SYNTHESIS AND STRUCTURAL ELUCIDATION OF NOVEL HIGHLY LIPOPHILIC C.N.S. AND ANTIVIRAL DRUGS

by

ROHANAH BINTE HUSSAIN
B.Sc. (Pharmacy) (Singapore); M.Phil. (Bath, U.K.)

A thesis submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

The School of Pharmacy
Faculty of Medicine
University of London
29-39 Brunswick Square
London WC1N 1AX

February 1992

To my family (especially Mum and Dad),
thank you very much for your support.
I love you all.

ABSTRACT

Many active drugs are restricted in their use pharmacologically due to their poor absorption. This dissertation deals with 2 different families of important drugs, the (i) C.N.S. (ii) antiviral compounds.

The lipidic amino acids and their oligomers have a potential use as a novel drug delivery system. Because of their bifunctional nature, they have the capacity to be chemically conjugated to different drugs with a wide variety of functional groups. The resulting conjugates may be either biologically stable or possess biological or chemical instability (the conjugates are prodrugs) and could possess a high degree of membrane-like character, sufficient to facilitate their passage across different membranes. The long hydrocarbon chain of this system, may also have an additional effect of protecting a labile parent drug from enzymatic attack and hence increase the metabolic stability.

This lipidic system was synthesised and to vary the physico-chemical properties, the unsubstituted and side chain substituted oligomers and copolymers substituted with highly hydrophilic compounds were also prepared.

The important neurotransmitter GABA does not cross the blood brain barrier. Coupling the lipophilic delivery system to GABA, conjugates of differing lipophilicities were synthesised in an attempt to increase the brain uptake. Two types of GABA conjugates were prepared involving ester and amide linkages. In order to carry out the biological *in vivo* examinations, tritiated and [^{14}C]-GABA conjugates were also prepared. *In vitro* studies were performed on the unlabelled GABA analogues. Ester conjugates of baclofen have also been used for pharmacological assessment.

The second part of the thesis deals with important antiviral compounds. To investigate the chemistry and the physico-chemistry of adenosine conjugates, compounds with ester linkage were synthesised. Finally the research was expanded

to the preparation of AZT conjugates.

One of the most important clinically approved drug for treating AIDS is 3'-azido-3'-deoxythymidine (AZT). Inadequate inhibition of virus production by macrophages may be the reason for failure to clear infectious virus. To direct larger amounts of the antiretroviral nucleosides to these cells, a major reservoir of HIV, lipidic amino acid and oligomer ester conjugates of AZT were synthesised by utilising the 5'-OH group of AZT for conjugation. To further examine the effect of chemical modification on the pharmacological activity, the 5'-hydroxyl of the AZT was also replaced with a thio group.

TABLE OF CONTENTS

Chapter 3

Chapter 4

ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr. Istvan Toth for his guidance and support and Prof. Willie Gibbons for his encouraging discussions.

Thanks are also due to Mr. Colin James and Dr. Graeme Anderson for carrying out some of the NMR studies, Mr. Kevin Welham and Dr. Dave Carter for skilled fast atom bombardment mass spectra and Mr. Wilfred Baldeo for microanalyses data.

To Prof. Norman Bowery, thank you for performing the pharmacological experiments on the GABA analogues and useful discussion on these biological data.

To the rest of the staff in Pharmaceutical Chemistry and my colleagues, you have been a fantastic "bunch" in friendship and discussion.

Not forgeting Dr. Alex Drake for proof-reading this thesis. Well, your train journey to and from Birkbeck College must have been well occupied. Thank you.

Finally, special thanks to The School of Pharmacy for offering the Sir Harry Jephcott Studentship to me, thus making this research possible.

PUBLICATIONS AND COMMUNICATIONS

Publications

Peptides 1990 (Proc. of 21st European Peptide Symposium, eds. E. Giralt, D. Andreu, ESCOM) p434-435.

- Lipidic peptide conjugates of GABA with improved bioavailability.
- **R Hussain**, I Toth, R A Hughes, N G Bowery and W A Gibbons.

Liposomes in Drug Delivery 1990.

- Lipidic amino acids and their homo-oligomers as a novel transmembrane delivery and formulation agents.
- W A Gibbons, I Toth, **R Hussain**, R A Hughes and I P Griffith.

16th Annual Lorne Conference on Protein Structure and Function 10-14 Feb 1991

- Novel lipidic drug and peptide delivery system.
- I Toth, R A Hughes, I P Griffith, **R Hussain** and W A Gibbons.

The Square Research Journal, July **1991**, 26-27.

- Synthesis and *in vitro* studies of lipophilic GABA conjugates.
- **R Hussain**.

Liebigs. Ann. Chem. **1991**, 963-966.

- Synthesis and structure elucidation of γ-aminobutyric acid conjugates with lipidic acids, lipidic amino acids and lipidic peptides.
- **R Hussain**, I Toth and W A Gibbons.

Peptides 1991 (Proc. 12th Amer. Peptide Symp.) (in press)

- Synthesis of lipidic peptide conjugates of nucleoside antiviral and cytostatic agents.
- **R Hussain**, I Toth and W A Gibbons.

33rd IUPAC Congress Budapest 17-22 August 1991, Hungary

- Novel lipidic drug and peptide delivery systems.
- I Toth, R A Hughes, I P Griffith, **R Hussain**, N G Bowery and W A Gibbons.

J. Pharm. Pharmacol. (1991) **43**, 129P

- Brain Uptake of lipophilic γ-aminobutyric acid conjugate.
- **R Hussain**, I Toth, N G Bowery and W A Gibbons.

VII Spanish Society Congress of Therapeutical Chemistry Jaca 25-27 September 1991, Spain

- Lipidic amino acids derivatives: Synthesis, structure and physicochemical properties.
- G J Anderson, W A Gibbons, **R Hussain**, E del Olmo, I Toth, P Ward, I P Wood.

Liebigs Ann. Chem. **1992** (in press).

- Synthesis ans structural elucidation of lipophilic Azidothymidine conjugates.
- **R Hussain**, I Toth and W A Gibbons.

Tetrahedron (1992) **48(5)**, 923-930.

- Synthesis, structural and physico-chemical elucidation of lipidic amino acid conjugates with hydrophilic compounds.
- I Toth, G J Anderson, **R Hussain**, I P Wood, E O Fernandez, P Ward and W A Gibbons.

Communications

Sir James Black Foundation Intercollegiate Seminar, London.

- Membrane transportable GABA conjugates. (September 1989)

University of London, The School of Pharmacy, Postgraduate Seminar, London.

- Lipophilic GABA conjugates. (April 1991)

The Pasteur Institute, Lille, France.

- Lipophilic γ-amino butyric acid and azidothymidine conjugates. (October 1991)

University of London, The School of Pharmacy, Departmental Seminar, London.

- Synthesis of highly lipophilic CNS and nucleosides conjugates. (November 1991).

CHAPTER 1

INTRODUCTION

A drug must reach its site or sites of action in pharmacological concentration to elicit a response. The concentration achieved is related to the amount of drug administered, the extent and rate of absorption from its site of administration, its distribution in the body, metabolism and excretion. Since the emergence of new techniques and methodologies, a more rational approach to drug design can be employed where various macromolecular structures (receptors, enzymes) are recognised as potential targets for activation, deactivation or modification as a means of allaying the principle symptoms of a disease, or attacking the root cause. Ideally, new drugs should be active compounds effecting a pharmacological response with minimal unwanted side effects reaching the required *in vivo* target site in therapeutic concentrations. This thesis explores some aspects of the effective delivery of medicinal drugs based on a group of peptides, the lipidic amino acids.

1.1 Drug Routes In The Body

1.1.1 Absorption

Apart from drugs introduced directly into the systemic circulation by intravenous injection, absorption from the site of administration is essential if a drug is to gain entry to the blood stream and reach its site of action. Thus the process of absorption is consequently of fundamental importance in determining the pharmacological and therapeutic activity of a drug response.

A drug must at least cross one cell membrane for the process of absorption to take place. The ease of the drug absorption will reflect the concentration achieved in the tissues and body fluids. Cell membranes can be considered to be bilayers of protein-lipid studded with water-filled pores (Singer and Nicholson 1972). Drugs therefore need to be transported across this membrane or be lipid soluble which are capable of naturally diffusing across the bilayer. The ability of a substance to dissolve in lipid can be assessed in terms of its partition coefficient (pK_a) between an aqueous and

immiscible non-aqueous phase such as n-octanol or chloroform. The permeability of the compounds to the membranes is proportionally related to the partition coefficient. Neutral or undissociated molecules, in general, are soluble in lipids while ions are not. Thus the dissociation constant of a drug plays an important part in determining its ability to cross cell membranes. This phenomenon is influenced by the pH of the environment. The interrelationship between the dissociation constant, pH of the environment and lipid solubility of a compound often dictates its absorption profile which is the basis of the pH-partition theory of drug absorption (Shore *et al.* 1957).

Accordingly, a solution of a weakly acidic drug existing in the non-ionised form which is lipid soluble, is rapidly absorbed in the stomach. On the other hand, the poor absorption of basic drugs in the stomach can be explained because they exist in the ionised form which hinders absorption (Schanker *et al.* 1957, Hogben *et al.* 1957). The absorption of weak acids and bases takes place more rapidly in the proximal intestine than in the stomach which appears to contradict the pH-partition theory. However, anatomical aspects have to be considered e.g. the large surface area offered by the intestine (Crouthamel *et al.* 1971) which reduces the necessity for a large fraction of the drug to be in its unionised form. Further setbacks to the simple pH-partition hypothesis are observed for compounds having similar pK_a values but different absorption rates (Martin 1981), due to the presence of unstirred mucus layers close to the gut lumen having both a different pH and permeability to the bulk intestinal contents (Gibaldi 1979). This requires a more detailed study and description of the drug absorption profile.

1.2 Drug Delivery Systems

Drug delivery systems are designed to assist, accelerate, and control the transport of pharmacologically active agents from sites of administration to specified targets in organs and tissues. They are intended to maintain continually efficacious drug concentrations *in vivo*, either locally or systemically, over longer time periods.

The avoidance of first-pass effect in the liver, spleen, and filtration organs, the

solubility and stability problems in certain formulations, as well as the reliability of long-term delivery within a single device are important considerations in clinical applications of controlled and targeted drug delivery systems.

Effective controlled and targeted drug delivery must enable a drug to locate its site of action more specifically faster, with higher affinity, and with greater probability than simple absorption of the drug alone. Between the pharmacon and the site of action, there exists a multitude of barriers to penetrate (gut, RES, cell membrane) before the drug successfully reaches its proper destination - factors which must be accounted for in the design of modern delivery systems.

1.3 Types of Drug Delivery Systems

Enhancement of transport across membrane and cellular uptake of drugs can be achieved by two ways classified as (i) the physical and (ii) the chemical methods. The physical strategies encompass the pharmaceutical formulations of the drug which is not chemically modified. This includes the use of liposomes, soluble and biodegradable polymers or microspheres. Chemical methods involve the chemical modification of the drug itself. This particular modification may be reversible or irreversible. The latter forms a newly modified drug that has its own intrinsic activity. In the reversible chemical strategy, the active compound is expected to be regenerated from the prodrug *in vivo* preferably after absorption. An introduction to the two methods of improving drug absorption is presented below.

1.3.1 Physical Methods

1.3.1.1 Liposomes

Liposomes were the first potential drug carrying agents introduced almost two decades ago (Gregoriadis and Ryman 1972) and have received wide interest in their application as a potential controlled drug delivery system (Gregoriadis 1980, Juliano 1981). The purpose of using liposomal carriers was to increase the uptake of drugs by specific

cells or tissues, thus enhancing the potency or reducing the toxicity of the encapsulated drugs.

Consisting of one or more phospholipid bilayers surrounding an aqueous internal phase they can be considered to be synthetic relatives of cell membranes (Gruner 1987). It should be possible either to encapsulate water soluble drugs in the aqueous core of the liposome, or include hydrophobic drugs within the phospholipid bilayer. They are formed spontaneously when amphipathic lipids are dispersed in excess water (Leserman and Barbet 1982). Lipophilic agents or derivatives of water soluble compounds can be incorporated into the lipid membranes.

Liposomes seem to be attractive carriers of drugs to macrophages as demonstrated by the application of liposomes in the treatment of certain infectious diseases or in the immunotherapy of cancer (Poste *et al.* 1982). Results have been reported on the application of liposomes for the local delivery of drugs, e.g. the intra-articular injection of liposomes-associated cortisol palmitate in the treatment of arthritis (De Silva *et al.* 1979), the intrapulmonary application in the therapy of the respiratory distress syndrome (Fujiwara *et al.* 1980) or the intralymphatic administration for the diagnosis or treatment of lymph node metastases (Kaledin *et al.* 1981).

Despite the favourable properties which would enable them to function as drug delivery systems, there are severe limitations such as the inability to cross the capillary endothelia cells in most organs except the liver (Poste *et al.* 1982), the limited potency of many cell types to phagocytose particles like liposomes. Fusion of liposomes with the plasma membrane has proven to be of little if any significance for any cell type (Weinstein *et al.* 1982, Toonen and Crommelin 1983, Connor *et al.* 1985).

1.3.1.2 Bioadhesive Polymers

The use of bioadhesive polymers in the oral controlled-release dosage forms provides very short-term adhesion between the drug delivery system and the mucus or epithelial

cell surface of the gastrointestinal (GI) tract. Bonding will therefore be that of secondary forces such as hydrogen bonds or van der Waals forces. Polymer candidates have to be non-toxic and non-adsorbable, adhere rapidly to wet tissues and able to release the incorporated drug in a controlled manner. This principle reduces patient non-compliance and improves drug therapy.

Tragacanth and dental adhesive powders were combined to form a vehicle for applying penicillin to the oral mucosa (Scrivener and Schantz 1947). This system was improved when carboxymethyl cellulose (CMC) and petrolatum were combined as the vehicle (Rothner *et al.* 1949). The ability to localise a drug delivery system in a selected region of the tract would lead to improvement in bioavailability particularly for drugs with a narrow margin of absorption and stability.

Development and use of synthetic polymers e.g. PVP as plasma expanders has lead to considerable investigation. Although PVP is not biodegradable, and accumulates in the body for some time after adminstration, it was given to over 500000 patients during the second world war without adverse reactions (Hulme *et al.* 1968). Studies on the immunogenicity of N-(2-hydroxypropyl)methacrylamide (HPMA) (Kopeček *et al.* 1973) and HPMA copolymers (Říhová *et al.* 1983, 1984) have shown that no antibodies are produced against the homopolymer and copolymers bearing peptide side-chains.

1.3.1.3 Microspheres

Microspheres are described as small particles ranging from tens of a nanometre to one hundred microns or more. Microcapsules are similar to microspheres but are comprised of small spheres that have an outer layer or membrane enclosing a core material that could be the drug itself (Tomlinson 1983). Lipid microspheres have been reported for drug targeting although these particles are defined as emulsions (Davis *et al.* 1987).

Microspheres for drug delivery can be prepared from a variety of different materials

and are of different physical characteristics, including albumin (Fujimoto *et al.* 1985), fibrinogen (Miyazaki *et al.* 1986), poly(lactic acid) (Krause *et al.* 1985), collagen (El-Samaligy and Rohdewald 1983), starch (Lindell *et al.* 1978), gelatin (Oppenheim *et al.* 1984) and many others. Factors affecting the choice of the microspheres are the drug, the intended destination, disease condition to be treated, duration of action etc. (Davis and Illum 1986, Juni and Nakano 1987).

1.3.1.4 Iontophoresis

The slow transport across the skin, and different enzymatic activity in the epithelium (Noonan and Wester 1985) are ideally suited for the delivery of peptides, which are digested in the gut and hence poorly absorbed orally. Peptides are not metabolised in the epidermis and are highly active in small quantities. However, they are normally too large and hydrophobic to be absorbed efficiently by this route. Some success has been achieved by using an electric field to drive peptides (Parasrampuria 1991, Chien *et al.* 1987, 1990, Lelawongs *et al.* 1990, Srinivasan *et al.* 1989) and non-peptides (Wearley *et al.* 1990, Sanderson *et al.* 1989) across the skin, a process termed iontophoresis.

1.3.2 Chemical Methods

1.3.2.1 Prodrugs

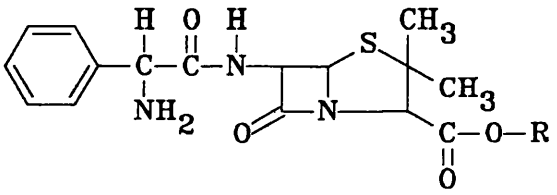
The prerequisite for the prodrug approach to be useful in solving drug delivery problems is the ready availability of chemical derivative types satisfying the prodrug requirements, the most prominent of these being reconversion of the prodrug to the parent drug *in vivo*. This prodrug-drug conversion may take place before absorption (e.g. in the gastrointestinal tract), during absorption, after absorption or at the specific site of drug action in the body, all dependent upon the specific goal for which the prodrug is designed. Prodrugs designed to overcome solubility problems in formulating intravenous injection solutions should preferably be converted immediately to drug following injection so that the concentration of circulating

prodrug would rapidly become insignificant in relation to that of the active drug. Conversely, if the objective of the prodrug is to produce a sustained drug action, the rate of the conversion should not be too fast.

The necessary conversion or activation of prodrugs to the parent molecules in the body can take place by a variety of reactions. The most common prodrugs are those requiring a hydrolytic cleavage mediated by enzymatic catalysis. Active drug species containing hydroxyl or carboxyl groups can often be converted to prodrug esters from which the active forms are regenerated by esterases within the body, e.g. in the blood. In other cases, active drug substances are regenerated from their prodrugs by biochemical reductive or oxidative processes. Sulindac, for example, is active only when reduced to its thioether form (Duggan *et al.* 1977, Duggan 1981) and a prodrug of the pyridinium quaternary compound, 2-PAM, is converted to the parent drug through an enzymatic oxidation process in the body (Bodor *et al.* 1976, Shek *et al.* 1976a, 1976b). Several types of bioreversible derivatives have been exploited for utilisation in designing prodrugs.

1.3.2.1.1 Esters as Prodrugs for Compounds Containing Carboxyl and Hydroxyl Groups

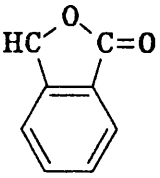
The possibility of using esters as a prodrug type for drugs containing carboxyl or hydroxyl functions (or thiol groups) stems primarily from the fact that the organism is rich in enzymes capable of hydrolysing esters. By appropriate esterification of molecules containing a hydroxyl or carboxyl group it is feasible to obtain derivatives with almost any desirable hydrophilicity or lipophilicity as well as *in vivo* lability, the latter being dictated by electronic and steric factors. This principle has been used successfully to improve the oral bioavailability of ampicillin (**1**), and no fewer than three ampicillin prodrug forms are now on the market, namely, the pivaloyloxymethyl ester (**2**) (pivampicillin) (Daehne *et al.* 1970), the phthalidyl ester (**4**) (talampicillin) (Clayton *et al.* 1974, Shiobara *et al.* 1974) and the ethoxycarbonyloxyethyl ester (**3**) (bicampicillin) (Bodin *et al.* 1975), the latter containing a terminal carbonate ester moiety.



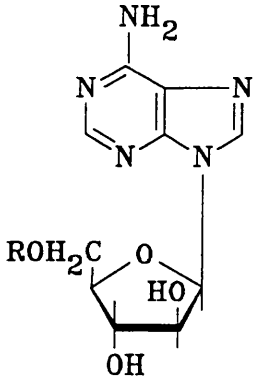
1 R=H

2 R=CH_2-OOC(CH_3)$_3$

3 R=CH(-O-CO-OC_2H_5)-CH_3

4 R= 

Vidarabine (5) has a low water solubility (0.5 mg/ml), primarily due to the occurrence of intermolecular hydrogen bonding in the crystalline state, as reflected in its melting point of 260 °C. By esterification of the 5'-hydroxyl group this bonding is reduced, and further, by choosing an only slightly lipophilic acyl group such as formyl, a vidarabine ester with greatly increased aqueous solubility has been obtained (Repta *et al.* 1975). The 5'-formate ester (6) is hydrolysed rapidly in human blood with a half-life of about 6 - 8 minutes, and it appears to be a useful parenteral delivery form of vidarabine (Repta *et al.* 1975).

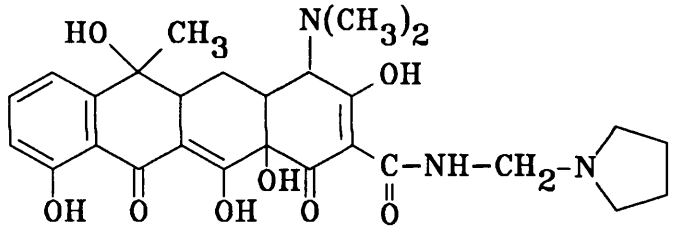


5 R= H

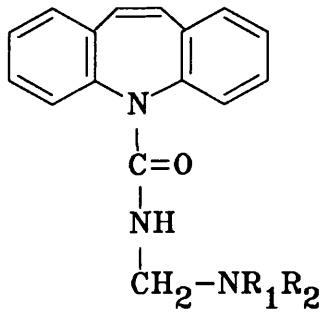
6 R= C(=O)-H

1.3.2.1.2 Prodrugs for Amides, Imides and Other NH-acidic Compounds

N-Mannich bases have been proposed as potentially useful prodrug candidates for NH-acidic compounds such as amides, imides, carbamates, hydantoins and urea derivatives as well as for aliphatic or aromatic amines (Bundgaard 1982, Bundgaard and Johansen 1980a, 1980b, 1980c, 1981a, 1981b, 1981c, Johansen and Bundgaard 1980a, 1980b, 1982). This concept has been utilised in the case of rolitetracycline (7). This highly water-soluble N-Mannich base of tetracycline and pyrrolidine, which is used clinically, is decomposed completely to tetracycline in neutral aqueous solution, the half life being 40 minutes at pH 7.4 and 37 °C (Vej-Hansen and Bundgaard 1981). Similarly, the various N-Mannich bases of carbamazepine (8) have been developed as water-soluble prodrugs for parenteral administration (Bundgaard *et al.* 1982).



7

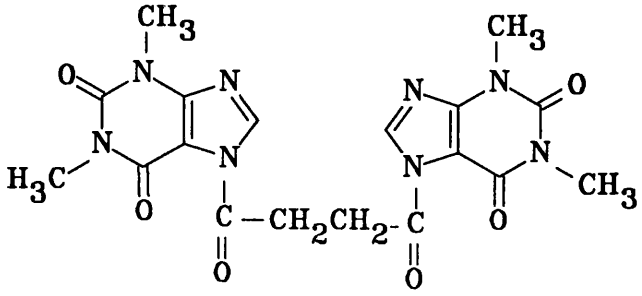


8

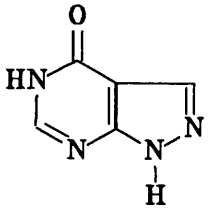
1.3.2.1.3 Prodrugs for Amines.

N-acylation of amines to give amide prodrugs has been used only to a limited extent due to the relative stability of amides *in vivo*. However, certain activated amides are sufficiently chemically labile e.g. 7-acylated derivatives of theophylline are activated amides (N-acyl imidazoles) (9) and they hydrolyse very rapidly under non-enzymatic conditions (Bodor *et al.* 1978, Lee *et al.* 1979). Certain amides formed with amino acids may be susceptible to enzymatic cleavage *in vivo* such as acylation of the

pyrazole moiety in allopurinol (**10**) giving rise to activated amide derivatives susceptible to a marked enzyme-catalysed hydrolysis by human plasma (Bundgaard and Falch 1985).



9



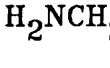
10

A new concept of obtaining site-specific and sustained release of amine drugs to the brain, involving N-acylation, has been developed recently by Bodor and coworkers (Bodor *et al.* 1981, 1983). Being highly protonated at physiological pH, several amine drugs are not sufficiently lipophilic to be able to pass through the blood brain barrier. Galzigna *et al.* (1978) reported that benzoyl or pivaloyl derivatives of γ-aminobutyric acid are hydrolysed in the presence of rat brain homogenates and that compounds, by having enhanced capacity to penetrate the blood brain barrier, should be evaluated as prodrugs. The buccal route has been explored for the delivery of peptides, and preliminary work in dogs demonstrated significant absorption of the hydrophobic lauroyl derivative of a tripeptide (Veillard *et al.* 1987).

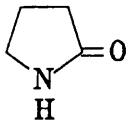
Schiff bases (imines) may in some particular cases be potentially useful as prodrug derivatives for primary or secondary amines. Their usefulness generally is limited, due to a facile hydrolysis in aqueous solution (Gout *et al.* 1984, Singh and Main 1983). The basic character of amines is greatly depressed, by formation of imines. Hence Schiff bases are less protonated at physiological pH than the parent amines. Accordingly the lipophilicity of an amine drugs can be increased and hence its membrane penetrating ability. Thus, in order to facilitate the passage of γ-aminobutyric acids or its amide derivative across membranes, the BBB Schiff bases derived from substituted benzophenone have been investigated (Kaplan *et al.* 1980).

1.3.2.1.4 Lactams and Pyrrolines

γ-aminobutyric acid (GABA) (**11**) and simple structural analogues such as 4-aminopentanoic acid are not capable of penetrating the blood brain barrier due to too low lipophilicity of the compounds. Lipophilic, bioreversible ester derivatives may be useful as brain delivery forms of γ-aminobutyric acid and its analogues but, more surprisingly some cyclic derivatives such as 2-pyrrolidones and Δ′-pyrrolines also appear to represent a chemical class of brain-penetrating prodrug derivatives. Thus Callery *et al.* (1979) have reported that intravenously administered 2-pyrrolidinone (**12**) penetrates readily into the CNS of mice and is converted enzymatically to γ-aminobutyric acid in the brain. In another study, Callery *et al.* (1982) showed that Δ′-pyrroline (**13**) and its analogues can serve as lipophilic prodrugs for γ-aminobutyric acid and its corresponding analogues. Liver and brain homogenates from mice were found to contain enzymes capable of oxidising the pyrrolines to the parent amino acids and, following intraperitoneal injection of the prodrugs to mice, substantial amounts of the parent amino acids were detected in the brain.



11

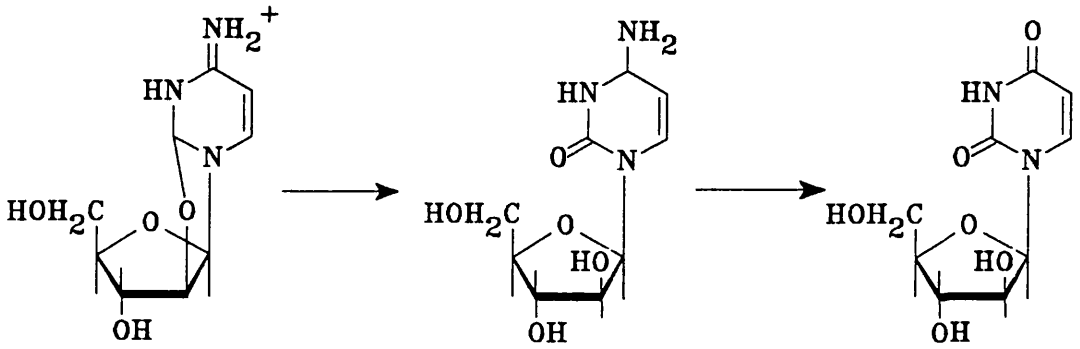


12

13

1.3.2.1.5 Cyclic Cytarabine Prodrug

Cytarabine (14) is an effective antileukaemic agent, but it has a very short half-life *in vivo* because of its rapid deamination by cytidine deaminase to give the inactive 1-β-arabinosyluracil (15). The cyclic compound ancitabine (16) has shown promise as a prodrug with improved pharmacokinetic properties. It is resistant to cytidine deaminase and has been found to extend the biological duration of cytarabine due to slow hydrolytic conversion to the drug *in vivo* (Himmelstein and Gross 1977, Kirsch and Notari 1984a). The hydrolysis is chemically, not enzymatically, mediated and it proceeds by hydroxide ion and water-catalysed reactions (Kirsch and Notari 1984b).



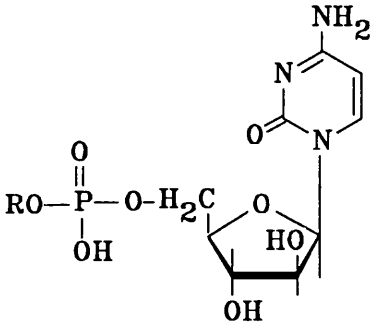
16 14 15

1.3.2.1.6 Prodrugs for the Phosphate Group

A vital step in the mode of action of purine and pyrimidine nucleosides against viral and neoplastic diseases is their conversion into their 5'-mono, di- or triphosphates by cellular or virus-induced kinases (Montgomery 1982, Robins 1984). The nucleoside form is the most often administered because of the ease with which it penetrates cells but, unfortunately, several nucleoside derivatives fail to undergo the necessary phosphorylation to the active nucleotide form (development of resistance) (Robins 1984). On the other hand, the nucleotides are not directly applicable as chemotherapeutic agents because of their poor penetration of cell membranes and

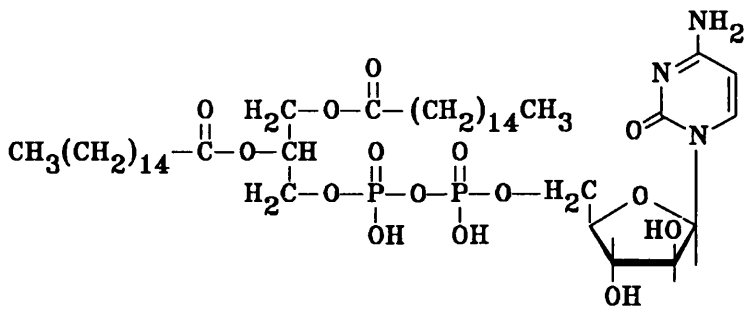
rapid dephosphorylation to the parent nucleosides by non-specific serum phosphohydrolases, such as alkaline and acid phosphatases (Leibman and Heidelberger 1955).

The ionic phosphate moiety can be converted to an ester function to reduce its less ionic character giving better cellular penetrability. Upon entry into cells, enzymatic hydrolysis would be expected to occur. Revankar *et al.* (1975) have described the synthesis and properties of the monomethyl phosphate ester of Ara-A (vidarabine) and Rosowsky *et al.* (1982) have evaluated a series of lipophilic 5'-alkyl phosphate esters of Ara-C, N^4-acyl Ara-C derivatives and anhydro-Ara-C. Only the 5'-glyceryl phosphate ester (**18**) showed activity comparable to the parent nucleotide (**17**). More successfully prodrug derivatives of Ara-C appear to be phospholipid compounds. Matsushita *et al.* (1981) have shown that the 5'-diphosphate-L,1,2,-dipalmitin derivative of Ara-C (**19**) exhibits a much increased efficacy relative to the parent drug against L1210 lymphoid leukaemia in mice. The aggregational and morphological characteristics of structure (**19**) and related liponucleotides have been studied and discussed in relation to the biological efficacy of these prodrugs (MacCoss *et al.* 1982).



17 R=H

18 R=CH_2OH-CHOH-CH_2-



19

1.3.2.2 Macromolecules

Since macromolecules were first proposed as potential drug-carriers (Ringsdorf 1975, De Duve *et al.* 1974) there has been growing interest in their development in clinical situations. This is further increased since liposomes have limited potential in therapeutic applications, particularly drug targeting. Other reported drug delivery systems include those based on protein carriers (Trouet *et al.* 1982b), dextran (Van Heeswijk *et al.* 1984) and inulin (Schacht *et al.* 1984, Remon *et al.* 1984) and antibodies (Thorpe and Ross 1982).

Binding drugs to macromolecules will alter their pharmacokinetics which can be used to advantage in at least three different therapeutic contexts (Duncan 1985). Drugs that exert their effect via cell surface receptors may have their action prolonged if conjugation to macromolecules serves to protect the drug from rapid metabolism or excretion. Drugs that act by interaction with intracellular receptors normally reach there by passing across cell membranes, conjugation inevitably prevents their normal penetration into cells.

In many cases, macromolecular drugs and carriers need to persist in the central blood compartment. This may be either because of a need to access a target (cell) within the cardiovascular system, or to remain in the central compartment long enough to be able to remain intact and to extravasate (via either passive or active means). Size,

surface charge, chemical stability and surface physical and photochemical stability are the most important features for achieving this persistence.

The use of macromolecules as the pro-moiety in prodrugs such as proteins, polypeptides, polysaccharides, nucleic acids, monoclonal antibodies, etc. (Sezaki and Hashida, 1984, Gros *et al.* 1981, Zaharko *et al.* 1979, Schacht *et al.* 1984) are currently being explored. In most cases such drug-carrier conjugates are only active after cleavage with release of the parent drug, and thus can be considered as prodrugs. Low molecular weight prodrugs can be used in the development of macromolecular prodrugs especially if the possibility of using spacer groups for linking the drug to the carrier is considered. For example, mitomycin C has been linked to dextran through the use of 6-aminohexanoic acid as a spacer (**Figure 1**) (Kojima *et al.* 1980, Hashida *et al.*, 1983, 1984, Takakura *et al.* 1984) and daunorubicin to albumin through an oligopeptidic spacer (Trouet *et al.* 1982a).

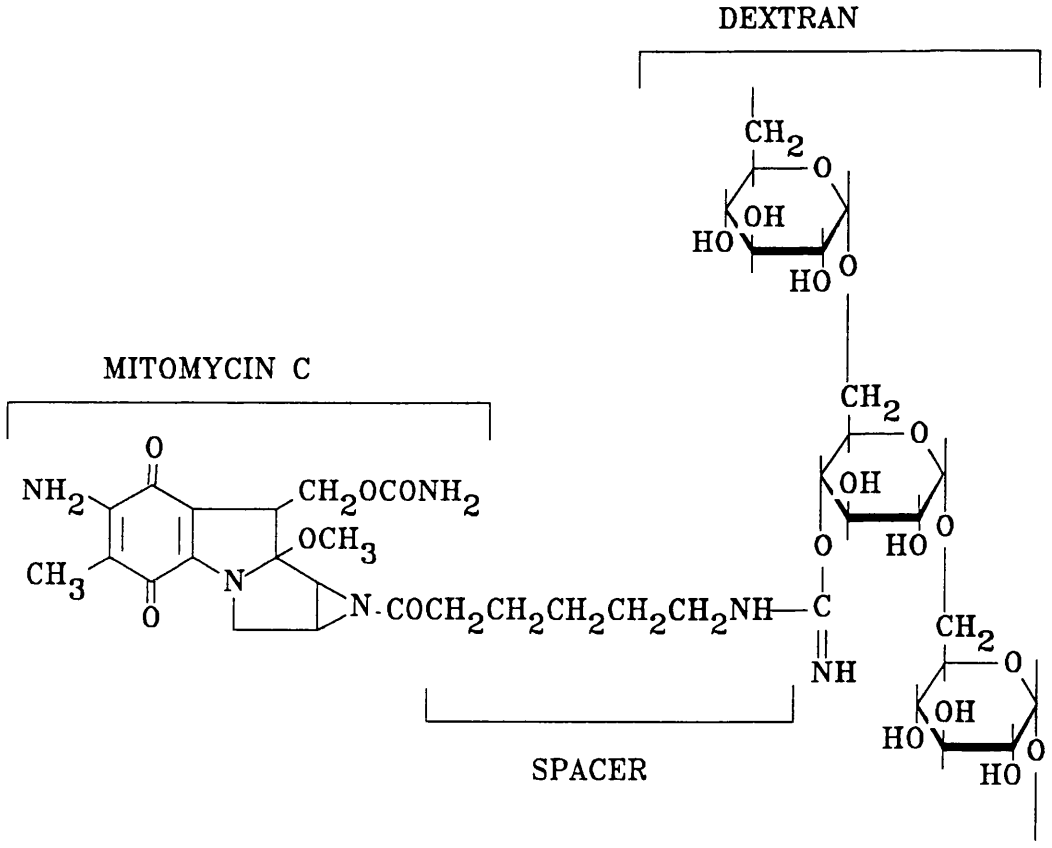


Figure 1

A lipophilic AZT analogue has been incorporated into liposomes for the treatment of human acquired immunodeficiency syndrome. The liposomal compounds are active in vitro, and may be important for the treatment of the macrophage-related pool of the viral cause of the disease (Hostetler *et al.* 1990). This form yields a novel prodrug which may be inactive during transport, improves drug solubility by lipid conjugation and provides direct interaction of drug with cell membrane targets through direct lipid exchange or endocytosis. Such a delivery system demonstrates both capabilities for drug function and target recognition.

1.3.2.2.1 Monoclonal Antibodies

Most research work in the field of antibody drug targeting are focused on the delivery of anticancer drugs where the greatest need for site-specific drug delivery arises. In order to achieve delivery of a drug to its target site by means of an antibody, the coupling procedure should fulfil certain criteria which includes the need for both the drug and the antibody to retain their respective activities and for the conjugate to remain stable in transit to the target site (Gallego *et al.* 1984).

Initial studies on developing soluble site-specific carriers using antibodies appear to be due to Ghose and Cerini (1969) - radioimmunolocalisation of tumours using antibodies, Ghose and Nigam (1972) - direct linking of chlorambucil to antibody.

The difficulty in drawing general conclusions about drug-to-antibody coupling methods when using monoclonals is that one monoclonal may behave quite differently from the other (Rowland *et al.* 1983). Using highly homogenous monoclonal preparations, each antibody must be individually evaluated for a particular type of drug coupling procedure which requires chemical manipulation (Arnon and Hurwitz 1985, Rowland and Simmonds 1985).

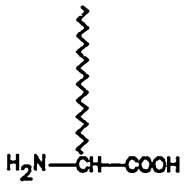
As a possible solution to some of the problems that may be encountered, we have chosen to explore a group of novel peptides, based on lipidic amino acids.

CHAPTER 2

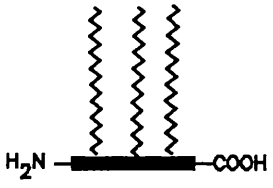
CHEMISTRY

2.1 Lipidic Amino Acids and Peptides

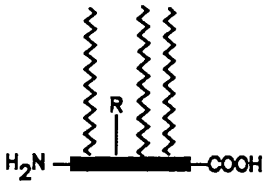
The α-amino acids with long alkyl side chains, the so-called lipidic amino acids **20** can be linked to form peptides. The peptides can take up several forms. These include the linear homo-oligomers **21a**, hetero-peptides containing other amino acids **21b** or substituted lipidic amino acids **21c** or cyclic liposome-like structure **21d**. They, thus, represent a class of compounds which combine structural features of lipids with those of amino acids and peptides or membranes (Gibbons *et al.* 1990).



20

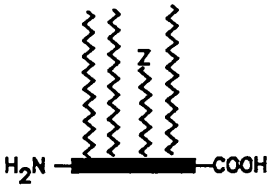


21a



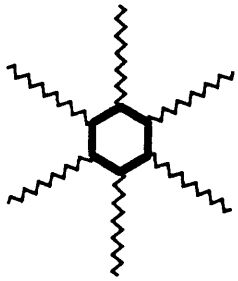
21b

R= Me, Ph, ···



21c

Z= Cl, OH, ···



21d

The length of the alkyl side chains can be varied or substituted with other functional groups and the number of the amino acid residues in the peptide changed. This will affect the hydrophilic/lipophilic character of the lipidic peptides. The physical properties can then be expected to be highly lipophilic due to the long alkyl side chains and yet exhibit polarity and conformations characteristic of amino acids and peptides.

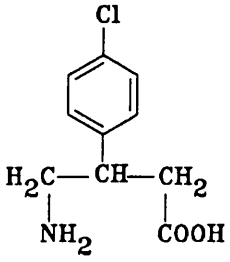
The potential use of lipidic amino acids as lubricants, polishes, cosmetics and surface improvers for ceramics (Kokai Tokkyo Koho 1987a, 1987b, 1987c, 1987d, 1989) has been explored. These lipidic amino acids **20** and peptides **21** could also be used as detergents and biocompatible and/or weatherproof coatings.

However, of special interest is their possible use as a drug delivery system and drug formulation (Gibbons 1988). The therapeutic use of many pharmacologically active peptides and drugs is restricted by their ability to cross biological membranes and the absence of an effective means of delivering them. The appropriately protected lipidic amino acids **20** and peptides **21a** and **21b** can then be employed by conjugating to or incorporated into poorly absorbed peptides and drugs. The passage of the biologically active compounds across the physiological membranes could hopefully be enhanced. For these reasons, the development and evaluation of these lipidic peptides for a drug delivery and formulation system of poorly absorbed drugs (as models), γ-aminobutyric acid (GABA) **11**, baclofen **22**, adenosine **23** and azidothymidine (AZT) **24**, will be dealt with in this thesis.

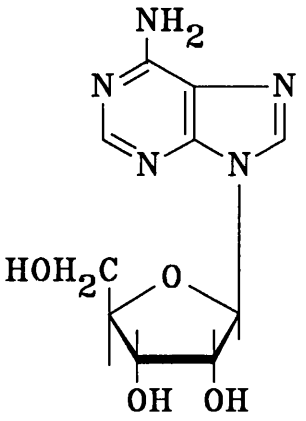
$H_2NCH_2CH_2CH_2COOH$

GABA

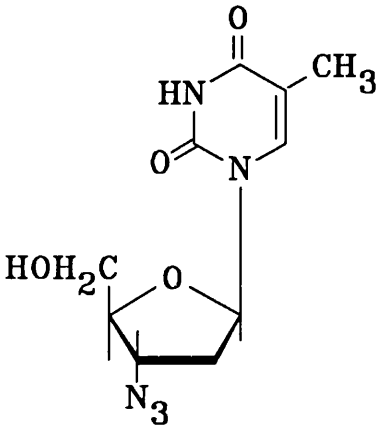
11



22



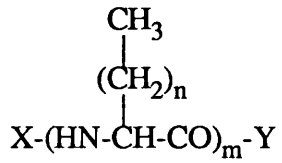
23



24

2.1.1 Unsubstituted α-Amino Acids

The lipidic amino acids can be prepared by treating the α-bromoalkanoic acid with ammonium hydroxide and left stirring for several days (Gerencevic *et al.* 1966, Birnbaum *et al.* 1953, Kimura 1962, Takino *et al.* 1989). Albertson (1946) reported an alternative method for synthesising the lipidic amino acids which was adopted for the preparation of the required lipidic amino acids **25a-e** (Gibbons *et al.* 1990).



