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The synthesis of mycolic acids

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The Synthesis of Mycolic Acids

A thesis submitted to Bangor University

for the degree of Doctor of Philisophy

by

Chioma Donubari Don-Lawson

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Table of Contents

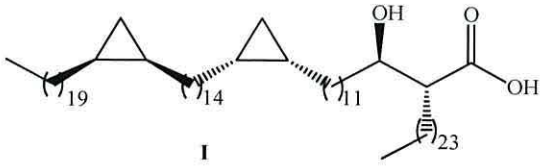
Abbreviation and acronyms

AIDS	Aquired immunodeficiency syndrome
APCs	Antigen-presenting cells
b	Broad
BCG	Bacillus Calmette-Guérin
m-CPBA	*m*-Chloroperbenzoic acid
d	Doublet
DHP	2,3-Dihydro-2*H*-pyran
ECDI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EI	Electron Impact
ELISA	Enzyme-linked immunosorbent assay
GC	Gas Chromatography
HIV	Human immunodeficiency virus
HMPA	Hexamethylphosphorotriamide
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IMS	Industrial Methylated Spirit
IR	Infra-Red
LDA	Lithium *N,N*-diisopropylamide
m	Multiplet
MA	Mycolic acid
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-Of-Flight
MCP	Monocyte Chemotatic Protien
MDR-TB	Multiple Drug Resistant tuberculosis
mol eq.	Molar equivalents
m.p.	Melting Point
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
OPD	*o*-Phenolindiamine
PAS	*p*-Aminosalicyclic acid
PBS	Phosphate Buffered Saline

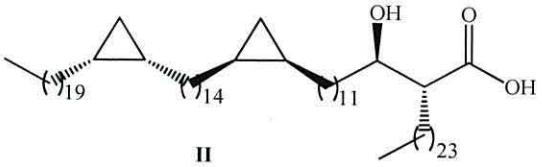
PCC	Pyridinium Chlorochromate
ppm	Parts per million
PPTS	Pyridinium *p*-toluenesulfonate
PTSA	*p*-Toluenesulfonic acid monohydrate
Pv	Pivaloyl
q	Quartet
R_f	Retardation factor
R.T.	Room temperature
s	Singlet
SAM	*S*-Adenosyl-*L*-methoine
sat.	Saturated
t	Triplet
T-cells	T Lymphocites
TB	Tuberculosis
TBAF	Tetra-n-Butylammonium fluoride
TBAI	Tetra-n-Butylammonium iodide
TBDMSCl	*tert*-butyldimethylsilyl chloride
TBDPSCl	*tert*-butyldiphenylsilyl chloridc
TDM	Trehalose dimycolate
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin-Layer Chromatography
TMM	Trehalose monomycolate
TNF	Tumor necrosis factor
p-TsCl	*para*-Toluene sulfonyl chloride
p-TsOH	*para*-Toluene sulfonic acid
UNICEF	United Nations Children's Fund (Previously United Nations International Children's Emergency Fund)
WHO	World health organisation
XDR-TB	Extensively Drug Resistant tuberculosis

Abstract

This project consists of three parts. In the first part the synthesis of two mycolic acid diastereomers (**I** and **II**) was achieved, thereby completing the full range of the *cis*-dicyclopropane α-mycolic acid diastereomers.

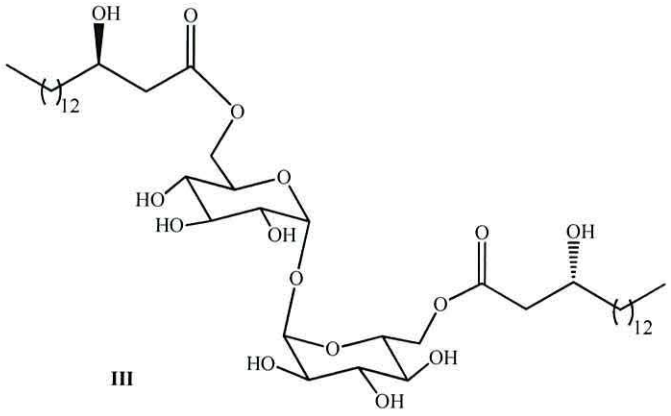


I



II

The second part comprised the development of a new method for the synthesis of the α-alkyl-β-hydroxy fragment of mycolic acids; two novel routes that involved fewer steps were targeted using L-malic as starting material. The first route which was via diethyl malate was achieved in fewer steps without a significant reduction in percentage yield. The second route via dimethyl malate was also completed successfully. Finally, a model cord factor **III** of a β-hydroxy carboxylic acid without an α-alkyl chain was prepared.



III

ELISA assays were carried out using the products as antigens to detect antibodies in serum of patients diagnosed with active tuberculois and higher antibody binding signal was observed for **II** in comparison to **I**. Good antibody recognition was also observed for **III**.

Introduction

Chapter 1

1. Overview

Mycolic acids (MA) are complex high molecular weight hydrophobic lipids or fatty acids found in all pathogenic mycobacteria including *Mycobacterium tuberculosis* (*M.tb*).[1-4] *M.tb* is known to be the causative agent of a chronic and infectious disease in human beings referred to as tuberculosis (TB).[5] These fatty acids are unique to mycobacteria and a few related species and generally contain high carbon numbers of 60 to 90, constituting 70% of the components of the cell envelope.[6,7] They are understood to contribute significantly to the exceptional hydrophobic nature of the mycobacterial cell wall, which prevents effective treatment of TB by resisting the entry of antibiotics and chemotherapeutic drugs.[7] Under these circumstances, mycolic acids are considered to be very relevant in TB research. It is believed that mycolic acids and their derivatives play important roles in the immune system. [8a] A report by Sekenka *et al* affirmed investigations which revealed that mycolic acid moieties are antigens to TB antibodies, and that free mycolic acids have good potential for serodiagnosis of TB and related illness. This was authenticated by the fact that the sera of individuals infected with active TB, were found to contain a high quantity of antibodies that were sensitive to mycolic acids.[9] However, for research purposes, synthetic analogues would be required since it is very difficult to isolate a single compound from the natural mycolic acids, these being a complex mixture of lipids with varying functionality and chain lengths. The chemical pathways involved in the preparation of synthetic mycolic acids, would also help to identify the exact structure, establish the stereochemistry and facilitate the understanding of their biological activities. Also, the antigenic properties of mycolic acids would be better understood and could be targeted for producing antibodies. This may offer more ideal methods in the detection and treatment of tuberculosis and in the treatment of asthma and other immune related diseases.

1.1 Tuberculosis

The genesis of the TB is not clearly understood but it is presumed to have originated in cattle which subsequently transferred it to human beings. It is actually believed that TB can also be contracted by eating meat from cattle infected with *Mycobacterium bovis*.[10-12]

Until the findings of Robert Knoch and Hermann Heinrich in 1882,[13,14] the cause of the disease remained unknown. However, it is believed that TB has been present in human beings since ancient times. One of the earliest specific detections was in the remains of bison from 18,000 years ago.[15] Evidence of deaths as a result of TB has also been discovered in Egyptian mummies over 5,000 years old.[16] In India and China, the presence of TB dates as far back as 3,300 years and 2,300 years respectively.[17,18] There is a lot of archaeological evidence of the occurrence of TB during the middle ages,[19] which spread progressively and globally and became endemic at this time. In the 17th and 18th centuries it reached its peak and was the primary cause of death in the developed world. Then in the 19th and 20th centuries it spread over Asia, Africa and South America and was accountable for one-quarter of all deaths.[20] Today TB is a major cause of preventable deaths by infection and is the leading cause of death in people who are infected with HIV/AIDS. In 2009, WHO reported, 9.4 million new incidences of TB, 14 million prevalent cases and 1.7 million TB deaths (of which 0.4 million were HIV positive). Approximately 80 % of the new cases were in the African region out of which 13 % were HIV positive people. According to the WHO report, funding for TB control was estimated at US$ 5 billion for 2011 and could increase to US$ 3 billion by 2015 in order to reduce the prevalence and death rates by 50% compared to the levels in 1990.[21]

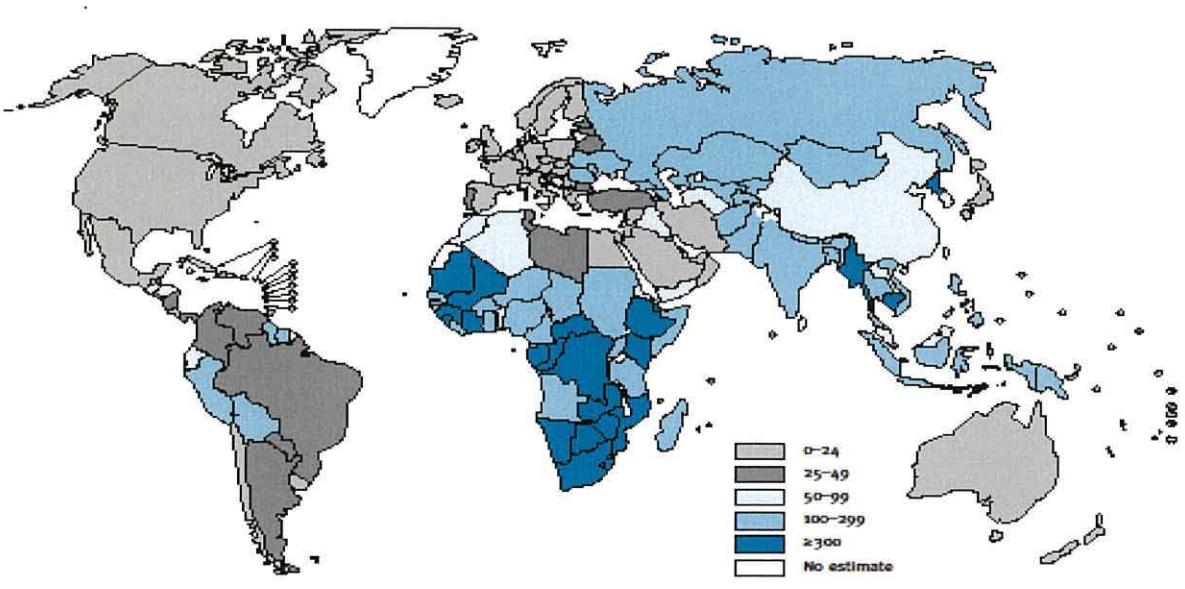


***Figure 1*: Estimated TB incidence rate per 100,000 population in the countries of the world.**[21]

The transmission of tuberculosis is mostly through the air, when people with active infection cough, sneeze, speak or spit in the environment.[5] By this occurrence, infectious aerosolised bacteria droplets of about 0.5 to 5 μm in diameter are expelled into the atmosphere.[22] The airborne bacteria pass into the upper airways and are inhaled into the lungs, causing tubercles or tuberculosis nodules, which are like sores. As a result of this infection, granulomas are formed in the lung tissues which often lead to necrosis or death of tissue cells.[23] Granulomas are immune system cells known as macrophages. They are usually generated when the immune system attempts to seal off infection.[24] The infectious dose of tuberculosis is very low. Essentially, the inhalation of a single bacterium in one droplet can cause an infection. However, the transmission of the disease in general is determined by the amount of infectious droplets expelled by the infected person, ventilation of the environment, the duration of exposure, and the virulence of the *M. tuberculosis* strain.[25,26] People with prolonged, frequent, or intense exposure to TB patients, such as health care workers, are believed to be at high risk of becoming infected. Others considered to be at risk include residents and employees in medically under-served and low-income population areas, where the use of drugs by injection with unsanitary needles is rampant.[27,28] Nevertheless, one major condition that makes people more susceptible to TB infection is HIV/AIDS. Quite often, AIDS patients who are immunologically depressed are co-infected with TB. This is because TB infection progresses when the bacilli can overcome the immune defence system and are able to multiply. Unquestionably, HIV virus is known to reactivate latent TB infection or even expedite its progression in already infected patients.[25,29]

The symptoms of tuberculosis are often latent and not obvious at the early stages but active tuberculosis is generally characterized by a prolonged chronic cough with blood-tinged sputum, chest pain, fever, chills, night sweats, lack of appetite coupled with weight loss and also fatigue.[30,31] Apart from the TB infection of the lungs which is referred to as pulmonary TB, other parts of the body can also be affected. The central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and the skin are all susceptible to non-pulmonary TB and extra-pulmonary TB, which happen to be non-infectious.[32,33] The former implies that the initial infection was not in the lungs and the later means that the source of infection started in the lungs and transferred to other parts of the body.[13]

Diagnosis of TB is carried out definitively by microscopic examination and microbiological culture of body fluids and other clinical samples such as sputum or pus, for the identification of *M. tuberculosis*. Diagnosis can also be made by chest X-rays, scans and tuberculin skin test. The conventional method of diagnosis has been the culture method but it is quite difficult to culture the slow-growing organism in the laboratory. Be that as it may, a complete medical evaluation for TB, which must include a medical history coupled with the aforementioned physical and microbiological examinations, requires not less than four weeks for accurate diagnosis.[34] For any individuals with HIV/AIDS infection, this time frame could be fatal. Studies have shown that late diagnosis of TB infection is estimated to be the cause of up to 85% of deaths of AIDS patients.[35] TB diagnosis may have been improved over time, but there has also been a corresponding increase in cost. This constitutes a fundamental problem to poorer countries, burdened with HIV/AIDS –TB.[36] Diagnostic tests should meet certain operational criteria that are suitable for developing third world countries as high technology tools are unlikely to make much impact. Alternatively, less complicated but quick and relatively cheap diagnostic tools that are non-invasive would be more appropriate.

Prevention and treatment are two aspects that must be harmonized in the battle against TB. Prevention of TB is generally implemented by vaccination with Bacillus Calmette-Guérin (BCG vaccine), which was developed from *M. bovis* in 1921 by Albert Calmette and Camelle Guérin.[37] Presently, it is available in the form of a freeze-dried vaccine. The vaccine is not considered effective epidemiologically, because it cannot prevent adults from contacting TB, but it provides some protection against severe forms of pediatric TB and is therefore clinically accepted for prevention in children.[14,38,39] Isolating patients with active disease and commencing effective anti-tuberculosis therapy is also known to be an effective prevention strategy. Regardless, treatment and cure of the infected person undoubtedly remains a sure means of reducing the spread of the disease. Effective treatment makes the TB non-contagious and prevents further spread. Due to the resilience of the *M.tb* to standard drugs and chemotherapy, the treatment of TB is difficult and involves long courses of multiple antibiotics.[40,41] The first drug proved to be effective against TB was streptomycin which was isolated from *Streptomyces griseus* in the 1940s. This was followed by the discovery of isoniazid in the 1950s. Subsequently, both drugs were used together in a multi-drug therapy.[13,42] Rifampicin, a natural compound extracted from *Streptomyces mediterranei* became available in the 1970s. Apparently, this had

several advantages over previous treatments especially with regards to its time of therapy, which was shorter.[43] These treatments soon became inadequate with the growing problem of antibiotic resistance, coupled with the emergence of Multi–Drug Resistant Tuberculosis (MDR-TB) and Extensively-Drug Resistant Tuberculosis (XDR-TB). The fatality rates recorded for these drug resistant strains were between 40 % and 60 %.[40,41] Recently, the four first-line drugs used in the treatment of TB, have been isoniazid, pyranzinamide, ethambul and rifampicin. A situation where the mycobacterial strain becomes resistant to the two most potent first-line drugs (i.e. isoniazid and rifampicin) leads to MDR-TB.[42,44] This can occur as a result of abuse, when the patient skips doses or fails to complete the treatment course. Administration of the wrong treatment could also cause MDR-TB. Treatment of MDR-TB takes a longer time and is more expensive. Essentially, it necessitates the use of second-line drugs which have more severe side-effects.[45] The second-line drugs include flouroquinolones, aminogycosides, cyclic peptides, cycloserine, thioamides, and PAS. They are usually administered in multi-drug therapy with the two other first-line drugs namely pyranzinamide and ethambul. Again, if the therapy is abused, then XDR-TB is likely to develop. Unfortunately, treatment options for XDR-TB are seriously limited because the strain is resistant to the major first-line drugs earlier mentioned and also resistant to flouroquinolone which is the key second-line drug. This greatly complicates the therapy, and diminishes the chances of cure.[45] Drug resistant strains are most prevalent in underdeveloped countries, where the compliance to the course of treatment is difficult to achieve due to poverty.[46] The treatment of TB as a co-infection with AIDS has been particularly troublesome, because most AIDS patients develop MDR-TB. This is due to the fact that isoniazid and rifampicin, have shown interaction with antiretroviral agents and are malabsorbed in the patients. As a consequence, AIDS patients require extended periods of therapy coupled with highly potent medications which incur more expense.[6,40,41,47]

Statistics have shown high incidence of HIV/AIDS-TB in Sub-Saharan Africa and other developing countries, where early and accurate diagnosis with effective therapy is still not easily accessible.[30,40,48,49] Invariably, the pathogenesis of the HIV/AIDS-TB pandemic presents an urgent challenge to global science.

1.2 Allergic diseases

Natural mycolic mixtures of mycolic acids and a number of single synthetic compounds have been shown to have strong effects on the immune system.[8b] This is discussed in more detail in **Section 1.2.3**. The role of the immune system is to harness its cellular and molecular components to work together to destroy antigens. The functions of the immune system involve the following processes; (i) acting as a barrier, (ii) recognising antigens introduced in the body as distinct from 'self' cells, (iii) eliminating the source of the foreign antigen and (iv) retaining a memory of immunological encounters which is referred to as sensitisation.[50] The accomplishment of these processes enables the entire body to launch a defensive action.

Antigens are foreign substances capable of inducing a specific immune response. They are generally introduced in the form of foreign proteins when the body contracts a bacterial or viral infection. The bacteria or viruses are not themselves antigens but they contain antigens. The antigens can be isolated and used to vaccinate against infection with the organism. On the other hand, an allergen is a type of antigen that is capable of inducing an immune response against foreign substances perceived to be harmful (but which are often normal and harmless), thereby causing allergic reactions. In other words, allergic reactions occur when the immune system goes beyond the normal trend and over reacts by vigorously defending the organism against substances that are not exactly real threats. This form of immune system disorder is known as hypersensitivity.[51]

Hypersensitivity leads to an excessive production of type 2 T-helper cells (Th2 cells) in the body. The T-helper cells or the Th0 cells are a sub-group of lymphocytes or white blood cells that are not capable of destroying any cells themselves but are known to activate and direct other immune cells to fight against any impending harmful entry and to maximize immune response. The Th0 cells would naturally mature into either Th1 or Th2 cells, based on genetic and environmental factors.[52] According to the "hygiene hypothesis," the Th1/Th2 paradigm predicts a negative association. Basically, it proposes that the production of Th1 inhibits Th2 and vice versa, such that they are mutually inhibitory.[53,54] For instance, in cases of tuberculosis and viral infections, Th1 cells are formed, whereas for allergies Th2 cell are formed.[55]

The Th2 cells produced as a result of the earlier mentioned hypersensitivity, stimulate the B cells (another sub-group of the lymphocyte in the bone marrow) to secrete massive amounts of IgE antibodies. There are two categories of antibodies, which are the defence immunoglobulins and other immunoglobulins. Immunoglobulin E (IgE) antibodies are categorized as "other immunoglobins". These protect the body against parasites and are responsible for allergic symptoms.[51] When IgE antibodies are massively generated, they in turn activate basophils and mast cells to produce histamines which cause symptoms observed in allergic conditions, such as sneezing, itchy and watering eyes, coupled with a protective inflammatory response.

Allergic reactions are predictable and can be very rapid. Allergies can affect different organs of the body and can manifest in different forms such as eye allergies, insect allergies, food allergies, skin allergies drug allergies and respiratory allergies. Respiratory allergies can be either nasal or bronchial, as found in asthma.[56] Amongst other conditions, hay fever, asthma and eczema, have been known to be allergic since the 20th century. The causes of allergy could be attributed to host or environmental factors. The most significant host factor is heredity. On the other hand, environmental factors may be due to pollution, allergen level, diet and so on.[57] Diagnosis for allergic conditions can be carried out by means of skin tests for responses to known allergens or by blood analyses to test for the presence and levels of allergen-specific IgE.[58] Common treatments for allergies include the use of anti-histamines, and steroids or reducing exposure to allergens.

1.2.1 Asthma

Asthma can be described as a chronic inflammation of the lungs. It is caused by the hypersensitivity of the bronchial immune system when allergens are inhaled.[59] The inhaled allergens are ingested in the airways through antigen-presenting cells [APCs], which distribute the allergens to the Th0 cells. The Th0 cells become transformed to Th2 and as a result the immune system is activated to produce antibodies against the inhaled allergen. In the process, chemicals are generated that cause the mucus producing cells to grow larger and to produce thicker mucus. As a result, the walls of the airway become thickened or inflamed. The inflammation of the airways obstructs the airflow, thereby increasing the hyper-reactiveness and causing bronchospasm which is accompanied by coughing, breathing difficulties and wheezing. In contrast to chronic pulmonary disease such as TB,

and chronic bronchitis, the inflammation of asthma is reversible and it affects the bronchi and not the alveoli.[51]

Symptoms of chronic asthma are often recognized by a sequence of experiences such as night-time coughing, shortness of breath with exertion, persistent 'throat-clearing' type cough, and tightness in the chest. An increase in the symptoms is usually an indication of the severity of the condition which could deteriorate to acute asthma. This condition can be easily mistaken for other chronic obstructive pulmonary disease such as chronic bronchitis, especially when wheezing is not obvious.[60,61] For some asthma patients, symptoms on exposure to triggers are rare but many others suffer obvious airflow obstruction and the conditions could be acute. Some severe asthma attacks are not responsive to standard treatments and sometimes the patient may turn blue, suffer chest pain or lose consciousness. Such cases are referred to as "status asthmaticus" and could be life-threatening, leading to respiratory arrest and death.[62]

The causes of asthma can be genetic or environmental and that could determine the severity of the condition.[63] Allergens arising from grass pollen, mold spores, and pet epithelial cells, house dust, mite and cockroaches, are all triggers of asthma.[64] In addition, allergens from tissue paper, clothing and furniture fabric, as well as volatile organic compounds such as perfumes and perfumed products (used in the home as cosmetics and for laundry and cleaning) fall under the category of indoor pollutants and are also classified as triggers.[60,64] In urban areas, asthmatic symptoms have been attributed to poor air quality due to various forms of industrial chemical wastes and traffic pollution.[65-68] Chlorinated swimming pools in urban areas are also believed to generate allergenic air pollution that induces asthma.[60] Therefore, it has been observed that hay fever or pollen allergies occur seasonally, and that pet allergies abate when the patient is outside the home. An opinion is also held that, occupational asthma may actually improve when affected persons are not within the work environment. In developed countries there has been an escalation in the number of asthma cases due to more use of antibiotics, caesarian sections, and cleaning products.[69,69,70,70-72] Studies have also shown that compared to children delivered by natural birth, those delivered by caesarean section are more susceptible to asthma as a result of modified bacterial exposure during birth.[69] Basically the use of antibiotics is largely considered to be a remote cause of the asthmatic symptoms, because the antibiotics generally reduce exposure to beneficial bacteria, thereby allowing

other immune system modulators to increase the risk for asthma and allergy.[65,70,71,73] Other environmental causes and triggers of asthma include psychological stress, respiratory infections, cold weather, high altitudes and intensive physical exercise, which expose the airway epithelium to cold dry air.[60,74] In some parts of the world, obesity has also been suggested to aggravate asthma and allergy symptoms.[75-77] Statistical evidence exists of hormonal changes in adolescent girls and pregnant women, being responsible for asthmatic attacks. In contrast, some women experience fewer asthmatic symptoms during pregnancy.[41] Furthermore, smoking during pregnancy has been associated with asthma in children.[65] Regardless of all the aforementioned causes, a family history of atopic disease still remains a very strong risk factor for developing asthma.[72] Genetic studies have shown that there are asthma related genes,[78] but on the other hand, some genetic factors do not qualify as causes except in association with some specific environmental exposures.[9] Presumably, allergies like asthma constitute a 21st century problem which has multiplied over the past two decades to emerge as a predominant First World ailment.[79,80]

Diagnosis of asthma is normally based on clinical examination and the family history of the patient. A patient with a history of asthma, atopic disease (e.g. eczema) and other forms of allergy is considered a potential asthma patient. Measurement of airway function is often used in adult patients but not in children as it is usually not possible to perform such tests. Nevertheless the most substantial observation in asthma diagnosis, especially in children, is the evidence of allergy trigger and wheezing upon exhalation. Diagnosis could also be confirmed when symptoms become worse with exercise, emotional stress, changes in the weather, colds, or exposure to irritants like smoke. Prior to diagnosis of an asthmatic condition, it is ensured that patients are not under treatment with any bronchoconstrictors that cause narrowing of the airways.[81] In addition, tests are carried out to eliminate the occurrence of other lung diseases such as chronic obstructive pulmonary disease and congestive heart failure, which present similar symptoms.

Treatment upon diagnosis can reverse asthmatic airway obstruction but reversibility could also be spontaneous. Treatment and control of asthmatic attacks include relief medication by the use of bronchodilators such as salbutamol inhalers. There are other short-acting, selective $beta_2$-adrenoceptor agonists, namely levalbuterol, terbutaline and bitolterol. For patients experiencing more severe attacks, long-acting $beta_2$-adrenoceptor agonists such as salmeterol, formoterol, bambuterol, and sustained-release oral albuterol are used, as well

as nebulized salbutamol, which provides a larger and continuous prolonged dose. Sometimes inhaled glucocorticoid preventers are used to supplement relief medication as combination inhalers in the treatment of severe attacks.[82] Glucocorticoids are widely used as prevention medication amongst many others. These also come as inhaler devices, some of which are beclomethasone, ciclesonide, budesonide, flunisolide, fluticasone, mometasone, and triamcinolone. Antihistamines are also commonly used as prevention treatment for allergies that accompany asthma. When an asthma attack is unresponsive to routine medication, other emergency treatment options are used in order to prevent respiratory arrest. These include the use of systemic steroids such as prednisolone and hydrocortisone, treatment by intubation as well as the use of mechanical ventilation. Oxygen can either be used by itself or as a mixture with helium (i.e. helox) to alleviate hypoxia. Other intense bronchodilators like intravenous salbutamol and intravenous magnesium sulfate are also employed.[82] In general, treatments for asthma can be broadly classified as relievers, preventers and emergency treatment, but medical treatment recommended to patients would still depend on the severity of their illness and the frequency of their symptoms. However, it is of essence that the doctors and their respective patients work together to establish a proactive management plan for the illness.

Prevention of the development of asthma and the prevention of asthma attacks in already established asthmatic patients are quite different. Allergy desensitization, also known as allergy immunotherapy, has been recommended as a possible means of preventing the development of asthma at the early stages, when allergens are confirmed to be the cause. This therapy has been proven to be more effective with early commencement.[83,84] Quite the opposite, one of the prime and effective ways to prevent episodes in already established cases of asthma is by limiting exposure to identified triggers.

According to research findings, the prevalence of asthma has been on the increase especially in children.[85] It has been observed that migration from rural to urban areas and from third-world countries to the developed world has accounted for the notable increase in prevalence.[86,87] It has also been revealed that asthma is most prevalent in, although not restricted to, affluent countries.[88] Nevertheless, mortality appears to be common in low to middle income countries of the world.[89] The prognosis for asthma is good, especially for children with mild disease, because there is a 50% chance of outgrowing the disease after a decade.[85,90] The extent of permanent lung damage in people with asthma is unclear but for

those who continue to suffer from symptoms, improved treatment methods offer the possibility of living a full life with fewer disabilities. Despite the fact that the mortality rate is relatively low,[47] better control of the condition as a result of enhanced research work could prevent some of these deaths.

1.2.2 Eczema

Eczema is a chronic but non-contagious form of dermatitis or mucosal inflammatory disorder of the skin which occurs as a result of hypersensitivity to irritants, food, or environmental allergens.[91] Inherently, hypersensitivity predisposes the immune system to form excessive IgE antibodies, which induces immune disorders that result in eczema. Eczema is not an allergy itself, rather allergies trigger eczema. It is believed to be hereditary and peculiar to persons with a family history of hay fever and asthma. The clinical forms of eczema include atopic eczema, irritant and allergic contact eczema, discoid eczema, venous eczema and pompholyx.[92,93]

Atopic eczema starts in the face, and spreads to the elbows and knees. The most commonly affected areas of the body are the flexural surfaces, such as the neck and the inner sides of the elbow and knee joints. Other affected areas are the head, scalp, and the buttocks. Regardless, any part of the body can be affected. Eczema is usually characterised by dry, flaky, itchy skin and red patches which develop to small blisters, known as vesicles. This causes weeping and crusting of the skin, and weakens its barrier function. Consequently, the skin becomes prone to further damage by external irritants and surface bacterial infections. Thickening (lichenification) and fissuring of the skin can also occur over time, as a result of scratching and rubbing. In some cases, complications occur due to secondary bacterial infection. Atopic eczema often occurs together with other atopic diseases and these symptoms can sometimes increase or decline over time.[94]

Although eczema is an inherited disease that is primarily aggravated by allergens, it can also be caused by other factors that upset the immune system such as stress or fatigue. Environmental factors such as nutrition or hygiene can also be part of the cause.[95] Common food allergens that can trigger an allergic reaction are found in milk, nuts, cheese, tomatoes, wheat, yeast, soy, and corn.[96] Tobacco smoke, the fur of household pets and dust amongst other irritants also trigger allergic reactions that end up as eczema. In

addition, humidity and sudden temperature swings from extreme coldness to excessive heat are also factors that provoke outbreaks.[97]

Apparently, there is no known cure for atopic eczema but it can be treated by combining prevention and drug therapy. The primary treatment of eczema is prevention and this includes avoiding or minimizing contact with known allergens and triggers, which can be identified by an allergy skin-patch or "scratch" test. There are also tropical treatments to reduce dryness and inflammation of the skin. For example, the dryness caused by eczema can be treated with a dermatologically approved intensive moisturizer, containing ingredients such as sodium hyaluronate which improves skin dryness. Non-soap cleansers, aqueous cream or other soap substitutes could also be used to keep the skin moisturized. In severe cases, topical corticosteroid ointments, creams or injections may be prescribed, but potent steroid creams are usually not applied where the skin is naturally thin and sensitive. Alternatively a less potent steroid such as hydrocortisone is used, and if there is infection on any area of the skin, antibiotics may be applied. Mucosal inflammatory disorders including atopic eczema have become dramatically more common. It has been found to be prevalent in children in industrialized countries such as the United States.[98] Presently, the cure of most types of dermatitis is not known and treating merely with cortisone and immunomodulation, only offers temporary relieve from the disease conditions. Therefore further research in the area of enhancing the immune system remains important.

1.2.3 Known effects of synthetic mycolic acids on immune signalling

Recent studies conducted by Grooten *et al*,[8a,8b] involved the intratracheal treatment of mice using single synthetic mycolic acid isomers with different oxygenation class and different orientation of the *cis* and α-methyl-*trans* proximal cyclopropane. This study revealed a strong dependence of mouse pulmonary inflammation on the meromycolic chemistries of the mycolic acids. The α-mycolic acid tested was inert, whereas the oxygenated mycolic acids with the *cis*-cyclopropane stereochemistry stimulated a strong inflammatory response in the case of the methoxy mycolic acid and a mild response for the keto-mycolic acid. However, in the *trans*-cyclopropane oreintation, the methoxy-mycolic acid did not show a significant inflammatory activity but the keto-mycolic acid showed an anti-inflammatory response which countered the *cis*-methoxy-mycolic acid induced airway inflammation.[8b] Based on the results of this work, the difference in the immune activities of the different

mycolic acids can be linked to the oxygenation class as well as the *cis* and the α-methyl-*trans* cyclopropane orientation and this reveals ways in which *M. tuberculosis* may affect the host immune response during infection. As such, synthetic mycolic acids constitute tools for studies on how *M.tb* can regulate inflammation and hosts immune response which can be useful in biomedical applications.

1.3 *Mycobacterium tuberculosis*

The variation in the types of bacteria depends on a number of factors such as colony shapes, colours, virulence, and temperature of growth. There are more than fifty different types of bacteria including *M. tuberculosis*, which is the source of TB in human beings. *M. bovis* is found in cattle and can also be a source of TB in humans.[12] Another pathogenic mycobacterium is *Mycobacterium avium* which causes diseases that are reminiscent of tuberculosis and can be found in water, soil environments and also in animals.[99] *Mycobacterium ulcerans* occurs mainly in water environments, and it infects fish and causes human skin lesions.[100] One other important bacterium is *Mycobacterium leprae* which is classified as non-tuberculous, but still causes the chronic infectious disease referred to as leprosy.[101]

Humans are the main hosts of *M. tuberculosis*, which is primarily a pathogen of the mammalian respiratory system. It can survive several years outside the human body but does not grow in soil or water. However, fowl and cattle are resistant to the bacterium. The size of the organism is between 2 and 4 micrometres. They are acid fast and as such resistant to acids (3% HCl) and alcohol (95%). Consequently they cannot be classified as gram-positive or gram-negative.[102] They are highly aerobic and require oxygen for growth, but the growth rate is slower than that of most other bacteria.[103] *M.tb* can survive for long periods in a dry environment without any effect on its growth. In fact, it is possible to add dyes or antibacterial substances into the growth medium and not interrupt growth. *M.tb* has proven to be more resistant to chemical reagents than most bacteria and this could be attributed to the definitive hydrophobic pattern of the cell wall.[104]

M. tuberculosis is characterized by a distinctive cell wall structure which consists of a membrane that is particularly impermeable to an array of standard drugs. The cell envelope as a whole is made up of the plasma membrane and a cell wall. In the cell wall, a

hydrophobic lipid layer is sandwiched between the cell membrane and an inner layer of long chain polymers (of sugars and amino acids) called peptidoglycan. Large amounts of fatty acids referred to as mycolic acid which are covalently bonded to arabinogalactan, are found in the lipid layer, together with many other components including lipoarabinomannan (LAM). The mycolic acids are bound as esters to the terminal penta-arabinose of arabinogalactan polysaccharides (consisting of arabinose and galactose). Also, the hydrocarbon chains of the bound mycolic acid molecules are aligned parallel to each other and perpendicularly to the surface of the cell wall. The arabinogalactan polysaccharides are in turn bound to the peptidoglycan polysaccharides thereby forming a peptidoglycan-arabinogalatan-mycolic acid skeleton which gives shape and rigidity to the cell wall.[105,106] This results in the interaction of the external lipid layer with free lipids to form a lipid bilayer (**Figure 2**).[1]

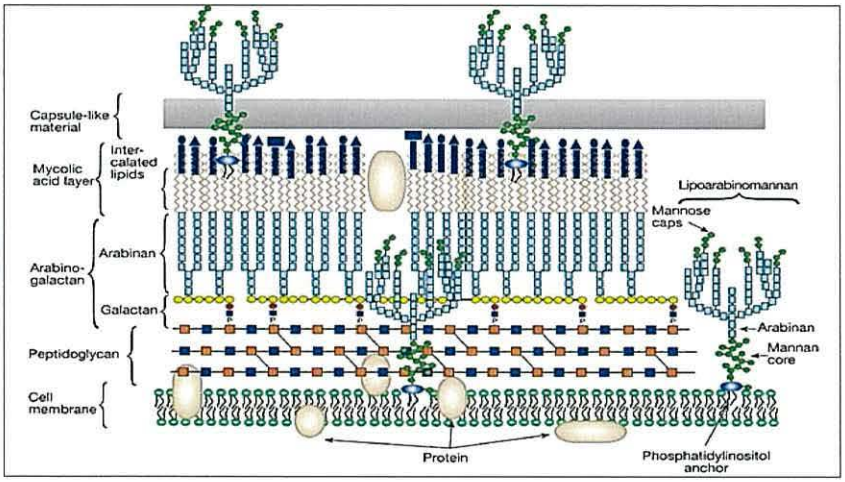


***Figure 2:* Schematic diagram of Mycobacterial cell wall.**[107]

The bilayer constitutes a permeability barrier such that fluidity increases towards the outer surface. This is because the outer surface of the lipid bilayer consists of the negative ends of the free lipids which are relatively more permeable in comparison to the inner layer. In any case, the inner layer has an exceptional thickness and ability to maintain a rigid cell shape with very low fluidity and permeability.[1,108,109] Evidence of these two distinct domains in the cell envelope was revealed in studies that determined the location of lipid domains in mycobacteria using fluorescent dyes of varying lipophilicity.[110] The cell envelope of *M.tb* is believed to contain a mixture of over 500 different mycolic acids with a varying combination of functional group type and chain length (**Figure 3**).[111a] Evidently, mycolic acids have a role in the permeability of the outer cell envelope of the bacteria, but

the stacking and arrangement of the long hydrocarbon chains of the acids within the cell wall is complicated. Nonetheless, it is known that these hydrocarbon chains are aligned parallel to each other and perpendicular to the surface of the cell wall and contain functional groups that vary in type, stereochemistry and spacing.[1,111b]

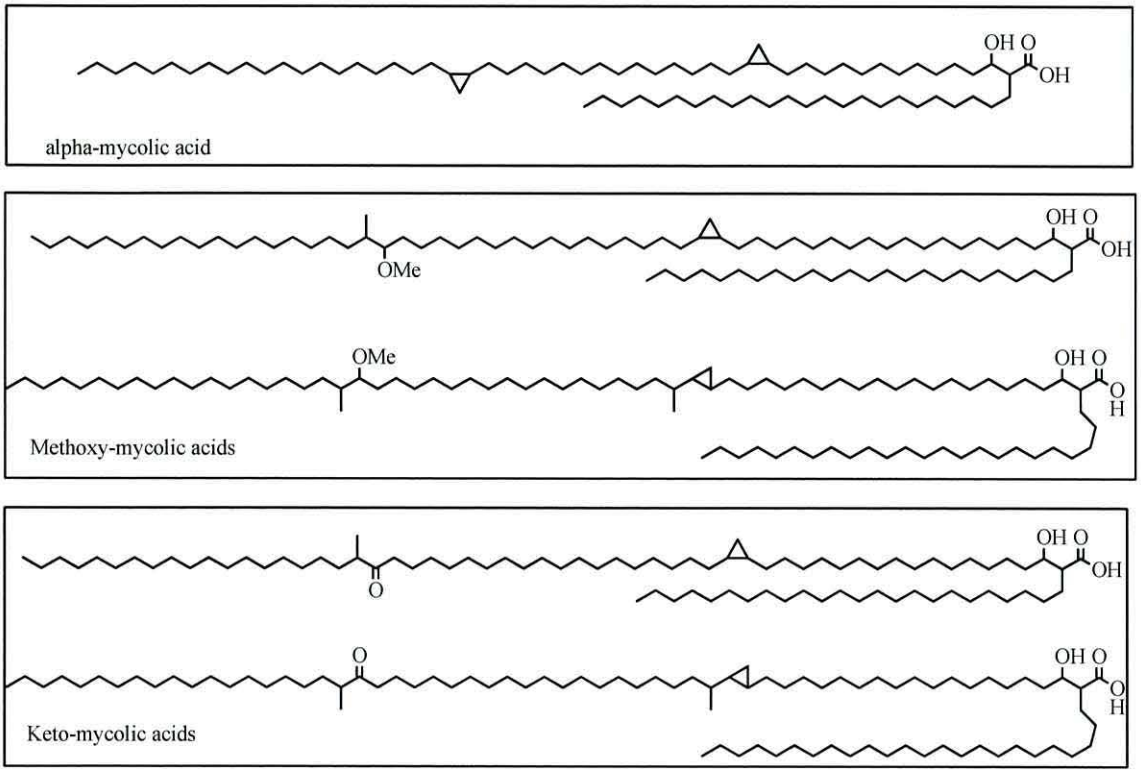


***Figure 3*: Different classes of mycolic acids in Mycobacterial cell wall**

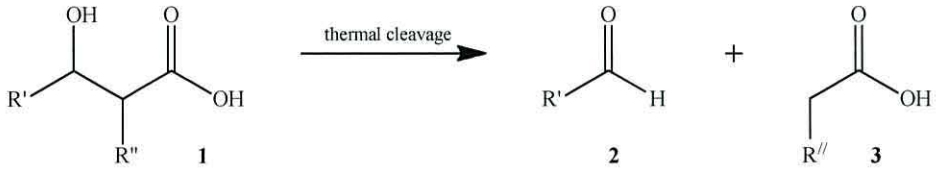
Each Mycobacterium has its own unique and complex mixture of the different classes of the mycolic acids which are based on different functional groups. Within each class there also exists a combination of homologues of different carbon chain lengths.[111b] The separation of a single mycolic acid is extremely difficult as a result of the varying chain lengths. Regardless, more detailed studies on pure individual molecular species of mycolic acids is imperative so as to obtain precise information about their conformational arrangement, which may have a bearing on their behaviour in the cell wall.[112a,112b]

1.4 Mycolic acids

1.4.1 Structure and Classification

Mycolic acids were first characterised by Anderson *et al.* as "hydroxyl acids of very high molecular weight" which contained complex mixtures with different compositions. They

proposed the name "Mycolic" acid and reported the presence of one carboxylic group, one hydroxyl group and a methoxy group. It was also reported that pyrolysis of the mycolic acid at 300 °C under vacuum gave hexacosanoic acid.[4,7,113] In addition, other mycobacterial strains such as *M.bovins* and *M.avium* were found to contain mycolic acids, which showed similar characteristics with respect to the presence of carboxylic **1**, hydroxyl and methoxy groups and also with respect to pyrolysis products.[114-116] However, pyrolysis at 300°C released a series of aldehydes **2** alongside the hexacosanoic acid **3**. This helped to determine the basic structure of mycolic acid as a β–hydroxyl fatty acids with a long alkyl chain in the α-position-[$R^{/}CH(OH)CH(CO_2H)R^{//}$][117] **(Scheme 1).**



***Scheme 1:* Pyrolysis of a mycolic acid**

Ultimately, two parts have been distinguished in the generalized structure of mycolic acids **4** in **Figure 4**. The fatty acid alpha branch is referred to as the mycolic motif [–$CH(OH)CH(CO_2H)R^{//}$] and the aldehyde branch on cleavage (**Scheme 1**) is referred to as the meromycolate moiety [$R^{/}$].

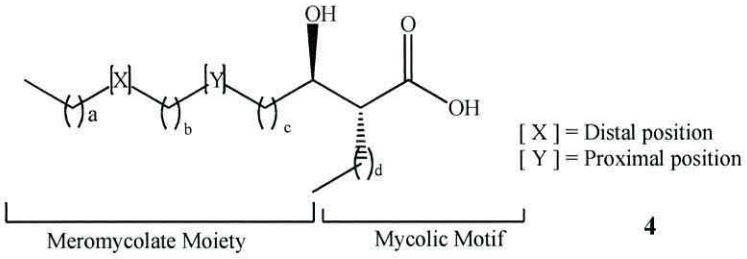
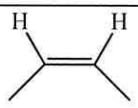
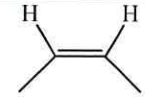
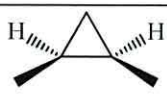
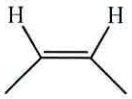
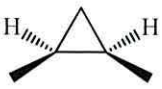
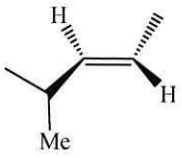
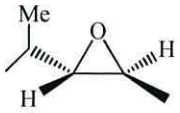
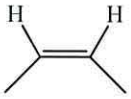
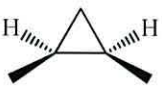
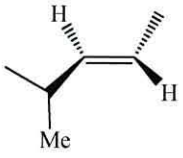
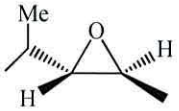
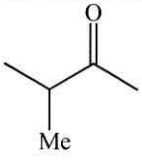
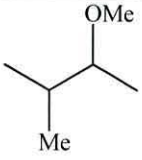


***Figure 4:* A generalized structure of mycolic acids.**

The mycolic motif is essentially the same for all mycolic acids with a common β-hydroxyl group and an α-alkyl chain [$R^{//}$] which might vary slightly in length for different compounds. On the other hand, the meromycolate [$R^{/}$] consists of homologues of different carbon chain lengths as well as the different functional groups, such as methoxy, keto, epoxy and cyclopropane. The different functional groups have varied roles and chemical properties in the acid and this forms the criterion for the classification of mycolic acids. As

indicated on **Table I** below, the difference in the classes of mycolates is primarily in the functional groups on the distal positions. For example, whereas the epoxy-mycolic acid contains an epoxy functional at the distal position, the keto- and the methoxy-mycolic acids contain keto and methoxy functional groups respectively at their own distal positions. Those on the proximal positions are similar and are mostly alkenes or cyclopropanes with variation in a few cases.

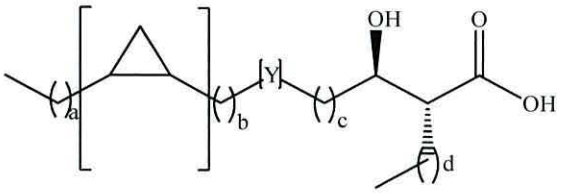
Table I: Examples of functional groups of various mycolates.

mycolate type	X	Y
α′	CH_2CH_2 –	
α	 	  
Epoxy		   
Keto		
Methoxy		

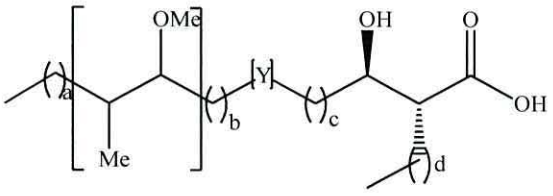
Analytical techniques such as chromatography, melting point, optical rotation and infrared spectroscopy have contributed towards the development of the structural understanding of mycolic acids. For instance, thin layer chromatography was used to ascertain the homogeneity and characteristic patterns of mycobacterial mycolates with respect to other components.[118] Other studies also proposed the existence of a remote long alkyl branch in addition to the alkyl branch on the α-position of the fatty acid.[119] Accurate molecular weights of the mycolic acids were determined by EI-MS,[120,121] but this technique was not

very adequate as it could not establish the presence of the cyclopropane in mycolates. Nonetheless, the problem with the technique was resolved by cleavage which generated suitable derivatives for mass spectroscopy.[108,122] The cyclopropane rings were also detected by NMR spectroscopy, which clarified the *cis* and *trans*-stereochemistry of the rings.[123] However, most of the structural interpretations of the mycolic acid have been based on the combination of NMR and EI-MS techniques.[108,122] In additional studies conducted by Watanabe *et al*, individual mycolic acids of selected mycobacterial species were separated and characterised and the positions of their respective functional groups were also located.[111b,124]

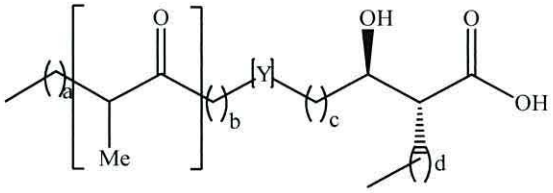
In the case of *M. tuberculosis*, three major classes of mycolic acids have been identified. The ones containing *cis* or *trans*–cyclopropanes and the ones containing a carbon to carbon double bond at the distal position, are classified as α–mycolic acids **5**. The α-mycolates are known to have a higher abundance in *M.tb* than any other subclass and the presence of the cyclopropane ring is a vital characteristic with regards to the pathogenesis of the bacterium.[111b,125] There are also those containing a methoxy group with a double bond or *cis*-cyclopropane and these are known as methoxymycolic acids **6**, and then those containing an alpha–methyl branched ketone which are classified as ketomycolic acids **7**.[126] "Y" in the structures is as represented on **Table 1 (Figure 5).**



alpha-mycolic acid (5)



methoxy-mycolic acid (6)



keto- mycolic acid (7)

***Figure 5*: Generalized structures of the major classes of mycolic acids in M.tb.**

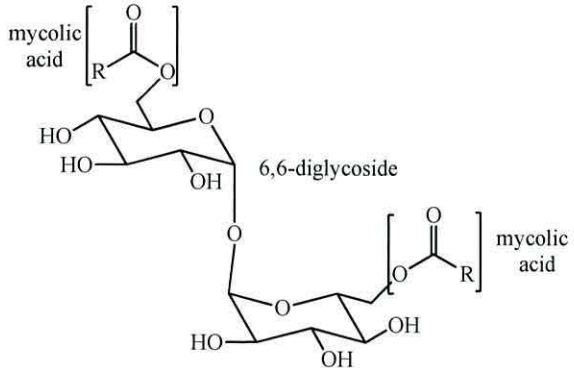
The saturated long chain structure of the alpha-branch, as well as the exceptional length of mycolic acid chain should strongly favour the parallel packing of hydrocarbon chains in the mycobacterial cell wall. However the keto, alkoxy, *cis*-double bond and *cis*- and *trans*–cyclopropane structures are expected to modulate the tight packing by producing twists in the chain. The groups that would disrupt the packing are located at the distal position from the carboxyl end while the *cis*-structures are located at the proximal position, yet the tight packing is not disrupted.[127] Studies have also shown that the presence of the distal cyclopropane ring on α–mycolic acids confers resistance against oxidative stress and stabilizes the bacteria in oxidizing environment.[127-130] The bacteria have been found to manipulate the ratio of keto and methoxy mycolic acids for better adaptation to their environment. The ketomycolic acids also encourage the growth of the bacterial cell. Their formation in the cell increases during the growth in macrophages and at low oxygen concentrations.[125] Methoxymycolic acids in *M. tuberculosis* increase in the stationary phase cells and in addition, the oxygenated mycolates in *M. tuberculosis* affect the growth rate of macrophages.[125] A case study has shown that the absence of keto and methoxymycolates reduces the activities of *M. tuberculosis* in mice.[131] As such keto and methoxy mycolic acids also have critical roles in the virulence of mycobacteria.[125,131,132]

The length of the hydrocarbon chain of the mycolic acid and the functional groups on the meromycolic unit, are believed to contribute to the high transition temperature of the mycobacterial cell wall. That of *M.tuberculosis* was found to be dramatically high, suggesting low fluidity at growth temperature.[133] The *trans*–double bond functional groups are known to promote the cell wall packing and increase the melting point of the cell wall which leads to high transition temperatures. However, *M. tuberculosis* does contain mycolates with *trans* double bond groups at low levels, but its high transition temperature has been attributed to the presence of the proximal cyclopropane groups on the mycolates. These instances suggest that an appropriate composition and structure of mycolates are important factors for growth, hence alteration in the proportion as well as the structure of these acids in the cell wall could produce significant changes in the fluidity, permeability and consequently toxicity of the bacteria.[133,134] If these effects of functional groups on the fluidity and permeability of the cell wall are understood, it may in turn give an insight into possible anti-mycobacterial agents.

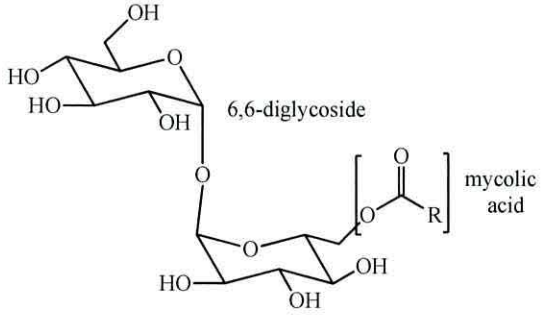
Some other mycobacterial species have been documented to contain various types of mycolic acids. For instance, *Mycobacterium fortuitum* and *Mycobacterium smegmatis* were found to produce epoxy-mycolates. *M. avium* contain wax ester mycolate. Also *Mycobacterium chelonae* was found to produce α'-mycolates with a single *cis*-double bond and a reduced molecular size.[2]

1.5 Cord factors

Although mycolic acids are unique to mycobacteria and are major components of the cell wall, *M. tuberculosis* has a variety of lipids within it cell wall,[43] some of which are extractable toxic glycolipids such as "cord factors." The cord factors are essentially sugar esters of non-wall-bound mycolic acids which derive their name from the incorrect belief that they are responsible for the cord-like appearance of the mycobacterial cell surfaces.[135] Cord factors are trehalose derivatives which consist of two classes, namely trehalose dimycolate (TDM) **8** and trehalose monomycolates (TMM) **9**. Each of these derivatives consists of a non-reducing, disaccharide sugar core which is trehalose (6,6-diglycoside). The trehalose contains two glucose molecules linked by an α,α-1,1-glycosidic linkage. In TDM, the trehalose is esterified with two mycolic acids on the 6,6' positions corresponding to both primary alcohol groups. In the TMM, the trehalose is esterified with one mycolic acid on the 6-position corresponding to one primary alcohol group (**Figure 6**).



Trehalose dimycolate (TDM) 8



Trehalose monomycolate (TDM) 9

***Figure 6*: Generalized structures of extractable mycolates**

It is understood that the axial linkage of the trehalose helps to prevent the damage that can be caused by heat, oxidation or stress and they are regarded as preservers and stabilizers.[136-138] They also function as mycolic acid donors for the formation of the

mycolic-arabinogalactan-peptidoglycan complex and they bind cells of *M. tuberculosis* together, making them more resistant to the immune system. Consequently, the virulence of the bacterium is linked to the amount of cord factor on its surface.[139]

The toxicity of cord factors has been shown by injecting them into mice and this has led to inflammation and inhibition of respiration.[135] On the contrary, cord factors are potent adjuvants which act as immunomodulators and boost the immune system and antibodies.[140] Trehalose dimycolates (TDM) has been suggested for use in treating moderate to severe eczema. The TDM works by blocking the immune system cells from creating chemical messages. It binds to the T cell receptors and inhibits the IgE sensitivity and the production of cytokine production, thereby slowing down or stopping the skin disease.[141] It has not only been found to show anti-parasitic and anti-bacterial properties, but has also shown anti-tumour properties and positive effects against cancers.[142-144] For instance, TDM proved to be active against bacterial infection and caused a regression of tumour growth in animals.[143,145,146] Other known uses of cord factor (TDM) are to increase resistance to TB and influenza and also in Th1 vaccine, to combat bacterial infection.[147] Additional biomedical applications include its use for wound healing and hair growth.[148]

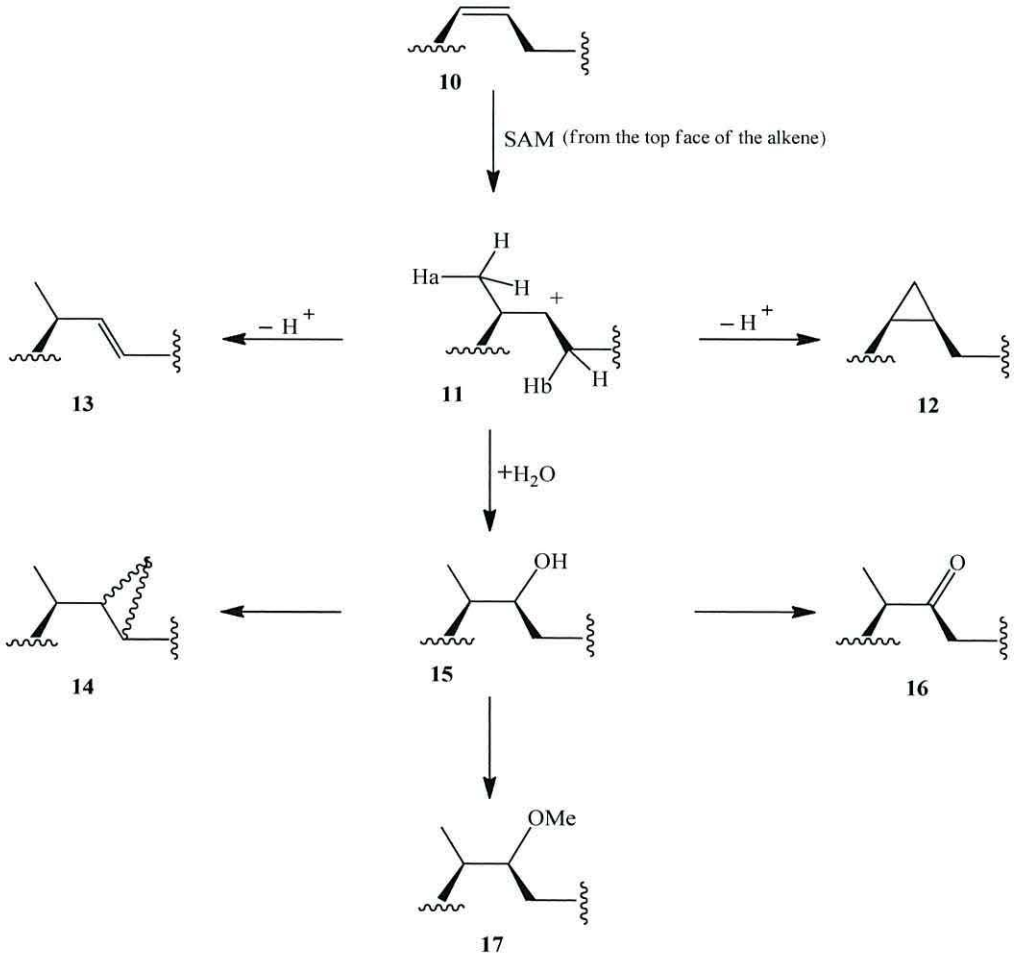
1.6. Synthesis and Biosynthesis of Mycolic acids and their derivatives

1.6.1 Mycolic acids

1.6.1.1 Biosynthesis

The biosynthesis of mycolic acids can be divided into four different steps which involve: (a) synthesis of the long chain saturated fatty acids which provide the main carbon skeleton; (b) synthesis of the carbon chain backbone of the meromycolate unit; (c) introduction of different functional groups in the meromycolate chain and (d) the final condensation to link the meromycolate unit to the mycolic motif. Several theories have been proposed for different mechanisms for the processes of cyclopropanation, oxygenation and methylation in the biosynthesis of mycolic acids. Studies have been conducted in which labelled methionine was incorporated in the growth of mycobacteria and it has been found that the methyl group of methionine can be directly integrated into mycolic acids. It has also been found that the linkage of the methylenes of the cyclopropane rings, the carbon of the methoxy and the methyl branches adjacent to *trans*-olefins, methoxy and keto moieties are all derived from methionine.[149-152] Investigations of

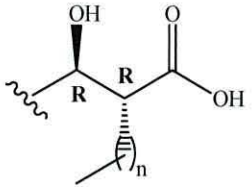
the mechanism for the biosynthesis of *cis*-cyclopropane, α-methyl-*trans*-cyclopropane and the α-methyl-β-alkoxy units suggest that when *Z*–alkene **10** is reacted with *S*–adenosyl–*L*–methionine, a methyl group is introduced which breaks the C – C double bond to form a carbocation **11**, as a common intermediate. This could be deprotonated to yield a *cis*–cyclopropane ring **12**, or an α–methyl–*trans* cyclopropane **14** unit via an α–methyl–*trans* olefin unit **13**. Deprotonation of the carbocation intermediate **11** occurs at two different positions. Deprotonation of H_a yields the *cis*-cyclopropane **12** whereas deprotonation of H_b yields the *trans*-olefin **13**. The carbocation intermediate could also undergo hydration to form the hydroxyl group **15** and then the keto– and methoxy–mycolate units **16** and **17** respectively. This shows all the sub-units formed as having the same absolute stereochemistry at the carbon bearing the methyl group and at the C-1 position of the *cis*-cyclopropane **15 (Scheme 2)**.[132,153]



***Scheme 2*: The mechanism for the formation of functional groups**

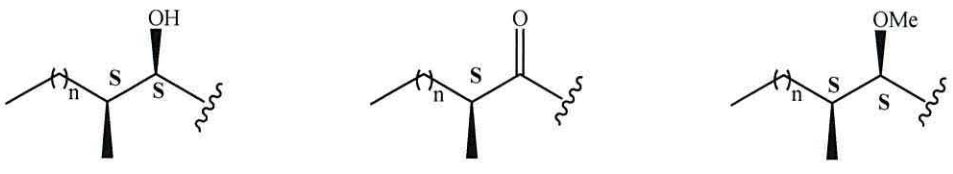
1.6.1.2 Synthesis and stereochemistry

In order to determine the actual structure and stereochemistry of naturally occurring mycolic acids and to identify whether the specific structure has any effect on their biological properties, the preparation of the synthetic analogues is of essence. Synthesis of single molecules of mycolic acids and determination of their properties would require understanding of the stereochemistry at each chiral position of the functional groups in the mycolic molecules. Much work is still needed with regards to understanding the stereochemistry of these functional groups but the α– and β-positions relative to the carboxylic group **18** have been found to possess the absolute *R,R*-stereochemistry for all mycolic acids, irrespective of the other functional groups (**Figure 7**).[149,154,155] The relative configuration between the α-alkyl chain and the β-hydroxyl group has been proven to affect the film molecular packing in the cell wall of mycobacteria, as a result of hydrogen bond formation between the hydroxyl group and the carboxylic group; this is believed to have a stabilizing effect on the alignment between two long mycolic acid chains.[112a, 112b,156,157] The configuration at these two chiral centres is also believed to have an effect on the recognition of T cells and immune response.[158] However, determination of the stereochemistry of the chiral centres in the meromycolate unit is quite difficult and as such not much has been reported on it. Studies based on degradation and molecular rotation have shown that oxygenated mycolic acids such as the methoxy-MAs **17**, keto-MAs **16** and hydroxyl-MAs **15** as derived from **Scheme 2** possess the *S,S*-configuration at the distal group of their meromycolate chains (**Figure 8**).[154,159] Little is known about the absolute stereochemistry of the *cis*-cyclopropane units in the α-mycolic acids.



18

***Figure 7*: R,R stereochemistry of mycolic acids at the α-alkyl-β-hydroxyl acid unit.**



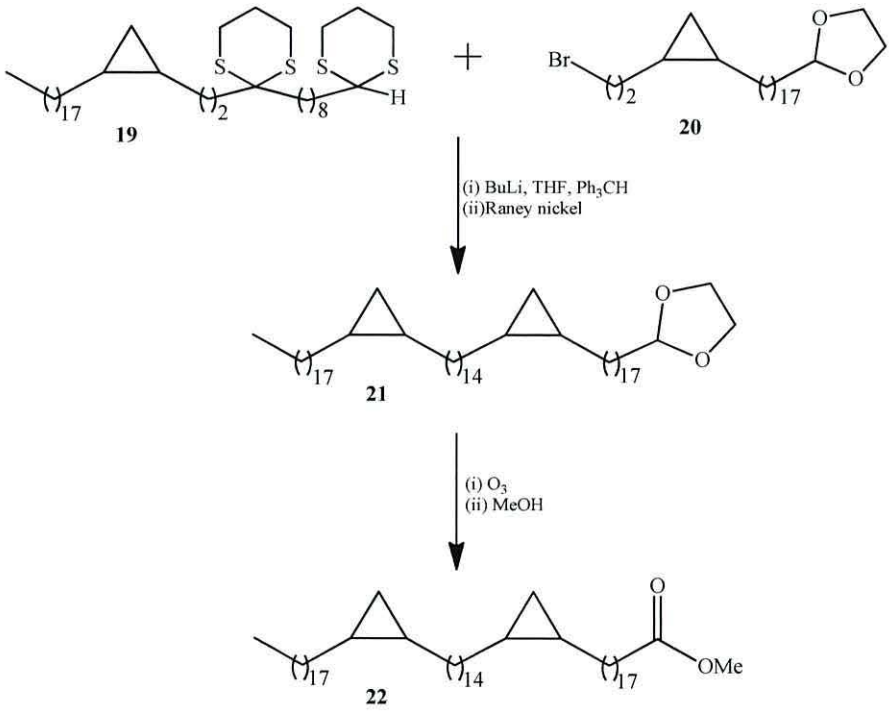
15 **16** **17**

***Figure 8*: Examples of S,S stereochemistries of functional groups.**

Synthesis of a full mycolic acid involves linking the different functional units (intermediates) in stages. This often involves repetitive cycles of alkylation and chain extension which is essentially a sequence of reactions such as protection, deprotection, oxidation, olefination, and hydrogenation and so on. As in the biosynthesis, these could be divided into stages as follows: (i) the synthesis of the long chain carboxylic acid with required substituents on the α and β positons, (ii) the synthesis of the meromycolate unit with different functional group and then (iii) the coupling reaction that joins the meromycolates to the mycolic motif.

1.6.1.2a Meromycolates

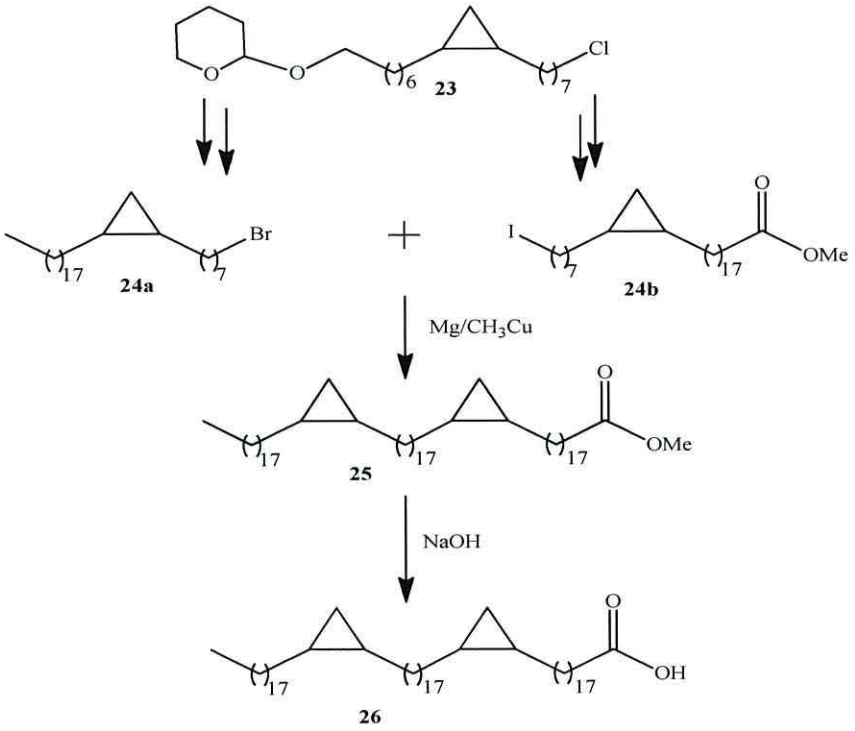
In the first synthesis of meromycolic acids conducted by Gensler *et al*,[160] a mixture of stereoisomers of the meromycolate **22** were formed in a number of steps via **21**, by coupling two intermediates **19** and **20** (**Scheme 3**).



***Scheme 3*: Synthesis of the first meromycolic acid**[160]

Using a shorter approach,[161] Gensler *et al* went further to prepare the second meromycolic acid **26**, by methods which combined different fragments. 1-Hydroxy-8-nonyne was converted into **23** in several steps and this gave two intermediates **24a** and **24b**. The

intermediates were coupled by Grignard reaction to give **25** which was followed by saponification. The product **26** obtained was as shown below **(Scheme 4)**.

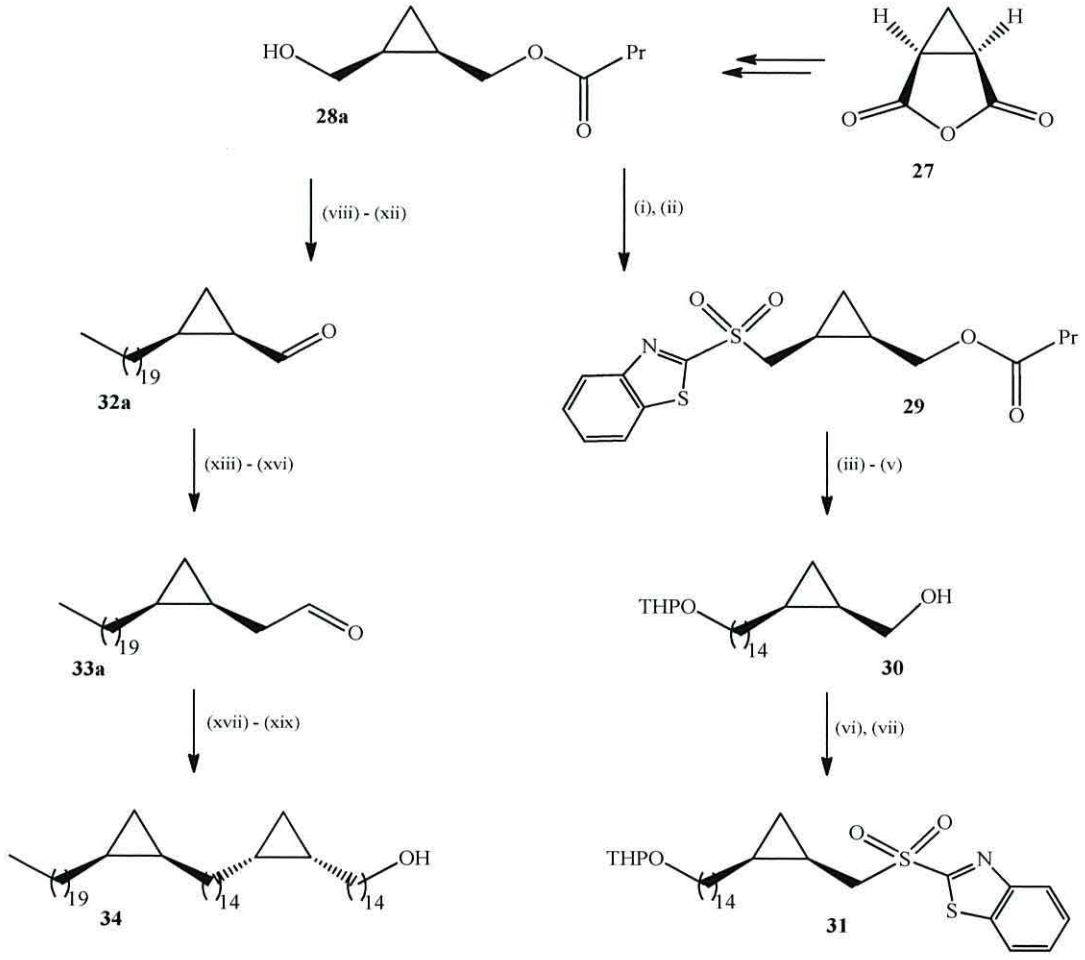


***Scheme 4*: The second synthesis of a meromycolic acid**[161]

This method was an improvement over the first approach, but gave a very poor yield. Several other side products were reported and only a small percentage of the crude product mixture was obtained as the desired product. Additionally, the absolute stereochemistry of the chiral centres of the cyclopropane rings was not controlled in Gensler's method.

