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SYNTHESIS OF SPHINGOSINE ANALOGUES AND EVALUATION OF ANTICANCER ACTIVITY

A Thesis submitted in part fulfilment of the requirements of the degree of Doctor of Philosophy

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Summary

Traditional anticancer agents are mainly targeted on the DNA replication process in the nucleus. However, with the advancements in modern biology, a new class of anticancer drugs has emerged. These compounds target on enzymes involved in the earlier part of the cancer cell cycle. Protein kinase C (PKC) is one such enzyme which has been under intensive investigation because of its ability to cause tumour promotion. It belongs to a family of enzymes which catalyse the phosphorylation of serine and threonine residues using ATP. As well as being a natural direct PKC inhibitor, sphingosine (A) can also inhibit PKC indirectly by inhibiting phosphatidic acid phosphohydrolase (PAP). PAP catalyses the hydrolysis of diacylglycerol, the main PKC activator. Since N-methylsphingosine and N,N-dimethylsphingosine are better PKC inhibitors than sphingosine, it appears that the bulkiness around the amino group is important for inhibition.

The first stage of the project was to establish reaction conditions for carrying out methylation, dimethylation and trimethylation of a primary amino group in compounds which are analogues of sphingosine. A selection of N-methylated, N,N-dimethylated and N,N,N-trimethylated analogues of sphingosine was synthesised from (R)-, and (S)-phenylalaninol and (1S,2S)-2-amino-1-phenylpropane-1,3-diol. They were tested on two PAP enzymes under the supervision of Dr Pamela Scott at the Department of Medical Oncology, University of Glasgow. The results suggest that there is no clear correlation between the amount of inhibition and the bulkiness around the amino group. However increasing the bulkiness around the amino group in some cases did lead to an improvement in the inhibition.

The tested compounds differ from sphingosine in the hydrophobic moiety. Since they had shown much less inhibitory effect on PAP than sphingosine, the length of the hydrophobic alkyl chain is likely to be a key structural feature for inhibition. A range of sphingosine analogues each containing a triple bond was synthesised diastereoselectively. Sphingosine was synthesised using the same route. However, only a small amount was synthesised, which was too small to be used for further reactions.
A novel enantioselective synthesis of sphingosine analogues was proposed and its effectiveness was tested by applying it to the synthesis of \((1R,2S)-2\text{-ethylamino}-1\text{-phenylpropane-1,3-diol}\) and \(N\text{-ethylsphingosine}\).

A selection of sphingosine analogues was tested on a range of cancer cell lines under the supervision of Dr A. T. McGown at the Paterson Institute, Manchester. The sphingosine analogues containing a triple bond had IC\(_{50}\) values in the region of 1 \(\mu\text{M}\) against these cell lines.
<table>
<thead>
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<tbody>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BOC</td>
<td>tert-Butoxycarbonyl</td>
</tr>
<tr>
<td>br</td>
<td>broad (IR spectroscopy)</td>
</tr>
<tr>
<td>d</td>
<td>doublet (NMR spectroscopy)</td>
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<td>dd</td>
<td>double of doublets</td>
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<td>ddt</td>
<td>double doublets of triplets</td>
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<tr>
<td>dt</td>
<td>doublet of triplets</td>
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<tr>
<td>DAG</td>
<td>Diacylglycerol</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
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<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>IC50</td>
<td>concentration required to reduce a parameter to 50% of that in a control literature value</td>
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<tr>
<td>lit</td>
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<tr>
<td>m</td>
<td>multiplet (NMR spectroscopy)</td>
</tr>
<tr>
<td>m.p.</td>
<td>melting point</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre(s)</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole(s)</td>
</tr>
<tr>
<td>PAP</td>
<td>Phosphatidic acid phosphohydrolase</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>PTSA</td>
<td>para-Toluenesulfonic acid</td>
</tr>
<tr>
<td>q</td>
<td>quartet (NMR spectroscopy)</td>
</tr>
<tr>
<td>s</td>
<td>sharp (IR spectroscopy)</td>
</tr>
<tr>
<td>s</td>
<td>singlet (NMR spectroscopy)</td>
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<tr>
<td>t</td>
<td>triplet (NMR spectroscopy)</td>
</tr>
<tr>
<td>TBDMS</td>
<td>tert-Butyldimethylsilyl</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<tr>
<td>v.br</td>
<td>very broad (IR spectroscopy)</td>
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CHAPTER ONE

Cancer - An Overview

1.1 History of Cancer

Cancer is the second highest killing disease of our time, after heart disease. In the USA it is estimated that one in three people will develop cancer at some point in their life. Even with the advanced technology in cancer therapy, cancer still has a profound effect on the population. Its statistics cast fear over people. The disease is not a new health problem because it has been present throughout the history of mankind. It started with the discovery of some Egyptian mummies diagnosed with bone cancers. During that period, the disease was believed to be a curse from God to punish sinners. Such misconception was held until the Middle Ages when questioning began. One of the first investigations into the cause of cancer was investigated by Sir Percival Pott around 1775. He discovered that young boys working as chimney sweeps had a high mortality rate due to scrotal cancer. The chimney soot was to blame but the development of cancer was found only to occur on chronic exposure. Such observation was important but the biological aspects of tumours were to remain a mystery until the discovery of the microscope in the 19th century. With this equipment, cancer was established as a cellular disease and most of the research was then focused into three areas: the cause, biological properties and treatment of cancer.

1.2 Causes of Cancer

Cancer is a disease at the cellular level as it causes abnormal cell proliferation. This is due to genetic alteration which results in a change in gene expression. But there is the question of what causes the genetic information to be altered. Epidemiological studies have shown that exposure to a variety of different external factors is responsible. These factors include viruses, a wide variety of chemicals, X-rays and ultraviolet radiation. Fuller knowledge of these factors coupled with their avoidance could prevent 90% of cancers.
1.2.1 Radiation

The majority of skin cancers is caused by solar radiation. It is the ultra-violet light that damages cells at their genetic level, causing a high frequency of mutations, DNA damage and chromosome abnormalities. Chronic exposure may result in melanoma, a form of skin cancer which is considered to be the most lethal due to its behaviour. This form of tumour metastasises, that is, it invades neighbouring tissues and organs, and therefore cannot be easily removed through surgical means. It is this form of skin cancer that mainly accounts for cancer mortality. However, prolonged exposure to sunlight does not always lead to death. Non-melanoma is the less harmful form of melanoma and can be treated effectively by surgery.

1.2.2 Medical X-Rays

X-rays were discovered by Roentgen in 1895. Initially, they were used to treat cancer. But after an X-ray technician, who was testing manufactured X-ray tubes, died from metastatic cancer, the use of X-rays was questioned. By 1908, animal experiments had showed that X-rays were carcinogenic. The risk was proportional to the degree of exposure. Despite this discovery, X-rays are still used to treat non-malignant diseases. In Israel, X-rays are administrated to the head and upper body to treat ringworm of the scalp. Israel now has the third highest incidence of thyroid cancer in the world. In contrast, Denmark, where X-rays have never been used to treat non-malignant diseases, has one of the lowest rates of thyroid cancer in the world. Nowadays, X-rays are used for the detection of skeletal deformities and for treatment of certain types of solid tumours. Since they are carcinogenic, they have to be used carefully and in small controlled doses.

1.2.3 Chemicals

In some work areas the handling of chemicals cannot be avoided. Employees in science and industry have new legislation (Control of Substances Hazardous to Health act) to help minimise the risk of working with chemicals. Even if we are not handling them we are still
at risk in society, mainly from the effects of tobacco. Cigarette smoke could be lethal because it contains 50 known carcinogens including arsenic, benzene, nitrosoamines and naphthylamine. This mixture is the main cause of lung cancer which accounts for one third of cancer mortality. There is a connection between the intake of smoke and life expectancy. A 30 year-old man smoking 40 cigarettes a day can expect to lose, on average, eight years from his life-span, as well as increasing his risk of cardiovascular and respiratory disease. Other cancers which have been linked to cigarette smoke are cancers of the oral cavity, pharynx, oesophagus, bladder, kidney and pancreas. The incidence of lung cancer is increasing as a result of changing lifestyles. This is due to the trend of more women smoking as well as more adolescents.

1.2.4 Medicines that Cause Cancer

Like medical X-rays, some medications have the ability to induce a second cancer as a result of their actions on normal cells. This is a side effect that a patient may have to face after treatment. These cancer-causing drugs have been removed from current practice but other potential carcinogens still remain in use. The benefits that these drugs bring generally outweigh the side effects.

1.2.4a Anticancer Drugs

Despite their ability to cause cancer, anticancer drugs are still used in chemotherapy. This will be discussed later in this chapter. Anticancer drugs are used to target all rapidly proliferating cells, that is, cancer cells, and any normal rapidly dividing cells. One example of a normal cell that is most affected by such drugs is the bone marrow forming cells. The effect of these drugs on normal cells might induce the development of a second cancer. Nevertheless, the benefits of these drugs outweigh the possibility that they will induce the development of a second cancer.
1.2.4b Hormones

In the early 1970s, incidences of vaginal and cervical cancer among young women were becoming noticeable. The mothers of these women had been treated with the synthetic oestrogen, diethylstilbestrol (DES), during their pregnancy back in the 1940s and 1950s. This practice no longer exists. However, oestrogen itself is used to treat menopause symptoms and osteoporosis (bone thinning). Postmenopausal hormone replacement therapy involves the use of high doses of oestrogen. Unfortunately this is a long-term treatment, and therefore carries the risk of developing endometrial cancer. This type of hormone replacement therapy is still practised because the risks can be reduced by the administration of another hormone, progesterone, as a co-drug.

1.2.5 Oncogenic Viruses

The common cold, herpes, polio, influenza and measles are all due to viral infections. Viruses invade cells to use the cells' biological machinery to reproduce. These oncogenic viruses incorporate their RNA into the DNA of a normal cell. This allows the virus to place its genetic make-up into the cells reproduction system to produce more RNA tumour viruses. The invaded cell then ruptures releasing virus progeny to infect neighbouring normal cells. An example of this oncogenic virus is Hepatitis B.

1.3 Cell Cycle and Growth

The range of causes of cancer increases the difficulty of finding the appropriate treatment. In order to do this, understanding of how a cell survives and reproduces is needed. Cellular processes are presented as a cell cycle in which there are four kinds of cellular events. Overall, a parental cell duplicates its genetic material and passes one copy of each to its two newly formed daughter cells.

When the parental cell is in the G1 phase, its metabolites become active and it is ready for DNA replication which happens in the S phase of the cycle (Diagram 1). By this time, the cell has two copies of its
genetic complement, and it is at its premitotic stage (G2 phase). In the remaining phase of the cycle (M phase), cell division occurs to form two daughter cells. Each cell contains one copy of the parental genetic material.

The lengths of the M, S and G2 phases do not vary considerably from one cell type to another. The length of the G1 phase varies depending on how soon newly formed cells are needed. Liver cells have a G1 phase of a few weeks since there is no urgent need for them to reproduce. In contrast, bone marrow cells have a G1 phase of a few hours in order to replace themselves efficiently. There are cells however which are permanently fixed at the G1 phase. These include nerve cells and adult brain cells. Therefore the rate of proliferation of a cell depends on how long it spends in the G1 phase. There are some cells that stay in the G1 phase and have entered a phase outside the cell cycle (G0 phase). They can leave the G0 phase and return to G1, thus entering the cell cycle, if the cell is induced by an external signal. A wound on a piece of connective tissue would induce the synthesis of skin fibroblasts.

After the M phase, normal daughter cells differentiate to undergo a programmed cell death since they are constantly being replaced by new cells. This keeps the cell population constant. With cancer cells,
they fail to die since they do not differentiate. Instead they go on to the S phase of the cell cycle having lost the ability to remain in the G1 phase. They go on to reproduce further daughter cells which also fail to differentiate. Thus, cancer cells are continuously proliferating.

1.4 Current Cancer Therapies

There are three treatments used in practice today: surgery, radiotherapy and chemotherapy. The choice of treatment depends on the nature of the cancer.

Surgery is effective against most localised tumours except brain tumours. It is useful in treatment of malignant melanoma which appears as lesions on the skin. Therefore, it can be easily seen and detected early. The success of surgery depends on the removal of all cancerous cells leaving none behind to continue the life cycle. This type of treatment is promising since there are no chemicals involved which may affect the body elsewhere. However it has its limitations. Once cancer cells have metastasised, it is difficult to ensure that all of them have been removed. Also, most cancers are undetected until they have metastasised. Therefore, it is often too late for surgical treatment. At this stage radiotherapy or chemotherapy is required.

Radiotherapy is sometimes used instead of surgery on localised tumours because it can kill the cancer cells as well as the invaded adjacent cells. However, some cancer cells can only be removed by a combination of radiotherapy and surgery. The role of radiotherapy is to induce mutations by damaging DNA. DNA damage can be lethal to any cell especially to those that are rapidly proliferating since they use DNA much quicker. Thus radiation targets selectively on cells with a short cell cycle. However this provides a limitation to its use. Not only are the cancer cells destroyed but also normal rapidly proliferating cells. It is the S phase of the cell cycle that is most likely to be affected. In practice, radiotherapy is spread over a month to allow any resting cancer cells left to move into the S phase, as well as giving the patient a chance to recover from the side effects of the treatment. A number of side effects are due to the killing of normal proliferating cells such as cells of the bone marrow, skin cells and the cells that form hair.
Sometimes, tumours have already metastasised by the time of diagnosis. Surgery and radiotherapy are no longer effective towards these tumours. At this stage, chemotherapy will be required. The basis of chemotherapy is to administer a combination of drugs to interfere with the process of cell division. The main drawback of this treatment is that the drugs are largely non-selective and can induce a second cancer. It is common to administer a combination of drugs during chemotherapy in case cancer cells develop resistance to any one of the drugs. Chemotherapy may be used in combination with surgery and radiotherapy and is the only real hope for patients with metastatic cancer.

1.5 Anticancer Drugs

It is of paramount importance to organic chemists to understand the mode of action of anticancer drugs to provide them with a foundation for the design of better ones. The three main groups of anticancer drugs are alkylating agents, antimetabolites and natural products.

1.5.1 Alkylating Agents

The largest group of DNA damaging drugs are the alkylating agents which originate from the use of mustard war gases. The parent compound, nitrogen mustard, forms covalent bonds with nucleophilic centres in DNA and other biological molecules. These centres include amine, phosphate and hydroxy groups on nucleic acids, nucleotides and enzymes. A modified nucleic acid is formed when alkylation occurs once. However, there are some drugs that have two alkylating sites. These agents crosslink two adjacent strands of DNA in its helical form, making it impossible for the double helix to unwind and replicate. The lone pair of nitrogen of the drug intramolecularly displaces the chloride to form an aziridinium ion (1) which reacts readily with an electron rich site to generate a modified nucleic acid (Scheme 1).
Nitrogen mustard is too reactive to be of much clinical use. Instead a derivative, cyclophosphamide (2), is widely used for the treatment of carcinoma of the breast and of the bronchus. In contrast to its parent compound, it can be administered orally since it remains inactive until its target site has been reached. Its stability is aided by the delocalisation of the nitrogen lone pair with the P=O bond.

After a combination of enzymatic and chemical activation, the drug is transformed into active phosphoramidate mustard alkylating agent (Scheme 2). If a single alkylation step only occurs then it could lead to mispairing of bases or strand breakage. However, the remaining chloroethyl group could react with another molecule of guanine in the opposite strand of DNA to form a crosslink. Ifosfamide, melphalan and chlorambucil are other drugs developed as a result of the success of cyclophosphamide.
Nitrosoureas are another group of alkylating agents. The most frequently one used in cancer therapy is bischloroethylnitrosourea (BCNU, 3).
Nirosoureas are lipid soluble and therefore are able to cross the blood-brain barrier and so are frequently used to treat brain tumours. BCNU, like cyclophosphamide, is inactive until it undergoes metabolism to release a number of cytotoxic compounds. An isocyanate is one of these metabolites, which can react to form covalent bonds with proteins, causing some cytotoxicity. But it is the alkylation step that is ultimately responsible for most of the cytotoxicity.

1.5.2 Antimetabolites

Generally, anticancer agents act in several different ways to inhibit cell division. Antimetabolites often act by interfering with DNA synthesis. When a cell has entered the S phase of its cycle, it has a reserve of metabolites from the G1 phase. The bindings of natural metabolites onto enzymes are essential for the synthesis of DNA. If analogues of these metabolites are present, they too will bind onto the enzymes making the enzyme inactive. Antimetabolites are administered to mimic the natural metabolites present in the S phase and thus compete for enzymes. Therefore, cells that are rapidly dividing will have a higher mortality rate. These cells are producing their metabolites quicker, which in turn means their enzymes are inactivated sooner.

Antimetabolites inhibit cellular enzymes involved in the synthesis of one or more of the nucleotides, the precursors of DNA. They also inhibit the synthesis of DNA itself. Methotrexate, 5-fluorouracil, cytosine arabinose, mercaptopurine and 6-thioguanine are all antimetabolites which inhibit DNA synthesis and are clinically used.
5-Fluorouracil (5-FU, 4) mimics the pyrimidine bases uracil (5) and thymine (6) of DNA. 5-FU is converted into its corresponding 5-FU nucleotide which inhibits thymidylate synthetase irreversibly once it has incorporated into the cell cycle (Diagram 2). This antimetabolite is used for the treatment of solid tumours and cancers of the breast and gastrointestinal tract. Bone marrow suppression and sickness are the side effects which may be severe in cases of high doses.

Tetrahydrofolic acid (THA) is a co-factor for thymidylate synthetase (Diagram 2). Removal of THA will indirectly block the synthesis of DNA. Methotrexate (7), an analogue of folic acid (8), is
used for reducing the production of THA by competing for dihydrofolate reductase, an enzyme that catalyses the reduction of dihydrofolate to tetrahydrofolate. Methotrexate is used to treat acute leukaemia and choriocarcinoma.

Other useful antimetabolites include cytosine arabinose, a nucleoside mimic used to treat myeloid leukaemia in adults; 6-mercaptopurine, a purine antagonist; and 6-thioguanine, which also inhibits purine synthesis.

1.5.3 Natural Products

Vincristine (9) and vinblastine (10) are two common alkaloids used in cancer therapy. These compounds inhibit the process of mitosis by binding to the microtubules protein tubulin. Before mitosis can take place, chromosomes are held in a certain orientation by these microtubules, also known as spindles. By binding to tubulin, these alkaloids disrupt the mitotic spindles and block the cell division at the
M phase of the cell cycle. Vincristine is used to treat leukaemias and lymphomas, and vinblastine is used for Hodgkin's disease.

Taxol (11), a taxane, is isolated from the bark of *Taxus brevifolia*. It is a potent anticancer drug and is mainly used for treating breast and ovarian cancers. Like vincristine and vinblastine, it affects the mitotic spindles. Taxol has been shown to stimulate the formation of microtubules and also to prevent their breakdown. Any interference in the spindle formation will lead to an imbalance of microtubules. Such imbalance prevents normal cell division from taking place. Cancer cells are most affected because they are rapidly proliferating. Although taxol is an effective anticancer drug, its use is limited because of its poor water solubility and the difficulty in transporting the drug to the site of action. Instead, a phosphate derivative (12) is used because it is more stable and is water soluble. Unfortunately, only small amounts of taxol can be isolated. Therefore taxol has to be synthesised. Due to its complexity, the total synthesis of taxol was not achieved until 1994 by the groups of Nicolaou\(^{11}\) and Holton.\(^{12}\) Analogues of taxol such as taxotere are proving to be very effective in treating cancers.
1.5.4 Miscellaneous Anticancer Agents

Some chemotherapeutic drugs cannot be categorised under the three previous headings. The enzyme asparaginase is used to treat acute leukaemias. It breaks down the amino acid asparagine which is important for both protein and DNA syntheses. Asparagine is generated in normal cells whereas leukaemic cells rely on the blood supply for asparagine for growth. Therefore treatment with asparaginase reduces the supply of asparagine to leukaemic cells and so controls their growth.

Cisplatin (13) is one of the few inorganic anticancer drugs. It is a square planar complex of platinium with two chlorides cis to one another. Its mode of action is very similar to that of alkylating agents. The chlorides can be replaced by nucleophilic sites on proteins or nucleic acids. Dialkylation can occur to produce DNA cross-linkage or monoalkylation can produce a modified protein or DNA molecule.
Although the mechanism is not fully known, N-7 of guanine is probably the base that undergoes the SN2 displacement. Interestingly, the trans-isomer appears to be completely unable to induce DNA cross-linkages of the double helix. Cisplatin is used for treatment of testicular cancer as well as being used in combination with other drugs in treating a variety of solid tumours.

![Cisplatin Structure](image)

Unlike alkylating agents which directly react with DNA molecules, there are some chemotherapeutic drugs which can cause DNA damage indirectly by interacting with the enzyme, topoisomerase II (Diagram 3). Topoisomerase II, acts to break and rejoin DNA strands within the cell, and is required for a number of cell processes including DNA replication. Etoposide and teniposide are chemotherapeutic drugs that are used to inhibit the rejoining process by forming a covalent complex with topoisomerase II and DNA. Thus any DNA already broken cannot be rejoined causing the DNA molecule to cleave permanently, leading eventually to cell death. Inhibition of topoisomerase II also occurs with the natural products daunomycin and doxorubicin.
1.6 Further Drug Development

Patients are sometimes misled to believe that cancer therapy will cure them from the disease. The truth is that over the past fifty years of intense research, there has been no single drug found which will banish cancer cells completely.

Whilst patients are undergoing therapy, they may also suffer the side effects of anticancer drugs. This is the price paid to extend the patient's life. Since the discovery of DNA, anticancer drugs have been designed to target on it. The nucleus of a cell was the main point of focus, that is, until more was known about how signals travel within the cell. Signal transduction pathways leading to cell division and sometimes tumour promotion were identified and so provided a fuller picture of the cell's behaviour. This expanded the range of drug design. If a certain biochemical pathway was found to lead to a faster rate of cell proliferation, a synthetic drug could be introduced to try to block this pathway. Thus a new field of targets was identified. The area of
focus shifted from the nucleus to other parts of the cell, in particular, the cytoplasmic enzymes involved in signal transduction pathways.

Future drugs will be developed to target tumour promoting enzymes, instead of DNA. DNA is at the heart of the cell and damaging or modifying it will ultimately cause side effects. Thus new drugs will be developed to reduce these side effects.
CHAPTER TWO

Cell Signalling and Cancer

Cell growth and differentiation of normal cells are carefully regulated by external signals. These external signal molecules are growth factors, neurotransmitters and hormones. Binding of these signals onto specific cell surface receptors triggers a series of reactions inside the cell (Diagram 4). This cascade of reactions serves to transmit a signal from the exterior surface of a cell to its nucleus. This will alter the gene expression in the nucleus which in turn will alter the cell behaviour.

![Diagram 4](image)

2.1 Growth Factors and Cell Signalling

Growth factors are a group of small secreted proteins, typically 100 amino residues long. They bind onto cell surface receptors (growth factor receptors) which serve as external sensors. Information in the form of a signal is transmitted from the extracellular matrix to the
interior of the cell. Overall, information on the cell environment is sent to its nucleus resulting in a change in gene expression. This in turn produces cell growth and division. Many growth factor receptors are members of a family of proteins that function as protein tyrosine kinases (PTKs).

Although the functions of many growth factors and their receptors have been established, the exact sequence of events in signal transduction is still not fully understood. This area of research is still active. Nevertheless, some of the cellular reactions involved in PTK mediated growth have been identified. The understanding of these pathways opens up a new area for chemotherapeutic drug development. A synthetic drug can be used to target a known receptor in the signal transduction pathways so that it can intercept the signal from reaching the nucleus. This will arrest the growth of transformed cells which is the ultimate aim of cancer therapy.

2.2 Role of Protein Kinase C in Cell Growth

The PTK receptor consists of two domains: intracellular and extracellular because it is partially embedded in the cell membrane (Diagram 5). Binding of a growth factor onto the receptor activates four distinct target molecules in the cytoplasm. Phospholipase C is the most important target molecule activated. It catalyses the hydrolysis of phosphatidylinositol 4,5-bisphosphate, generating diacylglycerol (DAG) and inositol triphosphate. These two compounds appear to play an important role in regulating cell division. Inositol triphosphate opens up the calcium channel allowing the calcium concentration to increase in the cytoplasm. An increase of calcium will lead to the activation of several other calcium-stimulated protein-serine/threonine kinases. DAG activates the enzyme protein kinase C (PKC), a protein-serine/threonine kinase.

PKC can stimulate cells to divide; thus it must be carefully regulated by the cell to prevent it from being overexpressed. Therefore the amounts of diacylglycerol present in the cell are crucial for cell division. The triol is only produced in small quantities by the hydrolysis of phosphatidyl 4,5-bisphosphate but most of it is derived
from the breakdown of phosphatidylcholine by the sequential action of phospholipase D and phosphatidic acid phosphohydrolyase. In conclusion, unregulated activity of PKC and the disruption of DAG production can contribute to tumour formation. Over the last few years some metabolites of sphingolipids have found to be regulators of both PKC and DAG.
2.3 Natural Inhibitors of Protein Kinase C

Sphingolipids are located in the cell membrane and, like many other lipids, were thought only to have a structural function, that is, they allowed desired nutrients to travel across the membrane. Over the last decade, metabolites of membrane lipids have been shown to play an important role in cell functions and to act as mediators to the cells' environment. In particular sphingosine and related compounds which are metabolites of sphingolipids, have attracted a lot of attention as a result of their ability to inhibit PKC. In the past few years sphingoid bases have emerged as a new of class of bioregulatory substances. Current evidence suggests exogenous sphingosine is involved in the signal transduction pathways, and at present there is little evidence for effects of endogenous sphingosine. The mode of action of exogenous sphingosine on protein kinases and the possible role of endogenous sphingosine in modulating signal transduction pathways will be discussed later in this chapter. Understanding of the biosynthesis of sphingolipids has provided a better insight into how endogenous sphingosine could be generated and how it could then be used to inhibit PKC indirectly.

2.4 Biochemistry of Sphingolipids and Sphingosine

The backbone of all sphingolipids is sphingosine, a long unsaturated hydrocarbon chain containing an aminodiol. The structure of sphingosine and its reported syntheses are discussed in chapter 3. The amino group of each sphingolipid is linked to a fatty acid by an amide bond. The difference between each lipid is the head group attached to the primary hydroxyl group. In sphingomyelin the primary hydroxyl group is substituted by a phosphorylcholine unit whereas in glycosphingolipids, a sugar molecule is linked to the hydroxyl group by a glycoside bond. Sphingolipids and their metabolites are found not only in mammalian tissues but also in plants. Phytosphingosine is one example that is present in both mammalian tissues (such as kidney\(^1\) and liver\(^1\)) and plants.\(^1\)

The biosynthesis of sphingolipids and sphingosine has been established and is well documented.\(^1\) Palmitoyl CoA and serine
condense to form 3-ketosphinganine which is then reduced to dihydrosphingosine (Scheme 3). Dihydrosphingosine can either be used directly in the biosynthesis of sphingolipids or after its conversion into sphingosine. The amino group of sphingosine is acylated with a long-chain acyl CoA to form ceramide (N-acylsphingosine). This is the key intermediate from which all sphingolipids are synthesised by coupling with various head groups to the primary hydroxyl group.

Scheme 3: The biosynthesis of sphingosine and sphingolipids
Sphingomyelinases catalyse the hydrolysis of sphingolipids to their corresponding ceramides and polar head groups or, to their lysosphingolipids and fatty acids. These enzymes are found in lysosomes and in plasma membranes. The latter require magnesium ions for activation. The action of a plasma membrane ceramidase on ceramide produces sphingosine. The excess of sphingosine can be reacylated and then is fed back into sphingolipid biosynthesis. On the other hand, sphingosine can be removed by undergoing phosphorylation and by subsequent degradation into phosphorylethanolamine and palmitaldehyde. 20

2.5 Role of Exogenous Sphingosine

The inhibitory effect of exogenous sphingosine and other naturally occurring sphingoid bases on PKC was discovered by Hannun and co-workers. 21, 22 Further studies show that lysosphingolipids are also potential inhibitors of PKC. With these discoveries, sphingoid bases provided a better insight into signal transduction pathways. With the knowledge that sphingosine inhibits PKC and the fact that it is used in more and more studies, its role in cell growth begins to become apparent. Interestingly, sphingosine was found to stimulate other protein kinases, in particular, the epidermal growth factor receptor tyrosine kinase and human epidermoid carcinoma cells. 23-26 An important discovery into the effects of some sphingosine analogues on protein kinases was noted by Igarashi et al. 26 They reported that N,N-dimethyl-L-erythro-sphingosine is a specific stimulator of epidermal growth factor receptor tyrosine kinase but N,N-dimethyl-D-erythro-sphingosine, D-erythro-sphingosine, and several lyso-sphingolipids are all inactive. In addition, they also reported that N,N-dimethyl-D-erythro-sphingosine is a better inhibitor than the pure synthetic D-erythro-sphingosine. 27 However, N,N-dimethyl-D-erythro-sphingosine stimulated another protein tyrosine kinase, src kinase while D-erythro-sphingosine inhibited its activity. In short, exogenous sphingosine has multiple effects on several protein kinases.
2.6 Endogenous Sphingosine

2.6.1 Production of Endogenous Sphingosine

A model of the sphingolipid cycle has been postulated\textsuperscript{21} to illustrate how sphingosine may be generated inside the cell (Diagram 6). Endogenous sphingosine may be obtained by hydrolysis of sphingolipids followed by deacylation of ceramide. The free sphingosine could then be used as a modulator of cell function. The action of sphingosine could be then terminated by either reacylation so that it could be reused in sphingolipid biosynthesis or by phosphorylation followed by degradation.

The main drawback in earlier studies of endogenous sphingoid bases as cell regulators was that there was no way of knowing whether or not endogenous free sphingoids existed inside the cell. Recently, Kobayashi \textit{et al.}\textsuperscript{28} developed a sensitive HPLC method for quantitation of endogenous sphingoid bases. This technique, along with the use of a radioenzymatic method, developed by Van Veldhoven \textit{et al.}\textsuperscript{29} confirmed that endogenous sphingoid bases are present in different
amounts depending on the cell type. Both methods also confirmed the biosynthetic pathways operating in the sphingolipid cycle. More studies were carried out on endogenous sphingoid base activation inside the cell. Of particular interest were the findings by Kolesnick et al. 30, 31 They were the first group to demonstrate that DAG, and not phorbol ester, stimulated the activity of an endogenous sphingomyelinase, causing an increase in ceramide and sphingosine levels. The activation of PKC by DAG seems to stimulate the breakdown of sphingolipid yielding the PKC inhibitor sphingosine.

