

**SYNTHESIS, STRUCTURAL ELUCIDATION
AND BIOLOGICAL EXAMINATION OF
NOVEL LIPOPHILIC DRUG CONJUGATES**

by

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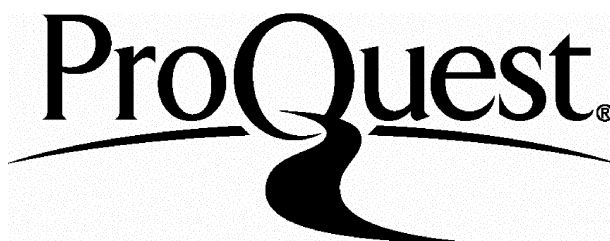
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Abstract.

The lipidic amino acids 33a-u and their homo-oligomers, the lipidic peptides 33v-au represent a class of compounds that are highly lipophilic, but show polar and conformational behaviour characteristics of amino acids and peptides. A series of lipidic amino acids/peptides was synthesised with a varying degree of lipophilicity due to modifications in the length of the alkyl side chain and/or the lipidic amino acid unit number.

Carboxyl protected lipidic amino acid 33r was conjugated to appropriately protected amino acids furnishing a series of dipeptides 53a-t as diastereomeric mixtures. Separation and structure-retention relationships of 16 dipeptides 53b-g and 53j-s were determined on reversed phase stationary phases using Spherisorb ODS and Supercosil™ LC-ABZ stationary phases.

Lipidic amino acids 33p, r, and oligomer af were coupled to highly lipophilic compounds furnishing conjugates 54a, d, c, f, g, h, i and k. After carbonyl deprotection, acids 54b, e and j were obtained. Physio-chemical investigations of the conjugates were undertaken using proton nuclear magnetic resonance. Plots of chemical shift versus concentration revealed the formation of aggregates or miscelles at high concentrations and monomers at low concentrations.

The cellular uptake of lipidic amino acid 33r and oligomers 33y, al, au were studied using *Ehrlich ascites* tumour cells to establish uptake by transformed mammalian cells. Cellular uptake of 33r was greater than the oligomers 33y, al and au.

Representative lipidic amino acids/peptides were conjugated to drug molecules with the aim of facilitating their passage across cell membranes resulting in an increase in uptake and/or modification of actions.

Lipidic amino acids and peptides were utilised in the synthesis of a series of chlorambucil conjugates 56a-f, 57a-e and 58a-b of varying lipophilicity and conjugate linkage. *In vitro* toxicity assessments were performed on conjugates 56a, b, c, e, 57a-c and 58a to examine and compare their toxicity towards ADJ/PC6 plasmacytoma cells. The lipophilicity and conjugate linkage had an effect on the toxicity of the compounds.

A series of benzoquinolizine lipidic amino acid/peptide conjugates 62a-f were synthesised. Initial *in vitro* experiments using *Caco-2* cell cultures were carried out, followed by *in vivo* oral absorption studies to investigate the preferred lipophilicity for optimal uptake of the conjugates. The conjugates showed varying degrees of uptake, all showing higher uptake than the parent.

The developement of an attractive synthetic pathway to produce 3,4-disubstitutedbenzyl cyanoprop-2-enoyl lipidic amino acid conjugates **72a-c**, **73a-f** and **67a-b** was undertaken with the aim of producing a membrane active signal transduction inhibitor.

Synthesis of BOC-aminotetradecanoyl-L-cysteine adenosine monophosphorodisulphide **80** was undertaken to demonstrate the feasibility of employing the lipidic amino acid system in the delivery of antisense oligonucleotides.

In summary, the lipidic amino acid system may be incorporated into a wide variety of drugs and peptides to enhance the passage of the pharmacologically active compounds across biological membranes and/or modify their biological stability and actions.

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Publications and Communications.

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Abbreviations.

Log P	- logarithmic value of the octanol/water partition coefficient.
pK _a	- dissociation constant.
PAAM	- phenylacetic acid mustard.
DeHCHL	- 3,4 dihydrochlorambucil.
DeC-PAAM	- monochloroethylated phenylacetic mustard.
DNA	- deoxyribonucleic acid.
ADP	- adenosine diphosphate.
RNA	- ribonucleic acid.
DCC	- dicyclohexylcarbodiimide.
EADC	- 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.
DCU	- N,N'-dicyclohexylurea.
HOBt	- 1-hydroxybenzotriazole.
BOC	- tertiary butoxycarbonyl.
TFA	- trifluoroacetic acid.
k'	- capacity ratio.
CMC	- critical micelle concentration.
DMF	- dimethylformamide.
Papp	- apparent permeability coefficients.
EATC	- <i>Ehrlich ascities</i> tumour cells.
DMEM	- Dulbecco's modification of eagles medium.
FCS	- foetal calf serum.

Chapter 1. Introduction.

1.1. Introduction.

In the past, medicinal chemists relied a great deal on empiricism in the discovery of new drugs. However, with the extension in knowledge of biological systems and an understanding of the physio-chemical properties of drug molecules which influence their absorption from the site of administration to the site of action, distribution in tissues, metabolism and excretion, a more rational approach to drug design is possible. Ideally a drug should be an active compound that can be easily formulated and which upon appropriate administration may reach its site of action at therapeutic concentrations to bring about the required pharmacological response, with absence of toxicity and side effects. Over the past three decades there has been increasing interest in the development and design of new drug delivery systems to optimise the usefulness of established therapeutic, diagnostic and preventative medicines. This dissertation examines some aspects relating to the effective delivery of medicinal drugs and explores the use of a group of peptides based on the lipidic amino acids to overcome some of the problems encountered.

1.2. Drug absorption.

It has been recognised that a drug may have high intrinsic activity but does not exhibit biological effects because its physio-chemical characteristics do not permit it to reach its site of action. Knowledge of the nature of biological membranes and their permeation by drugs is a great asset in understanding the absorption, distribution and excretion of drug molecules.

Cell membranes are barriers constructed almost entirely of proteins which serve as enzymes and lipids which provide the gross structural properties of the membrane. The concept of the cell membrane as a bimolecular lipid film originated from the work of Gorter and Grendel (1925), and was elaborated by Davison and Danielli (1935) who proposed that the basic cell membrane consists of a continuous lipid bilayer with absorbed protein films on either side. This biological membrane model was then refined by Robertson (1960) who put forward his "unit membrane" theory, that all cell membranes have a particular basic unit structure centred on a continuous lipid film. An alternate theory by Green and co-workers (1961, 1972) proposed that the membranes are made up of lipoprotein subunits with lipid molecules bonded to the protein by non-polar bonds. The structure of cell membranes has also been postulated to include

aqueous filled pores or channels, lined with fixed charges through which small polar molecules or ions may pass (Smyth 1964). Singer and Nicholson (1972) concluded that the only membrane model consistent with all the experimental data is a mosaic structure consisting of alternating globular proteins and phospholipid bilayers.

The majority of drug molecules cross biological membranes by passive diffusion from a region of high concentration (eg. the gastrointestinal tract) to a region of low concentration (eg. the circulatory system). The rate of diffusion depends upon the magnitude of the concentration gradient across the membrane and is a first order process (Zimmerman and Feldman 1990).

Lipid solubility is an important physical property governing the penetration of most drug molecules across membrane barriers of the skin, gastrointestinal tract, central nervous system, etc. and there is a relationship between the partition co-efficient of a drug and its ability to be absorbed in lipid. Experiments conducted (Höber and Höber 1937) have shown good agreement between the percentage of drug absorbed and the chloroform/water partition co-efficient of drugs. On the whole, most drugs are either weakly acidic or weakly basic and exist in equilibrium between unionised and ionised forms. The degree of ionisation is dependent upon the dissociation constant of the drug (pK_a) and the pH of the local environment. Unionised molecules have greater lipid solubility and can penetrate biological membranes more readily than the ionised form. Weak acids would largely be in the unionised form in acidic conditions ie. at low pH (eg. in the stomach) and would penetrate cell membranes. In an alkaline environment ie. high pH (eg. lower intestine) the drug would be ionised and passage across cell membranes would be inhibited. The interrelationship of dissociation constant and lipid solubility, pH at the local environment, and absorption characteristics of various compounds is known as the *pH-partition* theory (Shore *et al.* 1957). There are inconsistencies with the pH-partition hypothesis due to oversimplification, and more elaborate descriptions of drug absorption have been reported (Taylor *et al.* 1985).

Along with diffusion there are other ways by which drug molecules may cross biological membranes. Aqueous pores allow the passage of small polar molecules or ions (Smyth 1964). Pinocytosis may have a role in the uptake of small amounts of proteins and macromolecules. Many drugs are carried across biological membranes by active transport systems. These systems are responsible for the passage of lipid insoluble substances eg. amino acids and sugars into cells across a concentration gradient resulting in high intracellular concentrations. There is specificity for certain molecular structures, and thus drugs may compete for a carrier if

there is similarity in its structure and the normal transported molecule (Schanker and Jeffrey 1961, 1962).

A drug must pass across at least one, if not several, biological barriers to enable access to its site of action within a cell. This process i.e. absorption from the site of administration, plays a crucial role in the overall actions and efficiency of the drug. The process of absorption may be influenced by modification of the drug in one of three ways:

(1) Mode of administration.

The route of administration, time of administration as well as the time interval between doses can effect the response to a drug.

(2) Physical modification.

Usually a change in dosage form from which the drug is formulated.

(3) Chemical modification.

Alterations in the chemical structure to enhance or hinder biological effect.

1.3. Routes of administration.

With the exception of drugs that are injected intravascularly, or directly to the site of action, absorption from the site of administration is crucial if a drug is to attain entry into the bloodstream and reach its site of action at therapeutic levels. Thus the route of administration is an important factor in controlling the release of drugs.

Although the enteral route is the most frequently used method of administration of pharmaceutical agents, there are instances when an alternative route has to be employed; for example, the inappropriate adsorption of drugs from the gastrointestinal tract, or when patients are unable to consume oral dosage forms due to disability. The following is a list of the routes available for the effective delivery of drugs:-

Gastro-intestinal delivery.

Parenteral administration of injections, infusions and implants.

Nasal drug delivery.

Buccal and sublingual delivery of drugs.

Drug delivery to the respiratory tract.

Transdermal delivery.

Unique methods of drug delivery (intrauterine devices, impregnated bone cement etc.).

The gastro-intestinal tract is the most commonly used route to administer drugs and needs to be discussed briefly as this would be the route of choice for delivering the lipidic drug conjugates prepared in these studies.

1.3.1. Gastro-intestinal delivery.

The gastro-intestinal tract is currently the preferred site of absorption for most therapeutic agents with respect to ease of administration, patient compliance and cost effectiveness. Although there has been an upsurge in the development and use of other methods for drug administration, oral administration still predominates and will continue to do so for the foreseeable future. Factors affecting rate and extent of absorption can be separated into physical and physiological parameters (Table 1.1).

Physical Parameters	Physiological Parameters
solubility	regional and local pH
dissolution	drug binding and
molecular size	complexation
partition coefficient	intestinal permeability
sensitivity to chemical	drug metabolism – lumenal,
hydrolysis	mucosal and hepatic
delivery system	gastric and intestinal
	transit time

Table 1.1. Physical and physiological parameters affecting rate and extent of absorption.

Drugs administered orally may be absorbed from various regions of the gastrointestinal tract. In principle the stomach is not an absorbing organ, however the absorption of weak acids takes place due to the low pH reducing their charge, and thus increasing their absorption (Schanker *et al.* 1957, Hogben *et al.* 1957) the opposite taking place with basic drugs. The principal sites of drug absorption are located in the duodenum, jejunum and ileum. However absorption in the large intestine must not be overlooked as it may be employed as the site of absorption of drugs as with the case of sulphasalazine (Lennard-Jones and Powell-Tuck 1979).

The gastrointestinal tract is lined with the glycoprotein mucin, along with dead cells and cell debris. The mucous layer is highly hydrated and permits the passage of small molecular weight compounds. The mucous overlies the mucosa which consists of three layers: the epithelium, a single layer of columnar cells (which are concerned with absorption) and goblet cells; the lamina propria (which supports the epithelia on its basement membrane) with underlying connective tissue; the muscularis mucosae, consisting of connective tissue and the larger blood vessels. The first stage of the absorption process is the passage of drugs from the luminal fluid onto the mucous layer, the drug then diffuses through the mucous and enters the epithelium cells and exits the opposite side to enter the fluid of the lamina propria. The drug can then pass into the blood or lymph capillaries to be transported away from the intestine to the site of action.

Although drugs have been administered via the gastrointestinal tract for hundreds of years, only recently have methods of drug delivery been developed to maximise the effectiveness of drugs by increasing their absorption, protection against gastrointestinal fluids and also protecting the gastrointestinal tract from the administered drugs themselves. These developments will be discussed later.

1.4. Methods for improving drug delivery.

Techniques employed for improving drug delivery may be divided into physical and chemical methods. With physical methods the medicinal product is unaltered chemically and is usually "enclosed" or "entrapped" within a matrix or on a film, which allow the controlled release of the medicine. Other physical methods include the use of penetration enhancers. Chemical methods require chemical manipulation of the medicinal compound to modify its chemical properties to allow greater absorption. The chemical modification can be either irreversible, this leads to a new active chemical compound, or reversible, where the intention is to allow the regeneration of the active species at the site of action, i.e. a pro-drug. The following is a brief introduction to some physical and chemical methods employed to enhance the delivery and absorption of medicinal compounds.

1.5. Physical methods in drug delivery.

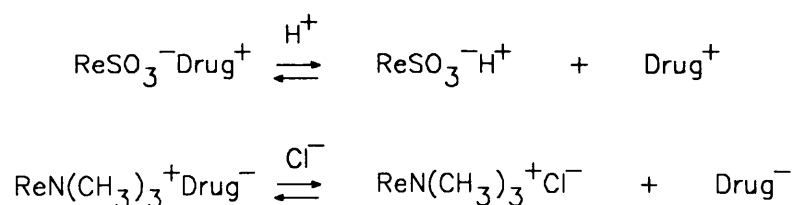
1.5.1. Bioadhesive polymers.

The majority of bioadhesive polymers studied for drug delivery adhere to epithelial tissues and to the mucous coat on the surface of the tissues, this is referred to as "mucoadhesion". Targeted mucosal tissues include the eye, mouth, gastrointestinal tract, nasal area, rectum, urinary tract and vagina. Bioadhesive polymers must be non-toxic, non-adsorbable, possess sufficient adherence to attachment sites and must release drug at a controlled rate. Hydrophobicity plays an important role in bioadhesion along with the molecular weight and chain length of the polymer. Natural and synthetic bioadhesive polymers that can adhere to hard or soft tissues have been used for many years in dentistry, orthopedics, ophthalmology and in surgical procedures.

1.5.2. Ion-exchange resin delivery systems.

Ion exchange resins have been defined as high molecular weight water insoluble polymers containing fixed positively or negatively charged functional groups in their matrix, which have an affinity for oppositely charged counter ions. Since most drugs possess a charge, there is the potential to attach drugs reversibly to the insoluble polymer, which may be an anion or cation exchange resin (Dofner 1972).

Drug release or dissociation may only occur through the displacement of the drug by another ion with the same charge since the exchange is an equilibrium process. The release is not instantaneous as the drug has to diffuse through the resin from the internal exchange sites.



In the stomach the latter occurs slowly, whereas the following occurs rapidly:



Complete dissolution will not occur due to equilibrium, however complete bioavailability can be assured by appropriate loading of the resin with drug. The insoluble resin will not be absorbed and will be eliminated in the faeces. Unless the resin is coated the ion exchange process initiates within seconds of ingestion, and the drive towards equilibrium will be very rapid. Resins, therefore, are often microencapsulated with an acid insoluble enteric coat to delay the ion exchange process. Ion exchange has been employed in the delivery of several drugs, for example dextromorphan, biphetamine and nicotine (Raghunathan *et al.* 1981, Ritschel, W.A. 1984, Jung *et al.* 1983).

1.5.3. Microparticulate delivery.

Several types of microparticulate carriers have been developed in recent years with most attention being shown to liposomes, microspheres and emulsions. Initial studies with a wide range of drugs revealed that generally these drug carriers have to be administered parenterally. Upon administration there are two major barriers to the distribution of microparticles *in vivo*; the endothelial barrier of the vasculature and phagocytic cells of the reticulo endothelial system.

Macromolecules up to a diameter of several hundred angstroms may cross the endothelial barrier by a process known as "trancytosis" (Simionescu and Simionescu 1983). Larger particles such as liposomes, most microspheres and emulsion particles remain impacted upon the luminal side of the capillary endothelium or may exit from the circulation via fenestrated sites situated along the sinusoidal vessels of the liver and spleen (Freudenberg *et al.* 1983).

The macrophages of the reticulo-endothelial system are an essential part of the defence functions of the body as they remove and engulf circulating pathogens, tissue debris and damaged macromolecules from the circulation (Altura and Saba 1981). Macrophages in the liver (Kupffer cells) and the splenic marophages readily engulf a variety of microparticles including

liposomes (Kao and Juliano 1981), microspheres (Arturson *et al.* 1983) as well as other colloidal particles (Altura and Saba 1981). Thus due to the endothelial fenestrations and abundance of macrophages (Roerdink *et al.* 1984) most of the injected microparticles will accumulate in organs such as liver and spleen.

1.5.3.1. Liposomes.

Liposomes are microscopic structures with internal aqueous compartments bounded by one or more concentric phospholipid bilayer membranes (Bangham 1972) and can be considered to be synthetic relatives of cell membranes (Gruner 1987). In 1972, Gregoriadis and Ryman proposed the use of liposomes as carriers of enzymes in the treatment of lysosomal storage diseases. Since that time the application of liposomes as a potential controlled drug delivery system has gained wide interest (Gregoriadis and Ryman 1972). Ampiphilic compounds such as sterols, sphingolipids, long chain fatty acids (Juliano and Layton 1980, Juliano 1983) may be used in conjunction with glycerophospholipids to make liposomes. The charge, stability, chemical reactivity and biological properties of the liposome preparation can be modified by combination of the chemical constituents. Along with encapsulating hydrophilic drugs in the aqueous core of liposomes, it is also possible to incorporate lipophilic agents into the phospholipid bilayer. The uptake of liposomes into cells takes place by endocytosis whereupon they end up in lysosomes where they are degraded by lysosomal (phospho) lipases, releasing the encapsulated drug contents (Dijkstra *et al.* 1984, 1985).

Interest has been generated in the use of liposomes as a carrier system for the selective delivery of drugs to cells of the reticuloepithelial system (Kramer 1974, Longo *et al.* 1982), and as carriers of antigens and adjuvants (Widder *et al.* 1982).

1.5.3.2. Microspheres.

The term microsphere can be used to describe small particles intended as carriers for drugs or other therapeutic agents; they are particles ranging from 100nm to 1µm that are usually solid monolithic devices in nature which may be prepared by one of several methods from proteins or polymers. Interest in their use as drug carriers is growing with several types having been developed. Albumen microspheres (Kramer 1974), protein microspheres coated with

polymers (Longo *et al.* 1982), acrylic microspheres (Arturson *et al.* 1983, Edman and Sjöholm 1981) and magnetically responsive albumen microspheres (Widder *et al.* 1982). Microcapsules are similar to microspheres but comprise of small spheres that have an outer layer or membrane entrapping a core material, usually the drug (Tomlinson 1983). They are normally larger than microspheres and are more restricted by routes of administration. The main advantage of microspheres over liposomes is their larger drug loading capacity (up to 10%), greater stability and ease of sterilisation.

Emulsions (Davis and Illum 1986) or "lipid microspheres" are different from solid microspheres as they are a dispersion of one liquid in another, stabilised by an emulsifying agent. They have common features governing the behaviour of all colloidal carriers.

1.5.4. Iontophoresis.

It has been reported that the skin has low levels of proteolytic enzymes (Pannatier *et al.* 1978) this would be advantageous for the delivery of peptide and protein drugs, providing a method to allow effective permeation through the skin barrier could be found. A method for achieving this goal is iontophoresis which uses an electric current to deliver ions and charged molecules across the skin. The enhancement of transdermal permeation of drugs under iontophoresis may be due to several factors. The molecular arrangement of skin components may be altered due to the applied electric potential resulting in changes in skin permeability (Jung *et al.* 1983), electro-osmotic flow, ionic conduction along with solute-solvent and solute-solute coupling (Chein *et al.* 1989, Wearley *et al.* 1989). The rate of iontophoretic delivery of drugs depends upon physiochemical properties of the formulation (Chein *et al.* 1989), and the electronic variables of the device itself. There has been some success using iontophoresis to deliver both peptides (Chein *et al.* 1987, Wearley *et al.* 1989) and non-peptides (Sanderson *et al.* 1989).

1.6. Chemical methods in drug delivery-Prodrugs.

Ideally a prodrug should be an inactive species which liberates the active drug species at the target site in sufficient quantities to elicit the desired pharmaceutical response. The prodrug form will protect the non-targeted cells from the active drug species and/or protect the drug until it reaches its destination at the targeted cells.

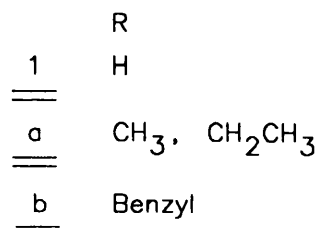
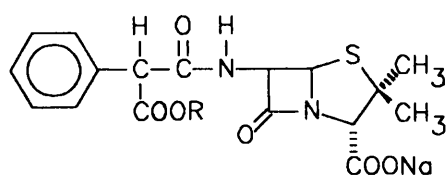
The site and rate of drug release will depend upon the specific delivery problems which are to be overcome by the prodrug design. Prodrugs have been designed to liberate the parent drug instantaneously to overcome formulation problems in intravenous preparations. Alternatively prodrugs with a slow rate of conversion to parent drug have been developed to produce sustained drug actions.

In the rational design of prodrugs, one has to consider the structural modifications necessary to eliminate the undesirable effects possessed by the parent molecule and what conditions are available *in vivo* to regenerate the parent molecule. Conversion or activation of prodrugs to the parent drug molecule can take place by a variety of reactions such as hydrolysis (Jansell and Russell 1965), reduction (Duggan *et al.* 1981) and oxidations (Shek *et al.* 1976) which may be catalysed by enzymes (Bauguess *et al.* 1975) or are pH dependent (Bundgaard and Johansen 1980).

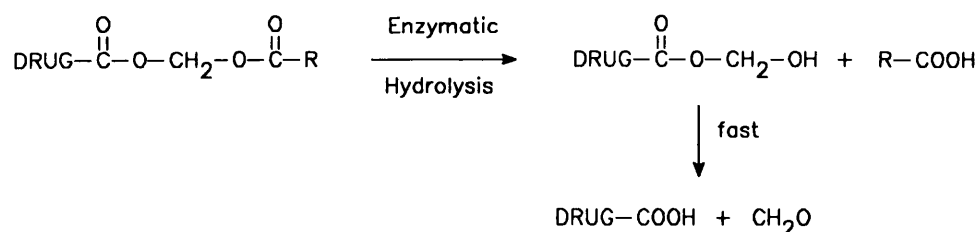
1.6.1. Esters.

The attractiveness of esters as prodrug linkages for drugs stems from the fact that the organism is rich in esterases. By appropriate esterification of parent drug molecules containing carbonyl, hydroxyl, thiol or phosphate groups it is possible to create derivatives with a range of hydrophilicities/lipophilicities and *in vivo* lability. Many examples of the use of esters can be found in the literature, some of which are summarised below.

The bioavailability of the β -lactam antibiotics is poor. Esters have been developed to improve the bioavailability problem with varying success. Alkyl (**1a**) and aryl (**1b**) esters of carbenicillin (**1**) are effectively absorbed by the oral route, and are probably hydrolysed *in vivo* furnishing the parent, however the esters exhibit antibiotic activity of their own (Clayton *et al.* 1975).

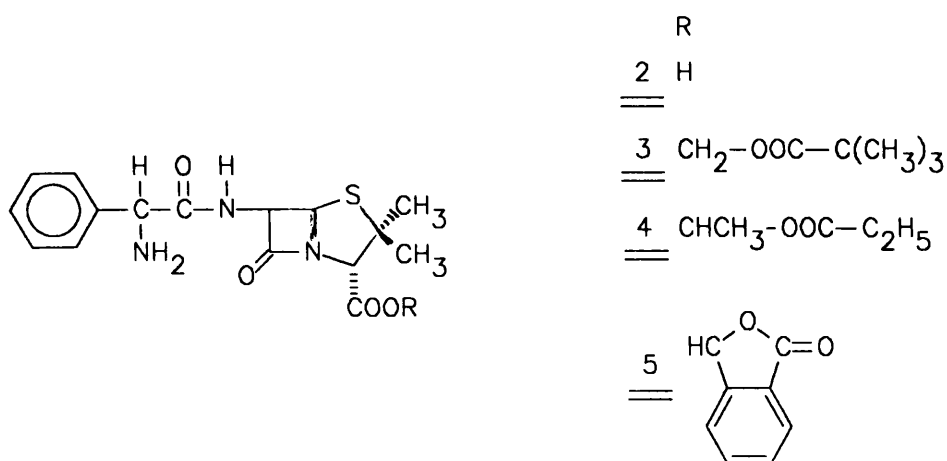


It was recognised that some simple aliphatic or aromatic esters were not sufficiently labile *in vivo* to ensure a sufficiently high rate and extent of prodrug conversion, as in the case with penicillin esters. Jansen and Russell (1965) solved the problem when they showed that a double ester (acyloxymethyl ester) of benzylpenicillin was hydrolysed in the blood and tissue of several species. The relatively sterically unhindered double esters are susceptible to enzymic hydrolysis of the terminal ester bond with formation of the unstable hydroxymethyl ester which spontaneously dissociates to the parent penicillin and formaldehyde (Scheme 1).

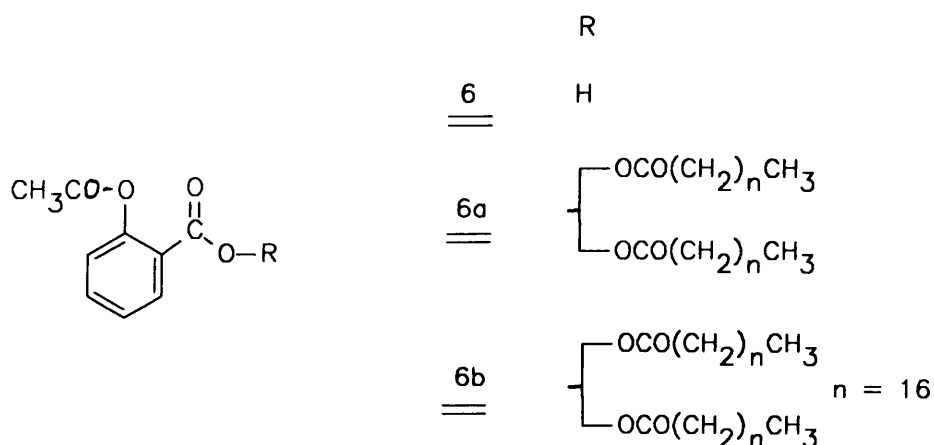


Scheme 1. Hydrolysis of Double Esters.

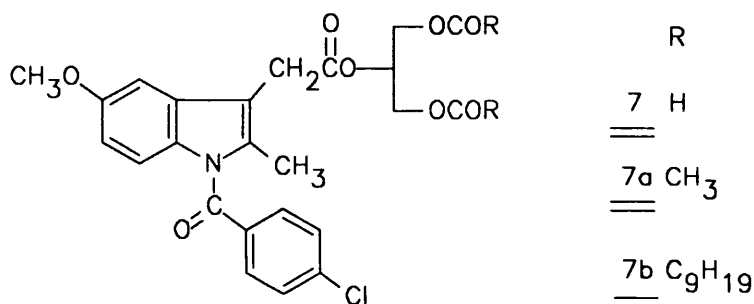
Successful double ester prodrugs of ampicillin (**2**) are used clinically including pivampicillin (**3**) (Daehne 1970), talampicillin (**4**) (Clayton *et al.* 1974) and bacampicillin (**5**) (Bodin *et al.* 1975).



The double ester approach has also been used to prepare prodrugs of non steroidal anti-inflammatory drugs, such as aspirin (**6**) and indomethacin (**7**). Aspirin is an effective analgesic anti-inflammatory agent, however it causes gastrointestinal bleeding in a high percentage of patients (Iuchi 1980). Triglyceride "prodrugs of aspirin" (**6a** and **6b**) have been synthesised and

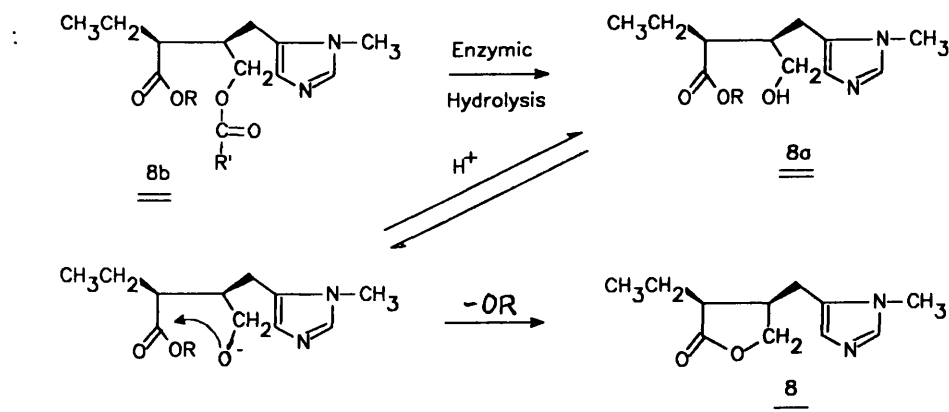


biological examination revealed that they possess essentially all the systemic activity of aspirin without producing gastric lesions, although the evidence is insufficient to show if the triglyceride derivatives are hydrolysed to aspirin, salicylic acid or both. The 2-triglycerides of indomethacin (**7a,7b**) showed an improvement in therapeutic index over the parent (Loftsson *et al.* 1981).

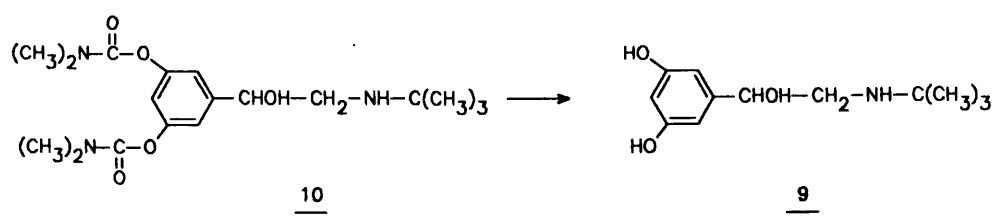


Bundgaard and Larsen (1980a) reported the potential use of esters as prodrug forms for the γ -lactone moiety which appears in several drugs such as pilocarpine (**8**). The ocular delivery of pilocarpine is very low due to poor corneal penetration, resulting from its low lipophilicity (Patton 1977, Lee and Robinson 1979). A series of alkyl and aralkyl esters of pilocarpic acid (**8a**) have been prepared which undergo lactonisation to produce pilocarpine. Although the pilocarpic acid esters being more lipophilic result in improved ocular bioavailability their solution stability is poor

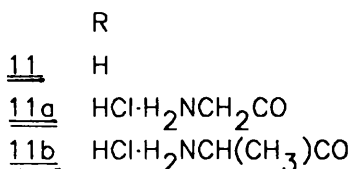
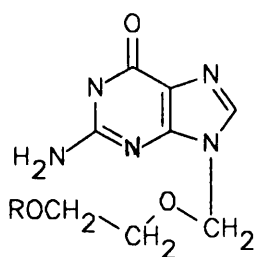
(Bundgaard *et al.* 1985). The problem was overcome by forming double esters (**8b**), along with solving the stability problem the derivatives showed enhanced absorption and longer therapeutic activity than the mono esters (**8a**) (Bundgaard *et al.* 1984). The double esters undergo hydrolysis of the o-acyl bond forming the mono ester, which upon spontaneous lactonization furnishes pilocarpine (Scheme 2).



Carbamate esters may prove useful for the delivery of phenolic drugs. N-Unsubstituted or N-monosubstituted carbamates derived from phenols were shown to be labile whereas the N-disubstituted carbamates proved highly stable (Digenis and Swintosky 1975). Olsson and Svensson (1984) showed that the bis-N,N-dimethylcarbamate derivative (**10**) of terbutiline (**9**) produced sustained blood levels after a single dose.

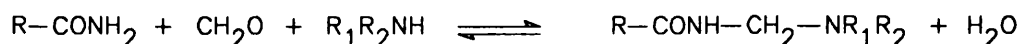


Esters are also used as an efficient means of increasing the aqueous solubility of drugs containing hydroxyl groups to allow their use for parenteral administration. The most commonly used esters for increasing the aqueous solubility of alcoholic drugs are hemisuccinates (Hegarty and Frost 1973), phosphates (Cho *et al.* 1982) and amino acids (Bundgaard *et al.* 1984a, 1984b). Amino acid derivatives (**11a,b**) of the antiviral drug acyclovir (**11**) (Colla *et al.* 1983) have been prepared with sufficient aqueous solubility to allow administration by injection.



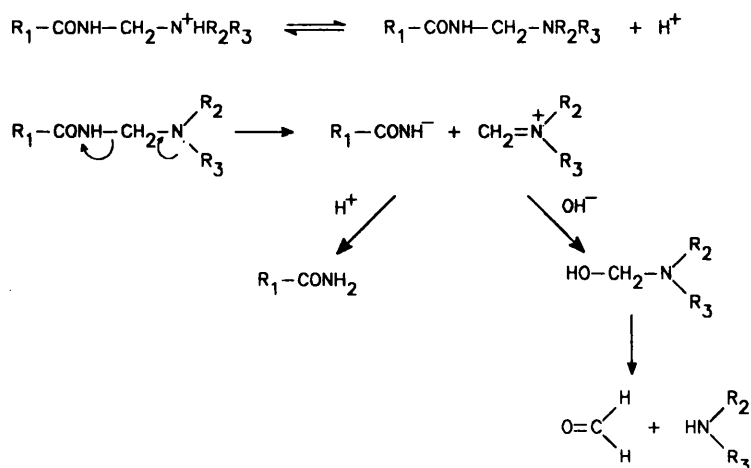
1.6.2. N-Mannich bases.

N-Mannich bases may be useful candidates for prodrug development of NH acidic compounds such as amides, imines, carbamates and hydantoins. The N-Mannich bases are formed by reacting a NH acidic compound with formaldehyde (usually) and a primary or secondary aliphatic/aromatic amine (Scheme 3).



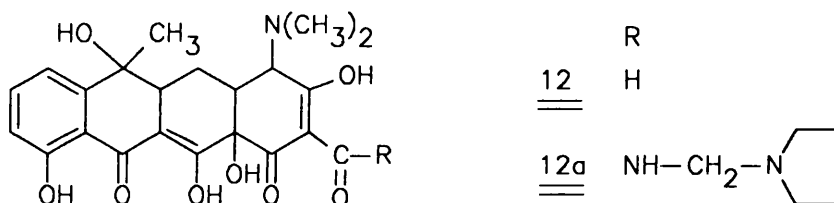
Scheme 3. N-Mannich Base Formation.

The breakdown of N-Mannich bases does not rely upon enzymatic catalysis but occurs through spontaneous decomposition. The proposed reaction mechanism (Bundgaard and Johansen 1980a, 1980b) involves an unimolecular N-C bond cleavage with formation of an amide (or imide) anion and an immonium cation. In subsequent fast steps, a solvent molecule transfers a proton to the anion and a hydroxide ion to the immonium ion, giving methylolamine, which rapidly dissociates to formaldehyde and amine (Scheme 4).



Scheme 4. Mechanism of N-Mannich Base Decomposition.

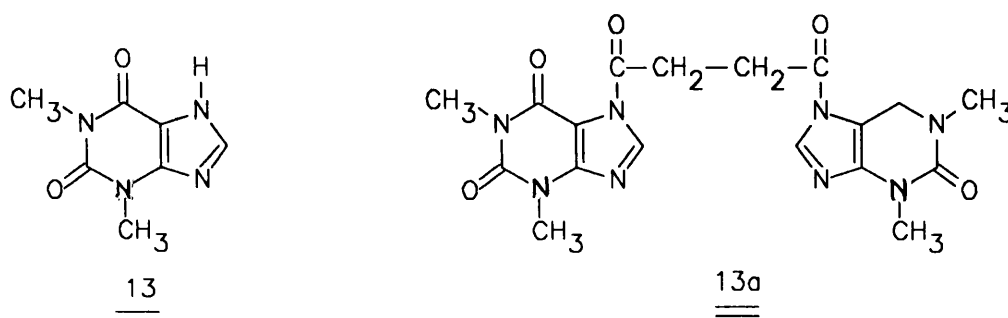
Prodrugs of a given amide-type drug may be prepared with varying lability, solubility, lipophilicity, dissolution rate, etc. by selection of an appropriate amine component. The N-Mannich base approach has been used to prepare rolitetracycline (**12a**) a more soluble derivative of tetracycline (**12**).



N-Mannich bases may also be used to form prodrugs of primary and secondary amines. The N-Mannich base may be used to increase the lipophilicity of the parent amine at physiological pH values by depressing protonation, thus allowing increase biomembrane-passage properties. However, the selection of biologically acceptable amide-type carrier groups is restricted. Salicylamide has been shown to produce N-Mannich bases of various amines with acceptable cleavage rates (Johansen and Bundgaard 1980).

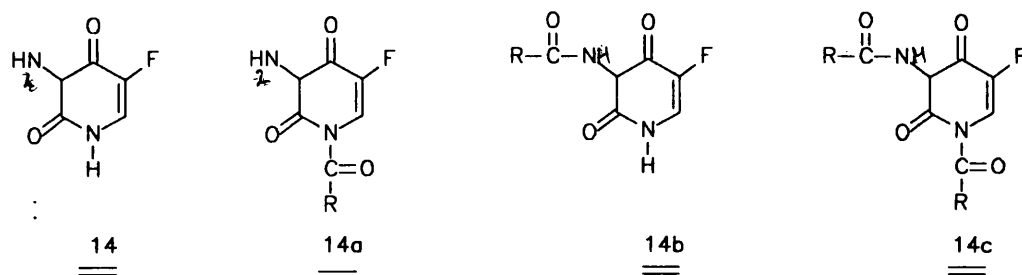
1.6.3. N-Acyl derivatives.

N-acylation of amide, imide and amines may be a useful approach for prodrug synthesis, although N-acylation of amines to form amide prodrugs is limited due to the relative stability of the amides formed. 7,7'-Succinlyditheophylline (**13a**) is an activated amide of theophylline (**13**) and has been developed as a sustained release prodrug of theophylline (Bansal *et al.* 1981).

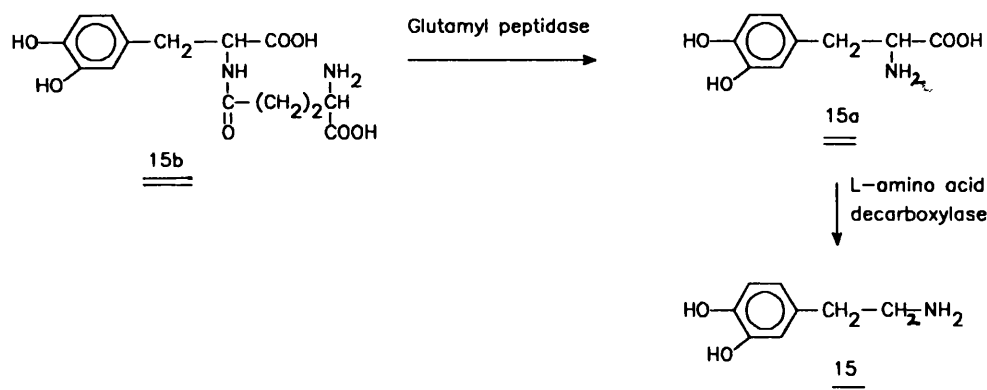


Buur and Bundgaard (1984, 1985) acylated 5-fluorouracil (**14**) with the aim of improving its absorption characteristics. Various N₁-acyl (**14a**), N₃-acyl (**14b**) and N₁,N₃-diacyl derivatives

(**14c**) were shown undergo hydrolysis, the rate being controllable by appropriate selection of the acyl group.



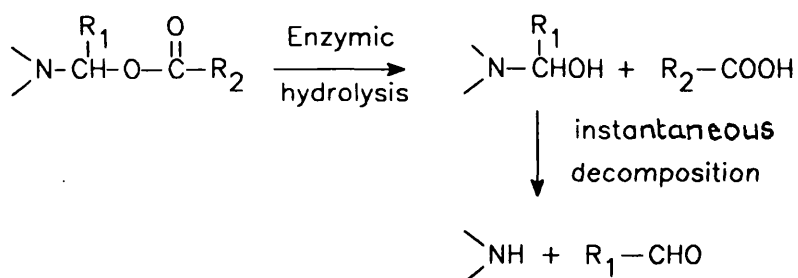
The kidney has a high concentration of γ -glutamyl transpeptidase that is capable of cleaving γ -glutamyl derivatives of amino acids and related compounds. γ -Glutamyl derivatives of dopamine (**15**) and L-dopa (**15a**) have been produced as kidney specific prodrugs (Wilk *et al.* 1978, Kyncl *et al.* 1979). The γ -glutamyl-L-dopa derivative (**15b**) is initially converted to L-dopa by γ -glutamyl peptidase, L-dopa is then decarboxylated by L-amino-acid decarboxylase furnishing dopamine. As the two enzymes are highly concentrated in the kidney compared to other tissues, systemic adrenergic effects are negligible (Scheme 5).



Scheme 5. Enzymic Cleavage of γ -Glutamyl-L-Dopa Derivative.

1.6.4. N-Acyloxyalkyl derivatives.

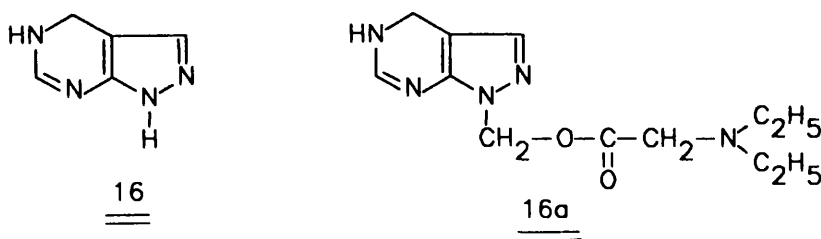
N-acyloxyalkylation is commonly used to obtain prodrugs of amides, imides, hydantoins and other NH acidic compounds. By varying the acyl moiety physiochemical properties can be manipulated to create appropriate lipophilicity, solubility and lability. They are cleaved *in vivo* by



Scheme 6. Enzyme Hydrolysis and Decomposition of N-Acyloxyalkyl Prodrug Derivatives.

enzyme-mediated hydrolysis of the ester grouping forming the N-hydroxyalkyl derivative, which is thought to decompose instantaneously into the corresponding aldehyde and parent NH-acidic drug (Scheme 6).

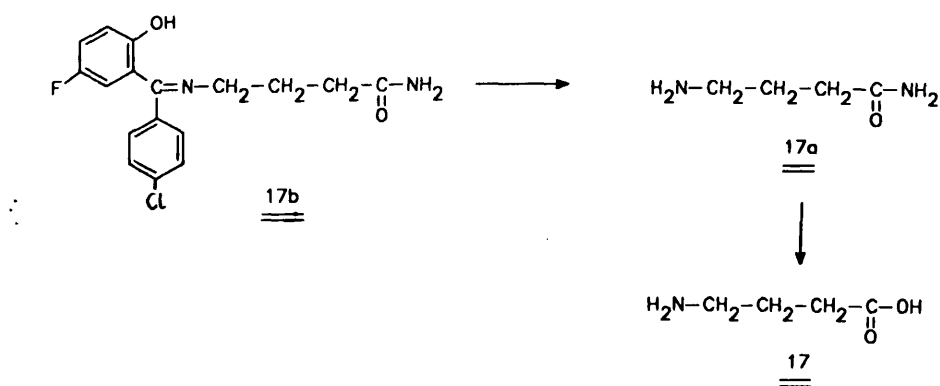
N-acyloxyalkylation has been used to increase the oral absorption of phenytoin (Yamaoka *et al.* 1983) and the dermal delivery of theophylline (Sloan *et al.* 1983) as derivatisation imparts increased lipophilicity to the parent drug. The N-acyloxyalkylated derivative **16a** of allopurinol **16** has an increased lipophilicity and water solubility due to a decrease in intermolecular hydrogen bonding (Prusiner and Sundaralingam 1972).



1.6.5. Schiff bases.

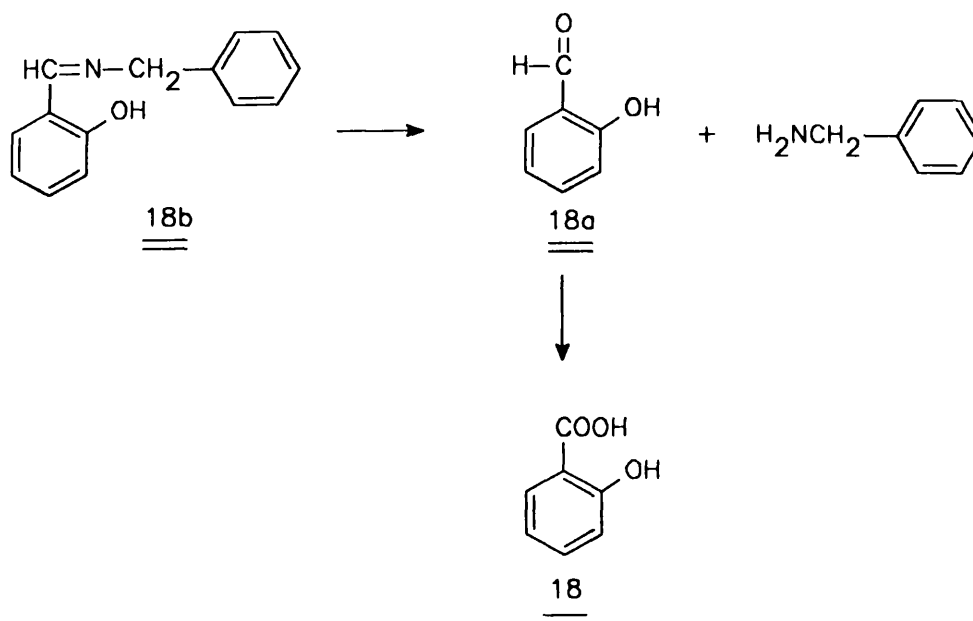
Schiff bases (imines) have been utilised to create prodrugs of selected amines and carboxyl compounds. Imine formation of amines decreases the basic character and therefore increases lipophilicity and hence increases membrane penetrating ability. This has been used to facilitate the passage of γ -aminobutyric acid (**17**) across the blood brain barrier. Progabide (**17b**) is the schiff base prodrug form of the amide derivative of γ -aminobutyric acid (**17a**). Due to the lipophilicity it passes the blood brain barrier, where upon the imine and amide bonds are cleaved releasing γ -amino- butyric acid in the brain (Kaplan *et al.* 1980) (Scheme 8). Progabide

is not a "true prodrug" as it has some pharmacological effects itself, eg. anticonvulsant (Worms *et al.* 1982).



Scheme 7. Hydrolysis of Progabide.

An example of Schiff base formation of a carbonyl containing drug is Saddamine (**18b**), which is a prodrug of salicylic acid (**18**) (Al-Ani *et al.* 1979).

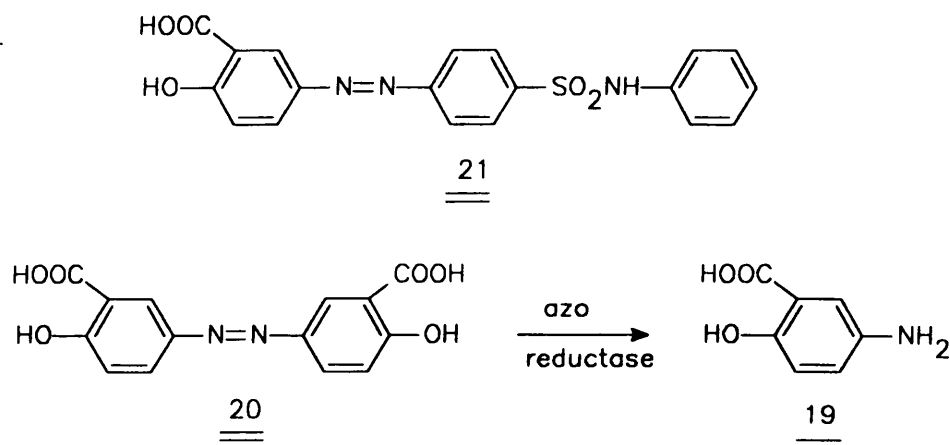


Scheme 8. Hydrolysis of Saddamine.

The Schiff base is probably hydrolysed in the gastrointestinal tract to benzylamine and salicylaldehyde (**18a**), the latter is then oxidised *in vivo* to salicylic acid (**18**) (Scheme 8).

1.6.6. Azo compounds.

The azo linkage has been employed to prepare prodrugs that can be cleaved by azo reductase enzymes, enabling site specific drug release to the lower gastrointestinal tract. Sulphasalazine (**21**) after oral administration is reduced by azo-reductase enzymes of the colonic

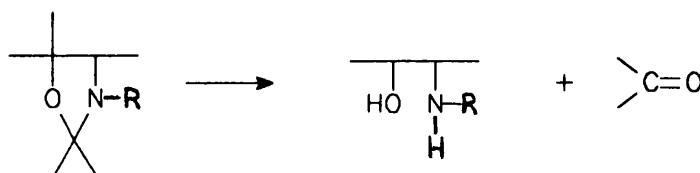


Scheme 9. Reduction of Azodisal Sodium.

bacteria resulting in the release of sulphapyridine and the active drug 5-aminosalicylic acid (**19**) at the target site (colon) in high concentrations. Due to the side effects related to sulphapyridine (Lennard-Jones and Powell-Tuck 1979) other derivatives have been prepared. Azodisal sodium (**20**) overcomes this problem, as azo reduction results in formation of 5-aminosalicylic acid (**19**) only (Willoughby *et al.* 1982) (Scheme 9).

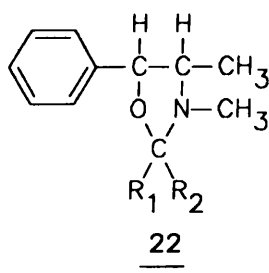
1.6.7. Oxalidines, thiazolidines and imidazolidenones.

Oxazolidines are cyclic condensation products of β -aminoalcohols with aldehydes and ketones and have potential as prodrug candidates for the β -aminoalcohol and carbonyl functional groups (Scheme 10). The β -aminoalcohol group appears in several drugs that exhibit delivery



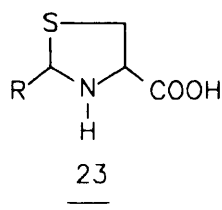
Scheme 10

problems due to unfavourable solubility or lipophilicity, such as the sympathomimetic amines and β -blockers. Several oxazolidine derivatives of ephedrine (**22**) have been prepared and their hydrolysis has been investigated (Johansen and Bundgaard 1983, Bundgaard and Johansen 1982). Oxalidines are weaker bases than the parent β -aminoalcohols and are therefore more lipophilic at physiological pH (Johansen and Bundgaard 1983). By altering the carbonyl moiety it is possible to control the lipophilicity and rate of hydrolysis of oxazolidine derivatives.