The first synthesis of a single enantiomer of a meromycolic unit **34** was conducted by Al Dulayymi *et al.*[162] As opposed to the Gensler methods, this involved an approach in which the chiral centres of the cyclopropane rings were controlled. According to the literature,[162] the anhydride of cyclopropane *cis*-1,2-dicarboxylic acid **27** via a combination of steps was converted into a half ester **28a**, from which two cyclopropane intermediates **31** and **33a** were prepared. To prepare **31**, the half ester **28a** was converted into a sufone **29**, which was oxidized and then homologated in a Julia- Kocienski reaction and subsequent hydrolysis and hydrogenation to give **30**. As before, compound **30** was further converted into its corresponding sulfone **31**. Again, compound **28a** was homologated on both sides to obtain **33a**. In order to achieve this, two Wittig reactions were carried out in sequence on

either sides of **28a** which led to **32a** and **33a** respectively. In the final sequence, the two cyclopropane units were coupled in a modified Julia–Kocienski reaction without the loss of stereochemistry to give an enantiomerically pure alcohol **34** (**Scheme 5**).



***Scheme 5*: *The synthesis of a single enantiomer of a meromycolic unit*[162]**

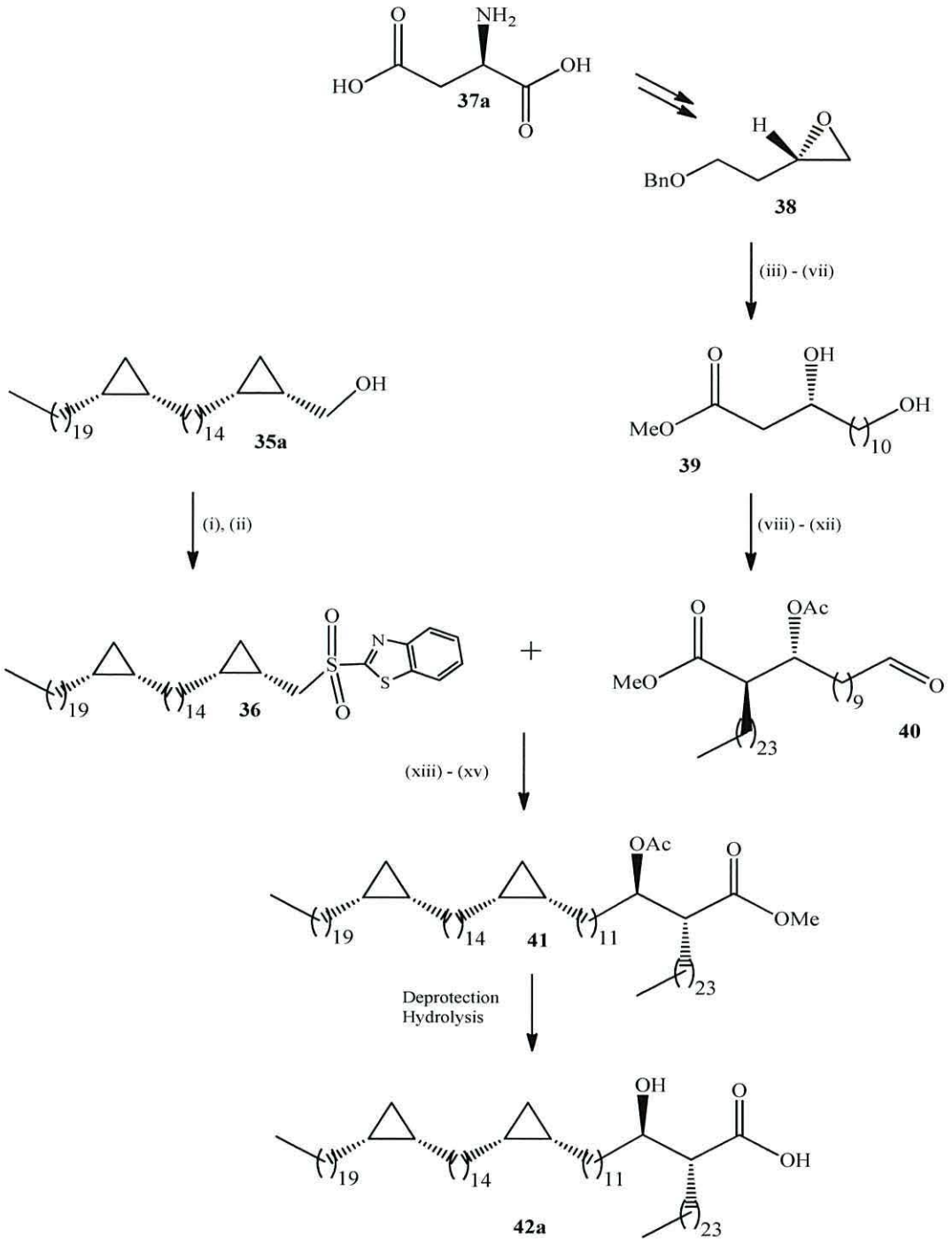
Reagents: (i) Benzthiazole, DEAD, PPh_3 (ii) MCPBA (iii) $THPO(CH_2)_{12}/NaN(SiMe_3)_2/THF$ (iv) $K_2CO_3/MeOH$ (v) $NH_2NH_2/CuSO_4/CH_3COOH/i\text{-}PrOH/NaIO_4$ (vi) Benzthiazole, DEAD, PPh_3 (vii) MCPBA (viii) PCC/CH_2Cl_2 (ix) $Me(CH_2)_{18}{}^{+}PPh_3Br^{-}$, BuLi (x) $LiAlH_4/THF$ (xi) $NH_2NH_2/CuSO_4/CH_3COOH/i\text{-}PrOH/NaIO_4$ (xii) PCC/CH_2Cl_2 (xiii) $MeOOC(CH_2)_{11}{}^{+}PPh_3Br^{-}$, BuLi (xiv) $LiAlH_4/THF$ (xv) $NH_2NH_2/CuSO_4/CH_3COOH/i\text{-}PrOH/NaIO_4$ (xvi) PCC/CH_2Cl_2 (xvii) (**31**)/$NaN(SiMe_3)_2$ (xviii) MeOH, THF, pTSA (xix) $NH_2NH_2/CuSO_4/CH_3COOH/i\text{-}PrOH/NaIO_4$

This method proved to be most effective with a combination of Wittig and Julia reactions in comparison to the previous methods by Gensler *et al.*[160,161] Different portions were built in at different stages as such the method can be applied in the synthesis of other meromycolates with varied chain lengths and functional groups. Further reaction to

convert **34** into a full mycolic acid was not completed at this stage but in more recent studies the synthesis full mycolic acids and their components, some of which are peculiar to *M. tuberculosis*, has been carried out.[163-166]

1.6.1.2b Full mycolic acids

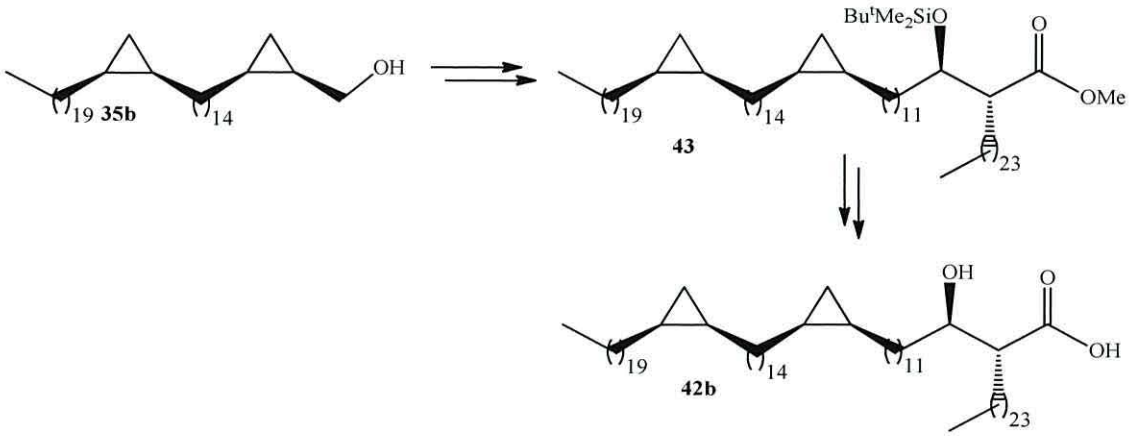
The synthesis of a single diastereomer of a protected α-mycolic acid **41** that can be found in *M.tuberculosis* has been reported **(Scheme 6a)**.[163] This contains di-*cis*-cyclopropane functional groups in the mermycolate chain, and is protected at the acid and alcohol positions in the mycolic motif. In this synthesis, a single enantiomer of the dicyclopropane unit **35a** was prepared and then converted into a sulfone **36** as in the previous method,[162] Furthermore, the α-alkyl-β-hydroxyl acid portion of a full mycolic acid was prepared from *R*-aspartic acid **37a**, which was converted into an epoxide intermediate **38** in several steps.[167a] The intermediate **38** was ring opened with a Grignard reagent followed by a combination of steps which led to the formation of an unprotected diol **39**. The diol **39** was in turn transformed into an aldehyde **40**, in five steps**.** Finally the sulfone **36** and aldehyde **40** were treated with a base in a Julia-Kocienski reaction, followed by hydrogenation to give a protected α-mycolic acid **41**. Deprotection of **41** to the free mycolic acid **42a** was not carried out. Notwithstanding, in recent work the free mycolic acid **42a** has been prepared, although this has not been published.[167b]



***Scheme 6a*: Synthesis of an α–mycolic acid of M.tuberculosis (42a)**[163, 167b]

Reagents: (i) Benzthiazole, DEAD, PPh_3 (ii) MCPBA (iii) $BrMg(CH_2)_9OTHP$, CuI (iv) imidazole, DMF, Bu^tSiMe_2Cl (v) H_2, Pd/C, MeOH (vi) $NaIO_4$, $RuCl_3$, H_2O, CH_3CN, H_2O, CCl_2 (vii) MeOH, H_2SO_4 (viii) Bu^tPh_2SiCl, DMAP, Et_3N (ix) LDA, $CH_3(CH_2)_{23}I$, HMPA (x) Ac_2O, pyridine (xi) F^- (xii) PCC/CH_2Cl_2 (xiii) (**26**), $LiN(SiMe_3)_2$ (xiv) $KO_2CN{=}NCO_2K$, CH_3COOH, THF.

Conversely, Al Dulayymi *et al*[168,169] also reported the preparation of the enantiomer **42b** through **35b** using similar procedures as in **Scheme 6a** but with the corresponding components of opposite stereochemistry (**Scheme 6b**).

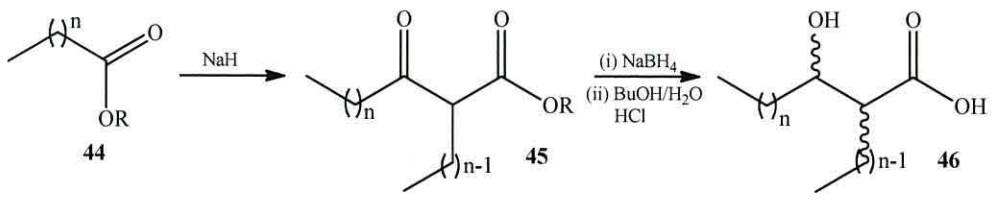


***Scheme 6a*: Synthesis of an α–mycolic acid of *M.tuberculosis* (42b)**[168,169]

This method can also be varied to modify the chain length or absolute stereochemistry of either functional group. However, the alkylation of the β-hydroxy ester in the preparation of the α-alkyl β-hydroxy portion proved the most difficult of all the reaction steps. It gave a low yield and was not always reproducible.

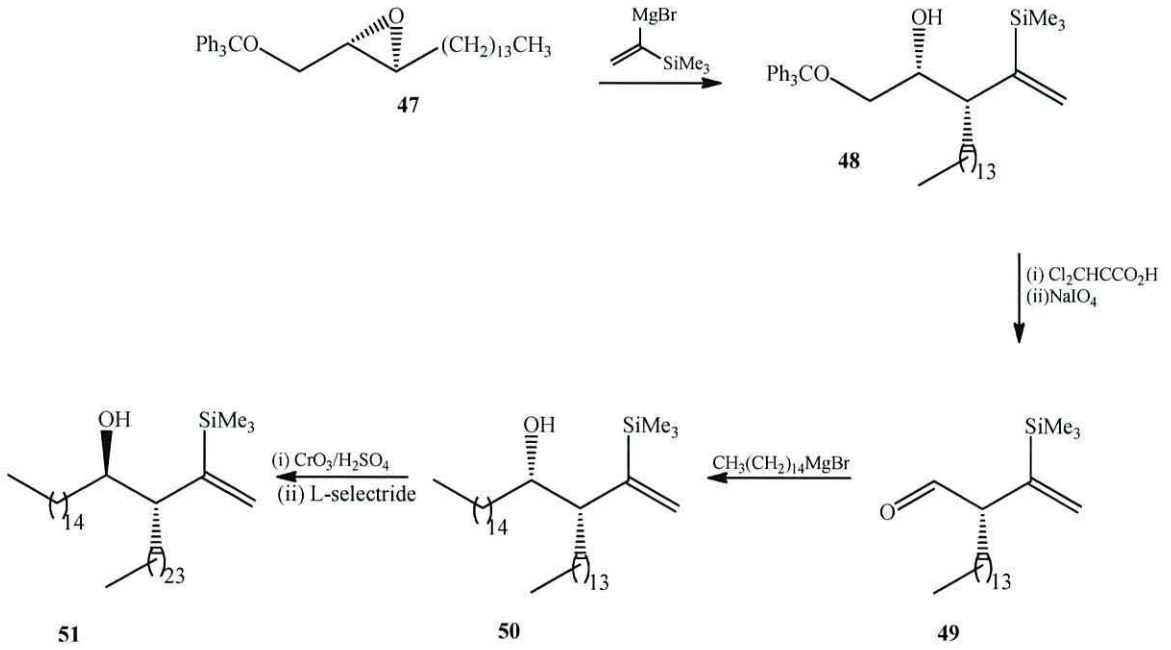
1.6.1.2c The α-alkyl-β-hydroxy fragment (mycolic motif)

Several studies have been conducted with respect to the preparation of the α-alkyl-β-hydroxy unit. One of the earliest attempts was by Lederer *et al.*[170] This involved the condensation of two fatty acid molecules **44** in the presence of sodium hydride to give β-keto-esters **45**. The esters were in turn reduced to give a mixture of α-alkyl-β-hydroxy diastereomers **45** which was difficult to separate (**Scheme 7**). However, the method was not stereoselective and as such the products were not desirable. Other preparations based on Claisen condensation have have been conducted which also yielded mixtures of diastereomers.[171,172]



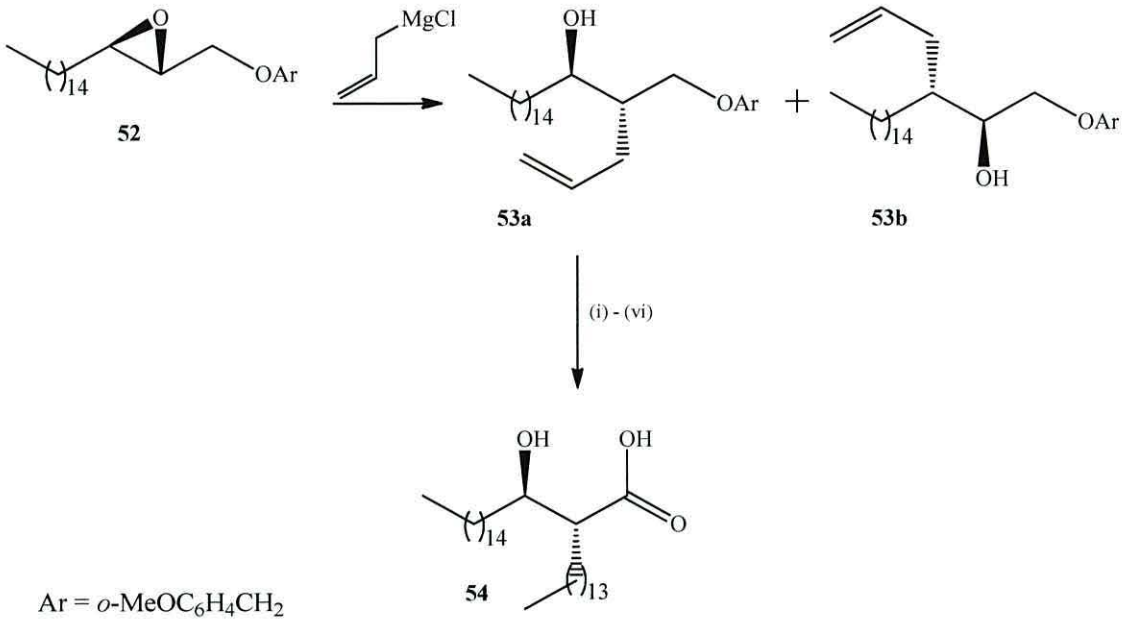
***Scheme 7*: Lederer et al method**[170]

The preparation of the first enantiomerically pure α-alkyl β-hydroxy unit was reported by Kitano *et al.*[173] In this method, a chiral epoxide **47** was ring opened stereoselectively using trimethylsilylvinylmagnesium bromide, which gave an optically active compound **48** with the correct stereochemistry at the α-position. Then the silyl compound **48** was oxidized to an aldehyde **49**, which was reacted with another Grignard reagent to yield the desired α-alkyl β-hydroxy derivative **50** (**Scheme 8**). Undesirably, the α- and β- chiral centres of the product were in the *threo*-configuration rather than the required *erythro*-configuration. This led to the need for further oxidation and stereoselective reduction to acquire compound **51** in the correct stereochemistry.



***Scheme 8*: Kitano et al method**[173]

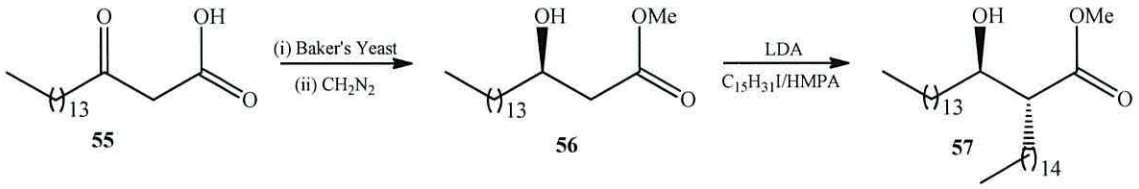
In a similar approach,[174] a chiral epoxide **52** was ring opened with a different kind of Grignard reagent and contrary to the previous method, the product mixture **53a** and **53b** had chiral centres in the *erytho*-configuration. A further chain extension was then carried out at the α-position of **53a** to give the desired compound **54** (**Scheme 9**). The disadvantage in this method was that the ring opening product was a mixture of regioisomers **53a** and **53b** of which the required compound **53a** was the minor isomer. In addition, the possibility of eventual scale up was quite remote as separation was only achieved by HPLC.



***Scheme 9:* Nishazawa et al approach**[174]

Reagents: (i) $NaH/PhCH_2Br/THF$-DMF (ii) $OsO_4/NaIO_4/THF$-H_2O (iii) $Ph_3PCH_2C_{11}H_{23}Br/BuLi/THF$ (iv) DDQ/CH_2Cl_2-H_2O (v) $DMSO/Et_3N/CH_2Cl_2$ (vi) $NaClO_2/NaH_2PO_4$/2-methyl-2butene/t-BuOH-H_2O

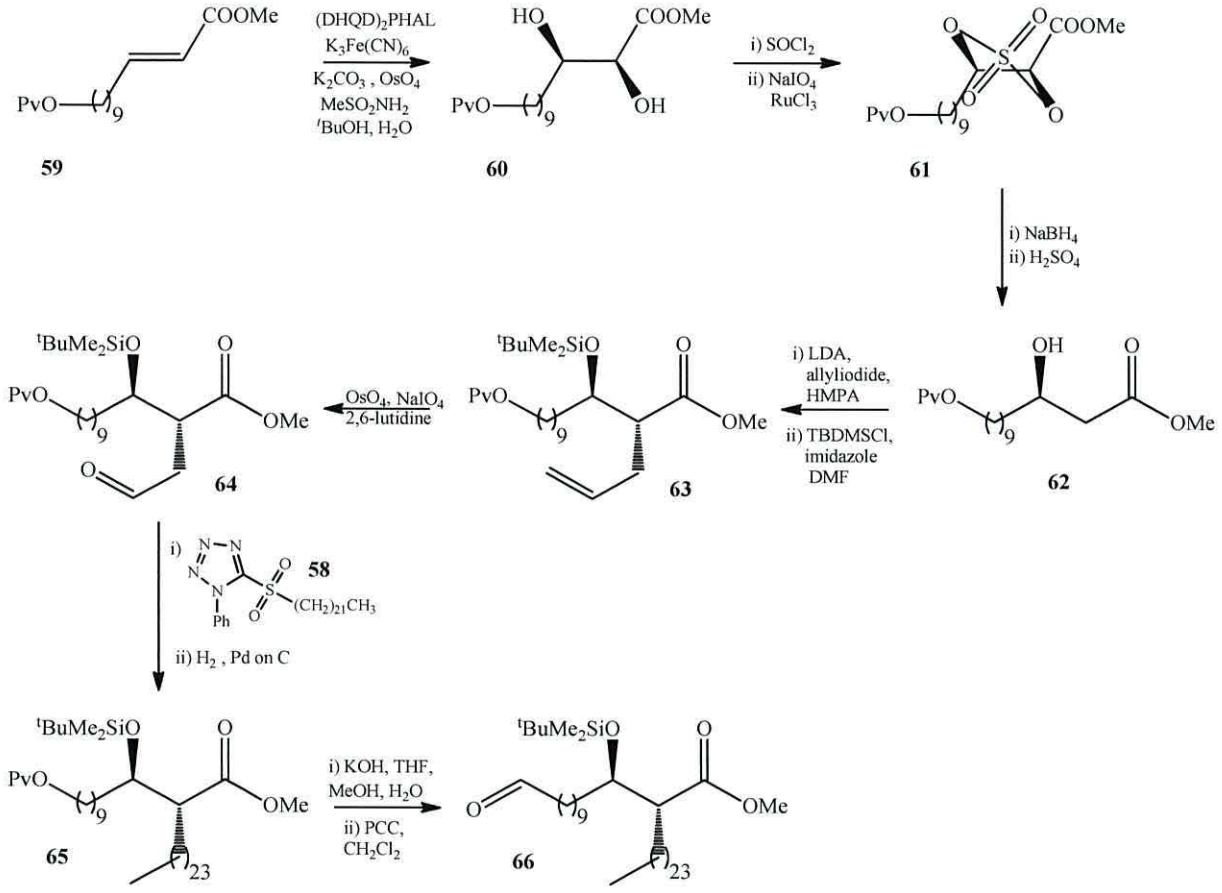
Utaka *et al*,[175] reported a different approach in which an alkyl chain was directly introduced in the α-position of a β-hydroxy ester **56** in a Fräter reaction to give **57**. The introduction of the alkyl chain was stereocontrolled due to the β-hydroxy group and the initial introduction of the hydroxy group at the β-position was by selective reduction of β-keto ester **55** with Baker's yeast (**Scheme 10**).



***Scheme 10:* Utaka et al method**[175]

As discussed earlier (**Scheme 6a**, page 28), Al Dulayymi *et al.*[163] also reported the direct alkylation method for preparing the α-alkyl-β-hydroxy derivative with the required chirality and the full alkyl chain length required, but this gave a low yield and was not reproducible. More recently, Baird *et al*,[176] put forward an improved method using a simple diol as the starting material to prepare an α,β-unsaturated ester **59.** The ester was

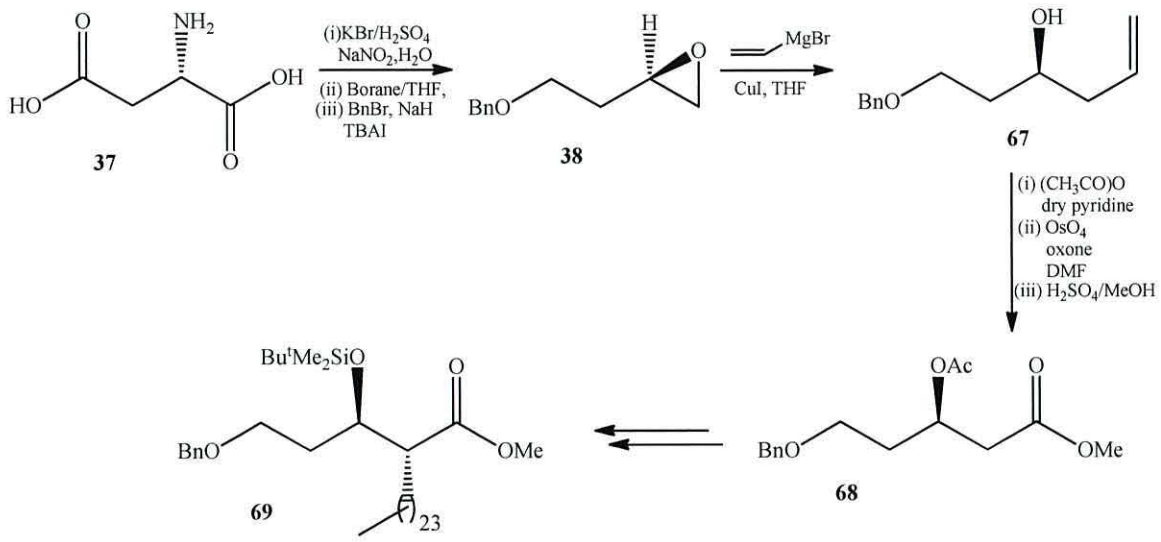
converted into an α,β-dihydroxy ester **60** which was then transformed into a cyclic sulfate **61**. The sulfate was further reduced and hydrolysed to (3*R*)-3-hydroxy ester **62** and this was followed by a Fräter allylation with a sulfone **58** at the α-position to give an (2*R*,3*R*)-2-allyl-3-hydroxy ester **63**. The allylated ester **63** was oxidized to an aldehyde **64** and the long chain at the β- position was formed by a Julia reaction with 1*H*-tetrazole, followed by hydrogenation of the unsaturated intermediate to give the desired compound **66,** after final deprotection of the primary alcohol of the diol **65 (Scheme 11)**.[176]



***Scheme11*: Improved synthesis of the α-alkyl β-hydxoxy unit**[176]

In a further development by Koza *et al* to prepare the mycolic motif **69**,[177] *L*-aspartic acid **37b** was the starting material instead of R-aspartic acid to obtain an epoxide intermediate **38** as described in Al Dulayymi *et al.*[163] This was because the *L*-aspartic acid was found to be much cheaper than the *R*-aspartic acid. The epoxide intermediate **38** was ring opened by treating with a Grignard reagent and then the derived compound **67** was converted into a β-hydroxy ester **68**. Rather than a direct long chain alkylation at the α-position, the steps employed by Baird *et al*,[176] in **Scheme 11** were repeated (a short chain Fräter allylation

was first carried out followed by a further Julia-Kocienski chain extension) to obtain the desired product **69** (**Scheme 12**).



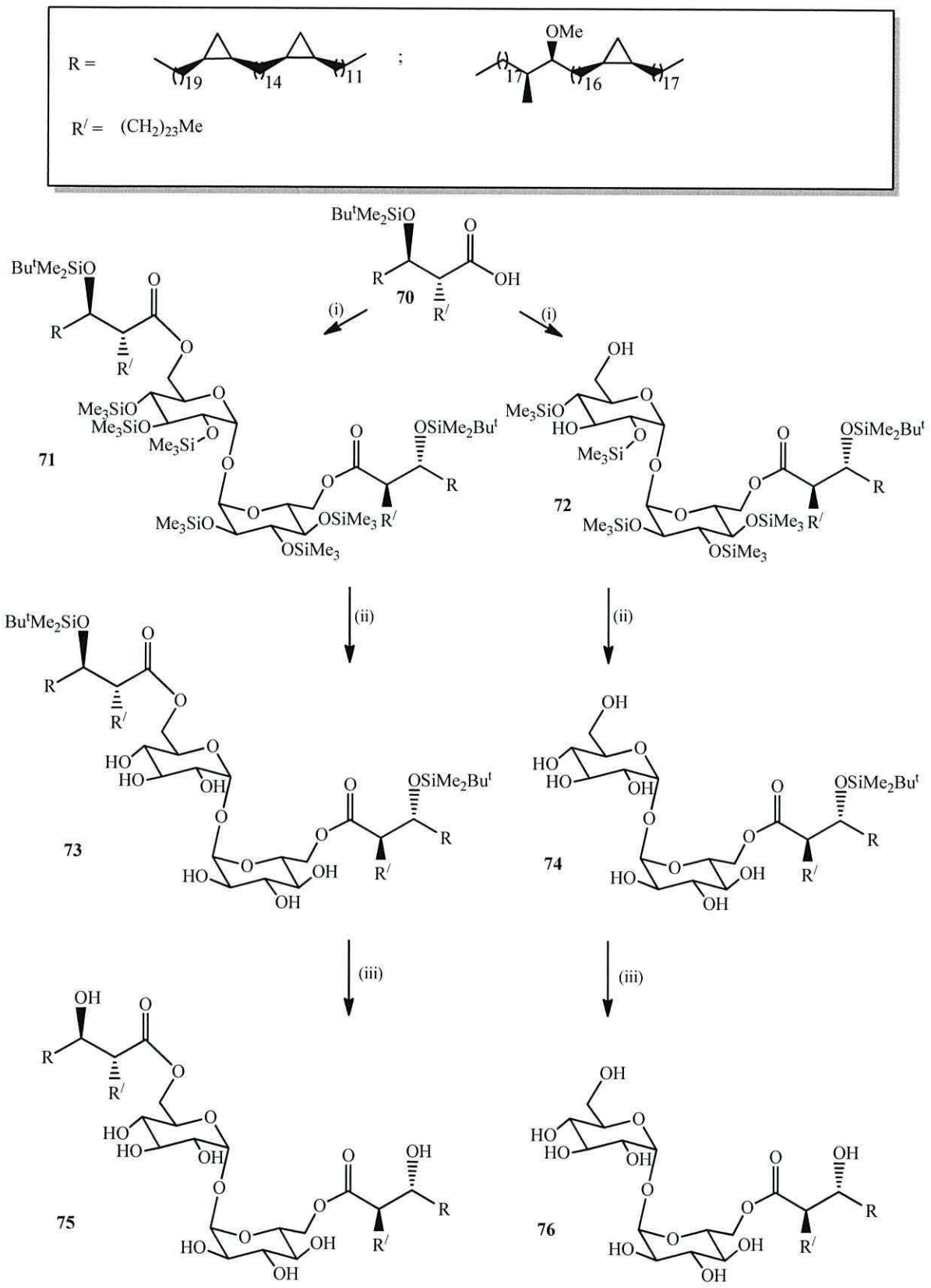
***Scheme 12*: *Koza et al method*[177]**

In the present project, synthetic approaches were examined using alternative routes which involved fewer steps starting from a cheaper starting material. This will be described in the results and discussion.

The synthesis of a number of α-, methoxy-, keto- and hydroxy mycolic acids that are identical to, or differ slightly in stereochemistry from, the natural ones found in other mycobacteria have been reported by Baird *et al* and these syntheses were carried out through similar methods to obtain enantiomerically pure compounds.[163-165,176,178]

1.6.2 Cord factors

Sugar esters have been reformed in different ways by reconstituting semi-synthetic compounds from naturally derived mycolic acids.[179-182] Synthetic TDMs made from behenic acid and corynomycolic acid have also been reported.[183,184] More recently, Baird *et al* converted a methoxy mycolic acid and an α-mycolic acid into their corresponding TDM and TMM (**Scheme 13**).[168,169]



***Scheme 13*: The first unique synthetic mycobacterial cord factor**[168]

Reagents: (i) ECDI, DMAP, CH_2Cl_2, 4 Å molecular sieves (ii) TBAF, THF (iii) pyridine, THF, HF-pyridine, $NaHCO_3$.

According to the reported procedure,[169] both mycolic acids were protected at the β-hydroxy acid position with TBDMS, prior to the coupling with the trehalose, in order to avoid reactions of the alcohol group. The protected mycolic acid **70** was coupled with protected trehalose to obtain a protected TDM **71** and a protected TMM **72**. The coupling was carried out by esterification, using ECDI and DMAP. Subsequently, deprotection was carried out in two stages. The first deprotection was done with TBAF, removing all the trimethylsilyl groups from the trehalose unit to give TBDMS-protected TDM **73** and TMM **74**. Further deprotection using HF pyridine removed TBDMS protection from the β-hydroxy position of the acid to yield the desired cord factors TDM **75** and TMM **76**. Two sets of cord factors corresponding to the α-mycolic acid, and the methoxy mycolic acid were obtained. High yields were recorded for the TDM and the TMM of the methoxy mycolic acid which were about the same, but lower yields were recorded in the case of the α-mycolic acid. Moreover, the yield for the α-mycolic TMM was about 50 % lower than its corresponding TDM.

1.7 The use of mycolic acids and cord factors in diagnosis

Enzyme-linked immunosorbent assay (ELISA) tests are widely utilized to detect substances that have antigenic properties. The test entails the use of components of the immune system and chemicals to detect immune responses in the body. The ELISA plate consists of tiny wells which hold the antigens. The first step in the test procedure involves coating the plate with the antigens (e.g. mycolic acid or cord factor), such that any free non-specific binding sites are blocked. Then the TB serum containing antibodies is layered over the antigen coating. The excess antibodies are washed away leaving only the antibodies which have bound onto the antigens. Subsequently, a secondary antibody is added to the well, which binds to the primary antibody and which also contains an enzyme. This is followed by washing to remove any excess secondary antibody. The coated plate is treated with a reagent which interacts with the enzyme to give a colour. The amount of antibodies binding to the antigen is determined by the intensity of the colour. This can be measured as absorbance which infers the level of antibodies in the serum sample.[185,186]

A number of mycolic acids and cord factors have been and are being tested to determine their biological activities and application to diagnosis. On account of their antigenic activities, they are both considered to be very potent signalling agents in the serodiagnosis

of TB.[9,187,188] Although the selectivity is not high enough for application, it has been found that natural mycolic acids can be used on an ELISA plate assay for the serodiagnosis for TB.[189,190] Beukes *et al* in 2011 reported a range of ELISA tests conducted on a natural *M.tb* mycolic acid, and unique synthetic mycolic acids (namely α-, keto-, hydroxyl- and methoxy-mycolic acids) which represent the three main functional classes in *M.tb*. The test results confirmed that free mycolic acids were recognized by antibodies and that the individual synthetic mycolic acids showed varying antigenic activities against human TB sera, which reflected the difference in their functional groups and stereochemistries. The oxygenated mycolic acids were found to be more antigenic than the α-mycolic acid. In descending order, the methoxy-mycolic acid was found to be the most antigenic, followed by the hydroxyl-, keto- and finally α-mycolic acids having shown the least recognition.[191]

In contrast to mycolic acids, natural cord factors which contain complex mixtures of isomers,[111a,111b] give a higher selectivity and sensitivity. Nonetheless, the analysis with natural TDM is complicated because many combinations of mycolic acids can be bonded to the trehalose, thereby increasing the number of possible structures which are believed to contribute to the variation of immune related effects.[192-195a]

Cord factors are also known to stimulate the immune system to produce a range of regulatory immune cells such as chemokines (e.g MCP-1) and cytokines (e.g TNF-α).[195b] For instance, the synthetic TMM and TDM species of α- and methoxy mycolic acids recently prepared by Baird *et al* [168] were tested against mouse macrophage cell line RAW 264.7 and analysed for cytokine and chemokine responses in order to determine their effects on the immune system. The TNF- alpha cytokine activity for the α-TDM was found to be three times higher than the commercially procured natural *M.tb*-TDM sample while the activities of the three other synthetic cord factors (methoxy-TDM, α-TMM and methoxy-TMM) were lower than the natural *M.tb*-TDM. The chemokine MCP-1 production for the α-TDM was twice more than the production for commercial sample, whereas the responses for the other synthetic cord factors were virtually the same as the commercial *M.tb*-TDM sample.[168] These results suggest that the testing of a wider range of TDM and TMM could generate interesting results.

1.8. Project Aim

This aim of this project was first to prepare two *cis*-α-mycolic acids **77a** and **77b** so as to have available all four possible *cis*-α-mycolic acid diastereomers containing di-cyclopropanes. Two other diastereomers **42a** and **42b** as earlier cited in this report have been previously prepared (**Figure 9**).[163,168] Preparation of all four possible diastereomers containing two cyclopropanes, would allow the effect of the groups on biological activity to be determined and hopefully lead to the stereochemistry of the natural material being defined. It would also show whether the stereochemistry had a major or minor effect on activity.

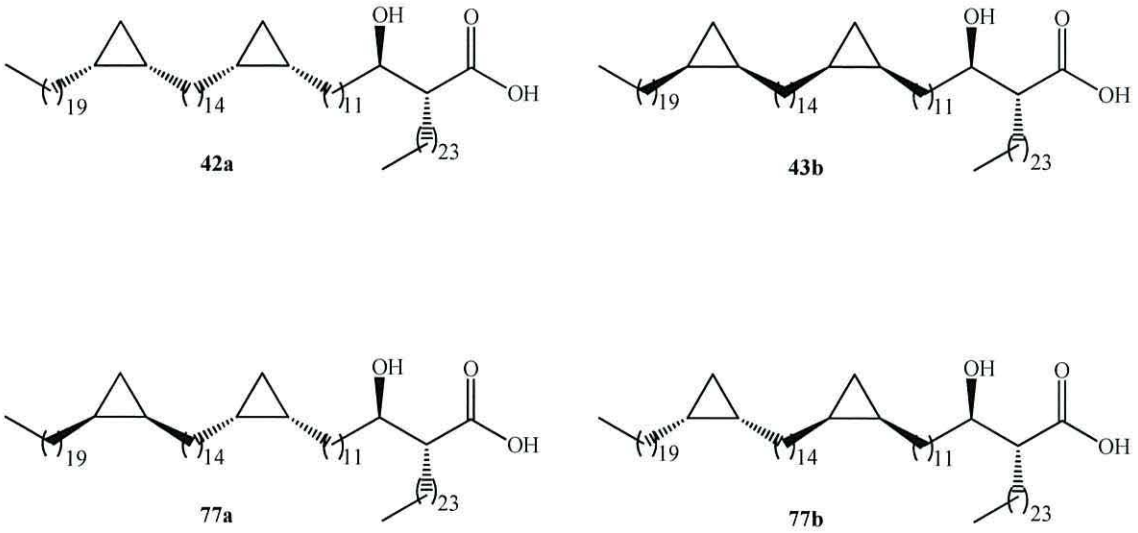


***Figure 9*: cis-Dicyclopropane α-mycolic acid diastereomers**

The second objective, directed at reducing the cumbersome and elaborate procedures currently involved in the synthesis of the mycolic motif, was to develop an improved method for the synthesis of the α-alkyl, β-hydroxy fragment (mycolic motif) **78** (**Figure 10**).

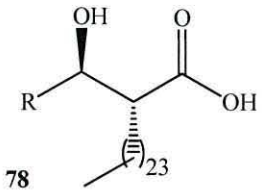


***Figure 10*: The α-alkyl, β-hydroxyl fragment of mycolic acids**

The improved method needed to be less expensive, involving fewer reaction steps. The α-alkyl-β-hydroxy fragment is a common unit in all mycolic acids and as such the method development for its synthesis would expedite the overall synthesis of all mycolic acid as well as cord factors.

For the third objective, the preparation of the model cord factors **78** and **79** of a β-hydroxy acid with no α-branch (**Figure 11**) was targeted. The β-hydroxy acid was derived from an intermediate compound in the synthetic route employed in the above-mentioned method development.

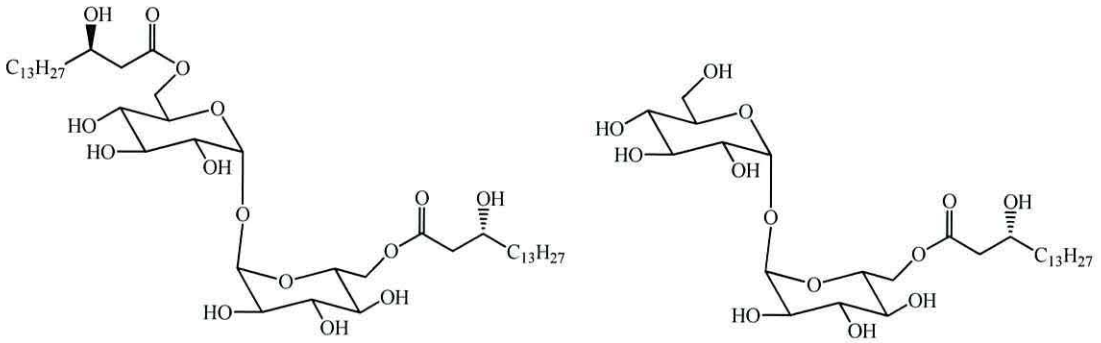


***Figure 11*: Target cord factors**

The derived cord factors would be tested in order to compare their biological activities with the cord factors of complete α-alkylated mycolic acids. It is anticipated that, if the results of the biological test are found to be identical, the α-alkylation procedure in the preparation of mycolic acids could be avoided. This would help in minimising the tedious work involved in mycolic acid synthesis.

Although there seem to be a lot of on-going mycolic acid syntheses, there are many more outstanding syntheses that have not been carried out. Preparation of more cord factors and mycolic acids of different classifications is essential, because they are very potent signalling agents of the immune system and are needed for testing the specific effects of structure on their biological activity.

Results and Discussion

Chapter 2

2. Synthesis of α-mycolic acids

The preparation of two diastereomers and of α-mycolic acids containing a dicyclopropane **42a** and **42b** has been reported (see page 28 and 29).[163,169] The first part of this project consists of the synthesis of the two other diastereomers **77a** and **77b**, in order to obtain the complete range of these α-mycolic acids. It was hoped that the availability of all four isomers would make it easier to determine through their biological activity which had the natural stereochemistry. Although the stereochemistry of some of the functional groups in mycolic acids has become clearer in recent years, partly through the specific rotations of fragments, this is not true for the *cis*-cyclopropane parts which essentially do not contribute to the specific rotations, because the cyclopropane is substituted with two similar long chains.

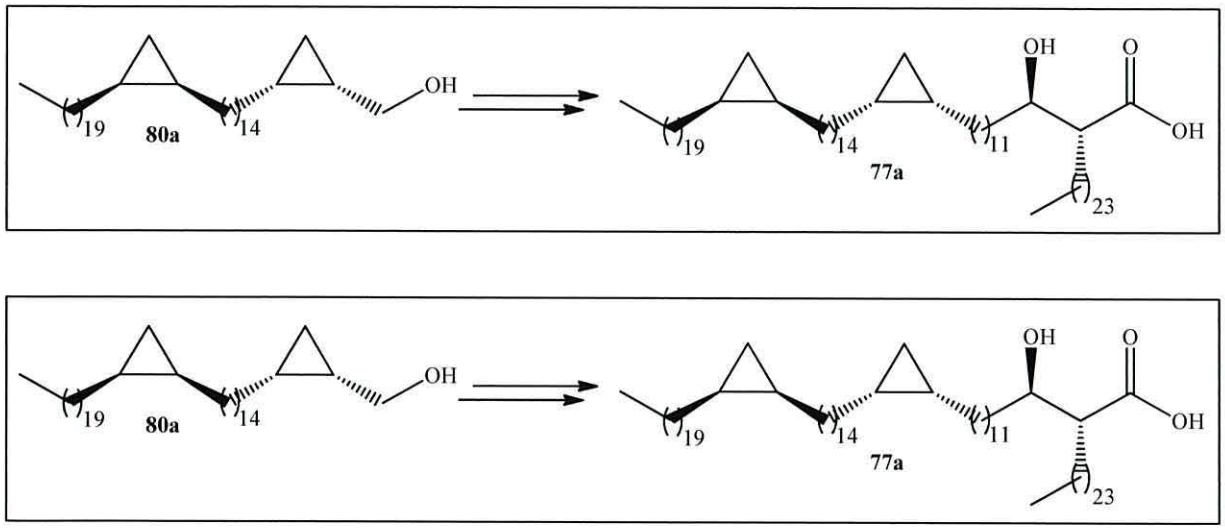


***Figure 12*: Target α-mycolic acids and their corresponding meromycolate precursors**

The target compounds (**77a** and **77b**) were prepared via their corresponding meromycolate precursors **80a** and **80b** respectively (**Figure 12**). The synthesis of the meromycolates entailed the preparation of two *cis*–cyclopropane units and chain extension reactions to enable the coupling of the two functional units in a modified Julia – Kocienski olefination reaction. Finally meromycolates were linked with the mycolic motif to form their corresponding full mycolic acids (**Figure 13**).

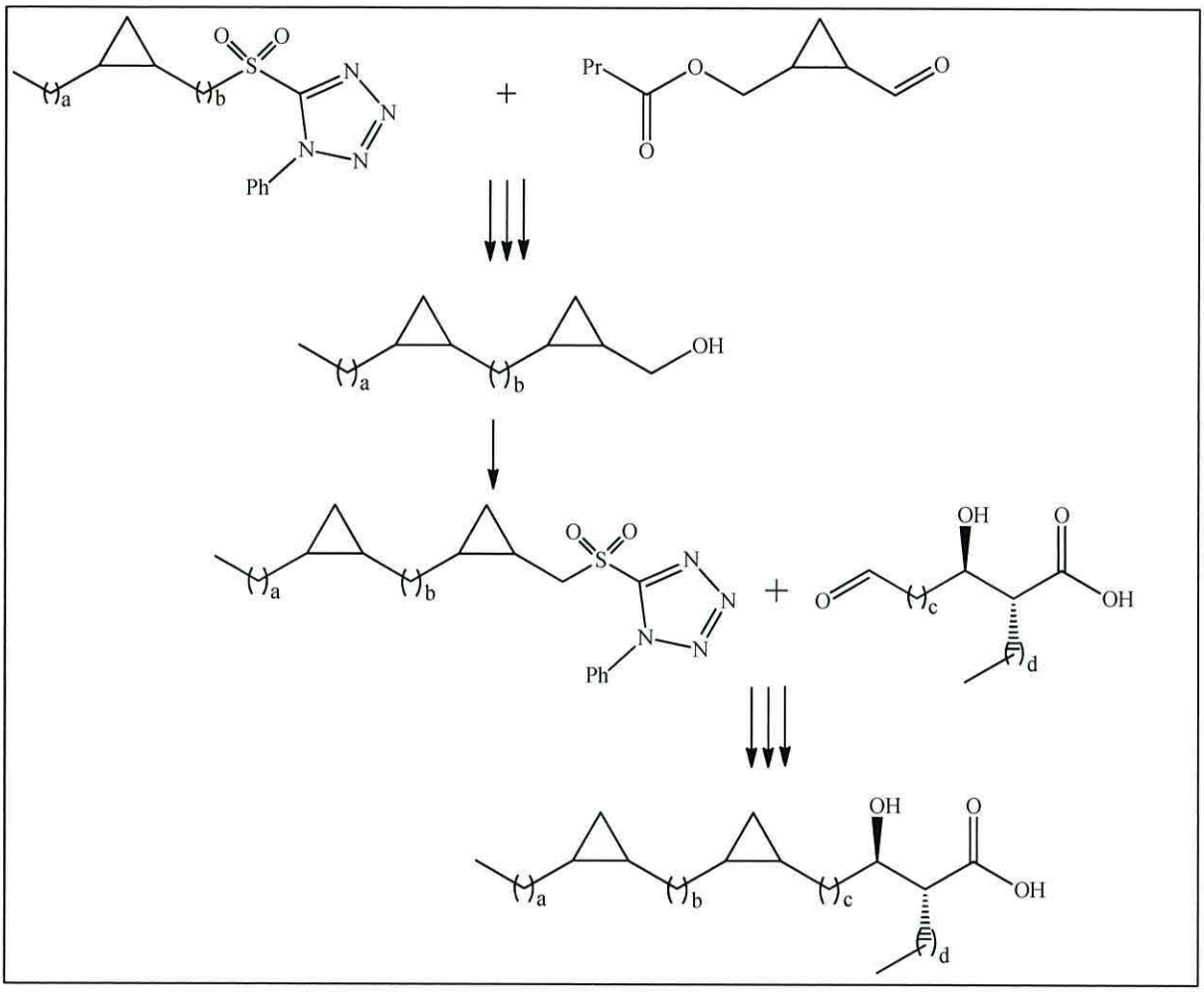


***Figure 13**: A general overview of the reaction steps for mycolic acid synthesis*

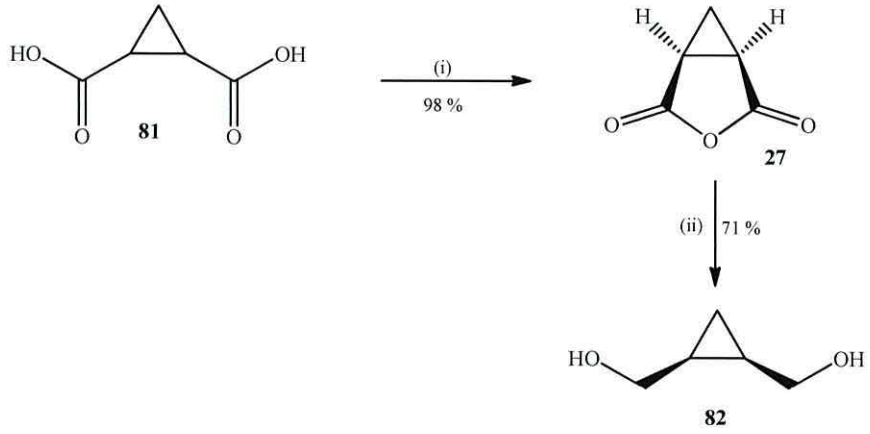
The general method used in this work is as described before in Sections **2.1.2.2** and **2.3** covering firstly the preparation and desymmetrisation of the cyclopropanes, then the chain extension and linking of the two cyclopropanes. Also the final coupling of these meromycolates to the mycolic motif is described in Sections **2.4** and **2.5**.

In order to make clear the steps in this process, the synthesis is divided into the following segments:

1. Preparation of single enantiomers of *cis*-cyclopropanes
2. Preparation of sulfones for chain extensions
3. Chain extension of the cyclopropanes
4. Linking the cyclopropanes to form the meromycolate
5. Chain extension of the mycolate motif
6. Linking of the meromycolcate to the mycolic motif

2.1 Preparation of the Cyclopropanes

By adapting methods described in the literature,[196,197] cyclopropane-1,2-dicarboxylic acid **81**[196] was treated with thionyl chloride and then the product of the reaction was washed repeatedly with hexane in order to remove excess reagent. A 98 % yield of cyclopropane dicarboxylic acid anhydride crystals **27** was obtained.[196] This was reduced using $LiAlH_4$ in THF to yield 71 % of pure cyclopropane diol **82**,[197] which gave one clear spot on TLC and a clean NMR. As such the product was not purified further (**Scheme 14**).



***Scheme 14*: Preparation of cis-1,2-disubstituted cyclopropane**[197]

Conditions and reagents: (i) $SOCl_2$, 24 hr reflux at 60 °C; (ii) $LiAlH_4$, THF, 2 hr at 100°C.

The ^1H NMR spectrum of the symmetrical diol **82** revealed signals for the cyclopropane protons at 0.07 (1H, q, *J* 5.3 Hz), 0.59 (1H, sext, *J* 4.3 Hz) and 1.20–1.14 (2H, m), whereas for the anhydride **27**, peaks for cyclopropane protons appeared further downfield at δ 1.70 – 1.64 (2H, m) and 2.78 (2H, dd, *J* 3.75, 8.2). These signals are particularly downfield because of the effect of oxygen (**Figure 14**).

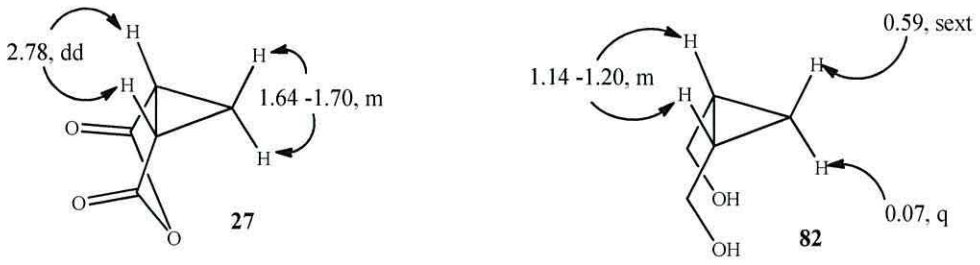


***Figure 14*: ^1H NMR Signals for cyclopropane protons**

Notably, signals for cyclopropane protons usually occur at high field, at about δ 1.00 or below, as a result of a ring current involving σ-electron delocalisation. **Figure 15** below shows typical peaks for cyclopropane protons, which is a pattern that was generally observed in the rest of this work.

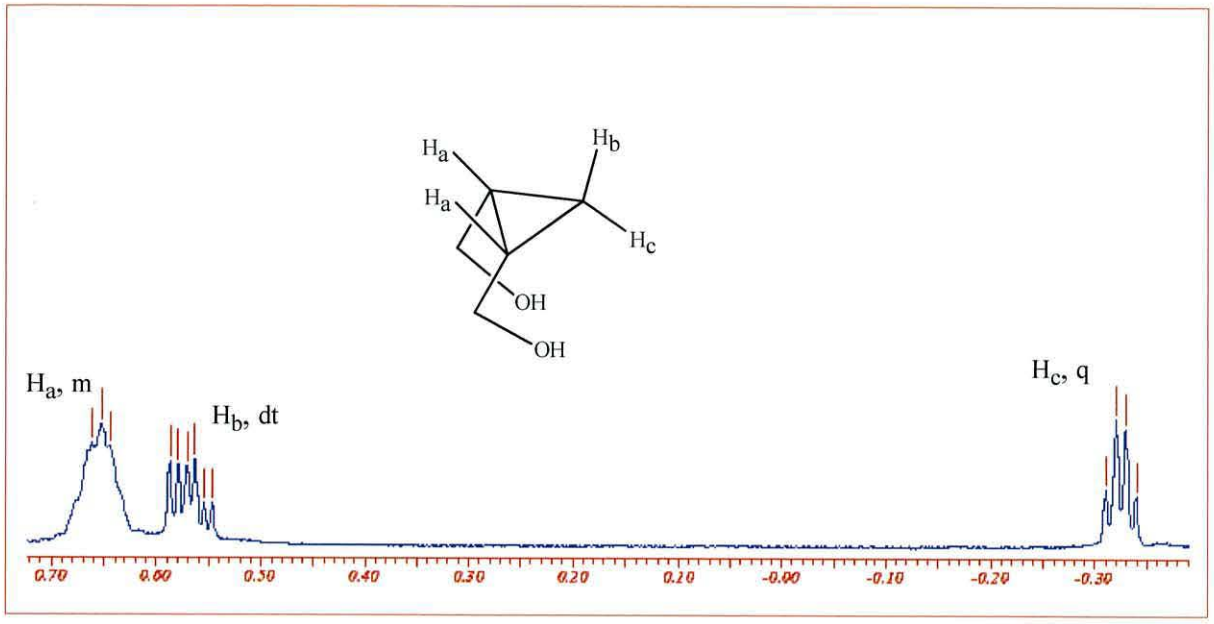
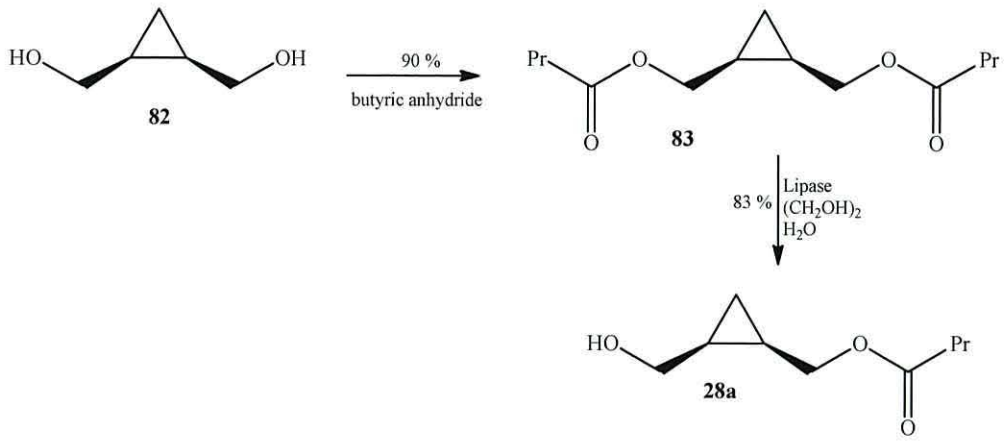


Figure 15*: *Typical signals of the four cyclopropane protons

2.1.1 Desymmetrisation to form the distal cyclopropane (28a)[163,197]

Prior to desymmetrisation, the diol **82** was protected by carrying out an esterification reaction with butyric anhydride, which led to the replacement of the –OH groups with propanoyl groups to form a meso-diester **83** (**Scheme15**). This was evident from the appearance of characteristic peaks for $R_2C{=}O$ and the $RCH_2{-}O$ at δ 173.7 and δ 64.3 in the carbon NMR spectrum and at 1736 and 1181cm^{-1} respectively in the IR spectrum. On the other hand, the proton NMR spectrum showed expected peak for propyl protons at δ 0.92 (6H, t, *J* 7.4 Hz), δ 1.62 (4H, sext, *J* 7.4 Hz), δ 2.25 (4H, t, *J* 7.4 Hz). Then desymmetrisation of the meso–diester **83** to a single enantiomer of a cyclopropyl monoester **28a** was achieved by reacting **83** with lipase in ethylene glycol and water under nitrogen, below 3 °C. The exothermic reaction was controlled by the use of cooling bath, and the pH was maintained at 6.5 to counter the production of butyric acid. This prevented the destruction of the enzyme due to high acidity and high temperature.



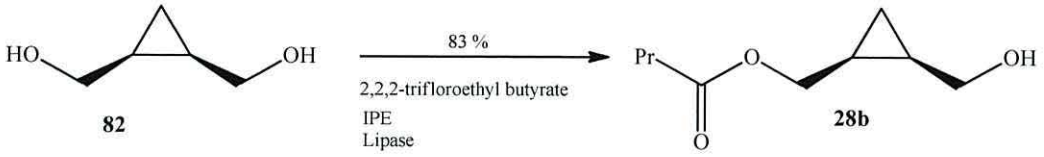
***Scheme 15*: Enzymatic desymmetrisation of the diester to monoester (28a)**[166,197]

The reaction yielded 83 % of the desired compound **28a**. The stereoselctivity of the reaction was a consequence of the chiral nature of the lipase. The NMR spectra of this compound were found to be similar to those of the previous compound **28a**. However, the proton NMR showed peaks corresponding to a propyl chain. A triplet appeared at δ 2.26 (2H, t, *J* 7.4 Hz) for the $-CH_2-$ protons next to the carbonyl group, a sextet at δ 1.61 (2H, sext, *J* 7.4 Hz) for the middle $-CH_2-$ and another triplet at δ 0.90 (3H, t, *J* 7.4 Hz) for the terminal methyl group. The integration of the propyl chain protons for this compound was half that of the previous compound **28a**. In addition, a broad O–H singlet also appeared at δ 2.39 (1H). This suggested that the one butyryloxyl group has been replaced by a hydroxyl group and that deprotection was successful. The hydroxyl group was also revealed at ν_{max} 3434 cm^{-1} in the IR spectrum. The specific rotation was $[\alpha]_D^{21}$ +22.1(c 1.3, $CHCl_3$) whereas in the literature it has been reported as $[\alpha]_D^{22}$ +18.2 (c 1.58, $CHCl_3$),[13] and $[\alpha]_D^{24}$ +19.8 (c 2.8, $CHCl_3$).[166]

2.1.2 Preparation of the proximal cyclopropane (28b)[166]

A solution of 2,2,2-triflouroethyl butyrate was freshly prepared with 2,2,2-trifluoroethanol in isopropyl ether, butyric anhydride and trimethylsilyl trifluoromethane sulphonate at ambient temperature, but initially at 4 °C because the reaction was exothermic. Cyclopropane diol **82** prepared in Section **2.1** was treated with the freshly prepared solution, using lipase as a catalyst. This led to the formation of a mono-ester **69b** (**Scheme 16**). The stereoselectivity of this reaction was again a result of the chiral structure of the lipase enzyme. The resulting compound **28b** had identical spectroscopic data to those for **28a**

(**Section 2.1.1**). The opposite stereochemistry was confirmed by the negative specific rotation value of **28b** that was measured as $[\alpha]_D^{20}$ –22.7 (c 1.5, $CHCl_3$) (literature[166] $[\alpha]_D^{24}$ – 18.1 (c 1.4, $CHCl_3$) in contrast to that of **28a,** which was $[\alpha]_D^{21}$ +22.1(c 1.3, $CHCl_3$).



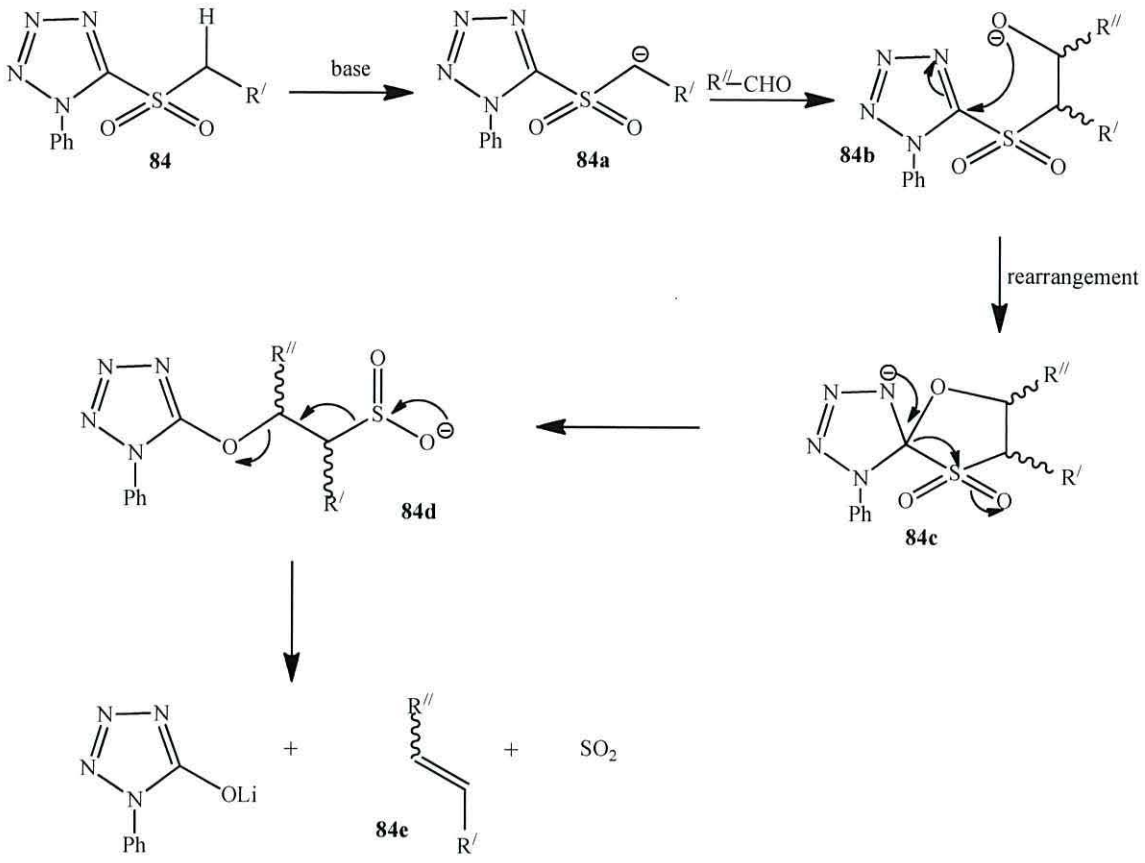
***Scheme 16*: Preparation of the second cyclopropane enantiomer (28b)**

2.2 The chain extension of the distal cyclopropane

In order to couple the two cyclopropane units, chain extensions were required on both sides of the distal cyclopropane. Key to this was the availability of chain extending sulfones with carbon chains of appropriate length. These were prepared in this section and utilized in a modified Julia–Kocienski reaction to achieve the chain extensions.

2.2.1 Julia- Kocienski olefination

The modified Julia-Kocienski olefination developed by M. Julia *et al*,[198] involved coupling an aldehyde with a sulfone, using lithium bis(trimethylsilyl)amide base, in dry THF to yield a mixture of unsaturated *E/Z* isomers. The base and the sulfone **84** form a metallated sulfone **84a** which reacts with the aldehyde to give an unstable β-alkoxysulfone **84b**. This in turn is converted by Smiles rearrangement into an intermediate **84c** that leads to the transfer of the heterocycle from sulfur to oxygen. Finally, sulfur dioxide, lithium 1-phenyl-1*H*-tetrazolene and a mixture of alkene isomers **84e** are formed via **84d** (**Scheme 17**). The kinetic control at the initial nucleophilic addition of aldehyde and sulfone is thought to be responsible for the formation of the two different isomers of olefins in different ratio.[199,200]

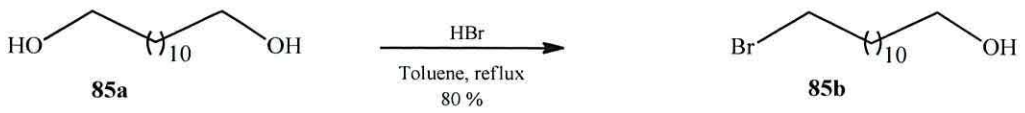


***Scheme 17*: The modified Julia-Kocienski olefination mechanism**

In previous syntheses by Al Dulayymi *et al*,[162,163] involving similar chain extension on cyclopropane, a Wittig reaction was used. In this instance, the Julia-Kocienski olefination was used as it was less cumbersome compared to the Wittig reaction which generates triphenyl phosphine oxide, which is known to be difficult to remove during the process of working up the reaction.

2.2.2 Preparation of the long chain Sulfones

To prepare the required sulfones of appropriate carbon chain length, 1–phenyl–1H–tetrazole–5–thiol was used in this work as opposed to 2-mercaptobenzothiazole used in previous syntheses,[163] because it is easily prepared from commercially available reagents and is less prone to self-condensation.[199,200] The starting material for the sulfones, 12-bromo-1-dodecanol,[201] was prepared from commercially obtained 1,12-dodecandiol **85a**. Using general methods,[201] the diol was easily converted into 12–bromo–1–dodecanol **85b**, with 40 % HBr by refluxing in toluene (**Scheme 18**).