2.6.2 Indirect Inhibition of Protein Kinase C by Endogenous Sphingosine

Sphingosine can modulate the level of DAG by inhibiting its production and activating its breakdown. It is a potent inhibitor of phosphatidic acid phosphohydrolase (PAP), the enzyme which hydrolyses phosphatidic acid to form DAG. Brindley et al. 32 have shown that there are two distinct types of PAP activities in rat liver. Phosphatidic acid phosphohydrolase 1 (PAPI) is dependent on magnesium ions for its activity and is involved in the synthesis of triacylglycerols, phosphatidylcholine and phosphatidylethanolamine. PAPI is believed to be inactive in the cytosol and only becomes active when it is translocated to the endoplasmic reticulum. The other type of phosphatidic acid phosphohydrolase, PAP2, does not require magnesium ions for its activity and is located in the plasma membrane. It is believed that PAP2 is involved in signal transduction.

The other enzyme which is affected by sphingosine, as recently shown by Sakane et al., 33 is diacylglycerol kinase isozyme. This isozyme converts DAG into phosphatidic acid and can be activated by sphingosine. Sphingosine may reduce most of the DAG levels by inhibiting PAP2 enzyme and by activating the DAG removal isozyme. Therefore it can inhibit PKC indirectly by decreasing the amount of DAG available for its activation. Kolesnick has proposed a model (Diagram 7) to help to explain how endogenous sphingosine terminates PKC activation. 31 According to this model, the levels of DAG and sphingosine are mutually dependent and are well balanced. For instance, the action of DAG may be terminated by activating a
membrane sphingomyelinase to generate free sphingosine, thus completing a complementary cycle.

Diagram 731: Dual action of sphingosine on diacylglycerol signal termination

The various routes which have been used to synthesise sphingosine and related compounds are discussed in the next Chapter.
CHAPTER THREE

Review of Sphingosine

3.1 The Structure of Sphingosine

Sphingosine (14) is termed as a regulatory compound\textsuperscript{21,22} and is the backbone of all sphingolipids. Such lipids are important in cell growth, cell adhesion and cell recognition. Since they constitute most of the cell membrane, they are compounds of increasing interest as they should provide a better insight into the role and function of cell surfaces. As discussed in Chapter 2, sphingosine is also important in the inhibition of Protein Kinase C. Unfortunately only small amounts of partially pure sphingosine and its derivatives can be extracted from plants and mammals. This fact is the driving force for organic chemists to set up some novel syntheses in which large amounts of sphingosine could be prepared in the laboratory, so that a variety of sphingolipids could be synthesised.

\[
\text{HO} \quad \text{NH}_{2} \\
\text{1} \quad \text{2} \quad \text{3} \quad \text{4} \quad \text{5} \quad \text{6} \quad \text{7} \quad \text{8} \quad \text{9} \quad \text{10} \quad \text{11} \quad \text{12} \quad \text{13} \quad \text{14} \quad \text{15} \quad \text{16} \quad \text{17} \quad \text{18}
\]

(14)

Sphingosine is a 2-amino-1,3-diol with an \textit{E}-double bond between C-4 and C-5, two chiral centres and a long alkyl chain. Its structure is basically made up of two parts: hydrophobic and hydrophilic moieties. Since there are two chiral centres present, there are four possible stereoisomers. Naturally occurring sphingosine has the (2\textit{S}, 3\textit{R})-\text{[D-erythro]}-configuration.
The three approaches that have been largely used for the synthesis of sphingosine involve: 1) the use of achiral materials to achieve synthesis of racemic material; 2) diastereoselective synthesis from simple chiral starting materials such as (S)-serine and sugar compounds; and 3) the use of versatile reactions such as Sharpless asymmetric epoxidation to achieve enantioselective synthesis. The most common disconnection (Scheme 4) is the breakage of the bond (b) as the aminodiol synthon is readily available from (S)-serine and many natural sugar compounds. Disconnection (a) remains most attractive and requires the synthesis of two new chiral centres in one or two steps. Asymmetric aldol reaction using a chiral auxiliary is frequently used to achieve the stereoselectivity. Racemic, diastereoselective, and enantioselective syntheses of sphingosine, in that order, are covered in the following review. The literature survey presented here covers the year 1952 onwards.
3.2 Synthesis of Racemic Sphingosine

This section reviews various ways in which the synthesis of racemic erythro-sphingosine and its derivatives were achieved. Most workers used an asymmetric synthesis to prepare racemic erythro-sphingosine except for the very early literature where the primary aim was probably to synthesise sphingosine, irrespective of its configuration.

Grob and Gadient\textsuperscript{34} were the first group to synthesise racemic sphingosine and a derivative in 1952. Their synthesis is one of the shortest in the literature but they made no attempt to tackle the stereochemical problem and thus all four stereoisomers were formed.
Condensation of 2-pentadecynal (15) with 2-nitroethanol gave all four stereoisomers of 2-nitro-4-octadecyn-1,3-diol (16) (Scheme 5). The *threo*-racemate was separated from the *erythro*-racemate by chromatography. The nitro group was then reduced with zinc and hydrochloric acid to the amino group. Partial reduction of the triple bond in the *erythro*-racemate with lithium aluminium hydride gave racemic *erythro*-sphingosine (17) whereas on prolonged exposure under hydrogen atmosphere, the triple bond was completely reduced to afford racemic *erythro*-dihydrosphingosine (18).

Scheme 5: Synthesis of (±)-*Z* and *E*-erythro-sphingosine and (±)-*erythro*-dihydrosphingosine by Grob and Gadient.34

An improved synthesis of racemic sphingosine was developed by Schmidt and co-workers starting with a C16 αβ-unsaturated aldehyde (Scheme 6).35,36 They had already established the reaction conditions, in which high diastereoselectivity would be obtained when the α-carbanion of the protected glycine was treated with a simple
aldehyde. These conditions were then applied to the condensation of a \( \text{C}_{16} \alpha\beta \)-unsaturated aldehyde with the same anion which afforded exclusively the \textit{erythro}-product (19). The stereospecificity may be due to the size of the disilylated amino group which disfavours the formation of the Z-enolate. The course of the reaction probably goes through a chair-like transition state which is held by the lithium ion. Reduction of the amino acid with lithium aluminium hydride followed by triacetylation gave racemic \( N,O,O \)-triacetylspingosine. It was important to carry out the acetylation step as this protected the amino group from being oxidised in air.

![Scheme 6: Synthesis of (±)-\textit{erythro}-\( N,O,O \)-Triacetylsphingosine by Schmidt et al. 35,36](image)

In contrast to the previous synthesis, Garigipati and Weinreb developed a more efficient route which employed an intramolecular Diels-Alder reaction to construct the relative configuration on the cyclic system. Myristic aldehyde was converted in three steps into (\( Z,E \))-carbamate (20), which upon treatment with thionyl chloride and pyridine afforded sulfinylcarbamate (21) (Scheme 7). Cycloaddition of the compound occurred at room temperature to give the Diels-Alder adduct (22). The bicyclic product, upon treatment with phenylmagnesium bromide, underwent a 2,3-sigmatropic rearrangement via a five-membered ring transition state to give the desired product with an \( E \)-double bond. Basic hydrolysis provided the racemic \textit{erythro}-spingosine.
Scheme 7: Synthesis of (±)-erythro-sphingosine by Garigipati and Weinreb.\textsuperscript{38}

In 1985 Cardillo et al.\textsuperscript{39-41} managed to achieve another diastereoselective synthesis of racemic sphingosine, using the same starting material as Weinreb's group. The dienol was transformed into its trichloroacetimidate (23). Regioselectivity of the cyclisation was uncertain, because compound (23) can either undergo a 5-exo or 6-endo closure in the presence of N-iodosuccinimide (Scheme 8). Since only the 6-membered oxazine ring was formed, the reaction must have been governed by an electronic factor. Cleavage of the oxazine followed by treatment with amberlyst A 26 in the carbonate form gave the corresponding unsaturated aziridine (24). Racemic \textit{erythro-}\textit{N}-acetylsphingosine was produced in two steps: protonation of the aziridine and regioselective opening of the ring with amberlyst A 26 in
the acetate form. Basic hydrolysis of racemic \textit{erythro-}N-acetylsphingosine afforded racemic \textit{erythro-}sphingosine.

![Scheme 8: Synthesis of (±)-erythro-sphingosine by Cardillo et al.\textsuperscript{39-41}](image)

3.3.3 Diastereoselective Synthesis of D-erythro-Sphingosine

This section reviews the various diastereoselective approaches that have been used to prepare D-\textit{erythro-}sphingosine and its derivatives. The idea was to start the synthesis with homochiral starting material so that it could be used to control the stereochemical outcome of the formation of the new chiral centre.

3.3.1 Diastereoselective Synthesis of D-\textit{erythro-}Sphingosine using Carbohydrates

Many carbohydrates are ideal homochiral starting materials in sphingosine syntheses. They are commercially available, inexpensive and their absolute configurations are known. In addition, their functional groups are useful for manipulation.

The diastereoselective synthesis of D-\textit{erythro-}sphingosine and its derivatives (Scheme 9) by Reist and Christie,\textsuperscript{42,43} commences from
readily available 3-amino-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-
allofuranose (25) which was transformed in three steps into its di-
protected aldehyde (26). Wittig condensation of the aldehyde with
tetradecyltriphenylphosphonium bromide afforded Z- and E-isomers
(27) as a mixture. Acid hydrolysis of the acetal followed by standard
sodium periodate oxidation and reduction reactions gave the
unsaturated diol (28). Hydrogenation of the diol using 10% palladium
on charcoal reduced the the double bond completely as well as
removing the benzyl group to afford D-erythro-dihydrosphingosine
(29). Interestingly, when the ethyl carbamate (30) was used instead of
benzyl carbamate, Wittig coupling in the presence of lithium salt gave
only the E-isomer. Using standard deprotection steps as before, the
corresponding diol (31) was formed. Basic hydrolysis of the crude
product with barium hydroxide afforded D-erythro-sphingosine (14).
Scheme 9: Synthesis of D-erythro-sphingosine and D-erythro-dihydrosphingosine by Reist and Christie.42,43

A similar approach was adopted by Ogawa et al. using 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose as starting material (Scheme 10).44,45 Wittig coupling was applied to introduce the double bond.
The ratio of \( E \)-alkene to its \( Z \)-isomer was 43:57. The crucial difference between this approach and that of Reist and Christie, is that the former used irradiation in order to improve the \( E \)- and \( Z \)-ratio to 94:6. The free secondary alcohol was converted into its mesylate before carrying out the three steps required for transforming the acetal into the diol. The diol was then protected as a macrocyclic structure (32). In order to establish the \( erythro \)-configuration, \( \text{SN}_2 \) displacement had to be performed on the mesylate group using sodium azide. Reduction of the azide, acylation and deprotection gave \( D \)-\( erythro \)-ceramide (33).
Gigg and co-workers made full use of oxazoline chemistry to prepare intermediates for the synthesis of sphingoglycolipids. The strategy of this approach (Scheme 11)\textsuperscript{46,47} involves cleaving a phenyloxazoline (34) to give a benzamide (35), which cyclises with the mesylate to generate a new oxazoline (36). Through the course of cyclisation the \textit{erythro}-configuration was established. The epoxide was made in 4 steps. Hydrolysis of the acetal followed by dimesylation and selective displacement of the primary mesylate by sodium benzoate gave the benzoate (37). The benzoate was readily converted into the oxirane (38) with sodium methoxide in chloroform-methanol. Alkylation with a Grignard reagent, followed by deprotection of the acetal gave the triol (39). This triol was then converted into the D-\textit{erythro}-sphingosine phenyloxazoline derivative (40).

Despite its length, the synthesis of (2S,3R)- and (2S,3S)-sphingosine developed by Obayashi and Schlosser\textsuperscript{48} is well designed. This is because the whole synthesis can be performed on a large scale without affecting the yields. D-(+)-Mannose was transformed into the bromodihydrofuran (41) (Scheme 12). This was the key intermediate which underwent dehalogenation in the presence of n-butyllithium and caused the 5-membered ring to collapse to generate the carbinol (42). Alkylation by the acetylide and \textit{SN}2 displacement of the mesylate by lithium azide gave the azide with the desired stereochemistry. The last step involved reduction of the triple bond and the azide as well as deprotection. The success of this synthesis was heightened by the fact that \textit{threo}-sphingosine was obtained via this route by using D-(+)-ribono-1,4-lactone as starting material.
Scheme 11: Synthesis of D-erythro-sphingosine by Gigg and co-workers.\textsuperscript{46,47}
Scheme 12: Synthesis of erythro- and threo-sphingosine by Obayashi and Schlosser.48

In addition to the contribution made in the synthesis of racemic sphingosine, Schmidt and Zimmermann49,50 developed a route to D-erythro-sphingosine starting from D-galactose (Scheme 13). This sugar was converted into its 4,6-0-benzylidene-D-galactose which upon treatment with sodium metaperiodate provided the desired 2,4-0-
benzylidene-D-threose (43). However, in the same year, Kiso et al.\textsuperscript{51} showed that 2,4-\textit{O}-benzylidene-D-threose could be obtained from two different sugars. Wittig coupling of the 2,4-\textit{O}-benzylidene-D-threose with hexadecanylidene triphenylphosphorane in the presence of lithium bromide gave exclusively the \textit{E}-eicosentriol (44). The mesylate of the alcohol was converted into the azide as before. Deprotection followed by reduction of the azide yielded D-\textit{erythro}-sphingosine.

In contrast to the above syntheses, Kamikawa et al. used a six-membered ring sugar, as opposed to five, as starting material and achieved diastereoselective synthesis of D-\textit{erythro}-sphingosine (Scheme 14).\textsuperscript{52} Mercuric ion-assisted acid hydrolysis of 3,4,6-tribenzyloxy-D-galactal yielded hydroxy-\textit{E}-enal (45). Reduction of the aldehyde gave the diol which was then protected as the diacetate (46). Coupling of dodecylmagnesium bromide cuprate with the allyl acetate gave the desired $\alpha$-substitution product. Displacement of the acetate group to obtain the azide with complete inversion of the stereochemistry was carried out via three steps: methanolysis, mesylation and formation of the azide. Reduction of the azide followed by deprotection gave D-\textit{erythro}-sphingosine.
Scheme 13: Synthesis of D-erythro-sphingosine by Schmidt and Zimmermann,\textsuperscript{49,50} and Kiso \textit{et al.}\textsuperscript{51}
Adopting a new approach, Inazu et al. carried out the synthesis of D-erythro-sphingosine by starting from 2,3-O-isopropylidene-D-glyceric acid methyl ester (47), which when coupled with dimethyl methylphosphonate, generated the phosphonate (48) (Scheme 15). Wittig condensation with myristic aldehyde in the presence of caesium carbonate gave exclusively the E-product (49). High diastereoselectivity in the reduction of the unsaturated ketone was achieved with L-Selectride. Hydrolysis of the acetal followed by treatment of the triol with benzaldehyde afforded the six-membered ring acetal. Displacement of the free hydroxyl group by sodium azide with inversion of stereochemistry was achieved in the usual manner (mesylation and formation of the azide). Reduction of the azide followed by deprotection gave D-erythro-sphingosine.
3.3.2 Diastereoselective Synthesis of D-erythro-Sphingosine using Serine

Another homochiral building block which has made an important impact in the diastereoselective synthesis of D-erythro-sphingosine is (S)-serine. Use of serine as a starting material has an advantage over most sugars. There is no need to carry out the typical three steps which convert the threo-triol into its erythro-aminodiol as the amino group is already present in serine.

Newman$^{54}$ reported a short and highly stereoselective synthesis of D-erythro-sphingosine in 1973. (S)-Serine was converted into the aldehyde (50) by $N$-phthaloylation, O-acetylation, acid chloride formation and catalytic hydrogenation (Scheme 16). Addition of trans-pentadecenyldiisobutylalane to the aldehyde gave a 4:1 mixture of erythro- and threo-isomers. The diastereoisomers were separated by chromatography. The conformation of the aldehyde controlled the
outcome of the diastereomeric mixture. Fortunately the preferred conformation, according to the proton NMR spectrum, was the one that gave predominantly the erythro-isomer when attacked by the organometallic reagent.

Scheme 16: Synthesis of D-erythro-sphingosine by Newman.\textsuperscript{54}

The approach adopted by Tkaczuk and Thornton\textsuperscript{55} was based on the sphingosine synthesis of Newman. (S)-Serine methyl ester\textsuperscript{56} was protected as an oxazoline which was then reduced to the aldehyde with diisobutylaluminium hydride (Scheme 17). Treatment of this aldehyde with E-pentadecenyldiisobutylalane gave the sphingosine oxazoline and its epimer as a 1:1 mixture. These isomers could be separated chromatographically. Acid hydrolysis of the erythro-isomer followed by N-acylation with p-nitrophenyl oleate afforded the corresponding D-erythro-N-oleoyl-1-O-benzoylsphingosine. Base hydrolysis provided D-erythro-ceramide (33).
Scheme 17: Synthesis of erythro- and threo-sphingosine derivatives by Tkaczuk and Thornton.⁵⁵

Kozikowski and Wa⁵⁷ were the first group to incorporate fluorine into sphingosine. Fluorine is a good replacement for an hydroxyl group because they are similar in size and fluorine can also act as a hydrogen bond acceptor. D-erythro-Sphingosine oxazoline was prepared using the procedure of Tkaczuk and Thornton.⁵⁵ Reaction of sphingosine oxazoline with 2-chloro-1,1,2-trifluorotiethylamine afforded the corresponding fluoro derivative (51) as well as the allylic
rearrangement product (52) (Scheme 18). Since the fluoro derivative (51) was isolated as a single stereoisomer, the reaction must have occurred via an SN2 mechanism. Hydrolysis of the oxazoline gave E-threo-2-amino-3-fluoro-octadec-4-en-1-ol (53).

Scheme 18: Synthesis of a fluorine-containing isostere of sphingosine by Kozikowski and Wa.57

A new improved procedure had been developed by Garner et al. for the synthesis of D-erythro-sphingosine from another stable protected serine aldehyde (Scheme 19).58 The efficacy of this aldehyde as a chiral building block was also demonstrated by Herold,59 Liotta et al.60 and Radunz et al.61 N-BOC-(S)-Serine methyl ester was transformed into the aldehyde (54) by acetalisation and reduction with diisobutylaluminium hydride. Treatment with 1-pentadecynyllithium gave the corresponding erythro-sphingosine 2,2-dimethylloxazolidine and its epimer as a 9:1 mixture. These diastereoisomers were readily separated chromatographically. Birch reduction followed by acid hydrolysis afforded D-erythro-sphingosine. However, on exposure to Lindlar hydrogenation cis-erythro-sphingosine was obtained. Interestingly, high threo-selectivity was observed on addition of 1-pentadecynyllithium to the aldehyde in the presence of anhydrous zinc bromide with dry ether as a solvent. It is noteworthy that the 2,2-
dimethyloxazolidine (55) and its epimer can be interchanged via a Mitsunobu inversion at the C-3 centre.

**Scheme 19: Synthesis of D-erythro-sphingosine by Garner et al.,**

In contrast to the synthesis of Garner et al., Rapoport and Boutin took two steps to synthesise the ynon (56) instead of one. α'-Amino-αβ-ynone was synthesised initially and then treated with sodium borohydride to achieve the diastereoselectivity (Scheme 20). N-Z-(S)-Serine was reacted with isobutylchloroformate to generate a mixed anhydride which was then quenched with isoxazolidine. N-Z-(S)-Serine isoxazolidide (57), being analogous to Weinreb’s amide deserves to be mentioned. This amide can only react with one mole of the nucleophile, due to the lithium ion complexing to both oxygens, giving rise to a stable intermediate which cannot undergo further alkylation. Alkylation of the amide (57) with an excess of 1-pentadecynyllithium afforded the corresponding αβ-ynone. Diastereoselective reduction of the ketone with sodium borohydride gave anti- and syn-isomers as a

Scheme 20: Synthesis of D-erythro-sphingosine by Rapoport and Boutin.62

3.4 Enantioselective Synthesis of Sphingosine

Enzymic reaction, Sharpless asymmetric epoxidation and asymmetric aldol reaction are the three approaches that have been frequently employed to introduce the stereochemical centres in one step. Only a few groups have employed enzymes to achieve enantioselective synthesis. In most cases, epoxidation and the aldol reaction are more favoured.

Earlier work of enantioselective synthesis of D-erythro-sphingosine via Sharpless asymmetric epoxidation was carried out by Vassella et al.63, 64 The enynol (58) was synthesised from epichlorohydrin as shown in Scheme 21. Sharpless asymmetric epoxidation of the enynol gave the desired epoxide and the free alcohol was then converted into a carbamate. This carbamate was then subjected to sodium hydride to generate the nitrogen anion. The key
step in this synthesis was the regioselective intramolecular opening of the oxirane by the nitrogen anion of the carbamate to afford the erythro-carbamate (59). Treatment with lithium and ethylamine afforded selective reduction of the triple bond and debenzylation. Basic hydrolysis of the oxazolidinone gave D-erythro-sphingosine.

Scheme 21: Synthesis of D-erythro-sphingosine by Vasella et al.\textsuperscript{63,64}

In an approach to (+)-aplidiasphingosine, Mori and Umemura prepared the key intermediate triene (60), from optically pure (R)-methyl citronellate in 16 steps (Scheme 22).\textsuperscript{65} The triene (60) was epoxidised with tert-butylhydroperoxide in the presence of titanium (IV) isopropoxide and (+)-diethyl tartrate to give the epoxy alcohol (61). Cleavage of the oxirane with ammonia gave a (3:1) mixture of diastereoisomers. Both isomers were purified by column chromatography. The absolute stereochemistry of the threo-aplidiasphingosine was confirmed by direct comparison with the natural product.
A new route was established by Katagawa et al. in which all sphingosine stereoisomers were synthesised from Z- and E-1,4-butenediol (Scheme 23). Z-1,4-Butenediol, used to prepare D-threo-sphingosine, was converted into its (2R, 3S)-epoxide in 4 steps: monoacetylation of the diol, protection of the free alcohol, alkaline hydrolysis of the acetate to generate the allyl alcohol system and Sharpless epoxidation. Upon treatment with Ti(OiPr)2(N3)2, the epoxide was opened regioselectively to give the 1,3-diol (62). It was converted into the aldehyde (63) through successive reactions: diprotection of the diol, reduction of the azide, N-acetylation, selective deprotection and Swern oxidation. Wittig condensation of the aldehyde (63) with tetradecyltriphenylphosphonium bromide gave a mixture of Z- and E-isomers. The ratio of this mixture was improved in favour of the E-isomer by irradiation of the crude product in the presence of diphenyldisulfide. Removal of the protecting groups afforded D-threo-
sphingosine. Interestingly opening of the (2R,3R)-epoxide with azide led to a mixture of 1,2-diol (64) and 1,3-diol (65) in the ratio of 1:14. Using the same conditions as before, the 1,3-diol was then transformed into D-erythro-sphingosine.

Scheme 23: Synthesis of erythro- and threo-sphingosine by Katagawa et al.66
Nicolaou et al. reported enantioselective synthesis of sphingosine using an asymmetric aldol reaction with an αβ-unsaturated aldehyde (Scheme 24). The strategy was based on the work of Evans and co-workers. The oxazolidinone derivative (66) was transformed into its boron enolate and condensed with C₁₆ αβ-unsaturated aldehyde to afford a mixture of syn-adducts. The diastereoisomers were separated by column chromatography. SN₂ displacement of the bromide with sodium azide gave the corresponding azide. Silylation of the secondary alcohol followed by reductive cleavage of the chiral auxiliary provided the monoprotected alcohol (67). Finally, D-erythro-sphingosine was obtained by desilylation and reduction of the azide.

The key step of the synthesis of Ito et al. was to employ the chiral (aminoalkyl)ferrocenylphosphine-gold (I) complex to catalyse the asymmetric aldol reaction (Scheme 25). Reaction of C₁₆ αβ-unsaturated...
aldehyde with methyl isocyanoacetate (68) in the presence of this complex gave a quantitative yield of methyl 5-[(E)-1-pentadecenyl]-2-oxazoline-4-carboxylate consisting of anti- and syn-isomers in a ratio of 89:11. A chiral shift reagent, Eu(dcm)$_3$, was used to calculate the enantiomeric excess. Hydrolysis and reduction, followed by acetylation provided the D-threo-N-acetylsphingosine. Synthesis of D-erythro-sphingosine was readily achieved from the threo-isomer by carrying out a Mitsunobu inversion at C-3.

Schollkopf et al. developed a new enantioselective synthesis of D-erythro-sphingosine via an asymmetric aldol reaction from the bislactim ether of cyclo-(L-Val-Gly) (69) (Scheme 26). Aldol addition of the lithiated derivative of the bislactim ether to an αβ-unsaturated aldehyde normally gave exclusively the syn-adduct with high diastereoselectivity. However, when the C$_{16}$ αβ-unsaturated aldehyde
was used, a 1:1 mixture of syn- and anti-adducts was observed. The yield of the hydrolysis step was improved when the hydroxyl group was protected. Reduction of the ester followed by debenzylation with lithium and ethylamine afforded D-erythro-sphingosine.

A lengthy enantioselective sphingosine synthesis via enzymic reaction was reported by Findeis and Whitesides (Scheme 27). Fumarase (E.C. 4.2.1.2) was used to transform achiral chlorofumaric acid into L-threo-chloromalic acid by stereospecific hydration of the carbon-carbon double bond. Reduction of the diacid followed by acetalisation gave the corresponding hydroxy acetonide which, upon treatment with benzyl isocyanate gave the carbamate (70). Deprotonation of the NH resulted in a direct SN2 displacement of the chloride. The acetal was converted into its aldehyde (71) in 5 steps. Reaction of the aldehyde with lithiated phenyl n-tetradecanlyl sulfone followed by acetylation yielded the acetoxy sulfone as a mixture of diastereoisomers, which on Na-Hg reduction produced predominantly the E-alkene. Debenzylation followed by basic hydrolysis gave D-erythro-sphingosine.
3.5 Conclusion

The importance of sphingosine is emphasised by the high number of papers published over the last 40 years. Since the work has been carried out worldwide, its importance must be fundamental. Although there are many different and reliable sphingosine syntheses, this area of research will remain active until the role of sphingosine derivatives becomes clear. Therefore organic chemists will continue to try to develop novel and more efficient syntheses, thus making sphingosine derivatives more readily available for biological evaluation.
CHAPTER FOUR

Synthesis and Biological Evaluation of Phenylalaninol and (1S,2S)-2-Amino-1-phenylpropane-1,3-diol Derivatives

4.1 Introduction

The initial aim of this project was to establish simple and reliable methods for carrying out acylation, methylation and dimethylation of a primary amino group in compounds which are analogues of sphingosine. Various groups attached to the amino group of sphingosine have been shown to play an important role in the inhibition of both PKC and PAP enzymes. N,N-Dimethylsphingosine for instance was found to be a better inhibitor of PKC and PAP enzymes than sphingosine itself. Sphingosine was not employed as the starting material in our work because of its cost. Cheaper alternatives were used instead. (R)-Phenylalaninol (72), (S)-phenylalaninol (73) and (1S,2S)-2-amino-1-phenylpropane-1,3-diol (74) were used to establish the key reactions. They are commercially available and were chosen because they have features in common with sphingosine such as functional groups in similar positions and a hydrophobic region.

![Chemical structures]

(72) = (R)-Phenylalaninol
(73) = (S)-Phenylalaninol
(74) = (1S,2S)-2-amino-1-phenylpropane-1,3-diol
(14) = (R)-N,N-Dimethylsphingosine

4.2 Synthesis of Phenylalaninol Analogues

N-Acetylation

The synthesis of these known compounds proved to be straightforward under standard conditions. (R)-N,N-Dimethylsphingosine (14) was synthesized using standard procedures. The reaction scheme involved acetylation of the amino group followed by methylation and dimethylation. The yields for these transformations were typically high, approaching 90%. The resulting derivatives were characterized by spectroscopic methods, including 1H NMR and MS, confirming their structures.
Diacetylphenylalaninol (75) and its enantiomer (76) were prepared using acetic anhydride and pyridine in THF. These compounds were isolated in greater than 80% yield. Being enantiomers, both have the same spectroscopic properties but different signs for the optical rotation. Their IR spectra showed strong absorptions at 1684 and 1772 cm\(^{-1}\) due to the amide carbonyl and the ester carbonyl respectively. Their 1H NMR spectra showed two singlets at 1.96 and 2.10 corresponding to the methyl groups.

**N-Methylation**

*N-Methylation* of the primary amine in compounds (72), (73) and (74) cannot be achieved selectively using methyl iodide and a base. The problem lies in the fact that the monomethylated products are likely to be more reactive than the starting material. The inductive effect of the methyl group makes the product able to react further to generate *N,N*-dimethylated and *N,N,N*-trimethylated products. Even with the use of one equivalent of methyl iodide, the problem is still not resolved because as soon as the product is formed, it will compete with the starting material for methyl iodide.

In order for mono-methylation to take place, the nitrogen of (S)-phenylalaninol (73) had to be protected as its butyloxy carbonyl derivative (Scheme 28). This protection was carried out as described with di-tert-dibutyl dicarbonate in dioxane and water. The product (77) was isolated in 96% yield after chromatography. The IR spectrum of the compound showed absorptions at 1686 and 3418 cm\(^{-1}\) corresponding to the carbamate carbonyl and the hydroxyl groups respectively. The BOC group gave a characteristic resonance in the 1H NMR spectrum of a singlet at 1.41 due to the tert-butyl group.
Coggins and Benoiton\textsuperscript{74} had prepared a variety of $N$-methylated derivatives from $N$-BOC derivatives using sodium hydride and methyl iodide in THF. Methylation of (S)-$N$-BOC-phenylalaninol using these conditions gave the desired $N$-methylated methyl ether (78) in 5\% yield. The IR spectrum of the amine showed a strong absorption at 2850 cm\(^{-1}\) due to the stretching of the CH. The $^1$H NMR spectrum showed two methyl singlets at $\delta$ 2.30 and 3.18 due to the $N$-methyl and $O$-methyl groups respectively.

However this reaction also gave two unexpected products: the cyclic carbamate (79) and $N$-BOC-$O$-methylphenylalaninol (80). Since three equivalents of sodium hydride were used, the dianion of $N$-BOC-phenylalaninol could have formed initially as an intermediate (81) which can be methylated on the nitrogen and oxygen atoms. $O$-Methylation, in theory, was more favoured because the nitrogen site is sterically hindered by the tert-butyl group as well as being less
nucleophilic than the alkoxide. However, once the oxygen atom was methylated (82), the yield of the product (78) suggested that the nitrogen was reluctant to carry out further alkylation. When $N$-methylation occurred first, the nucleophilic oxygen attacked either another mole of methyl iodide to give the product (78), or the carbamate carbon causing an expulsion of the tert-butoxide to afford the cyclic carbamate (79). The proposed mechanisms are shown in Scheme 29. The IR and $^1$H NMR spectra of $N$-BOC-O-methylphenylalaninol were similar to those of (78). The IR spectrum of the cyclic carbamate showed absorption at 1750 cm$^{-1}$ corresponding to the stretching of the carbamate carbonyl bond. The $^1$H and $^{13}$C NMR spectra of the carbamate showed no resonance in the region expected for the methyl groups of the tert-butyl group.

