 154(37.3), 120(11.8), 107(12.9) 91 (53.5), 56(20.6).

PHENYLALANYL-LEUCYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER HYDROCHLORIDE (CVII)

C (0.2037g, 0.363mM) in MeOH (4ml) and 30% HCl in MeOH (0.4ml) was refluxed for 20 mins. as in method G. Following evaporation yield = 0.1760g (97.2%).

NMR ($CDCl_3$) 0.91 (9H,m,CH_3 C_{10}, 2xCH_3 Leu); 1.28 (12H,s,CH_2 C_{10}); 1.59 (2H,m,$C_\beta H$ C_{10}, $C_\gamma H$ Leu); 1.80 (3H,m,$C_\beta H$ C_{10}, $C_\beta H_2$ Leu); 3.35 (2H,d,$C_\beta H_2$ Phe); 3.67 (3H,t,CH_3 Me ester); 4.38 (2H,broad m,$C_\alpha H$ C_{10}, $C_\alpha H$ Leu); 4.57 (1H,m,$C_\alpha H$ Phe); 7.31 (5H,m,C_6H_5 Phe); 7.78 (1H,m,NH Leu); 7.99 (½H,d,NH C_{10}); 8.06 (½H,d,NH C_{10}); 8.29 (2H,broad s, 2xNH Phe); 8.47 (1H,broad s, NH Phe)

MS m/e M^+ (%) $C_{26}H_{44}N_3O_4$ Cl (497/499) 484(M^+-HCl+Na)(7.9), 462(M^+-HCl+H)(100), 372(8.2), 340(9.7), 285(11.4), 271(8.6), 202(15.1), 160(23.7), 91(15.6), 55(5.3).

N-α-TERTIARYBUTOXYCARBONYL TYROSINYL (BENZYL HYDROXY) D-ALANYL-GLYCYL-PHENYLALANYL-LEUCYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER (CVIII)

CI (0.1999g, 0.4003mM), CII (0.1943g, 0.3906mM) EDAC (0.0767g, 0.4001mM), TEA (0.0405g, 0.4002mM) and HOBt (0.0541g, 0.3804mM) in EtOAc (10ml) and DCM (10ml) were reacted as in Method F. Following extraction and evaporation yield = 0.281 (77.4%).

NMR ($CDCl_3$) 0.87 (9H,m,CH_3 C_{10}, 2xCH_3 Leu); 1.22 (3H.d.CH_3 Ala); 1.25 (12H,s, 6 x CH_2 C_{10}); 1.39 (9H,s,$(CH_3)_3$ Boc); 1.60 (2H,m,$C_\gamma H$ Leu, $C_\beta H$ C_{10}) 1.76 (3H,m,$C_\beta H_2$ Leu, $C_\beta H$ C_{10}); 3.01 (4H,broad m, $C_\beta H_2$ Tyr, $C_\beta H_2$ Phe) 3.65 (3H,s,CH_3 Me ester); 3.87 (2H,m,CH_2 Gly); 4.12 (1H,m,$C_\alpha HC_{10}$);4.28 (2H,m,$C_\alpha H$ Ala,$C_\alpha H$ Leu); 4.43 (1H,m,$C_\alpha H$ Phe); 4.83 (1H,m,$C_\alpha H$ Tyr); 5.03 (2H,s,CH_2 Benzyl); 5.62 (1H,d,NH Tyr); 6.41 (1H,d,NH C_{10}); 6.60 (1H,broad s, NH Leu); 6.69 (1H,t,NH Gly); 6.89 (2H,d,C_6H_4 AB Tyr); 6.78 (1H,d,NH D Ala); 6.99 (1H,NH Phe); 7.12 (2H,d,C_6H_4 AB Tyr); 7.21 (5H,m,C_6H_5 Phe); 7.38 (5H,m,C_6H_5 Benzyl)

MS m/e M^+ (5) $C_{52}H_{74}N_6O_{10}$ (942) 966(M^++Na+H)(17.5), 908(9.4), 817(22.9), 717 (38.6), 572(33.8), 429(21.4), 338(23.6), 226(28.5), 15.7(11.9), 91(100), 57(68.3).

PHENYLALANYL-LEUCYL-dl-α2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER (HYDROCHLORIDE OR TRIFLUORIDE) (CIX)

CI (0.2060g, 0.2822mM) in MeOH (4.12ml) and 30% HCl in MeOH (0.412ml) were reacted as in Method G. Tlc indicated that deprotection was incomplete therefore 50% trifluoroacetic acid (TFA) in DCM was used for deprotection.

CI (0.2060g, 0.2022mM) in 50% TFA in DCM (3ml) was stirred for 30 mins at room temperature. Following evaporation yield = 0.2031g (96.9%)

NMR ($CDCl_3$) 0.90 (12H,broad s, CH_3 2 x C_{10}, 2 x CH_3 Leu); 1.25 (24H,s,12xCH_2, C_{10}); 1.67 (7H,broad m,$C_\gamma H$ Leu, 2x$C_\beta H_2C_{10}$, $C_\beta H_2$ Leu); 3.16 (2H,broad m,CH_2 Phe); 3.73 (3H,m,CH_3Me ester); 4.15 (1H,m,$C_\alpha H$ C_{10}); 4.45 (3H,broad m,$C_\alpha H$ C_{10}, $C_\alpha H$, Leu,$C_\alpha H$ Phe); 6.22 (½H,broad s,NH C_{10}); 6.42 (½H,broad s,NH C_{10}); 6.78 (½H,d,NH C_{10}); 7.02 (½H,broad s,NH C_{10}); 7.18 (5H,m,C_6H_5 Phe); 7.45 (½H,broad m, NH Leu); 7.68 (½H,broad s, NH Leu); 8.26 (3H,broad s, NH^+_3 Phe).

MS m/e M^+ (%) $C_{38}H_{63}N_4O_7F_3$ (744) 654(M^+-CF_3 CO+Na)(29.10), 632(M^++H) (100), 607(9.2), 542(12.2), 398(10.3), 309(7.9), 251(9.4), 197(17.7), 91(37.8), 82 (15.2), 55(11.3).

PHENYLALANYL-LEUCYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER TRIFLUOROACETATE (CX)

CII (0.10g, 0.1112mM) in MeOH (2ml) and 30% HCl in MeOH (0.2ml) were refluxed. Tlc indicated amino deprotection had not occurred therefore 50% TFA in DCM was used.

CII (0.100g, 0.1112mM) in 50% TFA in DCM (1.5ml) were stirred at room temperature for 30 mins. Yield = 0.1011g (99.6%).

NMR ($CDCl_3$) 0.91 (15H,t,CH_3 3 x C_{10} 2 x CH_3 Leu); 1.30 (36H,s,CH_2 x 18 C_{10} 2 x CH_3 Leu);1.68 (9H,broad m,$C_\gamma H$ Leu,3x$C_\beta H_2$ C_{10},$C_\beta H_2$ Leu); 3.23 (2H,d,$C_\beta H_2$ Phe); 3.67 (3H,d,CH_3 Me ester); 4.14 (1H,m,$C_\alpha H$ C_{10}), 4.42 (4H,broad m,2x$C_\alpha H$ C_{10},$C_\beta H$ Phe,$C_\alpha H$ Leu); 6.42 (1H,d,NH C_{10}); 6.81 (2H,d,NH C_{10}); 7.23 (5H,m,C_6H_5 Phe); 7.49 (½H,broad s, NH Leu); 7.66 (½H,m,NH Leu); 8.23 (3H,broad s,NH^+_3 Phe).

MS m/e M^+ (%) $C_{48}H_{82}N_5O_8F_3$ (913) 823(M^+-CF_3 CO5+Na)(6.9), 801(M^++H^+)

(100), 786( ), 67.2(7.4), 611(8,9), 520(12.2), 465(15.8), 408(9.1), 337(17.5), 208 (15.3), 110(11.2), 91(18.3), 72(57.5), 57(36.7).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY) D-ALANYL-GLYCYL-PHENYLALANYL-LEUCYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER (CXI)

CVI (0.1244g, 0.269mM), CIX (0.200g, 0.2688mM), TEA (0.0272g, 0.2688mM), HOBt (0.0364g, 0.2694mM), and EDAC (0.0516g, 0.2692mM) were reacted as in method F. Yield = 0.234g (70.1%)

NMR ($CDCl_3$) 0.86 (6H.t.$CH_3$2xC_{10}); 0.89 (6H,m,2xCH_3 Leu);1.20(3H,d,CH_3Ala); 1.23 (24H,s,CH_2x12 C_{10}); 1.37 (9H,s,$(CH_3)_3$ Boc); 1.56 (3H,m,2xC_βH C_{10}, C_γH Leu); 1.72 (4H,m,2xC_βH C_{10}, $C_\beta H_2$ Leu); 3.00 (4H,broad m.$C_\beta H_2$ Phe, $C_\beta H_2$ Tyr); 3.66 (3H,s,CH_3Me ester); 3.86 (2H,m,CH_2 Gly); 4.01 (1H,m,C_αH C_{10}); 4.10 (1H,m,C_αH C_{10}); 4.28 (2H,m,C_αH Ala, C_αH Leu); 4.42 (1H,m,C_αH Phe); 4.83 (1H,m,C_αH Tyr); 5.02 (2H,s,CH_2 Benzyl)5.62 (1H,d,NH Tyr); 6.38 (1H,d,NH C_{10}); 6.42 (1H,broad s,NH C_{10});6.60 (1H,broad s,NH Leu); 6.67 (1H,broad s, NH Gly); 6.88 (2H,d,C_6H_4 AB Tyr); 6.72 (1H,d,NH D Ala); 6.99 (1H,d,NH Phe); 7.10(2H,d,C_6H_4 AB Tyr);7.20 (5H,m,C_6H_5 Phe); 7.37 (5H,m,C_6H_5 Benzyl).

MS m/e M^+ (%) $C_{62}H_{93}N_4O_{11}$ (1111) 1134(M^++Na)(6.8), 998(36.3), 885(8.9), 792(13.4), 663(9.1), 552(11.6), 461(9.7) 40.3(7.3), 319(21.8), 263(14.2)

N-α-TERTIARYBUTOXYCARBONYL TYROSINYL (BENZYL HYDROXY)-D-ALANYL-GLYCYL-PHENYLALANYL-LEUCYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOYL-dl-α-2-AMINODECANOIC ACID METHYL ESTER (CXII)

CVI (0.0548g, 0.0198mM), CX (0.01000g, 0.1095mM), TEA (0.0111g,0.1097mM), HOBt (0.0148g, 0.1095mM), and sol. DCC (0.0210g, 0.1095mM) were reacted as in Method F. Yield = 0.089g (63.5%)

NMR ($DCDl_3$) 0.86 (9H,t,CH_3 3 x C_{10}); 0.89 6,m,2 x CH_3 Leu); 1.20 (3H,d,CH_3 Ala); 1.22 (36H,s, 6 x CH_2 3 x C_{10}); 1.37 (9H,s,$(CH_3)_3$ Boc); 1.56 (4H,m,3 x $C_\beta H$ C_{10}, $C_\gamma H$ Leu); 1.72 (5H,m,3 x $C_\beta H$ C_{10}, $C_\beta H_2$ Leu); 3.00 (4H,broad m, $C_\beta H_2$ Tyr, $C_\beta H_2$ Phe); 3.67 (3H,s,CH_3 Me ester); 3.86 (2H,m,CH_2 Gly); 4.01 (1H,m,$C_\alpha H$ C_{10}); 4.10 (2H,m,$C_\alpha H$ 2 x C_{10}); 4.28 (2H,m,$C_\alpha H$ Ala, $C_\alpha H$ Leu); 4.42(1H,m,$C_\alpha H$ Phe); 4.83 (1H,m,$C_\alpha H$ Tyr); 5.02 (2H,s,CH_2 Benzyl); 5.62 (1H,d,NH Tyr); 6.38 (1H,broad s, NH C_{10}); 6.43 (2H,broad s, NH 2 x C_{10}); 6.60 (1H,broad S, NH Leu); 6.67 (1H,broad s,NH Gly); 6.72 (1H,d, NH D Ala); 6.88 (2H,d,C_6H_4 AB Tyr); 6.99 (1H,broad s, Phe); 7.10 (2H,d,C_6H_4 AB Tyr); 7.20 (5H,m,C_6H_5 Phe); 7.37 (5H,m,C_6H_5 Benzyl).