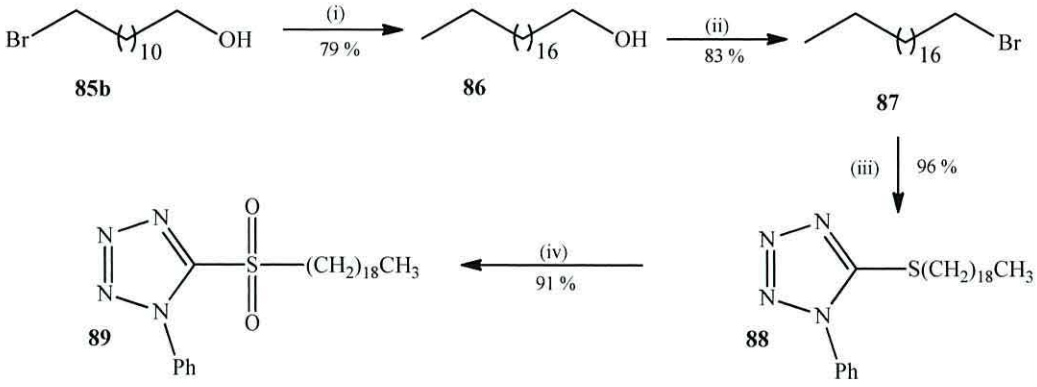


***Scheme 18*: Bromination of the diol**[201]

TLC gave two spots due to the presence of dibromide that was formed alongside the bromo-alcohol. However, this was removed by column chromatography to give an 80 % yield of the pure bromo-alcohol. The proton NMR spectrum showed a broad multiplet signal at δ 1.50–1.20 due to the protons of the long aliphatic chain and the hydroxyl proton. A CH_2–O signal appeared at δ 63.3 in the carbon NMR spectrum, and the IR spectrum also showed an O–H stretch at 3322cm^{-1}.

2.2.2.1 Preparation of C_{19} Sulfone (89)

In order to prepare nonadecyl sulfone **89**, the hydrocarbon chain of bromo-alcohol **85b** was extended to give 19-bromo-1-nonadecanol **87**.[163] This was carried out by first treating **85b** with a Grignard reagent and reconverting it into an alcohol **86**, which was in turn brominated to **87**. The bromide **87** was in turn reacted with 1–phenyl–1H–tetrazole–5–thiol to obtain a sulfane **88** which gave a sulfone **89** on oxidation (**Scheme 19**).



***Scheme 19*: Synthesis of C_{19} sulfone**

Conditions and reagents: (i) Mg, 1-bromoheptane, Li_2CuCl_4, dry THF; (ii) PPh_3, N-bromosuccinimide, CH_2Cl_2; (iii) 1–phenyl–H–tetrazole–5–thiol, K_2CO_3, acetone, 2.5 hr reflux, 80 °C; (iv) MCPBA, $NaHCO_3$, CH_2Cl_2, 18 hr stirring at R.T.

Following general procedures,[163] the Grignard reagent was generated under a very dry inert atmosphere from 1-bromoheptane and magnesium turnings in dry THF. Heating was

required at the initial stage of the exothermic reaction to adequately expose the reacting magnesium to the organic reagent.

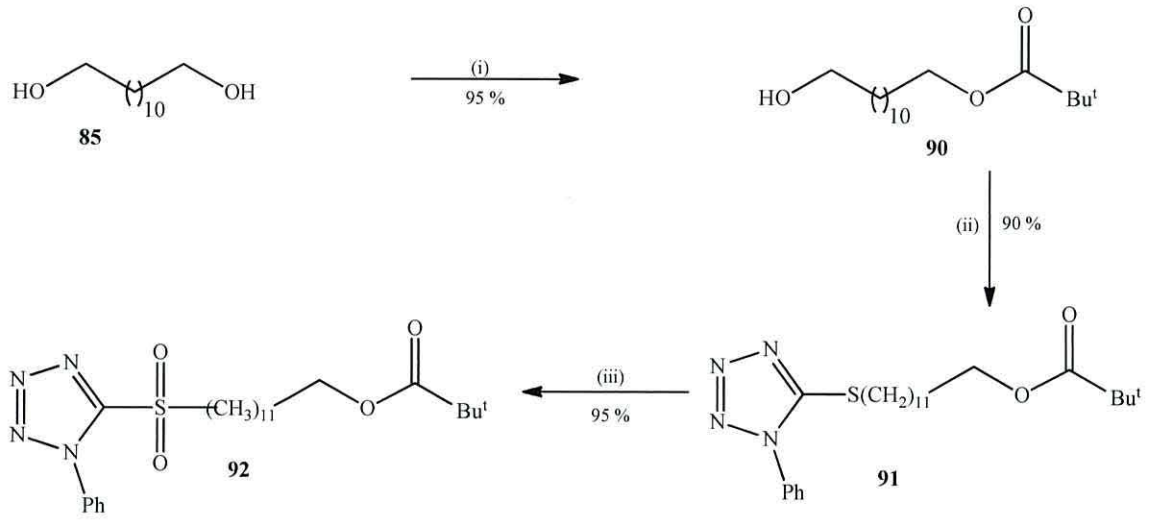
An excess of the derived Grignard reagent was reacted with 12–bromo–1–dodecanol **85b** in dry THF, in the presence of dilithum tetrachlorocuprate catalyst to obtain 1–nonadecanol **86** in a high yield of 79 %. The ^{1}H NMR spectrum showed a triplet at δ 0.93 (3H, t, *J* 7.1 Hz) to indicate the presence of a terminal methyl group and a triplet further down field at δ 3.67 (2H, t, *J* 7.2 Hz), due to the CH_2–O protons. This confirmed that the bromine at the end of the chain had been replaced by a methyl group.

The alcohol **86** was converted into 1-bromononadecane **87**,[163,164] by reacting with N-bromosuccinimide and triphenylphosphine in dichloromethane. The ^{1}H NMR spectrum of the product showed a peak at δ 0.92 (3H, t, *J* 6.8 Hz) reflecting the presence of a terminal –CH_3 group. An additional peak at δ3.50 (2H, t, *J* 6.8 Hz) was observed for CH_2–Br protons, which had moved further up field, compared to the CH_2–O peak in the spectrum for the starting material. Also the ^{13}C NMR spectrum did not show any peaks for CH_2–O protons as found on the spectrum of the starting material. Furthermore, the IR spectrum revealed a CH_2–Br band at ν_{max} between 1260 and 1217 cm^{-1}. These confirm that an –OH group of the alcohol has been substituted for a bromine.

Reaction of 19–bromononadecane **87** and 1–phenyl–H–tetrazole–5–thiol in the presence of K_2CO_3 and acetone yielded the sulfane **88**, which was oxidized with 3–chloroperbenzoic acid in the presence of sodium bicarbonate to obtain the corresponding sulfone **89**.[163,164] Conversion of the sulfane to the sulfone is an oxidation via a sulfoxide. The spectroscopic data obtained for the sulfane **88** and sulfone **89** were similar. However, the ^{1}H NMR for the sulfane revealed a multiplet signal at δ 7.58–7.52 (5H, m) representing five aromatic protons, and a triplet at δ 3.38 (2H, t, *J* 7.67Hz) for the two protons adjacent to the sulfur atom. Due to the presence of oxygen atoms in the sulfone, the five aromatic protons did not appear together, rather there were two sets of multiplets at δ 7.57–7.63 for three protons and δ 7.71–7.69 for two protons. Also, a characteristic peak for the two protons adjacent to the sulfur was shifted further down field to δ 3.74 (2H, t, *J* 7.8 Hz), due to the effect of oxygen. The IR spectrum for the sulfone also revealed an –S=O band at ν_{max} 1342 cm^{-1}, which was absent in that of the sulfane. This confirmed the oxidation of sulfane to a sulfone.

2.2.2.2 Preparation of C_{12} Sulfone (92)[202]

The sulfone **92** was prepared starting from 12-bromo-dodecan-1-ol **85b**. The hydroxy group was protected with trimethylacetyl chloride in the presence of pyridine and 4-dimethylaminopyridine (DMAP) to yield **90** (**Scheme 20 (i)**). This protecting group is easily removed using KOH and $LiAlH_4$.



***Scheme 20*: Preparation of C_{12} sulfone**

Conditions and reagents: (i) Et_3N, CH_2Cl_2, DMAP, Me_3COCl, 5 °C; (ii) 1–phenyl–1H–tetrazole–5–thiol, K_2CO_3, 2hr reflux at 70 °C; (iii) $NH_4Mo_7O_{24}.4H_2O$, H_2O_2, IMS, 10 °C, 18 hr at R.T.

The NMR spectra obtained for the trimethyl acetate were not complicated to interpret in comparison with those for THP protection used by Al Dulayymi *et al.*[162,163] In the ^1H NMR spectrum of the protected alcohol **90**, the *tert*-butyl protons appeared at δ 1.17 (9H, s). There was also a triplet at δ 4.02 (2H, t, *J* 6.6 Hz) corresponding to the protons adjacent to the carbonyl group, and then another triplet at δ 3.82 (2H, t, *J* 7.0 Hz) for the protons adjacent to the bromine group. The ^{13}C NMR spectrum showed a signal at δ 178.7 for the carbonyl carbon, a signal at δ 38.8 for the quaternary carbon of the protecting group, and a signal between 26.0 and 29.6 for the *tert*-butyl methyl carbons. In the IR spectrum, the O–H stretch of the bromo alcohol was replaced by peaks at 1157 and 1729 cm^{-1} for the C–O and C=O in the carboxylate group.

As discussed in Section **2.2.2.1**, the protected bromo alcohol **90** was reacted with 1-phenyl-1*H*-tetrazole-5-thiole to give the sulfane **91**, which was oxidized with ammonium

molybdate VI tetrahydrate in cold hydrogen peroxide to achieve the desired sulfone **92** (**Scheme 20 (ii) and (iii)**). Again, the spectroscopic data were alike for the sulfane **91** and sulfone **92**. Nevertheless the characteristic triplet for the two protons adjacent to the sulfur appeared at δ 3.70 (2H, t, *J* 7.9 Hz) for the sulfone, which was further downfield in comparison to that of the sulfane at δ 3.36 (2H, t, *J* 7.4 Hz). Additionally, in the sulfone **92**, separate signals for the phenyl ring corresponding to two proton and three protons respectively appeared between δ 7.67–7.55 (5H, m), as opposed to being together at δ 7.55–7.50 in the sulfane **77**. The IR spectrum also showed signal for S=O bond at 1336 and 1152 cm^{-1}.

When the sulfane was oxidized to the sulfone, the $-CH_2$ protons adjacent to the sulphur atom exhibited a distinctive splitting pattern, which occurs because the protons are not magnetically equivalent (**Figure 16**). A similar pattern was observed for the $-CH_2$ groups in all the sulfones in this work.

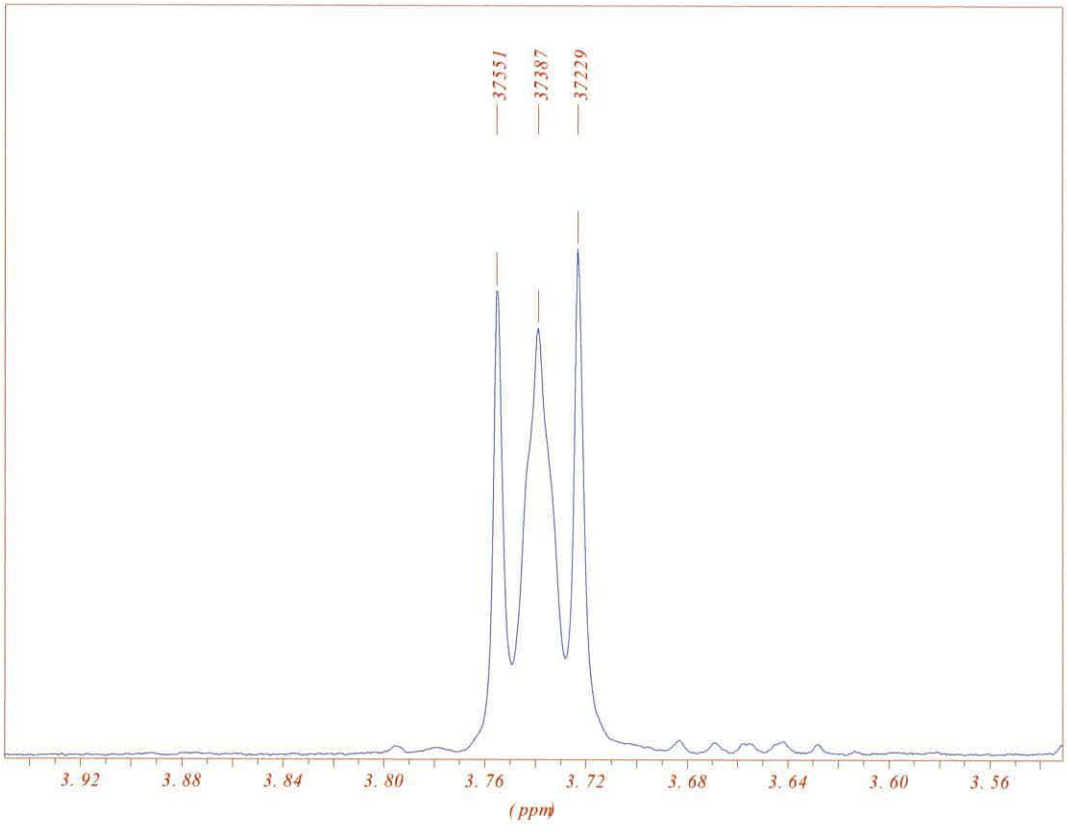


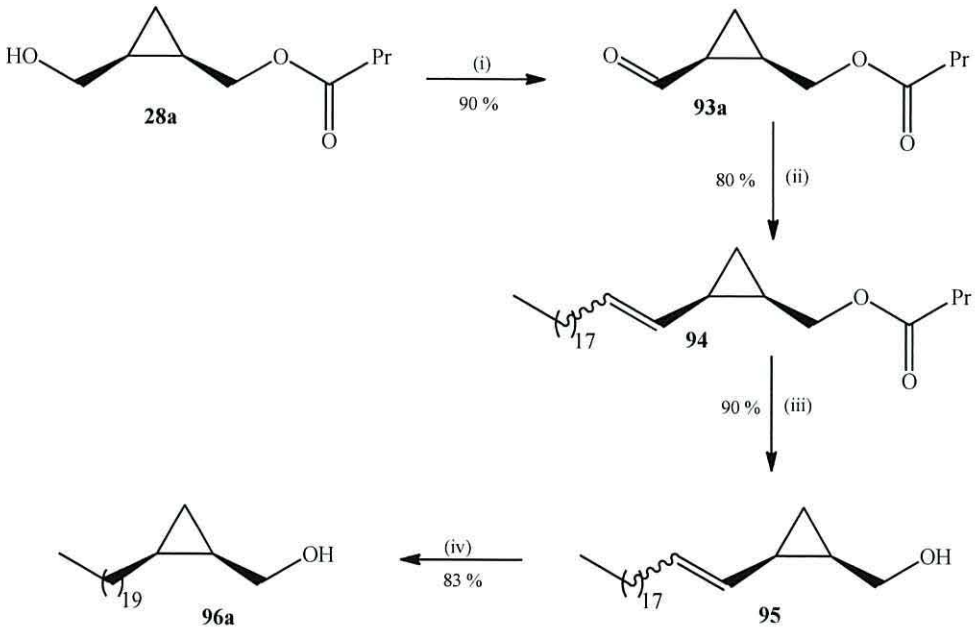
***Figure 16*: The characteristic signal of the protons adjacent to the sulfonyl group in 92**

This pattern was consistent throughout this work and is a typical example of the AA' BB' system depicted in Newman projection (**Figure 17**), which shows that A is not equivalent to A' and also that B and B' are non-equivalent. Thus, although H_b and $H_{b'}$ have the same chemical shift, H_a shows a *cis*-splitting pattern with H_b and a *trans*-splitting pattern with H_b'.

***Figure 17**: AA'BB' system in Newman projection*

2.2.3 The first chain extension

The chain extension involved oxidation of the alcohol group of the hydroxyl cyclopropyl monoester **28a** in order to convert it into an aldehyde **93a**. This was followed by coupling with sulfone **89** and the base to form an alkene **94**. Subsequently, the hydroxyl on the other side of the cyclpropane was deprotected to give **95**, and then the double bond was hydrogenated to obtain the desired compound **96a** (**Scheme 21**).



Scheme 21: Chain extension of the cyclopropane[163, 197]

Conditions and reagents: (i) PCC, CH_2Cl_2, 2 hr stirring at R.T; (ii) (**89**), $LiN(SiMe_3)_2$, dry THF, N_2, –10 °C; (iii) $LiAlH_4$, THF, 2 hr reflux at 100°C; (iv) N_2H_4, $NaIO_4$, $CuSO_4$, CH_3COOH, 2-propanol, 80 °C.

2.2.3.1 Oxidation of the cyclopropyl ester to an aldehyde (93a)[197]

The cylcopropyl ester **28a** was oxidized to aldehyde **93a** using pyridinium chlorochromate (PCC) in dichloromethane (**Scheme 21 (i)**), in a yield of 90 %. The ^{1}H NMR spectrum of the product showed a signal at δ 9.45 due to the CHO proton. Characteristic peaks reflecting the presence of both aldehyde and ester functional groups appeared at δ 199.88 and 173.44 respectively in the ^{13}C NMR spectrum. The IR showed no peak for an O–H stretch, but peaks for carbonyl functional groups appeared at 1736 and 1705 cm^{-1}. These results confirmed that the alcohol has been oxidized to an aldehyde. A prompt initiation of the next reaction step with the aldehyde **79a** was required because this is not stable and readily undergoes further oxidation to a carboxylic acid or oligomerisation

2.2.3.2 Julia-Kocienski reaction of the aldehyde

Following standard coupling methods, the derived aldehyde **93a** was homologated by treating with the sulfone **89** (earlier prepared in Section **2.2.2.1**) and $LiN(SiMe_3)_2$ as base, in dry THF (**Scheme 21(ii)**). The product was a mixture of *E/Z* isomers of unsaturated ester **94**, which appeared as two close spots on TLC. These were practically impossible to separate by column chromatography. The ^{1}H NMR spectrum showed signals for two sets of C=C protons between δ 6.00 and 5.00. In previous syntheses by Al Dulayymi *et al,*[162,163] the results were comparable. For instance, the yield obtained in this work was 80 % whereas in previous work it was 81 %.

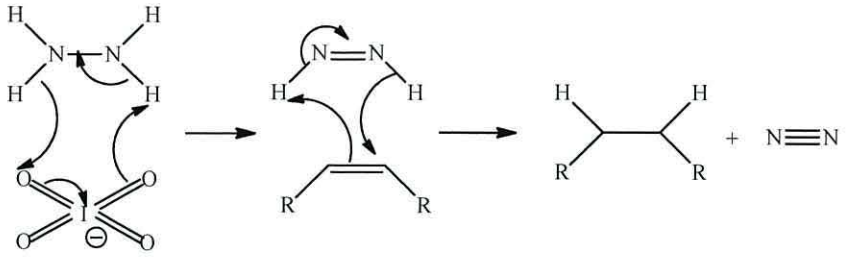
2.2.3.3 Deprotection of the alkene

The Julia-Kocienski olefination was followed by deprotection (**Scheme 21 (iii)**) and then hydrogenation (**Scheme 12 (iv)**). Lithium aluminium hydride in THF was used for the deprotection of **94**, thereby transforming the unsaturated ester to an unsaturated alcohol **95** (**Scheme 21 (iii)**). As before, two close spots appeared on TLC for a mixture of the *E/Z*–isomers. The products had a lower R_f than the starting material indicating that a hydxoxyl group had replaced the ester. Full characterization at this intermediate stage was again not carried out as it was a mixture of isomers.

2.2.3.4 Hydrogenation of the unsaturated alcohol

Hydrogenation of the unsaturated alcohol **95** using di–imide gave a homologated cyclopropyl alcohol **96a** in a yield of 83 % (**Scheme 21 (iv)**). The di–imide was generated

from hydrazine hydrate by oxidation with sodium (meta)periodate in the presence of glacial acetic acid and saturated aqueous copper sulfate (**Scheme 22**). The exothermic reaction was initiated by heating at the initial stage. This milder procedure was preferred to catalytic hydrogenation, in order to prevent ring opening of the cyclopropane.

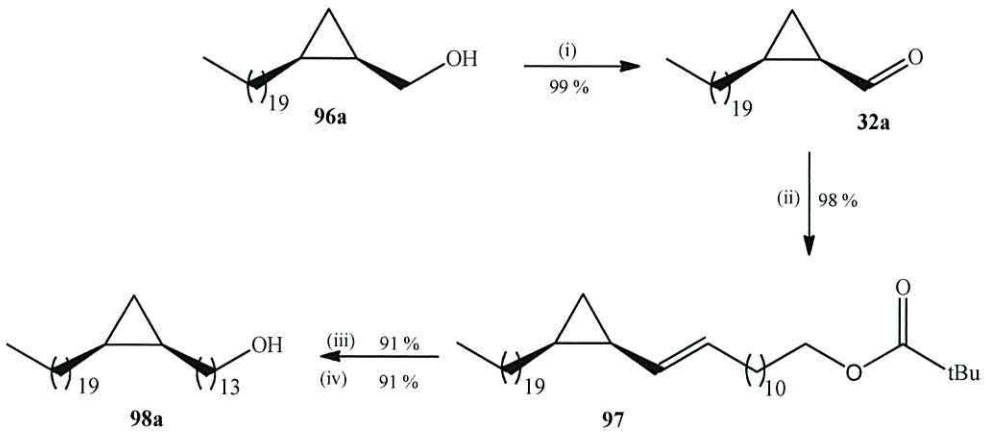


Scheme 22: Mechanism for hydrogenation by di –imide

The ^{1}H NMR spectrum of the product showed no peaks between δ 6.00 and 5.00. This suggested that the unsaturation had been removed. The IR spectrum revealed an O–H stretch at 3313 cm^{-1}. According to the literature,[162] this compound has been previously prepared, but the full characterisation was not recorded. Nonetheless, the synthesis of its enantiomer has been reported by Al Dulayymi *et al,*[163] and this showed identical spectroscopic properties. The melting point of compound **96a** in this work was 58 – 60 °C and the specific rotation was $[\alpha]_D^{26}$ +12.1 (c 1.1, $CHCl_3$), whereas in literature the corresponding values for the opposite enantiomer were 60 - 62 °C and $[\alpha]_D^{22}$ –7.5 (c 1.3, $CHCl_3$) respectively.[163]

2.2.4 The second chain extension of the cyclopropane

The second chain extension on the cyclopropane also entailed a modified Julia-Kocienski reaction between the sulfone **92** prepared in Section **2.2.2.2** and the aldehyde **32a** obtained from the oxidation of the cyclopropyl alcohol **96a**. As before, this was followed by deprotection and hydrogenation (**Scheme 23**).



***Scheme 23*: Chain extension of the cyclopropyl alcohol**

Conditions and reagents: (i) PCC, CH_2Cl_2, 2.5 hr stirring, R.T; (ii) (**92**), $LiN(SiMe_3)_2$, dry THF, –10 °C; (iii) $LiAlH_4$, THF, 2hr at 100 °C; (iv) N_2H_4, $NaIO_4$, $CuSO_4$, CH_3COOH, 2-propanol, 80 °C.

2.2.4.1 Oxidation of the alcohol to aldehyde (83)

In line with previously discussed procedures (in Section **2.2.2.1**), the oxidation of **96a** with PCC in dichloromethane resulted in **32a** (**Scheme 23 (i)**).[162] Due to the unstable nature of the aldehyde, it was purified by column chromatography and promptly used for the next reaction step. The aldehyde **32a** showed comparable spectroscopic data to those of **96a** but the IR spectrum revealed a C=O stretch at 2919 cm^{-1} and showed no signal for an O–H stretch. The ^1H NMR spectrum also showed a doublet corresponding to the proton next to the carbonyl at δ .9.36. In addition the ^{13}C NMR spectrum, a C=O signal appeared at δ 202.0.

2.2.4.2 The coupling reaction of the aldehyde

As in the earlier synthesis of diastereomeric products, the derived aldehyde **32a** and the sulfone **92** prepared in Section **2.2.2.2** were coupled in a modified Julia reaction, using lithium bis-(trimethylsilyl) amide as the base. The reaction was carried out in dry THF at –10°C, under nitrogen (**Scheme 23(ii)**). Again, an *E*/Z mixture of unsaturated esters **97** was obtained. These again appeared as two close spots on TLC, which were almost impossible to separate by column chromatography. The proton NMR spectrum had no signal for a carbonyl proton but signals corresponding to two sets of C=C protons, appeared between δ 6.00 and 5.00.

2.2.4.3 Deprotection and hydrogenation of the unsaturated ester

Deprotection of **97**, followed by reduction as in Sections **2.2.3.3** and **2.2.3.4** led to **98a** via an unsaturated alcohol (**Scheme 23 (iii), (iv)**).[162] Although this compound has been previously prepared by Al Dulayymi *et al*,[162] Wittig coupling was used in the place of the current Julia coupling, and also a full characterization of the compound was not reported, though more recently, the compound has been prepared and fully characterized by Maza Iglesias *et al*.[169] However, the spectroscopic data for the compound **98a** obtained in this work were very similar to those obtained in literature.[163,169] The ^{1}H NMR spectrum of the product showed a broad O–H singlet at δ 2.1 (1H) but there were no signals due to –HC=O in either ^{1}H NMR or ^{13}C NMR spectra. The IR spectrum also showed an O–H stretch at 2919 cm^{-1} and no peak for –RC=O. This confirmed the introduction of an O–H group and the absence of the aldehyde. There were also no peaks corresponding to –HC=CH– between δ 6.00 and δ 5.00, which showed that hydrogenation had been successful. Additionally, the chain extension was confirmed by the signals of the aliphatic chain protons at δ 1.38 – 1.13 (58H, m), 1.58 (4H, hept, *J* 5.6 Hz), 3.65 (2H, t, *J* 6.6 Hz) and 0.89 (3H, t, *J* 6.8 Hz) respectively. The melting point was 73–74 °C and the specific rotation was $[\alpha]_D^{26}$ +2.39 (c 1.2, $CHCl_3$). The specific rotation for the compound **98a** in this work was equal and opposite to that reported in literature for the enantiomer which was $[\alpha]_D^{22}$ –2.0 (c 1.0, $CHCl_3$).[163] However, Maza Iglesias *et al*[169] reported a specific rotation of $[\alpha]_D^{32}$ – 0.33 (c 2.03, $CHCl_3$) for the compound **98a**, which appears not to be consistent with the above. This difference could be as a result of instrumental error during measurement or one of the samples not being pure.

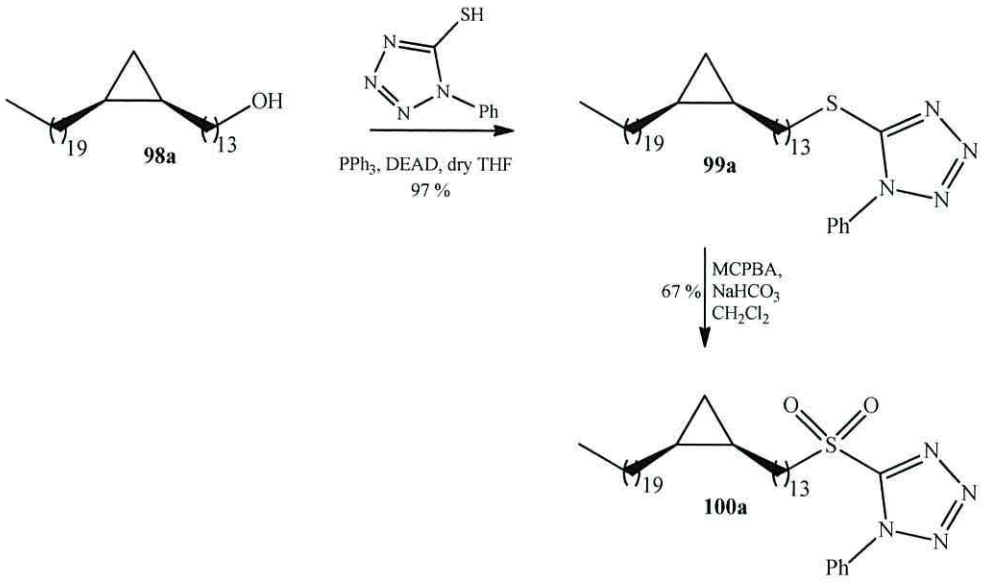
2.3 Coupling of the distal and proximal cyclopropanes

In this final sequence of reactions to form the meromycolate moiety, the homologated cyclopropane **98a** was converted into a sulfone **100a** in order to couple it with the required aldehyde in a modified Julia-Kocienski olefination reaction.

2.3.1 Preparation of sulfone (100a)

Compound **98a** was reacted with 1–phenyl–1H–tetrazole–5–thiol in the presence of diethyl azodicarboxylate and triphenylphosphine in dry THF to give a sulfane **99a**, which was in

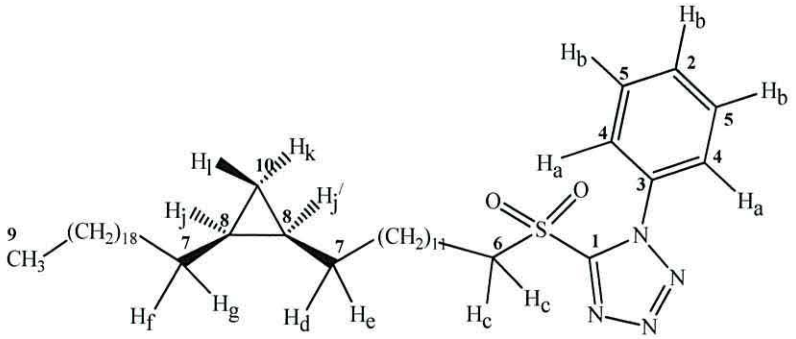
turn oxidized to **100a** using meta-chloroperbenzoic acid with sodium hydrogen carbonate in dichloromethane (**Scheme 24**).[164]



***Scheme 24*: Preparation of the cyclopropane sulfone**

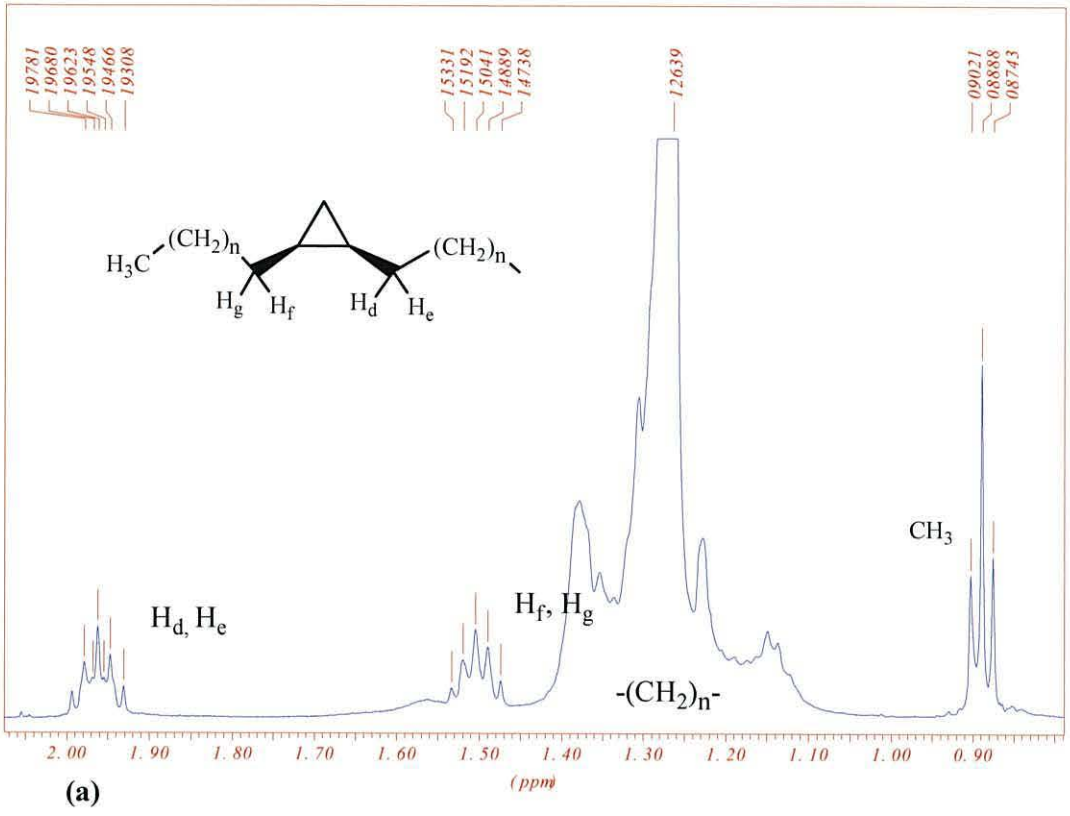
As discussed earlier, the spectroscopic data for the sulfane **99a** and the sulfone **100a** were similar except for the unique triplet signal due to the protons adjacent to the sulfur at δ 3.74 in the ^{1}H NMR spectrum of the sulfone **100a.** Meanwhile, in the ^{1}HMR spectrum of the sulfane **99a**, a triplet appeared upfield at δ 3.42. Besides the above-mentioned, both compounds had similar peaks for the ^{1}H NMR ^{13}C NMR and IR spectra. As in **99a**, compound **100a** showed characteristic ^{1}H NMR peaks corresponding to the cyclopropane protons at δ – 0.33 (1H, q, *J* 4.8 Hz), 0.57 (1H, dt, *J* 4.1, 8.5 Hz) and 0.66 – 0.64 (2H, m) (**Table II** and **Figure 18**). The phenyl protons gave signals between (δ) 7.74 – 7.60 (5H, m) and the ^{13}C NMR, peaks for the tetrazole carbon atom appeared at δ 154.5 and the phenyl carbon atoms showed peaks at δ 126.1, 130.6, 133.4 and 134.0 respectively. The specific rotation for the sulfone **100a** was $[\alpha]_D^{22}$ +1.7 (c 1.4, $CHCl_3$), whereas for the sulfane **99a** it was $[\alpha]_D^{22}$ –1.2 (c 1.3, $CHCl_3$) which shows that the specific rotation was reversed by the introduction of the oxygen atoms.

Table II: Selected ^{1}H and ^{13}C NMR signals for compound 100a



100a

Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
$2H_a$, $3H_b$	7.72 –7.70	m		1	154.5
$2H_c$	3.74	t	8.0	2	134
H_d, H_e	1.96	br.pent	7.8	3	133.4
H_f, H_g	1.50	br. pent	7.4	4	130.6
CH_3,	0.89	t	6.8	5	126.1
H_j and $H_j^{/}$	0.66–0.64	m		6	56.4
H_k	0.57	dt	4.1, 8.5	7	29.8
H_l	–0.33	q	4.8	8	16.1
				9	14.3
				10	11.0



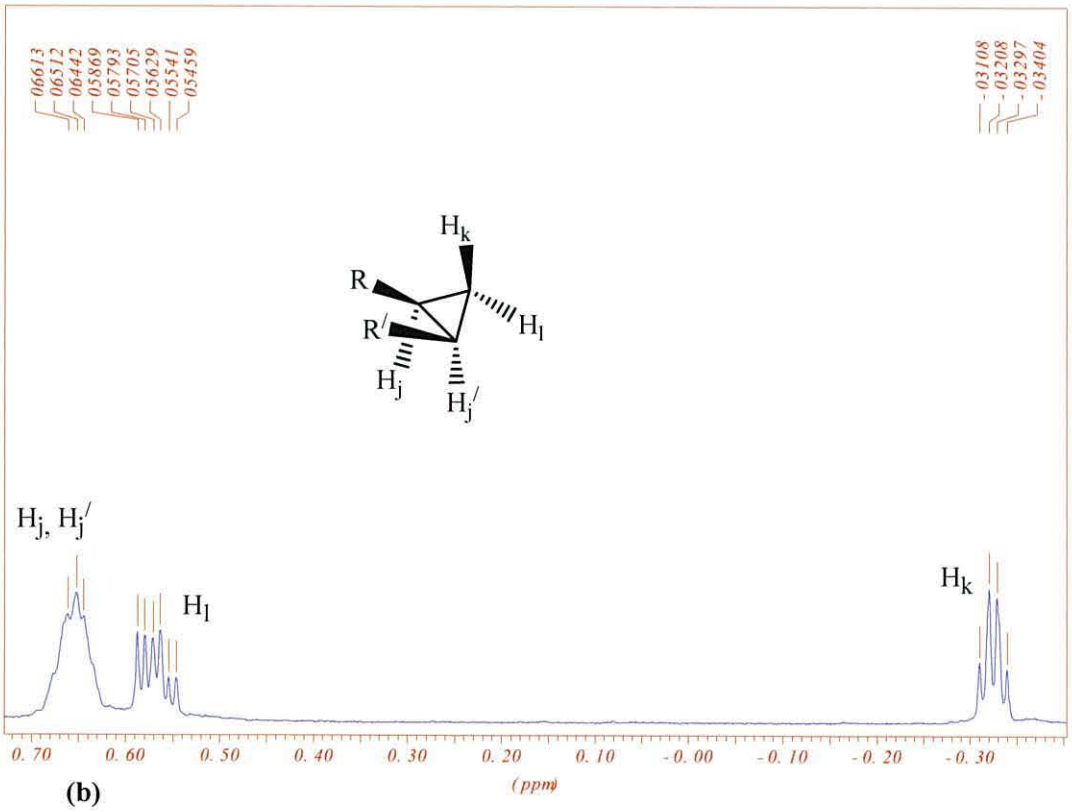
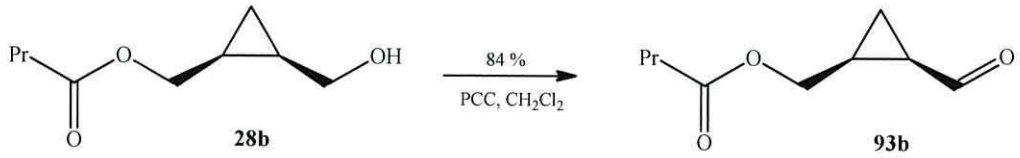


Figure 18: Selected signals in the H NMR spectrum of (100a)

(a) δ 2.00 to 0.87, (b) δ 0.70 to -0.34

2.3.2 Preparation of aldehyde (93b)

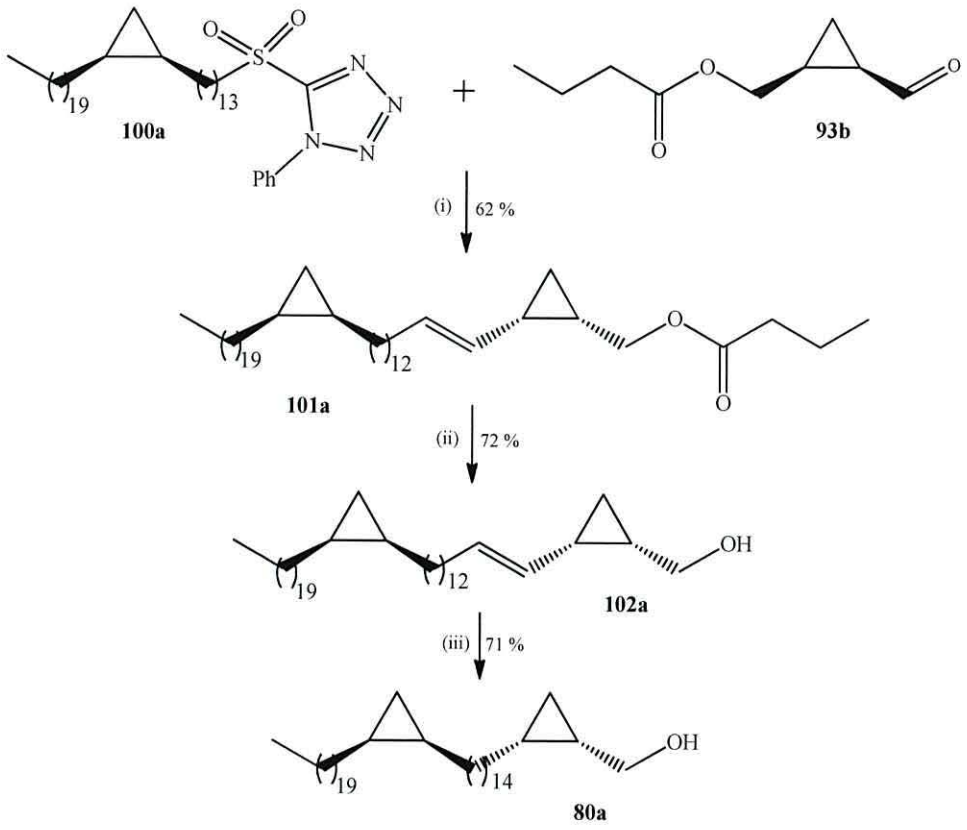
Using the same procedure as in Section **2.2.3.1**, oxidation of **28b** with pyridinium chlorochromate (PCC) in dichloromethane yielded 84 % of the corresponding aldehyde **93b** (**Scheme 25**). Again the specific rotation was measured to be $[\alpha]_D^{22}$ –72.6 (c 1.1g, $CHCl_3$), in contrast to that of the enantiomer **93a**, which was $[\alpha]_D^{22}$ + 73.1 (c 1.5, $CHCl_3$). The spectroscopic data of the two enantiomers **93a** and **93b** were also identical.



***Scheme 25*: Preparation of the cyclopropane aldehyde (93b)**[166]

2.3.3 Coupling for the formation of the meromycolic moiety (63a)

As before, Julia–Kocienski olefination was carried out between the butyryloxymethyl cyclopropane aldehyde **93b** derived in Section **2.3.2** and the cyclopropane sulfone **100a**, derived in Section **2.3.1 (Scheme 26a)**.



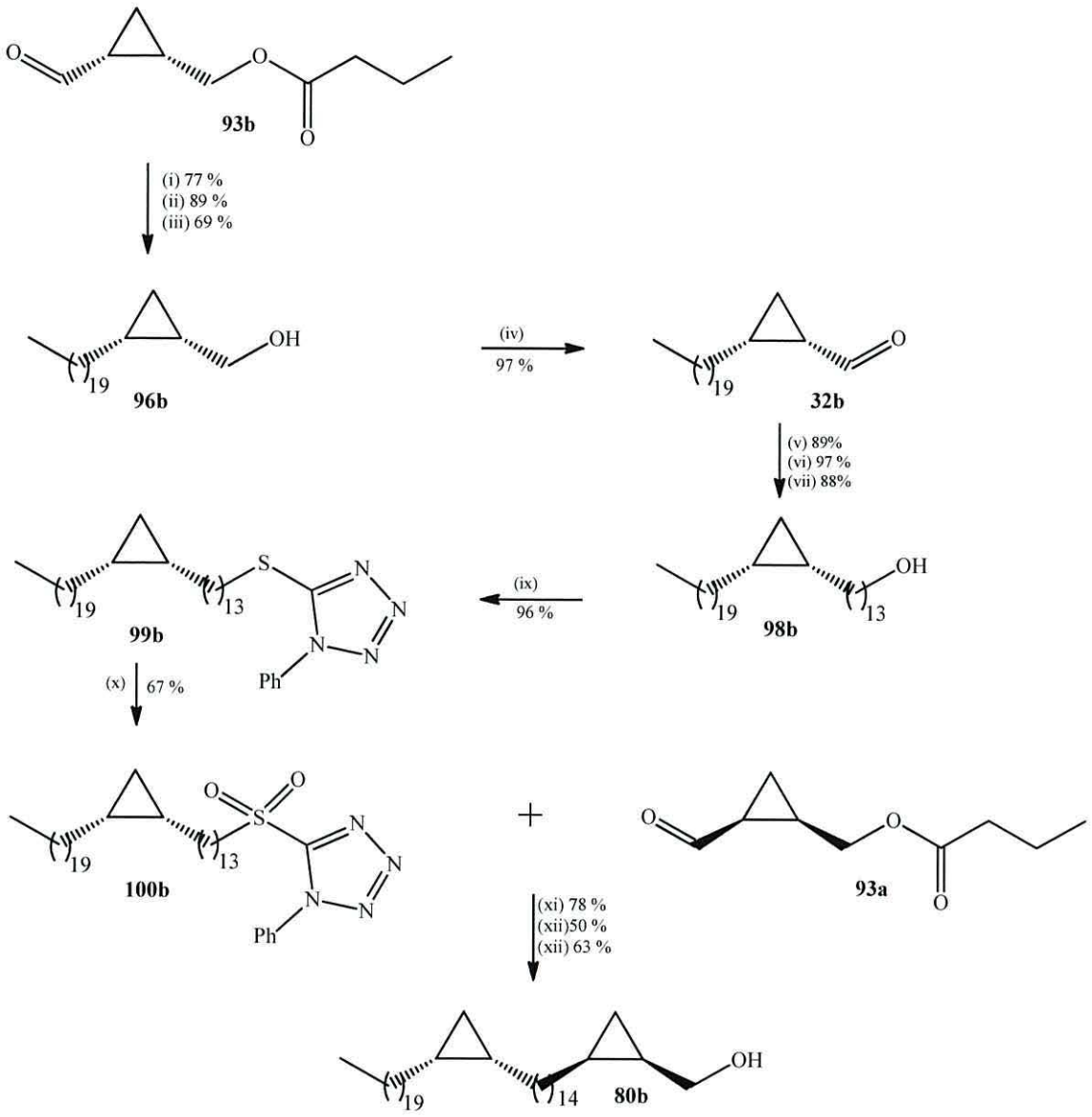
***Scheme 26a*: Coupling to form the meromycolate intermediate (63a) (Route 1)**

Conditions and reagents: (i) $LiN(SiMe_3)_2$, THF, –10 °C, N_2; (ii) $LiAlH_4$, THF, 2 hr reflux at 100 °C; (iii) N_2H_4, $NaIO_4$, $CuSO_4$, CH_3COOH, 2-propanol, 80 °C

This gave an unsaturated monoester **101a**, which was deprotected to give the corresponding alcohol **102a**, and then hydrogenated to yield the desired dicyclopropane intermediate **80a.** Characteristic proton NMR peaks at δ 6.00 – 5.00 confirmed the presence of the olefinic –HC=CH– group in the unsaturated monoester **101a**. On deprotecting, the IR spectrum showed a signal for O-H stretch 3370 cm^{-1} but none for a carbonyl group thereby confirming that the ester has been converted into an unsaturated alcohol **102a**. Subsequently hydrogenation was carried out as before, to obtain complete disappearance of the double bond signals at δ 6.00 – 5.00 in the proton NMR spectrum. However, the unsaturated alcohol was not readily soluble in the solvent used earlier, therefore the starting material was dissolved in 2-propanol at 70 – 80 °C, before commencing hydrogenation. A yield of 71 % was obtained. The melting point was 51 - 53 °C and the specific rotation was $[\alpha]_D^{21}$ –7.32 (c 1.0, $CHCl_3$). Signals for two sets of cyclopropane protons appeared at δ – 0.33 (1H, q, *J* 5.0 Hz), – 0.03 (1H, q, *J* 5.3 Hz), 0.57

(1H, dt, *J* 4.1, 8.5 Hz), 0.69 – 0.63 (2H, m), 0.72 (1H, dt, *J* 4.3, 8.3 Hz) and 0.92 – 0.83 (2H, m). In addition, hydrogenation of the olefinic bond was confirmed by the disappearance of the peaks between δ 5.00 and 6.00. This confirmed that coupling of two intermediate units via Julia olefination had been successful.

The enantiomer **80b** was also synthesized by repeating the same sequence of reactions as in the preparation of **80a** but using intermediate compounds of the opposite stereochemistry. The aldehyde **93b** was obtained from the cyclopropane half ester **28b** prepared in Section **2.1.2** (see Section **2.3.2**). As before, **93b** was homologated on both sides. The first chain extension involved a modified Julia- Kocienski coupling reaction of the aldehyde **93b** and a sulfone **89**, followed by deprotection and hydrogenation to give **96b** as described in Section **2.2.3**. For the second chain extension, the reaction steps in Section **2.2.4** were repeated; **96b** was oxidized to **32b** and coupled with a sulfone **92** in another modified Julia-Kocienski reaction, followed by deptection and hydrogenation which led to **98b**. Susequently, **98b** was reacted with 1–phenyl–1H–tetrazole–5–thiol in the presence of diethyl azodicarboxylate and triphenylphosphine in dry THF to give a sulfane **99b**, which was in turn oxidized to **100b** using meta-chloroperbenzoic acid with sodium hydrogen carbonate in dichloromethane as in Section **2.3.1**. As in Scheme **26a**, the sulfone **100b** was coupled in a modified Julia-Kocienski reaction with butyryloxymethyl cyclopropane aldehyde **93a** (derived in Section **2.2.3.1**) and then deprotection and hydrogenation were carried out to yield **80b** (**Scheme 26b**).



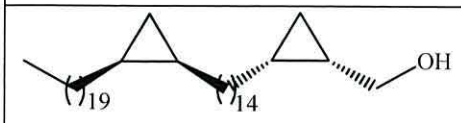
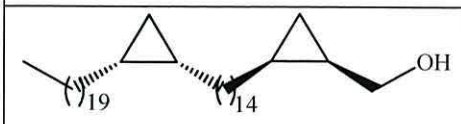
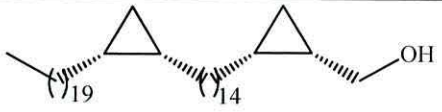
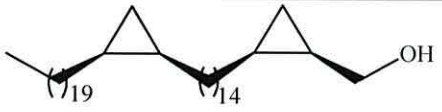
***Scheme 26b*: Coupling to form of the meromycolate intermediate (80b) (Route 1)**

Conditions and reagents: (i) (**89**), $LiN(SiMe_3)_2$, dry THF, N_2, –10 °C; (ii) $LiAlH_4$, THF, 2 hr reflux at 100°C; (iii) N_2H_4, $NaIO_4$, $CuSO_4$, CH_3COOH, 2-propanol, 80 °C, (iv) PCC, CH_2Cl_2, 2.5 hr stirring, R.T; (v) (**92**), $LiN(SiMe_3)_2$, dry THF, –10 °C; (vi) $LiAlH_4$, THF, 2hr at 100 °C; (vii) N_2H_4, $NaIO_4$, $CuSO_4$, CH_3COOH, 2-propanol, 80 °C; (viii) 1–phenyl–1H–tetrazole–5–thiol, PPh_3, DEAD, dry THF; (ix) MCPBA, $NaHCO_3$, CH_2Cl_2; (x) $LiN(SiMe_3)_2$, THF, –10 °C, N_2; (xi) $LiAlH_4$, THF, 2 hr reflux at 100 °C; (xii) N_2H_4, $NaIO_4$, $CuSO_4$, CH_3COOH, 2-propanol, 80 °C

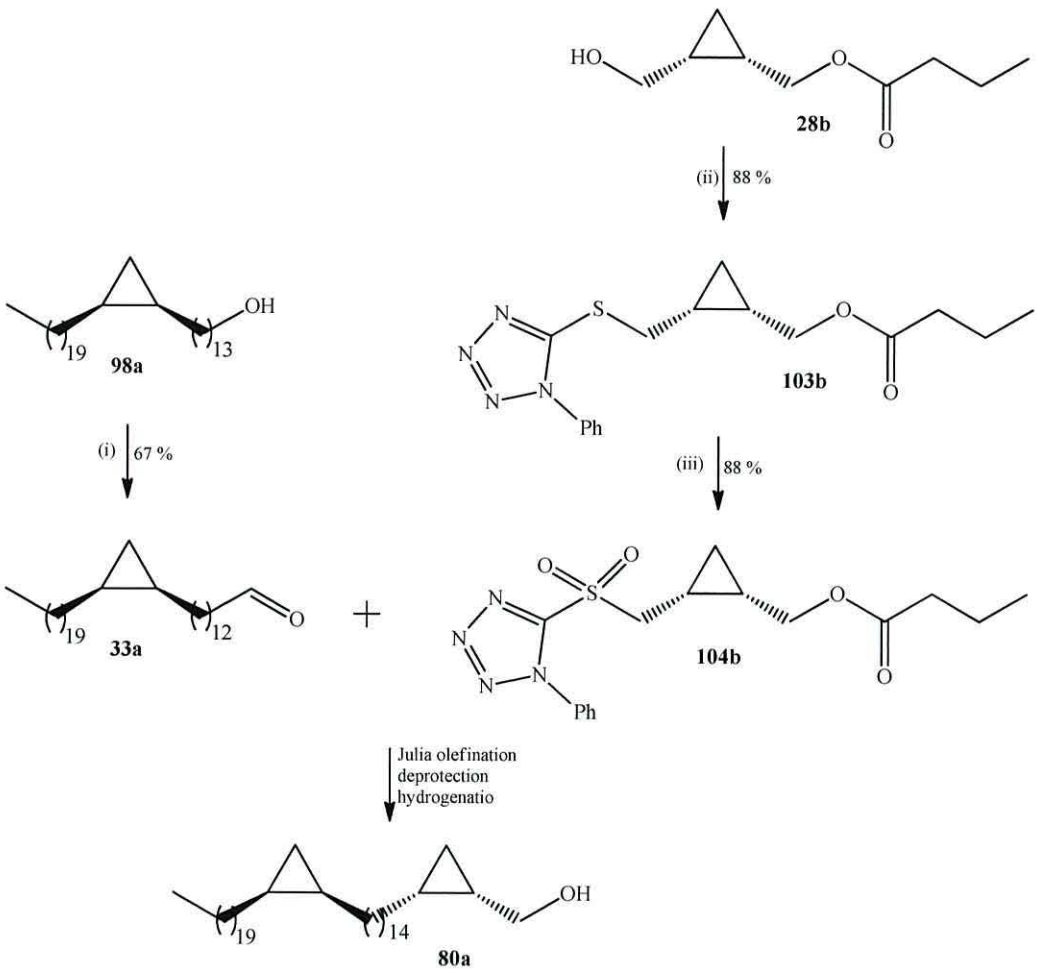
The spectroscopic data and physical properties for all the intermediates at all stages of the synthesis of **80b** were essentially identical to those obtained for **80a** except for the specific rotation values which were approximately equal and opposite. The specific rotation value for **80b** was $[\alpha]_D^{21}$ +7.59 (c 1.2, $CHCl_3$) whereas for **80a** it was $[\alpha]_D^{21}$ –7.32 (c 1.0, $CHCl_3$).

The two other diastereomers (**35a** and **35b**) of these dicyclopropane meromycolate intermediates have been prepared before. Al Dulayymi *et al*[163] reported the first one (**35a**) with a specific rotation of $[\alpha]_D^{22}$ –4.45 (c 1.17, $CHCl_3$) and a yield of 89 %. The second one (**35b**) has also been reported to have a specific rotation of $[\alpha]_D^{22}$ +8.33 (c 0.86, $CHCl_3$) and a yield of 63 %.[169] While the rotations of the diastereomers prepared in this work are equal and opposite as expected, those reported in literature are not and this could be as a result of instrumental error during measurement (**Table III**).

***Table III*: Meromycolate intermediates**

		Specific Rotation
	**80a**	$[\alpha]_D^{21}$ –7.32 (c 1.0, $CHCl_3$).
	**80b**	$[\alpha]_D^{21}$ +7.59 (c 1.2, $CHCl_3$)
	**35a**	$[\alpha]_D^{22}$ –4.45 (c 1.17, $CHCl_3$)
	**35b**	$[\alpha]_D^{22}$ +8.33 (c 0.86, $CHCl_3$)

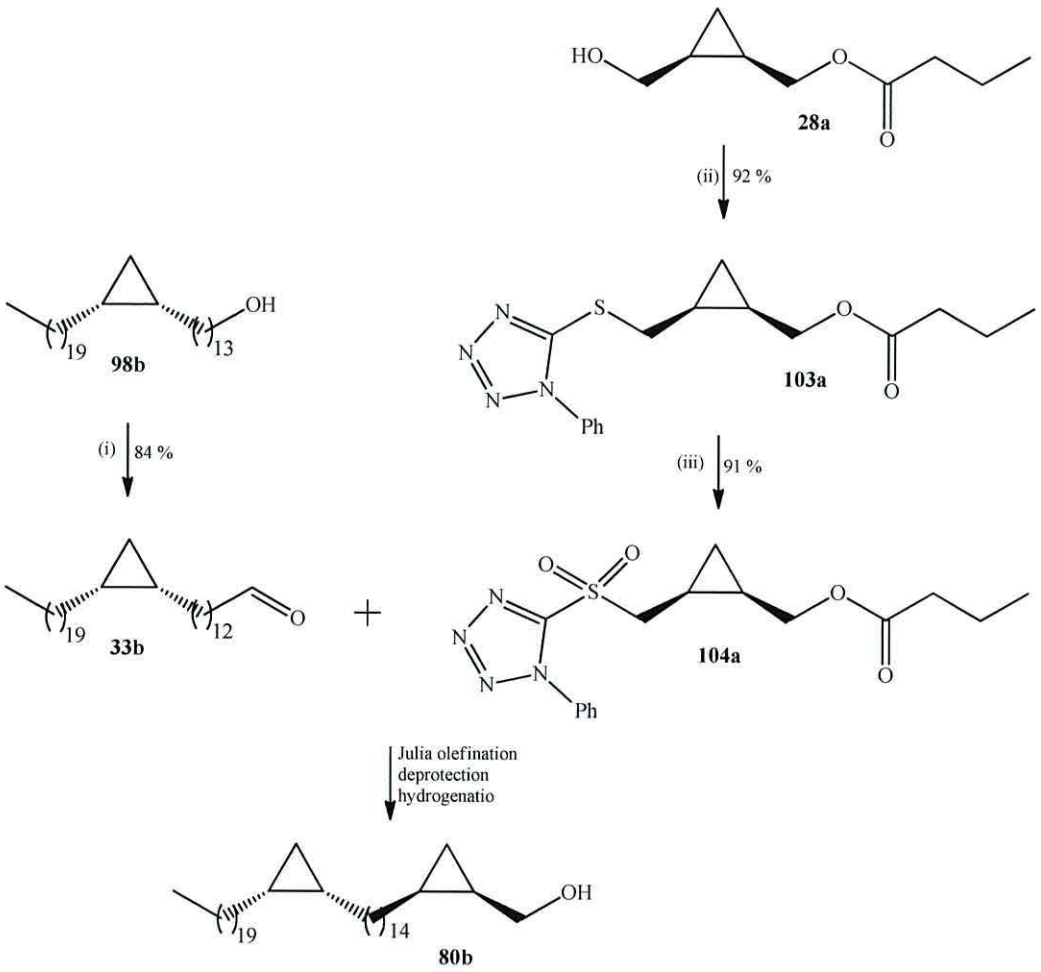
The preparation of these meromycolate intermediates (**80a** and **80b**) was also attempted via a slightly different route to those reported in previous literature.[163,169] Following general methods employed in this work, the alcohol **98a** derived in **Section 2.2.4.3** was converted into an aldehyde **33a** by PCC oxidation. Then the butyryloxymethyl cyclopropane ester **28b** from Section **2.1.2** was also converted into a sulfone **104b** via the corresponding sulfane **103b** by reacting it with 1–phenyl–1H–tetrazole–5–thiol. As in **Scheme 26a**, compound **33a** was coupled with **104b** in the presence of lithium bis(trimethylsilyl)amide to give an unsaturated ester, which was deprotected and then hydrogenated to give **80a** (**Scheme 27a**).



***Scheme 27a*: Coupling of the cyclopropane unit (Route2)**

Conditions and reagents: (i) PCC, CH_2Cl_2, 2.5 hr stirring, R.T; (ii) 1–phenyl–1H–tetrazole–5–thiol, PPh_3, DEAD, dry THF; (iii) MCPBA, $NaHCO_3$

The synthesis of the enantiomer **80b** was carried out precisely as **80a** though working with the enantiomers of opposite stereochemistries. Compound **33b** was prepared from **98a** via the same reaction steps that were involved in the preparation of **33a**. Likewise, **104a** was prepared from **28a** via **103b** (**Scheme 27b**).



***Scheme 27b*: Coupling of the cyclopropane unit (Route2)**

Conditions and reagents: (i) PCC, CH_2Cl_2, 2.5 hr stirring, R.T; (ii) 1–phenyl–1H–tetrazole–5–thiol, PPh_3, DEAD, dry THF; (iii) MCPBA, $NaHCO_3$

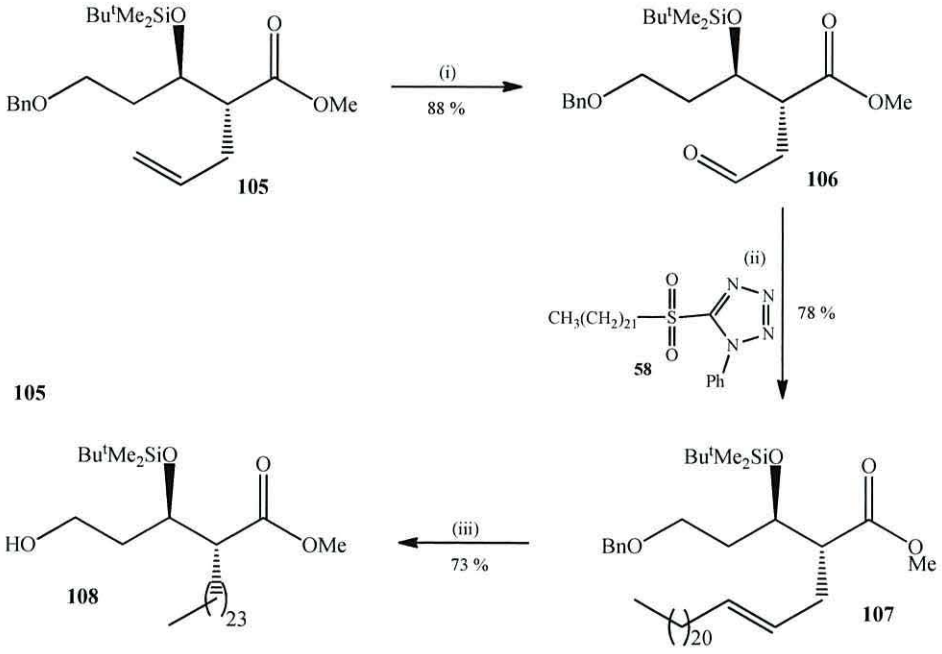
The data for the aldehyde enantiomers **33a** and **33b** were identical and matched those reported in the literature.[163,169] The sulfone enantiomers **104a** and **104b** also had identical characterisation which corresponded to the literature for the latter.[164] However, **103a** and **104a** were prepared for the first time in this work. The crude yields obtained at the final steps for compound **80a** and compound **80b** were 77 % and 86 % respectively, but the compounds were not pure. Although the spectroscopic data for both meromycolate intermediates were generally the same as the data of the intermediates obtained from route 1, the ^1H NMR spectra in this instance showed an aldehyde impurity and this proved very difficult to separate by column chromatography. Consequently the first route discussed in this work was preferred.

2.4 Preparation of a complete mycolic motif

An α-allyl-β-hydroxy ester **105** that had been prepared from L-aspartic acid (as in Koza *et al*)[177] was chain extended into a whole mycolic motif by oxidation at the α-position, followed by another chain extension at the meromycolate position.

2.4.1 Extension of the α-alkyl chain

Chain extensiion was carried out following standard methods (**Scheme28**).[177]



***Scheme 28*: Chain extension of the α-allyl-β-hydroxy ester**

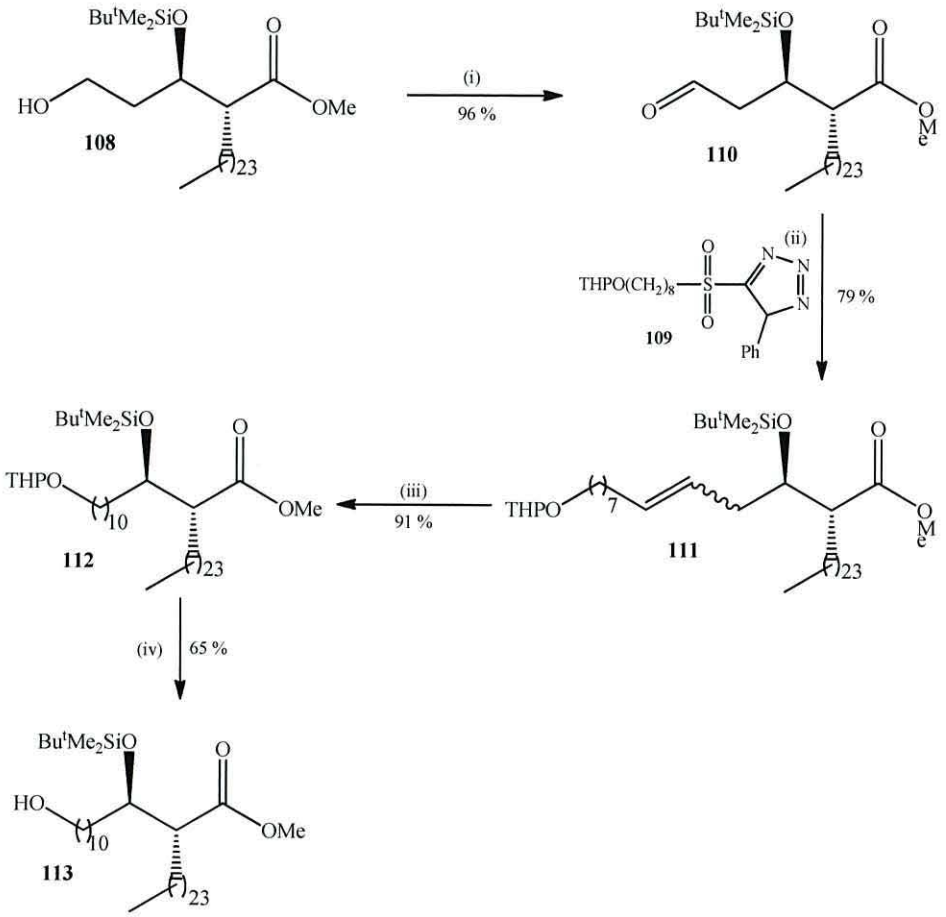
Conditions and Reagents: (i) 2,6-Lutidine, OsO_4 in 2-methyl-2-propanol, $NaIO_4$; (ii) (**58**), $LiN(SiMe_3)_2$, dry THF, -8°C, N_2; (iii)H_2/Pd, IMS/THF (1:1)

The alkene **105** was converted into an aldehyde **106** by oxidative cleavage with osmium tetraoxide and $NaIO_4$ in the presence of 2,6-lutidine. The 2,6-lutidine served as a base to inhibit side reactions and to improve the yield which was 88 %. According to the reaction mechanism proposed by Borhan *et al*,[203] the oxidative cleavage of alkenes with OsO_4 involves the formation of an osmate, which undergoes further oxidation by $NaIO_4$ to yield an intermediate which re-generates OsO_4 to produce two aldehydes. The aldehydes are unstable and can undergo further oxidation to yield carboxylic acids.

The ^{1}H NMR spectrum of the dark coloured crude product **106** included a singlet at δ 9.91. Subsequently, the compound was purified by column chromatography and promptly used for the next reaction in order to avoid decomposition. The purified aldehyde **107** was coupled with sulfone **58**[177] in the presence of a base in a Julia reaction to give a chain extended alkene compound **107** as a mixture of *E/Z* isomers. This was confirmed by the ^{1}HNMR spectrum, which showed multiplets at δ 5.27 – 5.17 and at δ 5.40 – 5.32, due to the double bond. However the double bond signals disappeared on hydrogenation. Hydrogenation of the alkene **107** was carried out in a solution of IMS/THF, under hydrogen gas and palladium catalyst to give **108** (**Scheme 28**). The reaction was allowed to go on for 3 days in order to ensure complete saturation and debenzylation of the final product. In the ^{1}H NMR spectrum of the derived alcohol **108**, there was a triplet of doublets at δ 4.29 for two protons next to the OH group and this was confirmed by the O–H band at ν_{max} 3435 cm^{-1} in the IR data.