25

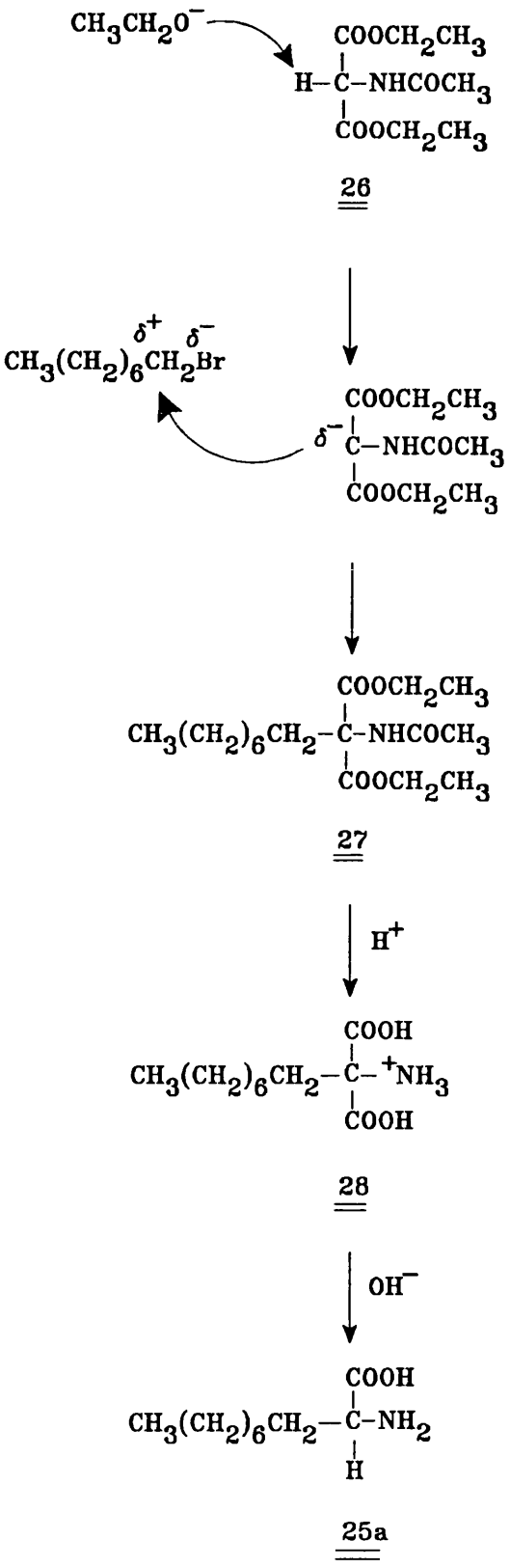
25	X	Y	n	m
a, b, c, d, e	H	OH	7, 9, 11, 13, 17	1
f, g, h	BOC	OH	7, 9, 11	1
i, j, k, l	H	OCH_3	7, 9, 11, 17	1

2.1.1.1 Mechanism of Reaction

The α-amino acid **25a**, a lipidic amino acid with a ten carbon atoms side chain, was synthesised by refluxing 1-bromooctane with diethyl acetamidomalonate (**26**) in the presence of sodium ethoxide (**Scheme 1**). The inductive effect of the carbonyl groups rendered the methine proton in position 2 of the malonate ester weakly acidic. This inductive property of the carbonyl functions causes the generation of carbanions in the presence of strongly basic conditions (sodium ethoxide). The resulting nucleophile (carbanion) can attack the electron deficient brominated carbon of bromooctane, due to the electron withdrawing effect of bromine, forming the octyl-substituted diethyl acetamido-octylmalonate **27**. The intermediate **27** underwent hydrolysis when refluxed with concentrated mineral acid, hydrochloric acid, forming 2-amino-2-carboxyl-decanoic acid **28**. This unstable intermediate **28** then decarboxylated easily yielding the α-aminodecanoic acid **25a**. The amino acids **25b** - **25e** were prepared in racemic form using the same procedure as in **25a** starting from the appropriate 1-bromoalkane. The lipidic peptides and conjugates were then synthesised from these racemic amino acids.

2.1.2 Hetero-oligomers and Homo-oligomers

The α-amino acids are bifunctional in nature. They contain both a free amino and carboxyl functional group. Therefore, to achieve a specific mode of reaction in synthesising the hetero-oligomers and homo-oligomers, the amino group of one reactant and the carboxyl group of the other reactant need to be protected. The reaction is thus directed to occur between one specific amino group of one reactant and a specific carboxyl group of the other reactant. After coupling of the protected amino acids, the protecting groups can then be removed and the process repeated until a peptide of desired length is obtained.



Scheme 1

Mechanism of reaction for the preparation of α-amino acid

2.1.2.1 Protecting Groups

The protecting groups should have certain characteristics such as:

i) they must be capable of being introduced under conditions in which side reactions (eg. racemisation) can be avoided.

ii) they should be inert in conditions when the peptide link (amide bond) is formed.

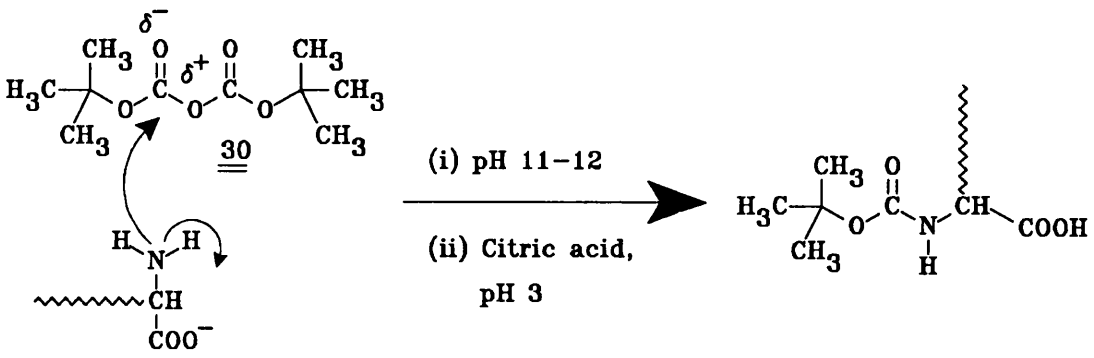
iii) they are removable under conditions which do not affect other bonds eg. the amide bonds or other protecting groups.

2.1.2.1.1 Protection of the Amino Group

There are a whole range of protecting groups for the amino function of the amino acids. They include the (i) acyl groups such as formyl (Fischer and Warburg 1905), chloroacetyl (Holley 1952) and phthalyl (Kidd and King 1948), (ii) alkyl groups, benzyl (Velluz *et al.* 1954) and triphenylmethyl (trityl) (Helferich *et al.* 1925), (iii) p-toluenesulphonyl (Ts) (Schönheimer 1926) and (iv) urethanes i.e. carbobenzoxy (Z) (Bergmann and Zervas 1932), tertiary butyloxycarbonyl (BOC) (Carpino 1957) and 9-fluorene methoxycarbonyl (Fmoc) (Carpino and Han 1972).

The BOC group was used for the protection of the amino function of the amino acids during the lipidic peptide synthesis because of ease and economy of synthesis, ease of removal with products which were sufficiently soluble in organic solvents.

The BOC-protected lipidic amino acids **25f**, **25g**, **25h**, were prepared in good yield of 60 - 80 % from the corresponding α-amino acids **25a**, **25b**, **25c** respectively. They were synthesised by reacting the symmetrical anhydride, ditertiary butyldicarbonate ($(BOC)_2O$) (**30**) (Tarbell *et al.* 1972, Moroder 1976) with the appropriate lipidic acid. The nucleophilic attack of the anhydride ($(BOC)_2O$) **30** resulted in the formation of the protected lipidic amino acid (**Scheme 2**).

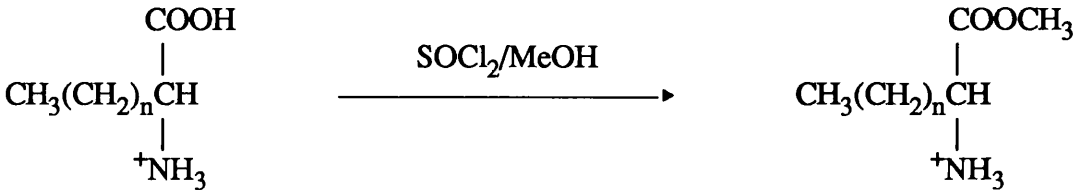


Scheme 2

Protection of the amino function

2.1.2.1.2 Carboxylic Acid Protection

In peptide synthesis, the widely used protecting groups for the carboxyl function of the amino acids are the methyl- (Brenner and Huber 1953), trichloroethyl- (Eckstein 1965), *tert.* butyl- (Roeske 1959) and phenyl- (Kenner 1959) groups. Carboxylic acid protection of lipidic amino acid using methyl ester as protecting group was used in the synthesis of lipidic peptides. The methyl ester HCl salts of **25i**, **25j** and **25k** were synthesised with excellent yield by treating the amino acids **25b**, **25c** and **25e** respectively with thionyl chloride in methanol (Brenner and Huber 1953) (**Scheme 3**).

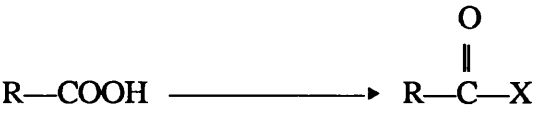


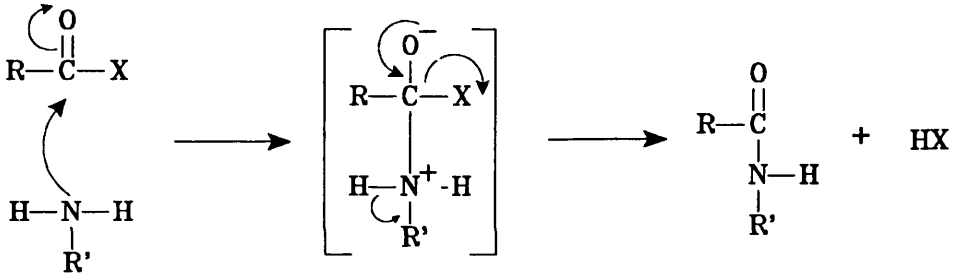
Scheme 3

Esterification of lipidic amino acid

2.1.2.2 The Amide Bond Formation

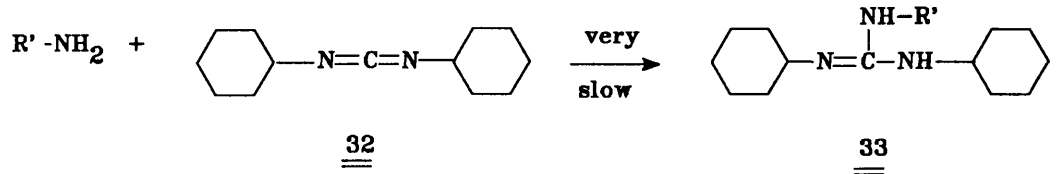
Carboxylic acids can be converted into acylating agents, increasing the polarising effect of the carbonyl group and thus the electrophilicity of its carbon atom, by replacing their hydroxyl group with an electron-withdrawing substituent (X). Hence the nucleophilic attack by the amino group (of the amino acid to be acylated) (31) is greatly facilitated:

Activation: 

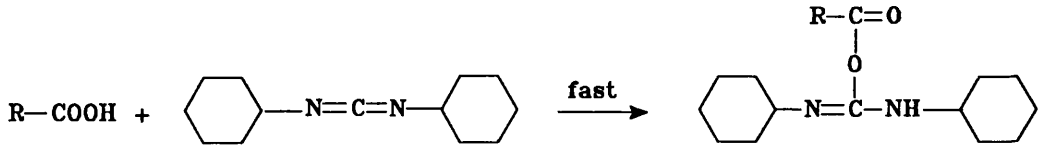
Coupling: 

31

Carbodiimides, particularly dicyclohexylcarbodiimide (DCC) (32), were introduced by Sheehan and Hess (1955) as coupling reagents. The features of these coupling reagents are that they can be added to the mixture of two reactants, the carboxyl component and the amine component. The amines do react with carbodiimides (yielding guanidine derivatives) (33), but the rate of this reaction is negligible compared with the addition of carboxylic acids to one of the double bonds of a carbodiimide, forming the O-acyl isoureas (34):

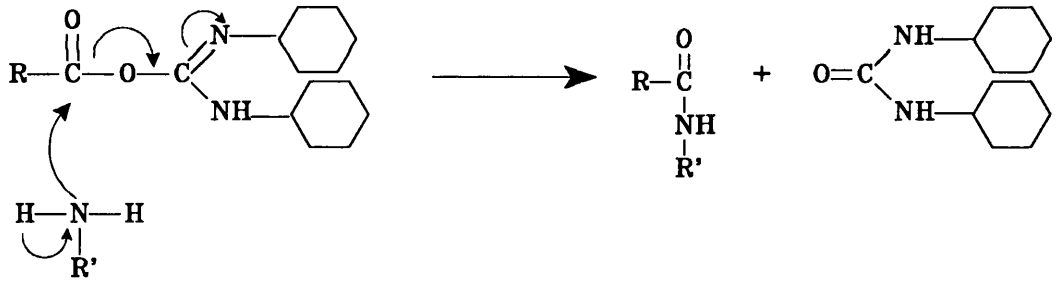


32 33



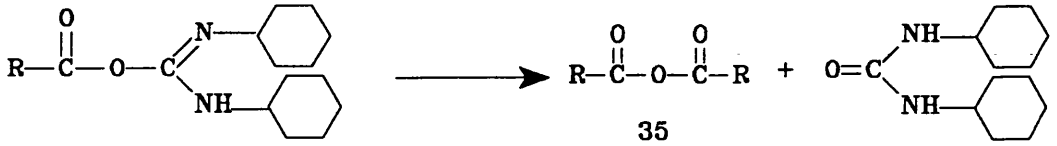
32 34

In O-acyl isoureas intermediates **34**, formed by the addition of carboxylic acids to the carbodiimides (the N=C group provides powerful activation) which leads to coupling:

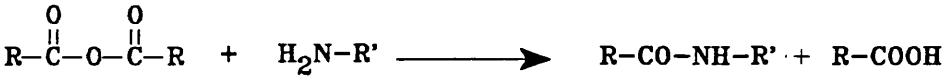


It is reasonable to attribute some basic character to O-acyl-isoureas and therefore general base catalysis can be evoked as an explanation of the surprisingly high reactivity observed in aminolysis.

A second pathway in the peptide bond formation is via symmetrical anhydrides **35** produced by the attack of an unreacted molecule of the carboxyl component on the O-acyl isourea intermediate:

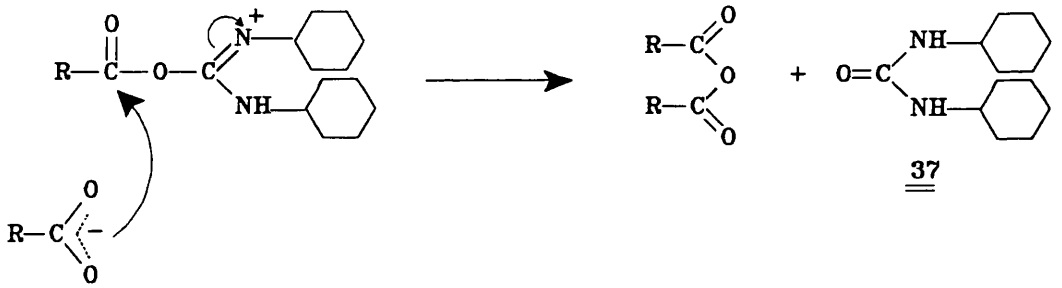


35



35

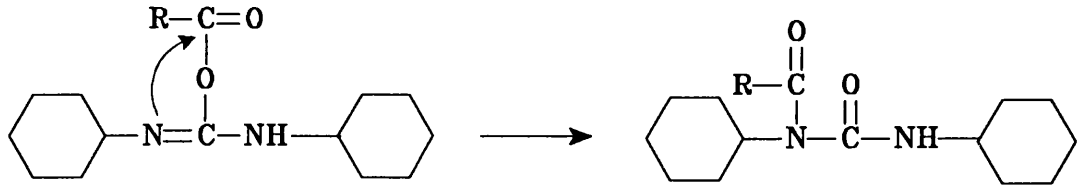
The rapid generation of symmetrical anhydrides **35** can be rationalised by the attack of the carboxylate anion **36** on the reactive carbonyl function of the O-acyl isourea **34**:



36

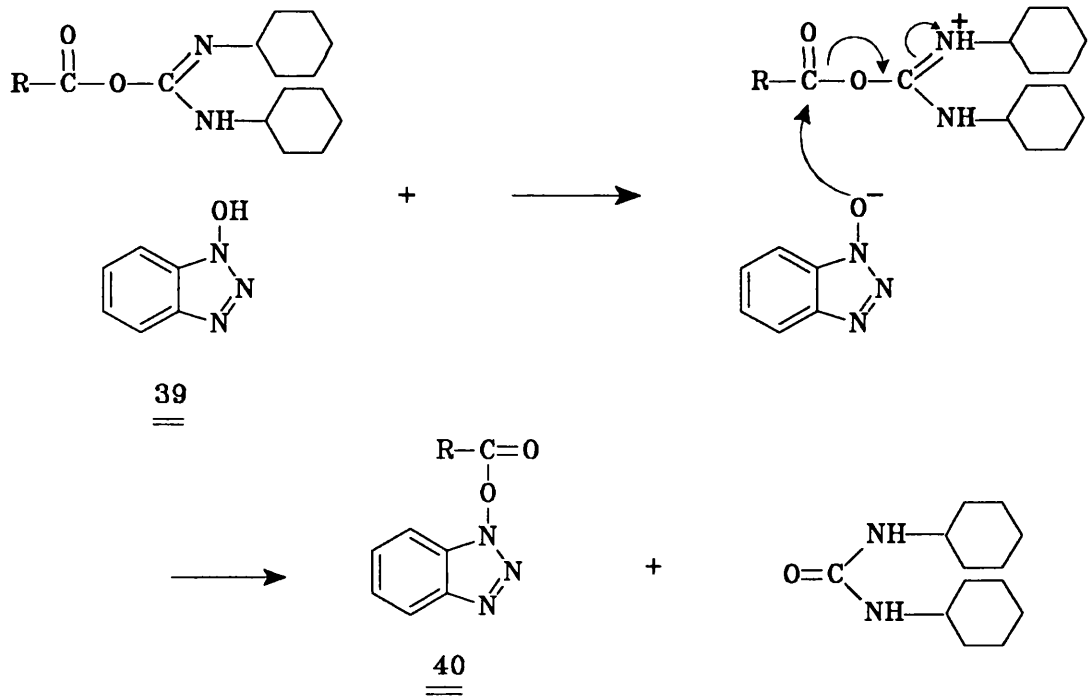
37

The fast execution of activation and coupling in a single operation and the simple removal of the insoluble by-product, N,N'-dicyclohexylurea (DCU) (**37**), by filtration made the DCC method generally popular. However, overactivation in the reactive intermediate results in some loss of enantiomeric purity when peptide segments are coupled. The nucleophilic centre on O-acylisoureas competes, as well, with the amine component for the acyl residue and this competition leads to the formation of unreactive by-products, N-acyl ureas **38**:



34 38

Both racemisation and N-acyl urea formation can be suppressed by the addition of auxiliary nucleophiles such as 1-hydroxybenzotriazole (HOBt) (**39**) proposed by König and Geiger (1970). Attack of the additive on the reactive intermediate yields an O-acyl-1-hydroxybenzotriazole (**40**), a powerful acylating agent:

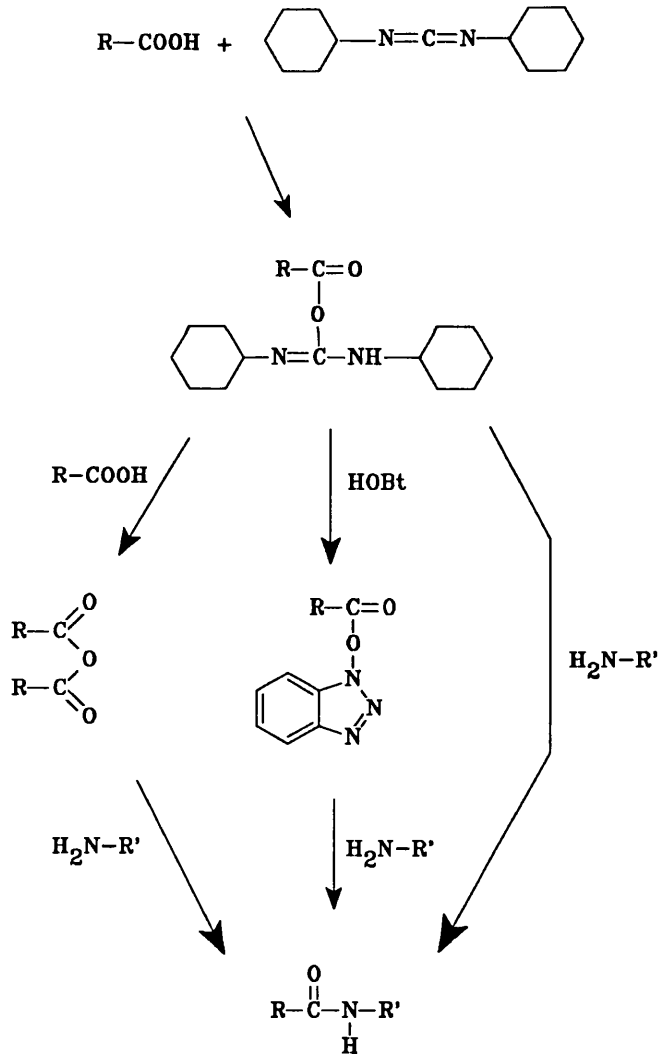


39

40

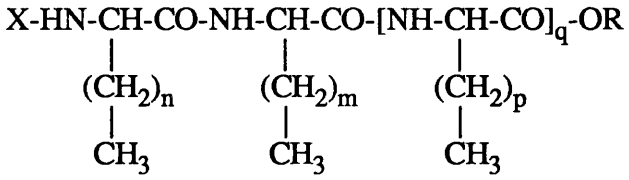
The presence of a second nucleophile **39** in the reaction mixture reduces the concentration of the O-acyl isourea **34** and thereby the extent of racemisation. HOBt (**39**) is also a weak acid, preventing proton abstraction from the chiral carbon atom and thus contributing to the preservation of enantiomeric purity in a second manner as well. The availability of HOBt shortens the lifetime of the overactivated O-acyl-isourea intermediate and hence diminishes the extent of O → N acyl-migration leading to N-acyl ureas **38**. HOBt is regenerated during acylation, maintaining the concentration almost constant during coupling.

A schematic summary of the coupling procedures is given below.



2.1.2.3 Synthesis of α-Amino Acid Homo- and Hetero-oligomers

Using the solution phase peptide synthetic procedure described above, a series of lipidic peptides was synthesised **41**.



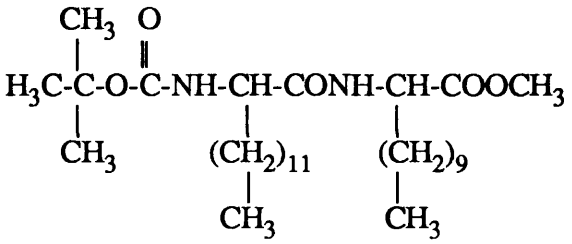
41

41	n	m	p	q	X	R
a	7	7	-	0	BOC	CH_3
b	11	9	-	0	BOC	CH_3
c	11	11	-	0	BOC	CH_3
d	11	17	-	0	BOC	CH_3
e	11	9	-	0	H	CH_3
f	11	11	-	0	H	CH_3
g	11	17	-	0	H	CH_3
h	7	7	-	0	BOC	H
i	11	9	-	0	BOC	H
j	11	11	-	0	BOC	H
k	11	17	-	0	BOC	H
l	7	7	7	1	BOC	CH_3
m	11	11	11	1	BOC	CH_3
n	11	17	11	1	BOC	CH_3
o	11	11	11	1	BOC	H

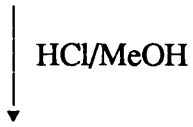
Oligomers **41b** - **d** were synthesised from N-BOC protected amino acid **25h** and C-protected amino acid **25j** - **l** respectively. Coupling compounds **25f** with **25i** yielded

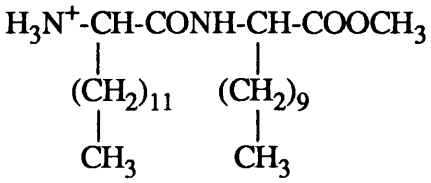
the fully protected dimer **41a**. The fully protected crude dimers were purified by flash chromatography on silica gel.

The lipidic amino acid trimers were subsequently prepared from the partially protected dimers and appropriately protected monomers. To synthesise trimeric amino acids **41l**, **41m** and **41n**, the fully protected dimers **41a**, **41c** and **41d** respectively have to be partially deprotected at the N-termini. The N-BOC group, since it is acid labile, could be cleaved by various means including the use of anhydrous trifluoroacetic acid (TFA) or dry hydrogen chloride in methanol (Carpino 1957, McKay and Albertson 1957). HCl in methanol was used to deprotect the amino function of the dimers **41b**, **41c** and **41d** to give N-deprotected dimers **41e**, **41f** and **41g** (**Scheme 4**) respectively, as hydrochloride salts.



41b



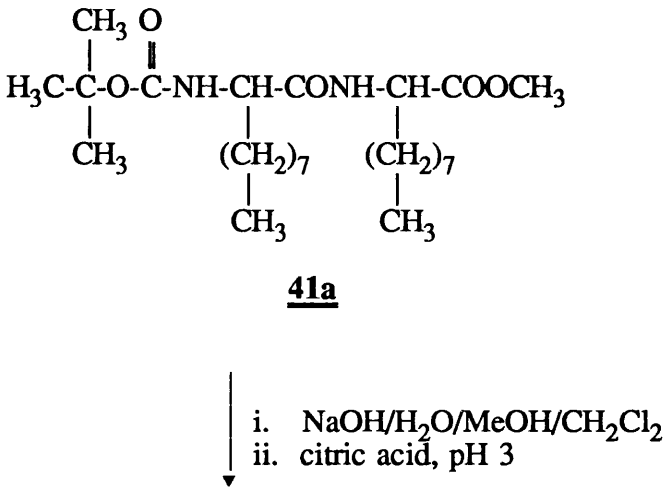


41e

Scheme 4

Removal of BOC group from 41b by acidolysis

The C-deprotected dimers **41h** , **41i**, **41j**, **41k** and trimer **41o** were prepared from the fully protected dimer **41a**, **41b**, **41c**, **41d** and trimer **41m** respectively by base catalysed hydrolysis (**Scheme 5**). Kenner and Seeley (1972) reported racemisation using base-catalysed hydrolysis of alkyl esters. This was not a problem since a racemic mixture of amino acid was used.



41a

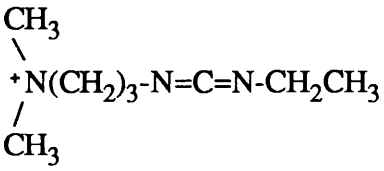
41h

Scheme 5

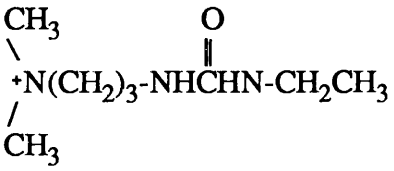
C-deprotection of 41a by saponification

The N-protected dimers **41h** and **41j** were coupled with C-protected amino acid methyl esters **25i** and **25k** respectively using HOBt and DCC or EDAC as described to yield fully protected trimers **41l** and **41m**. Compound **41n** was however prepared from C-deprotected dimer **41k** and C-protected **25k**. The trimers **41l**, **41m** and **41n** were purified by flash or preparative thin layer chromatography.

Although DCU (**37**) was claimed to be an insoluble by-product, it was found that small quantity of it did dissolve in the organic solvent used in the reaction and had a similar R_f value to some of the fully protected oligomers making purification difficult. In the synthesis of oligomers **41b** - **d** and **41l** - **n**, water soluble carbodiimide 3-[3-(dimethylamino)propyl]-1-ethylcarbodiimide (EDAC) (**42a**) hydrochloride salt was used instead of dicyclohexylcarbodiimide. The ease of removing the unreacted water soluble carbodiimide and urea (**42b**) from the reaction mixture by washing with water (Sheehan and Hlavka 1956), facilitated this purification process.



42a



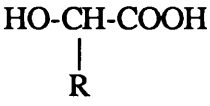
42b

2.1.3 Modified Lipidic Peptides

In trying to improve the transmembrane absorption of poorly-absorbed compounds, increased membrane-like character could be imparted by conjugation to lipidic amino acids and their oligomers. 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, the second was to conjugate the lipidic amino acids with hydrophilic molecules. A number of substituted lipidic acid derivatives, containing hydrophilic groups have been synthesised with the aim of enhancing their solubility in aqueous systems whilst maintaining membrane affinity characteristics. There are several hydrophilic species suitable for conjugation to lipidic amino acids and peptides, such as lactic acid, glycolic acid, glyceric acid and sugars. Of these, sugars represent the most efficient way of increasing water solubility, because of the many hydroxyl groups present on one molecule.

2.1.3.1 Lipidic Amino Acids Conjugates with Hydrophilic Compounds

To increase the hydrophilicity of the head group of lipidic amino acids, synthesis of lipidic amino acid oligomers, containing lactic and glycolic acid were carried out. Lactic acid (**43a**) was reacted with methyl 2-amino-tetradecanoate (**25k**) using dicyclohexylcarbodiimide (DCC) as a coupling reagent to yield **44a**.



43

43a R=CH_3

43b R=H

Because of the great difference between the reactivity of the hydroxyl and amino groups under the reaction conditions used, the hydroxyl function of the lactic acid does not need to be protected. Using the same procedure, glycolic acid conjugates **44c**, **44d** and **44f** were synthesised, starting from glycolic acid (**43b**) and esters **25i**, **25k** and **41f** respectively.

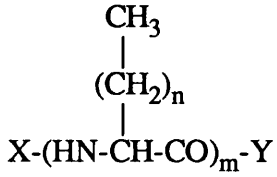
Base hydrolysis of methylesters **44a** and **44d** resulted in the free acids **44b** and **44e** with good yields. The coupling of unprotected D-glucuronic acid with methyl ester **25k** yielded the new product **44g**, but the mass spectrum showed 2 mass units less than the expected molecular ion $[M + H]^+$. There are examples in the literature (Baldwin *et al.* 1988), where under fast atom bombardment conditions, compounds having long alkyl chains may exhibit $[M - H]^+$ as the major quasimolecular ion species.

To further increase the conjugation possibilities and for physico-chemical investigations, several glyco-amino acid derivatives **44h** - **k** and glycopeptide **44l** were synthesised. The fully-protected **44h** was prepared by coupling the lipidic amino acid methylester **25i** to the hydroxyl-protected monosaccharide 1,2:3,4-di-O-isopropylidene-D-gulonic acid with the assistance of dicyclohexyl carbodiimide. The other fully-

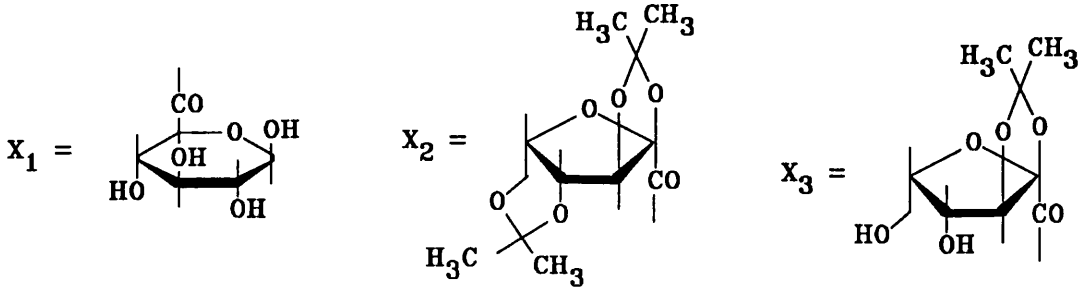
protected glycopeptides **44i** and **44l** were prepared in the same manner, starting from the amino acid methyl ester **25k** and the N-deprotected lipidic peptide dimer **41f** respectively. The free acid **44j** was obtained following saponification of the methyl ester **44i**. The carboxylic acid group of **44j** renders it suitable for further coupling to other compounds.

Attempts to remove the two isopropylidene groups protecting the sugar hydroxyl groups of **44j** were made with both acetic acid and TFA, each resulting primarily in the partially-deprotected glyco-amino acid **44k**. The second isopropylidene group was found to be resistant to cleavage even after six hours reflux with acetic acid or one day with TFA at room temperature.

44	n	m	X	Y
a	11	1	$HOCH(CH_3)CO$	OCH_3
b	11	1	$HOCH(CH_3)CO$	OH
c	7	1	$HOCH_2CO$	OCH_3
d	11	1	$HOCH_2CO$	OCH_3
e	11	1	$HOCH_2CO$	OH
f	11	2	$HOCH_2CO$	OCH_3
g	11	1	X_1	OCH_3
h	7	1	X_2	OCH_3
i	11	1	X_2	OCH_3
j	11	1	X_2	OH
k	11	1	X_3	OH
l	11	2	X_2	OCH_3



44



2.1.3.1.1 Physico-Chemical Investigations

NMR investigations of lipidic amino acid conjugates

Micelle formation in non-aqueous solvents:

The NMR studies were performed in the Department of Pharmaceutical Chemistry, The School of Pharmacy, by Dr. Graeme J. Anderson.

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. Many of these problems arise because of the smaller aggregates present in organic solvents. As the solutions have little surface activity and the aggregates are not ionized, it is not possible to use the two most commonly used methods to study aqueous systems, i.e. surface tension and conductivity.

It was decided to use ^{1}H-NMR to study the physico-chemical behavior of lipidic amino acid-systems in organic solvents.

Samples were dissolved in either chloroform ($CDCl_3$) or methanol (CD_3OD), at as

high a concentration as possible, as determined by the solubility of the compound, lower concentrations were achieved by subsequent dilution. All spectra were recorded in 0.6 mls of solvent, at 25 °C, as a function of concentration for each of the compounds. Sample concentrations varied from 320 mM to 2.50 mM for **44a**, 954 mM to 0.94 mM for **44d**, 657 mM to 0.87 mM for **44e**, 210 mM to 6.50 mM for **44j** and 124 mM to 0.97 mM for **44k**. Plots of log 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 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.

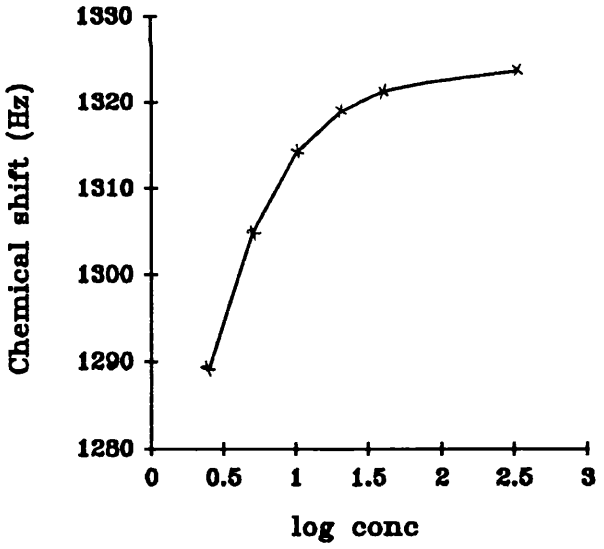


Figure 2: Chemical Shift of 2'–H of 44a

For **44a** in methanol (Figure 2) the chemical shift behavior was monitored over the 320 to 2.50 mM range. The chemical shift of the α-CH proton of the lactic acid moeity in compound **44a** was plotted against log concentration (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 (2.5 mM), 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 **44d**, **44e** were independent of concentration at low molarities, but began to change rapidly at higher molarities (Figure 3). 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 (254 mM and 657 mM respectively), showing that no fully aggregated, stable form was achieved. The additional methyl group present in **44a** appeared therefore to promote aggregation at the expense of the monomer relative to that of the two glycolic acid derivatives.

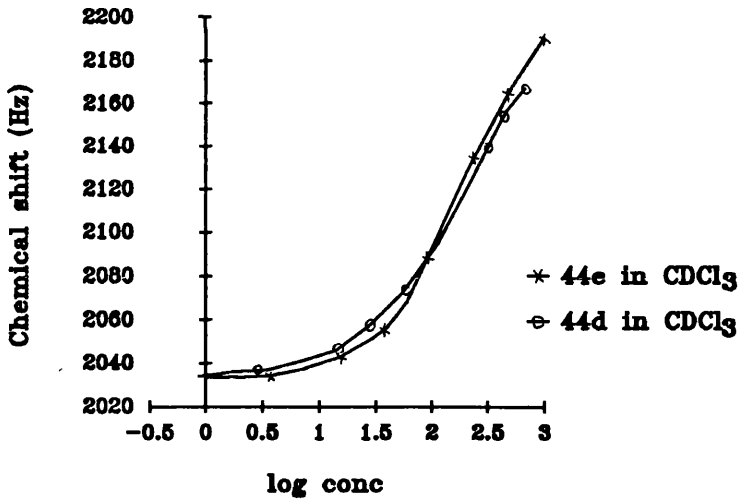


Figure 3: Chemical Shift of NH proton.

For monosaccharide derivatives with two and one protecting groups present, **44j** and **44k** respectively, the concentration studies yielded interesting results. Figure 4 shows the behavior 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 behavior. 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 ~16 mM for compound **44k** and ~40 mM for **44j**. The differences between the two CMC values could be explained by considering the polarity of the sugar head groups. The least polar moiety, **44j**, showed a tendency to remain in the monomer form rather than aggregate, whereas the removal of one of these protecting groups, to form **44k**, 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 **44k**.

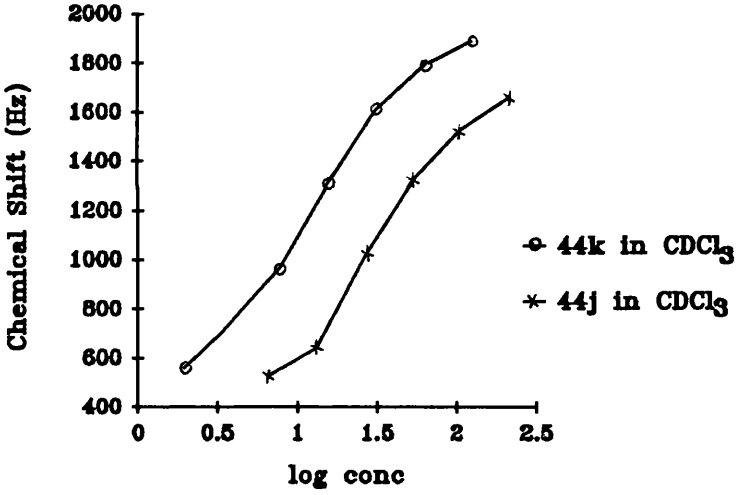


Figure 4: Chemical Shift of associated water

The positions of levelling-off of chemical shift *vs* log concentration plots (Figure 4) at low concentrations were also different, **44j** forming monomers at higher concentrations than **44k**, again most likely reflecting the increased interaction between molecules via their free -OH groups in **44k**.

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.

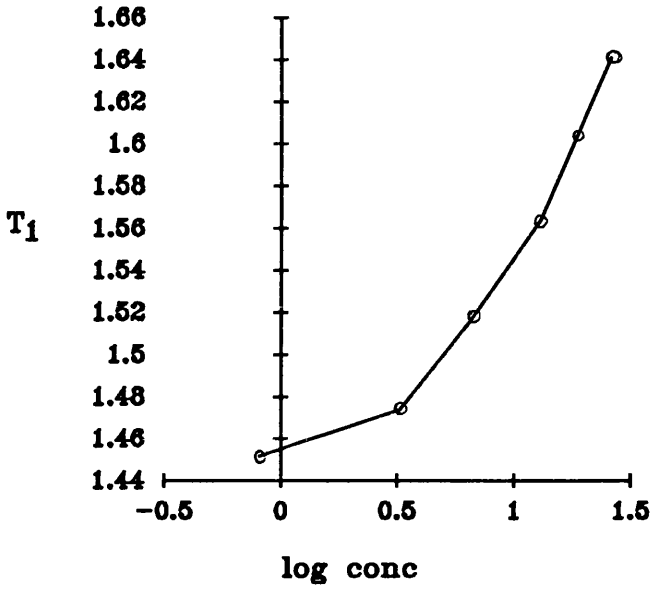


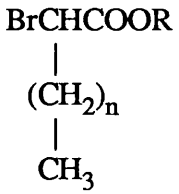
Figure 5: Spin Lattice Relaxation Time (T_1) of 44k.

For derivative **44k**, with one protecting group on the sugar moiety, the plot of T_1 versus log concentration (for the ω-CH_3 protons) is shown in Figure 5. This experiment gave excellent correlation with the shape of that in Figure 5, 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.

2.1.3.2 Halogen Substituted Lipidic Amino Acids and Oligomers.

Formation of an ester linkage between the delivery system and the parent peptide or drug can be achieved with the aid of halogen substituted lipidic acids **45**, amino acids and peptides **46**. A series of α-brominated fatty acids **45a** - **c** were protected at the carboxyl terminus by treatment with thionyl chloride in methanol under reflux

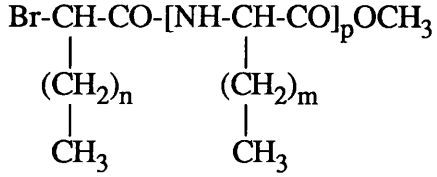
condition to yield a series of methyl esters **45d** - **f**. The conditions used were the same as that described in the carboxylic acid protection (**Scheme 3**, p 38). The methyl esters synthesised were in the form of oils in good yield.



45

45	n	R
a	5	H
b	11	H
c	13	H
d	5	CH_3
e	11	CH_3
f	13	CH_3

The lipidic systems **46** were prepared from C-protected amino acids (**25**) or oligomers (**41**) in solution phase using a coupling reagent dicyclohexylcarbodiimide (DCC) or water soluble carbodiimde, ethyl-3-[3-(dimethylamino)propyl]-1-ethylcarbodiimide (EDAC) (see Section 2.1.2.3, p 44). Compounds **46a** - **c** were prepared by reacting **45b** with **25j** - **l** respectively. Similarly, **46e** was obtained by coupling **45c** with **25k**. By increasing the number of amino acid units, compounds **46d** and **46f** were formed. This was done by reacting **45b** with N-deprotected dimer **41f** which gave rise to **46d**. Compound **46f** was synthesised in an analogous fashion by using **45c** instead. The lipidic peptide conjugates were then purified by flash chromatography in good yield using dichloromethane:methanol (100:3) as the eluent.



46

46	n	m	p
a	11	9	1
b	11	11	1
c	11	17	1
d	11	11	2
e	13	11	1
f	13	11	2

In the preparation of **46f**, a substantial amount of by-product ether **47** was formed, due to the inclusion of HOBT in the coupling reaction. NMR spectra indicated the presence of aromatic proton frequencies at 8.03 - 6.84 ppm attributed to the aromatic nucleus of HOBt (Fig. 6) and aliphatic proton frequencies in the highfield region of the spectrum at 4.98 - 0.90 ppm.