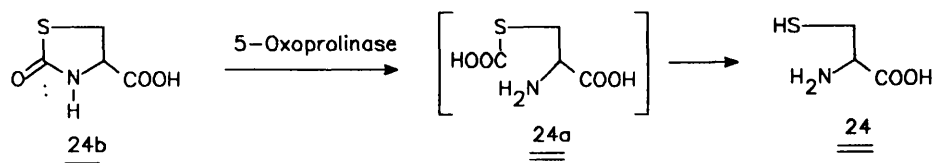


Oxazolidines may be considered as prodrugs for carbonyl drugs that have low oral bioavailability due to formulation problems resulting from their physical properties. Preparation of bioreversible oxazolidine derivatives has been used to increase the bioavailability of benzaldehyde. The oxazoline derivative of benzaldehyde is a crystalline solid that can be formulated easily allowing oral administration. Upon administration it hydrolyses spontaneously releasing the benzaldehyde in the gastrointestinal tract and/or in the blood after absorption (Bundgaard and Johansen 1982).

Thiazolidines may be prepared from β -amino thiols with ketones or aldehydes. Bodor and co-workers (Bodor *et al.* 1982, Bodor and Sloan 1982, Sloan *et al.* 1981) used thiazolines as bioreversible steroidal 3-ketone derivatives to improve dermal delivery of steroidal anti-inflammatory agents. Various 2-substituted thiazolidine-4(R)-carboxylic acids (**23**) have been proposed for the delivery of L-cysteine (**24**) for the protection of paracetamol overdose.

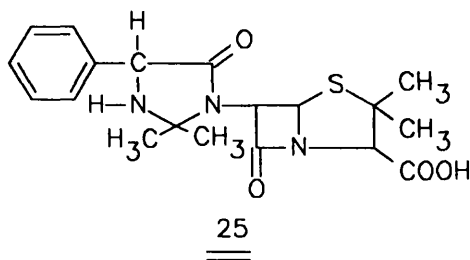


2-Oxothiazoline-4-(R)-carboxylic acid (**24b**) is hydrolysed by the intracellular enzyme 5-oxoprolinase (Williamson and Meister 1981) forming 5-carboxyl-L-cysteine (**24a**) which hydrolyses spontaneously to L-cysteine (**24**) (Scheme 11), which then stimulates the biosynthesis of glutathione resulting in detoxification of paracetamol metabolites.



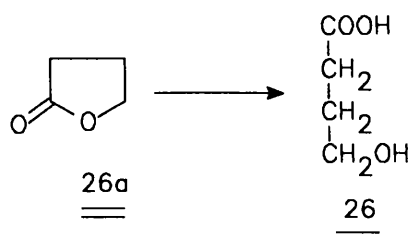
Scheme 11. Hydrolysis of 2-Oxothiazoline-4 (R)-Carboxylic Acid

Klixbüll and Bundgaard (1984) have suggested the use of 4-imidazolidenones as prodrugs for peptides. The imidazolidenones are derived from the action of aldehyde or ketone (usually acetone) on the α -aminoamine of dipeptides. They are much weaker bases than the parent and are therefore much more lipophilic at physiological pH (Klixbüll and Bundgaard 1984) and may be of advantage when low lipophilicity creates delivery problems. Hetacillin (**25**), a condensation product of ampicillin and acetone (Hardcastle *et al.* 1966), is more stable than ampicillin in concentrated solution and is hydrolysed to the parent in aqueous solution (Schwartz and Hayton 1972).



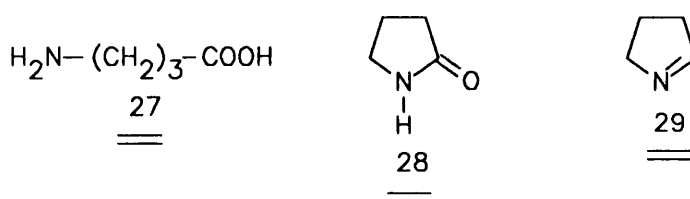
1.6.8. Lactones, lactams and pyrrolines.

Suitable prodrugs for open chain hydroxyacids or their esters may be the cyclic lactone derivatives. γ -Butyrolactone (**26a**), a prodrug of 4-hydroxybutyric acid (**26**) (Guidotti and Ballotti 1970, Gairman and Roth 1964) has a greater lipophilicity and resistance against first pass metabolism. The lactone is absorbed faster and to a greater extent than the parent which is furnished on hydrolysis by γ -lactonase enzyme (Scheme 12) resulting in a prolonged hypnotic effect (Roth and Giarman 1966).



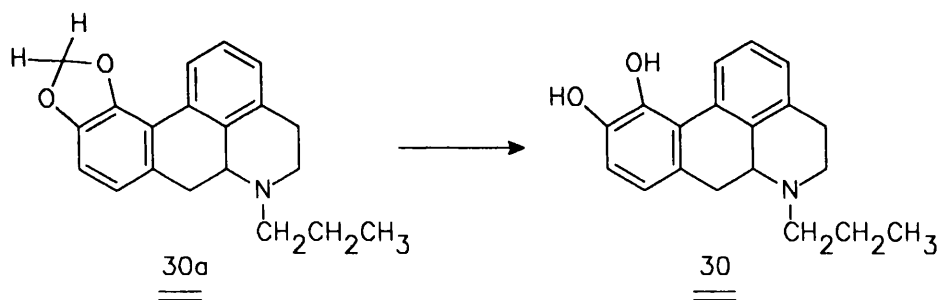
Scheme 12. Hydrolysis of γ -Butyrolactone.

γ -Aminobutyric acid (**27**) has low lipophilicity and is unable to penetrate the blood brain barrier. 2-Pyrrolidinones (**28**) and Δ^1 -pyrrolines (**29**) are brain penetrating prodrug derivatives that are converted enzymically in the brain to form the parent γ -aminobutyric acid.



1.6.9. Cyclic prodrugs for the catechol group.

The dopamine receptor agonist actions of N-*n*-propylnorapomorphine (**30**) are limited by poor oral bioavailability and short duration of action as a result of metabolism of the catechol system. 10,11 Methylenedioxy-N-*n*-propylnorapomorphine (**30a**) is a long acting orally effective prodrug (Baldessarini *et al.* 1977) which is converted to the parent by the action of hepatic microsomal enzymes (Scheme 13).

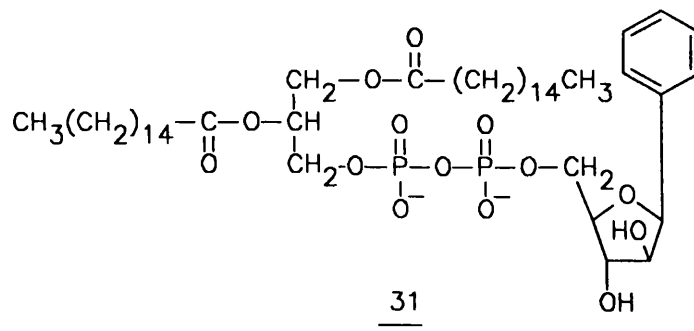


Scheme 13. Hydrolysis of Methylenedioxy Group.

1.6.10. Prodrugs for phosphate groups.

Purine and pyrimidine nucleosides employed for the treatment of viral and neoplastic conditions are normally administered as the nucleoside form, where upon they penetrate the cell and are phosphorylated to the active nucleotides (Montgomery 1982). However, several nucleoside derivatives do not undergo phosphorylation to the active form (Robins and Revankar 1984). Nucleotides are ineffective as chemotherapeutic agents as the highly hydrophilic phosphate group prevents sufficient penetration of cell membranes. The phosphate group is also rapidly dephosphorylated to the parent nucleoside by non-specific serum phosphohydrolases (Leibman and Heidelberger 1955, Roll *et al.* 1956). Considerable effort has been made to develop suitable biologically reversible phosphate protective groups.

Phosphate esters have been prepared to furnish a prodrug with less ionic character and enhanced cellular penetrability. Upon entry to cells, enzymic hydrolysis would release the parent nucleotide. The 5'-diphosphate-L-1,2-dipalmitin phospholipid derivative (**31**) of Ara-C was found to exhibit an increased antileukaemic activity in mice with respect to the parent Ara-C itself (Matsushita *et al.* 1981). Upon cellular uptake it is enzymically cleaved releasing Ara-C-5'monophosphate.



1.6.11. Macromolecules.

The proposal by Ringsdorf (1975) that macromolecules have a potential as drug carriers stimulated widespread attention and research development that has led to many drugs and hormones linked to natural and synthetic macromolecular drug molecules.

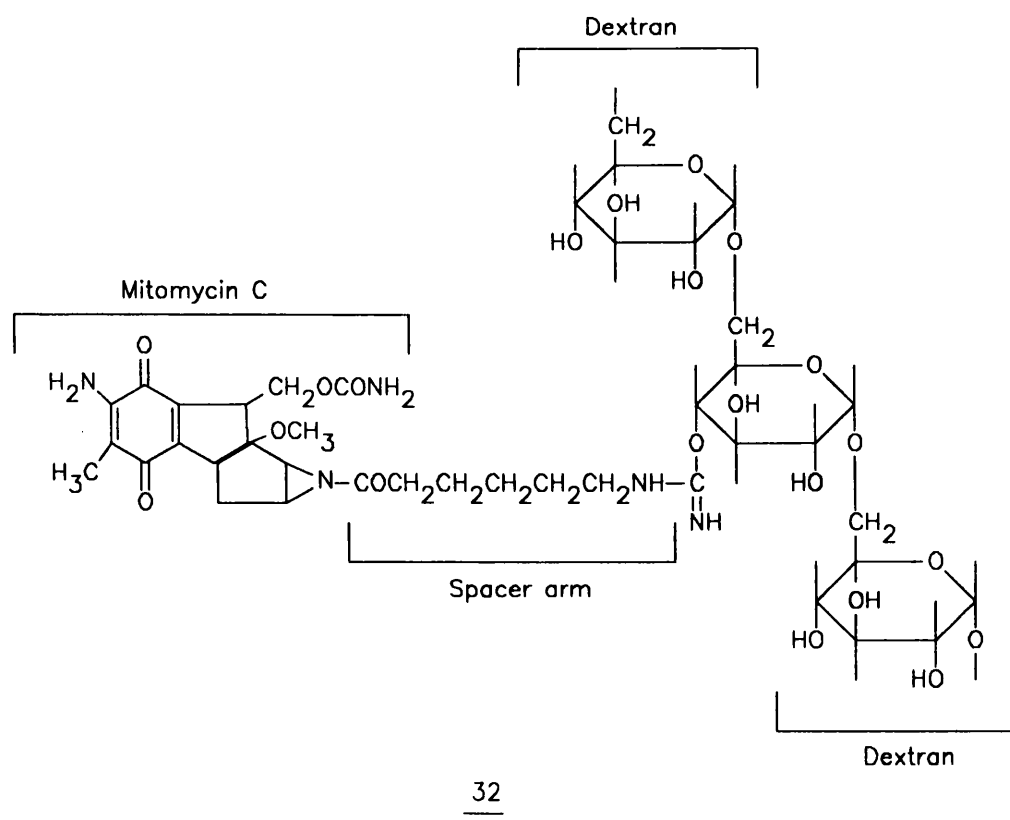
Advantages of macromolecular drug carrier systems over the parent drug molecule include favourable pharmaceutical, pharmacodynamic and pharmacokinetic characteristics.

There are disadvantages, the most important being the occurrence of adverse immunological reactions and retention within the body.

The majority of macromolecular drug conjugates rely upon the release of drug for activity and thus are examples of pro-drugs. Among the macromolecular carriers that have been employed for conjugating include poly (α -L-lysine) (Van Heeswijk *et al.* 1984), insulin (Schacht *et al.* 1984), antibodies (Sezaki and Hashida 1985), nucleic acids (Zaharko *et al.* 1979) and albumen (Trouet *et al.* 1982).

1.6.11.1. Mitomycin C-dextran conjugate.

Mitomycin C-dextran conjugate 32 bears the principle characteristics of macromolecule-drug conjugate systems, i.e. a polymer backbone that has been modified with a spacer molecule to allow attachment of the drug molecule.



The mitomycin C is chemically cleaved from the dextran in the body which is then

degraded. The liberated mitomycin C is then free to bring about its antitumour antibiotic effect (Kojima *et al.* 1980, Takakara *et al.* 1984).

1.6.11.2. Antibodies as carriers for drug delivery.

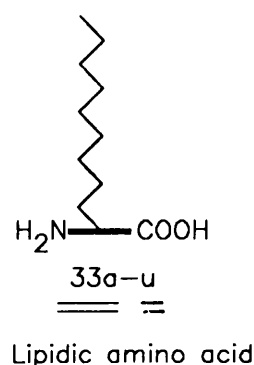
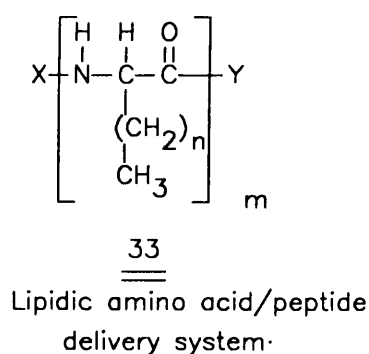
Antibodies were discovered at the beginning of the century (Ehrlich 1956), and there has been enormous advancement in the development of polyclonal and monoclonal antibodies with the majority of research aimed towards the immuno-targeting of antineoplastic drugs to tumour cells in cancer chemotherapy where the greatest need arises for site specific drug delivery.

The suitability of an antibody as a drug carrier relies on its high specificity, high binding affinity and avidity towards the target cell. Antibodies recognise the appropriate target site and thus will increase the local concentration of drug resulting in site-directed killing of the cells, with a lower systemic drug concentration. The major drawback with the use of antibodies is their heterogeneity due to a variation in amino acid sequence resulting in differences in size, charge, antigen specificity and affinity, they may also vary in their ability to couple to drug molecules and their biological half lives may also show variations. Upon coupling a drug to the antibody carrier it is imperative that their respective activities are maintained and the conjugate is stable whilst in transit to the target site (Gallego *et al.* 1984). The advantages of monoclonal antibodies are the result of their homogeneity of affinity which may be divided into (i) data interpretation, (ii) reproducibility of effects and (iii) tailored specificity (Neville 1985). Polyclonal antibodies may still offer advantages as they frequently exhibit higher avidity to the target cells and their antibody activities are less sensitive to drug binding procedures.

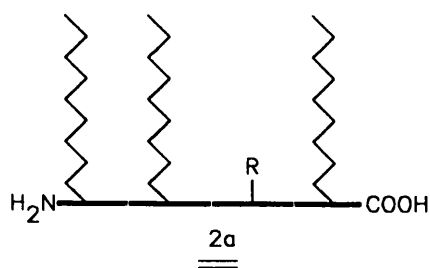
Many anti-cancer drugs have been conjugated to antibodies over the past four decades (Ghose and Nigam 1972, Rowland *et al.* 1977,1975, Hurwitz *et al.* 1975) showing various success. With the rapid development of antibody research and drug conjugating techniques, an antibody-drug system may be produced enabling specific immunochemotherapy in the near future.

1.7. The lipidic amino acid system.

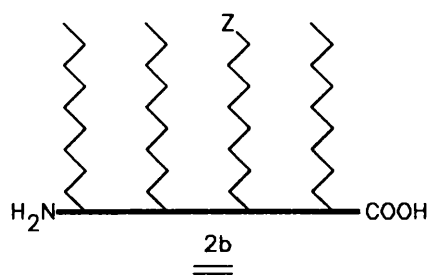
The lipidic amino acids and their homo-oligomers the lipidic peptides, represent a class of compounds which combine structural features of lipids with those of amino acids (Gibbons *et al.* 1990). Of particular interest is their potential use as a drug delivery system (Toth *et al.* 1991). The lipidic amino acids (33a-u, X=H, Y=OH, n=5-17, m=1) may be linked to form the corresponding homo-oligomers, the lipidic peptides (33v-au, m>1).



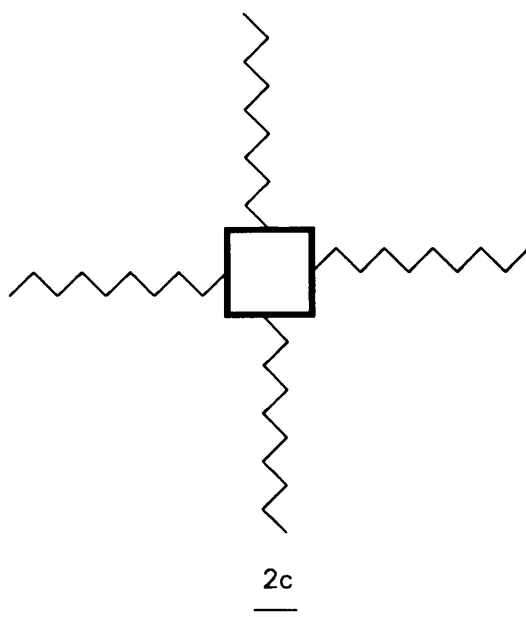
The lipidic peptides may be modified to allow the production of hetero-peptides 2a that contain other amino acids, substituted lipidic amino acids 2b or cyclic peptide structures 2c.



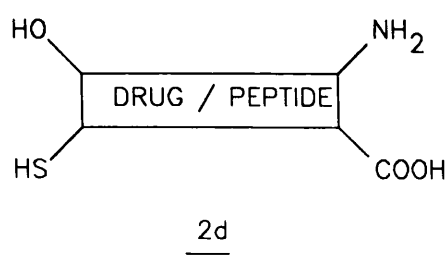
R = amino acid side chain residue



Z = halogen, OH, COOH, ..



By varying the length of the lipophilic side chain, number of lipidic amino acid residues and amino acid substitutions, etc. the relative hydrophilic/lipophilic nature of the lipidic amino acids/peptides can be controlled to assemble a family of compounds with a range of physicochemical parameters, being lipophilic due to the presence of the long alkyl side chain with the polar and conformational characteristics associated with amino acids and peptides. Due to their bifunctional nature, the fatty amino acids and peptides have the capacity to be chemically conjugated to drugs with a wide variety of functional groups **2d**. Table 1.2. displays the versatility of the system, illustrating the wide range of drug functional groups that can be employed in forming a conjugate bond. | Thus , conjugation may be utilised to modify uptake, activity and selectivity with the ultimate aim of increasing therapeutic indices of selected drugs.



Drug/peptide functional group	Conjugate linkage	Lipidic amino acid/peptide Functional group
-COOH	-CONH-	H ₂ N-
-NH ₂	-NHCO-	HOOC
-OH	-OOC-	HOOC-
-Br, -Cl	-OOC-	HOOC-
-COOH	-COO-Z-OOC- Z=spacer	HOOC-
		ω substituted derivatives
-OH	-O-	Br-, Cl-
-SH	-S-	Br-, Cl-
-SH	-S-S-	HS-
-COOH	-COO-	HO-

Table 1.2. Functional groups available for drug conjugation.

The linkage between drug and lipidic unit may either be biologically stable (ie. a new drug is formed) or possess biological or chemical instability (ie. the conjugate is a pro-drug). In either case, the resulting conjugates would be expected to possess a high degree of membrane-like character, which may be sufficient to facilitate their passage across membranes. The long alkyl side chains may also have the additional effect of protecting a labile parent drug from enzymatic attack.

This thesis aims to evaluate the potential of the lipidic amino acid/peptides as a drug delivery system by undertaking the following investigations on a number of selected lipidic conjugates.

Investigations undertaken to evaluate potential uses of the lipidic amino acid system.

1.7.1. Cellular uptake studies of a lipidic amino acid and its oligomers.

Cellular uptake of lipidic amino acid **33r**, and oligomers **33y**, **33al**, **33au** was studied using *Ehrlich ascities* tumour cells to establish uptake by transformed mammalian cells.

1.7.2. Structure-retention relationships of diastereomeric mixtures of lipidic dipeptides.

Under the conditions employed, synthesis of the lipidic amino acid system results in a racemic mixture which requires difficult chiral separation techniques to separate. When the racemic mixture is used for conjugation the synthesis results in products that are diastereomeric mixtures. The separation of diastereomeric mixtures does not require chiral separation conditions and therefore is easier and more economical. However the lipidic amino acid conjugates have amphiphilic and surface active character, so their separation still has difficulties, and the structure-retention relationship study can reveal their retention mechanism. The Supelcosil™ LC-ABZ column was used for the separation of the diastereomeric mixtures as it was designed specially for anionic, basic and zwitterionic compounds without silanophilic effects.

The retention parameters of 16 DL-,LL-lipidic dipeptide conjugate mixtures (**53b-g**, **53j-s**) were measured and the structure-retention relationships were studied. The chemical structure of the compounds was characterised by calculating log P values (the logarithmic values of the octanol/water partition coefficients) and the pK_a (dissociation constants). These may then be employed for method development in the separation of lipidic amino acid drug conjugate mixtures.

1.7.3. Physio-chemical elucidation of lipidic amino acid conjugates with hydrophilic compounds.

The major priority in trying to improve the transmembrane absorption of poorly-absorbed compounds was to impart them with increased membrane-like character by conjugation to lipidic amino acids and their oligomers (Toth *et al.* 1991, Hughes *et al.* 1991, Hussain *et al.* 1991). However, the resultant underivatised hydrophobic conjugates were often insufficiently soluble in water. The problem of how to increase the lipophilicity of the conjugates whilst maintaining adequate water solubility was addressed in two ways. The first was to modify the lipidic amino acids themselves (Gibbons *et al.* 1990), the second was to conjugate the lipidic amino acids with hydrophilic molecules. A number of substituted lipidic acid derivatives, containing hydrophilic groups **54a-l** were synthesised with the aim of enhancing their solubility in aqueous systems whilst maintaining membrane affinity characteristics. Hydrophilic species suitable for conjugation to lipidic amino acids and peptides are lactic acid, glycolic acid, glyceric acid and sugars. Of

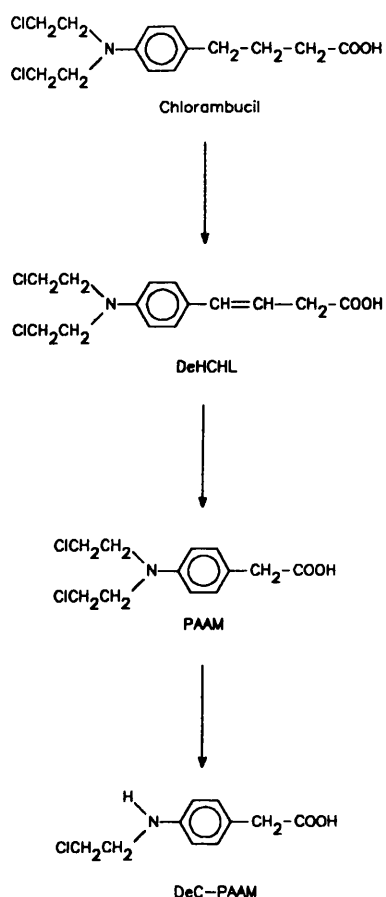
these, sugars represent the most efficient way of increasing water solubility, because of the multiple hydroxyl groups present on one molecule.

1.7.4. Synthesis and *in vitro* toxicity assessment of chlorambucil lipidic conjugates.

Chlorambucil {4-[4-Bis(2-chloroethyl)aminophenyl]butanoic acid} **55** was synthesised in 1953 (Everett *et al.* 1953), has been in clinical use for over 30 years and has become established along with the related nitrogen mustards, as a drug of major importance in the fight against malignant diseases (Lister *et al.* 1978, McElwain *et al.* 1977, Freckman *et al.* 1964, Lebhertz *et al.* 1965, Savitsky *et al.* 1977). Although chlorambucil is extensively used, factors underlying transport, action, metabolism and resistance to the compound, are incompletely understood.

Activity of chlorambucil is dependent on plasma membrane permeability enabling it to reach its site of action. Investigations using Yoshida ascites sarcoma cell lines (Harrap and Hill 1970, Hill *et al.* 1971, Hill 1972) and Chronic lymphocytic leucocytes (Hill and Harrap, 1972) indicated that uptake occurs by passive diffusion; however the plasma membranes of some tumours have reduced permeability resulting in resistance to some antitumour agents. Transport of the drug may also be affected by variations of pH between the inside and outside of cells; chlorambucil is a weak acid and will accumulate in cells whose intracellular pH is low relative to the extracellular pH, this may be of significance in the treatment of some solid tumours, where the interstitial pH is low (Mikklesen *et al.* 1985).

Chlorambucil is a reactive alkylating agent and hydrolyses in aqueous solution to (2-hydroxyethyl) derivative via a unimolecular process (Ehrsson *et al.* 1980). Chlorambucil is also metabolised extensively and rapidly via β -oxidation of its butyric acid side chain in both rats (Farmer *et al.* 1979, McClean *et al.* 1976) and man (Newell *et al.* 1979). In man only phenylacetic acid mustard (PAAM) has so far been identified, however in rats 3,4 dehydrochlorambucil (DeHCHL) and PAAM have been identified as major metabolic products, together with monodechloroethylated PAAM (DeC-PAAM) (Scheme 14). It has been shown that PAAM is more toxic and has a lower therapeutic index than chlorambucil (McClean *et al.* 1976), thus if β -oxidation of chlorambucil could be impaired, ideally stopped completely, then the therapeutic index of chlorambucil would be increased, a process known as metabolic switching (Farmer *et al.* 1979).



Scheme 14. Metabolism of Chlorambucil.

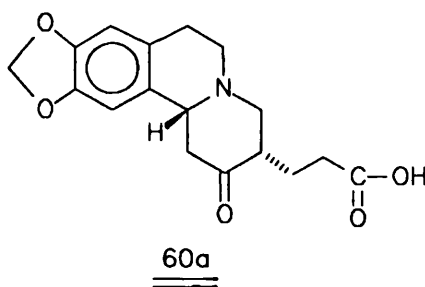
It is believed that the antitumour action of chlorambucil is due to its intracellular alkylation of DNA primarily at the N-7 position of guanine residues (Kohn *et al.* 1987). The chlorambucil molecule spans across the major groove of DNA between two guanine-N7 positions on opposite strands, damaging macromolecular structure and resulting in cell death. Resistance of tumour cells towards chlorambucil has been demonstrated, the mechanism of resistance being due to one of, or a combination of factors (Rutman *et al.* 1968, Harrap and Hill 1970, Ewig and Kohn 1977).

There have been a number of attempts to increase selectivity and improve the therapeutic efficiency of chlorambucil. Farmer (1979) synthesised chlorambucil with 2 deuterium atoms at the α -, or β - positions of the butyric acid side chain which showed no difference in therapeutic activity. β -Difluorochlorambucil was less potent than chlorambucil (Lee *et al.* 1986). Covalent and physical bonding to antibodies (Ross 1975a, 1975b, Ghose and Nigam 1972) has also been used, however the conjugates could not produce sufficient toxicity. Attempts have

been made to synthesise chlorambucil analogues with greater brain penetration. Greig studied the uptake of several lipophilic esters, however the brain concentration of chlorambucil remained low (Greig *et al.* 1990a). One ester that has been found to enter the brain is the t-butyl ester of chlorambucil (Greig *et al.* 1990b), and thus may be useful in the treatment of human brain tumours. The lipidic amino acid based delivery system was utilised in the synthesis of a series of novel chlorambucil lipidic conjugates to modify the delivery and cytotoxicity of chlorambucil.

1.7.5. Synthesis and *in vitro* and *in vivo* absorption investigations of benzoquinolizine lipidic conjugates.

Benzoquinolizine acid (**60a**) and its close relatives are a members of the alkaloid family. They possess anti-inflammatory, analgesic and gastric juice inhibition activity (Szantay *et al.* 1980a,b, Szabó *et al.* 1979). Thus, there is a potential for their use in arthritis and anti-inflammatory diseases of skeletal muscle (without ulcerogenic effect on prolonged use).



Benzoquinolizine compounds are poorly absorbed following oral administration and it was envisaged that conjugation of benzoquinolizine to the lipidic amino acid system may increase the permeability coefficient of the conjugate moiety to such an extent that its passage across the hydrophobic membrane of the gastro-intestinal tract is facilitated. The conjugates formed may be active themselves (i.e. new drug) or may be cleaved (prodrug), in both cases the conjugate being lipophilic should possess better absorption characteristics than the parent molecule. Benzoquinolizine acid **60a** was chosen as a representative model for the class of compounds. Benzoquinolizine acid was coupled to the lipidic amino acid system and the conjugates produced were studied both *in vitro* and *in vivo*.

1.7.6. Synthesis of (3,4 disubstitutedbenzyl)cyanoprop-2-enoyl lipidic conjugates.

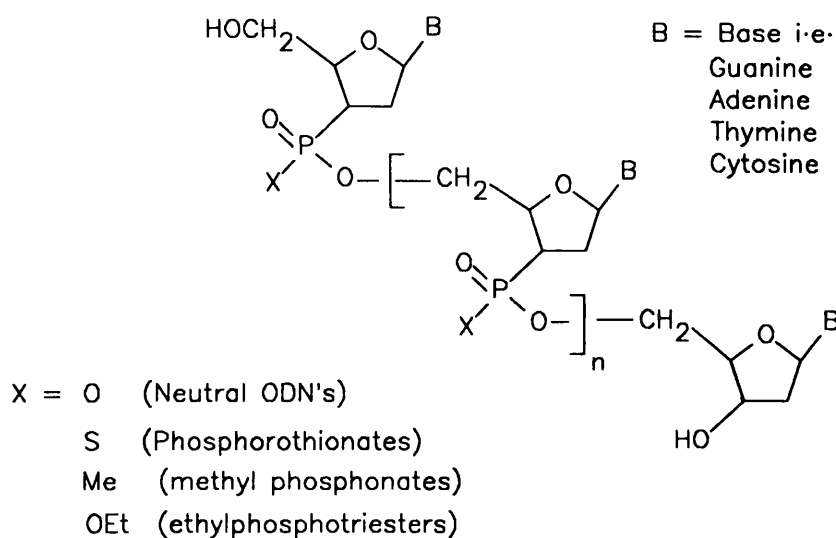
Carcinogenesis can be conceived in terms of disturbances in biochemical functions that normally control the expression or function of growth factors, receptors and pathways of signal transduction. It is the disturbance of signal transduction that I am particularly interested; the aim was to produce a series of membrane interactive signal transduction inhibitors, by developing an attractive synthetic pathway to produce 3,4 disubstituted benzyl cyanopropenoyl lipidic conjugates **64** based on the known poly ADP ribosylation inhibitor Benzamid.

1.7.7. Design of antisense oligonucleotides with therapeutic potential.

Many diseases are caused by an excess or lack of a particular protein or proteins. Traditionally drugs have been targeted to the proteins themselves, but targeting the DNA or RNA that encodes for a protein would be a more selective and efficient method of regulating protein levels. Antisense oligonucleotides have been shown to selectively inhibit gene expression, a property which confers enormous therapeutic potential.

Synthetic oligonucleotides were discovered to be potential inhibitors of gene expression several years ago (Stephenson and Zamecnik 1978, Zamecnik and Stephenson 1978) and attracted a great deal of interest, as they allow true rational drug design. The ideal requirements of an oligonucleotide drug include selectivity, tight binding to the target, stability to degradation (nucleases) and cell permeability.

Modifications to the sugar, base and phosphodiester groups have been used to improve the properties of antisense oligonucleotides **75a**. The main site of modification is substitution of the oxygen of the phosphodiester group; the best substituents being sulphur, methyl or ethyl groups (Uhlmann and Peyman 1990, Stec *et al.* 1984, Miller *et al.* 1982). Although modified oligonucleotides possess better binding strength and nuclease resistance characteristics the disadvantages are poor solubility in the case of methyl and ethyl phosphotriesters and poor cellular uptake of phosphorothionate derivatives (Uhlmann and Peyman 1990).



75a

Several methods have been used to increase the cellular uptake of synthetic oligonucleotides including conjugation to poly (α -L-lysine) (Uhlmann and Peyman 1990), cholesterol (Letsinger *et al.* 1989) and incorporating into liposomes (Thierry *et al.* 1992).

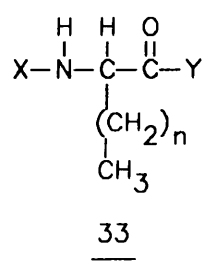
As the phosphorothionates possess the most favourable properties of the synthetic oligonucleotide derivatives, adenosine phosphorothionate was used to develop a synthetic route to produce aminoalkanoate-L-cysteine adenosine 5'monophosphorodisulphate.

Chapter 2. Chemistry Results and Discussion.

2.1. Synthesis of lipidic amino acids, peptides and derivatives.

2.1.1. Synthesis of lipidic amino acids (α -amino alkanoic acids).

The preparation of unsubstituted lipidic amino acids may be undertaken via one of two main synthetic routes. One route is to stir the appropriate α -bromoalkanoic acid with ammonium hydroxide for several days to produce the required lipidic amino acid in one step (Takino *et al.* 1988, Gerencevic *et al.* 1966, Kimura 1962, Birnbaum *et al.* 1953). The second route, reported by Albertson (1946) was selected for the synthesis of the lipidic amino acids **33a-33g**.

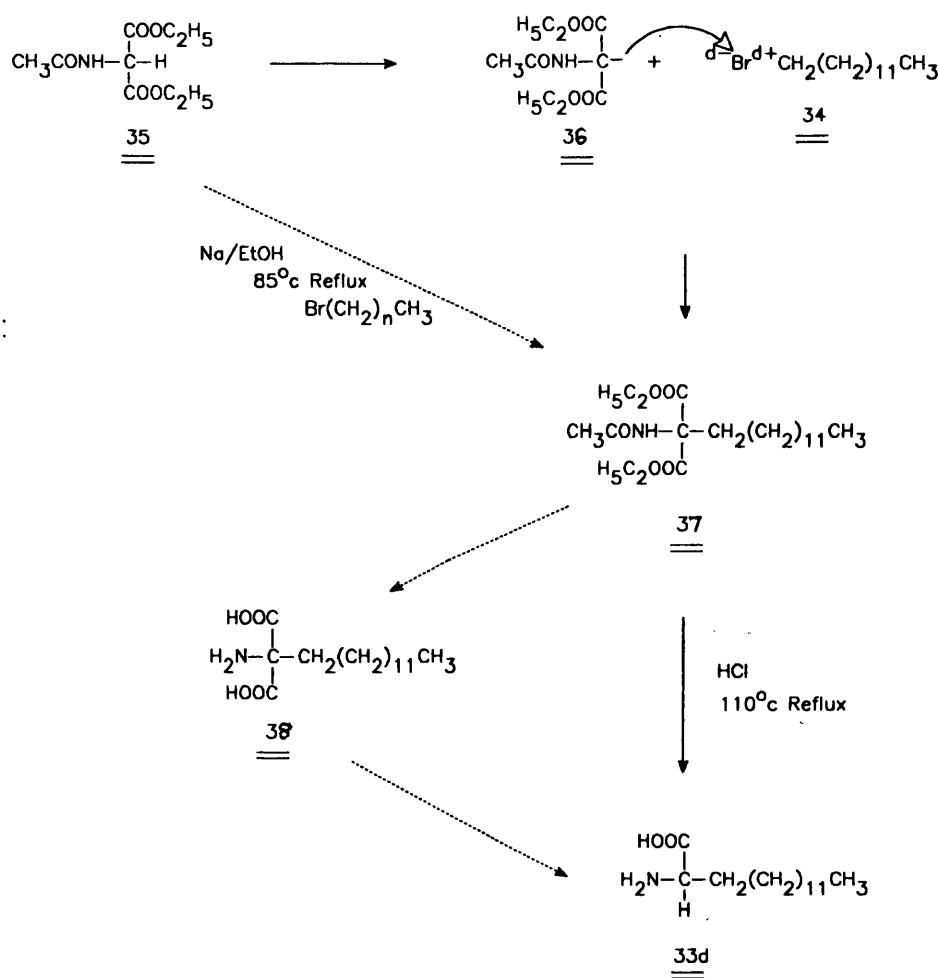


33	X	Y	n
<u>a,b,c,d,e,f,g</u>	H	OH	5,7,9,11,13,15,17
<u>h,i,j,k,l,m,n</u>	BOC	OH	5,7,9,11,13,15,17
<u>o,p,q,r,s,t,u</u>	H	OCH ₃	5,7,9,11,13,15,17

Table 2.1. α -Lipidic amino acids.

2.1.2. Mechanism of reaction

The fourteen carbon atom lipidic amino acid, α -aminotetradecanoic acid **33d** was synthesised by refluxing 1-bromododecane **34** with diethyl acetamidomalonate **35** in the presence of sodium ethoxide. The α -proton of the malonate ester is weakly acidic due to the inductive effects of the surrounding amido and acetate groups, this along with the strong basic conditions (sodium ethoxide) facilitates dissociation of the α -H. The carbanion **36** formed attacks the carbon C₁ of 1-bromododecane as it possesses a slight positive charge due to the



Mechanism 1. Synthesis of α-Aminotetradecanoic Acid.

electronegativity of bromine, to give the dodecyl-substituted diethyl acetamidomalonate intermediate **37**. The intermediate **37** was refluxed with the concentrated mineral acid, hydrochloric acid, resulting in the hydrolysis of the amide and two ester bonds to produce the geminal diacid, 2-amino-2-carboxyl tetradecanoic acid **38**. Spontaneous decarboxylation of the geminal diacid furnishes the required amino acid, α-aminotetradecanoic acid **33d**. The lipidic amino acids **33a-33g** were synthesised as racemic mixtures (D&L forms) in yields of 69% - 91% using the above procedure starting from the appropriate 1-bromoalkane. The racemic lipidic amino acids formed were then employed as starting materials for the synthesis of the lipidic peptides and conjugates.

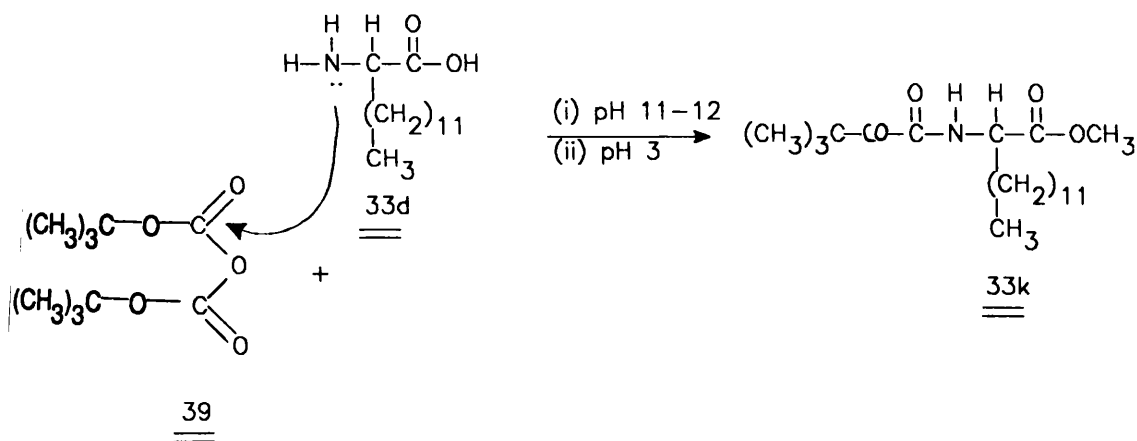
2.1.3. α -Lipidic amino acid functional group protection.

The lipidic amino acids possess both amine and carboxyl groups, i.e. they are bifunctional in nature. To produce specific homo-oligomers and hetero-oligomers, the lipidic amino acids must be condensed in a controlled, stepwise procedure, so as to prevent uncontrolled polymerisation resulting in a mixture of polymers of undefined molecular mass.

2.1.3.1. Protection of amino functional group.

The protection of the amino function may be achieved by the employment of any one of a large number of protecting groups that have developed over the years. They include: the acyl groups; formyl (Fisher and Warburg 1905), phthalyl (Kidd and King 1948) and chloracetyl (Holley and Holley 1952); the alkyl groups triphenylmethyl (trityl) (Helferich *et al.* 1925), *p*-tolouenesulphonyl (Tos) (Schönheimer 1926) and benzyl (Velluz *et al.* 1954); and the urethanes carbobenzoxy (Z) (Bergmann and Zervas 1932), tertiary butyloxycarbonyl (BOC) (Carpino 1957) and 9-fluorenylmethoxycarbonyl (Fmoc) (Carpino and Han 1972).

The tertiary butyloxycarbonyl group was employed for the protection of the amino functional group of the lipidic amino acids due to ease of synthesis, deprotection, purification and economical reasons.



Mechanism 2: Protection of Amino Group.

The BOC-protected lipidic amino acids **33h–33n** were prepared from the corresponding

et al.
dor (1976).

the methanol forming a methyl chlorosulphite which is a good leaving group and allows attack of the hydroxyl group.

2.1.4. Synthesis of α -lipidic amino acid homo-oligomers.

To achieve the synthesis of specific homo-oligomers and hetero-oligomers the coupling, or more correctly, the condensation reaction must be undertaken using appropriately protected lipidic amino-acid derivatives. The condensation reaction is directed to occur between the amino functional group of one monomer and the carboxylic functional group of a second monomer, resulting in the formation of the fully protected dimer species. The selective removal of either the amine or carboxyl protecting groups will permit further condensations to be undertaken. Repetition of the deprotection/condensation procedure ensures the synthesis of lipidic peptides of desired length is achieved.

<u>33</u>	X	Y	n	m
<u>v,w,x,y,z,aa,ab</u>	BOC	OCH ₃	5,7,9,11,13,15,17	2
<u>ac,ad,ae,af,ag,ah,ai</u>	H	OCH ₃	5,7,9,11,13,15,17	2
<u>aj,ak,al,am,an,ao</u>	BOC	OCH ₃	7,9,11,13,15,17	3
<u>ap,aq,ar,as,at</u>	H	OCH ₃	7,9,11,13,15	3
<u>au</u>	BOC	OCH ₃	11	4

Table 2.2. α -Lipidic amino acid homo-oligomers.

2.1.4.1. Synthesis of dimeric oligomers.

The synthesis of the fully protected dimeric-oligomers, i.e the fully protected lipidic dipeptides was achieved by coupling the appropriate amino and carboxylic acid protected lipidic amino acids using solution phase peptide synthesis methodology. Amide bond formation takes place due to nucleophilic attack by the free amino group towards a recipient carbonyl group. The efficiency of amide bond formation can be increased by substitution of the carboxylic acid hydroxyl group with an electron-withdrawing group (X), facilitating nucleophilic attack of the amino group (Mechanism 4).



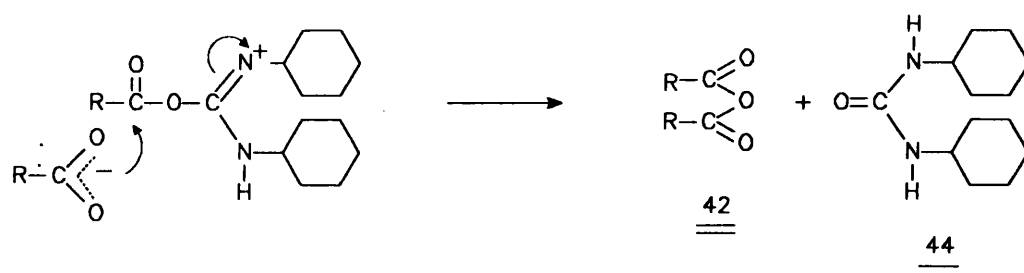
$$\text{R}-\text{COOH} + \text{Cyclohexyl}-\text{N}=\text{C}=\text{N}-\text{Cyclohexyl} \longrightarrow \text{Cyclohexyl}-\text{N}=\text{C}(\text{O}-\text{C}(=\text{O})\text{R})=\text{N}-\text{Cyclohexyl}$$

40
41

Scheme 14. Formation of O-Acyl Isourea Derivative.

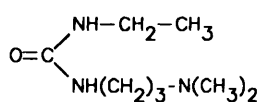
Mechanism 5.

Amide bond formation can also proceed via the symmetrical anhydride intermediates 42. Symmetrical anhydrides are formed by the attack of carboxyl compound towards the O-acyl isourea intermediate (Mechanism 6).



Mechanism 6.

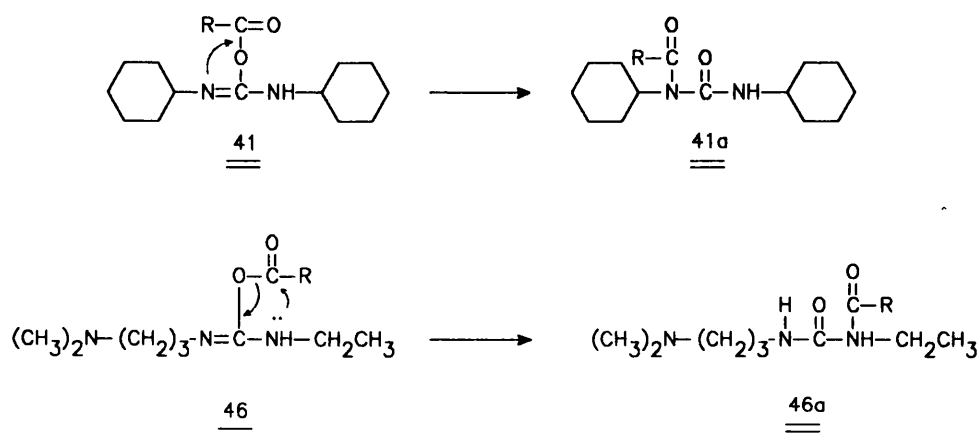
Coupling with DCC yields the almost insoluble byproduct N,N'-dicyclohexylurea (DCU) 44 which can be removed by filtration. Alternatively a water soluble derivative can be used such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EADC) which will form a water soluble byproduct 45 readily removable by washing with water.



Water soluble

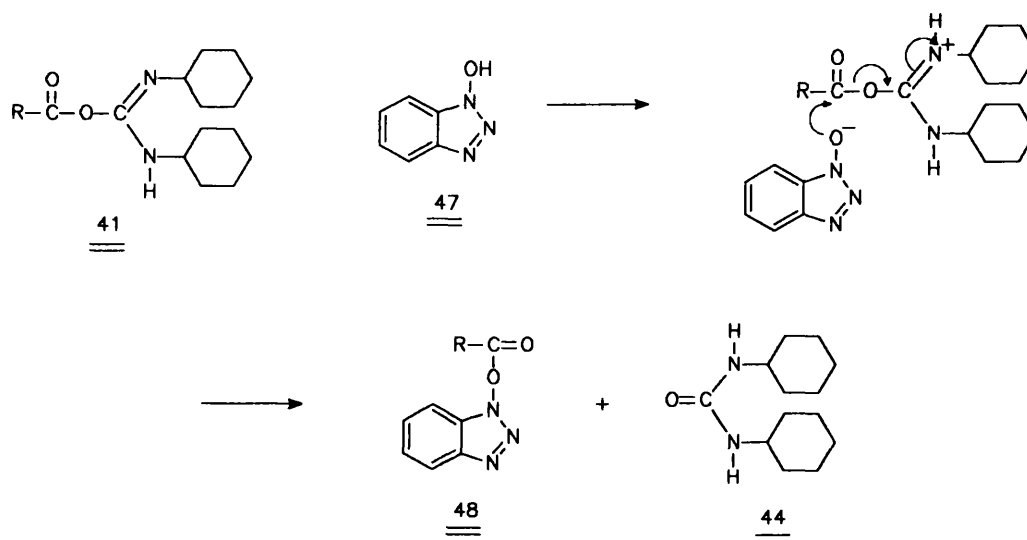
45

An undesired side reaction that occurs when using the carbodiimides DCC and EADC, is the rearrangement of the O-acyl isourea derivatives (41 and 46) to the undesired, unreactive N-acylurea derivatives (41a and 46a respectively).



Scheme 15. N-Acylurea Derivative Formation.

To prevent formation of the N-acylurea derivatives and the additional problem of some loss of enantiomeric purity an auxiliary nucleophile, such as 1-hydroxybenzotriazole (HOBt) **47** (Konig and Geiger 1970) is employed. The 1-hydroxybenzotriazole attacks the O-acyl isourea yielding the O-acyl-1-benzotriazole **48**, a powerful acylating agent (Scheme 16). The HOBt is used in an equimolar quantity, so there are two moles of nucleophile per mole of carboxyl function and carbodiimide, this reduces the lifetime of the highly reactive O-acylurea derivative.

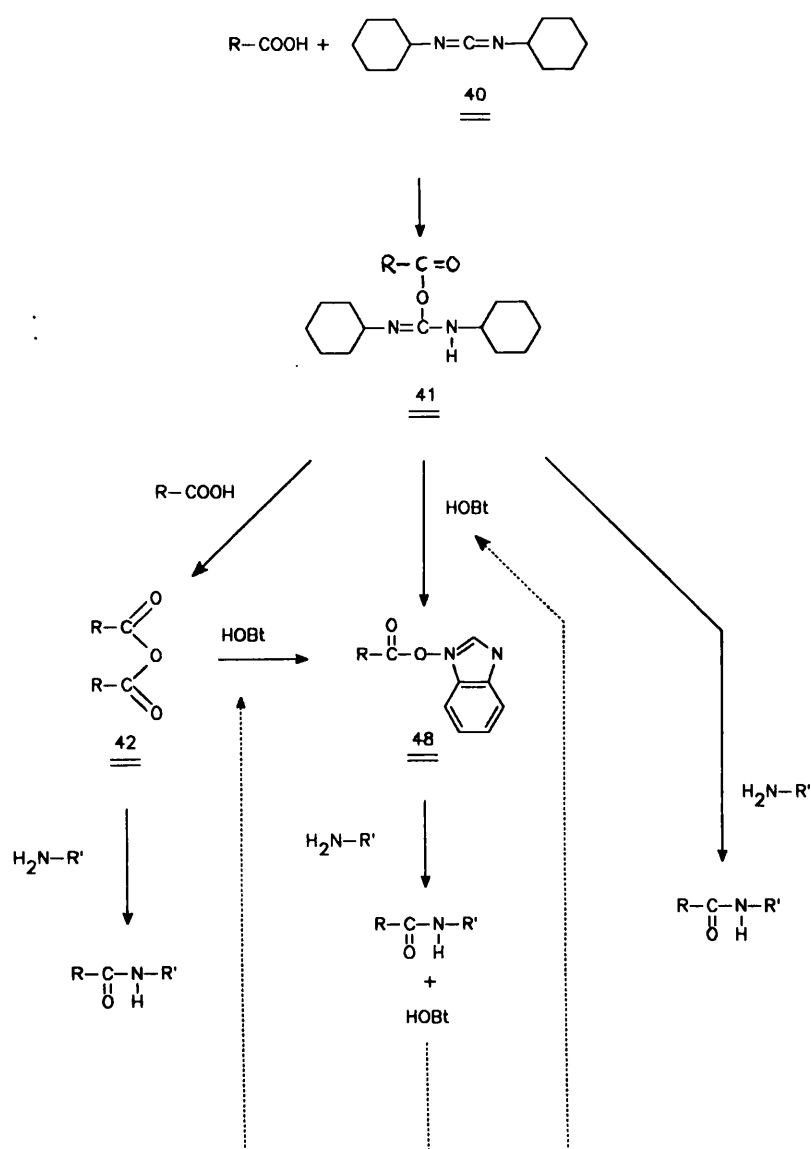


Scheme 16.

The HOBt provides multiple pathways for the reaction and is consistently regenerated. The active ester HOBt intermediate **48** has a lower activity than the O-acylurea derivative and is thus less prone to side reactions, but retains sufficient activity for satisfactory acylation rates.

The first stage in the coupling procedure is the addition of the appropriate BOC protected lipidic amino acid to DCC to form the O-acyl isourea derivative **41**. There are three pathways along which the intermediate O-acyl derivative may proceed to produce the desired fully protected lipidic dipeptide **33v-33ab** (Scheme 17).

- (i) reaction with another mole of BOC-protected lipidic amino acid producing the symmetrical anhydride **42** which acylates the methyl ester protected lipidic amino acid.
- (ii) reaction with 1-hydroxybenzotriazole producing the active ester **48** which undergoes nucleophilic attack by the methyl ester protected lipidic amino acid.
- (iii) direct acylation of the methyl ester protected lipidic amino acid.