2.4.2 The chain extension of the mycolic motif

To modify the derived compound **108** for coupling to the meromycolates prepared above and form a full mycolic acid, another chain extension was carried out at the meromycolate position in accordance to standard methods (**Scheme 29**).[177]



***Scheme 29*: Chain Extension**

Conditions and Reagents: (i) PCC, CH_2Cl_2, RT for 2hrs; (ii) (**109**), $LiN(SiMe_3)_2$, dry THF, -10°C, N_2; (iii) H_2/Pd, IMS/THF (1:1); (iv) PPTSA, MeOH, THF, H_2O, refluxing at 45°C

The alcohol **69** was oxidized to an aldehyde **110** using PCC in dichloromethane. In order to obtain the desired chain length between the meromycolate unit and mycolic motif, an eight carbon chain tetrahydropyran sulfone **109** was coupled with the aldehyde **110** and base in a modified Julia reaction, to give mixture of *E*/*Z* isomers **111.** The purified product gave a ^{1}H NMR spectrum which revealed peaks for unsaturation at δ 5.48 – 5.42 (2H, m). The TLC of the crude product also showed two very close spots, which were practically impossible to separate by column chromatography. As before, the alkene mixture **111** was hydrogenated in an atmosphere of hydrogen, in the presence of palladium catalyst to give a previously unsynthesized tetrahydropyran protected motif **112**. This was confirmed by characteristic ^{1}H NMR peaks indicative of the tetrahydropyran ring, which appeared at δ 3.26–3.23 (1H, m) and δ 3.67–3.61 (1H, m) for the protons adjacent to the oxygen atom, and at δ 4.33 (1H, bt, *J* 2.8 Hz) for the acetal proton. As expected, these peaks disappeared

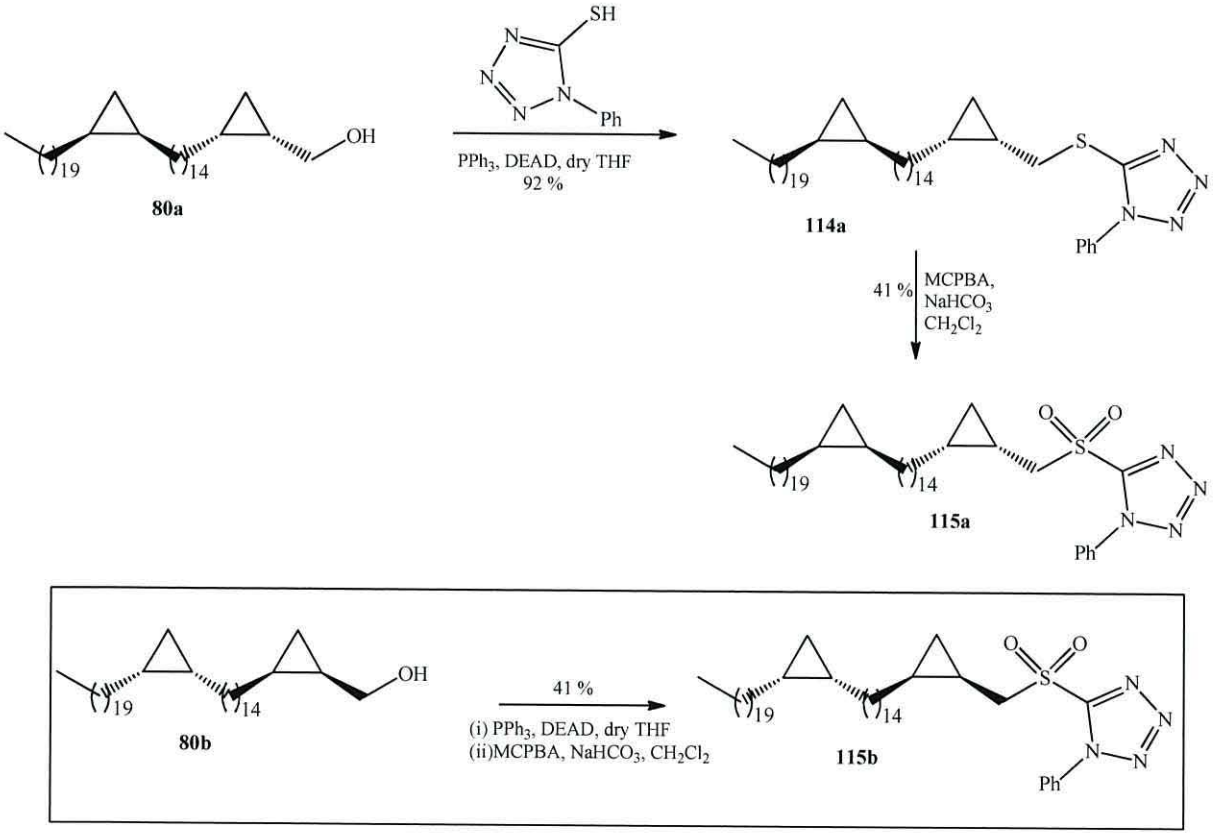
after the extended chain was deprotected in order to convert the tetrahydropyran group into the desired alcohol **113**.

2.5 Final coupling for the formation of full mycolic acids

The coupling reactions for the full mycolic acids were preceded by converting the meromycolates obtained above into their corresponding sulfones.

2.5.1 Preparation of the meromycolate sulfones

As described in Section **2.3.1**, the meromycolate intermediate **80a** was reacted with 1–phenyl–1H–tetrazole–5–thiol in the presence of diethyl azodicarboxylate and triphenylphosphine in dry THF, to give a sulfane **114a**. This was in turn oxidized to the corresponding sulfone **115a** using meta-chloroperbenzoic acid and sodium hydrogen carbonate in dichloromethane (**Scheme 30**). Likewise, the meromycolate enantiomer **80b** was converted into a sulfone **115b** via the corresponding sulfane **114b** with an overall yield of 41 %.



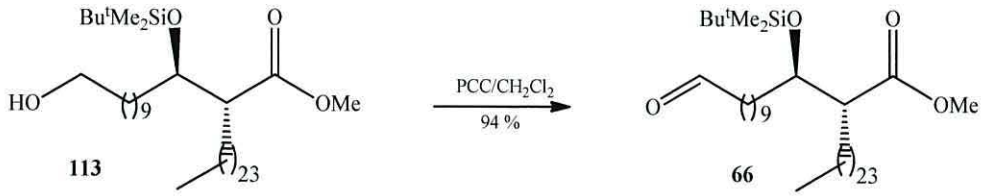
***Scheme 30*: Preparation of meromycolate sulfones**

The spectroscopic data for the sulfane **114a** and the sulfone **115a** were similar. In the ^{1}H NMR spectrum of the sulfone **115a**, peaks corresponding to two sets of cyclopropane protons appeared at 0.97–1.05 (1H, m), 0.65 (2H, m), 0.57 (1H, dt, *J* 4.1, 8.2 Hz), 0.25 (1H, q, *J* 5.6 Hz) and δ –0.33 (1H, q, *J* 5.0 Hz) and those for the phenyl protons appeared as multiplets at δ 7.71–7.60 (5H, m). The signals for the two protons next to sulfur were revealed as doublets of doublets at δ 3.98 (1H, dd, *J* 5.7, 14.9 Hz) and δ 3.57 (1H, dd, *J* 9.2, 14.5 Hz) as opposed to the ones for the sulfane **115a** that gave just one doublet at δ 3.49 (2H, d, *J* 7.9 Hz). The enantiomeric meromycolate sulfones (**115a** and **115b**) showed two sets of signals corresponding to the cyclopropane rings. Their ^{1}H NMR, ^{13}C NMR and IR spectra were identical and were also similar to those of their diastereomers (**36a)** and (**36b**) reported earlier.[169]

The specific rotation value for the sulfane **114a** was $[\alpha]_D^{20}$ –2.14 (c 1.7, $CHCl_3$) but for the sulfone **115a**, the value was $[\alpha]_D^{20}$ +16.67 (c 1.2, $CHCl_3$). There was a similar but opposite occurrence in the rotation values of the enantiomers, which were $[\alpha]_D^{20}$ +1.48 (c 1.4, $CHCl_3$) and $[\alpha]_D^{20}$ –18.37 (c 1.4, $CHCl_3$) for the sulfane **114b** and sulfone **115b** respectively.

2.5.2 Coupling of the meromycolate to the mycolic motif

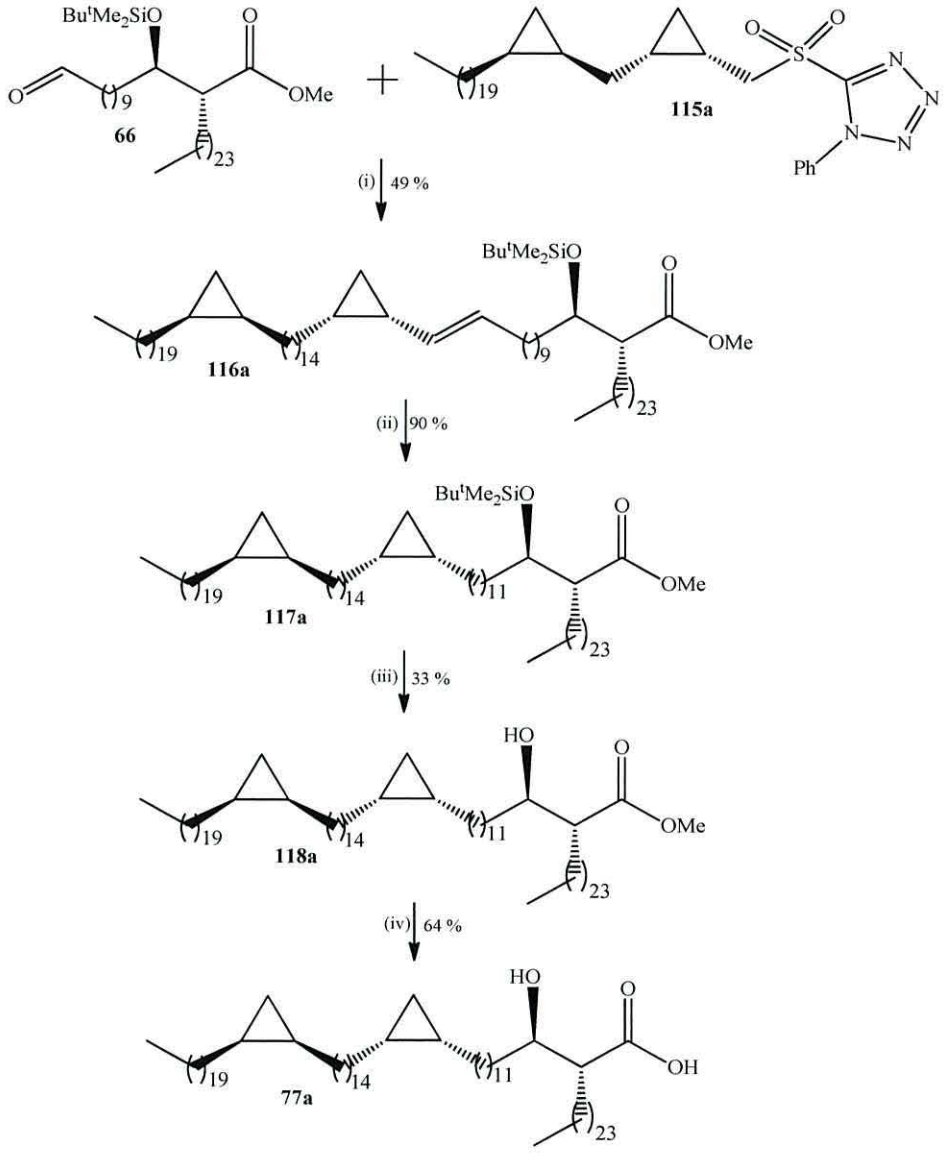
Prior to the coupling reaction, the alcohol **113** (from Section **2.4.2**) was converted into aldehyde **66** (**Scheme 31**).



***Scheme 31*: Oxidation to an aldehyde**

Oxidation was carried out using PCC in dichloromethane at room temperature and was confirmed by the ^{1}H NMR spectrum of the product, which showed a peak corresponding to

CHO at δ 9.77, but none for an OH group. The coupling was then done following standard methods.[163]



***Scheme 32a*: Final coupling for the formation of full mycolic acid (77a)**

Conditions and Reagents: (i) $LiN(SiMe_3)_2$, dry THF, -10°C, N_2; (ii) MeOH/THF, N_2, 0–5 °C, CH_3COOH, $KCO_2N{=}NCO_2K$; (iii) HF-pyridine, Pyridine, Dry THF, 40 °C; (iv) $LiOH.H_2O$, MeOH, H_2O, THF, 45 °C

The derived aldehyde **66** was reacted with the sulfone **115a** and base and subsequently hydrogenation was carried out, followed by deprotection and hydrolysis to give the final product **77a** via **116a**, **117a** and **118a** respectively **(Scheme 32a)**.

2.5.2.1 The Julia-Kocienski olefination of the mycolic motif

The modified Julia-Kocienski olefination, reaction was carried out as before with the aldehyde **66** and the sulfone **115a** (prepared in **2.4.3**) using lithium bis(trimethylsilyl)amide as base, to give an alkene **116a** (**Scheme 32a (i)**). The ^{1}H NMR spectrum of the product showed double bond signals at δ 5.20 – 5.15 and δ 5.54 – 5.38 which confirmed that the olefination reaction had taken place.

2.5.2.2 Hydrogenation of the unsaturated ester

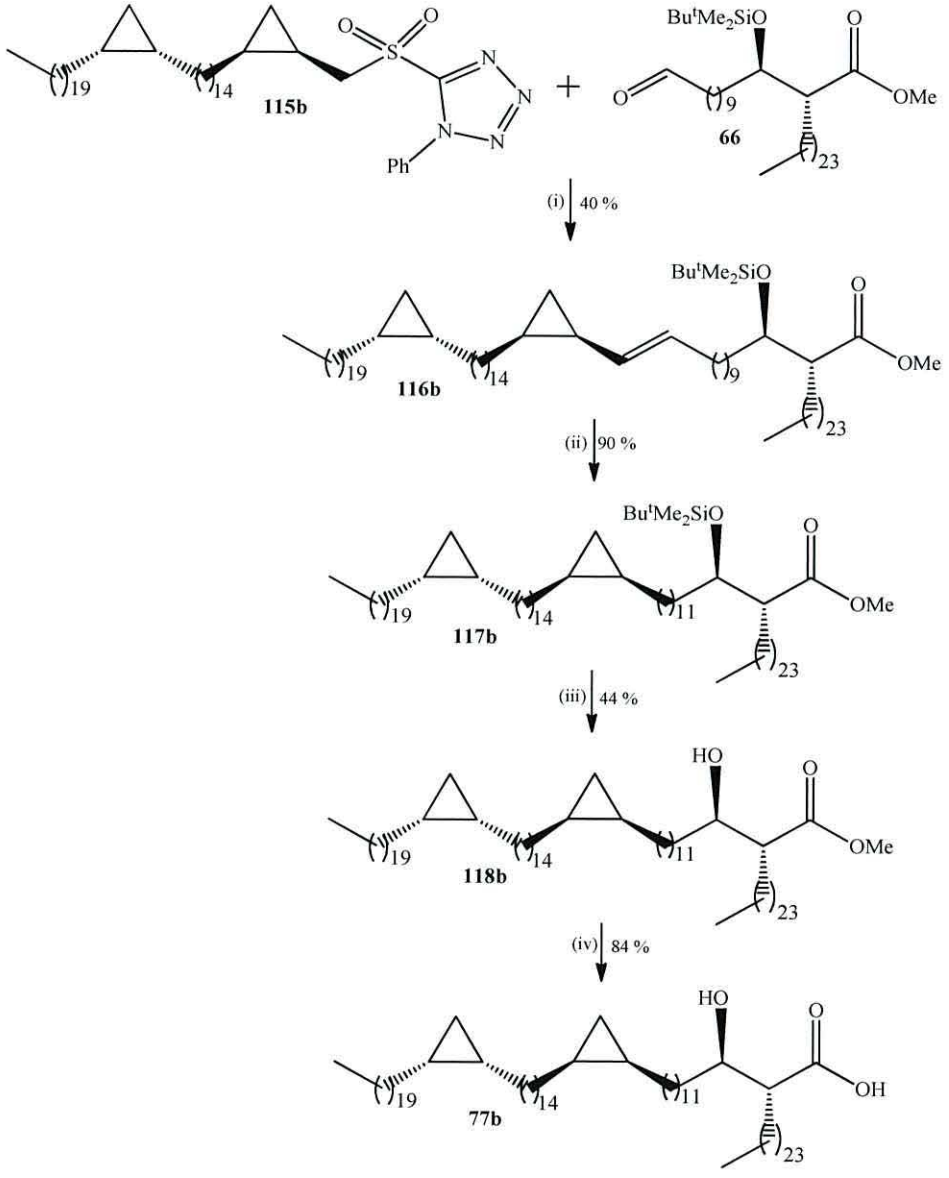
Hydrogenation of **116a** led to the saturation of the double bond to give **117a** (**Scheme 32a (ii)**) which retained a signal for three protons at δ 3.64 corresponding to the OMe, as well as signals at δ 0.02, δ 0.05 and δ 0.84 for the *tert*-butyldimethylsilyl group, but had lost the signals for the double bonds. In order to avoid opening of the cyclopropane this was not performed by the catalytic reduction with palladium and hydrogen. Consequently, the alkene was treated with di-imide, in this case generated from dipotassium azodicarboxylate and acetic acid in MeOH and THF. The dipotassium azodicarboxylate was prepared from potassium hydroxide and azodicarbonamide and is thermally unstable and so had to be handled under cold conditions prior to being used.[177]

2.5.2.3 Deprotection and Hydrolysis of the ester

The *tert*-butyldimethylsilyloxy group on compound **117a** was converted into a β-hydroxyl group by treating with HF.pyridine, giving a protected mycolic acid **118a** (**Scheme 32a (iii)**). This deprotection was carried out in a clean dry polyethylene vial and required careful handling of the reagent. A less sensitive method,[204] which involves the use of acetic acid, water, THF and HCl (2N) was not an option because of the reactivity of the cyclopropane ring to acid. Also deprotection of the *tert*-butyldimethylsilyl group with TBAF has been reported to be unsuccessful in previous work.[177] The NMR spectra of the product did not show any signals for the *tert*-butyldimethylsilyl group and other signals were as expected. Hydrolysis of the methyl ester in compound **118a** to an acid group was done by using LiOH to obtain the final α-mycolic acid **77a** with 64 % yield in this final step (**Scheme 32a (iv)**).

The second α-mycolic acid diastereomer **77b** was likewise obtained by repeating the same procedures for the preparation of **77a** in Scheme **32a**, but using intermediate compounds of

opposite stereochemistry. A modified Julia-Kocienski olefination, reaction was carried out as before with the aldehyde **66** and the sulfone **115b** (prepared in Section **2.4.3**) using lithium bis(trimethylsilyl)amide as base; this gave an alkene **116b**. Subsequently, hydrogenation of **116b** led to the saturation of the double bond to give **117b** and then the *tert*-butyldimethylsilyloxy group on compound **117b** was converted into a β-hydroxyl group by treating with HF.pyridine which gave a protected mycolic acid **118b**. In the final reaction, hydrolysis of the methyl ester in compound **118b** to an acid group was done by using LiOH. As a result, the α-mycolic acid diastereomer **77b** was obtained in a yield of 84 % in the final step (**Scheme 32b**).

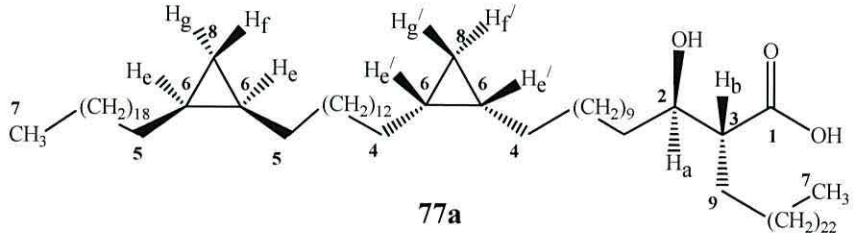


***Scheme 32b:* Final coupling for the formation of the full mycolic acid (77b)**

Conditions and Reagents: (i) $LiN(SiMe_3)_2$, dry THF, -10°C, N_2; (ii) MeOH/THF, N_2, 0–5 °C, CH_3COOH, $KCO_2N{=}NCO_2K$; (iii) HF-pyridine, Pyridine, Dry THF, 40 °C; (iv) $LiOH.H_2O$, MeOH, H_2O, THF, 45 °C

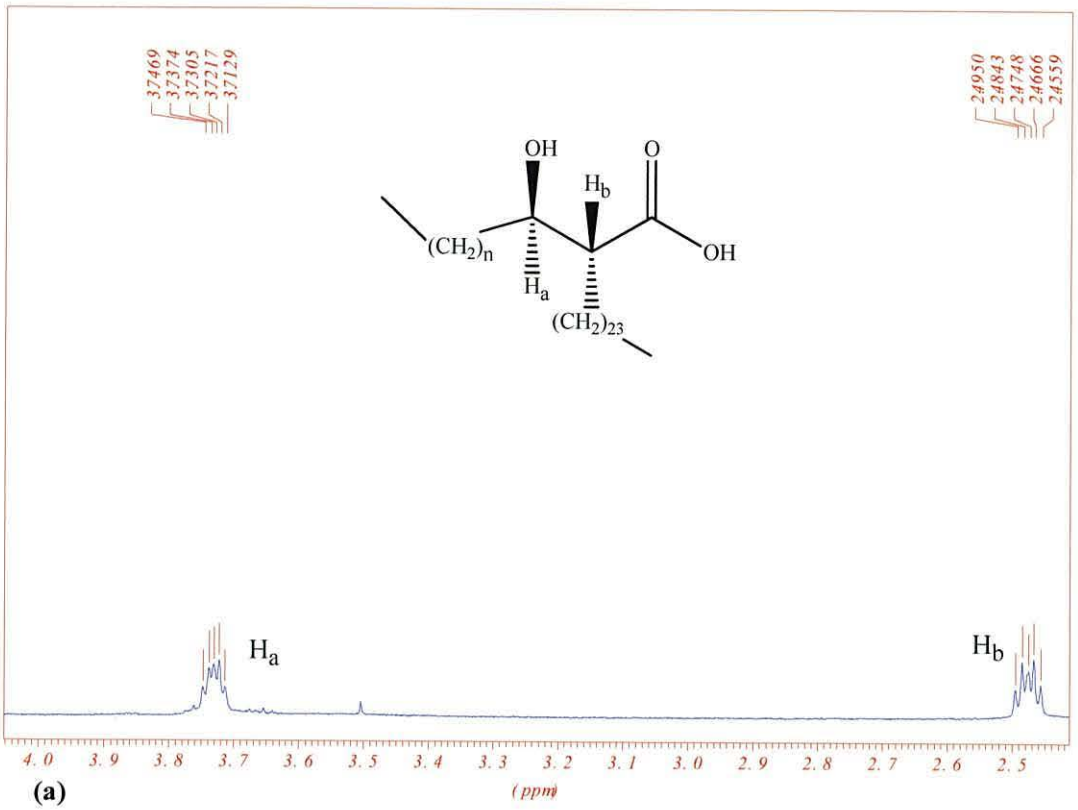
The ^{1}H NMR, ^{13}C NMR and the IR spectra which confirmed the structures of the novel alpha mycolic acids (**77a** and **77b**) were essentially identical, and corresponded to those reported in literature for the other two diastereomers.[163,169] For example, the ^{1}H NMR spectrum of **77a** (See **Table IV**) showed the peak for the proton on the β-position at δ 3.73 (1H, td). The peak corresponding to the proton on the α-position appeared at δ 2.48 (1H, td) and those for cyclopropane protons were revealed at δ –0.33 (2H, q, *J* 4.93 Hz), δ 0.57 (2H, dt, *J* 4.1, 8.2 Hz), δ 0.66 – 0.64 (4H, m). Also in the ^{13}C NMR spectrum for **77a**, the carboxylic acid signal was at δ 178.5, followed by the carbon on the beta hydroxy acid position resonating at δ 72.1. The α-carbon resonated at δ 50.7. The carbons adjacent to the cyclopropanes showed signals at δ 31.9 and δ 35.5 whereas the long chain $-CH_2$ carbons ranged from δ 30.2 – 29.7 Signals for the cyclopropane carbon atoms came between δ 15.8 and 10.9. Furthermore IR spectrum of **77a** showed the alcohol peak at 3317 cm^{-1} and the carbonyl at 1464 cm^{-1}.

***Table IV*: Selected ^{1}H and ^{13}C NMR signals for compound 77a**



77a

Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_a	3.73	td	4.8, 7.9	1	178.5
H_b	2.48	td	5.4, 8.9	2	72.1
2 x CH_3	0.89	t	6.9	3	50.7
2 x (H_e and $H_e^{/}$)	0.66– 0.64	m		4	36.6
H_f and $H_b^{/}$	0.57	dt	4.1, 8.2	5	31.9
H_a and $H_a^{/}$	– 0.33	q	4.9	6	15.8
				7	14.1
				8	10.9



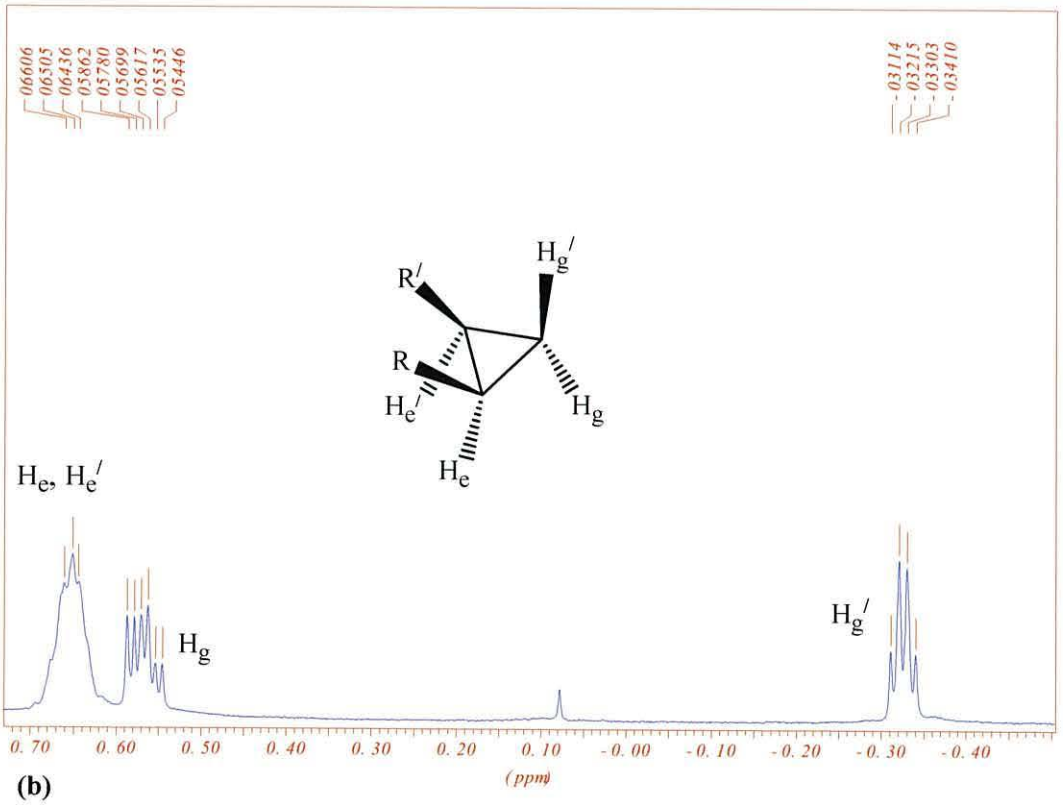
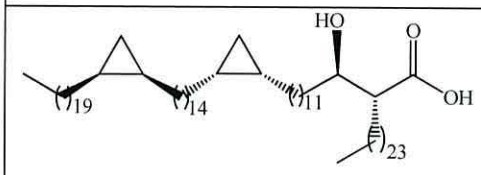
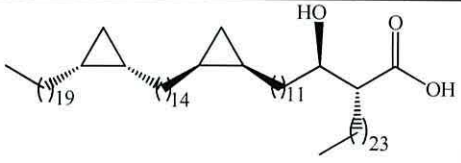
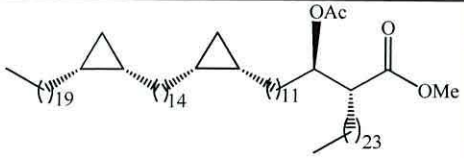
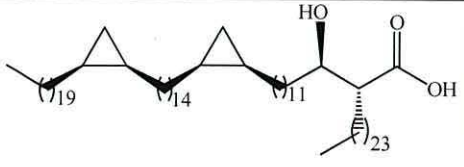


***Figure: 19:* Selected signals in the ^{1}H NMR spectrum of (77a)**

(a) δ 4.0 to 2.46 (b) δ 0.66 to –0.34

The specific rotation for **77a** was recorded as $[\alpha]_D^{21}$ +2.50 (c 1.4, $CHCl_3$) and the melting point was 53 °C, whereas for **77b** the rotation was $[\alpha]_D^{21}$ +2.46 (c 1.4, $CHCl_3$) and melting point was 52 – 53 °C. Additionally, the MALDI MS for **77a** gave an $[M+Na]^+$ = 1159.9 (expected value 1160.2), while **77b** gave an $[M+Na]^+$ = 1159.7. For the previously synthesized diastereomer **42b**, which was obtained in a yield of 93 % in the final step, the specific rotation was, $[\alpha]_D^{24}$ +2.01 (c 1.2, $CHCl_3$).[169] The specific rotations values were essentially the same which shows that the chirality of the *cis*-cyclopropane rings for **77a**, **77b** and **42b** does not make a significant contribution to the specific rotations (**Table V**). However, the specific rotation observed in the case of **42a** was $[\alpha]_D^{22}$ +4.43 (c 1.2, $CHCl_3$).[167b] Although the rotation of **42a** is in the same direction as the other three diastereomers, there is a difference in the magnitude of the rotation which might be due to the purity of the compound.

***Table V*: Specific rotations of α-mycolic acid diastereomers**

		Rotation $[\alpha]_D^T$	References
	**77a**	$[\alpha]_D^{21}$ +2.50 (c 1.4, $CHCl_3$)	This work
	**77b**	$[\alpha]_D^{21}$ +2.46 (c 1.4, $CHCl_3$)	This work
	**42a**	$[\alpha]_D^{22}$ +4.43 (c 1.2, $CHCl_3$)	167b
	**42b**	$[\alpha]_D^{24}$ +2.01 (c 1.2, $CHCl_3$)	169

The MALDI-MS of the alpha mycolic acids gave molecular ion patterns (**Figure 20** and **21**) which corresponded to the major isomer of the natural mixture of protected alpha mycolic acid homologues (**Figure 22**).

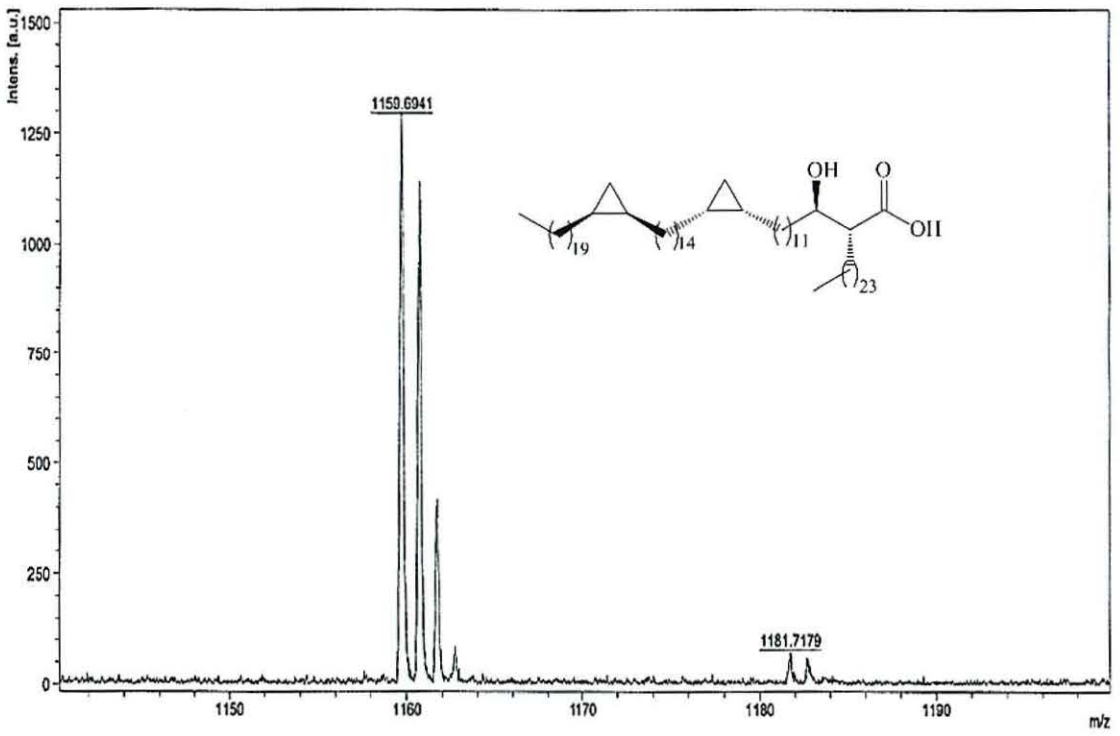


***Figure 20*: MALDI-MS of alpha mycolic acid diastereomer (77a)**

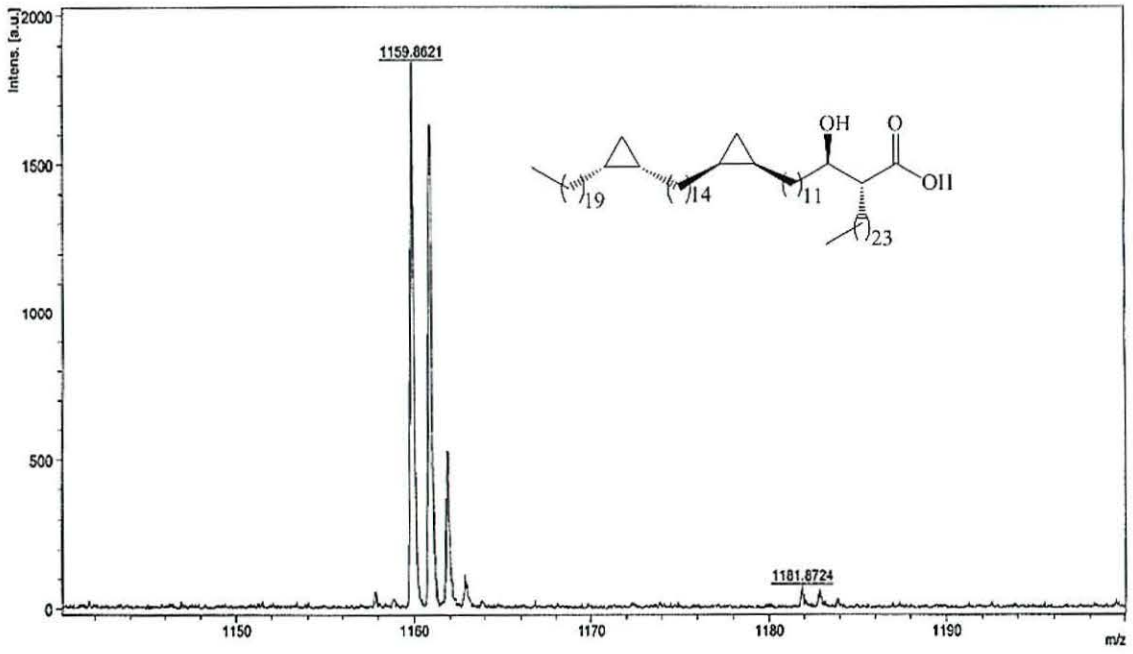


***Figure 21*: MALDI-MS of alpha mycolic acid diastereomer (77b)**

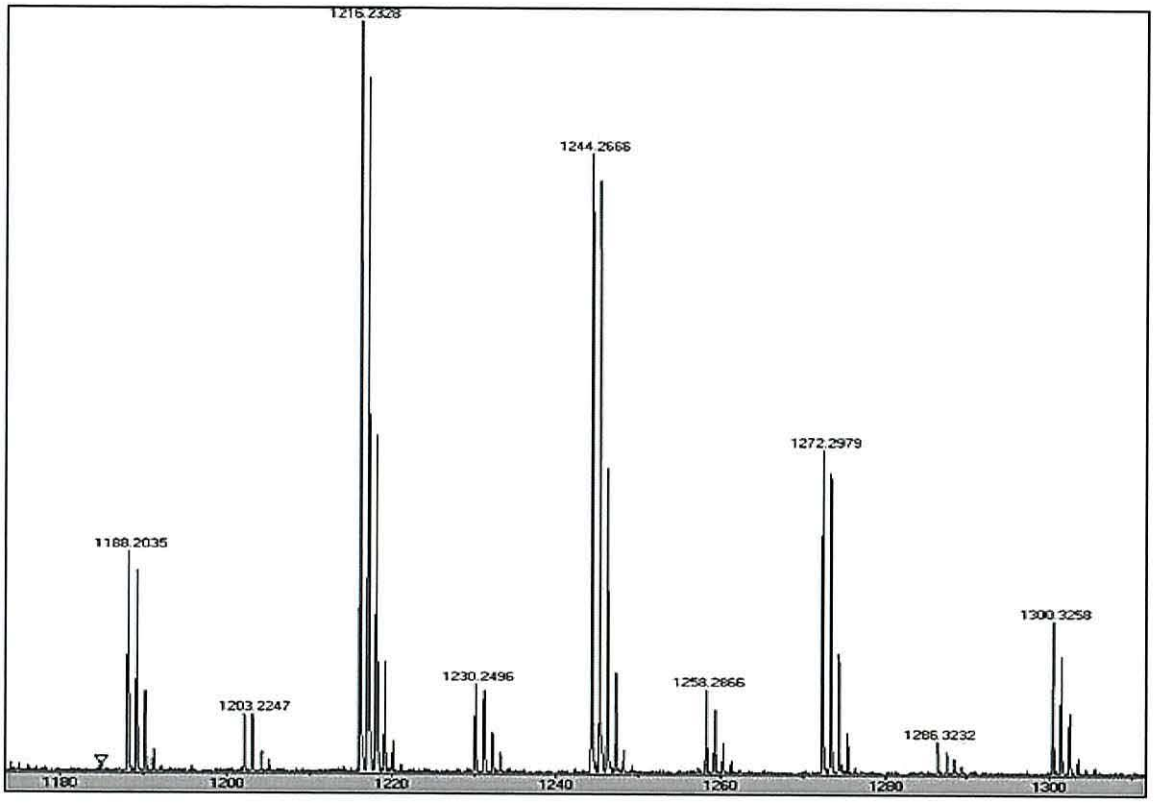


***Figure 22*: MALDI-MS of a natural mixture of protected α-mycolic acid homologues**[163]

2.6 Tests for antibody activity

Biological assays were carried out to study the recognition of the mycolic acids by lipid antibodies present in the serum of patients known to be infected with tuberculosis. The measurement of the response was made using enzyme-linked immunosorbent assay (ELISA). In ELISA, the surface of the well is coated with the mycolic acid (antigen), with casein/PBS buffer being used to block any free non-specific binding sites. The coated surface is then treated with sera containing specific antibodies, which may or may not bind to the antigen. Excess antibodies are removed by washing the surface with casein/PBS buffer, leaving only bound antibodies on the ELISA plate. Subsequently, a secondary antibody, which contains a peroxidase enzyme, is added to the wells, and this will in turn bind to any primary antibodies that are present. The wells are again washed with casein/PBS to remove any unbound secondary antibody, and a colour reagent (OPD/H_2O_2 solution) is added. Depending on the amount of secondary antibody present this gives a detectable colour, the absorbance of which is measured at 492nm. In ELISA tests, the proportion of actual TB positive sera which are correctly identified by the antigen to the total TB positive sera is refered to as the selectivity, while the proportion of the TB

negatives which are correctly identified to the total TB negative sera is refered to as the specificity.

ELISA tests have been carried out by Dr. A. Jones on using each of the four dicyclopropane α-mycolic acid diastereomers **77a**, **77b**, **42a** and **42b** as antigens and a set of sera from TB-indigenous countries provided by the WHO and diagnosed as TB-positive or TB-negative based on a range of assays. In line with previous tests conducted by Beukes *et al*,[191] the ELISA test results in this work indicated that their recognition by TB antibodies was generally low as shown in their average absorbance which was below 1.5. By selecting appropriate 'cut-off' values for the absorbance for each individual mycolic acid, the selectivity and specificity for all four diastereomers was also calculated; these were found to fall below the accepted minimum WHO standard of 85 % (**Table VI**).[21]

Table VI: ELISA data for dicyclocpropane α-mycolic acid diastereomers

	(77b)	**(77a)**	**(42a)**	**(42b)**
average absorbance for TB positive samples	0.73	1.49	0.76	0.73
Average absorbance for TB negative samples	0.49	0.87	0.58	0.61
selectivity %	14	43	14	29
specificity %	91	94	88	85

The average absorbance for **77b** appears to be higher than those for the other diastereomers (**Figure 23**). Compound **77b** also showed a higher percentage selectivity and specificity with respect to the other compounds. In addition, there appears to be a marked difference between the average abosorbance for the negative sera and the positive sera tested on compound **77b**, whereas for the other diastereomers the average absorbance for the positive sera and the negative sera were similar. This is an indication that **77b** may be more viable for making biological sensors for TB detection. It is also notable that there is a significant difference in the biological activities of **77a** and **77b** prepared in this work. In contrast, the other two diastereomers **42a** and **42b** have showed the same responses. It appears that the stereochemistries of the cyclopropane rings do contribute, albeit to a small

extent, to the antigenic properties of mycolic acids. Since the highest responses are for **77a**, this may provide some evidence that this has the natural stereochemistry.[191]

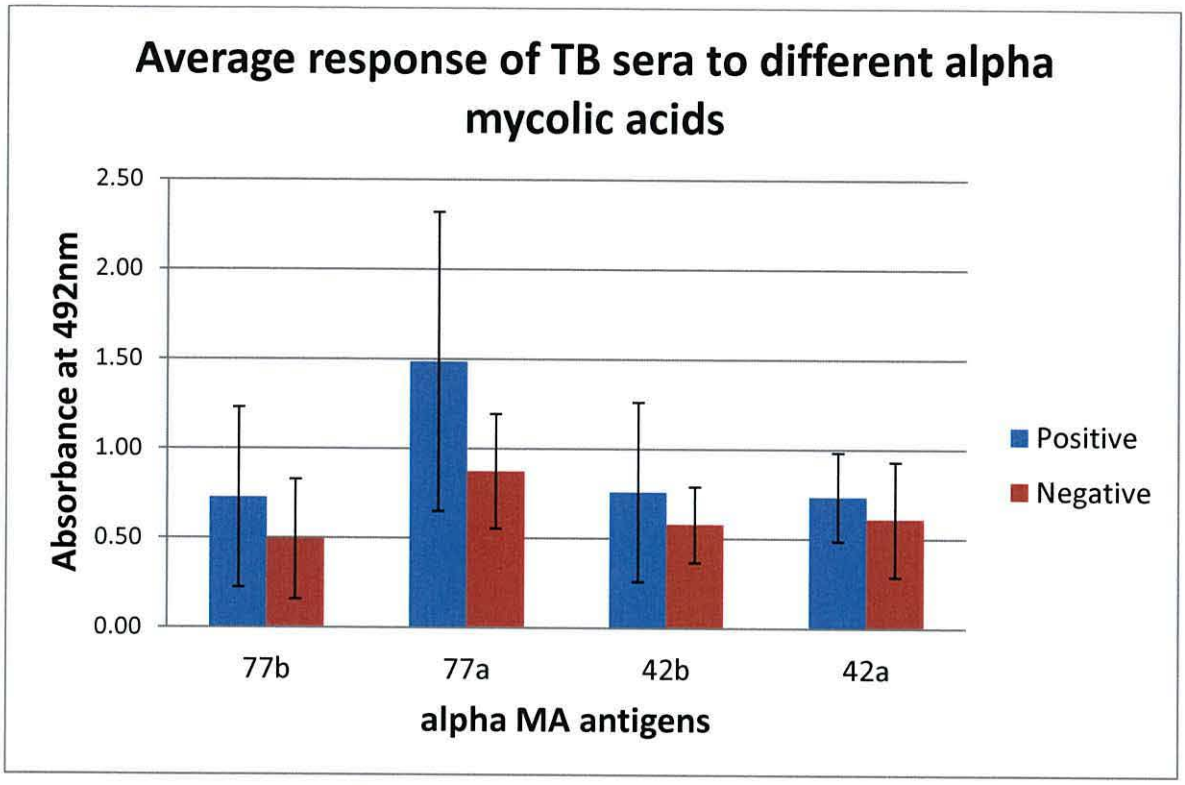


Figure 23: ***ELISA signals of antibody binding to both TB positive and TB negative sera for the α-mycolic acids diastereomers.*** (Data and standard deviations are for 14 TB-positive and 37 TB-negative samples from indigenous populations provided by WHO)

Further testing and assays of the effects of these dicyclopropane α-mycolic acid diastereomers on a range of cytokines and chemokines involved in the immune system are expected to be carried out in the near future.

Chapter 3

3 Method development for the synthesis of the mycolic motif

This part of the project involved the development of a new method for the synthesis of the α-alkyl-β-hydroxy fragment **78** of mycolic acids **(Figure 24)**, which is also known as the mycolic motif. The preparation of this unit is almost certainly the most tedious part of mycolic acid synthesis and as such necessitates some improvement. Improving this synthetic method is also fundamental as the α-alkyl-β-hydroxy unit is common to all mycolic acids and their derivatives such as cord factors.

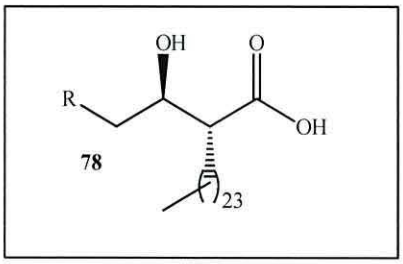


***Figure 24*: The α-alkyl-β-hydroxy fragment of mycolic acids**

Two basic methods relating to the preparation of the α-alkyl-β-hydroxy unit with *R,R* stereochemistry at the chiral α- and β- carbon atoms have so far been published.[176,205] The first one involves a direct alkylation of a long chain acid at the α-position relative to the carboxylic group. In the second method, a short chain allylation was initially carried out, followed by a further elongation by modified Julia-Kocienski reaction in order to achieve the desired carbon chain length. In the first method by Al Dulayymi *et al*, (2-benzeneoxyethyl) oxirane **38** was used as a chiral intermediate (see page 28). This was prepared from commercially available *R*-aspartic acid **37a** (which the same as *D*-aspartic acid) based on a method developed by Frick *et al*,[205,167a] The second method was put forward by Toschi *et al*[176] in which *cis*-olefin (derived from very simple materials such as 1,12-decanediol) was the starting material (see page 32). However, the first method was not repeatable and reliable and as such the second method was preferred. Further work was carried out by Koza *et al* in which *L*-aspartic acid **37b** was chosen as the starting material for the synthesis of the (2-benzeneoxyethyl)oxirane intermediate **38**. Then a short chain Fräter allylation followed by a further Julia-Kocienski chain extension was carried out on the α-position[177] (see page 33). This was achieved in 17 steps with an overall yield of 17 %

(**Figure 25**). In this project, synthetic approaches were examined using alternative routes starting from a cheaper starting material which was *L*-Malic acid **119**. These routes involved fewer steps.

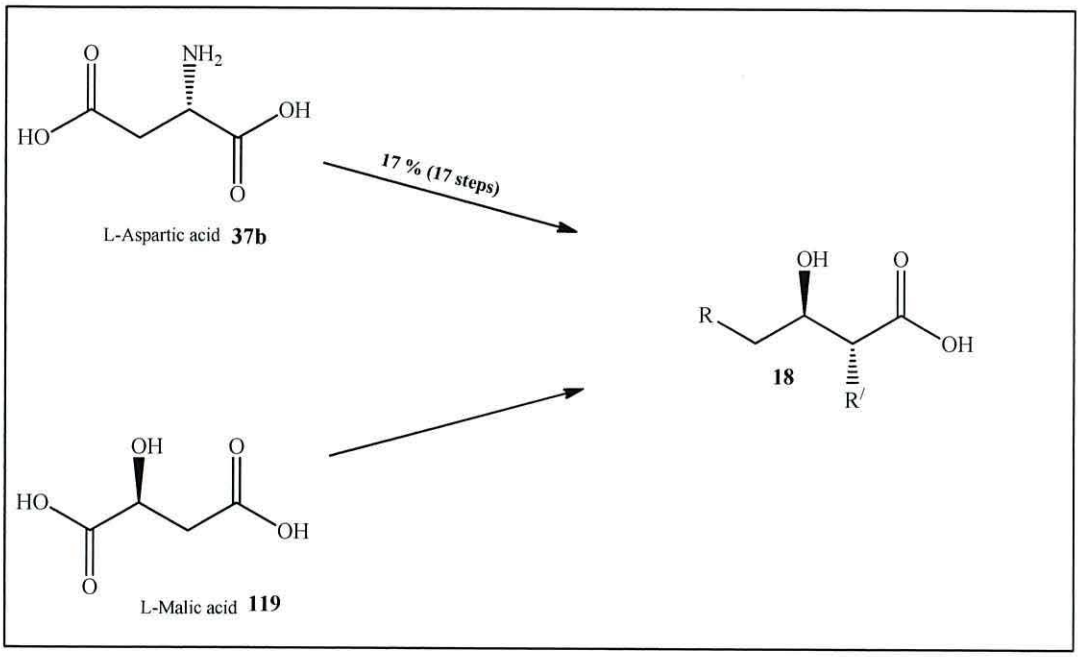
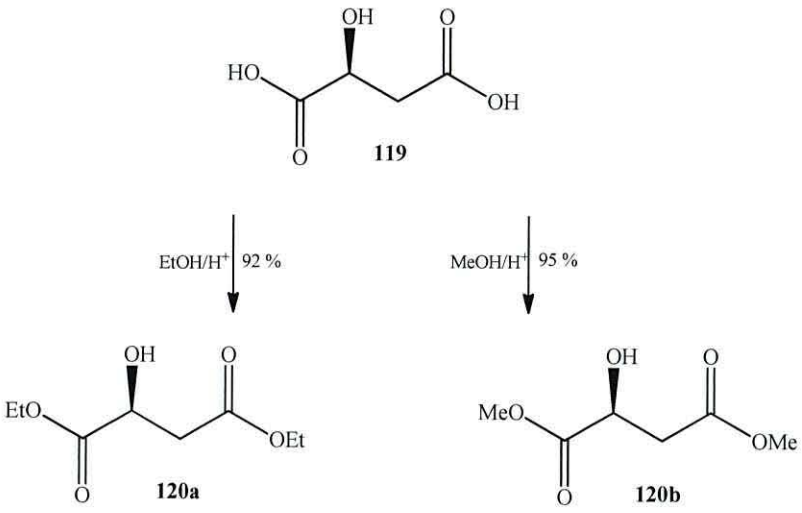


***Figure 25**: Alternative starting materials for the synthesis of the α-alkyl-β-hydroxy unit*

3.1 Esterification of L-malic acid to diesters[206,207]

Following general methods,[206,207] the *L*-malic acid **119** was esterified to diesters **120a** and **120b** in the presence of concentrated H_2SO_4, using EtOH and MeOH respectively (**Scheme 33**). Refluxing was carried out at 90 ℃ and reactions were monitored by TLC to confirm the absence of any starting material. The crude product was purified by flash distillation and the structures were confirmed by NMR spectra which were identical to the signals reported in literature.[206,208]



***Scheme 33*: Conversion of L-malic acid into diesters**

Specifically, the diethyl malate **120a** ^{1}H NMR spectrum revealed ethoxy signals at δ 1.28 and δ 1.30 corresponding to two terminal CH_3 groups, and then another set of $-CH_2$ signals at δ 4.16 and δ 4.24. On the other hand, the spectrum for the dimethyl malate showed methoxy signals at δ 3.59 and δ 3.69 for two sets of CH_3 groups next to oxygen. The ^{1}H NMR spectrum of both compounds also showed characteristic doublets of doublets corresponding to the two protons on the α-positon adjacent to the carbonyl group, which was a trend in all the subsequent compounds through this work. As a typical example, these signals in the case of diethyl malate appeared at δ 2.77 (*J* 4.5, 16.2 Hz) and 2.83 (*J* 6.3, 16.2 Hz). The small coupling constants were the vicinal coupling constants with the proton on the adjacent chiral carbon atom. The "*cis*" coupling was 4.5 Hz and the "*trans*" coupling was 6.3 Hz. The large germinal coupling constants (16.2 Hz) were equivalent. Excellent yields of 92 and 95 % were realized for the diethyl malate and the dimethyl malate respectively, which compares to yields of 90 – 95 % reported in literature.[206,207]

Subsequently, preparation of the α-alkyl-β-hydroxy unit was conducted via two routes (**Figure 26**).

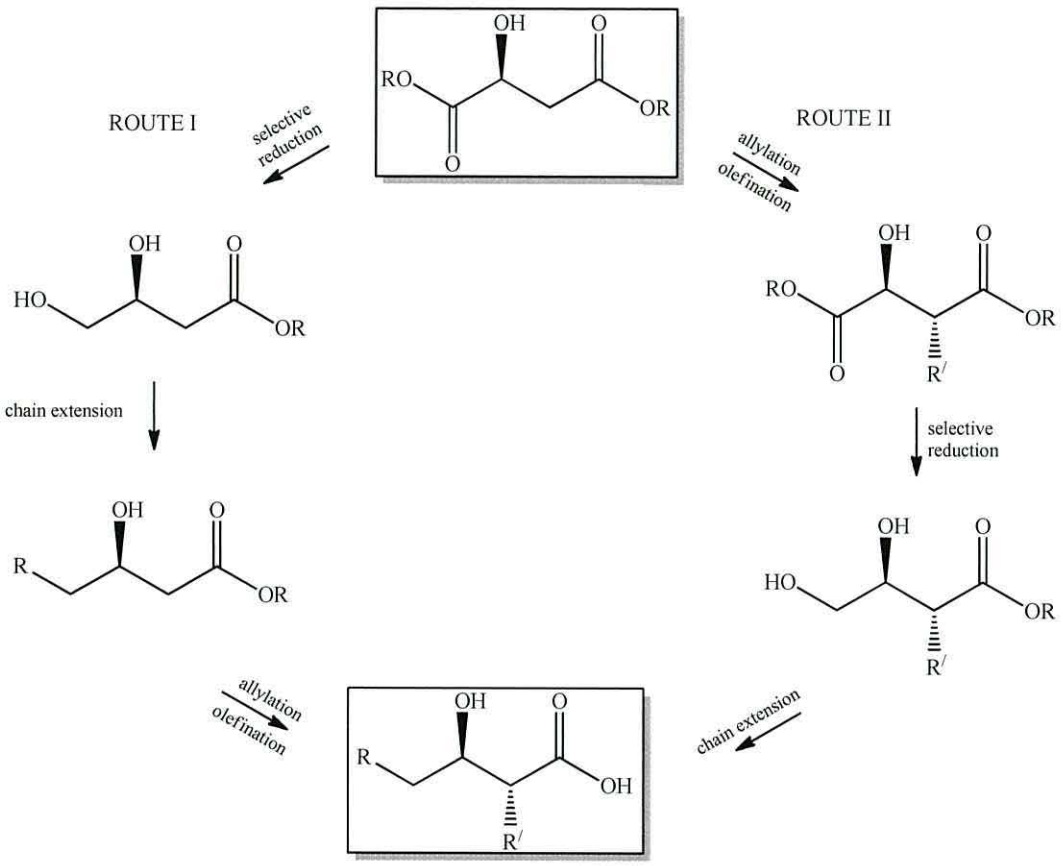


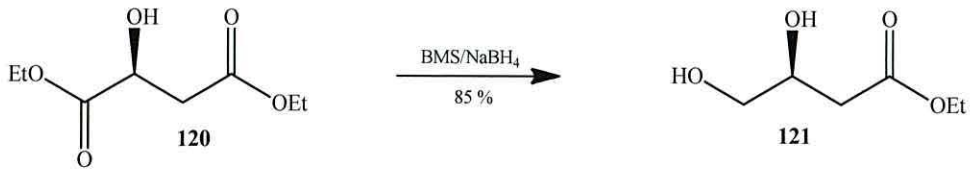
***Figure 26*: Generalized routes for the preparation of the α-alkyl-β-hydroxy unit**

3.2 Preparation via diethyl malate (route I)

Preparation of the α-alkyl-β-hydroxy fragment of the mycolic acid using diethyl malate (DEM) **120a** was achieved by an initial selective reduction of the malate, followed by chain elongation via a chiral epoxide intermediate **124**, and then alkylation on the α-position which involved the Fräter allylation and the Julia–Kocienski olefination respectively. The desired compound **126** was obtained in the correct stereochemistry after hydrogenation, deprotection and hydrolysis

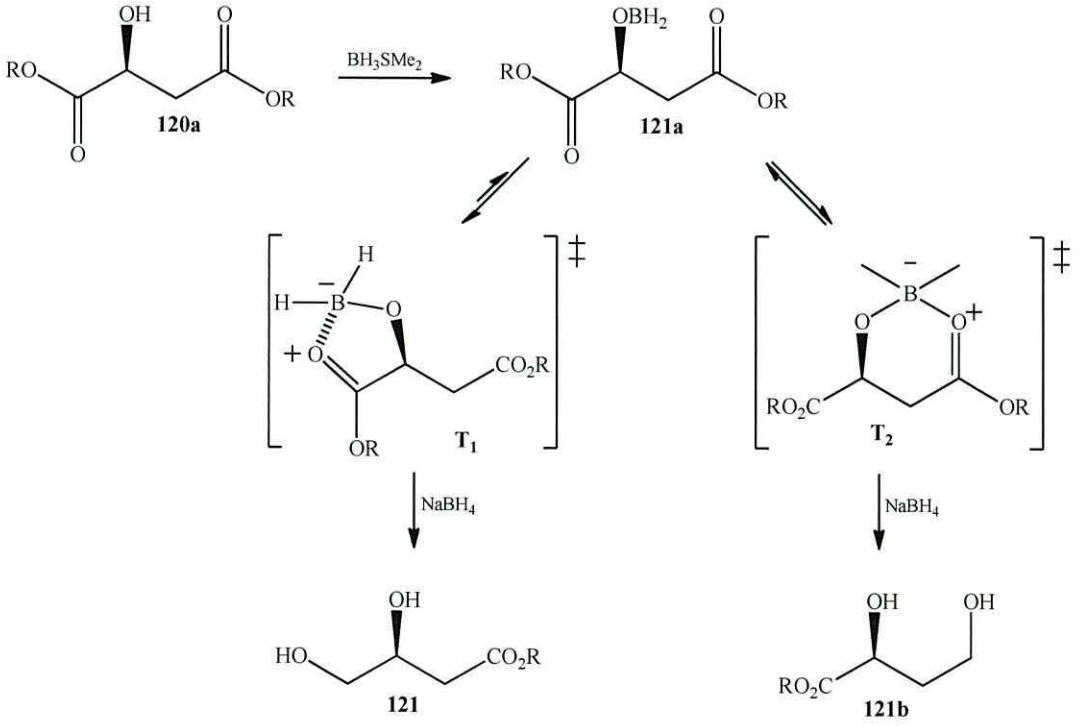
3.2.1 Selective reduction of diethyl malate

Following published procedures,[207,209,210] the ester group adjacent to the hydroxyl group in DEM **120a** was selectively reduced to give a diol **121** by treating with borane dimethyl sulfide complex (BMS) and catalytic $NaBH_4$ (**Scheme 34**).



***Scheme 34*: Selective reduction of diethylamine**

When the BMS was added to the DEM in dry THF at room temperature, evolution of hydrogen gas occurred for about 30 minutes. TLC showed only one spot at this stage, which corresponded to the starting material. This implied that no reduction had taken place. On addition of $NaBH_4$, TLC monitoring indicated the commencement of reduction which was completed in 1.5 hours at room temperature. The evolution of hydrogen gas was indicative of the coordination of borane with the ester group, which polarized the C–O bond for the effective transfer of the hydride from $NaBH_4$. In principle, the selective reduction first results in **121a** and the forms two different products via two transition states T_1 and T_2 respectively, the major product **121** being via T_1, and a very negligible minor product **121b** via T_2 (**Scheme 35**). Evidently, the T_1 transition state includes a five membered ring with lower energy than the T_2 six membered ring transition state.



***Scheme 35*: Mechanism for selective reduction**

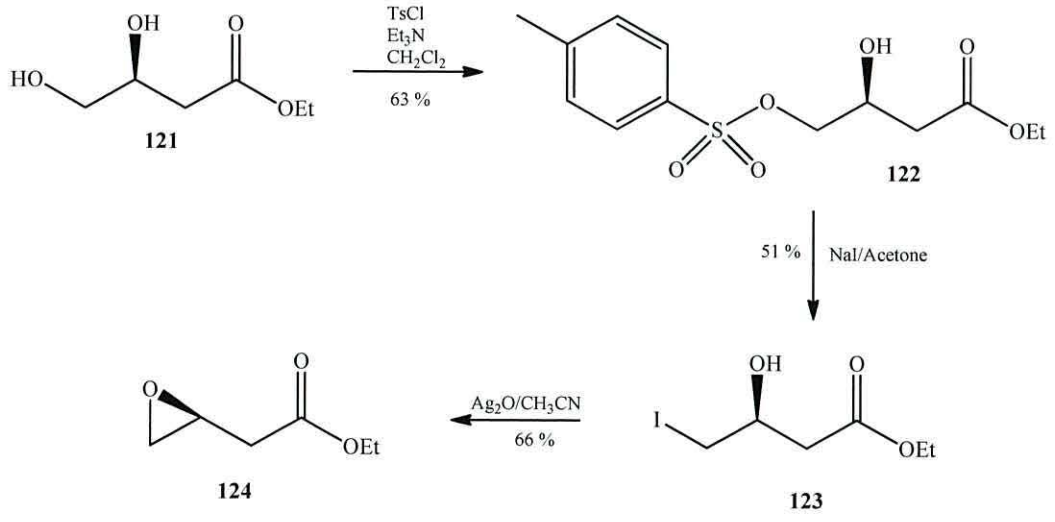
During the reaction, dimethyl sulfide complexed with borane was also discharged as a bi-product after the addition of $NaBH_4$ and this required the use of a well-ventilated fume cupboard. The reaction was quenched with EtOH, treated with p-TsOH and then toluene/IMS was added and the solvents were evaporated. This evaporation was done several times to eliminate $B(OEt)_3$. The purified product was obtained in a yield of 85 % whereas the literature value was 88 – 97 %.[210] The spectrocopic characterisation confirmed a successful selective reduction. The specific rotation was $[\alpha]_D^{23}$ – 6.23 (c 0.38, $CHCl_3$), whereas the literature value was $[\alpha]_D^{25}$ – 6.22 (c 1.22, $CHCl_3$).[210]

3.2.2 Chain extension of the diol

The chain extension was done by the transformation of the diol **121** into a chiral epoxide intermediate **124** via a tosylate **122** and an iodohydrin **123**. Subsequently, ring opening of the epoxide **124** was effected by reacting with a Grignard reagent to achieve the desired product.

3.2.2.1 Epoxidation to form the chiral intermediate (124)

In order to proceed with the chain extension of the diol **121**, the secondary alcohol group was protected in the presence of the primary one initiaitng sequence of steps which led to the formation of the epoxide **124** (**Scheme 36**).



Scheme 36: ***Preparation of the chiral epoxide intermediate***

3.2.2.1a Formation of the tosylate (122)

Following methods described in literature,[211] the tosylate **122** was prepared by dissolving the diol **121** in dry CH_2Cl_2 and treating with tosyl chloride in the presence of triethylamine. The reaction took place overnight at 0 ℃. A yield of 65 % was obtained. Although this tosylate has not been fully characterized before, it has been mentioned as a substrate for the preparation of azides in previous work by Saito *et al.*[212] Nonetheless, transformation into the tosylate was confirmed by the appearance of significant aromatic signals at δ 7.70 and δ 7.35 in the ^{1}HNMR and at δ 145.0, 132.5, 129.9, 127.9, in the ^{13}C NMR. In addition, there was an O–H band at 3453 cm^{-1} in the IR spectrum, which had been present in all the previous compounds beginning from the starting material.

3.2.2.1b Conversion of the tosylate into an iodohydrin (123)

The substitution with the iodide ion was done by treating the tosylate **122** with sodium iodide in acetone at 50 ℃. The NMR spectra for **122** and **123** were essentially alike, except that the aromatic signals corresponding to the tosyl group had disappeared. Apart from the doublet of doublet for the protons adjacent to the carbonyl as described in Section **3.1**, the ^{1}H NMR spectrum also showed two symmetrical doublet of doublets at δ 3.34 (*J* 5.35, 10.4 Hz) and δ 3.29 (*J* 5.65, 10.4 Hz) for the two protons next to iodine. The lower coupling constants were the vicinal coupling constants with the proton on the adjacent chiral carbon atom. A yield of 51 % was realised although this particular iodohydrin has been reported in literature with yields of 80 % and 63 % respectively, using a lactone, which is a more expensive staring material.[213,214] The ^{1}H NMR, ^{13}C NMR, and IR characterization of the iodohydrin also matched the literature values and in addition, the specific rotaion was $[\alpha]_D^{20}$ – 13.6 (c 2.9, $CHCl_3$) whereas in literature it was $[\alpha]_D^{20}$ – 10.7 (c 3.0, EtOH).[213]

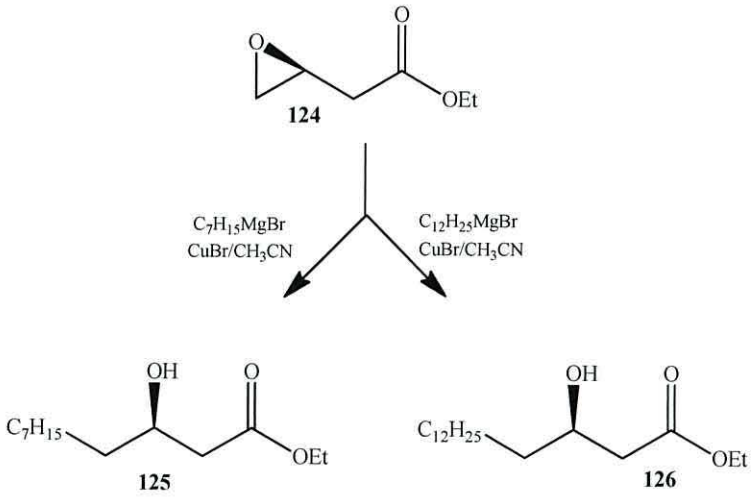
3.2.2.1c Formation of the epoxide (124)

The iodohydrin **115** was in turn converted into the chiral epoxide **123** using Ag_2O in acetonitrile at 100 ℃. Concentration of the product during work up and purification was carefully carried out at 25 – 30 °C using house vacuum, because of the high volatility of the epoxide. The ^{1}H NMR spectrum of the product showed peaks at δ 4.18 and δ 1.27 due to the presence of the ethoxy group. Additionally, the two doublets of doublets for the protons next to the iodine had disappeared. There was a multiplet at δ 2.56 – 2.54 which

corresponded to the two protons next to the carbonyl as well as one proton on the epoxide ring. The signals corresponding to the two other protons on the epoxide ring were revealed at δ 3.30 – 3.26 and 2.83. Also, there was still a peak for the carbonyl group in the ^{13}C NMR at δ 170, whereas the IR signal for the OH group had disappeared. These spectroscopic results were consistent with literature values. Also, a yield of 70 % was obtained in comparison to 77 % and 94 % recorded in the literature.[213,214] The specific rotaion was $[\alpha]_D^{24}$ –30.1 (c 0.79, $CHCl_3$) but it was $[\alpha]_D^{20}$ –25.3 (c 3.7, MeOH) in the literature.[213]

3.2.2.2 Coupling of the epoxide (124) with Grignard reagents

Formation of the epoxide intermediate **124** allows flexibility in ring opening with various Grignard reagents. Magnesium turnnings were reacted in turn with bromoheptane and bromododecane in anhydrous THF to give the corresponding Grignard reagents, heptyl magnesium bromide and dodecyl magnesium bromide.[215] These were in turn utilized for the ring opening reaction of the epoxide **124** in the presence of CuBr to give **125** and **126** respectively (**Scheme 37**). Both compounds had essentially identical spectroscopic data, which included signals for the alkoxy, carbonyl and the OH groups as in the previous steps. Notably, the ring opening was confirmed by the disappearance of the multiplet that was observed in **124** and the reappearance of the two doublets of doublets at δ 2.48 and 2.38 which corresponded to the protons next to the carbonyl. The yield for **125** was 62 %, the exact mass was $(M+Na)^+$ 253.1767 [$C_{13}H_{26}O_3Na$ requires: 253.1774] and the rotation was $[\alpha]_D^{19}$ –19.0 (c 1.7, $CHCl_3$). In the case of **126**, the yield was 79 %, the rotation was $[\alpha]_D^{20}$ –13.4 (c 1.3, $CHCl_3$) and the exact mass was $(M+Na)^+$ 323.2572 [$C_{18}H_{36}O_3Na$ requires: 323.2562]. The preparation of compound **126** has been reported in literature with a yield of 43 % and a specific rotaion of $[\alpha]_D^{20}$ –19.0 (c 1.0, $CHCl_3$).[216] Other reported characterization data corresponded with the ones for **126**. Only **126** was further alkylated at the α-position.