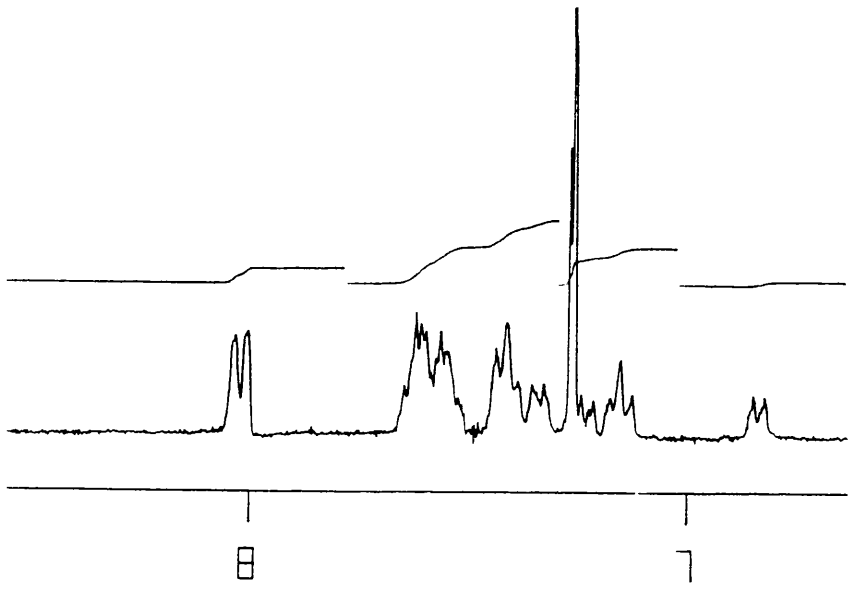


Figure 6

NMR spectrum (lowfield region) of HOBt-ether by-product

Mass spectrum also showed the natriated molecular ion 876 as the base peak (Fig. 7).

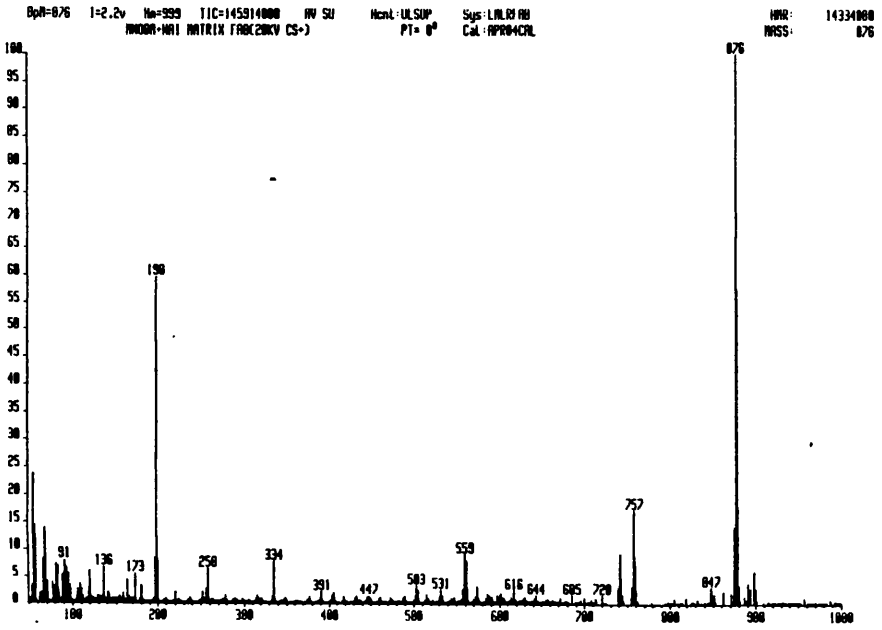
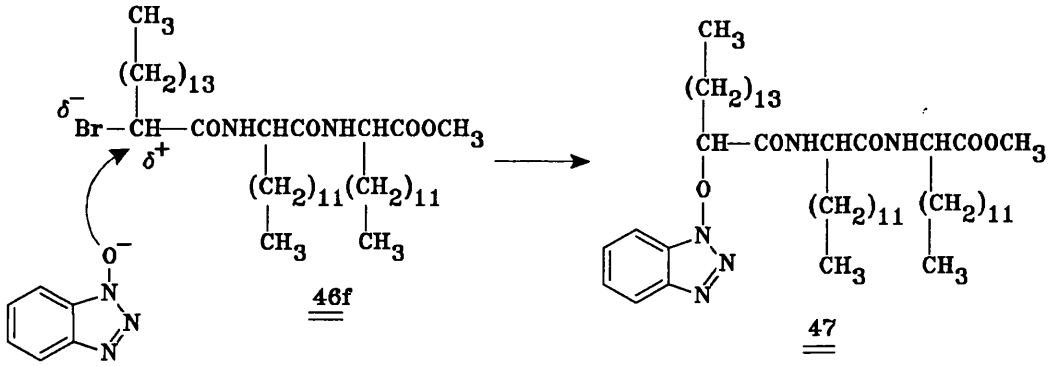


Figure 7

Mass Spectrum of 47

Because of the high electron withdrawing effect of the bromine group, its neighbouring carbon is electron deficient which facilitates nucleophilic attack by HOBt.



Scheme 6

Formation of HOBt-ether by-product

Unlike the O-acyl-1-hydroxybenzotriazole **40**, the HOBt-ether by-product **47** is stable. This side reaction greatly reduced the yield of the required halogenated conjugates. When the HOBt was omitted from the subsequent synthesis of other halogenated conjugates, the reaction proceeded at a slower rate but the desired products were obtained in higher yield.

2.2 Lipidic Drug Conjugates

The lipidic amino acids and peptides can be chemically conjugated to drugs with a wide variety of functional groups due to their bifunctional nature. The drug conjugates formed may be biologically stable or possess biological or chemical instability. The conjugates in any case will increase the lipophilicity of poorly-absorbed drugs, hopefully enhancing the absorption across a membrane due to its high degree of membrane-like character. Varying the length of the alkyl side chains and the amino acid units could change the degree of lipophilicity of the drug conjugates. This lipophilic conjugating unit may also act as a protecting group for the labile parent drug from enzymatic attack.

The free acid or amino group of the lipidic amino acids and peptides can be conjugated to an amino, carboxylic acids or hydroxyl substituent of the drug via ester or amide linkages.

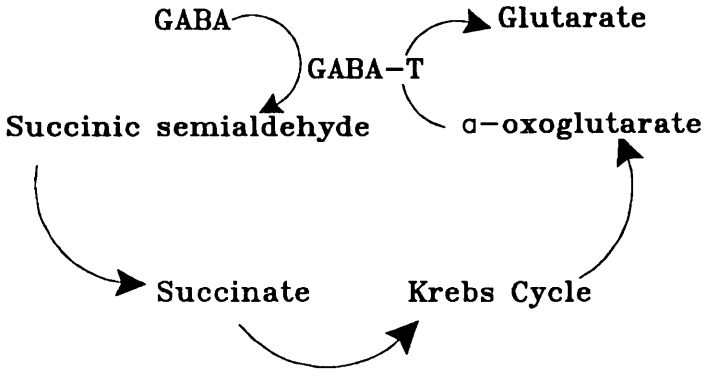
In selecting the drugs suitable for conjugation, the prerequisites include (i) the drugs have absorption problem, (ii) they possess functional groups where a range of linkages can be investigated and (iii) availability of *in vivo* and *in vitro* assays for determination of the effectiveness of the drug delivery system in the drug conjugates. Hence, drugs chosen as models are from the class of (1) CNS, neurotransmitter γ-aminobutyric acid (GABA) (**11**), and baclofen (**22**) (2) nucleosides, adenosine (**23**) and antiviral 3'-azadeoxythymidine (AZT) (**24**).

2.3 GABA Lipidic Conjugates

2.3.1 The Role and Delivery of GABA.

There is no single definition of GABA's action that embraces all of its effects. The function of GABA is ubiquitous i.e. it shows widespread inhibitory activities in both central and peripheral neurones of vertebrates (Ciesielski *et al.* 1979). The possible complicity of the central inhibitory neurotransmitter GABA in certain neurological and psychiatric disorders e.g. epilepsy and Huntington's disease (DeFeudis and Mandel 1981, Di Chiara and Gessa 1981, Krogsgaard-Larsen *et al.* 1979, Lal *et al.* 1980, Morselli *et al.* 1981 and Roberts *et al.* 1976) has led to an increase in neurochemistry research on GABA induced neuronal functions using GABA agonists, analogues and prodrugs.

GABA is metabolised in the brain (Ciesielski *et al.* 1979) by the enzyme, GABA-transaminase (GABA-T).



There are 2 distinct sub-types of GABA receptors: $GABA_A$ and $GABA_B$ (Hill and Bowery 1981).

Many ways have been investigated to effect an enhanced action of GABAergic systems in the CNS, either directly or indirectly. A very high concentration of GABA is needed in the blood if GABA is introduced directly. This necessitates the

administration of high doses of GABA which gives rise to side-effects prohibiting a more general use (Ciesielski *et al.* 1979). Indirect approaches to increase GABAergic action in CNS includes the use of GABA agonists e.g. baclofen, muscimol (Frey and Löscher 1980), GABA mimetics e.g. benzodiazepines (Meldrum 1981) or the administration of lipophilic GABA derivatives acting as prodrugs (Shashoua *et al.* 1984, Hesse *et al.* 1985, 1988, Jacob *et al.* 1985, 1987, Frey and Löscher 1980).

GABA has a poor ability to cross the BBB (Krogsgaard-Larsen 1980). This is consistent with the physicochemical properties of GABA in water:

- low partition coefficient (0.042) between octanol and water
- the predominant zwitterion structure **47** associated with the high molecular dipole moment (13 Debye) at physiological pH (Pardridge 1985a).

$$H_3N^+CH_2CH_2CH_2COO^-$$

47

Neither of these properties do favour a passive diffusion of the GABA molecule through such a lipidic structure, as the blood-brain barrier (BBB). The BBB (Section 2.3.2.1), therefore acts as an impermeable barrier for entry and exit of GABA into and from brain tissue under physiological conditions (Pardridge 1985a).

2.3.2 Endothelium

Microvascular endothelium is composed of single squamous epithelial cells that varies in thickness from 0.1 to 1.0 μm. Three types of endothelium are found in the microvasculature: continuous, fenestrated and discontinuous (Simionescu 1977, Smith and Kampine 1984). Continuous endothelium, comprised of closely apposed endothelial cells, are typical of the microvasculature of the heart, lungs, skeletal muscle, and the central nervous system. The endothelium of the renal glomerulus and the intestinal mucosa has relatively large transcellular openings called fenestrae and is referred to as fenestrated endothelium. Discontinuous endothelium possesses much larger transcellular openings or gaps between cells and is found in the spleen and

hepatic sinusoids (Simionescu 1977, Palade *et al.* 1979).

The microvascular endothelium plays a critical physiological role in forming a physical partition and semipermeable porous membrane between the blood and extravascular tissue. Thus it participates in the regulation of the exchange of fluid, water-soluble solutes (including drugs), macromolecules, and cells between the blood and the extravascular tissues (Simionescu 1977, Palade *et al.* 1979). Due to the anatomical and functional differences among the three types of endothelium, there is a variable microvascular permeability between different tissues. Discussion here will be limited to the continuous endothelium of the brain microvasculature known as the blood-brain barrier.

2.3.2.1 The Blood Brain Barrier (BBB).

The vertebrate brain is endowed with capillaries of unique morphological characteristics that make up the blood brain barrier (BBB). The continuous microvascular endothelium of the brain represents the most restrictive type of endothelium (Reese and Karnovsky 1967, Brightman and Reese 1969). This acts as a system-wide cellular membrane separating blood and brain interstitial space. Hydrophilic drugs that readily enter other tissues are barred from entry into the brain (Sinkula 1975).

Two major membrane systems segregating the brain extracellular space from that of the systemic extracellular space are the blood cerebrospinal fluid (CSF) barrier and the BBB which has a surface area 5000-fold greater than that of the blood-CSF barrier. This constitutes the principle diffusion barrier separating brain interstitial space from the main blood stream (Sinkula 1975).

The unique morphological characteristics of the brain capillaries making up the BBB include the epithelial-like high resistance tight junctions, which cement all the brain capillaries endothelia together and a few pinocytotic vesicles or transendothelial channels or fenestrations (Brightman 1977). Therefore, the BBB has a low filtration

coefficient and is impermeable to most polar solutes (Oldendorf 1974). However, circulating substances may gain access to the brain interstitial space, via one of four possible mechanisms:

-1) Lipid-mediation: Free diffusion of lipid-soluble substances
-2) Carrier-mediation: Transport of circulating water-soluble nutrients through the BBB, via the action of nutrient-specific carrier systems
-3) Pore-mediation: Transport of water through water-specific pores in the BBB
-4) Receptor-mediated transcytosis of circulating peptide-specific receptor systems (Sinkula 1975).

The endothelial cells of brain capillaries are surrounded by cell membranes which are lipophilic in nature. The cell membranes are made up of a bilayer of phospholipid molecules, containing long hydrophobic tails and short hydrophilic heads to their structure (Pardridge and Oldendorf 1977, Pardridge 1985a, 1985b). Thus the BBB exhibits selective permeability to circulating substances.

2.3.3 Strategies for Pharmaceutical Delivery through the BBB.

Category	Strategy
a) Invasive	Intraventricular catheter.
	Carotid injection of hypertonic media.
	Transplant peripheral nervous tissue.
b) Pharmacologic	Drug latentiation (Prodrugs).
	Liposomes.
c) Physiologic	Neutral amino acid drugs.
	Chimeric peptides.

The GABA molecule could be conjugated (covalently bound) to a lipophilic molecule, which readily passes through the BBB (Pardridge 1985a, Sinkula 1987). To penetrate CNS capillary endothelial cells, a drug in free solution (not bound to protein) in the blood plasma has to detach itself from the plasma and cytoplasmic water and then enter the lipid of the endothelial cell plasma membranes (Pardridge 1985b). The

relative affinity of a drug for water and lipid determines the degree of membrane penetration. Highly lipid-soluble drugs accumulate in the body fat maintaining a blood level of the drug for prolonged periods. An optimum lipid-water partition coefficient is required, of the derivatives, to gain immediate access to brain cells after intravenous administration. Drugs with lower lipid solubility will also penetrate the BBB but at a slower rate and so will have time to distribute to other tissue compartments, competing with the brain. Accordingly lipid solubility seems to be an important factor for neuropharmacological activity e.g. the glyceryl ester of GABA, with a low octanol:water partition coefficient, is not active (Pardridge 1985a).

An ideal prodrug consists of a bioactive parent molecule linked to a suitable transport group, that is sufficiently stable to deliver the molecule to the site of activity and is responsive to necessary enzymes, which would regenerate the parent molecule at the cellular site (Sinkula 1987, Galzigna *et al.* 1978).

Previous studies have been performed using lipophilic ester derivatives of GABA as prodrugs which include (Shashoua *et al.* 1984, Jacob *et al.* 1985, 1987, Hesse *et al.* 1988):

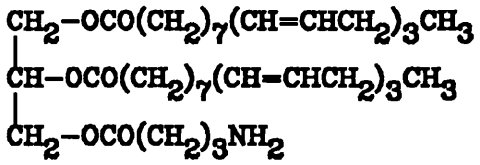
- Aliphatic and steroid esters of GABA
- Lipid esters of GABA
- C-18 glyceryl lipid esters of GABA with varying degrees of unsaturation.

Lipophilic esters such as the steroid, glyceryl lipid esters (Shashoua *et al.* 1984, Jacob *et al.* 1985, 1987) are very susceptible to esterases in the circulation (esterases are ubiquitously distributed around the body), although the high specificity of esterases for their substrate and the rapid circulation to the brain means that a high concentration can nevertheless reach the BBB before hydrolysis by esterases (Frey and Löscher 1980). These compounds e.g. glyceride lipid ester (Shashoua *et al.* 1984) once in the brain are found to have no pharmacological effect as GABA agonists by direct action on GABA-receptors. The presence of esterases, their enzymatic hydrolysis in CNS tissue and consequent release of GABA into the extracellular space agonise the GABA-receptors in the brain (Frey and Löscher 1980). This is supported

by experiments showing that the inhibitory effect of these GABA esters was significantly reduced by exposing the tissue tested to the esterase inhibitor, phenylmethylsulphonylfluoride (PMSF). This esterase inhibitor treatment had little or no effect on the response of the tissue to GABA (Galzigna *et al.* 1978).

Lipid esters of GABA have been shown to have a slow distribution and elimination which probably reflects slow absorption from the site of injection or slow secondary release of the highly insoluble molecules into the bloodstream from lipoid tissue stores (Jacob *et al.* 1985).

Due to the similarity of the lipid esters of GABA (especially dilipid ester compounds e.g. GABA ester of dilinolenoylglycerol **48** (Shashoua *et al.* 1984) to natural components of membrane lipids, it seems probable that the components become associated with brain membrane lipid bilayers, to provide a reservoir of the esters which subsequently release GABA following hydrolysis by the esterases present in the CSF and brain membranes (Jacob *et al.* 1987).



48

The pharmacological activity of the compounds is obtained by the release of GABA by esterase-mediated hydrolysis of the GABA esters in the brain. The conjugating unit could determine the rate of hydrolysis of a compound as well as affect the binding capacity of the GABA conjugates at the cell membranes close to GABA-receptor sites (Shashoua *et al.* 1984).

The effects of GABA esters were also found to be long lasting, indicating either a

delayed hydrolysis of the ester in the brain or a specific uptake of the GABA liberated into GABAergic neurones (Bowery *et al.* 1989). Toxicity studies of GABA ester derivatives, particularly the cetyl ester of GABA showed they were toxic when administered intravenously although oral administration showed they could be well tolerated (Frey and Löscher 1980). Deverre *et al.* (1989) reported *in vitro* filaricidal activity of a diglyceride prodrug of GABA.

Galzigna *et al.* (1978) have reported two acyl derivatives of GABA i.e. benzoyl GABA **49** and pivaloyl GABA **50** with increased lipophilic character. Increased amounts of the GABA derivatives **49** and **50** were obtained in rat brain after subcutaneous injection. These compounds **49** and **50** were found to be susceptible to Nagarse and α-chymotrypsin (proteolytic enzymes) to yield GABA. The derivatives contain amide linkages where the lipophilic moiety is bound to the γ-amino function of GABA.

C_6H_5-CONH-$(CH_2)_3$-COOH
49

$(CH_3)_3C$-CONH-$(CH_2)_3$-COOH
50

2.3.4 Synthesis of GABA Conjugates

GABA, itself crosses the blood brain barrier (BBB) very poorly, thus GABA agonists or prodrugs that might increase the uptake into the CNS are of considerable interest. Previous studies have demonstrated that lipophilic esters (Shashoua *et al.* 1984, Jacob *et al.* 1985, 1987, Hesse *et al.* 1988) and Schiff's base derivatives (Kaplan *et al.* 1980) enhanced the GABA uptake in the brain.

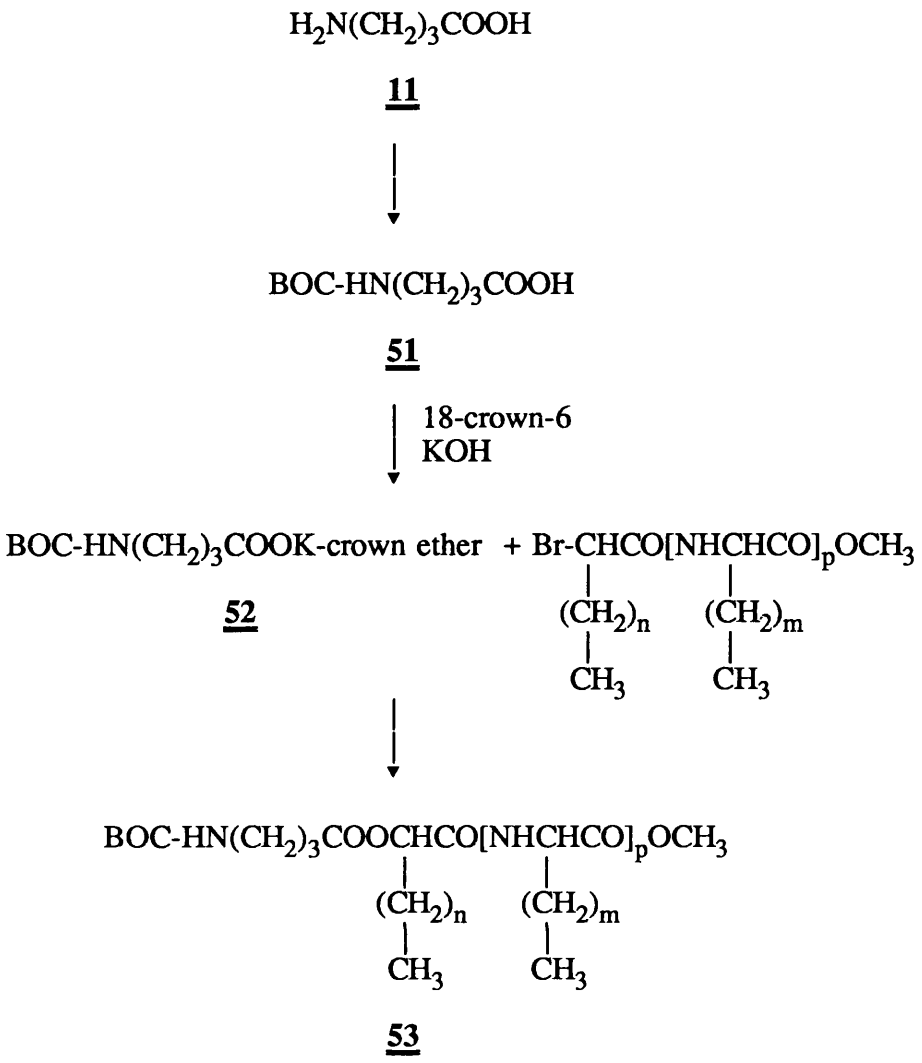
The lipidic amino acids **25** and peptides **41** have the capacity to be chemically conjugated to both functional groups of γ-aminobutyric acid (**11**). The linkage between the GABA and the lipidic units may either be biologically stable or possess biological or chemical instability. The resulting conjugate possesses a high degree of membrane-like character which should facilitate their passage across the cell

membranes e.g. blood-brain barrier (BBB). This novel lipophilic delivery system was therefore utilised in the synthesis of a series of GABA ester and amide conjugates to increase the lipophilic character of GABA.

Three types of conjugates were synthesised. GABA conjugates of ester linkage (**54a** - **i**) were prepared by coupling the lipidic acids and peptide conjugates to the carboxyl terminus of GABA. Compounds with an amide bond (**57f** - **h** and **58a** - **b**) containing the lipidic delivery system conjugated to the C- or N-terminus of GABA respectively were also synthesised. The oligopeptide contained from 1 - 3 lipidic amino acids and the alkyl side-chain ranged from 5 - 17 carbon atoms in length in an attempt to impart differing lipophilicities to the GABA conjugates.

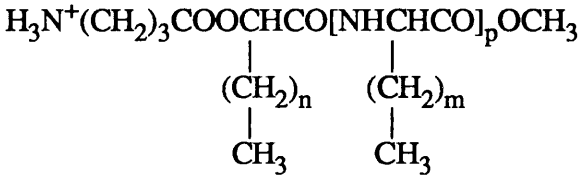
2.3.4.1 GABA Conjugates with Ester Linkages

In order to synthesise the GABA conjugates with an ester linkage, GABA must first be protected appropriately to avoid autopolymerisation. GABA **11** was protected at the amino terminus with a BOC group using the procedure described for amino function protection (**Scheme 2**, p 38). The BOC-GABA **51** was then treated with potassium hydroxide in ethanol-water (1:1) and the macrocyclic ether 18-Crown-6 to form the crown ether complex of the potassium salt of BOC-GABA **52**. This reaction was initiated to activate the carboxyl terminus of GABA which could then be used for further coupling.



53	n	m	p
a	5	-	0
b	11	-	0
c	13	-	0
d	11	9	1
e	11	11	1
f	11	17	1
g	11	11	2
h	13	11	1
i	13	11	2

The potassium salt of BOC-GABA crown ether **52** was reacted with fatty acid methyl esters **45d** - **f** in anhydrous dimethylformamide forming the fully protected monomeric conjugates **53a** - **c** respectively. The dimeric conjugates **53d** - **f** were synthesised from **46a** - **c** respectively with **52** whilst **53h** was prepared from **46e** and **52**. The fully protected trimeric conjugates of GABA, **53g** and **53i**, were synthesised in a similar manner by utilising the conjugating units **46d** and **46f** respectively with **52**. All conjugates were purified by flash column chromatography or preparative thin layer chromatography on silica gel and were obtained in good yield. The conjugates **53a** - **i** were then N-deprotected yielding compounds **54a** - **i**.

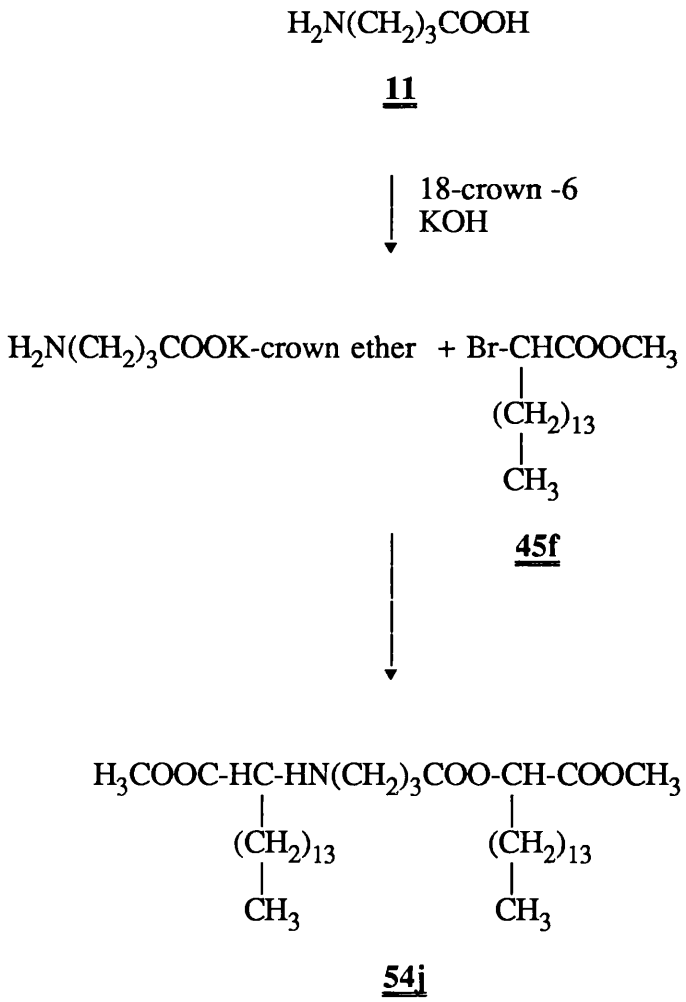


54

54	n	m	p
a	5	-	0
b	11	-	0
c	13	-	0
d	11	9	1
e	11	11	1
f	11	17	1
g	11	11	2
h	13	11	1
i	13	11	2

Treating the conjugates with methanolic HCl would cause the hydrolysis of the GABA-conjugate ester bond, thus the BOC protecting group was removed by treatment with trifluoroacetic acid (TFA) to give partially deprotected compounds **54a - i** as trifluoroacetate salts.

It was found that when GABA was not protected on the N-terminus, conjugation on the C- and N-termini was initiated. When GABA was activated on the carboxyl terminus by the formation of the crown ether potassium salt complex and coupled to the compound **45f**, the GABA analogue **54j** was obtained.



2.3.4.2 Synthesis of Aliphatic Alkyl Esters of GABA

Many reported works on GABA prodrugs are centred on using lipophilic conjugating units which give improved brain uptake of GABA. To evaluate the extend of the biological activities of these novel lipidic peptide conjugates **54**, the synthesis of aliphatic alkyl esters of GABA **56** was carried out. The GABA methyl ester (**56a**) was synthesised in good yield by reacting GABA with thionyl chloride in methanol. The pentyl ester **56b** and the octyl ester **56c** were prepared using the crown ether method. The crown ether potassium salt of BOC-GABA, **52**, was reacted with 1-bromo-pentane (**55a**) to yield compound **56b**. Similarly, treating **52** with 1-bromo-octane (**55b**) gave **56c**. The BOC protecting group was cleaved with TFA, yielding compounds **56d** and **56e**.

$Br\text{-}(CH_2)_nCH_3$ **55**

55a R=4

55b R=7

$XHN(CH_2)_3COOR$ **56**

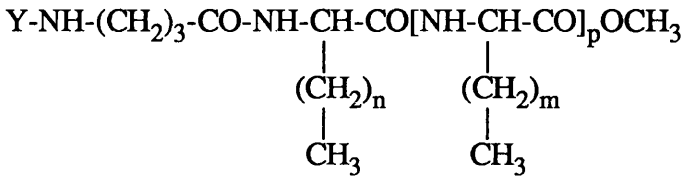
56	X	R
a	H	CH_3
b	BOC	$(CH_2)_4CH_3$
c	BOC	$(CH_2)_7CH_3$
d	H	$(CH_2)_4CH_3$
e	H	$(CH_2)_7CH_3$

2.3.4.3 Synthesis of GABA Conjugates with Amide Linkages

Another approach to the preparation of lipidic GABA conjugates involved amide linkages between the GABA and the lipophilic delivery system. Two types of GABA amide conjugates were prepared, namely those with a conjugation at the carboxyl terminus of GABA and those with a conjugation at the amino terminus of GABA.

2.3.4.4 Conjugation on Carboxyl Terminus of GABA

The BOC protected GABA (**51**) was reacted with a series of C-protected α-amino acids **25i**, **25k** and **25l** and C-protected lipidic peptides **41f** and **41e** respectively, using the DCC synthetic strategy mentioned before to yield compounds **57a** - **e** respectively. Compounds **57a** - **e** were purified by flash chromatography or preparative TLC. The BOC protected conjugates **57a** - **c** were then treated with TFA giving rise to the partially deprotected compounds **57f** - **h** respectively.



57

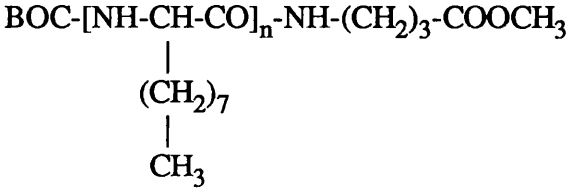
57	n	m	p	Y
a	7	-	0	BOC
b	11	-	0	BOC
c	17	-	0	BOC
d	11	11	1	BOC
e	11	9	1	BOC
f	7	-	0	H
g	11	-	0	H
h	17	-	0	H

2.3.4.5 Conjugation with Amino Terminus of GABA

For the synthesis of compounds **58a** and **58b**, the C-protected GABA methyl ester **56a** was reacted with N-protected α-amino acid **25f** and dimer **41h** to form the compounds **58a** and **58b** respectively, which were then purified by preparative TLC.

$H_2NCH_2CH_2CH_2COOCH_3$

56a

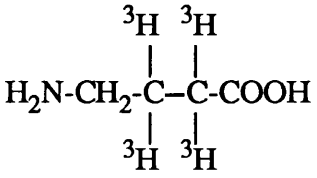


58

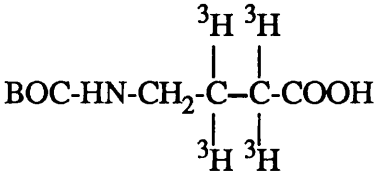
58	**n**
a	1
b	2

2.3.4.6 Tritiated GABA Conjugates

It was intended that the conjugates will have a higher brain uptake capacity than GABA itself. To assess this property by biological means, radiolabelled conjugates of GABA were synthesised. Using the same procedure as the synthesis of unlabelled GABA conjugates, tritiated GABA conjugate **63** was prepared containing tritium on the α and β carbons of the GABA molecule. The tritiated BOC-GABA was synthesised by combining a microquantity of unlabelled GABA and tritiated GABA **59** and reacting the radiolabelled mixture with di*tert.*butyldicarbonate in the same manner as introducing a BOC group previously described. The tritiated BOC-GABA **60** was then converted to the radioactive potassium salt of tritiated BOC-GABA crown ether **61** which was then reacted with the unlabelled C-protected fatty acid methyl ester **45e** in anhydrous dimethylformamide (Scheme 7). The radiolabelled conjugate **62** was then purified by preparative thin layer chromatography on silica gel.

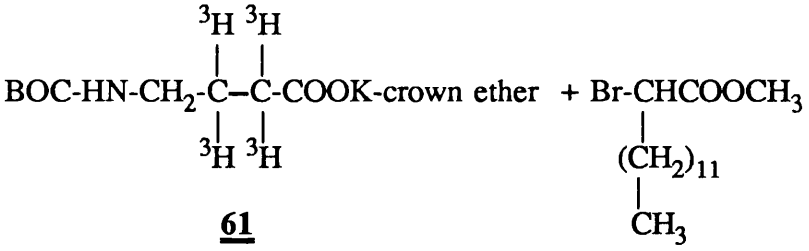


59

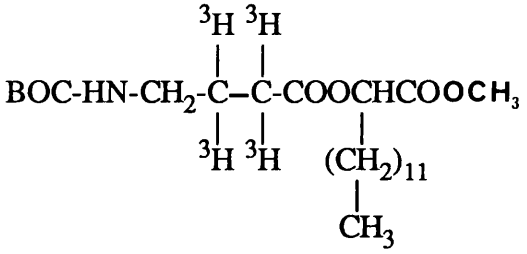


60

18-crown-6
KOH



61

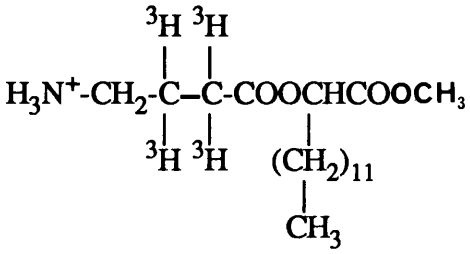


62

Scheme 7

Preparation of fully protected tritiated GABA conjugate 62

The BOC-group was cleaved using TFA yielding the partially deprotected tritiated GABA conjugate **63**.

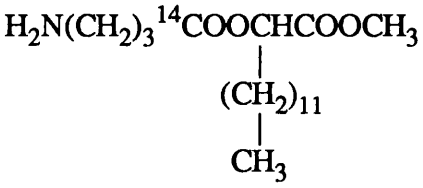


63

2.3.4.7 [^{14}C]-labelled GABA Conjugate

It was reported that tritium in tritiated GABA exchanges with the proton in water molecule forming tritiated water (Shashoua *et al.* 1984). Using tritiated GABA conjugates for *in vivo* studies led to misleading pharmacological results as will be discussed in Chapter 3. Due to this tritium exchange problem, synthesis of ^{14}C-labelled GABA conjugate was carried out to assess the brain uptake ability *in vivo* (Chapter 3).

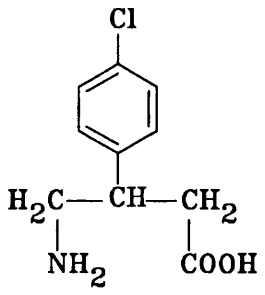
Radiolabelled GABA conjugate **64** was synthesised using radiolabelled GABA with ^{14}C on the carboxy carbon of GABA molecule. The synthetic procedure was similar to the preparation of the tritiated GABA conjugate above.



64

2.4 Baclofen Conjugates.

Though baclofen (22) enhances the GABAergic effect in the CNS, the amount taken up into the central nervous tissues is still very small. Baclofen is active at the $GABA_B$ receptor sites. However, the (-) isomer is a 100-fold more active than the (+) isomer (Bowery *et al.* 1980). For preliminary chemical and biological studies, racemic mixture of baclofen was used in the preparation of the conjugates. Lipophilicity can be modified by conjugation to a lipidic peptide delivery system to enhance the brain uptake of baclofen. Lipidic analogues of baclofen 67, 68 were accordingly synthesised.

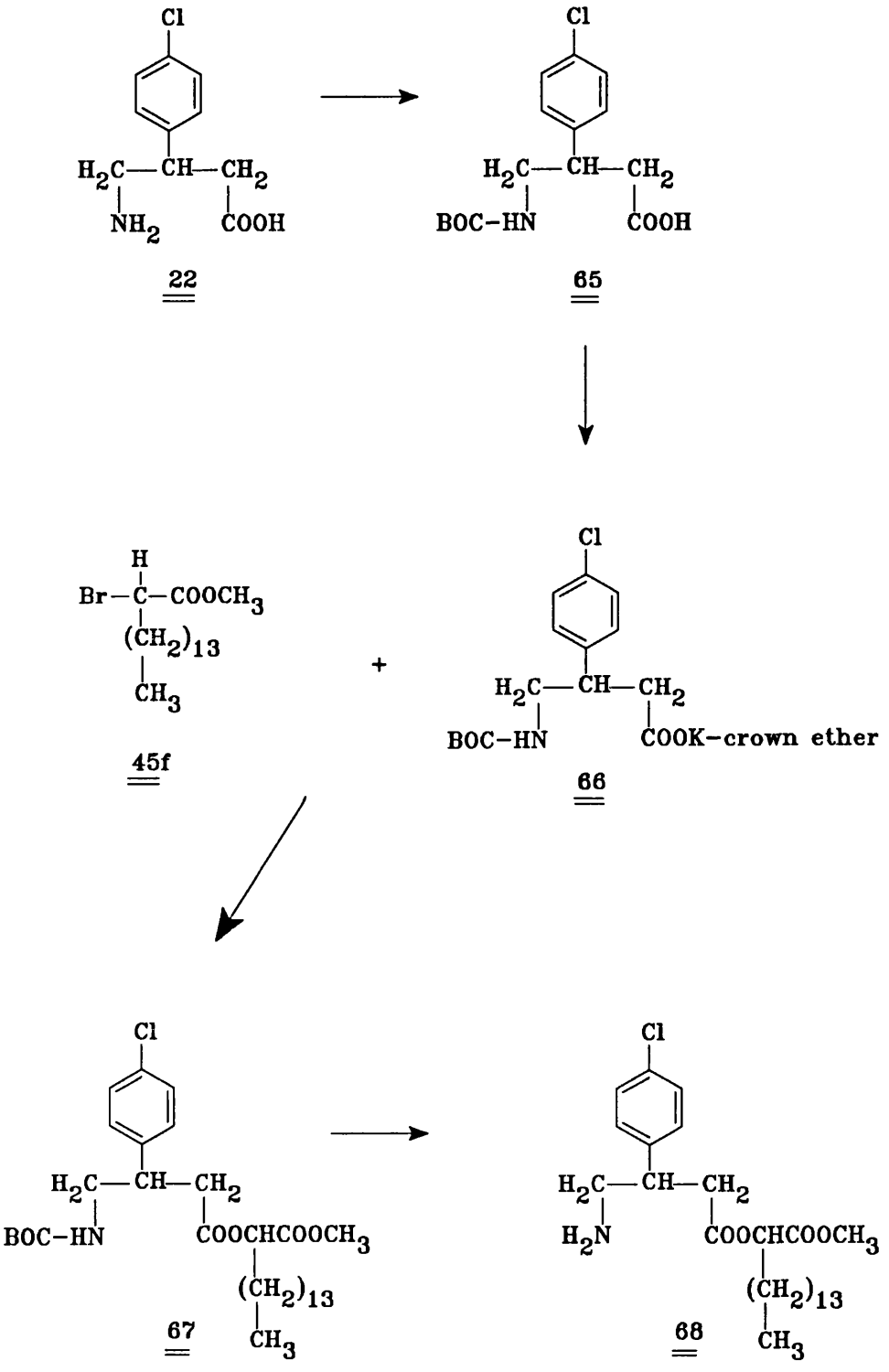


22

2.4.1 Lipidic conjugates of baclofen with ester linkage

The synthesis of baclofen conjugates utilised analogous synthetic procedures to those described earlier for the preparation of GABA conjugates.

Baclofen 22 was first protected at the N-terminus with the BOC protecting group to form the BOC-baclofen 65. Compound 65 was then treated with 18-crown-6 and potassium hydroxide solution giving rise to the activated species BOC-baclofen crown ether complex 66. The activated carboxyl group of 66 was further reacted with lipidic bromo-ester 45f in anhydrous dimethylformamide yielding the fully protected baclofen conjugate 67. Compound 67 was then partially deprotected on the N-terminus using trifluoroacetic acid to obtain the trifluoroacetate salt of conjugate 68 (**Scheme 8**).



Scheme 8

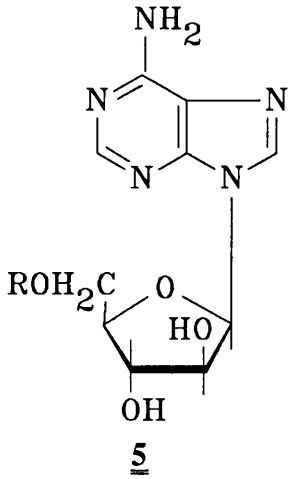
Preparation of lipophilic baclofen conjugates

2.5 Design of Nucleoside Analogues as Potential Antiviral Agents.

Viruses are composed of either RNA or DNA, encased in protein shells and often further mapped in polysaccharide or lipid-containing envelopes. Viruses multiply only within living cells, adapting the host cell to synthesize viral proteins and viral nucleic acids followed by final assembly into new virion particles. One of the successful treatment of viral diseases with antiviral agents requires the interruption of viral enzymes.

Nucleosides and nucleotides constitute the major class of compounds which exhibit significant *in vitro* and *in vivo* antiviral activity. Antiviral activities involve sufficient cellular uptake, phosphorylation in the cell and incorporation into viral DNA or RNA. Nucleotides are responsible for the antiviral activities. Direct administration of the active form, the nucleotides, would however be ineffective because of they do not penetrate the cell membranes (Leibman and Heidelberger 1955). On the other hand, nucleosides have been studied as potent antiviral agents because they do rapidly cross the cell plasma membranes by facilitated transport mechanisms (Hochsladt 1974, Berlin and Oliver 1975). An example of a nucleoside used as an antiviral is vidarabine **5**.

(9-β-D-Arabinofuranosyladenine Ara-A, vidarabine **5**)



5

Ara-A (R=H)

Ara-A was discovered as a nucleoside antiviral by Miller and co-workers (1968). However, Ara-A is rapidly deaminated by adenosine deaminase *in vivo* to give 9-β-D-arabinofuranosyl hypoxanthine (Ara-Hx), which is 10 times less active (North and Cohen 1979). Ara-A is phosphorylated in mammalian cells to Ara-AMP by adenosine kinase and deoxycytidine kinase, further phosphorylation to Ara-ADP and Ara-ATP also occurs. It was shown that herpes DNA synthesis is considerably more sensitive to Ara-A than the cellular DNA synthesis (Shipman *et al.* 1976).

2.5.1 Nucleosides Active Against Retroviruses and Persistent Viral Infections.

Retroviruses are unique RNA viruses characterised by the transcription of their single-stranded RNA into double-stranded DNA of the host cells by the viral enzyme, reverse transcriptase. One of the viral infections due to the cellular invasion of retroviruses is the acquired immunodeficiency syndrome (AIDS).

AIDS is a degenerative disease of the immune and central nervous systems for which no known cure exists. AIDS is an example of a slow or persistent viral infection with a long incubation period. The causative agent is the human immuno-deficiency virus (HIV) (Mitsuya and Broder 1987). Patients suffering from AIDS have cellular immuno-deficiency characterised by the depletion of helper/inducer ($T4^+$ or $CD4^+$) T cells (Gallo *et al.* 1984, Popovic *et al.* 1984). In addition to the effect on the immune system, such infections may be responsible for multiple sclerosis, subacute sclerosis, panencephalitis, Alzheimer's disease and many other dementia of unknown etiology (Haase 1986).

Infected monocytes/macrophages have been shown to play an important role in the pathogenesis of AIDS, in particular, in the traffic of the HIV across the blood-brain barrier and as a reservoir of HIV-I *in vivo* (Gartner *et al.* 1986, Koenig *et al.* 1986, Ho *et al.* 1986). Inadequate inhibition of virus production by macrophages may be the reason for failure to clear infectious virus.