MS m/e M^+ (%) $C_{72}H_{112}N_8O_{12}$ (1280) 1281(M^++H)(8.9), 1152(10.6), 1038(21.3), 1024(.5), 89.6(8.4), 839(11.8), 727(19.8), 636(17.9), 524(13.4), 393(15.6), 300 (12.2), 221(7.2).

N-α-TERTIARYBUTOXYCARBONYL PHENYLALANYL-dl-α-2-AMINOHEXADECANOIC ACID METHYL ESTER (CXIII)

Boc Phe OH (0.123g, 0.466mM), XXXXVI (0.15g, 0.466mM), EDAC (0.089g, 0.466mM), HOBT (0.063g, 0.466mM) and TEA (0.047g, 0.466mM) in DCM (15ml) were reacted as for method F. Yield = 0.236g (95.5%)

NMR ($CDCl_3$) 0.89 (3H,t,CH_3 C_{16}); 1.28 (24H,s,12 x CH_2 C_{16}); 1.43 (9H,s,$(CH_3)_3$ Boc); 1.61 (1H,m,$C_\beta H$ C_{16}); 1.76 (1H,m,$C_\beta H$ C_{16}); 3.07 (2H,d,$C_\beta H_2$ Phe);3.71 (3H,s,CH_3 Me ester); 4.36 (1H,broad s,$C_\alpha H$ C_{16}); 4.51 (1H,broad s,$C_\alpha H$ Phe); 5.00 (1H,broad s, NH, Boc Phe); 6.21 (½H,d,NH C_{10}); 6.30 (½H,d,NH C_{10}); 7.21 (5H,m,C_6H_5 Phe)

MS m/e M^+ (%) $C_{31}H_{52}N_2O_5$ (532) 554(M^++Na)(23.6), 540(6.2), 533(M^+ +) (17.1), 441(9.8), 433(15.5), 356(11.3), 301(12.5), 286(8.7), 250(9.4), 148(10.2), 88 (100).

PHENYLALANYL-dl-α-2-AMINOHEXADECANOIC ACID METHYL ESTER HYDROCHLORIDE (CXIV)

CXIII (0.2285, 0.429mM) in MeOH (4.57ml) and 30% HCl in MeOH (0.457ml) was refluxed for 20 mins as in Method G. Yield = 0.2002g (95.7%).

NMR ($CDCl_3$) 0.90 (3H,t,CH_3 C_{16}); 1.32 (24H,s,12xCH_2 C_{16}); 1.78 (2H,m,$C_\beta H_2 C_{16}$); 3.12 (2H,d,$C_\beta H_2$ Phe); 3.71 (3H,s,CH_3 Me ester); 4.45 (1H,broad s, $C_\alpha H$ C_{10}); 4.61 (1H,broad s, $C_\alpha H$ Phe); 7.32 (5H,m,C_6H_5 Phe); 7.60 (1H,d, NH C_{16}); 8.20 (3H,broad s, NH^+_3 Phe).

MS me/ M^+ (%) $C_{26}H_{45}N_2O_3Cl$ (468/470) 455(M^+-HCl+Na)(6.9), 433(M^+-HCl+H)(100), 419(6.5), 405(10.3), 314(15.6), 244(12.1), 202(7.8), 142(14.2), 112(13.3), 91(50.6) 55(27.7).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY) D-ALANYL-GLYCYL-PHENYLALANYL-dl-α-2-AMINOHEXADECANOIC ACID METHYL ESTER (CXV)

CVI (0.2134g, 0.4273mM), CVII (0.2002g, 0.4273mM) EDAC (0.019g, 0.4272mM), TEA (0.0432g, 0.4269mM), and HOBt (0.0577g, 0.4271mM) in DCM (25ml) were reacted as in Method F. Yield = 0.3462g (88.8%).

NMR ($CDCl_3$) 0.87 (3H,t,CH_3 C_{16}); 1.13 (3H,d,CH_3 Ala); 1.30 (24H,s,12 x CH_2 C_{16}); 1.40 (9H,s,$(CH_3)_3$ Boc); 1.56 (1H,m,$C_\beta H$ C_{16}); 1.80 (1H,m,$C_\beta H$ C_{16});3.05 (4H,m,$C_\beta H_2$ Phe, $C_\beta H_2$ Tyr); 3.67 (3H,s,CH_3Me ester); 3.88 (2H,m,CH_2 Gly); 4.02 (1H,m,$C_\alpha H$ C_{16}); 4.29 (2H,m,$C_\alpha H$ D Ala, $C_\alpha H$ Tyr); 4.56 (1H,m,$C_\alpha H$ Phe); 5.04 (2H,s,CH_2 benzyl); 5.64 (1H,d,NH Tyr); 6.22 (1H,m,NH C_{16}); 6.62 (1H,broad s,NH Ala); 6.74 (1H,t,NH Gly); 6.92 (2H,d,C_6H_4AB Tyr); 7.05 (1H,m,NH Phe); 7.13 (2H,d,C_6H_4 AB Tyr); 7.22 (5H,m,C_6H_5 Phe); 7.42 (5H,m,C_6H_5 Benzyl).

MS m/e M^+ (%) $C_{52}H_{75}N_5O_9$ (913) 936(M^++Na)(26.2), 836(16.7), 822(5.8), 734(11.1), 653(20.2), 540(9.7), 441(67.6), 332(18.3), 147(28.3), 91(66.4), 86(10.3), 57(38.2).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY) D-ALANYL-GLYCYL-PHENYLALANYL-METHYL ESTER (CXVI)

CVI (0.4145g, 0.83mM), Phe OMe HCl (0.1789g, 0.8302mM), TEA (0.084g, 0.8301mM), HOBt (0.1122g, 0.8304mM) and DCC (0.1712g, 0.8297mM) were reacted as for method C. Yield = 0.3656g (66.8%)

NMR ($CDCl_3$) 1.24 (3H,d,CH_3 Ala); 1.42 (9H,s,$(CH_3)_3$ Boc); 3.00 (2H,m,$C_\beta H_2$ Phe); 3.15 (2H,m,$C_\beta H_2$ Tyr); 3.70 (3H,s,CH_3 Me ester); 3.87 (2H,t,CH_2 Gly); 4.14 (1H,q,$C_\alpha H$ Ala); 4.34 (1H,m,$C_\alpha H$ Phe); 4.84 (1H,q,$C_\alpha H$ Tyr);5.04 (2H,s,CH_2 Benzyl); 5.25 (1H,broad s, NH Tyr); 6.16 (1H,d,NH Ala);6.74 (1H,broad s, NH Gly); 6.93 (2H,d,C_6H_4 AB Tyr); 7.01 (1H,broad s,NH Phe); 7.14 (5H,m,2HC_6H_4 AB Tyr, 3H,C_6H_5 Phe); 7.24 (2H,m,C_6H_5 Phe);7.43 (5H,m,C_6H_5 Benzyl).

MS m/e M^+ (%) $C_{35}H_{44}N_4O_8$ (660) 683(M^++Na)(3.5), 661(M^++H)(8.2), 561 (27.1), 543(8.2), 354(9.1), 307(15.9), 237(31.2), 180(22.4), 154(100), 135(90.6), 107(38.5), 91(69.7), 77(38.8), 57(24.1)

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY) D-ALANYL-GLYCYL-PHENYLALANINE (CXVII)

CXVI (0.3156g, 0.4782mM), was dissolved in MeOH (5ml) and IN NaOH (1ml) added, the reaction as in Method D. Yield = 0.2577g (83.4%)

NMR ($CDCl_3$) 1.20 (3H,d,CH_3 Ala); 1.38 (9H,s,$(CH_3)_3$ Boc); 3.04 (2H,m,$C_\beta H_2$ Phe); 3.22 (2H,m,$C_\beta H_2$ Tyr); 3.78 (2H,m,CH_2 Gly); 4.19 (1H.broad s,$C_\alpha H$ Ala); 4.23 (1H,broad s, $C_\alpha H$ Tyr); 4.65 (1H,broad s, $C_\alpha H$ Phe); 5.02 (2H,s,CH_2 Benzyl); 5.70 (1H,broad s, NH Tyr); 6.28 (1H,broad s, NH Ala); 6.67 (1H,broad s,NH Gly); 6.88 (2H,d,C_6H_4 AB Tyr); 7.02 (1H,broad s, NH Phe), 7.06 (2H,d,C_6H_4 AB Tyr); 7.23 (5H,m,C_6H_5 Phe); 7.38 (5H,m,C_6H_5 Benzyl).

MS m/e M^+ (%) $C_{35}H_{42}N_4O_8$ (646) 669(M^++Na)(14.4), 647(M^++H)(2.4),

547(6.5), 307(17.1), 289(17.6), 226(13.8), 154(100), 136(95.3), 107(36.5), 89 (38.2), 77(39.4), 65(16.2), 53(7.9).

LEUCYL-O-2-TETRADECANOATE METHYL ESTER TRIFLUOROACETATE (CXVIII)

XCIV (0.2154g, 0.4573mM) in 50% TFA in DCM (3,23ml) was stirred at room temperature for 2 hrs. Following evaporation yield = 0.22 (100%)

NMR ($CDCl_3$) 0.88 (3H,t,CH_3 C_{14}), 0.96 (6H,t,2 x CH_3 Leu); 1.32 (20H,s, 10 x CH_2 C_{14}); 1.88 (2H,m,$C_\gamma H$ Leu, $C_\beta H$ C_{14}); 2.01 (3H,m,$C_\beta H_2$ Leu, $C_\beta H$ C_{14});3.73 (3H,d,CH_3 Me ester); 4.41 (1H,broad s,$C_\alpha H$ C_{14}); 4.56 (1H broad s, C_α H Leu); 8.24 (3H,broad s,NH_3^+ Leu).

MS m/e M^+ (%) $C_{23}H_{42}NO_6F_3$ (485) 395(M^++Na)(3.5), 371(M^+-H)(100), 300(43.9), 277(13.7), 227(5.6), 199(10.3), 154(8.4), 130(57.8), 86(63.9), 56(68.7).

LEUCYL-O-HEXADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID METHYL ESTER TRIFLUOROACETATE (CXIX)

XCV (0.1004g, 0.1505mM) in 50% TFA in DCM (1.506ml) were stirred at room temperature for 3hrs. the deprotection being followed by tlc. Yield = 0.1021g, (99.8%)

NMR $CDCl_3$) 0.88 (6H,t,CH_3 C_{14} ,CH_3 C_{16}), 0.94 (6H,t,2 x CH_3 Leu), 1.30 (44H,s, 10 x CH_2 C_{14}, 12 x CH_2 C_{16}); 170 (3H,m,$C_\beta H$ C_{14}, $C_\beta H$ C_{16}, $C_\gamma H$ Leu); 1.86 (4H,m,$C_\beta H$ C_{14}, $C_\beta H$ C_{16}, $C_\beta H_2$ Leu); 3.70 (3H,m,CH_3 Me ester); 4.58 (3H,broad s, $C_\alpha H$ Leu, $C_\alpha H$ C_{14}, $C_\alpha HC_{16}$); 5.23 (1H,broad s, NH C_{14}); 8.22 (3H,broad s, $NH^+{}_3$ Leu).

MS m/e M^+ (%) $C_{39}H_{73}N_2O_7F_3$ (738) 648(M^+-CF_3COO^-+Na)(4.7), 591(13.5), 519(8.2), 415(17.3), 258(43.1), 198(100), 130(57.3), 86(16.7), 55(7.2)

LEUCYL-O-HEXADECANOYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID METHYL ESTER TRIFLUOROACETATE (CXX)

XCVI (0.1018g, 0.1073mM) in 50% TFA in DCM (1.527ml) was stirred at room temperature for 3 hrs. Yield = 0.1030g (99.7%)

NMR ($CDCl_3$) 0.88 (9H,t, CH_3 C_{16}, 2 x C_{14}); 0.96 (6H,m,2 x CH_3 Leu); 1.31 (64H,s 12 x CH_2 C_{16}, 20 x CH_2 C_{14}); 1.72 (4H,m,$C_\gamma H$ Leu, $C_\beta H$ C_{16}, $C_\beta H$ C_{14} x 2), 1.88 (5H,m,$C_\beta H$ C_{16}, $C_\beta H$ 2 xC_{14}, $C_\beta H_2$ Leu); 4.50 (3H,broad m, $C_\alpha H$ C_{16}, $C_\alpha H$ C_{14} x 2); 4.65 (1H,broad s, $C_\alpha H$ Leu); 6.37 (2H,broad NH C_{14} x 2), 8.07 (3H,broad s, NH^+_3 Leu);

MS m/e M^+ (%) $C_{53}H_{100}N_3O_8F_3$ (963) 873 M^+-CF_3 COO^-_3+Na), 812(7.7), 643 (16.1), 541(11.3), 470(7.6), 258(27.9), 198(100), 130(51.4), 86(33.5), 56(31.8).