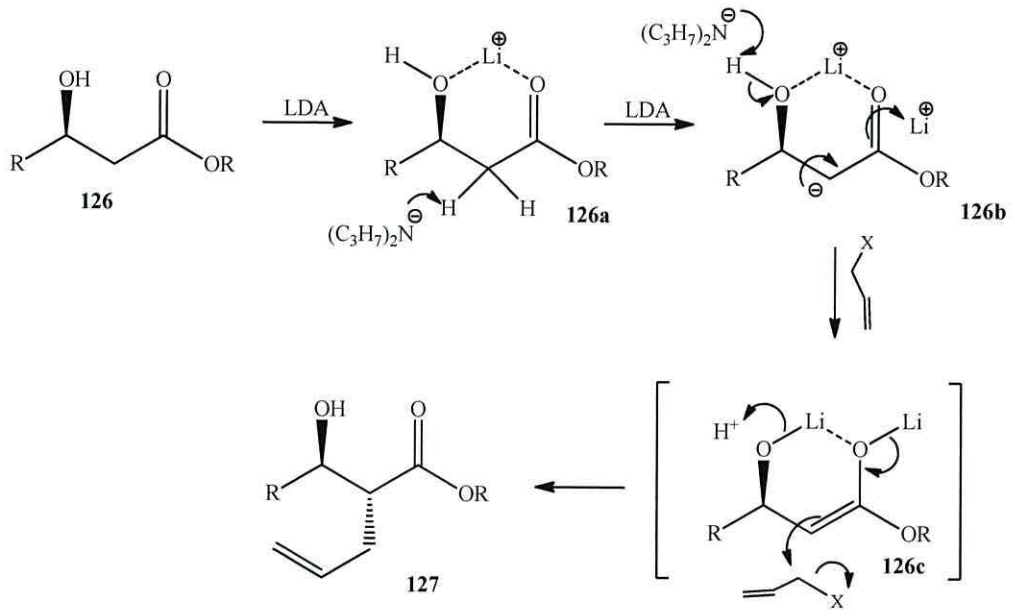
***Scheme 37:* Ring opening of the epoxide using the Grignard reagents**

3.2.3 Alkylation of the β-hydroxy ester

Alkylation of the β-hydroxy ester on the α-position was divided into two major steps. The first was the Fräter allylation reaction to attach the short α-alkenyl chain and the second was the Julia-Kocienski olefination to achieve the desired chain length.

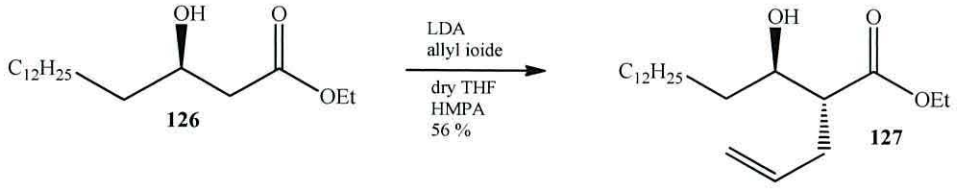
3.2.3.1 The Fräter allylation reaction

The Fräter allylation[217,218] has been reported to be highly diastereoselective and as such was considered most apppropraite for a stereo-controlled insertion of the alkyl chain at the α-position of the β-hydroxyl ester. According to the mechanism put forward by Seebach *et al*,[219] treatment of a β-hydroxyl ester substrate with 2 molar equivalents of lithium diisopropylamine (LDA), forms a stable chelated enolate intermediate **126c** via **126a** and **126b** respectively. This six-membered ring transition state coupled with an alkyl chain on the top face induces a steric effect which facilitates the diastereoselective addition from the bottom face to form the alkylated product in the correct stereochemistry (**Scheme 38**). Only one stereoisomer can be obtained in this asymmetric reaction, because of the chirality of the substrate.



***Scheme 38:* Formation of the chelated enolate intermediate**

Using the method in Koza *et al*,[177,205] the LDA was generated *in situ* with n-BuLi and di-isopropylamine in dry THF below 0 °C. After 30 minutes, it was cooled to – 78 °C and then reacted with the β-hydroxy ester **126**. In order to ensure the formation of the intermediate, the temperature was gradually raised to – 20 °C and the mixture was stirred at the same temperature for 25 to 30 minutes. It was again cooled to – 78 ℃ and treated with allyl iodide in the presence of HMPA as an aprotic additive to enhance the reaction. The reaction was slowly allowed to reach 0 °C and then worked up with NH_4Cl. The product **127** was obtained in a yield of 56 % (**Scheme 39**).



***Scheme 39*: The Fräter alkylation of the β-hydroxy ester.**

The ^1H NMR spectrum of the product showed a multiplet at δ 2.54 – 2.38 for one proton at the α-position and for the $-CH_2-$ protons adjacent to the alkene group. Also a doublet of doublets of triplets corresponding to the alkene proton adjacent to the $-CH_2-$ group appeared at δ 5.74 (*J* 17.4 Hz "*trans* coupling", 10.4 Hz "*cis* coupling", 6.0 Hz "vicinal

coupling"). The signals for the two other terminal alkene protons were revealed as doublets of doublets at δ 5.12 and δ 5.05 respectively. Additionally, there was a multiplet at δ 3.71–3.66 for the proton adjacent to the hydroxyl group. In addition, the ^{13}C NMR spectrum revealed a carbonyl carbon signal at δ 174.9, two olefinic signals at δ 135.0 and 117.1, one signal at δ 71.8 for the carbon adjacent to hydroxyl group, a signal at δ 60.5 for the ethoxyl carbon and another signal at δ 50.3 for the α-carbon. The specific rotation was recorded as $[\alpha]_D^{23}$ +1.1 (c 1.0, $CHCl_3$) and the exact mass was $(M+Na)^+$ 363.2870 [$C_{21}H_{40}O_3Na$ requires: 363.2875]. This procedure was attempted several times using MeLi but very low yields were obtained and a significant percentage of the starting material was often recovered. However, the main difficulty encountered in the allylation procedure in general was the recurrent cooling and warming processes and each time required gradual warming from –78 ℃.

3.2.3.2 Protection of the α-alkenyl-β-hydroxy ester

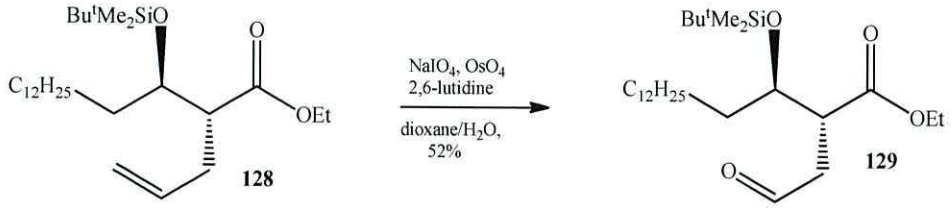
Prior to the chain extension, it was necessary to protect the β-hydroxy group of **127**, so as to oxidize it to an aldehyde in readiness for Julia-Kocienski olefination. Following general methods,[177] a mixture of **127** with *tert*-butyldimethylchlorosilane and imidazole was stirred in DMF at 45 °C for 18 hours to give **128** in 82 % yield (**Scheme 40**). As a proof of successful protection, the ^1H NMR spectrum showed one singlet at δ 0.88 for the *tert*-butyl group and two singlets at δ 0.06 and 0.05 for the two methyl groups. The specific rotation of the protected product was $[\alpha]_D^{23}$ –13.6 (c 1.2, $CHCl_3$)

***Scheme 40*: Protection of the β-hydroxy group**

3.2.3.3 Oxidation of the alkene to an aldehyde

As discussed in the previous chapter (**Section 2.4.1**), the alkene **128** was oxidised to an aldehyde **129** using $NaIO_4$ and OsO_4 in the presence of 2,6-lutidine in dioxane/water

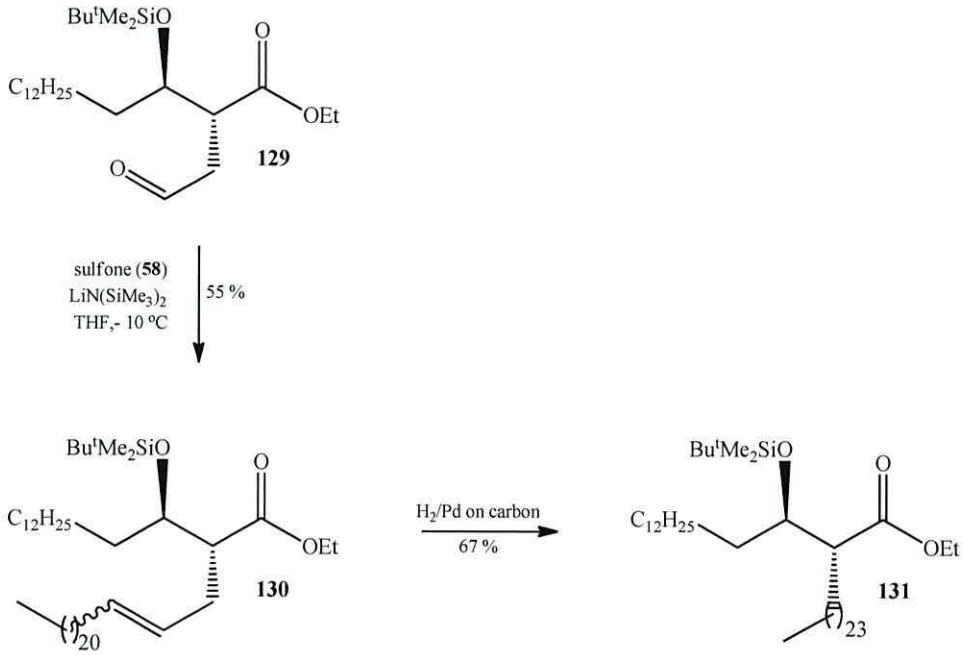
(**Scheme 41**). The yield of the oxidative cleavage reaction of olefins with OsO_4–$NaIO_4$ has been reported to improve with addition of 2,6-lutidine, because it impedes side reactions.[220] A successful transformation to an aldehyde in a yield of 52 % was confirmed by the ^1H NMR spectrum which showed a broad singlet at δ 9.81, and the ^{13}C NMR spectrum which also showed a signal at δ 200.7, both corresponding to the carbonyl group. The rotation was measured to be $[\alpha]_D^{22}$ –7.23 (c 2.2, $CHCl_3$).



***Scheme 41:* Oxidation to form an aldehyde**

3.2.3.4 Julia-Kocienski olefination of the aldehyde

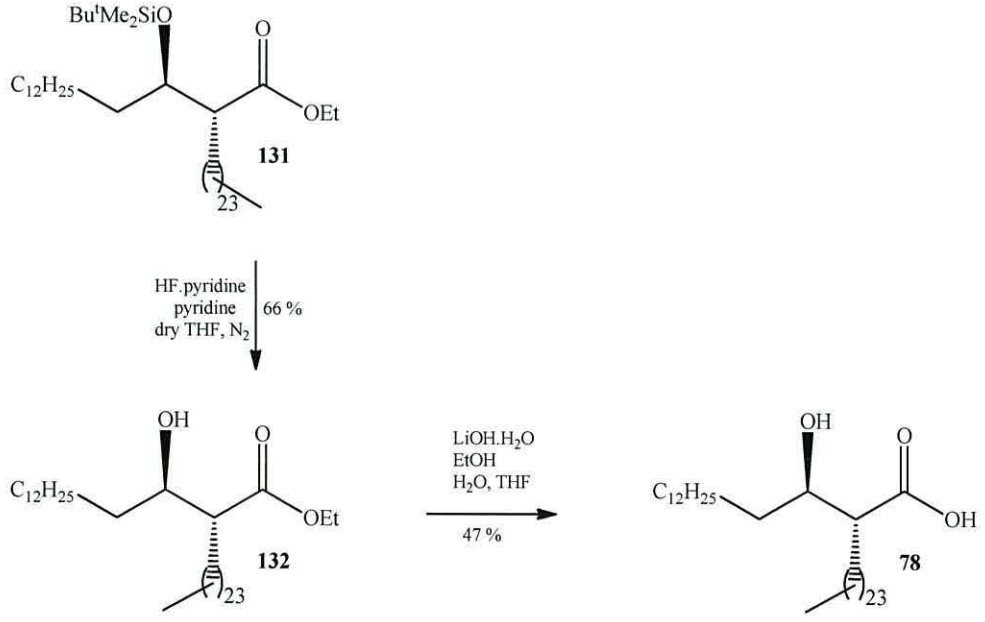
Following standard methods discussed earlier in Section **2.2.3**, the aldehyde **129** was coupled with a previously prepared sulfone **105,**[177] in a modified Julia-Kocienski reaction using $LiN(SiMe_3)_2$ as the base. A 55 % yield of a mixture of *E*/*Z*-isomers **130** was obtained. This was confirmed by the disappearance of the signal for the aldehyde proton in the ^1H NMR spectrum. Signals due to the protons of the alkene were seen between δ 6.00 – 5.00. This olefination reaction was followed by catalytic hydrogenation with palladium (on carbon) catalyst in THF/IMS to give the saturated product **131** in 47 % yield (**Scheme 42**). The saturated product **131** gave clean NMR spectra similar to that of **130** but without the signals for the C – C double bond protons. This indicated that hydrogenation had taken place. The specific rotation was $[\alpha]_D^{21}$ –3.30 (c 1.0, $CHCl_3$) and the exact mass was $(M+Na)^+$ 773.7177 [$C_{48}H_{98}O_3SiNa$ requires: 773.7183].



***Scheme 42*: Julia Kocienski olefination**

3.2.3.5 Deprotection and hydrolysis of the ester

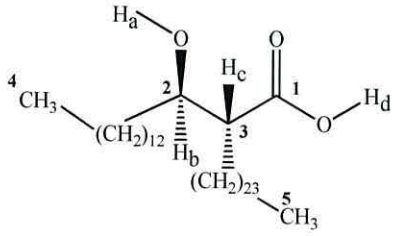
Deprotection was carried out as in previous sections. Compound **131** was treated with HF.pyridine and pyridine in dry THF under N_2 to give **132**. The NMR spectra of the product were the same as the ones for the previous compound **131** except there were no signals corresponding to the protons of the *tert*-butyldimethylsilyl protecting group. Subsequently, hydrolysis of the resulting compound **132** was done in THF, using $LiOH.H_2O$, water and EtOH to give the final product **78** (**Scheme 43**).



***Scheme 43*: Desilylation and hydrolysis**

The ^{1}H NMR spectrum of the final product **78** did not show any quartet corresponding to the $-CH_2-$ protons next to oxygen of the ethoxy group. This implied that hydrolysis had been successful. The structure of the α-alkyl-β-hydroxy carboxylic fragment **78** was further confirmed by the ^{1}H NMR, ^{13}C NMR and IR spectra. The significant peaks in the ^{1}H NMR were a triplet signal at δ 0.89 for six protons corresponding to two terminal alkyl groups (**Table VII**) and two multiplets at δ 2.50 – 2.45 and 3.68 – 3.64 for the α and β protons respectively. The $-CH_2-$ protons of the long aliphatic chains were revealed as a multiplet at δ 2.18 – 1.55. The ^{13}C NMR spectrum revealed a carbonyl signal at δ 175.0 in addition to other signals between δ 14.1 and 35.5 for the $-CH_2-$ and $-CH_3$ carbons. Also, the functional groups were confirmed by signals at 3392 and 1681cm^{-1} for the –OH group and C=O group respectively.

Table VII: Selected ^{1}H and ^{13}C NMR signals for compound 78

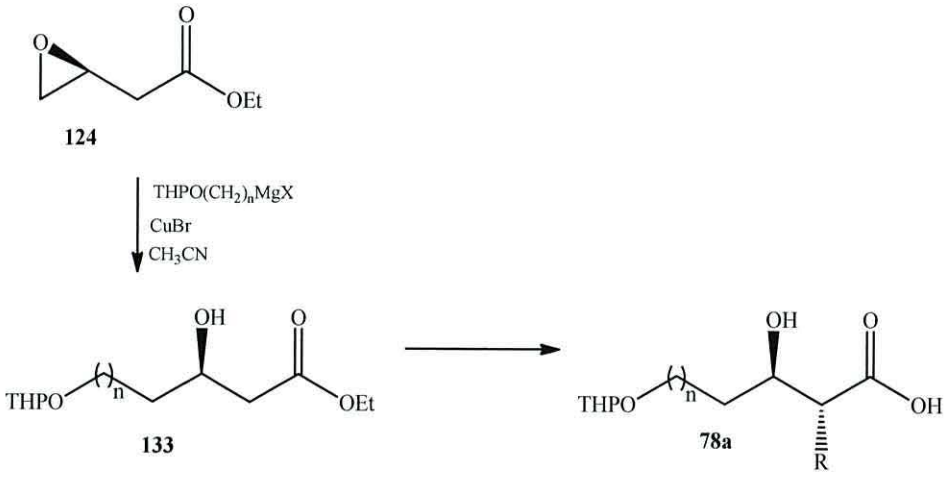


78

Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_a	3.74	bs		1	175
H_b	3.68 –3.64	m		2	71.6
H_c	2.50 –2.45	m		3	35.5 -22.7
H_d	2.41	bs		$-(CH_2)_n$	
$-(CH_2)_n-$	2.18 -1.55	m		4	14.1
2 x CH_3	0.89	t	5.0	5	14.0

The yield in the final step was 47 % but the overall yield for the entire scheme was 14 % and this was achieved in 12 steps. Essentially, a new method has been achieved for the synthesis of the α-alkyl-β-hydroxyl fragment of mycolic acids which entailed fewer steps.

In comparison to the current method by Koza *et* al, this new method is shorter by 5 steps without the need for any further chain extension required in order to couple with the meromycolate unit. Although the yield is lower, it is not by a very significant amount. It is also expected that the percentage yield achieved by this new method will improve in time with repetitions and proficiency. To enable the linking of various functional groups to the derived α-alkyl-β-hydroxy unit in this new method, the ring opening of the epoxide **124** (formed in Section **3.2.2.1**) using tetrahydropyranyl Grignard reagent is suggested (as in *Koza et al*[177]) so as to obtain a variety of mycolic acids (**Scheme 44**).



***Scheme 44*: Alternative ring opening of the epoxide**

Additionally, in the final step of the newly developed method, a short chain mycolic acid **78** was also obtained and biological testing is underway.

3.3 Preparation via dimethyl malate (route II)

The second route for the preparation of the α-alkyl-β-hydroxy fragment of the mycolic acid using dimethyl malate (DMM) **120b** was achieved by alkylation of the malate on the α-position. This entailed a Fräter allylation reaction followed by a modified Julia-Kocienski olefination. After the alkylation, selective reduction was carried out to give a diol in readiness for chain elongation on the meromycolate position.

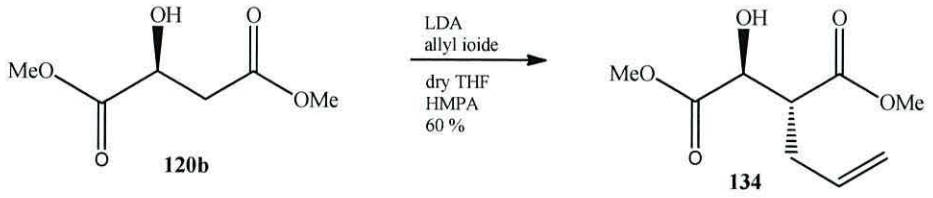
3.3.1 Alkylation of dimethyl malate

As in Section **3.2.3**, alkylation on the α-position involved two major steps. The first step was the Fräter allylation reaction to achieve a short α-alkenyl chain and the second step

was the modified Julia-Kocienski olefination for further extension to the desired chain length.

3.3.1.1 Fräter allylation of the diester

As in Section **3.2.3.1**, LDA was generated *in situ* with n-MeLi and diisopropylamine in dry THF below 0 °C. After 30 minutes, it was cooled to – 78 °C and then reacted with the diester **120b**. The temperature was gradually raised to – 20 °C and the mixture was stirred at that temperature for 25 to 30 minutes to ensure the formation of the chelated intermediate for a diastereoselective addition (see **Scheme 38**, page 89). Again the mixture was cooled to – 78℃ and treated with allyl iodide in the presence of HMPA. The temperature was slowly allowed to reach 0 °C and then the reaction was worked up with NH_4Cl. The product **126** was obtained in a yield of 60 % (**Scheme 45**).

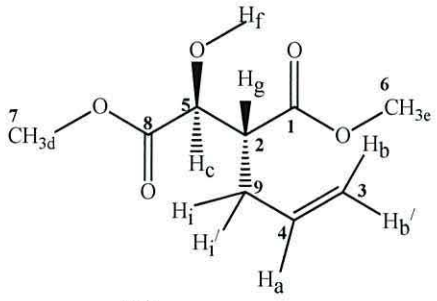


***Scheme 45*: The Fräter alkylation of the diester**

The allylation was confirmed by the ^1H NMR spectrum which showed a multiplet at δ 3.00 – 2.97 for the proton at the α-position (**Table VIII**). The two protons adjacent to the alkene group were also revealed as multiplets at δ 2.41 – 2.47 and 2.64 – 2.60 and two singlets corresponding to the methyl groups appeared at δ 3.8 and δ 3.68. For the alkene signals, a multiplet corresponding to one C=C proton adjacent to the $-CH_2-$ group appeared at δ 5.81 – 5.76 and the two other terminal C=C protons were revealed as doublets of doublets at δ 5.17 and 5.11 respectively. In addition, there was a peak at δ 3.4 for the hydroxyl proton and another one at δ 4.29 for the proton at the β-position. The ^{13}C NMR spectrum revealed two carbonyl carbon signals at δ 173.9 and 172.5, two olefinic signals at δ 134.6 and 117.9, and two methoxy signals at δ 51.9 and 52.7. Then one signal at δ 70.1 was revealed for the carbon adjacent to hydroxyl group, one signal at δ 48.1 for the α-carbon and a CH_2 – signal at δ 32.0. In the IR spectrum there were significant peaks for the hydroxyl, alkene

and carbonyl functional groups at v_{max} 3487, 1642 and1733 cm^{-1} respectively. The specific rotation was recorded as $[\alpha]_D^{18}$ +23.4 (c 1.5, CHCl3).

Table VIII: Selected ^1H and ^{13}C NMR signals for compound 134



134

Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_a	5.81-5.76	m		1	173.9
$H_b^{/}$	5.17	dd	1.25, 17.0	2	172.5
H_b	5.11	d	10	3	134.6
H_c	4.31	q	3.47	4	117.9
CH_{3d}	3.80	s		5	70.1
$3CH_{3e}$	3.68	s		6	52.7
H_f	3.4	d	7.25	7	51.9
H_g	3.00-2.97	m		8	48.1
$H_i^{/}$	2.64-2.60	m		9	32.0
H_i	2.47-2.41	m			

3.3.1.2 Protection of the alcohol

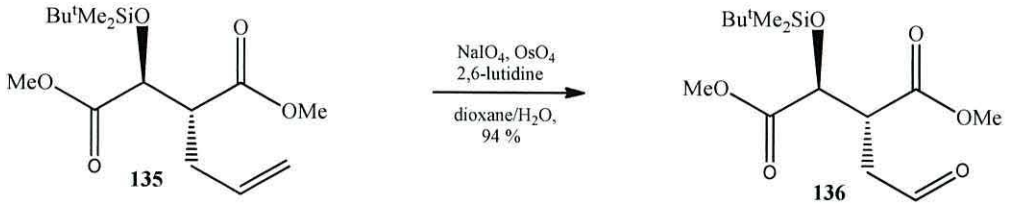
As in Section **3.2.3.2**, the β-hydroxyl group of compound **134** was protected by treating *tert*-butyldimethylchlorosilane and imidazole in DMF at 45 °C for 18 hours. This gave compound **135** in 83 % yield (**Scheme 46**). Successful protection was ascertained by the appearance of the peaks in the ^1H NMR spectrum, which corresponded to the protons of

the *tert*-butyldimethylsilyl group. A singlet was shown at δ 0.86 for the *tert*-butyl group and two singlets at δ 0.03 and 0.05 for the two methyl groups. The specific rotation of the protected product was $[\alpha]_D^{18}$ –36.5 (c 1.9, $CHCl_3$).

***Scheme 46*: Protection of the alcohol**

3.3.1.3 Oxidation of the olefin (135) to an aldehyde (136)

Oxidation of the alkene **135** to an aldehyde **136** was carried out as in Section **3.2.3.3** by using $NaIO_4$ and OsO_4 in dioxane/water and in the presence of 2,6-lutidine as an additive to suppress side reactions (**Scheme 47**). An excellent yield of 94 % was achieved and a successful conversion into an aldehyde was confirmed by signals at δ 9.80 in the ^{1}H NMR spectrum and at δ 199.5 in the ^{13}C NMR spectrum. The rotation was $[\alpha]_D^{19}$ –28.5 (c 1.4, $CHCl_3$).

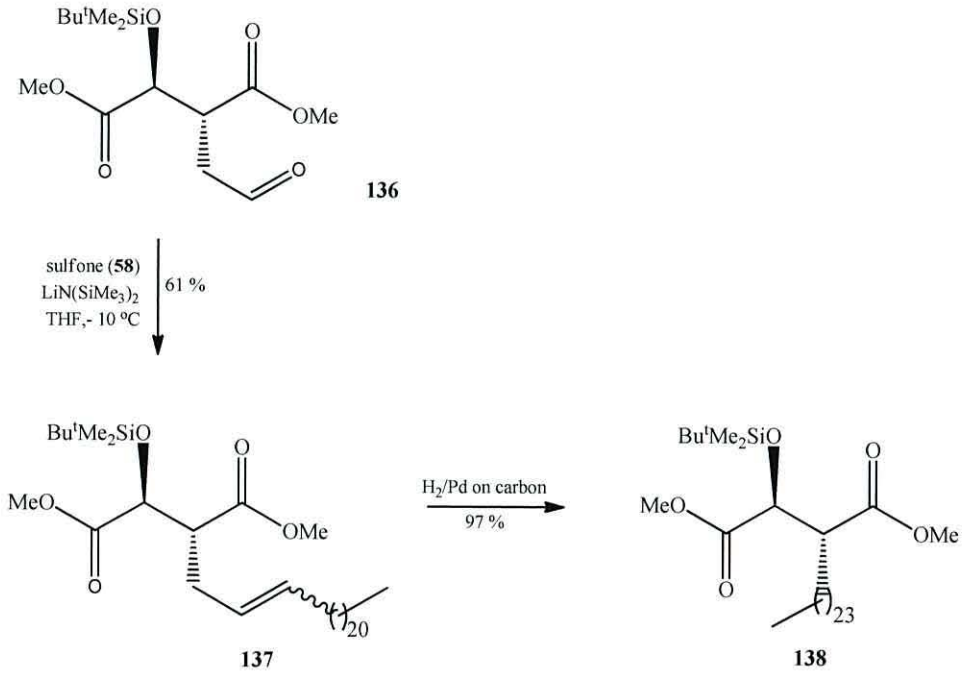


***Scheme 47*: Oxidation of the olefin to aldehyde**

3.3.1.4 Julia-Kocienski olefination of the aldehyde

As before, the aldehyde **136** was coupled with a previously prepared sulfone **105**[177] in a modified Julia-Kocienski reaction using $LiN(SiMe_3)_2$ as the base. An alkene mixture of *E*/*Z*-isomers **137** was obtained and the yield was 61 %. The ^{1}H NMR spectrum of the product showed no signal for the aldehyde proton but signals due to the protons of the C–C double bond were revealed at δ 5.34 – 5.26 and δ 5.51 – 5.43. This olefination reaction

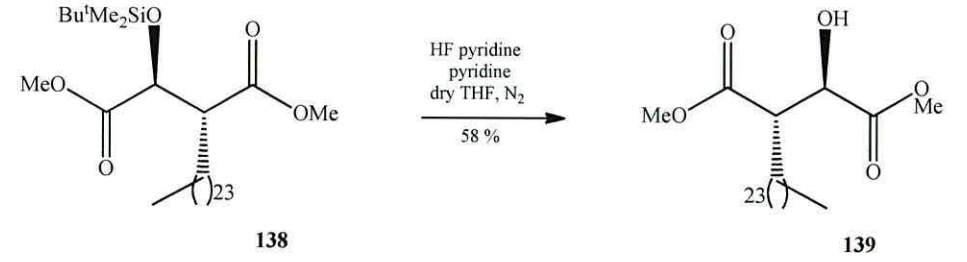
was followed by catalytic hydrogenation with palladium catalyst in THF/IMS solvent, which gave 97 % yield of the saturated product **138** (**Scheme 48**). The saturated product **130** gave clear NMR spectra similar to **137** but without the signals for the alkene protons. This indicated that hydrogenation has been successful. The specific rotation was $[\alpha]_D^{14}$ – 12.58 (c 0.3, $CHCl_3$).



***Scheme 48*:Alkylation at the α-position**

3.3.1.5 Desilylation of the protected α-alkyl diester

Desilylation was carried out as in previous sections. Compound **138** was treated with HF.pyridine and pyridine in dry THF under N_2 to give **139** in a 58 % yield (**Scheme 49**).

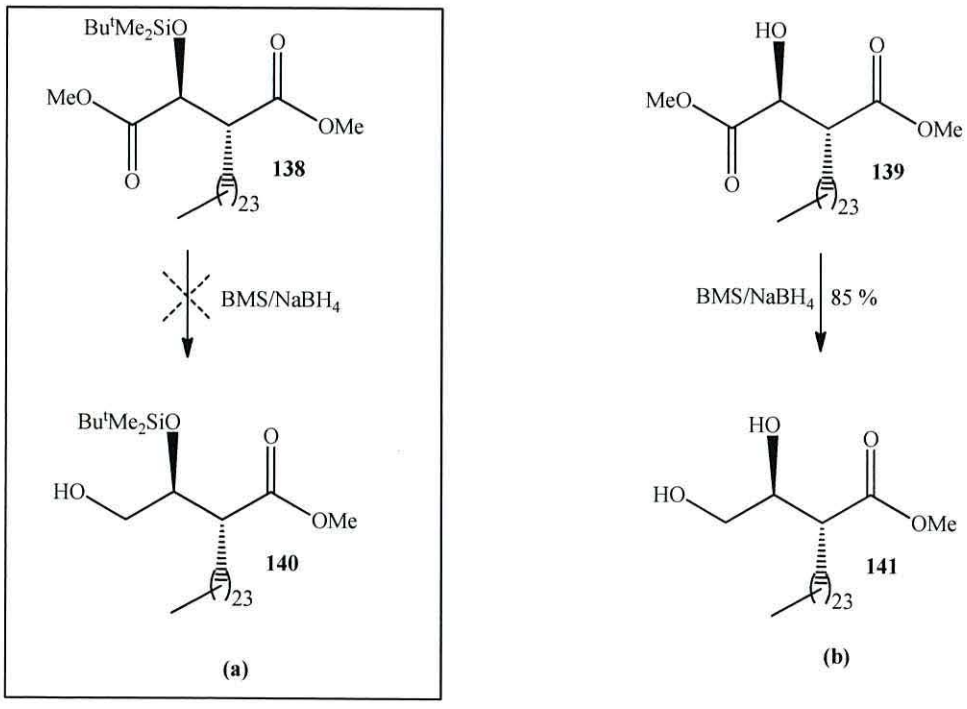


***Scheme 49*: Desilylation of protected diester**

The NMR spectra were similar to the ones for the previous compound **138** but there were no signals corresponding to the *tert*-butyldimethylsilyl protecting group. The specific rotation was $[\alpha]_D^{19}$ + 0.12 (c 1.2, $CHCl_3$) and the melting point was 50 – 52 °C

3.3.2 Selective reduction of the diester

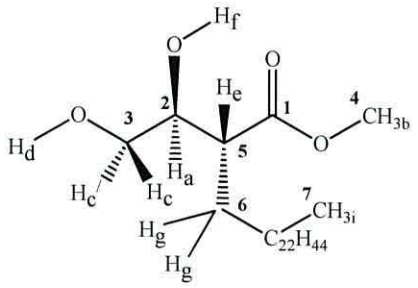
To effect the chain extension on the meromycolate position, selective reduction was carried out as in Section **3.2.1**, so that the secondary alcohol would be protected in the presence of the primary alcohol. An initial attempt to prepare **140** by selectively reducing the protected compound **138** using borane dimethyl sulfide complex (BMS) and catalytic $NaBH_4$ proved unsuccessful (**Scheme 50a**). The ^1H NMR peaks corresponding to the methoxy protons had disappeared, which implied that both methoxy groups had been reduced and that selective reduction was unsuccessful. Following the same procedure, selective reduction was successfully carried out with the deprotected compound **139** to give the diol **141** (**Scheme 50b**).



***Scheme 50*: Selective reduction**

A yield of 85 % was achieved and the spectroscopic characterisation confirmed a successful selective reduction. The ^{1}H NMR (**Table IX**) showed one signal corresponding to three methoxy protons at δ 3.74 instead of two signals in the starting material. The protns H_c and $H_c^{/}$ of the newly introduced alcohol each appeared as double doublets as expected. The exact mass was $(M+Na)^+$ 493.4204 [$C_{29}H_{58}O_4Na$ requires: 493.4233] and the specific rotaion was $[\alpha]_D^{19}$ + 0.18 (c 0.1.1, $CHCl_3$).

Table IX: Selected ^{1}H and ^{13}C NMR signals for compound 141



141

Proton	δ	multiplicity	*J* (Hz)	carbon	
Ha	3.86-3.82	m			δ
CH_{3b}	3,74	s		1	175.9
$H_c^{/}$	3.72	dd	3.5, 7.3	2	72.6
H_d	3.69	d	3.5	3	65.0
H_c	3.56	dd	6.6, 11.4	4	51.8
H_e	2.57	td	5.7, 9.2	5	47.5
H_f, H_g	1.75-1.55	m		6	31.9
$-(CH_2)_n$	1.26	m		$-(CH_2)_n-$	29.7-22.7
CH_{3i}	0.89	t	7.0	7	14.1

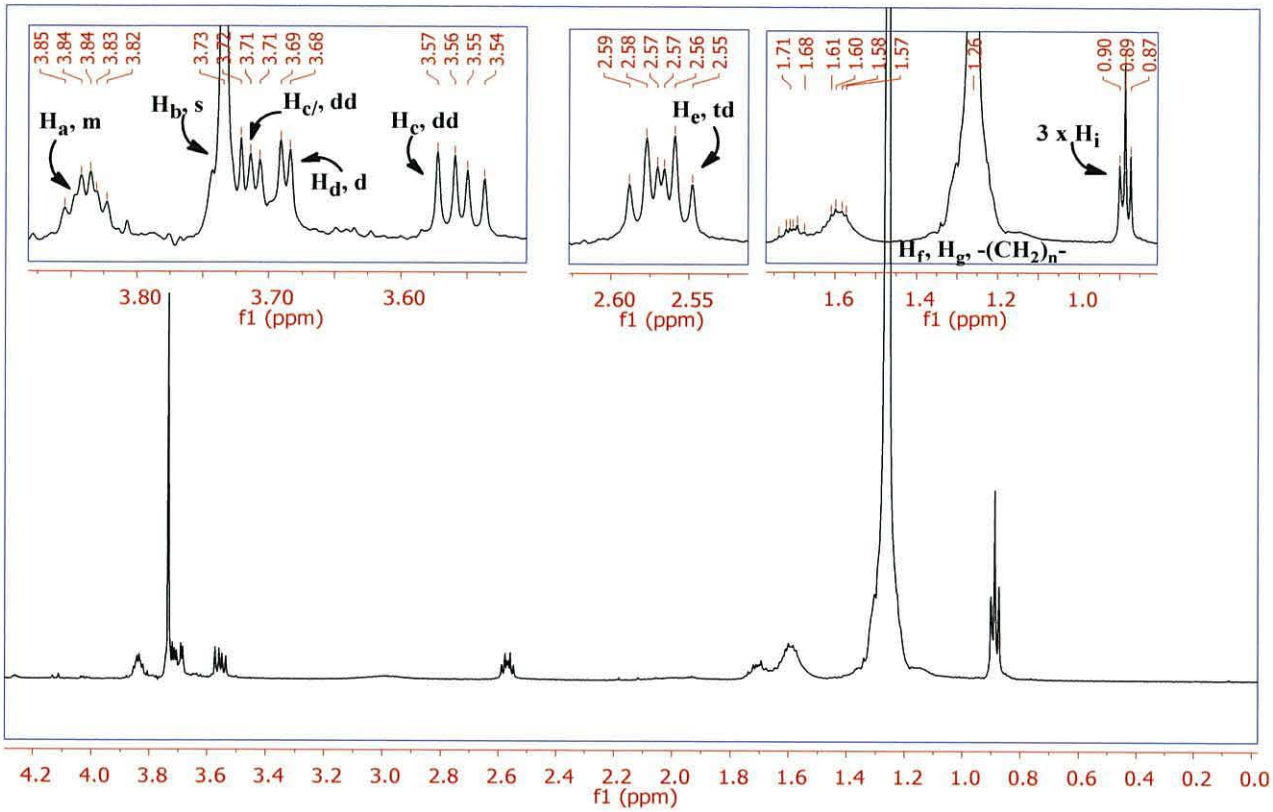


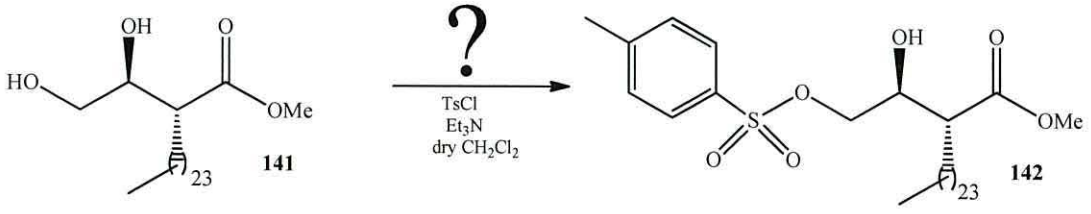
Figure 27: *^{1}H NMR spectrum of compound 141*

3.3.3 Chain extension of the diol

Prior to the chain extension on the diol **141** it was necessary to protect the secondary alcohol group in presence of the primary, before converting the primary alcohol group into an aldehyde in readiness for Julia coupling.

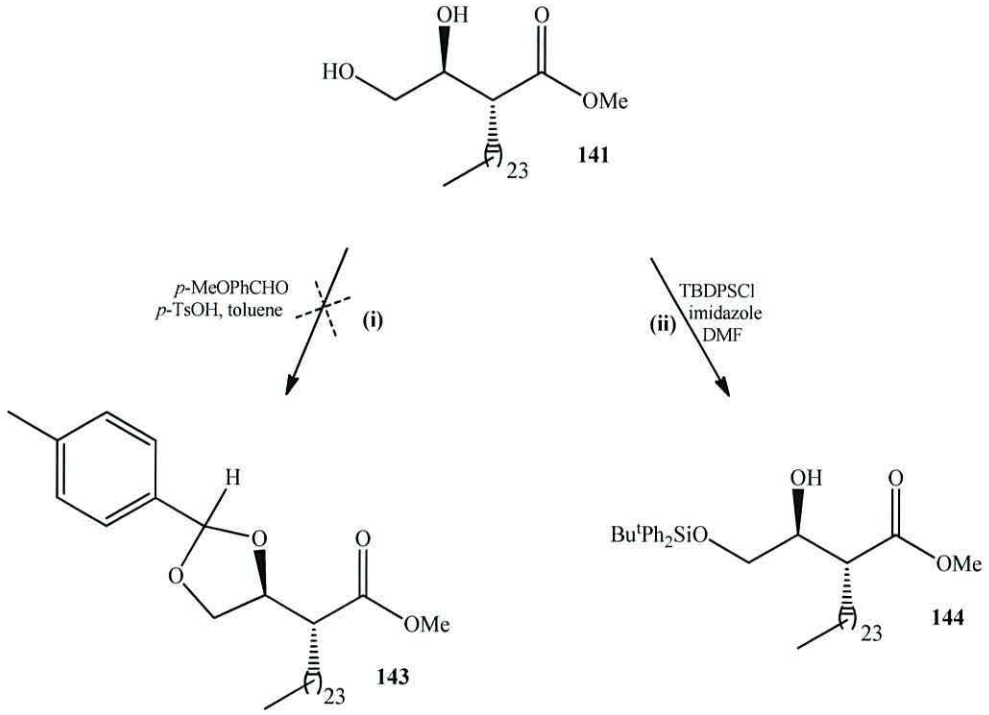
3.3.3.1 Protection of the primary hydroxyl of the diol

Simultaneous protection of the two hydroxyl groups by using a Grignard reagent on a chiral epoxide intermediate via a tosylate and iodohydrin respectively was attempted as in Section **3.2.2**. The first step in this process was the conversion of primary hydroxyl of the diol **141** into a tosylate **142** by treating with tosyl chloride and triethylamine in dry dichloromethane (**Scheme 51**).



Scheme 51: *Preparation of the tosylate(142)*

The solubility of the diol **141** in the reaction solvent was achieved with difficulty. However, at the end of the reaction a very poor yield of 27 % was obtained. In addition, it was very difficult to realize a pure product even after repeated purification by column chromatography and recrystallization. The tosylation was also attempted with added pyridine but was not successful. Protection with $MeSO_2Cl$ or CF_3SO_2Cl might have been successful although these experiments were not done. However, other possible methods for protecting the diol **141** were explored as shown in Scheme **52** below.



***Scheme 52:* Methods for protection of the diol 141**

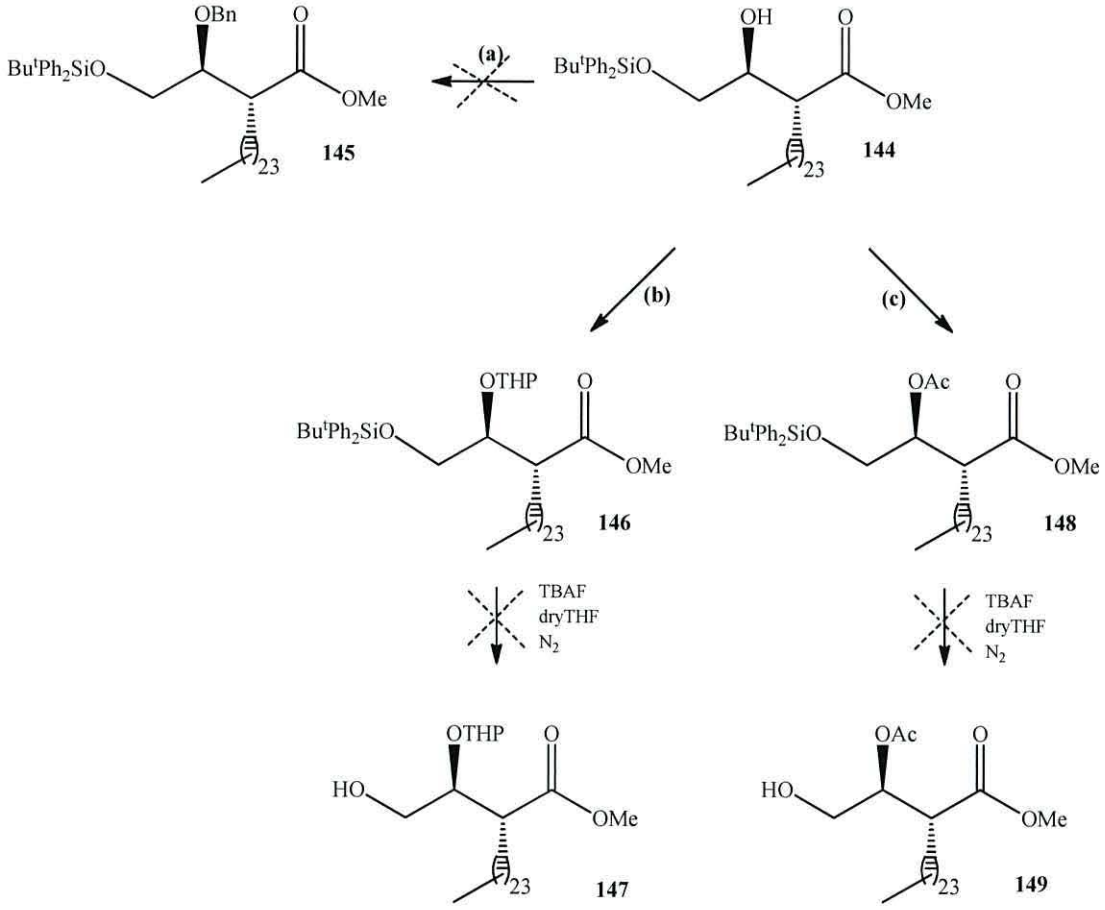
Protection of the diol **141** with 4-methoxybenzaldehyde and *p*-TsOH in toluene using a Dean-Stark apparatus (**Scheme 52 (i)**),[221a] was attempted. This was used in combination with a reflux condenser for continuous removal of the water produced during the reaction. However, the preparation of **143** was not successful as the extracted crude product was a complex dark mixture that could not be characterised by standard methods. Had the reaction worked, ring opening of the derived product **143** in dichloromethane using di-isobutylaluminium hydride in hexane[221b] was expected to break the acetal to produce a free primary alcohol group and a benzyl protected secondary alcohol; this would allow a chain

extension on the primary hydroxyl position while the secondary hydroxyl group remained protected.

Protecting the primary alcohol group with *tert*-butyldiphenylchlorosilane in the presence of imidazole and in DMF solvent (**Scheme 52 (ii)**) did prove very successful with a 99 % yield of compound **144**. The protection was confirmed by ^{1}H NMR peaks at δ 1.09 (9H, s) which corresponded to *tert*-butyl protons and at δ 7.47–7.38 (6H, m) and δ 7.67 (4H, d, *J* 7.6) for the phenyl protons.

3.3.3.2 Protection of the secondary hydroxyl and desilylation of (144)

With the protection of the primary hydroxyl in place, it was necessary to protect the secondary hydroxyl and again deprotect the primary hydroxyl for the Julia coupling (**Scheme 53**).



Scheme 53: ***Attempted protection of the secondary hydroxyl and deprotection of the primary hydroxyl of compound 144***

Reagents and conditions: (a) $C_6H_5CH_2Br$, TBAI, dry THF, NaH in 60 % oil (b) PPTS, 3,4-dihydropyran, dry CH_2Cl_2 (c) aceteic anhydride, pyridine, dry THF

3.3.3.2a Benzylation of (144)

Protection of the β-hydroxy group of **144** was first attempted following standard benzylation procedures,[222] which was conducted in dry THF under N_2, using sodium hydride (60 % dispersed in oil) and benzyl bromide in the presence of TBAF (**Scheme 53 (a)**) The reaction was worked to give a crude product **145**. The compound showed complicated NMR spectra that inferred that the reaction was not successful. Subsequently, two other approaches were employed. The first protection was with dihydropyran and the second with an acetyl group.

3.3.3.2b Dihydropyran protection and desilylation of (144)

Using standard procedures, compound **144** was treated with 3,4-dihydro-2*H*-pyran and pyridinium-*p*-toluene sulfonate in dry CH_2Cl_2 (**Scheme 53(b)**).[223] This gave 99 % yield of the product **146**. The presence of the OTHP group was shown in the ^{1}H NMR spectrum which revealed several signals between δ 5.00 and 2.80. Subsequently, deprotection of the primary hydroxyl was attempted using TBAF in dry THF at 0℃ under nitrogen. After working up the reaction, the ^{1}H NMR spectrum of the crude product showed that the signals corresponding to the OTHP group had disappeared but those for the *tert*-butyldiphenylsilyl group still appeared in the spectrum. This implied that deprotection had occurred for the secondary alcohol groups rather than for the primary. All other signals revealed in the ^{1}H NMR spectrum were unclear and the starting material (diol **133**) was not recovered.

3.3.3.2c Acetylation and deprotection of (144)

In the second protection method,[163] for compound **144**, pyridine and acetic anhydride in dry THF were used (**Scheme 53 (c)**) and a 61 % yield of the product **148** was obtained. This was confirmed by the ^{1}H NMR spectrum which revealed a singlet at δ 1.99 for the methyl protons of the introduced acetyl protecting group. At this stage desilylation was carried out with TBAF according to standard methods, but the reaction was not successful. The ^{1}H NMR spectrum of the crude product still showed signals corresponding to both the *tert*-butyldiphenylsilyl and the acetyl protection in addition to other unclear signals.

Although compound **144** is essentially an α-alkyl-β-hydroxyl unit (mycolic motif) it has not been possible to protect the secondary hydroxyl group at the β-position in order to

oxidize the primary hydroxyl group for chain extension and coupling to the meromycolate unit. However, there are still prospects of achieving this objective if further investigation can be carried out.

Chapter 4

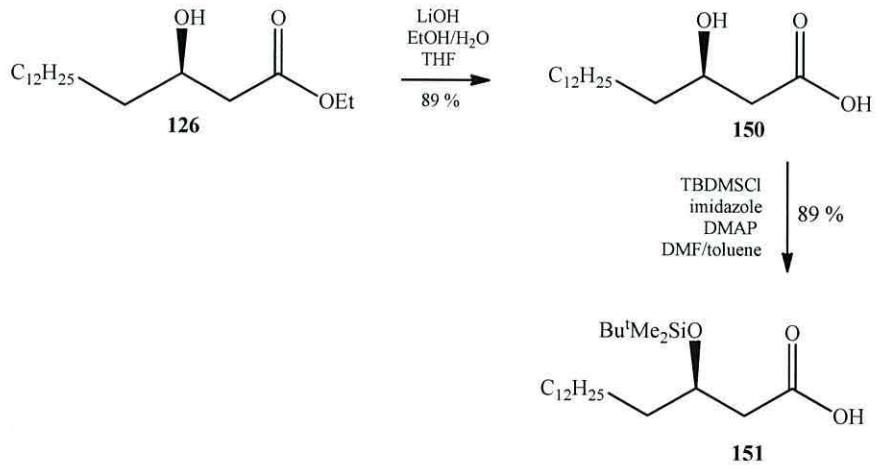
4 Preparation of cord factors

This section of the project was directed at the preparation of cord factors. In the course of the method development of the synthesis of the α-alkyl-β-hydroxy unit, a β-hydroxy ester **126** was prepared as a key intermediate (**Section 3.2**); this was used in the preparation of a cord factor. The aim was to prepare a novel cord factor of a β-hydroxycarboxylic acid as opposed to the cord factor of an α-alkyl-β-hydroxy carboxylic acid (mycolic acid). This was done with a view to comparing their biologigal activityand antigenicity. Perhaps it would be possible to avoid the α-alkylation step (which involves Fräter allylation) in mycolic acid synthesis, if the test results are found to be identical.

The synthesis of the cord factor involved the esterification of the protected acid with a protected trehalose. Alcohol protection was required on the trehalose at sites not involved in the coupling (such as the 3, 4, 5 positions in the sugar core) in order to avoid undesired alcohol reactions. For the same reason the intermediate **126** was also protected at the *β* hydroxy acid position prior to the esterification. After the esterification reaction, the final product was obtained by deprotection of the silyl groups on both sugar and acid.

4.1 Hydrolysis and protection of the β-hydroxyl ester

In order to convert the ester **126** into a carboxylic acid, hydrolysis was carried out according to standard methods, using LiOH, water and EtOH in THF. As a result compound **150** was obtained in 89 % yield **151** (**Scheme 54**).

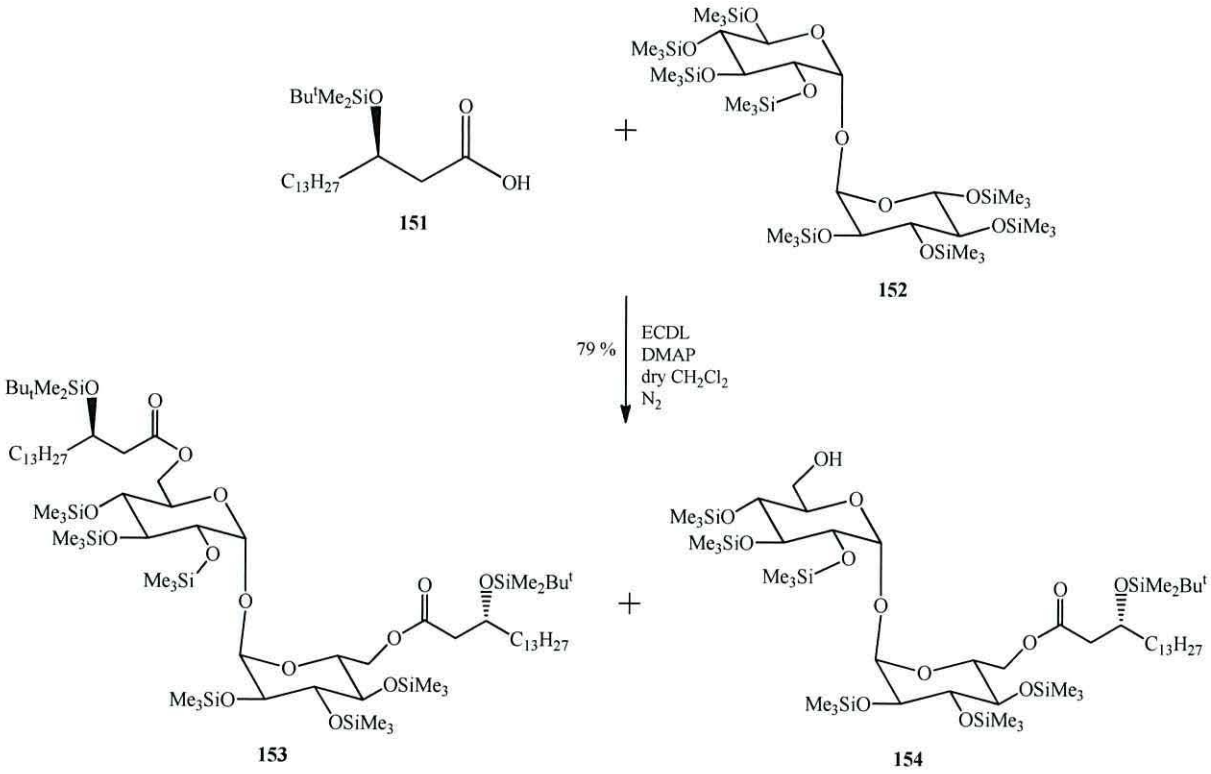


***Scheme 54*: Hydrolysis and protection**

Hydrolysis was evident in the ^{1}H NMR spectrum which showed no peaks corresponding to the ethoxy protons. Then standard silylation procedures were carried out on compound **150** so as to protect the β-hydroxyl group. This was conducted in dry DMF and dry toluene at room temperature in the presence of imidazole and DMAP to give the protected β-hydroxy acid. The most commonly used reagent for this protection is the *tert*-butyldimethylchlorosilane. The protection was confirmed in the ^{1}H NMR spectrum. It showed peaks at δ 0.08 (3H, s) and 0.09 (3H, s) which corresponded to the methyl groups next to the silicon atom in the protecting *tert*-butyldimethylsilyl group. The protons corresponding to the *tert*-butyl group also appeared at δ 0.92 (9H, s). A yield of 89 % was obtained and the specific rotation was $[\alpha]_D^{22}$ –13.9 (c 1.7, $CHCl_3$). The compound **151** has been prepared before by Jannet *et al* using a different method.[216] The the spectroscopic data were identical, the yield was 75 % and the rotation was $[\alpha]_D^{20}$ – 16.0 (c 1.0 $CHCl_3$).

4.2 Esterification the acid with the protected trehalose

The esterification of the trehalose **152** with the protected β-hydroxy acid **151** was carried out as described in the literature (**Scheme 55**).[184,169]

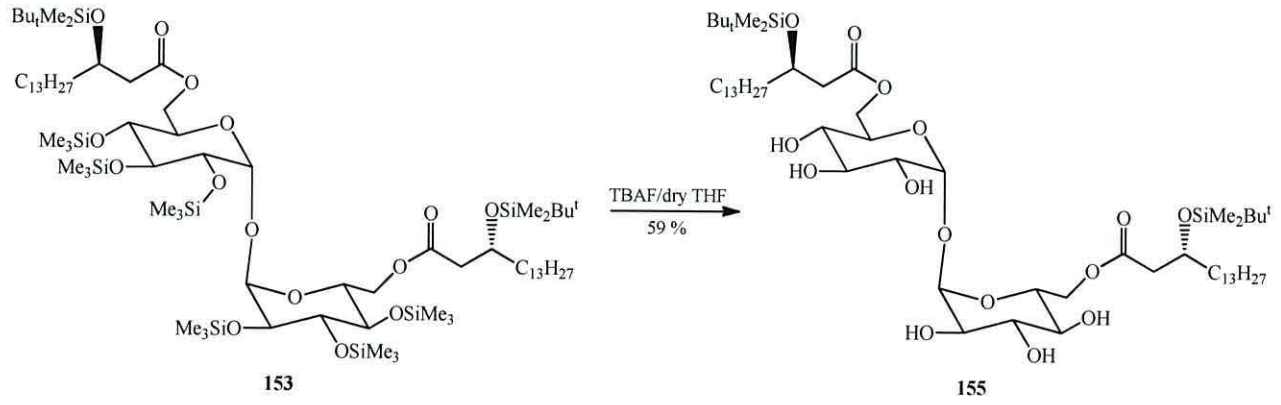


***Scheme 55*: Esterification of protected trehalose with acid**

The major product was TDM **153,** with a yield of 78 %. The yield for TMM **154** was less than 1 % which was not sufficient to continue with further steps. The TMM and the TDM were identified as two distinct spots on TLC and the first fraction from the column chromatography was the TDM **153**. The ^{1}H NMR spectrum showed signals for 5 sugar protons at 4.9 (1H, d, *J* 2.9 Hz), 4.27 (1H, dd, *J* 2.2, 12.0 Hz), 4.07 (1H, dd, *J* 4.7, 11.7 Hz), 4.02–3.99 (1H, m) and δ 3.91 (1H, t, *J* 9.0 Hz) respectively. The signals corresponding to the $-CH_2-$ protons adjacent to the oxygen linkage between the sugar and the acid appeared at δ 3.48 (1H, d, J 8.8 Hz) and 3.44 (1H, dd, J 3.2, 9.2 Hz). Additionally, the signals due to trimethylsilyl protecting groups on the sugar were revealed as singlets at δ 0.16 (9H, s), 0.14 (9H, s) and 0.13 (9H, s). The remaining signals corresponding to the protons of the acid part of the compound were the essentially the same as in compound **152**. On the other hand the ^{13}C NMR spectrum showed the characteristic downfield signal at δ 171.9 corresponding to the carboxylic acid. The sugar core carbon signals were found between δ 94.4 and 69.3. The next two signals resonating at δ 63.4 and 42.5 corresponded to the α-carbon and β-carbon atoms of the acid respectively. There were other signals due to aliphatic carbon atoms including the methyl carbon signals of the protecting silyl groups of the sugar which were seen up-field between δ 37.5 and -4.75. Mass spectrometry (MALDI MS) gave $(M+Na)^+$ 1534.4 [$C_{74}H_{158}O_{15}Si_8Na$ requires: 1534.0] and the specific rotation was $[\alpha]_D^{22}$ + 53.7 (c 0.74, $CHCl_3$). The second fraction was the TMM **154**. The ^{1}H NMR spectrum showed a very similar pattern of signals to the TDM **153**. However, the integration of the signals corresponding to the protons of the mycolic acid part of the compound was half of those for TDM **153** as expected. The signals for the sugar hydrogens were also more complicated due to the lack of symmetry. As mentioned before the yield of **155** obtained was not high enough to carry out full characterization and to proceed to the deprotection steps.

4.3 Removal of the trehalose protecting groups

The two step deprotection started with the removal of the trimethylsilyl groups on the sugar molecules in compound **153** using TBAF in dry THF. This gave a 59 % yield of compound **155** (**Scheme 56**). The ^{1}H NMR spectrum showed that the signals for the trimethyl protecting group had disappeared which was an indication that the deprotection has been successful. The specific rotation was $[\alpha]_D^{23}$ + 39.3 (c 0.29, $CHCl_3$).

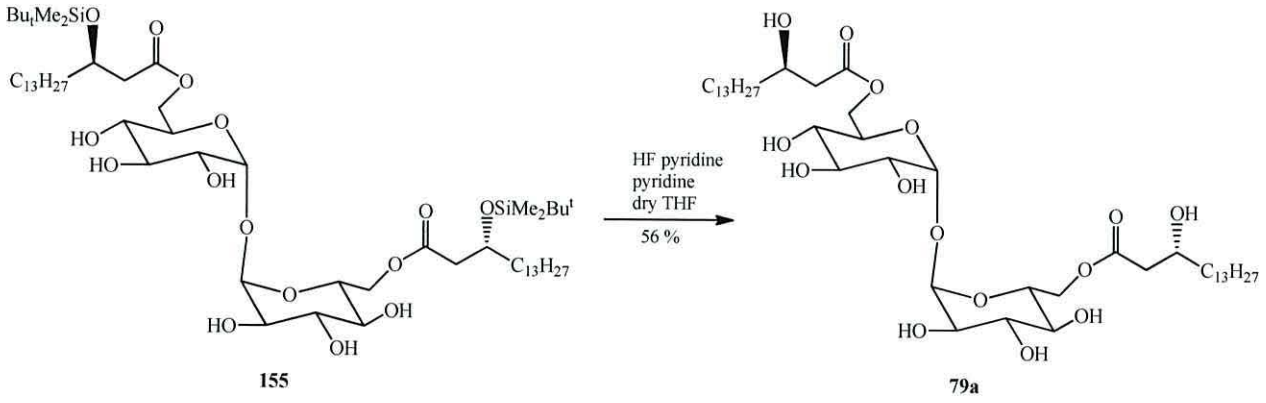


153 155

***Scheme 56*: Partial desilylation of the trehalose**

4.4 Deprotection of the hydroxy acid part

The second step involved deprotection of the *tert*-butyldimethylsilyl group on the β-hydroxy position of the acid part of compound **155**. The reaction was carried out in dry THF using HF.pyridine and pyridine and this gave a TDM (cord factor) **79a** in a yield of 56 % (**Scheme 57**).



155 79a

***Scheme 57*: Desilylation of the hydroxy acid part**

The ^{1}H NMR spectrum of the purified product was similar to that of the starting material but there were no signals for the protecting *tert*-butyldimethylsilyl group which proved that deprotection had taken place. This method of deprotection was preferred to the TBAF method because it has been reported that removal of tetrabutylammonium oxide at the end of the reaction was difficult as it runs at the same point as similar products on TLC and on a column.[169] The specific rotation of the product was $[\alpha]_D^{22}$ + 47.0 (c 1.2, $CHCl_3$) and the

MALDI MS (**Figure28**) gave $(M+Na)^+$ 873.1 while the value expected for the product was 873.6.

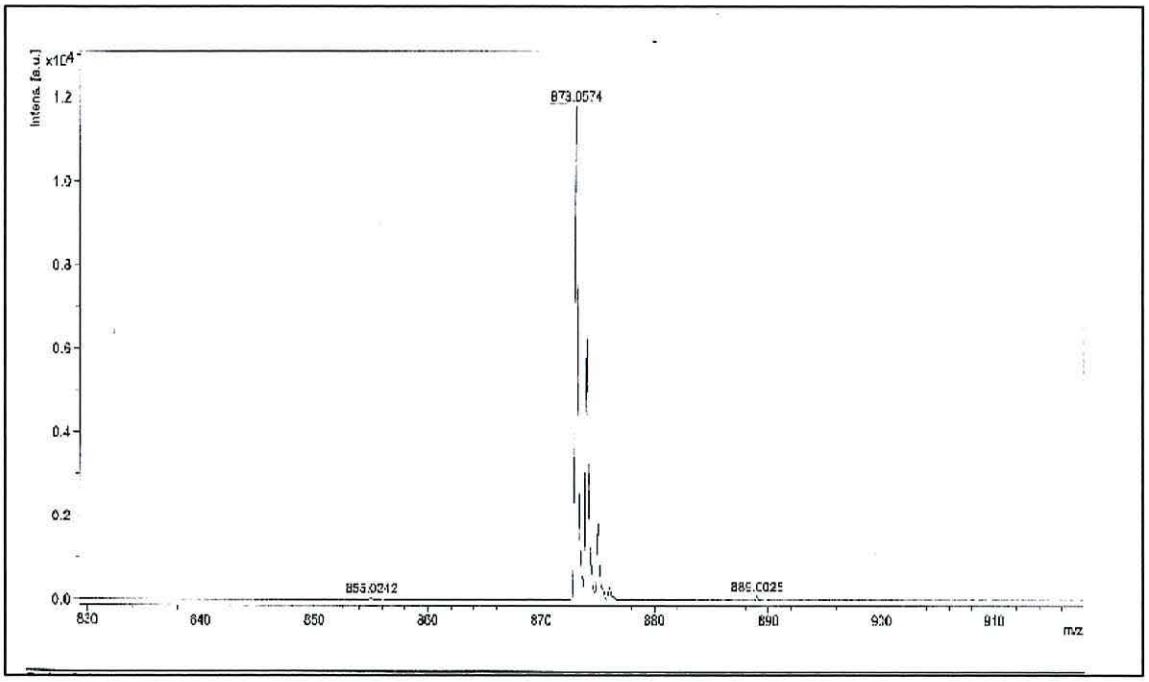


***Figure 28*: MALDI MS of TDM (79a)**

The model TDM **79a** has been tested as an antigen in ELISA with TB positive and negative serum samples as used earlier (see page 78) (**Figure 29**).

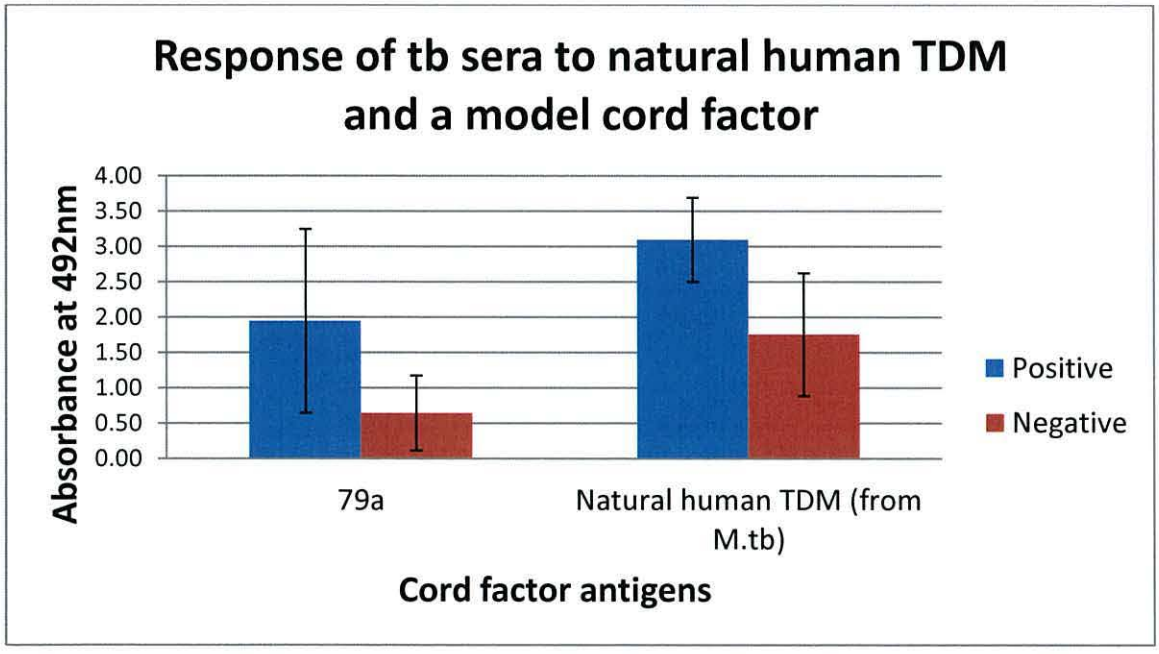


***Figure 29*: ELISA signals of antibody binding to both TB positive and TB negative sera for model cord factor (79a) and natural M.tb cord factor.** (Data and standard deviations are for 14 TB positive and 37 TB negative samples from indigenous populations provided by WHO)

In comparison with the natural human TDM from *M.tb*, the model TDM showed a lower average absorbance but a significant difference was observed between the absorbance of the negative sera and the positive sera. The selectivity and specificity of the model TDM were 71 % and 88 % respectively compared to 86 % and 88 % for the natural TDM (see appendix). Despite its relative simplicity the model TDM has relatively high selectivity and specificity. This might be an indication that the presence of the α-alkyl chain on the acid part of the cord factor has no significant effect on its antigenicity. If this can be confirmed by further investigations, there could be a chance of reducing the expense and the steps involved in the synthesis of mycolic acids and cord factors for use in the diagnosis of TB, as there will no longer be the need for alkylation at the α-position.

Conclusion

Chapter 5

The first aim of this project entailed the synthesis of α-mycolic acids containing dicyclopropane rings, with the purpose of acquiring a complete range of all the diastereomers in that category. Previously, two of these diastereomers have been prepared by *Al Dulayymi et al*[163,169] and in this work the synthesis of two others have been reported thereby completing the range of four *cis*-α-mycolic acid diastereomers containing dicycolpropane rings (**Figure 30**).