Progress has been made in the search for effective chemotherapeutic means against AIDS to prevent initiation or suppress progression of the disease in patients. Based on the virus replicative cycle (Fig. 8), different targets which serve as sites of chemotherapeutic attack include (DeClercq 1990):-

a) adsorption of the virus particle to the cell membrane
b) uncoating of the viral capsid
c) inhibition of the reverse transcriptase
d) integration of the proviral DNA into the cellular genome
e) processing of the viral precursor proteins
d) assembly and release ("budding") of the viral particles.

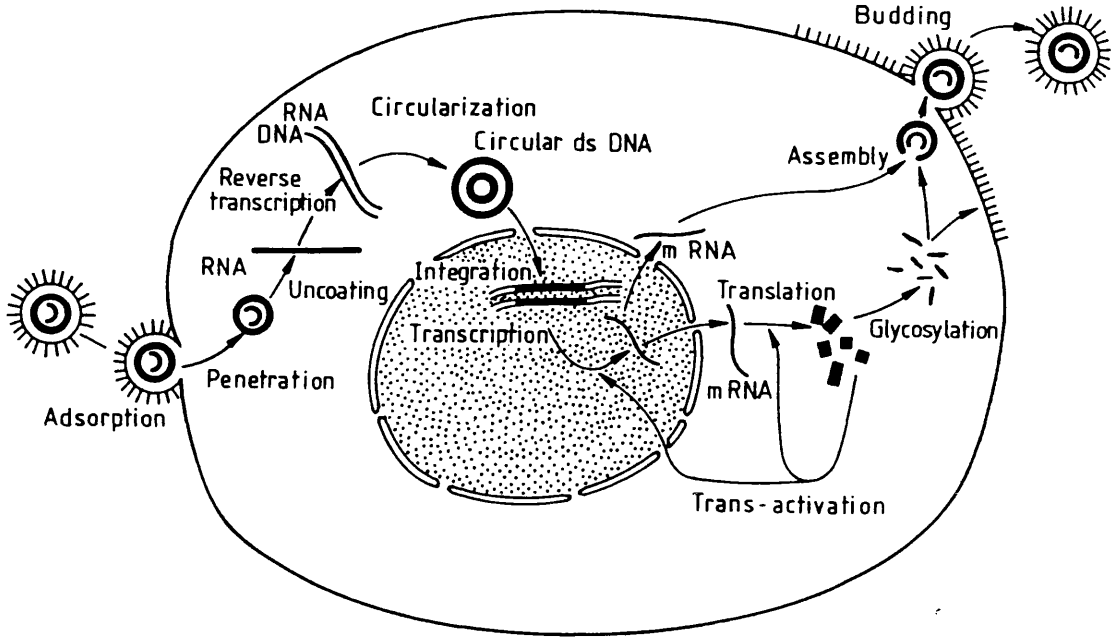


Figure 8

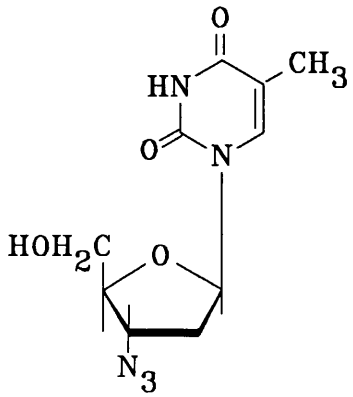
HIV Replicative Cycle

This search for effective inhibitors of HIV replication has led to the discovery of a variety of 2'-3'-dideoxynucleosides. Dideoxynucleosides analogues could suppress the replication of HIV in monocytes/macrophages *in vitro* (Perno *et al.* 1988). The

2'-3'-dideoxynucleoside analogues are successively phosphorylated in the cytoplasm of a target cell to yield ultimately 2'-3'-dideoxynucleoside-5'-triphosphate, although each drug may require a separate metabolic pathway (Mitsuya and Broder 1986, 1987, Yarchoan and Broder 1987, Yarchoan *et al.* 1989, Mitsuya *et al.* 1987b, Cooney *et al.* 1986, Furman *et al.* 1986, Ahluwalia *et al.* 1987, Johnson *et al.* 1988, Johnson and Fridland, 1989). In common, these compounds become analogues of the 2'-deoxynucleoside-5'-triphosphates that are the natural substrates for cellular DNA polymerases and the viral reverse transcriptase.

The dideoxynucleoside-5'-triphosphates can compete with normal nucleotides for binding to reverse transcriptase to become incorporated into the growing DNA chain. Termination of the viral DNA chain inevitably occurs as a normal 5'-3'phosphodiester linkage cannot be completed (Mitsuya and Broder 1986, 1987, Mitsuya *et al.* 1987a, 1987b).

The most studied nucleoside of the dideoxythymidine type is 3'-azido-3'-deoxythymidine (AZT) **24** which is until recently, the only clinically approved drug for treating AIDS.



24

AZT

AZT was first prepared by Horwitz *et al.* (1964) with *in vitro* activity against HIV being described by Mitsuya *et al.* (1985). It was also reported that AZT is non-selectively phosphorylated to AZT 5'-phosphate which selectively binds to HIV reverse transcriptase (Furman *et al.* 1986).

AZT prolongs life and delays the progression of the disease in patients with advanced human immuno-deficiency virus (HIV) infection. Although AZT is a powerful inhibitor of the reverse transcriptase (RT) enzyme (Furman *et al.* 1986) and suppresses the replication of HIV in monocytes/macrophages *in vitro* (Perno *et al.* 1988), it does not however eradicate the HIV from peripheral blood mononuclear cells *in vivo* (Merigan *et al.* 1989).

AZT is an excellent substrate for thymidine kinase and found to be very potent against divergent strains of HIV *in vitro* (Mitsuya *et al.* 1985). It undergoes anabolic phosphorylation to generate AZT-5'-triphosphate which competes with thymidine-5'-triphosphate (Furman *et al.* 1986) and also functions as a DNA-chain terminator.

It should be noted that antiretroviral activity of a nucleoside depends upon multiple factors including penetration into target cells, multistep anabolic phosphorylation, intracellular catabolism and relative affinities for reverse transcriptase and cellular DNA polymerases.

2.5.2 Efficacy, Resistance and Toxicity in Nucleoside Antiviral Therapy.

The lack of practical efficacy of a compound exhibiting a high degree of antiviral activity in cell culture may be due to:

- rapid loss of potency against a high multiplicity of infection;
- rapid metabolism of the drug to an inactive metabolite;
- failure of the drug to penetrate the cells.
- failure to be activated enzymatically within the particular infected cells (organ) to the 5'-triphosphate required for viral inhibition as may be the case for nucleosides.

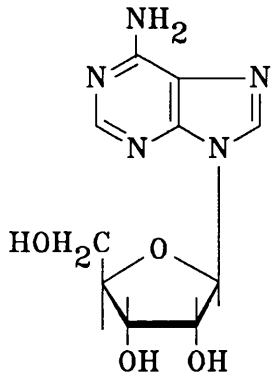
Resistance to nucleosides and nucleoside analogues can develop for many reasons. Acyclovir resistance has been reported to be mainly due to the loss of viral thymidine kinase activity (Crumpacker *et al.* 1982, McLaren *et al.* 1986).

Studies done indicated that 3-deazaguanine and 3-deazaguanosine are metabolised to the 5'-nucleotides (Saunders *et al.* 1986, Page *et al.* 1986) and are incorporated into DNA (Pieper *et al.* 1986) which is believed to be responsible for the toxicity noted. Great care must be taken to ensure the absence of incorporation into cellular DNA since even a small amount of nucleoside analogue would be cumulatively toxic especially if the toxicity is not reversed rapidly after removal (Prusoff *et al.* 1984, Kufe *et al.* 1984, Bubley *et al.* 1986).

The conjugation of nucleosides to form prodrugs or agonists to increase the nucleoside antiviral efficacy, reducing the development of viral resistance and the toxicity due to chemical modification of the nucleosides are of great interest and importance.

2.5.3 Adenosine Lipidic Conjugates

Adenosine **23** though not an antiviral, is a good model for following the chemical pathway in the production of lipidic conjugates of antiviral nucleosides. It has a similar chemical structure to most purine antivirals and its low cost substantiate its use to monitor the synthetic routes.



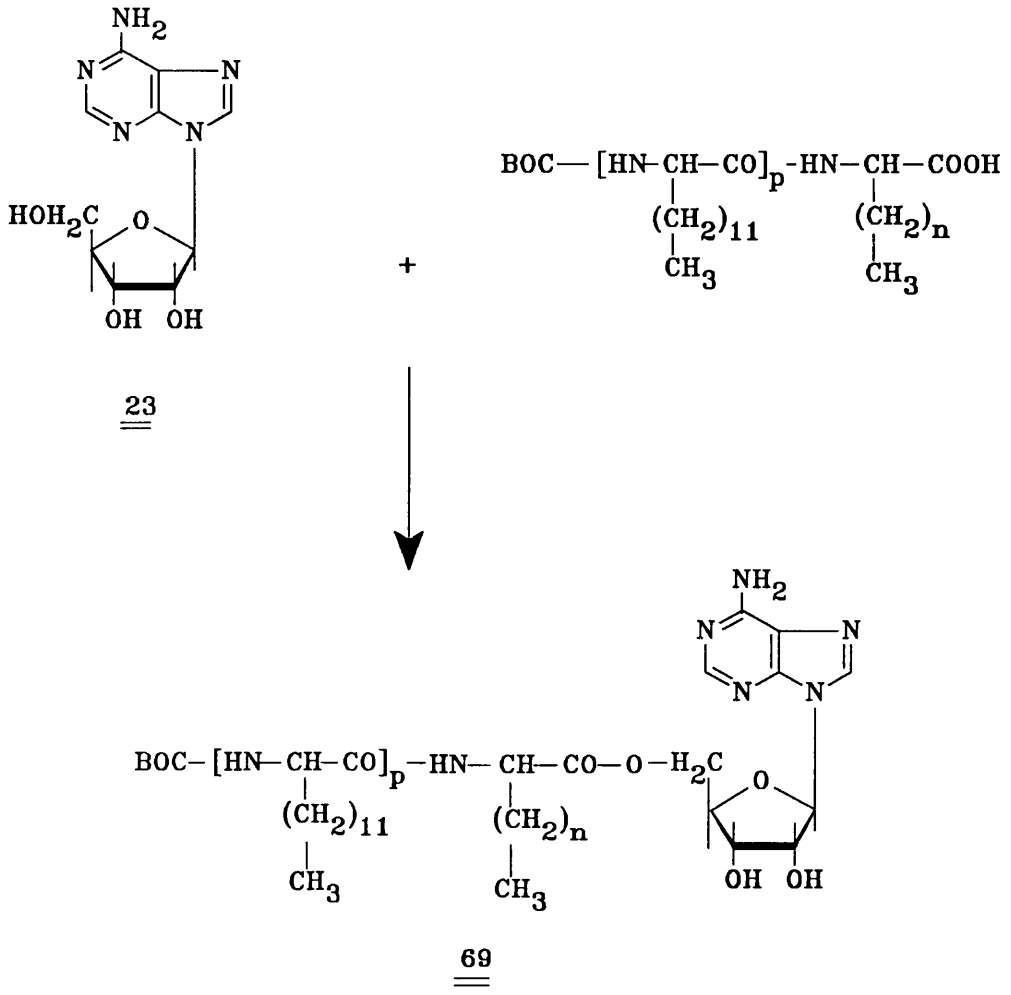
23

The primary alcoholic functions of adenosine can be utilised for chemical modifications which change the physico-chemical as well the pharmacological properties. This functional group could then be used with the lipidic delivery system forming lipophilic adenosine conjugates to produce products with increased stability chemically and enzymatically.

2.5.3.1 Synthesis of Lipidic Conjugates of Adenosine with Ester Linkages

Adenosine was reacted with the N-protected amino acid **25h** and N-protected oligomers **41j** and **41o** respectively yielding compounds **69a** - **c** respectively (**Scheme 9**). DCC and a catalyst, dimethylaminopyridine (DMAP) **70** were used in the condensation of the alcoholic function and the carboxyl group.

69	n	p
a	11	0
b	11	1
c	11	2



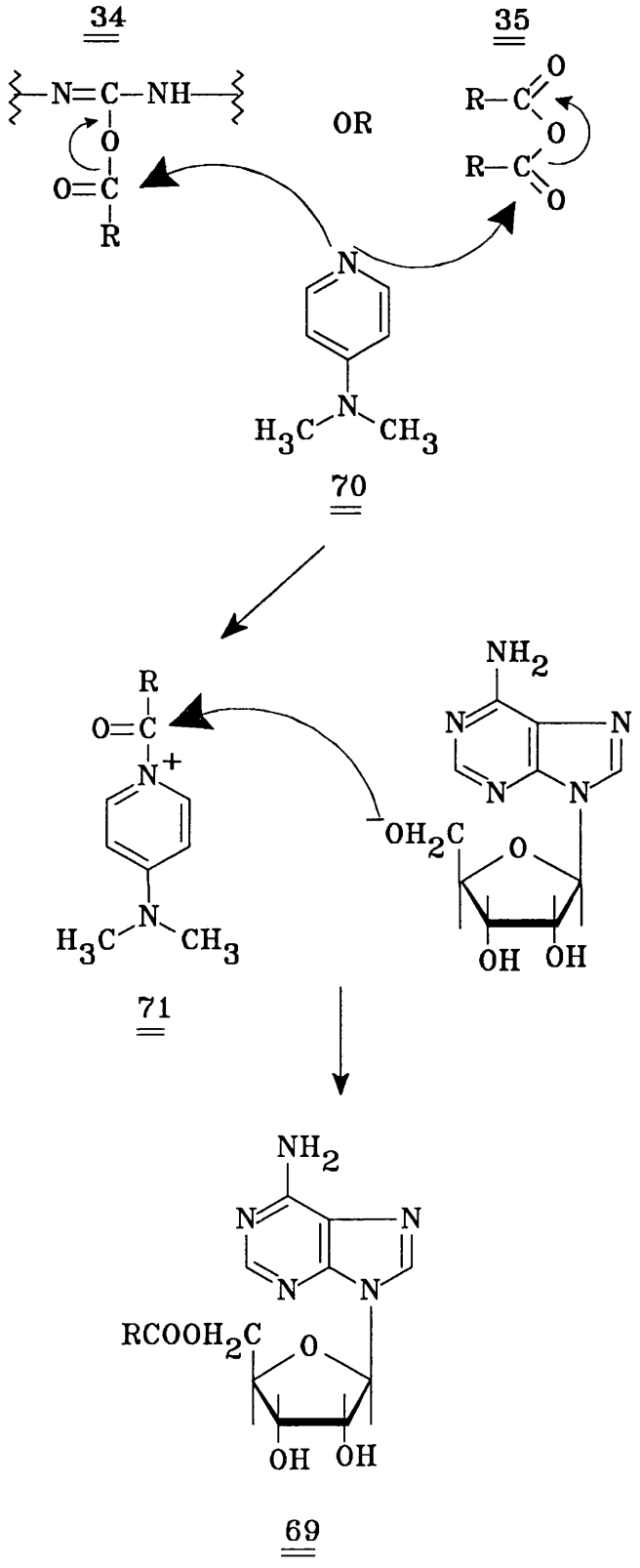
23

69

Scheme 9

Synthesis of Adenosine conjugates

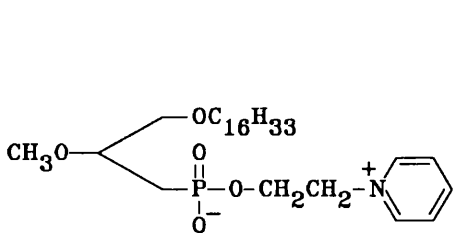
Esterification between the alcohol and the carboxylic acid group of the amino acid using the DCC method effected a very low yield. The yield of the reaction can be increased by the addition of bases preferably dimethylaminopyridine (DMAP) **70** (Neises and Steglich 1978, Hassner and Alexanian 1978, Ziegler and Berger 1979). DMAP has a high nucleophilic character and so reacts very readily with electrophilic reagents, in this case, the O-acyl isourea **34** or the symmetrical anhydride **35** forming the unstable acylated pyridinium ion **71**. Due to the charge of the acylated pyridinium salt, it is capable of transferring an acyl residue to a nucleophile (**Scheme 10**).



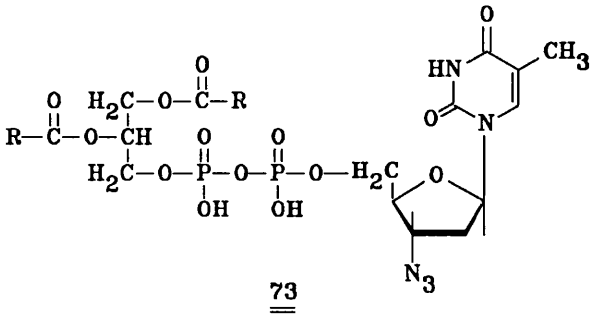
Scheme 10
Mechanism of reaction using DMAP

2.5.4 Synthesis of Azidothymidine Conjugates

Large amounts of parenterally administered liposomal AZT are taken up by macrophages (Hostetler *et al.* 1990). To direct larger amounts of the antiretroviral nucleosides to these cells, a major reservoir of HIV, AZT agonists (Piantadosi *et al.* 1991, Meyer *et al.* 1991) or prodrugs (Hostetler *et al.* 1990, Little *et al.* 1990) that show increased cellular uptake are of great interest as potential anti-aids drugs. An example of AZT agonist is the quatenary ammonium containing lipid **72** (Meyer *et al.* 1991) whilst the phospholipid analogues **73** would be typical AZT prodrugs (Hostetler *et al.* 1990)



72



73

Following the successful synthesis of the adenosine conjugates **69a** - **c**, lipidic AZT analogues were prepared using similar synthetic procedures.

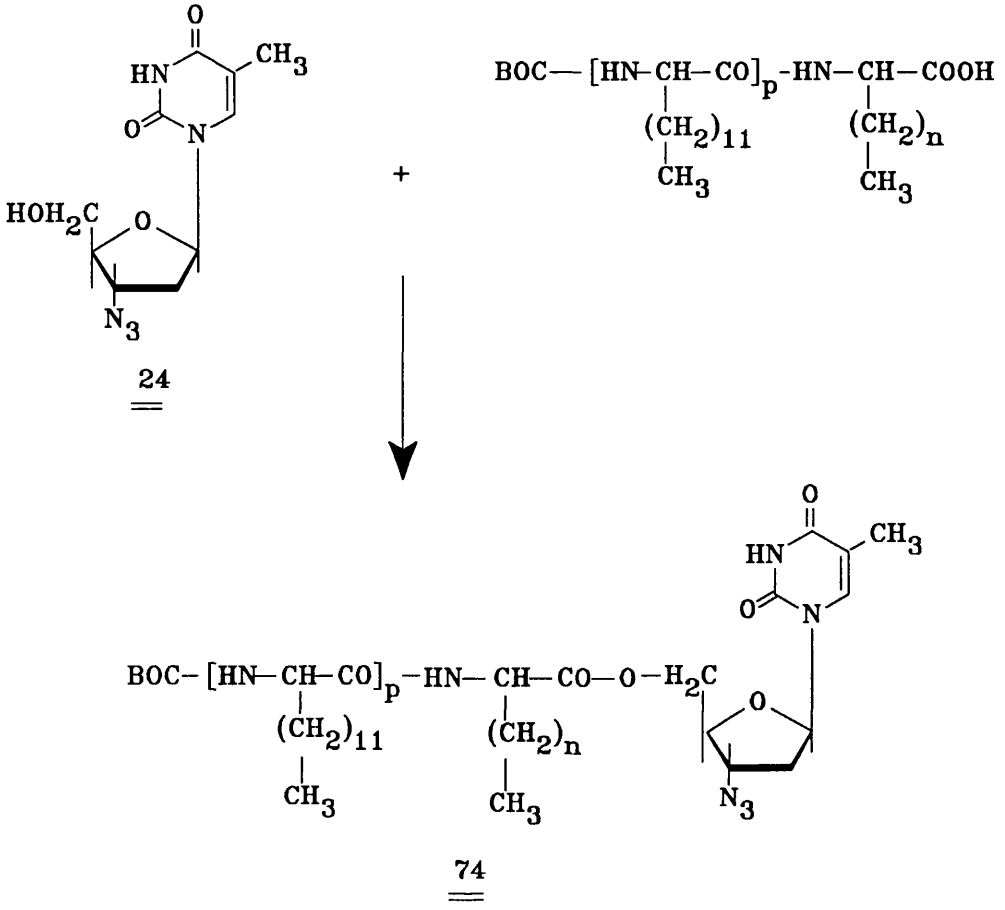
The 5'-OH of AZT can be utilised for conjugation with fatty amino acid (FAA), oligomers. It can also be modified to form lipophilic AZT with lipidic mercaptan forming lipophilic sulphide conjugate of AZT. The resulting conjugate possesses a high degree of membrane-like character which should facilitate their passage across the cell membranes e.g. peripheral blood mononuclear cells. This novel lipophilic delivery system and lipidic mercaptan were utilised in the synthesis of AZT conjugates in an attempt to increase the lipophilic character and cellular uptake of AZT.

Two types of conjugates were synthesised. AZT conjugates with ester linkages (**74a - e**) were prepared by coupling with the carboxyl terminus of the lipidic amino acids and peptide conjugating units at the 5'-hydroxyl group of AZT. Compound (**76**) containing a sulphide instead of the hydroxy function of the AZT was also synthesised. The oligopeptide contained from 1 - 3 lipidic amino acids with alkyl side chain kept to 12 carbon atoms while the mercaptan alkyl side chain is of 18 carbon atoms in length to impart differing lipophilicities to the AZT conjugates.

2.5.4.1 Lipidic Conjugates of AZT with Ester Linkages

Lipidic conjugation via an ester bond on the 5'-hydroxyl functional group of AZT was achieved by reacting AZT with the C-terminus of the N-BOC protected α-amino acid **25**. The AZT conjugate **74a** was synthesised by condensing the partially deprotected amino acid **25h** with AZT **24** with the aid of either dicyclohexylcarbodiimide (DCC) or water soluble 3-[3-(dimethylamino)propyl]-1-ethylcarbodiimide (EDAC) as a coupling agent and 4-dimethylaminopyridine (DMAP) as a catalyst in dichloromethane:benzene (1:1). Similarly the dimeric peptide conjugates of AZT **74b - d** were prepared by the coupling of **24** with N-protected dimers **41i**, **41j** and **41k** respectively. Using the same synthetic strategy as above, the peptidic trimer conjugate **74e** was formed by condensing the AZT with protected trimer **41o** (**Scheme 11**).

The synthesis of the AZT conjugates **74a - e** gave higher yield than the formation of adenosine conjugates **69a - c**. The yield was increased with the use of water soluble carbodiimide **42a** compared to DCC **32**. Using the water soluble carbodiimide also increased the efficiency of the purification process of the compounds since **42a** and its urea by-product **42b** could be eliminated from the reaction mixture by washing with water.



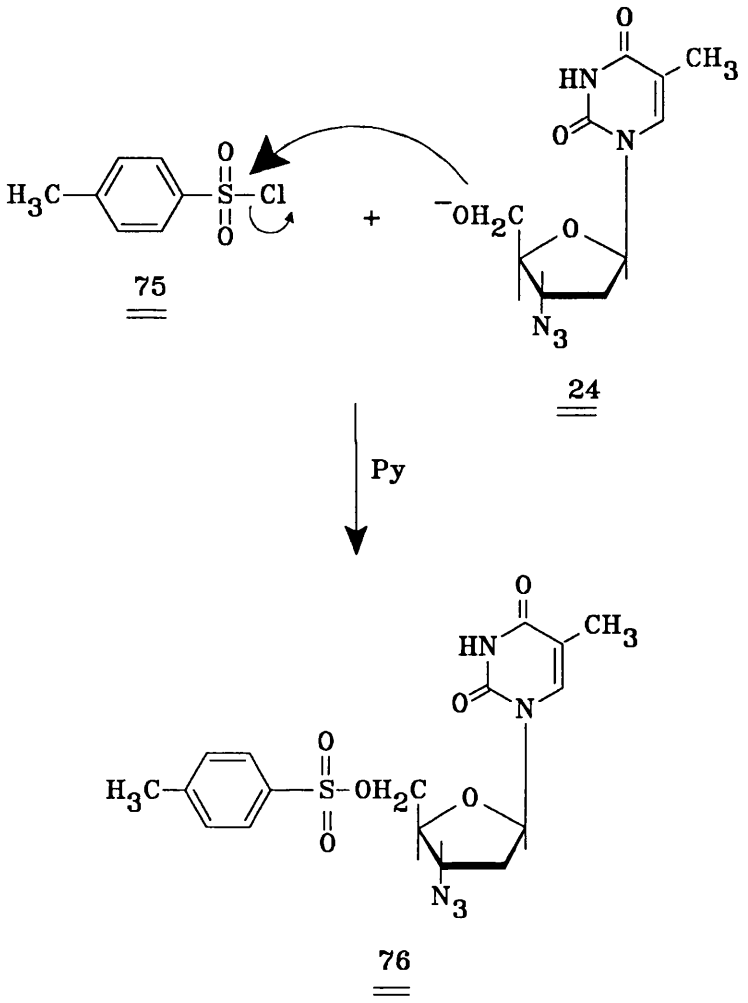
Scheme 11

Preparation of AZT ester conjugates

74	n	p
a	11	0
b	9	1
c	11	1
d	17	1
e	11	2

2.5.4.2 Sulphide Linkage Conjugation of AZT

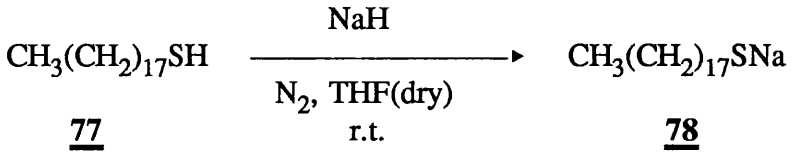
Another approach to the preparation of a lipidic AZT conjugate involves sulphide linkage between the 5'-dehydroxy-AZT and lipidic mercaptan. The hydroxyl function of AZT was first activated by the formation of a tosylate **76** which is a good leaving group for reaction with mercaptan. The 5'-AZT tosylate **76** was synthesised by reacting AZT **24** with tosyl chloride **75** in dry pyridine at room temperature (**Scheme 12**).



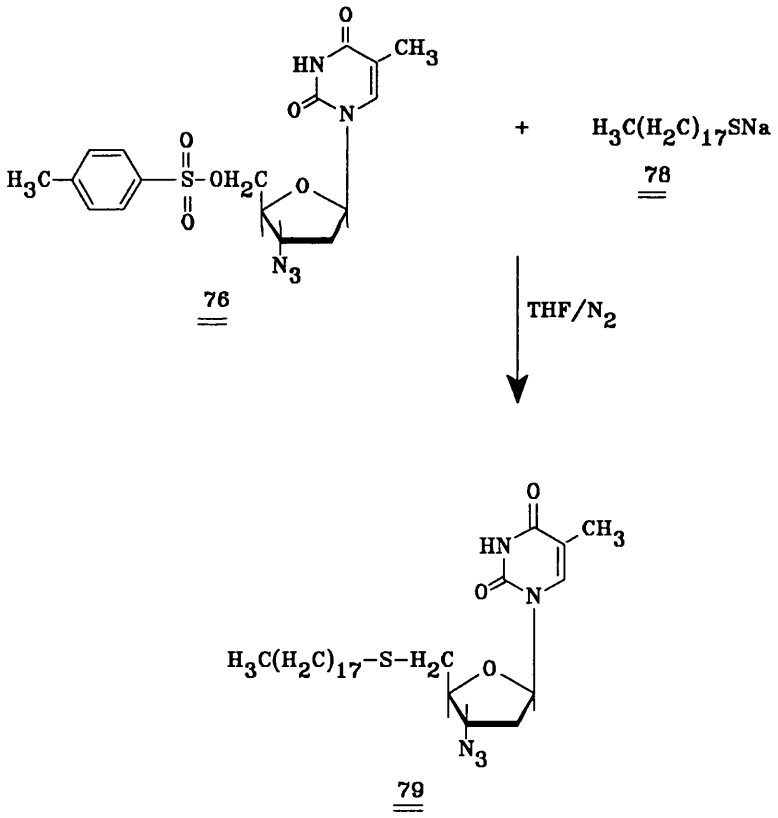
Scheme 12

Synthesis of AZT-tosylate

Williamson synthesis was used to prepare the sulphide conjugate of AZT. Compound **78**, a sodium salt was made by direct action of sodium hydride on the mercaptan **77**.



The tosylated AZT **76** was then reacted with the sodium salt of the mercaptan by nucleophilic substitution forming the required sulphide conjugate of AZT **79** with a yield of 30% (**Scheme 13**).



Scheme 13

Preparation of sulphide conjugate of AZT

CHAPTER 3

PHARMACOLOGY (APPENDIX)

The biological experiments were performed in the Department of Pharmacology, The School of Pharmacy, by Prof. Norman G. Bowery.

3.1 Biological Assessment of GABA Conjugates

GABA had been reported to cross the blood brain barrier very poorly (Krogsgaard-Larsen, 1980). GABA analogues with increased brain uptake ability are thus of considerable interest. Since GABA is a naturally occurring neurotransmitter, in order to carry out *in vivo* examinations, radiolabelled GABA conjugates were synthesised (Section 2.3.4.6 and 2.3.4.7, p 72 - 74). The results of these *in vivo* studies are discussed below. The effect of the analogues on the GABA receptor binding sites could also be investigated using the unlabelled compounds. The *in vitro* assays were hence performed on these GABA analogues.

3.1.1 Brain Uptake Studies of GABA Conjugates

To assess the brain penetration capacity of the GABA conjugates, mice were injected intravenously (IV) with the radiolabelled compounds and the amount present in the brain was measured from the amount of radioactivity present.

Initially a method was used to compare the uptake of ^{3}H-labelled GABA-C_{14}OMe ester **63** with [^{3}H]-GABA **59** in the brain of the mouse after a systemic injection. The radiolabelled compounds **59** and **63** were dissolved in 4 % BSA (bovine serum albumin) and injected intravenously. The animals were sacrificed at different times to assess the uptake. The time course of the uptake of tritiated GABA **59** and **63** could then be followed. The brain uptake capacity was expressed as the "brain penetration index" (BPI) which is the percentage equivalent to the amount of administered tritium present per weight in the brain tissue to that of the amount present per weight in liver tissue (Shashoua *et al.* 1984). The liver was taken as the reference point since it has no barrier to diffusible molecules present in the blood.

The results obtained, showed that GABA appeared to exhibit a BPI of 30 % 10 min postinjection which increased to 55 % after 30 min and decreased to 42 % 60 min postadministration of GABA (**Table 1**).

These high values were probably due to [^{3}H]-GABA **59** unlike **63** yielding large amounts of tritiated water, so that the uptake (as total radioactivity associated with the brain) was a reflection of tritium exchange rather than the uptake of GABA.

TABLE 1

BPI for ^{3}H-GABA and ^{3}H-(63) in mouse

Compound	Time/min	[Brain] dpm/g	[Liver] dpm/g	BPI %
^{3}H-GABA	10	142000	464000	30
59	30	197000	365000	55
	60	145000	334000	42
63	10	40000	429000	9
	30	58000	356000	17
	60	51000	380000	12.5

$$BPI = \frac{[Brain]}{[Liver]} \times 100$$

Thus it appeared, erroneously, that tritiated GABA **59** had better access to the brain than its lipophilic conjugate **63**. To overcome this problem, ^{14}C-labelled compounds were used. This necessitated the synthesis of ^{14}C-radiolabelled GABA conjugate **64**. Radiolabelled ^{14}C-GABA and ^{14}C-GABA conjugate **64** were administered to mice and the *in vivo* studies performed in the same manner as for the tritiated compounds.

^{14}C-GABA and conjugate **64** were assessed for their uptake at different times after intravenous administration. The mice were sacrificed at different times and the brain and liver were isolated, frozen overnight and then thawed and homogenised with water. Radioactivity levels of radiolabelled compounds administered present in the

brain and liver were determined by liquid scintillation spectrometry.

Comparative studies were performed simultaneously on ^{14}C-GABA and ^{14}C-GABA conjugate **64**. From the results shown in (**Table 2**), brain GABA uptake appeared to increase with time. Using the ^{14}C-GABA, the brain uptake studies performed gave BPI values ranging from 2.6 - 14.6 %.

TABLE 2

BPI and Time Course of Uptake of ^{14}C-GABA in Mouse Tissues

Time/min	Tissue	wt(g)	cpm/tissue	cpm/g	% injected	BPI(%)
5	Brain	0.4	7260	18150	0.15	2.6
	Liver	0.56	398614	711811	8.26	
10	Brain	0.42	3611	8598	0.08	3.1
	Liver	0.57	157565	276430	3.30	
15	Brain	0.43	492	1144	0.01	6.2
	Liver	0.46	8459	18389	0.18	
20	Brain	0.40	732	1830	0.015	6.2
	Liver	0.35	10332	29520	0.22	
30	Brain	0.40	5712	14280	0.12	14.6
	Liver	0.40	39156	97890	0.82	

Mice injected with 4769490 cpm in 4 % BSA.

The BPI values for radiolabelled GABA-C_{14}OMe ester **64** (**Table 3**) showed that the brain penetration was higher than that of GABA. 5 minutes after the administration of the conjugate, the BPI value was 12 % compared to 2.6 % for ^{14}C-GABA. A similar observation was made 10 minutes after the administration of both compounds with the conjugate **64** having a BPI of 50 % compared with a value for ^{14}C-GABA of 3 %. Even after longer periods, significant differences were observed in the BPI values for the two compounds. Although the ^{14}C-GABA conjugate **64** BPI values

decreased 15 and 20 minutes post administration, the brain penetration index remained much higher than that for ^{14}C-GABA which had a BPI of 6 % after 15 and 20 minutes. Increased BPI values were observed in both compounds 30 minutes after injection with the conjugate still showing the higher brain penetration index of 42 % compared to ^{14}C-GABA **57** with a BPI of 14 %.

TABLE 3

BPI and Time Course of Uptake of ^{14}C-64 in Mouse Tissue

Time/min	Tissue	wt(g)	cpm/tissue	cpm/g	% of injected	BPI(%)
5	Brain	0.4	672	1680	0.31	15.4
	Liver	0.5	5445	10890	2.53	
10	Brain	0.4	252	630	0.12	50.0
	Liver	0.35	441	1260	0.20	
15	Brain	0.43	258	600	0.12	15.2
	Liver	0.35	1386	3960	0.64	
20	Brain	0.43	308	716	0.14	22.7
	Liver	0.34	1071	3150	0.50	
30	Brain	0.4	2544	6360	1.18	42.2
	Liver	0.5	7530	15060	3.49	
60	Brain	0.45	675	1500	0.31	26.9
	Liver	0.45	2511	5580	1.17	

Amount injected 215512 cpm/50 µl 4 % BSA

The results in Table 1 demonstrate the need for the experiment to be performed with radiolabelled ^{14}C compounds. This agrees with the studies of Shashoua *et al.* (1984) and Hesse *et al.* (1985) which show that tritiated GABA yielded high amounts of tritiated water, thus the radioactivity was due to the tritium exchange rather than the uptake of the compound into the brain.

The results obtained from the time course study of ^{14}C-GABA (Table 2) showed that the BPI (2.6 %) 5 minutes after administration of ^{14}C-GABA was higher than the reported value of 0.96 % (Table 4). This is probably due to body system variation amongst the animals.

Table 4 summarizes the results of the application of this method to ^{14}C-labelled GABA (Shashoua *et al.* 1984).

TABLE 4

Uptake of ^{14}C-GABA by Mouse Tissues[a]

Dose µmol/kg	Injection route	[brain] nmol/g	[liver] nmol/g	BPI[c] (5 min) %
30	iv	0.04	4.2	0.95
60	ip	0.23	29	0.80
120	ip	0.45	46	1.00
160	ip	0.66	71	0.93
210	ip	0.83	83	1.00
270	sc	1.03	104	1.00
380	sc	1.81	165	1.10
mean ± SEM				0.96 ± 0.09

[a] All determination were carried out at 5 min after injection. The volumes of the injected doses into individual mice (body weight 18 - 24 g) for the iv, ip and sc doses were 0.15, 0.3 and 0.5 ml respectively. The measured concentrations in the brain and the liver represent the actual amounts of GABA found by thin layer chromatographic (TLC) analysis of the tissues homogenates. Each tissue was homogenised in 8 ml of pyridine in a glass homogeniser. The precipitated material was removed by centrifugation for 10 min at 12000 g and the supernatant was evaporated to dryness under N_2 and analysed by TLC in solvent chloroform:acetic acid 9:1. This procedure ensured that only labelled GABA and not its metabolites, was being measured. The

mean BPI after 5 min for labelled GABA was 0.96 ± 0.09 % (n = 7) and was apparently not dependent on the route of injection nor the dose (between 30 and 380 mol/kg).

[b] iv, intravenous; ip intraperitoneal; sc, subcutaneous injections.

[c] BPI (brain penetration index) = ([brain]/[liver])x100

(reproduced from Shashoua *et al.* 1984, p 660).

When the animals were sacrificed after longer post-administration periods, the BPI increased up to 14.6 % at 30 minutes post-injection. As GABA entered the brain tissues, it was probably metabolised by GABA-T which decreased the concentration of GABA in the brain. This then facilitated the entry of more GABA into the brain tissues which could explain the increase in the ^{14}C-GABA BPI (Table 2).

The BPI's determined for the radiolabelled ^{14}C-GABA conjugate **64** (Table 3), were generally higher than those of the ^{14}C-GABA. As discussed earlier the blood brain barrier is highly selective in the entry and exit of molecules in the brain tissues. Because of the presence of high amount of tight junctions in the brain capillary endothelia, passive diffusion is restricted to only small molecules such as water. The lipophilic nature of the blood brain barrier does not permit the entrance of ionic molecules. The GABA conjugate **64** due to its lipophilic nature could enter the blood brain barrier possibly by passive diffusion and partition into the brain endothelium membrane. Accordingly, higher amounts of the GABA conjugate would be expected to penetrate the blood brain barrier. This is in accordance with the observations from the *in vivo* radiolabelled experiments. It could then be postulated that the GABA conjugate **64** once in the brain, was hydrolysed to GABA by the circulating esterases and then be metabolised by GABA-T. The concentration gradient of GABA conjugate **64** would be potentiated leading to the difference in the uptake of the ^{14}C-GABA and the ^{14}C-GABA conjugate **64**.

3.1.2 *In vitro*: GABA Receptor Binding Studies.

GABA has two distinct receptors, the $GABA_A$ and $GABA_B$ receptors. The synthesised GABA conjugates **54**, **57** and **58** or their metabolites, should bind to both receptors. To test this hypothesis, a series of this synthesised GABA analogues were assayed for receptor binding capacity at $GABA_A$ receptors with the $GABA_B$ sites blocked. The assay utilised isoguvacine as a standard $GABA_A$ receptor agonist.

3.1.2.1 $GABA_A$ Receptor Binding Assay.

In this assay, the unlabelled samples dissolved in DMSO were mixed with buffer (Tris HCl + Ca^{2+} buffer). Analysis of the inhibition of ^{3}H-GABA binding for $GABA_A$ receptors were carried out on rat brain synaptosomal membranes (Hill and Bowery 1981). All incubations (incubation time = 10 mins) were performed in the presence of (-) baclofen (100 μM) which saturates the $GABA_B$ receptors leaving only the $GABA_A$ sites available. The amount of tritiated GABA displaced by isoguvacine at GABA receptor sites was expressed as 100 % displacement activity. Thus the percentage equivalent to the amount of tritiated GABA displaced by unlabelled conjugates to that of the amount displaced by isoguvacine defined the % displacement activity of the conjugates (equation below).

The results (Table 6) indicate that **54a**, **54b**, **54c**, **54d** and **54e** showed full displacement of GABA at $GABA_A$ receptor binding sites at a concentration of 1 μM. **54g** and **54i** effected a weaker binding ability. **54f** showed an even weaker agonist binding capacity whereas **54h** showed no binding activity at all.

TABLE 6

Displacement of ^{3}H-GABA at $GABA_A$ receptors in synaptosomal membranes for GABA conjugates with ester linkage

Compound	Size of conjugates			% displacement
	n	m	p	
54a	5	-	-	100
54b	11	-	-	100
54c	13	-	-	100
54d	11	9	1	100
54e	11	11	1	100
54f	11	17	1	22
54g	11	11	2	85
54h	13	11	1	0
54i	13	11	2	52

$$\% \text{ displacement} = \frac{[dpm]^3_{\text{H-GABA}} - [dpm]^3_{\text{H-GABA + conjugate}}}{[dpm]^3_{\text{H-GABA}} - [dpm]^3_{\text{H-GABA + isoguvacine}}} \times 100$$

The compounds with an ester linkage to GABA molecules showed differential $GABA_A$ binding activities depending on the size of the conjugates. With monomeric conjugating units attached to GABA as in compounds **54a** - **c**, full binding capacity at the receptors was observed. With increasing peptide units of the conjugates, the $GABA_A$ binding activity showed great variation from 100 % binding affinity to the binding sites seen for **54d** and **54e** to decreasing binding capacity for conjugates **54f**, **54g** and **54h** to no affinity to the $GABA_A$ receptors observed for **54h**.

TABLE 7

Displacement of ^{3}H-GABA at $GABA_A$ receptor in synaptosomal membranes of GABA conjugates with amide linkage

Compound	Size of Conjugates			% displacement
	n	m	p	
57f	7	-	-	94
57g	11	-	-	55
57h	17	-	-	61
58a	7	-	-	37
58b	7	7	1	19

GABA conjugates with an amide bond, whether the lipidic peptide delivery system was attached to the C-terminus of GABA (**57f** - **h**) or conjugated to the N-terminus of GABA, showed reduced GABA displacement capacity at the receptor sites with compound **58b** exhibiting the least displacement of binding.

It is assumed that for any of the GABA conjugates to show activity at the receptor sites, GABA itself has to be released in order to bind to the receptor. The ligand binding assay showed variation in the percentage of tritiated GABA displaced presumably due to the rate of hydrolysis of the conjugates by the esterases present in the synaptosomal membranes.

Compounds **54a** - **f** which showed total displacement of ^{3}H-GABA at $GABA_A$ receptors could possibly be hydrolysed to the parent active molecule GABA by the tissue esterases. With conjugates **54f** - **h**, the reduced activity at the receptors could be attributed to a rate of hydrolysis of the synthesised conjugates. This is also seen in the amide conjugates of GABA either at the C-terminus or at the N-terminus of GABA.

This observation on the variation of GABA displacement capacity was also found to be related to the size of the conjugating units to GABA. The type of linkage between GABA and the delivery system was also found to play a role in the binding activity at the receptors. For GABA conjugates with an ester linkage, the size of the alkyl side chain did not appear to be an important factor in the receptor binding activity in the analogues containing a monomeric unit of fatty acid conjugating groups as seen in **54a** - **c**.

However, when the conjugating units were increased to dimers, the pattern of receptor activities began to show some variation. The conjugates still showed full GABA displacement activities at the receptors when the alkyl side chain was varied from 10 (**54d**) to 12 (**54e**) carbon atoms in length. When the alkyl units was increased to 14 (**54h**), the receptor binding capacity decreased. This further reduction in the receptor activity was emphasised when the size of the alkyl side chain was further lengthened to 18 carbon atoms long (**54f**). Reduced receptor binding activities were also observed when the peptide units length were increased to trimers as seen in compounds **54g** and **54i**.