N-α-TERTIARYLBUTOXY TYROSINYL (BENZYL HYDROXY)-D-ALANYL-GLYCYL-PHENYLALANYL-LEUCINE-O-TETRADECANOIC ACID METHYL ESTER (CXXI)

CXVII (0.1773g, 0.2745mM), CXVIII (0.1326g, 0.2734mM), TEA (0.0278gm 0.2747mM), HOBt (0.0371g, 0.1245mM), EDAC (0.0526g, 0.2744mM) in DCM (5ml) and EtOAc (5ml) were reacted as in Method F. Yield = 0.1837g (64.0%)

NMR ($CDCl_3$) 0.90 (9H,m,CH_3C_{16}x 2 x CH_3 Leu); 1.10 (3H,d,d,CH_3 Ala);1.24 (20H,s,10xCH_2 C_{14}); 1.38 (9H,s,$(CH_3)_3$ Boc); 1.65 (2H,m,$C_\beta H$ C_{14}, $C_\gamma H$ Leu); 1.84 (1H,m,$C_\beta H$ C_{14}); 1.95 (2H,m,$C_\beta H_2$ Leu); 3.02 (4H,broad m, $C_\beta H_2$ Phe, $C_\beta H_2$ Tyr); 3.45 (1H,m,$C_\alpha H$ C_{14}); 3.68 3H,d,CH_3 Me ester); 3.93 (1H,m,$C_\alpha H$ Leu); 4.24 (2H,m,CH_2 Gly); 4.46 (1H,m,$C_\alpha H$ Ala); 4.65 (1H,m, $C_\alpha H$ Phe); 4.87 (1H,m,$C_\alpha H$ Tyr); 5.02 (2H,s,CH_2 Benzyl Tyr);5.59 (1H,s,NH Tyr); 6.67 (1H,broad s, NH Ala); 6.82 (1H,m,NH Gly);6.88 (2H,d,C_6H_4 AB Tyr); 7.02 (1H,d,NH Phe); 7.09 (3H,d,d,C_6H_4 (2H); AB Tyr, NH Leu); 7.19 (5H,m,C_6H_5 Phe); 7.30 (5H,m,C_6H_5 Benzyl)

MS m/e M^+ (%) $C_{56}H_{81}N_5O_{11}$ (999) 1022(M^++Na)(2.06), 1000(M^++H)(100), 900 (28.2), 867(18.5), 767(5.9), 679(13.5), 668(7.6), 642(8.2), 545(10.6)

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY) D-ALANYL-GLYCYL-PHENYLALANYL-LEUCYL-O-HEXADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID METHYL ESTER (CXXII)

CXVII (0.0878g,0.1359mM), CXIX (0.1000g, 0.1355mM), TEA (0.0138g, 0.1364mM), HOBt (0.0184g, 0.1262mM) and EDAC (0.0260g, 0.1356mM) in DCM (5ml) EtOAc (5ml) were reacted as in Method F. Yield = 0./100g (59.1%).

NMR ($CDCl_3$) 0.91 (12H,m,CH_3 C_{14}, C_{16}, 2 x CH_3 Leu); 1.10 (3H,d,d,CH_3 Ala);1.27 (44H,s,10 x CH_2 C_{14}, 12 x CH_2 C_{16}); 1.42 (9H,s,$(CH_3)_3$ Boc);1.66 (3H,m,C_β H C_{14}, C_βH C_{16}, C_γH Leu); 1.90 (4H,m,C_βH C_{14}, C_βH C_{16}, $C_\beta H_2$ Leu); 2.97 (2H,m,$C_\beta H_2$ Phe); 3.14 (2H,m,$C_\beta H_2$ Tyr); 3.48 (1H,m,C_αH C_{16}); 3.84 (3H,d,CH_3Me ester); 3.91 (2H,m,C_αH Leu, C_αH C_{14}); 4.21 (2H,m,CH_2 Gly); 4.42 (1H,m,C_αH Ala); 4.58 (1H,m,C_αH Phe); 4.84 (1H,m,C_αH Tyr); 5.03 (2H,s,CH_2 Benzyl); 5.21 (1H,m,NH Tyr); 5.52 (1H,m,NH C_{14}); 6.81 (1H,m,NH Ala); 6.89 (2H,d,2H C_6H_4 AB Tyr), 7.10 (3H,m,C_6H_4 AB Tyr, NH Gly); 7.19 (5H,m,C_6H_5 Phe); 7.35 (6H,m,C_6H_5 Tyr, NH Phe); 7.56 (1H,d,NH Leu).

MS m/e M^+ (%) $C_{72}H_{112}N_6O_{12}$ 1252) 1276(M^++Na+H)(100), 1176(17.6), 1148 (11.8), 978(13.2), 949(6.5), 923(5.9), 878(7.9), 850(6.2), 821(5.3), 793(5.9), 744 (5.9), 704(8.2), 682(5.9), 648(6.2), 623(14.7).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY)-D-ALANYL-GLYCYL-PHENYLALANYL-LEUCYL-O-HEXADECANOYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOIC ACID METHYL ESTER (CXXIII)

CXVII (0.0587g, 0.0909mM), CXX (0.100g, 0.1038mM), TEA (0.0092g, 0.090mM), HOBt (0.0123g, 0.0910mM) and EDAC (0.0174g, 0.0908mM) in DCM

(5ml) and EtOAc (5ml) were reacted as in method F. Yield = 0.0913g (68.0%)

NMR ($CDCl_3$) 0.92(15H,m,CH_3 2 x C14, CH_3 C_{16}, 2 x CH_3 Leu);1.11(3H,d, d,CH_3 Ala); 1.27 (64H,s, 20 x CH_2 C_{14}, 12 x CH_2 C_{16}); 1.39 (9H,d(CH_3)$_3$ Boc); 1.65 (4H,m,C_γH Leu, C_βH 2 x C_{14}, C_βH C_{16}); 1.89 (5H,m,$C_\beta H_2$ Leu, C_βH 2 x C_{14}, C_βH C_{16}); 2.97 (2H,m,$C_\beta H_2$ Phe); 3.13 (2H,m,C_βH Tyr); 3.46 (1H,m,C_αH C_{16}); 3.73 (4H,m,CH_3 Me ester, C_αH C_{14}); 3.87 (1H,m, C_αH C_{14}); 4.33 (3H,m,CH_2 Gly, C_αH Leu); 4.41 (1H,m,C_αH Ala); 4.60 (1H,m, C_αH Phe); 4.74 (1H,m,C_αH Tyr); 5.04 (2H,s,CH_2 Benzyl); 5.18 (1H,m,NH Tyr); 5.32 (½H,broad s, NH C_{14}); 5.56 (½H,broad s, NH C_{14});6.52 (1H,d,broad s, NH C_{14}); 6.77 (1H,m, NH Ala); 6.90 (2H,d,C_6H_4 AB Tyr); 7.08 (3H,m,C_6H_4 AB Tyr, NH Gly);7.21 (5H,m,C_6H_5 Phe); 7.39 (6H,m, C_6H_5 Tyr, NH Phe); 7.57 (1H,m, NH Leu).

MS m/e M^+ (%) $C_{86}H_{139}N_7O_{13}$ (1477) 1501(M^++Na+H)(50.0), 1374(14.1), 1276(60.6), 1148(24.4), 969(5.3), 915(10.0), 873(100.), 814(14.4), 760(40.3), 704(9.7), 648(19.1).

<u>N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY)-D-ALANYL-GLYCYL-PHENYLALANYL LEUCYL-O-METHYLENE-O-dl-α-2-AMINOHEXADECANOIC ACID N-α-TERTIARYBUTOXYCARBONYL</u>
(CXXIV)

XCI (0.150g, 0.3272mM), LXXXII (0.35g, 0.3298mM) in DMF (5ml) were stirred at room temperature for 5 days. EtOAc (10ml) was added and the solution washed with brine and $NaHCO_3$. Following evaporation yield = 0.22g (58.9%)

NMR ($CDCl_3$) 0.85(3H,t,CH_3 C_{16});0.95 (6H,m,CH_3 x 2 Leu);1.15 (3H,m,CH_3 Ala); 1.22 (24H,s, 12 x CH_2 C_{16}); 1.40 (9H,s,(CH_3)$_3$ Boc C_{16}); 1.43 (9H,s,(CH_3)$_3$ Boc Tyr); 1.57 (1H,m,C_βH C_{16}); 1.69 (2H,m,C_γH Leu, C_βH C_{16}); 1.94 (2H,m,$C_\beta H_2$ Leu); 2.91 (2H,m,$C_\beta H_2$ Phe); 3.09 (2H,m,$C_\beta H_2$ Tyr);3.44 (1H,m,OCH_2O); 3.67 (1H,m,OCH_2O); 3.87 (1H,m,C_αH C_{16});4.05 (1H,m,C_αH Ala); 4.25 (2H,m,CH_2 Gly); 4.50 (1H,broad s, C_αH Leu);4.70 (1H,m,C_αH

Phe); 4.83 (1H,m,C_αH Tyr); 5.02 (2H,s,CH_2 Benzyl);5.37 (½H,m,NH C_{16}); 5.52 (½H,m,NH C_{16}); 5.78 (1H,m,NH Tyr); 6.56 (½H,broad s,NH Ala); 6.90 (2½H,m,2H AB x C_6H_4 Tyr, ½ NH Ala); 7.07 (3H,m, 2H AB C_6H_4 Tyr,NH Gly); 7.22 (5H,m,C_6H_5 Phe); 7.39 (6H,m,C_6H_5 Benzyl Tyr, NH Phe); 7.50 (1H,m,NH Leu).

MS m/e M^+ (%) $C_{63}H_{94}N_6O_{13}$ (1142) 1166(M^++Na+H)(20.0), 1038(15.0), 881 (15.9), 859(15.3), 804(14.7), 778(100), 752(12.1), 704(42.9), 683(10.9), 636(10.9), 591(60.6), 566(19.4), 523(14.7), 468(79.4), 441(25.6)

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY)-D-ALANYL-GLYCYL-PHENYLALANYL-LEUCYL-O-METHYLENE-O-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOIC ACID-N-α-TERTIARYBUTOXYCARBONYL (CXXV)

XCII (0.100g, 0.1487mM), LXXXII (0.1575, 0.1484mM) in DMF (5ml) were reacted as in CXXIV. Yield = 0.1054 (50.8%).

NMR ($CDCl_3$) 0.77 (3H,m,CH_3 C_{16}); 0.88 (9H,m,CH_3 C_{16} 2 x CH_3 Leu);1.10 (3H,m,CH_3 D Ala); 1.23 (48H,m, 12 x CH_2 2 x C_{16}); 1.38 (9H,m,$(CH_3)_3$ Boc C_{16}); 1.44 (9H,m,$(CH_3)_3$ Boc Tyr); 1.58 (2H,m,C_βH C_{16} x 2); 1.68 (3H,m,C_γH Leu, C_βH C_{16} x 2); 1.92 (2H,m,$C_\beta H_2$ Leu); 2.93 (2H,m, CH_2 Phe); 3.14 (2H,m,CH_2 Tyr); 3.43 (1H,m, OCH_2O); 3.65 (2H,m, 1H OCH_2O C_αH C_{16}); 3.84 (1H,m, C_αH C_{16}); 4.22 (3H,broad m,CH_2 Gly, C_αH Ala); 4.50 (1H,m,C_αH Leu); 4.75 (2H,broad m,C_αH Phe, C_αH Tyr); 5.02 (2H,m,CH_2 Benzyl); 5.47 (1H,broad m,NH C_{16}); 5.58 (1H,m,NH Tyr); 6.54 (½H,d,NH C_{16}); 6.63 (½H,d,NH C_{16});6.77 (1H,d,NH Ala); 6.90 (2H,m,C_6H_4 AB Tyr); 7.09 (3H,m,2H C_6H_4 AB Tyr, NH Gly); 7.20 (5H,m,C_6H_5Phe); 7.40 (6H,m,C_6H_5 Tyr NH Phe); 7.55 (1H,m,NH Leu).