Scheme 29: Formation of $N$-methylated product and by-products
The final step was to deprotect the BOC group of (78) with 1M HCl in ethyl acetate to give (S)-N,O-dimethylphenylalaninol (83). The IR spectrum of the product showed a broad absorption ca. 3431 cm⁻¹ for the NH stretch, and the loss of the absorption corresponding to the carbamate carbonyl group. The ¹H and ¹³C NMR spectra also showed the disappearance of the BOC group.

To minimise the above side reactions it was necessary to protect the hydroxyl group of N-BOC-phenylalaninol. It was protected as the tert-butyldimethylsilyl ether (84) (Scheme 30) which was prepared using imidazole and tert-butyldimethylsilyl chloride (TBDMSCl) and DMF. The yield of the reaction was 89%. The IR spectrum of (84) showed absorptions at 1089 and 840 cm⁻¹ due to the stretching of the Si-O bond. The ¹H NMR spectrum showed a singlet at δ -0.03 due to the geminal dimethyl groups attached to the silicon, and a singlet at δ 0.88 corresponding to the methyl groups of the tert-butyl group.

Methylation of N-BOC-O-TBDMS-phenylalaninol (84) using sodium hydride and iodomethane was unsuccessful giving mainly the starting material. The conditions for this reaction were varied as shown in Table 1. As shown in Table 1, no success was achieved despite the range of solvents, temperature and time.
<table>
<thead>
<tr>
<th>Reagents</th>
<th>Conditions</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mel (7 equiv.), NaH (1.5 equiv.), THF</td>
<td>room temperature, 24 h</td>
<td>starting material, 80%</td>
</tr>
<tr>
<td>Mel (7 equiv.), NaH (3 equiv.), THF</td>
<td>room temperature, 24 h</td>
<td>starting material, 50%</td>
</tr>
<tr>
<td>Mel (7 equiv.), NaH (3 equiv.), THF</td>
<td>reflux 3 h, 24 h</td>
<td>starting material and product</td>
</tr>
<tr>
<td>Mel (7 equiv.), NaH (1.5 equiv.), DMF</td>
<td>room temperature, 24 h</td>
<td>starting material, 70%</td>
</tr>
<tr>
<td>Mel (7 equiv.), NaH (3 equiv.), DMF</td>
<td>reflux 3 h, 24 h</td>
<td>starting material and product</td>
</tr>
</tbody>
</table>

Grieco and Bahsas\textsuperscript{75} reported a novel method for the \textit{N}-methylation of a variety of amino acid derivatives and dipeptides via a retro aza Diels-Alder reaction, see Scheme 31. Two key features in this reaction make it an attractive method of \textit{N}-methylation. Firstly, it proceeds without racemisation and also, only the amino group will react so protection of the hydroxyl group is not required. The reaction was reported to proceed via the acid-catalysed formation of the imine adduct of the amino acid and formaldehyde, and subsequent trapping of this adduct via an aza Diels-Alder reaction. The retro aza Diels-Alder reaction then proceeded smoothly in the presence of trifluoroacetic acid. Triethylsilane reduced the liberated immonium ion to the \textit{N}-methyl compound.

![Scheme 31: N-Methylation of amino acids and dipeptides via retro aza Diels-Alder reactions by Grieco and Bahsas.\textsuperscript{75}](image-url)
Treatment of (S)-phenylalaninol (73) with formaldehyde, acetic acid and cyclopentadiene gave the corresponding aza Diels-Alder product (85) as a mixture of diastereoisomers (Scheme 32). These diastereoisomers did not need to be separated because they would give the same \( N \)-methylated product on reduction. The expected absorptions, due to the stretching of O-H and C=C groups, were found at 3401 and 1680 cm\(^{-1} \) respectively. The \(^1\)H NMR spectrum was complex but it was clear that the resonances at \( \delta \) 5.94 and 6.25 must be due to the alkene hydrogens. Fortunately, the \(^{13}\)C NMR spectrum of (85) was more informative. All the signals were accompanied by a smaller signal indicating that it was a diastereomeric mixture. The reduction step failed to generate the \( N \)-methylated compound. A variety of reducing agents was employed but none of them proved to be successful. This reaction was abandoned.

![Scheme 32: Attempted synthesis of \( N \)-methylphenylalaninol via a retro aza Diels-Alder reaction](image)

The last method employed to form the \( N \)-methylated product was reductive formylation of the amine. Meier and Ruechardt\(^{76} \) reported a general procedure for making \( N \)-acylated derivatives using carboxylic acids. In their procedures, salts were obtained when carboxylic acids were treated with ammonia or amines. The salts could be pyrolysed to
give amides. Treatment of (S)-phenylalaninol with excess formic acid and toluene under reflux gave the diformylated product (86)\textsuperscript{76} in 83% yield after purification by column chromatography (Scheme 33). The IR spectrum of (86) showed absorptions at 1668, 1718 and 3400 cm\textsuperscript{-1} corresponding to the stretching of amide carbonyl, ester carbonyl and N-H respectively. The \textsuperscript{1}H NMR spectrum showed a broad singlet at δ 8.07 with a relative intensity of 2 due to the two hydrogens adjacent to the carbonyls.

Reduction of the diformylated product (86) was carried out using lithium aluminium hydride in THF and gave the N-methylated product (87) in 74% yield after purification by column chromatography. The compound seemed to be pure by TLC but both its \textsuperscript{1}H and \textsuperscript{13}C NMR spectra showed that it was only partially pure. Further purification on silica column chromatography was attempted but without any success. The impurity was not the starting material since the \textsuperscript{1}H NMR spectrum proved the reduction had gone to completion as the resonance at δ 8.07 had disappeared. Also present in the \textsuperscript{1}H NMR spectrum was a sharp singlet at δ 2.33 corresponding to the N-methyl group. This resonance was accompanied by a small sharp singlet at δ 2.28 probably due to the impurity.
**N,N-Dimethylation**

Feldkamp *et al.*\(^7^7\) reported a simple one-pot method for *N,N*-dimethylation via reductive amination. The reaction was reported to proceed via the acid-catalysed formation of the imine adduct of the primary amine and formaldehyde, which was subsequently reduced with hydrogen gas over a palladium and charcoal catalyst. Due to the fact that the *N*-methylated product still contained an N-H group, the reductive amination could be repeated a second time to yield a *N,N*-dimethylated derivative. Treatment of (S)-phenylalaninol with acetic acid, formaldehyde, hydrogen, palladium and charcoal afforded the known *N,N*-dimethylated product (88)\(^7^8\) (Scheme 34). The product was isolated in 54% yield after column chromatography. The IR spectrum showed an absorption at 3390 cm\(^{-1}\) corresponding to the hydroxyl group. The \(^1\)H NMR spectrum showed a sharp singlet at \(\delta\) 2.56 due to the methyl groups attached to the nitrogen.

![Scheme 34: Synthesis of N,N-dimethylphenylalaninol](image)

An unexpected product, (S)-3-methyl-4-benzyloxazolidine (89), was also isolated in this reaction. One possible way this by-product was generated is shown in Scheme 35. Since 4-benzyloxazolidine (90) did not form as a by-product in this reaction, this may suggest that the reduction of the immonium ion was faster than the cyclisation of the alcohol to the imine. However, when the secondary immonium ion was
formed, the reduction was slower, and some of the secondary imine had cyclised to form (S)-3-methyl-4-benzyloxazolidine (89). The IR spectrum of this compound only showed absorptions of C-H stretches. The $^1\text{H}$ NMR spectrum showed a methyl singlet at $\delta$ 2.47 due to the $N$-methyl group. Also an AB pattern was observed at $\delta$ 4.01 and 4.39 with a geminal coupling constant of 4.4 Hz. These signals were slightly more deshielded than a typical $O$-methyl group, suggesting that the CH$_2$ group must be sandwiched between an oxygen atom and an electron withdrawing group. Similar results were obtained when the other enantiomer (72) was subjected to the reaction conditions of Feldkamp et al.\textsuperscript{77}

Scheme 35: Formation of $N,N$-dimethylphenylalaninol and by-product
(S)-N,N,N-Trimethylphenylalaninol iodide (91)\(^7\) was prepared in 61% yield by treatment of (88) with methyl iodide in diethyl ether (Scheme 36). The expected absorption due to the OH was found at 3200 cm\(^{-1}\). The \(^1\)H NMR spectrum showed a singlet at \(\delta 3.21\) for the methyl groups attached to the nitrogen. Although small traces of impurities were present according to the \(^1\)H spectrum, compound (91) was sufficiently pure for biological testing.

![Scheme 36: Synthesis of N,N,N-trimethylphenylalaninol iodide](image)

### 4.3 Synthesis of (1S,2S)-2-Amino-1-phenylpropane-1,3-diol Derivatives

With the reaction conditions established for \(N\)-acetylation, \(N\)-methylation, \(N,N\)-dimethylation and \(N,N,N\)-trimethylation of an aminol, it was of great interest to see whether these conditions could be applied to (1S,2S)-2-amino-1-phenylpropane-1,3-diol, an aminodiol. The extra hydroxyl group proved to be more problematic than anticipated. More side products were observed. \(N\)-Methylation using sodium hydride and methyl iodide proved to be unsuccessful as expected. In the case of reductive formylation, trifromylation of the aminodiol occurred. Although apparently pure by TLC, both \(^1\)H NMR and \(^13\)C NMR spectra showed that there were extra signals present. This mixture could not be purified further by column chromatography. It was decided to carry out the reduction with this partially purified product. The reduction step yielded a complex mixture from which the desired product could not be isolated. Although \(N\)-methylation of 2-amino-1-phenylpropane-1,3-diol did not occur with the established reaction conditions, a new synthesis was devised to carry out \(N\)-methylation specifically on an aminodiol. It was the success of this synthesis that opened up a new
route by which N-methylsphingosine could be synthesised. Details of this method are discussed in Chapter 6.

Out of the three established reaction conditions, \( N,N\)-dimethylation proved to be the most reliable. Treatment of \((1S,2S)-2\text{-amino-1-phenylpropane-1,3-diol}\) with acetic acid, formaldehyde, hydrogen gas, and a palladium and carbon catalyst afforded a complex mixture (Scheme 37). Fortunately, the known product \((92)^{80}\) was purified by careful column chromatography. The O-H stretch was found at 3400 cm\(^{-1}\). The \(^1\)H NMR spectrum showed a sharp singlet at \(\delta 2.34\) with a relative intensity of 6 due to the methyl groups attached to the nitrogen.

\[
\begin{align*}
\text{Scheme 37: Synthesis of } (1S,2S)-2\text{-dimethylamino-1-phenylpropane-1,3-diol} \\
\end{align*}
\]

4.4 Biological Testing of \((R)\) and \((S)\)-Phenylalaninol and \((1S,2S)-2\text{-Amino-1-phenylpropane-1,3-diol Analogues}\)

The biological testing of some of these compounds was carried out by Dr. Pamela Scott\(^{81}\) at the Department of Medical Oncology at the CRC Beatson Laboratories in Glasgow. Our initial aim was to study and compare the inhibitory effect of these derivatives with sphingosine on PAP enzymes.
4.5 Biological Assays

Determination of N-ethylmaleimide (NEM)-sensitive PAP (PAPI) activity

Each assay contained in a final volume of 0.1 ml: PAPI Cocktail (see below) (20 ml), H2O (45 ml), rat liver cytosol plasma membrane (PAPI) (10 ml), 3H-phosphatidate /phosphatidy1-choline (3:2) (20 ml), and drug (5 ml). The assays were started by addition of the test compound. The incubation was carried out for 60 minutes at 37 °C after which time the reactions were stopped by addition of chloroform/methanol (19:1) (2.2 ml) containing 0.08% olive oil and aluminium oxide (1 g). After centrifuging each test tube for 10 minutes to remove unreacted 3H-phosphatidate which precipitated with aluminium oxide, 1 ml of the top phase from each test tube was transferred to a scintillation vial which was then placed in a water bath at 90 °C so that the solvents would evaporate off. These vials now contained only the 3H-diacylglycerol and the radioactivity of each vial was measured in a Scintillation Counter (model 1600 TR; Canberra Packard). The above experiment was carried out with three different concentrations of each drug (0.2, 0.6, 1.2 mM), including propranolol as a reference and with 3 control assays that contained no enzyme.

Determination of (NEM)-insensitive PAP (PAP2) activity

Each assay consisted of PAP2 cocktail (see below) (20 ml), rat liver cytosol plasma membrane (45 ml), H2O (45 ml), 3H-phosphatidate (20 ml) and drug (5 ml). The reactions were started by addition of the test compound and the incubations were terminated after 60 minutes at 37 °C by addition of chloroform/methanol (19:1) (2.2 ml) containing 0.08% olive oil. The unreacted 3H-phosphatidate was removed by adding aluminium oxide (1 g). The radioactivity of the 3H-diacylglycerol was measured. The above experiment was carried out with three different concentrations of each drug (0.2, 0.6, 1.2 mM), including propranolol as a reference and with 3 control assays that contained no enzyme.
### PAPI and PAP2 cocktails

<table>
<thead>
<tr>
<th></th>
<th>PAP1</th>
<th>PAP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dithiothreitol (DTT)</td>
<td>80 ml</td>
<td>-----</td>
</tr>
<tr>
<td>Magnesium</td>
<td>30 ml</td>
<td>-----</td>
</tr>
<tr>
<td>Tris/maleate buffer</td>
<td>500 ml</td>
<td>500 ml</td>
</tr>
<tr>
<td>(pH 6.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-ethylmaleimide (NEM)</td>
<td>-----</td>
<td>6.8 mg</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>-----</td>
<td>500 ml</td>
</tr>
<tr>
<td>Water</td>
<td>1390 ml</td>
<td>990 ml</td>
</tr>
</tbody>
</table>

#### 4.5.1 Results of Biological Assays

The following Table 2 records the percentage of PAP inhibition for some of the compounds in the assays previously described.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>0.2</th>
<th>0.6</th>
<th>1.2</th>
<th>0.2</th>
<th>0.6</th>
<th>1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="72">Image of compound</a></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><a href="73">Image of compound</a></td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td><a href="74">Image of compound</a></td>
<td>62</td>
<td>86</td>
<td>89</td>
<td>8</td>
<td>29</td>
<td>41</td>
</tr>
<tr>
<td><a href="74">Image of compound</a></td>
<td>91</td>
<td>100</td>
<td></td>
<td>4</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>
From the Table, it is clear that most of the analogues show little inhibition of PAP2 activity compared to \(N,N,N\)-trimethylated sphingosine but have some significant inhibitory effects on PAP1 activity. Unfortunately, PAP2 is the enzyme that is involved in tumour promotion via signalling processes. The most promising inhibitors of
the PAPI enzyme were (R)- and (S)-4-benzyl-3-methyloxazolidine. (S)-4-Benzyl-3-methyloxazolidine (89) inhibited 45% of PAPI activity at 0.2 mmol. This compound was a better inhibitor than propanolol, a classical drug for the inhibition of PAP activities. (R)-4-Benzyl-3-methyloxazolidine displayed similar inhibitory effects to propanolol on both PAP enzymes. Evidently (R)- and (S)-phenylalaninols showed little inhibitory effect on both PAP enzymes. Converting the primary amine into either its secondary amine or amide increased the inhibitory effects on both enzymes. By analogy with the corresponding quaternised sphingosine, N,N,N-trimethylphenylalaninol iodide was expected to be the most potent inhibitor of the compounds prepared. However, it did not inhibit PAP2 activity at all. Consequently, we could not conclude that increasing the bulkiness around the amine group would increase the inhibitory effect on both PAP enzymes.

Also, it is difficult to relate these results from the cell-free PAP assays to what could happen in whole cells. Concentrations of the drug, substrate, enzyme and diacylglycerol in cells are much lower than the corresponding test tube assay. There are other factors that have to be taken into consideration. For instance the uptake of the drug by the cells and the purity of the enzymes. Unfortunately the enzymes used in the experiments are only partially pure which means that there are other enzymes present in the reaction that could interfere. Since the concentrations of phosphatidate and diacylglycerol are very low in the cells, it is better to examine the test compounds in a test tube assay initially to give some indication as to whether they have any affect on the PAP activities. If a test compound from the selection shows promising inhibitory effects on the PAP enzymes, then a further assay could be used.

In conclusion, a wide range of inhibition values was obtained from the above assays. There was no clear correlation between the inhibition and the bulkiness around the amino group. Nevertheless, in most cases increasing the bulkiness around the amino group did show an improvement in the inhibition. The results probably imply that the size around the amino group is not the only feature for the inhibition of PAP enzymes. A key feature which plays an important role in the inhibition of PAP and PKC enzymes may be the length of the
hydrophobic alkyl chain. We now directed our attention to the total synthesis of sphingosine. Once a reliable route had been established, it would be used to synthesise a variety of sphingosine analogues. The detail of this work is discussed in Chapter 5.
CHAPTER FIVE

Diastereoselective Synthesis of Sphingosine Analogues

5.1 Introduction

In the work described in Chapter 4, the main aim was to find the reaction conditions from which the amino group of sphingosine could be selectively methylated. Our next aim was to establish the methodology to synthesise sphingosine analogues with alkynyl chains of varying lengths. It was also of great interest to synthesise analogues of deoxysphingosine to check how such modifications would affect the inhibition of PKC. As previously mentioned in Chapter 3, diastereoselective and enantioselective approaches must be employed to synthesise optically active sphingosine. Most of our work was based on a diastereoselective strategy. However, an attempt was also made to synthesis sphingosine via an enantioselective approach. The details of the enantioselective approach will be discussed in the next Chapter.

(S)-Serine is the most frequently used starting material for the diastereoselective synthesis of sphingosine and is also the biological starting material for formation of sphingosine. As well as being cheap, it is also readily available in both enantiomeric forms. It had been reported that use of serine provided a shorter synthetic route to sphingosine than use of sugars. For these reasons, serine was chosen as the starting material for the following syntheses.

5.2 Preparation of Acyclic Diprotected Serinal

Serine, like many amino acids, is highly insoluble in organic solvents. To improve its solubility and carry out selective reactions it has to be protected. All the reported sphingosine syntheses using serine as the starting material involve transforming it into its acetonide serinal. Surprisingly, there is no evidence from the literature where acyclic diprotected serinal has been used for the synthesis of sphingosine.
Our aim was to establish reliable reaction conditions from which a substantial amount of acyclic diprotected serinal (93) could be prepared. This aldehyde would then be coupled with a selection of alkyne chains to give a range of sphingosine analogues. The rationale behind using acyclic diprotected serinal was to test whether or not it would improve the diastereoselective outcome.

The preparation of the acyclic diprotected serinal (93) is outlined in Scheme 38. (S)-Serine was first protected as its methyl ester using methanol and hydrochloride gas. The known ester (94)\(^82\) was isolated as a white solid in 94% yield. The IR spectrum showed the ester carbonyl absorption at 1747 cm\(^{-1}\). The \(^1\)H and \(^{13}\)C NMR spectra were both in accord with literature spectra of serine methyl ester.\(^60\) When serine methyl ester (94) was treated with triethylamine and di-tert-butyl dicarbonate in dichloromethane, it gave the known carbamate (95)\(^60\) in 64% yield after column chromatography. The tert-butyl protons of (95) were present as a large singlet at δ 1.42 in the \(^1\)H NMR spectrum. The carbamate carbonyl carbon showed clearly in the \(^{13}\)C NMR spectrum (δ 260) and the C=O stretching mode was present at 1712 cm\(^{-1}\) in the IR spectrum.

Chromatography also afforded an unexpected product: the di-BOC protected serine methyl ester (96). This compound was isolated as a white solid in 10% yield, and its melting point confirmed the identity of the compound, with mp 94 °C (lit.\(^83\) 94.5 °C).
Scheme 38: Preparation of acyclic diprotected serinal

Treatment of the monoprotected amino acid ester (95) with tert-butyldimethylsilyl chloride and imidazole in dry DMF at room temperature gave the diprotected ester (97) in 92% yield after purification by flash chromatography. The IR spectrum of this compound showed strong absorptions at 1752 and 1720 cm\(^{-1}\) corresponding to the ester carbonyl and the carbamate carbonyl respectively. Absorptions at 1256 and 1088 cm\(^{-1}\) corresponded to stretching of the SiMe\(_2\) group and Si-O bond respectively. The \(^1\)H NMR spectrum showed two singlets at \(\delta\) 0.00 and \(\delta\) 0.01 due to germinal methyl groups. Also a singlet at \(\delta\) 0.84 due to the methyl groups of the tert-butyl group. The signals of the silyl protecting group were distinctive in the \(^1\)H NMR spectrum. The geminal methyl groups are diastereotopic due to the chiral centre and give rise to two singlets at \(\delta\) 0.00 and 0.01.

The reduction of the ester into the corresponding aldehyde (93) using DIBAL-H in toluene proved to be more difficult than first
anticipated. A variety of reaction conditions was used and the results are shown in Table 3.

<p>| Table 3 |
|---------|---------|---------|---------|---------|</p>
<table>
<thead>
<tr>
<th>DIBAL-H</th>
<th>Solvent</th>
<th>Time</th>
<th>Temp (°C)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 equiv.</td>
<td>DCM</td>
<td>0.5 h</td>
<td>-78</td>
<td>mostly starting material</td>
</tr>
<tr>
<td>2 equiv.</td>
<td>DCM</td>
<td>2 h</td>
<td>-78</td>
<td>partial reduction</td>
</tr>
<tr>
<td>2 equiv.</td>
<td>DCM</td>
<td>2 h</td>
<td>Room temp</td>
<td>alcohol</td>
</tr>
<tr>
<td>1.1 equiv.</td>
<td>toluene</td>
<td>1 h</td>
<td>-78</td>
<td>partial reduction</td>
</tr>
<tr>
<td>1.5 equiv.</td>
<td>toluene</td>
<td>2 h</td>
<td>-78</td>
<td>mainly aldehyde + small traces of alcohol</td>
</tr>
<tr>
<td>2.5 equiv.</td>
<td>toluene</td>
<td>2 h</td>
<td>-78</td>
<td>ca 1:1 mixture of alcohol and aldehyde</td>
</tr>
</tbody>
</table>

It was found that using 1.5 equivalent of DIBAL-H in toluene produced the best result. No attempt was made to purify the aldehyde (93) from the alcohol using flash chromatography. Most amino carboxaldehydes are known to be unstable and can racemise on silica or if subjected to distillation conditions. Therefore the partially pure aldehyde was used directly for the next step to ensure the integrity of the chiral centre. The yield of the reduction could be estimated from the $^1$H NMR spectrum of the crude product. The aldehyde proton was distinctive in the $^1$H NMR spectrum showing a singlet at δ 9.59. The aldehyde carbonyl carbon showed up clearly in the $^{13}$C NMR spectrum (δ 199) and the C=O stretching mode was present at 1725 cm$^{-1}$ in the IR spectrum.
5.3 The Reaction of Acyclic Diprotected Serinal with the Lithium Acetylide of Alkynes

Addition of an excess of lithium acetylide of hex-1-yne to the aldehyde (93) gave a complex mixture (Scheme 39). TLC showed five spots, two of which were the starting materials. Two of the three remaining spots were very close to one another with large Rf values. These compounds (99) were too non-polar to be the desired carbinol (98). They were purified by careful column chromatography, and were found to have similar spectroscopic data. The IR spectra of (100) and (101) showed absorptions at ca 1248 and 1740 cm⁻¹ corresponding to the SiMe₂ group and the carbamate carbonyl respectively. Also there was no O-H absorption in the IR spectrum. While their ¹³C NMR spectra clearly indicated the presence of the carbamate carbonyl (δ ca.158), the O-tert-butyl group was absent in both ¹H and ¹³C NMR spectra. The only difference between their ¹H NMR spectra was the signal at δ ca. 5.0. This signal was due to the proton adjacent to the carbamate oxygen. Although each signal was a doublet of triplets, the coupling constants were different.

Scheme 39: Attempted synthesis of the carbinol (98)

In addition, two NMR homo-decoupling experiments were carried out to provide further structural information about these two
compounds (100) and (101). Irradiation of the signal of (100) at ca. \( \delta \) 3.56 caused the signals at \( \delta 3.82 \) and 4.92 to collapse from a doublet of triplets to a triplet and from a triplet to a singlet respectively. Also homo-decoupling of the signal at ca. \( \delta 4.90 \) caused each of the signals at \( \delta 3.82 \) and 2.25 to collapse to a triplet. The reaction must have been successful or else the proton at \( \delta 4.90 \) would not be coupled with the methylene protons (\( \delta 2.25 \)) nearest to the triplet bond. Similar experiments were carried out on (101) and the same findings were observed.

From the above observations, it was concluded that (100) and (101) must be the two diastereoisomers (99) formed in approximately equal amounts. Since there were no OH and O-tert-butyl groups present, the OH therefore must have cyclised with the carbamate. The mechanism of this reaction is shown in Scheme 40. The coupling of the acetylide of hex-1-yne with the aldehyde (93) resulted in the generation of an alkoxide ion which added to the carbamate carbonyl which then expelled the tert-butoxide group to give the cyclic carbamate mixture (99).
To differentiate between these two diastereoisomers, \(^1\)H NMR spectroscopy was employed as reported by Futagawa \textit{et al.} They found that the ring protons of the \textit{erythro}-isomer of 2-oxazolidine derivatives and 2-phenyl-2-oxazoline derivatives always had a larger coupling constant than those of the \textit{threo}-isomer. These findings are in agreement with use of the Karplus equation. The coupling constant for the \textit{erythro}-isomer will have a larger value because it has a dihedral angle of ca. 0°. Thus, the configurational identity of the \textit{threo}- and \textit{erythro}-isomers was assigned by analysis of the \(^1\)H NMR spectra of their 2-oxazolidone or 2-phenyl-2-oxazoline derivatives. The coupling constants for the ring protons of the diastereoisomers (99) were 3.3 and 5.5 Hz. We assigned the isomer with the coupling constant of 5.5 Hz to be the \textit{erythro}-form (101) and the isomer with the coupling constant of 3.3 Hz to be the \textit{threo}-isomer (100).
Although both diastereoisomers could be separated by careful column chromatography, the yield and the diastereoselectivity of this reaction were relatively poor. Therefore this route cannot be used generally to synthesise sphingosine analogues. In addition, when the reaction was scaled up, the reduction step only gave small traces of aldehyde. This problem coupled with the difficulty in purifying the acyclic diprotected serinal forced us to divert our attention to the synthesis of αβ-ynone (102) using the diprotected serine methyl ester (97), since it is more stable than its aldehyde. The αβ-ynone (102) might then be reduced diastereoselectively (Scheme 41).

5.4 The Reaction of Acyclic Diprotected Serine Methyl Ester with the Lithium Acetylide of an Alkyne

Upon treatment of the ester (97) with the lithium acetylide of hex-1-yne it was expected that the ester would undergo a nucleophilic displacement to give the corresponding αβ-ynone (102). Unfortunately this proved not to be the case. When the ester was treated with the lithium acetylide of hex-1-yne at -78 °C, only the starting material was obtained after the work up. The lack of success was probably due to the bulkiness of the protecting groups causing severe steric hindrance.
However, when the reaction was carried out at 0 °C, a mixture of the starting material and a side product were obtained. This side product will be discussed later in this chapter. In order to overcome the steric problem, different protecting groups were employed.

The next step was based on a recent paper by Evans et al. detailing the transformation of $N$-CBZ-serine methyl ester into its cyclic diprotected form. Treatment of (95) with 2,2-dimethylpropane and PTSA as a catalyst produced the known acetonide ester (103) in 70% yield. The 2,2-dimethylpropane acts as an acetone equivalent when treated under acidic conditions, see Scheme 42. These protecting groups should reduce the steric hindrance problem.

Scheme 42: Formation of the oxazolidine ring system

The IR spectrum of the oxazolidine (103) showed absorptions at 1725 and 1702 cm$^{-1}$ due to the ester carbonyl and carbamate carbonyl respectively. No O-H absorption was observed in the IR spectrum. The $^1$H NMR spectrum appeared more complex than the assigned structure
would indicate, see Diagram 8. The oxazolidine exists at room temperature as a mixture of rotamers. The two ring methyl groups appeared as four separate signals at $\delta$ 1.43, 1.46, 1.57 and 1.60, two for each methyl group. The heights of these signals are proportional to the proportional of molecules in that particular rotameric form. All the signals appeared as pairs except for the methyl group of the ester which is a singlet.

Treatment of the oxazolidine (103) with the lithium acetylide of hex-1-yne at $-78^\circ$C gave starting material, the desired $\alpha\beta$-ynone (104) and a known by-product (105). The $\alpha\beta$-ynone (104) was isolated in 17% yield after column chromatography. The IR spectrum showed absorptions at 1680 and 2225 cm$^{-1}$ corresponding to the ketone carbonyl and the triple bond, respectively. The ketone carbonyl carbon showed up clearly in the $^{13}$C NMR spectrum ($\delta$ 163.4). This value was low compared to the typical value for a ketone carbonyl because of the conjugation.

![Scheme 43: Synthesis of the $\alpha\beta$-ynone (104)](image)

The by-product isolated from the mixture was methyl 2-(tert-butyloxy carbonyl)aminoacrylate (105). This product was also found previously in the reaction of the acyclic diprotected ester with the lithium acetylide of hex-1-yne. It is UV active due to a conjugated system. The IR spectrum showed absorptions at 1680 and 1750 cm$^{-1}$.
Diagram 8: $^1$H NMR spectrum of methyl (4$S$)-3-tert-butyloxycarbonyl-2,2-dimethyloxazolidine-4-carboxylate (103)
corresponding to the $\alpha\beta$-unsaturated ketone and carbamate carbonyl, respectively. Both $^1$H and $^{13}$C NMR spectra showed that only the ring system had been cleaved and the rest of the functional groups remained intact. Only five singlets were found in the $^1$H NMR spectrum. The two new singlets at $\delta$ 6.08 and 6.10 were due to the olefinic protons. The formation of this by-product is shown in Scheme 44. The acetylide anion of hex-1-yne could either act as a nucleophile or a base. When acting as a base, it deprotonated the $\alpha$-hydrogen which subsequently caused the intermediate (106) to eliminate acetone to generate a stable conjugated compound (105).

![Scheme 44: Formation of the methyl 2-(tert-butyloxycarbonyl)aminoacrylate](image)

In order to try to prevent this by-product from being formed, the ester carbonyl was activated by a Lewis acid to enhance its reactivity, in the hope that the yield of the ynone would improve. However, addition of the Lewis acid to the reaction led to a complex mixture. The extra impurities resulted probably from the reaction of the nucleophile with the carbamate carbonyl. The carbamate carbonyl would have been more activated by the Lewis acid than the ester carbonyl as it has a higher electron density.
Since addition of the Lewis acid caused more problems than expected, it was decided to transesterify the methyl ester (103) into the phenyl ester. The phenyl ester is obviously a more reactive ester as phenoxyde is a better leaving group. If this did not prevent the formation of this by-product then the ester would have to be reduced to its aldehyde, which has been shown to be stable to silica.86

The standard method of transesterification using excess of phenol and PTSA with Dean-Stark conditions failed to give any product and only the starting material could be isolated. Another method was tried.