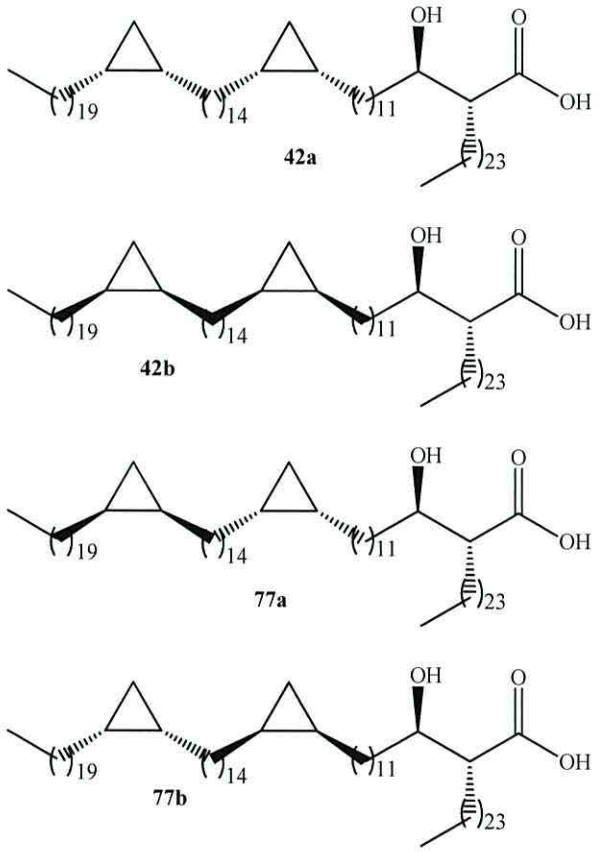


***Figure 30*: The complete range of dicyclopropane-α-mycolic acid diastereomers**

Of the four diastereomers shown in Figure **35**, the ones that were successfully synthesised for the first time, together with some key intermediates were (*S*,*R*), (*R*,*S*) *cis*-dicyclopropane α- mycolic acid (**77a**) and(*R*,*S*), (*S*,*R*) *cis*-dicyclopropane α- mycolic acid (**77b**).

In recent years, the stereochemistry of some of the functional groups in mycolic acids has become clearer, to some degree through the specific rotations of fragments. However, this

does not hold for the *cis*-cyclopropane parts because they do not contribute to the specific rotations due to the fact that the cyclopropane is substituted with very similar long chains. As discussed before, preliminary ELISA assays of the binding of all four α-mycolic acid diastereomers to antibodies in TB sera have been conducted and the responses were similar for the four diastereomers with the exception of compound **77b,** which showed a higher average absorbance of 1.49 for the TB positive sera and 0.87 for TB negative sera. The average absorbances for the other compounds were below 0.75 for both positive and negative sera (**Figure 25** section **2.3.3.3**). There was also a significant difference between the average absorbance of the TB positive sera and the TB negative sera for compound **77b**. In addition, the selectivity and specificity of **77b** were 43 % and 94 % respectively compared to the values recorded for the three other diastereomers which were relatively low (see **Table VII** page 78). According to the ELISA results, the previously prepared enantiomers **42a** and **42b** showed essentially the same antigenicity. On the contrary, the enantiomers **77a** and **77b** prepared in this work showed different antigenicities. The difference observed in the biological activities of **77b** could be futher investigated by the preparation of the cord factors of the four α-mycolic acid diastereomers. It could be that the stereochemistry of the cyclopropane rings contributes to the antigenic properties of mycolic acids. Essentially the availability of all four isomers would now make it easier to determine in subsequent investigations which had the natural stereochemistry via their different biological activities.

The synthesis of the mycolic acids in this work, as in all previous work, was quite a cumbersome process which involved many tedious steps. This justified the second objective which was the development of a method for the synthesis of α-alkyl-β-hydroxy fragment (mycolic motif) which is a common unit in all mycolic acids. The preparation of this intermediate unit has so far proved to be the most difficult part of mycolic acid synthesis and as such an improvement in the prodecures was deemed necessary so as to ease the hard work entailed in the overall synthesis of mycolic acids and cord factors. In the past, two basic approaches to the preparation of the α-alkyl-β-hydroxy unit have been reported in literature.[176,205] The first approach was a direct long chain alkylation on the α-position. Al Dulayymi *et al*,[205] in the first approach conducted the chain extension using (2-benzeneoxyethyl) oxirane as the chiral intermediate. The (2-benzeneoxyethyl) oxirane was prepared from commercially available L-aspartic acid, according to methods

developed by *Frick et al.*[167] In the second approach reported by Toschi *et al,*[176] a short chain allylation was initially carried out followed by a further elongation by modified Julia-Kocienski reaction to achieve the intended chain length. The starting material was *cis*-olefin obtained from 1,12-decanediol. However, in more recent work by Koza *et al,*[177] L-aspartic acid was chosen as the starting material for the synthesis of the (2-benzeneoxyethyl) oxirane intermediate and then a short chain Fräter allylation followed by a further Julia-Kocienski chain extension was carried out on the α-position. In this new approach, two alternative methods (**Figure 31**) that involved fewer steps were targeted, using a starting material, *L*-malic acid, which was cheaper.

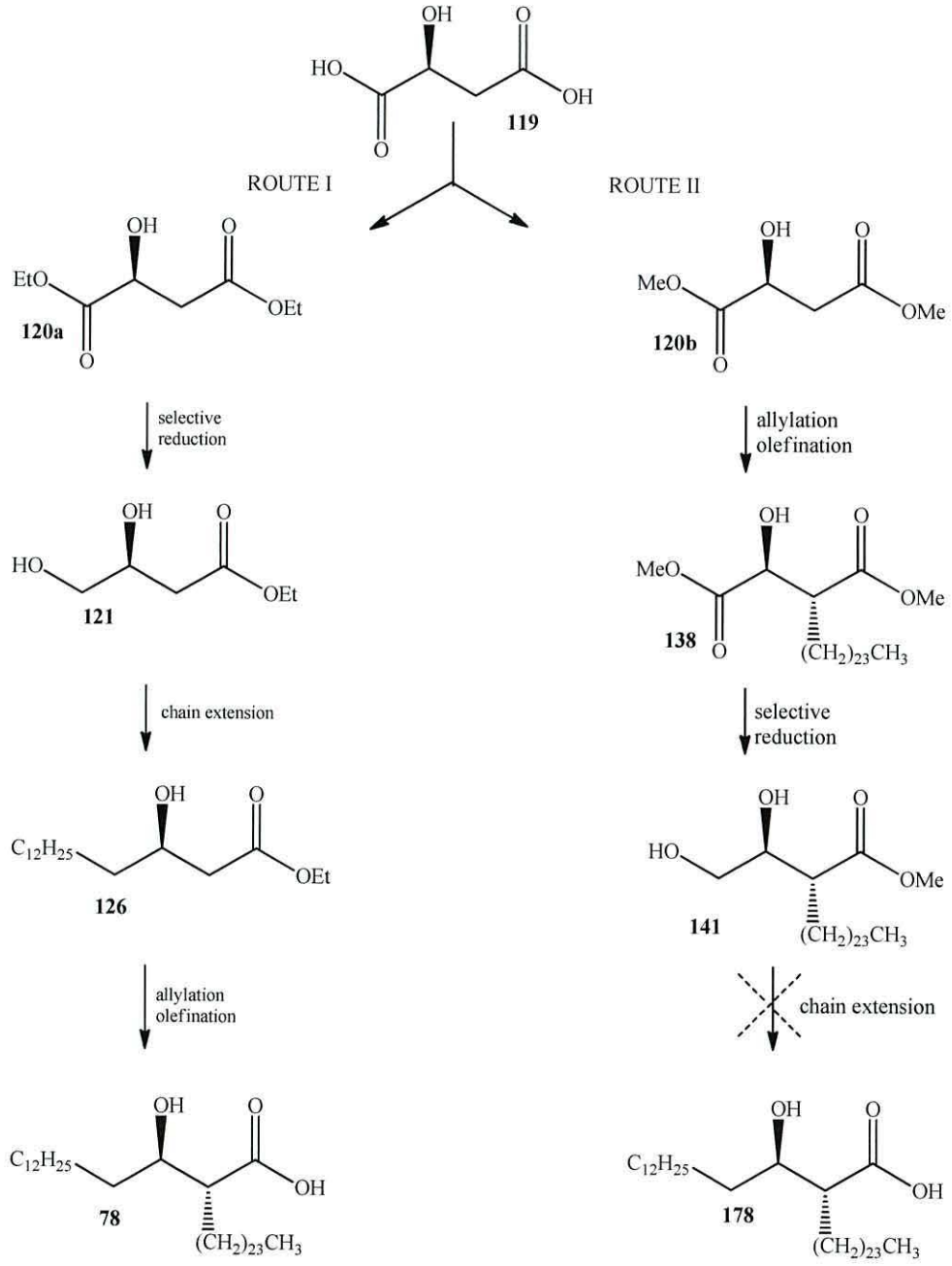


***Figure 31**: New methods for the preparation of the mycolic motif*

Route I was successfully completed in 6 steps (minus the Fräter allylation) with an overall yield of 14 %. Compared to the recent procedure by *Koza et al,*[177] which was achieved in 17 steps with an overall yield of 17 %, the number of reaction steps has been reduced by 5 steps without a significant reduction in percentage yield. Moreover, it is anticipated that a higher yield will be possible in time with improvement in the procedures. It is noteworthy that the final product (*R*)-2-((*R*)-1-hydroxytetradecyl)hexacosanoic acid (**78**) which is essentially a long chain mycolic acid was prepared for the first time and as such could be tested for biological activities. Additionally, it would be possible to chain extend the (2-benzeneoxyethyl) oxirane intermediate **124** to obtain **78a** with an oxytetrahydropyranyl Grignard reagent so as to achieve a similar compound which will enable linkage with other functional groups to give various mycolic acids as discussed in section **3.2.3.5** (see page 93).

In the case of **route II**, the alkylation at the α-position was successful and methyl -(*R*)-2-((*S*)-1,2-dihydroxyethyl)hexacosanoate (**141**) was prepared for the first time. However, several attempts under varied conditions and reagents to chain extend at the primary alcohol position were abortive. As a result, **route II** was not completed. Regardless, the derived compound **141** in this route represents the basic structure of the mycolic motif even though the chain extension for coupling to the meromycolates is still pending. It is believed that there are still prospects of resolving this challenge if more investigations can be conducted in the future.

Overall, two new methods for the synthesis of the mycolic motif have been devised. Although the Fräter allylation is a highly effective means to diastereoselectively introduce the α-alkyl substituent to the chiral β-hydroxy carboxylic acid, it still constituted a tedious step in the respective reaction schemes employed. Therefore, avoidance of the Fräter allylation as an added improvement of the preparation method would be appropriate for future work.

Another aim of this project entailed the preparation of model cord factors using a chiral β-hydroxy ester (a derived intermediate (**126**) in section **3.2.2.2** with no alkylation at the α-position). The synthesis was successfully carried out to obtain a TDM (**79a**) and a protected TMM (**154**). The TDM was obtained as the major product with a high yield of

78 % but the yield of the TMM was very negligible, such that the subsequent two step deprotection could not be carried out. ELISA tests have been conducted for the TDM **79a** in order to determine its biological activities. In comparison to the natural *M.tb*-TDM, the model TDM showed a lower antibody response. However, the test results for the model compound **79a** showed a significant difference between the average absorbance recorded for the TB positive sera and the TB negative sera. The selectivity and specificity values were 71 % and 88 % respectively and these figures are fairly close to the 85 % required by WHO for a diagnostic device. It could be that the α-alkyl chain has no significant effect on the biological activities. If this can be ascertained, it means that the α-alkylation step in the synthesis of mycolic acids can be avoided, thus reducing the tideous procedures involved in the synthesis of mycolic acids and cord factors.

The major areas suggested for future work include the following;

1. The preparation corresponding cord factors (**156**) and (**157**) (**Figure 32**) of the *cis*-dicyclopropane α-mycolic acids (**77a**) and (**77b**) according to the procedures in Section **4.1.2**, followed by testing so as to determine the physical and biological properties. It is anticipated that this would help in the development of the existing methods for detecting mycobacterial infection and may offer possibilities for the treatment of asthma and other immune diseases. Furthermore, the determination of the biological properties would also help to establish whether the stereochemistry of the *cis*-dicylopropane α-mycolic acids is critical to the biochemical effects in the natural material. Perhaps, preparing the different synthetic analogues could help in understanding the role of the acids in the drug permeability of the cell envelope based on their individual structural features.

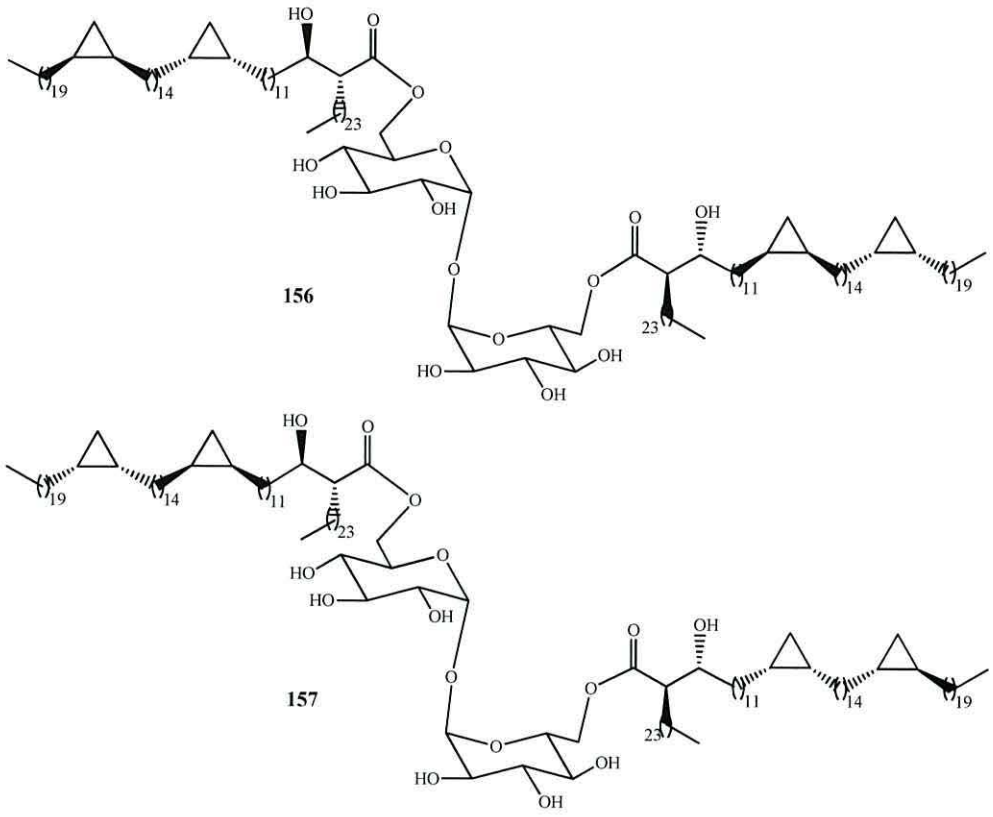


***Figure 32*: Proposed cord factors of cis-dicyclopropane α-mycolic acids**

2. The suggested approach for the preparation of the α-alkyl-β-hydroxy (**Figure 33**)

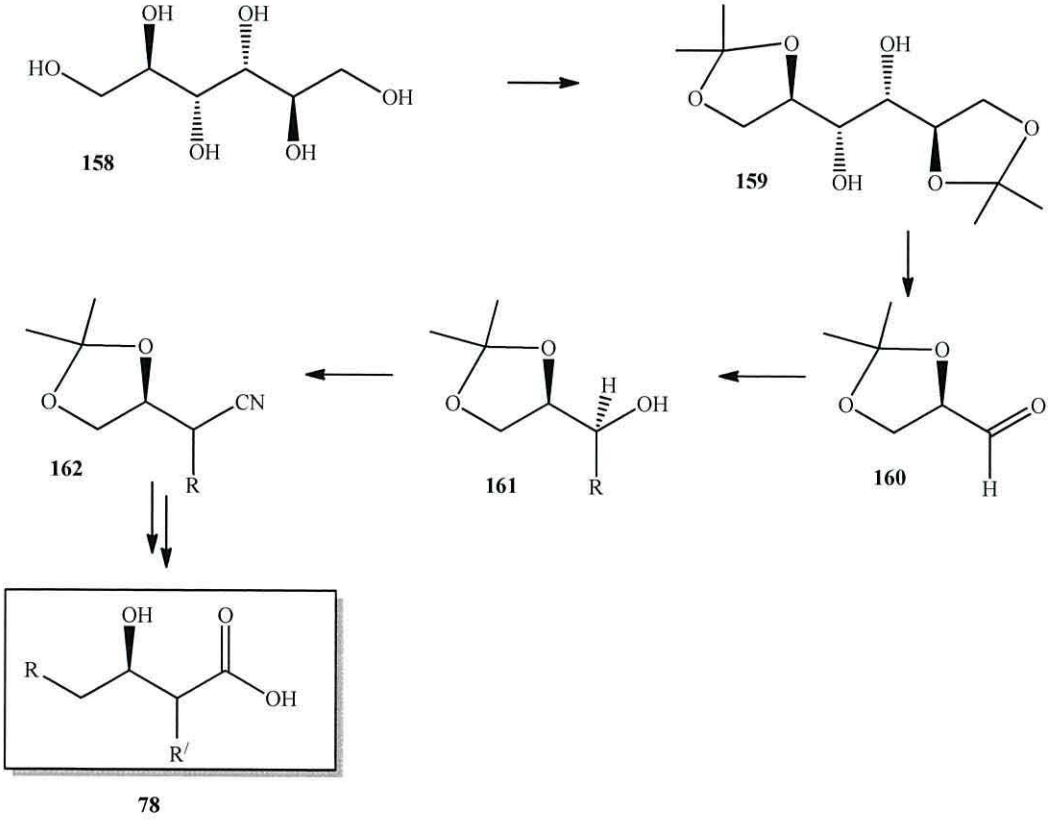


***Figure 33*: Proposed preparation of the mycolic motif from D-mannitol**

The suggested starting material in this method is *D*-mannitol which is cheap, and most of all it excludes the Fräter allylation step. The acid group is introduced via a nitrile replacement of the alcohol **161** The critical element of this method would be to control the stereochemistry of the formation of the alcohol **161** and the nitrile **162** or to be able to separate the isomers easily. If the desired product is achieved in the correct stereochemistry with this proposed scheme, the synthesis of mycolic acids and cord factors would be much easier.

Experimental section

Chapter 6

All chemicals used were purchased from commercial suppliers. Unless otherwise stated, all reagents and solvents were of reagent grade. However, solvents such as THF and diethyl ether were distilled over sodium wire and benzophenone under nitrogen, whereas dichloromethane was distilled over calcium hydride powder. Petroleum spirit was of boiling point 40–60 °C and was referred to as petrol. All organic solutions were dried over anhydrous magnesium sulfate. Solvents were removed by Buchi rotavapor at 14 mmHg and the residual traces were finally removed using the high vacuum evaporator at 0.1 mmHg. Reactions carried out under inert conditions were subjected to a slow stream of nitrogen using a nitrogen balloon. A cooling bath of methylated spirit and liquid nitrogen was used for reactions carried out at low temperatures. Pre-coated silica plates and silica gel were obtained from Aldrich and were used for TLC and column chromatography.

Optical rotations $[\alpha]_D^T$ were measured using a POLAR 2001 optical activity polarimeter and the solvent used was $CHCl_3$. Values were calculated using the following equation:

$$[\alpha]_D^T = \frac{10^4 \text{ x } \alpha}{l \text{ x } c}$$

α = observed angles of rotation

l = light path length in mm

c = concentration in g/100cm^3

T = temperature

Melting points were measured using a Gallenkamp melting point apparatus.

Infrared [IR] measurements were carried out on Perkin Elmer 1600 series FT-IR or Bruker Tensor 27 spectrometer as KBr discs (solids) or thin films on NaCl windows.

NMR spectra were recorded either on an Advance 500 spectrometer. The ^{1}H spectra were run at 500 MHz and ^{13}C spectra were run at 125 MHz. Deuterated chloroform ($CDCl_3$) was used as the reference solvent if not otherwise stated. Chemical shifts for ^{1}H and δ 7.27 ppm and δ 77.0 ppm respectively. Data were reported as follows: chemical shift,

integration, multiplicity (br, broad; s, singlet; d, doublet; t, triplet; q, quartet; pent, pentet; sext, sextet; hept, heptet, m, multiple), coupling constant.

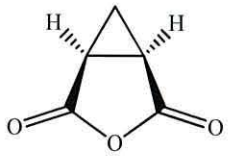
Mass spectra were obtained using a Bruker MicroTOF time of flight mass spectrometer and Matrix-assisted laser desorption ionisation (MALDI) were measured by using a Bruker Daltonics *Reflex IV*.

An assessment form for the control of substances hazardous to health (COSHH) was completed for each experiment according to health and safety regulations.

6 1 Synthesis of mycolic acids

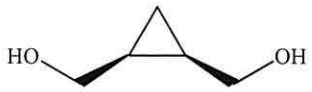
6.1.1 Synthesis of the meromycolates

Experiment 1: Cyclopropane-1,2-dicarboxylic acid anhydride (27)[196]



A mixture of *cis*-cyclopropane-1,2-dicarboxylic acid (130 g, 1.0 mol.) and thionyl chloride (90 mL) in dichloromethane (500 mL) was refluxed at 60 °C for 24 hours. The mixture was evaporated to dryness, re-dissolved in hexane (50 mL) and evaporated again. This process was repeated two more times in order to remove excess thionyl chloride. The solid residue was dissolved in dichloromethane (300 mL), filtered through a bed of celite, washing with warm dichloromethane (100 mL). A clear pale yellow solution was obtained, which was concentrated and recrystallization from hexane (300 mL) to give long needles of anhydride crystals (127.0 g, 98 %), m.p. 48 – 50 °C (lit. value[196] m.p. 58 – 59 °C). The compound showed δ_H, δ_C and ν_{max} identical to the literature.[196]

Experiment 2: ***cis*–2–Hydroxymethylcyclopropyl methanol (82)**[197]



THF (300 mL) was cooled to –5 ˚C and lithium aluminium hydride (25.5 g, 0.671 mol.) was gradually added so that no vigorous evolution of hydrogen occurred. Cyclopropane-1,2- dicarboxylic acid (50.0 g, 0.446 mol.) in THF (200 mL) was added dropwise causing an exothermic reaction but the temperature was kept under control below 20˚C. After the addition, the reaction was refluxed at 100˚C for 2.5 hours. Then it was cooled to 10˚ C and quenched with freshly prepared sodium sulfate decahydrate (60 mL) stirring until the colour changed from grey to white. This was filtered through a bed of celite and washed thoroughly with THF. TLC (1:1; petrol/ethyl acetate) showed one spot which had a lower R_f than the starting material When the filtrate was evaporated a colourless oil, *cis–2–hydroxymethylcyclopropyl methanol* (32.1 g; 71 %) was obtained. The compound showed δ_H, δ_C and ν_{max} identical to the literature.[197]

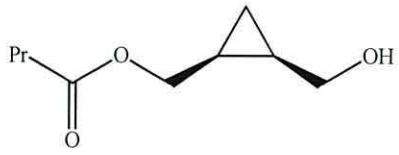
Experiment 3: Butyric acid (*cis*–2–butyryloxymethylcyclopropyl) methyl ester (83)[197]

A mixture of butyric anhydride (51.2 g, 0.324 mol.) and *cis*–2–hydroxymethylcyclopropyl methanol (15.0 g, 0.147 mol.) was refluxed at 120 °C for 1 hour and then cooled to room temperature. Aq. sodium hydroxide (10.5 g in 150 mL of water) was added, and the mixture was extracted with dichloromethane (300 mL). The aqueous layer was separated and re–extracted with dichloromethane (2 x 100 mL), and then the combined organic layers were washed with sat. aq. sodium bicarbonate. The solution was dried over magnesium sulfate, filtered and evaporated. The residue was purified by high vacuum flash distillation at 110–145°C to give a colourless oil, *butyric acid (1R,2S)-(cis–2–butyryloxymethylcyclopropyl) methyl ester* (32.0 g; 90 %), which showed δ_H and δ_C and ν_{max} identical to the literature.[197]

Experiment 4: Butyric acid (1*R*,2*S*)–*cis*–(2–hydroxylmethylcyclopropyl) methyl ester (28a)[166,197]

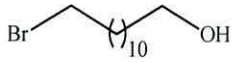
Ethylene glycol (120 mL) in distilled water (350 mL) was cooled to 3°C under nitrogen, lipase (3.0 g) was added and the pH was about 6.8. Butyric acid (*cis*–2–butyryloxymethylcyclopropyl) methyl ester (30 g, 0.124 mol.) was added, hydrolysis began and the pH became lowe due to the formation of butyric acid. The pH was restored to 6.5 by cautiously adding sodium hydroxide solution (1.0 M) but still maintaining the temperature below 3°C. Further lipase (0.75 g) was added to the reaction mixture after 1 hour, whilst the pH and temperature were still maintained at 6.5 and 3 °C respectively. A constant pH of 6.5 without addition of more sodium hydroxide was an indication that hydrolysis had stopped and that the reaction was complete. The total volume of sodium hydroxide solution used was 120 mL and the total time of the reaction was 5 hours. The mixture was filtered through a bed of celite, washed with water (80 mL) and then with ethyl acetate (100 mL). Sat. aq. sodium bicarbonate (100 mL) and sat. aq.NH_4Cl (100 mL) were added and the mixture was extracted with ether (3 x 300 mL). The combined organic layers were dried over magnesium sulfate, filtered and evaporated. The crude product was purified by column chromatography eluting with petrol/ether (1:1) to give a colourless oil, *butyric acid* (1*R*,2*S*)–*cis*(–2–*hydroxy-methylcyclopropyl) methyl ester* (17.7 g; 83 %), $[\alpha]_D^{21}$ +22.1(c 1.3, $CHCl_3$) (lit. values[166,197] $[\alpha]_D^{24}$ +19.8 (c 2.8, $CHCl_3$), $[\alpha]_D^{22}$ +18. 2 (c 1.58, $CHCl_3$)). The compound showed δ_H , δ_C and ν_{max} identical to the literature.[166, 197]

Experiment 5: Butyric acid (1*S*,2*R*)–*cis*–2–(hydroxylmethyl)cyclopropyl methyl ester (28b)[166]



A solution of 2,2,2-triflouroethyl butyrate in isopropyl ether was prepared by dissolving 2,2,2-trifluoroethanol (50 g, 0.50 mol.) in isopropyl ether (500 mL), and then butyric anhydride (92 mL, 0.58 mol.) was added. The mixture was cooled to about 4 °C, trimethylsilyl trifluoromethane sulfonate (2 mL) was added, causing an exothermic reaction which raised the temperature to 25 °C. The mixture was stirred at room temperature, over a period of 1.5 hours until GC showed the absence of butyric anhydride. Aqueous sodium hydroxide (350 mL, 1.25M) and sodium hydrogen carbonate (25 g) were added and the mixture was stirred for 15 minutes. The aqueous phase was separated and extracted with isopropanol (75 mL x 3). The combined organic phases were washed with brine (300mL), dried over magnesium sulfate and concentrated. Assuming a 90 % yield, the IPE solution was estimated to contain 76.5 g of 2,2,2-triflouroethyl butyrate. *cis*–2–Hydroxymethylcyclopropyl) methanol (26 g, 0.25 mol.) was dissolved in THF (120 mL) and added to the above isopropanol solution of 2,2,2-triflouroethyl butyrate (76.5 g, 0.45 mol.) and then lipase (25 g) was added. The reaction was stirred at room temperature for over 24 hours, after which GC showed a low diol content. Then it was filtered through a bed of celite, washed thoroughly with isopropanol and evaporated to give a yellow liquid residue, which was dissolved in dichloromethane (300 mL) and washed with sat. aq. sodium hydrogen carbonate (100 mL). The organic layer was separated, dried over $MgSO_4$ and evaporated. Colunm chromatography eluting with petrol/ethyl acetate (5:2) to give a clear liquid, *butyric acid (1S,2R)–2–cis–(hydroxymethyl)-cyclopropyl methyl ester* (28 g; 66 %), $[\alpha]_D^{20}$ –22.7 (c =1.5, $CHCl_3$) (lit. value[166] $[\alpha]_D^{24}$ – 18.1 (c 1.4, $CHCl_3$)), which showed δ_H, δ_C and ν_{max} identical to the literature.[166]

Experiment 6: 12–Bromo–1–dodecanol (85b)[201]



1,12–Dodecanediol (30 g, 0.148 mol.) was dissolved in toluene (300 mL) and hydrogen bromide (40%, 35 mL) was added. The mixture was heated under reflux at 120˚C for 17 hours. The toluene layer was separated and evaporated. The residue was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give a white solid, *12–bromo–1–dodecanol* (25.5 g, 80 %). The compound showed δ_H, δ_C and ν_{max} identical to the literature.[201]

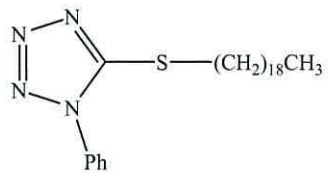
Experiment 7: 1–Nonadecanol (86)[163]

Magnesium turnings (9.0 g 0.375 mol.) were placed in dry THF (120 mL) and stirred at ambient temperature in a round bottom flask that was fitted with a reflux condenser. 1–Bromoheptane (60 g, 0.335 mol.) in dry THF (80 mL) was gradually added to the suspension at a steady rate that generated an exothermic reaction, enough to maintain a reflux. The refluxing was continued for 1 hour at 80 °C, after which the reaction was cooled to –10 °C, and then 12–bromo–1–dodecanol (27 g, 0.102 mol.) in dry THF (120 mL) was added dropwise. On further cooling to –60 °C, dilithum tetrachlorocuprate (24 mL, 0.1M) was added and the mixture was stirred overnight at room temperature. In order to quench the reaction, sat. aq. NH_4Cl (200 mL) was added at 0 °C, followed by ethyl acetate (200 mL). The bluish aqueous layer and solid precipitate were separated and re-extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with brine (200 mL), dried and evaporated to a pale yellow residue. Recrystallization from petrol gave a white crystalline, *1–nonadecanol* (15.0 g, 79 %), m.p. 58 – 60 °C (lit. value[163] m.p. 60 – 62 °C), which showed δ_H, δ_C and ν_{max} identical to the literature.[163]

Experiment 8: 1 – Bromononadecane (87)[163,164]

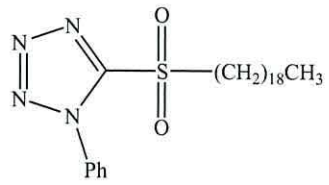
1–Nonadecanol (28 0 g, 0.099 mol.) and triphenylphosphine (33.6 g, 0.128 mol.) were dissolved in dichloromethane (800 mL) and cooled to 0 °C. N–bromosuccinamide (22.8 g, 0.128 mol.) was added in portions over 20 minutes, and the reaction was stirred for 2 hours at room temperature. When TLC showed no spot for the starting material, the reaction was quenched with sat. aq. sodiumbisulphite (250 mL). The aqueous layer was separated and re-extracted with dichloromethane (2 x 400 mL), and then the organic layer was washed with water (800 mL), dried and evaporated. The residue was dissolved in ethyl acetate (800 mL) and refluxed for 30 minutes and the triphenylphosphonium oxide was filtered off washing with excess ethyl acetate. Then the filtrate was evaporated and the residue was purified by column chromatography, eluting with petrol/ethyl acetate (5:2) to obtain a white solid, 1–*bromononadecanol* (28.5 g; 83.8 %), m.p. 36 – 38 °C (lit. value[163] m.p. 35 – 37 °C), which showed δ_H, δ_C and ν_{max} identical to the literature.[163]

Experiment 9: 5–(Nonadecyl–1–sulphanyl)–1–phenyl–1H–tetrazole (88)



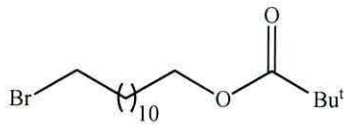
According to general methods,[198] 1–phenyl–1H–tetrazole–5–thiol (14.6 g, 0.082 mol.), 1–bromononadecane (28 g, 0.081 mol.) and K_2CO_3 (21.9 g, 0.159 mol.), were dissolved in acetone (300 mL) and refluxed at 80 °C for 2.5 hours. TLC was carried out to ensure the complete removal of thiol. Whilst the mixture was still hot, the inorganic salts were filtered off and washed with hot acetone. The filtrate was evaporated at high temperature, and the residue was dissolved in hot dichloromethane (200 mL) and then washed with water (350 mL). The aqueous layer was separated and re-extracted with dichloromethane (2 x 30 mL), and the combined organic layers were washed with water (350 mL), dried over $MgSO_4$ and evaporated. The crude product was recrystallized from methanol/acetone (2:1), filtered, washed and air dried, to give a white solid, *5–(nonadecyl–1–sulphanyl)–1–phenyl–1H–tetrazole* (34.5g. 95.8 %), m.p. 64–66 °C [Found $(M+Na)^+$ 467.3170. $C_{26}H_{44}N_4SNa$ requires: 467.3184], which showed δ_H (500 MHz, $CDCl_3$): 7.58–7.52 (5H, m), 3.38 (2H, t, *J* 7.67 Hz), 1.82 (2H, pent, *J* 7.6 Hz), 1.43 (2H, pent, *J* 6.5 Hz), 1.33–1.24 (30H, m), 0.88 (3H, t, *J* 6.9 Hz); δ_C (125 MHz, $CDCl_3$): 154.5, 133.8, 130.0, 129.7, 123.8, 33.4, 31.9, 29.6, 29.6, 29.6, 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 28, 22.6, 14.0; ν_{max} (cm^{-1}): 2919, 1502, 1092, 759.

Experiment 10: 5–(Nonadecyl–1–sulphonyl)–1–phenyl–1H–tetrazole (89)



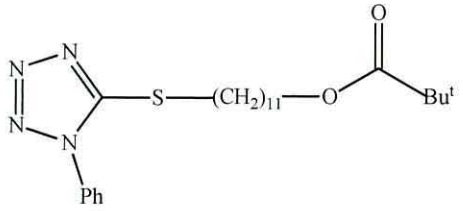
Using general methods,[198] sodium hydrogen carbonate (14.5 g, 0.172 mol.) was added to a stirred solution of 5–(nonadecyl–1–sulphanyl)–1–phenyl–1H–tetrazole (17.0 g, 0.038 mol.) in dichloromethane (250 mL), followed by the addition of dry 3–chloroperbenzoic acid (17.6g, 0.077 mol.) in dichloromethane (250 mL). The mixture was stirred overnight for 18 hours to give a whitish precipitate. Then it was quenched with sodium hydroxide (5%; 200mL) and diluted with dichloromethane (500 mL). After a shaking vigorously, the aqueous layer was separated and re–extracted with dichloromethane. The combined organic layers were washed with water (2 x 200 mL), dried over $MgSO_4$ and evaporated. The crude product was re–crystallized from methanol/acetone (1:1) to obtain a white solid *5–(nonadecyl–1–sulphonyl))–1–phenyl–1H–tetrazole* (16.5 g; 91 %), m.p. 60–68 °C [Found $(M+Na)^+$ 499.3045. $C_{26}H_{44}N_4O_2SNa$ requires: 499.3082], which showed δ_H (500 MHz, $CDCl_3$): 7.71–7.69 (2H, m), 7.63–7.57 (3H, m), 3.74 (2H, t, *J* 7.8 Hz), 1.96 (2H, pent, *J* 7.0 Hz), 1.50 (2H, pent, *J* 7.0 Hz), 1.35–1.25 (30H, br.m), 0.89 (3H, t, *J* 6.6 Hz); δ_C (125 MHz, $CDCl_3$): 153.5, 133.1, 131.5, 129.7, 125.1, 57.0, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.2, 28.9, 22.7, 22.0, 14.1; ν_{max} (cm^{-1}): 2919, 470, 1342, 1154, 771.

Experiment 11: 2,2–Dimethylpropanoic acid (12–bromo)dodecyl ester (90)[202]



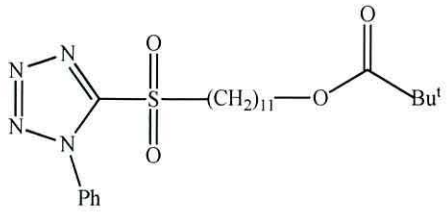
12–Bromo–1–dodecanol (26 0 g, 0.098 mol.) was dissolved in dichloromethane (250 mL), cooled to 5° C, and then triethylamine (34 mL, 0.245 mol.) was added. After 15 minutes, trimethylacetyl chloride (15 mL, 0.118 mol.) and 4-dimethylaminopyridine (0.5 g) were added and the reaction was stirred at room temperature for 3 hours, after which TLC showed no starting material. Water (250 mL) was added to the mixture to quench the reaction, followed by dichloromethane (300 mL). The aqueous layer was separated and re–extracted with dichloromethane (3 x 300 mL) and the combined organic layer was tested with litmus paper, which indicated that it was basic. As a consequence, hydrochloric acid (5 %, 20 mL) was added to neutralize the extract. The organic layer was washed with excess water, dried over $MgSO_4$ and evaporated. Column chromatography eluting with petrol/ethyl acetate (5:1) gave a clear oil, *2,2–dimethylpropanoic acid (12–bromo) dodecyl ester* (32.4 g; 95 %), which showed δ_H, δ_C and ν_{max} identical to the literature.[202]

Experiment 12: 2,2–Dimethylpropanoic acid 12–(1–phenyl–1H–tetrazole–5–ylsulfanyl) dodecyl ester (91)[202]



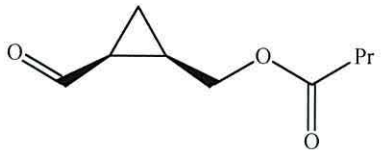
1–Phenyl–1H–tetrazole–5–thiol (16.2 g, 0.091 mol.), potassium carbonate (24.6 g, 0.178 mol.) and 2,2–dimethylpropanoic acid (12–bromo)dodecyl ester (31.0 g, 0.089 mol.) were dissolved in HPLC grade acetone (350 mL) and the mixture was stirred at room temperature for 1 hour, refluxed at 70 °C for the another 2 hours and then stirred overnight at room temperature. After TLC showed no starting material, the inorganic salts were filtered off washing with excess acetone. The filtrate was evaporated and the residue was treated with dichloromethane and water. The aqueous layer was separated and re-extracted with dichloromethane (3 x 150 mL) and the combined organic layer was washed with water (2 x 300 mL), dried and evaporated to give a thick oil, *2,2–dimethylpropanoic acid 12–(1–phenyl–1H–tetrazole–5–ylsulfanyl)dodecyl ester* (35.5 g, 90%) which showed δ_H, δ_C and ν_{max} identical to the literature.[202]

Experiment 13: 2,2–Dimethylpropanoic acid 12–(1–phenyl–1H–tetrazole–5–sulfonyl) dodecyl ester (92)[202]



Ammonium molybdate VI tetrahydrate (43.3 g, 0.035 mol.) was dissolved in cold hydrogen peroxide (35% w/w, 100 mL) and was carefully added to 2,2–dimethyl-propanoic acid 12–(1–phenyl–1H–tetrazole–5–ylsulfanyl)dodecyl ester (34.0 g, 0.076 mol.) in IMS (800 mL) at 10 °C. The reaction was stirred for 2 hours at room temperature, another portion of ammonium molybdate VI tetrahydrate (16.3 g, 0.013 mol.) in cold hydrogen peroxide was added, and then it was stirred overnight for over 18 hours. The mixture was poured into water (2.5 litres) and extracted with dichloromethane (3 x 400 mL). The combined organic layers were separated, washed with water (2 x 400 mL) and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by column chromatography eluting with petrol/ether (5:2), to give a white solid *2,2–dimethylpropanoic acid 12–(1–phenyl–1H–tetrazole–5–sulfonyl) dodecyl ester* (34.1 g, 94%), m.p. 35 – 37°C (lit. value[202] m.p. 34 – 36°C), which showed δ_H, δ_C and ν_{max} identical to the literature.[202]

Experiment 14: Butyric acid (1*R*,2*S*)–(cis–2–formyl cyclopropyl) methyl ester (93a)[166,197]



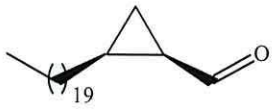
PCC (18.8 g, 0.087 mol.) was dissolved in dichloromethane (300 mL) and then butyric acid (1*R*,2*S*)–(*cis*–2–hydroxymethylcyclopropyl)methyl ester (6.0 g, 0.035 mol.) in dichloromethane (100 mL) was gradually added in small portions to the suspension and then stirred for 2 hours at room temperature. When TLC showed that the reaction was complete, it was diluted with ethyl acetate (400 mL) and filtered through a bed of celite and silica. The solvent was evaporated and the crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:2) to give a colourless oil, *butyric acid* (1*R*,2*S*)–*(cis–2–formylcyclopropyl)methyl ester* (5.3 g; 90 %), $[\alpha]_D^{22}$ + 73.1 (c 1.5, $CHCl_3$) (lit. values[166, 197] $[\alpha]_D^{24}$ + 88.9 (c 2.14, $CHCl_3$), $[\alpha]_D^{22}$ + 59.8 (c 2.68, $CHCl_3$)).The compound showed δ_H, δ_C and ν_{max} to the literature.[166,197]

Experiment 15: (1*R*,2*S*)–*cis*–2–Eicosylcyclopropyl methanol (96a)

5–(Nonadecyl–1–sulphonyl)–1–phenyl–1H–tetrazole (15.0 g, 0.032 mol.) and butyric acid (1*R*,2*S*)–(*cis*–2–formylcyclopropyl) methyl ester (4.8 g, 0.282 mol.) were dissolved in dry THF (250 mL) and cooled to –10°C. Lithium bis–(trimethylsilyl) amide (38.6 mL, 0.041 mol.) was carefully added and the reaction was stirred for 1.5 hours. TLC showed that the reaction was complete, and then water (100 mL) was added to quench it. The aqueous layer was separated and extracted with petrol/ether (1:1, 3 x 50 mL) and the combined organic layers were washed with sat. aq. sodium hydroxide (2 x 100 mL), separated and dried over $MgSO_4$ and filtered. The filtrate was evaporated to obtain a crude residue which was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give a colourless oil, *butyric acid (E/Z)–(1R,2S)–cis–2–eicos–1–enylcyclopropyl methyl ester* (9.5 g; 80 %), as a mixture of isomers. Lithium aluminium hydride (1.3 g, 0.034 mol.) in THF (60 mL) was reacted with the derived (1*R*,2*S*)–*cis*-2-ecos-1-enylcyclopropyl methyl ester (8.5 g, 0.020 mol.) in THF (100 mL) as before. The reaction was treated with freshly prepared sat. aq. sodium sulphate decahydrate (40 mL) and extracted with THF (4 x 40 mL). The filtrate was concentrated and the crude product was purified by column chromatography eluting with petrol/ ethyl acetate (5:2) to give a thick white oil, (*E*/*Z*)–(1*R*,2*S*)–*cis–2–eicos–1–enylcyclopropyl methanol* (6.4 g; 90 %). Hydrazine hydrate (80 mL), glacial acetic acid (5 mL) and sat. aq. copper sulphate (5 mL) were added in succession to the derived (*E*/*Z*)–(1*R*,2*S*)–*cis*–2–eicos–1–enylcyclopropyl methanol (6.0 g, 0.017 mol.) in 2–propanol (200 mL) at 70 – 80 °C. Subsequently, sodium (meta)periodate (73.4 g, 0.343 mol.) in hot water was added dropwise over a period of 2 hours, still maintaining the temperature at 70 – 80 °C. The reaction was stirred for 1 hour at room temperature and then quenched with sat. aq. ammonium chloride (100 mL). The aqueous layer was separated and extracted with petrol/ethyl acetate (1:1, 3 x 150 mL) and the combined organic layers were washed with brine, dried over anhydrous magnesium sulphate and filtered. The filtrate was evaporated and the crude product was recrystallized from petrol to give a white solid *(1R,2S)–cis–2–eicosylcyclpropyl methanol* (5.0 g; 83 %), m.p. 59°C, $[\alpha]_D^{26}$ +12.1 (c 1.1, $CHCl_3$) [Found $(M+Na)^+$ 373.34450. $C_{24}H_{46}ONa$ requires:

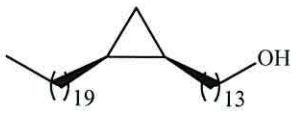
373.3446], which showed δ_H (500 MHz, $CDCl_3$): 3.65 (1H, dd, *J* 7.2, 11.0 Hz), 3.58 (1H, dd, *J* 7.9, 11.5 Hz), 1.67 (1H, bs), 1.50 – 1.38 (1H, m), 1.25 (38H, m), 1.11 – 1.03 (1H, m), 0.88 (3H, t, *J* 6.9Hz), 0.69 (1H, sext, *J* 4.5 Hz), –0.05 (1H,q, *J* 5.2, Hz) ; δ_C (125 MHz, $CDCl_3$): 63.5, 32.0, 30.5– 29.8, 28.8, 22.8, 18.5, 16.3, 14.0, 10.0; ν_{max} (cm^{-1}): 3313, 1463, 1377, 1040, 1008, 720.

Experiment 16: (1*R*,2*S*)–*cis*–2–Eicosylcyclopropane carbaldehyde (32a)[169]



The procedure in **Experiment 14** was repeated using (1*R*,2*S*)–*cis*–2–eicosylcyclopropyl methanol (4.5 g, 13.0 mmol.) in dichloromethane (200 mL), and pyridinium chlorochromate (7.0 g, 33.0 mmol.) in dichloromethane (20 mL). Diethyl ether (400 mL) was used to extract the organic layer. The filtrate was evaporated and the crude product was purified by column chromatography, eluting with petrol/ether (5:2) which gave (1*R*,2*S*)–cis–*2–eicosylcyclopropane carbaldehyde* (4.5 g, 99 %), m.p. 43 – 45°C, $[\alpha]_D^{26}$ +9.63 (c 1.4, $CHCl_3$); lit. value[169] $[\alpha]_D^{28}$ +3.76 (c 1.1, $CHCl_3$). The compound also showed δ_H, δ_C and ν_{max} identical to the literature.[169]

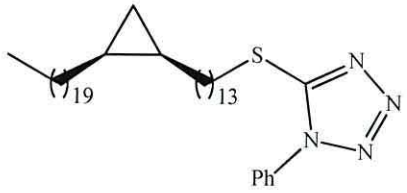
Experiment 17: 13–((1*R*,2*S*)–*cis*–2–Eicosylcyclopropyl)tridecan–1–ol (98a)[169]



A mixture of (1*R*,2*S*)–*cis*–2–eicosylcyclopropane carbaldehyde (4.0 g, 11.4 mmol.) and 2,2–dimethylpropanoic acid 12–(1–phenyl–1H–tetrazole–5–ylsulfonyl)dodecyl ester (7.1 g, 14.9 mmol.) in dry THF (200 mL) was treated with lithium bis-(trimethylsilyl) amide (18.2 mL, 19.3 mmol.) at –10℃ under nitrogen. The mixture was allowed to reach room temperature and was stirred for another 2 hours. Then the reaction was quenched with sat. aq. ammonium chloride (200 mL), the aqueous layer was separated and extracted with petrol/ether (1:1, 3 x 200 mL). The combined organic layers were dried and concentrated and the residue was purified by column chromatography, eluting with petrol/ether (20:1) to give a white solid, *2,2–dimethylpropionic acid (E/Z)–13–((1R,2S)–cis–2–eicosylcyclopropyl) tridece–12–enyl ester* (6.6 g, 98 %), as a mixture of isomers. The derived ester (6.0 g, 10.2 mmol.) in THF (20 mL) was gradually added to a stirred suspension of lithium aluminium hydride (0.77 g, 20.3 mmol.) in THF (50 mL) placed in a cooling bath, in order to control the exothermic reaction. Subsequently, the cooling bath was removed and the reaction was refluxed at 100℃ for 2 hours after which it was cooled, quenched with freshly prepared sat. aq. sodium sulfate decahydrate (30 mL). The mixture was stirred until it turned white and then it was filtered through a bed of celite washing with THF (3 x 50 mL). The filtrate was evaporated and the crude product was purified by column chromatography eluting with petrol/ ethyl acetate (5:2) to give a white solid *(E/Z)–13–((1R,2S)–cis–2–eicoscyl-cyclopropyl)tridece–12–en–1–ol* (5.0 g: 91 %). Hydrazine monohydrate (20 mL), acetic acid (2 mL), sat.aq. copper sulfate (2 mL) were added in succession to the derived alcohols (5.0 g, 9.7 mmol.) in iso–propanol (250 mL) at 80℃, and then and sodium metaperiodate solution (20 g, 97.0 mmol.) in hot water (60 mL) was carefully added dropwise, still maintaining the temperature at 80℃. After the addition, the reaction was allowed to cool down and then quenched with sat. aq. Ammonium chloride. The aqueous layer was seprated and re-extracted with petrol/ ether (1:1, 4 x 200 mL). The crude product was re-crystallized from chloroform to give *13–((1R,2S)–cis–2–eicoscylcyclo-propyl)tridecan–1–ol* (3.8 g; 75%), m.p. 74°C , $[\alpha]_D^{26}$ +2.39 (c 1.2, $CHCl_3$)

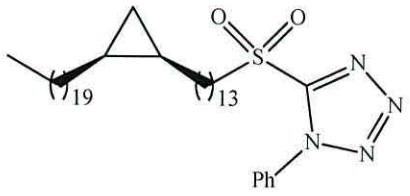
(lit. value[169] $[\alpha]_D^{32}$ – 0.33 (c 2.03, $CHCl_3$), which showed δ_H, δ_C and ν_{max} identical to the literature.[169]

Experiment 18: 5–[13–((1*R*,2*S*)-*cis*-2–Eicosylcyclopropyl)tridecylsulfanyl]–phenyl–1H–tetrazole (99a)



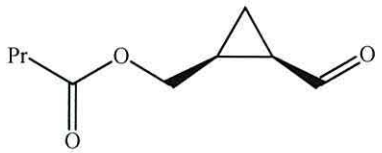
Following general methods,[163] 13–((1*R*,2*S*)–*cis*-2–eicosylcyclopropyl)tridecan–1–ol[169] (4.0 g, 7.7 mmol.) was dissolved in dry THF (10 mL) together with triphenylphosphine (2.6 g, 10.0 mol.) and 1–phenyl–1H–tetrazole–5–thiol (1.8 g, 10.0 mmol.). The mixture was cooled to 0 °C, followed by the addition of diethyl azodicarboxylate (1.7 mL, 10 mmol.) in dry THF (5 mL). The mixture was allowed to attain room temperature and then stirring was continued overnight. Subsequently, the solvent was evaporated, the residue was washed with petrol/ ether (10:1) and then filtered. The filtrate was evaporated and the product was purified by column chromatography eluting with chloroform to give 5–*[13–((1R,2S)–cis–2–eicosylcyclopropyl)tridecylsulfanyl]–phenyl–1H–tetrazole* (5.1 g; 97 %), m.p. 42–44°C, $[\alpha]_D^{22}$ –1.2 (c 1.3, $CHCl_3$) [Found $(M+Na)^+$ 703.5658. $C_{43}H_{76}N_4SNa$ requires: 703.5683], which showed δ_H (500 MHz, $CDCl_3$): 7.62–7.52 (5H, m), 3.42 (2H, t, *J* 7.4 Hz), 1.84 (2H, pent, *J* 7.5 Hz), 1.47 (2H, pent, *J* 7.3 Hz), 1.43–1.12 (57 H, m), 0.91 (3H, t, *J* 6.9 Hz), 0.71–0.64 (2H, m), 0.59 (1H, dt, *J* 4.1, 8.3 Hz), –0.35 (1H, q, *J* 4.9 Hz); δ_C (125 MHz, $CDCl_3$): 154.8, 134.2, 133.3, 130.1, 125.9, 56.5, 33.7, 29.9–28.9, 22.9, 15.6, 14.4, 11.1; ν_{max} (cm^{-1}): 1600, 1465, 1378, 1244, 1168, 1015, 693.

Experiment 19: 5–[13–((1*R*,2*S*)-*cis*-2–Eicosylcyclopropyl)tridecane–1–sulfonyl]–phenyl–1H–tetrazole (100a)



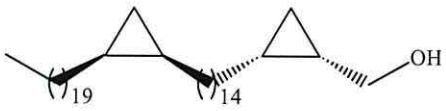
According to established methods,[163] sodium hydrogen carbonate (1.7 g, 19.9 mmol.) was added to a stirred solution of 5–[13–((1*R*,2*S*)-*cis*-2–eicosylcyclopropyl)tridecylsulfanyl]–phenyl–1H–tetrazole (3.0 g, 4.4 mmol.) in dichloromethane (50 mL), followed by the addition of a mixture of dry 3–chloroperbenzoic acid (2.0 g, 88.0 mmol.) and dichloromethane (50 mL). The reaction was stirred at room temperature for 18 hours to give a whitish precipitate. Subsequently, aq. sodium hydroxide (5%; 100mL) was added in order to quench the reaction. The aqueous layer was separated and extracted with dichloromethane (3 x 200 mL). The combined organic layers were washed with water (2 x 200 mL), dried over $MgSO_4$ and evaporated. The crude product was purified by recrystallizing from methanol/acetone (1:1), which gave *5–[13–(1R,2S)-cis-2–eicosyl-cyclopropyl)tridecane–1–sulfonyl]–phenyl–1H–tetrazole* (2.1 g; 67 %), m.p. 50–52 °C, $[\alpha]_D^{22}$ +1.7 (c 1.4, $CHCl_3$) [Found $(M+Na)^+$ 735.5596. $C_{43}H_{76}N_4SNa$ requires: 735.5581]. The compound showed δ_H (500 MHz, $CDCl_3$): 7.72–7.70 (5H, m), 3.74 (2H, t, *J* 8.0 Hz), 1.96 (2H, br.pent, *J* 7.8 Hz), 1.50 (2H, br.pent, *J* 7.4 Hz), 1.45–1.10 (57H, m), 0.89 (3H, t, *J* 6.8 Hz), 0.66–0.64 (2H, m), 0.57 (1H, dt, *J* 4.1, 8.5 Hz), –0.33 (1H, q, *J* 4.8 Hz); δ_C (125 MHz, $CDCl_3$): 154.5, 134.0, 133.4, 130.6, 126.1, 56.4,29.8–28.4, 22.8, 22.2, 16.1, 14.3, 11.0; ν_{max} (cm^{-1}): 1764, 1597, 1464, 1377, 1357, 1218, 1147, 1013, 685, 633.

Experiment 20: Butyric acid (1*S*,2*R*)–(*cis*–2–formylcyclopropyl) methyl ester (93b)[166]



The method in **Experiment 14** was repeated using PCC (38 g, 0.176 mol.) in dichloromethane (500 mL) and butyric acid (1*S*,2*R*)–(*cis*–2–hydroxymethylcyclopropyl)methyl ester (12 g, 0.07 mol.) in dichloromethane (150 mL). The reaction was worked up and the crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:2) to give a colourless oil, *butyric acid (1*S*,2R)–(cis–2–formylcyclopropyl) methyl ester* (10 g; 84 %), $[\alpha]_D^{22} = -72.6$ (c =1.8, $CHCl_3$) (lit. value[166] $[\alpha]_D^{20} - 66.0$ (c 1.45 $CHCl_3$)), which showed δ_H, δ_C and ν_{max} identical to the literature.[166]

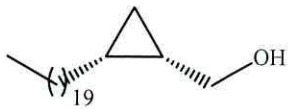
Experiment 21: (1*S*,2*R*)–2–[14–((1*R*,2*S*)–2–Eicosylcyclopropyl)tetradecyl]-cyclopropyl methanol (80a)



Using general methods,[163] butyric acid (1*S*,2*R*)–(*cis*–2–formylcyclopropyl)methyl ester[163] (0.5 g 3.0 mmol.) was dissolved in dry THF (30 mL), 5–[13–((1*R*,2*S*)–*cis*–2–eicosyl-cyclopropyl)tridecane–1–sulfonyl]–phenyl–1H–tetrazole (2.1 g, 30.0 mmol.) was added and the mixture was stirred at room temperature to obtain a clear solution. The stirred mixture was cooled to –10 °C and then lithium bis-(trimethylsilyl)amide (3.7 mL, 4.0 mmol.) in dry THF was added. The reaction was allowed to rise gradually to room temperature and stirring was continued for 1.5 hours. When TLC showed no remaining starting material, sat. aq. ammonium chloride (50 mL) was added and the mixture was extracted with petrol/ether (1:1, 3 x 50 mL). Column chromatography, eluting with petrol/ether (20:1) gave a white solid, *butyric acid (1S,2R)–2–[(E/Z)–14–((1R,2S)–2–eicosylcyclopropyl)tetradec–1–enyl]cyclopropyl methyl ester* (1.2 g; 62 %). Lithium aluminium hydride (0.14 g, 3.7 mmol.) was gradually added to THF (10 mL) at –10 °C and then the prepared ester (1.2 g, 1.8 mmol.) in THF (10 mL) was added dropwise to the stirred suspension, placed in a cooling bath such that the exothermic reaction was maintained below 20 °C. Subsequently, the cooling bath was removed and the reaction was refluxed at 100 °C for 2.5 hours and then treated with freshly prepared sat. aq. sodium sulfate decahydrate (20 mL) at 10 °C. Stirring was continued until the colour changed from grey to white. The mixture was filtered through a bed of celite and washed with THF (4 x 50 mL.). Again, the filtrate was evaporated and the residue was purified by column chromatography, eluting with petrol/ ethyl acetate (10:1) to give a white solid, *(1S,2R)–2–[(E/Z)–14–((1R,2S)–2–eicosylcyclopropyl)tetra-dec–1–enyl]cyclopropyl methanol* (0.77 g; 72 %). Hydrazine monohydrate (7 mL), acetic acid (1 mL), and aqueous copper sulfate (1 mL) were added in succession to the derived alcohol at 70 – 80 °C (0.77 g; 1.3 mmol.). Still maintaining the temperature at 80 °C, a solution of sodium metaperiodate (2.8 g, 13.1 mmol.) in hot water (10 mL) was carefully added dropwise. The reaction was allowed to cool to room temperature and after 1 hour, sat. aq. ammonium chloride was slowly added to quench the reaction. Then aqueous layer was separated and re-extracted with

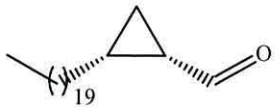
petrol/ether (1:1, 3 x 50 mL). The crude product was recrystallized from petrol to give a white solid, *(1S,2R)–2–[14–((1R,2S)–2–eicosylcyclopropyl)tetradecyl]cyclopropyl methanol* (0.55 g; 71 %), m.p. 51–53°C, $[\alpha]_D^{21}$ –7.32 (c 1.0, $CHCl_3$) [Found $(M+Na)^+$ 611.5916. $C_{41}H_{80}ONa$ requires; 611.6107] which showed δ_H (500 MHz, $CDCl_3$): 3.7 (1H, dd, *J* 7.3, 11.4 Hz), 3.59 (1H, dd, *J* 8.0, 11.2 Hz), 1.49–1.06 (67 H, m), 0.92–0.83 (5H, m including a triplet resonating at δ 0.89 with *J* 6.8 Hz), 0.72 (1H, dt, *J* 4.3, 8.3 Hz), 0.69–0.63 (2H, m), 0.57 (1H, dt, *J* 4.1, 8.5 Hz), –0.03 (1H, q, *J* 5.3 Hz), –0.33 ((1H, q, *J* 5.0 Hz); δ_C (125 MHz, $CDCl_3$): 63.6, 32.2, 30.5–28.8, 22.9, 18.4, 16.4; ν_{max} (cm^{-1}): 3368, 2852, 1771, 1464, 1370, 1170, 1064, 1037, 964, 932, 823.

Experiment 22: **(1*S*,2*R*)–*cis*–2–Eicosylcyclopropyl methanol (96b)**[163]



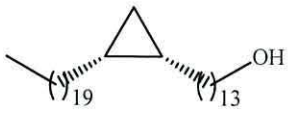
The procedures in **Experiment 15** were repeated. 5–(Nonadecyl–1–sulphonyl)–1–phenyl–1H–tetrazole (36.5 g, 76.5 mmol.) and butyric acid ((1*S*,2*R*)–*cis*–2–formylcyclopropyl) methyl ester (10.0 g, 58.8 mmol.), in dry THF (500 mL) were reacted with lithium bis–(trimethylsilyl)amide (94.0 mL, 99.4 mmol.) at –10°C under nitrogen. After 1 hour, the reaction was quenched with water (200 mL) and then the aqueous layer was separated and extracted with petrol/ethyl acetate (1:1, 2 x 100 mL). To neutralize any excess acid, the combined organic layers were washed with sat. aq. sodium hydroxide (2 x 100 mL), dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to obtain a yellowish oil, *butyric acid (E/Z)-((1S,2R)–cis–2–eicos–1–enylcyclopropyl) methyl ester* (19 g; 77 %), as a mixture of isomers. Lithium aluminium hydride (3.8g, 0.1 mol.) in THF (150 mL) was reacted with the derived esters (19 g, 0.045 mol.) in THF (250 mL) as in **15**. The reaction was treated with freshly prepared sat. aq. sodium sulfate decahydrate (100 mL), filtered through a bed of celite and washed thoroughly with THF (4 x 80 mL). The filtrate was concentrated and the residue was purified by column chromatography eluting with petrol/ ethyl acetate (5:2), to give a thick white oil, (E/Z)-*(1S,2R)–cis–2–eicos–1–enylcyclopropyl methanol* (14 g; 89 %). Hydrazine hydrate (30 mL), glacial acetic acid (3 mL), and sat. aq. copper sulfate (3 mL) were added to the derived alcohols (12 g, 34.3 mmol.) in 2–propanol (200 mL), and then sodium (meta)periodate (90.0 g, 0.42 mmol.) solution in hot water was carefully added over a period of 1.5 hours as in **15**. The reaction was quenched with sat. aq. ammonium chloride (100 mL), the aqueous layer was separated and extracted with petrol/ethyl acetate (1:1, 3 x 200 mL) and the combined organic layers were washed with brine (200 mL), dried over $MgSO_4$ and concentrated. The crude product was recrystallized from petrol to give a white solid *(1S,2R)–cis–2–eicosylcyclpropyl methanol* (9.7 g; 69 %), m.p. 59–60°C, $[\alpha]_D^{20}$ –10.6 (c = 1.2, $CHCl_3$) (lit. value[163] $[\alpha]_D^{22}$ – 7.5 (c 1.30, $CHCl_3$)), which showed δ_H, δ_C and ν_{max} identical to the literature.[163]

Experiment 23 (1S,2R)–cis–2–Eicosylcyclopropane carbaldehyde (32b)[163]



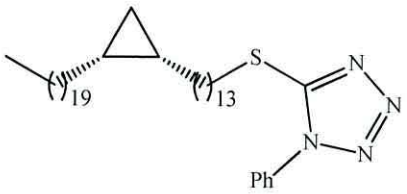
The procedure for the preparation of the corresponding enantiomer in **16** was repeated. (1*S*,2*R*)–*cis*–2–Eicosylcyclopropyl methanol (9.0 g 25.7 mmol.) was dissolved in dichloromethane (50 mL), and stirred in pyridinium chlorochromate (13.8 g, 63.9 mmol.) suspension in dichloromethane (300 mL) for 1.5 hours. The reaction was worked up and extracted with diethyl ether (400 mL). Column chromatography, eluting with petrol/ ethyl acetate (5:2) gave *(1S,2R)–cis–2–eicosylcyclopropanecarbaldehyde* (8.7 g, 97%), m.p. 45–47°C, $[\alpha]_D^{20}$ – 8.5 (c 1.64, $CHCl_3$) (lit. value[163] $[\alpha]_D^{23}$ – 3.9 (c 1.22, $CHCl_3$)). The compound showed δ_H, δ_C and ν_{max} identical to the literature.[163]

Experiment 24 **13–((1*S*,2*R*)–*Cis*–2–Eicosylcyclopropyl)tridecan–1–ol (98b)**[163]



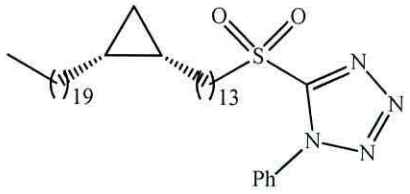
The procedures in **Experiment 17** was repeated using (1*S*,2*R*)–*cis*–2–eicosylcyclopropanecarbaldehyde (8.7 g, 24.9 mmol.), 2,2–dimethylpropanoic acid 12–(1–phenyl–1H–tetrazole–5–ylsulfonyl)dodecyl ester (13.1 g, 27.3 mmol.) in THF (400 mL), and lithium bis-(trimethylsilyl) amide (33.5 mL, 35.6 mmol.). The reaction was quenched with sat. aq. ammonium chloride (200 mL), the aqueous layer was separated and extracted with petrol/ether (1:1, 3 x 200 mL) and the combined organic layers were dried and concentrated. Column chromatography, eluting with petrol/ether (20:1) gave a white solid, *2,2–dimethylpropionic acid (E/Z)–13–((1S,2R)–cis–2–eicosylcyclopropyl)tridece–12–enyl ester* (13 g; 89 %), as a mixture of isomers. Lithium aluminium hydride (1.7 g, 44.0 mmol.) in THF (100 mL) and 2,2–dimethylpropionic acid was reacted with the derived esters (13.0 g, 22.0 mmol.) in THF (30 mL) as in **17**, and was treated with freshly prepared sat. aq sodium sulfate decahydrate (100 mL). The mixture was filtered through a bed of celite washed with THF (3 x 50 mL), and then evaporated. Column chromatography eluting with petrol/ ethyl acetate (5:2), gave *(E/Z)–13–((1S,2R)–cis–2–eicosylcyclopropyl)tridece–12–en–1–ol* (11.0 g: 97 %). Hydrazine monohydrate (50 mL), acetic acid (5 mL) and sat. aq. copper sulfate (5 mL) were added to the derived alcohols (11.0 g, 21.2 mmol.) in iso–propanol (500 mL), and sodium meta periodate solution (40.0 g, 21.2 mmol.) in hot water (150 mL) was carefully added over a period of 2.5 hours as in **14**. The reaction was worked up, the aqueous layer was separated and extracted with petrol/ethyl acetate (1:1, 3 x 500 mL) and the combined organic layers were dried concentrated. The crude product was re-crystallized from chloroform to give *13–((1S,RS)–cis–2–eicoscylcyclopropyl)tridecan–1–ol* (9.7 g; 88 %), m.p.73–75°C , $[\alpha]_D^{22}$ –2.13 (c 1.2, $CHCl_3$) (lit. value[163] $[\alpha]_D^{22}$ –2.04 (c 1.03, $CHCl_3$)) which showed δ_H, δ_C and ν_{max} identical to literature.[163]

Experiment 25: 5–[13–((1S,2R)-*cis*-2–Eicosylcyclopropyl)tridecylsulfanyl]–phenyl–1H–tetrazole (99b)



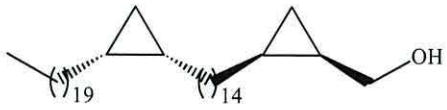
The reaction procedure in **Experiment 18** was repeated, using 13–((1*S*,2*R*)-*cis*-2–eicosylcyclopropyl)tridecan–1–ol[163] (4.0 g, 7.7 mmol.) and triphenylphosphine (2.6 g, 10.0 mmol.) and 1–phenyl–1H–tetrazole–5–thiol (1.8 g, 10.0 mmol.), in dry THF (50 mL). Diethyl azodicarboxylate (1.6 mL, 10.0 mmol.) in dry THF (5 mL) was added to the stirred mixture at 0 °C, which was allowed to reach room temperature and stirred overnight. The solvent was evaporated and the residue was washed with petrol/ ether (5:2) and filtered. The filtrate was evaporated and the product was purified by column chromatography, eluting with dichloromethane. *5–[13–((1S,2R)-cis-2-Eicosylcyclopropyl)tridecylsulfanyl]–phenyl–1H–tetrazole* was obtained (5.2 g; 96 %), m.p.. 44–45°C, $[\alpha]_D^{22}$ +1.5 (c 1.5, $CHCl_3$) [Found $(M+Na)^+$ 703.5643. $C_{43}H_{76}N_4SNa$ requires: 703.5683]. The compound showed δ_H (500 MHz, $CDCl_3$): 7.62–7.56 (5H, m), 3.42 (2H, t, *J* 7.4 Hz), 1.85 (2H, pent, *J* 7.5 Hz), 1.48 (2H, pent, *J* 7.5 Hz), 1.45–1.14 (57 H, m), 0.92 (3H, t, *J* 6.9 Hz), 0.71–0.65 (2H, m), 0.59 (1H, dt, *J* 4.2, 8.5 Hz), –0.34 (1H, q, *J* 4.9 Hz); δ_C (125 MHz, $CDCl_3$): 154.8, 139.9 133.2, 130.2, 33.9, 29.5–28.1, 22.7, 16.06, 14.5, 11.3 ; ν_{max} (cm^{-1}): 1600, 1461, 1377, 1239, 1168, 1011, 690.