From these data, it would appear that increasing the size of the alkyl side chain in dimeric conjugates and lengthening the peptide units reduced the enzymatic hydrolysis of the GABA conjugates. This could possibly be due to the protective effect of the alkyl side chain and the amino acid units of the delivery system when conjugated to GABA molecule. Increasing the length of the alkyl groups and the peptide backbone appeared to protect the ester bond of the GABA conjugates probably making it inaccessible stereochemically to the tissue esterases.

Interestingly, when the GABA conjugates were linked by amide bonds either on the C-terminus (**57f-h**) or N-terminus of GABA (**58a** and **58b**), the receptor binding activities were found to be reduced, though the size of the conjugating units used were not too bulky.

The monomeric conjugates as seen in compounds **57f** - **h** and **58a** showed reduced receptor binding activities in contrast to the monomeric conjugates of GABA with ester linkage (**54a** - **c**) which exhibited full GABA displacement capacity at the receptors. Similar observation was seen in the dimer conjugate of GABA **58b**. Although the alkyl side chain length was only 8 carbon atoms long in compund **57f**, it showed reduced receptor binding ability compared to the dimer conjugates of GABA with an ester linkage and a longer alkyl side chain e.g. **54e** (12 carbon atoms) long which exhibited full receptor binding capacities.

Thus from these assays, conjugates of amide linkage showed reduced receptor activities compared to GABA analogues of ester linkage probably due to the reduced rate of hydrolysis of the amide bonds in the GABA conjugates. As the amides were more stable than the esters, this suggests a more stable prodrug linkage with higher resistance to esterase-mediated hydrolysis.

3.1.3 Conclusion

From the pharmacological results obtained, various conclusions could be made concerning the effect of conjugating the delivery system to GABA.

In vivo studies showed that the GABA conjugate had improved BBB penetration capacity compared to GABA itself. The lipophilicity of the GABA molecule was increased by the presence of the conjugating unit attached to it. GABA did not penetrate easily unlike the conjugate **64** which showed a better ability to cross the brain capillary endothelia.

It was also possible that conjugating the lipidic peptide to GABA diminished the zwitterionic effect of GABA, thus reducing its high dipole moment (Section 2.3.1) which is one of the factors contributing to the poor brain uptake of GABA. With this factor being eliminated by the conjugation process, brain uptake of GABA could be enhanced as was observed in the *in vivo* studies.

The *in vitro* studies on the other hand, indicated that the type of bond employed in the conjugation played an important role in the receptor binding sites. Ester type conjugates being easily hydrolysed by the esterases present in the tissue generating the parent molecule, GABA **11**, which could then bind to the GABA receptors. Employing a more stable prodrug link such as the amide appeared to protect the conjugates from enzymatic hydrolysis.

The size of the alkyl side chain and the length of the peptide units also contributed to the biological effect of GABA. As shown in the preliminary *in vitro* experimental data, increases in both the alkyl side group and the amino acid units protected the conjugates from hydrolysis by the enzymes.

Thus the primary question as to whether brain uptake of GABA could be improved by using the novel lipidic peptide delivery system was answered from the data obtained. At the receptor level, the chemical constitution of the conjugates affects the biological activity of the analogues.

The *in vitro* data suggest that enzymatic degradation occurs, however, experiments with purified enzymes or with enzyme inhibitor are necessary to prove that the degree of degradation is responsible for the measured activity.

3.2 *In vitro* Biological Assessment of Baclofen Conjugate

Although baclofen is a specific $GABA_B$ agonist, it was thought to be useful to find out the effect of receptor activities when conjugation was done on the molecule itself. Baclofen was conjugated to the lipidic acid forming **68** which was then subjected to both $GABA_A$ and $GABA_B$ receptors activities assays. The procedure carried out was similar to that employed in assaying the receptor activity studies of GABA conjugates.

3.2.1 $GABA_A$ Receptor Binding Studies

The baclofen conjugate **68** was found to exhibit no $GABA_A$ receptor activities even at a concentration of 100 μM.

This result confirms that since baclofen is a $GABA_B$ agonist, its conjugate would be expected to behave similarly. Although higher concentrations of the baclofen analogue **68** were used compared to isoguvacine (1 μM) in these tritiated GABA displacement experiments, the absence of $GABA_A$ receptor activity and affinity was confirmed.

3.2.2 $GABA_B$ Receptor Binding Studies

Receptor activity studies were next performed with the baclofen conjugate **68** at 100 μM on the $GABA_B$ binding sites. Experimental details were analogous to the studies performed in $GABA_A$ binding assays. Isoguvacine (100 μM) was used to saturate the $GABA_A$ sites thus limiting binding to $GABA_B$ receptors. Specific binding was defined with (-) baclofen (10 μM).

The data obtained indicated that the baclofen conjugate exhibited 56 % displacement at 100 μM compared to 100 % by 10 μM baclofen.

Though baclofen is a specific $GABA_B$ agonist, its conjugate **68** even at a higher concentration did not show full $GABA_B$ receptor activity.

Comparing the results obtained from the $GABA_A$ receptor studies, it did indicate that the conjugate has selective affinity to $GABA_B$ but at a reduced capacity. As discussed in Section 3.1.2.1, it was possible that the receptor activities could be seen if the conjugates were cleaved to generate the parent compounds. Though the linkage of the baclofen conjugate is of the ester type, the rate of enzymatic hydrolysis of the conjugate by esterases was possibly not as rapid as that of the monomeric conjugates of GABA which showed full $GABA_A$ receptor activities at just 1 μM concentration.

The conjugating unit somehow, might be able to protect the baclofen conjugates **68** from esterase-mediated hydrolysis by structurally making it less accessible to the enzymes.

3.2.3 Conclusion

The conjugating unit has been shown to play a role in affecting the biological activities of baclofen even though a labile prodrug linkage was used. The interaction between the hydrophobic character of the baclofen aromatic ring and the lipophilic moiety of the conjugating unit may be responsible for making the ester bond inaccessible to the tissue esterases.

The results seem to be in accordance with the assumption that the receptor activities were brought upon by the parent molecules, thus indicating that the hydrolysis of the conjugates has to occur first before activities at the receptor sites could be detected.

To confirm this hypothesis, future experiments with esterase inhibitor could be performed to decide if the receptor activities were due to the parent compounds or the conjugates having their own intrinsic activities.

CHAPTER 4

4.1 CONCLUSIONS

The aim of the project was to synthesise a series of lipidic amino acids and peptides and conjugate them to poorly-absorbed drugs. It was envisaged that the lipidic amino acids and peptides would impart increased membrane-like character to drugs following conjugation, assisting the drug conjugates in their passage across cell membranes. In addition, the long alkyl side-chain of the lipidic amino acids and peptides might increase the stability of metabolically labile drugs, by physically shielding the drug from enzymatic attack.

4.1.1 Lipidic Amino Acids and Peptides

Unsubstituted lipidic amino acids **25a** - **e** were prepared. They possess a single, unfunctionalised, alkyl side-chain.

Condensation of appropriately amino- and carboxyl-protected lipidic amino acids yielded lipidic oligo-peptides. Two classes of oligomers were synthesised:

a) Homo-oligomers **41a**, **41c**, **41f**, **41h**, **41j**, **41l**, **41m**, **41o** consisting solely of unsubstituted lipidic amino acids.

b) Hetero-oligomers, in which unsubstituted amino acids were condensed with either:
   i) other unsubstituted lipidic amino acids (**41b**, **41d**, **41e**, **41g**, **41i**, **41k** and **41n**),
   ii) alkyl hydroxy-acids (**44a** - **f**),
   iii) sugars (**44g** - **l**),
   iv) α-brominated lipidic acids (**46a** - **f**).

NMR studies showed that the lipidic amino acids conjugated to the hydrophilic compounds (**44a**, **44d**, **44e**, **44j** and **44k**) formed aggregates and micelles at high concentration and existed as monomers at low concentration.

4.1.2 Lipidic Drug Conjugates

Compounds to be conjugated with lipidic amino acids and peptides were chosen from the two categories, the C.N.S (GABA and baclofen) and nucleosides (adenosine and AZT) compounds. The chemical nature of the linkage between the lipidic "carrier" and the active compound was expected to play a major role in determining the biological stability of the drug conjugates. Amide, ester and sulphide linkages were utilised to impart differing biological and/or chemical lability and activity of the drug conjugates.

4.1.2.1 GABA Conjugates

A series of γ-aminobutyric acid esters of lipidic acids, lipidic peptides and γ-aminobutyric acid amides of lipidic α-amino acids and oligomers were synthesised. The lipidic conjugating units were chosen with a range of alkyl side-chain and oligomer lengths in order to impart different lipophilicities to the GABA molecule conjugates. The GABA conjugates with ester linkages (**53a** - **i**, **54a** - **i**) were prepared by coupling the lipidic acids and peptide conjugates with GABA. Aliphatic alkyl esters of GABA, **56a** - **56e**, were also synthesised to compare biological activity between different types of GABA ester conjugates. Two types of GABA conjugates linked by amide bonds were synthesised. This class included compounds **57a** - **h** in which the amino group of the lipidic amino acid is condensed with the carboxyl function of GABA and compounds **58a** and **58b** with the carboxyl terminus of the α-amino acid coupled to the amino group of GABA.

To evaluate brain uptake of GABA conjugates *in vivo*, radiolabelled analogues **62**, **63** and **64** were synthesised. Pharmacological data showed that the brain uptake of lipidic GABA conjugate **64** was higher than GABA itself. The *in vitro* studies on the unlabelled conjugates depicted better $GABA_A$ receptor activity for GABA coupled to lipidic delivery system via ester than amide linkage. Within the lipidic ester conjugates of GABA, better displacement capacity of tritiated GABA at the receptor site was observed for analogues with smaller lipidic moiety. The type of linkage

plays a role in the activity of the analogues. The size of the delivery system also affect the binding ability of the conjugates. This is probably related to the rate of hydrolysis to the parent molecule (Chapter 3).

4.1.2.2 Baclofen Conjugates

To study the biological effect in increasing the lipophilicity of baclofen, lipidic analogues **67** and **68** were prepared. *In vitro* preliminary study showed that baclofen conjugate **68** had reduced $GABA_B$ receptor activity compared to the parent molecule. It is assumed that the activity was due to the parent molecule, thus the rate of hydrolysis of the conjugate determines the degree of activity at the receptor binding sites.

4.1.2.3 Adenosine Conjugates

Antiviral drugs, generally, have cellular uptake problems. Conjugating the nucleoside antivirals to the lipidic delivery system will impart some degree of lipophilicity to the parent molecules. This would then increase the cellular uptake. Adenosine lipidic conjugates of ester linkage **69a** - **c** were synthesised to investigate the chemistry and the physico-chemical properties when nucleoside are conjugating to the lipidic delivery system.

4.1.2.4 Azidothymidine Conjugates

In order to examine the effect of different lipophilicities on the activity of the AZT molecule conjugates, azidothymidine (AZT) esters of lipidic amino acid and oligomers **74a** - **e**, and AZT-5'lipidic sulphide **79** were synthesised. It was envisaged that conjugating AZT to the lipidic delivery system would enhance the cellular uptake of the analogues into monocytes/macrophages. It is also anticipated that the biological activity would be altered if the analogues were biologically or chemically stable. If positive pharmacological activity were achieved, the compounds could reduce the toxicity effect, the development of resistance and increase the efficacy of the

analogues as antiretroviral agents.

4.2 EXPERIMENTAL: Chemistry

Infra-red spectra were recorded with a Perkin Elmer 841 spectrophotometer. ^{1}H-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) ionisation. Reaction progress was monitored by thin layer chromatography (TLC) on Kieselgel PF_{254} using dichloromethane:methanol 10:1 as the mobile phase. Purification was achieved by flash chromatography through Kieselgel G (dichloromethane:methanol 10:0.5). Solvents were evaporated under reduced pressure with a rotatory evaporator. Melting points are not given for enantiomers.

Racemic mixtures of the bromo-fatty acids and fatty amino acids were used for conjugation. Due to the diastereomeric mixtures, the amide and ester carbonyl resonances overlapped giving broad signal at 1720 - 1740 cm^{-1} in the IR spectra (detailed of non-informative data are not given). Only characteristic IR signals were given. In the ^{1}H-NMR spectrum of the diastereomers **46b**, **46c**, **74a** and **74d**, the CONH protons gave overlapping doublets (seen as triplets). The OCONH protons in the diastereomeric mixtures **53a** - **i**, **74b** and **74e** were seen as multiplets and **74c** as a broad singlet due to overlapping triplets. In the NMR data, α, β, γ referred to the alkyl side chain, the α', β', γ' to that of GABA, 1, 2, 3, 4, 5, 6, to that of the thymine base and 1', 2', 3', 4', 5', to those of the ribose in the adenosine or AZT molecule.

4.2.1 Lipidic Amino Acids

α-Aminodecanoic acid (25a): Method A.

Sodium metal (2.5 g, 0.11 mol) in small pieces was added to absolute ethanol (85 ml) over a period of 45 minutes with stirring. Diethylacetamidomalonate (23.98 g, 0.11 mol) was then added to the solution with stirring. 1-bromo-octane (23.66 g, 0.11 mol) was then added to the mixture and refluxed overnight. The cooled mixture was poured into 1:1 ice water (160 ml). The resulting white precipitate was filtered, washed with water and dried by suction for 2 hours. Concentrated hydrochloric acid (33 %, 180 ml) was added and the suspension was heated under reflux for 18 hours. The cooled mixture was taken up in ethanol:water (3:1, 500 ml). The mixture was heated to dissolve and filtered and adjusted to pH 7 and the resulting white precipitate was chilled for 4 hours and then filtered with suction, washed with cold water (500 ml) and cold ethanol (200 ml) and dried in vacuo over P_2O_5 for 10 hours.
Yield: 8.0 g (38.9 %)

α-Aminododecanoic acid (25b)

Sodium metal (2.5 g, 0.11 mol), diethylacetamidomalonate (23.68 g, 0.11 mol) and 1-bromodecane (24.11 g, 0.11 mol) were used for the synthesis of **25b** using the procedure as described in Method A.
Yield: 12.85 g (54.8 %)

α-Aminotetradecanoic acid (25c)

Sodium metal (2.5 g, 0.11 mol), diethylacetamidomalonate (24.30 g, 0.11 mol) and 1-bromododecane (28.26 g, 0.11 mol) were used for the preparation of **25c** using the procedure as described in Method A.
Yield: 22.25 g (83.2 %)

α-Aminohexadecanoic acid (25d)

Sodium metal (2.5 g, 0.11 mol), diethylacetamidomalonate (24.30 g, 0.11 mol) and 1-bromotetradecane (30.47 g, 0.11 mol) were used to synthesise **25d** using the procedure as described in Method A.

Yield: 28.0 g (93.9 %)

α-Aminoeicosanoic acid (25e)

Sodium metal (2.5 g, 0.11 mol), diethylacetamidomalonate (24.3 g, 0.11 mol) and 1-bromo-octadecane (36.68 g, 0.11 mol) were used for the preparation of **25e** using procedure as described in Method A.

Yield: 20.73 g (57.6 %)

4.2.2 N-Protected Lipidic Amino Acid

α-(*tert*-Butoxycarbonylamino)decanoic acid (25f): Method B.

α-Aminodecanoic acid, **25a** (8.0 g, 0.042 mol) was dissolved in t-butanol-water (2:3, 90 ml). The mixture was stirred and pH adjusted to 13 with 8 M sodium hydroxide. Di*tert*-butyldicarbonate (9.31 g, 0.0428 mol) in t-butanol (15 ml) was added to the solution and the pH was kept between 11 - 12 for two hours by adding 8 M sodium hydroxide. The reaction mixture was diluted with water (30 ml), the pH adjusted to 3 with solid citric acid and extracted with ethyl acetate (3 x 20 ml). The combined organic phase was dried ($MgSO_4$) and evaporated.

Yield: 6.43 g (52 %)

MS m/z (%) $C_{15}H_{29}NO_4$ (287.41): 332 $[M + 2Na - H]^+$ (100), 310 $[M + Na]^+$ (60), 254 (24), 232 (72), 142 (9), 57 (20).

^1H NMR ($CDCl_3$, δ ppm): 5.00 (1 H, d, NH), 4.30 (1 H, d, α-CH), 1.60, 1.80 (2 H, m, β-CH_2), 1.40 [9 H, s, $C(CH_3)_3$], 1.25 (12 H, s, 6 CH_2), 0.90 (3 H, t, CH_3).

α-(*tert*-Butoxycarbonylamino)dodecanoic acid (25g)

α-Aminododecanoic acid, **25b** (4.5 g, 0.021 mol) in t-butanol:water (2:3, 45 ml) was reacted with di-*tert*-butyldicarbonate (4.66 g, 0.021 mol) in t-butanol (7.5 ml) as in Method B.

Yield: 5.90 g (89.2 %).

MS m/z (%) $C_{17}H_{33}NO_4$ (315.4): 361 $[M + 2Na]^+$ (18), 360 $[M - H + 2Na]^+$ (100), 339 (13), 338 $[M + Na]^+$ (66), 282 (20), 260 (48), 57 (30).

^1H NMR ($CDCl_3$, δ ppm): 4.98 (1 H, s, OCONH), 4.28 (1 H, m, α-CH), 1.84 - 1.67

(2 H, m, β-CH_2), 1.46 [9 H, s, $C(CH_3)_3$], 0.87 (3 H, t, CH_3).

α-(*tert*-Butoxycarbonylamino)tetradecanoic acid (25h)

α-Aminotetradecanoic acid, **25c** (16.0 g, 0.0658 mol) in t-butanol:water (2:3, 160 ml) was reacted with di-*tert*-butyldicarbonate (14.36 g, 0.0658 mol) in t-butanol (26.7 ml) as in Method B.

Yield: 18.30 g (81.1 %).

MS m/z (%) $C_{19}H_{37}NO_4$ (343): 389 $[M + H + 2Na]^+$ (15), 367 $[M + H + Na]^+$ (100), 343 $[M]^+$ (5), 310 (19), 288 (65), 259 (4), 244 (62), 215 (15), 198 (46), 176 (4), 154 (6), 137 (10), 119 (3), 107 (3), 89(2), 74 (6), 57 (45).

^1H NMR ($CDCl_3$, δ ppm): 4.95 (1 H, d, NH), 4.27 (1 H, m, α-CH), 1.84 (1 H, m, β-CH_2), 1.65 (1 H, m, β-CH_2), 1.44 (9 H, s, $C(CH_3)_3$), 0.87 (3 H, t, CH_3).

4.2.3 C-Protected Lipidic Amino acid

Methyl α-aminodecanoate (25i) HCl: Method C.

Thionyl chloride (17.8 g, 11.2 ml) was added dropwise to methanol (132 ml), cooled to 0 °C over period of 30 minutes. DL-α-aminodecanoic acid, **25a** (5.0 g, 0.027 mol) was added to the mixture of thionyl chloride and methanol and refluxed for 16 hours. The solvent was evaporated and the compound recrystallised from methanol.

Yield: 4.12 g (68.6 %)

MS m/z (%) $C_{11}H_{23}NO_2$ (201): 202 $[M + H]^+$ (100), 200 (4), 188 (1), 142 (24), 137 (1), 89 (2), 56 (3).

^1H NMR ($CDCl_3$, δ ppm): 8.82 (3 H, m, $^+NH_3$), 4.10 (1 H, t, α-CH), 3.80 (3 H, s, $COOCH_3$), 1.48 (2 H, m, β-CH_2), 1.26 (12 H, m, 6 CH_2), 0.86 (3 H, t, CH_3).

Methyl α-aminododecanoate (25j) HCl

α-Aminododecanoic acid, **25b** (10.0 g, 0.0465 mol), methanol (200 ml) and thionyl chloride (27.0 g, 17.0 ml) were reacted as in the procedure described in Method C.

Yield: 9.0 g (84.5 %)

MS m/z (%) $C_{13}H_{27}NO_2$ (229) HCl: 253 $[M + Na]^+$ (6), 230 $[M + H]^+$ (100), 170 (30), 154 (1), 137 (1), 102 (1), 89 (2), 77 (1), 69 (2), 56 (3), 43 (3).

^{1}H NMR ($CDCl_3$, δ ppm): 8.89 (3 H, m, $^+NH_3$), 4.05 (1 H, t, α-CH), 3.83 (3 H, s, $COOCH_3$), 1.90 (2 H, m, β-CH_2), 1.41 (2 H, m, CH_2), 1.31 (14 H, m, 7 CH_2), 0.89 (3 H, t, CH_3).

Methyl α-aminotetradecanoate (25k) HCl

α-Aminotetradecanoic acid, **25c** (10.0 g, 0.041 mol), methanol (200 ml) and thionyl chloride (27.0 g, 17.0 ml) were reacted as in the procedure described in Method C.
Yield: 8.5 g (74.4 %)
MS m/z (%) $C_{15}H_{31}NO_2$ (257): 280 $[M + Na]^+$ (6), 258 $[M + H]^+$ (100), 244 (2), 198 (26), 176 (2), 154 (3), 137 (2), 107(2), 89 (2), 77 (1), 69 (1), 56(3), 43 (3)
^{1}H NMR ($CDCl_3$, δ ppm): 8.86 (2 H, m, NH_2), 4.04 (1 H, t, α-CH), 3.82 (3 H, t, $COOCH_3$), 2.04 (2 H, m, β-CH_2), 1.54 (2 H, m, CH_2), 1.26 (18 H, m, 9 CH_2), 0.88 (3 H, t, CH_3).

Methyl α-aminoeicosanoate (25l) HCl

DL-α-aminoeicosanoic acid, **25e** (66.0 g, 0.0183 mol), methanol (90.3 ml) and thionyl chloride (7.6 ml) were reacted as in Method C.
Yield: 5.13g (74.3 %)
MS m/z (%) $C_{21}H_{43}NO_2$ (341): 342 $[M + H]^+$ (100), 328 (4), 314 (1), 282 (30), 136 (2), 123 (1), 107 (2), 95 (2), 89 (3), 83 (2), 77 (2), 69 (3), 56 (4).
^{1}H NMR ($CDCl_3$ + D_2O, δ ppm): 3.89 (1 H, m, α-CH), 3.79 (3 H, s, $COOCH_3$), 1.89 (2 H, m, CH_2), 1.26 (32 H, m, 16 CH_2), 0.89 (3 H, t, CH_3).

4.2.4 α-Amino Acid Homo- and Hetero-oligomers

α-*tert*-butoxycarbonyl-ω-methoxybis[imino(1-octyl-2-oxo-1,2-ethanediyl)] (41a): Method D.

BOC-α-aminodecanoic acid **25f** (1.80 g, 6.27 mmol), methyl α-aminodecanoate **25i** (1.26 g, 6.27 mmol), 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide hydrochloride (1.32 g, 6.90 mmol), triethylamine (1.33 g, 13.2 mmol) were reacted in dichloromethane (50 ml) for 16 hours. The reaction mixture was washed with brine (50 %, 20 ml), then with water (20 ml). The organic layer was then dried ($MgSO_4$)

and evaporated. The product mixture was purified by flash chromatography.

Yield: 1.31 g (46 %)

MS m/z (%) $C_{26}H_{50}N_2O_5$ (470.70): 493 $[M + Na]^+$ (100), 471 $[M + H]^+$ (5), 451 (4), 415 (12), 393 (26), 371 (21), 202 (19), 142 (82), 57 (30).

^1H NMR ($CDCl_3$, δ ppm): 6.50 (1 H, d, NH), 4.95 (2 H, s, NH), 4.55 (1 H, m, α-CH), 4.05 (1 H, m, α-CH), 3.75 (3 H, s, $COOCH_3$), 1.80 (2 H, m, β-CH_2), 1.50 (9 H, s, $C(CH_3)_3$), 1.25 (12 H, s, 6 CH_2), 0.90 (6 H, t, 2 CH_3).

Methyl 2-[2-(*tert*-butoxycarbamido)tetradecanamido]dodecanoate (41b)

A mixture of BOC-α-amino-tetradecanoic acid, **25h** (1.5 g, 4.37 mmol), methyl-N-α-amino-dodecanoate **25k** HCl (1.16 g, 4.37 mmol), triethylamine (0.88 g, 8.72 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide HCl (0.84 g, 4.37 mmol) was reacted in dichloromethane (30 ml) as described in Method D.

Yield: 1.5453 g (63.8 %).

MS m/z (%) $C_{32}H_{62}N_2O_5$ (554): 577 $[M + Na]^+$ (100), 477 (28), 455 (2), 413 (3), 307 (3), 230 (5), 198 (15), 170 (15), 119 (9), 57 (16), 42 (9).

^1H NMR ($CDCl_3$, δ ppm): 6.58, 6.51 (2 H, d, 2 NH), 4.98, 4.56 (2 H, m, 2 α-CH), 3.74 (3 H, s, $COOCH_3$), 1.82 (2 H, m, CH_2), 1.61 (4 H, m, 2 CH_2), 1.45 [9 H, s, $C(CH_3)_3$], 1.26 (30 H, m, 15 CH_2), 0.86 (6 H, t, 2 CH_3).

α-*tert*-butoxycarbonyl-ω-methoxybis[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (41c)

A mixture of BOC-α-aminotetradecanoic acid **25h** (2.0 g, 5.83 mmol), methyl α-aminotetradecanoate **25k** HCl (1.71 g, 5.83 mmol), triethylamine (1.18 g, 11.66 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide HCl (1.12 g, 5.83 mmol) was reacted in dichloromethane (30 ml) as described in Method D.

Yield: 1.9105 g (56.3 %).

MS m/z (%) $C_{34}H_{66}N_2O_5$ (582): 605 $[M + Na]^+$ (100), 553 (12), 505 (47), 198 (16), 119 (26), 88 (14), 77 (17), 57 (28), 44 (27), 42 (25).

^1H NMR ($CDCl_3$, δ ppm): 6.61, 6.52 (2 H, d, 2 NH), 5.0, 4.56 (2 H, m, 2 α-CH), 3.73 (3 H, s, $COOCH_3$), 2.81, 1.60 (4 H, m, 2 CH_2), 1.42 (9 H, s, $COOCH_3$), 1.24 (40 H, m, 20 CH_2), 0.89 (6 H, t, 2 CH_3).

Methyl 2-[2-(*tert*-butoxycarbamido)tetradecanamido]icosanoate (41d):

A mixture of BOC-α-amino-tetradecanoic acid **25h** (2.0 g, 5.83 mmol), methyl α-aminoicosanoate **25l** HCl (2.20 g, 5.83 mmol), triethylamine (1.18 g, 11.66 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide HCl (1.12 g, 5.83 mmol) was reacted in dichloromethane (50 ml) as described in Method D.

Yield: 1.8 g (61.8 %)

MS m/z (%) $C_{40}H_{78}N_2O_5$ (666): 690 $[M + H + Na]^+$ (55), 590 (19), 413 (100), 282 (9), 198 (6), 167 (8), 149 (49), 119 (9), 71 (15), 57 (36).

^1H NMR ($CDCl_3$, δ ppm): 6.54, 6.46 (2 H, d, 2 NH), 4.59, 4.24 (2 H, m, 2 α-CH), 3.75 (3 H, s, $COOCH_3$), 1.83, 1.69, 1.58 (8 H, m, 4 CH_2), 1.44 [9 H, s, $C(CH_3)_3$], 1.25 (48 H, m, 24xCH_2), 0.93, 0.88 (6 H, t, 2 CH_3).

Methyl-2-(2-aminotetradecanamido)dodecanoate (41e) HCl

Dimer **41b** (1.4 g, 2.53 mmol) was refluxed with methanolic HCl (3 %, 10 ml) for 20 minutes. The solvent was evaporated.

Yield: 1.25 g (100 %).

MS m/z (%) $C_{27}H_{54}N_2O_3$ (454) HCl: 477 $[M + Na]^+$ (39), 455 $[M + H]_+$ (28), 440 (17), 258 (28), 198 (100).

^1H NMR ($CDCl_3$, δ ppm): 8.05 (1 H, m, NH), 4.58 - 4.20 (2 H, m, 2 α-CH), 3.98 (3 H, s, OCH_3), 1.97 (4 H, m, 2 β-CH_2), 1.32 (36 H, m, 18 CH_2), 0.94 (6 H, t, 2 CH_3).

α-amino-ω-methoxybis[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (41f) HCl:

Dimer **41c** (1.0 g, 1.72 mmol) was refluxed with methanolic HCl (3 %, 10 ml) for 20 minutes. The solvent was then evaporated.

Yield: 0.89 g (99.8 %).

MS m/z (%) $C_{29}H_{58}N_2O_3$ (482) HCl: 505 $[M + Na]^+$ (35), 484 $[M + H]_+$ (26), 258 (24), 198 (100).

^1H NMR ($CDCl_3$, δ ppm): 8.19, 8.05 (3 H, br m, $^+NH_3$), 4.49 (1 H, m, NH), 4.40, 4.27 (2 H, m, 2 α-CH), 3.75 (3 H, s, OCH_3), 1.96, 1.82 (4 H, m, 2 CH_2), 1.29 (40 H, m, 20 CH_2), 0.89 (6 H, t, 2 CH_3).

Methyl-2-(2-aminotetradecanamido)icosanoate (41g) HCl

Dimer **41d** (1.5 g, 2.25 mmol) was refluxed with methanolic HCl (3 %, 10 ml) for 20 minutes. The solvent was then evaporated off.

Yield: 1.35 g (100 %).

MS m/z (%) $C_{35}H_{70}N_2O_3$ (566) HCl: 589 $[M + Na]^+$ (32), 567 $[M + H]_+$ (22), 551 (12), 258 (28), 198 (100).

^1H NMR ($CDCl_3$, δ ppm): 8.02 (1 H, m, NH), 4.61 - 4.24 (2 H, m, 2 α-CH), 3.97 (3 H, s, OCH_3), 1.94 (4 H, m, 2 β-CH_2), 1.34 (52 H, m, 26 CH_2), 0.92 (6 H, t, 2 CH_3).

α-*tert*-butoxycarbonyl-ω-hydroxybis[imino(1-octyl-2-oxo-1,2-ethanediyl)] (41h): Method E.

Dimer **41a** (0.24 g, 0.5 mmol) was left stirring in dichloromethane:methanol (3:7, 10 ml) with 1 M potassium hydroxide (1 ml) for 16 hours. The solvent mixture was then evaporated off and remaining solution was neutralised with 3 % hydrochloric acid to pH 4. The mixture was extracted into ether (3 x 10 ml), dried ($MgSO_4$), filtered and evaporated off.

Yield: 0.231 g (94 %)

MS m/e (%) $C_{25}H_{48}N_2O_5$ (456.67): 501 $[M + 2Na - H]^+$ (52), 479 $[M + Na]^+$ (49), 401 (100), 379 (46), 142 (62), 57 (31).

^1H NMR ($CDCl_3$, δ ppm): 9.25 (1 H, m, COOH), 7.05 (1 H, d, NH), 5.05 (1 H, m, OCONH), 4.75, 4.00 (2 H, m, α-CH), 1.75 (4 H, m, 2 β-CH_2), 1.40 (9 H, s, $C(CH_3)_3$), 1.20 (24 H, s, 12 CH_2), 0.90 (6 H, t, 2 CH_3).

2-[2-(*tert*-butoxycarbamido)tetradecanamido]dodecanoic acid (41i)

Dimer **41b** (0.145 g, 0.262 mmol) was left stirring in dichloromethane:methanol (5:5, 10 ml) with 1 M potassium hydroxide (1 ml) as in Method E.

Yield: 0.133 g (94 %)

MS m/e (%) $C_{31}H_{60}N_2O_5$ (540): 585 $[M + 2Na - H]^+$ (46), 563 $[M + Na]^+$ (52), 485 (100), 379 (46), 142 (62), 57 (31).

^1H NMR ($CDCl_3$, δ ppm): 8.5 (1 H, br s, COOH), 7.02 (1 H, m, NH), 5.35 (1 H, m, OCONH), 4.5 (2 H, m, 2 α-CH), 1.80, 1.74 (4 H, m, 2 β-CH_2), 1.42 [9 H, s, $C(CH_3)_3$], 1.20 (36 H, m, 18 CH_2), 0.82 (6 H, t, 2 CH_3).

α-*tert*-butoxycarbonyl-ω-hydroxybis[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (41j):

Dimer **41c** (0.67 g, 1.15 mmol) was left stirring in dichloromethane:methanol (5:5, 10 ml) with 1 M potassium hydroxide (1.2 ml) as in Method E.

Yield: 0.533 g (82 %)

MS m/z (%) $C_{33}H_{64}N_2O_5$ (568.9): 613 $[M + 2Na - H]^+$ (31), 591 $[M + Na]^+$ (7), 561 (39), 535 (43), 513 (100), 388 (21), 288 (45), 119 (20), 57 (42).

^1H NMR ($CDCl_3$, δ ppm): 7.05 (1 H, m, CONH), 5.40 (1 H, m, OCONH), 4.55 (2 H, m, 2 α-CH), 1.87 - 1.58 (4 H, m, 2 β-CH_2), 1.43 [9 H, s, $C(CH_3)_3$], 1.23 (40 H, s, 20 CH_2), 0.88 (6 H, t, 2 CH_3).

2-[2-(*tert*-butoxycarbamido)tetradecanamido]icosanoic acid (41k):

Dimer **41d** (0.172 g, 0.26 mmol) was left stirring in dichloromethane:methanol (5:5, 10 ml) with 1 M potassium hydroxide (1 ml) as in Method E.

Yield: 0.170 g (100 %)

MS m/e (%) $C_{39}H_{76}N_2O_5$ (652): 697 $[M + 2Na - H]^+$ (42), 675 $[M + Na]^+$ (11), 597 (100), 535 (46), 513 (40), 388 (21),

^1H NMR ($CDCl_3$, δ ppm): 9.05 (1 H, br s, COOH), 7.05 (1 H, m, NH), 5.40 (1 H, m, OCONH), 4.55 (2 H, m, 2 x α-CH), 1.87 - 1.58 (4 H, m, 2 β-CH_2), 1.43 [9 H, s, $C(CH_3)_3$], 1.23 (52 H, m, 26 CH_2), 0.88 (6 H, t, 2 CH_3).

α-*tert*-butoxycarbonyl-ω-methoxytris[imino(1-octyl-2-oxo-1,2-ethanediyl)] (41l)

N-protected dimer **41h** (1.26 g, 2.96 mmol), methyl-α-aminodecanoate **25i** HCl (0.851 g, 2.96 mmol), 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide hydrochloride (626 mg, 3.26 mmol), triethylamine (600 mg, 5.93 mmol) in dichloromethane (50 ml) were reacted as described in Method D.

Yield: 381 mg (67.1 %)

MS m/e (%) $C_{36}H_{69}N_3O_6$ (639.97): 663 $[M + Na]^+$ (100), 563 $[M - BOC + Na]^+$ (30), 493 (20), 464 (37), 432 (85), 393 (52), 371 (34), 259 (29), 219 (51), 173 (19), 142 (62), 92 (20), 57 (45).

^1H NMR ($CDCl_3$, δ ppm): 6.68, 6.56 (2 H, m, 2 CONH), 4.96 (1 H, m, OCONH),

4.51, 4.39, 4.02 (3 H, m, 3 α-CH), 3.71 (3 H, s, OCH_3), 1.75 (6 H, m, 3 β-CH_2), 1.43 [9 H, s, $C(CH_3)_3$], 1.25 (36 H, s, 18 CH_2), 0.85 (9 H, t, 3 CH_3).

α-[2-(4-*tert*-butoxycarbamido)tetradecanoyl]-ω-methoxybis[imino(1-dodecyl-2-oxo-ethanediyl)] (41m)

N-protected dimer **41j** (400 mg, 0.704 mmol), methyl-α-aminotetradecanoate **25k** HCl (235 mg, 0.80 mmol), 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide hydrochloride (153 mg, 0.8 mmol), triethylamine (162 mg, 1.6 mmol) in dichloromethane (20 ml) were reacted as described in Method D.

Yield: 381 mg (67.1 %)

MS m/z (%) $C_{48}H_{93}N_3O_6$ (807): 809 $[M + 2H]^+$ (5), 752 (6), 708 $[M - BOC]^+$ (4), 690 (1), 662 (1), 632 (1), 583 (2), 551 (2), 483 (12), 451 (3), 421 (5), 378 (2), 326 (2), 258 (80), 198 (100), 154 (7), 133 (22), 112 (9), 95 (13).

^1H NMR ($CDCl_3$, δ ppm): 6.72, 6.56 (2 H, m, 2 NH), 4.94 (1 H, s, OCONH), 4.51, 4.39, 4.02 (3 H, m, 3 α-CH), 1.75 (6 H, m, 3 β-CH_2), 1.44 [9 H, s, $C(CH_3)_3$], 1.25 (60 H, m, 30 CH_2), 0.87 (9 H, t, 3 CH_3).

Methyl-N-t-butoxycarbonyl-α-aminotetradecanoyl-α-aminoicosanoyl-α-aminotetradecanoate (41n)

N-protected dimer **41k** (170 mg, 0.26 mmol), methyl-α-aminodecanoate **25i** HCl (76 mg, 0.26 mmol), 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide hydrochloride (50 mg, 0.26 mmol), triethylamine (53 mg, 0.52 mmol) in dichloromethane (20 ml) were reacted as described in Method D.

Yield: 76 mg (32.8 %)

MS m/e (%) $C_{54}H_{105}N_3O_6$ (891): 915 $[M + H + Na]^+$ (92), 892 $[M + H]^+$ (100), 860(12), 836 (59), 792 (52), 760 (9), 718 (9), 690 (32), 668 (21), 635 (15).

NMR ($CDCl_3$, δ ppm): 6.71, 5.05, 4.60 (3 H, m, 3 x NH), 4.50, 4.42, 4.05 (3 H, m, 3 x α-CH), 3.73 (3 H, s, $COOCH_3$), 1.81, 1.60 (8 H, m, 4 CH_2), 1.42 (9 H, s, $C(CH_3)_3$), 1.26 (70 H, m, 35 x CH_2), 0.87 (9 H, t, 3 CH_3).

α-*tert*-butoxycarbonyl-ω-hydroxytris[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (41o):

Trimer **41m** (0.38 g, 0.471 mmol) was left stirring in dichloromethane:methanol (5:5, 10 ml) with 1 M potassium hydroxide (1 ml) as in Method E.

Yield: 0.23 g (62 %)

MS m/z (%) $C_{47}H_{91}N_3O_6$ (793): 817 $[M + H + Na]^+$ (7), 794 $[M + H]_+$ (1), 739 (2), 717 (1), 694 (2), 495 (2), 469 (2), 421 (1), 320 (1), 242 (5), 198 (100), 154 (3), 112 (2), 57 (27).

^1H NMR ($CDCl_3$, δ ppm): 7.44 - 6.78 (2 H, m, 2 NH), 5.05 (1 H, m, OCONH), 4.66 - 4.02 (4 H, m, 3 α-CH), 2.0 -1.5 (6 H, m, β-CH_2), 1.43 [9 H, s, $C(CH_3)_3$], 1.23 (60 H, s, 30 CH_2), 0.85 (9 H, t, 3 CH_3).

4.2.5 Modified Lipidic Peptides

4.2.5.1 Lipidic Amino Acid Conjugates with Hydrophilic Compounds

Methyl 2-(2-hydroxypropanamido)-tetradecanoate (44a): Method F.

To a mixture of lactic acid **43a** (360 mg, 3.42 mmol, 85 % water solution) and **25k** methyl ester HCl (1.0 g, 3.42 mmol), triethylamine (0.472 ml, 3.42 mmol and 1-hydroxybenzotriazole (0.462 g, 3.42 mmol) in ethylacetate (10 ml) and CH_2Cl_2 (5 ml), dicyclohexylcarbodiimide (1.409 g, 6.84 mmol) was added at 0 °C. The reaction mixture was stirred 2 hours at 0 °C and 1 day at room temperature, the solvents were evaporated. The residue was stirred in ethyl-acetate (20 ml) for 20 min. and the precipitate was filtered. The filtrate was extracted with brine, 10 % citric acid, brine, 3 % $NaHCO_3$ and brine again. The organic phase was dried, evaporated and the residue purified by flash chromatography.

Yield: 0.92 g (81.4 %)

MS m/z (%) $C_{18}H_{35}NO_4$ (329): 330 $[M + H]^+$ (100), 270 (23), 258 (24), 198 (31), 137 (2), 89 (1), 56 (2), 45 (2).

^1H NMR ($CDCl_3$, δ ppm): 6.89 (1 H, m, NH), 4.59 (1 H, m, α-CH), 4.26 (1 H, m, α'-CH), 3.72 (3 H, s, $COOCH_3$), 2.84 (1 H, br s, OH), 1.86 (2 H, m, CH_2), 1.69 (2 H, m, CH_2), 1.45 (3 H, 2 d, CH_3), 1.25 (18 H, m, 9 CH_2), 0.88 (3 H, t, CH_3).

Analysis $C_{18}H_{35}NO_4$ (329.5) Calcd. C 65.61 H 10.71 N 4.25
Found C 65.66 H 10.57 N 4.03

2-(2-hydroxypropanamido)tetradecanoic acid (44b): Method G.

44a (1.0 g, 3.04 mmol) was stirred in a mixture of MeOH (40 ml) 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.
Yield: 0.93 g (97.4 %)
MS m/z (%): 316 $[M + H]^+$ (77), 270 (39), 244 (38), 198 (100), 137 (10), 98 (9), 41 (20).
^{1} NMR ($CDCl_3$, δ ppm): 7.13, 7.05 (1 H, dd, NH), 4.54 (1 H, m, α-CH), 4.29 (1 H, m, CH-OH), 1.91 (2 H, m, CH_2), 1.72 (2 H, m, CH_2), 1.32 (18 H, m, 9 CH_2), 0.88 (3 H, t, CH_3).
Analysis $C_{17}H_{33}NO_4$ (315.5) Calcd. C 64.71 H 10.54 N 4.44
Found C 64.99 H 10.29 N 4.16

Methyl 2-(hydroxy-ethanamido)decanoate (44c):

A mixture of methyl α-amino-decanoate **25i** HCl (0.5 g, 2.10 mmol), glycolic acid **43b** (0.198 g, 95 %, 2.1 mmol), triethylamine (0.212 g, 2.10 mmol) and dicyclo hexylcarbodiimide (0.453 g, 2.10 mmol) and 1-hydroxybenzotriazole (0.283 g, 2.10 mmol) was reacted in dichloromethane (30 ml) using the procedure as described in Method F.
Yield: 0.50 g (91 %)
MS m/z (%) $C_{13}H_{25}NO_4$ (259): 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).
^{1}H NMR ($CDCl_3$, δ ppm): 6.86 (1 H, d, NH), 4.66 (1 H, m, α-CH), 4.15 (2 H, s, CH_2OH), 3.75 (3 H, s, $COOCH_3$), 1.86 (2 H, m, CH_2), 1.72 (2 H, m, CH_2), 1.30 (10 H, m, 5 CH_2), 0.87 (3 H, t, CH_3).
Analysis $C_{13}H_{25}NO_4$ (259.3) Calcd. C 60.21 H 9.72 N 5.40
Found C 59.99 H 9.84 N 5.18

Methyl 2-(hydroxy-ethanamido)tetradecanoate (44d):

A mixture of methyl-α-amino-tetradecanoate **25k** HCl (0.5 g, 1.70 mmol), glycolic acid **43b** (0.135 g, 95 %, 1.70 mmol), triethylamine (0.17 g, 1.70 mmol) and dicyclo hexylcarbodiimide (0.35 g, 1.70 mmol) and 1-hydroxybenzotriazole (0.229 g, 1.70 mmol) was reacted in dichloromethane (30 ml) using the procedure as described in Method F.