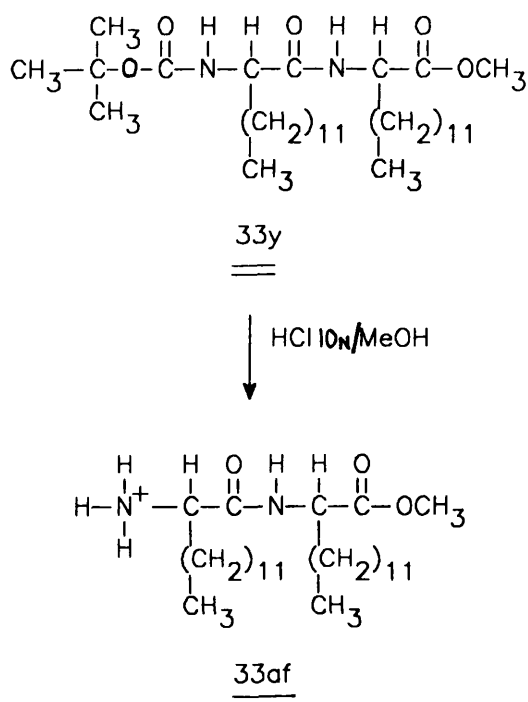
Scheme 17: Hydroxybenzotriazole Assisted Dicyclohexylcarbodiimide Coupling

2.1.4.2. Deprotection of lipidic dipeptides.

In order to synthesise the trimeric oligomers, i.e. the lipidic tripeptides the fully protected lipidic dipeptides have to be adequately deprotected to enable further coupling. Deprotection of can be undertaken on either the BOC group or the methyl ester group.

2.1.4.2.1. Acidolysis of BOC-protecting group.

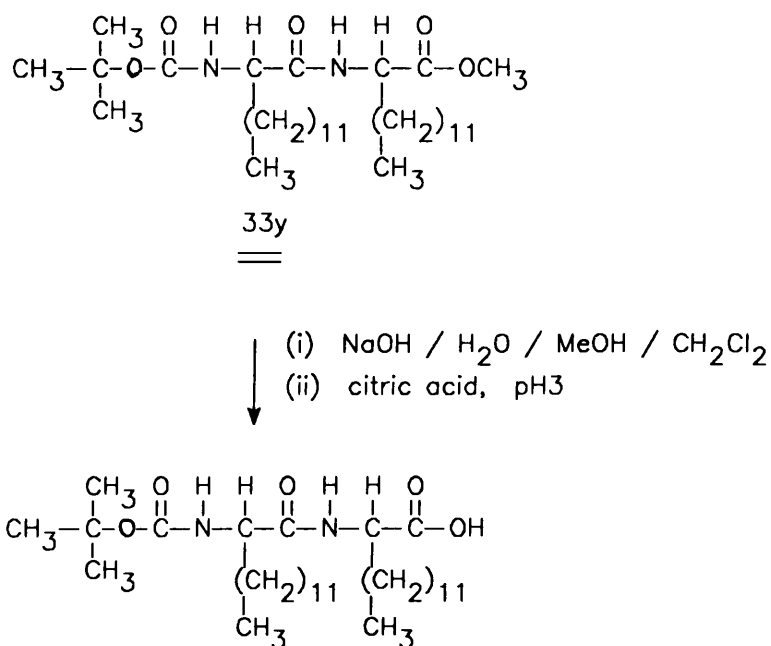
The BOC group is acid labile and can be removed by several reagents including anhydrous trifluoroacetic acid (TFA) and dry hydrogen chloride in methanol (Carpino 1957; McKay and Albertson 1957). Hydrochloric acid in methanol was employed to deprotect the amino function of the fully protected lipidic amino acids **33v-33ab** furnishing the N-deprotected dipeptides **33ac-33ai** as the hydrochloride salt (Scheme 18).



Scheme 18. Acidolysis of BOC Protecting Group.

2.1.4.2.2. Saponification of methyl ester protecting group.

The methyl ester protecting group can be removed from the fully protected lipidic dipeptides **33v-33ab** to liberate the C-deprotected lipidic dipeptides by base catalysed hydrolysis using sodium hydroxide (Scheme 19). As the lipidic amino acids are racemic mixtures the racemisation reported by Kenner and Seely (1972) following base-catalysed hydrolysis of alkyl esters does not present a problem.



Scheme 19. Saponification of Methyl Ester Protecting Group.

2.1.4.3. Synthesis of trimeric and tetrameric oligomers.

Either of two procedures can be employed to synthesise the Lipidic tripeptides tetrapeptides using the hydroxybenzotriazole assisted DCC coupling method described in 2.1.4.1.

(i) Coupling the BOC-protected lipidic amino acids to the appropriate N-deprotected lipidic dipeptides/tripeptides.

(ii) Coupling the methyl ester protected lipidic amino acids to the appropriate C-deprotected lipidic dipeptides/tripeptides.

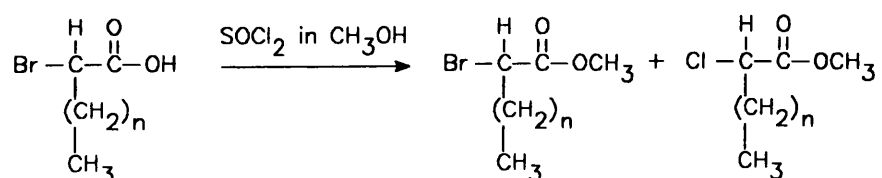
Procedure (i) was employed for the synthesis of the fully protected tripeptides **33aj-33ao** and tetrapeptide **33au**. Partial deprotection of the fully protected lipidic tripeptides/tetrapeptides as described in 2.1.4.2. allows further coupling to form higher oligomers and drug conjugates.

2.2. Synthesis of lipidic amino acid/oligomer halogenated derivatives.

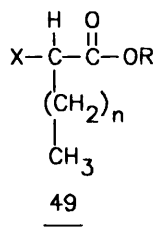
Ester linkages have been widely employed for creating pro-drugs and conjugating drugs to delivery systems (see introduction). The lipidic amino acid based delivery system can be modified allowing its conjugation to drugs and peptides via an ester linkage.

2.2.1. Synthesis of halogenated lipidic alkanoates.

A series of 2-bromoalkanoic acids **49a-49c** were protected at the carboxyl terminus by reflux with thionyl chloride in methanol as described for the preparation of lipidic amino acid methyl esters in 2.1.3.2. furnishing the halogenated lipidic alkanoates **49d-49f** as yellow brown oils in good yields (Scheme 20).



Scheme 20. Methylation of 2-Bromoalkanoic Acids.



<u>49</u>	X	n	R
<u>a</u>	Br	5	H
<u>b</u>	Br	11	H
<u>c</u>	Br	13	H
<u>di+dii</u>	Br + Cl	5	CH ₃
<u>ei+eii</u>	Br + Cl	11	CH ₃
<u>fi+fii</u>	Br + Cl	13	CH ₃

During methylation of the 2-bromoalkanoic acids exchange of bromine by chlorine occurs, resulting in a mixture of chlorinated and brominated lipidic alkanoate derivatives **49d-49f**.

Evidence for the exchange process can be shown in the $^1\text{H-NMR}$ (Fig. 2.1) and mass spectra (Fig. 2.2) of **49f**.

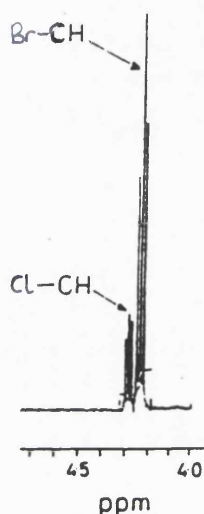


Figure 2.1. $^1\text{H-NMR}$ of **49f**.

Two $\alpha\text{-C-H}$ proton (triplet) peaks are observable at $\delta 4.28$ and $\delta 4.22$ attributed to the 2-bromo derivative **49fi** and the 2-chloro derivative **49fii** respectively.

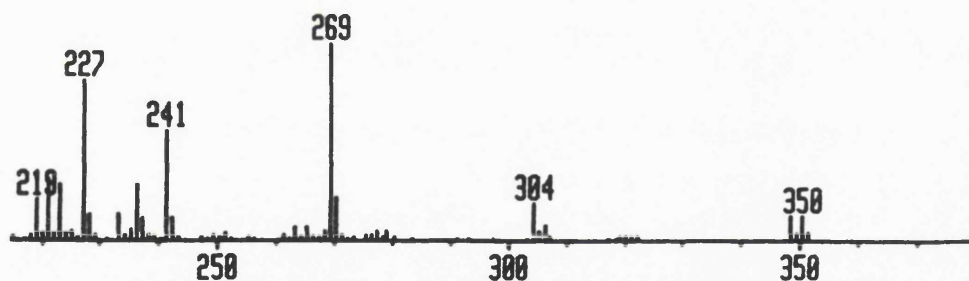


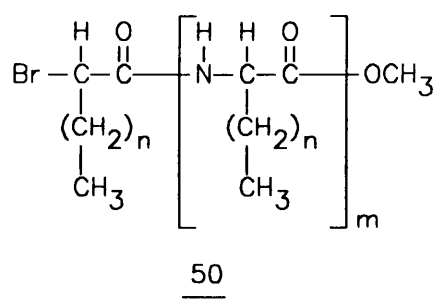
Figure 2.2. Mass Spectrum of **49f**.

The fast atom bombardment mass spectrum (FAB-MS) of **46f** show the presence of an isotopic molecular ion peak $[\text{M}+\text{H}]^+$ at m/e 350, 348 (1:1) relating to the 2-bromo derivative and an isotopic molecular ion peak $[\text{M}+\text{H}]^+$ at m/e 306, 304 (3:1) relating to the 2-chloro derivative.

The esters were not separated as both would be capable of participating in the esterification reactions to be undertaken.

2.2.2. Synthesis of halogenated lipidic peptides.

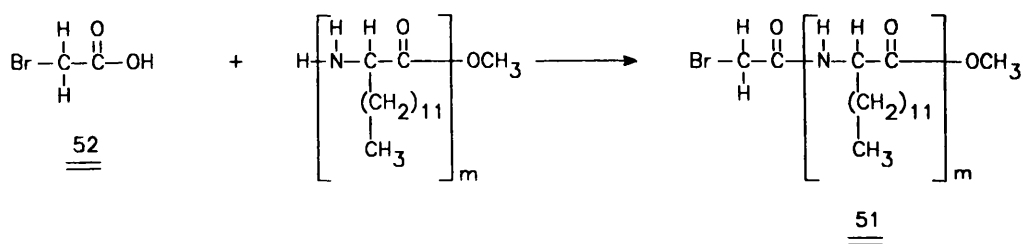
Bromonated lipidic oligomers **50a-50b** were prepared by condensing the appropriate 2-bromoalkanoic acid **49a-b** to the corresponding lipidic amino acid/oligomer methyl esters **33r-33af** using EADC assisted coupling. HOBt was omitted from the reaction mixture to prevent the formation of an ether side product (Hussain 1992). The products were purified using preparative TLC plates.



<u>50</u>	n	m
<u>a</u>	11	1
<u>b</u>	11	2

2.2.3. Synthesis of bromoacetyl lipidic amino acid/peptide conjugates.

Bromoacetyl lipidic amino acid **51a** and peptide **51b** were prepared by coupling bromoacetic acid **52** with lipidic methyl esters **33r** and **33af** respectively (Scheme 21) using the EADC coupling method described in 2.1.4.1.



Scheme 21.

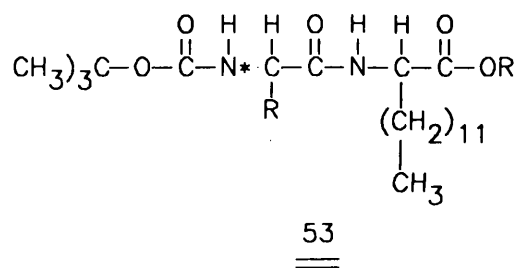
<u>51</u>	m
<u>a</u>	1
<u>b</u>	2

2.3. Synthesis and structure-retention relationships of diastereomeric mixtures of lipidic dipeptides on reversed phase stationary phases.

The quantitative structure activity correlation study (Valkó *et al.* 1992) revealed the role of the lipidic side chain in the in vivo activity of the lipidic amino acid conjugates of β -lactam antibiotics. As the racemic form of lipidic amino acids are used for conjugation the synthesis always results in diastereomeric mixtures. The separation of diastereomers does not require chiral separation conditions and therefore is easier and cheaper. However the lipidic amino acid conjugates have amphophilic and surface active character, so even their separation is not straight-forward, and the structure-retention relationship study can reveal their retention mechanism. As the Supelcosil™ LC-ABZ column was designed specially for anionic, basic and zwitterionic compounds without silanophilic effects it seemed worth trying for the separation of the diastereomeric mixtures.

In this investigation the retention parameters of 16 lipidic amino acid/amino acid conjugate mixtures were measured and the structure-retention relationships were studied. The chemical structure of the compounds was characterized by calculated log P values (the logarithmic values of the octanol/water partition coefficients) and the dissociation constants (pK_a).

The lipidic dipeptides **53a-t** were synthesized by coupling the appropriately protected acids to methyl α -amino tetradecanoate employing the 1-hydroxybenzotriazole assisted



carbodiimide coupling method as described in **2.1.4.1**. The chemical structure and their identification parameters can be seen in Table 2.3.

The compounds were dissolved in 1mg/ml concentration in 95% acetonitrile, 5% water and 0.1% TFA and 20 μ l of this solution was injected three times on the HPLC columns. The solvent peak was regarded as the dead time, and the log k' values were calculated from the average retention time obtained from the three injections.

The logarithmic values of the capacity ratio ($\log k'$) were determined for each compound on both stationary phases | using various concentrations of acetonitrile in the mobile phase. The $\log k'$ values were plotted against the acetonitrile concentration. In most cases straight lines were obtained with higher than 0.99 correlation coefficients. Only for compounds **53pi** and **pii** parabolic relationship was found as it can be seen in Fig.2.3 for the data obtained on Spherisorb ODS column.

As you can see from Table 2.3, compound **53p** has basic nitrogens which can interact with the free silanol groups of the Spherisorb ODS stationary phase, and that causes the parabolic relationship between the log k' values and the acetonitrile concentrations. Fig.2.4 shows the straight lines obtained for compound **53pi** and **pii** on the Supelcosil™LC-ABZ columns which does not have free silanol groups. The numbers **i** and **ii** denote to the separated diastereomers. Three diastereomer pairs could not be separated on the two columns at all, compounds **53k**, **53r** and **53s**. No satisfactory explanations can be given for the reason. Only their hydrophobicity do not differ so much to be able to differentiate between them under the studied chromatographic conditions. Compound **53a** is an enantiomeric mixture therefore no separation can be expected in non-chiral chromatographic conditions.

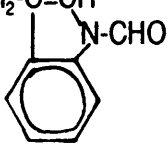
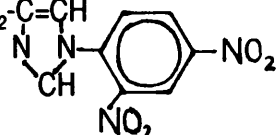
<u>53</u>	R
<u>a</u>	-H
<u>b</u>	-CH(CH ₃) ₂
<u>c</u>	-CH ₃
<u>d</u>	-CH ₂ -CH(CH ₃) ₂
<u>e</u>	-CH(CH ₃)C ₂ H ₅
<u>f</u>	-CH ₂ -CH ₂ -CH ₂ -N ⁺
<u>g</u>	-CH ₂ -C ₆ H ₅
<u>h</u>	-CH ₂ -C ₆ H ₄ -O-CO-CH ₂ -C ₆ H ₄ -p-Br
<u>i</u>	-CH ₂ -C=CH 
<u>j</u>	-CH ₂ -S-CH ₂ -NH-CO-CH ₃
<u>k</u>	-(CH ₂) ₂ -S-CH ₃
<u>l</u>	-CH(CH ₃)-O-CH ₂ -C ₆ H ₅
<u>m</u>	-CH ₂ -O-CH ₂ -C ₆ H ₅
<u>n</u>	-(CH ₂) ₄ -NH-CO-O-CH ₂ -C ₆ H ₅ -o-Cl
<u>o</u>	-(CH ₂) ₃ -NH-C(NH)-NH-SO ₂ -C ₆ H ₅ -p-CH ₃
<u>p</u>	-CH ₂ -C=CH 
<u>q</u>	-CH ₂ -CO-O-CH ₂ -C ₆ H ₅
<u>r</u>	-(CH ₂) ₂ -CO-O-CH ₂ -C ₆ H ₅
<u>s</u>	-CH ₂ -CO-NH ₂
<u>t</u>	-CH ₂ -CONH ₂

Table 2.3. The chemical structures of the investigated diastereomeric lipidic dipeptides 53a-t.

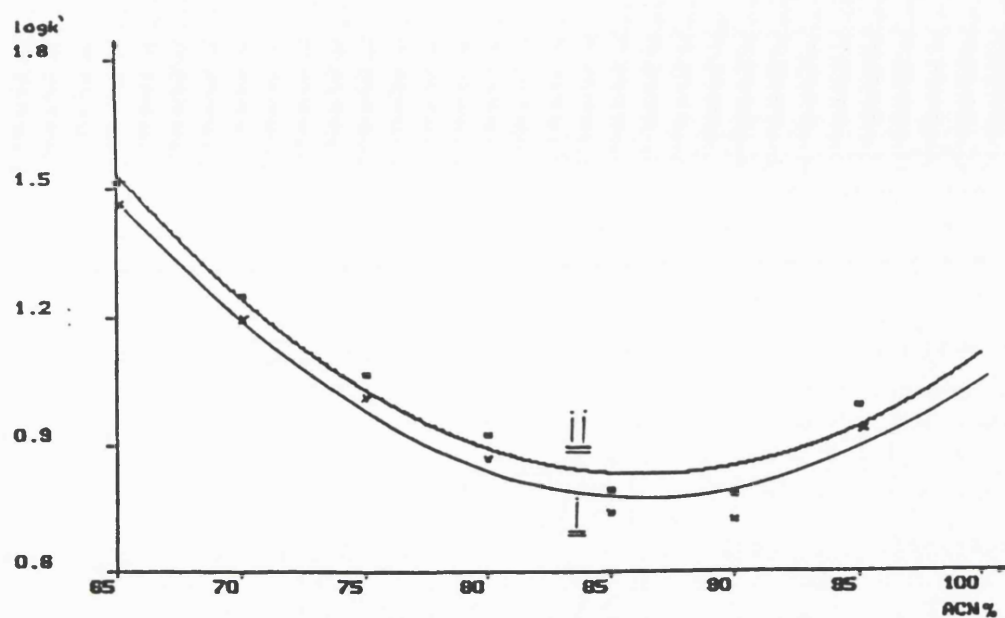


Figure 2.3. The plot of $\log k'$ vs acetonitrile concentration in the mobile phase for compounds **53pi** and **53pii** obtained on Spherisorb ODS column.

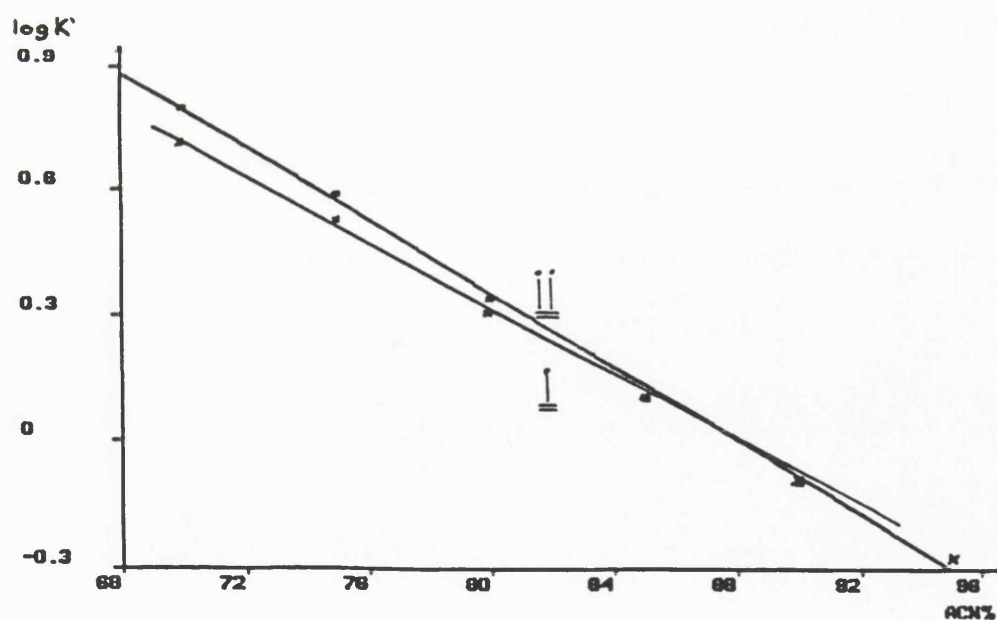


Figure 2.4. The plot of $\log k'$ vs acetonitrile concentration in the mobile phase for compounds **53pi** and **53pii** obtained on Supelcosil™ LC-ABZ column.

In general higher resolution of the diastereomers could be observed on Supelcosil™LC-ABZ column as can be seen on Figs.2.5-2.7. For compound **53l** and **53m** better separation was obtained on the Spherisorb ODS column. These two compounds were the only aromatic ether type in the series (Figs. 2.8 and 2.9). Tables 2.4 and 2.5 present the calculated slope (S) and intercepts ($\log k'_0$) values of the straight lines obtained by plotting $\log k'$ values against the acetonitrile concentration for each compound on the Spherisorb ODS and Supelcosil™ LC-ABZ columns, respectively. When parabolic relationship was observed only the linear portion of the relationship was considered in the calculation of the values. Table 2.5 also shows the calculated hydrophobicity values ($\log P$) and the dissociation constants (pK_a). In order to reveal the structure-retention relationships correlation analysis was carried out on the data shown in Tables 2.4 and 2.5. The correlation coefficients summarized in a correlation matrix can be seen in Table 2.6.

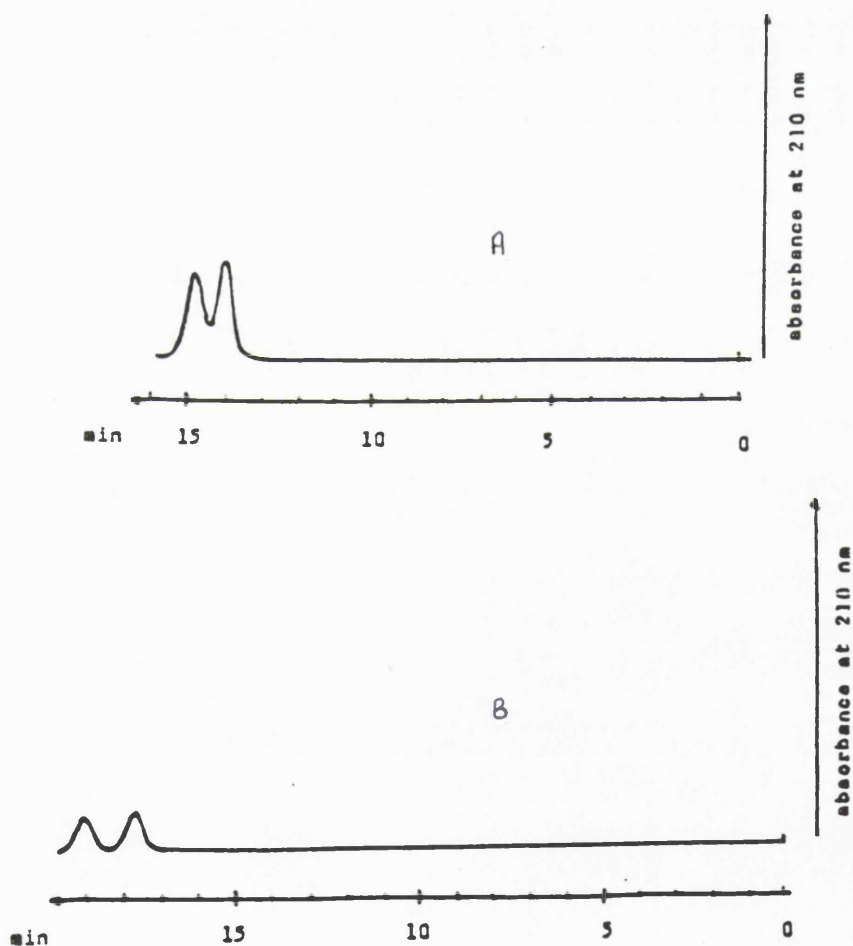


Figure 2.5. The separation of the diastereomeric mixture of compound **53b** by using 70% (v/v) acetonitrile, 0.1% TFA and 30% water as the mobile phase. Flow rate: 1.00 ml/min. Detection at 210 nm. A: Spherisorb ODS 5 μ m, 50 x 4.6 mm column; B: Supelcosil™ LC-ABZ 150 x 4.6 mm column.

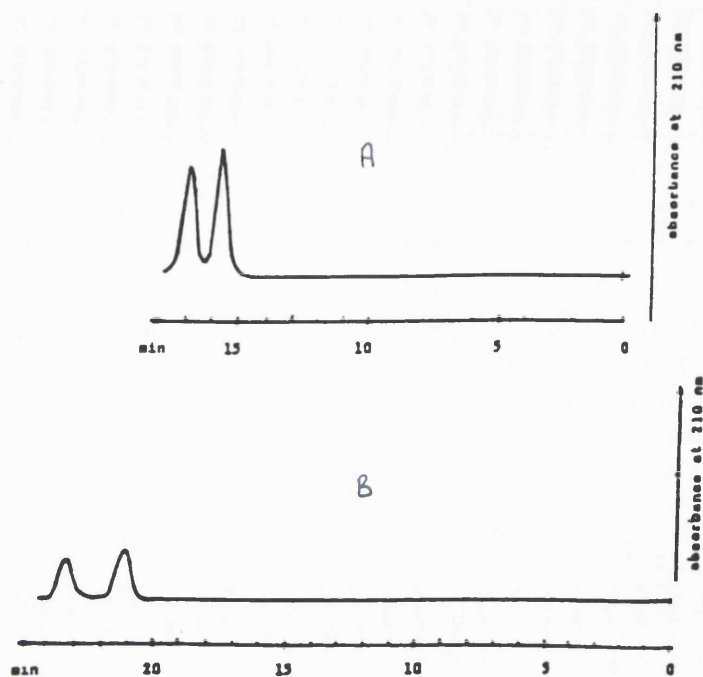


Figure 2.6. The separation of the diastereomeric mixture of compound **53d** by using 70% (v/v) acetonitrile, 0.1% TFA and 30% water as the mobile phase. Flow rate: 1.00 ml/min. Detection at 210 nm. A: Spherisorb ODS 5 μ m, 50 x 4.6 mm column; B: Supelcosil™ LC-ABZ 150 x 4.6 mm column.

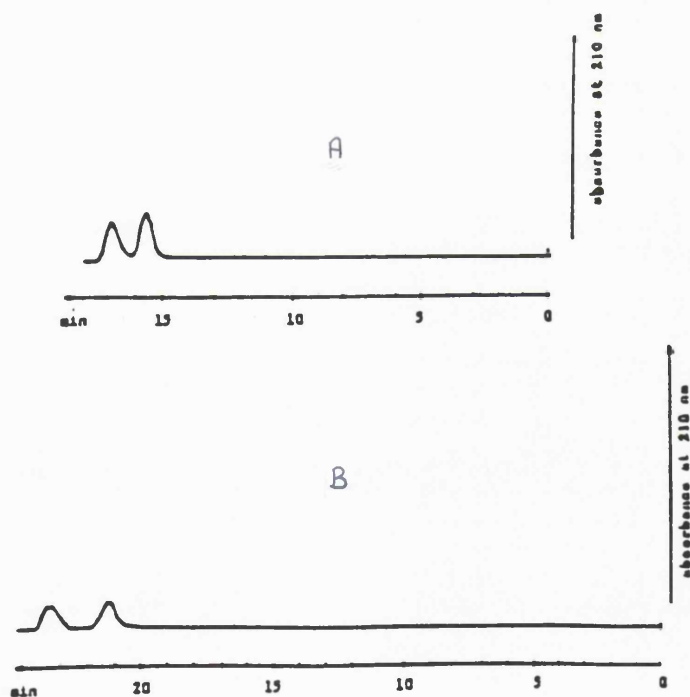


Figure 2.7. The separation of the diastereomeric mixture of compound **53e** by using 70% (v/v) acetonitrile, 0.1% TFA and 30% water as the mobile phase. Flow rate: 1.00 ml/min. Detection at 210 nm. A: Spherisorb ODS 5 μ m, 50 x 4.6 mm column; B: Supelcosil™ LC-ABZ 150 x 4.6 mm column.

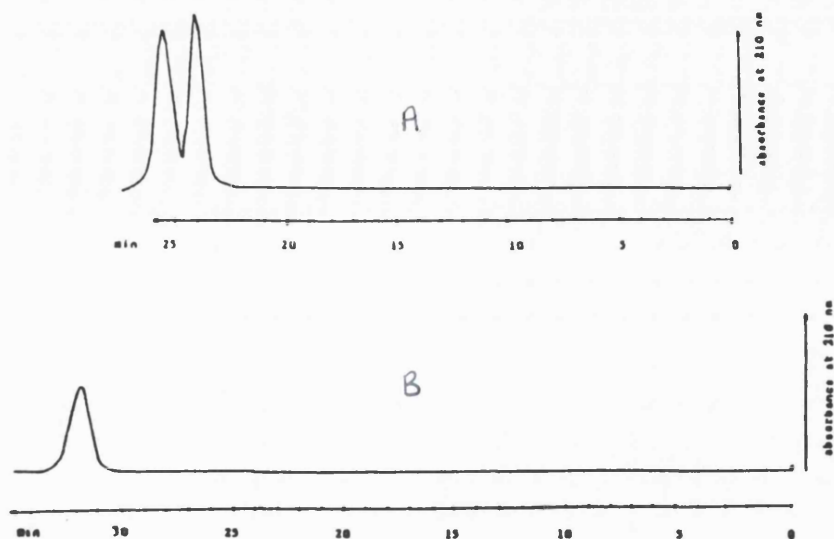


Figure 2.8. The separation of the diastereomeric mixture of compound **53l** by using 70% (v/v) acetonitrile, 0.1% TFA and 30% water as the mobile phase. Flow rate: 1.00 ml/min. Detection at 210 nm. A: Spherisorb ODS 5 μ m, 50 x 4.6 mm column; B: Supelcosil™ LC-ABZ 150 x 4.6 mm column.

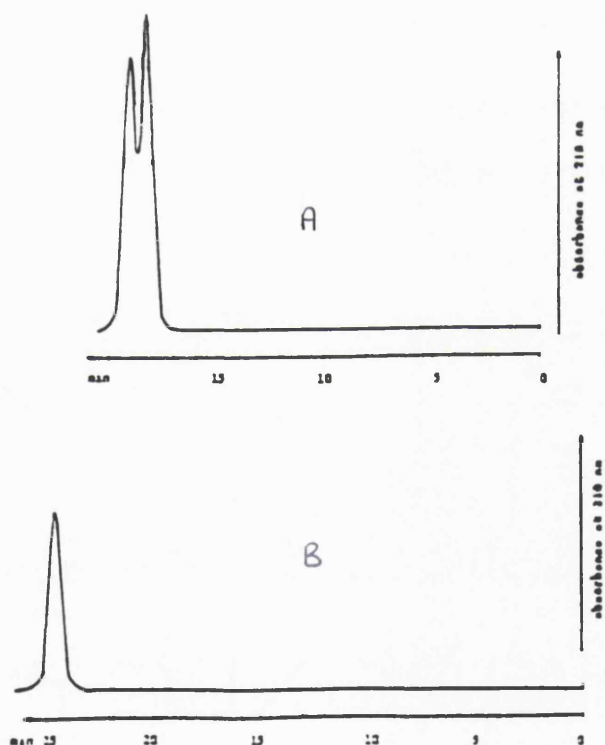


Figure 2.9. The separation of the diastereomeric mixture of compound **53m** by using 70% (v/v) acetonitrile, 0.1% TFA and 30% water as the mobile phase. Flow rate: 1.00 ml/min. Detection at 210 nm. A: Spherisorb ODS 5 μ m, 50 x 4.6 mm column; B: Supelcosil™ LC-ABZ 150 x 4.6 mm column.

Table 2.4. The slope (S), the intercept ($\log k'_0$) and the correlation coefficient (R) values of the compounds obtained on Spherisorb ODS (Sph) column according to equation: $\log k' = \log k'_0 - S\phi$.

<u>53</u>	S_{Sph}	$\log k'_{0\text{Sph}}$	R
<u>a</u>	36.3	3.366	0.999
<u>bi</u>	40.6	3.828	0.999
<u>bii</u>	40.3	3.830	0.999
<u>ci</u>	35.5	3.357	0.998
<u>cii</u>	35.5	3.357	0.998
<u>di</u>	40.4	3.853	0.998
<u>dii</u>	41.8	4.011	0.998
<u>ei</u>	42.9	4.056	0.999
<u>eii</u>	42.4	4.059	0.999
<u>fi</u>	31.7	3.254	0.998
<u>fii</u>	31.7	3.254	0.998
<u>gi</u>	43.0	4.048	0.995
<u>gii</u>	43.2	4.089	0.998
<u>hi</u>	34.8	3.154	0.996
<u>hii</u>	32.9	3.062	0.994
<u>ki</u>	41.1	3.829	0.998
<u>li</u>	46.5	4.497	0.999
<u>lii</u>	47.0	5.565	0.999
<u>mi</u>	42.6	4.065	0.996
<u>mii</u>	42.8	4.134	0.997
<u>ni</u>	43.5	4.075	0.997
<u>nii</u>	44.4	4.158	0.996
<u>oi</u>	38.9	3.455	0.996
<u>oii</u>	40.5	3.610	0.998
<u>pi</u>	30.4	3.308	0.999
<u>pii</u>	30.5	3.377	0.999
<u>qi</u>	43.5	4.080	0.990
<u>qii</u>	44.4	4.158	0.993
<u>r</u>	43.1	4.051	0.993
<u>s</u>	28.5	2.570	0.998

* In these cases parabolic relationships were found by plotting the $\log k'$ values as a function of the organic phase concentration. For the calculation of the S, $\log k'_0$ and R values, only the linear section of the curve was considered.

Table 2.5. The Slope (S), the intercept ($\log k'_0$) and the correlation coefficient values of the compounds obtained on Supelcosil™LC-ABZ (Sup) column according to the equation: $\log k' = \log k'_0 - S\phi$. The calculated hydrophobicity ($\log P$) and dissociation constants (pK_b) of the compounds investigated.

<u>53</u>	S_{Sup}	$\log k'_{0\text{Sup}}$	R	$\log P$	pK_b
<u>a</u>	40.4	3.459	0.999	7.25	3.3
<u>bi</u>	43.9	3.918	0.998	8.80	3.2
<u>bii</u>	45.0	4.042	0.995	8.80	3.2
<u>ci</u>	42.0	3.643	0.998	7.76	3.3
<u>cii</u>	42.9	3.723	0.998	7.76	3.3
<u>di</u>	45.5	4.124	0.999	9.32	3.3
<u>dii</u>	44.8	4.117	0.999	9.32	3.3
<u>ei</u>	45.6	4.133	0.998	9.32	3.3
<u>eii</u>	43.6	4.032	0.999	9.32	3.3
<u>fi</u>	38.4	3.433	0.999	6.52	2.2
<u>fii</u>	38.8	3.484	0.999	6.52	2.2
<u>gi</u>	47.0	4.234	0.999	8.90	3.7
<u>gii</u>	47.4	4.288	0.998	8.90	3.7
<u>ji</u>	39.6	3.275	0.999	7.29	3.6
<u>jii</u>	38.5	3.252	0.999	7.29	3.6
<u>ki</u>	44.4	3.946	0.999	8.29	3.4
<u>li</u>	47.9	4.483	0.999	9.46	3.6
<u>lii</u>	47.9	4.483	0.999	9.46	3.6
<u>mi</u>	51.1	4.597	0.990	8.94	3.6
<u>mii</u>	51.1	4.597	0.990	8.94	3.6
<u>ni</u>	48.5	4.281	0.998	10.75	3.3
<u>nii</u>	49.2	4.343	0.998	10.75	3.3
<u>oi</u>	43.9	3.716	0.996	9.76	8.1
<u>oii</u>	45.4	3.856	0.997	9.76	8.1
<u>pi</u>	40.0	3.511	0.999	7.27	3.3
<u>pii</u>	43.8	3.855	0.997	7.27	3.3
<u>qi</u>	49.4	4.466	0.998	9.23	3.8
<u>qii</u>	49.4	4.466	0.998	9.23	3.8
<u>r</u>	51.7	4.607	0.998	9.17	3.5
<u>s</u>	33.1	2.662	0.998	6.19	3.6

$\log P$ is the calculated logarithmic value of the octanol/water partition coefficients.
 pK_b is the calculated base dissociation constant.

	S_{Sph}	$\log k'_{Sph}$	S_{Sup}	$\log k'_{Sup}$	$\log P$	pK_b
S_{Sph}	1.00	-0.95	0.86	-0.88	-0.88	-0.15
$\log k'_{Sph}$	-0.95	1.00	-0.88	0.94	0.81	-0.02
S_{Sup}	0.86	-0.88	1.00	-0.98	-0.80	-0.13
$\log k'_{Sup}$	-0.88	0.94	-0.98	1.00	0.78	0.01
$\log P$	-0.88	0.81	-0.80	0.78	1.00	0.37
pK_b	-0.15	-0.02	-0.13	0.01	0.37	1.00

Table 2.6. The correlation coefficients between the chromatographic and physico-chemical parameters.

On both column the slope (S) and intercept ($\log k'_o$) values showed high correlation coefficients (-0.95 and -0.98) which means that the set of compounds behaved as structurally related (Valko 1987). As it was expected the dissociation constants (pK_b) alone did not show good correlations to the chromatographic parameters on the reversed phase columns where hydrophobicity governs the retention. The $\log P$ values showed better correlation to the chromatographic parameters obtained on the Spherisorb ODS column when the data obtained at higher organic concentrations, where the silanol effect could be observed were not included in the calculations. It suggests, that the hydrophobicity governed the retention. On the contrary in the retention data obtained on the Supelcosil™LC-ABZ column other types of interactions are possibly involved as well, as the stationary phase contains charged moieties among the long hydrophobic alkyl chains. When step-wise regression analyses were carried out on the data it was found that the calculated hydrophobicity values showed good correlations to the slope values (S) and the pK_b values as described by equation 1 and 2 for the data obtained on the Spherisorb ODS and the Supelcosil™LC-ABZ columns, respectively.

$$\log P = 1.936 (\pm 0.186) S_{Sph} + 0.23 (\pm 0.08) pK_b + 0.12 \quad (1)$$

$$n=30 \quad r=0.910 \quad s=0.517 \quad F=64.7$$

$$\log P = 2.086 (\pm 0.279) S_{Sup} + 0.26 (\pm 0.10) pK_b - 1.86 \quad (2)$$

$$n=30 \quad r=0.848 \quad s=0.660 \quad F=34.5$$

where $\underline{n}$ is the number of compounds, $\underline{r}$ is the multiple regression coefficient, $\underline{s}$ is the standard error of the estimate, $\underline{F}$ is the Fisher-test value (significant at 0.01% level). Usually the $\log k'_o$ values are expected to show good correlation with the partition coefficients of compounds. Similar, significant correlations can be found in our case as well (see equations 3 and 4), but the mathematical statistical parameters are slightly worse, than that of equations 1 and 2.

$$\log P = 2.124(\pm 0.22)\log k'_{o\text{Sph}} + 0.368(\pm 0.082)pK_b - 0.738 \quad (3)$$

$$n=30 \quad r=0.896 \quad s=0.552$$

$$\log P = 1.940(\pm 0.249)\log k'_{o\text{Sup}} + 0.346(\pm 0.095)pK_b - 0.386 \quad (4)$$

$$n=30 \quad r=0.857 \quad s=0.642$$

As the S and the $\log k'_o$ values show high correlations with each other, they are interchangeable. As it can be seen slightly better correlation was obtained on the Spherisorb ODS column (again data, where the silanol effect could be observed were not included in the calculations), which proves that on the SupelcosilTM LC-ABZ other than only hydrophobic interactions took place. The plots of the $\log P$ vs the estimated $\log P$ values by equation 1 and 2 are presented in Figs. 2.10 and 2.11, respectively.

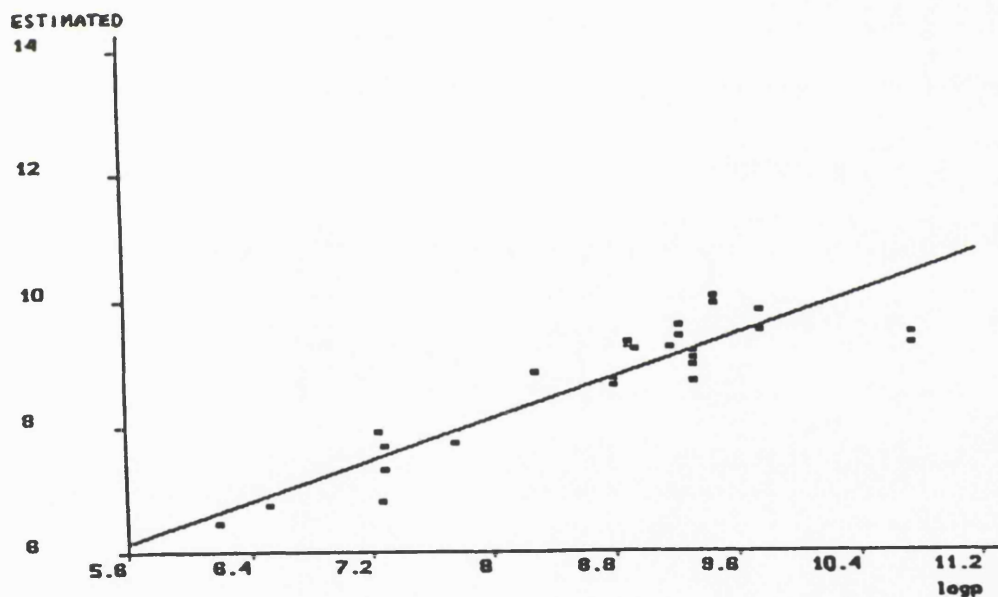


Figure 2.10. The plot of the $\log P$ values (logarithm of octanol/water partition coefficients) and the estimated values obtained on the Spherisorb ODS column by equation 1.

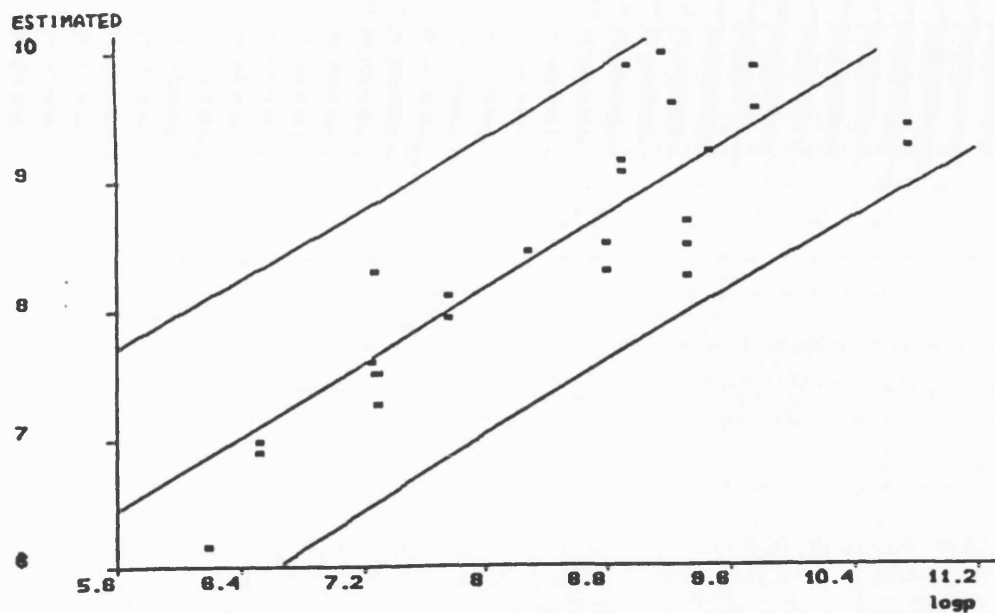


Figure 2.11. The plot of the $\log P$ values (logarithm of octanol/water partition coefficients) and the estimated values obtained on the SupelcosilTM LC-ABZ column by equation 2.

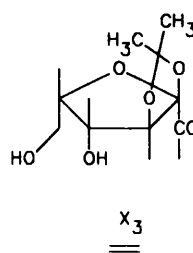
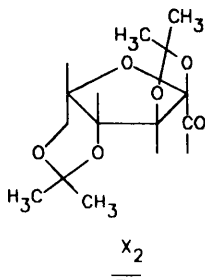
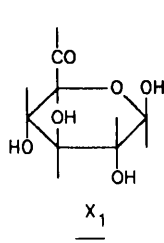
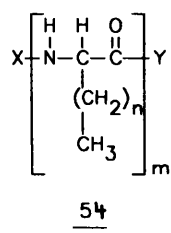
In conclusion , most of the diastereomeric lipidic amino acid mixtures could be separated in reversed phase conditions. The retention of the compounds was governed by hydrophobicity on the Spherisorb ODS column and the effect of the free silanol groups could be observed for basic derivatives. The SupelcosilTM LC-ABZ column did not show silanophilic effect, most cases better separation of the diastereomers could be observed and other than hydrophobic interactions governed the retention as well.

2.4. Physiochemical elucidation of lipidic amino acid conjugates with hydrophilic compounds.

2.4.1. Conjugation of lipidic amino acids and oligomers with hydrophilic compounds.

Lipidic amino acid/oligomer conjugates with hydrophilic compounds were prepared to increase the hydrophilicity of the lipidic amino acids with the aim of enhancing their solubility in aqueous systems, whilst maintaining their membrane affinity characteristics. Lactic acid **52a** was reacted with methyl 2-aminotetradecanoate **33r** using the HOBt assisted DCC coupling method as described in 2.1.4.1. to yield **54a**. Using the same procedure, glycolic acid was reacted with methyl esters **33p**, **33r** and **33af** furnishing conjugates **54c**, **54d** and **54f** respectively.

Saponification of methyl esters **54a** and **54d** furnished the free acids **54b** and **54e** in good yields. D-glucuronic acid was coupled to methyl ester **33r** furnishing product **54g**. The glyco-amino acid derivatives **54h** and **54i** and glycopeptide **54k** were synthesised by coupling hydroxyl protected monosaccharide 1,2:3,4-di-O-isopropylidene-D-gulonic acid with the methyl esters **33p**, **33r** and **33af** respectively. The free acid **54j** was obtained upon saponification of **54b**. Removal of the two isopropylidene groups protecting the sugar hydroxyl groups of **54j** with both acetic acid and trifluoroacetic acid resulted primarily in the partially-deprotected glyco-amino acid **54l**, the second isopropylidene group being resistant to cleavage after six hours reflux with acetic acid or one day with TFA at room temperature.



<u>54</u>	n	m	X	Y
<u>a</u>	11	1	HOCH(CH ₃)CO	OCH ₃
<u>b</u>	11	1	HOCH(CH ₃)CO	OH
<u>c</u>	7	1	HOCH ₂ CO	OCH ₃
<u>d</u>	11	1	HOCH ₂ CO	OCH ₃
<u>e</u>	11	1	HOCH ₂ CO	OH
<u>f</u>	11	2	HOCH ₂ CO	OCH ₃
<u>g</u>	11	1	X ₁	OCH ₃
<u>h</u>	7	1	X ₂	OCH ₃
<u>i</u>	11	1	X ₂	OCH ₃
<u>j</u>	11	1	X ₂	OH
<u>k</u>	11	2	X ₂	OCH ₃
<u>l</u>	11	1	X ₃	OH

2.4.2. Physio-chemical investigations.

The usual picture of the micelle in a non-aqueous solvent is that of the "inverted micelle". The polar head-groups of the surfactant monomer are present in the centre of the micelle with the hydrocarbon chains extending outwards into the solvent (Attwood and Florence 1983). The study of aggregation in organic solvents presents many more problems than are encountered with aqueous solutions. It was decided to use ¹H-NMR to study the physico-chemical behaviour of lipidic amino acid-systems in organic solvents.

Samples were dissolved in either chloroform (CDCl₃) or methanol (CD₃OD), at as high a concentration as possible, as determined by the solubility of the compound, then the solutions diluted to reach lower concentrations. All spectra were recorded in 0.6mls of solvent, at 25 °C, as a function of concentration for each of the compounds. Sample concentrations varied from - 320mM to 2.50mM for 54a, 954mM to 0.94mM for 54d, 657mM to 0.87mM for 54e, 210mM to 6.50mM for 54j and 124mM to 0.97mM for 54l. Plots of concentration versus chemical shift were obtained for each proton within the molecule, as well as for the water resonances which were associated with several of the compounds.

Recording the proton NMR spectrum as a function of concentration is an excellent means of monitoring the aggregation of fatty amino acid derivatives. The chemical shift is sensitive to

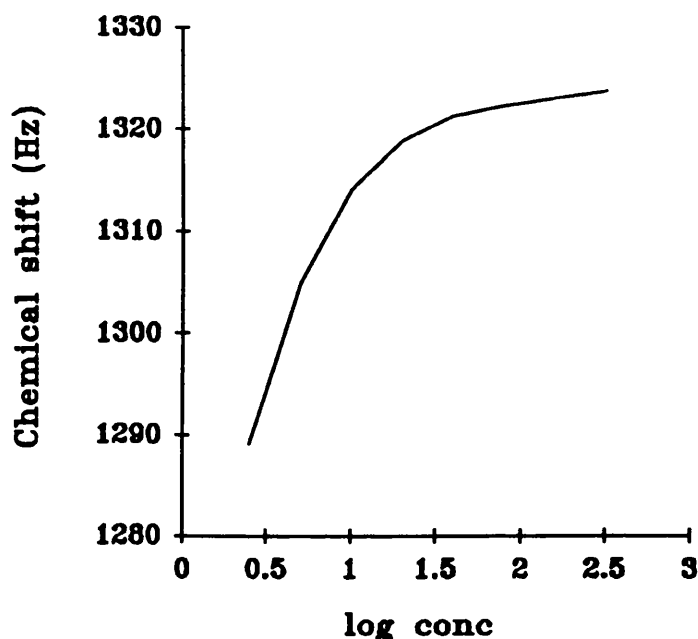


Fig.2.12 Chemical Shift of 2'-H of 54a

the chemical environment of each proton (Derome 1988) and can thus be used to observe changes in chemical environment due to liposome/micelle formation, aggregation or dissociation. For 54a in methanol (Fig.2.12) the chemical shift behaviour was monitored over the 320 to 2.50 mM range. The -CH proton from lactic acid was plotted in Fig.2.12 (identical results were obtained from other backbone protons). At the highest concentrations the chemical shift of this proton was independent of the concentration, but changed rapidly at lower concentrations. This type of curve was consistent with a fully aggregated form of the molecule at higher concentrations, with a gradual movement towards dissociation of the micelle (or aggregate) at lower concentrations. Even at the lowest concentrations (2.5mM), the curve did not level off, indicating that a stable plateau, consisting of monomers or the smallest stable forms of the aggregate, was not reached.

The chemical shift of the NH protons of 54d and 54e were independent of concentration at low molarities, but began to change rapidly at higher molarities (Fig.2.13). At low concentrations, these glycolic acid derivatives were monomers (or very small aggregates) which rapidly aggregated with increasing concentration. In this instance however, the curve did not

flatten at the highest concentrations (254mM and 657mM respectively), showing that no fully aggregated, stable form was achieved. The additional methyl group present in **54a** appeared therefore to promote aggregation at the expense of the monomer relative to that of the two glycolic acid derivatives.