Experiment 26: 5–[13–((1*S*,2*R*)-*cis*-2–Eicosylcyclopropyl)tridecane–1–sulfonyl]–phenyl–1H–tetrazole (100b)



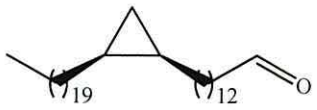
Repeating the procedure in **Experiment 19**, sodium hydrogen carbonate (2.8 g, 33.1 mmol.) was added to a stirred solution of 5–[13–((1*S*,2*R*)-*cis*-2–eicosylcyclopropyl)-tridecylsulfanyl]–phenyl–1H–tetrazole (5.0 g, 7.4 mmol.) in dichloromethane (50 mL), followed by the addition of dry 3–chloroperbenzoic acid (3.4 g, 14.7 mmol.) in dichloromethane (50 mL). The reaction was stirred at room temperature for 18 hours to give a whitish precipitate. It was quenched with sodium hydroxide (5%; 100mL) and diluted with dichloromethane (200 mL). The aqueous layer was separated and re–extracted with dichloromethane (2 x 150 mL). The combined organic layers were washed with water (2 x 200 mL), dried over $MgSO_4$ and evaporated. The crude product was purified by recrystallizing from methanol/acetone (1:1) which gave a whitish solid, *5–[13–((1S,2R)-cis-2–eicosylcyclopropyl)tridecane–1–sulfonyl]–phenyl–1H–tetrazole* (3.5 g; 67 %), m.p. 51–53°C, $[\alpha]_D^{22}$ –1.9 (c 1.3, $CHCl_3$) [Found $(M+Na)^+$ 735.5613. $C_{43}H_{76}N_4SNa$ requires: 735.5581], which showed the following: δ_H (500 MHz, $CDCl_3$): 7.75–7.60 (5H, m) 3.74 (2H, t, *J* 8.0 Hz), 1.97 (2H, pent, *J* 7.8 Hz), 1.52 (2H, pent, *J* 7.5 Hz), 1.45–1.10 (57 H, m), 0.91 (3H, t, *J* 6.8 Hz), 0.70–0.62 (2H, m), 0.58 (1H, dt, *J* 4.2, 8.5 Hz) –0.34 ((1H, q, *J* 4.8 Hz); δ_C (125 MHz, $CDCl_3$): 155.0, 135.1, 133.5, 129.9, 125 8, 56.5, 29.9–28.5, 22.8, 22.3, 16.3, 14.3, 11.1; ν_{max} (cm^{-1}): 1760, 1595, 1461, 1372, 1352, 1218, 1143, 1013, 681.

Experiment 27: (1*R*,2*S*)–2–[14–((1*S*,2*R*)–2–Eicosylcyclopropyl)tetradecyl]-cyclopropyl methanol (80b)



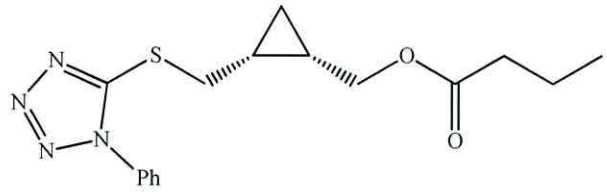
The reaction steps in **Experiment 21** were repeated by using butyric acid (1*R*,2*S*)–(*cis*–2–formylcyclopropyl)methyl ester[163](0.85 g, 5.0 mmol.) and 5–[13-((1*S*,2*R*)–*cis*-2–eicosyl-cyclopropyl)tridecane–1–sulfonyl]–phenyl–1H–tetrazole (3.56 g, 5.0 mmol.) in dry THF (50 mL), and lithium bis-(trimethylsilyl) amide (6.1 mL, 6.5 mmol.). The reaction was quenched with sat. aq. ammonium chloride (50 mL) and extracted with petrol/ether (1:1, 3 x 50mL). Column chromatography eluting with petrol/ether (20:1) gave a white solid, *butyric acid (1R,2S)–2–[(E/Z)–14–((1S,2R)–2–eicosylcyclopropyl)tetradec–1–enyl]cyclo-propylmethyl ester* (2.5 g; 78 %). Lithium aluminium hydride (0.29 g, 7.62 mmol.) in THF (20 mL) was reacted with the derived ester (2.5 g, 3.81 mmol.) in THF (20 mL). The reaction was treated with freshly prepared sat. aq. sodium sulfate decahydrate (30 mL) and then filtered through a bed of celite, washed with THF (4 x 50 mL) and evaporated to give *(1R,2S)–2–[(E/Z)–14–((1S,2R)–2–eicosylcyclopropyl)tetradec–1–enyl]cyclopropyl methanol* (1.1 g; 50 %). Hydrazine monohydrate (10 mL), acetic acid (1 mL) and sat. aq. copper sulfate (1 mL) were added in succession to the unsaturated alcohol (1.1 g, 1.88 mmol.) in iso–propanol (30 mL), and the mixture was treated with a solution of sodium metaperiodate (4.0 g, 18.8 mmol.) in hot water (10 mL) at 80 °C. The reaction was worked up as in before and the crude product was extracted with petrol/ether (1:1, 3 x 50 mL) and then recrystallized from petrol to give a white solid, *(1R,2S)–2–[14–((1S,2R)–2–eicosylcyclopropyl)tetradecyl]cyclopropyl methanol* (0.7 g; 63 %), m.p. 59–61°C, $[\alpha]_D^{21}$ +7.59 (c 1.2, $CHCl_3$) [Found $(M+Na)^+$ 611.9000. $C_{41}H_{80}ONa$ requires: 611.6206], which showed δ_H (500 MHz, $CDCl_3$): 3.7 (1H, dd, *J* 7.5, 11.5 Hz), 3.59 (1H, dd, *J* 8.0, 11.3 Hz), 1.50–1.06 (67 H, m), 0.94–0.84 (2H, m including a triplet resonating at δ 0.89 with *J* 6.7 Hz), 0.73 (1H, dt, *J* 4.5, 8.4 Hz), 0.68–0.63 (2H, m), 0.58 (1H, sext, *J* 4.1 Hz), –0.03 (1H, q, *J* 5.5 Hz), –0.33 ((1H, q, *J* 5.0 Hz); δ_C (125 MHz, $CDCl_3$): 63.8, 32.4, 30.6–29.0, 22.9, 18.6, 16.6; ν_{max} (cm^{-1}): 3365, 2850, 1770, 1462, 1370, 1169, 1062, 1035, 963, 930, 821.

Experiment 28: 13-((1*R*,2*S*)-2-*cis*-Eicosylcyclopropyl)tridecanal (33a)[169]



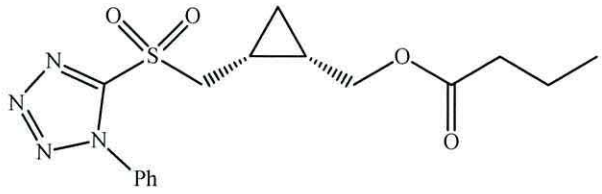
13–(1*R*,2S)–cis–(2–eicoscylcyclopropyl)tridecan–1–ol (3.0 g, 5.8 mmol.) in dichloromethane (20 mL) was added to a stirred suspension of pyridinium chlorochromate (4.0 g, 17.4 mmol.) in dichloromethane (100 mL) in portions at room temperature. The mixture was stirred for 2 hrs and when TLC showed no starting material left, the mixture was diluted with petrol/ether 2:1 (300mL) and filtered through a pad of celite on silica, then wash thouroughly with warm ether (400mL). The filtrate was evaporated to give a residue which was recrystallized in petrol to obtain a white solid *13-((1R,2S)-2-cis-eicosylcyclopropyl)tridecanal* (2.0 g, 67 %), mp 64–66 °C, $[\alpha]_D^{22}$ +3.27 (c 1.1, $CHCl_3$) (lit. value[169] $[\alpha]_D^{25}$ +1.59 (c 1.2, $CHCl_3$) which showed δ_H, δ_C and ν_{max} identical to the literature.[169]

Experiment 29: (1*S*,2*R*)–2–(1–Phenyl–1H–tetrazole–5–sulphanylmethyl)cyclopropyl-methyl butyrate (103b)



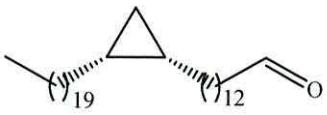
Following standard methods as before, butyric acid (1*S*,2*R*)–2–*cis*–(hydroxymethyl) cyclopropylmethyl ester (10.0 g, 58.1 mmol.), triphenylphosphine (18.4 g, 69.8 mmol.) and 1–phenyl–1H–tetrazole–5–thiol (12.43 g, 69.8 mmol.) were dissolved in dry THF (250 ml) and cooled to 0 °C. While stirring, diethyl azodicarboxylate (12.2 g, 69.8 mmol.) in dry THF (20 ml) was added. The mixture was allowed to reach room temperature and left to stir overnight. The solvent was evaporated and the residue treated with petrol/ ether (5:2, 250 ml) and filtered. The filtrate was again evaporated and chromatography was carried out using petrol/ethyl acetate (5:2) as eluting solvent. *(1S,2R)–2–(1–phenyl–1H–tetrazole–5–sulphanylmethyl)cyclopropylmethyl butyrate* was obtained (17.0 g; 88 %), $[\alpha]_D^{22}$ +1.99, (c 1.5, CHCl3) [Found $(M+Na)^+$ 355.1190. $C_{16}H_{20}O_2SN_4$ requires: 355.1199], which showed δ_H (500 MHz, $CDCl_3$): 7.61–7.55 (5H, m), 4.35 (1H, dd, *J* 6.7, 12.5 Hz), 3.95 (1H, dd, *J* 9.1, 12.1 Hz), 3.60 (1H, dd, *J* 7.9, 13.6 Hz), 3.43 (1H, dd, *J* 7.9, 13.6 Hz), 2.28 (2H, t, *J* 7.8 Hz), 1.65 (2H, sext, *J* 7.6 Hz), 1.51 (1H, b.pent, *J* 5.7, 8.1 Hz), 1.44–1.37 (1H, m), 0.97 (1H, dt, *J* 5.4, 8.2 Hz), 0.94 (3H, t, *J* 7.3 Hz), 0.41 (1H, q, *J* 5.7 Hz); δ_C (125 MHz, $CDCl_3$): 174.0, 154.2, 133.6, 130.0, 129.8, 123.8, 63.5, 36.4, 34.2, 18.4, 16.5, 15.5, 13.4, 11.1; ν_{max} (cm^{-1}): 2970, 2876, 1735, 1520, 1171, 985, 760.

Experiment 30: (1*S*,2*R*)–2–(1–Phenyl–1H–tetrazole–5–sulphonylmethyl)cyclopropylmethyl butyrate (104b)



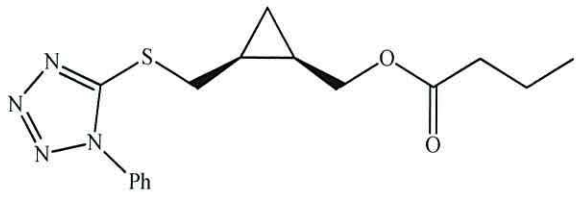
In line with previous procedures used by Al Dulayymi *et al*,[164] ammonium molybdate VI tetrahydrate (31.6 g, 25.6 mmol.) was dissolved in hydrogen peroxide (35%,w/w, 50 ml) and was gradually added to a stirred solution of (1*S*,2*R*)–2–(1–phenyl–1H–tetrazole–5–sulphanyl-methyl)cyclopropylmethyl butyrate (17.0 g, 51.1mmol.) in THF/IMS (100/300 ml) below 10 °C. The reaction was allowed to reach room temperature and stirred for 2 hours, after which another portion of ammonium molybdate VI tetrahydrate (16.6 g, 13.4 mmol.) in cold hydrogen peroxide (30 ml) was added. The reaction was stirred overnight for over 18 hours and then neutralized with water (2.5 litres). The product was extracted with dichloromethane (3 x 400 ml) and washed with more water (2 x 400 ml). Crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:2). A thick clear liquid *(1S,2R)–2–(1–phenyl–1H–tetrazole–5–sulphonylmethyl)cyclopropylmethyl butyrate* (15 g, 88 %) was obtained, $[\alpha]_D^{22}$ –53.0 (c 2.5, $CHCl_3$) [Found $(M+Na)^+$ 387.1067 $C_{16}H_{20}O_4SN_4$ requires: 387.1097], which showed δ_H (500 MHz, $CDCl_3$): 7.71–7.69, (2H, m), 7.70–7.59 (3H, m), 4.3 5(1H, dd, *J* 5.7, 12.1 Hz),4.04 (1H, dd, *J* 5.3, 15.0 Hz), 3.90 (1H, dd, *J* 8.1, 11.9 Hz), 3.69 (1H, dd, *J* 9.0, 15.0 Hz), 2.30 (2H, t, *J* 7.5 Hz), 1.67 (2H, sext, *J* 7.7 Hz), 1.52–1.45 (2H, m), 1.01 (1H, dt, *J* 5.7,8.5 Hz), 0.98 (3H, t, *J* 7.7 Hz), 0.62 (1H, q, *J* 5.9 Hz); δ_C (125 MHz, $CDCl_3$): 173.3, 153.7, 133.1, 131.0, 129.5, 125.4, 63.4, 56.9, 36.1, 18.2,14.5, 13.8, 9.9, 8.6; ν_{max} (cm^{-1}): 2969, 1730, 1339,1151, 770.

Experiment 31 **13-((1*S*,2*R*)-2-*cis*-Eicosylcyclopropyl)tridecanal (33b)**[163]



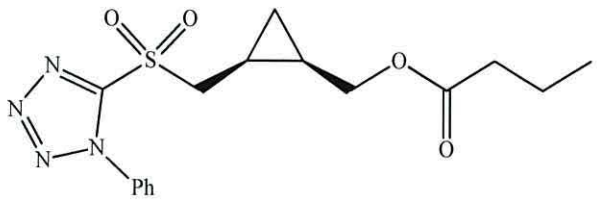
As in **Experiment 28**, 13–(1*S*,2R)–*cis*–(2–eicoscylcyclopropyl)tridecan–1–ol (5.0 g, 9.6 mmol.) in dichloromethane (20 mL), was stirred into a suspension of pyridinium chlorochromate (6.2 g, 28.9 mmol.) in dichloromethane (100 mL) at room temperature. The mixture was stirred for 2 hrs and when TLC showed that there was no starting material left, petrol/ether 2:1 (300mL) was added to the reaction, which was filtered through a bed of celite on silica, then wash thoroughly with warm ether (400mL). The filtrate was evaporated to give a residue which was recrystallized in petrol to obtain a white solid *13–((1S,2R)–2–cis-eicosylcyclo-propyl)tridecane carbaldehyde*. (2.0 g, 84 %), mp 63–65 °C, $[\alpha]_D^{22}$ –1.52 (c 1.1, $CHCl_3$) (lit. value[163] $[\alpha]_D^{22}$ –1.69 (c 1.2, $CHCl_3$) which showed δ_H, δ_C and ν_{max} identical to the literature.[163]

Experiment 32 Butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazol-5-ylsulfanylmethyl)-cyclopropylmethyl ester (103a)[164]



As in **Experiment 29** butyric acid (1*R*,2*S*)–2–*cis*–(hydroxymethyl)cyclopropylmethyl ester (10.0 g, 58.1 mmol.), triphenylphosphine (18.4 g, 69.8 mmol) and 1–phenyl–1H–tetrazole–5–thiol (12.4 g, 69.8 mmol.) were dissolved in dry THF (250 ml) and cooled to 0 °C. While stirring, diethyl azodicarboxylate (12.2 g, 69.8 mmol) in dry THF (20 ml) was added. The mixture was allowed to reach room temperature and left to stir overnight. The solvent was evaporated and the residue treated with petrol/ ether (5:2, 250 ml) and filtered. The filtrate was again evaporated and chromatography was carried out using petrol/ethyl acetate (5:2) as eluting solvent. *(1R,2S)–2–(1–phenyl–1H–tetrazole–5–sulphanylmethyl)cyclopropylmethyl butyrate* was obtained (17.0 g; 92 %), $[\alpha]_D^{20}$ –2.98, (c 1.5, $CHCl_3$) (lit. value.[164] $[\alpha]_D^{24}$ –1.2, (c 1.06, $CHCl_3$) which showed δ_H, δ_C and ν_{max} identical to the literature.[164]

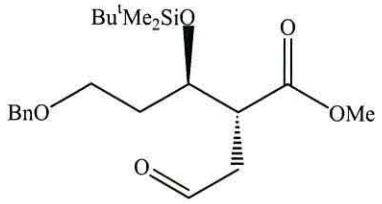
Experiment 33 Butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazole-5-sulfonylmethyl)-cyclopropylmethyl ester (104a)[164]



As in **Experiment 30** solution of ammonium molybdate (VI) tetrahydrate (31.6 g, 25.6 mmol) in hydrogen peroxide (50 ml, 35 % w/w) was cooled to –10 °C and added to a stirred solution of butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazol-5-ylsulfanylmethyl)-cyclopropylmethyl ester (17 g, 51.1 mmol) in THF (100 ml) and IMS (300 ml) at –10 °C. The mixture was stirred at 15 – 20 °C for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (16.6 g, 13.5 mmol, 0.2 mol. equiv.) in –10 °C hydrogen peroxide (30 ml, 35 % w/w) was added and the reaction stirred for 18 hrs. The reaction mixture was then poured into water (1 l) and extracted with dichloromethane (3 x 200 ml), dried and evaporated. The crude product was purified via column chromatography eluting with petrol/ethyl acetate (5:2) to give a thick clear oil, butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazole-5-sulfonylmethyl)-cyclopropylmethyl ester (17.0 g, 91 %), $[\alpha]_D^{20}$ +51.0, (c 1.3, $CHCl_3$) (lit. value.[164] $[\alpha]_D^{24}$ +52.7, (c 1.45, $CHCl_3$) which showed δ_H, δ_C and ν_{max} identical to the literature.[164]

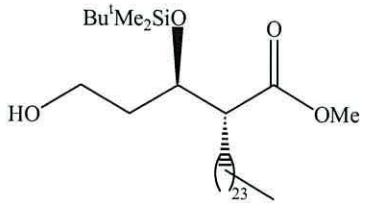
6.1.2 Synthesis of the full mycolic acids

Experiment 34: (2*R*,3*R*)-5-Benzyloxy-3-(*tert*-butyl-dimethyl-silanyloxy)-2-(2-oxo-ethyl)-pentanoic acid methyl ester (106)[177]



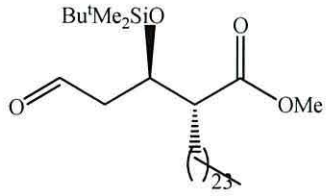
2,6-Lutidine (2.4 g, 21.9 mol.), osmium tetroxide (25 %) in 2-methyl-2-propanol (2 mL, 0.2 mmol.) and sodium (meta) periodide (9.4 g, 44.0 mmol.) were added to (*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyl-dimethyl-silanyloxy)-propyl]pent-4-enoic acid methyl ester (4.0 g, 11.0 mmol.) in 1,4-dioxane/water (3:1) at room temperature. The mixture was stirred for 2 hours, after which TLC showed no starting material. Then water (200 mL) was added in order to dilute the reaction. The aqueous layer was separated and extracted with dichloromethane (3 x 150 mL), and the combined organic layers were washed with brine (200 mL), dried over $MgSO_4$ and filtered. The filtrate was evaporated and the crude product was purified by column chromatography, eluting with petrol/ ethyl acetate (2:1) to give a dark oil *(2R,3R)-5-benzyloxy-3-(tert-butyldimethylsilanyloxy)-2-(2-oxo-ethyl)-pentanoic acid methyl ester* (3.5 g, 88 %), $[\alpha]_D^{21}$ –20.1 (c 1.8, $CHCl_3$) (lit. value[177] $[\alpha]_D^{26}$ – 18.4 (c 0.97, $CHCl_3$)), which showed δ_H, δ_C and ν_{max} identical to the literature.[177]

Experiment 35: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-hydroxypropyl] hexacosanoic acid methyl ester (108)[177]



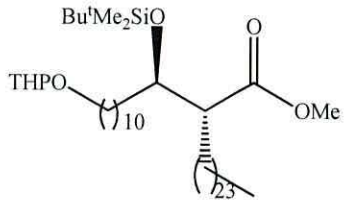
(2*R*,3*R*)-5-Benzyloxy-3-(*tert*-butyldimethylsilanyloxy)-2-(2-oxoethyl)pentanoic acid methyl ester (3.5 g, 8.9 mmol.) and 5-(docosyl-1-sulfonyl)-1-phenyl-1H-tetrazole (6.0 g, 11.6 mmol.) were dissolved in dry THF (200 mL), and then lithium bis-(trimethylsilyl) amide (14.2 mL, 15.1 mmol.) was added at –8 °C under nitrogen,. After 1 hour, TLC showed that the reaction was complete, and then it was quenched with sat. aq ammonium chloride (150 mL). The aqueous layer was separated and extracted with petrol/ethyl acetate (10:1, 3 x 150 mL), and the combined organic layers were dried over $MgSO_4$, and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a clear yellow liquid, *(E/Z)-(R)-2-[(R)-3-Benzyloxy-1-(tert-butyldimethylsilanyloxy)propyl]-hexacos-4-enoic acid methyl ester* (4.0 g; 79 %), as a mixture of isomers. Palladium 10 % on carbon was added to the derived esters (4 g, 5.97 mmol.) in THF/IMS (90mL, 1:1). The mixture was stirred under hydrogen for 5 days, and then filtered over a bed of celite, washing with excess ethyl acetate. The filtrate was concentrated purified by column chromatography, eluting with petrol/ethyl acetate (10:1) to give a very thick oil, *(R)-2-[(R)-1-(tert-butyl-dimethyl-silanyloxy)-3-hydroxy-propyl]-hexacosanoic acid methyl ester* (2.6 g, 73 %), $[\alpha]_D^{18}$ – 6.34 (c 2.6, $CHCl_3$) (lit. value[177] $[\alpha]_D^{24}$ –8.3 (c 0.4, $CHCl_3$)) which showed δ_H, δ_C and ν_{max} identical to the literature.[177]

Experiment 36: (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-3-oxo-propyl] hexacosanoic acid methyl ester (110)[177]



Repeating the method used in **Experiment 14**, small portions of (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethylsilanyloxy)-3-hydroxypropyl]-hexacosanoic acid methyl ester (2.5 g, 4.2 mmol.) in dichloromethane (30 mL) were gradually added to a stirred suspension of PCC (2.3 g, 10.4 mmol.) in dichloromethane (100 mL). The mixture was stirred for 2 hours at room temperature, after which TLC showed that the reaction was complete. The reaction was diluted with ethyl acetate (100 mL) and filtered through a bed of celite and silica. The filtrate was evaporated to obtain the crude product, and then column chromatography, eluting with petrol/ethyl acetate (5:2) gave a colourless oil, *(R)-2-[(R)-1-(tert-butyldimethylsilanyloxy)-3-oxopropyl]-hexacosanoic acid methyl ester* (2.4 g; 96 %), $[\alpha]_D^{18}$ –2.4 (c 1.9, $CHCl_3$); lit. value[177] $[\alpha]_D^{28}$ –5.0 (c 1.23, $CHCl_3$) which showed δ_H, δ_C and ν_{max} identical to the literature.[177]

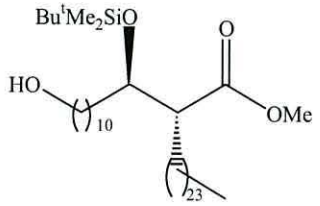
Experiment 37: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-(tetrahydro-pyran-2-yloxy)-undecyl]-hexacosanoic acid methyl ester (112)



Following established methods,[163] 5–(7–(tetrahydropyran–2–yloxy)heptane–1–sulphonyl)–1–phenyl–1H–tetrazole[224] (2.21 g, 5.20 mmol.) and (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-oxo-propyl]-hexacosanoic acid methyl ester[177] (2.4 g, 4.0 mmol.) were dissolved in dry THF (25 mL) and cooled to –8 °C under nitrogen, and then lithium bis–(trimethylsilyl)amide (6.8 mL) was added to the mixture which turned light yellow. Stirring was continued for 1.5 hours after which the reaction was allowed to attain room temperature. TLC showed that the reaction was complete, and then it was quenched with water (100 mL). The mixture was extracted with petrol/ether (1:1, 3 x 50 mL) and the combined organic layers were washed with sat. aq. sodium hydroxide (2 x 50 mL), dried over $MgSO_4$ and filtered. The filtrate was evaporated and the residue was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give *(R)-2-[(E/Z)-(R)-1-(tert-butyldimethylsilanyloxy)-11-(tetrahydropyran-2-yloxy)-undec-3-enyl]-hexacosa-noic acid methyl ester* (2.5 g; 79 %) which showed δ_H (500 MHz, $CDCl_3$): 5.47–5.42, (1H, m) 4.57 (1H, br.t, *J* 3.5 Hz), 3.94–3.85 (2H, m), 3.73 (1H, dt, *J* 7.0, 9.5 Hz), 3.65 (3H, s), 3.52–3.47 (1H, m), 3.37 (1H, dt, *J* 7.0, 9.5 Hz), 2.52–2.49 (1H, m), 2.30–2.18 (2H, m), 2.0 (1H, m), 1.86–1.80 (1H, m), 1.74–1.69 (1H, m), 1.60–1.52 (6H, m), 1.21–1.31 (56H, m), 0.88 (3H, t, *J* 6.6 Hz), 0.84 (9H, s), 0.05 (3H,s), 0.01 (3H,s); δ_C (125 MHz, $CDCl_3$): 175.0, 174.9, 133.6, 132.0, 124.8, 124.4, 98.8, 73.2, 67.6, 62.3, 51.2, 37.4, 32.7, 31.9, 30.8, 29.8–29.1, 26.2, 25.7, 25.5, 22.6, 19.7, 17.9, 14.1, -4.3, -5.1. Palladium (10 % on carbon,1.0 g) was added to the derived unsaturated ester (2.4 g, 3.03 mmol.) in THF/IMS (50mL, 1:1), and the mixture was stirred under hydrogen for 2 days. Subsequently, it was filtered over a bed of celite, washed with excess ethyl acetate and evaporated. Column chromatography eluting with petrol/ethyl acetate (10:1) gave a colourless oil, *(R)-2-[(R)-1-(tert-butyl-dimethyl-silanyloxy)-11-(tetrahydropyran-2-yloxy)-undecyl]-hexacosanoic acid methyl*

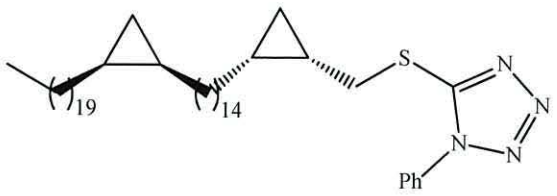
ester (2.2 g, 91 %), $[\alpha]_D^{19}$ –14.1 (c 1.8, $CHCl_3$) [Found $(M+Na)^+$ 817.775. $C_{49}H_{98}O_5SiNa$ requires: 817.7081] which showed δ_H (500 MHz, $CDCl_3$): 4.33 (1H, br.t, *J* 2.8 Hz), 3.67–3.61 (2H,m), 3.49 (1H, dt, *J* 6.9,9.8 Hz), 3.41 (3H, s), 3.23–3.26 (1H, m), 3.14 (1H, dt *J* 7.0, 9.6 Hz), 2.28 (1H, ddd, *J* 3.8, 7.2, 11.5 Hz), 1.61–1.56 (1H, m), 1.49–1.45 (1H, m), 1.49–1.45 (1H, m), 1.36–0.85 (62H, m), 0.63(3H, t, *J* 6.6 Hz), 0.62 (9H, s), -2.0 (3H, s),-0.23 (3H, s); δ_C (125 MHz, $CDCl_3$): 175.1, 98.8, 73.2, 67.7, 62.3, 51.3, 33.7, 31.9, 30.8, 29.7, 27.7, 25.7, 23.7, 22.7, 19.7, 17.9, 14.1; ν_{max} (cm^{-1}): 2923, 2854, 2360, 1742, 1465, 1361, 1254, 1304.

Experiment 38: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-hydroxyundecyl] hexacosanoic acid methyl ester (113)[225]



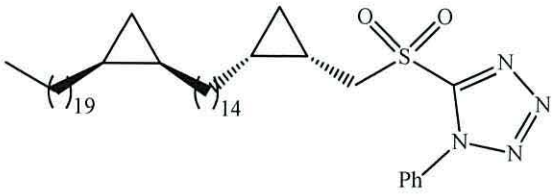
(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-(tetrahydropyran-2-yloxy)undecyl]-hexacosanoic acid methyl ester (2.1 g, 2.65 mmol.) was dissolved in THF (15mL). Pyridinium-p-toluene sulfate (0.3g, 1.19 mmol.) was dissolved in a mixture of methanol (2 mL) and water (0.5mL) and added to the ester andstirred at 45 °C overnight. TLC showed that the reaction was complete, and then sat. aq. $NaHCO_3$ (10 mL) was added, followed by water (20 mL), to neutralize the acid. The aqueous layer was separated and extracted with ethyl acetate (3 x 25mL) and the combined organic layers were dried over $MgSO_4$ and evaporated. The crude residue was purified by column chromatography, eluting with petrol/ethyl acetate (first 5:1, followed by 3:1 and then 5:1) to give a thick whitish oil, *(R)-2-[(R)-1-(tert-butyldimethylsilanyloxy)-11-hydroxyundecyl]hexacosanoic acid methyl ester* (0.58 g, 65 %), $[\alpha]_D^{17}$ –5.73 (c 1.9, $CHCl_3$) (lit. value[225] $[\alpha]_D^{23}$ –5.04 (c 1.232 µmol, $CHCl_3$)), which showed δ_H, δ_C and ν_{max} identical to the literature.[225]

Experiment 39: 5-[(1*S*,2*R*)-2-[14-((1*R*,2*S*)-2-Eicosyl-cyclopropyl)tetra- decyl]-cyclopropylmethylsulfanyl]-1-phenyl-1*H*-tetrazole (114a)



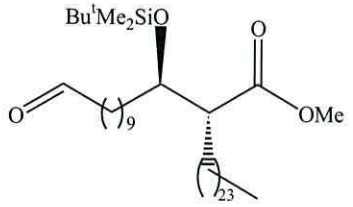
Using established methods,[163] (1*S*,2*R*)–2–[14–((1*R*,2*S*)–2–eicosylcyclopropyl)tetradecyl]-cyclopropyl methanol (0.55 g, 0.85 mmol.) was dissolved in dry THF (5 mL) and then triphenylphosphine (0.3 g, 1.11 mmol.) and 1–phenyl–1H–tetrazole–5–thiol (0.2 g, 1.11 mmol.) were added. The mixture was cooled to 0 °C, diethyl azodicarboxylate (0.18 mL, 1.11 mmol.) in dry THF (5 mL) was added and the reaction was allowed to attain room temperature and then stirred overnight. The solvent was evaporated and the residue was washed with petrol/ ether (5:2) and filtered. The filtrate was evaporated to obtain the crude product which was purified by column chromatography eluting with dichloromethane to give *5-[(1S,2R)-2-[14-((1R,2S)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropylmethy-lsulfanyl]-1-phenyl-1H-tetrazole* (0.7 g, 92 %), m.p. 43 – 50 °C, $[\alpha]_D^{20}$ –2.14 (c 1.7, $CHCl_3$) [Found $(M+Na)^+$ 771.469. $C_{48}H_{84}N_4SNa$ requires: 771.6314] which showed δ_H (500 MHz, $CDCl_3$): 7.63–7.55 (5H, m) 3.49 (2H, d, *J* 7.9 Hz), 1.60 -1.11 (67H, including a multiplet at 1.47–1.53 for one cyclopropane proton), 0.94 (1H, ddd, *J* 2.2, 8.2, 14.2 Hz), 0.89 (3H, t, *J* 6.9 Hz), 0.84 (1H, dt, 4.7, 8.2 Hz), 0.65 (2H, m), 0.57 (1H, dt, *J* 3.4, 8.2 Hz), 0.23 (1H, q, *J* 5.4 Hz), –0.32 (1H, q, *J* 5.4 Hz); δ_C (125 MHz, $CDCl_3$): 133.8, 130, 123.8, 35.0, 31.9, 30.9, 29.5, 28.5, 22.7, 21.0, 18.0, 15.8, 14.6, 14.1, 12.5, 10.9.; ν_{max} (cm^{-1}): 3058, 2923, 1462, 1316, 1274, 1169,1087, 1017.

Experiment 40: 5-[(1*S*,2*R*)-2-[14-((1*R*,2*S*)-2-Eicosylcyclopropyl)-tetradecyl]-cyclopropylmethanesulfonyl]-1-phenyl-1H-tetrazole(115a)



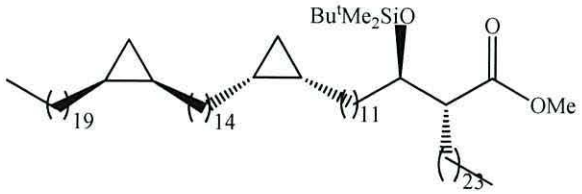
Using an established procedure,[163] ammonium molybdate VI tetrahydrate (2.9 g, 2.34 mmol.) was dissolved in cold hydrogen peroxide (35% w/w, 10 mL) and was added gradually to a stirred mixture of 5-[(1*S*,2*R*)-2-[14-((1*R*,2*S*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropylmethylsulfanyl]-1-phenyl-1H-tetrazole (0.35 g, 0.468 mmol.) in IMS/THF (30:10 mL) at 5 - 10 °C. The reaction was allowed to attain room temperature and stirred for another 2 hours after which another portion of ammonium molybdate VI tetrahydrate (1.2 g, 0.935 mmol.) in cold hydrogen peroxide (5 mL) was added. The reaction was stirred for 18 hours and then poured into water (200 mL) and extracted with dichloromethane (3 x 50 mL). The extracted organic layers were washed with more water (2 x 50 mL), dried over $MgSO_4$ and concentrated. Column chromatography was carried out on the crude product eluting with petrol/ether (5:2) to obtain *5-[(1S,2R)-2-[14-((1R,2S)-2-eicosylcyclopropyl)tetradecyl]-cyclopropylmethanesulfony]-1-phenyl-1H-tetrazole* (0.15 g, 41 %), m.p. 68 – 69 °C, $[\alpha]_D^{20}$ +16.67 (c 1.2, $CHCl_3$) [Found $(M+Na)^+$ 803.5452. $C_{48}H_{84}O_2N_4SNa$ requires: 803.621] which showed δ_H (500 MHz, $CDCl_3$): 7.71–7.61 (5H, m) 3.98 (1H, dd, *J* 5.7, 14.9 Hz), 3.57 (1H, dd, *J* 9.5, 14.9 Hz), 1.38 -1.13 (68H, m), 1.05–0.97 (1H, m), 0.89 (3H, t, *J* 6.6 Hz), 0.65 (2H, m), 0.57 (1H, dt, *J* 4.1, 8.2Hz), 0.25 (1H, q, *J* 5.6 Hz), –0.33 (1H,q, *J* 5.0 Hz); δ_C (125 MHz, $CDCl_3$): 131.4, 129.7, 125.2, 57.1, 31.9, 30.2, 29.7, 29.5, 29.1, 28.7, 22.7, 15.9, 14.1, 11.4, 10.9, 8.0.; ν_{max} (cm^{-1}) : 2923, 2854, 1462, 1377, 1338, 1156.

Experiment 41 (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-11-oxo-undecyl] hexacosanoic acid methyl ester (66)[225]



The oxidation reaction procedure in **Experiment 14** was repeated. (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethylsilanyloxy)-11-hydroxyundecyl]hexacosanoic acid methyl ester (0.5 g, 0.704 mmol.) in dichloromethane (2 mL) was carefully added in small portions to a suspension of PCC (0.38 g, 1.76 mmol.) in dichloromethane (20 mL), and the mixture was stirred at room temperature for 2 hours. The reaction was monitored by TLC until there was no starting material. Then it was diluted with ethyl acetate (20 mL) and filtered through a bed of celite and silica. The filtrate was evaporated and the crude product was purified by column chromatography, eluting with petrol/ethyl acetate (10:1) to give a colourless oil, *(R)-2-[(R)-1-(tert-butyldimethylsilanyloxy)-11-oxoundecyl]hexacosanoic acid methyl ester* (0.47 g, 94 %), $[\alpha]_D^{17}$ –3.41 (c 1.4, $CHCl_3$) (lit. value[225] $[\alpha]_D^{22}$ –4.41 (c 1.184 μmol., $CHCl_3$)), which showed δ_H, δ_C and ν_{max} identical to the literature.[225]

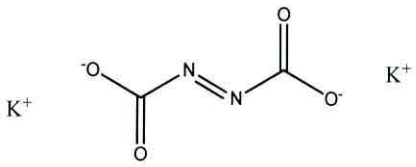
Experiment 42: (*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-[(1*S*,2*R*)-2-[14-((1*R*,2*S*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester (117a)



The reaction was carried out using standard procedures.[163] (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-11-oxo-undecyl]-hexacosanoic acid methyl ester (0.12 g, 0.169 mmol.) and 5-[(1*S*,2*R*)-2-[14-((1*R*,2*S*)-2-eicosylcyclopropyl)tetradecyl]-cyclopropyl-methanesulfonyl]-1-phenyl-1H-tetrazole (0.15 g, 0.186 mmol.) were dissolved in dry THF (5 mL) and the mixture was cooled to –10 °C under nitrogen. Lithium bis–(trimethylsilyl)amide (0.3 mL, 0.242 mmol.) was added to the mixture, which was allowed to attain room temperature, then stirring was continued for 1.5 hours when TLC showed a faint spot for the starting material, and then water (10 mL) was added to quench the reaction. The mixture was extracted with petrol/ether (1:1, 3 x 10 mL) and the combined organic layers were washed with sat. aq. sodium hydroxide (2 x 5 mL), dried over $MgSO_4$ and filtered. The filtrate was concentrated and the residue was purified by column chromatography, eluting with petrol/ethyl acetate (20:1). This gave, *(R)-2-((E/Z)-(R)-1-(tert-butyl-dimethyl-silanyloxy)-12-[(1S,2R)-2-[14-((1R,2S)-2-eicosylcyclo-propyl)-tetradecyl]-cyclopropyl]-dodec-11-enyl)-hexacosanoic acid methyl ester* (0.12 g; 49 %) Freshly prepared dipottasium azodicarboxylate[165] (2.0 g, 10.3 mmol.) was added to a stirred solution of the derived esters (0.11 g, 0.087 mmol.) in THF (3 mL) and methanol (1.5 mL) at 0 to 5 °C under nitrogen. Glacial acetic acid (0.5mL) was dissolved in THF (1.0 mL), and then added dropwise to the reaction mixture, which was stirred overnight at room temperature. The process was repeated using the same amount of glacial acetic acid in THF until there was a change in colour from bright yellow to a whitish colour. Subsequently, the reaction was quenched by careful addition of sat. aq. sodium bicarbonate (20 mL) and the aqueous layer was separated and extracted with petrol/ ethyl acetate (10:1, 3 x 25 mL). The combined organic layers were washed with water, dried over $MgSO_4$, filtered and concentrated to obtain the crude product. Column chromatography, eluting

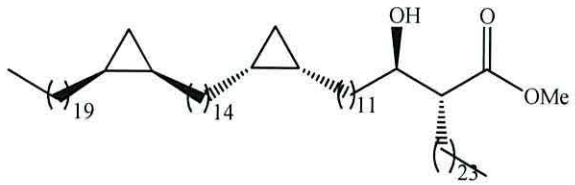
with petrol/ ethyl acetate (10:1) gave a very thick oil, *(R)-2-((R)-1-(tert-butyl-dimethyl-silanyloxy)-12-[(1S,2R)-2-[14-((1R,2S)-2-eicosyl-cyclopropyl)-tetradecyl-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester* (0.1 g, 90 %), $[\alpha]_D^{19}$ –2.06 (c 1.2, $CHCl_3$) [Found $(M+Na)^+$ 1288.574. $C_{85}H_{168}O_3SiNa$ requires: 1288.2661] which showed δ_H (500 MHz, $CDCl_3$);3.89 (1H, td, *J* 4.8, 6.6 Hz), 3.64 (3H, s), 2.51 (1H, ddd, *J* 3.8, 7.2, 11.1 Hz), 1.55–1.24 (136H, m), 0.85 (6H, t, *J* 7.0 Hz), 0.84 (9H, s), 0.65–0.62 (4H, m), 0.55(2H, dt, *J* 3.5, 7.9 Hz), 0.05 (3H,s), 0.02 (3H, s), – 0.35 (2H, q, *J* 5.0 Hz); δ_C (125 MHz, $CDCl_3$):175.1, 73.2, 51.4, 33.7, 31.9, 30.2, 29.7, 28.7, 27.4, 25.8, 23.7, 22.7, 18.0, 15.8, 14.1, 10.9.; ν_{max} (cm^{-1}): 2923, 2853, 1742, 1464, 1254.

Experiment 42a **Di-potassium azodicarboxylate**[177]



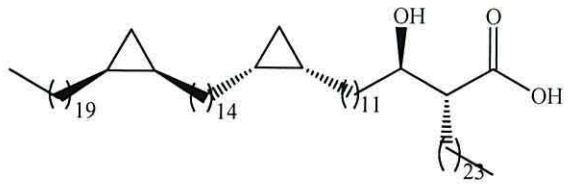
Potassium hydroxide (15 g, 0.268 mol.) was dissolved in water (15 mL), and then azodicarbonamide (7.5 g, 0.065 mol.) was added at –10 °C to give a bright yellow solution which was stirred for 1 hour below 5 °C. A thick bright yellow precipitate of dipotassium salt was formed which was filtered through a sintered glass funnel and washed in cold methanol (60 mL). The yellow precipitate was dissolved in water (40 mL) at room temperature and the resulting yellow solution was poured into cold IMS to give some more yellow precipitate. This was in turn filtered, washed with cold methanol (50 mL) and cold petrol (50 mL). The yellow solid was dried on the sintered funnel by vacuum and transferred into a pre-cooled round bottom flask and stored in the freezer.

Experiment 43: (*R*)-2-((*R*)-1-Hydroxy-12-[(1*S*,2*R*)-2-[14-((1*R*,2*S*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester (118a)



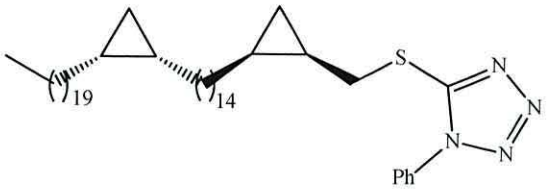
Using established methods,[163] (*R*)-2-((*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-12-[(1*S*,2*R*)-2-[14-((1*R*,2*S*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester (0.1 g, 0.079 mmol.), was dissolved in dry THF (4.0 mL) at room temperature and placed in a dry polyethylene vial under nitrogen. Pyridine (0.1mL) and HF pyridine (0.7 mL) were added, and then the reaction was stirred for 17 hours at 40 °C. It was diluted with petrol/ ethyl acetate (10:1, 30 mL) and neutralized by the addition of sat. aq. sodium bicarbonate until the liberation of carbon dioxide ceased. The aqueous layer was separated and re-extracted with ethyl acetate (10:1, 3 x 25 mL). The organic layer was washed with brine and dried over $MgSO_4$. The excess solvent was evaporated and the residue was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to obtain a white solid, *(R)-2-((R)-1-hydroxy-12-[(1S,2R)-2-[14-((1R,2S)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester* (0.03 g, 33%), m.p. 57 °C, $[\alpha]_D^{19}$ +2.45 (c 1.7, $CHCl_3$) [Found $(M+Na)^+$ 1174.403. $C_{89}H_{154}O_3Na$ requires: 1174.1796], which showed δ_H (500 MHz, $CDCl_3$); 3.71 (3H, s), 3.66 (1H, m), 2.46–2.42 (1H, m), 1.74–1.70 (1H, m), 1.61–1.57 (1H, m), 1.48–1.15 (134H, m,), 0.89 (6H, t, *J* 6.6 Hz), 0.55 (2H, dt, *J* 4.1, 8.2 Hz), –0.33 (2H, q, *J* 4.9 Hz) ; δ_C (125 MHz, $CDCl_3$): 176.2, 72.3, 51.2, 35.7, 31.9, 30.2, 29.7, 28.7, 27.4, 25.7, 22.7, 15.8, 14.1, 10.9; ν_{max} (cm^{-1}): 3520, 2924, 2855, 2367, 1712, 1461, 1377, 1165, 721.

Experiment 44: (*R*)-2-((*R*)-1-Hydroxy-12-[(1*S*,2*R*)-2-[14-((1*R*,2*S*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid (77a)



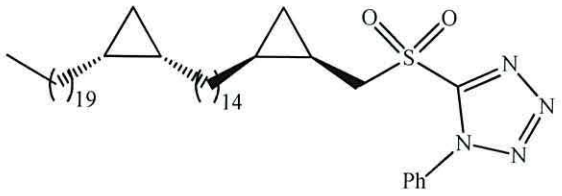
Using general methods,[163] (*R*)-2-((*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-[14-((1*R*,2S)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester (0.03 g, 0.026 mmol.) was dissolved in THF (4.0 mL), methanol (0.5 mL) and water (0.7 mL), and then lithium hydroxide monohydrate (0.02 g, 0.476 mmol.) was added to the stirred mixture at room temperature. The reaction was heated under reflux at 45 °C for 18 hours, after which TLC showed a very faint spot for the starting material. The reaction was cooled to room temperature and diluted with petrol/ethyl acetate (7:2, 5 mL), followed by dropwise addition of sat. aq, potassium hydrogen sulfate (20 mL) which brought the mixture to a pH of 1. The aqueous layer was separated and re-extracted with petrol/ethyl acetate (7:2, 3 x 10mL). The combined organic layers were dried and concentrated to a crude product which was purified by column chromatography, eluting with petrol/ethyl acetate (7:2) to give a white solid, *(R)-2-((R)-1-hydroxy-12-[(1S,2R)-2-[14-((1R,2S)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid* (0.019 g, 64 %), m.p. 53 °C, $[\alpha]_D^{21}$ + 2.5 (c 1.4) [Found $(M+Na)^+$ 1159.8621. $C_{78}H_{152}O_3Na$ requires: 1160.1639] which showed δ_H (500 MHz, $CDCl_3$); 3.73 (1H, td, 4.8, 7.9), 2.48 (1H, td, *J* 5.4, 8.9), 1.76–1.71 (1H, m), 1.63–1.60 (2H, m), 1.54–1.47 (4H, m), 1.26 (130H, m), 0.89 (6H, t, *J* 6.9 Hz), 0.66–0.64 (4H, m), 0.57 (2H, dt, *J* 4.1, 8.2 Hz), –0.33 (2H, q, *J* 4.9 Hz); δ_C (125 MHz, $CDCl_3$):178.6, 72.1, 50.8, 36.6, 31.9, 30.2, 29.7, 29.6, 29.5, 29.4, 28.7, 27.3, 25.7, 22.7, 15.8, 14.1, 10.9.; ν_{max} (cm^{-1}): 3281, 2919, 2852, 1709, 1466, 1377, 721.

Experiment 45: 5-[(1*R*,2*S*)-2-[14-((1*S*,2*R*)-2-Eicosyl-cyclopropyl)-tetradecyl]-cyclopropylmethylsulfanyl]-1-phenyl-1*H*-tetrazole (114b)



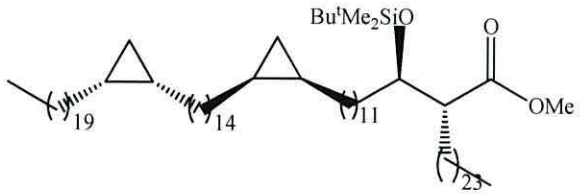
The procedure in **Experiment 39**, was repeated by dissolving (1*R*,2*S*)–2–[14–((1*S*,2*R*)–2–eicosylcyclopropyl)tetradecyl]cyclopropyl methanol (0.70 g, 1.19 mmol.) in dry THF (5 mL) followed by the addition of triphenylphosphine (0.41 g) and 1–phenyl–1H–tetrazole–5–thiol (0.28 g, 1.55 mmol.). The stirred mixture was cooled to 0 °C, diethyl azodicarboxylate (0.24 mL, 1 55 mmol.) in dry THF (5 mL) was added and the reaction was allowed to attain room temperature and then stirred overnight. The solvent was evaporated and the residue was dissolved in petrol/ ether (5:2), filtered and concentrated to obtain the crude product which was purified by column chromatography eluting with dichloromethane to give *5-[(1R,2S)-2-[14-((1S,2R)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropylmethylsulfanyl]-1-phenyl-1H-tetrazole* (0.8 g, 89 %), m.p. 43 – 50 °C, $[\alpha]_D^{20}$ +1.48 (c 1.4, $CHCl_3$) [Found $(M+Na)^+$ 771.6308. $C_{48}H_{84}N_4SNa$ requires: 771.6314], which gave δ_H (500 MHz, $CDCl_3$): 7.62–7.54 (5H, m), 3.50 (2H, d, *J* 8.0 Hz), 1.60 -1.10 (67H, including a multiplet at 1.46–1.51 for one cyclopropane proton), 0.92–0.97 (1H, m), 0.89 (3H, t, *J* 6.6 Hz), 0.83 (1H, dt, 4.7, 8.1 Hz), 0.66 (2H, m), 0.57 (1H, dt, *J* 3.8, 7.9 Hz), 0.23 (1H, q, *J* 5.4 Hz), –0.33 (1H, q, *J* 5.4 Hz) ; δ_C (125 MHz, $CDCl_3$): 133.8, 129.9, 123.8, 35.0, 31.9, 30.1, 29.5, 28.7, 22.7, 17.9, 15.8, 14.6, 14.1, 12.5, 10.9; ν_{max} (cm^{-1}) : 3059, 2920, 1463, 1339, 1275, 1169,1088, 1018.

Experiment 46: 5-[(1*R*,2*S*)-2-[14-((1*S*,2*R*)-2-Eicosylcyclopropyl)tetradecyl]-cyclopropylmethanesulfonyl]-1-phenyl-1H-tetrazole (115b)



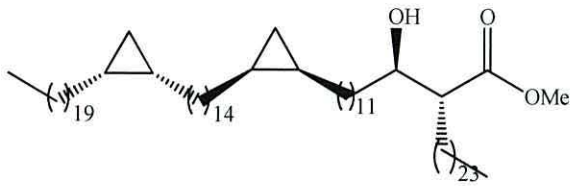
The procedure in **Experiment 40** was repeated using ammonium molybdate VI tetrahydrate (5.0 g, 4.01 mmol.) which was dissolved in cold hydrogen peroxide (35%,w/w, 10 mL), 5-[(1*R*,2*S*)-2-[14-((1*S*,2*R*)-2-eicosylcyclopropyl)tetradecyl]-cyclopropylmethyl sulfanyl]-1-phenyl-1H-tetrazole (0.6 g, 0.802 mmol.) in IMS/THF (30:10 mL) and a further portion of ammonium molybdate VI tetrahydrate (2.0 g, 1.60 mmol.) in cold hydrogen peroxide (5 mL). The reaction was worked up as before to give a whitish solid, *5-[(1R,2S)-2-[14-((1S,2R)-2-eicosylcyclopropyl)tetradecyl]-cyclopropyl-methanesulfony]-1-phenyl-1H-tetrazole* (0.2 g, 46 %), m.p. 65 – 66 °C, $[\alpha]_D^{20}$ –18.37 (c 1.4, $CHCl_3$) [Found $(M+Na)^+$ 803.486. $C_{48}H_{84}O_2N_4SNa$ requires: 803.6212], which showed δ_H (500 MHz, $CDCl_3$): 7.60–77.1 (5H, m), 3.98 (1H, dd, *J* 5.7, 14.9 Hz), 3.57 (1H, dd, *J* 9.2, 14.5 Hz), 1.38 -1.18 (68H, m), 1.05–0.97 (1H, m), 0.89 (3H, t, *J* 6.7 Hz), 0.65 (2H, m), 0.57 (1H, dt, *J* 4.1, 8.2 Hz), 0.25 (1H, q, *J* 5.6 Hz), –0.33 (1H, q, *J* 5.0 Hz); δ_C (125 MHz, $CDCl_3$): 131.4, 129.7, 125.2, 57.1, 31.9, 30.2, 29.7, 29.5, 29.1, 28.7, 22.7, 15.9, 14.1, 11.4, 10.9, 8.0; ν_{max} (cm^{-1}) : 2922, 2852, 1464, 1377, 1339, 1156.

Experiment 47: (*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-[(1*R*,2*S*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester (117b)



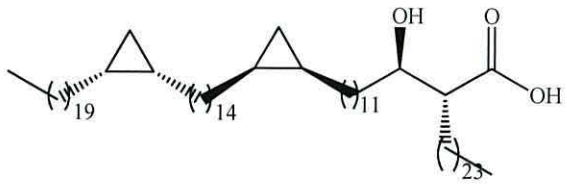
The steps in **Experimet 42** were repeated, using lithium bis–(trimethylsilyl)amide (0.32 mL, 0.343 mmol.), (*R*)-2-[(*R*)-1-(tert-butyl-dimethyl-silanyloxy)-11-oxo-undecyl]-hexacosanoic acid methyl ester (0.17 g, 0.24 mmol.) and 5-[(1*R*,2*S*)-2-[14-((1*S*,2*R*)-2-eicosylcyclopropyl)tetradecyl]-cyclopropylmethanesulfony]-1-phenyl-1H-tetrazole (0.20 g, 0.264 mmol.) in dry THF (5 mL) at –10 °C, under nitrogen, to obtain *(R)-2-((E/Z)-(R)-1-(tert-butyl-dimethyl-silanyloxy)-12-[(1R,2S)-2-[14-((1S,2R)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodec-11-enyl)-hexacosanoic acid methyl ester* (0.13 g; 40 %). The derived ester (0.11 g, 0.087 mol) in THF (3mL) and methanol (1.5 mL) was treated with di-potassium azodicarboxylate (2 g 10.3 mmol.) at 0 to 5 °C under nitrogen, followed by a mixture of glacial acetic acid (0.5 mL) and THF (1.0 mL). The reaction was worked up as before to give a thick oil, *(R)-2-((R)-1-(tert-butyl-dimethyl-silanyloxy)-12-[(1R,2S)-2-[14-((1S,2R)-2-eicosyl-cyclopropyl)-tetradecyl-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester* (0.1 g, 90 %), $[\alpha]_D^{19}$ –1.01 (c 1.1, $CHCl_3$) [Found $(M+Na)^+$ 1288.506. $C_{85}H_{168}O_3SiNa$ requires: 1288.2661] which showed δ_H (500 MHz, $CDCl_3$); 3.85 (1H, td, *J* 4.8, 6.5 Hz), 3.66 (3H, s), 2.54 (1H, ddd, *J* 3.8, 7.3, 10.8 Hz), 1.56–1.14 (136H, m), 0.89 (6H, t, *J* 6.6 Hz), 0.85 (9H, s), 0.66–0.65 (4H, m), 0.55 (2H, dt, *J* 4.1, 8.2 Hz), 0.05 (3H, s), 0.03 (3H, s), –0.33 (2H, q, *J* 4.9 Hz); δ_C (125 MHz, $CDCl_3$):175.1, 73.3, 51.4, 33.7, 31.9, 30.2, 29.7, 28.7, 27.8, 25.8, 23.7, 22.7, 18.0, 15.8, 14.1, 10.9; ν_{max} (cm^{-1}): 2924, 2853, 1742, 1465, 1254.

Experiment 48: (*R*)-2-((*R*)-1-Hydroxy-12-[(1*R*,2*S*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester (118b)



(*R*)-2-((*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-12-[(1*R*,2*S*)-2-[14-((1*S*,2*R*)-2-eicosylcyclo-propyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester (0.1 g, 0.079 mmol.), in dry THF (4.0 mL) was reacted with pyridine (0.1mL) and HF pyridine according the procedure described in **Experiment 43**, to give a white solid *(R)-2-((R)-1-hydroxy-12-[(1R,2S)-2-[14-((1S,2R)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester* (0.04 g, 44%), m.p. 57 °C, $[\alpha]_D^{19}$ +2.40 (c 2.0, $CHCl_3$) [Found $(M+Na)^+$ 1174.381. $C_{80}H_{154}O_3Na$ requires: 1174.1796], which showed δ_H (500 MHz, $CDCl_3$); 3.72 (3H, s), 3.66 (1H, m), 2.46–2.41 (1H, m), 1.75–1.68 (1H, m), 1.62–1.57 (1H, m), 1.49–1.14 (134H, m,), 0.88 (6H, t, *J* 7.3 Hz), 0.58 (2H, dt, *J* 3.8, 8.1 Hz), –0.33 (2H, q, *J* 5.0 Hz); δ_C (125 MHz, $CDCl_3$):176.2, 72.3, 51.2, 35.7, 31.9, 30.2, 29.7, 28.7, 27.4, 25.7, 22.7, 15.8, 14.1, 10.9; ν_{max} (cm^{-1}): 3520, 2924, 2855, 2367, 1712, 1461, 1377, 1165, 721.

Experiment 49: (*R*)-2-((*R*)-1-Hydroxy-12-[(1*R*,2*S*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid (77b)

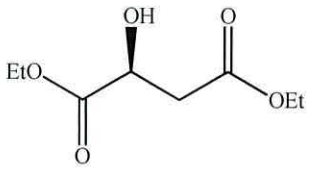


Repeating the procedure in **Experiment 44**, (*R*)-2-((*R*)-1-hydroxy-12-[(1*R*,2*S*)-2-[14-((1*S*,2R)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid methyl ester (0.04 g, 0.035 mmol.) was dissolved in THF (4.0 mL), methanol (0.5 mL) and water (0.7 mL), and then lithium hydroxide monohydrate (0.02 g, 0.476 mmol.) was added to the stirred mixture at room temperature. The mixture was refluxed at 45 °C for 18 hours and then worked up as before to obtain a white solid, *(R)-2-((R)-1-hydroxy-12-[(1R,2S)-2-[14-((1S,2R)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl]-dodecyl)-hexacosanoic acid* (0.033 g, 84 %), m.p. 52–53 °C, $[\alpha]_D^{21}$ + 2.46 (c 1.7, $CHCl_3$) [Found $(M+Na)^+$: 1159.8621. $C_{78}H_{152}O_3Na$ requires: 1160.1639], which showed δ_H (500 MHz, $CDCl_3$); 3.74 (1H, td, *J* 4.8, 7.9), 2.47 (1H, td, *J* 5.4, 8.8), 1.78–1.72 (1H, m), 1.67–1.60 (2H, m), 1.54–1.47 (4H, m), 1.45–1.07 (130H, m), 0.88 (6H, t, *J* 6.9 Hz), 0.67–0.64 (4H, m), 0.57 (2H, dt, *J* 4.1, 8.2 Hz), –0.33 (2H, q, *J* 4.9 Hz); δ_C (125 MHz, $CDCl_3$): 179.5, 72.1, 50.7, 35.5, 31.9, 30.2, 29.8, 29.6, 29.5, 29.4, 28.6, 27.4, 25.7, 22.6, 15.9, 14.0, 10.8; ν_{max} (cm^{-1}): 3317, 2922, 2852, 1681, 1464, 1377, 721.

6.2: Method development for the synthesis of α-alkyl-β-hydroxy fragment of mycolic acids

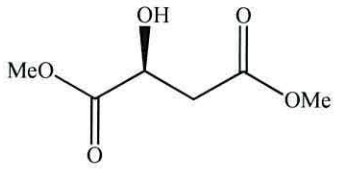
6.2.1 Route 1

Experiment 50: Diethyl- (*S*)-2-hydroxysuccinate (120a)[208]



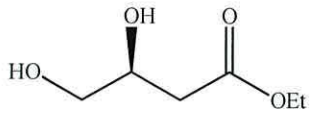
L-Malic acid (50 g, 0.373 mol.) was dissolved in ethanol (300 mL), conc. H_2SO_4 (3 mL) was added and the mixture was refluxed for 3hrs at 92 °C. The reaction was monitored by TLC until there was no more staring material. The excess ethanol was evaporated and then sat. aq. $NaHCO_3$ (100 mL) was added to neutralize excess acid. The aqueous layer was separated and extracted with dichloromethane (3 x 200 mL) and then the combined organic layer were dried over $MgSO_4$ and concentrated. The crude product was purified by flash distillation at 90 – 120 °C to give a clear liquid, *diethyl (S)-2-hydroxysuccinate* (46 g, 90 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$) and ν_{max} (cm^{-1}) identical to the literature.[208]

Experiment 51 Dimethyl-(*S*)-2-hydroxysuccinate (120b)[206]



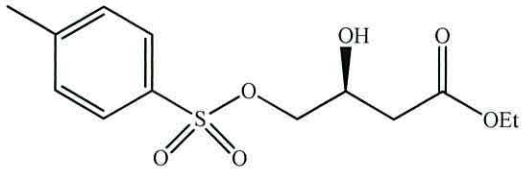
Conc. H_2SO_4 (3 mL) was added to a mixture of *L*-malic acid (50 g, 0.373 mol.) in methanol (300 mL) and the mixture was refluxed for over 3hrs at 90 °C. When TLC showed there was no more staring material, the excess methanol was evaporated and the acid in the mixture was neutralized by the addition of sat. aq. $NaHCO_3$ (100 mL). The aqueous layer was separated and extracted with dichloromethane (3 x 100ml) and the combined organic layers were dried over $MgSO_4$ and then evaporated. The crude product was purified by flash distillation at 90 – 120 °C to give a clear liquid, *dimethyl (S)-2-hydroxysuccinate* (46 g, 93 %), which showed δ_H, δ_C and ν_{max} identical to the literature.[206]

Experiment 52: Ethyl (*S*)-3,4-dihydroxybutanoate (121)[210]



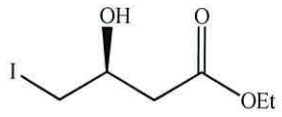
Diethyl (*S*)-2-hydroxysuccinate (10 g, 53 mmol.) in dry THF (100 mL) was placed in a two–necked round bottom flask that has been fitted with a reflux condenser. Borane-dimethylsulphide complex (27.3 mL, 54.6 mmol., 2M) was added dropwise over 10 minutes at 20 °C. The reaction was stirred at room temperature for 30 minutes until the evolution of hydrogen ceased and then cooled to 10 °C in an ice bath, stirring vigorously for 10 minutes. $NaBH_4$ (0.1 g, 2.65 mmol.) was added in one portion and this caused an exothermic reaction which was allowed to subside before the cooling water bath was removed. The reaction was stirred at room temperature until the starting material disappeared as shown by TLC, and then it was cooled to 5 °C. Ethanol (50 ml) was added, followed by p-TsOH (0.5 g, 2.65 mmol.). After stirring for 30 minutes, the mixture was evaporated and the residue was re-dissolved in toluene/IMS (1:1, 50 mL) and evaporated again (this process was repeated three times). The final residue was purified by column chromatography, eluting with ethyl acetate (1:19).to give a clear liquid, *ethyl (S)-3,4-dihydroxybutanoate* (6.6 g; 85 %), $[\alpha]_D^{23}$ +6.23 (c 0.4, $CHCl_3$) (lit. value[210] $[\alpha]_D^{25}$ +6.22 (c 1.2, $CDCl_3$)), which showed δ_H, δ_C and ν_{max} identical to the literature.[210]

Experiment 53: Ethyl (*S*)-3-hydroxy-4-(tosyloxy)butanoate (122)



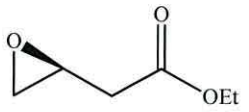
In line with standard procedures,[212] a solution of 3,4-dihydroxybutyric acid ethyl ester (1.0 g, 6.76 mmol.) and triethylamine (1.37 g, 13.5 mmol.) in dry dichloromethane (25 mL) was cooled to –20 °C under nitrogen, and stirred for 20 minutes. Toluene sulfonyl chloride (1.55 g, 8.13 mmol.) was added in one portion and the solution was kept in the fridge overnight. The solvent was evaporated, and the residue was washed with water and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried and concentrated to obtain the crude product. Column chromatography, eluting with petrol/ethyl acetate gave a thick clear yellow liquid, *ethyl (S)-3-hydroxy-4-(tosyloxy)butanoate* (1.3 g; 65 %), $[\alpha]_D^{24}$ – 6.37 (c 2.7, $CHCl_3$) [Found $(M+Na)^+$ 325.0723. $C_{13}H_{18}O_6SNa$ requires: 325.0716], which showed δ_H (500MHz, $CDCl_3$); 7.70 (2H, d, *J* 8.2 Hz), 7.35 (2H, d, *J* 8.2 Hz), 4.22–4.26 (1H, m), 4.14 (2H, q, *J* 7.1 Hz), 4.03 (2H, d, *J* 5.4 Hz), 3.02 (1H, br.s), 2.54 (IH, dd, *J* 4.7, 16.7 Hz), 2.49 (1H, dd, *J* 7.6, 16.7 Hz), 2.40 (3H, s), 1.24 (3H, t, *J* 6.9 Hz); δ_C (125 MHz, $CDCl_3$): 171.3, 145.0, 132.5, 129.9, 127.9, 72.0, 65.9, 61.0, 37.3, 21.6, 14.0; ν_{max} cm^{-1}: 3453, 2984, 1731, 1598, 1360, 1177, 1097, 985.

Experiment 54: Ethyl (*S*)-3-hydroxy-4-iodobutanoate (123)[213]



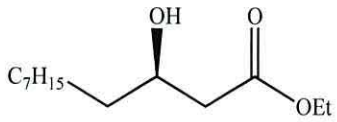
Ethyl (*S*)-3-hydroxy-4-(tosyloxy)butanoate (5.0 g, 16.6 mmol) was stirred into a solution of sodium iodide (7.5 g, 49.3 mmol.) in acetone (30 mL) and then refluxed at 50 °C for 5 hours. The reaction mixture was dissolved in water (to which a small amount of sodium thiosulfate had been added, so as to remove the colour). The aqueous layer was separated and extracted with dichloromethane (3 x 50 mL) and the combined organic layers were washed with water, followed by brine solution. The extract was dried over magnesium sulfate and evaporated to obtain the crude product. Column chromatography, eluting with petrol/ethyl acetate (1:1) gave a brownish liquid, *ethyl (S)-3-hydroxy-4-iodobutanoate* (2.2 g; 51 %), $[\alpha]_D^{20}$ –13.6 (c 2.9, $CHCl_3$) (lit. value[213] $[\alpha]_D^{24}$ – 10.7 (c 3.0, EtOH)), which showed δ_H, δ_C and ν_{max} identical to the literature.[213]

Experiment 55: Ethyl-(*S*)-2-(oxiran-2-yl) acetate (124)[213]



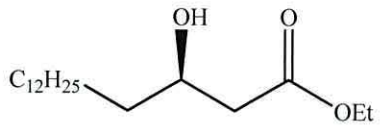
Ethyl (*S*)-3-hydroxy-4-iodobutanoate (20 g, 80.7 mmol.) was dissolved in acetonitrile (300 ml) and then silver II oxide (19.6 g, 84.7 mmol.) was added. The mixture was refluxed overnight at 100 °C, after which it was filtered and washed with excess acetonitrile. The solvent was removed by evaporating under house vacuum at 25 °C. The crude product was purified by column chromatography (using triethylamine on the silica gel), eluting with petrol/ethyl acetate (5:1) and evaporating at 25 °C under house vacuum to give a clear yellow liquid, *ethyl (S)-2-(oxiran-2-yl)acetate* (7.1 g; 70 %), $[\alpha]_D^{24}$ –30.9 (c 0.8, $CHCl_3$) (lit.value[213] $[\alpha]_D^{24}$ –25.3 (c 3.7, MeOH)), which showed δ_H, δ_C and ν_{max} identical to the literature.[213]

Experiment 56: Ethyl (*R*)-3-hydroxyundecanoate (125)



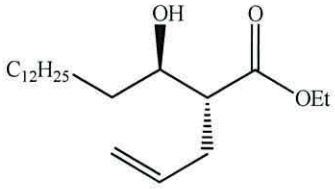
A Grignard solution was prepared according to the general procedure,[215] using n-bromoheptane in dry THF and magnesium turnings. A solution of the n-bromoheptane (1.72 g, 9.61mmol.) in dry THF (5 mL) was gradually stirred into a suspension of magnesium turnings (0.35 g, 14.42 mmol.) in dry THF (5mL) which was contained in a two neck flask that was fitted with a condenser. The addition was carried out at ambient temperature at a steady rate that was sufficient to maintain a reflux, which occurred as a result of the exothermic reaction. Then the mixture was heated under reflux for 1 hour at 80 °C. Following coupling procedures described in literature,[214] the derived Grignard reagent was added dropwise to a stirred suspension of copper(I) bromide (0.83 g, 5.77 mmol) in dry THF (15 ml) at –30 °C under nitrogen. With the temperature still maintained at –30 °C, the reaction was stirred for 30 minutes and then oxiranyl-acetic acid ethyl ester[214] (0.5 g, 3.85 mmol.) in dry THF (2 mL) was added dropwise. Stirring was continued at –30 °C for another 1 hour and then the reaction was allowed to warm up to room temperature and was stirred overnight. It was quenched with sat.aq. ammonium chloride (20 mL) and the aqueous layer was separated and re-extracted with ethyl acetate (3 x 25mL). The organic extracts were combined and washed with brine and then dried over magnesium sulfate. The suspension was filtered and the solvent was evaporated to obtain the crude product. This was followed by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a clear liquid *ethyl (R)-3-hydroxyundecanoate* (0.55 g, 62 %), $[\alpha]_D^{19}$ –0.19 (c 1.7, $CHCl_3$) [Found $(M+Na)^+$ 253.1767. $C_{13}H_{26}O_3Na$ requires: 253.1774], which showed δ_H (500MHz, $CDCl_3$): 4.16 (2H, q, *J* 7.4 Hz), 4.01–3.96 (1H, m), 3.00 (1H, s), 2.49 (1H, dd, *J* 8.2, 16.4 Hz), 2.39 (IH, dd, *J* 9.2, 16.4 Hz), 1.54–1.48 (1H, m), 1.43–1.41 (1H, m), 1.26 (15H, m, including one triplet for a CH_3), 0.87 (3H, t, *J* 6.9 Hz); δ_C (125 MHz, $CDCl_3$): 172.9, 67.9, 60.5, 41.3, 36.5, 31.7, 29.3,25.4, 22.5, 14.1, 14.0; ν_{max}/ cm^{-1}: 3445, 2926, 1732, 1467, 1372, 1180, 1034, 929, 852.