Upon treatment of the ester (103) with phenol and a tiny piece of lithium metal in dry DMF at 95 °C, the phenoxide anion should displace the methoxy group which would then abstract a proton from the phenol to give methanol and phenoxyde anion. Thus lithium phenoxyde should act as a catalyst and the equilibrium could be displaced by distilling off the methanol. Unfortunately, this proved not to be case. Treatment of the ester (103) with a catalytic amount of lithium phenoxyde in DMF and phenol at 95 °C gave the methyl 2-(tert-butyloxycarbonyl)aminoacrylate (105). This product was isolated in 95% yield. It came as a surprise that the phenoxyde anion was able to deprotonate the \(\alpha\)-hydrogen as it is a relatively weak base. In conclusion, the \(\alpha\)-hydrogen of the ester must be relatively acidic which causes the ring to collapse. Therefore alkynylation of the ester (103) was abandoned.

5.5 The Use of the Acetonide of Serinal with the Lithium Acetylide of an Alkyne

Garner et al.86 had reported that the ester (103) could be transformed into its aldehyde (107) by carrying out the reduction with DIBAL in toluene at -78 °C. This aldehyde was found to be relatively stable and could be purified by column chromatography or distillation. The reduction of the ester (103) to the acetonide (107) of serinal was achieved in 62% yield. Chromatography was carried out to purify the aldehyde. The \(^1\)H NMR spectrum of (107) showed the aldehyde proton at 8 9.47 and 9.52. The \(^13\)C NMR spectrum showed the aldehyde
carbon at δ 199.1 and 199.3. Due to the presence of rotameric isomers, all the signals were observed as pairs.

At this point, this aldehyde (107) was adopted as the starting material for a new approach to the synthesis of sphingosine analogues. This synthesis is detailed in Scheme 45.

Scheme 45: Attempted synthesis of sphingosine analogues

Careful dropwise addition of vinylmagnesium bromide to a stirred solution of (S)-N-(tert-butoxycarbonyl)serinal acetonide (107) was successful in producing a mixture of diastereoisomeric vinyl alcohols (108) which was isolated in 77% yield after chromatography. Attempts
to separate these diastereoisomers by flash chromatography failed. The signals for the alkene hydrogens were found at $\delta$ 5.1 for the terminal CH$_2$ group and $\delta$ 5.7 for the alkene CH in the $^1$H NMR spectrum. The $^{13}$C NMR spectrum was complex. The purity of these diastereoisomers was confirmed by microanalysis.

If these diastereoisomers could have been separated, the *erythro-*isomer would have been subjected to ozonolysis to give the $\alpha$-hydroxyl aldehyde (109). The E-double bond would then be introduced by Wittig reaction. By varying the Wittig reagents, a selection of sphingosine analogues would have been synthesised. Such an aldehyde would be most useful as an optically active building block for the synthesis of sphingosine analogues.

In order to achieve the synthesis of sphingosine analogues in the time constraints, the method of Garner *et al.*$^{58}$ was employed (Scheme 46). They reported that condensation of the serinal acetonide (107) with the lithium acetylide of pentadec-1-yne gave good diastereoselectivity and both diastereoisomers were separable. Treatment of the acetonide aldehyde (107) with the lithium acetylide of pentadec-1-yne did indeed give the desired diastereoisomers (110) which were separated by careful column chromatography. The yield of the major product was 68%. The optical rotation was identical to the published value$^{58}$ for the *erythro*-isomer. The IR spectrum showed the carbamate carbonyl and OH absorptions at 1704 and 3277 cm$^{-1}$ respectively. The microanalysis confirmed the purity of the compound.
Scheme 46: Attempted synthesis of sphingosine analogues

The use of the methodology of Garner et al. was extended to prepare the erythro-isomers (111) and (112). After purification by column chromatography, erythro-isomer (111) was isolated in 58% yield. The IR spectrum showed absorptions at 1693 and 3308 cm\(^{-1}\) due to the carbamate carbonyl and OH respectively. The \(^1\)H NMR spectrum showed a broad doublet at \(\delta\) 4.70 for the CH of the alcohol group, and a broad singlet at \(\delta\) 3.90 for the -OH signal. The \(^{13}\)C NMR spectrum showed signals at \(\delta\) 77.8 and 86.1 for the triple bond carbons.

The erythro-isomer (112) was isolated in 63% yield after purification by column chromatography. This compound possessed spectroscopic data similar to other erythro-isomers.
The diastereoselectivity could be explained by assumption of a transition state according to the Felkin-Anh model. That is, the α-amino moiety is orthogonal to the plane of the carbonyl group. The nucleophile then approaches the carbonyl in the direction which gives the least steric interaction. Such an approach would favour the formation of the erythro-isomer, see Diagram 9.

Diagram 9: Felkin-Anh model (not chelation controlled)

The next step was to reduce the triple bond partially using Birch conditions as reported. Treatment of (110) with lithium and ammonia at -78 °C gave only small traces of the trans-product (113), when compared to the value of 68% from Garner's procedure. The conditions for the Birch reduction were varied as shown in Table 4.
| Table 4 |
|-----------------|-----------------|-----------------|-----------------|
| Temp (°C)       | Solvent         | Time            | Result          |
| 2.5 equiv. of   | -78             | 2 h             | traces of       |
| sodium          | NH₃             |                 | product         |
| 3.0 equiv. of   | -78             | 3 h             | traces of       |
| sodium          | NH₃             |                 | product         |
| 3.0 equiv. of   | -78             | 8 h             | complex         |
| sodium          | NH₃             |                 | mixture         |
| 4.0 equiv. of   | -78             | 4 h             | traces of       |
| sodium          | NH₃             |                 | product         |
| 2.5 equiv. of   | Reflux          | 3 h             | mainly          |
| sodium          | NH₃ and THF     |                 | starting        |
|                 |                 |                 | material        |
| 2.5 equiv. of   | Reflux          | 3 h             | mainly          |
| sodium          | EtNH₂ and       |                 | starting        |
|                 | THF             |                 | material        |
| 2.5 equiv. of   | Reflux then RT  | 3 h at reflux    | complex         |
| sodium          | EtNH₂ and       | followed by 8 h  | mixture         |
|                 | THF             | at RT           |                 |

Despite the variety of reaction conditions tested, none of them succeeded in producing a better yield of the trans-product (113). The lack of success was probably due to the lack of solubility of (110).

When (111) was subjected to Birch reduction, the trans-product (114) was isolated in 10% yield after purification by column chromatography. The expected absorption due to the stretching of the C=C bond was found at 1645 cm⁻¹. The $^1$H NMR spectrum showed a doublet of doublets at δ 5.34 for the olefinic proton nearest to the alcohol group and a doublet of triplets at δ 5.66 for the other olefinic proton. The coupling constant between the olefinic protons was found to be 15.4 Hz, suggesting it must be an E-double bond. Treatment of (114) with concentrated HCl in ethyl acetate caused the compound to polymerise. Thus a milder deprotection step must be employed to prevent the compound from undergoing polymerisation.
Since the partial reduction of the triple bond to the E-double bond was not as straightforward as anticipated, it was decided to keep the triple bond in these sphingosine analogues. This would give us the benefit of seeing whether or not the E-geometry of the double bond was an essential criterion for PKC inhibition.

In the typical deprotection of the NBOC-acetonide ring, there are two steps involved. Methanol and PTSA were used initially to open the ring followed by treatment with HCl in ethyl acetate to cleave the BOC protecting group. The use of HCl in ethyl acetate was not ideal as previously found. Such strong conditions might cause polymerisation. It was found by accident that treatment of (110) with an excess of PTSA in methanol at room temperature for 48 h gave the known aminodiol (115) which was isolated as a yellow oil in 87% yield (Scheme 47). The IR spectrum showed absorptions at 2360 cm\(^{-1}\) due to the carbon to carbon triple bond. Another absorption at 3356 cm\(^{-1}\) (very broad) indicating the OH and NH stretches was also found in the IR spectrum. Also no absorption was found for the carbamate carbonyl in the IR spectrum. Both \(^1\)H NMR and \(^{13}\)C NMR spectra had no signals for the ring methyls and the BOC protecting group.

![Scheme 47: Synthesis of sphingosine analogues with a triple bond](image)

(110); \(R = C_{13}\)
(111); \(R = C_9\)
(112); \(R = C_{11}\)

(115); \(R = C_{13}\)
(116); \(R = C_9\)
(117); \(R = C_{11}\)

(118); \(R = C_9\)
(119); \(R = C_{11}\)
This deprotection procedure was used to prepare the new compounds (2S,3R)-2-amino-1,3-pentadec-4-ynediol (116) and (2S,3R)-2-amino-1,3-octadec-4-ynediol (117). Both compounds exhibited similar spectroscopic data to compound (115). The molecular ions for all three aminodiols could not be detected because they lost water instantaneously when injected into the mass spectrometer.

Aminodiols in general are relatively unstable and have a tendency to decompose when exposed to air. In order to prevent this from taking place, these compounds are normally protected as the corresponding triacetates. Treatment of (111) with triethylamine and acetic anhydride afforded the corresponding triacetate (118). The triacetate was isolated as a yellow oil in 21% yield. The IR spectrum showed absorptions at 1547, 1659 and 1749 cm\(^{-1}\) correlating to the amide carbonyl and ester carbonyls respectively. The \(^1\)H NMR spectrum showed three singlets at \(\delta\) 1.97, 2.00 and 2.01 due to the \(\alpha\)-methyl groups. The high resolution mass spectrum gave a value for the molecular ion of 339.2065 compared to the calculated value of 339.2046.

When (112) was exposed to triethylamine and acetic anhydride, the triacetate (119) was isolated as a white solid in 94% yield. The \(^1\)H NMR spectrum showed three singlets at \(\delta\) 1.97, 2.00 and 2.02 for the \(\alpha\)-methyl groups. The high resolution mass spectrum gave a value for the molecular ion of 381.2532 in accordance with the theoretical value of 381.2515.

Liotta et al.\(^{60}\) reported the synthesis of sphingosine from unprotected aminodiol (117) using either Birch reduction or lithium aluminium hydride reduction. Birch reduction of (117) proved to be unsuccessful. However, when (117) was treated with lithium aluminium hydride in dimethoxyethane at reflux, partially pure sphingosine (14) was isolated as an oil in 20% yield. Attempts to remove the impurity by column chromatography failed. The IR spectrum showed the C=C absorption at 1680 cm\(^{-1}\). The \(^1\)H and \(^13\)C NMR spectra were both in accord with the literature spectra of sphingosine.\(^{60}\)
In order to facilitate purification, it was decided to transform sphingosine into its known triacetate\textsuperscript{58} which should result in an easily purified crystalline derivative. The triacetylation of (14) using acetic anhydride and triethylamine proceeded in 70\% crude yield to give an oil, which was partially purified by flash chromatography. A further attempt to purify the triacetate by silica column chromatography caused it to decompose. Time constraints prevented further development of this route.

5.6 Attempted Synthesis of (R)-2-Amino-octadec-4\textit{E}-en-1-ol

A recent paper by Sibi et al.\textsuperscript{89} detailed a general synthesis of 4-substituted oxazolidinones. Cleavage of the oxazolidinone ring would generate the corresponding aminol. Such a method was adopted for the synthesis of (R)-2-amino-octadec-4\textit{E}-en-1-ol (120) as shown in Scheme 48. The starting material for the synthesis was (S)-serine methyl ester.

\begin{center}
Scheme 48: Attempted synthesis of (R)-2-amino-octadec-4\textit{E}-en-1-ol (120)
\end{center}
(S)-Serine methyl ester was first protected as its oxazolidin-2-one (121) using phosgene under basic conditions. The oxazolidin-2-one was isolated as an oil in 80% yield. The IR spectrum of (121) showed the presence of the ester carbonyl (1767 cm\(^{-1}\)) and carbamate carbonyl (1740 cm\(^{-1}\)) absorptions. The carbamate carbonyl carbon was evident in the \(^{13}\)C NMR spectrum and the correct molecular ion (\(m/z = 145\)) was present in the mass spectrum.

The reduction of (121) to the known alcohol (122) was achieved in 63% yield using sodium borohydride in ethanol. Chromatography was required to purify the alcohol. The \(^1\)H NMR spectrum showed two sets of doublets of doublets at \(\delta 3.60\) for the methylene protons of the alcohol group.

In order to transform the alcohol (122) into a better leaving group it was converted into the tosylate (123) by treatment with tosyl chloride in triethylamine. The product was isolated by flash chromatography in 64% yield. The \(^1\)H NMR spectrum showed a singlet at \(\delta 2.33\) and an AA'BB' system at \(\delta 7.73\) due to the methyl group and the p-substituted benzene ring respectively.

Chromatography also gave the ditosylated product (124), not reported previously. The product showed two methyl signals (at \(\delta 2.35, 2.37\)) and two sets of AA'BB' systems (at \(\delta 7.26-7.88\)) in the \(^1\)H NMR spectrum. Microanalysis confirmed the purity of the compound.

Akynylation of (123) with the lithium acetylide of pentadec-1-yne only led to the recovery of both starting materials. No improvement was achieved with the use of an extra equivalent of lithium acetylide. It appeared that once deprotonation had occurred, the salt precipitated and failed to react further. It was thought that the problem might be overcome by using a different organometallic reagent. However, the reaction was still not successful even with the use of an organocuprate. Several attempts were made but none proved to be successful. The lack of success was probably due to the fact the cuprate did not form in the reaction mixture. Transmetallation of the lithium acetylide of an alkyne into its cuprate is known to be a difficult process.
However, addition of the lithium acetylide of pentadec-1-yne to a stirred solution of the ditosylated product (124) was successful in producing compound (125) in a yield of 23% (Scheme 49). The IR spectrum of (125) showed an absorption at 2236 cm\(^{-1}\) corresponding to the carbon to carbon triple bond stretch. The \(^{13}\)C NMR spectrum clearly showed the triple bond carbons at \(\delta 72.3\) and 90.9. High resolution mass spectrometry gave a molecular ion of 461.2575 in accordance with the theoretical value of 461.2599. There was not enough compound (125) made to continue the synthesis and time constraints prevented this reaction from being repeated on a larger scale.

![Scheme 49: Towards (R)-2-amino-octadec-4 E-en-1-ol (120)](image)

To synthesise optically active sphingosine, a diastereoselective or enantioselective approach must be employed. In this chapter, we have tried to devise a novel synthesis of sphingosine via a diastereoselective route. Unfortunately there were several drawbacks, such as the difficulty in separating the diastereoisomers of (108) and the Birch reduction of the triple bond in (117), which could not be overcome. Instead of continuing to try to find better conditions for the Birch reduction, an alternative approach to synthesis of sphingosine analogues was attempted and is discussed in the next Chapter.
6.1 Introduction

Our next aim was to devise enantioselective routes by which optically active aminodiol compounds with one or more alkyl groups attached to the amino group could be synthesised. \((1R,2S)-2-N\)-Alkylamino-1-phenylpropane-1,3-diol (126) and \(N,N\)-dialkylamino-1-phenylpropane-1,3-diol (127) were the chosen targets. These compounds have features in common with sphingosine, such as functional groups in similar positions and a hydrophobic region. Once the preparations of these aminodiols were established, their routes would then be applied to the total synthesis of monomethylated (128) and dimethylated sphingosine (129). As discussed in Chapter 4, monoalkylation of (1S,2S)-2-amino-1-phenylpropane-1,3-diol could not be carried out using standard methods and as for the dialkylation it occurred only in poor yield. Therefore to synthesise \((1R,2S)-2-N\)-alkylamino-1-phenylpropane-1,3-diol and \(N,N\)-dialkylamino-1-phenylpropane-1,3-diol enantioselectively would be a bonus.

\[
\begin{align*}
(126); & \quad R_1 = \text{alkyl group; } R_2 = \text{H} \\
(127); & \quad R_1 = R_2 = \text{alkyl group} \\
(128); & \quad R_3 = \text{alkyl group; } R_4 = \text{H} \\
(129); & \quad R_3 = R_4 = \text{alkyl group}
\end{align*}
\]

6.2 Attempted Enantioselective Synthesis of \((1R,2S)-2-\text{Diethylamino-1-phenylpropane-1,3-diol}\)

Rayner and his co-workers reported a novel method for preparing 2,3-diaminoalcohols via Lewis acid induced rearrangement of 2,3-epoxyamines (Scheme 50). The procedure deserves a mention
because the stereochemical outcome was controlled. The initial step utilised Sharpless asymmetric epoxidation to prepare optically active 2,3-epoxyalcohol (130) which was then transformed into its tosylate (131) by treatment with triethylamine and tosyl chloride. The tosylate was then displaced by diethylamine to afford the corresponding 2,3-epoxyamine (132). Treatment of (132) with trimethylsilyl trifluoromethanesulfonate caused the nitrogen lone pair to attack the epoxide ring regioselectively to give the aziridinium trifluoromethanesulfonate (133). The less hindered position (C-1) of (133) was regiospecifically attacked by a nucleophile which is normally an amine, to give a protected diaminol (134). If the nucleophile was water then the final product would be an aminodiol instead of a diaminol. This was basis for the attempted enantioselective synthesis of the dialkylated aminodiol (127).

Scheme 50: General synthesis of diaminol by Rayner et al.91

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97
To synthesise the $N,N$-dialkylamino-1-phenylpropane-1,3-diol (127), (E)-3-phenylpropen-2-ol was chosen as the starting point for the synthesis, as outlined in Scheme 51. Sharpless asymmetric epoxidation of (E)-3-phenylpropen-2-ol by the published procedure$^{92}$ gave the desired epoxide (135) which was isolated in 64% yield after purification by column chromatography. The IR spectrum of the epoxide showed absorptions at 3342 and 1256 cm$^{-1}$ due to the OH and C-O respectively. Its $^1$H NMR spectrum showed a doublet at $\delta$ 3.90 for the CH nearer to the phenyl ring and a multiplet at $\delta$ 3.23 for the other epoxide CH. The $^{13}$C NMR spectrum also showed the presence of the two CH groups at $\delta$ 55.6 and 62.6 and high resolution mass spectrometry gave a molecular ion of 150.0680 in accordance with a theoretical value of 150.0683. An experiment was not carried out to confirm the optical purity of the 2,3-epoxy alcohol (135) using the Mosher method. It was assumed, at this stage, that the enantiomeric excess for the Sharpless asymmetric epoxidation was the same as the value quoted in the literature for the same compound.$^{92}$

![Scheme 51: Attempted synthesis (1R,2S)-2-diethylamino-1-phenylpropane-1,3-diol](image)
The 2,3-epoxyalcohol (135) was then transformed into its known tosylate (136) by treatment with tosyl chloride and triethylamine. The tosylate was isolated as white crystals in 80% yield. The $^1$H NMR spectrum of (136) showed a singlet at $\delta$ 2.44 for the methyl group and an AA'BB' system at $\delta$ 7.28-7.80 for the p-substituted benzene ring. The high resolution mass spectrum of (136) gave a molecular ion of 304.0769 in accordance with a theoretical value of 304.0782.

Treatment of the tosylate (136) with diethylamine and potassium iodide in dry DMF afforded a complex mixture. This mixture was purified by flash chromatography to give the new 2,3-epoxyamine (137) in 22% yield. The IR spectrum of the compound still showed the presence of the epoxide ring. Its $^1$H NMR spectrum showed a triplet at $\delta$ 0.97 for the methyl groups and a multiplet at $\delta$ 2.51 for the methylene protons. The epoxide CH groups were also found in the expected region of the $^1$H NMR spectrum and high resolution mass spectrometry gave a value for the molecular ion of 205.1500 compared with a theoretical value of 205.1517.

The key step was the Lewis acid induced rearrangement of the 2,3-epoxyamine (137) to give the corresponding 2-trimethylsiloxymethylaziridinium ion which would then be opened regioselectively by water to form the aminodiol (138). The rearrangement followed by the opening of the ring should provide the erythro-configuration. However, when the epoxyamine (137) was treated with trimethylsilyl trifluoromethanesulfonate for 30 minutes before water was introduced, work up gave a complex mixture. Although it could not be purified by column chromatography, the $^1$H NMR spectrum of the mixture suggested that the epoxide ring had been opened. The epoxide cleavage was also evident in the $^{13}$C NMR spectrum. Chemical shifts of the CH groups suggested that the epoxide ring was opened not by the nitrogen lone pair but by the water molecule to give the corresponding amino-2,3-diol (139). From the spectroscopic evidence, the intermediate 2-trimethylsiloxymethylaziridinium ion, had not been generated. Another attempt was carried out where the 2,3-epoxyalcohol was stirred with trimethylsilyl trifluoromethanesulfonate for 10 hours before the nucleophile was introduced. After the work-up, a complex mixture was again isolated.
The $^1$H and $^{13}$C NMR spectra of the crude product were almost identical to the previous spectra. The route was abandoned due to the difficulty in performing the key step.

### 6.3 Synthesis of (1R,2S)-2-Ethylamino-1-phenylpropane-1,3-diol

It is known that 2,3-epoxyalcohols are useful intermediates in the synthesis of carbohydrates, and related compounds. In a recent report by Roush and Adam, 2,3-epoxyalcohols were transformed into their 2-amino-1,3-diols, see Scheme 52. They reported that the reaction of the 2,3-epoxyalcohols with an isocyanate, followed by deprotonation of the carbamate (140) with sodium hydride, generated a nitrogen nucleophile which would then open the epoxide ring regioselectively to afford the cyclic carbamate (141). Basic hydrolysis of (141) gave the 2-amino-1,3-diol (142).

Scheme 52: Synthesis of monomethylated 2-amino-1,3-diol (142) by Roush and Adam.
In the procedure of Roush and Adam, every step gave a yield of at least 80% and the cyclisation of the nitrogen anion with the epoxide ring was highly regiospecific. These two characteristics of the procedure made it ideal for the synthesis of (1R,2S)-2-ethylamino-1-phenylpropane-1,3-diol (145). The synthesis of (1R,2S)-2-ethylamino-1-phenylpropane-1,3-diol is shown in Scheme 53. Treatment of (2R,3R)-2,3-epoxy-3-phenylpropan-1-ol (135) with ethyl isocyanate and triethylamine afforded the carbamate (143) in 93% yield after column chromatography. The IR spectrum of this compound showed an absorption at 1689 cm\(^{-1}\) due to the carbamate carbonyl. Its \(^1\)H NMR spectrum showed a triplet at \(\delta\) 1.05 for the methyl group and a quartet at \(\delta\) 3.05 for the methylene protons of the ethyl group. The \(^{13}\)C NMR spectrum clearly showed the presence of the carbamate carbonyl at \(\delta\) 155.9.

**Scheme 53: Synthesis of monomethylated 2-amino-1,3-diol.**
The epoxyalcohol (135) was assumed to be optically active and at this point it was decided that we should carry out a range of experiments to find out if the epoxycarbamate (143) was still optically active too. The optical rotation was found to be +54.5 and further experiments were carried out to find out the optical purity of (143). The initial experiment was to examine the $^1\text{H}$ NMR spectrum of the compound after addition of 3% (by mole) and 6% of the chiral shift reagent europium (III) tris[3-(heptafluoropropylhydroxymethylene)-(+)camphorato] (146). There was no splitting of any signals but they had moved downfield as a result of paramagnetic properties of the europium atom. Another $^1\text{H}$ NMR experiment was carried out, this time with a different chiral shift reagent, (+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol (147). Again no splitting of the signals was observed in the $^1\text{H}$ NMR spectrum.

These NMR experiments with chiral shift reagents were not sufficient proof that the epoxycarbamate (143) was a single enantiomer. The last experiment involved the use of chiral column gas chromatography. Only one compound was observed. These experiments established that the optical purity of this compound must be greater than 95%.

The next step was the reaction of the epoxycarbamate (143) with sodium hydride in THF. This provided the erythro-hydroxycarbamate (144) in 80% yield after recrystallisation. Its optical rotation was -52.5 in contrast to value of +54.5 for (143). The IR spectrum of (144) showed absorptions at 1760 and 3300 cm$^{-1}$ due to the carbamate carbonyl and OH respectively. Ring strain of the cyclic ring caused the
carbamate carbonyl stretch to shift to a higher frequency. Its $^1$H NMR spectrum suggested that the epoxide ring was cleaved. The $^{13}$C NMR spectrum showed that the carbonyl carbon was deshielded slightly (from δ 155.8 to 158). The compound gave a correct elemental composition.

Roush and Adam suggested that the cyclisation of the nitrogen anion with the epoxide ring was highly regiospecific giving only the carbamate with the five-membered ring. We decided to investigate whether or not such regioselectivity was observed in our experiment. It is likely that there is hardly any difference in the spectroscopic data between the carbamate with the five- (144) and six-membered ring (148).

Since both compounds are isomers, microanalysis and mass spectrometry provided little help. In theory, the most valuable piece of spectroscopic data which should establish whether it was a carbamate with a six- or a five-membered ring is from IR spectra. The carbamate carbonyl stretch for different ring sizes should be different. Such absorption could be used to distinguish clearly between the carbamate carbonyl groups of five- and six-membered rings. However, a literature search provided little evidence for the existence of an absorption frequency for a six-membered carbamate. Thus IR spectrometry is not ideal to be used to establish whether the cyclic carbamate was a five- or six-membered ring. The $^1$H NMR spectrum of (144) might be the solution providing that the CH next to the alcohol group coupled with the OH. A D$_2$O exchange experiment was carried out. The $^1$H NMR spectrum of (144) showed only one signal was affected. It could be concluded that the signal must be the CH next to
the alcohol group. On D2O exchange, this signal was resolved from a multiplet to a doublet. This suggested that the compound must be the carbamate with a five-membered ring otherwise the CH next to the alcohol group would have remained a multiplet on D2O exchange, if it was in the six-membered ring.

The last step of the synthesis of (1R,2S)-2-ethylamino-1-phenylpropane-1,3-diol (145) was hydrolysis of the hydroxycarbamate (144). Hydrolysis was effected using lithium hydroxide in a mixture of water and ethanol to give the 2-ethylamino-1,3-diol (145) in 93% yield. The IR spectrum of this compound showed absorptions at 3448, 3286 and 3083 cm\(^{-1}\) due to the NH and two OH groups respectively and the compound gave a correct elemental composition.

Compound (145) was recrystallised in a conical flask from analytical grade ethyl acetate and hexane giving small cubic crystals. These were submitted to Miss K. McCormark (University of Glasgow) for X-ray crystallographic analysis. The displacement ellipsoid structure of this compound is shown in Diagram 10.

As expected the molecule has several hydrogen bonding interactions (Diagram 11). The oxygen and hydrogen atoms of the hydroxyl groups, and the nitrogen and hydrogen atoms of the amino group are all involved in H-bonding. Each functional group is H-bonded to two other identical molecules. The unit cell of the crystal is depicted in Diagram 12.
Diagram 10: The displacement ellipsoid structure of (1R,2S)-2-ethylamino-1-phenylpropane-1,3-diol (145)
Diagram 12: The unit cell of (1R,2S)-2-ethylamino-1-phenylpropane-1,3-diol (145)
Since the overall yield for the synthesis of (1R,2S)-2-ethylamino-1-phenylpropane-1,3-diol was excellent, it was decided to apply the procedure of Roush and Adam to the synthesis of N-ethylsphingosine. However, this methodology required the use of an allyl alcohol as a starting material. Therefore, the ene-allyl alcohol (149) required for the synthesis of N-ethylsphingosine had to be prepared from tetradecanal (153). This material is commercially available, but was found to be too impure and difficult to purify because of its instability. Therefore fresh material had to be prepared from tetradecanoic acid (150). The preparation of (149) and its use in the synthesis are outlined in Scheme 54.
Scheme 54: Attempted synthesis of N-ethylsphingosine
To prepare the hydroxydiene (149), tetradecanoic acid was treated with dry HCl in methanol to afford the known methyl ester (151) in 95% yield. The IR spectrum of the methyl ester (151) showed a absorption at 1744 cm\(^{-1}\) due to the ester carbonyl. Its \(^1\)H NMR spectrum showed a singlet at \(\delta\) 3.53 corresponding to the methoxy group and the high resolution mass spectrum gave a value for the molecular ion of 242.2237 compared with a theoretical value of 242.2245.

Reduction of the methyl ester (151) with lithium aluminium hydride gave the known alcohol (152) in 94% yield. The expected absorption due to the stretching of the OH was found at 3421 cm\(^{-1}\). The \(^1\)H NMR spectrum of (152) showed a triplet at \(\delta\) 3.52 for the methylene protons nearest to the hydroxyl group.

Swern oxidation of (152) to the known aldehyde (153) was achieved in 84% yield using dimethylsulfoxide, oxalyl chloride and triethylamine. Flash chromatography was required to purify the aldehyde. The IR spectrum showed an absorption at 1720 cm\(^{-1}\) for the aldehyde carbonyl. The aldehyde was evident from signals at \(\delta\) 9.70 in the \(^1\)H NMR spectrum, and at \(\delta\) 202.1 in the \(^{13}\)C NMR spectrum.

Wittig condensation was then required to convert the aldehyde into the known diene methyl ester (154). The first step involved deprotonation of methyl (E)-4-dimethylphosphono-2-butenolate followed by coupling with the aldehyde to afford the diene methyl ester. The diene methyl ester was isolated in 62% yield. The IR spectrum of the diene methyl ester (154) showed absorptions at 1650 and 1718 cm\(^{-1}\) for the C=C and \(\alpha\beta\)-unsaturated ester carbonyl. Its \(^1\)H NMR spectrum showed a singlet at \(\delta\) 3.70 for the methoxy group and a multiplet at \(\delta\) 5.81 - 7.72 for the alkene hydrogens. Due to the complexity of this multiplet, the coupling constants could not be estimated. The \(^{13}\)C NMR spectrum showed that it was only partially pure and consisted of a mixture of (2E,4Z)- and (2E,4E)-isomers. These isomers could not be separated using column chromatography and had to be used directly in the next step with the possibility that the products of the next step would give a better separation on TLC.
The reduction of (154) to the known alcohol (149) was achieved in 80% yield using lithium aluminium hydride. The expected OH stretch was found at 3318 cm\(^{-1}\). The \(^1\)H NMR spectrum showed a doublet at \(\delta 4.05\) for the methylene protons nearest to the hydroxyl group. The splitting patterns of alkene protons were complex and so the coupling constants could not be determined. A small trace of the (2\(E\),4\(Z\))-isomer was shown by the \(^{13}\)C NMR spectrum. Further attempts to separate these isomers by column chromatography failed. Nevertheless the mixture was used in the next reaction.