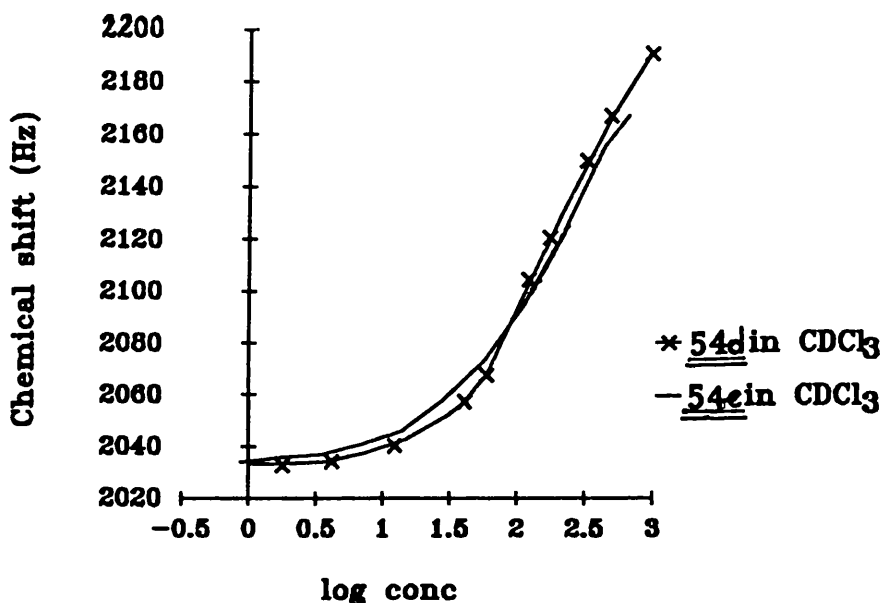


Fig.2.13 Chemical Shift of NH proton.

For monosaccharide derivatives with two and one protecting groups present, **54i** and **54e** respectively, the concentration studies yielded interesting results. Fig.2.14 shows the behaviour of the chemical shift of the associated water molecules over the whole of the concentration range studied. The two curves were identical in shape, indicating that both derivatives were exhibiting similar behaviour. The 'S' shaped curve was consistent with the formation of a large, stable aggregate at high concentrations, with a gradual reduction in size with decreasing concentration until monomers, or small aggregates were formed. The approximate critical micelle concentrations (CMC) were determined to be ~16mM for compound **54i** and ~40mM for **54j**. The differences between the two CMC values could be explained by considering the polarity of the sugar head groups. The least polar moiety, **54j**, showed a tendency to remain in the monomer form rather than aggregate, whereas the removal of one of these protecting groups, to form **54i**, allowed the possibility of hydrogen-bond formation and electrostatic interaction, via the free hydroxyl groups of the sugar ring. This resulted in micelle (or aggregate) formation at lower CMC values for **54i**.

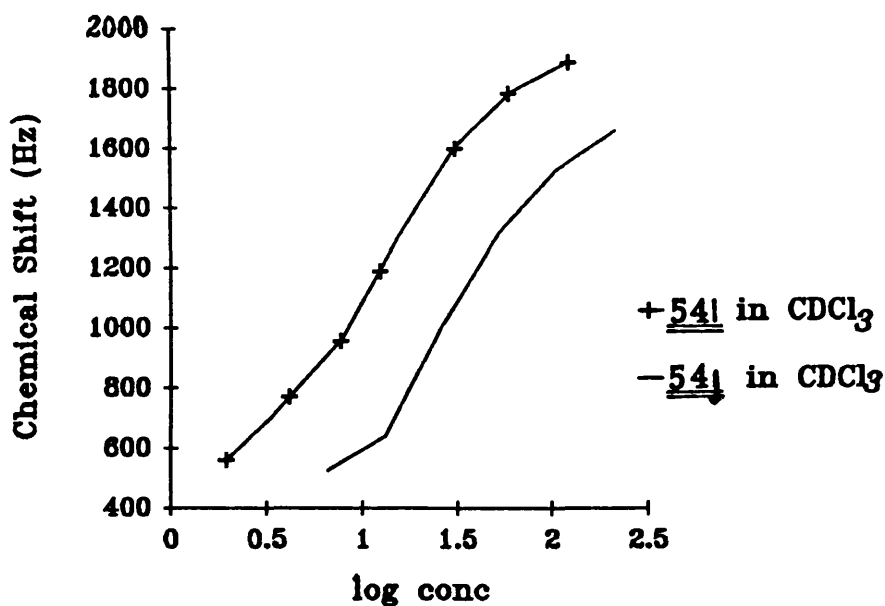


Fig.2.14 Chemical Shift of associated water.

The positions of levelling-off of chemical shift vs concentration plots (Fig.2.14) at low concentrations were also different, 54I forming monomers at lower concentrations than 54I, again most likely reflecting the increased interaction between molecules via their free -OH groups in 54I.

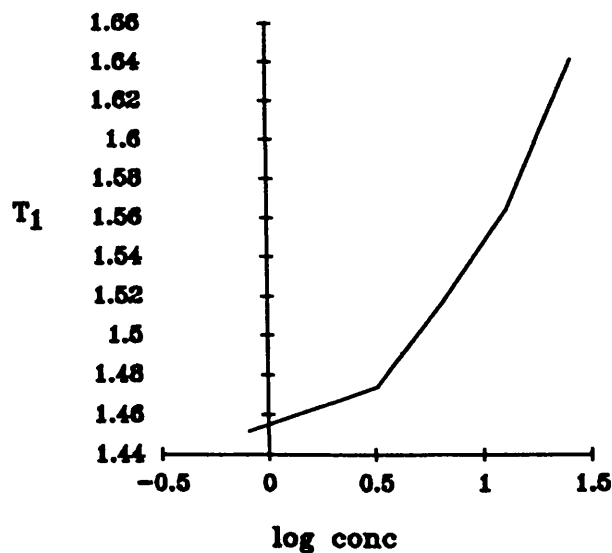


Fig.2.15 Spin Lattice Relaxation Time (T₁) of 54I

The chemical shift parameter however, is merely a reflection of changing chemical environment surrounding an atom, and is not a direct measure of molecular size. A more reliable

measure of this aggregation was to determine the spin-lattice relaxation time (Farrar and Becker 1971), or T_1 , at each concentration. The resulting graph of T_1 vs concentration should mirror that of the chemical shift vs concentration.

For derivative **54i**, with one protecting group on the sugar moiety, the plot of T_1 versus concentration (for the $-CH_3$ protons) is shown in Fig.2.15. This experiment gave excellent correlation with the shape of that in Fig.2.14, proving that the dimensions of the micelles were increasing with increasing concentration and that the observed changes in chemical shift were a reflection of this process.

2.5. Synthesis of chlorambucil lipidic conjugates.

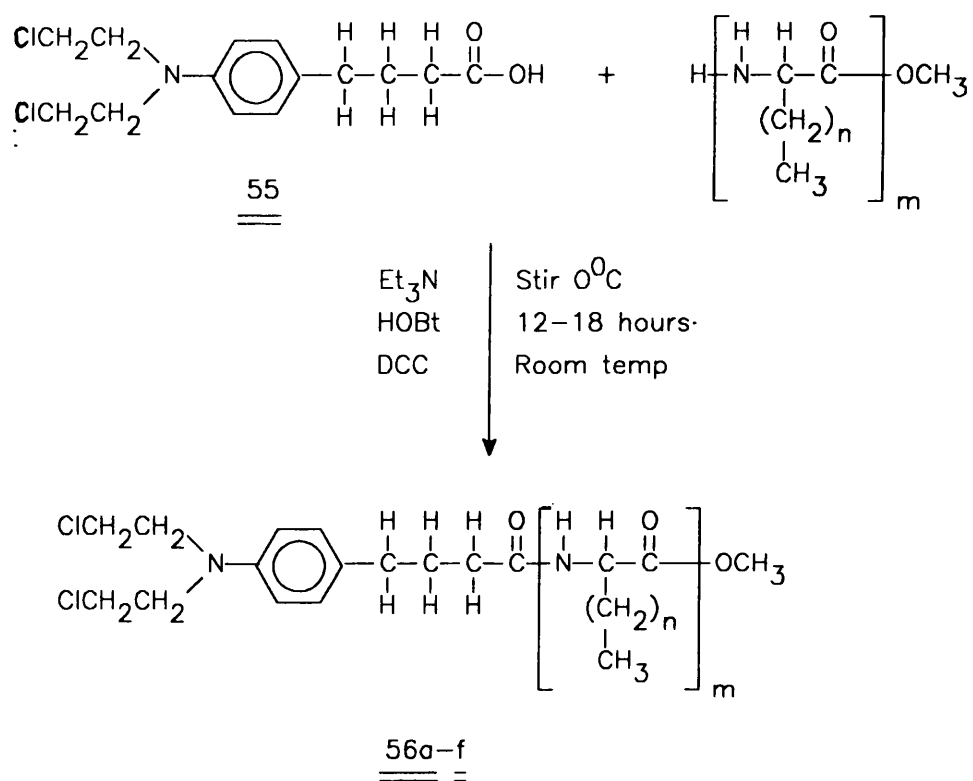
Chlorambucil **55** crosses the plasma membrane by passive diffusion (Harrap and Hill, 1970), and although its oral absorption is high (McLean *et al.* 1979) several attempts have been made to modify its uptake to increase selectivity and reduce toxicity to increase its therapeutic index (Farmer *et al.* 1979, Lee *et al.* 1986).

The lipidic amino acids and oligomers have the capacity to be chemically conjugated to the butyric acid functional group of chlorambucil via several forms of linkage that may be biologically stable, or unstable. The conjugate formed will possess membrane-like character and should maintain the ability to pass across the cell membrane.

Three types of novel chlorambucil lipidic conjugates were synthesised. Chlorambucil conjugates with amide linkages **56a-f**, chlorambucil conjugates with ester linkages **57a-e** and a conjugate consisting of a spacer molecule **58a-b**, resulting in an ester/amide bifunctional linkage.

2.5.1. Synthesis of chlorambucil lipidic amino acid/peptide conjugates.

Chlorambucil **55** was reacted with a series of C-protected lipidic amino acids **33p**, **33r**, **33s** and C-protected lipidic peptides **33af** and **33ag**, employing the EADC synthetic strategy described in 2.1.4.1. to furnish conjugates **56a-56f** (Scheme 22). The conjugates were purified on preparative TLC.



Scheme 22. Synthesis of Chlorambucil conjugates 56a–f.

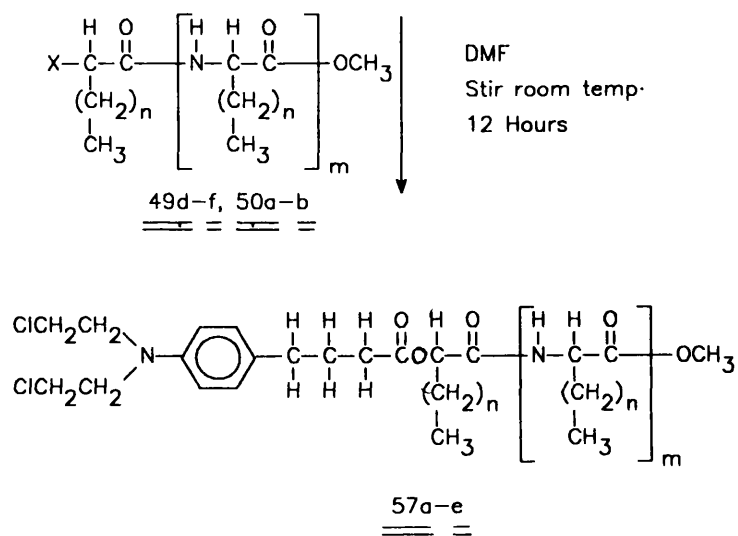
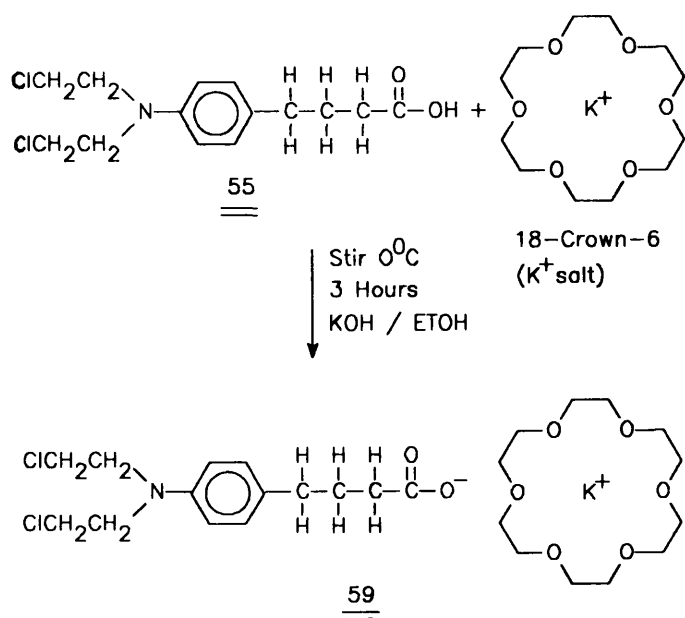
<u>56</u>	n	m
<u>a</u>	7	1
<u>b</u>	11	1
<u>c</u>	13	1
<u>d</u>	11	2
<u>e</u>	13	2
<u>f</u>	11	3

2.5.2. Synthesis of chlorambucil lipidic acid conjugates.

To aid the formation of the ester bond between chlorambucil **55** and the appropriate methyl-2-bromoalkanoate **49d-f** and methyl-2-bromo lipidic peptides **50a-b** the carboxylic acid terminus of chlorambucil was activated. Chlorambucil **55** was treated with potassium hydroxide in ethanol (96%) in the presence of the macrocyclic ether 18-crown-6, to produce a crown ether complex of the potassium salt of chlorambucil **59**. This potassium salt of chlorambucil crown ether **59** was reacted with the methyl-2-haloalkanoates **49d-f** and bromonated lipidic peptides **50a** and **b** in anhydrous dimethylformamide furnishing the chlorambucil lipidic acids **57a-c** and chlorambucil lipidic peptides **57d-e** respectively (Scheme 23). The conjugates were purified by preparative TLC in good yield and purity.

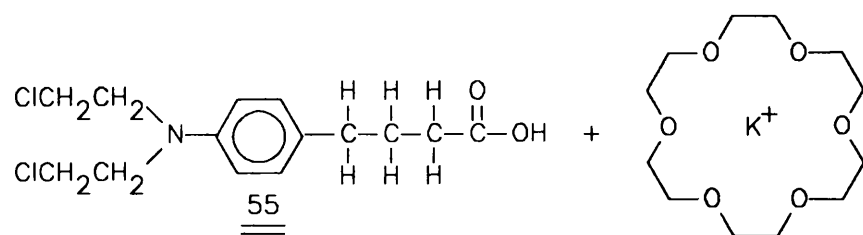
2.5.3. Synthesis of chlorambucil lipidic amino acid/peptide with spacer moiety.

The first stage in the synthesis was the formation of methyl-2-bromoacetyl derivatives **51a** and **51b** by coupling bromoacetic acid to methyl protected lipidic amino acid **33r** and peptide **33af** respectively employing the DCC coupling strategy described in 2.1.4.1. The second stage was the activation of the carboxyl terminus of chlorambucil by crown ether complexation as described in 2.3.2. For the final stage the potassium salt of chlorambucil crown ether **59** was reacted with methyl-2-bromoacetyl derivatives **51a** and **51b** in anhydrous DMF to furnish chlorambucil lipidic conjugates with spacer moieties **58a** and **b**, which was purified by preparative TLC.



Scheme 23. Crown ether assisted coupling of chlorambucil to methyl-2-haloalkanoates.

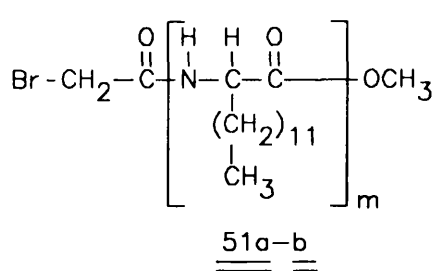
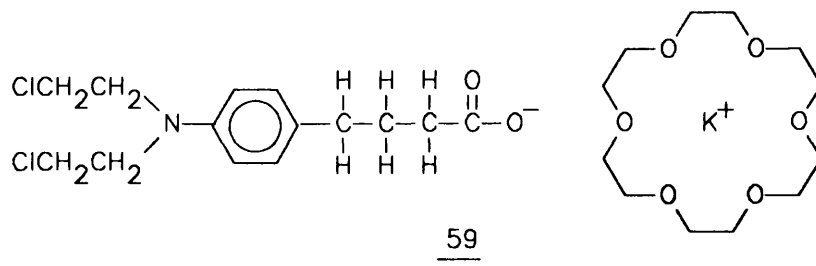
<u>57</u>	n	m
<u>a</u>	5	-
<u>b</u>	11	-
<u>c</u>	13	-
<u>d</u>	11	1
<u>e</u>	11	2



Stir 0°C
 3 Hours
 KOH/ETOH

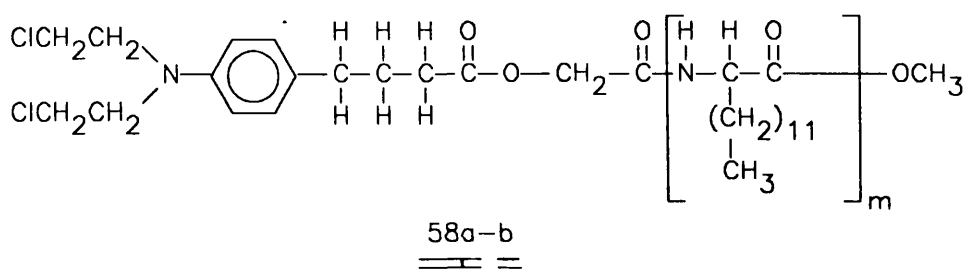
↓

18-Crown-6
 (K⁺ salt)



DMF
 Stir room temp.
 12 Hours

↓



Scheme 24. Synthesis of Chlorambucil conjugates with spacer moiety

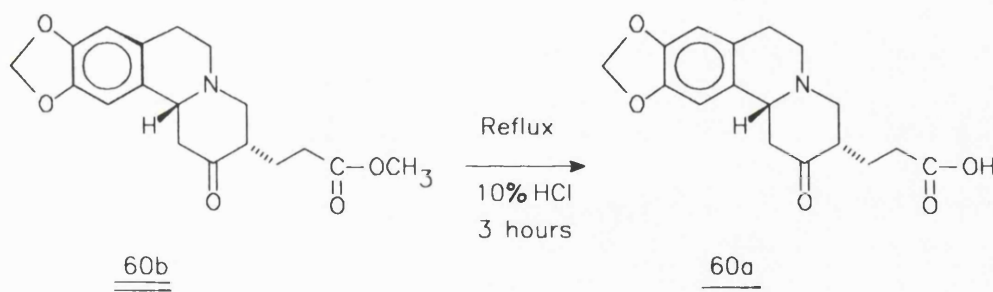
<u>58</u>	n	m
<u>a</u>	11	-
<u>b</u>	11	1

2.6. Synthesis of benzoquinolizine lipidic conjugates.

Benzoquinolizine acid **60a** and its methyl ester **60b** are members of a family of alkaloids their therapeutic potential being inhibited by their poor oral absorption (Toth *et al.* 1991). Benzoquinolizine is best stored as its methyl ester to limit decomposition, the free acid can be obtained from the methyl ester derivative by hydrolysis. Benzoquinolizine acid has a free acid functional group located on the propyl side chain which may be employed for conjugation to the free amino group of the appropriate lipidic amino acid/peptide methyl esters. Benzoquinolizine is a good model system to investigate the ability of lipidic amino acids and peptides to aid absorption of poorly absorbed drugs. Several conjugates were synthesised to encompass a range of lipophilicities so as to enable biological investigations to be undertaken to establish the optimal lipidic amino acid side chain length (n)/residue number (m) required for maximum uptake under both *in vitro* and *in vivo* environments.

2.6.1. Acid hydrolysis of benzoquinolizine methyl ester.

Benzoquinolizine methyl ester **60b** was treated with hydrochloric acid under reflux to furnish benzoquinolizine acid **60a**.

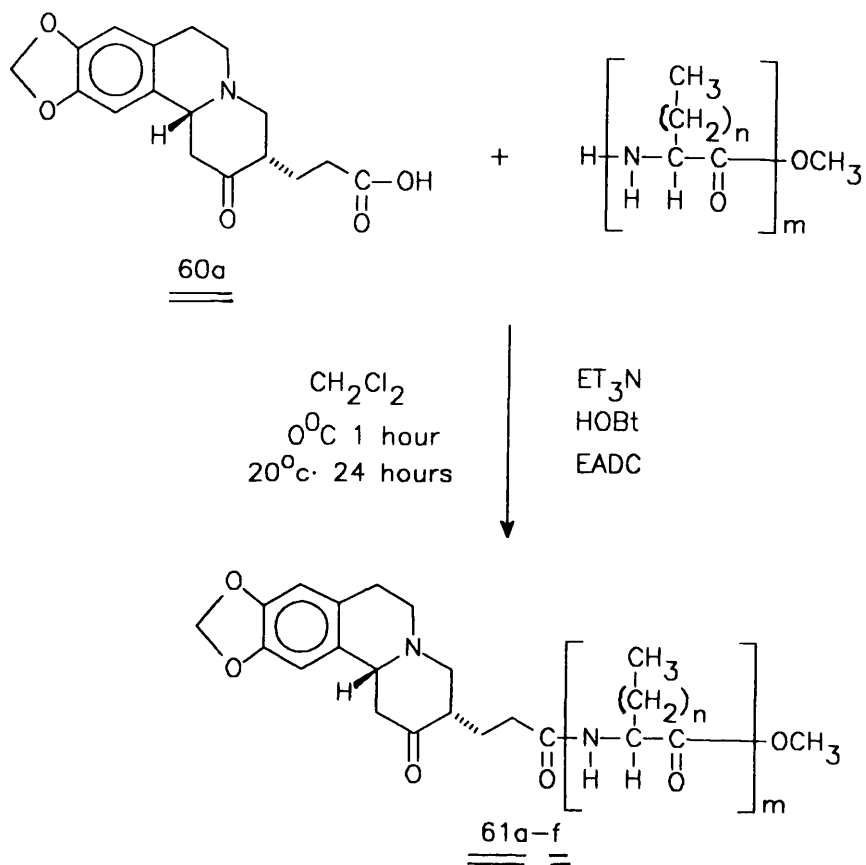


Scheme 25. Hydrolysis of methyl ester group

2.6.2. Synthesis of benzoquinolizine lipidic amino acid/peptide conjugates.

Benzoquinolizine acid **60a** was coupled to the appropriate C-protected lipidic amino acid/peptide **33o**, **33p**, **33r**, **33ad**, **33af** and **33ar** utilizing the EADC/HOBt assisted coupling method described in 2.1.4.1. The crude reaction mixtures were purified by preparative TLC

furnishing the desired conjugates **61a-f** (Scheme 26).



Scheme 26. Conjugation of benzoquinolizine to the lipidic amino acid system

<u>61</u>	n	m
<u>a</u>	5	1
<u>b</u>	7	1
<u>c</u>	11	1
<u>d</u>	7	2
<u>e</u>	11	2
<u>f</u>	11	3

2.6.3. Reduction of benzoquinolizine lipidic conjugates to alcohols.

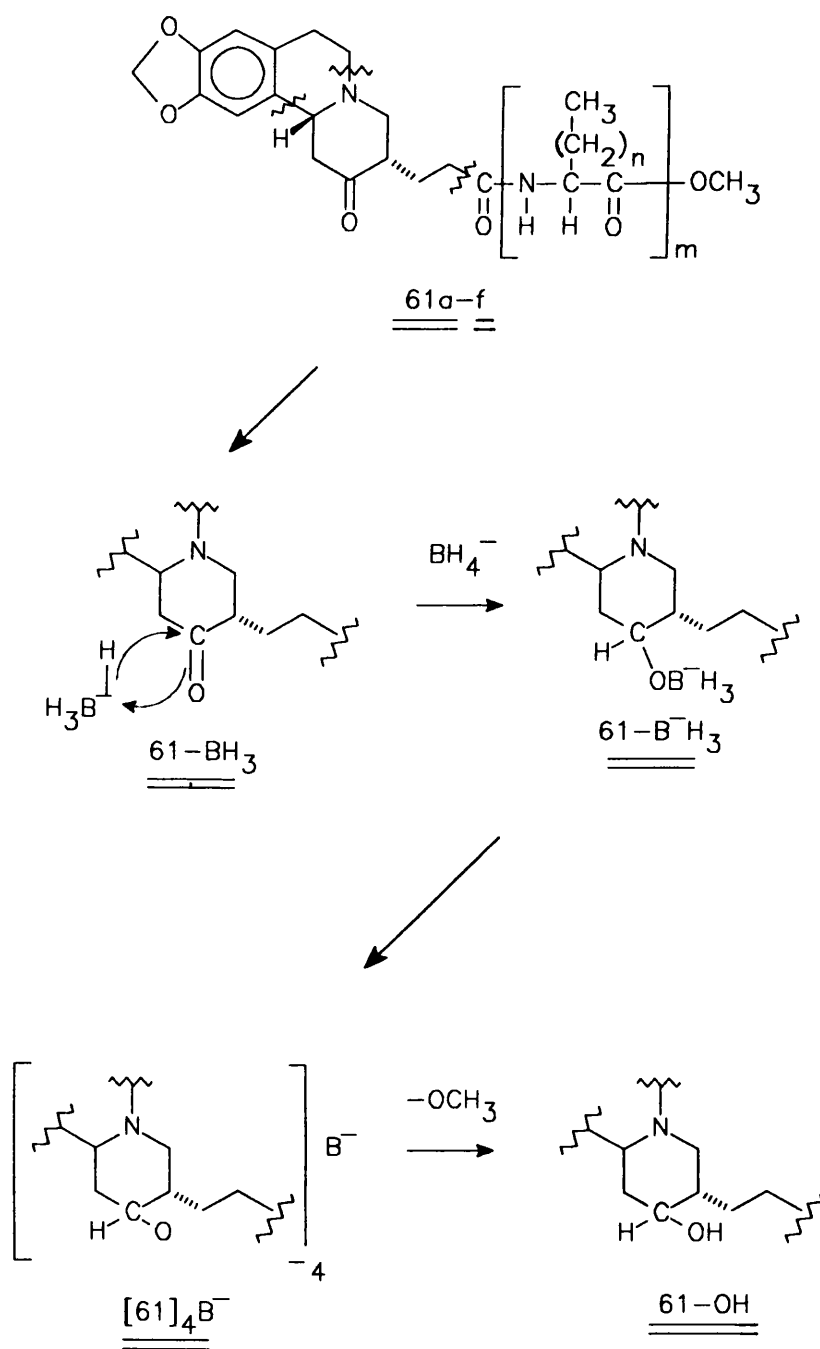
Alcohol derivatives of the benzoquinolizine conjugates were prepared by reduction with sodium borohydride of the secondary ketone function at the C2 position of the benzoquinolizine molecule (Scheme 27) and also reduction of the methyl ester function to an alcohol (Scheme 28).

2.6.3.1. Reduction of ketone function at C2 position of benzoquinolizine moiety.

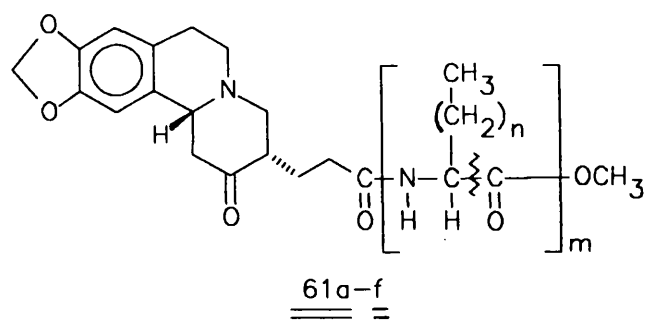
The mechanism of the reduction is shown in Scheme 27. Sodium borohydride is a powerful reducing agent and the BH_4^- complex formed in the reaction mixture acts as a powerful hydride ion (H^-) donor. The nucleophile BH_4^- irreversibly donates H^- to the carbonyl carbon atom C2 of the benzoquinolizine conjugates **61a-f**, the residual BH_3 then complexes with its oxygen atom to form a complex **61-B'H₃**. The complex then dissociates to form **[61]_AB⁻** and BH_4^- . Intermediate **[61]_AB⁻** is converted into the alcohol product **61-OH** on treatment with a protic solvent. Thus, one of the two protons in the reduction is provided by BH_4^- the other by the solvent.

2.6.3.2. Reduction of the methyl ester function.

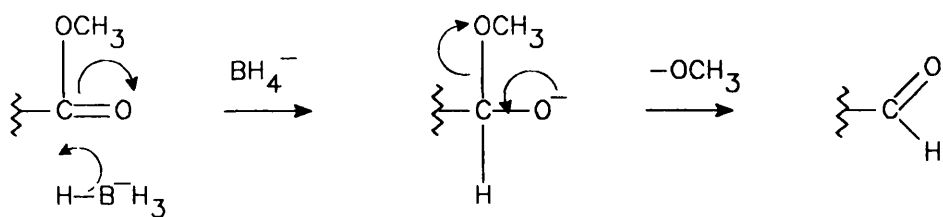
The methyl ester functional group of the benzoquinolizine lipidic conjugates **61a-f** was reduced to the corresponding primary alcohol by the excess sodium borohydride employed to ensure complete reduction, resulting in the formation of conjugates **62a-f**. The methyl ester function is first reduced to an aldehyde which is further reduced to a primary alcohol as described in 2.6.3.1. (Scheme 28).



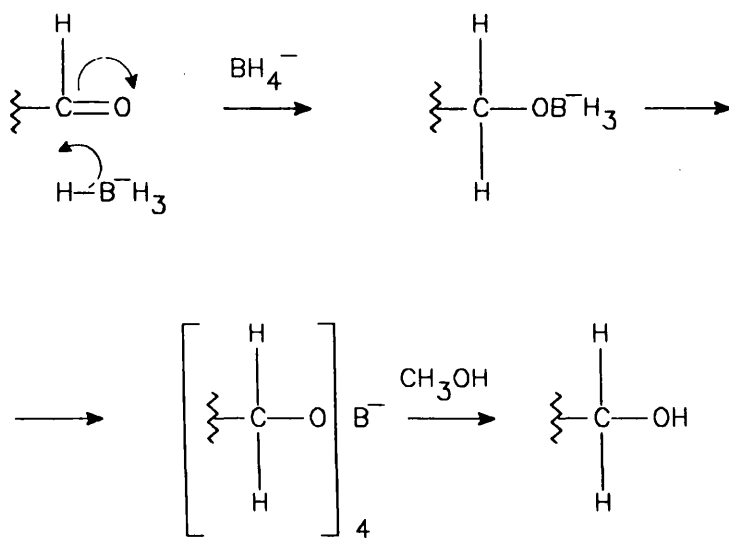
Scheme 27. Reduction of keto function
to secondary alcohol



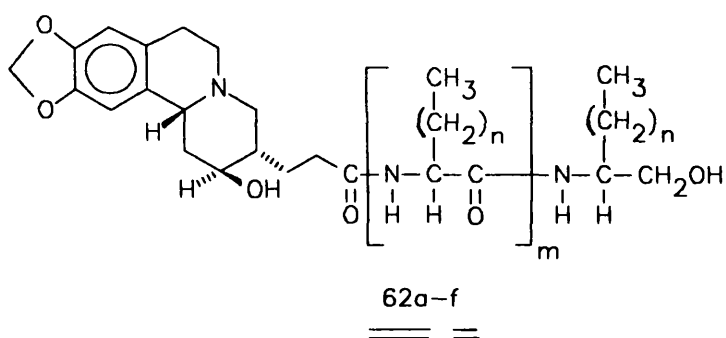
a. Reduction to aldehyde.



b. Reduction of the aldehyde to a primary alcohol.



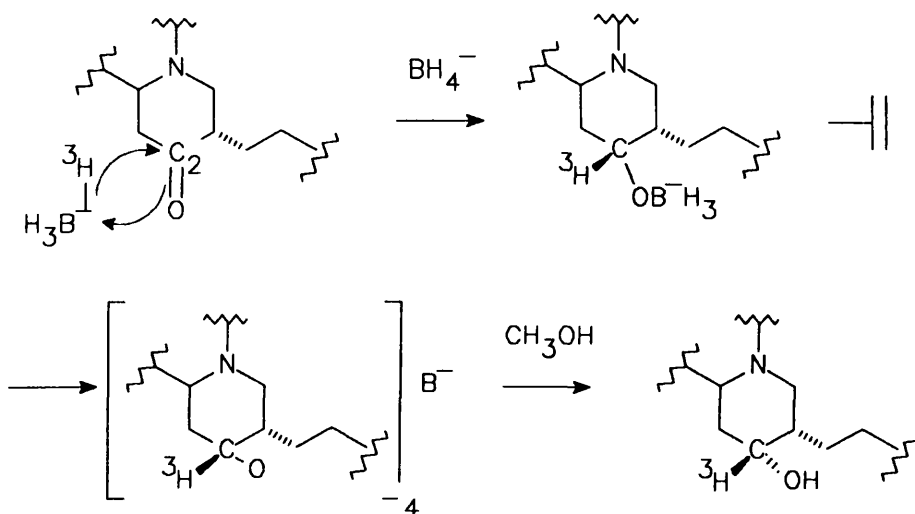
Scheme 28. Reduction of the methyl ester group



<u>62</u>	n	m
<u>a</u>	5	-
<u>b</u>	7	-
<u>c</u>	11	-
<u>d</u>	7	1
<u>e</u>	11	1
<u>f</u>	11	2

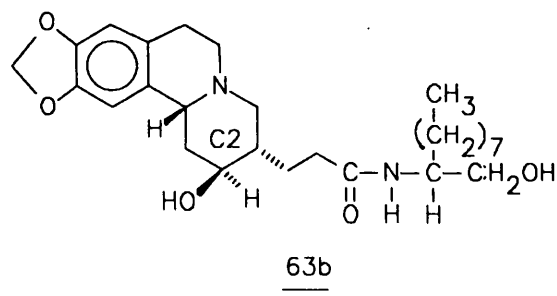
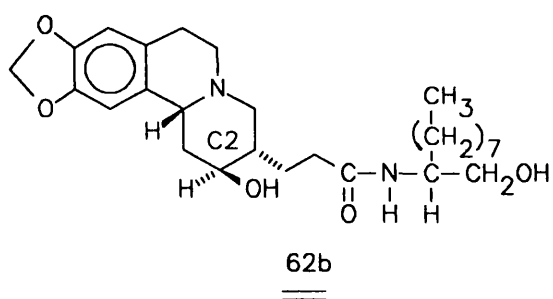
2.6.4. Tritiated benzoquinolizine derivatives.

To enable biological evaluations of the benzoquinolizine derivatives to be undertaken a radiolabel was introduced into the molecule. Radiolabel was introduced by employing a small quantity of tritiated sodium borohydride for the reduction of a small fraction of the benzoquinolizine conjugates present in the reaction mixture (Scheme 29). The ready exchangability of the alcoholic proton allows almost exclusive attachment of the radiolable to the carbon ring atom in position C2 of the benzoquinolizine conjugate. An excess of cold sodium borohydride was added at a later stage to complete reduction of all the benzoquinolizine conjugate molecules present as described in 2.6.3 furnishing the radiolabelled benzoquinolizine lipidic conjugates **62a^{*}-f^{*}** respectively.



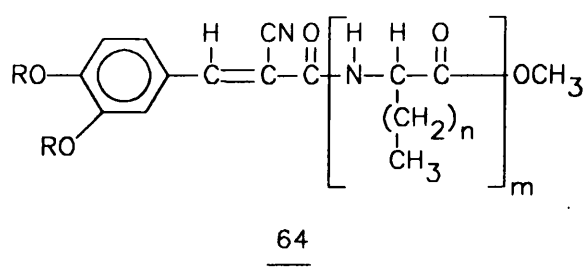
Scheme 29. Introduction of radiolabel by reduction.

The configuration of the hydroxyl group at position C2 of conjugates **62a-f** was equatorial, as demonstrated by the chemical shift (3.4-3.5ppm) and large axial-axial coupling constant of the proton in position C2 (Toth *et al.* 1978) in the ^1H -NMR spectrum of **62b**. The semi-purified product revealed a minor resonance at 3.9ppm which was indicative of the presence of a small amount of the thermodynamically less favoured, axially-substituted hydroxyl product **63b**.



2.7. Membrane active signal transduction inhibitors.

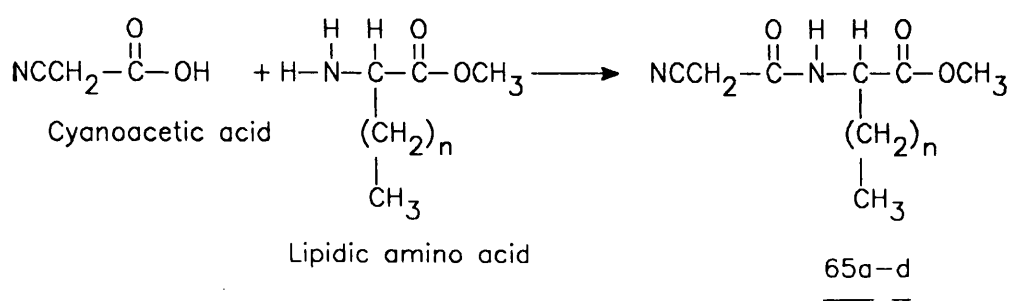
The aim of the work undertaken was to produce a series of compound that were envisaged to be membrane active signal transduction inhibitors. The desired general structure of the compounds required the presence of a 3,4-disubstitutedbenzyl-, cyano- and lipidic group (**64**). The lipidic amino acid moiety will enable the molecule to pass into/onto the membrane (Gibbons 1988, Toth *et al.* 1992a,b) whilst the 3,4 disubstitutedbenzyl (especially 3,4 dihydroxybenzyl) and cyano groups are required for the inhibitory activity (Lyll *et al.* 1989).



To produce compounds of the general structure **64** exploratory experiments were performed with the starting materials 3,4-disubstituted benzaldehyde derivatives, cyano acetic acid and methyl protected lipidic amino acids to establish the most appropriate route of synthesis.

2.7.1. Synthesis of methyl(N-cyanoethanoyl)iminoalkanoates.

Cyanoacetic acid was conjugated to the C-protected lipidic amino acids **33p-s** using HOBt assisted EADC coupling method as described in **2.1.4.1**, furnishing the methyl(N-cyanoethanoyl)iminoalkanoates **65a-d** in good yields (Scheme 30).



Scheme 30.

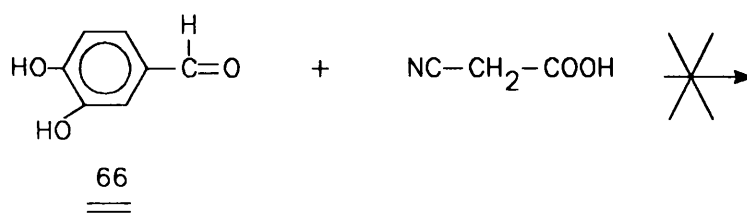
<u>65</u>	n
<u>a</u>	7
<u>b</u>	9
<u>c</u>	11
<u>d</u>	13

2.7.2. Carbonyl-methylene condensation reaction.

The approach employed for conjugating the benzaldehyde functional group to the cyanoethanoyl functional group was a carbonyl-methylene condensation reaction (Hein *et al.* 1960, Patai and Isreali 1960a,b; Patai and Zabicky 1960a,b, Patai *et al.* 1960) based on the Knoevangel-Doebner condensation (Patai *et al.* 1954, Cope *et al.* 1941).

2.7.2.1. 3,4-Dihydroxybenzaldehyde and cyanoacetic acid.

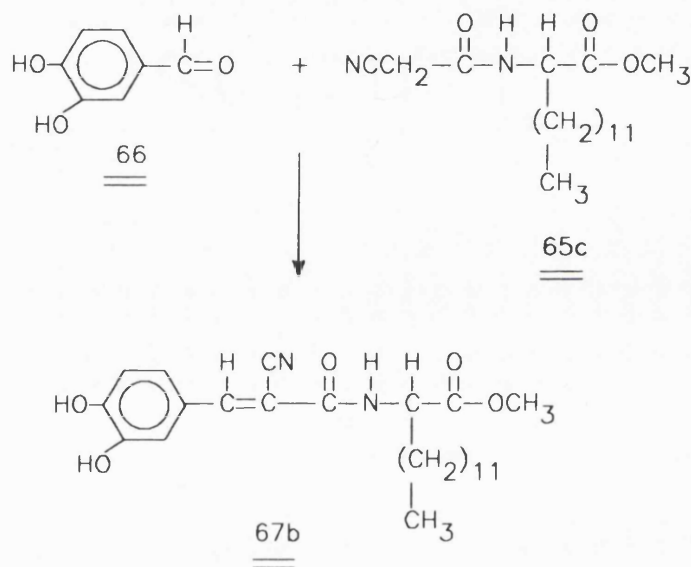
No condensation occurred between 3,4-dihydroxybenzaldehyde 66 and cyanoacetic acid (Scheme 31) under the conditions listed in Table 5.1.



Scheme 31.

2.7.2.2. 3,4-Dihydroxybenzaldehyde and methyl-(N-cyanoethanoyl) iminotetradecanoate.

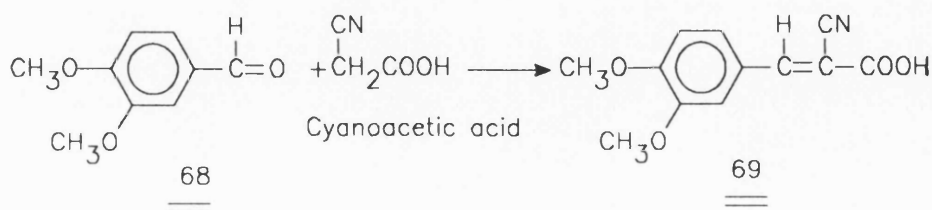
3,4-Dihydroxybenzaldehyde **66** was reacted with methyl-(N-cyanoethanoyl) iminotetradecanoate **65c** under the conditions shown in Table 5.1. The yields obtained were extremely low 0-7% thus, the method was not suitable for synthesising methyl-3-[(3,4-dihydroxybenzyl)-2-cyanoprop-2-enoyl]-iminoalkanoates (Scheme 32).



Scheme 32.

2.7.2.3. 3,4-Dimethoxybenzaldehyde and cyanoacetic acid.

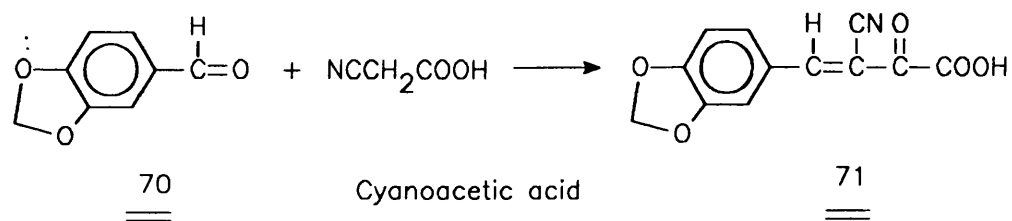
3,4-Dimethoxybenzaldehyde **68** was condensed with cyanoacetic acid successfully under the conditions shown in Table 5.1 to produce 3-(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoic acid **69** (Scheme 33).



Scheme 33.

2.7.2.4. 3,4-Methylenedioxybenzaldehyde and cyanoacetic acid.

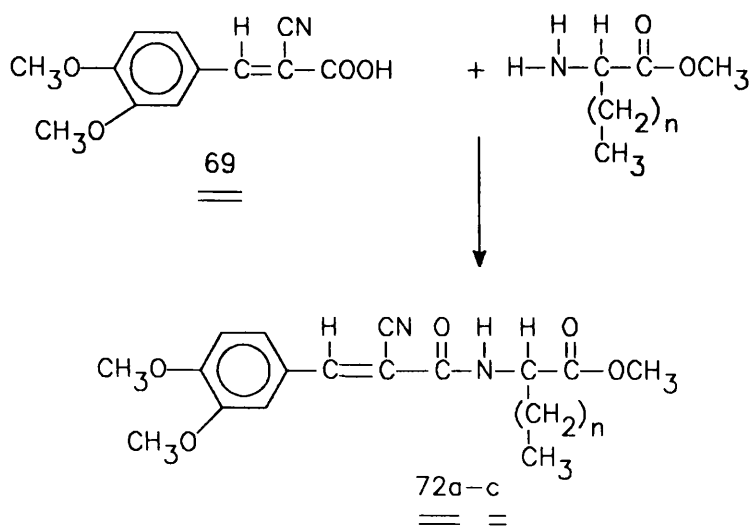
Under selected conditions shown in table 5.1 3,4-Methylenedioxybenzaldehyde **70** was condensed with cyanoacetic acid successfully under the conditions shown in Table 2.8 to produce 3-(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoic acid **71** (Scheme 34).



Scheme 34.

2.7.3.1. Coupling of 3-(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoic acid to lipidic amino acid methyl esters.

3-(3,4-Dimethoxybenzyl)-2-cyanoprop-2-enoic acid **69** was coupled to the lipidic amino acid methyl esters **33p**, **33r** and **33u** employing 1-hydroxybenzotriazole assisted coupling as described in 2.1.4.1. furnishing methyl-3-[(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoyl]-iminoalkanoates **72a-c** (Scheme 35).

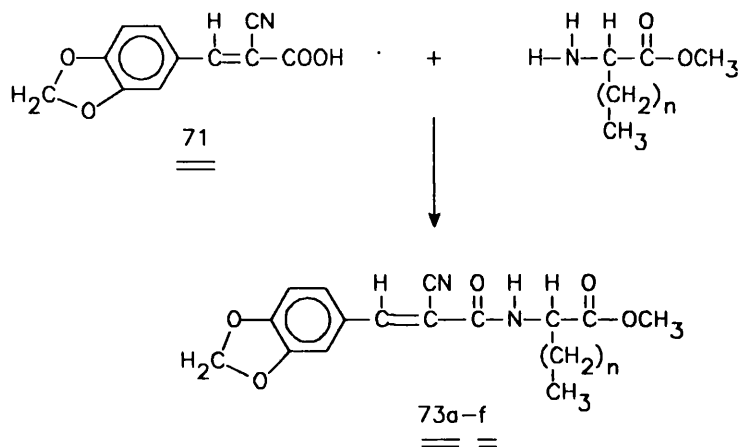


Scheme 35.

<u>72</u>	n
<u>a</u>	7
<u>b</u>	11
<u>c</u>	17

2.7.3.2. Coupling of 3-(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoic acid to lipidic amino acid methyl esters.

3-(3,4-Methylenedioxybenzyl)-2-cyanoprop-2-enoic acid **71** was coupled to the lipidic amino acid methyl esters **33o-s** and **33u** employing 1-hydroxybenzotriazole assisted coupling as described in 2.1.4.1. furnishing methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]-iminoalkanoates **73a-f** (Scheme 36).



Scheme 36.

<u>73</u>	n
<u>a</u>	5
<u>b</u>	7
<u>c</u>	9
<u>d</u>	11
<u>e</u>	13
<u>f</u>	17

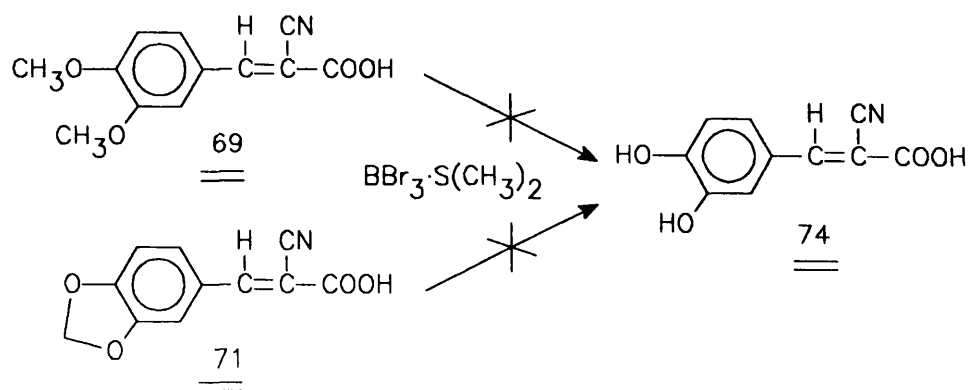
2.7.4. Cleavage of ether linkage.

The ether bonds of the methylenedioxy and methoxy functional groups have to be cleaved with a reagent that would not attack other functional groups present in conjugates **72a-c** and **73a-f**. Boron tribromide and boron tribromide dimethylsulphide complex were used in exploratory experiments with 3-(3,4 dimethoxybenzyl)-2-cyanoprop-2-enoic acid (**69**) and 3-(3,4 methylenedioxybenzyl)-2-cyanoprop-2-enoic acid (**71**) in an attempt to produce 3-(3,4 dihydroxybenzyl)-2-cyanoprop-2-enoic acid (**74**). Boron tribromide has been regarded as a reagent of choice for the cleavage of ethers as it is able to cleave ether protecting groups without affecting a large number of other functional groups (Blatt and Kulkarni 1983). One of the difficulties in the use of boron tribromide is its tendency to fume profusely in air. To overcome these difficulties boron tribromide-methyl disulphide complex was employed for the initial reactions.

2.7.4.1. Boron tribromide dimethylsulphide complex.

Boron tribromide dimethylsulphide complex is a solid, stable in air and therefore safer and easier to handle than boron tribromide itself. Boron tribromide is liberated from the complex in solution (Willard and Fryhle 1980).

Boron tribromide dimethylsulphide complex (3 equivalents) was added to 3-(3,4 dimethoxybenzyl)-2-cyanoprop-2-enoic acid (**69**) and 3-(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoic acid (**71**) in a nitrogen atmosphere under the selected conditions shown in Table 5.2 (Scheme 36), based on the general procedure of Willard and Fryhle (1980).



Scheme 37.

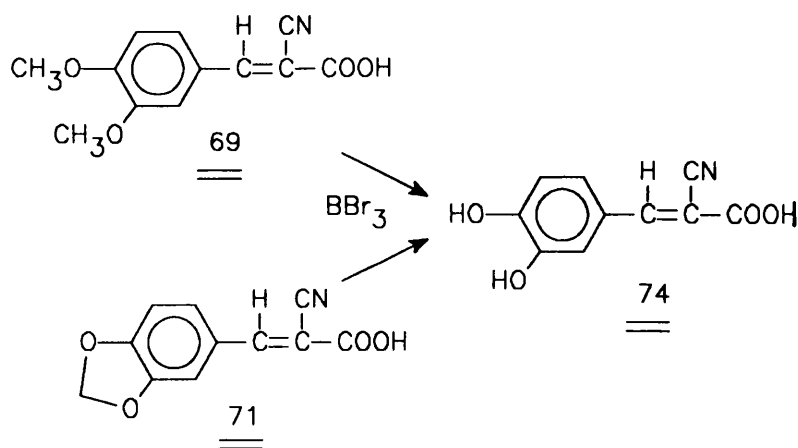
Unfortunately neither compound was cleaved, thus boron tribromide in dichloromethane 1M solution under a nitrogen atmosphere within a glove box was employed.

2.7.4.2. Boron tribromide.

Boron-tribromide was first used to cleave ether bonds by Benton and Dillon (1942), the method was then reintroduced by McOmie and co-workers in 1963. The reaction is carried out in an appropriate solvent at room temperature, work-up involves hydrolysis with water and extraction with organic solvent. If milder conditions are required, the reactants are mixed at temperatures ranging from -80°C to -20°C and allowed to warm to room temperature (Benton and Dillon 1942, McOmie and Watts 1963, McOmie *et al.* 1969).

2.7.4.2.1. Cleavage of (3,4-dimethoxybenzyl)-2-cyanoprop-2-enoic acid and (3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoic acid using boron tribromide.