Yield: 0.43 g (81 %)

MS m/z (%) $C_{17}H_{33}NO_4$ (315): 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).

^1H NMR ($CDCl_3$, δ ppm): 6.89 (1 H, m, NH), 4.64 (1 H, m, α-CH), 4.15 (2 H, s, CH_2OH), 3.76 (3 H, s, $COOCH_3$), 2.82 (1 H, m, OH), 1.87 (2 H, m, CH_2), 1.68 (2 H, m, CH_2), 1.27 (18 H, m, 9 CH_2), 0.88 (3 H, t, CH_3).

Analysis $C_{17}H_{33}NO_4$ (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 (44e):

44d (0.3 g, 0.95 mmol) was stirred in a mixture of MeOH (10 ml) and N NaOH (3 ml) as in Method F.

Yield: 0.27 g (95.5 %)

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

^1H NMR ($CDCl_3$, δ ppm): 7.18 (1 H, dd, NH), 4.59 (1 H, d, CH_3), 4.54, 4.45 (1 H, m, α-CH), 4.07 (2 H, s, CH_2OH), 3.68 (3 H, s, $COOCH_3$), 1.82 (2 H, m, CH_2), 1.68 (2 H, m, CH_2), 1.25 (18 H, m, 9 CH_2), 0.84 (3 H, t, CH_3).

Analysis $C_{16}H_{31}NO_4$ (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)] (44f):

A mixture of dimer **41f** HCl (0.5 g, 0.96 mmol), glycolic acid **43b** (0.80 g, 95 %, 1.0 mmol), triethylamine (0.10 g, 1.0 mmol) and dicyclohexylcarbodiimide (0.21 g, 1.0 mmol) and 1-hydroxybenzotriazole (0.14 g, 1.0 mmol) was reacted in dichloromethane (20 ml) using the procedure as described in Method F.

Yield: 0.16 g (31 %)

MS m/z (%): 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).

^1H NMR ($CDCl_3$, δ ppm): 7.37, 6.95, 6.46 (2 H, 3 m, 2 NH), 4.59-4.37 (2 H, m, 2 α-CH), 4.14 (2 H, s, CH_2OH), 3.70 (3 H, s, $COOCH_3$), 3.20 (1 H, m, OH), 1.87 (4 H, m, 2 CH_2), 1.70 (4 H, m, 2 CH_2), 1.28 (36 H, m, 18 CH_2), 0.85 (6 H, t, 2 CH_3).

Analysis $C_{31}H_{60}N_2O_5$ (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 (44g):

A mixture of methyl-α-amino-tetradecanoate **25k** HCl (0.5 g, 1.70 mmol), D-glucuronic acid (0.33 g, 1.70 mmol), triethylamine (0.17 g, 1.70 mmol) dicyclohexyl-carbodiimide (0.35 g, 1.70 mmol) and 1-hydroxybenzotriazole (0.229 g, 1.70 mmol) was reacted in dichloromethane (30 ml) using the procedure as described in Method F.

Yield: 0.33 g (45 %)

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

^1H NMR ($CDCl_3$, δ ppm): 3.84 (1 H, m, C_2,-H), 3.75 - 3.43 (3 H, m, C_4,-H, C_3,-H, α-CH), 3.37 (3 H, s, $COOCH_3$), 3.30 (1 H, m, C_5,-H), 1.85, 1.65 (4 H, 2 m, 2 CH_2), 1.22 (18 H, m, 9 CH_2), 1.84 (3 H, t, CH_3).

Analysis $C_{21}H_{39}NO_8$ (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 (44h):

A mixture of methyl α-amino-decanoate **25i** HCl (0.5 g, 2.10 mmol), 1,2:3,4-di-O-isopropylidene-D-gulonic acid (0.575 g, 2.10 mmol), triethylamine (0.212 g, 2.10 mmol) dicyclohexylcarbodiimide (0.453 g, 2.10 mmol) and 1-hydroxybenzotriazole (0.283 g, 2.10 mmol) was reacted in dichloromethane (30 ml) using the procedure as described in Method F.

Yield: 0.82 g (81 %)

MS m/z (%): 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).

^1H NMR ($CDCl_3$, δ ppm): 7.46 (1 H, m, NH), 4.59 (1 H, d, C_3H), 4.57 (1 H, m, α-CH), 4.30 (1 H, dd, C_4-H), 4.16 (1 H, t, C_5-H), 4.11 (2 H, d, CH_2O), 1.87 (2 H, m, CH_2), 1.70 (2 H, m, CH_2), 1.58, 1.54, 1.44, 1.36 (12 H, 4 s, 4 CH_3), 1.27 (18 H, m, 9 CH_2), 0.88 (3 H, t, CH_3).

Analysis $C_{23}H_{39}NO_8$ (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 (44i):

A mixture of methyl-α-amino-tetradecanoate **25k** HCl (0.5 g, 1.70 mmol), 1,2:3,4-di-O-isopropylidene-D-gulonic acid (0.465 g, 1.70 mmol), triethylamine (0.17 g, 1.70 mmol) dicyclohexylcarbodiimide (0.35 g, 1.70 mmol) and 1-hydroxybenzotriazole (0.229 g, 1.70 mmol) was reacted in dichloromethane (30 ml) using the procedure as described in Method F.

Yield: 0.70 g (81 %)

MS m/z (%): 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), 69 912), 59 (15).

^1H NMR ($CDCl_3$, δ ppm): 7.44 (1 H, m, NH), 4.58 (1 H, d, C_3H), 4.57 (1 H, m, α-CH), 4.30 (1 H, dd, C_4-H), 4.17 (1 H, dt, C_5-H), 4.13 (2 H, d, CH_2O), 1.86 (2 H, m, CH_2), 1.69 (2 H, m, CH_2), 1.56, 1.53, 1.44, 1.34 (12 H, 4 s, 4 CH_3), 1.25 (18 H, m, 9 CH_2), 0.88 (3 H, t, CH_3).

Analysis $C_{27}H_{47}NO_8$ (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 (44j):

44i (0.3 g, 0.58 mmol) was stirred in a mixture of MeOH (10 ml) and N NaOH (2 ml) as in Method G.

Yield: (95.6 %)

MS m/z (%): 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).

^1H NMR ($CDCl_3$, δ ppm): 7.47 (1 H, dd, NH), 4.61 (1 H, d, C_3-H), 4.55 (1 H, m, α-CH), 4.34 (1 H, dd, C_4-H), 4.18 (1 H, dt, C_5-H), 4.13 (2 H, d, CH_2O), 1.92 (2 H, m, CH_2), 1.76 (2 H, m, CH_2), 1.53, 1.47, 1.35 (12 H, s, 4 CH_3), 1.27 (18 H, m, 9 CH_2), 0.87 (3 H, t, CH_3).

Analysis $C_{26}H_{45}NO_8$ (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 (44k):

Acid **44j** (1.0 g, 2.0 mmol) was stirred in a mixture of methanol (10 ml) and 70 % acetic acid (10 ml) at 40 °C for 24 hours. The mixture was diluted with brine (50 ml) and extracted with ethylacetate (3 x 50 ml). The organic phase was dried, evaporated and the residue purified by flash chromatography.

Yield: 0.76 g (82.7 %)

MS m/z (%): 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).

^1H NMR ($CDCl_3$, δ ppm): 8.00 - 7.54 (1 H, m, NH), 4.6 - 4.0 (5 H, m, CH_2O, α-CH, 2 sugar-H), 3.75 (1 H, m, sugar-H), 1.90 (2 H, m, CH_2), 1.82 (2 H, m, CH_2), 1.54, 1.46 1.44 (6 H, 3 s, 2 CH_3), 1.22 (18 H, m, 9 CH_2), 0.88 (3 H, t, CH_3).

Analysis $C_{23}H_{41}NO_8$ (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)] (44l):

A mixture of dimer **41f** HCl (0.5 g, 0.96 mmol), 1,2:3,4-di-O-isopropylidene-D-gulonic acid (0.26 g, 0.96 mmol), triethylamine (0.10 g, 1.0 mmol) dicyclohexyl-carbodiimide (0.21 g, 1.0 mmol) and 1-hydroxybenzotriazole (0.14 g, 1.0 mmol) was reacted in dichloromethane (20 ml) using the procedure as described in Method F.

Yield: 0.56 g (80 %)

MS m/z (%): 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).

^1H NMR ($CDCl_3$, δ ppm): 7.37 (1 H, m, NH), 6.63, 6.40 (1 H, 2 m, NH), 4.59 (1 H, d, C_3H), 4.54, 4.45 (2 H, 2 m, 2 x α-CH), 4.34 (1 H, dd, C_4-H), 4.16 (1 H, t, C_5-H), 4.13 (2 H, d, CH_2O), 1.92 (4 H, m, 2 CH_2), 1.77 (4 H, m, 2 CH_2), 1.56, 1.53, 1.44, 1.34 (12 H, 3 s, 1 m, 4 CH_3), 1.23 (36 H, m, 18 CH_2), 0.88 (6 H, t, 2 CH_3).

Analysis $C_{41}H_{74}N_2O_9$ (739.0) Calcd. C 66.63 H 10.09 N 3.79
Found C 66.55 H 9.89 N 3.56

4.2.5.2 Halogen Substituted Lipidic Amino Acids and Oligomers

Methyl 2-bromooctanoate (45d):

As described in Method C, **45d** was prepared using 2-bromo-octanoic acid **45a** (6.0 g, 0.0269 mmol), methanol (132.8 ml) and thionyl chloride (11.17 ml).

Yield: 4.24g (66.5%) (oil).

MS m/z (%): 239 $[M + H]^+$ (2), 238 $[M]^+$ (7), 237 $[M + H]^+$ (8), 236 $[M]^+$ (8), 223 (7), 221 (5), 209 (78), 207 (84), 205 (15), 195 (96), 193 (100), 181 (17), 179 (19), 177 (10), 175 (6).

^1H NMR ($CDCl_3$, δ ppm): 4.20 (1 H, m, α-CH), 3.75 (3 H, s, $COOCH_3$), 1.95 (2 H, m, β-CH_2), 1.38 (2 H, m, γ-CH_2), 1.27 (6 H, m, 3 CH_2), 0.84 (3 H, t, CH_3).

Analysis $C_9H_{17}O_2Br$ (237.1) Calcd. C 45.58 H 7.23 Br 33.35
Found C 45.84 H 7.09 Br 33.58

Methyl 2-bromotetradecanoate (45e).

A mixture of thionyl chloride (15.5 ml), methanol (100 ml, cooled to 0 °C) and 2-bromo-tetradecanoic acid (**45b**) (5.0 g, 16.3 mmol) was reacted as in Method C.

Yield: 3.60 g (69.0 %).

MS m/z (%): 323 $[M + H]^+$ (51), 321 $[M + H]^+$ (56), 279 (32), 277 (100), 243 (26), 241 (47), 209 (5), 199 (6), 179 (4), 154 (10), 137 (30), 123 (6), 109 (10), 95 (15), 87 (45), 81 (17), 69 (27), 55 (40).

^1H NMR ($CDCl_3$, δ ppm): 4.24 (1H, m, α-CH), 3.77 (3 H, s, $COOCH_3$), 2.0 (2 H, m, β-CH_2), 1.44 (2 H, m, γ-CH_2), 1.27 (18 H, m, 9 CH_2), 0.89 (3 H, t, CH_3).

Analysis $C_{15}H_{29}BrO_2$ (321.3) Calcd. C 56.07 H 9.10 Br 24.87
Found C 56.35 H 9.35 Br 24.61

Methyl 2-bromohexadecanoate (45f):

2-Bromo-hexadecanoic acid, **45c** (5.0 g, 0.015 mmol), methanol (74 ml) and thionyl chloride (9.85 g, 6.2 ml) were reacted as in Method C.

Yield: 4.2 g (80.3 %).

MS m/z (%): 351 $[M + H]^+$ (43), 349 $[M + H]^+$ (55), 303 (14), 305 (64), 269 (56), 267 (8), 137 (29), 123 (13), 109 (22), 95 (39), 87 (74), 81 (44), 69 (65), 55 (100).

^1H NMR ($CDCl_3$, δ ppm): 4.26 (1 H, m, α-CH), 3.77 (3 H, s, $COOCH_3$), 2.00 (2 H, m, β-CH_2), 1.45 (2 H, m, γ-CH_2), 1.29 (22 H, m, 11 CH_2), 0.89 (3 H, t, CH_3).

Analysis $C_{17}H_{33}O_2Br$ (349.4) Calcd. C 58.44 H 9.52 Br 22.87
Found C 58.66 H 9.75 Br 23.12

Methyl 2-(2-bromotetradecanamido)dodecanoate (46a):

A mixture of methyl-α-amino-dodecanoate **25j** HCl (1.73 g, 6.51 mmol), 2-bromo-tetradecanoic acid (2.0 g, 6.51 mmol), triethylamine (1.32 g, 13.02 mmol) and 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide HCl (1.25 g, 6.51 mmol) was reacted in dichloromethane (30 ml) using the procedure as described in Method D.

Yield: 2.85 g (84.5 %)

MS m/z (%): 520 $[M + H]^+$ (18), 518 $[M + H]^+$ (20), 419 (7), 391 (17), 170 (43), 127 (10), 113 (15), 95 (17), 85 (27), 71 (63), 57 (90).

^1H NMR ($CDCl_3$, δ ppm): 6.79 (1 H, m, CONH), 4.58 (1 H, m, BrCH), 4.31 (1 H, m, α-CH), 3.74 (1 H, s, $COOCH_3$), 2.08, 1.87 (4 H, m, 2 β-CH_2), 1.69, 1.44 (4 H, m, 2 γ-CH_2), 1.27 (38 H, m, 16 CH_2), 0.90 (6 H, t, 2 CH_3).

Analysis $C_{27}H_{52}NO_3Br$ (518.6) Calcd. C 62.53 H 10.11 N 2.70 Br 15.41
Found C 62.70 H 9.85 N 2.96 Br 15.70

Methyl 2-(2-bromotetradecanamido)tetradecanoate (46b).

A mixture of methyl ester **25k** HCl (1.91 g, 6.5 mmol), 2-bromo-tetradecanoic acid (**45b**) (2.0 g, 6.5 mmol), triethylamine (1.32 g, 13.0 mmol) and 1-ethyl-3-(3-dimethyl amino-propyl)carbodiimide HCl (1.25 g, 6.5 mmol) was reacted in dichloromethane

(50 ml) as described in Method D.

Yield: 3.5 g (99.8 %).

MS m/z (%): 546 $[M + H]^+$ (10), 489 (8), 258 (16), 198 (100), 170 (7), 136 (8), 109 (7), 136 (8), 109 (7), 95 (14), 81 (18), 69 (27).

^1H NMR ($CDCl_3$, δ ppm): 6.82 (1 H, t, CONH), 4.60 (1 H, m, BrCH), 4.32 (1 H, m, α-CH), 3.78 (3 H, s, $COOCH_3$), 2.08, 1.89 (4 H, m, 2 β-CH_2), 1.71 (2 H, m, γ-CH_2), 1.29 (38 H, m, 19 CH_2), 0.89 (6 H, t, 2 CH_3).

Analysis $C_{29}H_{56}NO_3Br$ (546.7) Calcd. C 63.71 H 10.33 N 2.56 Br 14.62
Found C 64.04 H 10.18 N 2.71 Br 14.91.

Methyl 2-(2-bromotetradecanamido)icosanoate (46c):

A mixture of 2-bromotetradecanoic acid **45b** (1.0 g, 3.25 mmol), methyl α-aminoeicosanoate **25l** HCl (1.23 g, 3.25 mmol), triethylamine (0.66 g, 6.5 mmol) and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (0.63 g, 3.25 mmol) was reacted in dichloromethane (25 ml) as described in Method D.

Yield: 1.4833 g (72.4 %)

MS m/z (%): 654 $[M + Na]^+$ (13), 652 $[M + Na]^+$ (14), 606 (5), 577 (25), 477 (10), 441 (22), 282 (13), 149 (33), 125 (13), 69 (42), 55 (91), 42 (100).

^1H NMR ($CDCl_3$, δ ppm): 6.78 (1 H, t, CONH), 4.58 (1 H, m, BrCH), 4.32 (1 H, m, α-CH), 3.76 (1 H, s, $COOCH_3$), 2.08, 1.85 (4 H, m, 2 β-CH_2), 1.69, 1.44 (4 H, m, 2 γ-CH_2), 1.27 (48 H, m, 24 CH_2), 0.90 (6 H, t, 2 CH_3).

Analysis $C_{35}H_{68}NO_3Br$ (630.8) Calcd. C 66.64 H 10.87 N 2.70 Br 12.67
Found C 66.89 H 10.87 N 2.41 Br 12.39

α-(2-bromotetradecanoyl)-ω-methoxybis[imino(1-dodecyl-2-oxo-1,2-ethanediyl)] (46d):

A mixture of C-protected dimer **41f** HCl (0.87 g, 1.71 mmol), 2-bromo-tetradecanoic acid **45b** (0.53 g, 1.71 mmol), triethylamine (0.345 g, 3.42 mmol) and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (0.328 g, 1.71 mmol) was reacted in dichloromethane (25 ml) using the procedure as described in Method D.

Yield: 0.85 g (64.5 %)

MS m/z (%): 795 $[M + Na]^+$ (35), 793 $[M + Na]^+$ (37), 745 (23), 713 (10), 505 (84),

483 (27), 258 (30), 198 (100), 138 (10), 55 (36), 42 (33).

^{1}H NMR ($CDCl_3$, δ ppm): 6.81, 6.35 (2 H, m, 2 CONH), 4.55, 4.39, 4.24 (3 H, m, 3 α-CH), 3.73 (3 H, s, $COOCH_3$), 2.00, 1.56 (6 H, m, 3 β-CH_2), 1.29 (60 H, m, 30 CH_2), 0.87 (9 H, t, 3 CH_3).

Analysis $C_{43}H_{83}N_2O_4Br$ (772.0)	Calcd.	C 66.89	H 10.84	N 3.63	Br 10.35
	Found	C 67.04	H 10.98	N 3.40	Br 10.08

Methyl 2-(2-bromohexadecanamido)tetradecanoate (46e):

Methyl α-aminotetradecanoate (**25k**) (2.0 g, 6.8 mmol), 2-bromo-hexadecanoic acid (2.37 g, 7.08 mmol), triethylamine (1.374 g, 13.6 mmol) and 1-ethyl-3-(3-dimethyl amino-propyl)-carbodiimide HCl (1.303 g, 6.8 mmol) were reacted in dichloromethane (50 ml) as described in Method D.

Yield: 3.02 g (77.4 %)

MS m/z (%): 576 (20), 575 $[M + H]^+$ (9), 574 (23), 516 (9), 514 (10), 258 (17), 198 (100), 136 (16), 95 (17), 81 (18), 69 (28), 55 (40).

^{1}H NMR ($CDCl_3$): δ = 6.77 (1 H, m, CONH), 4.56(1 H, m, BrCH), 4.33 (1 H, m, α-CH), 3.74 (3 H, s, $COOCH_3$), 2.08, 1.85 (4 H, m, 2 β-CH_2), 1.65 (2 H, m, γ-CH_2), 1.24 (42 H, m, 21 CH_2), 0.85 (6 H, t, 2 CH_3).

Analysis $C_{31}H_{60}NO_3Br$ (574.7)	Calcd.	C 64.78	H 10.52	N 2.44	Br 13.90
	Found	C 64.95	H 10.75	N 2.31	Br 14.18

α-(2-bromohexadecanoyl)-ω-methoxybis[imino(1-dodecyl-2-oxo-ethanediyl)] (46f):

C-protected dimer **41f** HCl (0.651 g, 1.25 mmol), 2-bromo-hexadecanoic acid **45c** (0.42 g, 1.25 mmol), 1-hydroxybenzotriazole (0.169 g, 1.25 mmol), triethylamine (0.252 g, 2.5 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (0.24 g, 1.25 mmol) was reacted in dichloromethane (20 ml) using the procedure as described in Method D.

Yield: 0.531 g (53.1 %).

MS m/z (%): 823 (88), 822 $[M + H]^+$ (27), 821 (100), 741 (26), 651 (20), 559 (13), 413 (52), 384 (31), 198 (76), 173 (32), 92 (17).

^{1}H NMR ($CDCl_3$, δ ppm): 6.80, 6.40 (2 H, m, 2 CONH), 4.58, 4.43, 4.30 (3 H, m,

3 α-CH), 3.75 (3 H, s, $COOCH_3$), 2.15, 2.01, 1.90, 1.80, 1.65, 1.42 (6 H, m, 3 β-CH_2), 1.27 (60 H, m, 30 CH_2), 0.87 (9 H, t, 3 CH_3).

Analysis $C_{45}H_{87}N_2O_4Br$ (800.1) Calcd. C 67.55 H 10.96 N 3.50 Br 9.99

Found C 67.72 H 10.78 N 3.65 Br 10.25

47 Minor product R_f **46f** > R_f **47**

Yield: 264 mg (24.7 %)

MS m/z (%) $C_{51}H_{91}N_5O_5$ (854.28): 876 $[M + Na]^+$ (100), 757 (18), 559 (9), 334 (9), 258 (8), 198 (59), 91 (9).

NMR ($CDCl_3$, δ ppm): 8.03 - 6.84 (4 H, m, aromatic-H), 4.98, 4.91, 4.80 (2 H, m, 2 NH), 4.60 - 4.45 (3 H, m, 3 α-CH), 3.78, 3.71, 3.68 (3 H, s, $COOCH_3$), 2.12 (2 H, m, β-CH_2), 1.9, 1.7 (4 H, m, CH_2), 1.28 (64 H, m, 32 CH_2), 0.9 [9 H, s, $C(CH_3)_3$].

4.2.6 GABA Conjugates

4.2.6.1 Lipidic Conjugates with Ester Linkage

4-(*tert*-butoxycarbamido)butanoic acid (51)

GABA **11** (3.0 g, 29 mmol) was dissolved in t-butanol-water (2:3, 60 ml) and reacted with di*tert*-butyl-dicarbonate (6.33 g, 29 mmol) in t-butanol (10 ml) as described in Method B.

Yield: 5.9 g (99 %).

MS m/z (%) $C_9H_{17}NO_4$ (203): 226 $[M + Na]^+$ (45), 204 $[M + H]^+$ (24), 103 (100), 87 (21), 28 (12).

NMR ($CDCl_3$, δ ppm): 4.75 (1 H, m, NH), 3.68 (2 H, t, γ'-CH_2), 2.40 (2 H, t, α'-CH_2), 1.82 (2 H, tt, β'-CH_2C), 1.43 [9 H, s, $C(CH_3)_3$].

Methyl 2-[4-(*tert*-butoxycarbamido)butanoyl]octanoate (53a). Method H:

Potassium salt crown-ether complex 52: A mixture of BOC-GABA (**51**) (5.9 g, 29 mmol) in ethanol (13.6 ml), water (13.6 ml), potassium hydroxide (1.62 g, 29 mmol) and 18-Crown-6 (7.71 g, 29 mmol) was stirred for 4 hours at room temperature. The solvent was evaporated and the residue dried in vacuo.

Esterification: The crown-ether complex prepared above (4.38 g, 8.43 mmol) and **45d**

methyl-2-bromo-octanoate (2.0 g, 8.43 mmol) were stirred in dry dimethylformamide (10 ml) for 24 hours at room temperature. The reaction mixture was diluted with brine (50 ml) and extracted with ether (3 x 30 ml). The organic phase was dried ($MgSO_4$), evaporated and purified by flash chromatography.

Yield: 2.02 g (66.6 %).

MS m/z (%): 382 $[M + Na]^+$ (100), 326 (35), 281 (11), 219 (10), 195 (8), 170 (3), 148 (3), 119 (4), 77 (6), 57 (10), 42 (6).

^1H NMR ($CDCl_3$, δ ppm): 4.69 (1 H, m, OCONH), 4.16 (1 H, m, α-CH), 3.73 (3 H, s, $COOCH_3$), 3.16 (2 H, m, γ'-CH_2), 2.42 (2 H, m, α'-CH_2), 1.82 (2 H, m, β'-CH_2), 1.58 (2 H, m, β-CH_2), 1.42 [9 H, s, $C(CH_3)_3$], 1.27 (8 H, m, 4 CH_2), 0.85 (3 H, t, CH_3).

Analysis $C_{18}H_{33}NO_6$ (359.5) Calcd. C 60.14 H 9.25 N 3.90
Found C 60.28 H 9.13 N 4.02

Methyl 2-[4-(*tert*-butoxycarbamido)butanoyl]tetradecanoate (53b)

BOC-GABA Crown ether potassium salt **52** (3.23 g, 6.23 mmol) and methyl 2-bromo-tetradecanoate **45e** (2.0 g, 6.23 mmol) were reacted in dry dimethylformamide (10 ml) as described in Method H.

Yield: 2.67 g (97.1 %).

MS m/z (%): 466 $[M + Na]^+$ (100), 410 (32), 344 (15), 279 (9), 248 (5), 208 (6), 173 (26), 149 (9), 130 (8), 112 (8), 86 (31), 57 (38), 42 (28).

^1H NMR ($CDCl_3$, δ ppm): 5.00 (1 H, t, OCONH), 4.25 (1 H, m, α-CH), 3.68 (3 H, s, $COOCH_3$), 3.17 (2 H, m, γ'-CH_2), 2.43 (2 H, m, α'-CH_2), 1.97 (2 H, m, β'-CH_2), 1.80 (4 H, m, β-CH_2, γ-CH_2), 1.35 [9 H, s, $C(CH_3)_3$], 0.87 (3 H, t, CH_3).

Analysis $C_{24}H_{45}NO_6$ (443.6) Calcd. C 64.97 H 10.22 N 3.16
Found C 64.78 H 10.10 N 3.05

Methyl 2-[4-(*tert*-butoxycarbamido)butanoyl]hexadecanoate (53c):

BOC-GABA Crown ether potassium salt **52** (2.97 g, 5.73 mmol) and methyl 2-bromo-hexadecanoate **45f** (2.0 g, 5.73 mmol) were reacted in dry dimethylformamide (10 ml) as described in in Method H.

Yield: 1.73 g (64.2 %) (oil)

MS m/z (%): 494 $[M + Na]^+$ (100), 438 (32), 393 (2), 372 (5), 352 (2), 331 (4), 307 (7), 248 (4), 208 (5), 181 (8), 153 (4), 119 (4), 86 (9), 77 (2), 69 (2).

^1H NMR ($CDCl_3$, δ ppm): 5.0 (1 H, t, OCONH), 4.27 (1 H, m, α-CH), 3.73 (3 H, s, $COOCH_3$), 3.17 (2 H, m, γ'-CH_2), 2.42 (2 H, m, α'-CH_2), 1.88 (4 H, m, β'-CH_2, β-CH_2), 1.42 [9 H, s, $C(CH_3)_3$], 1.37 (24 H, m, 12 CH_2), 0.89 (3 H, t, CH_3).

Analysis $C_{26}H_{49}NO_6$ (471.7) Calcd. C 66.20 H 10.47 N 2.97
Found C 66.42 H 10.35 N 2.83

Methyl 2-{2-[4-(*tert*-butoxycarbamido)butanoyl]tetradecanamido}-dodecanoate (53d):

BOC-GABA crown ether potassium salt **52** (1.0 g, 1.93 mmol) was reacted the same way as described in Method H with **46a** dimer (1.0 g, 1.93 mmol) in dry dimethylformamide (10 ml).

Yield: 1.28 g (100%)

MS m/z (%): 663 $[M + Na]^+$ (100), 595 (57), 540 (34), 520 (7), 460 (24), 306 (18), 248 (11), 170 (18), 148 (8), 127 (8), 55 (10).

^1H NMR ($CDCl_3$, δ ppm): 6.80 (1 H, m, CONH), 4.95 (2 H, m, OCONH), 4.54, 4.29 (2 H, m, 2 α-CH), 3.75 (3 H, s, $COOCH_3$), 3.19 (2 H, m, γ'-CH_2), 2.39 (2 H, m, α'-CH_2), 2.08 (2 H, m, β'-CH_2), 1.82 (2 H, m, β-CH_2), 1.69 (2 H, m, γ-CH_2), 1.42 [9 H, s, $C(CH_3)_3$], 1.25 (36 H, m, 18 CH_2), 0.86 (6 H, t, 2 CH_3).

Analysis $C_{16}H_{68}N_2O_7$ (640.9) Calcd. C 67.46 H 10.69 N 4.37
Found C 67.60 H 10.75 N 4.51

Methyl 2-{2-[4-(*tert*-butoxycarbamido)butanoyl]tetradecanamido}tetra decanoate (53e)

BOC-GABA crown ether potassium salt **52** (0.950 g, 1.83 mmol) was reacted with **46b** dimer (1.0 g, 1.83 mmol) using the procedure described in Method H.

Yield: 1.1 g (90.0 %)

MS m/z (%): 691 $[M + Na]^+$ (100), 635 (25), 560 (5), 528 (6), 504 (7), 488 (5), 406 (2), 334 (4), 248 (7), 133 (23), 57 (8), 42 (4).

^1H NMR ($CDCl_3$, δ ppm): 6.82 (1 H, m, CONH), 5.15 (1 H, t, OCONH), 4.63 (2 H, m, 2 α-CH), 3.74 (3 H, s, $COOCH_3$), 3.19 (2 H, m, γ'-CH_2), 2.45 (2 H, m, α'-CH_2),

1.84 (6 H, m, β'-CH_2, 2 β-CH_2), 1.45 [9 H, s, $C(CH_3)_3$], 1.24 (40 H, m, 20 CH_2), 0.87 (6 H, t, 2 CH_3).

Analysis $C_{38}H_{72}N_2O_7$ (669.0) Calcd. C 68.22 H 10.84 N 4.19
Found C 68.38 H 10.71 N 4.31

Methyl2-{2-[4-(*tert*-butoxycarbamido)butanoyl]tetradecanamido}icosanoate (53f):

BOC-GABA crown ether potassium salt **51** (0.988 g, 1.90 mmol) was reacted with **46c** dimer (1.2 g, 1.90 mmol) using the procedure as described in Method H.

Yield: 1.47 g (100 %)

MS m/z (%): 776 $[M + H + Na]^+$ (3), 729 (5), 707 (50), 655 (13), 652 (16), 572 (5), 413 (49), 398 (49), 363 (4), 282 (4), 164 (16), 141 (13), 92 (35), 63 (100).

^1H NMR ($CDCl_3$, δ ppm): 6.76 (1 H, m, CONH), 4.95 (1 H, m, OCONH), 4.56, 4.29 (2 H, m, 2 α-CH), 3.75 (3 H, s, $COOCH_3$), 2.08 (2 H, m, γ'-CH_2), 1.86 (2 H, m, α'-CH_2), 1.70 (4 H, m, β'-CH_2-, β-CH_2), 1.56 [9 H, s, $C(CH_3)_3$], 1.42 (2 H, m, β-CH_2), 1.25 (52 H, m, 26 CH_2), 0.88 (6 H, t, 2 CH_3).

Analysis $C_{44}H_{84}N_2O_7$ (753.1) Calcd. C 70.17 H 11.24 N 3.72
Found C 70.08 H 11.29 N 3.81

α-{2-[4-(*tert*-butoxycarbamido)butanoyl]tetradecanoyl}-ω-methoxybis-[imino(1-dodecyl-2-oxo-ethanediyl)] (53g):

BOC-GABA crown ether complex **52** (0.27 g, 0.52 mmol) was reacted with **46d** trimer (0.4 g, 0.52 mmol) as in Method H.

Yield: 0.2774 g (59.7 %)

MS m/z (%): 916 $[M + Na]^+$ (10), 792 (21), 748 (12), 713 (5), 504 (26), 441 (12), 413 (19), 258 (23), 198 (58), 149 (21), 70 (57), 42 (100).

^1H NMR ($CDCl_3$, δ ppm): 6.81 (2 H, m, 2 CONH), 6.29 (1 H, m, OCONH), 4.56, 4.37, 4.26 (3 H, m, 3 α-CH), 3.73 (3 H, s, $COOCH_3$), 2.05 (4 H, m, γ'-CH_2, α'-CH_2), 1.82 (2 H, m, β'-CH_2), 1.65 (2 H, m, β-CH_2), 1.58 [9 H, s, $C(CH_3)_3$], 1.40 (4 H, m, 2 β-CH_2), 1.27 (60 H, m, 30 CH_2), 0.87 (9 H, t, 3 CH_3).

Analysis $C_{52}H_{99}N_3O_8$ (894.1) Calcd. C 69.83 H 11.16 N 4.70
Found C 69.68 H 11.30 N 4.55

Methyl 2-{2-[4-(*tert*-butoxycarbamido)butanoyl]hexadecanamido}tetradecanoate (53h):

BOC GABA crown-ether complex **52** (439 mg, 0.845 mmol) was reacted with **46e** dimer (477 mg, 0.845 mmol) using the procedure described in Method H.

Yield: 410 mg (69.6 %)

MS m/z (%): 719 $[M + Na]^+$ (100), 663 (13), 597 (9), 532 (4), 258 (4), 198 (17), 176 (5), 112 (5), 86 (16), 57 (16).

^1H NMR ($CDCl_3$ δ ppm): 6.83 (1 H, m, CONH), 5.15 (1 H, m, OCONH), 4.76 - 4.68 (1 H, m, α-CH), 4.58 (1 H, m, α-CH), 3.74 (3 H, s, $COOCH_3$), 3.20 (2 H, m, γ'-CH_2), 2.50 (2 H, m, α'-CH_2), 1.86 (4 H, m, β'-CH_2, β-CH_2), 1.72 (2 H, m, β-CH_2), 1.45 [9 H, s, $C(CH_3)_3$], 1.28 (44 H, m, 22 CH_2), 0.87 (6 H, t, 2 CH_3).

Analysis $C_{40}H_{76}N_2O_7$ (697.0) Calcd. C 68.92 H 10.99 N 4.02
Found C 69.05 H 10.82 N 4.11.

α-{2-[4-(*tert*-butoxycarbamido)butanoyl]hexadecanoyl}-ω-methoxybis-[imino-(1-dodecyl-2-oxo-ethanediyl)] (53i):

BOC GABA crown-ether potassium salt **52** (64.8 mg, 0.125 mmol) was reacted with **46f** trimer using the procedure described in Method H.

Yield: 71 mg (61.6%)

MS m/z (%): 945 $[M + Na]^+$ (100), 917 (7), 889 (9), 823 (14), 758 (6), 742 (5).

^1H NMR ($CDCl_3$, δ ppm): 7.15 - 6.5 (3 H, m, 2 CONH, OCONH), 5.05 (1 H, m, α-CH), 4.83 (1 H, m, α-CH), 4.5 (1 H, m, α-CH), 3.75, 3.42 (3 H, 2 s,$COOCH_3$), 3.25 - 3.15 (2 H, m, γ'-CH_2), 2.52 - 2.42 (2 H, m, α'-CH_2), 1.95 (2 H, m, β'-CH_2), 1.80 (2 H, m, β-CH_2), 1.63 (4 H, m, 2 β-CH_2), 1.42 [9 H, s, $C(CH_3)_3$], 1.28 (64 H, m, 32 CH_2), 0.87 (9 H, t, 3 CH_3).

Analysis $C_{54}H_{103}N_3O_8$ (922.4) Calcd. C 70.31 H 11.26 N 4.56
Found C 70.48 H 11.32 N 4.67

Methyl 2-(4-aminobutanoyl)octanoate (54a) TFA: Method I.

53a (190 mg, 0.53 mmol) was stirred in trifluoroacetic acid in dichloromethane (1:1, 1 ml) for 30 min at room temperature. The solvent was removed in vacuo and the residue recrystallised in methanol/ether.

Yield: 128 mg (65 %)

MS m/z (%): 281 $[M - H + Na]^+$ (14), 260 $[M + H]^+$ (16), 219 (9), 195 (4), 159 (15), 133 (5), 101 (15), 77 (10), 55 (9).

^1H NMR ($CDCl_3$, δ ppm): 7.80 (3 H, m, $^+NH_3$), 5.00 (1 H, t, α-CH), 3.71 (3 H, s, $COOCH_3$), 3.11 (2 H, m, γ'-CH_2), 2.58 (2 H, m, α'-CH_2), 2.05 (2 H, m, β'-CH_2), 1.84 (2 H, m, β-CH_2), 1.31 (8 H, m, 4 CH_2), 0.87 (3 H, t, CH_3).

Analysis $C_{15}H_{26}NO_6F_3$ (373.4) Calcd. C 48.25 H 7.02 N 3.75
Found C 48.59 H 7.35 N 3.51.

Methyl 2-(4-aminobutanoyl)tetradecanoate (54b) TFA.

Trifluoroacetic acid:dichloromethane (1:1, 1 ml) was added to **53b** (110 mg, 0.25 mmol) and reacted the same way as Method I.

Yield: 114 mg (100.0 %).

MS m/z (%): 345 $[M + 2H]^+$ (100), 331 (11), 154 (3), 137 (3), 104 (4), 86 (25), 69 (7), 55 (8).

^1H NMR (D_2O, δ ppm): 3.73 (3 H, s, $COOCH_3$), 3.05 (2 H, t, γ'-CH_2), 2.58 (2 H, m, α'-CH_2), 2.00 (2 H, m, β'-CH_2), 1.87 (2 H, m, β-CH_2), 1.29 (20 H, m, 10CH_2), 0.89 (3 H, m, CH_3).

Analysis $C_{21}H_{38}NO_6F_3$ (457.5) Calcd. C 55.13 H 8.37 N 3.06
Found C 55.51 H 8.61 N 2.84.

Methyl 2-(4-aminobutanoyl)hexadecanoate (54c) TFA:

GABA conjugate **53c** (1.0 g, 2.12 mmol) was reacted with trifluoroacetic acid (1 ml) in dichloromethane (1 ml) as described in Method I.

Yield: 1.03 g (100 %)

MS m/z (%): 372 $[M + Na]^+$ (100), 305 (5), 104 (6), 86 (38), 69 (7), 55 (10).

^1H NMR (D_2O, δ ppm): 4.93 (1 H, m, α-CH), 3.72 (3 H, s, $COOCH_3$), 3.04 (2 H, m, γ'-CH_2), 2.51 (2 H, m, α'-CH_2), 1.96 (2 H, m, β'-CH_2), 1.79 (2 H, m, β-CH_2), 1.30 (24 H, m, 12 CH_2), 0.86 (3 H, t, CH_3).

Analysis $C_{23}H_{42}NO_6F_3$ (485.6) Calcd. C 56.89 H 8.72 N 2.89
Found C 57.14 H 8.58 N 2.84

Methyl 2-[2-(4-aminobutanoyl)tetradecanamido]dodecanoate (54d) TFA:

Compound **53d** (105 mg, 0.16 mmol) in trifluoroacetic acid:dichloromethane (1:1, 1 ml) was reacted the same way as Method I.

Yield: 117 mg (100 %).

MS m/z (%): 542 $[M + 2H]^+$ (15), 519 (12), 459 (6), 439 (5), 416 (9), 381 (3), 345 (100), 331 (6), 305 (3), 285 (3), 231 (15), 204 (7), 170 (80), 136 (15), 120 (8), 104 (8), 95 (9), 86 (39), 69 (25), 55 (38).

^1H NMR ($CDCl_3$, δ ppm): 8.08 (3 H, br s, $^+NH_3$), 6.84 (1 H, m, CONH), 4.58, 4.33 (2 H, m, 2 α-CH), 3.76 (3 H, s, $COOCH_3$), 2.09 (2 H, m, γ'-CH_2), 1.87 (2 H, m, α'-CH_2), 1.71 (2 H, m, β'-CH_2), 1.45 (4 H, m, 2 β-CH_2), 1.24 (36 H, m, 18 CH_2), 0.87 (6 H, t, 2 CH_3).

Analysis $C_{33}H_{61}N_2O_7F_3$ (654.8)	Calcd.	C 60.52	H 9.39	N 4.28
	Found	C 60.85	H 9.61	N 4.03

Methyl 2-[2-(4-aminobutanoyl)tetradecanamido]tetradecanoate (54e) TFA:

53e (100 mg, 0.15 mmol) was reacted with TFA:dichloromethane (1:1, 1 ml) as in Method I.

Yield: 66.5 mg (65 %).

MS m/z (%): 591 $[M+Na]^+$ (100), 569 $[M+H]^+$ (88), 555 (10), 506 (10), 484 (5), 312 (5), 258 (29), 244 (5), 198 (69), 176 (6), 104 (8), 86 (22), 69 (10), 55 (12).

^1H NMR ($CDCl_3$, δ ppm): 8.00 (3 H, m, $^+NH_3$), 6.85 (1 H, m, CONH), 4.58 (1 H, m, α-CH), 4.47 (1 H, m, α-CH), 3.75 (3 H, s, $COOCH_3$), 3.09 (2 H, m, γ'-CH_2), 2.58 (2 H, m, α'-CH_2), 2.05 (2 H, m, β'-CH_2), 1.82, 1.65 (4 H, m , 2 β-CH_2), 1.29 (40 H, m, 20 CH_2), 0.89 (6 H, t, 2 CH_3).

Analysis $C_{35}H_{65}N_2O_7F_3$ (682.9)	Calcd.	C 61.55	H 9.59	N 4.10
	Found	C 61.19	H 9.25	N 3.85

Methyl 2[2-(4-aminobutanoyl)tetradecanoyl]icosanoate (54f) TFA:

53f (169 mg, 0.23 mmol) and trifluoroacetic acid:dichloromethane (1:1, 1 ml) were reacted the same way as in Method I.

Yield: 174 mg (100 %)

MS m/z (%): 685 $[M + Na]^+$ (5), 652 $[M]^+$ (29), 632 (6), 572 (14), 418 (8), 340 (10),

304 (5), 282 (100), 198 (11), 176 (13), 154 (7), 136 (15), 109 (10), 95 (19), 81 (22), 69 (42), 55 (68).

^{1}H NMR ($CDCl_3$, δ ppm): 6.85 (1 H, m, CONH), 4.58 (1 H, m, α-CH), 4.32 (1 H, m, α-CH), 3.77 (3 H, s, $COOCH_3$), 2.10 (2 H, m, γ'-CH_2), 1.89 (2 H, m, α'-CH_2), 1.71 (2 H, m, β'-CH_2), 1.45 (4 H, m, 2 β-CH_2), 1.27 (52 H, m, 26 CH_2), 0.89 (6 H, t, 2 CH_2).

Analysis $C_{41}H_{77}N_2O_7F_3$ (767.1)	Calcd.	C 64.20	H 10.12	N 3.65
	Found	C 64.56	H 10.36	N 3.32

α-[2-(4-aminobutanoyl)tetradecanoyl]-ω-methoxybis[imino(1-dodecanoyl-2-oxo-ethanediyl)] (54g) TFA:

53g (60 mg, 0.067 mmol) was left stirring in trifluoroacetic acid:dichloromethane (1:1; 1 ml) as in Method I.

Yield: 60 mg (99 %)

MS m/z (%): 816 $[M + Na]^+$ (100), 794 $[M + H]^+$ (5), 731 (22), 575 (22), 505 (4), 334 (3), 312 (3), 290 (3), 258 (14), 198 (100), 176 (6), 136 (6), 55 (47).