Asymmetric epoxidation of the hydroxydiene (149) gave the new \(E\)-ene-epoxyalcohol (155) in 5% yield using Sharpless conditions. The IR spectrum of (155) showed absorptions at 1245, 1645 and 3200 cm\(^{-1}\) due to the C-O, C=C and OH respectively. The epoxide protons were observed in the \(^1\)H NMR spectrum as a multiplet at \(\delta 3.03\) for the epoxide proton nearest to the hydroxyl group and a double of doublets at \(\delta 3.30\) for the other epoxide proton. Also present in the \(^1\)H NMR spectrum were a double doublets of triplets at \(\delta 5.11\) for the alkene proton adjacent to the epoxide, and a double of triplets at \(\delta 5.89\) for the other alkene proton. The coupling constant between the alkene protons was found to be 15.4 Hz indicating it must be a \(E\)-double bond.

The reaction also gave the new \(Z\)-ene-epoxyalcohol (156) which was isolated in 10% yield. There was little spectroscopic difference between (155) and (156) apart from their \(^1\)H NMR coupling constants. The alkene protons of the \(Z\)-ene-epoxyalcohol were found to have a coupling constant of 11 Hz. A homo-decoupling experiment was carried out on the \(E\)-isomer (155) to ensure that the epoxidation did indeed occur at the allylic double bond. Irradiation of the signal at \(\delta 5.89\) caused the signal (saturated methylene protons) at \(\delta 2.01\) to collapse from a multiplet to a triplet. This decoupling, however, had no effect on the methylene protons adjacent to the hydroxyl group. Thus it could be concluded that Sharpless asymmetric epoxidation must have occurred at the allylic double bond. The same finding was also observed for the \(Z\)-isomer.
Although the enantioselective route was successful in the synthesis of (1R,2S)-2-ethylamino-1-phenylpropane-1,3-diol (145), the route to the corresponding N-ethylsphingosine came to a halt at the Sharpless epoxidation stage. The yield was poor for the Sharpless asymmetric epoxidation because the starting material had a tendency to crystallise out of the reaction mixture at low temperature. But from room temperature to 0 °C, the starting material readily dissolved in dichloromethane. Unfortunately, it was necessary to carry out the Sharpless asymmetric epoxidation around -30 °C in order to achieve the best selectivity and to ensure the formation of the titanium complex. Such a problem has to be overcome as this is the key step of the synthesis. One possible solution may be to perform the epoxidation with a co-solvent. However, time constraints prevented further development of this route towards N-ethylsphingosine. We believe that the success of this synthesis probably relies solely on improving the yield of the Sharpless epoxidation as the three remaining steps appear straightforward.

6.5 Biological Testing of a Selection of Sphingosine Analogues

A selection of compounds was tested in vitro on two different cancer lines by Dr A. T. McGown at the Paterson Institute in Manchester. These cell lines were human ovarian carcinoma and chronic myelogenous leukaemia. Table 5 records the IC50 values of these compounds. Comparison of IC50 values for both assays show that the order of potency increases when the hydrophobic part is an alkyl chain. Compounds (115), (116) and (117) were the most potent for both cell lines having IC50 values of about 1 µM.

<table>
<thead>
<tr>
<th>Structure</th>
<th>IC50 values (µM)</th>
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<tbody>
<tr>
<td></td>
<td>A2780 cell line</td>
</tr>
<tr>
<td>CH1 (158)</td>
<td>&gt;50</td>
</tr>
<tr>
<td>CH2 (88)</td>
<td>&gt;50</td>
</tr>
<tr>
<td>CH3</td>
<td></td>
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<td>------</td>
<td>---</td>
</tr>
<tr>
<td>(79)</td>
<td></td>
</tr>
<tr>
<td>CH4</td>
<td></td>
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<tr>
<td>(144)</td>
<td></td>
</tr>
<tr>
<td>CH5</td>
<td></td>
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<tr>
<td>(145)</td>
<td></td>
</tr>
<tr>
<td>CH6</td>
<td></td>
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<tr>
<td>(116)</td>
<td></td>
</tr>
<tr>
<td>CH7</td>
<td></td>
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<tr>
<td>(117)</td>
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<tr>
<td>CH8</td>
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<tr>
<td>CH9</td>
<td></td>
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<td>(118)</td>
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<tr>
<td>CH10</td>
<td></td>
</tr>
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<td>(119)</td>
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</table>

Further testing was carried out by Dr McGown on the most active alkynes (115), (116) and (117). They were tested *in vitro* on mouse
P388 lymphoid neoplasm with a high PKC expression, a P388 cell line with a low PKC expression (ca. 10%) resistant to bryostatin (Br/D) and a human ovarian carcinoma resistant to adriamycin (A2780ADR). The results shown in Table 6 demonstrate that these alkynes (115), (116) and (117) are effective against a range of cancer cell lines with IC50 values of about 1µM. In addition, decreasing the PKC expression (ca. 10%) in the P388 cell line decreases the IC50 values of compounds (116) and (117) by five fold, indicating that these compounds are exerting some effect on PKC.

<table>
<thead>
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<th>IC50 values (µM)</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P338</td>
<td>Br/D</td>
<td>A2780ADR</td>
<td></td>
</tr>
<tr>
<td>CH 6</td>
<td>6.56</td>
<td>1.5</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>(116)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH 7</td>
<td>7.87</td>
<td>1.18</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>(117)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH 8</td>
<td>1.53</td>
<td>0.59</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td>(115)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-erythro-</td>
<td>3.8</td>
<td>4.4</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td>sphingosine</td>
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</table>

6.6 Future Work

Since the three sphingosine analogues containing a triple bond showed very promising cytotoxic effects on different cancer lines, it will be of great interest to synthesise the corresponding N-methylated compounds to see how such a modification will affect the anti-cancer activity. As discussed in Chapter 4, N-methylation of the aminodiol proved to be more difficult than first anticipated. Such a problem may be overcome by using the retro aza Diels-Alder procedure. The main attraction of this procedure was the fact that the reaction could proceed in the presence of other functional groups. Although (S)-N-methylphenylalaninol could not be synthesised using this procedure because of the mixture of products obtained in the reduction step, we believe that once the conditions and reagents for this reduction have been established, this procedure will be a powerful way to carry out N-methylation.
Sphingosine was only produced in small amount in the reduction step of the diastereoselective synthesis. It was too low to be used to synthesise sphingosine analogues. The yield of the reduction step must be improved. As for the enantioselective approach, the problem was with the insolubility of the starting material for the Sharpless epoxidation. Achieving enantioselective synthesis was more desirable than the diastereoselective synthesis because the final product of the enantioselective approach would be N-alkylsphingosine instead of sphingosine itself. The alkyl group of N-alkylsphingosine could be varied simply by using different isocyanates in the carbamate formation step. It would be desirable to have a route in which N-methylsphingosine could be synthesised, providing an alternative to the usual N-methylation of sphingosine.

The synthesis of deoxysphingosine slowed to a halt until (S)-3-(4-toluenesulfonyl)-4-(4-toluenesulfonyloxymethyl)oxazolidin-2-one (124) was discovered. The use of (124) in the alkynylation step was encouraging even though the yield was not that good. Unfortunately there was insufficient product to be used for the last step which would be the Birch reduction.
CHAPTER SEVEN

Experimental to Chapters 4 - 6

7.1 General

Melting points (m.p.) were measured with a Gallenkamp apparatus. Infra-red (IR) spectra were obtained on a Perkin Elmer 983 spectrophotometer or a Perking Elmer PU 9800 FT-IR spectrophotometer. $^1H$ and $^{13}C$ NMR spectra were obtained on a Bruker WP200-SY spectrometer operating at 200MHz and 50MHz respectively. All coupling constants quoted are measured in Hz. The numbering schemes shown are used for ease of assigning the NMR spectra. Mass spectra (MS) were recorded on AEI MS12 or MS902 spectrometers. Thin layer chromatography (TLC) was carried out on silica gel G plates of 0.25mm thickness developed with hexane - ethyl acetate unless stated otherwise and compounds were detected with vanillin, iodine or by ultra-violet radiation. Column chromatography was carried out on silica gel, 70-230 mesh with the same solvent system.

7.2 Experimental to Chapters 4

\[
\begin{align*}
(S)-N,O-Diacetylphenylalaninol (7) & 72 \\

\end{align*}
\]

Pyridine (1.3 g, 16.6 mmol) and acetic anhydride (1.69 g, 16.6 mmol) were added sequentially to a solution of (S)-phenylalaninol (1.0 g, 6.62 mmol) in dry THF (20 ml). After the reaction mixture was stirred at room temperature for 10 h, it was diluted with ethyl acetate (40 ml) and washed with 1 M hydrochloric acid (3 x 30 ml) and then with saturated sodium bicarbonate solution (3 x 30 ml). The organic layer was dried with magnesium sulfate and concentrated to give white needles (1.3 g, 84%); mp 112-113 °C [lit.,$^72$ 114-115 °C]; Rf 0.6 (ethyl
acetate); $\left[\alpha\right]_{D}^{22} -13.0$ (c 1 in acetone) \{lit., $\left[\alpha\right]_{D}^{22} -13$ (c 1 in acetone)\};

$\delta_H$ (200MHz, CDCl3) 1.96 (3H, s, 13-H3), 2.10 (3H, s, 11-H3), 2.85 (2H, ABX, 3-H2, J 4.6, 11.4 Hz), 4.10 (2H, ABX, 1-H2, J 6.4, 13.7 Hz), 4.38 (1H, m, 2-H), 5.67 (1H, br d, NH), 7.26 (5H, m, aromatic H); $\delta_C$ (50 MHz, CDCl3) 20.8 (C-13), 23.3 (C-11), 37.4 (C-3), 49.4 (C-2), 64.7 (C-1), 126.7 (C-7), 128.5 (C-5 and -9 or C-6 and -8), 129.1 (C-5 and -9 or C-6 and -8) 136.9 (C-4), 169.8 (C-12), 171.0 (C-10); $\nu_{max}$ (KBr disc)/cm$^{-1}$ 1772 (COO), 1684 (CON), 1271, 1261, 1045, 1033; $m/z$ 235 (M$^+$, 1.5%), 176 (14.6, M - OCOCH3) 134 (9.6, M - OCCH3, - NHCOCH3), 120 (20.9), 102 (51.2), 84 (72.9), 60 (100%); (found M$^+$, 235.1211. Calc. for C$_{13}$H$_{17}$NO$_3$, 235.1280). (Found: C, 66.13; H, 7.36; N, 5.90. Calc. for C$_{13}$H$_{17}$NO$_3$: C, 66.00; H, 7.20; N, 5.90 %).

(R)-N.O-Diacetyll2henylalaninol (75)$^{72}$

The title compound was prepared in the same way as for the (S)-isomer except that (R)-phenylalaninol (1.0 g, 6.62 mmol) was used. The compound was isolated as white needles (1.3 g, 84%); mp 113-114 °C \{lit., $\left[\alpha\right]_{D}^{22} 114-115$ °C\}; Rf 0.6 (ethyl acetate); $\left[\alpha\right]_{D}^{22} +13.0$ (c 1 in acetone) \{lit., $\left[\alpha\right]_{D}^{22} +13$ (c 1 in acetone)\}; $\delta_H$ (200MHz, CDCl3) 2.10 (3H, s, 13-H3), 2.10 (3H, s, 11-H3), 2.85 (2H, ABX, 3-H2, J 4.5, 11.5 Hz), 4.10 (2H, ABX, 1-H2, J 6.3, 13.5 Hz), 4.38 (1H, m, 2-H), 5.67 (1H, br d, NH), 7.26 (5H, m, aromatic H); $\delta_C$ (50 MHz, CDCl3) 20.8 (C-13), 23.3 (C-11), 37.4 (C-3), 49.4 (C-2), 64.7 (C-1), 126.7 (C-7), 128.5 (C-5 and -9 or C-6 and -8), 129.1 (C-5 and -9 or C-6 and -8), 136.9 (C-4), 169.8 (C-12), 171.0 (C-10); $\nu_{max}$ (KBr disc)/cm$^{-1}$ 1772 (COO), 1684 (CON), 1271, 1261, 1045, 1033; $m/z$ (M$^+$, 1.3%), 176 (14.6, M - OCOCH3) 134 (9.6, M - OCCH3, - NHCOCH3), 120 (21.9), 102 (51.2), 84 (74.9), 60 (100%); (found M$^+$, 235.1214. Calc. for C$_{13}$H$_{17}$NO$_3$, 235.1208). (Found: C, 66.19; H, 7.42; N, 5.97. Calc. for C$_{13}$H$_{17}$NO$_3$: C, 66.00; H, 7.20; N, 5.90%).
Dioxan (12 ml) and di-tert-butyldicarbonate (2 g, 8.6 mmol) were added to a solution of (S)-phenylalaninol (1.0 g, 6.62 mmol) in water (25 ml) with continuous stirring at room temperature for 18 h. The solvents were then removed with ethanol in vacuo and the residue was chromatographed (ethyl acetate-hexane; 1:3) to give an oil (1.66 g, 97%); Rf 0.4 (ethyl acetate-hexane; 1:1); δH (200MHz, CDCl₃) 1.41 (9H, s, 11-, 12- and 13-H3), 2.80 (2H, d, 3-H₂, J 7.2 Hz), 2.89 (1H, br s, OH), 3.56 (2H, ABX, 1-H₂, J 3.7, 11.0 Hz), 3.86 (1H, m, 2-H), 4.76 (1H, br d, NH), 7.26 (5H, m, aromatic H); δC (50 MHz, CDCl₃) 28.2 (C-11, -12 and -13), 37.8 (C-3), 50.5 (C-2), 65.0 (C-1), 79.3 (C-14), 126.5 - 129.2 (aromatic CH), 137.2 (C-4), 155.1 (C-10); νmax (film) / cm⁻¹ 3418br (OH), 2914, 1686 (OCON); m/z 251 (M⁺, 0.3%)178 (10.9, M - OC₄H₉), 160 (19.2, M - COOC₄H₉), 91 (42.7, M - OHCH₂CHNHCOOC₄H₉), 57 (100%); (found M⁺, 251.1530. Calc. for C₁₄H₂₁N0₃, 251.1521).

(S)-2-[(tert-Butyloxy carbonylamino)-N-methyl]-O-methylphenylalaninol (78)
(using the procedure of Coggins and Benoiton74)

Sodium hydride (95%, 0.35 g, 21.6 mmol) was added to a stirred solution of N-BOC-phenylalaninol (77) (1.8 g, 7.17 mmol) and iodomethane (4 ml, 21.2 mmol) in dry THF (20 ml). After the mixture
was stirred for 15 h at room temperature, the mixture was poured into ice water and extracted with ethyl acetate (3 x 40 ml). The combined extracts were dried with magnesium sulfate and concentrated under reduced pressure to afford a yellow oil. TLC (ethyl acetate-hexane; 1:1) showed three spots with Rf values of 0.4, 0.6 and 0.7. Purification of the oil by column chromatography eluting with ethyl acetate-hexane (1:4) gave (S)-3-methyl-4-benzyloxazolin-2-one (79), N-tert-butyloxy carbonyl-O-methylphenylalaninol (80), and the title compound.

The title compound was obtained as a yellow oil (0.1 g, 5%); Rf 0.7; \( \delta_H \) (200MHz, CDCl3) 1.3 (9H, s, 11-, 12- and 13-H3), 2.30 (3H, s, 16-H3), 2.71 (2H, m, 3-H2), 3.16 (2H, m, 1-H2), 3.18 (3H, s, 15-H3), 3.81 (1H, m, 2-H), 7.12 (5H, m, aromatic H); \( \delta_C \) (50 MHz, CDCl3) 28.3 (C-11, -12 and -13), 33.8 (C-16), 37.7 (C-3), 51.5 (C-15), 58.7 (C-2), 72.5 (C-1), 79.4 (C-14) 126.2 (C-7), 128.4 (C-5 and -9 or C-6 and -8), 129.3 (C-5 and -9 or C-6 and -8), 138.2 (C-4), 155.0 (C-10); \( \nu_{max} \) (film) / cm\(^{-1}\) 2850, 1745 (OCON); m/z 206 (9.1, M- OC4H9), 188 (23.5, M- COOC4H9), 134 (11.2, M - N(CH3)COOC4H9, - CH3); (found M+ - OC4H9, 206.1179. Calc. for C12H16NO2, 206.1181).

(S)-3-Methyl-4-benzyloxazolin-2-one (79) was obtained as white needles (0.30 g, 21%).

\[
\text{m.p. 90 °C; Rf 0.4; } [\alpha]^D_{22} -58.1 \text{ (c 1.5 in CHCl3); } \delta_H \text{ (200MHz, CDCl3) 2.75 (2H, m, 3-H2), 2.77 (3H, } s, \text{ 11-H3), 3.82 (2H, m, 1-H2), 4.02 (1H, m, 2-H), 7.11 (5H, m, aromatic H); } \delta_C \text{ (50 MHz, CDCl3) 29.4 (C-11), 38.2 (C-3), 58.2 (C-2), 66.52 (C-1), 126.2 (C-7), 128.4 (C-5 and -9 or C-6 and -8), 129.3 (C-5 and -9 or C-6 and -8), 135.5 (C-4), 158.42 (C-10); } \nu_{max} \text{ (KBr disc) / cm}\(^{-1}\) 1750 (OCON); m/z 191 (M+, 0.8), 117 (1.2), 100 (100, M - C6H5CH2), 91 (13.9, M - CHCH2OCONMe); (found M+, 191.0956. Calc. for C11H13N02, 191.0946). \text{ (Found: } C, 69.26; H, 6.98; N, 7.14. Calc. for C11H13N02: C, 69.11; H, 6.77; N, 7.32%).
(S)-N-tert-Butyloxy carbonyl-O-methylphenylalaninol (80) was obtained as a yellow oil (1.1 g, 57%).

Rf 0.6; δH (200MHz, CDCl3) 1.3 (9H, s, 11-, 12- and 13-H3), 2.71 (2H, m, 3-H2), 3.16 (2H, ABX, 1-H2, J 3.7, 11.2 Hz), 3.18 (3H, s, 15-H3), 3.81 (1H, m, 2-H), 4.92 (1H, br d, NH), 7.11 (5H, m, aromatic H); δC (50 MHz, CDCl3) 28.3 (C-11, -12 and -13), 37.7 (C-3), 51.5 (C-15), 58.7 (C-2), 72.5 (C-1), 79.7 (C-14), 126.2 (C-7), 128.3 (C-5 and -9 or C-6 and -8), 129.3 (C-5 and -9 or C-6 and -8), 138.2 (C-4), 155.0 (C-10); vmax (film) / cm⁻¹ 1745 (OCON); m/z 192 (18.9, M - OC₄H₉), 174 (5.9, M - COOC₄H₉), 134 (14.9, M - HNCOOC₄H₉, - CH₃); (found M - OC₄H₉, 192.1018. Calc. for C₁₁H₁₄NO₂, 192.1024).

(S)-N.O-Dimethylphenylalaninol (83)

A solution of (S)-2-[(tert-butyloxycarbonylamino)-N-methyl]-O-methylphenylalaninol (78) (0.1 g, 38.4 mmol) in 1 M HCl in ethyl acetate (30 ml) was stirred at room temperature for 5 h. The reaction mixture was diluted with ethyl acetate (20 ml), washed with 1 M sodium hydroxide (2 x 50 ml), dried with magnesium sulfate and concentrated to give the title compound (0.04 g, 78%) as a yellow oil; Rf 0.4 (ethyl acetate); [α]D²² -1.4 (c 1.7 in ethanol); δH (200MHz, CDCl₃) 2.38 (3H, s, 11-H₃), 2.56 (1H, br s, NH), 2.72 - 2.81 (3H, m, 1-H₂ and 2-H), 3.23 (2H, m, 3-H₂), 3.29 (3H, s, 10-H₃), 7.26 (5H, m, aromatic H); δC (50 MHz, CDCl₃) 33.9 (C-11), 37.3 (C-3), 58.8 (C-10), 60.6 (C-2), 73.2 (C-1), 126.1 (C-7), 128.5 (C-5 and -9 or C-6 and -8), 129.2 (C-5 and -9 or
C-6 and -8), 138.7 (C-4); $\nu_{\text{max}}$ (KBr disc) / cm$^{-1}$ 3431m (NH); $m/z$ 179 (M$^+$, 3.0%), 134 (50, M - NHCH$_3$, - CH$_3$), 81 (100, M - C$_6$H$_5$CH); (found M$^+$, 179.1320. Calc. for C$_{11}$H$_{17}$NO, 179.1320).

\[(S)-N\text{-tert-Butyloxy carbonyl-}\text{-O-tert-Butyldimethylsilylphenylalaninol}\]

\[(84)\]

(\text{using the procedure of Corey et al.})

\[\text{N-BOC-phenylalaninol (77) (3.24 g, 13.0 mmol), imidazole (1.3 g, 20.0 mmol), and tert-butyl-dimethylsilyl chloride (2.35 g, 14.3 mmol) in dry DMF were placed in a 250 ml round bottom flask. The reaction was stirred for 1 h then 1 M hydrochloric acid (30 ml) was added. The aqueous layer was extracted with ethyl acetate (3 x 30 ml). The combined extracts were dried with magnesium sulfate and concentrated under reduced pressure to give an oily solid (4.2 g, 89%); Rf 0.6 (ethyl acetate-hexane; 1:2); $\delta_H$ (200MHz, CDCl$_3$) -0.03 (6H, s, 15- and 16-H$_3$), 0.88 (9H, s, 17-, 18- and 19-CH$_3$), 1.43 (9H, s, 11-, 12- and 13-H$_3$), 2.76 (2H, d, 3-H$_2$, J 8.0 Hz), 3.41 (2H, m, 1-H$_2$), 3.52 (1H, m, 2-H), 4.73 (1H, br d, NH), 7.13 (5H, m, aromatic H); $\delta_C$ (50 MHz, CDCl$_3$) -5.5 (C-15 and -16), 18.1 (C-20), 25.7 (C-17, -18 and -19), 28.3 (C-11, -12 and -13), 37.3 (C-3), 53.0 (C-2), 62.9 (C-1), 79.1 (C-14), 121.5 (C-7), 134.6 (C-5 and -9 or C-6 and -8), 134.8 (C-5 and -9 or C-6 and -8), 138.2 (C-4), 155.4 (C-10); $\nu_{\text{max}}$ (film) / cm$^{-1}$ 2953, 1695 (OCON), 1256 (Si(CH$_3$)$_2$), 1056, 840 (SiO); $m/z$ 292 (2.1, M - OC$_4$H$_9$), 251 (100, MH - Si(CH$_3$)$_2$C(CH$_3$)$_3$), 173 (50.1), 90 (87.6); (found M - OC$_4$H$_9$, 292.1690. Calc. for C$_{16}$H$_{26}$SiNO$_2$, 292.1684).
Attempted preparation of (S)-2-[(tert-butyloxycarbonylamino)-N-methyl]-O-tert-butylidimethylsilylphenylalaninol
(using the procedure of Coggins and Benoiton)

![Chemical structure image]

Sodium hydride (95%, 0.1 g, 4.2 mmol) was added to a solution of (S)-N-BOC-O-TBDMS-phenylalaninol (84) (1 g, 2.7 mmol) in dry DMF under nitrogen. Once the hydrogen gas had stopped evolving, methyl iodide (1 g, 7.0 mmol) was added dropwise to the resulting mixture. After the addition was complete, the flask was stirred overnight at room temperature. Ethyl acetate (50 ml) was added followed by water (30 ml). The aqueous layer was extracted with ethyl acetate (4 x 30 ml) and the combined extracts were washed with brine (3 x 30 ml), water (3 x 30 ml), dried, filtered and concentrated to give a yellow oil. TLC and all spectral properties were identical to N-BOC-O-TBDMS-phenylalaninol (84). This reaction was repeated several times under different conditions, all of which were unsuccessful. Table 1 summarises the outcomes.

Azabicyclic (2.2.1) heptene (85)
(using the procedure of Grieco and Bahsas)
Cyclopentadiene (1.3 g, 19.6 mmol) was added to a stirred solution of (S)-phenylalaninol (1 g, 6.62 mmol), trifluoroacetic acid (0.75 g, 6.62 mmol) and formaldehyde (37%, 1.2 g, 13.2 mmol) in water (30 ml). After 1 h at room temperature, the heterogeneous mixture was washed with hexane and neutralised with saturated sodium bicarbonate solution. The resulting solution was then extracted with methylene chloride (3 x 40 ml). The combined organic extracts were dried over magnesium sulfate and concentrated to give an oil which was purified by column chromatography eluting with methylene chloride/methanol/ammonium hydroxide (96: 3: 1) to afford the title compound (1.0 g, 67%); the 1H NMR spectrum was complex; δC (50 MHz, CDCl3) 42.8 (C-2), 47.5 (C-6), 51.1 (C-8), 51.5 (C-5), 52.1 (C-1), 61.4 (C-7), 128.3 (C-13), 129.3 (C-11 and -15 or C-12 and -14), 130.2 (C-11 and -15 or C-12 and -14), 130.4 (C-3 or -4), 136.8 (C-3 or -4), 137.3 (C-15); νmax (film) / cm⁻¹ 3401, 1680.

**Attempted synthesis of (S)-N-methylphenylalaninol**

![Structure](image)

Trifluoroacetic acid (0.25 g, 2.18 mmol) and triethylsilane (0.50 g, 4.4 mmol) were added to a solution of (121) (0.5 g, 2.18 mmol) in chloroform. The reaction mixture was stirred at room temperature under nitrogen. After 20 h, the solvent was removed under pressure. The crude product was redissolved in chloroform (50 ml), treated with 1 M HCl (50 ml) and washed with ether (3 x 30 ml). The aqueous layer was neutralised with saturated sodium bicarbonate solution and extracted with methylene chloride (3 x 50 ml). The organic extracts were dried over magnesium sulfate and concentrated to give a complex mixture.
(S)-N,O-Diformylphenylalaninol (86)

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\text{N}
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\text{11}
\end{array}}
\]

Formic acid (1.2 g, 31 mmol) was added to a solution of (S)-phenylalaninol (1 g, 6.6 mmol) in toluene (10 ml) and the mixture was heated at 90 - 98 °C for 13 h. After cooling to room temperature, the mixture was diluted with ethyl acetate (20 ml) and washed with saturated sodium bicarbonate solution and then brine. The organic layer was dried with magnesium sulfate, filtered and concentrated under reduced pressure to give a yellow oil which was chromatographed (ethyl acetate-hexane; 1:1) to afford a white solid (1 g, 83%); mp 98 - 99 °C; Rf 0.3 (ethyl acetate-hexane; 1:1); δH (200MHz, CDCl₃) 2.91 (2H, d, 3-H₂), 4.16 (2H, d, 1-H₂), 4.54 (1H, m, 2-H), 6.17 (1H, br d, NH), 7.20 (5H, m, aromatic H), 8.07 (2H, br s, 10-H); δC (50 MHz, CDCl₃) 37.1 (C-3), 47.9 (C-2), 65.1 (C-1), 126.9 (C-7) 128.5 (C-5 and -9 or C-6 and -8), 129.2 (C-5 and -9 or C-6 and -8), 136.4 (C-4), 160.7 - 160.9 (C-10 and -11); νmax (KBr disc) / cm⁻¹ 3400, 1718 (OCO), 1668 (CON); m/z 207 (M⁺, 4.2%), 162 (100, M - OCOH), 133 (18.3, M - OCOH, - CHO), 115 (7.4, M - OCOH, - NHCHO), 91 (80, M - HCOOCH₂CHNHCHO); (found M⁺, 207.0870. Calc. for C₁₁H₁₃NO₃, 207.0895).

(S)-N-Methylphenylalaninol (87)

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\]

Lithium aluminium hydride (0.55 g, 14.4 mmol) and dry THF (30 ml) were placed in a flame dried three-necked flask. A solution of (S)-N,O-diformylphenylalaninol (86) (1 g, 4.8 mmol) in dry THF (20 ml) was then added to the suspension at 0 °C. The reaction mixture was stirred for 1 h at this temperature and then quenched by addition of saturated
ammonium chloride solution (50 ml). The resulting mixture was extracted with ethyl acetate (3 x 50 ml), dried with magnesium sulfate, filtered and concentrated to give a yellow oil. The oil was partially purified by flash chromatography eluting with ethyl acetate-ethanol (9:1) to afford the title compound (0.65 g, 74%).

\[(\text{1S,2S})-\text{N.O.O-Triformyl-2-amino-1-phenylpropane-1,3-diol (157)}\]

Formic acid (1.4 g, 3.0 mmol) was added to a solution of (1S, 2S)-2-amino-1-phenylpropane-1,3-diol (1 g, 6.0 mmol) in toluene (10 ml) and the mixture was heated at 90 - 98 °C for 13 h. After cooling to room temperature, the mixture was diluted with ethyl acetate (20 ml) and washed with saturated sodium bicarbonate solution and then brine. The organic layer was dried with magnesium sulfate, filtered and concentrated under reduced pressure to give a yellow oil which was chromatographed (ethyl acetate-hexane; 1:1) to afford an oil (1.3 g, 86%); Rf 0.4 (ethyl acetate-hexane; 1:1); δH (200MHz, CDCl3) 3.80 (1H, dd, 1-H, J 5.4, 11.4 Hz), 4.10 (1H, dd, 1-H, J 6.3, 11.0 Hz), 4.67 (1H, m, 2-H), 5.93 (1H, d, 3-H, J 6.9 Hz), 7.25 (5H, m, aromatic H), 8.73, 7.95, 8.02 (3H, 3 x s, 10-, 11- and 12-H); δC (50 MHz, CDCl3) 49.7 (C-2), 62.0 (C-1), 73.0 (C-3), 126.0 (C-5 and -9 or C-6 and -8), 128.2 (C-5 and -9 or C-6 and -8), 128.9 (C-7), 135.5 (C-4), 159.3, 160.3, 161.4 (C-10, -11 and -12); νmax (KBr disc) / cm⁻¹ 1718 v. b (OCO), 1668 v. b (CON); m/z 251 (M+, 0.5%), 177 (5.8), 132 (14.7), 107 (23.8), 71 (100); (found M+, 251.0808. Calc. for C12H13N05, 251.0810).
Attempted synthesis of (1S,2S)-2-methylamino-1-phenylpropane-1,3-diol

\[
\text{OH} \quad \text{OH} \\
\text{HN}_
\]

Lithium aluminium hydride (0.68 g, 20 mmol) and dry THF (30 ml) were placed in a flame dried three-necked flask. A solution of (157) (1 g, 4.0 mmol) in dry THF (20 ml) was then added to the suspension at 0 oC. The reaction mixture was stirred for 1 h at this temperature and then quenched by addition of saturated ammonium chloride solution (50 ml). The resulting mixture was extracted with ethyl acetate (3 x 50 ml), dried with magnesium sulfate, filtered and concentrated to give a complex mixture.