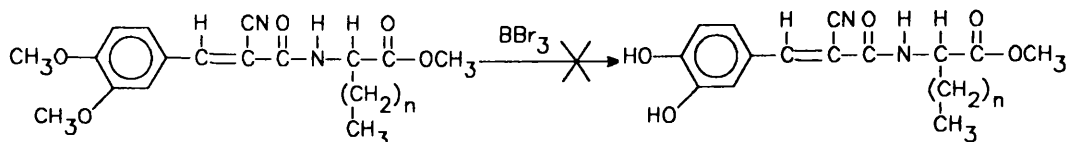
Boron tribromide in dichloromethane was added to 3-(3,4 dimethoxybenzyl)-2-cyanoprop-2-enoic acid (**69**) and 3-(3,4 methylenedioxybenzyl)-2-cyanoprop-2-enoic acid (**71**) respectively in a nitrogen atmosphere in an effort to furnish the dihydroxy product. Both reactions furnished the product in low yields.



Scheme 38.

2.7.4.2.2. Boron tribromide cleavage of methyl[(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoyl]-iminoalkanoates.

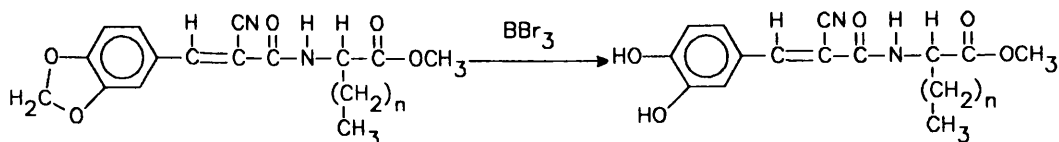
Boron tribromide in dichloromethane (1M) (4 equivalents) was added to 3-(3,4 dimethoxybenzyl)-2-cyanoprop-2-enoyl]-iminoalkanoates **72a** and **72b** in a nitrogen atmosphere and refluxed for 12hrs (Scheme 39). Unfortunately no pure product could be isolated from the reaction mixture.



Scheme 39

2.7.4.2.3. Boron tribromide cleavage of methyl-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]-iminoalkanoates.

Boron tribromide in dichloromethane (1M) (4 equivalents) was added to 3-(3,4 methylenedioxybenzyl)-2-cyanoprop-2-enoyl]-iminoalkanoates **73b** and **73d** in a nitrogen atmosphere and refluxed for 12hrs (Scheme 40) furnishing the dihydroxy products **68a** and **68b** in very low yields and poor purity.



Scheme 40

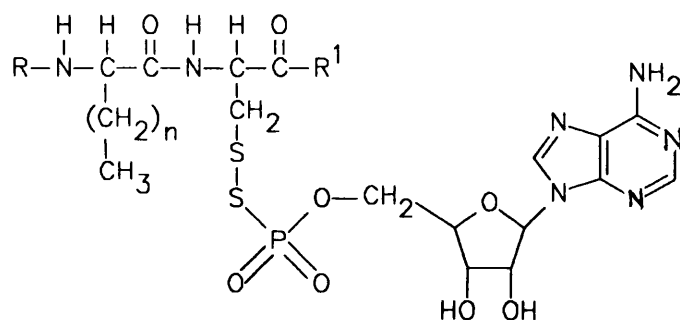
2.7.5. Coupling of 3-(3,4-dihydroxybenzyl)-2-cyanoprop-2-enoic acid to lipidic amino acid methyl esters.

3-(3,4 dihydroxybenzyl)-2-cyanoprop-2-enoic acid was coupled to lipidic amino acid methyl esters **33p** and **33r** using the DCC coupling as described in 2.1.4.1. to furnishing the products **67a** and **67b** respectively in very low yields (Scheme 41).



2.8. Antisense oligonucleotides with therapeutic potential.

103

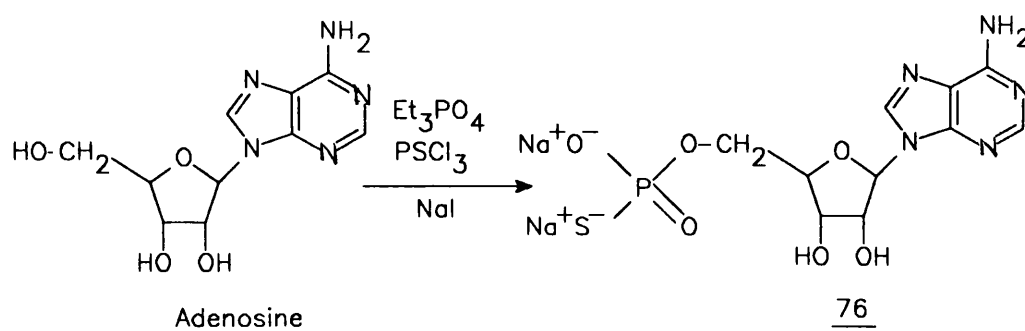


where R = protecting group or H
 R^1 = protecting group or OH

75

2.8.1. Synthesis of adenosine 5'monophosphorothionate 76.

Adenosine 5'monophosphorothionate 76 was synthesised from adenosine using the method of Murry and Atkinson (1963), which was based on the work of Yoshikawa *et al.* (1967) where nucleoside 5'phosphates have been produced from unprotected nucleosides by phosphorylation with POCl_3 in the presence of triethylphosphate (Scheme 42).



Scheme 42. Synthesis of adenosine 5'monophosphorothionate

2.8.2. Disulphide bond formation between cysteine ethyl ester, adenosine 5'monophosphorothionate and model thiol compounds.

There are several methods of oxidising thiols to form a disulphide bridge including air oxidation (Reid *et al.* 1958), halogens (bromine and iodine) (Sandler and Karo 1972),

dimethylsulphoxide (Yiannios and Karabinos 1963) and lead tetra-acetate (Field and Lawson 1958). The method employed was air oxidation in alkaline solution, and several synthesis were undertaken between model thiol compounds (propanethiol and octadecylmercaptan), cysteine and adenosine 5'monophosphorothionate to establish if a mixed disulphide could be produced in satisfactory yields (Scheme 43).



R = Cysteine derivatives

R' = Propanethiol

Benzymercaptan

Octadecylmercaptan

Adenosine 5'monophosphorothionate

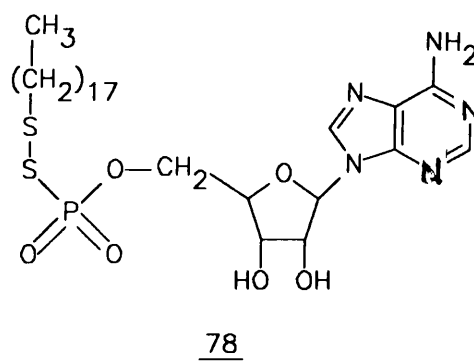
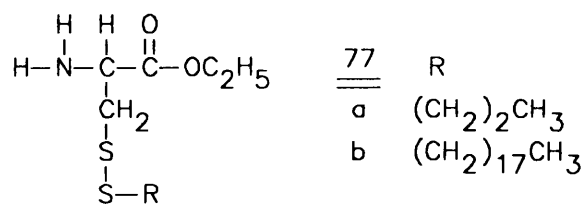
Scheme 43. disulphide bond formation.

2.8.2.1. Disulphide bond formation between cysteine ethyl ester and model thiol compounds.

Mixed disulphides **77a** and **b** were obtained by reacting cysteine ethyl ester with propanethiol and octadecylmercaptan respectively in the presence of air in an alkaline environment.

2.8.2.2. Disulphide bond formation between octadecylmercaptan and adenosine 5'monophosphorothionate.

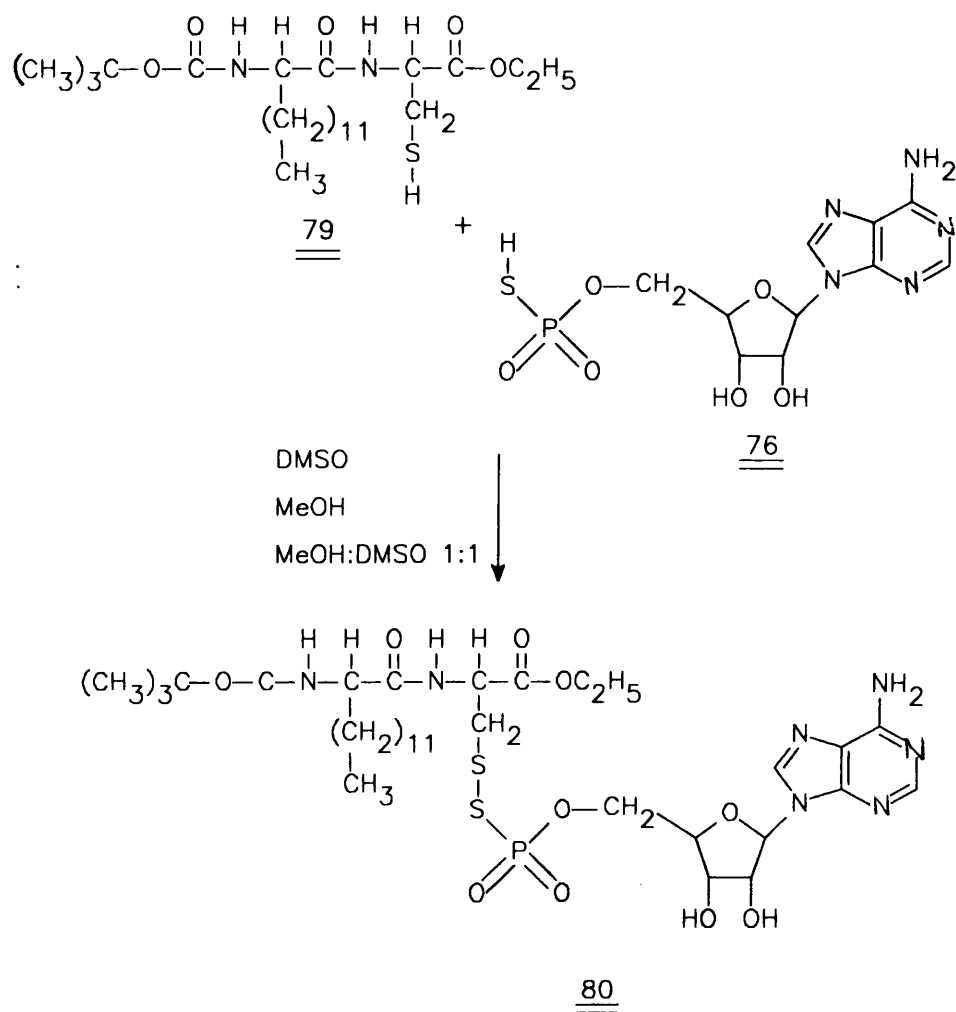
Octadecyl-adenosine 5'phosphorodisulphide **78** was synthesised by reacting octadecylmercaptan with adenosine 5'monophosphorothionate as described in **2.8.2.1**.



The method described in **2.8.2.** above was successful in producing mixed disulphides between mixtures of cysteine ethyl ester, adenosine 5'monophosphorothionate and mercaptans (propanethiol and octadecylmercaptan), therefore it was used in the attempt to synthesise BOC-tetradecanoyl-L-cysteine ethyl ester-adenosine 5'monophosphorodisulphide **80**.

2.8.3. Synthesis of BOC-tetradecanoyl-L-cysteine ethyl ester-adenosine 5'monophosphorodisulphide 80.

BOC-tetradecanoyl-L-cysteine ethyl ester was stirred with adenosine 5'monophosphorothionate as described in **2.8.2.** using methanol, dimethylsulphoxide and methanol:dimethylsulphoxide 1:1 as the solvent (Scheme 44).



Scheme 44. Disulphide bond formation between adenosine 5'-monophosphorothionate and Boc-tetradecanoyl-L-cysteine ethyl ester

Unfortunately only a small percentage of the desired product was isolated from the reaction mixture (methanol solvent), the majority of Boc-tetradecanoyl-L-cysteine ethyl ester oxidising to itself. Thus, other oxidising conditions are required to enable synthesis of **80** in sufficient yields to extend the use of the lipidic amino acids as a delivery system for antisense oligonucleotides.

Chapter 3. Biology, Results and Discussion.

3.1. Cellular uptake studies of lipidic amino acid and oligomers on *Ehrlich ascites* tumour cells.

The results of cellular uptake experiments on *Ehrlich ascites* tumour cells are in good correlation with that of the results obtained previously on rat erythrocytes, rat hepatocytes and *Escherichia coli* (Toth *et al.* 1991). The absorption was dependent upon the concentration of the compounds, the incubation time and the nature of the lipidic amino acids and oligomers. The cellular uptake of the lipidic amino acid **33r** was substantially greater than that of the dimer **33y**, trimer **33al** and the tetramer **33au**. This trend was observed at all concentrations (Fig. 3.1-3.3). The uptake of **33r** increased over 45 minutes (Fig. 3.1-3.3) after which either the monomer was removed from the cell, or bound activity decreased due to decomposition. This 45 min turning point in uptake was also evident in the uptake of dimer **33y**, trimer **al** and tetramer **au**.

Temperature experiments were carried out at all concentrations for a time interval of 10 min. Temperature had little effect on the uptake profile of the compounds indicating that the compounds were taken up by a diffusion mechanism. Diffusion does not require an input of energy which at low temperatures is not generated but works under the principle of molecular motion.

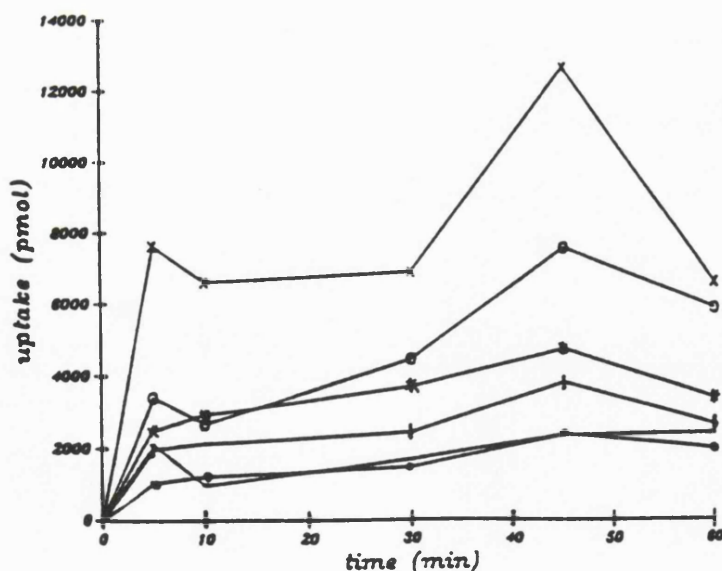


Figure 3.1. Cellular uptake of **33r**. (x) 200μM, (o) 100μM, (*) 70μM, (+) 49μM, (-) 34μM, (•) 24μM.

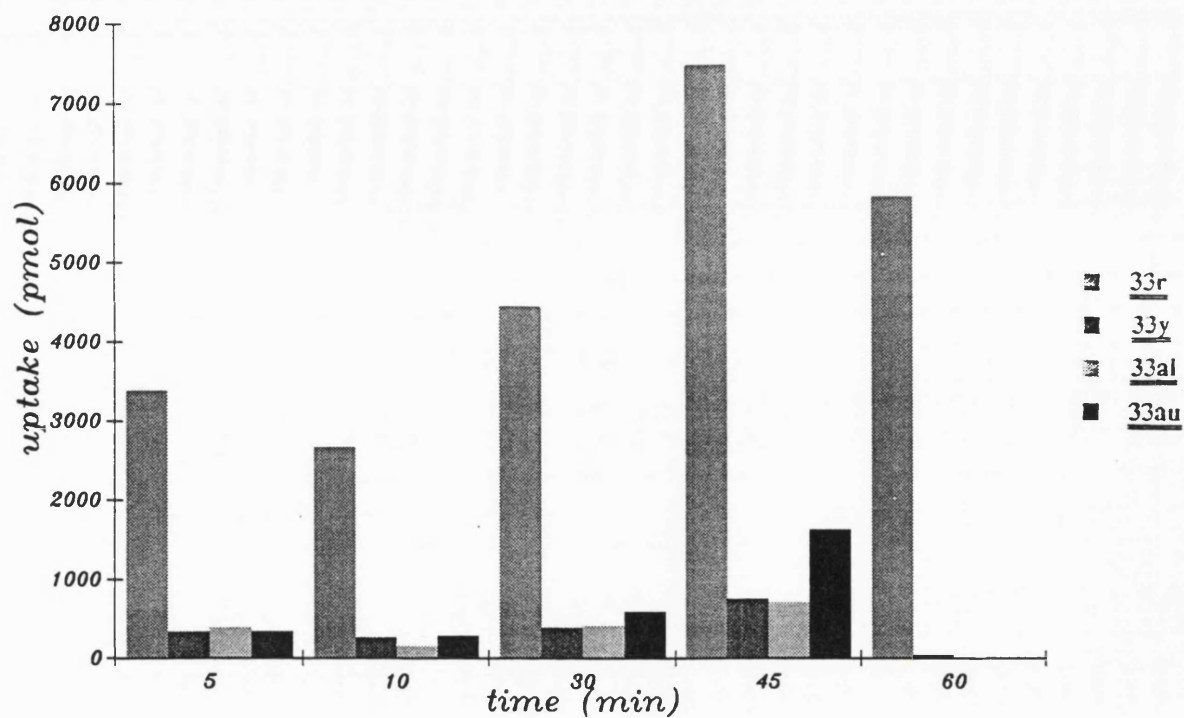


Figure 3.2. Cellular uptake of ^{33}r , ^{33}y , ^{33}al and ^{33}au . 100 μM .

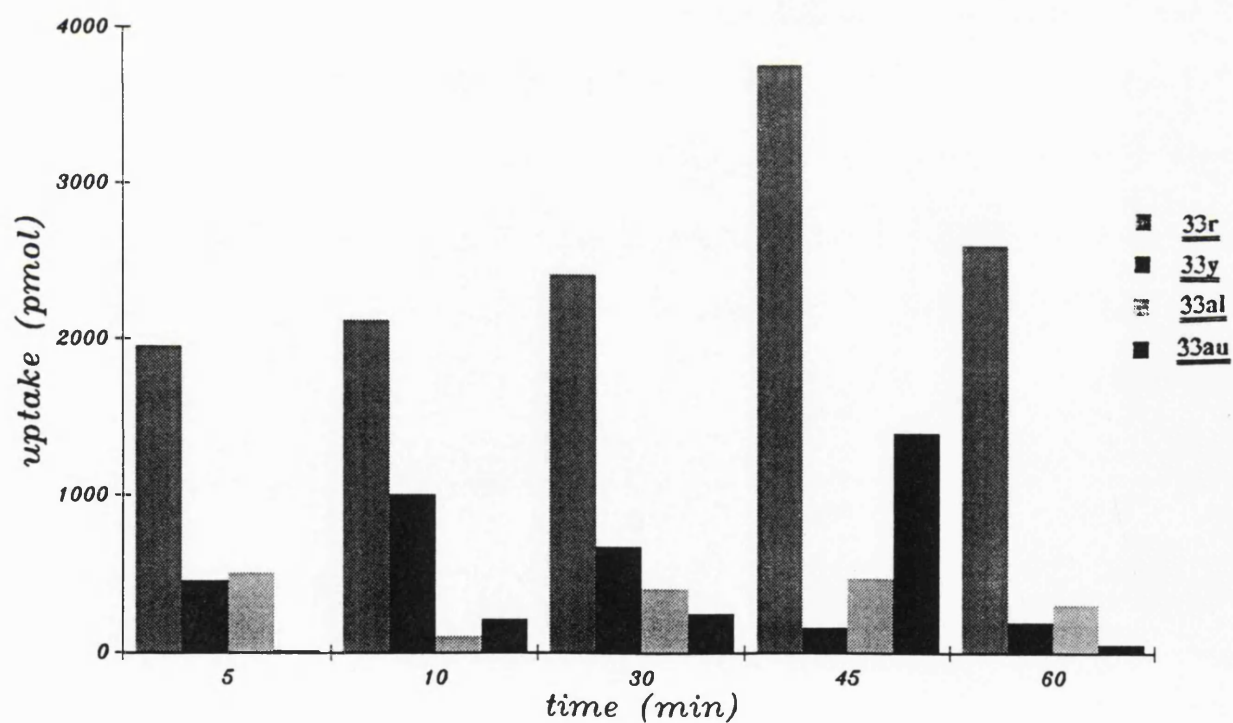


Figure 3.3. Cellular uptake of ^{33}r , ^{33}y , ^{33}al and ^{33}au (+) 49 μM .

As the cells were washed with cold compound after uptake, it is believed that most of the surface compound is displaced and therefore the results reflect the actual cellular uptake. The monomer **33r** was taken up much more rapidly and in higher amount, than the larger dimer **33y**, trimer **33al** or tetramer **33au**. It is believed that the monomer **33r** was transported across the membrane via a simple diffusion process. The dimer **33y**, trimer **33al** and the tetramer **33au** due to their increased lipophilic structures are retained in the plasma membrane due to lipophilic interactions.

3.2. *In vitro* toxicity assessment of chlorambucil conjugates with lipidic acids, lipidic amino acids and their oligomers to ADJ/PC6 plasmacytoma cells.

The *in vitro* toxicity of chlorambucil conjugates **56a**, **b**, **c**, **e**, **57a-c** and **58a** towards ADJ/PC6 plasmacytoma cells was probably due to the release of free chlorambucil in the cell, since the conjugates themselves did not crosslink with naked DNA. The chlorambucil conjugates **56a**, **b**, **c** and **e** showed varying degrees of toxicity (Fig. 3.4) as assessed by ³H-thymidine incorporation into DNA. However, the biological activity was not in linear correlation with the lipophilicity (i.e. length of alkyl side chain) of the conjugates. A rebound DNA synthesis may explain the increased ³H-thymidine incorporation observed for low concentrations of conjugate **56e**. Conjugates **57a-c** exhibited a decrease in toxicity with increase in the alkyl chain length (Fig. 3.5). Although the long alkyl chain may increase the uptake of the compounds, it may also cause steric hindrance of the conjugating bond, reducing the rate of release of the parent chlorambucil. The cytotoxicity of conjugates **56b**, **57b** and **58a** were compared (Fig. 3.6) and we found that the conjugating linkage had a great influence on the toxicity of the chlorambucil conjugates. The most toxic compound was conjugate **58a** with an ester linkage and a small spacer between active parent and conjugate moiety; the least toxic compound, as expected, was conjugate **57b** with an amido linkage.

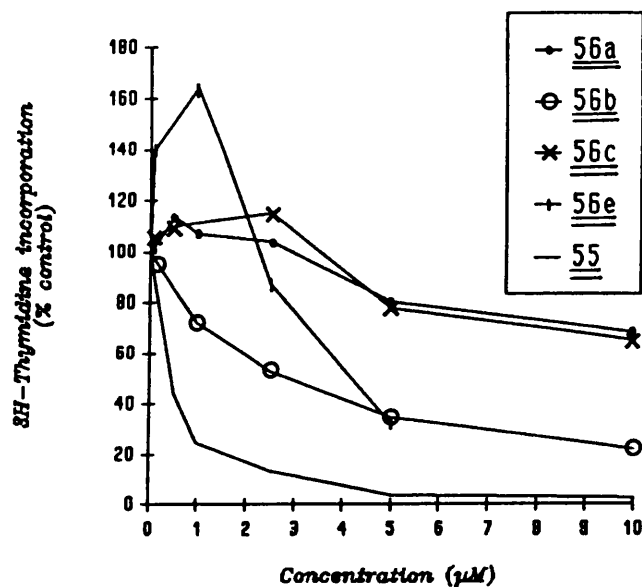


Figure 3.4. In vitro toxicity of compounds 55, 56a,b,c and e to ADJ/PC6 plasmacytoma cells

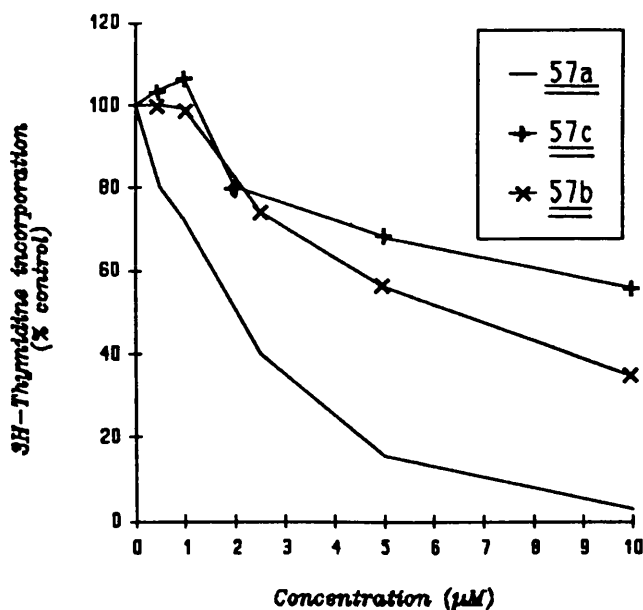


Figure 3.5. In vitro toxicity of compounds 57a-c to ADJ/PC6 plasmacytoma cells

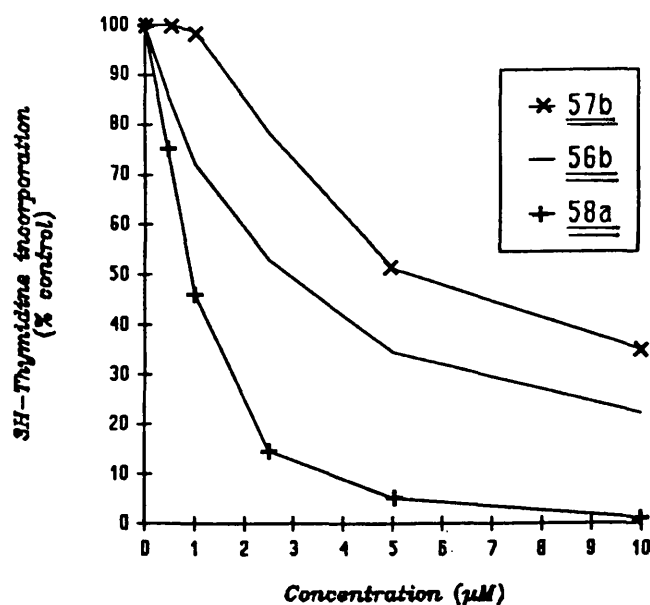


Figure 3.6. In vitro toxicity of compounds 56b, 57b and 58a to ADJ/PC6 plasmacytoma cells

The series of chlorambucil conjugates 56a, b, c, e, 57a-c and 58a showed varying degrees of toxicity to ADJ/PC6 plasmacytoma cells, and we suggest that the conjugates are hydrolysed releasing the parent chlorambucil. The observations in the case of compounds 57a-c was that increasing the length of the alkyl side chain brought about a decrease in toxicity. The longer alkyl side chains may decrease the rate of hydrolysis due to increase in steric hindrance. The linkage in the chlorambucil conjugates also influences their toxicity. Conjugates 56b, 57b and 58a had the same length of alkyl side chain, conjugate 58a with ester and amide linkage showed the highest toxicity followed by 56b with ester linkage, then compound 57b which had an amide linkage. By varying the lipophilicity and linkage of the chlorambucil conjugates we were able to modify chlorambucil toxicity towards ADJ/PC6 plasmacytoma cells.

3.3. *In vitro* and *in vivo* absorption of benzoquinolizine lipidic amino acid/peptide conjugates.

Compounds **62a**¹-**62c**¹ represented a series in which the alkyl side chain contained six, eight and 12 carbon atoms, respectively, so that the compounds exhibited a gradual increase in lipophilicity. Compounds **62d**¹ and **62e**¹ were more lipophilic, containing two alkyl chains with eight and twelve carbons, respectively; compound **62f**¹ was the most lipophilic, containing three alkyl chains of twelve carbon atoms. The increased lipophilicity was reflected in increased partition co-efficients of the conjugates (Table 3.8), which were calculated using an Eluex Version 3.0 program of Compudrug Chemistry Ltd. Budapest, Hungary.

3.3.1. *Caco-2* cell experiments.

Conjugates **62a**¹-**f**¹ were transported rapidly across the *Caco-2* monolayers (Table 3.1). The apparent permeability coefficients (P_{app}) of conjugates **62d**¹-**f**¹ were approximately twice as high as the less lipophilic conjugates **62a**¹-**c**¹ (p <0.01). This increase in transport rate of the conjugates correlated with the increase in the calculated partition coefficients (Table 3.8).

Compound 62	P _{app} , cm/s x 10 ⁷	Resistance, ohm cm ²
-	-	314±16
a ¹	1.18±0.05	273±29
b ¹	1.11±0.05	341±26
c ¹	1.18±0.02	407±42
d ¹	1.91±0.05	206±42
e ¹	2.46±0.07	269±78
f ¹	2.66±0.06	173±3

¹ see materials and methods. Mean values ±S.D.

Table 3.1. Apparent permeability coefficients of benzoquinolizine conjugates and transepithelial electrical resistance after the transport experiments in *Caco-2* monolayers¹

Conjugates **62d⁺-f⁺** reduced the transepithelial electrical resistance across the monolayers by up to approximately 40% (compound **62f⁺** as compared to controls exposed to buffer only) while no effect was observed for conjugates **62a⁺-c⁺** (Table 3.1). This suggests that compounds **62d⁺-f⁺** increased the permeability of the intestinal epithelium *in vitro* and enhanced their own absorption. In general, the absorption of the conjugates to the monolayer/filters was low and only 2-5% of the radioactivity was found in the filters at the end of the experiments (data not shown). Therefore, no correction of the Papp-values for filter associated radioactivity was performed.

3.3.2. Absorption of ³H-labelled lipidic conjugates following oral administration.

The *in vitro* cell culture model suggested that conjugation to lipidic peptides could enhance oral absorption, therefore the experiments were extended to investigate *in vivo* absorption. The ³H-labelled **62a⁺-f⁺** were dissolved in a corn oil vehicle and administered by gavage to fasted rats. Animals were sacrificed 2, 6 and 24hrs after dosing. Stomach, small intestine with mesenteric node, large intestine, liver, kidney and spleen were removed for analysis. The amount of radiolabel present in the different organs selected, in the blood, urine and faeces (if applicable) was determined. Tables 3.2-3.7 and Fig. 3.7 summarise the results of these experiments.

The radioactivity detectable in the blood and organs following oral administration of the parent benzoquinolizine acid (Toth *et al.* 1991) was no greater than background.

In contrast, the lipidic conjugates showed significant oral uptake. The extent of oral uptake increased with increasing lipophilicity of the conjugates, with maximum absorption occurring for conjugate **62c⁺**. The oral uptake decreased for the more highly lipophilic conjugates. The organ uptake of the least lipophilic conjugate, compound **62a⁺**, decreased from 19.2% (3hr), to 1.9% (24hr); the percentage of the administered dose in the blood increased from 0.2% to 6.5% in this time interval (Table 3.2, Fig. 3.7). The increased lipophilicity of compounds **62b⁺** (8 carbon side chain) and **62c⁺** (12 carbon side chain) increased the oral uptake over the 24hr period with a total uptake of 31% for compound **62b⁺** after 24hr and 37.1% for compound **62c⁺** after 24hr (Tables 3.3 and 3.4, Fig. 3.7).

Further increases in the lipophilicity of the conjugates resulted in a decrease in the oral uptake. Compound **62d⁺** (2 alkyl chains with 8 carbon atoms) showed a decrease in uptake from 4.75% (3hr) to 1.66% (24hr). Compound **2e⁺** (2 alkyl chains with 12 carbon atoms) showed a

decrease from 6.61% (3hr) to 2.23% (24hr). Conjugate **2f** (3 alkyl chains with 12 carbon atoms) showed a decrease from 3.3% (3hr) to 1.29% (24hr) (Tables 3.5, 3.6 and 3.7, Fig. 3.7). In the 24hr experiment the compounds appeared in the urine and the faeces.

Organ	% Uptake of <u>62a</u>		
	1 (3hr)	2 (6hr)	3 (24hr)
Stomach	6.9	1.1	0.3
S.I.	9.8	3.3	0.1
L.I.	0.2	0.9	0.6
Liver	1.8	1.1	0.3
Spleen	0.4	0.1	0.3
Kidney x 2	0.1	0.1	0.3
Organ Uptake (%)	19.2	6.6	1.9
Blood	0.2	0.2	6.5
Total Uptake (%)	19.4	6.8	8.4

Table 3.2. Oral uptake of compound 62a.

Organ	% Uptake of <u>62b</u>		
	1 (3hr)	2 (6hr)	3 (24hr)
Stomach	2.3	0.7	2.1
S.I.	8.6	4.8	3.2
L.I.	0.1	6.9	2.6
Liver	1.5	5.1	7.1
Spleen	0.3	0.7	2.0
Kidney x 2	1.5	0.6	4.0
Organ Uptake (%)	14.3	18.8	21.0
Blood	2.0	6.2	10
Total Uptake (%)	16.3	25.0	31.0

Table 3.3. Oral uptake of compound 62b.

Organ	% Uptake of 62c		
	1 (3hr)	2 (6hr)	3 (24hr)
Stomach	4.5	2.1	1.2
S.I.	4.4	6.4	2.2
L.I.	6.3	0.6	1.3
Liver	1.3	3.1	3.6
Spleen	0.2	0.2	3.6
Kidney x 2	0.2	0.5	3.1
Organ Uptake (%)	16.9	12.9	15.0
Blood	4.1	22.9	22.1
Total Uptake (%)	21.0	35.8	37.1

Table 3.4. Oral uptake of compound 62c^{*}.

Organ	% Uptake of 62d		
	1 (3hr)	2 (6hr)	3 (24hr)
Stomach	2.50	0.42	0.08
S.I.	1.50	0.52	0.19
L.I.	0.23	0.47	0.31
Liver	0.24	0.16	0.13
Spleen	0.02	0.02	0.05
Kidney x 2	0.03	0.06	0.14
Organ Uptake (%)	4.52	1.65	0.90
Blood	0.23	-	0.76
Total Uptake (%)	4.75	1.65	1.66

Table 3.5. Oral uptake of compound 62d^{*}.

Organ	% Uptake of 62e		
	1 (3hr)	2 (6hr)	3 (24hr)
Stomach	3.84	0.76	1.19
S.I.	2.07	1.08	0.05
L.I.	0.09	0.08	0.07
Liver	0.13	0.23	0.24
Spleen	0.01	0.05	0.06
Kidney x 2	0.02	0.14	0.11
Organ Uptake (%)	6.16	2.34	1.72
Blood	0.45	0.18	0.51
Total Uptake (%)	6.61	2.52	2.23

Table 3.6. Oral uptake of compound 62e.

Organ	% Uptake of 62f		
	1 (3hr)	2 (6hr)	3 (24hr)
Stomach	1.45	1.02	0.11
S.I.	1.48	1.48	0.09
L.I.	-	0.19	0.14
Liver	0.08	0.07	0.21
Spleen	0.40	0.02	0.22
Kidney x 2	0.01	0.04	0.06
Organ Uptake (%)	3.42	2.82	0.83
Blood	0.08	0.44	0.46
Total Uptake (%)	3.50	3.26	1.29

Table 3.7. Oral uptake of compound 62f.

Maximum oral uptake of the lipidic system occurred when the logD was 6.16. For a water-lipid-water model of intestinal permeability, the permeability should depend on the partition coefficient of the solute. An increased partition coefficient leads to enhanced concentrations within the lipid membrane, greater solute flux and hence greater permeability (Leahy *et al.* 1989). For very lipophilic solutes (as in conjugates **62d'-f'**), the permeability through the lipid membrane is very high, so that transmembrane flux becomes limited by diffusion across the unstirred water layer. The low water solubility of these highly lipophilic conjugates could therefore be the cause of the lower uptake *in vivo*. Other factors contributing to the reduced *in vivo* uptake of conjugates **62d'-f'** include the increased tendency of these conjugates to aggregate and precipitate, their higher molecular weight and possible interactions with the mucus layer.

62	logD	ac.pKa	bas.pKa
a'	3.04	none	9.6
b'	4.08	none	9.6
c'	6.16	none	9.6
d'	7.17	none	9.6
e'	11.32	none	9.6
f'	18.07	none	9.6

Table 3.8. Calculated partion cefficents of compounds **62a'-f'.**

The *Caco-2* cell culture model served as a valid initial screening test for the lipidic conjugates, prior to *in vivo* absorption studies. The results of the oral absorption studies of compounds **62a'-c'** were largely in agreement with the results of the *Caco-2* cell culture experiments: the *Caco-2* cell apparent permeability was high and the compounds were taken up orally. These compounds did not show any toxicity.

In the *Caco-2* experiments, the highly lipophilic conjugates **62d'-f'** showed greatest uptake. This was in contrast to the *in vivo* situation and may be due to their low water solubility and the other factors discussed.

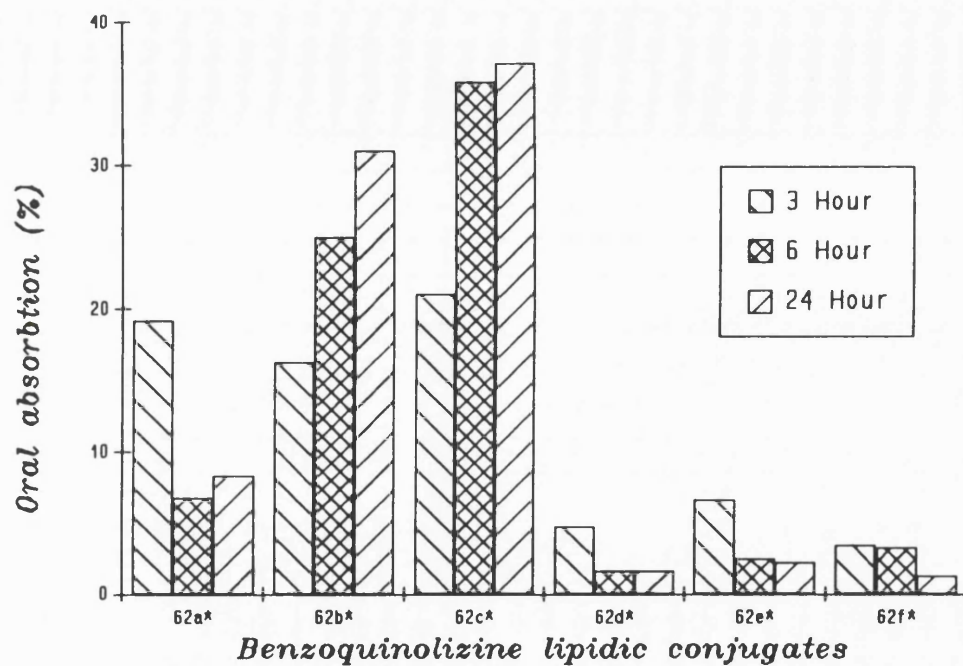


Figure 3.7. Oral absorbtion of benzoquinolizine lipidic conjugates 62a*-f*

Increasing the lipophilicity of the conjugates increased their oral absorption, up to a maximum lipophilicity obtained by conjugation with a 12 carbon alkyl chain lipidic peptide. Further increases in the lipophilicity resulted in a decrease in the oral uptake.

Chapter 4. Conclusions.

4.1. Lipidic amino acids and peptides.

The aim of the project was to synthesize a series of lipidic amino acids **33a-z** and their corresponding homo-oligomers **33z-av** the lipidic peptides. The lipidic amino acids/peptides were then examined to establish if they would be suitable candidates for a drug delivery system. The lipidic amino acids/peptides possess membrane like character due to their high lipophilicity and it was envisaged that they could be employed as a drug carrier. By manipulating the length of lipidic amino acid side chain, or the number of lipidic amino acid units in a peptide, their lipophilicity may be altered. Also, hetero-oligomers were synthesised to impart hydrophilicity to the system. Thus, by manipulation of the system, a carrier of desired lipophilicity/hydrophilicity may be produced and successfully conjugated to a drug via an appropriate conjugate linkage.

4.1.1. Cellular uptake studies of a lipidic amino acid and its oligomers on *Ehrlich ascites* tumour cells.

The monomer **33r** was taken up by the *Ehrlich Ascites* tumour cells much more rapidly and in higher amount, than the larger dimer **33y**, trimer **33al** or tetramer **33au**. It is believed that the monomer **33r** was transported across the membrane via a simple diffusion process. The dimer **33y**, trimer **33al** and the tetramer **33au** due to their increased lipophilic structures are retained in the plasma membrane due to lipophilic interactions.

4.1.2. Structure - retention relationships of diastereomeric mixtures of lipidic amino acid conjugates on reversed phase stationary phases.

A series of diastereomeric lipidic dipeptide conjugates **53a-t** were synthesised, the majority of which could be separated in reversed phase conditions. The retention of the compounds was governed by hydrophobicity on the Spherisorb ODS column and the effect of the free silanol groups could be observed for basic derivatives. The Supelcosil™LC-ABZ column did not show silanophilic effect, most cases better separation of the diastereomers could be observed and other mechanisms along with hydrophobic interactions governed the retention as well.

4.1.3. Conjugation of lipidic amino acids and oligomers with hydrophilic compounds.

Lipidic amino acids **33p**, **33r** and lipidic dipeptide **33af** were conjugated to several hydrophilic compounds, then physio-chemical investigations were carried out on them using proton nuclear magnetic resonance. Plots of chemical shift versus concentration revealed the conjugates existed as monomers at low concentrations, but formed aggregates or micelles at high concentrations.

4.2. Lipidic drug conjugates.

Lipidic amino acids and peptides were to be conjugated to selected compounds that are known to possess, or may possess therapeutic potential. Compounds used for conjugation were: the cytotoxic agent chlorambucil, the anti-inflammatory agent benzoquinolizine, 3,4-dihydroxybenzaldehyde derivatives and an adenosine 5'monophosphorothionate derivative. It was envisaged that the conjugate linkage between lipidic moiety and "drug" moiety may play an important part in the activity of the conjugate. Thus, several conjugate linkages were used and examined to determine their stability and suitability.

4.2.1. Synthesis and *In vitro* toxicity assessment of chlorambucil conjugates with lipidic acids, lipidic amino acids and their oligomers.

A series of lipidic amides of chlorambucil **56a-f**, lipidic esters of chlorambucil **57a-e** and chlorambucil conjugates containing both ester and amide linkage **58a-b** were synthesised successfully. *In vitro* toxicity assessments of selected members of the series revealed varying degrees of toxicity towards the ADJ/PC6 plasmacytoma cells which was related to the length of the alkyl side chain of the lipidic amino acid, the number of lipidic amino acid units and the conjugating linkage itself. By varying the lipophilicity and linkage of the chlorambucil conjugates it was possible to modify chlorambucil toxicity towards ADJ/PC6 plasmacytoma cells. Thus, the lipidic amino acid based delivery system may be used as a tool in the development of alkylating agents, such as chlorambucil, with increased selectivity and increased therapeutic index.

4.2.2. Synthesis and *In vitro* and *in vivo* studies of benzoquinolizine lipidic conjugates.

³H-radiolabelled benzoquinolizine lipidic conjugates **62a⁺-f⁺** were successfully synthesised and their lipophilicity determined by calculating partition co-efficients. The conjugates were then studied both *in vitro* and *in vivo* to establish the optimum lipophilicity for oral uptake.

In the Caco-2 experiments, the highly lipophilic conjugates **62d⁺-f⁺** showed greatest uptake. This was in contrast to the *in vivo* situation and may be due to factors such as high molecular weight, low water solubility, tendency to aggregate and precipitate, and the other factors discussed.

The results of the oral absorption studies of compounds **62a⁺-c⁺** were largely in agreement with the results of the Caco-2 cell culture experiments. Increasing the lipophilicity of the conjugates increased their oral absorption, up to a maximum lipophilicity obtained by conjugation with an 12 carbon alkyl chain lipidic peptide. The Caco-2 cell apparent permeability was high and the compounds were taken up orally.

The results suggested that conjugation to lipidic amino acids and peptides is a useful approach to improving the absorption of poorly-absorbed drugs. The optimal lipophilicity of any drug moiety can be determined by conjugation of the drug to lipidic peptides which vary in the constituent length and number of alkyl chains.

4.2.3. Synthesis of membrane active signal transduction inhibitors.

The fully protected methyl-[(3,4-disubstituted benzyl) cyanoprop-2-enoyl] iminoalkanoates **72a-c** and **73a-c** were synthesised in reasonable yields, by first condensing cyanoacetic acid to **68** and **70** respectively employing a methylene-condensation reaction; then coupling the products (**69** and **71**) to the appropriate methyl protected lipidic amino acid (**33o-s** and **u**).

Unfortunately, it proved difficult to synthesise the methyl-[(dihydroxybenzyl)cyanoprop-2-enoyl]iminoalkanoates **67a-b** in reasonable yields and purity, as dihydroxybenzyl derivatives could not be condensed using the conditions employed. Also, cleavage of the ether groups (methyl and dimethoxy) proved more difficult than anticipated. Thus to furnish the desired compounds in reasonable yields the methods employed have to be further manipulated.

One further point to add is that compounds 72a-c and 73a-c may show the desired inhibitory effects, as cleavage of ether groups has been shown to take place *in vivo* (Sperk *et al.* 1982, Balderessi *et al.* 1977).

4.2.4. Oligonucleotide conjugates.

Only small quantities of the desired product could be isolated. Thus, other oxidising conditions are required to enable synthesis of 80 in sufficient yields. To extend the use of the lipidic amino acids as a delivery system for antisense oligonucleotides the reactions must be improved substantially to allow quantitative yields of the desired product. Also, consideration as to how the conjugation of lipidic amino acids to antisense oligonucleotides can be achieved in the automated systems now employed in the synthesis of antisense oligonucleotides themselves.

Chapter 5. Biology Experimental, Materials and Methods.

5.1. *Ehrlich ascites* tumour cell uptake studies.

Source of cells: *Ehrlich ascities* tumour cells (EATC) were obtained from The School of Pharmacy, University of London from Dr. M. Jones (Institute of Cancer Research, Sutton). Flow Lab Composition of Medium for Culturing: Dulbecco's Modification of Eagles Medium (DMEM)/10% Foetal Calf Serum (FCS); DMEM (Stock solution x 10 concentration) (50.0ml); NaHCO₃ 75% w/v (1.85g/500ml) (25.0ml); Foetal Calf Serum (x 10 concentration) (50.0ml); Penicillin/streptomycin antibiotics (5.0ml); Glutamine (stock 1mM) (2.5 ml); Hepes Buffer (12.5ml); Sterile distilled water (355.0ml). Specific activities:

33r 50 nmol=53472 DPM, **33y** 50nmol=62442 DPM, **33al** 50nmol=80994.5DPM, **33au** 50nmol=71318DPM

Cell preparation: The *Ehrlich ascites* tumor cells (EATC) were cultivated under sterile conditions (5%CO₂, 37°C) in Dulbecco's Modification of Eagles Medium (DMEM)/10% Foetal Calf Serum (FCS). The cells were harvested by trypsinisation (0.25% trypsin in 0.9% NaCl, 10 min, 37°C) followed by the addition of 10ml DMEM/10%FCS. The cells were centrifuged (1000rpm, 5 min), the medium removed and the cells resuspended in a further 10ml DMEM/10%FCS. A coulter counter was used to count the cell suspension (100µl cell suspension, 9.9 ml Isoton) and the suspension diluted to give the final density of 1x10⁵ cells/ml. Aliquots of 0.5ml were added wells of 24 well multicell culture plates and incubated overnight to form monolayer. Uptake experiments: Conjugates **33r**, **33y**, **33al** and **33au** were dissolved in DMSO (3 drops) and diluted with DMEM/FCS (200 times). The solutions were filtered and with further dilutions lower concentrations were prepared. The solutions of the conjugates (0.2ml) were added to the wells and incubated for set times. (Each well was washed with 1ml of a 1mM solution of unlabelled drug made up in 4% BSA using non sterile 0.9% NaCl solution). After the incubation period, 400µl of 1M NaOH was added to each well, and left overnight. 300µl of solution was taken from the wells into a scintillation vial, neutralised with 300µl of 1M HCl, Aquasol scintillation fluid (4ml) was added and the radioactivity was determined. The radioactivity of the original drug solution was also measured to calculate the specific activity. The radioactivity counts were converted to pmoles.

5.2. *In vitro* toxicity studies using ADJ/PC6 plasmacytoma cells.

Source of cells: Murine ADJ/PC6 plasmacytoma cells were provided by Dr. L. Kelland, Institute of Cancer Research, 15 Cotswold Rd. Belmont, Sutton.

Cell preparation: Approx. 5×10^4 ADJ/PC6 tumour cells were seeded into wells of 24-well multiwell culture dishes together with different concentrations of the conjugates 56a, b, c, e, 57a-c and 58 being tested. The final volume in each well was 1ml, and all incubations were carried out in DMEM/10% FCS (Dulbecco's modification of eagles medium supplemented with 10% foetal calf serum) in a 5% CO₂ atmosphere at 37°C, under sterile conditions. The cells were incubated with the conjugates for 70Hr: then 0.5µCi [³H]thymidine was added to each well and incubated for a further 2hr. Cells were transferred to 75x12mm tubes, centrifuged at 1000 rpm to spin down the cells and the radioactive supernatant discarded. The cells were washed twice with 0.9% NaCl containing 1mM thymidine (unlabelled) to remove unbound radiolabelled thymidine. 10% trichloroacetic acid (1ml) was added to the cell pellet to precipitate protein (4°C, 10min) and then centrifuged at 2000 rpm for 5min. The supernatant was discarded and the precipitate was dissolved in 300µl of 1M NaOH. The solution was neutralised with 300µl of 1M HCl, transferred to scintillation vials and 4ml Aquasol (scintillation fluid) added to each vial. After capping and mixing the samples were analysed on the β-counter. The amount of labelled [³H]thymidine incorporated into the DNA of the cells gave an indication of their viability. Results for each concentration of conjugate were expressed as a percentage of the radioactivity incorporated into untreated control cells. A 72hr incubation was used to enable cells to go through a number of cell cycles, allowing the DNA damage inflicted by the conjugates to be manifested as cell death.

5.3. Caco-2 cell uptake studies.

Source of cells: Caco-2 cells were obtained from the American Type Culture Collection, Rockville, MD. The cells were cultivated on polycarbonate filters (Transwell, Costar, Badhoevedorp, The Netherlands) for transport experiments. The cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 1% nonessential amino acids, benzylpenicillin (10 IU/ml) and streptomycin (10µg/ml). Cells of passage 97 were used.

Integrity of the cell monolayers: The integrity of the cell monolayers was tested after 21-22 days in culture by measurement of transepithelial electrical resistance and transport of ¹⁴C-

mannitol. Control monolayers had a transepithelial electrical resistance of $\geq 300 \text{ ohm cm}^2$ and mannitol permeability of $9.6 \pm 0.6 \times 10^{-8} \text{ cm/s}$. The integrity of the monolayers was also checked after the transport experiments by measurement of transepithelial electrical resistance.

Transport experiments: The cell monolayers were used in transport experiments after 21-22 days. All transport experiments were performed in Hank's balanced salt solution containing 25 mM N-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HBSS). The ^3H -labelled benzoquinolizine derivatives (**62a-f**) were dissolved in DMSO and the solutions were diluted in HBSS to the final concentrations of $2.0\text{--}4.7 \times 10^{-4} \text{ M}$. The final concentration of DMSO was 0.1-0.5 %. The solutions were sterile filtered and added to the apical side of the Caco-2 monolayers. At regular intervals (30, 60, 90 and 120 min), the cell culture inserts were transferred rapidly to new basolateral chambers and samples were withdrawn and analyzed in a liquid scintillation counter. Apparent permeability coefficients (P_{app}) were calculated. After measurement of the transepithelial resistance the filters were washed rapidly three times with ice cold HBSS and the filter associated radioactivity was determined. Statistics: Unpaired twotailed Student's t-test was used to test the statistical significance between two independent means.

5.4. *In vivo* oral dosing studies.

Animal preparation: Male Wistar rats (inbred, 200-225g, 8 weeks old) were housed in metabolic cages to prevent coprophagia, to allow monitoring the growth curves and to enable the collection of urine and faeces. The rats were fed a commercial diet (Maintenance Expanded Rat and Mouse Pellet Diet, Bantin and Kingman, Hull U.K.) and maintained in an air conditioned environment at 20°C , under a 12hr light: 12hr dark schedule.