Experiment 57: Ethyl (*R*)-3-hydroxyhexadecanoate (126)[216]



The preparation was carried out as described by Huang *et al.*[214] A solution of n-bromododecane (1.72g, 9.61 mmol.) in dry THF (5mL) was gradually stirred into a suspension of turnnings (0.35 g, 14.42 mmol.) in dry THF (5mL) in a two neck flask that was fitted with a condenser. The addition was carried out at ambient temperature at a steady rate that was sufficient to maintain a reflux, which occurred as a result of the exothermic reaction. Then the mixture was heated under reflux for 1 hour at 80 °C. The derived Grignard reagent was added dropwise to a stirred suspension of copper I bromide (0.83 g, 5.77 mmol.) in dry THF (15 ml) at –30 °C under nitrogen. With the temperature still maintained at –30 °C, oxiranyl-acetic acid ethyl ester[213, 214] (0.5 g, 3.85mmol.) in dry THF was added dropwise, 30 minutes later. Stirring was continued at a –30 °C for another 1 hour and then the reaction was allowed to attain room temperature and was stirred overnight. It was quenched with sat.aq. ammonium chloride (20 mL) and the aqueous layer was separated and re-extracted with ethyl acetate (3 x 25mL). The organic extracts were combined and washed with brine and then dried over magnesium sulphate. The suspension was filtered and the solvent was evaporated to obtain the crude product. This was followed by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a clear liquid, *ethyl (R)-3-hydroxyhexadecanoate* (0.91 g, 79 %), $[\alpha]_D^{20}$ –13.4 (c 1.3, $CHCl_3$) (lit.value[216] $[\alpha]_D^{20}$ –19.0 (c 1.0, $CHCl_3$)), which showed δ_H, δ_C and ν_{max} identical to the literature.[216]

Experiment 58: Ethyl (2*R*,3*R*)-2-allyl-3-hydroxyhexadecanoate (127)



According to standard methods,[177] dry diisopropylamine (0.45 g, 4.45 mmol.) was dissolved in dry THF (3 mL) and the stirred solution was cooled to –10 °C. Butyllithium (1.75 mL, 4.67 mmol, 2.5M) was slowly added to the mixture, which was allowed to reach room temperature. After 30 minutes the mixture was cooled to –78 °C and ethyl (*R*)-3-hydroxyhexadecanoate (0.5 g, 1.67 mmol.) in dry THF (3.0 mL) was added dropwise. The mixture was stirred at -78 °C for 1 hour, the temperature was allowed to increase to –20 °C for 1 hour and then stirring was continued for another 25 to 30 minutes whilst maintaining the temperature at –20 °C. The reaction was re-cooled to –78 °C, and a mixture of allyl iodide (0.23 mL, 2.50 mmol.) and HMPA (0.64 mL, 3.66 mmol.) in dry THF (2 mL) was slowly added. The reaction was stirred for 2 hours during which time the temperature increased to 0 °C. The reaction was quenched with sat.aq. ammonium chloride (20 mL) and extracted with diethyl ether (3 x 100 mL). The combined organic layers were washed with water (3 x100 mL), dried over $MgSO_4$ and evaporated. Column chromatography eluting with petrol/ethyl acetate (5:1) gave a semi-solid, pure *ethyl (2R,3R)-2-allyl-3-hydroxyhexadecanoate* (0.3 g, 56 %), $[\alpha]_D^{23}$ +1.1 (c 1.0, $CHCl_3$) [Found $(M+Na)^+$ 363.2870, $C_{21}H_{40}O_3Na$ requires: 363.2875], which showed δ_H (500MHz, $CDCl_3$); 5.74 (1H, ddt, *J* 6.0, 10.4, 17.4 Hz), 5.12 (1H, dd, *J* 1.6, 17.1 Hz), 5.05 (1H, dd, *J* 0.95, 10.1 Hz), 4.19 (2H, q, *J* 7.2 Hz), 3.71–3.66 (1H, m), 2.54–2.38 (1H, m), 1.60 (1H, s), 1.50–1.44 (2H, m), 1.28 (3H, t, *J* 6.9 Hz), 1.26 (24H, m), 0.89 (3H, t, *J* 6.6 Hz); δ_C (125 MHz, $CDCl_3$): 174.9, 135.0, 117.1, 71.8, 60.5, 50.3, 35.6, 33.9, 29.6, 25.7, 22.7, 14.3; ν_{max} (cm^{-1}): 3463, 3067, 2925, 2854, 1716, 1643, 1466, 1379, 1036, 917.

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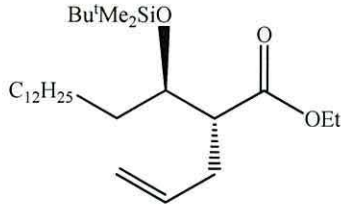
Experiment 59: Ethyl (2*R*,3*R*)-2-allyl-3- ((*tert*-butyldimethylsilyl)oxy)hexadecanoate (128)

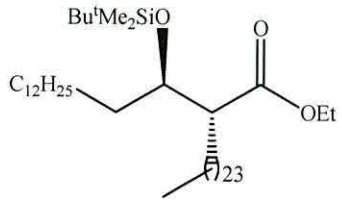


Using standard methods,[177] ethyl (2*R*,3*R*)-2-allyl-3-hydroxyhexadecanoate (0.8 g, 2.35 mmol.) was dissolved in dry DMF (3.0 mL) and imidazole (0.48 g, 7.05 mmol.) was added to the stirred solution at room temperature. The mixture was cooled to 0 °C followed by the addition of *tert*-butyldimethylchlorosilane (1.24 g, 8.24 mmol.). The cooling bath was removed and the reaction was stirred at 65 °C for 24 hours. TLC showed the reaction was complete, and then DMF was removed by flash distillation. The residue was dissolved in water (25 mL) and extracted with dichloromethane (3 x 20 mL). The combined organic layers were washed with some more water, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography, eluting with petrol/ ethyl acetate (40:1), to give a clear yellow oil, *ethyl (2R,3R)-2-allyl-3-((tert-butyldimethylsilyl)oxy)-hexadecanoate* (0.9 g, 82 %), $[\alpha]_D^{23}$ –13.6 (c 1.2, $CHCl_3$) [Found $(M+Na)^+$ 477.3734, $C_{27}H_{54}O_3SiNa$ requires: 477.3740], which showed δ_H (500MHz, $CDCl_3$): 5.80– 5.72 (1H, m), 5.07 (1H, dd, *J* 1.5, 17.0), 4.99 (1H, dd, *J* 0.9, 10.1 Hz), 4.13 (2H, q, *J* 7.2 Hz), 3.95 (1H, q, *J* 5.5 Hz), 2.63–2.59 (1H, m), 2.27–2.22 (1H, m), 1.27 (22H, m), 1.25 (3H, t, *J* 7.3), 0.89 (3H, t, *J* 6.9 Hz), 0.88 (9H, s), 0.06 (3H, s), 0.05 (3H, s); δ_C (125 MHz, $CDCl_3$): 173.54, 136.10, 116.22, 60.10, 51.31, 33.68, 31.74, 29.59, 25.77, 24.29, 22.69, 18.01, 14.26; ν_{max} (cm^{-1}): 3080, 2927, 2856, 1737, 1643, 1464, 1377, 1255, 1179, 1097, 1068, 1006, 914, 836, 775.

Experiment 60: Ethyl (2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-(2-oxoethyl)hexadecanoate (129)

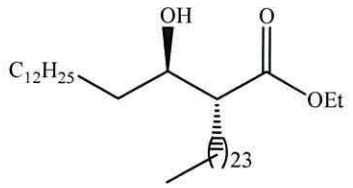
Using standard methods,[177] ethyl (2*R*,3*R*)-2-allyl-3-((*tert*-butyldimethylsilyl)oxy)-hexadecanoate (0.67 g, 1.48 mmol.), 2,6-lutidine (0.61 g, 2.95 mmol.), osmium tetroxide (25 %) in 2-methyl-2-propanol (0.29 mL, 0.030 mmol.) and sodium (meta) periodate (1.26 g, 5.9 mmol.) were stirred into a mixture of 1,4-dioxane and water (40 mL, 3:1) at room temperature. The reaction was stirred for 2 hours and monitored by TLC until it was complete. Water (50 mL) was added to quench it and the mixture was extracted with dichloromethane (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography, eluting with petrol/ ethyl acetate (2:1) to recover starting material (0.23 g, 26 %) and a thick oil, pure *ethyl (2R,3R)-3-((tert-butyldimethylsilyl)oxy)-2-(2-oxoethyl)hexadecanoate* (0.35 g, 52 %), $[\alpha]_D^{22}$ –7.23 (c 2.2, $CHCl_3$) [Found $(M+Na)^+$ 479.3472, $C_{26}H_{52}O_4SiNa$ requires: 479.3533], which showed δ_H (500MHz, $CDCl_3$): 9.81 (1H, bs), 4.14–4.06 (2H, m), 4.05–4.02 (1H, m), 3.18 (1H, td, *J* 3.5, 10.7 Hz), 2.96 (1H, dd, *J* 10.6, 17.8 Hz), 2.66 (1H, dd, *J* 3.5, 18 Hz), 1.25 (25H, m, including one triplet from CH_3), 0.88 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.07 (3H, s), 0.06 (3H, s); δ_C (125 MHz, $CDCl_3$): 200.7, 172.1, 71.9, 68.0, 60.8, 45.4, 40.0, 33.6, 31.9, 29.6, 25.7, 22.7, 18.0, 14.1; ν_{max} (cm^{-1}): 2927, 2855, 2720, 1733, 1464, 1379, 1312, 1254, 1182, 1095, 1045, 1006, 939, 837, 810, 776.

Experiment 61: Ethyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)tetradecyl)hexacosanoate (131)



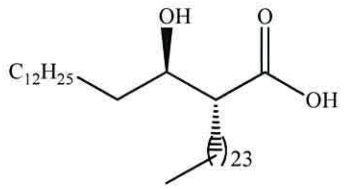
As described in **Experiment 42**, lithium bis-(trimethylsilyl) amide (1.33 mL, 1.41 mmol.) was added to a stirred solution of ethyl (2*R*,3*R*)-3-((tert-butyldimethylsilyl)oxy)-2-(2-oxoethyl)hexadecanoate (0.33 g, 0.723 mmol.) and 5-(docosyl-1-sulfonyl)-1-phenyl-1H-tetrazole (0.49 g, 0.94 mmol.) in dry THF (5 mL) at –10 °C under nitrogen. After 1 hour, TLC showed that the reaction was complete and it was quenched with sat. aq. ammonium chloride (20 mL). The mixture was extracted with petrol/ethyl acetate (20:1, 3 x 20 mL), dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick oil, *ethyl (R)-(E/Z)-2-((R)-1-((tert-butyldimethylsilyl)oxy)tetradecyl)hexacos-4-enoate* (0.30 g; 55 %). Palladium (10 % on carbon) (0.2 g) was added to the derived ethyl (*R*)-(*E/Z*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)tetradecyl)hexacos-4-enoate (0.3 g, 0.382 mmol.) in IMS/THF (4mL, 1:1). The suspension was stirred overnight under hydrogen until there was no further absorption. This was followed by filtration over a bed of celite, washing with excess ethyl acetate and then evaporating. The crude product obtained was purified by flash chromatography eluting with petrol/ethyl acetate (10:1), to give a thick oil, *ethyl (R,E)-2-((R)-1-((tert-butyldimethylsilyl)oxy)tetradecyl)hexacos-4-enoate* (0.2 g, 67 %), $[\alpha]_D^{21}$ –3.30 (c 1.0, $CHCl_3$) [Found $(M+Na)^+$ 773.7177. $C_{48}H_{98}O_3SiNa$ requires: 773.7183], which showed δ_H (500MHz, $CDCl_3$): 4.17–4.08 (2H, m), 3.91 (1H, td, *J* 5.0, 10.0 Hz),0.03 (3H, s), 2.53–2.48 (1H, m), 1.26 (73H, m including one triplet corresponding to one terminal CH_3 group), 0.87–0.90 (15H, including a triplet which resonated at δ 0.89 with *J* 5.0 Hz, integrated for six protons, and a singlet which resonated at δ 0.87, corresponding to three CH_3 groups on the *tert*-butyl), 0.05 (3H, s); δ_C (125 M Hz, $CDCl_3$): 174.7, 60.0, 51.6, 33.6, 31.9, 29.6, 27.6, 25.8, 23.8, 22.7, 18.0,14.1, 4.7; ν_{max} (cm^{-1}): 2924, 2854, 1737, 1465, 1378, 1254, 1178, 1096, 1005, 969, 836, 810, 775, 721.

Experiment 62: Ethyl (*R*)-2-((*R*)-1-hydroxytetradecyl)hexacosanoate (132)



Using the deprotection method described in **Experiment 43**, ethyl (*R*)-(*E/Z*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)tetradecyl)hexacos-4-enoate (0.2 g, 0.266 mmol.) in dry THF (8 mL) was placed in a well dried polyethylene vial and cooled to 0 °C under nitrogen. Pyridine (0.1 mL) and HF.pyridine (0.5 mL) were added and the mixture was stirred for 20 hours at 40 °C. TLC showed that the reaction was complete and then sat. aq. sodium bicarbonate was added until the liberation of carbon dioxide ceased. The mixture was extracted with ethyl acetate (10:1, 4 x 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated to give the crude product. This was purified by flash chromatography, eluting with petrol/ethyl acetate (10:1) to give a white solid, pure *ethyl (R)-2-((R)-1-hydroxytetradecyl)hexacosanoate* (0.11 g, 66 %), m.p. 44 °C, $[\alpha]_D^{23}$ +5.23 (c 1.1, $CHCl_3$) [Found $(M+Na)^+$ 659.6313. $C_{42}H_{84}O_3Na$ requires: 659.6318], which showed δ_H (500MHz, $CDCl_3$): 4.24–4.15 (2H, m), 3.88–3.77 (1H, m), 2.44–2 39, (1H, m), 1.58 (1H, br.s), 1.42–1.26 (73H, m, including a triplet from a terminal $-CH_3$), 0.89 (6H, t, *J* 7.5 Hz); δ_C (125 MHz, $CDCl_3$): 174.8, 31.92, 29.7, 22.7, 14.1; ν_{max} (cm^{-1}): 3522, 2955, 2918, 2850, 1706, 1473, 1463, 1377, 1180, 1163, 1138, 1026, 730, 720.

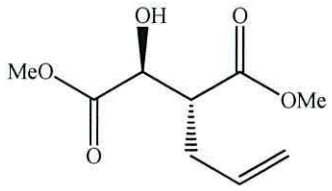
Experiment 63: (*R*)-2-((*R*)-1-hydroxytetradecyl)hexacosanoic acid (78)



As described in **Experiment 44**, ethanol (1.5 mL), water (2 mL), and then lithium hydroxide monohydrate (0.1 g, 2.36 mmol.) were added to a stirred solution of ethyl (*R*)-2-((*R*)-1-hydroxytetradecyl)hexacosanoate (0.10 g, 0.157 mmol.) in THF (10 mL) at room temperature. The reaction was refluxed at 45 °C for 18 hours. Subsequently, it was cooled to room temperature, diluted with petrol/ethyl acetate (5:1) and acidified to pH 1 by dropwise addition of dilute HCl. The aqueous layer was separated and extracted with petrol/ethyl acetate (5:1; 20 mL x 3), and the combined organic layers were dried over $MgSO_4$ filtered and evaporated. Column chromatography eluting with petrol/ethyl acetate (2:1) gave a white solid, *(R)-2-((R)-1-hydroxytetradecyl)hexacosanoic acid* (0.45 g, 47 %), m.p. 50 – 52 °C, $[\alpha]_D^{22}$ + 5.45 (c 1.0, $CHCl_3$) [Found $(M+Na)^+$ 631.6000, $C_{40}H_{80}O_3Na$ requires: 631.6005], which showed δ_H (500MHz, $CDCl_3$): 3.74 (1H, br.s), 3.68 – 3.64 (1H, m), 2.50 – 2.45 (1H, m), 2.41 (1H, bs), 2.18 – 1.55 (70H, m), 0.89 (6H, t, *J* 5.0 Hz); δ_C (125 MHz, $CDCl_3$): 175, 71.6, 53.9, 35.5 – 22.7, 14.1, 14.0; ν_{max} (cm^{-1}): 3585, 3392, 2916, 2849, 1682, 1465, 1381, 1047.

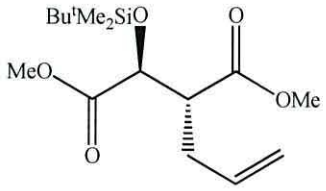
6.2.2 Route 2

Experiment 64: Dimethyl (2*R*,3*S*)-2-allyl-3-hydroxysuccinate (134)



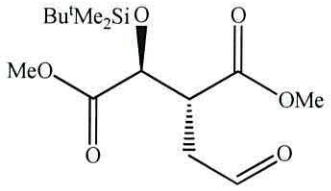
Following the procedure described in **Experiment 58** methyl lithium (56.8 mL, 85.3 mmol 1.5M) was added dropwise to a stirred dry diisopropylamine (9.56 mL, 68.2 mmol.) in dry THF (50 mL) at –78 °C than allowed to reach +16 °C and stirred for 30 minutes. The reaction was cooled to –78 °C and dimethyl (*S*)-2-hydroxysuccinate (5.0 g, 30.9 mmol.) in dry THF (30 mL) was slowly added. The temperature rose gradually to –45 °C and was stirred for 1 hour at –45 °C, then warmed up to –20 °C, and stirred for another 30 minutes at –20 °C. The reaction was re-cooled to –78 °C and a mixture of allyl iodide (4.25 mL, 46.3 mmol.) and HMPA (11.8 mL, 67.9 mmol.) in dry THF (10 mL) was slowly added. Again, the temperature gradually rose to –45 °C and the reaction was stirred for 1 hour at –45 °C, then for 30 minutes between –45 and –20 °C and then for an additional 30 minutes between –20 and 0 °C. The reaction was quenched with sat. aq. ammonium chloride (50 mL), extracted with ethyl acetate (3 x 100 mL) and the organic layers were dried over $MgSO_4$ and evaporated. Column chromatography eluting with petrol/ethyl acetate (2:1) gave a clear oil, pure *dimethyl (2R,3S)-2-allyl-3-hydroxysuccinate* (2.4 g, 60 %), $[\alpha]_D^{18}$ +23.4 (c 1.5, $CHCl_3$) [Found $(M+Na)^+$ 225.0733. $C_9H_{14}O_5Na$ requires: 225.0841], which showed δ_H (500MHz, $CDCl_3$); 5.81–5.76 (1H, m), 5.17 (1H, dd, *J* 1.25, 17.00 Hz), 5.11 (1H, d, *J* 10.00 Hz), 4.31 (1H, q, *J* 3.47 Hz), 3.68 (1H, s), 3.80 (1H, s), 3.40 (1H, d, *J* 7.25), 3.00–2.97 (1H, m), 2.64 – 2.60 (1H, m), 2.47 – 2.41 (1H, m); δ_C (125MHz, $CDCl_3$):173.9, 172.5, 134.6, 117.9, 52.7, 51.9, 48.1, 32.0; ν_{max} (cm^{-1}): 3487, 3080, 2955, 1733, 1643, 1440, 1217, 921.

fExperiment 65: Dimethyl (2*R*,3*S*)-2-allyl-3-((*tert*-butyldimethylsilyl)oxy) succinate (135)



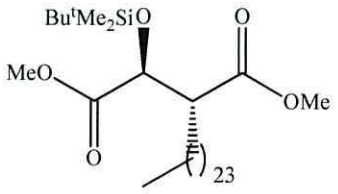
As in **Experiment 59**, imidazole (4.66 g, 68.4 mmol.) was added to a stirred solution of dimethyl (2*R*,3*S*)-2-allyl-3-hydroxysuccinate (5.5 g, 27.4 mmol.) in dry DMF (30 mL) at room temperature, the mixture was cooled to 0 °C, and then *tert*-butyldimethylchlorosilane (5.36 g, 35.6 mmol.) was added. The cooling bath was removed and the reaction was stirred at 45 °C for 20 hours when TLC showed the reaction was complete. The DMF was removed by flash distillation. Water (250 mL) was added and the mixture was extracted with dichloromethane (3 x 100 mL). The combined organic layers were washed with water, dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography eluting with petrol/ ethyl acetate (10:1), to give a clear yellow oil, *dimethyl (2R,3S)-2-allyl-3-((tert-butyldimethylsilyl)oxy)succinate* (5.33 g, 83 %), $[\alpha]_D^{18}$ – 36.5 (c 1.4, $CHCl_3$) [Found $(M+Na)^+$ 339.1588. $C_{15}H_{28}O_5SiNa$ requires: 339.1598]. This showed δ_H (500MHz, $CDCl_3$): 5.77–5.69 (1H, m), 5.07 (1H, dd, *J* 1.3, 17.1), 5.03 (1H, dd, *J* 0.9, 16.1 Hz), 4.35 (1H, d, *J* 6.0 Hz), 3.72 (3H, s), 3.64 (3H, s), 2.95 (1H, td, *J* 6.0, 8.5 Hz), 2.43 (1H, pent, *J* 7.4 Hz), 2.22 (1H, pent, *J* 6.9 Hz), 0.86 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 172.3, 172.2, 134.8, 117.2, 72.9, 51.9, 51.6, 50.0, 31.8, 25.5, 18.0; ν_{max} (cm^{-1}): 3081, 2967, 2859, 1750, 1644, 1437, 1363, 1267, 1167, 995, 919, 850, 783.

Experiment 66: Dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl) succinate (136)



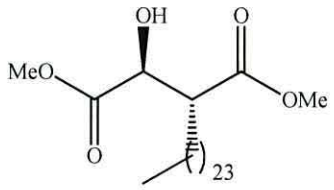
Using the method in **Experiment 60**, dimethyl (2*R*,3*S*)-2-allyl-3-((*tert*-butyldimethylsilyl)-oxy)succinate (5.0 g, 15.8 mmol.) was dissolved in 1,4-dioxane and water (160 mL, 3:1) and then 2,6-lutidine (3.4 g, 31.6 mmol.), osmium tetroxide (25 %) in 2-methyl-2-propanol (4.0 mL, 0.32 mmol.) and sodium (meta) periodate (13.5 g, 63.2 mmol.) were stirred into the solution at room temperature. The mixture was stirred for 2 hours and monitored by TLC until it was complete. It was quenched with water (200 mL) and the mixture was extracted with dichloromethane (3 x 150 mL). The combined organic layers were washed with brine (200 mL), dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography, eluting with petrol/ ethyl acetate (2:1) to give *dimethyl (2S,3R)-2-((tert-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate* (4.0 g, 80 %), $[\alpha]_D^{19}$ –28.5 (c 1.4, $CHCl_3$). The compound showed δ_H (500MHz, $CDCl_3$): 9.80 (1H, s), 4.52 (1H, d, *J* 4.4 Hz), 3.73 (3H, s), 3.69 (3H, s), 3.49 (1H, td, *J* 4.4, 9.2 Hz), 3.01 (1H, dd, *J* 8.8, 18.3 Hz), 2.68 (1H, dd, *J* 4.5, 18.3 Hz), 0.88 (9H, s), 0.09 (3H, s), 0.05 (3H, s); δ_C (125 MHz, $CDCl_3$): 199.5, 171.7, 171.0, 72.1, 67.1, 60.3, 52.1, 44.0, 25.6, 21.0, 18.1, 14.2, ; ν_{max} (cm^{-1}): 2955, 2931, 2859, 1743, 1494, 1437, 1363, 1253, 1207, 1667, 1051, 1006, 833, 783.

Experiment 67: Dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosyl succinate (138)



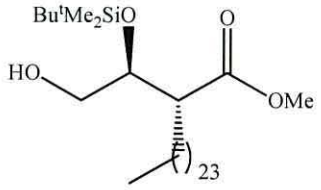
Using general methods as in **Experiment 61**, dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate (4.0 g, 12.6 mmol.) and 5-(docosyl-1-sulfonyl)-1-phenyl-1H-tetrazole (8.5 g, 16.4 mmol.) were dissolved in dry THF (150 mL), cooled to –10 °C under nitrogen whilst stirring, and then lithium bis-(trimethylsilyl) amide (23.2 mL, 24.6 mmol.) was added. After 1 hour, TLC showed the reaction was complete and sat. aq. ammonium chloride (100 mL) was added to quench the reaction. The aqueous layer was separated and extracted with petrol/ethyl acetate (20:1, 3 x 100 mL) and the combined organic layers were dried with $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick yellow oil, *dimethyl (2S,3R)-2-((tert-butyldimethylsilyl)oxy)-3-((E/Z)-tetracos-2-en-1-yl)succinate* (4.7 g; 61 %) as a mixture of isomers, which showed δ_H (500MHz, $CDCl_3$): 5.51 – 5.43 (1H, m), 5.34 – 5.26 (1H, m), 4.37 (1H, dd, *J* 6.5, 16.9 Hz), 3.74 (3H, s), 3.65 (3H, s), 2.93 – 2.87 (1H, m), 2.44 – 2.22 (1H, m), 2.18 – 2 13 (1H, m), 2.01 – 1.94 (1H, m), 1.26 (39H, m), 0.88 (12H, s including one triplet corresponding to CH_3), 0.06 (3H,s), 0.04 (3H, s); δ_C (125 MHz, $CDCl_3$): 172.5, 172.4, 133.8, 132.8, 125.8, 125.2, 73.0, 51.9, 50.7, 41.4, 32.5 – 11.4, -5.0, -5.4. Palladium (10 % on carbon) (1.0 g) was added to the above succinate (7.0 g, 11.4 mmol.) in THF/IMS (200mL, 1:1) and the suspension was stirred overnight under hydrogen. When there was no further absorption of hydrogen, the suspension was filtered over a bed of celite, washing with excess ethyl acetate. The filtrate was concentrated and purified by column chromatography eluting with petrol/ethyl acetate (2:1) to give a thick oil, *dimethyl (2S,3R)-2-((tert-butyldimethylsilyl)oxy)-3-tetracosylsuccinate* (6.8 g, 97 %), $[\alpha]_D^{14}$ –12.58 (c 0.3, $CHCl_3$) [Found $(M+Na)^+$ 635.5013. $C_{36}H_{72}O_5SiNa$ requires: 635.5047]. This showed δ_H (500MHz, $CDCl_3$): 4.34 (1H, d, *J* 7.3 Hz), 3.74 (3H, s), 3.67 (3H, s), 2.81–2.85 (1H, m), 1.66–162 (1H, m), 1.26 (9H, m), 0.87 (12H, s, including a triplet for a CH_3), 0.05 (3H, s), 0.04 (3H, s); δ_C (125 MHz, $CDCl_3$): 172.2, 172.4, 51.9, 51.5, 50.6, 31.9, 30.0, 29.4, 27.7, 27.3, 25.5, 22.7, 18.0, 14.1; ν_{max} (cm^{-1}): 2923, 2851, 1767, 1746, 1472, 1434, 1350, 1250, 1202, 1153, 1122, 909, 839, 783, 734.

Experiment 68: Dimethyl (2*S*,3*R*)-2-hydroxy-3-tetracosylsuccinate (139)



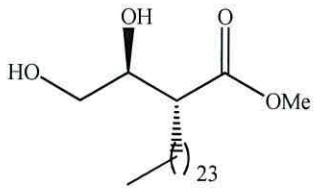
Following general deprotection methods used in this work, dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosylsuccinate (6.8 g, 11.1 mmol.) was dissolved in dry THF (30 mL) in a dry polyethylene vial and then cooled to 0 °C, under nitrogen. Pyridine (2 mL) and HF.pyridine (2 mL) were added and the reaction was stirred for 17 hours at 40 °C. TLC was carried out to determine the end of the reaction. Then it was diluted with petrol/ ethyl acetate (10:1, 30 mL) and neutralized by careful addition of sat. aq. sodium bicarbonate, until the liberation of carbon dioxide ceased. The aqueous layer was separated and extracted with ethyl acetate (10:1, 3 x 50 mL) and the combined organic layers were washed with brine solution, dried over $MgSO_4$ and concentrated to give the crude product. Flash chromatography eluting with petrol/ethyl acetate (2:1) gave a yellow solid, pure *dimethyl (2S,3R)-2-hydroxy-3-tetracosylsuccinate* (4.9 g, 58 %), m.p. 50 – 52 °C, $[\alpha]_D^{19}$ +0.12 (c 1.2, $CHCl_3$) [Found $(M+Na)^+$ 521.4171. $C_{30}H_{58}O_5Na$ requires: 521.4182] which showed δ_H (500MHz, $CDCl_3$): 4.29 (1H, d, *J* 2.5 Hz), 3.81 (3H, s), 3.71 (3H, s), 3.18 (1H, d, *J* 7.3 Hz), 2.89–2.86 (1H, m), 1.86–1.80 (1H, m), 1.70–1.63 (1H, m), 1.58 (1H, br.s), 0.89 (3H, t, *J* 6.6 Hz); δ_C (125 MHz, $CDCl_3$): 174.0, 173.5, 71.0, 52.7, 51.9, 48.6, 31.9, 28.1, 27.4, 22.7, 14.1; ν_{max} (cm^{-1}): 3379, 2919, 2850, 1738, 1717, 1463, 1377, 1223, 1166, 1147, 1102, 1074, 1017, 720.

Experiment 69: Attempted preparation of methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl)hexacosanoate (140)



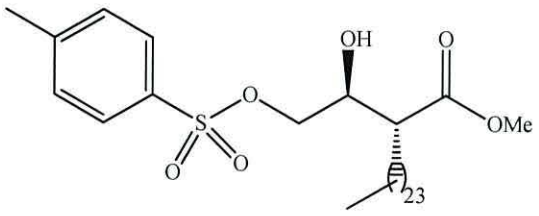
Following procedures described by Saito *et al*,[209, 210] (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosylsuccinate (1.0g, 4.0 mmol.) in dry THF (20 mL) was placed in a two-necked round bottom flask, which was fitted with a reflux condenser, and then borane-dimethylsulphide complex (2.08 mL, 4.2 mmol, 2M) was added dropwise at 20 °C over a period of 10 minutes. The solution was stirred vigorously at room temperature for 30 minutes until the evolution of hydrogen ceased and then it was cooled to 10 °C for 10 minutes followed by addition of $NaBH_4$ (30 mg, 0.794 mmol.) in one portion. When the exothermic reaction had subsided, the cooling water bath was removed and the reaction was stirred at room temperature, until TLC showed no starting material. Ethanol (10 mL) and p-TSOH (40 mg, 0.210 mmol.) were added at 5 °C, and then the reaction was stirred for 30 minutes and concentrated by evaporation. The residue was dissolved in toluene/IMS (1:1, 50 mL) and evaporated and this process was carried out three times. The TLC showed one spot and the ^{1}H NMR of the crude showed a broad multiplet peak for a long chain hydrocarbon and no peak for methoxy protons.

Experiment 70: Methyl (*R*)-2-((*S*)-1,2-dihydroxyethyl)hexacosanoate (141)



As in **Experiment 69**, (2*S*,3*R*)-dimethyl-2-hydroxy-3-tetracosylsuccinate (2.0g, 4.01 mmol.) in dry THF (20 mL) was treated with borane-dimethylsulphide complex (2.07 mL, 4.13 mmol, 2M) which was added dropwise at room temperature for 10 minutes. The solution was stirred vigorously for 30 minutes, and when the evolution of hydrogen ceased it was cooled to 10 °C and then $NaBH_4$ (30 mg, 0.794 mmol.) was added in one portion. When the exothermic reaction had subsided, the cooling water bath was removed. The reaction was monitored by TLC whilst stirring at room temperature. When there was no more starting material, the reaction was cooled to 5 °C and then ethanol (10 mL) and p-TsOH (40 mg, 0.210 mmol.) were added. After 30 minutes the mixture was concentrated by evaporation, the residue was dissolved in toluene/IMS (1:1, 50 mL) and evaporated again. This process was repeated three times. Then the product was purified by flash chromatography, eluting with ethyl acetate to give a white solid *methyl (R)-2-((S)-1,2-dihydroxyethyl)hexacosanoate* (1.6 g; 85 %), m.p. 64 °C, $[\alpha]_D^{19}$ +0.18 (c 1.1, $CHCl_3$) [Found $(M+Na)^+$ 493.4204. $C_{29}H_{58}O_4Na$ requires: 493.4233]. This showed δ_H (500MHz, $CDCl_3$): 3.86–3.82 (1H, m), 3.74 (3H, s), 3.72 (1H, dd, *J* 3.5, 7.3 Hz), 3.69 (1H, d, *J* 3.5 Hz), 3.56 (1H, dd, *J* 6.6, 11.4 Hz), 2.57 (1H, td, *J* 5.7, 9.2 Hz), 1.75–1.55 (1H, m), 1.26 (46H, m), 0.89 (3H, t, *J* 7.0 Hz); δ_C (125 MHz, $CDCl_3$): 175.9, 72.6, 65.0, 51.8, 47.6, 31.9, 29 7, 27.2, 22.7, 14.1; ν_{max} (cm^{-1}): 3392, 2923, 2852, 1731, 1463, 1377, 1168, 720.

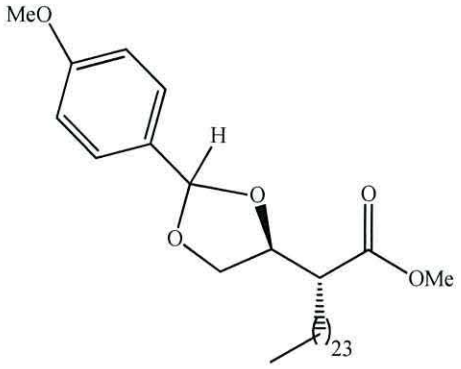
Experiment 71: Methyl (*R*)-2-((*S*)-1-hydroxy-2-(tosyloxy)ethyl)hexacosanoate (142)



As in **Expriment 53,** methyl (*R*)-2-((*S*)-1,2-dihydroxyethyl)hexacosanoate (0.1 g, 0.213 mmol.) was dissolved in anhydrous pyridine (1.7 mL) and anhydrous toluene (0.9 mL) and then cooled to 6 °C under nitrogen. p-Toluenesulfonyl chloride (0.09 g, 0.478 mmol.) in dry toluene (0.3 mL) was added in one portion to the stirred solution and the temperature rose to 7.3 °C. After 45 minutes stirring, the mixture was kept in the refrigerator overnight. The solvent was evaporated and the residue was washed with water, the aqueous layer was separated and re-extracted with ethyl acetate (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Column chromatography eluting with petrol/ethyl acetate (1:1), gave a white solid, *methyl (R)-2-((S)-1-hydroxy-2-(tosyloxy)ethyl)hexacosanoate* (0.05 g, 27 %) [Found $(M+Na)^+$ 647.4316. $C_{36}H_{64}O_6SNa$ requires: 647.4321], which showed δ_H (500MHz, $CDCl_3$); 7.79 (2H, d, *J* 8.5 Hz), 7.36 (2H, d, *J* 8.2 Hz), 4.05 (2H, d, *J* 5.1 Hz), 3.92 (1H, q, *J* 5.0 Hz), 3.67 (1H, s), 2.57 (1H, dt, *J* 5.7, 8.8 Hz), 2.46 (3H, s), 2.19 (1H, br.s), 1.70–1.63 (IH, m), 1.53–1.49 (1H, m), 1.26 (44H, m), 0.88 (3H, t, *J* 6.9 Hz); δ_C (125 MHz, $CDCl_3$): 175.4, 145.0, 138.1, 129.8, 128.0, 71.6, 51.9, 47.1, 29.7 – 27.2, 22.7, 14.1; ν_{max} (cm^{-1}): 3556, 2952, 2922, 2853, 22727, 1783, 1728, 1598, 1464, 1456, 1190, 1177, 1697, 973, 163, 842, 818, 720, 676, 667

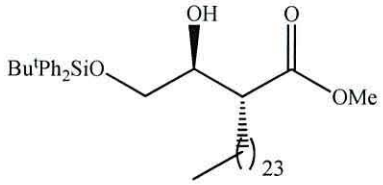
.

Experiment 72: Attempted preparation of methyl (2*R*)-2-((4*S*)-2-(4-methoxyphenyl)-2-methyl-1,3-dioxolan-4-yl)hexacosanoate (143)



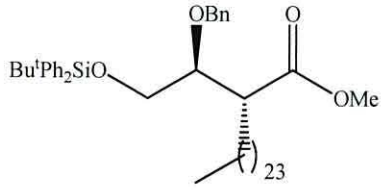
Following an established method,[221] methyl (*R*)-2-((*S*)-1,2-dihydroxyethyl)hexacosanoate (0.1 g, 0.213 mmol.) was dissolved in toluene (20 mL), then 4-methoxybenzaldehyde (0.04 mL, 0.319 mmol.) and p-TsOH (25 mg, 0.131 mol) were stirred into the mixture, which was refluxed at 140 °C for 28 hours, using a Dean-Stark apparatus. The reaction was monitored by TLC to ensure it was complete and then it was quenched with sat. aq. $NaHCO_3$. The aqueous layer was separated and re-extracted with petrol/ethyl acetate (5:1; 3 x50 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated. The TLC of the crude product revealed multiple spots that appeared very close together and the ^1H NMR spectrum was complicated.

Experiment 73: Methyl (*R*)-2-((S)-2-((tert-butyldiphenylsilyl)oxy)-1-hydroxy-ethyl) hexacosanoate (144)



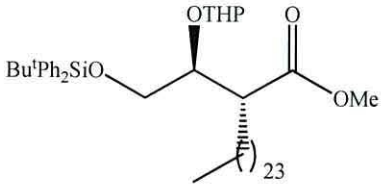
Following protection procedures described in previous Experiments, methyl (*R*)-2-((*S*)-1,2-dihydroxyethyl)hexacosanoate (0.3 g, 0.638 mmol.) was dissolved in dry DMF (5 mL) and imidazole (0.2 g, 2.94 mmol.) was stirred into the solution at room temperature. The stirred mixture was cooled to 0 °C and then *tert*-butyldiphenylchlorosilane (0.23 g, 0.829 mmol.) was added. The cooling bath was removed and the reaction was stirred for 20 hours at 45 °C when TLC indicated that there was no remaining starting material, and then the DMF was removed by flash distillation. The residue was dissolved in water (20 mL) and extracted with dichloromethane (3 x 10 mL). The combined organic layers were washed with water, dried and concentrated. The crude product was purified by column chromatography eluting with petrol/ ethyl acetate (5:2), to give a thick white oil, *methyl (R)-2-((S)-2-((tert-butyldiphenylsilyl)oxy)-1-hydroxyethyl)-hexacosanoate* (0.45 g; 99 %), $[\alpha]_D^{20}$ + 2.44 (c 1.2, $CHCl_3$) [Found $(M+Na)^+$: 731.5405. $C_{45}H_{76}O_4SiNa$ requires: 731.5411], which showed δ_H (500MHz, $CDCl_3$): 7.67 (4H, d, *J* 7.6), 7.47–7.38 (6H, m), 3.81 (1H, q, *J* 5.5 Hz), 3.72–3.63 (5H, m including a singlet resonating at δ 3.67 corresponding to 3 protons for a methoxy group), 2.67–2.65 (1H, m), 1.62 – 1.57 (1H, m), 1.43–1.38 (1H, m), 1.27 (45H, m including the signal for 1 proton associated with the–OH group), 1.09 (9H, s), 0.81 (3H, t, *J* 7.0 Hz); δ_C (125 MHz, $CDCl_3$): 174.8, 135.5, 135.6, 134.8, 129.8, 129.6, 127.8, 72.7, 65.8, 51.6, 48.0, 31.9, 29.7, 29.3, 27.4, 26.8,26.5, 22.7, 19.2, 19.0, 14.1; ν_{max} (cm^{-1}): 3449, 3072, 3050, 3015, 2999, 1958, 1889, 1822, 1718, 1590, 1465, 1428, 1391, 1375, 1362, 1261, 1196, 1170, 1114, 1008, 999, 939, 855, 822, 759, 740, 702, 607.

Experiment 74: Attempted preparation of methyl (*R*)-2-((*S*)-2-(benzyloxy)-2-(hydroxy) ethyl) hexacosanoate (145)



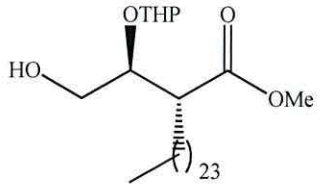
As described in the literature,[222] sodium hydride (60 % dispersed in oil) (0.075 1.86 mmol.) was placed in a dry flask and washed with petrol (2.0 ml x 3) to remove the oil. The petrol was vacuumed off and dry THF (10 mL) was added. The suspension was cooled to –10 °C under nitrogen, then methyl (*R*)-2-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)-1-hydroxyethyl) hexacosanoate (0.44 g, 0.621 mmol.) in dry THF (5.0 mL) was added dropwise, and the mixture was stirred for 30 minutes, maintaining the temperature at –10 °C. Benzyl bromide (0.3 mL, 2.54 mmol.) and tetrabutylammonium iodide (100 mg) were added; 10 minutes later, the cooling bath was removed, and the reaction was stirred overnight at room temperature. Subsequently it was cooled and quenched with sat. aq. ammonium chloride (10 mL) and extracted with ethyl acetate (20 mL x3). The combined organic layers were dried over $MgSO_4$ and concentrated. The TLC of the crude product revealed multiple spots and a complicated ^{1}H NMR spectrum was obtained.

Experiment 75: Methyl (2*R*)-2-((1*S*)-2-((tert-butyldiphenylsilyl)oxy)-1-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)hexacosanoate (146)



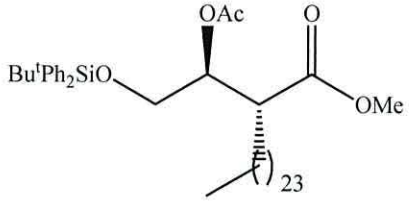
Using standard procedures,[223] methyl (*R*)-2-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)-1-hydroxyethyl)hexacosanoate (0.44 g, 0.621 mmol.) was dissolved in dry dichloromethane (5 mL) at room temperature under nitrogen and then 3,4-dihydro-2*H*-pyran (0.312 g, 3.71 mmol.) and pyridinium-*p*-toluene sulfonate (0.1 g, 0.398 mmol.) were added. The reaction was stirred at room temperature for 3 hours, after which it was filtered through a bed of celite, washing thoroughly with dichloromethane. The excess solvent was evaporated and column chromatography eluting with petrol/ ethyl acetate (10:1) gave a clear oil, *methyl (2R)-2-((1S)-2-((tert-butyldiphenylsilyl)oxy)-1-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)hexacosanoate* (0.49 g, 99 %), $[\alpha]_D^{25}$ – 14.4 (c 0.7, $CHCl_3$) [Found $(M+Na)^+$ 815.5980. $C_{50}H_{84}O_5SiNa$ requires: 815.5986], which showed δ_H (500MHz, $CDCl_3$): 7.71–7.68 (8H, m), 7.46–7.28 (12H, m), 4.98 (2H, t, *J* 3.5 Hz), 4.78 (1H, t, *J* 3.2 Hz), 4.64 (1H, t, *J* 3.3Hz), 4.16–4.09 (2H, m), 3.94–3.91 (1H, m), 3.90–3.80 (7H, m), 3.74–3.67 (12H, m including 2 singlets resonating at δ 3.68 and δ 3.70 respectively, corresponding to 6 proton of two methoxy groups), 3.52–3.46 (3H, m), 3.32–3.28 (1H, m), 3.01–2.98 (1H, m), 2.90–2.86 (1H, m), 200–1.93 (2H, m), 1.83–173 (2H, m), 1.71–1.28 (88H, m), 1.10 (9H, s), 1.08 (9H, s), 0.91 (3H, t, *J* 6.8 Hz); δ_C (125 MHz, $CDCl_3$): 174.8, 135.6, 129.6, 127.5, 100.2, 96.1, 94.0, 64.8, 62.6, 62.4, 62.1, 61.8, 51.3, 48.1, 33.0, 31.9, 30.9, 29.7, 29.4, 26.8, 25.5, 22.7, 19.4, 19.4, 19.0, 14.1; ν_{max} (cm^{-1}): 3450, 3071, 3050, 2919, 2851, 1737, 1590, 1465, 1428, 1362, 1259, 1201, 1162, 1113, 1077, 1021, 989, 932, 872, 822, 740, 702, 612.

Experiment 76: Attempted preparation of methyl (2*R*)-2-((1*S*)-2-hydroxy-1-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)hexacosanoate (147)



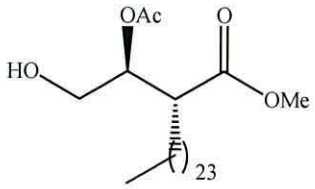
Following an established method,[177] tetrabutylammonium fluoride (0.23 mL, 0.804 mmol.) was added to a stirred solution of methyl (*R*)-2-((1*S*)-2-((*tert*-butyldiphenylsilyl)oxy)-1-((tetrahydro-2H-pyran-2-yl)oxy)ethyl)-hexacosanoate (0.49 g, 0.618 mmol.) in dry THF (5.0 mL) at 0 °C, under nitrogen. The reaction was stirred for 18 hours, during which it was monitored by TLC. When TLC showed the reaction was complete, the mixture was diluted with petrol/ethyl acetate (1:1), cooled to 5 °C and quenched with sat. aq. ammonium hydroxide. The aqueous layer was separated and re-extracted with more petrol/ethyl acetate (1:1, 3 x 30 mL). The combined organic layer was washed with brine, separated and dried over $MgSO_4$. Then the solvent was evaporated to give the crude product. The TLC showed two main spots, but the ^1H NMR spectrum showed no signals corresponding to the OTHP group.

Experiment 77: Methyl (*R*)-2-((*S*)-1-acetoxy-2-((*tert*-butyldiphenylsilyl)oxy)-ethyl) hexacosanoate (148)



Using standard procedures,[163] methyl (*R*)-2-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)-1-hydroxyethyl)hexacosanoate (0.5 g, 0.71 mmol.) was dissolved in dry THF (0.2mL) at room temperature under nitrogen, followed by the addition of pyridine (5 mL) and acetic anhydride (5 mL). The reaction was stirred overnight after which it was evaporated. The residue was neutralized with dilute acid (10 ml) and extracted with dichloromethane (20 mL x 3). The combined organic layers were dried over $MgSO_4$ and concentrated. Then the crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a clear thick liquid, *methyl (R)-2-((S)-1-acetoxy-2-((tert-butyldiphenylsilyl)oxy)ethyl)hexacosanoate* (0.32 g, 61 %), $[\alpha]_D^{23}$ – 7.1 (c 2.1, $CHCl_3$) [Found $(M+Na)^+$ 773.5511. $C_{47}H_{78}O_5SiNa$ requires: 773.5516], which showed δ_H (500MHz, $CDCl_3$): 7.73 (4H, dd, *J* 1.3, 7.6 Hz), 7.44–7.37 (6H, m), (1H, broad pent, *J* 4.0 Hz), 3.80 (2H, ddd, *J* 3.5, 11.7, 18.6 Hz), 3.66 (3H, s), 296–3.00 (IH, m), 1.99 (3H, s), 1.25 (46H, m), 1.06 (9H, s), 0.89 (3H, t, *J* 7.0 Hz); δ_C (125 MHz, $CDCl_3$): 135.6, 134.8, 74.6, 62.9, 51.6, 46.3, 31.9, 29.7, 28.3, 27.3, 22.7, 20.9, 19.3,14.0; ν_{max} (cm^{-1}): 3524, 2925, 2855, 1746, 1464, 1426, 1372, 1235, 1114, 823, 740, 702, 607.

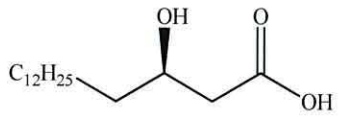
Experiment 78: Attempted preparation of methyl (2*R*)-2-((1*S*)-1-acetoxy-2-(hydroxyl)ethyl)hexacosanoate (149)



As in **Experiment 76**, tetrabutylammonium flouride (0.15 mL, 0.520 mmol.) was added to a stirred solution of methyl (*R*)-2-((*S*)-1-acetoxy-2-((*tert*-butyldiphenylsilyl)oxy)ethyl)-hexacosanoate (0.3 g, 0.40 mmol.) in dry THF (2.0 mL) at 0 °C, under nitrogen. The reaction was stirred for 18 hours at room temperature during which it was monitored by TLC. When TLC showed that the reaction was complete, it was diluted with petrol/ethyl acetate (1:1), cooled to 5 °C and quenched with sat. aq. ammonium hydroxide. The aqueous layer was separated and re-extracted with more petrol/ethyl acetate (1:1, 3 x 30 mL). The combined organic layer was washed with brine, separated and dried over $MgSO_4$ and solvent evaporated to give the crude product which showed complicated NMR spectra, which did not correspond to either starting material or the expected product. The TLC showed one spot.

6.3 Synthesis of cord factors

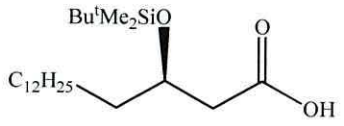
Experiment 79: (*R*)-3-hydroxyhexadecanoic acid (150)



As described in **Experiment 44**, ethanol (1.5 mL), water (1.5 mL), and then lithium hydroxide monohydrate (0.88 g, 2.1 mmol.) were stirred into a solution of ethyl (*R*)-3-hydroxyhexadecanoate (2.1 g, 6.99 mmol.) in THF (20 mL) at room temperature and then refluxed at 45 °C for 18 hours. The reaction was cooled to room temperature, diluted with petrol/ethyl acetate (5:1) and acidified to pH 1 by dropwise addition of dilute H_2SO_4. The aqueous layer was separated and extracted with petrol/ethyl acetate (5:1; 50 mL x 3), dried over $MgSO_4$ and evaporated. The crude product was recrystallized with petrol to obtain a white solid, *(R)-3-hydroxyhexadecanoic acid* (1.6 g, 89 %), m.p. 51 °C, $[\alpha]_D^{22}$ + 14.9 (c 1.1, $CHCl_3$) [Found $(M+Na)^+$ 295.2244. $C_{16}H_{32}O_3Na$ requires: 295.2249], which showed δ_H (500MHz, $CDCl_3$): 4.07–4.02 (1H, m), 2.59 (1H, dd, *J* 3.15, 16.7 Hz), 2.49 (1H, dd. *J* 9.1, 16.7 Hz), 1.59–154 (1H, m), 1.50–143 (2H, m), 1.27 (23H, m), 0.89 (3H, t, *J* 6.6 Hz); δ_C (125 MHz, $CDCl_3$): 177.3, 68.0, 41.0, 36.5, 31.9, 29.6, 25.4, 22.7, 14.1; ν_{max} (cm^{-1}): 3391, 2925, 2855, 1739, 1679, 1456, 1376, 1243, 1095, 1051, 918, 881, 737, 699

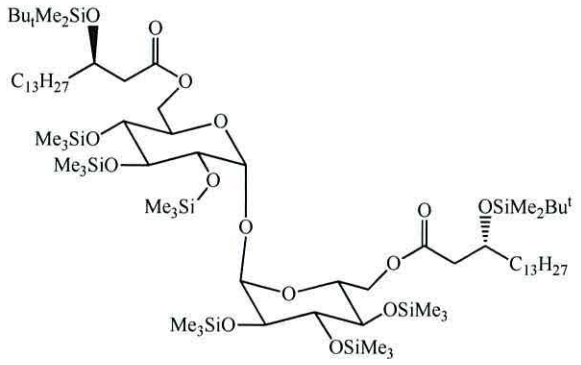
.

Experimrnt 80: **(*R*)-3-((*tert*-butyldimethylsilyl)oxy)hexadecanoic acid (151)**[216]



Following standard protection methods[169] (*R*)-3-hydroxyhexadecanoic acid (1.5 g, 5.51 mmol.) was dissolved in dry DMF (15 mL) and dry toluene (10 mL) at room temperature. Then imidazole (1.88 g, 27.6 mmol.), *tert*- butyldimethylsilychloride (4.2 g, 27.6 mmol.) and 4-methylaminopyridine (0.35 g, 2.87 mmol.) were added one after the other. The reaction was stirred at 70℃ for 24 hours and then at room temperature for another 18 hours, after which TLC showed that there was no starting material. The solvent was evaporated under high vacuum and the residue was dissolved in water (50 mL) and extracted with petrol/ethyl acetate (5:1; 50 mL x 3).The combined organic layers were washed with water (50 mL), dried over $MgSO_4$ and concentrated. The residue was dissolved in THF (50 mL), water (10 mL) and methanol (10 mL), and then potassium carbonate (1.5 g, 10.9 mmol.) was added to the mixture which was refluxed at 45℃. This was monitiored by TLC which showed that no starting material was left after 18 hours. The reaction was again diluted with petrol/ethyl acetate (5:1, 50 mL) and water (10 mL) and then acidified to pH 2 by the addition of sat. aq. potassium hydrogen carbonate. The aqueous layer was separated and re-extracted with petrol/ethyl acetate (5:1; 3 x 50 mL). The combined organic layers were washed with water, dried and evaporated to give the crude product. Column chromatography eluting with petrol/ethyl acetate (5:1) gave a clear liquid, pure *(R)-3-((tert-butyldimethylsilyl)-oxy) hexadecanoic acid* (1.9 g, 89 %), $[\alpha]_D^{22}$ – 13.9 (c 1.7, $CHCl_3$) (lit.value[216] $[\alpha]_D^{20}$ –16.0 (c 1.0, $CHCl_3$), which showed δ_H, δ_C and ν_{max} identical to the literature[216]

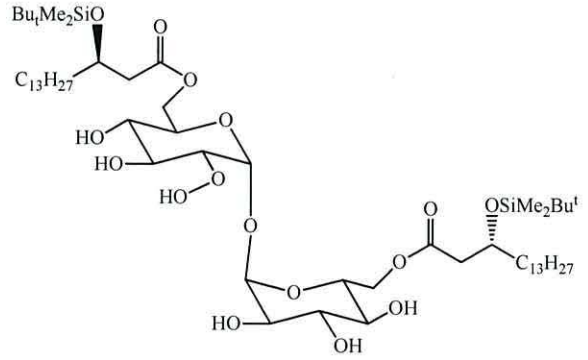
Experiment 81: (3*R*,3'*R*)-((2*R*,2'*R*,3*R*,3'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-6,6'-oxybis-(3,4,5-tris((trimethylsilyl)oxy)tetrahydro-2H-pyran-6,2-diyl))bis(methylene)bis (3-((*tert*-butyldimethylsilyl)oxy)hexadecanoate) (153)



153

Following established methods,[169, 184] (*R*)-3-((*tert*-butyldimethylsilyl)oxy)hexadecanoic acid (0.34 g, 0.880 mmol.) and 2,3,4,2',3',4'-hexakis-O-(trimethylsilyl)-α,α'-trehalose (0.27 g, 0.352 mmol.) were dissolved in dry dichloromethane (2.0 mL) at room temperature, under nitrogen. 4 Å molecular sieve (0.2g) was added to the stirred solution, followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodimidehydrochloride (0.27 g, 1.41 mmol.) and 4-dimethylaminopyridine (0.14 g, 1.23 mmol.). The reaction was stirred at room temperature for 6 days, after which TLC showed that no starting material remained. It was diluted with dichloromethane, filtered and evaporated under reduced pressure to obtain a crude product. This was purified by column chromatography eluting with petrol/ethyl acetate ((20:1) to give a thick clear oil, *(3R,3'R)-((2R,2'R,3R,3'R,4S,4'S,5R,5'R,6R,6'R)-6,6'-oxybis(3,4,5-tris((trimethylsilyl)oxy)tetrahydro-2H-pyran-6,2-diyl))bis(methylene)bis (3-((tert-butyl dimethylsilyl)oxy)hexadecanoate)* (0.42 g, 78 %), $[\alpha]_D^{22}$ + 53.7 (c 0.74, $CHCl_3$) [Found $(M+Na)^+$ 1534.4. $C_{74}H_{158}O_{15}Si_8Na$ requires: 1534.0], which showed δ_H (500MHz, $CDCl_3$): 4.9 (1H, d, *J* 2.9 Hz), 4.27 (1H, dd, *J* 2.2, 12.0 Hz), 4.17–4.11 (1H, m), 4.07 (1H, dd, *J* 4.7, 11.7 Hz), 4.02–3.99 (1H, m), 3.91 (1H, t, *J* 9.0 Hz,), 3.48 (1H, d, *J* 8.8 Hz), 3.44 (1H, dd, *J* 3.2, 9.2 Hz), 2.53 (1H, dd, *J* 6.9, 15.5 Hz), 2.44 (1H, dd, *J* 5.7, 15.2 Hz), 1.26 (22H, m), 1.47 (2H, m), 0.88 (3H, t, *J* 7.3 Hz), 0.87 (9H, s), 0.16 (9H, s), 0.14 (9H, s), 0.13 (9H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C (125 MHz, $CDCl_3$): 171.9, 94.4, 73.5, 72.7, 72.0, 70.7, 69.3, 63.5, 42.5, 37.6, 31.9, 29.7, 25.8, 25.0, 22.7, 18.0, 14.1, 0.90, 0.17, –4.8; ν_{max} (cm^{-1}): 2957, 2927, 2856, 1743, 1463, 1378, 1312, 1252, 1167, 1112, 1100, 1078, 1010, 966, 937, 899, 873, 840, 776, 749, 683.

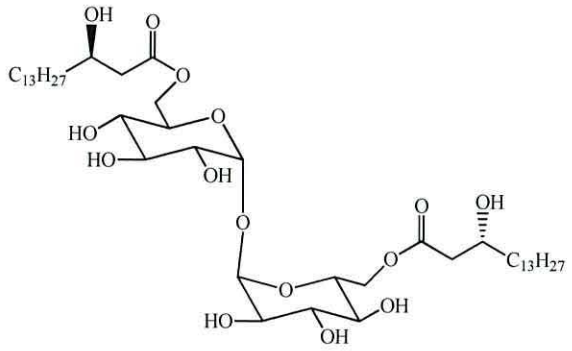
Experiment 82: (3*R*,3'*R*)-((2*R*,2'*R*,3*S*,3'*S*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-6,6'-oxybis(3,4,5-trihydroxytetrahydro-2H-pyran-6,2-diyl))bis(methylene)bis(3-((*tert*-butyl-dimethylsilyl)oxy)hexadecanoate) (155)



155

Using known procedures,[169] **153** (0.20 g, 0.132 mmol.) was dissolved in dry THF (10 mL), cooled to 5 °C and then tetrabutylammonium fluoride (0.4 mL.0.397 mmol.) in THF (5.0 mL) was added dropwise, still maintaining the temperature at 5 °C. The cooling bath was removed and the reaction was stirred at room temperature for approximately 1 hour, after which TLC showed that there was no starting material. The reaction was cooled again to 5 °C, quenched with sat. aq. sodium bicarbonate (10 mL) and diluted with chloroform (50 mL). The aqueous layer was separated and re-extracted with chloroform (50 mL x 3). The combined organic layers were dried and concentrated to obtain the crude product which was purified by column chromatography, eluting with chloroform/methanol (10:1) to give a semi-solid pure ***155*** (0.084 g, 59 %), $[\alpha]_D^{23}$ + 39.3 (c 0.29, $CHCl_3$) [Found $(M+Na)^+$ 1101.5. $C_{56}H_{110}O_{15}Si_2Na$ requires: 1101.7], which showed δ_H (500MHz, $CDCl_3$): 5.11 (1H, d, *J* 3.5 Hz), 4.36–4.30 (2H, m), 4.12 (1H, br.pent, *J* 5.9 Hz), 4.02–3.95 (2H, m), 3.62 (1H, dd, *J* 2.2, 11.1 Hz), 3.38 (1H, t, *J* 9.2 Hz), 2.49 (2H, t, *J* 6.6 Hz), 1.27 (22H, m), 1.48 (2H, m), 0.89 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.06 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 172.5, 93.4, 73.1, 71,7, 70.4, 69.4, 63.1, 60.0, 42.5, 37.6, 29.7, 25.8, 25.0, 22.7, 18.0, 14.1, –4.7; ν_{max} (cm^{-1}): 3413, 2927, 2856, 1738, 1639, 1464, 1377, 1255, 1150, 1079, 989, 836, 809, 776.

Experiment 83: (3*R*,3'*R*)-((2*R*,2'*R*,3*S*,3'*S*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-6,6'-oxybis(3,4,5-trihydroxytetrahydro-2H-pyran-6,2-diyl))bis(methylene)bis(3-hydroxyhexadecanoate) (79a)



79a

Following established methods,[169] **155** (0.08 g, 0.074 mmol.) was dissolved in dry THF (7.0 mL), placed in a well dried polyethylene vial under nitrogen at room temperature. Pyridine (0.1 mL) and HF pyridine (0.6 mL) were added, and the mixture was stirred for 17 hours at 40 °C. TLC was carried out severally to determine the end of the reaction. Then it was neutralized by careful addition of sat. aq. sodium bicarbonate, until the liberation of carbon dioxide subsided. The mixture was extracted with chloroform (3 x 50 mL) and the combined organic layers were washed with water, dried over $MgSO_4$ and concentrated to give the crude product. Column chromatography eluting with chloroform/methanol (10:1) gave a semi-solid ***79a*** (0.035 g, 56 %), $[\alpha]_D^{22}$ + 47.0 (c 1.2, $CHCl_3$) [Found $(M+Na)^+$ 873.1. $C_{44}H_{82}O_{15}Na$ requires: 873.6], which showed δ_H (500MHz, $CDCl_3$): 5.04 (1H, br.s), 4.58 (1H, br.d, *J* 7.9 Hz), 4.09–4.05 (3H, m), 3.87 (1H, broad pent, *J* 9.2 Hz), 3.54 (1H, m), 3.31 (1H, m), 2.50 (1H, d, *J* 15.4 Hz), 2.38 (1H, dd, *J* 10.1, 15.4 Hz), 1.48 (1H, br.s), 1.39 (2H, m), 1.23 (22H, m), 0.85 (3H, t, *J* 6.8 Hz); δ_C (125 MHz, $CDCl_3$): 172.7, 93.9, 73.1, 71.3, 70.7, 69.9, 68.3, 64.0, 41.6, 37.0, 31.9, 29.7, 29.4, 25.6, 22.7, 14.1 ν_{max} (cm^{-1}): 3394, 2918, 2850, 1725,1467, 1078.

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Appendix

Data sheet for biological activities from ELISA assays

		77b	77a	42a	42b	79a	Natural human TDM (from M.tb)
		IgG (whole molecule)	IgG (whole molecule)	IgG (whole molecule)	IgG (whole molecule)	IgG (Fc specific)	IgG (whole molecule)
8-01273	TB+	0.44	1.32	0.52	0.67	1.90	3.50
8-01201	TB+	0.59	1.01	0.81	0.87	0.50	3.01
8-01214	TB+	0.50	1.67	0.58	0.74	3.02	3.85
12-02151	TB+	0.43	0.71	0.50	0.65	1.94	3.36
12-02247	TB+	0.43	1.88	0.67	0.70	3.49	2.93
3-02371	TB+	0.46	2.04	0.72	0.71	1.84	3.60
11-03719	TB+	0.64	0.64	0.73	0.75	0.36	3.33
11-01917	TB+	0.37	0.46	0.44	0.43	0.42	1.81
1-00317	TB+	1.56	3.58	2.46	1.52	4.41	3.09
1-00370	TB+	0.85	1.03	0.65	0.62	1.30	3.61
6-00962	TB+	0.68	1.24	0.70	0.77	1.74	3.11
9-01426	TB+	0.42	2.19	0.72	0.59	3.79	3.01
9-01549	TB+	0.69	0.89	0.46	0.60	0.70	1.86
9-01559	TB+	2.13	2.13	0.68	0.66	1.83	3.29
12-02103	TB-	0.41	0.64	0.72	0.96	0.45	1.56
3-02420	TB-	0.40	0.85	0.58	0.64	0.56	2.20
3-02492	TB-	0.31	0.62	0.54	0.58	0.47	0.74
10-01703	TB-	0.38	0.71	0.58	0.67	0.43	1.51
10-01714	TB-	0.43		0.82	0.61		0.81
10-01641	TB-	0.43	1.01	0.58	0.51	0.91	1.40
10-01744	TB-	0.32	0.96	0.38	0.47	0.70	2.91
10-01695	TB-	0.47	0.81	0.65	0.74	0.30	1.03
10-01785	TB-	0.42	0.99	0.64	0.93	1.32	1.68
10-01675	TB-	0.30	0.85	0.56	0.65	0.43	2.45
10-01807	TB-	0.38	0.97	0.52	0.50	1.21	2.96
10-01829	TB-	0.31	0.46	0.25	0.35	0.24	0.81
10-01689	TB-	0.48	0.69	0.55	0.51	0.53	0.96
10-01842	TB-	0.35	0.41	0.35	0.36	0.45	0.92
1-00335	TB-	1.03	1.58	0.99	1.00	0.37	1.96
1-00354	TB-	0.74	0.89	0.69	0.77	0.41	1.93
1-00380	TB-	0.56	0.77	0.60	0.61	0.44	1.85
1-00394	TB-	1.34	1.47	0.93	0.44	0.74	3.83
1-00467	TB-	0.49	0.92	0.66	0.67	0.63	2.20
1-00473	TB-	0.52	1.08	0.56	0.45	0.90	2.76
1-00481	TB-	1.92	1.64	1.31	0.68	1.27	2.81
1-00494	TB-	0.32	1.35	0.67	0.46	0.90	3.12
4-00423	TB-	0.49	0.94	0.51	0.58	0.49	2.47
5-00516	TB-	0.65	1.14	0.70	0.73	2.73	2.84
5-00538	TB-	0.33	0.61	0.39	0.38	0.27	0.92
5-00545	TB-	0.33	0.70	0.52	0.46	0.17	0.99
5-00552	TB-	0.26	0.58	0.49	0.44	0.22	1.09
5-00560	TB-	0.43	1.17	0.50	0.57	0.41	1.35
6-01016	TB-	0.26	0.41	0.32	0.34	0.19	0.51
6-01023	TB-	0.50	1.11	0.69	2.14	1.76	2.70
6-01029	TB-	0.28	0.49	0.33	0.38	0.44	1.25
6-01035	TB-	0.31	0.52	0.31	0.37	0.29	0.98
6-01042	TB-	0.26	0.86	0.45	0.38	0.25	0.99
6-01074	TB-	0.31	0.68	0.34	0.40	0.28	1.05
Averages	TB+	0.73	1.49	0.76	0.73	1.94	3.10
	TB-	0.49	0.87	0.58	0.61	0.64	1.75
	Selectivity	14	43	14	29	71	86
	Specificity	91	94	88	85	88	88