MS m/e M^+ (%) $C_{79}H125N_7O_{14}$ (1395) 1420(M^++Na+2H)(15.0), 1291(12.1), 1031(53.2), 957(18.5), 844(33.8), 778(46.8), 690(76.1), 660(53.5), 596(99.8), 570 (100), 506(38.5), 478(24.4).

N-α-TERTIARYBUTOXYCARBONYL-TYROSINYL (BENZYL HYDROXY)-D-ALANYL-GLYCYL-PHENYLALANYL-LEUCYL-O-METHYLENE-O-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOYL-dl-α-2-AMINOHEXADECANOIC ACID-N-α-TERTIARYBUTOXYCARBONYL (CXXVI)

XCIII (0.100g, 0.1080mM), LXXXII (0.1145g, 0.1079mM) in DMF (5ml) were reacted as in CXXIV Yield = 0.86 (48.4%).

NMR ($CDCl_3$) 0.85 (15H,m,CH_3 C_{16} x 3, 2 x CH_3 Leu); 1.10 (3H,m,CH_3 D Ala); 1.29 (72H,s, 12 x CH_2 3 x C_{16}); 1.39 (9H,d,$(CH_3)_3$ Boc); 1.45 (9H,d,$(CH_3)_3$ Boc); 1.58 (3H,m,$C_\beta H$ C_{16} x 3);1.71 (4H,m,$C_\beta H$ C_{16} x 3, $C_\gamma H$ Leu); 1.91 (2H,m,$C_\beta H_2$ Leu); 2.94 (2H,m, $C_\beta H_2$ Phe); 3.13 (2H,m,$C_\beta H_2$ Tyr); 3.48 (1H,m,-OCH_2O); 3.74 (2H,m,-OCH_2O 1H, $C_\alpha H$ C_{16}); 4.13 (1H,m,$C_\alpha H$ C_{16}); 4.22 (2H,m,$C_\alpha H$ C_{16}, $C_\alpha H$ Ala); 4.30 (1H,m,CH Gly); 4.39 (1H,m,CH Gly); 4.54 (1H,m,$C_\alpha H$ Leu);4.73 (1H,m,$C_\alpha H$ Tyr); 4.81 (1H,m,$C_\alpha H$ Phe); 4.98 (1H,m,NH Boc C_{16});5.03 (0.5H,s,CH_2 Benzyl); 5.37 (0.5H,m,NH Tyr); 5.78 (0.5H,m,NH Tyr); 6.50 (1H,m,NH C_{16}); 6.59 (1H,m,NH C_{16}); 6.77 (1H,m,NH Ala); 6.90 (2H,m,C_6H_4 AB Tyr); 7.09 (3H,m,C_6H_4 AB Tyr NH Gly); 7.24 (5H,m,C_6H_5 Phe); 7.38 (5H,m,C_6H_5 Benzyl Tyr); 7.53 (1H,m,NH Phe); 7.78 (1H,m,NH Leu)

MS m/e M^+ (%) $C_{95}H_{156}N_8O_{15}$ (1648)1673 (M^++Na+2H)(13.5), 1544(10.6), 1419(26.5), 1291(23.5), 943(594), 913(77.4), 844(47.9), 804(28.2), 690(68.2), 660 (100), 618(72.1), 570(91.8), 525(20.8), 450(57.6).

ELASTASE INHIBITORS

N-(ρ-METHYOXYCINNAMOYL)-5-AMINO-HEXANOIC ACID METHYL ESTER (CXXVII)

ρ-methoxycinnamic acid (57.7g, 32.4 mM) 5-amino-hexanoic acid methyl ester (5.89,32.4mM), HOBt (4,38, 32.4mM) and TEA (3.27g, 4.5ml, 32.4mM) were reacted as in method F. Yield = 9.16g (92.6%).

NMR ($CDCl_3$) 1.33 (2H,m,CH_2); 1.6 (4H,m,2xCH_2); 2.31 (2H,t,NCH_2);
3.32 (2H,t,CH_2CO); 3.64 (3H,s,OCH_3); 3.81 ((3H,s,$COOCH_3$);
6,33.6,25 (1H,d,ArCH); 7,45.7,42.6,87.6,84 (4H,ddAB, 4 x aromatic CH);
7.60,7.52 (1H,d,=CHCO)

MS m/e M^+ (%) $C_{17}H_{23}NO_4$ (305.36), 328$(M+Na)^+$(38), 247(3), 173(15), 102(100), 92(4), 63(3), 55(1).

N-(ρ-METHYOXYCINNAMOYL)-5-AMINO-HEXANOIC ACID (CXXVIII)

CXXVII (9.16g, 30mM) in MeOH (70ml) and IN NaOH (60ml) was reacted for 3 hours and then IN HCl (30ml) was added. The MeOH was evaporated and the pH adjusted to 2.5 with INHCl. The precipitate was filtered, washed with water and recrystallised from MeOH: ether 2:1. Yield = 6.29g (72%).

NMR ($CDCl_3$) 1.22 (2H,m,CH_2); 1.50 (4H,m, 2 x CH_2), 2.19 (2H,s,NCH_2);
3.19 (2H,t,CH_2CO); 3.69 (3H,s,OCH_3), 6.20, 6.27 (1H,d,$ArCH_2$);
6,75.6.79.7.33.7.36(4H,ddAB,4xaromatic CH), 7.37,7.43 (1H,d,=CHCO).

MS m/e M^+ (%) $C_{16}H_{21}NO_4$ (291) 314(M+Na)(27), 235(11), 159(16), 88(98), 78(15).

N-MYRISTOYL-ALANINE ETHYL ESTER (CXXIX)

Alanine ethyl ester hydrochloride (5.0g, 32.55mM) and myristic acid (7.43g, 32.55M) were reacted as in method F. Yield = 11.1g (100%).

NMR ($CDCl_3$) 0.88 (3H,t,CH_3); 1.26 (22H,m,11 x CH_2); 1.38,1.43 (3H,d,CH_3 Ala);

1.61 (3H,t,CH_3 Et ester); 2.17(2H,t,CH_2CO); 4.19(2H,q,$COOCH_2$); 4.60(1H,m,$C_\alpha H$ Ala); 6.05 (1H,d,NH).

MS m/e M^+ (%) $C_{19}H_{37}NO_3$ (327.50) 328$(M+H)^+$ (100), 278(6), 255(10), 172(4), 159(5), 137(4), 118(88), 68(8), 57(13), 44(55).

N-MYRISTOYL ALANINE (CXXX)

CXXIX (11.0g, 33.6mM) was saponified as in method D. Yield = 99%).

NMR ($CDCl_3$) 0.87 (3H,t,CH_3); 1.21 (20H,m, 10 x CH_2); 1.40,1.43 (3H,s,CH_3 Ala); 1.55 (2H,m,CH_2); 2.16 (2H,t,CH_2CO); 4.35 (1H,m,$C_\alpha H$ Ala).

MS m/e M^+ (%) $C_{17}H_{33}NO_3$ (299.44); 344$(M+2Na-H)^+$ (70), 322$(M+Na)^+$(100), 300$(M+H)^+$(22), 198(7), 176(10), 90(37), 57(19).

N-OLEOYL ALANINE ETHYL ESTER (CXXXI)

Alanine ethyl ester hydrochloride (5.0g, 32.55mM) and oleic acid (9.19g, 32.55mM) were reacted as in method F. Yield = 11.17g (90%).

NMR ($CDCl_3$) 0.89 (3H,t, CH_3); 1.25 (20H,m, 10 x CH_2); 1.37,1.39 (3H,d,CH_3 Ala); 1.60 (3H,t, CH_3 ester); 1.63(2H,m,CH_2);2.0(4H,m,2xCH_2); 2.18(2H,t,CH_2CO); 4.19(2H,q,$COOCH_2$); 4.58 (1H,m,$C_\alpha H$); 5.32 (2H,m,CH=CH); 6.05 (1H,d,NH).

MS m/e M^+ (%) $C_{23}H_{43}NO_3$ (381) 404$(M+Na)^+$(17), 384$(M+H)^+$(100), 337(10), 266(6), 198(5), 132(9), 55(18).

N-OLEOYL-ALANINE (CXXXII)

CXXXI (6,7g, 17.55mM) was hydrolysed as in method D. Yield = 5.65g.

NMR ($CDCl_3$) 0.89 (3H,t,CH_3); 1.26 (20H,m,10 x CH_2); 1.35,1.37 (3H,d,CH_3 Ala) 1.57(2H,m,CH_2);1.97(4H,m,2xCH_2);2.17(2H,m,CH_2CO);4.46(1H,m,$C_\alpha H$);

5.28 (2H,m,CH=CH); 6.54 (1H,d,NH).

MS m/e M^+ (%) $C_{21}H_{39}NO_3$ (353.53) 376 $(M+Na)^+$ (13), 354 $(M+H)^+$ (35), 316 (13), 161 (4), 131 (7), 109 (9), 90 (100), 82 (25), 69 (35), 55 (54).

N-(N-METHOXYCINNAMOYL-6-AMINO HEXANOYL) ALANINE ETHYL ESTER (CXXXIII)

Alanine ethyl ester hydrochloride (1.48g, 9.61mM) and CXXVIII (2.8g, 9.61mM) were reacted as in method F. Yield = 3.0g (80%).

NMR ($CDCl_3$) 1.26 (3H,t,CH_3 Ala); 1.38 (2H,d,CH_2); 1.60 (3H,m,CH_3 ester); 1.71 (4H,m,2 x CH_2); 2.22 (2H,t,NCH_2); 3.39 (2H,t,CH_2CO); 3.81 (3H,s,OCH_3); 4.19 (2H,q,$COOCH_2$); 4.52 (1H,m,$C_\alpha H$); 5.90 (1H,m,NH); 6.10 (1H,m,NH Ala); 6.24,6.31 (1H,d,ArCH); 7.45,7.42,6.90, 6.87 (4H,dd AB, 4 x aromatic CH); 7.60,7.53 (1H,d,=CHCO).

MS m/z M^+ (%) $C_{21}H_{30}N_2O_5$ (390.47), 391$(M+H)^+$(100), 225(9), 161(90), 136(10), 118(4), 107(4), 89(4), 77(4).

N-(N-METHOXYCINNAMOYL-6-AMINO HEXANOYL ALANINE (CXXXIV)

CXXXIII (2.8g, 7.18mM) was saponified as in method D. Yield = 2.4g (92.2%).

NMR($CDCl_3$-CD_3OD) 1.25 (3H,t,CH_3 Ala); 1.30(2H,m,CH_2); 1.46(2H,m,CH_2); 1.56(2H,m,CH_2); 2.15 (2H,t,NCH_2); 3.21(2H,t,CH_2CO); 3.74(3H,s,OCH_3); 4.35(1H,m,$C_\alpha H$); 6.29,6.22 (1H,d,ArCH); 7.36,7.33,6.82,6.72 (4H,dd,AB,4xaromaticCH); 7.44,7.37 (1H,d,=CHCO).

MS m/e M^+ (%) $C_{19}H_{26}N_2O_5$ (362.42), 385 $(M+Na)^+$(8), 363$(M+H)^+$(49), 225(15), 201 (4), 176(3), 161(100), 147(3), 133(9), 121(8), 107(3), 98(8), 69(9), 55(9).

N-α-TERTIARYBUTOXYCARBONYL-PROLYL-VALINE METHYL ESTER (CXXXV)

(a) To N-α-tboc proline (6.45g, 30mM), and triethylamine (TEA) (3.04g, 4.18ml, 30mM), in EtOAc (70ml), -10°C IBCF (4.10g, 3.89ml, 30mM) was added. The mixture was stirred for 10mins. The EtOAc (50ml), valine methyl ester hydrochloride (5.87g, 35mM), and TEA (4.87g, 6.70ml, 35mM) were added as in method B. The reaction was followed by TLC (system A). Yield = 9.6g (97%). (b) Valine methyl ester hydrochloride (2.52g, 15mM), TEA (1.52g, 95ml, 15mM), HOBt (2.026g, 15mM), N-α-tboc-proline (3.27g, 15mM), and DCC (3.09g, 15mM) in EtOAc (30ml) were reacted as in method F. Yield=4.5g (91.5%). m.p.=46-50°C.