Dosing technique: A single dose of $4 \mu\text{mol}$ compound in 1mL corn oil vehicle was administered to each rat by gavage, using a blunt tipped feeding needle inserted into the stomach. The animals were fasted for twelve hours prior to dosing and free access to water was given at all times. Animals were sacrificed 2, 6 and 24hr after dosing. A control group were administered normal saline by gavage.

Blood Sample Collection: The rats were anaesthetized with halothane and 1mL of blood was withdrawn from the abdominal aorta. The sample was solubilized in 10mL Scintran at room temperature for 48hr and by heating at 50°C for 20 min. The samples were decolourised with 0.2ml H_2O_2 (30%) and evaporated to dryness using N_2 flow at 50°C . The sample was left to

redisperse in Optiphase Safe scintillation fluid for 24hr and the radioactivity measured.

Organ Sample Collection: Stomach, small intestine with mesenteric node, large intestine, liver, kidney and spleen were removed for analysis. The stomach was cut longitudinally, the stomach contents removed and the stomach tissue washed gently with distilled water in an effort to remove any particles that were not absorbed, but were adhering to the surface of the tissue. Intestinal luminal contents were removed by cutting the tissue into 2 cm strips and forcing the contents out with a forceps. The intestinal tissue was also longitudinally dissected and washed to try and clear the tissue of adhering, unabsorbed particles. All dissected organs were washed and weighed. Distilled water was added to the individual samples (roughly 1:4 ratio based on the weight of the sample) and the final volume noted. The samples were homogenised for 5 min. An aliquot part of the homogenate was diluted with Scintran and the sample was left for 48hr at room temperature. An aliquot of the solubilized sample was decolorised with 0.2 ml H_2O_2 (30%) and evaporated to dryness using N_2 flow at 50°C . The samples were redissolved in scintillant for 24hr and the radioactivity measured.

Urine Sample Collection: The urine was collected from each animal at 12hr intervals and the volume noted. A sample from each 12hr interval was removed, decolorised using 0.2 ml H_2O_2 (30%), diluted scintillant and the radioactivity measured.

Faeces Sample Collection: Faeces were collected after 24hr and weighed. 200ml of water was added to each sample and the samples were homogenised for 10 min. An aliquot of the homogenate was solubilized in 5ml of Scintran for 48hr and the samples were placed in an ultrasonic bath for 1hr. An aliquot from each sample was decolorized using 0.2 ml H_2O_2 (30%), scintillant added and the radioactivity measured.

Data Calculation: Samples were prepared in triplicate and measured for a period of 2 minutes on the scintillation counter. Results were corrected for background counts, control counts and dilution factors. Results were expressed as (i) the percentage uptake of the administered dose in the organ or blood sample and (ii) the percentage recovery in the urine and faecal samples.

Chapter 6. Chemistry Experimental.

Experimental.

Infra-red spectra were recorded with a Perkin Elmer 841 spectrophotometer. Melting points are not given for diastereomeric mixtures. ^1H -NMR spectra were obtained on Varian XL-300 and Bruker AM500 instruments operating at fields of 300 and 500 MHz respectively; chemical shifts are reported in ppm downfield from internal TMS. Mass spectra were run on a VG Analytical ZAB-SE instrument, using fast atom bombardment (FAB) techniques. Reaction progress was monitored by thin layer chromatography (TLC) on Kieselgel PF₂₅₄ using appropriate solvent systems as the mobile phase. Purification was achieved by TLC using Kieselgel PF₂₅₄₊₃₆₆ (Merck) on 20 x 20cm plates of thickness 1.5mm, or column or flash chromatography through Kieselgel G, using appropriate solvent systems. Solvents were evaporated under reduced pressure with a rotary evaporator. Infra-red spectra gave characteristic NH, carbonyl and aromatic absorbances. Purity of the compounds was determined by thin layer and high pressure liquid chromatography. HPLC separation was carried out on a Whatman Partisal 5 RAC silica column. HPLC grade dichloromethane (Aldrich) and methanol (Rathburn) were filtered through a 25 μm membrane filter and degassed with helium flow prior to use. Separation was achieved with a solvent gradient beginning with 0% methanol, increasing constantly to 50% methanol at 15 minutes and decreasing steadily to 0% methanol from 17 to 20 minutes at a constant flow of 3ml min⁻¹. The gradient was effected by two microprocessor-controlled Gilson 302 single piston pumps. Compounds were detected with a Holochrome UV-VIS detector at 254nm. Chromatographs were recorded with an LKB 2210 single channel chart recorder.

6.2. α -Amino alkanolic acids, N- and C- protected α -amino alkanolic acids.

α -Amino-tetradecanoic acid (**33r**) [Method A].

To a pre-cooled 500ml round bottom flask containing absolute ethanol (85ml) was added sodium pellets (5g, 0.22mol) and stirred until dissolved. Diethylacetamidomalonate (48.6g, 0.22mol) was added and allowed to dissolve then 1-bromododecanoate was added and the mixture refluxed overnight. The cooled mixture was precipitated over a 1:1 ice water (320ml) mixture and the precipitate was filtered, washed with water and dried under suction for 2 hours. The precipitate was placed into a 2l round bottom flask then concentrated hydrochloric acid (360ml) added along with dimethylformamide (10ml) and the mixture refluxed for 18 hours. To the cooled mixture was added ethanol:water (3:1, 1000ml) and heated to redissolve the solid. The hot solution was filtered, cooled and neutralised with ammonia solution (35%). The precipitate formed was filtered, washed with water followed by alcohol, dried under suction then over phosphorous pentoxide.

Yield : 78.9%.

$C_{14}H_{29}O_2N$ (243.29).

α -Aminoalkanoates **33a**, **b**, **c**, **e**, **f** and **g** were synthesised by reacting sodium (1 eq.), the appropriate 1-bromoalkane (1.5eq.) and diethylacetamidomalonate (1 eq.) as described in method A.

α -Aminooctanoic acid (**33a**).

Yield : 45.7%.

$C_8H_{17}O_2N$ (159.17).

α -Aminodecanoic acid (**33b**).

Yield : 68.0%.

$C_{10}H_{21}O_2N$ (187.21).

α -Aminododecanoic acid (**33c**).

Yield : 74.0%.

$C_{12}H_{25}O_2N$ (215.25).

α -Aminohexadecanoic acid (33e).

Yield : 82.0%.

$C_{16}H_{33}O_2N$ (271.33).

α -Aminooctadecanoic acid (33f).

Yield : 86.4%.

$C_{18}H_{37}O_2N$ (299.37).

α -Amineicosanoic acid (33g).

Yield : 86.3%.

$C_{20}H_{41}O_2N$ (327.41).

α -(Tertiary-butoxycarbonylimino)tetradecanoic acid (33k) [Method B].

DL- α -aminotetradecanoic acid 33d (15g, 61.7mmol) was suspended in a mixture of *tert*.butanol:water (2:3, 240ml) and 8M sodium hydroxide added dropwise to attain a pH13 upon which the solid dissolved. Di-*tert*.butyldicarbonate (20g, 91.6mmol) dissolved in 40ml *tert*.butanol was added, the pH was maintained at pH11-12 and the reaction stirred for 2 hours. The reaction mixture was diluted with addition of water (60ml), solid citric acid was added to pH4 and the oil formed extracted with ethyl acetate (3x150ml). The combined organic phase was dried (anhydrous $MgSO_4$), evaporated, the residue triturated with cold acetonitrile and the product filtered and dried.

Yield : 89.3%.

1H -NMR ($CDCl_3$) δ : 4.95 (H, s, OCONH), 4.25 (H, m, α -CH), 1.65-1.35 (2H, 2xm, CH_2), 1.43 (9H, s, $C(CH_3)_3$) 1.25 (20H, m, $10 \times CH_2$), 0.87 (3H, t, CH_3).

MS m/e (%) $C_{19}H_{37}NO_4$ (343.5) : 389 $[M+H+2Na]^+$ (11), 367 $[M+H+Na]^+$ (100), 343 $[M]^+$ (14), 244(72), 215(12), 198(24), 153(15).

α -(Tertiary-butoxycarbonylimino)alkanoic acids 33h, i, j, l, m and n were synthesised by suspending the appropriate DL- α -aminoalkanoic acid 33a, b, c, e, f and g (1 eq.) in a mixture of *tert*.butanol:water 2:3 and reacting with di-*tert*.butyldicarbonate (1.5 eq.) dissolved in *tert*.butanol as described in method B.

α -(Tertiary-butoxycarbonylimino)octanoic acid (33h).

Yield : 70.2%.

$^1\text{H-NMR}$ (CDCl_3) δ : 4.95 (H, s, OCONH), 4.25 (H, m, α -CH), 1.65-1.35 (2H, 2xm, CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$) 1.25 (8H, m, $4\times\text{CH}_2$), 0.87 (3H, t, CH_3).

MS m/e (%) $\text{C}_{15}\text{H}_{25}\text{NO}_4$ (283.3) : 330.3 $[\text{M}+\text{H}+2\text{Na}]^+$ (8), 307.3 $[\text{M}+\text{H}+\text{Na}]^+$ (100), 283 $[\text{M}]^+$ (7), 113(27).

α -(Tertiary-butoxycarbonylimino)decanoic acid (33i).

Yield : 84.2%.

$^1\text{H-NMR}$ (CDCl_3) δ : 4.90 (H, s, OCONH), 4.21 (H, m, α -CH), 1.45-1.35 (2H, 2xm, CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$) 1.25 (12H, m, $6\times\text{CH}_2$), 0.86 (3H, t, CH_3).

MS m/e (%) $\text{C}_{15}\text{H}_{29}\text{NO}_4$ (287.4) : 332 $[\text{M}+\text{H}+2\text{Na}]^+$ (100), 310 $[\text{M}+\text{Na}]^+$ (74), 254(72), 232(12), 142(24).

α -(Tertiary-butoxycarbonylimino)dodecanoic acid (33j).

Yield : 92.5%.

$^1\text{H-NMR}$ (CDCl_3) δ : 4.97 (H, s, OCONH), 4.27 (H, m, α -CH), 1.75-1.45 (2H, 2xm, CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$) 1.25 (16H, m, $8\times\text{CH}_2$), 0.86 (3H, t, CH_3).

MS m/e (%) $\text{C}_{17}\text{H}_{33}\text{NO}_4$ (315.4) : 361(23), 360 $[\text{M}+\text{Na}]^+$ (100), 338 $[\text{M}+\text{H}]^+$ (57), 282(25), 260(32).

α -(Tertiary-butoxycarbonylimino)hexadecanoic acid (33l).

Yield : 82%.

$^1\text{H-NMR}$ (CDCl_3) δ : 4.95 (H, s, OCONH), 4.25 (H, m, α -CH), 1.65-1.35 (2H, 2xm, CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$) 1.25 (24H, m, $12\times\text{CH}_2$), 0.86 (3H, t, CH_3).

MS m/e (%) $\text{C}_{21}\text{H}_{41}\text{NO}_4$ (371.55) : 394 $[\text{M}+\text{Na}]^+$ (100), 372 $[\text{M}+\text{H}]^+$ (19), 371 $[\text{M}]^+$ (6), 294(72).

α -(Tertiary-butoxycarbonylimino)octadecanoic acid (33m).

Yield : 79.3%.

$^1\text{H-NMR}$ (CDCl_3) δ : 4.95 (H, s, OCONH), 4.23 (H, m, α -CH), 1.65-1.35 (2H, 2xm, CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$) 1.25 (28H, m, $14\times\text{CH}_2$), 0.87 (3H, t, CH_3).

MS m/e (%) $\text{C}_{23}\text{H}_{45}\text{NO}_4$ (399.6) : 447 $[\text{M}+\text{H}+2\text{Na}]^+$ (9), 424 $[\text{M}+\text{H}+\text{Na}]^+$ (100), 400 $[\text{M}]^+$ (21),

254(24).

α -(Tertiary-butoxycarbonylimino)eicosanoic acid (33n).

Yield : 78.6%.

$^1\text{H-NMR}$ (CDCl_3) δ : 4.95 (H, s, OCONH), 4.25 (H, m, α -CH), 1.65-1.4 (2H, 2xm, CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.25 (32H, m, $16\times\text{CH}_2$), 0.87 (3H, t, CH_3).

MS m/e (%) $\text{C}_{25}\text{H}_{49}\text{NO}_4$ (427.6) : 475 [$\text{M}+\text{H}+2\text{Na}$] $^+$ (8), 452 [$\text{M}+\text{H}+\text{Na}$] $^+$ (100), 428 [M] $^+$ (13), 382(59), 367(15).

Methyl α -aminotetradecanoate (33r) HCl salt [Method C].

To a 1l round bottom flask was added methanol (374ml) which was cooled to 0°C , thionyl chloride (73.4g, 42ml) was then added dropwise over a period of 45 minutes. DL- α -aminotetradecanoic acid 33d (15g, 61.7mmol) was added and the reaction mixture refluxed for 24 hours. Solvent (approx.90%) was removed *in vacuo* and the precipitated compound filtered and recrystallised from methanol.

Yield : 92.6%.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.86 (2H, m, NH_2), 4.1 (H, m, α -CH), 3.75 (3H, s, COOCH_3), 1.5 (2H, 2xm, CH_2), 1.25 (20H, m, $10\times\text{CH}_2$), 0.87 (3H, t, CH_3).

MS m/e (%) $\text{C}_{15}\text{H}_{32}\text{NO}_2\text{Cl}$ (293.9) : 259(16), 258 [$\text{M}+\text{H}$] $^+$ (100), 198(24), 153(15).

Methyl α -aminooctanoate (33o) HCl salt.

DL- α -aminooctanoic acid 33a (9.9g, 61.7mmol), methanol (374ml) and thionyl chloride (73.4g, 42ml) were reacted as described in method C.

Yield : 64.3%.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.82 (2H, m, NH_2), 4.15 (H, m, α -CH), 3.76 (3H, s, COOCH_3), 1.45 (2H, 2xm, CH_2), 1.27 (8H, m, $4\times\text{CH}_2$), 0.85 (3H, t, CH_3).

MS m/e (%) $\text{C}_9\text{H}_{20}\text{NO}_2\text{Cl}$ (209.7) : 196 [$\text{M}+\text{Na}$] $^+$ (100), 173 [$\text{M}+\text{H}$] $^+$ (81), 123(21).

Methyl α -aminodecanoate (33p) HCl salt.

DL- α -aminodecanoic acid 33b (15g, 80.2mmol), methanol (486ml) and thionyl chloride (95g, 54ml) were reacted as described in method C.

Yield : 84.2%.

¹H-NMR (CDCl₃) δ : 8.86 (2H, m, NH₂), 4.1 (H, m, α-CH), 3.77 (3H, s, COOCH₃), 1.65 (2H, 2xm, CH₂), 1.25 (12H, m, 6xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₁₁H₂₄NO₂Cl (237.8) : 203(17), 224 [M+Na]⁺ (100), 202 [M+H]⁺ (11), 142(24).

Methyl α-aminododecanoate (33g) HCl salt.

DL-α-aminododecanoic acid **33c** (21.5g, 0.1mol), methanol (606ml) and thionyl chloride (119g, 68ml) were reacted as described in method C.

Yield : 84.6%.

¹H-NMR (CDCl₃) δ : 8.84 (2H, m, NH₂), 4.1 (H, m, α-CH), 3.76 (3H, s, COOCH₃), 1.75-1.35 (2H, 2xm, CH₂), 1.28 (16H, m, 8xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₁₃H₂₈NO₂Cl (265.8) : 252 [M+Na]⁺ (100), 267 [M+H]⁺ (81), 170(24), 125(15).

Methyl α-aminohexadecanoate (33s) HCl salt.

DL-α-aminohexadecanoic acid **33e** (25g, 90mmol), methanol (546ml) and thionyl chloride (107g, 62ml) were reacted as described in method C.

Yield : 78.5%.

¹H-NMR (CDCl₃) δ : 8.86 (2H, m, NH₂), 4.15 (H, m, α-CH), 3.78 (3H, s, COOCH₃), 1.85-1.75 (2H, 2xm, CH₂), 1.35-1.10 (24H, m, 12xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₁₇H₃₆NO₂Cl (321.9) : 309 [M+Na]⁺ (100), 287 [M+H]⁺ (81), 226(35).

Methyl α-aminooctadecanoate (33t) HCl salt.

DL-α-aminooctadecanoic acid **33f** (29.9g, 0.1mol), methanol (606ml) and thionyl chloride (119g, 68ml) were reacted as described in method C.

Yield : 84.8%.

¹H-NMR (CDCl₃) δ : 8.85 (2H, m, NH₂), 4.15 (H, m, α-CH), 3.73 (3H, s, COOCH₃), 1.85-1.75 (2H, 2xm, CH₂), 1.35-1.10 (28H, m, 14xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₁₉H₄₀NO₂Cl (349.9) : 336 [M+Na]⁺ (100), 314(10), 313 [M+H]⁺ (29), 254(24).

Methyl α-amineicosanoate (33u) HCl salt.

DL-α-amineicosanoic acid **33g** (20g, 61mmol), methanol (294ml) and thionyl chloride (73g, 42ml) were reacted as described in method C.

Yield : 82.6%.

¹H-NMR (CDCl₃) δ : 8.93 (2H, m, NH₂), 4.0 (H, m, α-CH), 3.73 (3H, s, COOCH₃), 1.55 (2H, 2xm, CH₂), 1.28 (32H, m, 16xCH₂), 0.87 (3H, t, CH₃).

MS m/e (%) C₂₁H₄₄NO₂Cl (378) : 343(24), 342 [M+H]⁺ (100), 282 (38), 198(16), 153(13).

6.3. α-Amino alkanolic acid homo-oligomers.

α-Tert.butoxycarbonyl-ω-methoxybis[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (33y) [Method D].

Methyl α-aminotetradecanoate 33r HCl salt (4.24g, 14.43mmol) was added to dichloromethane (40ml) followed by triethylamine (1.46g, 14.43mmol), 1-hydroxybenzotriazole hydrate (1.95g, 14.43mmol), α-(tert.butoxycarbonylimino)tetradecanoic acid 33k (4.95g, 14.43mmol) and dicyclohexylcarbodiimide (3.27g, 15.87mmol) which were stirred at 0°c for 1 hour then at room temperature for 18 hours. The solvent was evaporated *in vacuo*, ethyl acetate (30ml) added and left to stir for 20 min. The precipitate formed was filtered and the filtrate washed with brine (50%, 30ml), citric acid 10%, brine, 3% NaHCO₃ and brine again. The organic phase was dried (anhydrous MgSO₄) and evaporated. The residue was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.25).

Yield : 76.8%.

¹H-NMR (CDCl₃) δ : 6.42 (H, s, CONH), 4.93 (H, s, OCONH), 4.56, 4.15 (2H, m, 2xα-CH), 3.73 (3H, s, COOCH₃), 1.75-1.55 (4H, 2xm, 2xCH₂), 1.43 (9H, s, C(CH₃)₃), 1.28 (40H, m, 20xCH₂), 0.87 (6H, t, 2xCH₃).

MS m/e (%) C₃₄H₆₆N₂O₅ (582.66) : 605 [M+Na]⁺ (100), 583 [M+H]⁺ (26), 505(28), 198(36), 153(13).

Fully protected lipidic dipeptides 33v, w, x, z, aa and ab were synthesised by coupling BOC protected α-amino alkanolic acid 33h, i, j, l, m and n (1 eq.) with the appropriate methyl α-aminoalkanoate 33o, p, q, s, t and u (1 eq.) using triethylamine (1 eq.), 1-hydroxybenzotriazole hydrate (1 eq.) and dicyclohexylcarbodiimide (1.1 eq.) as described in method D.

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-hexyl-2-oxo-1,2-ethanediyl)] (33v).

Yield : 83.4%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.43 (H, s, CONH), 4.92 (H, s, OCONH), 4.57, 4.15 (2H, m, $2x\alpha\text{-CH}$), 3.73 (3H, s, COOCH_3), 1.75-1.55 (4H, 2xm, $2x\text{CH}_2$), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.27 (16H, m, $8x\text{CH}_2$), 0.87 (6H, t, $2x\text{CH}_3$).

MS m/e (%) $\text{C}_{22}\text{H}_{42}\text{N}_2\text{O}_5$ (414.14) : 437 $[\text{M}+\text{Na}]^+$ (100), 415 $[\text{M}+\text{H}]^+$ (12), 237(18), 113(16).

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-octyl-2-oxo-1,2-ethanediyl)] (33w).

Yield : 78.3%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.44 (H, s, CONH), 4.93 (H, s, OCONH), 4.56, 4.16 (2H, m, $2x\alpha\text{-CH}$), 3.73 (3H, s, COOCH_3), 1.75-1.55 (4H, 2xm, $2x\text{CH}_2$), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.27 (24H, m, $12x\text{CH}_2$), 0.87 (6H, t, $2x\text{CH}_3$).

MS m/e (%) $\text{C}_{26}\text{H}_{50}\text{N}_2\text{O}_5$ (470.5) : 493 $[\text{M}+\text{Na}]^+$ (100), 471 $[\text{M}+\text{H}]^+$ (23), 141(21), 126(12).

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-decyl-2-oxo-1,2-ethanediyl)] (33x).

Yield : 73.9%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.44 (H, s, CONH), 4.93 (H, s, OCONH), 4.53, 4.14 (2H, m, $2x\alpha\text{-CH}$), 3.73 (3H, s, COOCH_3), 1.76-1.5 (4H, 2xm, $2x\text{CH}_2$), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.27 (32H, m, $16x\text{CH}_2$), 0.87 (6H, t, $2x\text{CH}_3$).

MS m/e (%) $\text{C}_{30}\text{H}_{58}\text{N}_2\text{O}_5$ (526.58) : 493 $[\text{M}+\text{Na}]^+$ (100), 471 $[\text{M}+\text{H}]^+$ (23), 141(21), 126(12).

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-tetradecyl-2-oxo-1,2-ethanediyl)] (33z).

Yield : 84.3%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.44 (H, s, CONH), 4.93 (H, s, OCONH), 4.53, 4.14 (2H, m, $2x\alpha\text{-CH}$), 3.73 (3H, s, COOCH_3), 1.76-1.5 (4H, 2xm, $2x\text{CH}_2$), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.27 (48H, m, $24x\text{CH}_2$), 0.87 (6H, t, $2x\text{CH}_3$).

MS m/e (%) $\text{C}_{38}\text{H}_{74}\text{N}_2\text{O}_5$ (638.74) : 661 $[\text{M}+\text{Na}]^+$ (100), 639 $[\text{M}+\text{H}]^+$ (34), 225(19), 210(12).

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-hexadecyl-2-oxo-1,2-ethanediyl)] (33aa).

Yield : 84.3%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.44 (H, s, CONH), 4.93 (H, s, OCONH), 4.53, 4.14 (2H, m, $2x\alpha\text{-CH}$), 3.73 (3H, s, COOCH_3), 1.76-1.5 (4H, 2xm, $2x\text{CH}_2$), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.27 (56H, m, $28x\text{CH}_2$), 0.87

(6H, t, 2xCH₃).

MS m/e (%) C₄₂H₈₂N₂O₅ (694.82) : 717 [M+Na]⁺ (100), 695 [M+H]⁺ (24), 253(19), 238(9).

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-octadecyl-2-oxo-1,2-ethanediyl)] (33ab).

Yield : 75.3%.

¹H-NMR (CDCl₃) δ : 6.44 (H, s, CONH), 4.93 (H, s, OCONH), 4.53, 4.14 (2H, m, 2x α -CH), 3.73 (3H, s, COOCH₃), 1.76-1.5 (4H, 2xm, 2xCH₂), 1.43 (9H, s, C(CH₃)₃), 1.27 (64H, m, 32xCH₂), 0.87 (6H, t, 2xCH₃).

MS m/e (%) C₄₆H₉₀N₂O₅ (750.9) : 773 [M+Na]⁺ (100), 751 [M+H]⁺ (13), 281(23), 266(19).

α -Amino- ω -methoxybis[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (33af) [Method E].

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] **33y** (3g, 5mmol) was dissolved in methanol (60ml), hydrochloric acid in methanol (approx.10M, 3ml) added and the resulting solution refluxed for 25mins. The reaction mixture was cooled, and the solvent removed *in vacuo*. The residue was crystallised from cold methanol.

Yield : 91.3%.

¹H-NMR (CDCl₃) δ : 7.85 (H, m, CONH), 4.56, 4.10 (2H, m, 2x α -CH), 3.73 (3H, s, COOCH₃), 1.75-1.55 (4H, 2xm, 2xCH₂), 1.28 (40H, m, 20xCH₂), 0.87 (6H, t, 2xCH₃).

MS m/e (%) C₂₉H₅₉N₂O₃Cl (519.24) : 506 [M+Na]⁺ (43), 484 [M+H]⁺ (26), 258(28), 198(100), 153(23).

α -Amino- ω -methoxybis[imino-(1-hexyl-2-oxo-1,2-ethanediyl)] (33ac).

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-hexyl-2-oxo-1,2-ethanediyl)] **33v** (2.07g, 5mmol) was dissolved in methanol (60ml) then refluxed with methanolic HCl (10M, 3ml) as described in method E.

Yield : 89.2%.

¹H-NMR (CDCl₃) δ : 7.9 (H, m, CONH), 4.56, 4.10 (2H, m, 2x α -CH), 3.73 (3H, s, COOCH₃), 1.75-1.55 (4H, 2xm, 2xCH₂), 1.28 (16H, m, 8xCH₂), 0.87 (6H, t, 2xCH₃).

MS m/e (%) C₁₇H₃₅N₂O₃Cl (350.8) : 337 [M+Na]⁺ (47), 315 [M+H]⁺ (26), 113(100), 98(90).

α -Amino- ω -methoxybis[imino-(1-octyl-2-oxo-1,2-ethanediyl)] (33ad).

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-octyl-2-oxo-1,2-ethanediyl)] 33w (2.35g, 5mmol) was dissolved in methanol (60ml) then refluxed with methanolic HCl (10M, 3ml) as described in method E.

Yield : 79.2%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.85 (H, m, CONH), 4.56, 4.10 (2H, m, $2\times\alpha\text{-CH}$), 3.73 (3H, d, COOCH_3), 1.75-1.55 (4H, 2xm, $2\times\text{CH}_2$), 1.27 (24H, m, $12\times\text{CH}_2$), 0.87 (6H, t, $2\times\text{CH}_3$).

MS m/e (%) $\text{C}_{21}\text{H}_{43}\text{N}_2\text{O}_3\text{Cl}$ (406.88) : 393 $[\text{M}+\text{Na}]^+$ (24), 371 $[\text{M}+\text{H}]^+$ (54), 141(71), 126(100).

α -Amino- ω -methoxybis[imino-(1-decyl-2-oxo-1,2-ethanediyl)] (33ae).

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-decyl-2-oxo-1,2-ethanediyl)] 33x (5.26g, 10mmol) was dissolved in methanol (120ml) then refluxed with methanolic HCl (10M, 6ml) as described in method E.

Yield : 84.7%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.85 (H, m, CONH), 4.54, 4.15 (2H, m, $2\times\alpha\text{-CH}$), 3.73 (3H, d, COOCH_3), 1.75-1.55 (4H, 2xm, $2\times\text{CH}_2$), 1.27 (32H, m, $16\times\text{CH}_2$), 0.87 (6H, t, $2\times\text{CH}_3$).

MS m/e (%) $\text{C}_{25}\text{H}_{51}\text{N}_2\text{O}_3\text{Cl}$ (462.96) : 449 $[\text{M}+\text{Na}]^+$ (35), 427 $[\text{M}+\text{H}]^+$ (57), 169(100), 154(51).

α -Amino- ω -methoxybis[imino-(1-tetradecyl-2-oxo-1,2-ethanediyl)] (33ag).

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-decyl-2-oxo-1,2-ethanediyl)] 33z (6.38g, 10mmol) was dissolved in methanol (120ml) then refluxed with methanolic HCl (10M, 6ml) as described in method E.

Yield : 88.6%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.85 (H, m, CONH), 4.54, 4.15 (2H, m, $2\times\alpha\text{-CH}$), 3.73 (3H, d, COOCH_3), 1.75-1.55 (4H, 2xm, $2\times\text{CH}_2$), 1.27 (48H, m, $24\times\text{CH}_2$), 0.87 (6H, t, $2\times\text{CH}_3$).

MS m/e (%) $\text{C}_{33}\text{H}_{67}\text{N}_2\text{O}_3\text{Cl}$ (575.12) : 561 $[\text{M}+\text{Na}]^+$ (35), 539 $[\text{M}+\text{H}]^+$ (57), 225(100), 210(63).

α -Amino- ω -methoxybis[imino-(1-hexadecyl-2-oxo-1,2-ethanediyl)] (33ah).

α -Tert.butoxycarbonyl- ω -methoxybis[imino(1-decyl-2-oxo-1,2-ethanediyl)] 33aa (3.48g, 5mmol) was dissolved in methanol (60ml) then refluxed with methanolic HCl (10M, 3ml) as described in method E.

Yield : 81.7%.

¹H-NMR (CDCl₃) δ : 7.85 (H, m, CONH), 4.54, 4.15 (2H, m, 2xα-CH), 3.73 (3H, s, COOCH₃), 1.75-1.55 (4H, 2xm, 2xCH₂), 1.27 (56H, m, 28xCH₂), 0.87 (6H, t, 2xCH₃).

MS m/e (%) C₃₇H₇₅N₂O₃Cl (632.21) : 618 [M+Na]⁺ (35), 596 [M+H]⁺ (57), 253(100), 238(32).

α-Amino-ω-methoxybis[imino-(1-octadecyl-2-oxo-1,2-ethanediyl)] (33ai).

α-Tert.butoxycarbonyl-ω-methoxybis[imino(1-decyl-2-oxo-1,2-ethanediyl)] 33ab (3.75g, 5mmol) was dissolved in methanol (60ml) then refluxed with methanolic HCl (10M, 3ml) as described in method E.

Yield : 85.8%.

¹H-NMR (CDCl₃) δ : 7.85 (H, m, CONH), 4.54, 4.15 (2H, m, 2xα-CH), 3.73 (3H, s, COOCH₃), 1.75-1.55 (4H, 2xm, 2xCH₂), 1.27 (64H, m, 32xCH₂), 0.87 (6H, t, 2xCH₃).

MS m/e (%) C₄₁H₈₃N₂O₃Cl (687.28) : 674 [M+Na]⁺ (35), 652 [M+H]⁺ (22), 337(100), 217(32), 188(11).

α-Tert.butoxycarbonyl-ω-methoxytris[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (33ai)

[Method F].

α-Amino-ω-methoxybis[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] 33af HCl salt (3g, 5.8mmol), triethylamine (0.585g, 5.8mmol), 1-hydroxybenzotriazole hydrate (0.78g, 5.8mmol), α-(tert.butoxycarbonylimino)tetradecanoic acid 33k (1.98g, 5.8mmol), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (1.15g, 6mmol) were stirred in dichloromethane at 0^oc for 1 hour and 20^oc overnight. The reaction was washed with water (2x50ml) and the organic layer dried (anhydrous MgSO₄) and evaporated. The residue was triturated with MeOH and the product filtered and purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:1).

Yield : 79.8%.

¹H-NMR (CDCl₃) δ : 6.71, 6.56 (2H, 2xm, 2xCONH), 4.93 (H, s, OCONH), 4.56, 4.31, 4.15 (3H, 3xm, 3xα-CH), 3.73 (3H, m, COOCH₃), 1.75-1.45 (6H, 3xm, 3xCH₂), 1.43 (9H, s, C(CH₃)₃), 1.28 (60H, m, 30xCH₂), 0.87 (9H, t, 3xCH₃).

MS m/e (%) C₄₈H₉₃N₃O₆ (808.25) : 832 (13), 831 [M+Na]⁺ (100), 809 [M+H]⁺ (26), 731(21), 258(9), 258(24), 198(38).

Fully protected lipidic tripeptides 33aj, ak, am, an and ao were synthesised by coupling BOC protected α -aminoalkanoic acids 33i, j, l, m and n (1 eq.) with the appropriate methyl protected lipidic dipeptides 33d, e, g, h and ai (1 eq.) using triethylamine (1 eq.), 1-hydroxybenzotriazole hydrate (1 eq.) and dicyclohexylcarbodiimide (1.1 eq.) as described in method F.

α -*Tert*.butoxycarbonyl- ω -methoxytris[imino(1-octyl-2-oxo-1,2-ethanediyl)] (33aj).

Yield : 91.8%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.79, 6.56 (2H, 2xm, 2xCONH), 4.94 (H, s, OCONH), 4.54, 4.30, 4.16 (3H, 3xm, 3x α -CH), 3.73 (3H, s, COOCH_3), 1.75-1.45 (6H, 3xm, 3x CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.28 (54H, m, 18x CH_2), 0.87 (9H, t, 3x CH_3).

MS m/e (%) $\text{C}_{36}\text{H}_{69}\text{N}_3\text{O}_6$ (639.94) : 664 (12), 663 $[\text{M}+\text{Na}]^+$ (100), 641 $[\text{M}+\text{H}]^+$ (26), 562(21), 202(9), 142(79).

α -*Tert*.butoxycarbonyl- ω -methoxytris[imino(1-decyl-2-oxo-1,2-ethanediyl)] (33ak).

Yield : 91.8%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.77, 6.54 (2H, 2xm, 2xCONH), 4.94 (H, s, OCONH), 4.53, 4.30, 4.16 (3H, 3xm, 3x α -CH), 3.73 (3H, s, COOCH_3), 1.75-1.45 (6H, 3xm, 3x CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.28 (72H, m, 24x CH_2), 0.87 (9H, t, 3x CH_3).

MS m/e (%) $\text{C}_{42}\text{H}_{81}\text{N}_3\text{O}_6$ (723.81) : 748 (8), 747 $[\text{M}+\text{Na}]^+$ (100), 725 $[\text{M}+\text{H}]^+$ (21), 647(47), 169(93), 154(42).

α -*Tert*.butoxycarbonyl- ω -methoxytris[imino(1-tetradecyl-2-oxo-1,2-ethanediyl)] (33am).

Yield : 88.8%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.77, 6.54 (2H, 2xm, 2xCONH), 4.94 (H, s, OCONH), 4.53, 4.30, 4.16 (3H, 3xm, 3x α -CH), 3.73 (3H, s, COOCH_3), 1.75-1.45 (6H, 3xm, 3x CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.28 (72H, m, 36x CH_2), 0.87 (9H, t, 3x CH_3).

MS m/e (%) $\text{C}_{54}\text{H}_{105}\text{N}_3\text{O}_6$ (892.05) : 916 (13), 915 $[\text{M}+\text{Na}]^+$ (100), 893 $[\text{M}+\text{H}]^+$ (32), 815(67), 253(21), 225(93), 210(41).

α -Tert.butoxycarbonyl- ω -methoxytris[imino(1-hexadecyl-2-oxo-1,2-ethanediyl)] (33an).

Yield : 85.6%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.77, 6.54 (2H, 2xm, 2xCONH), 4.94 (H, s, OCONH), 4.53, 4.30, 4.16 (3H, 3xm, 3x α -CH), 3.73 (3H, s, COOCH_3), 1.75-1.45 (6H, 3xm, 3x CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.28 (84H, m, 42x CH_2), 0.87 (9H, t, 3x CH_3).

MS m/e (%) $\text{C}_{60}\text{H}_{117}\text{N}_3\text{O}_6$ (976.17) : 1000 (7), 999 $[\text{M}+\text{Na}]^+$ (100), 977 $[\text{M}+\text{H}]^+$ (42), 899(63), 281(9), 253(73), 238(28).

α -Tert.butoxycarbonyl- ω -methoxytris[imino(1-octadecyl-2-oxo-1,2-ethanediyl)] (33ao).

Yield : 82.7%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.77, 6.54 (2H, 2xm, 2xCONH), 4.94 (H, s, OCONH), 4.53, 4.30, 4.16 (3H, 3xm, 3x α -CH), 3.73 (3H, s, COOCH_3), 1.75-1.45 (6H, 3xm, 3x CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.28 (84H, m, 42x CH_2), 0.87 (9H, t, 3x CH_3).

MS m/e (%) $\text{C}_{66}\text{H}_{129}\text{N}_3\text{O}_6$ (1060.29) : 1084 (17), 1083 $[\text{M}+\text{Na}]^+$ (100), 1061 $[\text{M}+\text{H}]^+$ (37), 983(57), 309(5), 281(79), 266(35).

α -Amino- ω -methoxytris[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (33ar).

α -Tert.butoxycarbonyl- ω -methoxytris[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] **33al** (2.02g, 2.5mmol) was dissolved in methanol (30ml) then refluxed with methanolic HCl (10M, 0.3ml) as described in method E.

Yield : 91.8%.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.21, 7.85 (2H, 2xm, 2xCONH), 4.56, 4.32, 4.10 (3H, 3m, 3x α -CH), 3.73 (3H, s, COOCH_3), 1.75-1.55 (6H, 3xm, 3x CH_2), 1.28 (60H, m, 30x CH_2), 0.87 (9H, t, 3x CH_3).

MS m/e (%) $\text{C}_{43}\text{H}_{86}\text{N}_3\text{O}_4\text{Cl}$ (744.31) : 731 $[\text{M}+\text{Na}]^+$ (33), 258(7), 198(100), 176(16), 69(13).

α -Amino- ω -methoxytris[imino-(1-octyl-2-oxo-1,2-ethanediyl)] (33ap).

α -Tert.butoxycarbonyl- ω -methoxytris[imino(1-octyl-2-oxo-1,2-ethanediyl)] **33j** (0.78g, 1.25mmol) was dissolved in methanol (15ml) then refluxed with methanolic HCl (10M, 0.75ml) as described in method E.

Yield : 90.1%.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.2, 7.8 (2H, 2xm, 2xCONH), 4.52, 4.32, 4.15 (3H, 3m, 3x α -CH), 3.73 (3H, s, COOCH_3), 1.75-1.55 (6H, 3xm, 3x CH_2), 1.28 (36H, m, 18x CH_2), 0.87 (9H, t, 3x CH_3).

MS m/e (%) $C_{31}H_{62}N_3O_4Cl$ (576.07) : 562 $[M+Na]^+$ (33), 540 $[M+H]^+$ (7), 141(100), 126(12).

α -Amino- ω -methoxytris[imino-(1-decyl-2-oxo-1,2-ethanediyl)] (33aq).

α -Tert.butoxycarbonyl- ω -methoxytris[imino(1-decyl-2-oxo-1,2-ethanediyl)] **33k** (0.9g, 1.25mmol) was dissolved in methanol (15ml) then refluxed with methanolic HCl (10M, 0.75ml) as described in method E.

Yield : 93.2%.

1H -NMR ($CDCl_3$) δ : 8.2, 7.8 (2H, 2xm, 2xCONH), 4.52, 4.32, 4.15 (3H, 3m, 3x α -CH), 3.73 (3H, s, $COOCH_3$), 1.75-1.55 (6H, 3xm, 3x CH_2), 1.28 (48H, m, 24x CH_2), 0.87 (9H, t, 3x CH_3).

MS m/e (%) $C_{37}H_{74}N_3O_4Cl$ (660.19) : 646 $[M+Na]^+$ (33), 624 $[M+H]^+$ (7), 169(100), 154(19).

α -Amino- ω -methoxytris[imino-(1-tetradecyl-2-oxo-1,2-ethanediyl)] (33as).

α -Tert.butoxycarbonyl- ω -methoxytris[imino(1-tetradecyl-2-oxo-1,2-ethanediyl)] **33m** (2.2g, mmol) was dissolved in methanol (30ml) then refluxed with methanolic HCl (10M, 1.5ml) as described in method E.

Yield : 87.3%.

1H -NMR ($CDCl_3$) δ : 8.25, 7.81 (2H, 2xm, 2xCONH), 4.52, 4.32, 4.15 (3H, 3m, 3x α -CH), 3.73 (3H, s, $COOCH_3$), 1.75-1.55 (6H, 3xm, 3x CH_2), 1.28 (72H, m, 36x CH_2), 0.87 (9H, t, 3x CH_3).

MS m/e (%) $C_{49}H_{98}N_3O_4Cl$ (828.43) : 814 $[M+Na]^+$ (23), 792 $[M+H]^+$ (18), 253(5), 225(100), 210(9).

α -Amino- ω -methoxytris[imino-(1-hexadecyl-2-oxo-1,2-ethanediyl)] (33at).

α -Tert.butoxycarbonyl- ω -methoxytris[imino(1-tetradecyl-2-oxo-1,2-ethanediyl)] **33n** (1.22g, 1.25mmol) was dissolved in methanol (15ml) then refluxed with methanolic HCl (10M, 0.75ml) as described in method E.

Yield : 85.7%.

1H -NMR ($CDCl_3$) δ : 8.21, 7.85 (2H, 2xm, 2xCONH), 4.48, 4.31, 4.15 (3H, 3m, 3x α -CH), 3.73 (3H, s, $COOCH_3$), 1.75-1.55 (6H, 3xm, 3x CH_2), 1.28 (84H, m, 42x CH_2), 0.87 (9H, 3t, 3x CH_3).

MS m/e (%) $C_{55}H_{110}N_3O_4Cl$ (912.55) : 898 $[M+Na]^+$ (39), 876 $[M+H]^+$ (21), 281(8), 253(100), 238(14).

α -Tert.butoxycarbonyl- ω -methoxytetra[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (33au**).**

α -Amino- ω -methoxytris[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (**33af**) HCl salt (223mg, 0.3mmol) was added to dichloromethane (10ml) followed by triethylamine (30mg, 0.3mmol), 1-hydroxybenzotriazole hydrate (41mg, 0.3mmol), α -(tert.butoxycarbonylimino)tetradecanoic acid **33k** (103mg, 0.3mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (63mg, 0.33mmol) as described in method **D**.

Yield : 78.8%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.76-6.47 (3H, m, 3xCONH), 4.93 (H, s, OCONH), 4.56-3.96 (4H, m, 4x α -CH), 3.73 (3H, s, COOCH_3), 1.75-1.55 (8H, m, 4x CH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.28 (80H, m, 40x CH_2), 0.87 (12H, t, 4x CH_3).

MS m/e (%) $\text{C}_{62}\text{H}_{120}\text{N}_4\text{O}_7$ (1033.62) : 1055 [$\text{M}+\text{Na}$] $^+$ (40), 1034 [$\text{M}+\text{H}$] $^+$ (5), 943(22), 830(30), 607(13), 592(17), 591(43), 491(15), 414(20), 413(70), 330(23), 329(100), 327(34), 304(22), 242(10).

6.4. Halogenated lipidic acids, lipidic amino acids and oligomers.

Methyl-2-halotetradecanoate 49ei+ii HCl salt.

2-Bromotetradecanoic acid (1g, 3.25mmol), methanol (20ml) and thionyl chloride (2.2ml) were reacted as described in method **C**.

Yield : 74.6% (oil).

$^1\text{H-NMR}$ (CDCl_3) δ : 4.24 (H, 2xd, α -CH), 3.76 (3H, s, COOCH_3), 1.75 (2H, 2xm, CH_2), 1.28 (20H, m, 10x CH_2), 0.86 (3H, t, CH_3).

MS m/e (%) $\text{C}_{15}\text{H}_{30}\text{O}_2\text{ClBr}$ (357.75), $\text{C}_{15}\text{H}_{30}\text{O}_2\text{Cl}_2$ (313.2) : 323, 321 [$\text{M}+\text{Na}$] $^+$ (52, 54), 299, 297 [$\text{M}+\text{Na}$] $^+$ (43, 14), 242(24), 211(35), 183(100).

Methyl-2-halooctaoate 49fi+ii HCl salt.

2-Bromooctanoic acid (0.73g, 3.25mmol), methanol (20ml) and thionyl chloride (2.2ml) were reacted as described in method **C**.

Yield : 79.4% (oil).

$^1\text{H-NMR}$ (CDCl_3) δ : 4.24 (H, 2xd, α -CH), 3.76 (3H, s, COOCH_3), 1.65 (2H, 2xm, CH_2), 1.28 (8H, m, 4x CH_2), 0.86 (3H, t, CH_3).

MS m/e (%) $\text{C}_9\text{H}_{18}\text{O}_2\text{ClBr}$ (273.63), $\text{C}_9\text{H}_{18}\text{O}_2\text{Cl}_2$ (229.08) : 261, 259 [$\text{M}+\text{Na}$] $^+$ (64, 67), 217, 215

[M+Na]⁺ (55, 18), 157(12), 126(35), 98(100).

Methyl-2-halohexadecanoate 49gi+ii HCl salt.

2-Bromohexadecanoic acid (1.09g, 3.25mmol), methanol (20ml) and thionyl chloride (2.2ml) were reacted as described in method C.

Yield : 63.74%.

¹H-NMR (CDCl₃) δ : 6.75 (H, 2xd, NH), 4.6, 4.3 (H, m, α-CH), 3.75 (3H, s, COOCH₃), 2.1-1.6 (4H, 2xm, 2xCH₂), 1.3 (24H, m, 12xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₁₇H₃₄O₂ClBr (385.79), C₁₇H₃₄O₂Cl₂ (341.24): 350, 348 [M+H]⁺ (3, 3), 306, 304 [M+H]⁺ (1.5, 4), 270(5), 269(16), 241(56), 227(13), 177(15), 163(21), 154(33), 108(97), 87(100).

α-(1-Bromo-1-dodecyl-2-oxo-1,2-ethandiyl)-ω-methoxy[lmino-(1-dodecyl-2-oxo-1,2-ethanediyl)] 50a.

2-Bromotetradecanoic acid (1g, 3.25mmol), methyl α-aminotetradecanoate 33r HCl salt (0.96g, 3.25mmol), triethylamine (0.33g, 3.25mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (0.74g, 3.6mmol) were reacted as described in method F. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.25).

Yield : 65.7%.

¹H-NMR (CDCl₃) δ : 6.43 (H, s, CONH), 4.56, 4.15 (2H, 2xm, 2xα-CH), 3.73 (3H, s, COOCH₃), 1.75-1.55 (4H, 2xm, 2xCH₂), 1.43 (9H, s, C(CH₃)₃), 1.28 (40H, m, 20xCH₂), 0.87 (6H, t, 2xCH₃).

MS m/e (%) C₂₉H₅₆O₃NBr (547.56) : 571, 569 [M+Na+H]⁺ (18, 21), 570, 568 [M+Na]⁺ (54, 61), 549, 547 [M+H]⁺ (6, 8), 548, 546 [M]⁺ (6, 8), 488(24), 466(9), 198(80), 133(100).

α-(1-Bromo-1-dodecyl-2-oxo-1,2-ethandiyl)-ω-methoxybis[lmino-(1-dodecyl-2-oxo-1,2-ethanediyl)] 50b.

2-Bromotetradecanoic acid (0.5g, 1.63mmol), α-amino-ω-methoxybis[lmino-(1-dodecyl-2-oxo-1,2-ethanediyl)] 33af (0.79g, 1.63mmol), triethylamine (0.165g, 1.63mmol), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (0.37g, 1.79mmol) were reacted as described in method F. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.25).

Yield : 65.7%.

¹H-NMR (CDCl₃) δ : 6.54, 6.43 (2H, 2xm, 2xCONH), 4.56, 4.31, 4.15 (3H, 3xm, 3xα-CH), 3.73

(3H, s, COOCH₃), 1.75-1.55 (6H, 3xm, 3xCH₂), 1.28 (60H, m, 30xCH₂), 0.86 (9H, t, 3xCH₃).

MS m/e (%) C₄₃H₈₃O₄N₂Br (771.83) : 795, 793 [M+Na]⁺ (41,39), 713(9), 412(24), 398(42), 198(46), 176(100).

α -Bromoethanoyl- ω -methoxy[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] 51a.

Bromoacetic acid (1g, 7.2mmol), methyl α -aminotetradecanoate **33r** (2.1g, 7.2mmol), triethylamine (720mg, 7.2mmol) and dicyclohexylcarbodiimide (1.48g, 7.2mmol) as described in method **D**. The crude product was purified by flash chromatography (solvent CH₂Cl₂:C₆H₆ 6:4). Yield : 74.8%.

¹H-NMR (CDCl₃) δ : 7.0-6.85 (H, m, NH), 4.6 (H, m, CH), 3.9 (2H, s, CH₂Br), 3.75 (3H, s, COOCH₃), 1.85 (2H, m, CH₂), 1.7 (2H, m, CH₂), 1.4-1.2 (18H, m, 2xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₁₇H₃₂O₃NBr : 667(4), 555(5), 433(10), 380,378 [M+H]⁺ (8, 8), 336(32), 334(100), 276(19), 274(54), 198(30), 137(27), 89(12), 69(20), 55(42).

α -Bromoethanoyl- ω -methoxybis[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] 51b.

Bromoacetic acid (91.4mg, 0.66mmol), α -amino- ω -methoxybis[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] **33af** (343mg, 0.66mmol), triethylamine (134mg, 0.66mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (126mg, 0.66mmol) as described in method **D**. The crude product was purified by preparative TLC (solvent CH₂Cl₂:C₆H₆ 6:4).

Yield : 64.5%.

¹H-NMR (CDCl₃) δ : 7.0-6.85 (2H, m, 2xNH), 4.6-4.45 (2H, 2xm, 2xCH), 3.9 (2H, s, CH₂Br), 3.75 (3H, s, COOCH₃), 1.8-1.7 (4H, 2xm, 2xCH₂), 1.4-1.2 (40H, m, 20xCH₂), 0.85 (6H, t, 2xCH₃).

MS m/e (%) C₃₁H₅₉O₄N₂Br (602.58) : 603,601 [M+H]⁺ (13, 14), 559(20), 537(60), 225(31), 494(12), 198(100), 69(20).

6.5. Hetero-oligomers containing amino-acids.

α -[*Tert*.butyloxycarbonyl-glycyl]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethanediyl] 53a.

Glycine (150mg, 0.5mmol), methyl α -aminotetradecanoate hydrochloride **33r** HCl (147mg, 0.5mmol), triethylamine (51mg, 0.5mmol), 1-hydroxybenzotriazole hydrate (68mg, 0.5mmol), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (106mg, 0.55mmol) as described in method **F**. The crude material was purified by preparative TLC (solvent CH₂Cl₂:MeOH

10:0.75).

Yield : 78.6%.

¹H-NMR (CDCl₃) δ : 6.54 (H, d, NH), 5.29 (H, bs, NH), 4.59 (H, m, α-CH), 3.86-3.76, (2H, m, CH₂CO), 3.74 (3H, s, COOCH₃), 1.83 (H, m, CH), 1.45 (9H, s, 3xCH₃), 1.33-1.2 (20H, m, 10xCH₂) 0.86 (3H, t, CH₃).

MS m/e (%) C₂₂H₄₂O₅N₂ (414.4) : 438 [M+Na+H]⁺ (24), 437[M+Na]⁺ (100), 359(11), 337(30), 315(5), 198(26).

Conjugates 53b-t were synthesised by reacting the appropriately protected amino acid (0.5mmol) with methyl α-aminotetradecanoate 33r (147mg, 0.5mmol) as described for conjugate 53a.

α-[*Tert*.butyloxycarbonyl-L-valyl]-ω-methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53b.

Yield : 72.8%.

¹H-NMR (CDCl₃) δ : 6.25 (H, m, NH), 5.0 (H, bs, NH), 4.57 (H, m, α-CH), 4.00-3.85 (H, m, α-CH), 3.74 (3H, s, COOCH₃), 2.25-2.1 (H, m, CH), 1.64 (H, m, CH), 1.44 (9H, s, 3xCH₃), 1.30-1.24 (20H, m, 10xCH₂), 0.97 (6H, 2xd, 2xCH₃), 0.87 (3H, t, CH₃).

MS m/e (%) C₂₅H₄₈O₅N₂ (456.48) : 479 [M+Na]⁺ (5), 457 [M+H]⁺ (2), 401(5), 379(5), 357(16), 258(13), 198(29), 116(12), 72(100).