\text{(S)-N.N-Dimethylphenylalaninol (88)}
\text{(using the procedure of Feldkamp \textit{et al.}77)}

Formaldehyde (36%, 0.8 g, 8.0 mmol) was added to a solution of (S)-phenylalaninol (1 g, 6.7 mmol), palladium/carbon catalyst (10%, 0.1 g) and glacial acetic acid (0.8 g, 13.4 mmol) in water (30 ml). Nitrogen gas was bubbled through the reaction mixture for 5 min before hydrogen gas was introduced. The reaction was stirred for 10 h after which the catalyst was removed through Celite. The residue was diluted with diethyl ether and slowly made basic with 2 M sodium hydroxide solution. The ether layer was separated and the aqueous layer was extracted with diethyl ether (3 x 30 ml). The combined extracts were dried with magnesium sulfate and concentrated under reduced pressure to give a yellow oil. TLC (ethyl acetate-pet ether; 1:1) showed two spots with Rf values of 0.2 and 0.5. Purification of the oil by column chromatography eluting with ethyl acetate-pet ether (1:1)
The title compound was obtained as a yellow oil (0.65 g, 54%); Rf 0.2;  
[α]D22 -1.8 (c 1.56 in ethanol) ([α]D22 -2.1 (c 1.56 in ethanol)); δH  
(200 MHz, CDCl3) 2.56 (6H, s, 10 and 11-H3), 2.81 (2H, m, 3-H2), 3.2 (3H,  
m, 1-H2 and 2-H), 3.81 (1H, br s OH), 7.12 (5H, m, aromatic H); δC (50  
MHz, CDCl3) 30.5 (C-3), 40.1 (C-10 and -11), 60.3 (C-1), 66.8 (C-2),  
126.1 (C-7) 128.4 (C-5 and -9 or C-6 and -8), 128.9 (C-5 and -9 or C-6  
and -8), 139.38 (C-4); vmax (film) / cm⁻¹ 3390, 2936, 778; m/z 179  
(M⁺, 0.1%), 148 (7.7, M - (CH₃)₂, - H), 133 (2.1), 88 (21.7), 59 (100);  
(found M⁺, 179.1328. Calc. for C₁₁H₁₇NO, 179.1310).

(S)-3-Methyl-4-benzyloxazolidine (89) was obtained as a white soild  
(0.2 g, 17%).

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\text{N} \\
\text{C} \\
\text{N} \\
\text{C} \\
\text{C} \\
\text{C} \\
\end{array}
\]

Rf 0.5; [α]D22 -2.4 (c 1.7 in ethanol); δH (200 MHz, CDCl₃) 2.47 (3H, s, 10-  
H₃), 2.52 (1H, m, 2-H), 2.82 (2H, m, 3-H₂), 3.62 (2H, m, 1-H₂), 4.01 (1H,  
d, 11-H, J 4.4 Hz), 4.39 (1H, d, 11-H, J 4.4 Hz), 7.13 (5H, m, aromatic H);  
δC (50 MHz, CDCl₃) 38.7 (C-3), 40.0 (C-10), 66.8 (C-2), (C-1), 88.2 (C-11),  
126.1 (C-7) 128.4 (C-5 and -9 or C-6 and -8), 128.9 (C-5 and -9 or C-6  
and -8), 138.7 (C-4); vmax (KBr disc) / cm⁻¹ 2944, 2856, 2792; m/z 177  
(M⁺, 0.2%), 147 (1.4, M - OCH₂), 91 (13, M - N(CH₃)CH₂OCH₂CH, 86 (100,  
M - C₆H₅CH₂); (found M⁺, 177.1138. Calc. for C₁₁H₁₅NO, 177.1154).

(R)-N.N-Dimethylphenylalaninol (158)  
(using the procedure of Feldkamp et al.⁷⁷)

\[
\begin{array}{c}
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\text{N} \\
\text{C} \\
\text{N} \\
\text{C} \\
\text{C} \\
\text{C} \\
\end{array}
\]

The title compound was prepared in the same way as the S-isomer  
except that (R)-phenylalaninol (1 g, 6.7 mmol) was used. TLC also
showed two spots with the same Rf values as the S-isomer. The mixture was purified by column chromatography eluting with ethyl acetate-pet ether (1:1) to afford partially pure \((R)-3\text{-}methyl\text{-}4\text{-}benzyloxazolidine\) and the title compound.

The title compound was obtained as a yellow oil (0.70 g, 64%); Rf 0.2; \([\alpha]_D^{22} +1.9\) (c 1.56 in ethanol) \(\{\text{lit.} 78 [\alpha]_D^{22} +2.1\) (c 1.56 in ethanol)\); \(\delta_H^\text{H} (200\text{MHz}, \text{CDCl}_3) 2.56 (6\text{H}, \text{s}, 10\text{ and }11\text{-H}_3), 2.81 (2\text{H}, \text{m}, 3\text{-H}_2), 3.2 (4\text{H}, \text{m}, 1\text{-H}_2, \text{OH}\text{ and }2\text{-H}), 7.12 (5\text{H}, \text{m}, \text{aromatic }H); \delta_C (50\text{ MHz}, \text{CDCl}_3) 30.5 (C-3), 40.1 (C-10\text{ and }-11), 60.3 (C-1), 66.8 (C-2), 126.1 (C-7) 128.4 (C-5 and -9 or C-6 and -8), 128.9 (C-5 and -9 or C-6 and -8), 139.38 (C-4); \nu_{\text{max}} (\text{film}) / \text{cm}^{-1} 3390, 2936, 778; m/z 179 (M+, 0.1%), 148 (7.7, M -(CH_3)_2, - H), 133 (2.1), 88 (21.7), 59 (100); (found M+, 179.1328. Calc. for C_{11}H_{17}NO, 179.1310).

\((R)-3\text{-}methyl\text{-}4\text{-}benzyloxazolidine\) (159) was obtained as a yellow solid (0.25 g, 21%).

![Chemical structure](image)

\(Rf\) 0.5; \([\alpha]_D^{22} +2.3\) (c 1.7 in ethanol); \(\delta_H (200\text{MHz}, \text{CDCl}_3) 2.47 (3\text{H}, \text{s}, 10\text{-H}_3), 2.52 (1\text{H}, \text{m}, 2\text{-H}), 2.82 (2\text{H}, \text{m}, 3\text{-H}_2), 3.62 (2\text{H}, \text{m}, 1\text{-H}_2), 4.01 (1\text{H}, \text{d}, 11\text{-H}, J 4.4 \text{ Hz}), 4.39 (1\text{H}, \text{d}, 11\text{-H}, J 4.4 \text{ Hz}), 7.13 (5\text{H}, \text{m}, \text{aromatic }H); \delta_C (50\text{ MHz}, \text{CDCl}_3) 38.7 (C-3), 40.0 (C-10), 66.8 (C-2), (C-1), 88.2 (C-11), 126.1 (C-7) 128.4 (C-5 and -9 or C-6 and -8), 128.9 (C-5 and -9 or C-6 and -8), 138.7 (C-4); \nu_{\text{max}} (\text{KBr disc}) / \text{cm}^{-1} 2944, 2856, 2792; m/z 177 (M+, 0.2%), 147 (1.4, M - OCH_2), 91 (13, M - N(CH_3)CH_2OCH_2CH, 86 (100, M - C_6H_5CH_2); (found M+, 177.1138. Calc. for C_{11}H_{15}NO, 177.1154).
Formaldehyde (36%, 0.72 g, 7.2 mmol) was added to a solution of (1S, 2S)-2-amino-1-phenylpropan-1,3-diol (1 g, 6.0 mmol), palladium/carbon catalyst (10%, 0.1 g) and glacial acetic acid (0.72 g, 12 mmol) in water (30 ml). Nitrogen gas was bubbled through the reaction mixture for 5 min before hydrogen gas was introduced. The reaction was stirred for 10 h after which the catalyst was removed through Celite. The residue was diluted with diethyl ether and slowly made basic with 2 M sodium hydroxide solution. The ether layer was separated and the aqueous layer was extracted with diethyl ether (3 x 30 ml). The combined extracts were dried with magnesium sulfate and concentrated under reduced pressure to give a yellow oil. Purification of the oil by flash chromatography eluting with ethyl acetate afforded the title compound (0.3 g, 26%) as a yellow oil; Rf 0.1 (ethyl acetate); \( \delta_{\text{H}} \) (200MHz, CDCl3) 2.34 (6H, s, 10- and 11-H3), 2.46 (1H, m, 2-H), 3.16 (2H, m, 1-H2), 4.15 (3H, br d, 3-H, 3-OH, 1-OH), 7.13 (5H, m, aromatic H); \( \delta_{\text{C}} \) (50 MHz, CDCl3) 41.1 (C-10 and -11), 57.7 (C-1), 70.8 (C-2 and -3), 126.9 (C-7), 128.3 (C-5 and -9 or C-6 and -8), 128.5 (C-5 and -9 or C-6 and -8), 141.9 (C-4); \( \nu_{\text{max}} \) (film) / cm\(^{-1}\) 3400, 3300, 2930; m/z 195 (M\(^+\), 0.4%), 164 (12.7, M - CH2OH), 134 (3.6, M - CH2OH, - (CH3)2, 89 (51), 58 (100, M - HN(CH)(CH3)2); (found M\(^+\), 195.1281. Calc. for C\(_{11}\)H\(_{17}\)NO2, 195.1259).

\[\text{N.N.N-Trimethylphenylalaninol iodide (91)}\]
A solution of \(N,N\)-dimethylphenylalaninol (88) (1 g, 5.64 mmol) and methyl iodide (1.69 g, 11.3 mmol) in diethyl ether (30 ml) was stirred for 24 h at room temperature. The solid was filtered and washed with diethyl ether (3 x 40 ml) to give a yellow solid (1.1 g, 61%); \(\delta_H\) (200MHz, CD\(_3\)OD) 3.33 (9H, s, 11-, 12- and 13-H3), 3.67 (2H, m, 3-H2), 4.06 (1H, m, 2-H), 4.80 (3H, m, 1-H2, -OH), 7.13 (5H, m, aromatic H); \(\delta_C\) (50 MHz, CD\(_3\)OD) 31.9 (C-3), 53.1 and 53.7 (C-10, -11 and -12), 57.7 (C-1), 77.1 (C-2), 128.2 (C-7), 129.8 (C-5 and -9 or C-6 and -8), 130.1 (C-5 and -9 or C-6 and -8), 137.3 (C-4); \(v_{max}\) (film) / cm\(^{-1}\) 3348; \(m/z\) 148 (43.9, M - C\(_2\)H\(_6\)OI), 133 (1.2), 119 (3.3), 88 (100); (found M - C\(_2\)H\(_6\)OI, 148.1106. Calc. for C\(_{10}\)H\(_{14}\)N, 148.1104).
7.3 Experimental to Chapter 5

(S)-Serine methyl ester hydrochloride (94)

\[
\text{Cl} \quad \text{H}_3\text{N} \quad \text{CO}_2\text{Me} \quad \text{OH}
\]

Hydrochloride gas was bubbled through a solution of (S)-serine (10 g, 95.2 mmol) and methanol (150 ml) until all the serine had dissolved. After stirring the solution at room temperature for 5 h, methanol was evaporated off under reduced pressure to afford the title compound (14.0 g, 94%) as a white solid. The white solid was stored in a desiccator under vacuum overnight with pellets of potassium hydroxide to ensure the complete removal of HCl. \( \delta_H \) (200MHz, D\(_2\)O) 3.57 (3H, s, 4-H3), 3.73 (1H, dd, 3-H, J 3.6, 12.5 Hz), 3.85 (1H, dd, 3-H, J 4.0, 12.5 Hz), 4.01 (1H, t, 2-H, J 3.8 Hz); \( \delta_C \) (50 MHz, D\(_2\)O) 50.3 (C-4), 51.3 (C-3), 56.0 (C-2), 167.9 (C-1); \( \nu_{\text{max}} \) (KBr) / cm\(^{-1}\) 3417, 2958, 1747. (Found: C, 30.8; H, 6.4; N, 9.0. Calc. for C\(_4\)H\(_{10}\)NClO: C, 30.73; H, 6.52; N, 8.92%).

(S)-N-tert-Butyloxy carbonylserine methyl ester (95)

Di-tert-butyldicarbonate (17.8 ml, 77.2 mmol) was added to a solution of triethylamine (18 ml, 12.9 mmol) and (S)-serine methyl ester hydrochloride (94) (10 g, 64.3 mmol) in dry dichloromethane (100 ml). After the reaction mixture was stirred for 10 h at room temperature, it was diluted with dichloromethane (50 ml) and washed with water (3 x 50 ml). The organic layer was dried with magnesium sulfate and concentrated to give a yellow oil. This was purified by column chromatography (hexane-ethyl acetate; 9:1) to give the title compound (9 g, 64%) and (S)-N-tert-butyloxy carbonyl-O-tert-butyloxy carbonyl-serine methyl ester (96) (2 g, 11%).
The title compound was isolated as a clear oil; Rf 0.1 (hexane-ethyl acetate; 9:1); \([\alpha]_D^{22} -6.0 \text{ (c 3.6 in CHCl}_3) \) \{lit., \([\alpha]_D^{22} -6.5 \text{ (c 3.6 in CHCl}_3) \); \(\delta_H \text{ (200MHz, CDCl}_3) 1.37 \text{ (9H, s, 6-}, 7- \text{ and 8-H}_3), 3.37 \text{ (1H, br t, OH), 3.78 (3H, s, 9-H}_3), 3.81 (2H, m, 3-H}_2), 4.29 (1H, m, 2-H), 5.61 (1H, d, NH); \(\delta_C \text{ (50 MHz, CDCl}_3) 28.2 \text{ (C-6, -7 and -8), 52.5 (C-9), 55.8 (C-2), 63.0 (C-3), 80.1 (C-5), 155.8 (C-4), 171.5 (C-1); } \nu_{\max} \text{ (film) / cm}^{-1} 3400, 2978, 1713 \text{ br (OCON and CO}_2\text{Me); } m/z 160 \text{ (23, M - CO}_2\text{Me), 146 (21.5, M - OC}_4\text{H}_9), 133 (79), 104 (32), 88 (39), 60 (100); \text{ (found } M^- \text{ CO}_2\text{Me, 160.0985. Calc. for C}_7\text{H}_14\text{N}_0\text{O}_3, 160.0973).}

\((S)-N, O\text{-Di-tert-butyloxycarbonylserine methyl ester (96) was isolated as a white solid; mp 94 °C (lit., 83 94.5 °C)}

\[
\begin{align*}
\text{Rf 0.3 (hexane-ethyl acetate; 9:1); } [\alpha]_D^{22} -33.0 \text{ (c 1.1 in CHCl}_3); \delta_H \text{ (200MHz, CDCl}_3) 1.42 \text{ (6-, 7- and 8-H}_3 \text{ or 12-, 13- and 14-H}_3), 1.44 \text{ (6-, 7- and 8-H}_3 \text{ or 12-, 13- and 14-H}_3), 3.74 \text{ (3H, s, 1-H}_3), 4.25 \text{ (1H, dd, 2-H, J 3.5, 10.9 Hz), 5.30 (1H, d, NH); } \delta_C \text{ (50 MHz, CDCl}_3) 27.6 \text{ (C-6, -7 and -8 or C-12, -13 and -14), 28.2 (C-6, -7 and -8 or C-12, -13 and -14), 52.6 (C-9), 52.8 (C-3), 66.3 (C-2), 80.2 (C-5 or -11), 82.7 (C-5 or -11), 153.2 (C-4 or C-10), 156.2 (C-4 or C-10) 171.3 (C-1); } \nu_{\max} \text{ (KBr disc) / cm}^{-1} 2933, 1740, 1712, 1526; \text{ m/z 260 (0.8, M - CO}_2\text{Me), 205 (1.1, M - (C}_4\text{H}_9)_2), 146 (5.3), 104 (19.2), 57 (100); (found } M^+ \text{, 206.1507. Calc. for C}_12\text{H}_22\text{N}_0\text{O}_5, 260.1498). \text{ (Found: C, 52.6; H, 7.8; N, 4.3. Calc. for C}_14\text{H}_25\text{N}_0\text{7: C, 52.44; H, 7.76; N, 4.34%).}
\end{align*}
\]
(S)-N-tert-butyloxycarbonyl-O-tert-butyltrimethylsilyloxyserine methyl ester (97)
((using the procedure of Corey et al.98)

A solution of compound (95) (1 g, 4.57 mmol), imidazole (0.62 g, 9.14 mmol) and tert-butyldimethylsilyl chloride (0.9 g, 5.94 mmol) in dry DMF (30 ml) was stirred at room temperature for 15 h. The mixture was then diluted with ethyl acetate (100 ml) and washed with brine (5 x 50 ml). The organic layer was dried with magnesium sulfate, filtered and concentrated to a crude oil. The oil was purified by flash chromatography eluting with hexane-ethyl acetate (9:1) to give a clear oil (1.4 g, 92%); Rf 0.8 (ethyl acetate-hexane; 1:2); δH (200MHz, CDCl3) 0.00 (3H, s, 9- or 10-H3), 0.01 (3H, s, 9- or 10-H3), 0.84 (9H, s, 11-, 12-, and 13-H3), 1.43 (9H, s, 6-, 7- and 8-H3), 3.71 (3H, s, 15-H3), 3.76 (1H, dd, 3-H, J 3.0, 10 Hz), 4.00 (1H, dd, 3-H, J 2.5, 10 Hz), 4.34 (1H, m, 2-H), 5.34 (1H, br d, NH); δC (50 MHz, CDCl3) -5.7 (C-9 or -10), -5.6 (C-9 or -10), 18.1 (C-14), 25.6 (C-11, -12 and -13), 28.2 (C-6, -7 and -8), 52.1 (C-15), 55.5 (C-2), 63.6 (C-3), 79.7 (C-5), 155.3 (C-4), 171.3 (C-1); vmax (film) / cm−1 2970, 1752 (COOMe), 1720 (OCON), 1256 (SiMe2), 1088, 840 (SiO); m/z 260 (13.5, M - OC4H9), 220 (55.4), 176 (4.7), 89 (100); (found M - OC4H9, 260.1312. Calc. for C11H22NO4Si, 260.1318). (Found: C, 54.1; H, 9.3; N, 4.2. Calc. for C15H31NO5Si: C, 53.94; H, 9.26; N, 4.41%).
A stirred solution of compound (97) (1.4 g, 4.2 mmol) in anhydrous toluene (30 ml) was cooled to -78 °C and diisobutylaluminium hydride (1.5M, 4.8 ml, 7.1 mmol) was added dropwise, keeping the internal temperature below -70 °C. After the mixture had been stirred at -78 °C for a further 2 h, saturated ammonium chloride solution (30 ml) was added. The mixture was poured into cold 1 M HCl (50 ml) and extracted with ethyl acetate (3 x 50 ml). The combined extracts were washed with brine (2 x 50 ml), dried with magnesium sulfate, filtered and concentrated to afford a crude oil. This oil was sufficiently pure and had to be used directly for the next step; Rf 0.75 (hexane-ethyl acetate; 2:1); δH (200MHz, CDCl3) 0.00 (6H, s, 9- and 10-H3), 0.81 (9H, s, 11-, 12- and 13-H3), 1.41 (9H, s, 6-, 7- and 8-H3), 3.90 (3H, m, 2-H and 3-H2), 4.30 (1H, br d, NH), 9.59 (1H, s, 1-H); δC (50 MHz, CDCl3) -5.6 (C-9 and -10), 18.1 (C-14), 25.6 (C-11, -12 and -13), 28.2 (C-6, -7 and -8), 61.3 (C-3), 61.5 (C-2) 79.7 (C-5), 155.2 (C-4), 199.3 (C-1); v max (film) / cm⁻¹ 1725 (CHO), 1720 (OCON), 1250 (SiMe2), 839 (SiO).
BuLi (1.5 M, 4 ml, 6.0 mmol) was added to a solution of 1-hexyne (0.54 g, 6.6 mmol) in dry THF (30 ml) at -78 °C. After stirring at -78 °C for 30 min, (S)-2-[(tert-butyloxycarbonyl)amino]-3-tert-butyldimethylsilyloxypropanal (93) (1 g, 3.3 mmol) in anhydrous TIF (10 ml) was added dropwise. The resulting mixture was stirred at this temperature for a further 2 h and then allowed to warm up to room temperature overnight. Saturated ammonium chloride solution (40 ml) was added and the mixture was extracted with ethyl acetate (3 x 50 ml). The combined organic extracts were washed with brine (3 x 50 ml), dried with magnesium sulfate, filtered and concentrated to give a yellow oil. The mixture of diastereoisomers was separated by careful chromatography eluting with hexane-ethyl acetate (9:1).

(4S,5R)-4-tert-Dimethylsilyloxyethyl-5-(1-hexynyl)oxazolidine-2-one (100) was isolated as a yellow oil (0.21 g, 20%).

![Chemical structure](image)

Rf 0.3 (hexane-ethyl acetate; 9:1); [α]D22 -43.0 (c 1.1 in CHCl3); δH (200MHz, CDCl3) 0.00 (6H, s, 11- and 12-H3), 0.82 (12H, m, 1-, 14-, 15- and 16-H3), 1.39 (4H, m, 2- and 3-H2), 2.14 (2H, dt, 4-H, J 1.8, 6.6 Hz), 3.56 (2H, d, 10-H2, J 3.3 Hz), 3.82 (1H, m, 9-H), 5.20 (1H, dt, 7-H, J 1.9 and 7.7 Hz), 6.42 (1H, br s, NH); δC (50 MHz, CDCl3) -5.5 (C-11 and -12), 13.5 (C-1), 18.1 (C-13), 18.3, 21.8 (C-2 and -3), 25.7 (C-14, -15 and -16), 30.1 (C-4), 55.9 (C-9), 63.4 (C-10), 68.1 (C-7), 72.0, 91.1 (C-5 and -6), 158.4 (C-8); νmax (film) / cm⁻¹ 1740, 1248 (SiMe2), 1092 (SiO); m/z 254 (35, M - C4H9), 224 (30, M - C4H9, - C2H6), 210 (60), 168 (40), 115 (3.5), 75 (100); (found M - C4H9, 254.1243. Calc. for C12H20NO3Si, 254.1212).

(4S,5S)-4-tert-Dimethylsilyloxyethyl-5-(1-hexynyl)oxazolidine-2-one (101) was isolated as a yellow oil (0.31 g, 30%).
Rf 0.25 (hexane-ethyl acetate; 9:1); [α]D22 -11.2 (c 1.4 in CHCl3); δH (200MHz, CDCl3) 0.00 (6H, s, 11- and 12-H3), 0.82 (12H, m, 1-, 14-, 15- and 16-H3), 1.40 (4H, m, 2- and 3-H2), 2.25 (2H, dt, 4-II, J 1.8, 6.9 Hz), 3.56 (2H, d, 10-H2, J 4.7 Hz), 3.82 (1H, m, 9-H), 4.90 (1H, dt, 7-II, J 1.9 and 5.2 Hz), 6.42 (1H, br s, NH); δC (50 MHz, CDCl3) -5.5 (C-11 and -12), 13.5 (C-1), 18.1 (C-13), 18.3, 21.8 (C-2 and -3), 25.6 (C-14, -15 and -16), 30.2 (C-4), 60.9 (C-9), 63.5 (C-10), 68.3 (C-7), 75.8, 89.2 (C-5 and -6), 158.8 (C-8); νmax (film) / cm⁻¹ 1740, 1248 (SiMe2), 1092 (SiO); m/z 254 (46.7, M - C4H9), 210 (78), 168 (36.9), 115 (3.5), 75 (100); (found M - C4H9, 254.1203. Calc. for C12H2ONO3Si, 254.1212).

Methyl (4S)-3-tert-butyloxycarbonyl-2,2-dimethyloxazolidin-4-carboxylate (103)86

A solution of (S)-N-tert-butyloxycarbonylserine methyl ester (95) (3 g, 13.7 mmol) in anhydrous benzene (150 ml) with 2,2-dimethoxypropane (2.5 ml, 20.5 mmol) and p-toluenesulfonic acid (0.3 g) was heated under Dean-Stark conditions for 2 h. The deep yellow coloured reaction mixture was poured into saturated sodium bicarbonate solution (100 ml) and extracted with ether (3 x 50 ml). The combined organic extracts were washed with water (2 x 50 ml), dried with magnesium sulfate, filtered and concentrated to afford a crude oil. The oil was purified by column chromatography to give the title compound (2.5 g, 70%) as a light yellow oil; Rf 0.4 (hexane-ethyl acetate; 4:1); [α]D22 -46.0
(c 1.3 in CHCl₃) \{lit., \([\alpha]_D^{22} = -46.7 \) (c 1.3 in CHCl₃)\}; \(\delta_H\) (200MHz, CDC₁₃) 1.34 (5H, s, 9-, 10- and 11-H₃), 1.43 (5H, s, 9-, 10- and 11-H₃ & 5- or 6-H₃), 1.46 (2H, s, 5- or 6-H₃), 1.57 (1H, s, 5- or 6-H₃), 1.60 (2H, s, 5- or 6-H₃), 3.69 (3H, s, 12-H₃), 4.04 (2H, m, 3-H₂), 4.30 (0.5H, dd, 2-H), 4.40 (0.5H, dd, 2-H); \(\delta_C\) (50 MHz, CDC₁₃) 24.2, 24.8, 25.0, 25.9 (C-5 and -6), 28.1 (C-9, -10 and -11), 52.1, 52.2 (C-12), 59.1 (C-2), 65.9, 66.1 (C-3), 80.1, 80.7 (C-8), 94.3, 94.9 (C-4), 155.1 (C-7), 171.2, 171.6 (C-12); \(v_{\text{max}}\) (film) / cm⁻¹ 3065, 2978, 1725 (COOMe), 1702 (OCON); \(m/z\) 244 (8.6, M - CH₃), 200 (2.0, M - CO₂Me), 186 (5.1, M - OC₄H₉), 144 (100), 100 (1.1), 84 (84); (found M - CH₃, 244.1171. Calc. for C₁₁H₁₈NO₅, 244.1185).

\((4S)-3\text{-}\text{tert-Butyloxy carbonyl}-2,2\text{-dimethyl}-4\text{-hex-5-ynylcarbonyloxazolidine (104)}\)

BuLi (1.5M, 4 ml, 4.6 mmol) was added dropwise at -78 °C to a solution of 1-hexyne (0.4 g, 5 mmol) in dry THF (20 ml). The solution was stirred for 30 min before a solution of methyl \((4S)-3\text{-}\text{tert-}
\text{butyloxy carbonyl-2,2-dimethyloxazolidine-4-carboxylate (103)}\) (1 g, 3.9 mmol) in dry THF (10 ml) was added. The resulting solution was stirred at -78 °C for a further 4 h. Saturated ammonium chloride solution (20 ml) was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (2 x 30 ml) and the combined extracts were dried with magnesium sulfate and concentrated to give an oil. The oil was purified by column chromatography eluting with hexane-ethyl acetate (6:1) to give the title compound, starting material and methyl \((\text{tert-}
\text{butyloxy carbonyl)aminoacrylate (105).}\)
The title compound was isolated as a yellow oil (0.2 g, 17%); Rf 0.3 (hexane-ethyl acetate; 3:1); δH (200MHz, CDCl3) 0.81 (3H, t, 17-H3), 1.35 (19H, m, 15-H2, 16-H2, 5-, 6-, 8-, 9- and 10-H3), 2.30 (2H, t, 14-H2), 4.04 (2H, m, 3-H2), 4.28 (0.6, dd, 2-H, J 3.9, 7.1 Hz), 4.47 (0.4H, app. t, 2-H); δC (50 MHz, CDCl3) 13.3 (C-17), 18.7, 21.8 (C-15 and -16), 24.1, 24.9, 25.0, 26.0 (C-5 and -6), 28.0, 28.3 (C-9, -10 and -11), 29.4 (C-14), 65.2, 65.6 (C-3), 66.5 (C-2), 80.6 (C-8), 80.8 (C-12), 95.2 (C-13), 155.9 (C-7), 163.4 (C-1); vmax (KBr disc) / cm⁻¹ 2200, 2705, 1680.

Methyl 2-(tert-butyloxycarbonyl)aminoacrylate (105) was isolated as a clear oil (0.15 g, 19%).

\[
\begin{align*}
\text{Rf} & \ 0.7 \ \text{(hexane-ethyl acetate; 3:1); } \\
\delta_H & \ (200\text{MHz, CDCl}_3) \ 1.35 \ (9H, s, 6-, 7- \text{and 8-H3}), \ 3.75 \ (3H, s, 9-H3), \ 5.65 \ (1H, br d, NH), \ 6.08 \ (1H, d, 3-\text{H, } J 1.5 \text{ Hz}), \ 6.08 \ (1H, br s, 3-H); \ \\
\delta_C & \ (50 \text{MHz, CDCl}_3) \ 28.2 \ (C-6, -7 \text{ and -8}), \ 52.8 \ (C-9), \ 80.3 \ (C-5), \ 118.1 \ (C-3), \ 155.9 \ (C-4), \ 164.4 \ (C-1); \ v_{\text{max}} \ (\text{KBr disc}) / \text{cm}^{-1} \ 1680, 1750.
\end{align*}
\]

First attempted preparation of phenyl (4S)-3-tert-butyloxycarbonyl-2,2-dimethyloxazolidine-4-carboxylate

\[
\begin{align*}
\text{A solution of methyl (4S)-3-tert-butyloxycarbonyl-2,2-dimethyloxazolidine-4-carboxylate (103) (0.5 g, 1.9 mmol) in anhydrous benzene (150 ml) with phenol (0.5 g, 5.7 mmol) and } p-\text{toluenesulfonic acid (0.3 g) was heated under Dean-Stark conditions for 6 h. The deep yellow coloured reaction mixture was poured into saturated sodium bicarbonate solution (100 ml) and extracted with}
\end{align*}
\]
ether (3 x 50 ml). The combined organic extracts were washed with water (2 x 50 ml), dried with magnesium sulfate, filtered and concentrated to afford a crude oil. TLC analysis and NMR spectra were identical to those of starting material.

Second attempted preparation of phenyl (4S)-3-tert-butyloxy carbonyl-2,2-dimethyl oxazolidine-4-carboxylate

Lithium (13 mg, 0.9 mmol) was added to a solution of phenol (0.36 g, 3.8 mmol) in dry DMF (25 ml). A solution of (4S)-3-tert-butyloxy carbonyl-2,2-dimethyl oxazolidine-4-carboxylate (103) (0.5 g, 1.9 mmol) in DMF (5 ml) was then added. The reaction was set up as a distillation. The resulting solution was heated at 95 °C for 4 h and then allowed to cool down to room temperature. The mixture was poured into ethyl acetate (30 ml) and water (30 ml). The organic layer was separated and the aqueous layer was washed with ethyl acetate (3 x 30 ml). The combined organic extracts were washed with brine (4 x 50 ml), dried with magnesium sulfate and concentrated to give an oil. The oil was purified by flash chromatography eluting with hexane-ethyl acetate (5:1) to give methyl 2-(tert-butyloxy carbonyl)aminoacrylate (105) (0.35 g, 92%).