NMR ($CDCl_3$): 0.88 (6H, t, $(CH_3)2$ val) 1.45 (9H, s, $(CH_3)_3$ boc); 1.91 (2H, broad s, $C_\gamma H_2$ pro); 2.16 (2H, m, $C_\beta H$ val,$C_\beta H$ pro); 2.34 (1H, broad s, $C_\beta H_1$ pro); 3.38 (2H, broad m, $C_\delta H_2$ pro); 3.71 (3H, s, OCH_3 Me ester); 4.29 (1H,broad m, $C_\alpha H$ val) 4.52 (1H, broad s, $C_\alpha H$ pro); ; 6.52 (1H, broad s, NH val).

MS m/e (%): $C_{16}H_{28}N_2O_5$ (328.39), 351(M+Na)(8.89), 329(M+H)(23.89), 273(23.33), 255(4.44), 241(2.22), 229(100), 213(3.89), 195(8.33), 170(8.89), 132(4.44), 114(17.5), 70(38.89), 57(13.33).

PROLYL-VALINE METHYL ESTER TRIFLUOROACETATE (CXXXVI)

CXXXV (4g, 12mM), was treated with trifluoroacetic acid(TFA) (25ml.) at room temperature for 30 mins the reaction was followed by TLC (system A). When no starting material remained, the solvent was evaporated, ether added and evaporated. MeOH was added to the oil then evaporated and the oil dried over sodium hydroxide pellets, yield =4.10g.(100%).

NMR ($CDCl_3$) 0.91 (6H,d,d,2xCH_3 val), 2.10 (4H,m,$C_\beta H_2$ pro, $C_\gamma H_2$ pro), 2.64 (1H,m,$C_\beta H$ val), 3.54 (2H,m,$C_\delta H_2$ pro), 3.70 (3H,s,OCH_3 Me ester), 4.35 (1H,m,$C_\alpha H$ val), 4.91 (1H,m,$C_\alpha H$ pro), 7.79 (1H, broad s, NH Pro), 8.97 (1Hm,d, NH val), 10.48 (1H, broad s, NH pro).

MS m/e M^- (%) $C_{13}H_{21}NO_{25}F_3$ (342) 228($M-CF_3COO)^+$(100), 185(21), 170(3), 131(3), 70(18).

N-α-TERTIARYBUTOXYCARBONYL-ALANYL-PROLYL-VALINE METHYL ESTER (CXXXVIIa)

CXXXVI (4.95g, 14mM), N-α-tboc alanine (2.65g, 14mM), HOBT (1.89g, 14mM), TEA (1.95g, 14mM), and DCC (2.89g, 14mM), in EtOAc (.30ml) were reacted in method F. TLC (system A) indicated the product was impure. Yield=3.2g (57%)

PROLYL-VALINE METHYL ESTER HYDROCHLORIDE (CXXXVIII)

CXXXV (9.2g, 28mM) was dissolved in 1M HCl/methanol (120ml) and refluxed for 15mins. Tlc. (system A), indicated no starting material remained. The solvent was evaporated at room temperature and addition of ether gave a yellow oil. Yield=7.39g (99.8%).

NMR ($CDCl_3$) 0.96 (6H, d.d. CH_3x2 val); 2.03 (3H, m, $C_\beta H_2$ pro, $C_\gamma H$ pro); 2.22 (1H, m, $C_\gamma H_1$ pro); 2.61 (1H, m, $C_\beta H_1$ val); 3.47 (2H, broad s, $C_\delta H_2$ pro); 3.67 (3H, s, CH_3 Me ester); 4.28 (1H, m, CαH val); 4.92 (1H, broad s, CαH pro); 7.92 (1H, broad s, NH pro); 8.47 (1H, d, NH val).

MS m/e (%) $C_{11}H_{21}N_2O_3Cl$ (264) 229(M^++1-HCl)(100), 186(1.6), 169(1.2), 130(1.8), 70 (17.5).

N-α-TERTIARYBUTOXYCARBONYL-ALANYL-PROLYL-VALINE METHYL ESTER (CXXXVIIb)

CXXXVIII (7.77g, 29.3mM), tboc-alanine (5.48g, 29.3mM), HOBT (3.92g, 29.3mM), TEA (4.073g, 29.3mM), and DCC (5.98g, 29.3mM) in EtOAC (60ml) were reacted as in method F and purified by silicon gel chromatography (system A) Yield = 8.1g (69.29%).

NMR ($CDCl_3$): 0.87 (6H, t, $(CH_3)_2$ val).1.30 (3H,d, CH_3 ala.); 1.42 (9H, s, $(CH_3)_3$

Boc); 1.90 (2H, m, $C_\gamma H_2$ pro); 2.11 (2H, m, $C_\beta H$ pro $C_\beta H$ val); 2.38 (1H, m, $C_\beta H$ pro); 3.53 (1H, m, $C_\delta H$ pro); 3.68 (1H, m, $C_\delta H$ pro); 3.72 (3H, s, OCH_3 ester); 4.43 (2H, m, $C_\alpha H$ val CαH ala); 4.67 (1H, m, $C_\alpha H$ pro); 5.40 (1H, d, NH ala); 7.31 (1H, d, NH val);

MS m/z (%) $C_{19}H_{33}N_3O_6$ (399.32), 422 $(399+Na)^+$ (2.78), 400$(399+H)^+$ (28.33), 344(23.33), 300(81.11), 265(7.22), 229(68.33), 213(23.33), 195(7.22), 185(11.67), 169(21.11), 141(12.78), 126(5.56), 116(3.89), 98(3.90), 88(7.80), 70(100), 57(28.06).

D-VALINE METHYL ESTER HYDROCHLORINE (CXXXIX)

D-Valine (23.43g, 0.2M), was suspended in MeOH and cooled to O°C, and thionyl chloride (21.66ml, 0.25M), added dropwise as in method A. Recrystallisation from methanol/ether. Yield=29.2g (88%).
Mp.168-170°C (lit. value= 171-173°C)

NMR ($CDCl_3$) 1.16 (6H, d.d, 2xCH_3 val); 2.45 (1H, m, $C_\beta H$ val); 3.80 (3H, s, CH_3 Me ester); 3.95 (1H, d, $C_\alpha H$ val); 8.87 (3H, broad s, NH_3^+ val).

MS m/e (%) $C_6H_{14}NO_2Cl$ (167, 169) 132 (M^+-HCl salt)(100), 118(3.5), 107(3.8), 89(3.9), 72(16.5).

N-α-TERTIARYBUTOXYCARBONYL-PROLYL-D-VALINE METHYL ESTER (CXL)

N-α-tboc-proline (9.81g, 45mM), D-valine methyl ester hydrochloride (7.55g, 45mM), HOBT (6.08g, 45mM), TEA (4.55g, 5.48ml. 45mM), and DCC (9.27g, 45mM), in EtOAc (140ml.) and dichloromethane (DCM) (50ml) were reacted as in method F.
Yield=12.7g (86%). Mp.=102-104°C

NMR ($CDCl_3$): 0.88-0.90 (3H, d, CH_3 val.) 0.94-0.97 (3H, d, CH_3 val); 1.47 (9H, s,$(CH_3)_3$ boc); 1.83 (1H, m, $C_\gamma H$ pro); 1.87 (2H, m, $C_\gamma H_1$ pro $C_\beta H_1$ val);

2.14 (2H, broad m, $C_\delta H_2$ pro); 3.48 (2H, broad m, $C_\delta H_2$ pro); 3.71 (3H, s, OCH_3 ester); 4.30 (1H, broad m, $C_\alpha H$ val); 4.53 (1H, broad m, $C_\alpha H$ pro); 6.52 (1H, broad s, NH val).

MS m/e (%) $C_{16}H_{28}N_2O_5$ (328) 351(M^++Na)(100), 329(M^++1)(3.6), 251(27.6), 229(30.2), 114(11.8), 70(30.9), 57(11.8).

PROLYL-D-VALINE METHYL ESTER HYDROCHLORIDE (CXLI)

CXL methyl ester (6.56g, 20mM), was dissolved in 1N HCl in MeOH (90ml) and refluxed for 20 mins. Evaporation of the solvent at room temperature gave an oil. Yield=5.39g (100%).

NMR ($CDCl_3$) 0.91 (6H, d.d, 2xCH_3 val); 2.09 (4H, m, $C_\beta H_2$ pro $C_\gamma H_2$ pro); 2.64 (1H, m,$C_\beta H$ val); 3.52 (2H, m, $C_\delta H_2$ pro); 3.70 (3H, s, OCH_3 Me ester); 4.36 (1H,m, $C_\alpha H$ val); 4.90 (1H, m, $C_\alpha H$ pro); 7.77 (1H, broad s, NH pro); 8.98 (1H,d, NH val); 10.46 (1H, broad s, NH pro).

MS m/e (%) $C_{11}H_{21}N_2O_3Cl$ (264, 268)229(M^++1-HCl)(100), 186(1.6), 169(1.2), 130(1.8), 70(17.5).

N-α-TERTIARYBUTOXYCARBONYL-ALANYL-PROLYL-D-VALINEMETHYL ESTER (CXLII)

CXLI (5.39g, 20mM), with tBoc-Ala (3.78g, 20mM), HOBt(2.70g, 20mM), TEA (2.02g, 2.78ml, 20mM),and DCC (4.13g, 20mM) in EtOAc (60ml) were reacted and observed by TLC (system M). Yield=7.20g (90%).

NMR ($CDCl_3$) 0.88 (6H, d.d. 2xCH_3 val); 1.33 (3H, d, CH_3 ala); 1.43 (9H, s, $(CH_3)_3$ Boc); 1.90 (1H, m, $C_\gamma H$ pro); 2.05 (1H, m, $C_\gamma H$ pro); 2.16 (2H, m, $C_\beta H$ pro, $C_\beta H$ val); 2.40 (1H, m, $C_\beta H$ pro); 3.54 (1H, m, $C_\delta H$ pro); 3.68 (1H, m, $C_\delta H$ pro); 3.77 (3H, s, OCH_3 Me ester); 4.46 (1H, q, $C_\alpha H$ val); 4.50 (1H, m, $C_\alpha H$ ala); 4.70 (1H, d.d, $C_\alpha H$ pro); 5.41 (1H, d, NH ala); 7.33 (1H, d, NH val).

MS m/e (%) $C_{19}H_{33}N_3O_6$ (339) 422(M^++Na)(100), 400(M^++1)(9.7), 322(22.8), 300(16.1), 229(16.5), 70(51.8), 57(15.9)

ALANYL-PROLYL-VALINE METHYL ESTER HYDROCHLORIDE (CXLIII)

CXXXVII (3.1g, 7.7mM), was refluxed in 1N HCl in methanol (35ml.) for 20 mins, TLC (system 1) indicating deprotection was complete. The solvent was evaporated giving an oil. Yield= 2.60g (100%).

NMR ($CDCl_3$): 0.91 (6H, q, $(CH_3)_2$ val), 1.42 (3H, d, CH_3 ala); 1.82-2.05 (3H, m, $C_\beta H$ pro $C_\gamma H_2$ pro); 2.06-2.19 (1H, m, $C_\beta H$ val); 2.32 (1H, m, $C_\beta H$ pro); 3.29 (3H, broad m, NH_3^+); 3.52 (2H, m, $C_\delta H_2$ pro); 3.70 (3H, s, OCH_3 Me ester); 3.91 (1H, broad m, $C_\alpha H$ ala); 4.44 (1H, q, $C_\alpha H$ val); 4.67 (1H, m, $C_\alpha H$ pro);7.43 (1H, d, NH val);

MS m/e (%) $C_{14}H_{26}N_3O_4Cl$ (334.5), 300(-HCl+H)(100), 286(14.4), 243(5.3), 229(25.3), 201(35.4), 187(7.5), 169(10), 141(9.7), 130(15.29), 104(6.2), 70(38.2), 57(6.2), 44(27.1).