^{1}H NMR ($CDCl_3$, δ ppm): 6.63 (2 H, m, 2 CONH), 4.53, 4.44, 4.18 (3 H, m, 3 α-CH), 3.76 (3 H, s, $COOCH_3$), 2.77 (2 H, m, γ'-CH_2), 2.35 (2 H, m, α'-CH_2), 2.05 (2 H, m, β'-CH_2), 1.84, 1.68 (12 H, m, 3 β-CH_2, 3 γ-CH_2), 1.27 (54 H, m, 27 CH_2), 0.89 (9 H, t, 3 CH_3).

Analysis $C_{49}H_{92}N_3O_8F_3$ (908.2)	Calcd.	C 64.79	H 10.21	N 4.63
	Found	C 64.98	H 10.47	N 4.41

Methyl 2-[2-(4-aminobutanoyl)hexadecanamido]tetradecanoate (54h) TFA:

Conjugate **53h** (80 mg, 0.115 mmol) was reacted as in Method I in trifluoroacetic acid:dichloromethane (1:1, 1 ml).

Yield: 82 mg (100 %)

MS m/z (%): 620 $[M + H + Na]^+$ (100), 598 $[M + 2H]^+$ (69), 517 (2), 413 (2), 258 (6), 198 (21), 154 (4), 136 (5), 104 (7), 86 (18), 69 (8), 55 (8).

^{1}H NMR ($CDCl_3$, δ ppm): 7.32, 6.84 (1 H, m, CONH), 5.04 (1 H, m, α-CH), 4.57, 4.45 (1 H, m, α-CH), 3.63 (3 H, s, $COOCH_3$), 3.09 (2 H, m, γ'-CH_2), 2.56 (2 H, m, α'-CH_2), 2.05 (2 H, m, β'-CH_2), 2.73 (8 H, m, 2 β-CH_2, 2 γ-CH_2), 1.27 (40 H, m,

20 CH_2), 0.86 (6 H, t, 2 CH_3).

Analysis $C_{37}H_{69}N_2O_7F_3$ (710.9) Calcd. C 62.50 H 9.78 N 3.94
Found C 62.82 H 9.98 N 3.70.

α-[2-(4-aminobutanoyl)hexadecanonyl]-ω-methoxybis[imino(1-dodecyl-2-oxo-ethanediyl)] (54i) TFA:

53i (36 mg, 0.039 mmol) was reacted with trifluoroacetic acid:dichloromethane (1:1, 1 ml) as described in Method I.

Yield: 40 mg (100 %)

MS m/z (%): 845 $[M + H + Na]^+$ (98), 823 $[M + 2H]^+$ (79), 760 (14), 689 (3), 560 (3), 506 (4), 340 (5), 258 (20), 198 (100), 104 (8), 86 (32), 69 (10), 55 (13).

^1H NMR ($CDCl_3$, δ ppm): 6.62 (2 H, m, 2 CONH), 4.51, 4.42, 4.18 (3 H, m, 3 α-CH), 3.76 (3 H, s, $COOCH_3$), 2.77 (2 H, m, γ'-CH_2), 2.35 (2 H, m, α'-CH_2), 2.05 (2 H, m, β'-CH_2), 1.84, 1.68 (12 H, m, 3 β-CH_2, 3 γ-CH_2), 1.27 (54 H, m, 27 CH_2), 0.89 (9 H, t, 3 CH_3).

Analysis $C_{51}H_{96}N_3O_8F_3$ (879.30) Calcd. C 69.66 H 11.01 N 4.79
Found C 69.98 H 11.35 N 4.52

Methyl-hexadecanoate-2-yl-γ-(methyl-hexadecanoate-2-yl-amino)-butylate (54j):

GABA crown-ether:

In ethanol (10 ml) and water (5 ml) mixture GABA (2.0 g, 10.63 mmol), potassium-hydroxide (598 mg, 10.63 mmol) and 18-crown-6 (2.907 g, 11.00 mmol) were stirred for 2 hours at room temperature. The solvent was evaporated and the residue dried in vacuo.

Esterification:

GABA-crown-ether complex (500 mg, 1.02 mmol) and **45f** methyl 2-bromo-hexadecanoate (357 mg, 1.02 mmol) were stirred in dimethylformamide (3 ml) for 24 hours. The reaction mixture was diluted with brine (10 ml) and extracted with ether (3 x 10 ml). The organic phase was dried and evaporated. The residue was purified by flash chromatography.

Yield: 450 mg (68.9 %)

MS m/z (%) $C_{38}H_{73}NO_6$ (639.97): 641 $[M + H]^+$ (100), 581 (6), 372 (23), 287 (22), 227 (9), 154 (24), 137 (30), 69 (14), 55 (18).

^1H NMR ($CDCl_3$, δ ppm): 4.15 (2 H, 2 t, 2 αCH), 3.75 (6 H, s, 2 $COOCH_3$), 3.70 (2 H, m, γ'-CH_2), 2.75 (2 H, m, α'-CH_2), 1.80 (2 H, m, β'-CH_2), 1.65 (4 H, m, CH_2), 1.25 (48 H, m, 24 CH_2), 0.85 (6 H, t, CH_3).

4.2.6.2 Aliphatic Alkyl Esters of GABA

Methyl-γ-amino-butylate (56a):

Thionyl chloride (33.0 g, 268.0 mmol) was reacted with γ-aminobutyric acid **11** (6.0 g, 58.0 mmol) in methanol (100ml) as described in Method C.

Yield: 2.06 g (30 %)

MS m/z (%) $C_5H_{11}NO_2$ (117.15): 118 $[M + H]^+$ (100), 104 (15), 101 (17), 86 (9).

NMR ($CDCl_3$, δ ppm): 8.10 (3 H, br m, $^+NH_3$), 3.65 (3 H, s, $COOCH_3$), 3.00 (2 H, t, γ-CH_2), 2.40 (2 H, t, α-CH_2), 2.00 (2 H, t, β-CH_2).

Pentyl-4-(*tert*-butoxycarbamido)butanoate (56b)

BOC-GABA Crown ether potassium salt **52** (1.28 g, 2.46 mmol) was reacted with 1-bromo-pentane **55a** (0.37 g, 2.46 mmol) as in Method H in dry dimethylformamide (10 ml).

Yield: 0.47 g (62 %)

MS m/z (%) $C_{14}H_{27}NO_4$ (273): 296 $[M + Na]^+$ (100), 274 $[M + H]^+$ (4), 240 (10), 218 (14), 199 (1), 176 [M + H - BOC] (10), 174 (9), 154 (5), 137 (3), 112 (1), 86 (4), 76 (1), 68 (1), 56 (6).

NMR ($CDCl_3$, δ ppm): 4.63 (1 H, br s, NH), 4.05 (2 H, t, γ'-CH_2), 3.16 (2 H, m, α'-CH_2), 2.35 (2 H, t , β'-CH_2), 1.81 (2 H, m, α-CH_2), 1.63 (2 H, m, β-CH_2), 1.63 (2 H, m, γ-CH_2), 1.43 [9 H, s, $C(CH_3)_3$], 1.31 (4 H, m, 2 CH_2), 0.92 (3 H, t, CH_3).

Octyl-4-(*tert*-butoxycarbamido)butanoate (56c)

BOC-GABA Crown ether potassium salt **52** (1.28 g, 2.46 mmol) and 1-bromo-octane **55b** (0.48 g, 2.46 mmol) were reacted in dry dimethylformamide (10 ml) as described in Method H.

Yield: 0.55 g (65 %)

MS m/z (%) $C_{17}H_{33}NO_4$ (315): 338 $[M + Na]^+$ (56), 316 $[M + H]^+$ (5), 282 (17), 260 (34), 216 $[M - BOC]^+$ (25), 181 (4), 148 (5), 130 (18), 112 (34), 86 (82), 69 (15), 57 (100).

NMR ($CDCl_3$, δ ppm): 4.66 (1 H, m, NH), 4.06 (2 H, t, γ'-CH_2), 3.15 (2 H, m, α'-CH_2), 2.32 (2 H, t, β-CH_2), 1.82 (2 H, m, α-CH_2), 1.61 (2 H, m, CH_2), 1.42 [9 H, s, $C(CH_3)_3$], 1.26 (10 H, m, 5 CH_2), 0.85 (3 H, t, CH_3).

Pentyl-4-aminobutanoate (56d) TFA:

56b (76 mg, 0.028 mmol) was reacted with trifluoroacetic acid:dichloromethane (1:1, 1 ml) as described in Method I.

Yield: 80 mg (100 %).

MS m/z (%) $C_9H_{19}NO_2$ (287) base (173): 174 $[M + H]^+$ (100), 157(4), 147 (4), 133 (12), 129 (10), 104 (8), 95 (5), 87 (27), 86 (24), 81 (7), 71 (13), 69 (17), 57 (12), 55 (20).

^1H NMR ($CDCl_3$, δ ppm): 4.00 (2 H, m, α-CH_2), 2.88 (2 H, m, β'-CH_2), 2.37 (2 H, m, α'-CH_2), 1.55 (2 H, m, β-CH_2), 1.55 (2 H, m, β-CH_2), 1.25 (4 H, m, 2 CH_2), 0.80 (3 H, m, CH_3).

Octyl-4-aminobutanoate (56e) TFA:

56c (60 mg, 0.019 mmol) was reacted with trifluoroacetic acid:dichloromethane (1:1, 1 ml) as described in Method I.

Yield: 63 mg (100 %).

MS m/z (%) $C_{12}H_{25}NO_2$ (329) base (215): 431 $[2M + H]^+$ (7), 216 $[M + H]^+$ (100), 200 (1), 186 (1), 172 (1), 158 (1), 145 (1), 129 (1), 104 (4), 86 (10), 79 (1), 69 (3), 57 (4).

^1H NMR ($CDCl_3$, δ ppm): 3.95 (2H, m, α-CH_2), 2.85 (2 H, d, γ'-CH_2, 2.33 (2 H, m, α'-CH_2), 1.84 (2 H, m, β'-CH_2), 1.52 (2 H, m, β-CH_2), 1.17 (10 H, m, 5 CH_2), 0.75 (3 H, d, CH_3).

4.2.6.3 Amide Conjugates on the C-terminus of GABA

Methyl 2-[4-(*tert*-butoxycarbamido)butanamido]decanoate (57a):

A mixture of BOC-GABA **51** (1.5 g, 7.39 mmol), **25i** HCl salt (1.76 g, 7.39 mmol), triethylamine (1.5 g, 14.78 mmol) and 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide HCl (1.42 g, 7.39 mmol) was reacted in dichloromethane (50 ml) as described in Method D.

Yield: 1.94 g (68 %)

MS m/z (%): 409 $[M + Na]^+$ (32), 316 (61), 284 (26), 256 (20), 232 (16), 202 (12), 142 (48), 103 (24), 77 (61), 57 (57), 42 (49).

^1H NMR ($CDCl_3$, δ ppm): 6.65 (1 H, m, CONH), 4.74 (1 H, m, OCONH), 4.51 (1 H, m, α-CH), 3.55 (3 H, s, $COOCH_3$), 3.18 (2 H, m, γ'-CH_2), 2.82 (2 H, m, α'-CH_2), 2.31 (2 H, m, β'-CH_2), 1.82, 1.67 (4 H, m, β-CH_2, γ-CH_2), 1.45 [9 H, s, $C(CH_3)_3$], 0.89 (3 H, t, CH_3).

Analysis $C_{20}H_{38}N_2O_5$ (386.5) Calcd. C 62.14 H 9.91 N 7.25
Found C 62.39 H 9.85 N 7.14

Methyl 2-[4-(*tert*-butoxycarbamido)butanamido]tetradecanoate (57b):

A mixture of BOC-GABA **51** (1.5 g, 7.39 mmol), **25k** HCl salt (2.17 g, 7.39 mmol), triethylamine (1.49 g, 14.78 mmol) and 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide HCl (1.42 g, 7.39 mmol) was reacted in dichloromethane (50 ml) as described in Method D.

Yield: 1.63 g (49.9 %)

MS m/z (%): 465 $[M + Na]^+$ (13), 442 (2), 413 (6), 390 (21), 372 (30), 340 (15), 312 (13), 288 (11), 258 (7), 198 (29), 149 (23); 103 (14), 77 (37), 42 (65).

^1H NMR ($CDCl_3$, δ ppm): 6.64 (1 H, m, CONH), 4.80 (1 H, m, OCONH), 4.49 (1 H, m, α-CH), 3.71 (3 H, s, $COOCH_3$), 3.17 (2 H, m, γ'-CH_2), 2.78 (2 H, m, α'-CH_2), 2.29 (2 H, m, β'-CH_2), 1.78, 1.64 (4 H, m, β-CH_2, γ-CH_2), 1.39 [9 H, s, $C(CH_3)_3$], 1.27 (18 H, m, 9 CH_2), 0.85 (3 H, t, CH_3).

Analysis $C_{24}H_{46}N_2O_5$ (442.6) Calcd. C 65.12 H 10.48 N 6.33
Found C 65.37 H 10.53 N 6.43

Methyl 2-[4-(*tert*-butoxycarbamido)butanamido]eicosanoate (57c):

A mixture of BOC-GABA **51** (1.5 g, 7.39 mmol), **25l** HCl (2.79 g, 7.40 mmol), triethylamine (1.5 g, 14.78 mmol) and 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide HCl (1.42 g, 7.39 mmol) was reacted in dichloromethane (50 ml) as described in Method D.

Yield: 2.43 g (62.5 %).

MS m/z (%): 549 $[M + Na]^+$ (100), 497 (5), 449 (12), 413 (5), 309 (3), 296 (4), 282 (2), 251 (2), 180 (3), 166 (2), 153 (3), 133 (6), 119 (5), 77 (4), 57 (5).

^1H NMR ($CDCl_3$, δ ppm): 6.57 (1 H, m, CONH), 5.73 (1 H, m, OCONH), 4.54 (1 H, m, α-CH), 3.71 (3 H, s, $COOCH_2$), 3.18 (2 H, m, γ'-CH_2), 2.75 (2 H, m, α'-CH_2), 2.25 (2 H, t, β'-CH_2), 1.79, 1.65 (4 H, m, β-CH_2, γ-CH_2), 1.43 [9 H, s, $C(CH_3)_3$], 1.25 (30 H, m, 15 CH_2), 0.86 (3 H, t, CH_3).

Analysis $C_{30}H_{58}N_2O_5$ (528.6) Calcd. C 68.40 H 11.10 N 5.32
Found C 68.68 H 11.02 N 5.18

α-[4-(*tert*-butoxycarbamido)butanamido]-ω-methoxybis[imino(1-dodecyl-2-oxo-ethanediyl)] (57d):

A mixture of BOC-GABA **51** (39 mg, 0.19 mmol), **41f** HCl (0.10 mg, 0.19 mmol), triethylamine (38.5 g, 0.38 mmol) and 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide HCl (36.4 g, 0.19 mmol) was reacted in dichloromethane (10 ml) as described in Method D.

Yield: 16 mg (13 %).

MS m/z (%) $C_{38}H_{73}N_3O_6$ (667): 690 $[M + Na]^+$ (100), 674 (2), 634 (6), 616 (3), 590 (16), 588 (7), 559 (8), 534 (4), 503 (3), 478 (3), 461 (2), 434 (2), 405 (7), 353 (2), 334 (6), 305 (3), 258 (8), 198 (57), 173 (3), 154 (9), 136 (12), 107 (10), 69 (16), 57 (27).

^1H NMR ($CDCl_3$, δ ppm): 6.78 - 6.49 (2 H, m, 2 NH), 5.76 (1 H, m, NH), 4.56, 4.44 (2 H, m, 2 α-CH), 3,73 (3 H, s, $COOCH_3$), 3.16 (2 H, m, γ'-CH_2), 2.25 (2 H, t, α'-CH_2), 1.82 (2 H, m, β-CH_2), 1.67 (4 H, m, 2 β-CH_2), 1.44 [9 H, s, $C(CH_3)_3$], 1.25 (36 H, m, 18 CH_2), 0.89 (3 H, t, CH_3).

Methyl 2-{2-[4-(*tert*-butoxycarbamido)butanamido]tetradecanamido}-dodecanoate (57e):

A mixture of BOC-GABA **51** (41 mg, 0.204 mmol), **41e** HCl (0.1 mg, 0.204 mmol), triethylamine (41 mg, 0.41 mmol) and 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide HCl (39 mg, 0.204 mmol) was reacted in dichloromethane (10 ml) as described in Method D.

Yield: 16 mg (12 %).

MS m/z (%) $C_{36}H_{69}N_3O_6$ (639): 662 $[M + Na]^+$, (55), 633 (3), 606 (6), 588 (6), 562 (19), 531 (6), 506 (3), 477 (4), 413 (16), 377 (5), 306 (9), 282 (5), 251 (5), 230 (12), 198 (65), 170 (38), 149 (30), 109 (18), 95 (32), 81 (38), 69 (63), 55 (100).

^1H NMR ($CDCl_3$, δ ppm): 6.60 (2 H, m, 2 NH), 4.75 (1 H, m, OCONH), 4.55, 4.38 (2 H, m, 2 α-CH), 3.73 (3 H, s, $COOCH_3$), 3.16 (2 H, m, γ'-CH_2), 2.44 (2 H, t, α'-CH_2), 1.82 (2 H, m, β'-CH_2), 1.45 (8 H, m, 4 CH_2), 1.27 [9 H, s, $C(CH_3)_3$], 0.89 (6 H, t, 2 CH_3).

Methyl 2-(4-aminobutanamido)decanoate (57f) TFA:

57a (55 mg, 0.014 mmol) was reacted with trifluoroacetic acid:dichloromethane (1:1, 1 ml) as described in Method I.

Yield: 58 mg (100 %)

MS m/z (%) $C_{15}H_{30}N_2O_3$ (286) TFA: 287 $[M + H]^+$ (50), 252 (14), 202 (30), 150 (42), 142 (100), 136 (40), 136 (40), 107 (21), 89 (24), 69 (27), 55 (30).

^1H NMR ($CDCl_3$, δ ppm): 9.00 (3 H, m, $^+NH_3$), 6.88 (1 H, m, CONH), 4.45 (1 H, m, α-CH), 3.71 (3 H, s, $COOCH_3$), 3.17 (2 H, m, γ'-CH_2), 2.52 (2 H, m, α'-CH_2), 2.05 (2 H, m, β'-CH_2), 1.74 (4 H, m, β-CH_2, γ-CH_2), 1.26 (10 H, m, 5 CH_2), 0.88 (3 H, t, CH_3).

Analysis $C_{17}H_{31}N_2O_5F_3$ (400.4)	Calcd	C 50.99	H 7.80	N 7.00
	Found	C 51.25	H 7.98	N 6.38

Methyl 2-(4-aminobutanamido)tetradecanoate (57g) TFA:

57b (100 mg, 0.226 mmol) was reacted with trifluoroacetic acid:dichloromethane (1:1, 1 ml) as described in Method I.

Yield: 102 mg (100 %)

MS m/z (%): 365 $[M + Na]^+$ (12), 312 (4), 288 (7), 258 (5), 222 (2), 198 (23), 173 (5), 150 (4), 128 (4), 77 (7), 54 (12).

^1H NMR ($CDCl_3$, δ ppm): 7.87 (2 H, br. s, NH_2), 6.87 (1 H, m, CONH), 4.22 (1 H, m, α-CH), 3.76 (3 H, s, $COOCH_3$), 3.16 (1 H, m, γ'-CH_2), 2.78 (1 H, m, γ'-CH_2), 2.56 (1 H, m, α'-CH_2), 2.04 (1 H, m, α'-CH_2), 1.82 (2 H, m, β'-CH_2), 1.67 (2 H, m, β-CH_2), 1.27 (20 H, m, 10 CH_2), 0.89 (3 H, t, CH_3).

Analysis $C_{21}H_{39}N_2O_5F_3$ (456.5) Calcd. C 55.24 H 8.61 N 6.14
Found C 55.49 H 8.95 N 6.38

Methyl 2-(4-aminobutanamido)eicosanoate (57h) TFA:

57c (114 mg, 0.216 mmol) was reacted with trifluoroacetic acid:dichloromethane (1:1, 1 ml) as described in Method I.

Yield: 120 mg (100 %)

MS m/z (%): 427 $[M + H]^+$ (100), 413 (19), 384 (7), 342 (8), 282 (30), 198 (3), 174 (4), 149 (8), 136 (12), 120 (4), 107 (9), 86 (9), 69 (11), 55 (11).

^1H NMR ($CDCl_3$, δ ppm): 6.87 (1 H, m, CONH), 4.47 (1 H, m, α-CH), 3.74 (3 H, s, $COOCH_3$), 3.18 (2 H, m, γ'-CH_2), 2.79 (2 H, m, α'-CH_2), 2.55 (2 H, m, β'-CH_2), 1.81, 1.69 (4 H, m, β-CH_2, γ-CH_2), 1.26 (30 H, m, 15 CH_2), 0.87 (3 H, t, CH_3).

Analysis $C_{27}H_{51}N_2O_5F_3$ (540.7) Calcd. C 59.97 H 9.51 N 5.18
Found C 60.28 H 9.88 N 4.87

4.2.6.4 Amide conjugate on the N-terminus of GABA

Methyl 4-[2-(*tert*-butoxycarbamido)decanamido]butanoate (58a):

BOC-α-aminodecanoic acid 25f (2.45 g, 8.54 mmol), methyl-4-aminobutanoate 56a HCl (1.0 g, 8.54 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.32 g, 6.90 mmol) and triethylamine (1.33 g, 13.20 mmol) were reacted as in Method D.

Yield: 0.780 g (24 %)

MS m/z (%): 409 $[M + Na]^+$ (100), 309 (33), 294 (15), 142 (20), 129 (22), 84 (27), 58 (56).

^1H NMR ($CDCl_3$, δ ppm): 5.40 (1 H, m, CONH), 5.10 (1 H, m, OCONH), 4.20 (1

H, m, α-CH), 3.75 (3 H, s, $COOCH_3$), 3.50 (2 H, m, γ'-CH_2), 3.40 (2 H, m, β'-CH_2), 3.05 (2 H, m, α'-CH_2), 1.65 (2 H, m, β-CH_2), 1.40 [9 H, s, $C(CH_3)_3$], 1.15 (12 H, s, 6 CH_2), 0.90 (3 H, t, CH_3).

Analysis $C_{20}H_{38}N_2O_5$ (386.5) Calcd. C 62.14 H 9.91 N 7.25
Found C 62.38 H 10.52 N 7.54

Methyl 4-{2-[2-(*tert*-butoxycarbamido)decanamido]decanamido}butanoate (58b):

Dimer **41h** (230 mg, 0.506 mmol), methyl-4-aminobutanoate **56a** HCl (60 mg, 0.506 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (106 mg, 0.557 mmol) and triethylamine (102 mg, 1.1 mmol) were reacted the same way as in Method D in dichloromethane (50 ml).

Yield: 114 mg (40 %)

MS m/z (%): 578 $[M + Na]^+$ (100), 479 (35), 273 (15), 176 (64), 142 (68), 102 (36), 57 (31).

^1H NMR ($CDCl_3$, δ ppm): 6.90 (2 H, m, 2 CONH), 5.20 (1 H, m, OCONH), 4.40 (1 H, m, α-CH), 4.00 (1 H, m, α-CH), 3.70 (3 H, s, $COOCH_3$), 3.40 (2 H, m, γ'-CH_2), 3.30 (2 H, m, α'-CH_2), 2.20 (2 H, t, β'-CH_2), 1.60 (4 H, m, 2 β-CH_2), 1.40 [9 H, s, $C(CH_3)_3$], 1.20 (24 H, m, 12 CH_2), 0.90 (6 H, t, 2 CH_3).

Analysis $C_{30}H_{57}N_3O_6$ (555.8) Calcd. C 64.83 H 10.34 N 7.56
Found C 64.98 H 10.52 N 7.31

4.2.6.5 Tritiated GABA Conjugates

4-(*tert*-butoxycarbamido)[2,3-^{3}H]butanoic acid (60)

GABA **11** (20 mg, 0.196 mmol) and ^{3}H-GABA **59** (500 μCi/500 μl 2 % aq. ethanol, 5.1 μmol) was dissolved in t-butanol-water (2:3, 1 ml) and reacted with di*tert*-butyl-dicarbonate (43 mg, 0.20 mmol) in t-butanol (0.2 ml) as described in Method B.

Yield: 45 g (100 %)

Methyl 2-{4-(*tert*-butoxycarbamido)[2,3-^{3}H]butanoyl}tetradecanoate (62):

Crown-ether complex: A mixture of BOC-GABA (61) (20 mg, 0.099 mmol) in ethanol (0.14 ml), water (0.14 ml), potassium hydroxide (1 M, 0.14 mmol) and 18-Crown-6 (26 mg, 0.099 mmol) was stirred for 4 hours at room temperature. The solvent was evaporated and the residue dried in vacuo.

Esterification: The crown-ether complex prepared above (50 mg, 0.099 mmol) and methyl 2-bromo-tetradecanoate (45e) (35 mg, 0.108 mmol) were stirred in dry dimethylformamide (2 ml) for 24 hours at room temperature. The reaction mixture was diluted with brine (10 ml) and extracted with ether (3 x 10 ml). The organic phase was dried ($MgSO_4$), evaporated and purified by preparative thin layer chromatography.

Yield: 34 mg (78 %)

Methyl 2-(4-amino[2,3-^{3}H]butanoyl)tetradecanoate (63) TFA.

62 (34 mg, 195 µCi, 0.077 mmol) was stirred in trifluoroacetic acid in dichloromethane (1:1, 0.5 ml) for 30 min at room temperature. The solvent was removed in vacuo and the residue recrystallised in methanol/ether.

Yield: 35 mg (100 %)

4.2.6.6 ^{14}C-GABA Conjugates

4-(*tert*-butoxycarbamido)[carboxy-^{14}C]butanoic acid:

GABA 11 (20 mg, 0.196 mmol) and ^{14}C-GABA (250 µCi/250 µl in 10 % aq. ethanol, 16.13 µmol) was dissolved in t-butanol-water (2:3, 1 ml) and reacted with di*tert*-butyl-dicarbonate (66 mg, 0.302 mmol) in t-butanol (0.5 ml) as described in Method B.

Yield: 42 g (100 %)

Methyl 2-[4-(*tert*-butoxycarbamido)[carboxy-^{14}C]butanoyl]tetradecanoate:

Crown-ether complex: A mixture of BOC-[^{14}C]GABA (42 mg, 0.21 mmol) in ethanol (0.3 ml), potassium hydroxide (1 M, 0.22 ml) and 18-Crown-6 (66 mg, 0.25 mmol) was stirred for 4 hours at room temperature. The solvent was evaporated and the residue dried in vacuo.

Esterification: The crown-ether complex prepared above (98 mg, 0.19 mmol) and methyl 2-bromo-tetradecanoate **45e** (71 mg, 0.21 mmol) were stirred in dry dimethylformamide (2 ml) for 24 hours at room temperature. The reaction mixture was diluted with brine (10 ml) and extracted with ether (3 x 10 ml). The organic phase was dried ($MgSO_4$), evaporated and purified by preparative thin layer chromatography.

Yield: 87 mg (93 %)

Methyl 2-(4-amino[^{14}C]butanoyl)tetradecanoate (64) TFA.

The fully protected ^{14}C-GABA conjugate prepared above (87 mg, 195 μCi, 0.077 mmol) were stirred in trifluoroacetic acid in dichloromethane (1:1, 1 ml) for 30 min at room temperature. The solvent was removed in vacuo and the residue recrystallised in methanol/ether.

Yield: 80 mg (80 %)

4.2.7 Baclofen Conjugates

3-(4-chlorophenyl)-4-*tert*-butoxycarbamido-butanoic acid (65):

Baclofen **22** (0.50 g, 2.34 mmol) was dissolved in t-butanol-water (2:3, 10 ml) and reacted with di*tert*-butyl-dicarbonate (0.76 g, 3.5 mmol) in t-butanol (2.5 ml) as described in Method B.

Yield: 0.48 g (64 %)

MS m/z (%) $C_{15}H_{20}NO_4Cl$ (313.5): 360 $[M - H + 2Na]^+$ (8), 358 $[M - H + 2 Na]^+$ (22), 338 $[M + Na]^+$ (36), 336 $[M + Na]^+$ (100), 314 (4), 302 (3), 289 (3), 280 (10), 258 (23), 240 (3), 214 (6), 196 (11), 154 (45), 136 (33), 120 (6), 107 (11), 89 (10), 77 (9), 69 (5), 57 (36).

^{1}H NMR ($CDCl_3$, δ ppm): 7.30, 7.13 (4 H, d, Ar-H), 4.50 (1 H, m, OCONH), 3.49 (1 H, m, γ'-CH_2), 3.30 (2 H, m, β'-CH), 2.74, 2.61 (2 H, m, α'-CH_2), 1.41 [9 H, s, $C(CH_3)_3$].

Methyl 2-[3-(4-chlorophenyl)-4-(*tert*-butoxycarbamido)butanoyl]-hexadecanoate (67):

Crown-ether complex 66: A mixture of BOC-baclofen **65** (180 mg, 0.575 mmol) in ethanol (2 ml) and water (2 ml), potassium hydroxide (32 mg, 0.575 mmol) and 18-Crown-6 (152 mg, 0.575 mmol) was stirred for 4 hours at room temperature. The solvent was evaporated and the residue dried in vacuo and used for the esterification below.

Esterification: The crown-ether complex prepared above and methyl 2-bromo-hexadecanoate **45f** (200 mg, 0.575 mmol) were stirred in dry dimethylformamide (5 ml) for 24 hours at room temperature. The reaction mixture was diluted with brine (20 ml) and extracted with ether (3 x 20 ml). The organic phase was dried ($MgSO_4$), evaporated and purified by preparative TLC (eluent, dichloromethane:methanol, 100:1).

Yield: 108 mg (32 %)

R_f = 0.50.

MS m/z (%): 606 $[M + Na]^+$ (19), 604 $[M + Na]^+$ (47), 571 (3), 548 (13), 482 (27), 448 (2), 307 (4), 281 (5), 240 (13), 196 (75), 176 (13), 138 (21), 73 (34), 57 (100).

^1H NMR ($CDCl_3$, δ ppm): 7.28, 7.15 (2 H, d, Ar-H), 4.9 (1 H, m, α-CH), 4.51 (1 H, m, OCONH), 3.69, 3.64 (3 H, s, $COOCH_3$), 3.45 (1 H, m, β'-CH), 3.30 (2 H, m, γ'-CH_2), 2.74 (2 H, m, α'-CH_2), 1.72 (2 H, m, β-CH_2), 1.40 [9 H, s, $C(CH_3)_3$], 0.88 (3 H, t, CH_3).

Methyl 2-[3-(4-chlorophenyl)-4-aminobutanoyl]hexadecanoate (68) TFA:

The fully protected baclofen conjugate **67** (58 mg, 0.10 mmol) were stirred in trifluoroacetic acid in dichloromethane (1:1, 1 ml) for 2 hours at room temperature. The solvent was removed in vacuo and the residue recrystallised in methanol/ether.

Yield: 57 mg (96 %)

MS m/z (%) $C_{27}H_{44}NO_4Cl$ (595.5) TFA: 507 $[M + H + Na]^+$ (21), 505 $[M + H + Na]^+$ (55), 485 $[M + 2H]^+$ (38), 483 $[M + 2H]^+$ (100), 469 (2), 449 (2), 309 (2), 196 (22), 179 (4), 154 (6), 117 (2), 69 (4), 55 (5).

^1H NMR ($CDCl_3$, δ ppm): 7.82 (3 H, br s, $^+NH_3$), 7.31, 7.15 (4 H, d, Ar-H), 4.93 (1 H, m, α-CH), 3.69 (3 H, s, $COOCH_3$), 3.51, 3.38 (2 H, m, γ'-CH_2), 3.22 (1 H, m, β'-

CH), 2.85 (2 H, m, α'-CH_2), 1.76 (2 H, m, β-CH_2), 1.25 (24 H, m, 12 CH_2), 0.87 (3 H, t, CH_3).

4.2.8 Adenosine Conjugates

5'-O-[2-(*tert*-butoxycarbonylamino)tetradecanoyl]-adenosine (69a): Method J.

A mixture of adenosine **23** (100 mg, 0.37 mmol), BOC-2-amino-tetradecanoic acid (**25h**) (128 mg, 0.37 mmol), 4-dimethylaminopyridine (46 mg, 0.37 mmol) and dicyclohexylcarbodiimide (84 mg, 0.41 mmol) in benzene (20 ml) was stirred 16 hours and dichloromethane (15 ml). The solvent was evaporated and the residue was purified by preparative TLC (solvent system dichloromethane:methanol, 10:1).

Yield: 30 mg (14 %)

MS m/e (%) $C_{29}H_{48}N_6O_7$ (592): 613 $[M - 2H + Na]^+$ (2), 591 $[M - H]^+$ (2), 572 (5), 513 (6), 491 $[M - H - BOC]^+$ (3), 469 (3), 388 (3), 369 (3), 325 (10), 288 (8), 266 (2), 242 (13), 214 (6), 198 (65), 136 (47), 119 (3), 97 (22), 81 (17), 69 (10), 57 (100).

NMR (CD_3OD, δ ppm): 8.12, 7.65 (2 H, m, Ar-H), 6.20 (1 H, m, 1'-H), 4.20 (1 H, m, α-CH), 3.68 (1 H, m, 2'-H), 3.52 (1 H, m, 3'-H), 3.18 (2 H, m, 4'-H), 2.88 (1 H, m, 4'-H), 1.82 (4 H, m, 2 CH_2), 1.38 [9 H, s, $C(CH_3)_3$], 1.29 (18 H, m, 9 CH_2), 0.94 (3 H, t, CH_3).

5'-{2-[2-(*tert*-butoxycarbonylamino)tetradecanamido]tetradecanoyl}-adenosine (69b):

A mixture of adenosine **23** (61 mg, 0.23 mmol), dimer **41j** (130 mg, 0.23 mmol), 4-dimethylaminopyridine (38 mg, 0.30 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (57 mg, 0.3 mmol) and triethylamine (30 mg, 0.3 mmol) was stirred in dichloromethane:benzene (20 ml; 1:1) overnight. The reaction mixture was washed with brine (50 %, 20 ml). The organic layer was then dried ($MgSO_4$) and evaporated. The product mixture was purified by preparative TLC (solvent system, dichloromethane:methanol 10:1).

Yield: 24 mg (13 %)

MS m/e (%) $C_{43}H_{75}N_7O_8$ (817): 816 (2), 759 (2), 647 (35), 619 (18), 592 (2), 547

(15), 519 (8), 496 (3), 449 (3), 390 (3), 320 (3), 291 (3), 242 (9), 198 (100), 176 (8), 136 (7).

NMR (CD_3OD, δ ppm): 8.05, 7.55 (2 H, m, Ar-H), 6.50 (1 H, m, 1'-H), 4.25, 4.06 (2 H, m, 2 α-CH), 3.50 (1 H, m, 2'-H), 3.40 (1 H, m, 3'-H), 3.25 (2 H, m, 4'-H), 2.70 (1 H, m, 4'-H), 1.75 (8 H, m, 4 CH_2), 1.45 [9 H, s, $C(CH_3)_3$], 1.27 (36 H, m, 18 CH_2), 0.90 (6 H, t, 2 CH_3).

α-(5'-adenosin-5'-yloxy)-{ω-*tert*-butoxycarbonyltris[imino(1-dodecyl-2-oxo-ethanediyl)] (69c):

A mixture of adenosine **23** (20 mg, 0.0749 mmol), N-protected trimer **41o** (50 mg, 0.23 mmol), 4-dimethylamino pyridine (8 mg, 0.08 mmol) and 1-ethyl-3-(3-dimethyl amino-propyl)carbodiimide hydrochloride (16 mg, 0.084 mmol) and triethylamine (8.5 mg, 0.084 mmol) was stirred in dichloromethane:benzene (10 ml, 1:1) overnight. The reaction mixture was washed with brine (50 %, 20 ml). The organic layer was then dried ($MgSO_4$) and evaporated. The product mixture was purified by preparative TLC (solvent system, dichloromethane:methanol 10:1).

Yield: 25 mg (32 %).

MS m/e (%) $C_{57}H_{102}N_8O_9$: 914 (2), 871 (8), 831 (33), 772 (2), 731 (9), 574 (2), 483 (2), 421 (2), 334 (2), 258(12), 198 (100), 154 (3), 123 (4).

NMR ($CDCl_3/CD_3OD$, δ ppm): 8.05, 7.57 (2 H, m, Ar-H), 6.50 (1 H, m, 1'-H), 4.90, 4.50, 4.39 (3 H, m, 3 α-CH), 4.00 (1 H, m, 2'-H), 3.70 (2 H, m, 5'-H), 3.50 (1 H, m, 3'-H), 3.20 (1 H, m, 4'-H), 1.85, 1.62 (12 H, m, 6 β-CH_2), 1.45[9 H, s, $C(CH_3)_3$], 1.25 (54 H, m, 27 CH_2), 0.90 (9 H, t, 3 CH_3).

4.2.9 Azidothymidine Conjugates

4.2.9.1 Lipidic AZT Conjugates with Ester Linkage

3'-Azido-5'-O-[2-(*tert*-butoxycarbonylamino)tetradecanoyl]-3'-deoxythymidine (74a):

A mixture of 3'-azido-3'-deoxythymidine **24** (100 mg, 0.37 mmol), 2-BOC-tetradecanoic acid **25h** (128 mg, 0.37 mmol), 4-dimethylaminopyridine (5 mg 0.041

mmol) and dicyclohexylcarbodiimide (84 mg, 0.41 mmol) in dry benzene (10 ml) and dichloromethane (15 ml) was reacted as in Method J. The solvent was evaporated off and the residue was purified by preparative TLC.

Yield: 130 mg (60 %) oily solid.

R_f = 0.72.

MS m/z (%): 638 $[M + 2Na]^+$ (6), 615 $[M + Na]^+$ (13), 589 (1), 572 (13), 537 (3), 493 $[M + H - BOC]^+$ (2), 388 (4), 369 (6), 325 (20), 288 (8), 269 (2), 242 (12), 225 (13), 198 (57), 171 (11), 149 (12), 127 (23), 98 (25), 81 (66), 69 (15), 57 (100).

^1H NMR ($CDCl_3$, δ ppm): 8.86 (1 H, d, 3-H), 7.4, 7.18 (1 H, d, 6-H), 6.15 (1 H, td, 1'-H), 5.0 (1 H, d, OCONH), 4.74 (1 H, d, α-CH), 4.40 (1 H, d, 3'-H), 4.20 (3 H, m, 4'-H, 5'-H), 2.42 (1 H, m, 2'-H), 1.94 (3 H, d, 5-CH_3), 1.70 (4 H, m, β-CH_2, γ-CH_2), 1.40 [9 H, s, $C(CH_3)_3$], 1.26 (18 H, m, 9 CH_2), 0.88 (3 H, t, CH_3).

Analysis $C_{29}H_{48}N_6O_7$ (592.72) Calcd. C 58.76 H 8.16 N 14.18

Found C 58.48 H 8.45 N 14.51

3'-Azido-5'-O-{2-[2-(*tert*-butoxycarbonylamino)tetradecanoylamino]-dodecanoyl}-3'-deoxythymidine (74b):

A mixture of 3'-azido-3'-deoxythymidine **24** (60 mg, 0.22 mmol), 2-[2-(*tert*-butoxycarbamido)tetradecanamido]dodecanoic acid **41i** (120 mg, 0.22 mmol), 4-dimethylaminopyridine (5 mg, 0.03 mmol) and dicyclohexylcarbodiimide (50 mg, 0.24 mmol) was reacted overnight in dry benzene (10 ml) and dichloromethane (10 ml) as in Method J.

Yield: 70 mg (40 %) oily solid.

R_f = 0.78.

MS m/z (%): 836 $[M + 2Na + H]^+$ (5), 813 $[M + Na + H]^+$ (33), 766 (3), 735 (3), 685 (2), 645 (2), 606 (2), 585 (2), 545 (2), 505 (2), 485 (3), 441 (10), 413 (12), 360 (4), 326 (10), 291 (3), 242 (4), 199 (21), 176 (100), 136 (15), 107 (8), 81 (42).

^1H NMR ($CDCl_3$, δ ppm): 10.26 (1 H, br s, 3-H), 7.76 (1 H, m, 6-H), 7.00 (1 H, s, NH), 5.53 (1 H, br d, 1'-H), 5.07 (1 H, br d, OCONH), 4.75, 4.60 (2 H, m, 2 α-CH), 4.42 (1 H, m, 3'-H), 4.22, 3.91 (2 H, m, 5'-H), 2.91 (1 H, m, 4'-H), 2.42 (2 H, m, 2'-H), 1.93 (3 H, s, 5-CH_3), 1.80 (4 H, m, 2 CH_2), 1.51 [9 H, s, $C(CH_3)_3$], 1.31 (36 H, m, 18 CH_2), 0.89 (6 H, t, 2 CH_3).

Analysis $C_{41}H_{71}N_7O_8$ (790.03) Calcd. C 62.33 H 9.06 N 12.41
Found C 62.61 H 8,79 N 12.30

3'-Azido-5'-O-{2-[2-(*tert*-butoxycarbonylamino)tetradecanoylamino]tetradecanoyl}-3'-deoxythymidine (74c):

A mixture of 3'-azido-3'-deoxythymidine **24** (40 mg, 0.15 mmol), dimer **41j** (85 mg, 0.15 mmol), 4-dimethylaminopyridine (15 mg, 0.12 mmol) and dicyclohexylcarbodiimide (34 mg, 0.17 mmol) was reacted for 16 hours in dry benzene (10 ml) and dichloromethane (10 ml) as described in Method J.

Yield: 60 mg (49 %) oily solid.

R_f = 0.79.

MS m/z (%): (817) 840 $[M+Na]^+$ (45), 817 $[M]^+$ (2), 718 $[M+H\text{-}BOC]^+$ (19), 633 (2), 549 (5), 513 (2), 469 (2), 344 (1), 278 (3), 242 (12), 198 (100), 176 (7), 154 (5), 127 (9), 96 (8), 81 (47), 56 (31).

^1H NMR ($CDCl_3$, δ ppm): 8.40 (1 H, br s, 3-H), 7.42 (1 H, s, 6-H), 6.24 (1 H, t, NH), 6.07 (1 H, m, 1'-H), 4.95 (1 H, m, OCONH), 4.64, 4.44 (2 H, m, 2 α-CH), 4.27, 4.05 (2 H, m, 5'-H), 3.49 (1 H, m, 4'-H), 2.44 (2 H, m, 2'-H), 1.93 (3 H, s, 5-CH_3), 1.65 (8 H, m, 2 β-CH_2, 2 γ-CH_2), 1.45 [9 H, s, $C(CH_3)_3$], 1.27 [36 H, m, 18 CH_2], 0.89 (6 H, t, 2 CH_3).

Analysis $C_{43}H_{75}N_7O_8$ (818.08) Calcd. C 63.13 H 9.24 N 11.99
Found C 62.81 H 9.48 N 11.72

3'-Azido-5'-O-{2-[2-(*tert*-butoxycarbonylamino)tetradecanoylamino]icosanoyl}-3'-deoxythymidine (74d):

A mixture of 3'-azido-3'-deoxythymidine **24** (77 mg, 0.29 mmol), dimer **41k** (189 mg, 0.29 mmol), 4-dimethylaminopyridine (5 mg, 0.03 mmol) and dicyclohexylcarbodiimide (66 mg, 0.32 mmol) was reacted for 16 hours in dry benzene (10 ml) and dichloromethane (10 ml) as described Method J.