(4S)-3-tert-Butyroxy carbonyl-2,2-dimethyl oxazolidine-4-carboxaldehyde (107)

A stirred solution of (4S)-3-tert-butyloxy carbonyl-2,2-dimethyl oxazolidine-4-carboxylate (103) (2.2 g, 8.5 mmol) in anhydrous toluene (30 ml) was cooled to -78 °C and diisobutylaluminum hydride (1.5 M, 9.6 ml, 14.5 mmol) was added dropwise, keeping the internal temperature below -70 °C. After the mixture had been stirred at -78 °C for a further 2 h, methanol (20 ml) was slowly added with the internal temperature kept below -70 °C. The reaction mixture was poured into ice cold 1 M HCl (50 ml) and
extracted with ethyl acetate (3 x 50 ml). The combined organic extracts were washed with brine (3 x 50 ml), dried with magnesium sulfate, filtered and concentrated to afford a yellow oil. This oil was purified by flash chromatography (hexane-ethyl acetate; 4:1) to give title compound (1.2 g, 62%) as a clear oil; Rf 0.36 (hexane-ethyl acetate; 4:1); [α]D22 -90.5 (c 1.34 in CHCl3) (lit.,58 [α]D22 -91.7 (c 1.34 in CHCl3)); δH (200MHz, CDCl3) 1.36 (5H, s, 9-, 10- and 11-H3), 1.39 (5H, s, 9-, 10- and 11-H3 & 5- or 6-H3), 1.43 (2H, s, 5- or 6-H3), 1.44 (1H, s, 5- or 6-H3), 1.48 (2H, s, 5- or 6-H3), 4.01 (2H, m, 3-H2), 4.11 (0.5H, m, 2-H), 4.27 (0.5H, m, 2-H), 9.47 (0.5H, d, 1-H, J 2.3 Hz), 9.52 (0.5H, d, 1-H, J 2.3 Hz); δC (50 MHz, CDCl3) 23.7, 24.5, 25.6, 27.0 (C-5 and -6), 28.1 (C-9, -10 and -11), 63.3, 63.8 (C-3), 64.6 (C-2), 80.9, 81.2 (C-8), 94.7, 94.7 (C-4), 155.1, 155.4 (C-7), 199.1, 199.3 (C-1); αmax (film) / cm⁻¹ 3072, 2976, 1725 (CHO), 1720 (OCON); m/z 200 (10.5, M - CHO), 156 (1.3, M - OC₄H₉), 146 (53), 100 (66), 84 (100), 72 (14.2); (found M - CHO, 200.1278. Calc. for C₁₀H₁₈NO₃, 200.1287).

(4S)-3-tert-Butyloxycarbonyl-4-[(1R,S)-1-hydroxyallyl]-2,2-dimethyloxazolidine (108)

A solution of compound (107) (1.2 g, 5.2 mmol) in anhydrous THF (30 ml) was added dropwise over 30 min into vinylmagnesium bromide (1M, 7.9 ml, 7.9 mmol) at -78 °C. The resulting mixture was stirred at this temperature for a further 1 h before saturated ammonium chloride solution (30 ml) was added. The organic layer was separated and the aqueous layer was extracted with ether (3 x 30 ml). The combined organic extracts were washed brine (30 ml), dried with magnesium sulfate, filtered and concentrated to leave an oil. Purification by column chromatography (hexane-ethyl acetate; 3:1) afforded the title compound (1.1 g, 77%) as a clear oil; Rf 0.28 (hexane-ethyl acetate; 4:1); δH (200MHz, CDCl3) 1.38 (15H, m, 5-, 6-, 9-, 10- and 11-H3), 3.90 (3H, m, 2-H, 3-H₂), 4.21 (1H, br s, 1-H), 5.10 (2H, m, 13-H₂), 5.7 (1H, m, 12-
H); the carbon spectrum was complex; $v_{\text{max}}$ (KBr disc) / cm$^{-1}$ 3415, 1740, 1696; $m/z$ 200 (3.1, M - C$_4$H$_9$), 184 (4.2, M - OC$_4$H$_9$), 144 (20), 57 (100); (found M - C$_4$H$_9$, 200.0938. Calc. for C$_9$H$_{14}$NO$_4$, 200.0923).

(Found: C, 60.7; H, 8.9; N, 5.4. Calc. for C$_{13}$H$_{23}$N$_2$O$_4$: C, 59.92; H, 8.88; N, 5.29%).

General procedure 1 for alkynylation of aldehyde (107)

(using the procedure of Garner et al. 58)

BuLi (1.5M, 0.014 mmol) was added to a -23 °C solution of the alkyne (0.015 mmol) in dry THF (100 ml) under nitrogen. The resulting suspension of lithium acetylide was stirred at the same temperature for a further 1 h and then a solution of the aldehyde (107) (0.01 mmol) in dry THF (20 ml) was added. The reaction was complete (monitored by TLC) after 2 h at this temperature and then was quenched by addition of saturated ammonium chloride solution (50 ml). The organic layer was separated and the aqueous layer was extracted with ether (3 x 30 ml). The combined organic layers were washed with brine (2 x 50 ml), dried with magnesium sulfate, filtered and concentrated to give a yellow oil. TLC in hexane-ethyl acetate (4:1) showed formation of both diastereoisomers. The major diastereoisomer was separated by careful chromatography eluting with hexane-ethyl acetate (9:1).

(4S)-3-tert-Butyloxycarbonyl-4-[(1R)-1-hydroxy-2-nonynyl]-2,2-dimethylloxazolidine (111)

Following general procedure 1, the title compound was prepared with these reagents: BuLi (1.5M, 4 ml, 4.1 mmol), 1-nonyne (0.81 g, 6.6 mmol), aldehyde (107) (1 g, 4.4 mmol). The compound was isolated as
a clear oil (0.9, 58%); Rf 0.36 (ethyl acetate-hexane; 1:4); [α]D22 -40.2 (c 2.4 in CHCl3); δH (200MHz, CDCl3) 0.81 (3H, t, 20-H3), 1.18 (10H, m, 15-H2 to 19-H2), 1.42 (15H, m, 5-, 6-, 9-, 10- and 11-H3), 2.12 (2H, dt, 14-H2, J 1.7 and 6.8 Hz), 3.90 (1H, br s, OH), 4.10 (2H, m, 3-H2), 4.40 (1H, m, 2-H), 4.70 (1H, br d, 1-H, J 8.2 Hz); δC (50 MHz, CDCl3) 214.0 (C-20), 18.5, 22.4, 28.4, 28.7 (C-15 to C-19), 25.2, 25.6 (C-5 and -6), 28.2 (C-9, -10 and -11), 31.6 (C-14), 60.3 (C-2), 63.8 (C-1), 64.9 (C-3), 77.7, 86.1 (C-12 and -13), 80.9 (C-8), 94.7 (C-4), 155.1 (C-7); νmax (film) / cm⁻¹ 3308 (OH), 2932, 2233, 1693 (OCON); m/z 297 (2.8, MH - C4H9), 200 (27.4), 144 (29.6), 100 (100); (found MH - C4H9, 297.1931. Calc. for C16H27NO4, 297.1940).

(4S)-3-tert-Butyloxycarbonyl-4-[(1R)-1-hydroxy-2-dodecynyl]-2,2-dimethyloxazolidine (112)

General procedure 1 was used with the following reagents: BuLi (1.5M, 4 ml, 4.1 mmol), 1-dodecyne (1.1 g, 6.6 mmol), aldehyde (107) (1 g, 4.4 mmol). The compound was isolated as a light yellow oil (1.1 g, 63%); Rf 0.38 (hexane-ethyl acetate; 4:1); [α]D22 -37.8 (c 1.3 in CHCl3); δH (200MHz, CDCl3) 0.80 (3H, t, 23-H3), 1.15 (16H, m, 15-H2 to 22-H2), 1.45 (15H, m, 5-, 6-, 9-, 10- and 11-H3), 2.13 (2H, m, 14-H2), 3.86 (1H, br s, OH), 4.03 (2H, m, 3-H2), 4.56 (1H, m, 2-H), 4.70 (1H, br d, 1-H, J 8.2 Hz); δC (50 MHz, CDCl3) 14.0 (C-23), 18.6, 22.5, 28.5, 28.8, 29.0, 29.2, 29.5, 30.8 (C-15 to C-22), 25.4 (C-5 and -6), 28.6 (C-9, -10 and -11), 31.8 (C-14), 62.7 (C-2), 63.9 (C-1), 64.9 (C-3), 77.7, 86.1 (C-12 and -13), 80.9 (C-8), 94.8 (C-4), 15.3 (C-7); νmax (film) / cm⁻¹ 3245, 2945, 2270, 1695; m/z 339 (4.9, MH - C4H9), 280 (6.3), 200 (73.9), 156 (3.8), 144 (100); (found MH - C4H9, 339.2411. Calc. for C19H33NO4, 339.2409).
(4S)-3-tert-Butyloxycarbonyl-4-[(1R)-1-hydroxy-2-pentadecynyl]-2,2-dimethyloxazolidine (110)

General procedure 1 was used with the following reagents: BuLi (1.5M, 4 ml, 4.1 mmol), 1-pentadecyne (1.4 g, 6.6 mmol), aldehyde (107) (1 g, 4.4 mmol). The compound was isolated as a light yellow oil (1.3 g, 68%); Rf 0.4 (hexane-ethyl acetate; 4:1); [α]D²² -41.2 (c 1.4 in CHCl₃) {lit., ref7, [α]D²² -40.1 (c 1.4 in CHCl₃)}; δH (200MHz, CDCl₃) 0.91 (3H, t, 26-H₃), 1.21 (22H, m, 15-H₂ to 25-H₂), 1.45 (15H, m, 5-, 6-, 9-, 10- and 11-H₃), 2.15 (2H, br dt, 14-H₂, J 1.6 and 6.7 Hz), 3.90 (1H, br s, OH), 4.20 (2H, m, 3-H₂), 4.49 (1H, m, 2-H), 4.70 (1H, br d, 1-H, J 8.3 Hz); δC (50 MHz, CDCl₃) 14.0 (C-26), 18.7, 22.6, 28.5, 28.8, 29.1, 29.3, 29.5, 29.6, 31.5, 31.9 (C-15 to C-25), 25.3 (C-5 and -6), 28.6 (C-9, -10 and -11), 31.8 (C-14), 62.7 (C-2), 64.0 (C-1), 65.0 (C-3), 78.4, 87.2 (C-12 and -13), 81.0 (C-8), 94.8 (C-4), 155.8 (C-7); νmax (film) / cm⁻¹ 3277 (OH), 2856, 2243, 1704 (OCON); m/z 381 (1.2, M - C₄H₉), 200 (22.4), 144 (33.8), 100 (100); (found M - C₄H₉, 381.2883. Calc. for C₂₂H₃₉NO₄, 381.2879). (Found: C, 71.3; H, 10.8; N, 3.2. Calc. for C₂₆H₄₇N₄O₄: C, 71.14; H, 10.63; N, 3.38%).
(4S)-3-tert-Butyloxycarbonyl-4-[(1R)-1-hydroxy-2-nonynyl]-2,2-dimethyloxazolidine (113)

A solution of (4S)-3-tert-butyloxycarbonyl-4-[(1R)-1-hydroxy-2-nonylnyl]-2,2-dimethyloxazolidine (111) (1 g, 2.8 mmol) in dry THF (10 ml) was added to a blue solution of Li (0.04 g, 5.6 mmol) in ammonia (50 ml) at -78 °C. After it was stirred at -78 °C for 4 h, the reaction was quenched at -78 °C with saturated ammonium chloride solution (20 ml) and the ammonia was allowed to evaporate at room temperature overnight. The residue was redissolved in ethyl acetate (100 ml), washed with 1 M HCl (3 x 50 ml), dried with magnesium sulfate and concentrated to give a yellow oil. The oil was purified by careful chromatography eluting with (hexane-ethyl acetate; 9:1) to give the title compound as a yellow oil (0.1 g, 10%) and starting material (0.6 g); Rf 0.38 hexane-ethyl acetate, (4:1); δH (200 MHz, CDCl3) 0.81 (3H, t, 20-H3), 1.18 (10H, m, 15-H2 to 19-H2), 1.42 (15H, m, 5-, 6-, 9-, 10- and 11-H3), 2.20 (2H, m, 14-H2), 4.00 (4H, m, OH, 2-H, 3-H2), 5.34 (1H, dd, 12-H, J 5.6 and 15.5 Hz), 5.66 (1H, dt, 13-H, J 6.6, 15.4 Hz); δC (50 MHz, CDCl3) 14.1 (C-20), 22.6, 29.1, 29.2, 31.8 (C-15 to C-19), 25.7, 26.2 (C-5 and -6), 28.7 (C-9, -10 and -11), 32.4 (C-14), 62.3 (C-2), 65.2 (C-3), 74.1 (C-1), 81.1 (C-8), 94.4 (C-4), 128.0 (C-12), 133.4 (C-13), 155.4 (C-7); νmax (film) / cm⁻¹ 3301, 1704, 1645.
Attempted preparation of (4S)-3-tert-butyloxycarbonyl-4-[(1R)-1-hydroxy-2-pentadecenyl]-2,2-dimethyloxazolidine

A solution of (4S)-3-tert-butyloxycarbonyl-4-[(1R)-1-hydroxy-2-pentadecenyl]-2,2-dimethyloxazolidine (110) (1 g, 2.8 mmol) in dry THF (10 ml) was added to a blue solution of Li (0.04 g, 5.6 mmol) in ammonia (50 ml) at -78 °C. After it was stirred at -78 °C for 4 h, the reaction was quenched at -78 °C with saturated ammonium chloride solution (20 ml) and the ammonia was allowed to evaporate at room temperature overnight. The residue was redissolved in ethyl acetate (100 ml), washed with 1 M HCl (3 x 50 ml), dried with magnesium sulfate and concentrated to give a yellow oil. NMR spectroscopy of the crude product showed that the major component was starting material but there was a small trace of trans-double bond product formed.

General procedure 2 for deprotection of 3-tert-butyloxycarbonyl-2,2-dimethyloxazolidine
(lit. procedure was modified)

The 3-tert-butyloxycarbonyl-2,2-dimethyloxazolidine (1 mmol) was dissolved in methanol (40 ml) and toluene-p-sulfonic acid (4 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 48 h after which methanol was removed under reduced pressure. The crude oil was redissolved in ethyl acetate (100 ml), and the solution was washed with saturated sodium bicarbonate solution (3 x 50 ml), brine (2 x 50 ml), dried with magnesium sulfate and concentrated to give the aminodiol.
(2S, 3R)-2-Amino-1,3-dodec-4-ynediol (116)

General procedure 2 was used with the following reagents: Toluene-\(p\)-sulfonic acid (1.4 g, 6.4 mmol), (4S)-3-\textit{tert}-butyloxy carbonyl-4-[(1R)-1-hydroxy-2-nonynyl]-2,2-dimethyloxazolidine (111) (0.5 g, 1.4 mmol), methanol (50 ml). The compound was isolated as a clear yellow oil (0.3 g, 87%); 

\[
\text{Rf} \ 0.1 \ \text{(ethyl acetate-ethanol; 9:1); } [\alpha]_D^{22} -22.6 \ \text{(c 0.7 in CHCl}_3) \]

\[
\delta_H \ (200MHz, CDCl}_3) \ 0.80 \ (3H, t, 12-H3), 1.20 - 1.40 \ (10H, m, 7-H_2 \ \text{to} \ 11-H_2), 2.14 \ (2H, t, 6-H_2, J 6.1 Hz), 2.82 \ (1H, m, 2-H), 3.90 \ (4H, br s, OH, NH_2), 3.60 \ (2H, br d, 1-H_2, J 4.8 Hz), 4.32 \ (1H, br d, 3-H, J 4.7Hz); \delta_C \ (50 MHz, CDCl}_3) \ 14.0 \ (C-12), 18.6, 22.4, 28.4, 28.7 \ (C-7 \ \text{to} \ C-11), 31.6 \ (C-6), 56.9 \ (C-2), 63.1 \ (C-1), 64.3 \ (C-3), 78.4, 87.2 \ (C-4 \ \text{and} \ -5); \nu_{\text{max}} \ (\text{KBr disc}) \ / \ \text{cm}^{-1} \ 3356 \ \text{v.br}, 2932, 2360; m/z \ 196 \ (1.2 \ \text{MH} - \text{H}_2\text{O}), 100 \ (100), 83 \ (26.5), 60 \ (7.8); \ \text{(found MH} - \text{H}_2\text{O}, 196.1732. \ \text{Calc. for C}_{12}H_{22}N\text{O}, 196.1701).

(2S, 3R)-2-Amino-1,3-pentadec-4-ynediol (117)

Following general procedure 2, the title compound was prepared with these reagents: Toluene-\(p\)-sulfonic acid (1.73 g, 6.4 mmol), (4S)-3-\textit{tert}-butyloxy carbonyl-4-[(1R)-1-hydroxy-2-dodecynyl]-2,2-dimethyloxazolidine (112) (0.7 g, 1.8 mmol), methanol (50 ml). The compound was isolated as a white solid (0.41 g, 89%); 

\[
\text{Rf} \ 0.1 \ \text{(ethanol-ethyl acetate; 1:9); } [\alpha]_D^{22} -8.8 \ \text{(c 1.1 in CHCl}_3) \]

\[
\delta_H \ (200MHz, CDCl}_3) \ 0.80 \ (3H, t, 15-H3), 1.18 - 1.40 \ (16H, m, 7-H_2 \ \text{to} \ 14-H_2), 2.13 \ (2H, t, 6-H_2, J 7.0 Hz), 2.82 \ (1H, m, 2-H), 3.45 \ (4H, br s, OH, OH, NH_2), 3.68 \ (2H, br d, 1-H_2, J 5.0 Hz), 4.32 \ (1H, m, 3-H); \delta_C \ (50 MHz, CDCl}_3) \ 14.0 \ (C-15), 18.6, 22.5, 28.5, 28.8, 29.0, 29.2, 29.5, 30.8 \ (C-7 \ \text{to} \ C-14), 31.8 \ (C-6), 57.0 \ (C-2), 62.9 \ (C-1),
64.2 (C-3), 78.3, 87.1 (C-4 and -5); \( \nu_{\text{max}} \) (film) / cm\(^{-1}\) 3357 \( \nu_{\text{br}} \), 2973, 2338; m/z 238 (0.9, MH - H\(_2\)O), 100 (100), 9.3 (12.7), 8.3 (14.0); (found MH - H\(_2\)O, 238.2166. Calc. for C\(_{15}\)H\(_{28}\)NO, 238.2171).

(2S, 3R)-2-Amino-1,3-octadec-4-ynediol (115)

General procedure 2 was employed with the following reagents: Toluene-\( p \)-sulfonic acid (2.1 g, 8.8 mmol), (4S)-3-tert-butyloxy-carbonyl-4-[(1R)-1-hydroxy-2-pentadecynyl]-2,2-dimethyloxazolidine (110) (1.0 g, 2.2 mmol), methanol (50 ml). The compound was isolated as a yellow solid (0.6 g, 92%); Rf 0.1 (ethyl acetate-ethanol; 9:1); \([\alpha]_D^{22}\) -6.1 (c 0.7 in CHCl\(_3\)); \( \delta_\text{H} \) (200MHz, CDCl\(_3\)) 0.81 (3H, t, 18-H\(_3\)), 1.25 (22H, m, 7-H\(_2\) to 17-H\(_2\)), 2.14 (2H, t, 6-H\(_2\)), 2.83 (1H, br s, 2-H\(_2\)), 3.18 (4H, br s, OH, OH, NH\(_2\)), 3.62 (2H, br d, 1-H\(_2\)), 4.32 (1H, br s, 3-H\(_2\)); \( \delta_\text{C} \) (50 MHz, CDCl\(_3\)) 14.1 (C-18), 18.7, 22.6, 28.6, 28.9, 29.1, 29.3, 29.5, 29.6 (C-7 to C-17), 31.9 (C-6), 56.9 (C-2), 63.2 (C-1), 64.4 (C-3), 78.3, 87.4 (C-4 and -5); \( \nu_{\text{max}} \) (KBr disc) / cm\(^{-1}\) 3398, 3280, 3100, 2232; m/z 280 (2.1, M - ClH\(_3\)), 279 (0.3, M - H\(_2\)O), 153 (2.1), 102 (100), 85 (68); (found M - NH\(_3\), 280.2390. Calc. for C\(_{18}\)H\(_{32}\)O\(_2\), 280.2402).

(2S, 3R)-2-Acetamino-1,3-diacetoxydodec-4-yn (118)

(using the procedure of Garner et al.\(^{58}\))
A solution of (2S,3R)-2-amino-1,3-dodec-4-ynediol (116) (0.3 g, 1.4 mmol), acetic anhydride (0.7 ml, 7 mmol) and 4-dimethylaminopyridine (0.03 g) in triethylamine (20 ml) was stirred at room temperature for 8 h. The excess of triethylamine was removed under reduced pressure and the residue was redissolved in ethyl acetate (50 ml). The organic layer was washed with 1 M HCl (3 x 50 ml), saturated sodium bicarbonate solution (3 x 50 ml), brine (50 ml), dried with magnesium sulfate and concentrated to give an oil which was purified by flash chromatography eluting with hexane-ethyl acetate (5:1) to afford the title compound as an yellow oil (0.1 g, 21%); Rf 0.35 (hexane-ethyl acetate; 1:1); δH (200MHz, CDCl3) 0.80 (3H, t, 12-H2), 1.45 (10H, m, 7-H2 to 11-H2), 1.97, 2.00, 2.01 (9H, 3 s, 14-, 16- and 18-H2), 2.18 (2H, br dt, 6-H2, J 2.0, 7.1 Hz), 4.01 (1H, dd, 1-H, J 5.8, 11.3 Hz), 4.25 (1H, dd, 1-H, J 6.8, 11.3 Hz), 4.48 (1H, m, 2-H), 5.46 (1H, dt, 3-H2, J 2.0, 3.8 Hz), 5.71 (1H, br d, NH); δC (50 MHz, CDCl3) 14.0 (C-12), 16.6, 22.5, 28.2, 28.6, 28.8 (C-7 to C-11), 20.7, 20.8, 23.3 (C-14, -16 and -18), 31.6 (C-6), 50.6 (C-2), 62.5 (C-1), 63.8 (C-3), 74.2, 89.2 (C-4 and -5), 169.6, 169.9, 170.2 (C-13, -15 and -17); vmax (KBr disc) / cm⁻¹ 3282, 2360, 1747, 1659, 1547; m/z 339 (M⁺, 0.9%), 296 (1.5), 280 (5.4), 144 (23.4), 103 (39.7), 84 (100); (found M⁺, 339.2065. Calc. for C₁₈H₂₉NO₅, 339.2046).

\[(2S,3R)-2-Acetamino-1,3-diacetoxypentadec-4-yne (119)\]

A solution of (2S,3R)-2-amino-1,3-pentadec-4-ynediol (117) (0.5 g, 2.0 mmol), acetic anhydride (1 ml, 10 mmol) and dimethylaminopyridine (0.05 g) in triethylamine (25 ml) was stirred at room temperature for 8 h. The excess of triethylamine was removed under reduced pressure and the residue was redissolved in ethyl acetate (50 ml). The organic layer was washed with 1 M HCl (3 x 50 ml), saturated sodium bicarbonate solution (3 x 50 ml), brine (50 ml), dried with magnesium sulfate and concentrated to give an oil. The oil was triturated with
hexane (20 ml) to afford the title compound as a white solid (0.72 g, 94%); mp 98-99 °C; [α]D22 -48.1 (c 1.2 in CHCl3); δH (200MHz, CDCl3) 0.80 (3H, t, 15-H3), 1.35 (18H, m, 7-H2 to 14-H2), 1.97, 2.01, 2.02 (9H, 3 s, 17-, 19- and 21-H3), 2.15 (2H, dt, 6-H2, J 2.0, 7.1 Hz), 4.05 (1H, dd, 1-H, J 5.8, 11.3 Hz), 4.25 (1H, dd, 1-H, J 6.4, 11.3 Hz), 4.45 (1H, m, 2-H), 5.48 (1H, dt, 3-H, J 2.0, 3.8 Hz), 5.98 (1H, br d, NH); δC (50 MHz, CDCl3) 13.9 (C-15), 18.6, 22.6, 28.2, 28.8, 28.9, 29.2, 29.3, 29.4 (C-7 to C-14), 20.7, 20.9, 23.1 (C-17, -19 and -21), 31.8 (C-6), 50.6 (C-2), 62.3 (C-1), 65.8 (C-3), 73.5, 89.0 (C-4 and -5), 169.5, 170.0, 170.7 (C-16, -18 and -20); νmax (KBr disc) / cm⁻¹ 3290, 2370, 1747, 1660, 1545; m/z 381 (M⁺, 0.4%), 338 (4.7), 322 (4.4), 278 (3.0), 144 (90.1), 102 (100); (found M⁺, 381.2532. Calc. for C21H35N05, 381.2515).

D-erythro-Sphingosine (14)⁶⁰

A solution of (2S,3R)-2-amino-1,3-octadec-4-ynediol (115) (0.3 g, 1 mmol) and lithium aluminium hydride (0.11 g, 3 mmol) in DMF (20 ml) was heated under reflux for 10 h and then allowed to cool down to room temperature. The reaction was quenched by careful addition of saturated ammonium chloride solution (20 ml) followed by ethyl acetate (30 ml). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 30 ml). The combined organic extracts were washed with brine (30 ml), dried with magnesium sulfate, filtered and concentrated to afford a yellow oil. Purification of the oil by flash chromatography eluting with ethanol-ethyl acetate (1:9) afforded the title compound as a yellow oil (60 mg, 20%); Rf 0.0 (ethyl acetate-ethanol; 9:1); δH (200MHz, CDCl3) 0.81 (3H, t, 18-H3), 1.13 (22H, m, 7-H2 to 17-H2), 1.94 (2H, m, 6-H2), 2.73 (1H, m, 2-H), 3.65 (1H, dd, 1-H, J 3.3, 11.4 Hz), 3.75 (1H, dd, 1-H, J 4.4, 11.2 Hz), 4.23 (1H, m, 3-H), 4.47 (4H, br s, OH, OH, NH2), 5.38 (1H, dd, 4-H, J 5.8, 15.3 Hz), 5.72 (1H, dt, 5-H, J 6.7, 15.2 Hz); δC (50 MHz, CDCl3) 14.8 (C-18), 22.6, 29.0, 29.2, 29.3, 29.4, 29.6, 31.9 (C-7 to C-17), 32.4 (C-6), 48.1 (C-2), 59.7 (C-1),
A solution of D-erythro-sphingosine (14) (60 mg, 0.2 mmol), acetic anhydride (0.2 ml, 2 mmol) and 4-dimethylaminopyridine (0.05 g) in triethylamine (25 ml) was stirred at room temperature for 8 h. The excess of triethylamine was removed under reduced pressure and the residue was redissolved in ethyl acetate (50 ml). The organic layer was washed with 1 M HCl (3 x 50 ml), saturated sodium bicarbonate solution (3 x 50 ml), brine (50 ml), dried with magnesium sulfate and concentrated to give an oil. The oil was partially purified by flash chromatography eluting with hexane-ethyl acetate (4:1) to afford the title compound (60 mg, 70%); Rf 0.41 (hexane-ethyl acetate; 4:1); δH (200MHz, CDC13) 0.78 (3H, t, 18-H3), 1.18 (22H, m, 7-H2 to 17-H2), 2.04 (1H, m, 6-H2, 20-22 and 24-H3), 2.89 (1H, m, 2-H), 3.99 (2H, m, 1-H2), 5.22 (2H, m, 3-H and 4-H), 5.70 (1H, dt, 5-H, J 6.7, 15.3 Hz); δC (50 MHz, CDC13) 14.0 (C-18), 20.1, 23.0, 23.1 (C-20, -22 and -24), 22.6, 28.6, 29.1, 29.3, 29.4, 29.5, 29.6, 30.9, 31.9 (C-7 to C-17), 32.2 (C-6), 56.6 (C-2), 63.7 (C-1), 74.3 (C-3), 124.4 (C-4), 136.8 (C-5), 170.1, 170.9 (C-19, -21 and -23). Attempted further purification on a silica column caused the title compound to decompose.
(S)-Serine methyl ester hydrochloride (95) (10 g, 64.3 mmol) and potassium hydrogen carbonate (6.7 g, 66.8 mmol) were dissolved in water (110 ml) and the solution was stirred at room temperature for 10 min. Potassium carbonate (9.4 g, 67.8 mmol) was added to the solution which was then cooled to 0 °C. A solution of phosgene in toluene (2M, 44 ml, 85.4 mmol) was added dropwise and the resulting solution was stirred for 2 h at 0 °C. The solvent was removed under reduced pressure to leave a white solid which was extracted with dichloromethane (3 x 100 ml). The extracts were dried with magnesium sulfate and concentrated to give a clear oil (7.5 g, 80%); Rf 0.45 (ethyl acetate); [α]D22 -18.6 (c 4.3 in CH2Cl2); δH (200MHz, CDCl3) 3.73 (3H, s, 6-H3), 4.39 (1H, dd, 3-H, J 4.8, 9.6 Hz), 4.52 (1H, dd, 3-H, J 4.8, 9.1 Hz), 4.67 (1H, app. t, 2-H, J 9.6 Hz), 6.88 (1H, br s, NH); δC (50 MHz, CDCl3) 51.3 (C-2), 52.1 (C-6), 65.2 (C-3), 157.8 (C-4), 169.3 (C-1); νmax (KBr disc) / cm⁻¹ 3323, 1767, 1740; m/z 145 (M⁺, 5.4%), 87 (5), 86 (100); (found M⁺, 145.0380. Calc. for C5H7NO4, 145.0375).

(R)-4-Hydroxymethyl oxazolidin-2-one (122)89

Sodium borohydride (1.36 g, 35.7 mol) was added in portions to a solution of compound (121) (5 g, 34.2 mmol) in ethanol (100 ml) at 0 °C. The reaction mixture was allowed to warm to room temperature. After it was stirred for 3 h at this temperature, saturated aqueous
ammonium chloride solution (30 ml) was added. The white solid was filtered off and the filtrate was concentrated to give a white paste. The white paste was purified by flash chromatography eluting with methanol-ethyl acetate (1:4) to afford the title compound as a white solid (2.5 g, 63%); Rf 0.28 (ethyl acetate-hexane; 9:1); δ_H (200MHz, D_2O) 3.60 (2H, 2 x dd, 1-H_2), 4.01 (1H, m, 2-H), 4.19 (1H, dd, 3-H, J 5.1, 8.7 Hz), 4.45 (1H, t, 3-H, J 8.7 Hz); δ_C (50 MHz, D_2O) 54.4 (C-2), 63.2 (C-1), 68.8 (C-3), 159.6 (C-4); ν_max (KBr disc) / cm⁻¹ 3370, 1740.