α-[*Tert*.butyloxycarbonyl-L-alanyl]-ω-methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53c.

Yield : 76.8%.

¹H-NMR (CDCl₃) δ : 6.6-6.5 (H, 2xbd, N-H), 4.95 (H, bs, NH), 4.55 (H, m, α-CH), 4.15 (H, m, α-CH), 3.68 (3H, s, COOCH₃), 1.85 (H, m, CH), 1.65 (H, m, CH), 1.45 (9H, s, 3xCH₃), 1.34 (3H, m, CH₃), 1.30-1.15 (20H, m, 10xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₂₃H₄₄O₅N₂ (428.44) : 453 [M+Na+H]⁺ (25), 452 [M+Na]⁺ (100), 430 [M+H]⁺ (3), 374(7), 352(3), 330(3), 199(13), 176(10).

α-[*Tert*.butyloxycarbonyl-L-leucyl]-ω-methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53d.

Yield : 72.8%.

¹H-NMR (CDCl₃) δ : 6.35-6.25 (H, m, NH), 5.05 (H, bs, NH), 4.57 (H, m, α-CH), 3.95 (H, m, α-CH), 3.74 (3H, s, COOCH₃), 2.45 (2H, m, CH₂), 2.25-2.1 (H, m, CH), 1.82 (H, m, CH), 1.63 (H,

m, CH), 1.44 (9H, s, 3xCH₃), 1.30-1.24 (20H, m, 10xCH₂), 0.95 (6H, 2xd, 2xCH₃), 0.87 (3H, t, CH₃).

MS m/e (%) C₂₆H₅₀O₅N₂ (390.5) : 494 [M+Na+H]⁺ (28), 493 [M+Na]⁺ (100), 415(10), 393(28), 371(16), 258(10), 198(28), 130(12), 86(43).

α -[*Tert*.butyloxycarbonyl-L-isoleucyl]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53e.

Yield : 67.4%.

¹H-NMR (CDCl₃) δ : 6.35 (H, 2xd, NH), 5.1 (H, d, NH), 4.6 (H, m, α -CH), 3.95 (H, m, α -CH), 3.75 (3H, s, COOCH₃), 1.95-1.6 (3H, m, CHCH₂), 1.45 (9H, s, 3xCH₃), 1.35-1.25 (20H, m, 10xCH₂), 0.95-0.75 (9H, m, 3xCH₃).

MS m/e (%) C₂₆H₅₀O₅N₂ (470.5) : 495 [M+Na+H]⁺ (28), 494 [M+Na]⁺ (100), 472 [M+H]⁺ (3), 416(9), 198(21), 130(18), 86(53).

α -[*Tert*.butyloxycarbonyl-L-prolyl]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53f.

Yield : 68.6%.

¹H-NMR (CDCl₃) δ : 7.05 (H, m, NH), 4.65-4.5 (2H, 2xm, 2x α -CH), 3.65 (3H, s, COOCH₃), 3.3 (2H, t, CH₂), 2.1 (2H, m, CH₂), 1.9 (2H, m, CH₂), 1.8-1.6 (2H, m, CH₂), 1.45 (9H, s, 3xCH₃), 1.35-1.15 (20H, m, 10xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₂₅H₄₆O₅N₂ (454.46) : 478 [M+Na]⁺ (38), 477 [M+Na-H]⁺ (100), 377(37), 198(15), 144(34).

α -[*Tert*.butyloxycarbonyl-L-phenylalanyl]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53g.

Yield : 51.2%.

¹H-NMR (CDCl₃) δ : 7.35-7.2 (5H, 2xm, 5xaro.H), 6.25 (H, 2xd, NH), 5.0 (H, s, NH), 4.55 (H, m, α -CH), 4.35 (H, bd, α -CH), 3.7 (3H, s, COOCH₃), 3.1 (2H, m, CH₂), 1.75-1.60 (2H, m, CH₂), 1.40 (9H, s, 3xCH₃), 1.35-1.2 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₂₉H₄₈O₅N₂ (504.48) : 528 [M+Na+H]⁺ (35), 527 [M+Na]⁺ (100), 499(5), 427(37), 405(23), 258(11), 198(32), 120(55).

α -[*Tert*.butyloxycarbonyl-L-(2-bromobenzyloxycarbonyl)-tyrosyl]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53h.

Yield : 49.4%.

¹H-NMR (CDCl₃) δ : 7.6 (H, d, aro.H), 7.5 (H, d, aro.H), 7.35 (H, m, aro.H), 7.2 (2H, m, 2xaro.H), 7.15 (2H, m, 2xaro.H), 6.4 (H, d, NH), 5.85 (2H, s, CH₂O), 4.95 (H, bs, NH), 4.55 (H, m, α-CH), 4.35 (H, m, α-CH), 3.7 (H, d, COOCH₃), 3.35 (2H, t, bz.CH₂), 3.05 (2H, m, bz.CH₂), 1.75 (2H, m, CH₂), 1.40 (9H, s, 3xCH₃), 1.35-1.2 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₃₇H₅₅O₈N₂Br (732.6) : 757, 755 [M+Na]⁺ (32,31), 656(4), 336(19), 258(12), 198(66), 171(97), 169(100), 92(60), 69(100).

α-[Tert.butyloxycarbonyl-L-tryptophyl]-ω-methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53i.

Yield : 49.5%.

¹H-NMR (CDCl₃) δ : 8.05-7.65 (4H, m, 4xaro.H), 7.3 (H, bs,CH) 6.95 (H, s, NH), 5.75 (H, m, NH), 4.7-4.45 (2H, 2xm, 2xα-CH), 3.65 (3H, s, COOCH₃), 1.45 (9H, s, 3xCH₃), 1.35-1.1 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₃₂H₄₈O₆N₃ (570.48) : 595 [M+Na+H]⁺ (57), 594 [M+Na]⁺ (100), 494(75), 258(19), 198(65), 158(330), 130(48).

α-[Tert.butyloxycarbonyl-L-acetamidocystinyl]-ω-methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53j.

Yield : 65.5%.

¹H-NMR (CDCl₃) δ : 7.15-6.6 (2H, 2xd, 2xNH), 5.75-5.5 (H, m, NH), 4.5 (2H, m, 2xα-CH), 3.75 (3H, d, COOCH₃), 3.0-2.75 (4H, m, 2xS-CH₂), 2.05 (3H, d, COCH₃), 1.90-1.65 (2H, m, CH₂), 1.45 (9H, s, 3xCH₃), 1.45-1.25 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₂₆H₄₉O₆N₃S (531.8) : 555 [M+Na+H]⁺ (33), 554 [M+Na]⁺ (100), 454(44), 405(9), 361(16), 258(16), 198(31).

α-[Tert.butyloxycarbonyl-L-methionyl]-ω-methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53k.

Yield : 65.6%.

¹H-NMR (CDCl₃) δ : 6.65 (H, 2xd, NH), 5.15 (H, bs, NH), 4.5 (H, m, α-CH), 4.25 (H, m, α-CH) 3.75 (3H, m, COOCH₃), 2.60-2.45 (2H, m, S-CH₂), 2.15 (3H, s,CH₃), 1.95 (2H, m, CH₂), 1.85-1.65 (2H, m, CH₂), 1.45 (9H, s, 3xCH₃), 1.35-1.15 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₂₅H₄₈O₅N₂S (488.59) : 512 [M+Na+H]⁺ (12), 511 [M+Na]⁺ (49), 411(12), 389(36), 258(28), 198(68), 133(43).

α -[*Tert*.butyloxycarbonyl-(L-benzyl-threonyl)]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl]

53l.

Yield : 76.5%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.35-7.25 (5H, m, 5xaro.H), 7.1-6.9 (H, 2xd, NH), 5.5 (H, m, NH), 4.47-4.5 (3H, m, α -CH, CH_2O), 4.3 (H, m, α -CH), 4.15 (9H, s, $3\times\text{CH}_3$), 3.7 (3H, d, COOCH_3), 1.8-1.5 (2H, m, CH_2), 1.45 (9H, s, $3\times\text{CH}_3$), 1.35-1.2 (20H, m, $10\times\text{CH}_2$), 1.15 (3H, m, CH_3), 0.85 (3H, t, CH_3).
MS m/e (%) $\text{C}_{31}\text{H}_{52}\text{O}_6\text{N}_2$ (548.8) : 572 [$\text{M}+\text{Na}+\text{H}$] $^+$ (36), 571 [$\text{M}+\text{Na}$] $^+$ (99), 493(7), 471(43), 449(30), 258(5), 256(6), 198(25), 164(11), 91(100)

α -[*Tert*.butyloxycarbonyl-(L-benzoyl-seryl)]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl]

53m.

Yield : 80.1%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.35-7.25 (5H, m, 5xaro.H), 7.05-6.85 (H, 2xd, NH), 4.9 (H, bs, NH), 4.55 (3H, m, CH_2 , α -CH), 4.3 (H, bs, α -CH), 3.7 (3H, s, COOCH_3), 3.6 (2H, m, CH_2), 2.8-1.6 (H, 2xm, CH_2), 1.45 (9H, s, $3\times\text{CH}_3$), 1.35-1.15 (20H, m, $10\times\text{CH}_2$), 0.85 (3H, t, CH_3).
MS m/e (%) $\text{C}_{30}\text{H}_{51}\text{O}_6\text{N}_2$ (534.7) : 558 [$\text{M}+\text{Na}+\text{H}$] $^+$ (28), 557 [$\text{M}+\text{Na}$] $^+$ (63), 457(32), 435(18), 198(17), 15(8), 91(100).

α -[*Tert*.butyloxycarbonyl-(L-2-chlorobenzoxy-carbonyl-lycyl)]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53n.

Yield : 58.9%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.6 (H, d, aro.H), 7.5 (H, d, aro.H), 7.35-7.2 (2H, m, 2xaro.H), , 6.6-6.2 (2H, m, 2xNH), 5.85 (2H, s, CH_2O), 4.95 (H, bs, NH), 4.55-4.35 (2H, m, 2x α -CH), 3.7 (H, d, COOCH_3), 2.85 (2H, m, CH_2), 1.75-1.6 (4H, m, 2x CH_2), 1.40 (9H, s, $3\times\text{CH}_3$), 1.35-1.2 (26H, m, $13\times\text{CH}_2$), 0.85 (3H, t, CH_3).
MS m/e (%) $\text{C}_{34}\text{H}_{56}\text{O}_7\text{N}_3\text{Cl}$ (654.3) : 678,676 [$\text{M}+\text{Na}+\text{H}$] $^+$ (25,63), 578,576 [$\text{M}+\text{Na}$] $^+$ (10,27), 556,554(13,33), 367(13), 258(9), 198(135), 127,125(33,100).

α -[*Tert*.butyloxycarbonyl-(L-tosyl-arginyl)]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53o.

Yield : 63.9%.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.4 (H, bs, NH), 7.7 (H, bs, NH), 7.51 (2H, d, aro.H), 7.33 (2H, d, aro.H), 7.05 (H, m, NH), 5.3 (H, m, NH), 4.55 (2H, m, 2x α -CH), 3.7 (H, d, COOCH_3), 2.86 (2H, m, CH_2),

1.65-1.55 (2H, m, CH₂), 1.45 (9H, s, 3xCH₃), 1.35-1.15 (24H, m, 12xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₃₃H₅₇O₇N₅S (667.9) : 712 [M+2Na-H]⁺ (6), 691 [M+Na+H]⁺ (37), 690 [M+Na]⁺ (100), 591(10), 590(31), 568(5), 419(15), 198(7), 176(7), 91(12), 70(44).

α -[*Tert*.butyloxycarbonyl-(L-2,4-dinitrophenyl-histidyl)]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53p.

Yield : 77.3%.

¹H-NMR (CDCl₃) δ : 8.75 (H,s, aro.H), 8.55 (H,m, aro.H), 7.75-7.45 (H, 2xd, aro.H), 7.6 (H, d, CH), 7.1-6.9 (2H, 2xm, 2xNH), 6.2-5.95 (H, m, NH), 4.55 (2H, m, 2x α -CH), 3.65 (3H, d, COOCH₃), 3.25-3.0 (2H, m, CH₂), 1.75-1.6 (2H, 2xm, CH₂), 1.45 (9H, s, 3xCH₃), 1.35-1.20 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₃₂H₄₈O₉N₆ (661.79) : 684 [M+Na+H]⁺ (38), 683 [M+Na]⁺ (98), 667(16), 583(86), 561(13), 477(14), 377(14), 276(100), 198(36), 176(33).

α -[*Tert*.butyloxycarbonyl-(L-aspartylbenzyl ester)]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53q.

Yield : 65.2%.

¹H-NMR (CDCl₃) δ : 7.4-7.3 (5H, m, 5xaro.H), 7.0 (H, bs, NH), 5.2-5.1 (2H, m, CH₂), 4.55 (2H, m, α -CH), 3.7 (H, d, COOCH₃), 3.0-2.75 (2H, 2xm, CH₂), 1.8-1.6 (2H, m, CH₂), 1.45 (9H, s, 3xCH₃), 1.35-1.15 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₃₁H₅₀O₇N₂ (562.7) : 586 [M+Na+H]⁺ (30), 585 [M+Na]⁺ (87), 485(42), 463(15), 198(18), 178(6), 91(100).

α -[*Tert*.butyloxycarbonyl-(L-glutamyl benzyl ester)]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53r.

Yield : 74.3%.

¹H-NMR (CDCl₃) δ : 7.35 (5H, m, 5xaro.H), 7.1-6.8 (H, m, NH), 5.4 (H, m, NH), 4.55 (2H, m, 2x α -CH), 3.7 (H, d, COOCH₃), 3.1 (2H, m, CH₂), 1.65-1.5 (4H, m, 2xCH₂), 1.45 (9H, s, 3xCH₃), 1.4-1.2 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₃₂H₅₁O₇N₂ (576.69) : 601 [M+Na+H]⁺ (23), 600 [M+Na]⁺ (100), 578(10), 500(30), 198(24), 178(15), 107(10).

α -[*Tert*.butyloxycarbonyl-L-asparaginy]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53s.

Yield : 64.1%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.55 (H, d, NH), 5.5 (H, m, NH), 5.3 (H, m, NH), 4.9 (H, m, α -CH), 4.65 (H, m, α -CH), 3.7 (H, s, COOCH_3), 3.2 (2H, m, CH_2), 1.8-1.6 (2H, m, CH_2), 1.45 (9H, s, $3\times\text{CH}_3$), 1.35-1.2 (20H, m, $10\times\text{CH}_2$), 0.85 (3H, t, CH_3).

MS m/e (%) $\text{C}_{24}\text{H}_{45}\text{O}_6\text{N}_2$ (471.65) : 495 [$\text{M}+\text{Na}+\text{H}$] $^+$ (25), 494 [$\text{M}+\text{Na}$] $^+$ (100), 394(34), 198(7), 176(11), 109(10).

α -[*Tert*.butyloxycarbonyl-L-Glutamy]- ω -methoxy[1-dodecyl-2-oxo-1,2-ethane-diyl] 53t.

Yield : 59.6%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.65 (H, d, NH), 5.55 (H, m, NH), 5.35 (H, m, NH), 4.85-4.45 (2H, 2xm, $2\times\alpha$ -CH), 3.7 (H, s, COOCH_3), 3.2 (2H, m, CH_2), 1.9(2H, m, CH_2), 1.75-1.6 (2H, m, CH_2), 1.45 (9H, s, $3\times\text{CH}_3$), 1.35-1.2 (20H, m, $10\times\text{CH}_2$), 0.85 (3H, t, CH_3).

MS m/e (%) $\text{C}_{25}\text{H}_{47}\text{O}_6\text{N}_2$ (485.7) : 511 [$\text{M}+\text{Na}+\text{H}$] $^+$ (23), 510 [$\text{M}+\text{Na}$] $^+$ (100), 488(11), 198(20), 176(11).

6.6. Hetero-oligomers containing hydrophilic compounds.

Methyl 2-(2-hydroxypropamido)tetradecanoate 54a.

To a mixture of lactic acid (360mg, 3.42mmol, 85 % water solution) and methyl ester 33r HCl (1.0g, 3.42mmol), triethylamine (0.472ml, 3.42mmol), and 1-hydroxybenzotriazole (0.462g, 3.42mmol) in ethylacetate (10ml) and CH_2Cl_2 (5ml), dicyclohexylcarbodiimide (1.409g, 6.84mmol) was added at 0°C . The mixture was reacted as described in method **D**. The crude product was purified by flash chromatography (solvent CH_2Cl_2 :MeOH 10:0.5).

Yield : 81.4%.

$^1\text{H-NMR}$ (CDCl_3) δ : 6.89 (H, m, NH), 4.59 (H, m, α -CH), 4.26 (H, m, CH-OH), 3.72 (3H, s, COOCH_3), 1.86 (2H, m, CH_2), 1.69 (2H, m, CH_2), 1.45 (3H, 2xd, CH_3), 1.25 (18H, m, $9\times\text{CH}_2$), 0.88 (3H, t, CH_3).

MS m/e (%) : 330 [$\text{M}+\text{H}$] $^+$ (100), 270(23), 258(24), 198(31), 137(2), 89(1), 56(2), 45(2).

$\text{C}_{18}\text{H}_{35}\text{NO}_4$ (329.5)

Calcd. C 65.61 H 10.71 N 4.25

Found C 65.66 H 10.57 N 4.03

2-(2-Hydroxypropamido)tetradecanoic acid (54b) [Method G].

Methyl 2-(2-hydroxypropamido)tetradecanoate 54a (1.0g, 3.04mmol) was stirred in a mixture of MeOH (40ml) and N NaOH (10 ml) at room temperature for 5 hours. The methanol was evaporated, the pH of the residue was adjusted to 4 with 3 % HCl, extracted with ether and the organic phase, after drying, was evaporated and the residue purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 97.4%.

¹H-NMR (CDCl₃) δ : 7.13, 7.05 (H, dd, NH), 4.54 (H, m, α-CH), 4.29 (H, m, CH-OH), 1.91 (H, m, CH₂), 1.72 (H, m, CH₂), 1.32 (18H, m, 9xCH₂), 0.88 (3H, t, CH₃).

MS m/e (%) : 316 [M+H]⁺ (77), 270(39), 244(38), 198(100), 137(10), 98(9), 41(20).

C₁₇H₃₃NO₄ (315.5)

Calcd. C 64.71 H 10.54 N 4.44

Found C 64.99 H 10.29 N 4.16

Methyl 2-(2-hydroxyacetamido)decanoate (54c).

A mixture of methyl α-aminodecanoate 33p HCl (0.5g, 2.1mmol), glycolic acid (0.198g, 95%, 2.1mmol), triethylamine (0.212g, 2.1mmol) and dicyclohexylcarbodiimide (0.453g, 2.1mmol) and 1-hydroxybenzotriazole (0.283g, 2.1mmol) was reacted in dichloromethane (30ml) as described in Method D. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 91%.

¹H-NMR (CDCl₃) δ : 6.86 (1 H, d, NH), 4.66 (1 H, m, α-CH), 4.15 (2 H, s, CH₂OH), 3.75 (3 H, s, COOCH₃), 1.86 (2 H, m, CH₂), 1.72 (2 H, m, CH₂), 1.30 (10 H, m, 5 CH₂), 0.87 (3 H, t, CH₃).

MS m/e (%): 282 [M+Na]⁺ (88), 260 [M+H]⁺ (85), 244(5), 225(11), 200(100), 142(82), 128(5), 115(7), 102(9), 88(10), 69(11), 55(28).

C₁₃H₂₅NO₄ (259.3)

Calcd. C 60.21 H 9.72 N 5.40

Found C 59.99 H 9.84 N 5.18

Methyl 2-(2-hydroxyethanamido)tetradecanoate (54d).

A mixture of methyl α-aminotetradecanoate 33r HCl (0.5g, 1.7mmol), glycolic acid (0.135g, 95%, 1.7mmol), triethylamine (0.17g, 1.7mmol), 1-hydroxybenzotriazole (0.229g,

1.7mmol) and dicyclohexylcarbodiimide (0.35g, 1.7mmol) were reacted as described in method **D**. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 81%.

¹H-NMR (CDCl₃) δ : 6.89 (H, m, NH), 4.64 (H, m, α-CH), 4.15 (H, s, CH₂OH), 3.76 (3H, s, COOCH₃), 2.82 (H, m, OH), 1.87 (2H, m, CH₂), 1.68 (2H, m, CH₂), 1.27 (18H, m, 9xCH₂), 0.88 (3H, t, CH₃).

MS m/e (%) : 338 [M+ Na]⁺ (100), 316 [M+H]⁺ (5), 256(6), 198(5), 182(2), 173(2), 147(1), 133(1), 124(1), 92(1), 77(1), 69(1), 55(2).

C₁₇H₃₃NO₄ (315.5)

Calcd. C 64.71 H 10.54 N 4.44

Found C 64.66 H 10.32 N 4.23

2-(2-Hydroxyethanamido)tetradecanoic acid (54e).

Methyl 2-(2-hydroxyethanamido)tetradecanoate 54d (0.3g, 0.95mmol) was stirred in a mixture of MeOH (10ml) and N NaOH (3ml) for 16 hours as described in method **G**. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 95.5%.

¹H-NMR (CDCl₃) δ : 7.18 (H, dd, NH), 4.59 (3H, d, CH₃), 4.54, 4.45 (H, m, α-CH), 4.07 (2H, s, CH₂OH), 3.68 (3H, s, COOCH₃), 1.82 (2H, m, CH₂), 1.68 (2H, m, CH₂), 1.25 (18H, m, 9xCH₂), 0.84 (3H, t, CH₃).

MS m/e (%) : 302 [M+H]⁺ (100), 282(2), 268(2), 256(45), 244(4), 225(26), 198(25), 98(3), 56(3).

C₁₆H₃₁NO₄ (301.4)

Calcd. C 63.76 H 10.36 N 4.65

Found C 63.55 H 10.09 N 4.41

α-(2-Hydroxyacetyl)-ω-methoxybis[imino(1-dodecyl-2-oxo-ethanediyl)] (54f).

A mixture of α-amino-ω-methoxybis[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] 33af HCl (0.5g, 0.96mmol), glycolic acid (0.8g, 95%, 1mmol), triethylamine (0.1g, 1mmol) and dicyclohexylcarbodiimide (0.21g, 1mmol), and 1-hydroxybenzotriazole (0.14g, 1mmol) was reacted in dichloromethane (20ml) as described in method **D**. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 31%.

¹H-NMR (CDCl₃) δ : 7.37-6.56 (2H, 3xm, 2xNH), 4.59-4.37 (2H, m, 2xα-CH), 4.14 (2H, s, CH₂OH), 3.70 (3H, s, COOCH₃), 3.20 (H, m, OH), 1.87 (4H, m, 2xCH₂), 1.70 (4H, m, 2xCH₂), 1.28 (36H, m, 18xCH₂), 0.85 (6H, t, 2xCH₃).

MS m/e (%) : 563 [M+Na]⁺ (100), 541 [M+H]⁺ (2), 533(2), 505(4), 491(2), 432(8), 407(7) 390(4), 364(4), 334(2), 278(3), 256(6), 239(1), 198(17), 176(4), 149(5), 95(5).

C₃₁H₆₀N₂O₅ (540.8)

Calcd. C 68.84 H 11.18 N 5.18

Found C 68.59 H 10.96 N 4.93

Methyl 2-(2-glucoronamido)tetradecanoate (54g).

A mixture of methyl α-aminotetradecanoate **33r** HCl (0.5g, 1.7mmol), D-glucuronic acid (0.33g, 1.7mmol), triethylamine (0.17g, 1.7mmol), 1-hydroxybenzotriazole (0.229g, 1.7mmol) and dicyclohexylcarbodiimide (0.35g, 1.7mmol) were reacted in dichloromethane (30ml) as described in method **D**. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 45%.

¹H-NMR (CDCl₃) δ : 3.84 (H, m, C₂-H), 3.75-3.43 (3H, m, C₄-H, C₃-H, α-CH), 3.37 (3H, s, COOCH₃), 3.30 (H, m, C₅-H), 1.85, 1.65 (4H, 2xm, 2xCH₂), 1.22 (18H, m, 9xCH₂), 1.84 (3H, t, CH₃).

MS m/e (%) : 432 [M-H]⁺ (100), 350(14), 268(5), 198(6), 133(7), 112(4), 81(5), 69(3), 55(8).

C₂₁H₃₉NO₈ (433.5)

Calcd. C 58.18 H 9.07 N 3.23

Found C 57.93 H 8.89 N 3.23

Methyl 2-[2-(2,3:4,6-di-O-isopropylidene-2-keto-L-gulonamido)]decanoate (54h).

A mixture of methyl α-aminodecanoate **33b** HCl (0.5g, 2.1mmol), 1,2:3,4-di-O-isopropylidene-D-gulonic acid (0.575g, 2.1mmol), triethylamine (0.212g, 2.1mmol), 1-hydroxybenzotriazole (0.283g, 2.1mmol) and dicyclohexylcarbodiimide (0.453g, 2.1mmol) were reacted in dichloromethane (30ml) as described in method **D**. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 81%.

¹H-NMR δ (CDCl₃) : 7.46 (H, m, NH), 4.59 (H, d, C₃H), 4.57 (H, m, α-CH), 4.30 (H, dd, C₄-H),

4.16 (H, t, C₅-H), 4.11 (2H, d, CH₂O), 1.87 (2H, m, CH₂), 1.70 (2H, m, CH₂), 1.58, 1.54, 1.44, 1.36 (12H, 4xs, 4xCH₃), 1.27 (18H, m, 9xCH₂), 0.88 (3H, t, CH₃).

MS m/e (%) : 458 [M+H]⁺ (67), 442(43), 400(100), 342(10), 282(6), 240(6), 200(9), 171(8), 142(11), 113(6), 95(6), 85(6), 69(8), 59(10).

C₂₃H₃₉NO₈ (457.5)

Calcd. C 60.34 H 8.59 N 3.06

Found C 60.61 H 8.59 N 3.00

Methyl 2-[2-(2,3:4,6-di-O-isopropylidene-2-keto-L-gulonamido)]tetradecanoate (54i).

A mixture of methyl α-aminotetradecanoate 33r HCl (0.5g, 1.7mmol), 1,2:3,4-di-isopropylidene-D-gulonic acid (0.465g, 1.7mmol), triethylamine (0.17g, 1.7mmol), 1-hydroxybenzotriazole (0.229g, 1.7mmol) and dicyclohexylcarbodiimide (0.35g, 1.7mmol) were reacted in dichloromethane (30ml) as described in method D. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 81%.

¹H-NMR (CDCl₃) δ : 7.44 (H, m, NH), 4.58 (H, d, C₃H), 4.57 (H, m, α-CH), 4.30 (H, dd, C₄-H), 4.17 (H, dt, C₅-H), 4.13 (2H, d, CH₂O), 1.86 (2H, m, CH₂), 1.69 (2H, m, CH₂), 1.56, 1.53, 1.44, 1.34 (12H, 4xs, 4xCH₃), 1.25 (18H, m, 9xCH₂), 0.88 (3H, t, CH₃).

MS m/e (%) : 514 [M+H]⁺ (38), 498(36), 456(100), 398(13), 256(15), 216(9), 198(32), 171(19), 133(11), 113(11), 95(11), 85(11), 69(12), 59(15).

C₂₇H₄₇NO₈ (513.7)

Calcd. C 63.12 H 9.22 N 2.72

Found C 63.00 H 8.99 N 2.68

2-[2-(2,3:4,6-Di-O-isopropylidene-2-keto-L-gulonamido)]tetradecanoic acid (54j).

Methyl 2-[2-(2,3:4,6-di-O-isopropylidene-2-keto-L-gulonamido)]tetradecanoate 55i (0.3g, 0.58mmol) was stirred in a mixture of MeOH (10ml) and N NaOH (2ml) as in method G. Yield : 95.6%. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

¹H-NMR (CDCl₃) δ : 7.47 (H, dd, NH), 4.61 (H, d, C₃-H), 4.55 (H, m, α-CH), 4.34 (H, dd, C₄-H), 4.18 (H, 2xt, C₅-H), 4.13 (2H, d, CH₂O), 1.92 (2H, m, CH₂), 1.76 (2H, m, CH₂), 1.53, 1.47, 1.35 (12H, s, 4xCH₃), 1.27 (18H, m, 9xCH₂), 0.87 (3H, t, CH₃).

MS m/e (%) : 522 [M+Na]⁺ (6), 500 [M+H]⁺ (24), 484(26), 442(100), 426(6), 396(9), 384(10), 338(5), 296(7), 216 (7), 198(37), 133(21), 113(10), 95(11), 85(10), 69(14), 59(18).

C₂₆H₄₅NO₈ (499.6)

Calcd. C 62.50 H 9.08 N 2.80

Found C 62.51 H 9.33 N 2.69

2-[2-(2,3-O-Isopropylidene-2-keto-L-gulonamido)]tetradecanoic acid (54i)

[Method H].

2-[2-(2,3:4,6-Di-O-isopropylidene-2-keto-L-gulonamido)]tetradecanoic acid 54i (1.0g, 2.0mmol) was stirred in a mixture of methanol (10ml) and 70 % acetic acid (10ml) at 40°C for 24hours. The mixture was diluted with brine (50ml) and extracted with ethylacetate (3x50ml). The organic phase was dried, evaporated and the residue purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 82.7%.

¹H-NMR (CDCl₃) δ : 8.00-7.54 (H, m, NH), 4.6 - 4.0 (5H, m, CH₂O, α-CH, 2xsugar-H), 3.75 (H, m, sugar-H), 1.90 (2H, m, CH₂), 1.82 (2H, m, CH₂), 1.54, 1.46 1.44 (6H, 3xs, 2xCH₃), 1.22 (18H, m, 9xCH₂), 0.88 (3H, t, CH₃).

MS m/e (%) : 504 [M+2Na-H]⁺ (100), 482 [M+Na]⁺ (98), 466(6), 436(12), 411(24), 389(16), 282(27), 256(12), 198(59), 176(11), 164(12), 149(18), 137(11), 81(18), 69(37), 55(69).

C₂₃H₄₁NO₈ (459.6)

Calcd. C 60.10 H 8.99 N 3.05

Found C 59.86 H 9.11 N 2.94

α-(2,3:4,6-Di-O-isopropylidene-2-keto-L-gulonyl)-ω-methoxybis[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (54k).

A mixture of dimer 33af (0.5g, 0.96mmol), 1,2:3,4-di-O-isopropylidene-D-gulonic acid (0.26g, 0.96mmol), triethylamine (0.1g, 1mmol), 1-hydroxybenzotriazole (0.14g, 1mmol) and dicyclohexylcarbodiimide (0.21g, 1mmol) were reacted in dichloromethane (20ml) as described in method **D**. The crude product was purified by flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 80%.

¹H-NMR (CDCl₃) δ : 7.37 (H, m, NH), 6.63, 6.40 (H, 2xm, NH), 4.59 (H, d, C₃H), 4.54, 4.45 (2H,

2xm, 2x α -CH), 4.34 (H, dd, C₄-H), 4.16 (H, t, C₅-H), 4.13 (2H, d, CH₂O), 1.92 (4H, m, 2xCH₂), 1.77 (4H, m, 2xCH₂), 1.56, 1.53, 1.44, 1.34 (12H, 3xs, m, 4xCH₃), 1.23 (36H, m, 18xCH₂), 0.88 (6H, t, 2xCH₃).

MS m/e (%) : 762 [M+Na]⁺ (100), 682(6), 631(4), 605(7), 476(9), 396(8), 322(9), 258(9), 198(49), 171(5), 133(19), 55(16).

C₄₁H₇₄N₂O₉ (739.0)

Calcd. C 66.63 H 10.09 N 3.79

Found C 66.55 H 9.89 N 3.56

6.7. Chlorambucil lipidic acids, lipidic amino acids and oligomers.

α -{4-[4-Bis(2-chloroethyl)aminophenyl]butanoyl}- ω -methoxy[imino-(1-octyl-2-oxo-1,2-ethanediy)] (**56a**) [Method I].

Chlorambucil **55** (150mg, 0.49mM), triethylamine (50mg, 0.49mM), 1-hydroxybenzotriazole hydrate (67mg, 0.49mM), methyl α -aminodecanoate **33p** HCl (119.3mg, 0.49mM) and dicyclohexylcarbodiimide (102mg, 0.49mM) were stirred in dichloromethane at 0°C for 1 hour then 20°C for a further 4 hours. The reaction mixture was filtered, the solvent removed in vacuo, the residue washed with ethyl acetate then filtered and the ethyl acetate removed by vacuo. The crude material was purified by preparative TLC (solvent system CH₂Cl₂:MeOH 10:1). Yield : 64%.

¹H-NMR (CDCl₃) δ : 7.06 (2H, d, aro.H), 6.65 (2H, d, aro.H), 5.86 (H, d, NH), 4.63 (H, m, α -CH), 3.75 (3H, s, COOCH₃), 3.68(4H, t, 2xCH₂N), 3.62 (4H, t, 2xCH₂Cl), 2.58 (2H, t, bzCH₂), 2.28 (2H, t, CH₂CO), 1.94 (2H, t, CH₂), 1.83-1.65 (2H, m, β -CH₂), 1.26 (12H, m, 6xCH₂), 0.88 (3H, t, CH₃). MS m/e (%) C₂₅H₄₀O₃N₂Cl₂ (487.3) : 491,489,487 [M+H]⁺ (9,60,82), 454(52), 437(56), 290,288,286 [CLB-OH]⁺ (10,60,100), 250(25), 230(38), 194(48), 142(75), 118(35), 55(45).

Chlorambucil lipidic conjugates **56b-f** were synthesised by coupling chlorambucil **55** (1 eq.) to the appropriate methyl protected lipidic amino acid/peptide **33r, s, af, ag, al** (1 eq.) using triethylamine (1 eq.), 1-hydroxybenzotriazole (1 eq.) and dicyclohexylcarbodiimide (1.1 eq.) as described in method I.

α -{4-[4-Bis-(2-chloroethyl)aminophenyl]butanoyl}- ω -methoxy[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (56b)

Yield : 43.2%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.08 (2H, d, aro.H), 6.63 (2H, d, aro.H), 5.85 (H, d, NH), 4.62 (H, m, α -CH), 3.74 (3H, s, COOCH_3), 3.68 (4H, t, $2\times\text{CH}_2\text{N}$), 3.62 (4H, t, $2\times\text{CH}_2\text{Cl}$), 2.58 (2H, t, bzCH_2), 2.40, 2.21 (2H, 2xt, CH_2CO), 1.94 (2H, t, CH_2), 1.83-1.60 (4H, m, $2\times\beta\text{CH}_2$, $2\times\gamma\text{-CH}_2$), 1.25 (22H, m, $11\times\text{CH}_2$), 0.879 (3H, t, CH_3).

MS m/e (%) $\text{C}_{29}\text{H}_{48}\text{O}_3\text{N}_2\text{Cl}_2$ (543.38) : 570, 568, 556 [$\text{M}+\text{Na}+\text{H}$] $^+$ (3, 24, 35), 569, 567, 565 [$\text{M}+\text{Na}$] $^+$ (12, 73, 100), 532(12), 507(15), 291(1), 289(6), 286(9), 250(13).

α -{4-[4-Bis(2-chloroethyl)aminophenyl]butanoyl}- ω -methoxy[imino-(1-tetradecyl-2-oxo-1,2-ethanediyl)] (56c)

Yield : 66.3%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.05 (2H, d, aro.H), 6.65 (2H, d, aro.H), 5.85 (H, d, NH), 4.6 (H, m, α -CH), 3.75 (3H, s, COOCH_3), 3.70 (4H, t, $2\times\text{CH}_2\text{N}$), 3.62 (4H, t, $2\times\text{CH}_2\text{Cl}$), 2.55 (2H, m, bzCH_2), 2.2 (2H, m, CH_2CO), 1.8-1.55 (4H, m, $4\times\text{CH}$), 1.25 (24H, m, $12\times\text{CH}_2$), 0.875 (3H, t, CH_3).

MS m/e (%) $\text{C}_{31}\text{H}_{51}\text{O}_3\text{N}_2\text{Cl}_2$ (570.41) : 598, 596, 594 [$\text{M}+\text{Na}+\text{H}$] $^+$ (4, 23, 37), 597, 595, 593 [$\text{M}+\text{Na}$] $^+$ (10, 65, 100), 290(5), 288(22), 286(35), 250(26), 194(26), 188(22), 69(30).

α -{4-[4-Bis(2-chloroethyl)aminophenyl]butanoyl}- ω -methoxybis[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (56d).

Yield : 86.4%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.05 (2H, d, aro.H), 6.65 (2H, d, aro.H), 6.55-5.96 (H, m, NH), 5.98 (H, m, NH), 4.55-4.90 (2H, m, $2\times\alpha$ -CH), 3.75 (3H, s, COOCH_3), 3.70 (4H, m, $2\times\text{CH}_2\text{N}$), 3.60 (4H, m, $2\times\text{CH}_2\text{Cl}$), 2.55 (2H, m, bzCH_2), 2.85-2.15 (2H, m, CH_2CO), 1.95 (2H, m, CH_2), 1.85-1.50 (4H, m, $2\times\text{CH}_2$), 1.5 (40H, m, $20\times\text{CH}_2$), 0.85 (6H, t, $2\times\text{CH}_3$).

MS m/e (%) $\text{C}_{43}\text{H}_{74}\text{O}_4\text{N}_3\text{Cl}_2$ (768.7) : 772, 770, 768 [$\text{M}+\text{H}$] $^+$ (2, 5, 13), 771, 769, 767 [$\text{M}+\text{H}$] $^+$ (2, 6, 18), 722, 720, 718 (2, 6, 18), 511(6), 290, 288, 286 (8, 46, 75), 258(23), 230(21), 198(100), 118(16), 91(12).

α -{4-[4-Bis(2-chloroethyl)aminophenyl]butanoyl}- ω -methoxybis[imino-(1-tetradecyl-2-oxo-1,2-ethanediyl)] (55f).

Yield : 82%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.05 (2H, d, aro.H), 6.65 (2H, d, aro.H), 6.55-5.96 (H, m, NH), 5.98 (H, m, NH), 4.55-4.90 (2H, m, $2\times\alpha\text{-CH}$), 3.75 (3H, s, COOCH_3), 3.70 (4H, m, $2\times\text{CH}_2\text{N}$), 3.60 (4H, m, $2\times\text{CH}_2\text{Cl}$), 2.55 (2H, m, bzCH_2), 2.85-2.15 (2H, m, CH_2CO), 1.95 (2H, m, CH_2), 1.85-1.50 (4H, m, $2\times\text{CH}_2$), 1.5 (48H, m, $24\times\text{CH}_2$), 0.85 (6H, t, $2\times\text{CH}_3$),

MS m/e (%) $\text{C}_{47}\text{H}_{82}\text{O}_4\text{N}_3\text{Cl}_2$ (823.72) : 850,848,846 $[\text{M}+\text{Na}]^+$ 15,74,100, 809(25), 744(7), 748(7), 733(10), 613 (13), 290(3), 288(10), 286(19), 250(61), 226(70), 173(72), 73(100).

α -{4-[4-Bis(2-chloroethyl)aminophenyl]butanoyl}- ω -methoxytris[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (56f).

Yield : 75.8%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.05 (2H, d, aro.H), 6.65 (2H, d, aro.H), 6.55-5.96 (H, m, NH), 5.98 (H, m, NH), 4.55-4.90 (2H, m, $2\times\alpha\text{-CH}$), 3.75 (3H, s, COOCH_3), 3.70 (4H, m, $2\times\text{CH}_2\text{N}$), 3.60 (4H, m, $2\times\text{CH}_2\text{Cl}$), 2.55 (2H, m, bzCH_2), 2.85-2.15 (2H, m, CH_2CO), 1.95 (2H, m, CH_2), 1.85-1.50 (6H, m, $3\times\text{CH}_2$), 1.5 (60H, m, $30\times\text{CH}_2$), 0.85 (6H, t, $2\times\text{CH}_3$).

MS m/e (%) $\text{C}_{57}\text{H}_{101}\text{O}_5\text{N}_4\text{Cl}_2$ (992.91) : 997,995,993 $[\text{M}+\text{H}]^+$ (4,23,37), 996,994,992 $[\text{M}+\text{H}]^+$ (2,6,18), 927,945,943(3,18,26), 290,288,286(8,46,75), 258(29), 230(27), 198(100), 118(6), 91(17).

α -{4-[4-bis(2-chloroethyl)aminophenyl]butanoate}- ω -methoxy(1-dodecyl-2-oxo-1,2-ethanediyl) (57b) [Method J].

Chlorambucil **55** (199.8mg, 0.66mmol) and 18-crown-6 (173.7mg, 0.66mmol) were added to an ethanolic potassium hydroxide solution 5mgml^{-1} , (22.3ml) and stirred at 0°C for 3 hours. The solvent was evaporated in vacuo then the residue lyophilised. The resulting chlorambucil crown ether complex and methyl 2-halotetradecanoate **49e** (211mg, approx.0.66mmol) were dissolved in 5ml dimethylformamide and stirred for 12 hours. The dimethylformamide was evaporated off under high vacuo and the crude product was purified by preparative TLC (solvent system $\text{CH}_2\text{Cl}_2:\text{MeOH}$ 10:1, followed by $\text{CH}_2\text{Cl}_2:\text{C}_6\text{H}_6$ 6:4).

Yield : 63.7%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.05 (2H, d, aro.H), 6.62 (2H, d, aro.H), 4.23 (H, m, $\alpha\text{-CH}$), 3.60 (3H, s, COOCH_3), 3.67 (4H, m, $2\times\text{CH}_2\text{N}$), 3.61 (4H, m, $2\times\text{CH}_2\text{Cl}$), 2.58 (2H, m, bzCH_2), 2.48 (2H, m,

CH₂CO), 1.98 (2H, m, CH₂), 1.96-1.80 (4H, m, 2xCH₂), 1.25 (18H, m, 9xCH₂) 0.85 (3H,t,CH₃).
 MS m/e (%) C₂₉H₄₇O₄NCl₂ (544.37) : 570,568,566 [M+Na]⁺ (6,22,35), 547,545,543 [M]⁺ (7,65,80),
 517(46), 496(47), 494(69), 290(10), 288(58), 286(100), 259(22), 230(40), 194(28), 168(22), 188
 (38).

α-[4-[4-bis(2-chloroethyl)aminophenyl]butanoate]-ω-methoxy(1-hexyl-2-oxo-1,2-ethanediyl) (57a).

Chlorambucil crown ether complex (0.33mmol) and methyl 2-haloctanoate **49d** (85mg, approx.0.33mmol) were dissolved in 5ml dimethylformamide and reacted as described in method J. The dimethylformamide was evaporated off under high vacuo and the crude product was purified by preparative TLC (solvent system CH₂Cl₂:MeOH 10:1, followed by CH₂Cl₂:C₆H₆ 6:4). Yield : 69.2%.

¹H-NMR (CDCl₃) δ : 7.06 (2H, d, aro.H), 6.62 (2H, d, aro.H), 4.24 (H, m, α-CH), 3.80 (3H, s, COOCH₃), 3.65 (4H, m, 2xCH₂N), 3.61 (4H, m, 2xCH₂Cl) 2.58 (2H, m, bzCH₂), 2.48 (2H, m, CH₂CO), 1.98 (2H, m, CH₂), 1.90-1.80 (2H, m, βCH₂), 1.26 (8H, m, 4xCH₂), 0.85 (3H, t, CH₃).
 MS m/e (%) C₂₃H₃₅O₄NCl₂ (460.25) : 524,522,520 [M+Na] (11,59,100), 501,449,447 [M]⁺ (3,17,28), 290(4), 288(22), 286(37), 250(19), 230(42).

α-[4-[4-bis(2-chloroethyl)aminophenyl]butanoate]-ω-methoxy(1-tetradecyl-2-oxo-1,2-ethanediyl) (57c).

Chlorambucil crown ether complex (0.33mmol) and methyl 2-halohexadecanoate **49f** (120mg, approx.0.33mmol) were dissolved in 5ml dimethylformamide and reacted as described in method J.

Yield : 67%.

¹H-NMR (CDCl₃) δ : 7.06 (2H, d, aro.H), 6.62 (2H, d, aro.H), 4.25 (H, m, α-CH), 3.82 (3H, s, COOCH₃), 3.65 (4H, m, 2xCH₂N), 3.61 (4H, m, 2xCH₂Cl), 2.57 (2H, m, bzCH₂), 2.47 (2H, m, CH₂CO), 1.98 (2H, m, CH₂), 1.9-1.81 (4H, m, 2xCH₂), 1.27 (22H, m, 11xCH₂), 0.85 (3H, t, CH₃).
 MS m/e (%) C₃₁H₅₁O₄NCl₂ (572.41) : 575,573,571 [M]⁺ (20,61,100), 536(35), 522(46), 290(10), 288(60), 286(87), 230(30), 194(20), 118(17).

α -(1-{4-[4-bis(2-chloroethyl)aminophenyl]butanoate}-1-dodecyl-2-oxo-1,2-ethandiyl)- ω -methoxy[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (57d).

Chlorambucil crown ether complex (0.4mmol) and α -(1-bromo-1-dodecyl-2-oxo-1,2-ethandiyl)- ω -methoxy[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] 50a (218.6mg, 0.4mmol) were dissolved in 5ml dimethylformamide and reacted as described in method J.

Yield : 32.1%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.1 (2H, d, aro.H), 6.65 (2H, d, aro.H), 6.5 (H, m, NH), 4.6-4.25 (2H, m, 2x α -CH), 3.82 (3H, s, COOCH_3), 3.65 (4H, m, 2x CH_2N), 3.61 (4H, m, 2x CH_2Cl), 2.57 (2H, m, bz CH_2), 2.47 (2H, m, CH_2CO), 1.98 (2H, m, CH_2), 1.9-1.81 (4H, m, 2x CH_2), 1.27 (40H, m, 20x CH_2), 0.85 (3H, t, CH_3)

MS m/e (%) $\text{C}_{42}\text{H}_{74}\text{O}_4\text{N}_2\text{Cl}_2$ (739.62) : 767-761 [$\text{M}+\text{Na}$] $^+$ (1-10), 598,596(34,100), 476(12), 286(4), 173(66).

α -(1-{4-[4-bis(2-chloroethyl)aminophenyl]butanoate}-1-tetradecyl-2-oxo-1,2-ethandiyl)- ω -methoxy[imino-(1-tetradecyl-2-oxo-1,2-ethanediyl)] (57e).

Chlorambucil crown ether complex (0.3mmol) and α -(1-bromo-1-tetradecyl-2-oxo-1,2-ethandiyl)- ω -methoxy[imino-(1-tetradecyl-2-oxo-1,2-ethanediyl)] 50b (232mg, 0.3mmol) were dissolved in 5ml dimethylformamide and reacted as described in method J.

Yield : 43.2%.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.05 (2H, d, aro.H), 6.6 (2H, d, aro.H), 6.75-6.25 (2H, m, 2xNH), 4.6-4.15 (3H, 3xm, 3x α -CH), 3.78 (3H, s, COOCH_3), 3.65 (4H, m, 2x CH_2N), 3.61 (4H, m, 2x CH_2Cl), 2.57 (2H, m, bz CH_2), 2.47 (2H, m, CH_2CO), 1.98 (2H, m, CH_2), 2.1-1.6 (6H, m, 3x CH_2), 1.5-1.1 (60H, m, 30x CH_2), 0.85 (3H, t, CH_3).

MS m/e (%) $\text{C}_{42}\text{H}_{74}\text{O}_4\text{N}_2\text{Cl}_2$ (739.62) : 1021,1019,1017 [$\text{M}+\text{Na}$] $^+$ (8,44,56), 849(7), 795(13), 713(11), 290,288,286(4,22,34), 250(37), 198(100).

α -(4-[4-Bis(2-chloroethyl)aminophenyl]butanoate)-ethanoyl- ω -methoxy[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (58a).

Chlorambucil crown ether complex (0.66mmol) and α -bromoethanoyl- ω -methoxy[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] 51a (250mg, 0.66mmol) were dissolved in 5ml dimethylformamide and reacted as described in method J.

Yield : 53.4%.

¹H-NMR (CDCl₃) δ : 7.6-7.1 (4H, m, 4xaro.H), 7.15 (H, m, NH), 6.75 (H, m, NH), 4.65 (H, m, α-CH), 4.55 (2H, d, COOCH₂CO), 3.75 (3H, s, COOCH₃), 3.70 (4H, t, 2xCH₂N), 3.60 (4H, t, 2xCH₂), 2.60 (2H, t, bzCH₂), 2.45 (2H, t, CH₂CO), 2.00 (2H, m, CH₂), 1.85-1.75 (2H, m, CH₂), 1.30 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₃₁H₅₁O₅N₂Cl₂ (602.41) : 605, 603, 601 [M+H]⁺ (4, 28, 35), 551 (15), 433 (10), 274 (100), 290 (2), 288 (12), 286 (17), 198 (41).

α-{4-[4-Bis(2-chloroethyl)aminophenyl]butanoate}-ethanoyl-ω-methoxybis[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (58b).

Chlorambucil crown ether complex (0.66mmol) and α-bromoethanoyl-ω-methoxybis[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] **51b** (199mg, 0.33mmol) were dissolved in 5ml dimethylformamide and reacted as described in method J.

Yield : 43.1%.

¹H-NMR (CDCl₃) δ : 7.6-7.1 (4H, m, 4xaro.H), 7.15 (H, m, NH), 6.75 (H, m, NH), 4.95-4.65 (2H, 2xm, 2xα-CH), 4.55 (2H, d, COOCH₂CO), 3.75 (3H, s, COOCH₃), 3.70 (4H, t, 2xCH₂N), 3.60 (4H, t, 2xCH₂), 2.60 (2H, t, bzCH₂), 2.45 (2H, t, CH₂CO), 2.00 (2H, m, CH₂), 1.85-1.75 (4H, m, 2xCH₂), 1.30 (40H, m, 20xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₄₅H₇₈O₆N₃Cl₂ (827.68) : 851, 849 [M+H]⁺ (13, 18), 813 (400), 680 (15), 581 (16), 561 (8), 323 (12), 250 (115), 198 (23), 173 (100).

6.8. Benzoquinolizine lipidic amino acid/oligomer conjugates.

2-[9,10-(Methylenedioxy)-2β-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl-propionic acid 60a [Method K].

Benzoquinolizine methyl ester **60b** (2g, 6.04mmol) was added to hydrochloric acid (10%, 30ml) and refluxed for 3 hours. Upon cooling the reaction mixture was neutralised with ammonium hydroxide. The crystals formed were filtered under vacuum and dried overnight.

Yield : 92%.

¹H-NMR (CDCl₃) δ : 6.58 (H, s, C₁₁-H), 6.52 (H, s, C₈-H), 5.9 (2H, s, OCH₂O), 3.1 (H, m, C_{11b}-H), 3.01 (H, m, H₄).

MS m/e (%) C₁₇H₁₉O₅N (317.36) : 317 [M]⁺ (5), 316 [M-H]⁺ (13), 299 (3), 274 (8), 245 (8), 175 (100), 173 (92), 148 (27), 124 (22), 116 (31), 96 (57), 89 (66).

Methyl α -[9,10-(Methylenedioxy)-2-oxo-1,3 β ,4,6,7,11 β α -hexahydrobenzo[a]quinolizine-3-yl-propanoyl]iminotetradecanoate (61c**) [Method J].**

9, 10-(methylenedioxy)-2-oxo-1,3 β ,4,6,7,11 β α -hexahydro-benzo[a]quinolizine-3-yl-propionic acid (**60a**) (302mg, 0.95mmol), methyl α -tetradecanoate (**33r**) HCl (300mg, 0.95mmol), triethylamine (96mg, 0.95mmol), 1-hydroxybenzotriazole (129mg, 0.95mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (200mg, 1.05mmol) were reacted as described in method F. The crude product was purified by preparative TLC (solvent CH₂Cl₂:MeOH 10:1). Yield : 67.3%.