Yield: 26 mg (10 %) oily solid.

R_f = 0.71.

MS m/z (%): 950 (4), 906 (9), 870 (22), 841 (3), 821 (3), 777 (5), 739 (3), 691 (57), 590 (22), 567 (10), 532 (4), 506 (3), 472 (5), 414 (5), 391 (4), 365 (5), 318 (5), 282

(59), 247 (40), 225 (42), 198 (82), 173 (58), 119 (25), 67 (100).

^{1}H NMR ($CDCl_3$, δ ppm): 8.39 (1 H, br s, 3-H), 7.43 (1 H, s, 6-H), 6.54 (1 H, m, NH), 6.07 (1 H, t, 1'-H), 4.95 (1 H, br s, OCONH), 4.55, 4.38 (2 H, m, 2 α-CH), 4.12 (1 H, m, 3'-H), 3.95 (1 H, m, 4'-H), 3.45 (2 H, m, 5'-H), 2.43 (2 H, m, 2'-H), 1.89 (3 H, s, 5-CH_3), 1.64 (8 H, m, 2 β-CH_2, 2 γ-CH_2), 1.41 [9 H, s, $(CH_3)_3$], 1.16 (48 H, m, 24 CH_2), 0.88 (6 H, t, 2 CH_3).

Analysis $C_{49}H_{87}N_7O_8$ (902.24) Calcd. C 65.23 H 9.72 N 10.87
Found C 65.53 H 10.01 N 10.99

α-(3'-Azido-3',5'-deoxythymidin-5'yloxy)-{ω-*tert*-butoxycarbonyltris[imino(1-dodecyl-2-oxo-ethanediyl)] (74e):

A mixture of 3'-azido-3'-deoxythymidine **24** (74 mg, 0.28 mmol), trimer **41o** (220 mg, 0.28 mmol), 4-dimethylaminopyridine (38 mg, 0.3 mmol), 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide HCl (60 mg, 0.31 mmol) and triethylamine (30 mg, 0.30 mmol) was stirred in dichloromethane:benzene (30 ml, 1:1) overnight. The reaction mixture was washed with brine (50 %, 20 ml). The organic layer was then dried ($MgSO_4$) and evaporated. The product mixture was purified by preparative TLC.

Yield: 200 mg (70 %) oily solid.

R_f = 0.75.

MS m/z (%): 1066 $[M+H+Na]^+$ (7), 1044 $[M+2H]^+$ (25), 1018 (100), 1012 (17), 989 (11), 975 (11), 957 (9), 945 (51), 918 (17), 902 (13), 890 (12), 869 (7), 850 (10), 836 (7), 823 (10).

^{1}H NMR ($CDCl_3$, δ ppm): 8.50 (1 H, br s, 3-H), 7.38 (1 H, m, 6-H), 7.10, 6.68 (2 H, m, 2 NH), 5.93 (1 H, m, 1'-H), 5.05 (1 H, m, OCONH), 4.63 (1 H, m, α-CH), 4.40 (3 H, m, 2 α-CH, 3'-H), 4.25 (1 H, m, 4'-H), 4.02, 3.92 (2 H, m, 5'-H), 2.45 (2 H, m, 2'-H), 1.92 (3 H, d, 5-CH_3), 1.81, 1.61 (6 H, m, 3 β-CH_2), 1.47 [9 H, s, $C(CH_3)_3$], 1.30 (60 H, m, 30 CH_2), 0.88 (9 H, t, 3 CH_3).

Analysis $C_{57}H_{102}N_8O_9$ (1043.44). Calcd. C 65.61 H 9.85 N 10.74
Found C 65.92 H 10.11 N 10.98

4.2.9.2 Lipidic AZT Conjugate with Sulphide Linkage

3'-Azido-3',5'-dideoxythymidine-5'-tosylate (76):

3'-Azido-3'-deoxythymidine 24 (100 mg, 0.37 mmol) was dissolved in pyridine (10 ml) and stirred at 0 °C. Tosyl chloride (80 mg, 0.37 mmol) was then added to the mixture and left stirring at room temperature for 4 days. The pyridine was then evaporated and the residue was then titurated with ethyl acetate and dil. sulphuric acid (1 M). Ethyl acetate extract was dried ($MgSO_4$) and evaporated.

Yield: 150 mg (97 %) oil.

R_f = 0.78.

IR (thin film): υ = 2120 cm^{-1} (N_3 stretch), 1700 cm^{-1} (C=O stretch).

MS m/z (%): 466 $[M + 2Na - H]^+$ (7), 444 $[M + Na]^+$ (35), 422 $[M + H]^+$ (6), 413 (10), 360 (6), 329 (12), 308 (5), 281 (3), 245 (8), 217 (5), 199 (7), 176 (100), 154 (37), 136 (40), 127 (25), 120 (10), 107 (20), 91 (24), 81 (46), 69 (47), 58 (6).

^1H NMR ($CDCl_3$, δ ppm): 9.10 (1 H, s, 3-H), 7.80, 7.40 (4 H, d, OTs-H), 7.35 (1 H, d, 6-H), 6.20 (1 H, t, 1'-H), 4.33 (2 H, m, 5'-H), 4.25 (1 H, m, 3'-H), 4.00 (1 H, m, 4'-H), 2.40 (2 H, m, 2'-H), 1.95 (3 H, d, 5-CH_3).

Analysis $C_{17}H_{19}N_5O_6S$ (421.42) Calcd. C 48.45 H 4.54 N 16.62
Found C 48.72 H 4.79 N 16.81

3'-Azido-3',5'-dideoxythymidin-5'-yl Octadecyl Sulphide (79):

Octadecanethiol 77 (72 mg, 0.25 mmol) and sodium hydride (50 % in oil, 25 mg, 0.31 mmol) were stirred in dry THF (1.5 ml) under nitrogen for 2 h. 3'-azido-3'-deoxythymidine-5'-tosylate 76 (100 mg, 0.25 mmol) was then added to the reaction mixture and stirred under nitrogen for 2 days. The THF was evaporated off and the reaction mixture was redissolved in dichloromethane (1 ml) and filtered. The compound was then purified by preparative TLC using eluent CH_2Cl_2/MeOH (9:1).

Yield: 40 mg (30 %) oil.

R_f = 0.74.

MS m/z (%): 580 $[M + 2Na - H]^+$ (16), 558 $[M + Na]^+$ (50), 531 (3), 516 (3), 410 (12), 382 (8), 299 (94), 282 (4), 265 (3), 243 (5), 196 (4), 176 (13), 149 (40), 127 (100), 97 (32).

^{1}H NMR ($CDCl_3$, δ ppm): 8.95 (1 H, s, 3-H), 7.37 (1 H, d, 6-H), 6.15 (1 H, t, 1'-H), 4.25 (1 H, m, 3'-H), 4.05 (1 H, m, 4'-H), 2.90, 2.83 (2 H, m, 5'-H), 2.60 (2 H, m, α S-CH_2), 2.45, 2.35 (2 H, m, 2'-H), 1.95 (3 H, d, 6-CH_3), 1.60 (2 H, m, β S-CH_2), 1.37 (2 H, m, γ S-CH_2), 1.25 (28 H, m, 14 CH_2), 0.90 (3 H, t, CH_3).

Analysis $C_{28}H_{49}N_5O_3S$ (535.77) Calcd. C 62.77 H 9.22 N 13.07

Found C 63.05 H 9.53 N 13.35

4.3 EXPERIMENTAL: Pharmacology

4.3.1 *In vivo*: Determination of Brain Penetration Index in mouse.

Mice (25 g) were injected intravenously (tail vein) with ^{14}C-Gaba conjugate of specified radioactivity (Chapter 3) in 4 % BSA. The mice were killed after 5 min, 10 min, 12 min, 20 min and 30 min. The brain and liver were removed and frozen overnight at -20 ^{o}C. These organs were then thawed and homogenised (Potter-Elvejhem homogeniser) with 0.2 ml water per 100 µg. 100 µl aliquots were counted by Liquid Scintillation Spectrometry.

4.3.2 *In vitro* Assay:

4.3.2.1 Analysis of the displacement of tritiated GABA at $GABA_A$ receptor binding site to rat brain synaptosomal membranes by GABA and baclofen conjugates.

Crude synaptic membranes were prepared by homogenization of whole rat brain in 10 vol ice-cold 0.32 M sucrose. The homogenate was centrifuged at 1000 g for 10 min and the supernatant collected and recentrifuged at 20000 g for 20 min. The pellet obtained from this second centrifugation was dispersed in distilled water (volume equal to original sucrose solution) and centrifuged at 8000 g for 20 min. The supernatant together with the buffy layer on the pellet was then centrifuged at 50000 g for 20 min. The final pellet was stored at -15 ^{o}C. For the assay, pellets which had been frozen for at least 16 hours were allowed to thaw for 20 min at room temperature before resuspension in buffer (Tris-HCl solution containing 2.5 mM $CaCl_2$). The pellet obtained from the equivalent of one rat brain was resuspended in 10 ml buffer. The suspension was incubated for 45 min at 20 ^{o}C before centrifugation at 7000 g for 10 min. This washing procedure was repeated three more times allowing 15 min incubation with each addition of buffer. The final pellet was resuspended in the buffer for the assay. Samples in DMSO and aliquot were mixed with buffer (Tris HCl + Ca^{2+} buffer) to give 1 % DMSO. To each 0.8 ml aliquot of

membrane suspension 0.1 ml buffer containing unlabelled GABA conjugates at final concentration of 1 μM were added to each incubation mixture together with 0.1 ml buffer containing a fixed concentration of ^{3}H-GABA, baclofen (100 μM) and isoguvacine (1 μM final concentration). The mixture was incubated for 10 min at 20 ^{o}C and then centrifuged at 7000 g for 10 min. The supernatant was aspirated off and the pellet blotted dry before the addition of 100 μl Soluene. The tritium level of each pellet was determined by liquid scintillation spectrometry. As the total amount bound was < 3 % of the radiolabel in the incubation medium, the 'free' concentration was taken as equivalent to the total tritium concentration. Data derived from experiments performed in triplicate at each concentration.

4.3.2.2 Analysis of the displacement of tritiated GABA on the $GABA_B$ receptor site to rat brain synaptosomal membranes by baclofen conjugate.

Assays were performed according to the method described as in $GABA_A$ binding experiment. Samples of conjugates of concentration 100μM, (±)-baclofen (10μM) and isoguvacine (100 μM) were used.

REFERENCES

Ahluwalia, G., Cooney, D.A., Mitsuya, H., Fridland, A., Flora, K.P., Hao, Z., Dalal, M., Broder, S. and Johns, D.G. (1987) *Biochem. Pharmacol.* **36**, 3797 - 3800.

Albertson, N.F.J. (1946) *J. Am. Chem. Soc.* **68**, 450.

Arnon, R. and Hurwitz, E. (1985) In: *Monoclonal Antibodies for Cancer Detection and Therapy* (Acad. Press, Lond.) p 367 - 383.

Attwood, D. and Florence, A.T. (1983) In: *Surfactant Systems: Their Chemistry, Pharmacy and Biology* (Chapman and Hall, London, N.Y.).

Baldwin, M.A, Welham, K.J., Toth, I. and Gibbons, W.A. (1988) *Org. Mass Spectrometry*, **23**, 697 - 699.

Bergmann, M. and Zervas, L. (1932) *Ber. dtsch. Chem. Ges.* **65**, 1192.

Berlin, R.D. and Oliver, J.M. (1975) *Int. Rev. Cytol.* **42**, 287.

Birnbaum, S.M., Fu, S.-C.J. and Greenstein, J.P. (1953) *J. Biol. Chem.* **203**, 333.

Bodin, N.O., Ekström, B., Forsgren, U., Jalar, L.P., Magni, L., Ramsey, C.H. and Sjöberg, B. (1975) *Antimicrob. Agents Chemother.* **9**, 528 - 525.

Bodor, N. and Brewster, M.E. (1983) *Pharmacol. Therap.* **19**, 337 - 386.

Bodor, N., Farag, H.H. and Brewster, M.E. (1981) *Science* **214**, 1370 - 1372.

Bodor, N., Shek, E. and Higuchi, T. (1976) *J. Med. Chem.* **19**, 102 - 107

Bodor, N., Sloan, K.B., Kuo, Y.-N. and Higuchi, T. (1978) *J. Pharm. Sci.* **67**, 1045 - 1050.

Bowery, N.G. (1989) *Trends in Pharmacol. Sci. Reviews.* **10**, 401 - 407.

Bowery, N.G., Hill, D.R., Hudson, A.L., Doble, A., Middlemiss, D.N., Shaw, J. and Turnbull, M. (1980) *Nature*, **283**, 92 - 94.

Brenner, M. and Huber, W. (1953) *Helv. Chim. Acta* **36**, 1109.

Brightman, M.W., and Reese, T.S. (1969) *J. Cell. Biol.* **40**, 648.

Brightman, M.W. (1977) *Exp. Eye Res.* **25**, 1.

Bubley, G.J., Crumpacker, C.S. and Schnipper, L. (1986) *Antimicrob. Agents Chemother.* **29**, 716.

Bundgaard, H. (1982) In: *Optimisation of Drug Delivery* (eds. H. Bundgaard, A.B. Hansen, and H. Kofod, Munksgaard, Copenhagen) p 178 - 197.

Bundgaard, H. and Falch, E. (1985) *Int. J. Pharm.* **23**, 223 - 237.

Bundgaard, H. and Johansen, M. (1980a) *J. Pharm. Sci.* **69**, 44 - 46.

Bundgaard, H. and Johansen, M. (1980b) *Arch. Pharm. Chem., Sci. Ed.* **8**, 29 - 52.

Bundgaard, H. and Johansen, M. (1980c) *Int. J. Pharm.* **7**, 129 - 136.

Bundgaard, H. and Johansen, M. (1981a) *Int. J. Pharm.* **8**, 183 - 192.

Bundgaard, H. and Johansen, M. (1981b) *Int. J. Pharm.* **9**, 7 - 16.

Bundgaard, H. and Johansen, M. (1981c) *Acta Pharm. Suec.* **18**, 129 - 134.

Bundgaard, H., Johansen, M., Stella, V. and Cortese, M. (1982) *Int. J. Pharm.* **10**, 181 - 192.

Callery, P.S., Stogniew, M. and Geelhaar, L.A. (1979) *Biomed. Mass Spectrometry* **6**, 23 - 26.

Callery, P.S., Geelhaar, L.A., Nayar, M.S.B., Stogniew, M. and Rao, K.G. (1982) *J. Neurochem.* **38**, 1063 - 1067.

Carpino, L.A. (1957) *J. Am. Chem. Soc.* **79**, 98.

Carpino, L.A. and Han, G.Y. (1972) *J. Org. Chem.* **37**, 3404.

Chien, Y.W., Siddiqui, O., Sun, Y., Shi, W.M. and Liu, J.C. (1987) *Ann. N. Y. Acad. Sci.* **507** (Bio. Approaches Controlled Delivery Drugs), 32 - 51.

Chien, Y.W., Lelawongs, P. Siddiqui, O., Sun, Y. and Shi, W.M. (1990) *J. Controlled Release* **13 (2-3)**, 263 - 278.

Clayton, J.P., Cole, M., Elson, S.W. and Ferres, H. (1974) *Antimicrob. Agents Chemother.* **5**, 670 - 671.

Ciesielski, L., Similer, S., Gensburger, C., Mandel, P., Taillandier, G., Benoit-Guyod, J.L., Boucherie, A., Cohen-Addad, C. and Lajzeowicz, J. (1979) In: *GABA Biochemistry and CNS Functions.* (eds. P. Mandel and F.V. Defeudis), 21 - 43.

Connor, J., Sullivan, S. and Huang, L. (1985) *Pharmac. Ther.* **28**, 341 - 365.

Cooney, D.A., Dalal, M., Mitsuya, H., McMahon, J.B., Nadkarni, M., Balzarini, J., Broder, S. and Johns, D.G. (1986) *Biochem. Pharmacol.* **35**, 2065 - 2068.

Crouthamel, W.G., Tan, G.H., Dittert, L.W. and Doluisio, J.T. (1971) *J. Pharm. Sci.* **60**, 1160 - 1164.

Crumpacker, C.S., Schnipper, L.E., Marlowe, S.I., Kowalsky, P.N., Hershey, B.J. and Levin, M.J. (1982) *New Eng. J. Med.* **306**, 343.

Daehne, W.V., Frederiksen, E., Gundersen, E., Lund, F., Mørch, P., Petersen, H.I., Roholt, K., Tybring, L. and Godtfredsen, W.O. (1970) *J. Med. Chem.* **13**, 607 - 612.

Davis, S.S., Washington, C., West, P., Illum, L., Liversidge, G., Sternson, L. and Kirsh, R. (1987) In: *Biological Approach to the Controlled Delivery of Drugs: Barriers, technologies and therapies.* (N.Y. Acad. Sci.).

Davis, S.S. and Illum, L. (1986) In: *Site Specific Drug Delivery (Cell Biology, Medical and Pharmaceutical Aspects).* (eds. E. Tomlinson and S.S. Davis) p 93 - 110.
De Clercq, E., ed. (1990) In: *Design of Anti-Aids* (Elsevier Science Publishing, N.Y.) p 1 - 24.

De Duve, C., De Barsey, T., Poole, B., Trouet, A., Tulkens, P. and Van Hoof, F. (1974) *Biochem. Pharmacol.* **23**, 2495.

DeFeudis, F.V. and Mandel, P., eds. (1981) *Amino Acid Neurotransmitters* (Raven Press, New York).

Derome, A.E. (1988) In: *Modern NMR Techniques for Chemistry Research* (Pergamon Press, Oxford) p 63 - 76.

De Silva, M., Hazleman, B.L., Page Thomas, D.P. and Wraight, P. (1979) *Lancet.* **1**, 1320 - 1322.

Deverre, J.R., Loiseau, P., Couvreur, P., Letourneux, Y., Gayral, P. and Benoit, J.P. (1989) *J. Pharm. Pharmacol.* **41**, 191 - 193.

Di Chiara, G. and Gessa, G.L., editors (1981) In: *GABA and the Basal Ganglia* (Raven Press, New York).

Duggan, D.E., Hooke, K.F., Risley, E.A., Shen, T.Y. and Van Arman, C.G. (1977) *J. Pharmacol. Exp. Therap.* **201**, 8 - 13.

Duggan, D.E. (1981) *Drug Metab. Rev.* **12**, 325 - 337.

Duncan, R. (1985) *CRC Critical Reviews in Therapeutic Drug Carrier Systems* **1(4)**, 281 - 310.

Eckstein, F. (1965) *Angew. Chem. Int. Ed.* **4**, 876.

El-Samaligy, M.S. and Rohdewald, P. (1983) *J. Pharm. Pharmacol.* **35**, 537 - 539.

Farrar, T.C. and Becker, E.D., eds. (1971) In: *Pulse and Fourier Transform NMR* (Academic Press, N.Y.).

Fischer, E. and Warburg, D. (1905) *Ber. dtsch. Chem. Ges.* **38**, 3997.

Frey, H.-H. and Löscher, W. (1980) *Neuropharmacology*, **19**, 217 - 220.

Furman, P.A., Fyfe, J.A., St. Clair, M.H., Weinhold, K., Rideout, J.L., Freeman, G.A., Nusinoff Lehrman, S.m Bolognesi, D.O., Broder, S., Mitsuya, H. and Barry, D.W. (1986) *Proc. Natl. Acad. Sci. USA.* **83**, 8333 - 8337.

Fujimoto, S. Miyazaki, M., Endoh, F., Takahashi, O., Okui, K. and Morimoto, Y. (1985) *Cancer Drug Delivery*, **2**, 173 - 181.

Fujiwara, T., Maeta, H., Chida, S., Morita, T., Watabe, Y. and Abe, T. (1980) *Lancet.* **1**, 55 - 59.

Gallego, J., Price, M.R. and Baldwin, R.W. (1984) *Int. J. Cancer.* **33**, 737 - 744.

Gallo, R.C., Salahuddin, S.Z., Shearer, G.M., Kaplan, M., Haynes, B.F., Palker, T.J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P. and Markham, P.D. (1984) *Science* **224**, 500 - 503.

Galzigna, L., Garbin, L., Bianchi, M. and Marzotta, A. (1978) *Arch. Int. Pharmacodyn.* **235**, 73 - 85.

Gartner, S., Markovits, P. Karkovits, D.M., Kaplan M.H., Gallo, R.C. and Popovic, M. (1986) *Science* **233**, 215 - 219.

Gerencevic, N., Castek, A., Sateva, M., Pluscec, J. and Prostenik, M. (1966) *Monatsch. Chem.* **97**, 331.

Ghose, T. and Cerini, M.C (1969) *Nature* **222**, 993 - 994.

Ghose, T. and Nigam, S.P. (1972) *Cancer* **29**, 1398 - 1400.

Gibaldi, M. (1979) In: *Drug Absorption* (eds. C.F. Prescott and W.S. Nimmo, Adis Press, Sydney) p 1 - 5.

Gibbons, W.A. (April 19, 1988) *Brit. Pat. Appl.* GB 2217319.

Gibbons, W.A., Hughes, R.A., Charalambous, M., Christodoulou, Szeto, A. Aulabaugh, A.E., Mascagni, P. and Toth I. (1990) *Liebigs Ann. Chem.* 1175 - 1183.

Gout, E., Zador, M. and Béguin, C.G. (1984) *Nouv. J. Chimie* **8**, 243 - 250.

Gregoriadis, G. and Ryman, B.E. (1972) *Eur. J. Biochem.* **24**, 485 - 491.

Gregoriadis, G. (1980) In: *Liposomes in Biological Systems.* (eds. G. Gregoriadis and A.C. Allison)

Gros, L., Ringsdorf, H. and Shupp, H. (1981) *Angew. Chem. Int. Ed. Engl.* **20**, 305 - 325.

Gruner, S.M. (1987) In: *Liposomes* (ed. M. Ostro, Dekker, N.Y.) p 1 - 38.

Haase, A.T. (1986) *J. Infect. Dis.* **153**, 441.

Hashida, M., Takakura, Y., Matsumoto, S., Sasaki, H., Kato, A., Kojima, T., Muranishi, S. and Sezaki, H. (1983). *Chem. Pharm. Bull.* **31**, 2055 - 2063.

Hashida, M., Kato, A., Takakura, Y. and Sezaki, H. (1984) *Drug Metab. Disp.* **12**, 492 - 499.

Hassner, A. and Alexanian, V. (1978) *Tetrahedron Lett.* 4475.

Helferich, B. Moog, L. and Jünger, A. (1925) *Ber. dtsch. Chem. Ges.* **58**, 872.

Hesse, G.W., Shashoua , V.E. and Jacob, J.N. (1985) *Neuropharmacology,* **24**, 139 - 146.

Hesse, G.W., Jacob, J.N. and Shashoua, V.E. (1988) *Neuropharmacology,* **27**, 637 - 640.

Hill, D.R. and Bowery N.G. (1981) *Nature Lond.* **290**, 149 - 152

Himmelstein, K.J. and Gross, J.F. (1977) *J. Pharm. Sci.* **68**, 1441 - 1444.

Ho, D.D., Rota, T.R. and Hirsch, M.S. (1986) *J. Clin. Invest.* **77**, 1712 - 1715.

Hochsladt, J. (1974) *Crit. Rev. Biochem.* **2**, 259.

Hogben, C.A.M., Schanker, L.S., Tocco, D.J and Brodie, B.B. (1957) *J. Pharmacol. Exp. Ther.* **120**, 540 - 545.

Holley, R.W. and Holley, A.D. (1952) *J. Am. Chem. Soc.* **74**, 3069.

Horwitz J.P., Chua J. and Noel, M. (1964) *J. Org. Chem.* **29**, 2076.

Hostetler, K.Y., Stuhmiller, L.M., Lenting, H.B.M. van den Bosch, H. and Richman, D.D. (1990) *J. Biol. Chem.* **265**, 6112 - 6117.

Hulme, B., Dykes, P.W., Appleyard, I. and Arkall, D.W. (1968) *J. Nucl. Med.* **9**, 389.

Jacob, J.N., Shashoua, V.E., Campbell, A. and Baldessarini, R.J. (1985) *J. Med. Chem.* **28**, 106 - 110.

Jacob, J.N., Hesse, G.W. and Shashoua V.E. (1987) *J. Med. Chem.* **30**, 1573 - 1576.

Johansen, M. and Bundgaard, H. (1980a) *Arch. Pharm. Chem., Sci. Ed.* **8**, 141 - 151.

Johansen, M. and Bundgaard, H. (1980b) *Int. J. Pharm.* **7**, 119 - 127.

Johansen, M. and Bundgaard, H. (1982) *Arch. Pharm. Chem., Sci. Ed.* **10**, 112 - 121.

Johnson, M.A., Ahluwalia, G., Connelly, M.C., Cooney, D.A., Broder, S., Johns, D.G. and Fridland, A. (1988) *J. Biol. Chem.* **30**, 15354 - 15357.

Johnson, M.A. and Fridland, A. (1989) *Mol. Pharmacol.* **36**, 291 - 295.

Juni, K. and Nakano, M. (1987) In: *CRC Critical Reviews, Therapeutic Drug Carrier Systems* (CRC Press Inc., Boca Raton) **3**, 209 - 232.

Juliano, R.L. (1981) *Trends in Pharmacol. Sci.* **2**, 39 - 41.

Kaledin, V.I., Matienko, N.A., Nikolin, V.P., Grutenko, Y.V. and Budker, V.G. (1981) *J. Natl. Cancer Inst.* **66**, 881 - 887.

Kaplan, J.P., Raizon, B.M., Desarmenien, M., Feltz, P., Headley, P.M., Worms, P., Lloyd, K.G. and Bartholini, G. (1980) *J. Med. Chem.* **23**, 702 - 704.

Kenner, G.W. (1959) *Angew. Chem.* **71**, 741.

Kenner, G.W. and Seeley, J.H. (1972) *J. Am. Chem. Soc.* **94**, 3259.

Kidd, D.A.A. and King, F.E. (1948) *Nature* **162**, 776.

Kimura, Y. (1962) *Chem. Pharm. Bull.* **10**, 1152.

Kirsch, L.E. and Notari, R.E. (1984a) *J. Pharm. Sci.* **73**, 728 - 732.

Kirsch, L.E. and Notari, R.E. (1984b) *J. Pharm. Sci.* **73**, 896 - 902.

Kojima, T., Hashida, M., Muranishi, S. and Sezaki, H. (1980) *J. Pharm. Pharmacol.* **32**, 30 - 34.

Koenig, S., Gendelman, H.E., Orenstein, J.M., Dal Canto, M.C., Pezeshpour, G.H., Yungbluth, M., Janotta, F., Aksamit, A., Martin, M.A. and Fauti, A.S. (1986) *Science* **233**, 1089 - 1093.

Kokai Tokkyo Koho (H. Takino, K. Sagawa, N. Kitamura, M. Kilahara Inv.), Jpn. Pat. 62/151495 (July 6, 1987) [*Chem. Abstr.* **107** (1987a) p 220318c].

Kokai Tokkyo Koho (N. Kitamura, K. Sagawa, M. Takehara Inv.), Jpn. Pat. 62/4211 (January 10, 1987) [*Chem. Abstr.* **106** (1987b) p 182464s].

Kokai Tokkyo Koho (K. Sagawa, M. Takehara Inv.), Jpn. Pat. 62/30171 (February 9, 1987) [*Chem. Abstr.* **107** (1987c) p 796672e].

Kokai Tokkyo Koho (K. Sagawa, M. Takehara Inv.), Jpn. Pat. 62/65964 (March 25, 1987) [*Chem. Abstr.* **107** (1987d) p 63607b].

Kokai Tokkyo Koho (H. Takino, K. Sagawa, M. Takehara Inv.), Jpn. Pat. 63/3078 (January 8, 1988) [*Chem. Abstr.* **109** (1989) p 151601s].

König, W. and Geiger, R. (1970) *Chem. Ber.* **103**, 788, 2024, 2034.

Kopeček, J., Šprincl L. and Lim, D. (1973) *J. Biomed. Mater. Res.* **7**, 179.

Krause, H.-J., Schwarz, A. and Rohdewald, P. (1985) *Int. J. Pharm.* **27**, 145 - 155.

Krogsgaard-Larsen, P., Scheel-Krüger, J. and Jofod, H., editors (1979) In: *GABA-Neurotransmitters. Pharmacochemical, Biochemical and Pharmacological Aspects.* (Munksgaard, Copenhagen).

Krogsgaard-Larsen, P. (1980) *Ann. Rep. Med. Chem.* **5**, 41 - 49.

Kufe, D., Herrick, D., Crumpacker, C.S. and Schnipper, L. (1986) *Antimicrob. Agents Chemother.* **29**, 716.

Lal, H., Fieldinf, S., Malick, J., Roberts, E., Shah, N. and Usdin, E., eds. (1980) In: *GABA Neurotransmission: Current Developments in Physiologic and Neurochemistry* (Ankho International Inc., Fayetteville, New York).

Lee, H.K., Lambert, H., Stella, V.J., Wang, D. and Higuchi, T. (1979) *J. Pharm. Sci.* **68**, 288 - 295.

Leibman, K.C. and Heidelberger, C. (1955) *J. Biol. Chem.* **216**, 823 - 830.

Lelawongs, P., Liu, J.C. and Chien, Y.W. (1990) *Int. J. Pharm.* **61 (3)**, 179-188.

Leserman, L.D. and Barbet, J. (eds.) (1982) *Liposome Methodology.* **107**, editions INSERM, Paris.

Lindell, B., Aronsen, K.-F., Nosslin, B. and Rothman, U. (1978) *Ann. Surg.* **187**, 95 - 99.

Little, R., Bailey, D., Brewster, M.E., Estes, K.S., Clemmons, R.M., Saab, A. and Bodor, N. (1990) *J. Biopharm. Sci.* **1**, 1 - 18.

MacCoss, M., Edwards, J.J., Seed, T.M. and Sprahh, S.P. (1982) *Biochim. Biophys. Acta* **719**, 544 - 555.

Martin, Y.C. (1981) *J. Med. Chem.* **24**, 229 - 239.

Matsushita, T., Ryu, E.K., Hong, C.I. and MacCoss, M. (1981) *Cancer Res.* **41**, 2707 - 2713.

McKay, F.C. and Albertson, N.F. (1957) *J. Am. Chem. Soc.* **79**, 4686.

McLaren, C., Chen, M.S., Ghazzouli, I., Sarral, R. and Burns, W.H. (1986) *Antimicrob. Agents. Chemother.* **28**, 740.

Meldrum, B. (1981) In: *GABA and Benzodiazepine Receptors*, p 207 - 215.

Merigan, T.C., Skowron, G., Bozzette, S.A., Richman, D., Uttamchandani, R., Fischl, M., Schooley, R., Hirsch, M., Soo, W., Petinelli, C., Schaumburg, H. and ddC Study Group of the AIDS Clinical Trials Group (1989) *Ant. Int. Med.* **110**, 189 - 194.

Meyer, K.L., Marasco, C.J., Morris-Natschke, S.L., Ishaq, K.S. and Piantadosi, C. (1991) *J. Med. Chem.* **34**, 1377 - 1383.

Miller, F.A., Dixon, G.J., Ehrlich, J., Sloan, B.J. and McLean, Jr., I.W. (1968) *Antimicrob. Agents Chemother.* **9**, 120.

Mitsuya, H., Weinhold, K.J., Furnam, F.A., St. Clair, M.H., Lehrman, S.N., Gallo, R.C., Bolognesi, D., Barry, D.W. and Broder, S. (1985) *Proc. Natl. Acad. Sci. USA.* **82**, 7096 - 7100.

Mitsuya, H. and Broder, S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1911 - 1915.

Mitsuya, H. and Broder, S. (1987) *Nature* **325**, 773 - 778.

Mitsuya, H., Jarrett, R.F., Matsukura, M., Di Marzo Veronese, F., Devico, A.L. Sarngadharan, M.G., Johns, D.G., Reitz, M.S. and Broder, S. (1987a) *Proc. Natl. Acad. Sci. USA.* **84**, 2033 - 2037.

Mitsuya, H., Matsukura, M. and Broder, S. (1987b) In: *AIDS:Modern Concepts and Therapeutic Challenges* (ed. S. Broder, Marcel Dekker Inc., New York) p 303 - 333.

Miyazaki, S., Hashiguchi, N., Sugiyama, M., Takada and Morimoto, Y. (1986) *Chem. Pharm Bull.* **34**, 1370 - 1375.

Montgomery, J.A. (1982) *Med. Res. Rev.* **2**, 271 - 308.

Moroder L., Hallett, A., Wünsch, E., Keller, O. and Wersin, G. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 1651.

Morselli, P.L., Lloyd, K.G., Löscher, W., Meldrum, B. and Reynolds, E.H., editors (1981) In: *Neurotransmitters, Seizures and Epilepsy* (Raven Press, New York).

Neises, B. and Steglich, W. (1978) *Angew. Chem., Int. Ed. Engl.* **17**, 522.

North, T.W. and Cohen, S.S. (1979) *Pharmacol. Ther.* **4**, 81.

Noonan and Wester (1985) *Cutaneous xenobiotics in: Percutaneous absorption* p 65 - 85.

Oldendorf, W.H. (1974) *Proc. Soc. Exp. Bio. Med.* **147**, 813 - 816.

Oppenheim, R., Gipps, E.M., Forbes, J.F. and Whitehead, R.H. (1984) In: *Pharmaceutical Immunological and Medical Aspects* (eds. S.S. Davis, L. Illum, J.G. McVie and E. Tomlinson, Elsevier Science Publishers B.V., Armsterdam) p 117 - 128.

Page, T.M., Jacobsen, S.J., Nigam, W.L., Mangum, J.H. and Robins, R.K. (1986) *Int. J. Biochem.* **18**, 957.

Palade, G.E., Simionescu, M. and Simionescu, N. (1979) *Acta Physiol. Scand., Suppl.* **463**, 11.

Parasrampuria, D. and Parasrampuria, J. (1991) *J. Clin. Pharm. Ther.* **16 (1)**, 7 - 17.

Pardridge, W.M. and Oldendorf, W.H., (1977) *J. Neurochem.* **28**, 5.

Pardridge, W.M. (1985a) *Annual Reports in Medicinal Chemistry*, **20**, 305 - 311.

Pardridge, W.M. (1985b) In: *Directed Drug Delivery*, p 83 - 96.

Perno, C.-F., Yarchoan, R., Cooney, D.A., Hartman, N.R., Gartner, S., Popovic, M., Hao, Z., Gerard, T.L., Wilson, Y.A., Johns, D.G. and Broder, S. (1988) *J. Exp. Med.* **168**, 1111 - 1125.

Piantadosi, C., Marasco, C.J., Morris-Natschke, S.L., Meyer, K.L. Gumus, F., Surles, J.R. and Ishaq, K.S. (1991) *J. Med. Chem.* **34**, 1408 - 1414.

Pieper, R.O., Barrows, W.R. and Mandel, H.G. (1986) *Cancer Res.* **46**, 4960.

Popovic, M., Sarngadharan, M.G., Read, E. and Gallo, R.C. (1984) *Science* **224**, 497 - 500.

Poste, G., Bucana, C., Raz, A., Bugelski, P., Kirsh, R. and Fidler, I.J. (1982) *Cancer Res.* **42** 1412 - 1422.

Prusoff, W.H., Mancini, W.R., Lin, T.S. Lee, J.J., Siegel, S.A. and Otto, M.J. (1984) *Antiviral Res.* **4**, 303.

Reese, T.S. and Karnovsky, M.J. (1967) *J. Cell Biol.* **34**, 207.

Remon, J.P., Duncan, R. and Schacht, E. (1984) *J. Controlled Release*, **1**, 47.

Repta, A.J., Rawson, B.J., Shaffer, R.D., Sloan, K.B., Bodor, N. and Higuchi, T. (1975) *J. Pharm. Sci.* **64**, 392 - 396.

Revankar, G.R., Huffman, J.H., Allen, L.B., Sidwell, R.W., Robins, R.K. and Tolman, R.L. (1975) *J. Med. Chem.* **18**, 721 - 726.

Ringsdorf, H. (1975) *J. Polymer Sci. Polymer Symp.* **51**, 135.

Říhová, B., Ulbrich, K., Kopeček, J. and Mančal, P. (1983) *Folia Microbiol.* **28**, 217.

Říhová, B., Kopeček, J., Ulbrich, K., Popišil, M. and Mančal, P. (1984) *Biomaterials* **5**, 143.

Roberts, E., Chase, T.N. and Tower, D.B., editors (1976) In: *GABA in Nervous System Function* (Raven Press, New York).

Robins, R.K. (1984) *Pharm. Res.*, 11 - 18.

Roeske, R. (1959) *Chem. Ind. (London)*, 1121.

Rosowsky, A., Kim, S.-H., Ross, J. and Wick, M.M. (1982) *J. Med. Chem.* **25**, 171 - 178.

Rothner, J.T., Cobe, H.M., Rosenthal, S.L. and Bailin, J. (1949) *J. Dent. Res.* **28**, 544 - 548.

Rowland, G.F., Simmonds, R.G., Corvalan, J.R.F., Baldwin, R.W., Brown, J.P., Embleton, M.J., Ford, C.H.J., Hellström, K.E., Hellström, I., Kemshead, J.T., Newman, C.E. and Woodhouse, C.S. (1983) *Protides of the Biol. Fluids.* **30**, 375 - 379.

Rowland, G.F. and Simmonds, R.G. (1985) In: *Monoclonal Antibodies for Cancer Detection and Therapy* (Acad. Press, Lond.) p 345 - 364.

Sanderson, J.E., De Riel, S. and Dixon, R. (1989) *J. Pharm. Sci.* **78 (5)**, 361 - 4.

Saunders, P.P., Tan, M.T., Spindler, C.D., Robins, R.K. and Plunkett, W. (1986) *J. Biol. Chem.* **261**, 6410 - 6415.

Schacht, E., Ruys, L., Vermeersch, J. and Remon, J.P. (1984) *J. Controlled Release* **1**, 33 - 34.

Schanker, L.S., Shore P.A., Brodie, B.B. and Hogben, C.A.M. (1957) *J. Pharmacol. Exp. Ther.* **120**, 528.

Schönheimer, R. (1926) *Hoppe-Seyler's Z. Physiol. Chem.* **154**, 203.

Scrivener, C.A. and Schantz, C.W. (1947) *J. Am. Dental Assoc.* **35**, 644 - 647.

Sezaki, H. and Hashida, M. (1984) *CRC Crit. Rev. Therap. Drug Carrier Systems* **1**, 1 - 38.

Shashoua, V.E., Jacob, J.N., Ridge, R., Campbell, A. and Baldessarini, R.J. (1984) *J. Med. Chem.* **27**, 659 - 664.

Sheehan, J.C. and Hess, G.P. (1955) *J. Amer. Chem. Soc.* **77**, 1067.

Sheehan, J.C. and Hlavka, J.J. (1956) *J. Org. Chem.* **21**, 439 - 441.

Shek, E., Bodor, N. and Higuchi, T. (1976a) *J. Med. Chem.* **19**, 108 - 112.

Shek, E., Bodor, N. and Higuchi, T. (1976b) *J. Med. Chem.* **19**, 113 - 117.

Shiobara, Y., Tachibana, A., Sasaki, H., Watanabe, T. and Sado, T. (1974) *J. Antibiot.* **27**, 665 - 673.

Shipman, Jr., C., Smith, H.S., Carlson, R.H. and Drach, J.C. (1976) *Antimicrob. Agents Chemother.* **9**, 120.

Shore, P.A., Brodie, B.B. and Hogben, C.A. (1957) *J. Pharmacol. Exp. Ther.* **119**, 361.

Simionescu, N. and Simionescu, M. (1977) In: *Histology*, p 373 - 431.

Singer, S.J. and Nicholson, G.L. (1972) *Science*, **175**, 720 - 731.

Singh, R.M.B. and Main, L. (1983) *Aust. J. Chem.* **36**, 2327 - 2332.

Sinkula, A.A. (1975) *Ann. Rep. Med. Chem.* **31**, 306 - 314.

Sinkula, A.A. (1987) In: *Biological Approaches to the Controlled Delivery of Drugs.* p 281 - 287.

Smith, J.J. and Kampine (1984) In: *Circulatory Physiology*, p 127 - 137.

Srinivasan, V., Higuchi, W.I., Sims, S.M., Ghanem, A.H. and Behl, C.R. (1989) *J. Pharm. Sci.* **78 (5)**, 370 - 375.

Takakura, Y., Matsumoto, S., Hashida, M. Sezaki, H. (1984) *Cancer Res.* **44**, 2505 - 2510.

Takino, H., Sagawa, K. and Takehara, M. (1989) *Chem Abstr.* **109**, 151601s.

Tarbell, D.S., Uamamoto, Y. and Pope, B.M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 730.

Toonen, P.A.H.M. and Crommelin, D.J.A. (1983) *Pharmac. Weekblad. Scientific Edition.* 269 - 280.

Thorpe, P.E. and Ross, W.C.J. (1982) *Immun. Rev.* **62**, 119.

Tomlinson, E. (1983) *Int. J. Pharm. Tech. Prod. Mgr.* **4**, 49 - 57.

Trouet, A., Masquelier, M., Baurain, R. and Deprez-De-Campeneere, D. (1982a) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 626 - 629.

Trouet, A., Baurain, R., Deprez-De-Campeneere, D., Masquelier, M. and Pirson, P. (1982b) In: *Targeting of Drugs* (eds. G. Gregoriadis, J. Senior and A. Trouet) p 19 - 30.

Van Heeswijk, W.A.R., Stoffer, T., Eenik, M.J.D., Potman, W., van der Vijgh, W.J.F., Vander Poort, J., Pinedo, H.M., Lelieveld, P. and Feijen, J. (1984) In: *Recent Advances in Drug Delivery Systems* (eds. J.M. Anderson, and S.W. Kim, Plenum Press, N.Y.) p 77 - 100.

Veillard, M.M, Longer, M.A., Martens, T.W. and Robinson, J.R. (1987) *J. Controlled Release* **6**, 123 - 131.

Velluz, L., Amiard, G. and Heymes, R. (1954) *Bull. Soc. Chim. France* 1012.

Vej-Hansen, B. and Bundgaard, H. (1981) *Arch. Pharm. Chem., Sci. Ed.* **7**, 65-77.

Wearley, L., Tojo, K. and Chien Y.W. (1990) *J. Pharm. Sci.* **79 (11)**, 992 - 998.

Weinstein, J.N., Leserman, L.D., Henkart, P.A. and Blumenthal, R. (1982) "Antibody-mediated targeting of liposomes. In: *Targeting of Drugs* (eds. G.Gregoriadis, J. Senior and A. Trouet, Plenum Press, N.Y.) p 185 - 202.

Yarchoan, R. and Broder, S. (1987) *New. Engl. J. Med.* **316**, 557 - 564.

Yarchoan, R., Mitsuya, H., Meyr, C.E. and Broder, S. (1989) *New. Engl. J. Med.* **321**, 726 - 738.

Zaharko, D.S., Przybylski, M. and Oliverio, V.T. (1979) *Methods Cancer Res.* **16**, 347 - 380.

Ziegler, F.E. and Berger, G.D. (1979) *Synth. Commun.* 539.