(Tosyl chloride (6.3 g, 32.8 mmol) was added at 0 °C to a solution of the compound (122) (2.5 g, 21.3 mmol) in dry triethylamine (20 ml). The reaction was stirred at room temperature overnight after which triethylamine was removed under reduced pressure. The solid was redissolved in dichloromethane (100 ml), washed with 1 M HCl (2 x 50 ml) and the organic layer was dried with magnesium sulfate, filtered and concentrated to give a yellow oil. The oil was purified by column chromatography to give the title compound and (S) -3-(4-toluenesulfonyl)-4-(4-toluenesulfonyloxymethyl)oxazolidin-2-one (124).

The title compound was obtained as a crystalline solid (3.5 g, 64%); mp 97 °C (lit., 99 96-99 °C); Rf 0.28 (methanol-ethyl acetate; 1:9); [α]_D 22 +6.0 (c 0.54 in CHCl_3); δ_H (200MHz, CDCl_3) 2.33 (3H, s, 1-H_3), 4.04 (4H, m, 8- and 10-H_2), 4.37 (1H, m, 9-H), 6.33 (1H, br s, NH), 7.27 (2H, d, 2- and 4-H), 7.73 (2H, d, 5- and 7-H); δ_C (50 MHz, CDCl_3) 21.6 (C-1), 50.8 (C-9), 66.4 (C-8 or -10), 69.7 (C-8 or -10), 127.9 (C-2 and -4), 130.3 (C-5 and -7), 131.7 (C-3), 145.6 (C-6), 159.3 (C-11); ν_max (KBr disc) / cm⁻¹ 3445, 3300, 1770. (Found: C, 48.7; H, 4.86; N, 5.16. Calc. for C_{11}H_{13}NSO_5: C, 48.65; H, 4.63; N, 5.02%).
(S)-3-(4-toluenesulfonyl)-4-(4-toluenesulfonyloxymethyl)oxazolidin-2-one (124) was obtained as a fluffy white solid (1.0 g).

\[ \text{Attempted preparation of (R)-4-(2-hexadecynyl)oxazolidin-2-one} \]

BuLi (1.5M, 3 ml, 4.6 mmol) was added dropwise at -78 °C to a solution of 1-pentadecyne (1.3 ml, 4.6 mmol) in dry THF (30 ml). The solution was allowed to warm to room temperature and stirred for 30 min before cooling back down to -78 °C. CuI (0.45 g, 2.4 mmol) was then added. After the grey suspension was stirred for 2 h at -78 °C, a solution of compound (123) (0.5 g, 1.8 mmol) in dry THF was added dropwise via a dropping funnel. This suspension was stirred at -78 °C for 30 min and then at room temperature overnight. The organic
solvent was removed under reduced pressure. The resulting blue solution was extracted with ethyl acetate (3 x 50 ml), and the combined extracts were dried with magnesium sulfate and concentrated to give an oil. All spectral data were identical to those of starting material.

(R)-3-(4-Toluenesulfonyl-4-(2-hexadecynyl)oxazolidin-2-one (125)

![Chemical structure of (R)-3-(4-Toluenesulfonyl-4-(2-hexadecynyl)oxazolidin-2-one (125)]

BuLi (1.5M, 0.64 ml, 0.96 mmol) was added dropwise at -78 °C to a solution of 1-pentadecyne (0.3 ml, 0.96 mmol) in dry THF (20 ml). The solution was allowed to warm to room temperature and stirred for 30 min before cooling back down to -78 °C. A solution of compound (124) (0.2 g, 0.48 mmol) in dry THF (10 ml) was added to this mixture. The resulting solution was stirred at -78 °C for 2 h and at room temperature overnight. Saturated ammonium chloride solution (20 ml) was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (2 x 30 ml) and the combined extracts were dried with magnesium sulfate and concentrated to give an oil. The oil was purified by column chromatography eluting with hexane-ethyl acetate (2:1) to give the title compound as a yellow oil (50 mg, 23%); Rf 0.4 (hexane-ethyl acetate; 2:1); δH (200MHz, CDCl3) 0.80 (3H, t, 1-H3), 1.18 (22H, m, 2-H2 to 12-H2), 2.20 (4H, m, 13- and 16-H2), 2.37 (3H, s, 26-H3), 2.93 (1H, m, 17-H), 3.91 (1H, dd, 18-H, J 6.1, 1.2 Hz), 4.10 (1H, dd, 18-H, J 4.7, 1.2 Hz), 7.26 (2H, d, 22- and 24-H), 7.75 (2H, d, 21- and 25-H); δC (50 MHz, CDCl3) 14.0 (C-1), 18.6, 22.6, 27.4, 28.7, 28.9, 29.2, 29.3, 29.5, 30.9 (C-2 to C-12), 31.1 (C-13 or -16), 31.9 (C-13 or -16), 36.8 (C-17), 64.1 (C-18), 72.3 (C-14 or -15), 90.9 (C-14 or -15), 127.9 (C-22 and -24), 129.7 (C-21 and -25), 134.4 (C-23), 144.7 (C-20), 152.9 (C-19); vmax (KBr disc) / cm⁻¹ 2927, 2236, 1715; m/z 461 (M⁺, 1.6%), 349 (20), 306 (30.8), 276 (7.6), 235 (100), 210 (20), 155 (100); (found M⁺, 461.2575. Calc. for C26H39NO4S, 461.2599).
A flame-dried four-necked flask fitted with a thermometer and two dropping funnels was charged with (-)-diethyl tartrate (0.46 g, 2 mmol) and dichloromethane (250 ml). After the mixture was cooled to -20 °C, activated powdered 4A molecular sieves (1.5 g), titanium tetraisopropoxide (0.38 g, 1.4 mmol) and tert-butylhydroperoxide (11 ml of 5-6 M solution in decane; ca. 55 mmol) were added sequentially. The mixture was stirred at -20 °C for 1 h and was then treated with a solution of freshly distilled (E)-3-phenylpropen-2-ol (3.5 g, 26 mmol) in dichloromethane (10 ml). After 3 h at -20 °C, the reaction was quenched with a 10% solution of sodium hydroxide saturated with sodium chloride. Before removing the cold bath, ether (20 ml) was added and the stirred mixture was allowed to warm up to 10 °C, whereupon magnesium sulfate (1 g) and Celite (0.1 g) were added. The mixture was then filtered through Celite, washed with diethyl ether and concentrated to give a yellow oil. The oil was purified by column chromatography eluting with hexane-ethyl acetate; (2:1) to give white crystals (2.5 g, 64%); mp 50-51 °C (lit.,92 51-53 °C); Rf 0.3 (hexane-ethyl acetate; 2:1); δH (200MHz, CDCl3) 3.16 (1 H, br s, OH), 3.23 (1 H, m, 2-H), 3.75 (1H, dd, 1-H, J 4.2 and 12.8 Hz), 3.90 (1H, d, 3-H, J 2.2 Hz), 4.00 (1H, dd, 1-H, J 2.2 and 12.8), 7.25 (5H, m, aromatic H); δC (50 MHz, CDCl3) 55.6 (C-2), 61.3 (C-1), 62.6 (C-3), 125.7 (C-5 and -9 or C-6 and -8), 127.6 (C-7), 128.4 (C-5 and -9 or C-6 and -8), 136.7 (C-4); v_max (KBr disc) / cm⁻¹ 3442 (OH), 1256; m/z 150 (M⁺, 7.1%), 132 (28.2, M - H₂O), 119 (28.7), 91 (100, M - CHOCH₂OH); (found M⁺, 150.0683. Calc. for C₉H₁₀O₂: C, 71.72; H, 6.76%).
(2R,3R)-1-[(p-Toluenesulfonyl)oxy]-2,3-epoxy-3-phenylpropane (136)

(using the procedure of Awad et al.100)

Tosyl chlorde (2.8 g, 14.6 mmol) was added to a solution of (2R,3R)-2,3-epoxy-3-phenylpropan-1-ol (135) (2 g, 13.3 mmol) in triethylamine (20 ml). After it was stirred overnight at room temperature, the reaction mixture was diluted with saturated aqueous ammonium chloride solution (30 ml) and extracted with ethyl acetate (3 x 30 ml). The combined extracts were dried with magnesium sulfate, filtered and concentrated to a white solid. Recrystallisation from hexane-ethyl acetate gave white crystals (3.2 g, 80%); mp 68 °C (lit., ref 68-69 °C); Rf 0.8 (hexane-ethyl acetate; 2:1); δH (200MHz, CDCl3) 2.44 (3H, s, 16-CH3), 3.23 (1H, m, 2-H), 3.75 (IH, d, 3-1H, J 1.9 Hz), 4.14 (1H, dd, 1-H, J 5.5 and 11.5 Hz), 4.34 (1H, dd, 1-H, J 3.6 and 11.5 Hz), 7.28 (5H, m, aromatic H, 11- and 15-H), 7.80 (2H, d, 12- and 14-H); δC (50 MHz, CDCl3) 21.6 (C-16), 56.3 (C-2), 59.5 (C-3), 69.5 (C-1), 125.6 - 132.5 (aromatic CII), 135.4 (C-4), 136.1 (C-13), 145.2 (C-10); νmax (KBr disc) / cm⁻¹ 2945, 1356 (SO20), 1175, 960; m/z 304 (M⁺, 2.0%), 155 (47.2, M - SO2OC6H5CH3), 133 (21.9), 91 (100); (found M⁺, 304.0782. Calc. for C16H16SO4, 304.0769).

(2R,3R)-1-(N,N-Diethylamino)-2,3-epoxy-3-phenylpropane (137)

(using the procedure of Rayner et al.91)

A flame dried three-necked flask under nitrogen was charged with (2R,3R)-1-[(p-toluenesulfonyl)oxy]-2,3-epoxy-3-phenylpropane (136) (2 g, 6.6 mmol), potassium iodide (1.2 g, 7.3 mmol) and dry dimethylformamide (20 ml). Diethylamine (0.96 g, 13.2 mmol) was
then added dropwise to the mixture. After it was stirred overnight at room temperature, the mixture was poured into ice water and extracted with ethyl acetate (3 x 20 ml). The combined extracts were washed with saturated sodium bicarbonate solution, dried with magnesium sulfate and concentrated to give an oil. Purification of the oil by flash chromatography eluting with ethyl acetate afforded a yellow oil (0.3 g, 22%); Rf 0.05 (hexane-ethyl acetate; 2:1); δH (200MHz, CDCl3) 0.97 (6H, t, 11- and 13-H3), 2.51 (5H, m, 10- & 12-H2 and 1-H), 2.81 (1H, dd, 1-H, J 4.1 and 11.5 Hz), 3.05 (1H, m, 2-H), 3.52 (1H, d, 3-H, J 1.9 Hz), 7.22 (5H, m, aromatic H); δC (50 MHz, CDCl3) 10.1 (C-11 and -13), 45.9 (C-10 and -12), 53.4 (C-1), 55.4 (C-2), 59.8 (C-3), 123.9 (C-5 and -9 or C-6 and -8), 126.4 (C-7), 126.8 (C-5 and -9 or C-6 and -8), 135.7 (C-4); vmax (film) / cm⁻¹ 2934, 1203; m/z 205 (M⁺, 0.6%), 91 (8.3), 86 (100, M - C₆H₅CHCH), 72 (10.7, M- C₆H₅CHCHCH₂); (found M⁺, 205.1517. Calc. for C₁₃H₁₉NO, 205.1466).

**Attempted preparation of (1R,2S)-2-N,N-diethylamino-1-phenylpropan-1,3-diol**

(using the procedure of Rayner et al.⁹¹)

![diagram]

Trimethylsilyl trifluoromethanesulfonate (0.24 g, 1.1 mmol) was added to a solution of (2R,3R)-1-(N,N-diethylamino)-2,3-epoxy-3-phenylpropane (137) (0.2 g, 0.9 mmol) in dry dichloromethane (10 ml) at -78 ºC under nitrogen. After 30 min, water (1 ml) was added to the solution which was then allowed to warm to room temperature and stirred for 24 h. Ethyl acetate (30 ml) and water (20 ml) were added. The organic layer was separated, washed with saturated sodium bicarbonate solution, dried with magnesium sulfate and concentrated to give an oil. NMR spectroscopic analysis indicated a complex mixture but the epoxide ring was opened.
Freshly distilled ethyl isocyanate (1.68 ml, 21.3 mmol) was added to a solution of (2R,3R)-2,3-epoxy-3-phenylpropan-1-ol (135) (2 g, 13.3 mmol) and triethylamine (3.8 ml, 26.6 mmol) in dry dichloromethane (20 ml). After it was stirred overnight at room temperature, the reaction mixture was diluted with saturated aqueous ammonium chloride solution (20 ml) and extracted with ethyl acetate (3 x 30 ml). The combined extracts were washed with brine, dried with magnesium sulfate, filtered and concentrated. The resulting crude product was purified by column chromatography eluting hexane-ethyl acetate (4:1), to give the title compound (2.3 g, 93%) as a white solid which proved to be > 95% optically pure by chiral column gas chromatography and use of shift reagents (+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol and tris[3-(heptafluoroprolylhydroxymethylene)-(−)-camphorato], europium (III) derivative; mp 46 °C; Rf 0.4 (hexane-ethyl acetate; 4:1); [α]D22 + 54.5 (c 0.29 in CHCl3); δH (200MHz, CDCl3) 1.05 (3H, t, 12-H3), 3.05 (3H, m, 11-H2 and 2-H), 3.70 (1H, d, 3-H, J 1.9 Hz), 4.01 (1H, dd, 1-H, J 5.8 and 12.3 Hz), 4.53 (1H, dd, 1-H, J 3.0 and 12.2 Hz), 7.19 (5H, m, aromatic H); δC (50 MHz, CDCl3) 15.1 (C-12), 35.9 (C-11), 56.2 (C-2), 59.8 (C-3), 64.3 (C-1), 125.7 (C-5 and C-9 or C-6 and -8), 128.4 (C-7), 128.5 (C-5 and -9 or C-6 and -8), 136.3 (C-4), 155.9 (C-10); νmax (KBr disc) / cm⁻¹ 3316 (CONH), 2972, 1689 (OCON); m/z 226 (M⁺, 0.6), 150 (7.5%, M - CONCH₂CH₃), 134 (8.8), 107 (100, M - C₆H₅OH). 91 (66.7); (found M⁺, 221.1050. Calc. for C₁₂H₁₅N₂O₃, 221.1050). (Found: C, 65.2; H, 6.7; N, 6.3. Calc. for C₁₂H₁₅N₂O₃: C, 65.12; H, 6.60; N, 6.33%).
(2S,3R)-3-Ethyl-4-(1-hydroxybenzyl)-2-oxazolidinone (144)  
(using the procedure of Roush et al., 94)

\[
\begin{align*}
\text{OH} & \\
\text{8} & \\
\text{9} & \\
\text{10} & \\
\text{11} & \\
\text{12} & \\
\text{1} & \\
\text{2} & \\
\text{3} & \\
\text{4} & \\
\text{5} & \\
\text{6} & \\
\text{7} & \\
\end{align*}
\]

Sodium hydride (95%, 0.88 g, 36 mmol) was added to a solution of (2R,3R)-1-[(N-ethylaminocarbonyl)oxy]-2,3-epoxyphenylpropane (143) (2 g, 9 mmol) in dry THF (30 ml). After the reaction mixture had been stirred for 6 h, it was quenched by addition of saturated aqueous ammonium chloride solution (30 ml) and extracted with ethyl acetate (3 x 30 ml). The combined extracts were dried with magnesium sulfate and concentrated to give crystalline crude product. Recrystallisation from ethyl acetate-hexane afforded light brown needles (1.6 g, 80%); mp 98.5 °C; Rf 0.1 (hexane-ethyl acetate; 4:1); [α]D \text{22} = 52.5 (c 0.84 in CHCl3); δH (200MHz, CDCl3) 0.98 (3H, t, 12-H3), 2.91 (IH, dq, 11-H2), 3.50 (1H, dq, 11-H), 3.91 (2H, ABX, 1-H2), 4.25 (1H, m, 2-H), 4.85 (1H, m, 3-H) (became d on exchange with D2O), 7.25 (5H, m, aromatic H) δC (50 MHz, CDCl3) 12.4 (C-12), 36.7 (C-11), 59.8 (C-2), 62.6 (C-1), 69.8 (C-3), 125.8 (C-5 and -9 or C-6 and -8), 127.9 (C-7), 128.6 (C-5 and -9 or C-6 and -8), 139.3 (C-4), 158.0 (C-10); νmax (KBr disc) / cm\textsuperscript{-1} 3300, 2964, 1760 (OCON); m/z 114 (100, M - C5HgNO2), 107 (53.7, M - C6H5ClO1H), 77 (59.3). (Found: C, 65.2; H, 6.7; N, 6.3. Calc. for C12H15N03: C, 65.15; H, 6.74; N, 6.27%).

(1R,2S)-2-Ethylamino-1-phenylpropane-1,3-diol (145)  
(using the procedure of Roush et al., 94)

\[
\begin{align*}
\text{OH} & \\
\text{8} & \\
\text{9} & \\
\text{1} & \\
\text{2} & \\
\text{3} & \\
\text{4} & \\
\text{5} & \\
\text{6} & \\
\text{7} & \\
\text{10} & \\
\text{11} & \\
\end{align*}
\]

A solution of (2S,3R)-3-ethyl-4-(1-hydroxybenzyl)-2-oxazolidinone (144) (1 g, 4.5 mmol) and lithium hydroxide (3.3 g, 0.14 mol) in
aqueous ethanol (30%, 20 ml) was heated under reflux for 15 h. After the reaction mixture was cooled to room temperature, it was diluted with brine (30 ml) and extracted with ethyl acetate (3 x 30 ml). The combined extracts were dried with magnesium sulfate, filtered and concentrated to give a white solid. Recrystallisation from ethyl acetate-hexane afforded white needles (0.82 g, 93%); mp 75-76 °C; [α]D22 - 40.9 (c 0.86 in CHCl3); δH (200MHz, CDCl3) 0.97 (3H, t, 11-H3), 2.56 (3H, m, 10-H2 and 2-H), 3.40 (2H, ABX, 1-H2), 3.71 (1H, br s, NH), 4.80 (1H, d, 3-H, J 4.5 Hz), 7.19 (5H, m, aromatic H); δC (50 MHz, CDCl3) 15.0 (C-11), 41.4 (C-10), 59.9 (C-1), 63.0 (C-2), 73.6 (C-3), 125.8 (C-5 and -9 or C-6 and -8), 127.3 (C-7), 128.3 (C-5 and -9 or C-6 and -8), 141.9 (C-4); νmax (KBr disc) / cm⁻¹ 3448, 3286, 3083, 2973; m/z 88 (100, M - C₄H₁₀NO), 77 (13.6, M - C₆H₅CHOH). (Found: C, 67.7; H, 8.7; N, 7.1. Calc. for C₁₁H₁₇N₀₂: C, 67.66; H, 8.85; N, 7.09%).

Methyl tetradecanoate (151)³⁹⁵

Hydrochloride gas was kept bubbling through a solution of tetradecanoic acid (10 g, 0.04 mmol) and methanol (300 ml) until all the solid dissolved. After stirring the solution at room temperature for 5 h, methanol was evaporated off under reduced pressure to afford a white solid. The white solid was redissolved in ethyl acetate (150 ml) and water (100 ml). The organic layer was washed with saturated sodium bicarbonate solution (3 x 100 ml), dried with sodium sulfate, filtered and concentrated to give the title compound as a clear oil (10.5 g, 95 %); δH (200MHz, CDCl3) 0.66 (3H, t, 14-H3), 1.04 - 1.39 (22H, m, 3-H2 to 13-H2), 2.07 (2H, t, 2-H2), 3.43 (3H, s, 15-H3); δC (50 MHz, CDCl3) 13.8 (C-14), 22.5, 24.8, 29.0, 29.1, 29.2, 29.3, 29.4, 31.8, 33.8 (C-3 to C-13), 34.1 (C-2), 50.9 (C-15), 173.7 (C-1); νmax (film) / cm⁻¹ 2924, 2854, 1744; m/z 242 (M+, 0.6%), 154 (10.8), 112 (43.2), 87 (31.3, 74 (100); (found M+, 242.2237. Calc. for C₁₅H₃₀O₂, 242.2245).
Tetradecan-1-ol (152)
(using the procedure of Weinreb et al.\textsuperscript{38})

A solution of methyl tetradecanoate (151) (5 g, 0.019 mmol) in dry THF (20 ml) was added dropwise at 0 °C to a stirred suspension of lithium aluminum hydride (0.72 g, 0.019 mmol) in dry THF (100 ml). After the reaction was stirred at this temperature for 2 h, it was quenched by addition of saturated ammonium chloride solution (20 ml). The mixture was extracted with ethyl acetate (3 x 50 ml) and the combined extracts were dried with magnesium sulfate, filtered and concentrated to afford the title compound as a white solid (3.8 g, 94%); Rf 0.2 (hexane-ethyl acetate; 9:1); \(\delta_H\) (200MHz, CDCl\textsubscript{3}) 0.78 (3H, t, 14-H\textsubscript{3}), 1.18 (2H, br s, 3-H\textsubscript{2} to 13-H\textsubscript{2}), 1.47 (2H, m, 2-H\textsubscript{2}), 2.67 (1H, br s, OH), 3.52 (2H, t, 1-H\textsubscript{2}); \(\delta_C\) (50 MHz, CDCl\textsubscript{3}) 14.0 (C-14), 22.6, 25.7, 29.3, 29.4, 29.6, 31.9 (C-3 to C-13), 32.6 (C-2), 62.6 (C-1); \(v_{\text{max}}\) (KBr) / cm\textsuperscript{-1} 3421, 2924, 2853; \(m/z\) 196 (12.2, M - H\textsubscript{2}O), 168 (13.2), 111 (30.6), 97 (62.6), 55 (100); (found M - H\textsubscript{2}O, 196.2191. Calc. for C\textsubscript{14}H\textsubscript{28}, 196.2191).

Tetradecanal (153)
(using the procedure of Swern et al.\textsuperscript{101})

Dimethylsulfoxide (2.6 ml, 0.028 mol) in dry dichloromethane (20 ml) was added dropwise via dropping funnel to a cooled -50 °C solution of oxalyl chloride (1.48 ml, 15.4 mmol) in anhydrous dichloromethane (150 ml). The reaction was stirred for 10 min before tetradecan-1-ol (152) (3 g, 0.014 mol) in dry dichloromethane (20 ml) was introduced. Stirring at this temperature was continued for 1 h and triethylamine (10 ml, 0.14 mol) was then added. The resulting solution was allowed to warm to room temperature and stirred for a further 30 min. Saturated ammonium chloride solution was added and the whole mixture was extracted with dichloromethane (3 x 80 ml). The combined
extracts were dried with magnesium sulfate, filtered and concentrated to give a yellow oil. The oil was purified by flash chromatography eluting with hexane-ethyl acetate (9:1) to give the title compound as a waxy solid (2.5 g, 84%); δ_H (200MHz, CDCl3) 0.82 (3H, t, 14-H3), 1.30 - 1.52 (22H, m, 3-H2 to 13-H2), 2.30 (2H, dt, 2-H2, J 1.8 and 7.2 Hz), 9.70 (1H, t, 1-H, J 1.8 Hz); δ_C (50 MHz, CDCl3) 13.6 (C-14), 22.6, 24.5, 29.3, 29.4, 29.5, 29.6, 29.7, 31.7, 32.4 (C-3 to C-13), 43.8 (C-2), 202.6 (C-1); ν_max (KBr) / cm⁻¹ 2924, 2864, 1720; m/z 212 (M⁺, 0.5), 178 (10), 84 (40), 74 (100); (found M⁺, 212.2314. Calc. for C₁₄H₂₈O₃, 212.2349).

Methyl (2E,4E)-octadecadienoate (154)

A solution of methyl 4-dimethylphosphono-2-butenoate (1.7 g, 9.4 mmol) in dry THF (20 ml) was slowly added to a stirred, cooled (0 °C) solution of LDA prepared in dry THF (30 ml) from diisopropylamine (1.14 g, 11.3 mmol) and butyllithium (1.6 M, 7.05 ml, 11.3 mmol) for 1 h at 0 °C. The red solution was allowed to reach -40 °C and tetradecanal (153) (2 g, 9.4 mmol) dissolved in dry THF (20 ml) was added dropwise to the reaction mixture. After 2 h the reaction was quenched by addition of water (100 ml) and extracted with ether (3 x 80 ml). The combined extracts were dried with magnesium sulfate, filtered and concentrated to afford an oily solid. The residue was partially purified by column chromatography eluting with hexane-ethyl acetate (20:1) to give the title compound as a clear oil (1.7 g, 62%); δ_H (200MHz, CDCl3) 0.88 (3H, t, 18-H3), 1.30 (22H, m, 7-H2 to 17-H2), 1.7 - 2.3 (2H, m, 6-H2), 3.70 (3H, s, 19-H3), 5.81 - 7.72 (4H, m, 2-, 3-, 4- and 5-H); δ_C (50 MHz, CDCl3) 14.1 (C-18), 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.7, 31.8 (C-7 to C-17), 34.4 (C-6), 51.4 (C-19), 118.5, 128.2, 144.9 (C-2, -3 and -4), 120.0, 126.3, 137.8, 141.7 (cis-isomer), 145.3 (C-5), 167.7 (C-1); ν_max (KBr) / cm⁻¹ 1718, 1650; m/z 294 (M⁺, 10%), 264 (21), 195 (100); (found M⁺, 294.2534. Calc. for C₁₉H₃₄O₂, 294.2558).
A solution of methyl (2E, 4E)-octadecadienoate (154) (1 g, 3.4 mmol) in dry ether (20 ml) was slowly added to a cooled 0 °C stirred suspension of lithium aluminum hydride (0.12 g, 3.4 mmol) in dry ether (30 ml). After it was stirred for 0.5 h at -10 °C, the mixture was quenched by addition of saturated ammonium chloride solution (50 ml) and then extracted with ether (3 x 50 ml). The organic layer was washed with water, dried over magnesium sulfate, filtered and concentrated to give a solid. The solid was partially purified by column chromatography eluting with hexane-ethyl acetate (9:1) to give the title compound (0.72 g, 80%) as a white solid; mp 45 °C (lit., ref 51 °C); δH (200MHz, CDCl3) 0.80 (3H, t, 18-H3), 1.18 (22H, m, 7-H2 to 17-H2), 1.8 - 2.1 (2H, m, 6-H2), 1.90 (1H, br s, OH), 4.05 (2H, d, 1-H2, J 6.0 Hz), 5.51 - 6.40 (4H, m, 2-, 3-, 4- and 5-H); δC (50 MHz, CDCl3) 14.1 (C-18), 22.6, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.7, 31.9 (C-7 to C-17), 32.6 (C-6), 63.4 (C-1), 126.7, 127.5, 131.5, 133.1 (cis-isomer), 129.3, 131.9, 135.7 (C-2, -3, -4 and -5); νmax (KBr) / cm⁻¹ 3318, 2921, 1645.

A flame-dried four-necked flask was fitted with a thermometer and two dropping funnels and charged with (-)-diethyl tartrate (0.46 g, 2 mmol) and dichloromethane (100 ml). After the mixture was cooled to -20 °C, activated powdered molecular sieves (0.5 g), titanium tetraisopropoxide (0.38 g, 1.4 mmol) and tert-butylhydroperoxide (11 ml of 5-6 M solution in decane; ca. 55 mmol) were added sequentially. The mixture was stirred at -20 °C for 1 h and then treated with a solution of (2E,4E)-octadecadien-1-ol (149) (0.2 g, 0.75 mmol) in
dichloromethane (10 ml). After 3 h at -20 °C, the reaction was quenched with a 10% solution of sodium hydroxide saturated with sodium chloride. After ether (20 ml) was added, the cold bath was removed and the stirred mixture was allowed to warm up to 10 °C, whereupon magnesium sulfate (1 g) and Celite (0.1 g) were added. The mixture was then filtered through Celite, washed with diethyl ether and concentrated to give a yellow oil. The oil was purified by column chromatography eluting with hexane-ethyl acetate; (9:1) to give the title compound (10 mg, 5%), starting material (0.1 g) and (2R,3R)-4Z-2,3-epoxy-3-octadec-4-ene-1-ol (156) (20 mg, 10%) (57).

The title compound was isolated as an oily solid; Rf 0.23 (hexane-ethyl acetate; 9:1); δH (200MHz, CDCl3) 0.78 (3H, t, 18-H3), 1.16 - 1.56 (22H, m, 7-H2 to 17-H2), 2.01 (2H, m, 6-H2), 3.03 (1H, m, 2-H), 3.30 (1H, dd, 3-H, J 2.2 and 8.4 Hz), 3.70 (2H, m, 1-H2), 5.11 (1H, ddt, 4-H, J 8.4 and 15.5 Hz), 5.89 (1H, dt, 5-H, J 6.8 and 15.4 Hz); δC (50 MHz, CDCl3) 14.1 (C-18), 22.6, 29.2, 29.4, 29.5, 29.8, 30.9, 31.9 (C-7 to C-17), 32.4 (C-6), 55.9 (C-2), 59.9 (C-3), 61.2 (C-1), 126.2 (C-4), 138.1 (C-5); vmax (KBr disc) / cm⁻¹ 3200, 1645, 1245, 1340; m/z 282 (M⁺, 4.2%), 251 (36.5), 149 (5.4), 83 (100); (found M⁺, 282.2548. Calc. for C₁₈H₃₄NO₂, 282.2559).

(2R,3R)-4Z-2,3-Epoxy-3-octadec-4-ene-1-ol (156) was isolated as a solid.

Rf 0.25 hexane-ethyl acetate, (9:1); δH (200MHz, CDCl3) 0.80 (3H, t, 18-H3), 1.16 - 1.59 922H, m, 7-H2 to 17-H2), 2.20 (2H, m, 6-H2), 3.09 (1H, m, 2-H), 3.60 (2H, m, 1-H2), 3.90 (1H, br dd, 3-H), 5.00 (1H, ddt, 4-H, J 9.0 and 11 Hz), 5.67 (1H, dt, 5-H, J 7.7 and 11 Hz); δC (50 MHz, CDCl3) 14.1 (C-18), 22.6, 22.8, 29.2, 29.4, 29.7, 29.8, 29.9, 31.6, 31.9 (C-7 to C-
17), 31.9 (C-6), 51.3 (C-2), 59.7 (C-3), 61.0(C-1), 125.6 (C-4), 137.7 (C-5); \nu_{\text{max}} \text{ (KBr disc)} / \text{cm}^{-1} 3250, 1640, 1340, 1236; m/z 282 (M+, 0.6%), 269 (32.0), 239 (32.7), 137 (15.4), 109 (58), 95 (100); (found M+, 282.2535. Calc. for C_{18}H_{34}NO_{2}, 282.2559).
References


4) S. L. Whelan, D. M. Parkin and E. Masuyer, *Patterns of Cancer in Five Continents*, IARC Scientific Purifications, Lyon, no. 102, p. 43.


8) Diagram taken from ref. 7, p. 594.

9) Diagram taken from ref. 7, p. 597.


13) Diagram taken from ref. 1, p. 248.

15) Diagram taken from ref. 1, p. 184.