¹H-NMR (CDCl₃) : 6.58 (H, s, C₁₁-H), 6.52 (H, s, C₈-H), 5.98 (H, dd, NH), 5.9 (2H, s, OCH₂O), 4.57 (H, m, α -CH), 3.73 (3H, d, COOCH₃), 3.1 (H, m, C_{11 β} -H), 3.01 (H,m, H₄), 1.3 (22H, m, 11xCH₂), 0.87 (3H, t, CH₃).

MS m/e (%) C₃₂H₄₇O₆N₂ (556.79) : 579 [M+Na]⁺ (100), 557(23), 298(26), 256(15), 242(12), 189(12), 176(22).

Benzoquinolizine lipidic amino acids\peptides **61a, **b**, **d**, **e** and **f** were synthesised by coupling 2-[9,10-(methylenedioxy)-2 β -oxo-1,3 β ,4,6,7,11 β α -hexahydrobenzo[a]quinolizine-3-yl-propionic acid **60a** (1 eq.) using triethylamine (1 eq.), 1-hydroxybenzotriazole (1 eq.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.1 eq.) employing method J as described for conjugate **61c**.**

Methyl α -[9,10-(Methylenedioxy)-2-oxo-1,3 β ,4,6,7,11 β α -hexahydrobenzo[a]quinolizine-3-yl-propanoyl]iminooctanoate (61a**).**

Yield : 67.3%.

¹H-NMR (CDCl₃) : 6.58 (H, s, C₁₁-H), 6.52 (H, s, C₈-H), 6.0 (H, dd, NH), 5.9 (2H, s, OCH₂O), 4.57 (H, m, α -CH), 3.73 (3H, d, COOCH₃), 3.1 (H, m, C_{11 β} -H), 3.01 (H,m, H₄), 1.3 (10H, m, 5xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₂₆H₃₆O₆N₂ (472.62) : 472 [M]⁺ (17), 455(9), 493(6), 429(14), 298(26), 258(44), 244(79), 216(27), 189(70), 175(100), 144(45), 69(32), 55(44).

Methyl α -[9,10-(Methylenedioxy)-2-oxo-1,3 β ,4,6,7,11 β α -hexahydrobenzo[a]quinolizine-3-yl-propanoyl]iminodecanoate (61b**).**

Yield : 65.3%.

¹H-NMR (CDCl₃) : 6.58 (H, s, C₁₁-H), 6.52 (H, s, C₈-H), 6.0 (H, dd, NH), 5.9 (2H, s, OCH₂O), 4.57 (H, m, α-CH), 3.73 (3H, d, COOCH₃), 3.1 (H, m, C_{11b}-H), 3.01 (H, m, H₄), 1.3 (14H, m, 7xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₂₈H₄₀O₆N₂ (500.68) : 523 [M+Na]⁺ (100), 501 [M+H]⁺ (36), 298(17), 256(9), 176(17).

α-[9, 10-(Methylenedioxy)-2-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl-propanoyl]-ω-methoxybis[imino(1-octyl-2-oxo-1,2-ethanediyl)] (61d).

Yield : 43.4%.

¹H-NMR (CDCl₃) : 6.58 (H, s, C₁₁-H), 6.52 (H, s, C₈-H), 6.3-6.05 (2H, 2m, 2xNH), 5.9 (2H, s, OCH₂O), 4.54-4.38 (2H, 2xm, 2xα-CH), 3.73 (3H, d, COOCH₃), 3.1 (H, m, C_{11b}-H), 3.01 (H, m, H₄), 1.5-1.2 (28H, m, 14xCH₂), 0.87 (3H, t, CH₃).

MS m/e (%) C₃₈H₅₉O₇N₃ (669.97) : 692 [M+Na]⁺ (100), 670 [M+H]⁺ (14), 517(9), 475(14), 447(10), 300(13), 298(23), 256(15), 242(17).

α-[9, 10-(Methylenedioxy)-2-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl-propanoyl]-ω-methoxybis[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (61e).

Yield : 56.9%.

¹H-NMR (CDCl₃) : 6.58 (H, s, C₁₁-H), 6.52 (H, s, C₈-H), 6.3-6.05 (2H, 2m, 2xNH), 5.9 (2H, s, OCH₂O), 4.54-4.38 (2H, 2xm, 2xα-CH), 3.73 (3H, d, COOCH₃), 3.1 (H, m, C_{11b}-H), 3.01 (H, m, H₄), 1.5-1.2 (44H, m, 22xCH₂), 0.87 (6H, t, 2xCH₃).

MS m/e (%) C₄₆H₇₅O₇N₃ (781.75) : 804 [M+Na]⁺ (100), 772 [M+H]⁺ (22), 629(9), 587(13), 298(12), 176(32).

α-[9, 10-(Methylenedioxy)-2-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl-propanoyl]-ω-methoxytris[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (61f).

Yield : 40.6%.

¹H-NMR (CDCl₃) : 6.58 (H, s, C₁₁-H), 6.52 (H, s, C₈-H), 6.6-6.05 (3H, 3xm, 3xNH), 5.9 (2H, s, OCH₂O), 4.54-4.35 (3H, 3xm, 3xα-CH), 3.73 (3H, d, COOCH₃), 3.1 (H, m, C_{11b}-H), 3.01 (H, m, H₄), 1.5-1.2 (66H, m, 33xCH₂), 0.87 (9H, t, 3xCH₃).

MS m/e (%) C₆₀H₁₀₂O₈N₄ (1006.62) : 1029 [M+Na]⁺ (100), 854(8), 812(12), 772(10), 300(27), 256(13), 198(100), 176(40).

2-[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β α -heptahydrobenzo[a]quinolizine-3-yl-propanoyl]iminooctan-1-ol (62a) [Method L].

Keto-ester conjugate 61a (47mg, 0.01mmol) was dissolved in dichloromethane (4ml) and the solution cooled to 0°C. Sodium borohydride (8.6mg, 0.2mmol) was added and stirred at 0°C for 1 hour, methanol (2 drops) was added and the mixture allowed to stir for a further 1 hour. The mixture was neutralised with acetic acid (1/2 drop), the solvent evaporated *in vacuo* and the residue treated with 2% sodium hydrogen carbonate (5ml) and extracted with dichloromethane (3x5ml), dried (MgSO₄) and evaporated *in vacuo*.

Yield : 93.2%.

¹H-NMR (CDCl₃) : 6.65 (H, s, C₁₁-H), 6.53 (H, s, C₈-H), 6.25-6.05 (H, 3xd, NH), 5.88 (2H, s, O-CH₂-O), 4.54 (H, m, α -CH), 3.95 (H, m, CH₂-OH), 3.75-3.65 (2H, bd, CH₂-OH), 3.41 (H, m, C₂-H), 3.10 (H, m, C_{11 β} -H), 3.01 (H, m, C₄-H), 1.25 (10H, m, 5xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) : 469 [M+Na]⁺ (100), 447 [M+H]⁺ (36), 427 (26), 302 (12), 240 (60), 176 (66).

Anal. C₂₅H₃₈O₅N₂ (446.63).

Calcd. C 67.22 H 8.59 O 17.91 N 12.54.

Found C 67.17 H 8.54 O 17.87 N 12.52.

Conjugates 62b-f were synthesised by reducing keto-ester conjugates 61b-f (1 eq.) respectively, using sodium borohydride (2.4 eq.) as described in method L.

2-[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β α -heptahydrobenzo[a]quinolizine-3-yl-propamido]decan-1-ol (62b).

Yield : 92.2%.

¹H-NMR (CDCl₃) : 6.65 (H, s, C₁₁-H), 6.53 (H, s, C₈-H), 6.05 (H, m, NH), 5.88 (2H, s, O-CH₂-O), 4.35 (H, m, α -CH), 3.90 (H, m, CH₂-OH), 3.75-3.55 (2H, bd, CH₂-OH), 3.40 (H, m, C₂-H), 3.10 (H, m, C_{11 β} -H), 3.01 (H, m, C₄-H), 1.26 (14H, m, 7xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) : 497 [M+Na]⁺ (100), 475 [M+H]⁺ (42), 455 (26), 240 (35), 226 (20), 176 (34), 149 (11).

Anal. C₂₇H₄₂O₅N₂ (474.69).

Calcd. C 68.31 H 8.94 O 16.85 N 5.90.

Found C 68.28 H 8.91 O 16.76 N 5.84.

2-[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β α -heptahydro-benzo[a]quinolizine-3-yl-propamido]tetradecan-1-ol (62c).

Yield : 94.1%.

$^1\text{H-NMR}$ (CDCl_3) : 6.64 (H, s, $\text{C}_{11}\text{-H}$), 6.53 (H, s, $\text{C}_8\text{-H}$), 6.45-6.01 (H, 3xd, NH), 5.88 (2H, s, $\text{O-CH}_2\text{-O}$), 4.60 (H, m, $\alpha\text{-CH}$), 3.92 (H, m, $\text{CH}_2\text{-OH}$), 3.75-3.60 (2H, bd, $\text{CH}_2\text{-OH}$), 3.41 (H, m, $\text{C}_2\text{-H}$), 3.10 (H, m, $\text{C}_{11\beta}\text{-H}$), 3.01 (H, m, $\text{C}_4\text{-H}$), 1.28 (22H, m, $11\times\text{CH}_2$), 0.87 (3H, t, CH_3).

MS m/e (%) : 553 [$\text{M}+\text{Na}$] $^+$ (100), 532 [$\text{M}+\text{H}$] $^+$ (37), 512 (26), 302(14), 240 (59), 228 (32), 189 (33), 176 (66), 149 (18).

Anal. $\text{C}_{31}\text{H}_{50}\text{O}_5\text{N}_2$ (530.81).

Calcd. C 70.14 H 9.51 O 15.07 N 5.27.

Found C 70.12 H 9.45 O 15.04 N 5.24.

α -[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β α -heptahydro-benzo[a]quinolizine-3-yl-propionyl]- ω -(2-iminodecan-1-ol)[imino-(1-octyl-2-oxo-1,2-ethanediyl)] (62d).

Yield : 90.1%.

$^1\text{H-NMR}$ (CDCl_3) : 6.65 (H, s, $\text{C}_{11}\text{-H}$), 6.53 (H, s, $\text{C}_8\text{-H}$), 6.25-6.05 (2H, bd, $2\times\text{NH}$), 5.88 (2H, m, $\text{O-CH}_2\text{-O}$), 4.54-4.25 (2H, 2m, $2\times\alpha\text{-CH}$), 3.90 (H, m, $\text{CH}_2\text{-OH}$), 3.75-3.55 (2H, bd, $\text{CH}_2\text{-OH}$), 3.40 (H, m, $\text{C}_2\text{-H}$), 3.10 (H, m, $\text{C}_{11\beta}\text{-H}$), 3.01 (H, m, $\text{C}_4\text{-H}$), 1.26 (28H, m, $7\times\text{CH}_2$), 0.86 (6H, t, $2\times\text{CH}_3$).

MS m/e (%) : 666 [$\text{M}+\text{Na}$] $^+$ (84), 644 [$\text{M}+\text{H}$] $^+$ (20), 622 (17), 447 (9), 419 (10), 298 (18), 240 (38), 176 (52), 142 (100).

Anal. $\text{C}_{37}\text{H}_{61}\text{O}_6\text{N}_3$ (643.98).

Calcd. C 70.14 H 9.51 O 15.07 N 5.27.

Found C 70.12 H 9.45 O 15.04 N 5.24.

α -[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β α -heptahydrobenzo[a]quinolizine-3-yl-propionyl]- ω -(2-iminotetradecan-1-ol)-[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (62e).

Yield : 91.3%.

$^1\text{H-NMR}$ (CDCl_3) : 6.63 (H, s, $\text{C}_{11}\text{-H}$), 6.53 (H, s, $\text{C}_8\text{-H}$), 6.10-6.00 (2H, bd, $2\times\text{NH}$), 5.88 (2H, s, $\text{O-CH}_2\text{-O}$), 4.54-4.25 (2H, 2xm, $2\times\alpha\text{-CH}$), 3.90 (H, m, $\text{CH}_2\text{-OH}$), 3.75-3.55 (2H, bd, $\text{CH}_2\text{-OH}$), 3.40 (H, m, $\text{C}_2\text{-H}$), 3.10 (H, m, $\text{C}_{11\beta}\text{-H}$), 3.01 (H, m, $\text{C}_4\text{-H}$), 1.27 (44H, m, $22\times\text{CH}_2$), 0.86 (6H, t, $2\times\text{CH}_3$).

MS m/e (%) : 779 [$\text{M}+\text{Na}$] $^+$ (100), 757 [$\text{M}+\text{H}$] $^+$ (31), 739 (17), 302 (22), 242 (24), 200 (52), 176 (69), 149 (15).

Anal. C₄₅H₇₇O₆N₃ (756.22).

Calcd. C 71.47 H 10.28 O 12.69 N 5.55.

Found C 71.43 H 10.23 O 12.61 N 5.48.

α -[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β -heptahydrobenzo[a]quinolizine-3-yl-propionyl]- ω -(2-iminotetradecan-1-ol)-bis[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (62f).

Yield : 92.1%.

¹H-NMR (CDCl₃) : 6.66 (H, s, C₁₁-H), 6.55 (H, s, C₈-H), 6.45-6.10 (3H, bd, 3xNH), 5.88 (2H, s, O-CH₂-O), 4.40-4.15 (3H, 3xm, 3x α -CH), 3.85 (H, m, CH₂-OH), 3.75-3.55 (2H, bd, CH₂-OH), 3.40 (H, m, C₂-H), 3.10 (H, m, C_{11b}-H), 3.01 (H, m, C₄-H), 1.25 (66H, m, 33xCH₂), 0.87 (9H, t, 3xCH₃).

MS m/e (%) : 1004 [M+Na]⁺ (100), 982 [M+H]⁺ (14), 785 (9), 745 (13), 302 (17), 242 (19), 198 (69), 176 (42).

Anal. C₃₈H₅₉O₇N₃ (981.63).

Calcd. C 72.19 H 10.70 O 11.41 N 5.70.

Found C 72.11 H 10.63 O 11.36 N 5.65.

2-[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β -heptahydrobenzo[a]quinolizine-3-yl-propanoyl]iminooctan-1-ol (62a).

Keto-ester **61a** (4.7mg, 0.001mmol) was reduced with tritiated sodium borohydride followed by cold sodium borohydride (2mg, 10-20mCi) using the method described in method L.

Yield : 93.2%.

¹H-NMR (CDCl₃) : 6.65 (H, s, C₁₁-H), 6.53 (H, s, C₈-H), 6.25-6.05 (H, 3xd, NH), 5.88 (2H, s, O-CH₂-O), 4.54 (H, m, α -CH), 3.95 (H, m, CH₂-OH), 3.75-3.65 (2H, bd, CH₂-OH), 3.41 (H, m, C₂-H), 3.10 (H, m, C_{11b}-H), 3.01 (H, m, C₄-H), 1.25 (10H, m, 5xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) : 469 [M+Na]⁺ (100), 447 [M+H]⁺ (36), 427 (26), 302 (12), 240 (60), 176 (66).

Anal. C₂₅H₃₈O₅N₂ (446.63).

Calcd. C 67.22 H 8.59 O 17.91 N 12.54.

Found C 67.17 H 8.54 O 17.87 N 12.52.

Conjugates **62b-f** were synthesised by reducing keto-ester conjugates **61b-f** (0.001mmol) respectively, using sodium borohydride (2mg, 10-20mCi) as described for **61a** employing method L.

2-[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β α -heptahydrobenzo[a]quinolizine-3-yl-propamido]decan-1-ol (62b**).**

Yield : 92.2%.

¹H-NMR (CDCl₃) : 6.65 (H, s, C₁₁-H), 6.53 (H, s, C₈-H), 6.05 (H, m, NH), 5.88 (2H, s, O-CH₂-O), 4.35 (H, m, α -CH), 3.90 (H, m, CH₂-OH), 3.75-3.55 (2H, bd, CH₂-OH), 3.40 (H, m, C₂-H), 3.10 (H, m, C_{11 β} -H), 3.01 (H, m, C₄-H), 1.26 (14H, m, 7xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) : 497 [M+Na]⁺ (100), 475 [M+H]⁺ (42), 455 (26), 240 (35), 226 (20), 176 (34), 149 (11).

Anal. C₂₇H₄₂O₅N₂ (474.69).

Calcd. C 68.31 H 8.94 O 16.85 N 5.90.

Found C 68.28 H 8.91 O 16.76 N 5.84.

2-[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β α -heptahydro- benzo[a]quinolizine-3-yl-propamido]tetradecan-1-ol (62c**).**

Yield : 94.1%.

¹H-NMR (CDCl₃) : 6.64 (H, s, C₁₁-H), 6.53 (H, s, C₈-H), 6.45-6.01 (H, 3xd, NH), 5.88 (2H, s, O-CH₂-O), 4.60 (H, m, α -CH), 3.92 (H, m, CH₂-OH), 3.75-3.60 (2H, bd, CH₂-OH), 3.41 (H, m, C₂-H), 3.10 (H, m, C_{11 β} -H), 3.01 (H, m, C₄-H), 1.28 (22H, m, 11xCH₂), 0.87 (3H, t, CH₃).

MS m/e (%) : 553 [M+Na]⁺ (100), 532 [M+H]⁺ (37), 512 (26), 302(14), 240 (59), 228 (32), 189 (33), 176 (66), 149 (18).

Anal. C₃₁H₅₀O₅N₂ (530.81).

Calcd. C 70.14 H 9.51 O 15.07 N 5.27.

Found C 70.12 H 9.45 O 15.04 N 5.24.

α -[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β α -heptahydro-benzo[a]quinolizine-3-yl-propionyl]- ω -(2-iminodecan-1-ol)[imino-(1-octyl-2-oxo-1,2-ethanediyl)] (62d**).**

Yield : 90.1%.

¹H-NMR (CDCl₃) : 6.65 (H, s, C₁₁-H), 6.53 (H, s, C₈-H), 6.25-6.05 (2H, bd, 2xNH), 5.88 (2H, m,

O-CH₂-O), 4.54-4.25 (2H, 2m, 2x α -CH), 3.90 (H, m, CH₂-OH), 3.75-3.55 (2H, bd, CH₂-OH), 3.40 (H, m, C₂-H), 3.10 (H, m, C_{11b}-H), 3.01 (H, m, C₄-H), 1.26 (28H, m, 7xCH₂), 0.86 (6H, t, 2xCH₃).
MS m/e (%) : 666 [M+Na]⁺ (84), 644 [M+H]⁺ (20), 622 (17), 447 (9), 419 (10), 298 (18), 240 (38), 176 (52), 142 (100).

Anal. C₃₇H₆₁O₆N₃ (643.98).

Calcd. C 70.14 H 9.51 O 15.07 N 5.27.

Found C 70.12 H 9.45 O 15.04 N 5.24.

α -[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β -heptahydrobenzo[a]quinolizine-3-yl-propionyl]- ω -(2-iminotetradecan-1-ol)-[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (62e).

Yield : 91.3%.

¹H-NMR (CDCl₃) : 6.63 (H, s, C₁₁-H), 6.53 (H, s, C₈-H), 6.10-6.00 (2H, bd, 2xNH), 5.88 (2H, s, O-CH₂-O), 4.54-4.25 (2H, 2xm, 2x α -CH), 3.90 (H, m, CH₂-OH), 3.75-3.55 (2H, bd, CH₂-OH), 3.40 (H, m, C₂-H), 3.10 (H, m, C_{11b}-H), 3.01 (H, m, C₄-H), 1.27 (44H, m, 22xCH₂), 0.86 (6H, t, 2xCH₃).
MS m/e (%) : 779 [M+Na]⁺ (100), 757 [M+H]⁺ (31), 739 (17), 302 (22), 242 (24), 200 (52), 176 (69), 149 (15).

Anal. C₄₅H₇₇O₆N₃ (756.22).

Calcd. C 71.47 H 10.28 O 12.69 N 5.55.

Found C 71.43 H 10.23 O 12.61 N 5.48.

α -[9,10-(Methylenedioxy)-2 β -hydroxy-1,2,3 β ,4,6,7,11 β -heptahydrobenzo[a]quinolizine-3-yl-propionyl]- ω -(2-iminotetradecan-1-ol)-bis[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (62f).

Yield : 92.1%.

¹H-NMR (CDCl₃) : 6.66 (H, s, C₁₁-H), 6.55 (H, s, C₈-H), 6.45-6.10 (3H, bd, 3xNH), 5.88 (2H, s, O-CH₂-O), 4.40-4.15 (3H, 3xm, 3x α -CH), 3.85 (H, m, CH₂-OH), 3.75-3.55 (2H, bd, CH₂-OH), 3.40 (H, m, C₂-H), 3.10 (H, m, C_{11b}-H), 3.01 (H, m, C₄-H), 1.25 (66H, m, 33xCH₂), 0.87 (9H, t, 3xCH₃).
MS m/e (%) : 1004 [M+Na]⁺ (100), 982 [M+H]⁺ (14), 785 (9), 745 (13), 302 (17), 242 (19), 198 (69), 176 (42).

Anal. C₃₈H₅₉O₇N₃ (981.63).

Calcd. C 72.19 H 10.70 O 11.41 N 5.70.

Found C 72.11 H 10.63 O 11.36 N 5.65.

6.9. Disubstituted benzaldehyde lipidic amino acid/oligomer conjugates.

Methyl-(N-cyanoethanoyl)iminotetradecanoate (**65c**).

Methyl aminotetradecanoate **33r** (3.82g, 13mmol) and cyanoacetic acid (1.11g, 13mmol) were coupled using triethylamine (1.315g, 13mmol), 1-hydroxybenzotriazole (1.757g, 13mmol) and dicyclohexylcarbodiimide (2.93g, 14.3mmol) in dichloromethane as described in method D. The product was purified using flash chromatography (solvent CH₂Cl₂:MeOH 10:0.5).

Yield : 73.7%.

¹H-NMR (CDCl₃) δ : 6.7 (H, s, NH), 4.75 (H, m, α-CH), 3.78 (3H, s, COOCH₃), 3.65 (2H, s, CNCH₂CO), 1.6-1.48 (2H, m, CH₂), 1.25 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₁₈H₃₂O₃N₂ (324.32) : 347 [M+Na]⁺ (100), 325 [M+H]⁺ (14), 319(19), 176(36), 154(16), 137(21).

Methyl-(N-cyanoethanoyl)iminoalkanoates **65a**, **b** and **d** were synthesised by coupling methyl α-aminoalkanoates **33p**, **q** and **s** (1 eq.) to cyanoacetic acid (1eq.) using triethylamine (1 eq.), 1-hydroxybenzotriazole (1 eq.) and dicyclohexylcarbodiimide (1.1 eq.) as described for **65c** employing method D.

Methyl-(N-cyanoethanoyl)iminodecanoate (**65a**).

Yield : 63.6%.

¹H-NMR (CDCl₃) δ : 6.7 (H, s, NH), 4.75 (H, m, α-CH), 3.78 (3H, s, COOCH₃), 3.65 (2H, s, CNCH₂CO), 1.6-1.48 (2H, m, CH₂), 1.25 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₁₄H₂₄O₃N₂ (268.24) : 291 [M+Na]⁺ (100), 269 [M+H]⁺ (17), 225(15), 209(30), 133(74), 98(23).

Methyl-(N-cyanoethanoyl)iminododecanoate (**65b**).

Yield : 67.3%.

¹H-NMR (CDCl₃) δ : 6.7 (H, s, NH), 4.75 (H, m, α-CH), 3.78 (3H, s, COOCH₃), 3.65 (2H, s, CNCH₂CO), 1.6-1.48 (2H, m, CH₂), 1.25 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₁₆H₂₈O₃N₂ (296.28) : 319 [M+Na]⁺ (100), 297 [M+H]⁺ (27), 237(19), 176(18), 137(14).

Methyl-(N-cyanoethanoyl)iminohexadecanoate (65d).

Yield : 75.4%.

¹H-NMR (CDCl₃) δ : 6.7 (H, s, NH), 4.75 (H, m, α-CH), 3.78 (3H, s, COOCH₃), 3.65 (2H, s, CNCH₂CO), 1.6-1.48 (2H, m, CH₂), 1.25 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₂₀H₃₆O₃N₂ (352.36) : 375 [M+Na]⁺ (100), 353 [M+H]⁺ (16), 293(7), 154(16), 136(17).

Carbonyl-methylene condensation reaction [Method M].

To a pre-flame dried Dean-Stark apparatus benzaldehyde derivative (1eq), cyanomethylene derivative (1eq) and a catalyst (0.1eq) were heated at the boiling point of the solvent employed for up to 24hrs. The combination of conditions used are shown in Table 5.1.

3,4-Dihydroxybenzyl-2-cyanoprop-2-enoic acid 74.

3,4-Dihydroxybenzaldehyde (2.76mg, 20mmol) and cyanoacetic acid (1.71mg, 20mmol) were condensed under conditions **Ma,b,d,f,g,h** and **i** however no reaction took place.

Methyl-3-[(3,4-Dihydroxybenzyl)-2-cyanoprop-2-enoyl]iminotetradecanoate 67b.

3,4 Dihydroxybenzaldehyde (2.76mg, 20mmol) and methyl (N-cyanoethanoyl)iminotetradecanoate **65c** (6.49g, 20mmol) were treated under conditions **Ma,b,c,d,h** and **i**. There was no coupling in reactions **Ma-d**.

Condition **Mh**. Yield : <5%.

Coupling occurred. The solvent was removed under *vacuo*, the solid residue washed with dichloromethane (10ml x 2), filtered, washed with water (10ml x 2), filtered, dried in an oven overnight then purified on preparative TLC (solvent CH₂Cl₂:CH₃CN:CH₃COOH 10:1:0.5).

Condition **Mi**. Yield : 7%.

Coupling occurred. The solvent was removed under *vacuo*, the solid residue washed with dichloromethane (10ml x 2), filtered, washed with water (10ml x 2), filtered, dried in an oven overnight then purified on preparative TLC (solvent CH₂Cl₂:CH₃CN:CH₃COOH 10:1:0.5).

¹H-NMR (CDCl₃) δ : 8.25 (H, s, CH), 7.89 (H, s, aro.H), 7.5 (H, d, aro.H), 6.98 (H, d, aro.H), 6.7 (H, s, NH), 4.75 (H, m, α-CH), (3H, s, COOCH₃), 1.6-1.48 (2H, m, CH₂), 1.25 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₂₅H₃₆O₅N₂ (444.4) : 467 [M+Na]⁺ (42), 345 [M+H]⁺ (14), 413(26), 347(19), 329(100),

Condition M.	Solvent	Catalyst.	Temperature °c.
a	Pyridine	NH ₄ COOCH ₃	100
b	Pyridine	Piperidine	100
c	Pyridine	NH ₄ COOCH ₃	40
d	Pyridine	Piperidine	40
e	Benzene	NH ₄ COOCH ₃	80
f	Benzene	Piperidine	80
g	Hexane	NH ₄ COOCH ₃	70
h	Dichloromethane	NH ₄ COOCH ₃	40
i	Dichloromethane	Piperidine	40

Table 5.1. Reaction conditions for method **M**.**3-(3,4-Dimethoxybenzyl)-2-cyanoprop-2-enoic acid 69.**

3,4-Dimethoxybenzaldehyde **68** (3.32g, 20mmol) was coupled to cyanoacetic acid (1.7g, 20mmol) under conditions **Ma** and **h**.

Condition **Ma**. Yield : 31.4%.

Coupling occurred. The solvent was removed under *vacuo*, the solid residue washed with dichloromethane (10ml x 2), filtered, washed with water (10ml x 2), filtered, recrystallised from acetonitrile and dried in an oven overnight.

Condition **Mh**. Yield : 70.3%.

Coupling occurred. The solvent was removed under *vacuo*, the solid residue washed with dichloromethane (10ml x 2), filtered, washed with water (10ml x 2), filtered, recrystallised from acetonitrile and dried in an oven overnight.

¹H-NMR (CDCl₃) δ : 8.25 (H, s, CH), 7.89 (H, s, aro.H), 7.5 (H, d, aro.H), 6.98 (H, d, aro.H), 4.02 (6H, s, 2xCH₃O).

¹H-NMR (CD₃OD) δ : 8.2 (H, s, CH), 7.8 (H, s, aro.H), 7.55 (H, d, aro.H), 7.05 (H, d, aro.H), 5.4 (H, s, COOH), 3.9 (6H, d, 2xCH₃O).

¹H-NMR (CD₃OD + D₂O decoupled at 1689 DP.32L pre.sat.) δ : 8.2 (H, s, CH), 7.8 (H, s, aro.H),

7.55 (H, d, aro.H), 7.05 (H, d, aro.H), 3.9 (6H, d, 2xCH₃O).

MS m/e (%) C₁₂H₁₁O₄N (233.1) : 326(14), 235 [M+2H]⁺ (24), 234 [M+H]⁺ (100), 233 [M]⁺ (41), 216(19), 185(12), 93(33).

3-(3,4-Methylenedioxybenzyl)-2-cyanoprop-2-enoic acid 71.

3,4-methylenedioxybenzaldehyde 70 (3g, 20mmol) was coupled to cyanoacetic acid (1.7g, 20mmol) under condition h. The solvent was removed under *vacuo*, the solid residue washed with dichloromethane (10ml x 2), filtered, washed with water (10ml x 2), filtered, recrystallised from acetonitrile and dried in an oven overnight.

Yield : 84.6%.

¹H-NMR (CDCl₃) δ : 8.15 (H, s, CH), 7.65 (H, s, aro.H), 7.55 (H, d, aro.H), 7.01 (H, d, aro.H), 6.15 (2H, s, OCH₂O).

MS m/e NH₃/DCI (%) C₁₁H₇O₄N (217.1) : 235 [M+NH₃]⁺ (100), 217 [M]⁺ (45), 193(55), 191(96), 175(36), 173(74), 15(50).

Methyl-3-[(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoyl]iminotetradecanoate 72b.

3-(3,4-Dimethoxybenzyl)-2-cyanoprop-2-enoic acid 69 (210mg, 0.9mmol) and methyl aminotetradecanoate 33r (265mg, 0.9mmol) were coupled using triethylamine (88mg, 0.9mmol), 1-hydroxybenzotriazole (116mg, 0.9mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (192mg, 1.0mmol) in dichloromethane (10ml) as described in method F. The product was purified by preparative TLC. (solvent CH₂Cl₂:MeOH 10:1).

Yield : 42.8%.

¹H-NMR (CDCl₃) δ : 8.25 (H, s, CH), 7.75 (H, s, aro.H), 7.5 (H, d, aro.H), 6.95 (H, d, aro.H), 6.7 (H, s, NH), 4.75 (H, m, α-CH), 3.95 (6H, m, 2xCH₃O), 3.8 (3H, s, COOCH₃), 1.8 (2H, m, CH₂), 1.25 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₂₇H₄₀O₅N₂ (472.4) : 517 [M+2Na-H]⁺ (9), 495 [M+Na]⁺ (97), 473 [M+H]⁺ (17), 413(13), 216(100), 176(76), 154(23), 136(23).

Methyl-3-[(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoyl]iminoalkanoates 72a and c

Methyl-3-[(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoyl]iminoalkanoates 72a and c were synthesised by coupling 3-(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoic acid 69 (1 eq.) and methyl α -aminoalkanoates 33p and u (1 eq.) respectively using triethylamine (1 eq.), 1-hydroxybenzotriazole (1 eq.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.1 eq.) as described for 72b employing method F.

Methyl-3-[(3,4-Dimethoxybenzyl)-2-cyanoprop-2-enoyl]iminodecanoate 72a.

Yield : 76.9%.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.25 (H, s, CH), 7.75 (H, s, aro.H), 7.5 (H, d, aro.H), 6.95 (H, d, aro.H), 6.7 (H, s, NH), 4.75 (H, m, α -CH), 3.95 (6H, m, $2\times\text{CH}_3\text{O}$), 3.8 (3H, s, COOCH_3), 1.8 (2H, m, CH_2), 1.25 (12H, m, $6\times\text{CH}_2$), 0.85 (3H, t, CH_3).

MS m/e (%) $\text{C}_{23}\text{H}_{32}\text{O}_5\text{N}_2$ (416.3) : 439 $[\text{M}+\text{Na}]^+$ (43), 416 $[\text{M}]^+$ (11), 356(6), 217(28), 216(100), 188(13), 151(19), 133(61).

Methyl-3-[(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoyl]iminoeicosanoate 72c.

Yield : 56.2%.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.25 (H, s, CH), 7.75 (H, s, aro.H), 7.5 (H, d, aro.H), 6.95 (H, d, aro.H), 6.7 (H, s, NH), 4.75 (H, m, α -CH), 3.95 (6H, m, $2\times\text{CH}_3\text{O}$), 3.8 (3H, s, COOCH_3), 1.8 (2H, m, CH_2), 1.6-0.9 (32H, m, $16\times\text{CH}_2$), 0.85 (3H, t, CH_3).

MS m/e (%) $\text{C}_{33}\text{H}_{52}\text{O}_5\text{N}_2$ (556.5) : 579 $[\text{M}+\text{Na}]^+$ (100), 557 $[\text{M}+\text{H}]^+$ (26), 497(21), 260(54), 238(18), 216(90), 190(9).

Methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]iminoalkanoates 73a-e

Methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]iminoalkanoates 73a-e were synthesised by coupling 3-(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoic acid 71 (1 eq.) and methyl α -aminoalkanoates 33o-s (1 eq.) respectively using triethylamine (1 eq.), 1-hydroxybenzotriazole (1 eq.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.1 eq.) as described for 72b employing method F.

Methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]iminooctanoate 73a.

Yield : 45.7%.

¹H-NMR (CDCl₃) δ: 8.17 (H, s, CH), 7.61 (H, s, aro.H), 7.35 (H, d, aro.H), 6.85 (H, d, aro.H), 6.7 (H, d, NH), 6.07 (2H, s, OCH₂O), 4.59 (H, m, α-CH), 3.78 (3H, s, COOCH₃), 1.8-1.65 (2H, m, CH₂), 1.25 (8H, m, 4xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₂₀H₂₄O₅N₂ (272.24) : 290 [M+NH₄]⁺ (12), 273 [M+H]⁺ (100), 272 [M]⁺ (24), 200(24), 141(18), 113(7).

Methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]iminodecanoate 73b.

Yield : 39.6%.

¹H-NMR (CDCl₃) δ: 8.17 (H, s, CH), 7.61 (H, s, aro.H), 7.35 (H, d, aro.H), 6.85 (H, d, aro.H), 6.7 (H, d, NH), 6.07 (2H, s, OCH₂O), 4.68 (H, m, α-CH), 3.77 (3H, s, COOCH₃), 1.9-1.75 (2H, m, CH₂), 1.28 (12H, m, 6xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₂₂H₂₈O₅N₂ (400.28) : 600(19), 418 [M+NH₄]⁺ (10), 402 [M+2H]⁺ (43), 401 [M+H]⁺ (100), 400 [M]⁺ (31), 341(16), 288(5), 249(5), 200(19).

Methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]iminododecanoate 73c.

Yield : 45.9%.

¹H-NMR (CDCl₃) δ: 8.2 (H, s, CH), 7.61 (H, s, aro.H), 7.39 (H, d, aro.H), 6.85 (H, d, aro.H), 6.7 (H, d, NH), 6.07 (2H, s, OCH₂O), 4.68 (H, m, α-CH), 3.77 (3H, s, COOCH₃), 1.8 (2H, m, CH₂), 1.28 (16H, m, 8xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₂₄H₃₂O₅N₂ (428.32) : 452 [M+Na+H]⁺ (27), 451 [M+Na]⁺ (100), 429 [M+H]⁺ (7), 200(20), 176(8), 170(6).

Methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]iminotetradecanoate 73d.

Yield : 67.5%.

¹H-NMR (CDCl₃) δ: 8.2 (H, s, CH), 7.6 (H, s, aro.H), 7.39 (H, d, aro.H), 6.95 (H, d, aro.H), 6.7 (H, m, NH), 6.15 (2H, s, OCH₂O), 4.69 (H, m, α-CH), 3.8 (3H, s, COOCH₃), 1.8 (2H, m, CH₂), 1.6-0.9 (32H, m, 16xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₂₆H₃₆O₅N₂ (456.36) : 479 [M+Na]⁺ (100), 457 [M+H]⁺ (8), 397(8), 170(25).

Methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]iminohexadecanoate 73e.

Yield : 64.2%.

¹H-NMR (CDCl₃) δ : 8.2 (H, s, CH), 7.61 (H, s, aro.H), 7.39 (H, d, aro.H), 6.85 (H, d, aro.H), 6.7 (H, d, NH), 6.07 (2H, s, OCH₂O), 4.68 (H, m, α-CH), 3.77 (3H, s, COOCH₃), 1.7 (2H, m, CH₂), 1.22 (24H, m, 12xCH₂), 0.86 (3H, t, CH₃).

MS m/e (%) C₂₈H₄₀O₅N₂ (484.4) : 529 [M+2Na+H]⁺ (18), 507 [M+Na]⁺ (100), 485 [M+H]⁺ (11), 413(17), 200(70), 176(61).

3-(3,4-Dihydroxybenzyl)-2-cyanoprop-2-enoic acid (74) by cleavage using borontribromide dimethylsulphide complex.**i. Cleavage of 3-(3,4-Dimethoxybenzyl)-2-cyanoprop-2-enoic acid 69 [Method N].**

3-(3,4-Dimethoxybenzyl)-2-cyanoprop-2-enoic acid 69 (536mg, 2.3mmol) was dissolved in 5ml dichloromethane (5ml) and added to a flame-dried 10ml round bottom flask, boron tribromide dimethylsulphide complex (1.43g, 4.6mmol) was added under nitrogen and heated for 12hrs under the conditions shown in Table 5.2. The reaction was quenched with water (20ml) followed by ether extraction (3x20ml). 1M NaOH (15ml) was added to the ether extract, the aqueous layer removed and acidified with 1M HCl. Pure product could not be isolated from the crude mixture.

Conditions N.	Solvent.	Temperature 0°c.
a	Dichloromethane	40
b	Benzene	80
c	Dimethylformamide	100

Table 5.2. Conditions employed for method N.

ii. Cleavage of 3-(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoic acid 71.

3-(3,4-Methylenedioxybenzyl)-2-cyanoprop-2-enoic acid 71 (499mg, 2.3mmol) was reacted with boron tribromide dimethylsulphide complex (1.43g, 4.6mmol) as described in method **Ma-c**. Pure product could not be isolated from the crude mixture.

3-(3,4-Dihydroxybenzyl)-2-cyanoprop-2-enoic acid (74) by cleavage using borontribromide [Method O].

i. Cleavage of 3-(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoic acid 69.

To a flame-dried 10ml round bottom flask was added 3-(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoic acid 69 (1.074g, 4.61mmol) dissolved in dichloromethane (5ml). Boron tribromide in dichloromethane (1M) (13.8ml, 13.8mmol) was added at -40°C under nitrogen, the temperature was raised to 20°C and stirred for 12hrs. The reaction mixture was quenched with water (20ml) and extracted with ether (3 x 20ml). NaOH (1M) (10ml) was added to the ether extract and the aqueous layer removed then acidified with dilute HCl. Ethyl acetate extraction of the acidified aqueous layer furnished semi-pure product. The ethyl acetate was dried (anhydrous MgSO₄) and removed *in vacuo*. The product was purified using flash chromatography (solvent butanol:water:acetic acid 8:2:0.5).

Yield : 34%.

ii. Cleavage of 3-(3,4-Methylenedioxybenzyl)-2-cyanoprop-2-enoic acid 71.

3-(3,4-Methylenedioxybenzyl)-2-cyanoprop-2-enoic acid 71 (1.0g, 4.61mmol) dissolved in dichloromethane (5ml) was reacted with boron tribromide in dichloromethane (1M) (13.8ml, 13.8mmol) as described in method Oi.

Yield : 49%.

¹H-NMR (DMSO) δ : 7.85 (H, s, aro.H), 7.44 (H, s, aro.H), 7.22 (H, d, aro.H), 6.83 (H, d, aro.H).

MS m/e (%) C₁₀H₇O₄N (205.07) : 233 [M+NH₄]⁺ (16), 179(100), 161(19), 105(6).

3-[(3,4-Dihydroxybenzyl)-2-cyanoprop-2-enoyl]iminotetradecanoic acid 67b.

i. From methyl-3-[(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoyl]iminotetradecanoate 73b.

Methyl-3-[(3,4-dimethoxybenzyl)-2-cyanoprop-2-enoyl]iminotetradecanoate 73d (638mg, 1.36mmol) in dichloromethane (8ml), was reacted with boron tribromide in dichloromethane (1M) (5.5ml, 5.44mmol) as described in method Oi. Pure product could not be isolated.

ii. From methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]iminotetradecanoate 73d.

Methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]iminotetradecanoate 73d

(620mg, 1.36mmol) in dichloromethane (8ml), was reacted with boron tribromide in dichloromethane (1M) (5.5ml, 5.44mmol) as described in method Oi.

Yield : 7%.

¹H-NMR (DMSO) δ : 8.25 (H, s, CH), 7.89 (H, s, aro.H), 7.5 (H, d, aro.H), 6.98 (H, d, aro.H), 6.7 (H, s, NH), 4.75 (H, m, α-CH), 3.78 (3H, s, COOCH₃), 1.6-1.48 (2H, m, CH₂), 1.25 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₂₅H₃₆O₅N₂ (444.4) : 489(24), 467 [M+Na]⁺ (61), 432(17), 413(15), 351(100), 322(43), 258(21), 198(57), 176(76).

Methyl-3-[(3,4-dihydroxybenzyl)-2-cyanoprop-2-enoyl]iminooctanoic acid 67a.

Methyl-3-[(3,4-methylenedioxybenzyl)-2-cyanoprop-2-enoyl]iminooctanoate 73a (500mg, 1.36mmol) in dichloromethane (8ml), was reacted with boron tribromide in dichloromethane (1M) (5.5ml, 5.44mmol) as described in method Oi.

Yield : 5%.

¹H-NMR (DMSO) δ : 8.25 (H, s, CH), 7.89 (H, s, aro.H), 7.5 (H, d, aro.H), 6.98 (H, d, aro.H), 6.7 (H, s, NH), 4.65 (H, m, α-CH), 3.75 (3H, s, COOCH₃), 2-1.40 (2H, m, CH₂), 1.25 (8H, m, 4xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₁₉H₂₄O₅N₂ (360.24) : 405(13), 383 [M+Na]⁺ (22), 176(38), 114(100).

Methyl-3-[(3,4-dihydroxybenzyl)-2-cyanoprop-2-enoyl]iminotetradecanoate 67b [Method P].

3-(3,4-Dihydroxybenzyl)-2-cyanoprop-2-enoic acid 74 (150mg, 0.73mmol), methyl α-aminotetradecanoate 33r (215mg, 0.73mmol), triethylamine (74mg, 0.73mmol), 1-hydroxybenzotriazole (99mg, 0.73mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (155mg, 0.81mmol) were reacted in dimethylformamide as described in method F. The DMF was removed *in vacuo*, the residue dissolved in dichloromethane, washed with water, dried (anhydrous MgSO₄) and purified by preparative TLC. (solvent CH₂Cl₂:MeOH 10:1).

Yield : 7%.

¹H-NMR (DMSO) δ : 8.25 (H, s, CH), 7.89 (H, s, aro.H), 7.5 (H, d, aro.H), 6.98 (H, d, aro.H), 6.7 (H, s, NH), 4.75 (H, m, α-CH), 3.78 (3H, s, COOCH₃), 1.6-1.48 (2H, m, CH₂), 1.25 (20H, m, 10xCH₂), 0.85 (3H, t, CH₃).

MS m/e (%) C₂₅H₃₆O₅N₂ (444.4) : 489(24), 467 [M+Na]⁺ (61), 432(17), 413(15), 351(100), 322(43), 258(21), 198(57), 176(76).

Methyl-3-[(3,4-dihydroxybenzyl)-2-cyanoprop-2-enoyl]iminooctanoate 67a.

3-(3,4-Dihydroxybenzyl)-2-cyanoprop-2-enoic acid 74 (150mg, 0.73mmol), methyl α -aminooctanoate 33o (152mg, 0.73mmol), triethylamine (74mg, 0.73mmol), 1-hydroxybenzotriazole (99mg, 0.73mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (155mg, 0.81mmol) were reacted in dimethylformamide as described in method **P**.

Yield : 4%.

$^1\text{H-NMR}$ (DMSO) δ : 8.25 (H, s, CH), 7.89 (H, s, aro.H), 7.5 (H, d, aro.H), 6.98 (H, d, aro.H), 6.7 (H, s, NH), 4.65 (H, m, α -CH), 3.75 (3H, s, COOCH_3), 2-1.40 (2H, m, CH_2), 1.25 (8H, m, $4\times\text{CH}_2$), 0.85 (3H, t, CH_3).

MS m/e (%) $\text{C}_{19}\text{H}_{24}\text{O}_5\text{N}_2$ (360.24) : 361 $[\text{M}+\text{Na}]^+$ (14), 301(6), 283(7), 188(25), 114(100).

6.10. Thio-conjugates.

Adenosine 5'monophosphorothionate 76 [Method Q].

Adenosine (534mg, 2mmol) was added to triethylphosphate (5ml) and heated to 100°C. The mixture was then cooled to 0°C and thiophosphoryl chloride added (0.6ml, 5.8mmol). After 12 hrs. at 2-4°C, the resultant suspension was mixed with 20ml 10% aqueous barium acetate and held at 20°C for 45mins. The solution was adjusted to pH9 with triethylamine (4ml) and ethanol (95%, 60ml) added producing a precipitate which was filtered, washed with ethanol (70%, 3x40ml) and dried. Water (3x50ml) was used to extract the product and the aqueous extract was passed through an ion-exchange column (25x200mm of HCO_3^- DEAE-cellulose) using a solvent gradient of 100% water to 100% NH_4HCO_3 (total solvent volume 500ml). Fractions were collected (5-10ml) and analysed under UV light. The product was dissolved in dry methanol (7ml) and mixed with NaI in dry acetone (1M, 6ml) containing 1% mercaptoethanol. Addition of acetone (54ml) precipitated adenosine 5'monophosphorothionate as the sodium salt. The precipitate was washed with acetone (4x30ml) and dried at 55°C.

Yield : 49.2%.

$^1\text{H-NMR}$ (D_2O) : 8.48 (H, s, aro.H), 8.12 (H, s, CH), 6.0 (H, d, CH), 4.39 (H, t, CH), 4.26 (H, s, CH), 3.98 (2H, q, OCH_2), 2.08 (H, s, CH).

MS m/e (%) $\text{C}_{10}\text{H}_{13}\text{O}_6\text{N}_5\text{SPNa}_2$ (363.14) : 386 $[\text{M}+\text{Na}]^+$ (35), 364 $[\text{M}+\text{H}]^+$ (71), 343(75), 330(63), 317(43), 295(38), 251(100), 238(49).

L-Cysteine ethyl ester propanedisulphide 77a [Method R].

Triethylamine (2-3 drops) were added to methanol (25ml) followed by simultaneous addition of L-cysteine ethyl ester (751mg, 4mmol) and propanethiol (0.36ml, 4mmol). Air was bubbled through the solution for 24 hours. The solvent was removed by *vacuo* and the crude product separated by preparative TLC (solvent isopropanol:CH₃OH 1:1).

Yield : 21%.

¹H-NMR (CD₃OD) : 4.55 (H, m, α-CH), 3.1 (2H, m, SCH₂), 2.9 (2H, m, SCH₂), 2.10 (2H, q, OCH₂), 1.25 (2H, m, CH₂), 0.9 (3H, t, CH₃), 0.87 (3H, t, CH₃).

MS m/e (%) C₈H₁₇O₂NS₂ (223.17) : 270(31), 246 [M+Na]⁺ (16), 181(8), 170(11), 91(100).

L-Cysteine ethyl ester octadecyldisulphide 77b

L-Cysteine ethyl ester (375.5mg, 2mmol) was reacted with octadecylmercaptan (573mg, 2mmol) as described in method R.

Yield : 35%.

¹H-NMR (CD₃OD) : 4.55 (H, m, α-CH), 3.1 (2H, m, SCH₂), 2.8 (2H, m, SCH₂), 2.10 (2H, q, OCH₂), 1.25 (32H, m, 16CH₂), 0.9 (3H, t, CH₃), 0.87 (3H, t, CH₃).

MS m/e (%) C₂₃H₄₇O₂NS₂ (433.47) : 434 [M+H]⁺ (100), 391(35), 317(16), 307(23), 279(32), 251(82), 244(22), 238(40), 232(17), 205(25).

Octadecyl-adenosine 5'monophosphorodisulphide 78

Adenosine 5'monophosphorothionate (50mg, 0.14mmol) was reacted with octadecylmercaptan (39mg, 0.14mmol) as described in method R.

Yield : 29%

¹H-NMR (CD₃OD) : 8.48 (H, s, aro.H), 8.12 (H, s, CH), 6.0 (H, d, CH), 4.39 (H, t, CH), 4.26 (H, s, CH), 3.98 (2H, q, OCH₂), 2.8 (2H, m, SCH₂), 2.08 (H, s, CH), 1.25 (32H, m, 16CH₂), 0.87 (3H, t, CH₃).

MS m/e (%) C₂₈H₅₁O₆N₅S₂P (648.51) : 692 [M+2Na]⁺ (53), 670 [M+Na]⁺ (13), 649 [M+H]⁺ (21), 343(15), 330(63), 317(32), 286(80), 251(37), 238(41).

α-(*Tert*.butoxycarbonylimino)tetradecanoyl-ω-L-cysteine ethyl ester 79.

α-(*Tert*.butoxycarbonylimino)tetradecanoic acid 33k (100mg, 0.29mmol), to L-cysteine ethyl ester (34mg, 0.29mmol) triethylamine (29mg, 0.29mmol), 1-hydroxybenzotriazole (39mg,

0.29mmol) and 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (61mg, 3.2mmol) were reacted as described in method F. The crude product was purified by flash chromatography (solvent CH₂Cl₂:CH₃OH 10.0.5).

Yield : 48%

¹H-NMR (CD₃OD) : 6.5-6.18 (2H, 2xm, 2xNH), 4.55-4.26 (2H, 2xm, 2xα-CH), 2.11 (2H, q, OCH₂), 1.45 (9H, s, 3xCH₃), 1.3 (2H, m, CH₂), 1.25 (20H, m, 10xCH₂), 0.9 (3H, t, CH₃), 0.87 (3H, t, CH₃).

MS m/e (%) C₂₄H₄₆O₅N₂S (474.46) : 497 [M+Na]⁺ (100), 475 [M+H]⁺ (67), 326(35), 225(21), 198(21).

α-(*Tert*.butoxycarbonylimino)tetradecanoyl-ω-l-cysteine ethyl ester-adenosine 5'monophosohorodisulphide 80.

Adenosine 5'monophosphorothionate 76 (50mg, 0.14mmol) was reacted with l-cysteine aminotetradecanoate-octadecydisulphide (65mg, 0.14mmol) as described in method R.

Yield : 6%

¹H-NMR (CD₃OD) : 8.48 (H, s, aro.H), 8.12 (H, s, CH), 6.5-6.18 (2H, 2xm, 2xNH) 6.0 (H, d, CH), 4.55 (H, m, α-CH), 4.39 (H, t, CH), 4.26 (H, s, CH), 4.21 (H, m, α-CH), 3.98 (2H, q, OCH₂), 2.11 (2H, q, OCH₂), 2.08 (H, s, CH), 1.45 (9H, s, 3xCH₃), 1.3 (2H, m, CH₂), 1.25 (20H, m, 10xCH₂), 0.9 (3H, t, CH₃), 0.87 (3H, t, CH₃).

MS m/e (%) C₃₄H₅₉O₁₁N₇S₂P (837.6) : 838 [M+H]⁺ (22), 837 [M]⁺ (8), 363(10), 475(43), 326(73), 251(100), 238(49), 226(24), 225(31), 198(100), 153(14).

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