OLEOYL-ALANYL-ALANYL-PROLYL-VALINE METHYL ESTER (CXLIV)

CXLIII (2.72g, 8.1mM), CXXXII (2.83g, 8.1mM), HOBt (1.08g, 8.1mM), TEA (0.81g, 1.11ml, 8.1mM), and DCC (1.65g, 8.1mM), in EtOAc (20ml) DCM (10ml), were reacted as per method F. Yield =5.3g (97.5%).

TLC (system A and B) indicated the presence of impurities. Purification was by flash chromatography (system A). Yield=4.9g (90%).

NMR ($CDCl_3$) 0.92 (9H, m, CH_3 oleoyl, CH_3 val x 2) 1.20-1.48 (28H, m, 11xCH_2 oleoyl, CH_3 alax 2); 1.55-1.78 (3H, broad m, $C_\beta H_1$ val, $C_\beta H_1$ pro, $C\gamma H_1$ pro); 2.00 (4H, m, $\underline{CH_2}CH=CH\underline{CH_2}$ oleoyl); 2.09 (2H, m, CH_2CO oleoyl), 2.16 (1H, t, $C_\gamma H$ pro);2.25 (1H, m, $C_\beta H$ pro); 3.66 (2H, m, $C_\delta H_2$ pro); 3.73 (3H, s, OCH_3 ester); 4.47 (1H, q, $C_\alpha H$ pro); 4.59-4.84 (3H, broad m, $C_\alpha H$ alax2, $C_\alpha H$ val); 5.35(2H, m, CH=CH oleoyl); 6.21 (1H, d, NH ala); 7.35 (1H, d, NH ala); 7.44 (1H, d, NH val);

MS m/e (%) $C_{35}H_{62}N_4O_6$ (634.84) 657(M^++Na)(100%), 597(3.3), 557(1.4), 503(2.5), 447(5.3), 413(6.9), 391(4.2), 376(1.5), 306(3.1), 249(8.8), 208(6.7), 180(2.2), 137(5.3), 122(2.2), 88(7.5), 63(14.2).

OLEOYL-ALANYL-ALANYL-PROLYL-VALINE (CXLV)

CXLIV (1.96g, 2.9mM), was hydrolyzed with 1N NaOH (3.5ml) in MeOH (13ml) as per method D. TLC (system A) after 2hrs. indicated the presence of ester therefore 1N NaOH (1ml.) was added and the mixture stirred for a further hour. TLC (system A) indicated de-esterification had occurred. Yield=1.42g (74.5%).

NMR ($CDCl_3$) 0.90 (9H, CH_3 oleoyl CH_3 valx2) 1.32 (28H, m, 11 x CH_2 oleoyl, CH_3 ala x2); 1.46 (1H, $C_\beta H$ val); 1.59 (2H, m, $C_\beta H_1$ pro, $C_\gamma H_1$ pro); 1.98 (4H, m, $\underline{CH_2}CH=CH\underline{CH_2}$ oleoyl); 2.02 (4H, m, $C_\beta H_1$ pro, $C_\gamma H_1$ pro, $\underline{CH_2}CO$ oleoyl); 4.10 (broad s OH); 3.66 (2H, m, $C_\delta H_2$ pro); 4.49 (1H, m, $C_\alpha H$ pro); 4.70 (1H, m, $C_\alpha H$ ala); 4.81 (2H, m, $C_\alpha H$ val, $C_\alpha H$ ala); 5.33 (2H, m, CH=CH oleoyl); 6.47 (1H, d, NH ala); 7.42 (1H, d, NH ala);7.83 (1H, d, NH val).

MS m/e (%) $C_{34}H_{60}N_4O_6$ (620), 687(M^++3Na) (21.2), 665(M^++2Na)(100), 644(M^++Na) (4.41), 621(M^++H)(2.35), 598(5.3), 491(6.8), 469(31.5), 420(8.8), 398(60), 330(5.3), 315 (5.3), 281(16.8), 257(26.8), 216(23.5), 188(13.5), 160(7.1), 118(14.1), 88(23.8), 55(54.4).

ALANYL-PROLYL-D-VALINE METHYL ESTER HYDROCHLORIDE (CXLVI)

CXLII (7.1g, 17.8mM), was dissolved in 1N HCl in methanol (80ml.) and refluxed for 20 mins, as in method E (II). Yield = 5.35g (89.6%).

NMR ($CDCl_3$) ppm 1.00 (6H, d.d, 2xCH_3 val); 1.60 (3H, d, CH_3 ala); 2.00 (2H, broad m, $C_\beta H$ pro, $C_\gamma H$ pro); 2.19 (3H, m, $C_\beta H$ pro, $C_\gamma H$ pro, $C_\beta H$ val); 3.51 (1H, m, $C_\delta H$ pro); 3.71 (3H, s, CH_3 Me ester); 3.80 (1H, m, $C_\delta H$ pro); 4.32 (2H, $C_\alpha H$ pro, $C_\alpha H$ val); 5.10 (1H, m, $C_\alpha H$ ala); 7.80 (1.5H, broad s, NH_3^+ ala); 8.09 (1.5H, broad s, NH_3^+ ala); 8.31 (0.5H, d, NH val); 8.40 (0.5H, d,

NH val).

MS m/e (%) $C_{14}H_{26}N_3O_4Cl$ (335,337) 322(M^++Na-HCl) (44.4), 300(M^++1-HCl)(100), 229 (24.4), 176(9.3), 154(8.1), 70(26.6).

OLEOYL-ALANYL-ALANYL-PROLYL-D-VALINE METHYL ESTER (CXLVII)

CXLVI methyl ester hydrochloride (4.03g, 12mM), CXXXII (4.24g, 12mM), HOBt (1.62g, 12mM), TEA (1.21g, 1.67ml.),and DCC (2.48g, 12mM), in EtOAc (20ml) DCM (15ml) and were reacted as per method F. Following filtration, extraction and evaporation an oil was obtained. Yield=6.30g (78.2%). TLC (system A) indicated the presence of impurities, therefore the oil was dissolved in petroleum ether(60-80), and the insoluble material removed by filtration. Evaporation of the filtrate, gave an oil, pure by tlc. Yield = 6.03g (74.8%).

NMR ($CDCl_3$) 0.91 (9H, 2xCH_3 val, CH_3 oleoyl) 1.33 (28H, CH_2 x 11 Oleoyl, CH_3 ala x 2); 2.00 (5H, $C_\beta H$ val, $\underline{CH_2}CH=CH\underline{CH_2}$); 2.08 (2H,m,$CH_2$ Co oleoyl); 2.18 (3H, m, $C_\beta H_1$ pro, $C_\gamma H_2$ pro); 2.33 (1H, m, $C_\beta H_1$ pro); 3.61 (2H, m, $C_\delta H_2$ pro); 3.67 (3H, s, OCH_3 ester); 4.48 (1H, q, $C_\alpha H$ pro); 4.59 (1H, m, $C_\alpha H$ ala); 4.73 (2H, m, $C_\alpha H$ val, $C_\alpha H$ ala); 5.35 (2H,m, CH=CH oleoyl); 6.21 (1H, d, NH ala); 7.16 (1H, d, NH ala); 7.37 (1H, d, NH val);

MS m/z (%) $C_{35}H_{62}N_4O_6$ (634) 679 (M^++2Na)(5.9), 657 (M^++Na)(100), 461(13.5), 398 (17.4), 249(10.6), 208(7.1), 88(8.2), 77(6.8), 55(10.2).

MYRISTOYL-ALANYL-ALANYL-PROLYL-VALINE METHYL ESTER (CXLVIII)

CXXX (2.99g, 10mM), $CHCl_3$ (65ml.), was reacted with CXLIII (3.36g, 10mM), HOBt (1.35g, 10mM), TEA (1.01g, 1.39ml., 10mM), and DCC (2.06g, 10mM), as in method F. Yield = 5.5g (94.8%). Tlc. (system A), indicated the presence of impurities and purification was carried out using flash chromatography. Yield = 3.95g (68.1%).

NMR ($CDCl_3$) (40°C) 0.90 (9H, t, CH_3 x 2 val, CH_3 myr) 1.32 (28H, m, CH_3 ala x 2,11xCH_2 myr); 1.62 (3H, m, $C_\beta H_1$ val, $C_\beta H_1$ pro, $C_\gamma H_1$ pro); 2.03 (2H, m, $C_\beta H_1$ pro, $C_\gamma H_1$ pro); 2.21 (2H, t, CH_2CO myr); 3.61 (2H, m, $C_\delta H_2$ pro); 3.74 (3H, s, OCH_3 ester); 4.85 (4H, m, $C_\alpha H$ ala, ala, pro, val); 6.07 (1H, d, NH myr-ala); 6.16 (1H, d, NH ala); 6.85 (1H, d, NH val).

MS m/e M^+(%) $C_{31}H_{56}N_4O_6$ (580.76) 603(M^++Na) (36.6), 581(M^++H)(19.4), 504(17.8), 482(14.4), 300(21.6), 282(24.1), 229(100), 201(23.4), 130(56.3), 104(13.8), 70(67.5), 57 (13.1).

p-METHOXYCINNAMOYL-5-AMINO-HEXANOYL-ALANYL-ALANYL-PROLYL-VALINE METHYL ESTER (CXLIX)

CXXXIV (2.29g, 6.33mM) CXLIII (2.35g, 7mM), HOBt (0.86g, 6.33mM), TEA (0.71g, 0.97ml., 7mM), and DCC (1.31g, 6.33mM) in DMF (10ml) were reacted as in method F. A small amount of the reaction mixture was added to water, but there was no precipitation.

The DMF was evaporated under high vacuum (3-4mmHg) and the resulting residue dissolved in EtOAc. The DCU obtained was removed by filtration and the organic phase extracted and evaporated giving an oil. TLC (system A) indicated the presence of impurities, therefore the oil was dissolved in chloroform and the solid produced removed by filtration. Evaporation gave a white foam like solid, tlc. indicated it was pure. Yield = 1.87g (46%).

NMR ($CDCl_3$) 0.96 (6H, t, 2xCH_3 val); 1.37 (12H,m, CH_3x2 ala, 3xCH_2 moxy); 1.62 (6H, m, $C_\beta H_1$ pro, $C_\gamma H_1$ pro, 2xCH_2 moxy); 2.05 (2H, m, $C_\beta H$ pro, $C_\gamma H$ pro); 2.24 (1H, t, $C_\beta H$ val); 3.39 (3H, s, OCH_3 moxy); 3.64 (1H, m, $C_\delta H$ pro); 3.69 (1H, m, $C_\delta H$ pro); 3.81 (3H, s, CH_3 Me ester); 4.17 (1H, m, $C_\alpha H$ val); 4.36 (1H, m, $C_\alpha H$ ala); 4.54 (1H, m, $C_\alpha H$ ala); 4.63 (1H, m, $C_\alpha H$ pro); 5.88 (1H, d,NH ala); 6.48 (2H,d.d CH=CH); 6.63 (1H, d. NH ala); 6.85 (1H, d, NH val); 6.93 (2H, d, AB moxy); 7.48 (2H, d, AB moxy), 8.18 (1H, m, NH moxy).

MS m/e M^+ (%) $C_{33}H_{49}N_5O_8$ (643) 644 (M^++H)(13.4), 612(22.8), 512(14.8), 415(17.5), 344 (19.1), 176(33.9), 154(18.2)

OLEOYL-ALANYL-ALANYL-PROLYL-D-VALINE (CL)

CXLVII (3.02g, 4.5mM) was dissolved in methanol (10ml), 1N NaOH (5ml) added and reacted as in method D. TLC (system A) indicated the presence of impurities yield = 2.4g (81%). Purification was carried out using column chromatography (system A) Yield = 1.93g (65%).

NMR ($CDCl_3$) 0.88 (9H,m, 2xCH_3 val, CH_3 oleoyl) 1.31 (28H, t, CH_2x11 oleoyl, CH_3 alax2); 1.67 (2H, $C_\beta H_1$ pro, $C_\gamma H_1$ pro), 1.98 (5H, m, $C_\beta H$ val, $\underline{CH_2}CH=CH\underline{CH_2}$); 2.10 (4H, m, $C_\beta H_1$ pro, $C_\gamma H_1$ pro, CH_2CO oleoyl); 3.61 (2H, m, $C_\delta H_2$ pro); 4.15 (broad s, OH), 4.48 (1H, m, $C_\alpha H$ pro); 4.70 (1H, m, $C_\alpha H$ ala); 4.71 (2H, m, $C_\alpha H$ val, $C_\alpha H$ ala); 5.33 (2H, m, CH=CH oleoyl); 6.47 (1H, d, NH ala); 7.24 (1H, d, NH ala); 7.75 (1H, d, NH val).

MS m/e M^+ (%) $C_{34}H_{60}N_4O_6$ (632)665 (M^++2Na)(100), 643(M^++Na)(10.8), 597(16.3), 583 (8.8), 498(12.8), 469(27.2), 441(16.3), 413(20), 393(10.2), 322(24.4), 275(10.9), 257 (16.3), 216(13.8), 176(48.4), 149(28.4), 107(12.7), 95(18.1), 81(24.7), 69(43.6), 55 (66.6).

MYRISTOYL-ALANYL-ALANYL-PROLYL-VALINE (CLI)

CXLVIII (3.80g, 6.5mM) was dissolved in MeOH (15ml) cooled to 0°C and 1N NaOH (7.2ml.) was added. On addition of NaOH precipitation of the starting material occurred, therefore MeOH (10ml) was added, the reaction was allowed to proceed and was followed by TLC (system A). Yield = 3.25g (88.2%). following recrystallisation from methanol mpt.= 65-67°C.

NMR ($CDCl_3$) 0.89 (9H, m, CH_3x2 val, CH_3 myr); 1.33 (22H, s, CH_2x11 myr); 1.42 (6H, d.d, CH_3x2 ala); 1.63 (2H, m, $\underline{CH_2}CO$ myr); 1.91 (1H, m, $C_\beta H$ pro); 2.07 (1H, m, $C_\gamma H$ pro); 2.22 (3H, m, $C_\beta H$ pro, $C_\gamma H$ pro, $C_\beta H$ val); 3.69 (2H, m, $C_\delta H_2$ pro); 4.49 (1H, m, $C_\alpha H$ ala); 4.51 (1H, m, $C_\alpha H$ ala); 4.69 (1H,

m, $C_\alpha H$ val); 4.78 (1H, m, $C_\alpha H$ pro); 6.52 (1H, d, NH myr ala); 6.79 (1H, d, NH ala); 8.15 (1H, d, NH val).

MS m/z M+(%) $C_{30}H_{54}N_4O_6$ 566 612 (M^++Na)(22.4), 590(M^++Na)(7.1), 512(14.1), 490 (9.4), 444(11.2), 415(18.5), 344(20.6), 266(14.1), 232(6.5), 176(32.9), 154(18.2), 132(6.5), 107(10.0).

N-α-TERTIARYBUTYLOXYCARBONYL-ALANYL-ALANYL-PROLYL-VALINE METHYL ESTER (CLII)

tBoc-alanine (5.67g, 30mM), CXLIII (10.07g, 30mM), HOBt (4.05g, 30mM), TEA (3.04g, 4.17ml., 30mM), and DCC (6.19g, 30mM) in EtOAc (20ml) were reacted as per method F. Yield = 11.5g (81.4%).

NMR ($CDCl_3$) 0.89 (6H, d.d., 2xCH_3 val); 1.36 (6H, t, CH_3 alax2); 1.45 (9H, s, $(CH_3)_3$ Boc); 1.93 (2H, m, $C_\beta H$ pro, $C_\gamma H$ pro); 2.15 (2H, m, $C_\beta H$ pro, $C_\gamma H$ pro); 2.39 (1H, m, $C_\beta H$ val); 3.60 (1H, m, $C_\delta H$ pro); 3.67 (1H, m, $C_\delta H$ pro); 3.73 (3H, s, OCH_3 Me ester); 4.20 (1H, m, $C_\alpha H$ val); 4.47 (1H, q, $C_\alpha H$ ala); 4.67 (1H, d.d, $C_\alpha H$ ala); 4.74 (1H, m, $C_\alpha H$ pro); 5.04 (1H, d, NH Boc-ala); 7.04 (1H, d, NH ala), 7.24 (1H, d, NH val).

MS m/z M+(%) $C_{22}H_{38}N_4O_7$ 470, 493(M^++Na)(100), 471(M^++1)(3.8), 393(29.2), 297 (12.6), 229(54.8), 197(13.4), 183(55.3), 70(88.2), 57(32.8).

ALANYL-ALANYL-PROLYL-VALINE METHYL ESTER HYDROCHLORIDE (CLIII)

CLII (10g, 24mM), was dissolved in 1N HCl in methanol (90 ml.) and refluxed for 20mins. following evaporation an oil was obtained . Yield = 9.9g (100%).

NMR ($CDCl_3$) 0.92 (6H, d, 2xCH_3 val); 1.62 (6H, m, CH_3 alax2); 2.13 (5H, m, $C_\beta H_2$ pro, $C_\gamma H_2$ pro, $C_\beta H$ val); 3.68 (3H, s, OCH_3 Me ester); 3.81 (2H, m, $C_\delta H_2$ pro); 4.41 (2H, m, $C_\alpha H$ ala, $C_\alpha H$ val); 4.76 (2H, m, $C_\alpha H$ ala, $C_\alpha H$ pro);

7.61 (1H, broad s, NH ala); 8.22 (3H, broad m, NH_3^+ ala); 8.49 (1H, broad d, NH val).

MS m/z M+(%) $C_{17}H_{31}N_4O_5Cl$ 406/408, 393 (M^++Na HCl) (3.5), 371(53.7), 300(18.3), 229 (100), 175(17.1), 149(8.8), 115(11.2), 104(10.2), 70(79.7).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANOYL-l-ALANINE ETHYL ESTER (CLIV)

XXXXII (1.7g, 0.004M), alanine ethyl ester hydrochloride (0.61g, 0.004M), HOBt (0.54g, 0.004M), and TEA (0.04g, 0.004M) and DCC (0.82g, 0.004M) in EtOAc (50ml) and DCM (200ml) were reacted as in method F. Yield =1.9g (90%). Purification was carried out by flash chromatography. Yield =1.34g (64%).

NMR ($CDCl_3$) ppm 0.87 (3H, t, CH_3 C_{14}) 1.30 (23H,m,$(CH_2)_{10}$ C_{14}, CH_3 ethyl ester); 1.40 (3H, d,CH_3 ala); 1.43 (9H, s, $(CH_3)_3$Boc); 1.51 (1H, m, $C_\beta H_1$ C_{14}); 1.81 (1H, m, $C_\beta H_1$ C_{14}); 4.05 (1H, m, $C_\alpha H$ C_{14}); 4.18 (2H, q, $\underline{CH_2}CH_3$); 4.56 (1H, m, $C_\alpha H$ ala); 4.95 (1H, broad s, NH C_{14}); 6.50 (0.5H, d, NH ala). 6.59 (0.5H, d, NH ala);

MS m/e M^+ (%) $C_{24}H_{46}N_2O_5$ (442), 465(M^++Na)(10.6), 443(28.4), 387(70.9), 343(99.4), 242(15.5), 198(100), 133(28.2), 118(40.5), 57(44.5).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANOYL-l-ALANINE (CLV)

CLIV (1.3g, 2.93mM), was hydrolyzed in MeOH (5.5ml) and 1N NaOH (6.5ml.) as in method D. The reaction was followed by TLC (system A). Yield = 1.25g (98%).

NMR ($CDCl_3$) 0.87 (3H, t, CH_3 C_{14}) 1.27 (20H, m, $(CH_2)_{10}$ C_{14}); 1.34(3H,d,CH_3 ala); 1.50 (9H, s, $(CH_3)_3$ Boc); 1.59 (1H, m, $C_\beta H$ C_{14}); 1.72 (1H, m, $C_\beta H_1$ C_{14}); 4.12 (1H, m, CαH C_{14}); 4.30 (1H, m, CαH ala); 4.50 (1H, broad s, OH); 5.35 (0.5H, m, NH C_{14}); 5.59 (0.5H, m, NH C_{14}); 7.26 (0.25H, m, NH ala);

7.39 (0.25H, m, NH ala); 7.53 (0.25H, m, NH ala); 7.70 (0.25H, m, NH ala).

MS m/e M^+ (%) $C_{22}H_{42}N_2O_5$ (414), 895($2M^+$+3Na-2H)(11.2), 459(M^++2Na) (13.7), 437 (M^++Na)(17.1), 381(20.4), 359(96.5), 337(18.2), 198(12.9), 149(22.6), 118(11.4), 88 (20.6), 71(25.2), 57(100).

N-α-TERTIARYBUTOXY-dl-α-2-AMINOTETRADECANOYL-L-ALANYL-L-ALANYL-L-PROPYL-L-VALINE METHYL ESTER (CLVI)

CXLIII (1.065g, 3.17mM) CLV (1.25g, 3.17mM), HOBt (0.43g, 3.17mM), TEA (0.32g, 3.17mM), and DCC (0.65g, 3.17mM), in EtOAc (30ml) and DCM (10ml) were reacted as in method F. Yield =2.15g (93%). 0.2g was purified by preparative TLC (system A) to give the pure pentapeptide.

NMR ($CDCl_3$) 0.84 (9H, m, (CH_3)2 val, CH_3 C_{14}), 1.26 (20H, s, $(CH_2)_{10}$.C_{14}); 1.35 (6H,m, CH_3 alax2); 1.46 (9H, s, $(CH_3)_3$ Boc); 1.59 (1H, m, $C_\beta H$ C_{14}); 1.82 (1H,m, $C_\beta H$ C_{14}); 1.98 (2H, m, $C_\beta H$ pro, $C_\gamma H$ pro); 2.15 (2H, m, $C_\beta H$ val, $C_\gamma H$ pro); 2.33 (1H, m, $C_\beta H$ pro); 3.58 (1H, m, $C_\delta H$ pro), 3.66 (1H, m, $C_\delta H$ pro); 3.73 (3H, s OCH_3 Me ester); 4.06 (1H, broad s, $C_\alpha H$ C_{14}); 4.45 (1H, q, $C_\alpha H$ val); 4.58 (1H, m, $C_\alpha H$ ala); 4.68 (1H, m, $C_\alpha H$ pro); 4.77 (1H, m, $C_\alpha H$ ala); 4.98 (0.5H, broad s, NH C_{14}); 5.12 (0.5H, broad s, NH C_{14}); 6.68 (1H, m, NH ala); 7.30 (2H, m, NH val, NH ala).

MS m/e M^+ (%) $C_{36}H_{65}N_5O_8$ (695) 740(M^++2Na), 718(M^++Na)(100), 619(32.1), 462 (3.6), 393(4.7), 362(2.9), 306(3.8), 249(5.6), 229(10.3), 208(4.8), 137(3.2), 70(4.7), 57 (6.2).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOYL-L-ALANINE ETHYL ESTER (CLVII)

l-Alanine ethyl ester hydrochloride (0.21g, 1.40mM), TEA (0.14g, 1.40mM), HOBt (0.19g, 1.40mM), LII (0.78g, 1.40mM) and EDAC (0.27g, 1.40mM) in DCM (10ml) were reacted as in method D. Yield=0.71g (77%). TLC (system B)

indicated the product to be pure.

NMR ($CDCl_3$) 0.88 (6H, t, CH_3 C_{14}x2), 1.27 (49H, m, $(CH_2)_{10}$ C_{14}x2 CH_3 Et ester); 1.43 (12H, m,$(CH_3)_3$ Boc, CH_3 ala); 1.83 (4H, m, $C_\beta H_2$ 2 x C_{14},); 4.02 (1H, m, $C_\alpha H$ Boc C_{14}); 4.18 (2H, m, CH_2 ethyl ester); 4.38 (1H,m, C_{14}); 4.51 (1H, m, $C_\alpha H$ ala);4.94 (1H, broad s, NH Boc C_{14}); 6.53 (1H, m, NH C_{14}); 6.58 (0.5H, broad d, NH Ala); 6.82 (0.5H, broad d, NH ala).

MS m/e M^+ (%) $C_{38}H_{73}N_3O_6$ (667) 690(M^++Na)(25.2), 668(M^++H)(1.5), 612(1.8), 590 (8.2), 560(1.5), 495(1.8), 421(1.8), 365(1.2), 343(5.9), 242(2.9), 198(100), 154(1.8), 118 (21.8), 95(2.9), 69(5.3), 57(22.4).

<u>N-α-TERTIARYBUTOXY-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOYL-dl-α-AMINOTETRADECANOIC ACID</u> (CLVIII)

LVI (0.685g, 2.334mM) was dissolved in DCM (23ml) then TEA (0.47g, 4.66mM), HOBt (0.315g, 2.331mM), LII (1.30g, 2.334mM) and EDAC (0.447g, 2.332mM) were added. Yield = 1.62g (87.1%).

NMR ($CDCl_3$) 0.85 (9H, t, CH_3x 3 C_{14}); 1.23 (60H,m, 30 x CH_2 C_{14}); 1.43 (9H,s, $(CH_3)_3$ Boc); 1.73 (6H,m, 3 x $C_\beta H_2 C_{14}$); 4.19, 4.32, 4.50 (3H,m, 3 x $C_\alpha H$ C_{14}); 5.42 (1H,s, Boc NH); 7.32, (2H,m, 2 x NH)

MS m/e M^+ (%) $C_{47}H_{91}N_3O_6$ (793) 837(M^++2Na)(4.7). 760(14.1), 738(43.2). 613(10.6), 561(13.5), 535(27.6), 513(66.8), 490(12.9), 342(15.3), 313(11.2), 286(19.6), 198(100), 176(12.3), 117(24.7), 88(24.4), 57(97.1).

FOUND	C, 70.98; H, 11.11; N, 5.22
CALCULATED FOR:	$C_{48}H_{93}N_6O_6$, C.71.32; H, 11.60; N, 5.32

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOYL-l-ALANINE ETHYL ESTER (CLIX)

CLVIII (0.42g, 0.529mM) alanine ethyl ester hydrochloride (0.07g, 0.53mM), HOBt (0.07g, 0.53mM), TEA (0.054g, 0.53mM), and EDAC (0.10g, 0.53mM) in DCM (10ml) EtOAc (10ml) were reaced as in method F. Yield = 0.40g (84.7)

NMR ($CDCl_3$) ppm 0.88 (9H, t, CH_3 C_{14} x 3); 1.26 (63H,m, $(CH_2)_{10}$ C_{14} x 3, CH_3 ethyl ester); 1.42 (12H, m, $(CH_3)_3$Boc,CH_3 ala); 1.84 (6H, broad s, $C_\beta H_2$ C_{14} x 3); 3.99 (1H, broad s, C_α H Boc C_{14}); 4.16 (2H, m, CH_2 ethyl ester); 4.30 (1H, m, $C_\alpha H$ C_{14}); 4.42 (1H, m, $C_\alpha H$ C_{14}); 4.50 (1H, m, $C_\alpha H$ ala); 4.94 (1H, broad s, NH boc C_{14}); 6.61 (1H, broad s, NH C_{14}); 6.79 (1H, broad s,NH C_{14}), 6.93 (1H, broad s, NH ala).

MS m/e M^+ (%) $C_{52}H_{100}N_4O_7$ (892) 916(M^++Na)(100), 815(25.7), 690(21.3), 590(19.4), 198(46.9), 173(11.7), 88(9.5), 57(16.6)

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOYL-ALANINE (CLX)

CLIX (0.61g, 0.928mM) was hydrolyzed in MeOH (10ml), DCM (10ml) amd 1N NaOH (2ml). The reaction was followed by TLC. Yield = 0.5g (84.3).

NMR ($CDCl_3$) ppm 0.88 (6H, t, CH_3 C_{14} x 2), 1.28 (40H, s, $(CH_2)_{10}$ x C_{14}); 1.45 (12H, m, $(CH_3)_3$ boc, CH_3 ala); 1.59 (2H, $C_\beta H$ C_{14}); 1.86 (2H, broad s, $C_\beta H$ C_{14}); 4.05 (1H, m, CαH boc C_{14}), 4.51 (2H, broad m, $C_\alpha H$ ala, $C_\alpha H$ C_{14}); 5.03 (0.5H, broad s, NH boc C_{14}); 5.13 (0.5H, broad s, NH boc C_{14}); 6.71 (0.5H, broad s, NH C_{14}); 6.80 (0.5H, broad s, NH C_{14}); 7.06 (1H, d, NH ala).

MS m/e M^+ (%) $C_{36}H_{69}O_6N_3$ (639) 1302(2M+Na+H)(13.7), 685(M+2Na)(17.5), 662(M+Na)$^+$(19.3), 606(21.2), 562(20.1), 539(12.8), 483(11.6), 371(35.1), 198(57.6), 57(43.89).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOYL-ALANYL-ALANYL-PROLYL-VALINE METHYL ESTER (CLXI)

CLX (0.5g, 0.782mM), CXLIII (0.26g, 0.78mM), HOBt (0.11g, 0.78mM), TEA (0.08g, 0.78mM), and EDAC (0.15g, 0.78mM) in DCM (10ml) were reacted as in method F. Yield = 0.72g (100%). (System B)

NMR ($CDCl_3$) ppm 0.88 (12H, CH_3 x 2 val, CH_3 C_{14} x 2) 1.28 (40H,m, $(CH_2)_{10}$ C_{14} x 2); 1.35 (6H, m, CH_3 ala x 2); 1.46 (9H, m, $(CH_3)_3$ Boc); 1.67 (2H, m, $C_\beta H_2$ C_{14}); 1.88 (2H, m, $C_\beta H_2$ C_{14}); 2.05 (2H, m, $C_\gamma H$ pro, $C_\beta H$ pro); 2.15 (2H, m, $C_\beta H$ val, $C_\gamma H$ pro); 3.59 (1H, m, $C_\delta H$ pro 2.36 (1H, m, $C_\beta H$ pro), 3.68 (1H, m, $C_\delta H$ pro); 3.74 (3H, d, CH_3 methyl ester); 4.01 (1H, m, C_α H boc C_{14}); 4.35 (1H, m, $C_\alpha H$ C_{14}); 4.46 (2H, m, C_α H ala x 2); 4.69 (2H, m, C_α H val, C_α H pro); 4.98 (1H, m, NH Boc C_{14}); 6.57 (1H, d, NH ala); 6.72 (1H, d, NH C_{14}); 6.86(1H, broad s, NH ala); 7.36 (1H, broad s, NH val);

MS m/e M^+ (%) $C_{50}H_{92}N_6O_9$ (920), 944(M^++Na)(72.6), 844(18.5), 748(16.9), 647(15), 229(38.9), 198(100).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-TETRAAMINODECANOYL-ALANINE (CLXII)

CLIX (0.37g, 0.415mM), was hydrolyzed in MeOH (5ml) DCM (10ml) and 1N NaOH (0.91ml) as in method D. The reaction was followed by tlc. (system 2) and on completion (3hrs), the solvent was evaporated water (10ml.) added and the solution acidified to pH4 with 3% HCl. The solution was extracted with ether and evaporation gave an oil yield = 0.23g (63.2%).

NMR ($CDCl_3$) ppm, 0.88 (9H,t,CH_3 x 3 C_{14}), 1.26 (60H,m, $(CH_2)_{10}$ x 3); 1.43 (12H,m, $(CH_3)_3$ Boc x CH_3 ala); 1.86 (6H,m,$C_\beta H_2$ C_{14} x 3); 4.00 (1H,m broad s, $C_\alpha H$ C_{14}); 4.52 (2H,m,$C_\alpha H$ C_{14}); 4.52 (1H,m,$C_\alpha H$ ala); 5.04 (1H, broad s,

NH C_{14}); 6.80 (1H, broad s, NH C_{14}); 6.91 (1H, broad s, NH C_{14}); 7.53 (1H, broad s, NH ala).

MS m/e M^+ (%) $C_{50}H_{96}N_4O_7$ (864) 909$(M+2Na-H)^+$ (15.1), 887$(M+Na)^+$(17.5), 865$(M+H)^+$(11.2), 787(13.7), 765(20.3), 742(11.4), 686(22.6), 568(12.3), 463(17.1), 407(33.1), 296(36.4), 198(37.3), 57(53.5).

N-α-TERTIATYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOYL-dl-α-2-AMINOTETRADECANOYL-ALANYL-ALANYL-PROLYL-VALINE METHYL ESTER (CLXIII)

CLXII (0.22g, 0.255mM), CXLIII (0.09g, 0.255mM), HOBt (0.03g, 0.255mM), TEA (0.03g, 0.255mM), and EDAC (0.05g, 0.255mM) in DCM (10ml) and EtOAc (5ml) were reacted as in method F. Yield = 0.30g (100%). 0.1g was purified by preparative TLC (system 2).

NMR ($CDCl_3$) ppm 0.88 (15H, CH_3 C_{14} x 3, CH_3 x 2 val) 1.27(60H,M, 10 X CH_2 C_{14} x 3); 1.34(6H,m,CH_3 ala x 2); 1.48 (12H, m, $(CH_3)_3$ Boc, CH_3 ala); 1.67 (6H, broad m, $C_\beta H_2$ C_{14} x 3); 2.10 (5H, broad m, $C_\beta H_2$ pro, $C_\gamma H_2$ pro, $C_\beta H$ val); 3.61 (2H, m, $C_\delta H_2$ pro); 3.71 (3H, m, CH_3 ester); 4.06 (1H, m, $C_\alpha H$ boc C_{14}); 4.21 (1H, $C_\alpha H$ ala); 4.29 (1H, m, C_α H ala); 4.46 (1H, m, C_α H val), 4.82 (3H, broad m, C_α H C_{14},x 2 $C_\alpha H$ pro); 5.13 (1H, broad s,NH Boc C_{14}); 6.58 (1H, broad s, NH C_{14}); 6.70 (1H, broad s, NH C_{14}); 6.83 (1H broad s, NH ala); 7.50 (1H, m, NH ala); 7.70 (1H, m, NH val);

MS m/e M^+ (%) $C_{64}H_{119}N_7O_{10}$ (1145), 1169(M^++Na)(100), 1069(19.4), 973(17.1), 944 (17.8), 873(9.4), 844(10), 810(5.7), 742(5.4), 648(6.0), 490(14.6), 447(6.3).

N-α-TERTIARYBUTOXYCARBONYL-dl-α-2-AMINOTETRADECANOYL-ALANYL-ALANYL-PROLYL-VALINE (CLXIV)

CLVI (0.1g, 0.14mM), was dissolved in methanol (10ml.), and 1N NaOH (0.3ml) added as in method D. The reaction was follwed by TLC (system B) and after 4hrs was found to be complete. Yield = 0.098g (100%). Purity was

checked by HPLC. A gradient was run 0-70% isopropanol, this indicated the compound to be pure.

NMR ($CDCl_3$) ppm 0.88 (9H, m, CH_3 x 2 val, CH_3 C_{14}), 1.27 (16H, m, $(CH_2)_{10}$ C_{14}, CH_3 ala x 2); 1.44 (9H, m, $(CH_3)_3$ Boc), 1.54 (1H, m, $C_\beta H$ C_{14}); 1.77 (1H, m, $C_\beta H$ C_{14}); 2.00 (2H, m, $C_\beta H$ pro, $C_\gamma H$ pro); 2.18 (2H, m, $C_\beta H$ val, $C_\gamma H$ pro); 2.31 (1H, m, $C_\beta H$ pro); 3.43 (broad s, OH); 3.62 (1H, m, $C_\delta H$ pro); 3.69 (1H, m, $C_\delta H$ pro); 4.32 (1H, m, $C_\alpha H$ C_{14}); 4.48 (1H, m, $C_\alpha H$ val); 4.59 (1H, m, $C_\alpha H$ ala); 4.69 (1H, m, $C_\alpha H$ pro); 4.78 (1H, m, $C_\alpha H$ ala); 5.13, 5.23 (1H, m, NH Boc C_{14}); 7.03, 6.94,(1H, m, NH ala); 7.56, 7.45 (1H, m, NH ala); 7.88, 7.71 (1H, m, NH val).

MS m/e M^+ (%) $C_{35}H_{63}N_5O_8$ (681) 726 (M^++2Na)(24.8), 704(M^++Na)(100), 682 (M^++H)(2.9), 626(16), 604(17.7), 508(12.7), 341(10.3), 215(39.5), 198(23.6), 149(21.7), 85(10.3), 71(21.1), 57(40.1).

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