DOCTOR OF PHILOSOPHY

The synthesis of mycolic acids

Schubert-Rowles, Richard

Award date:
2010

Awarding institution:
Bangor University

The Synthesis of Mycolic Acids

A thesis presented for the degree of

Doctor of Philosophy

in the

School of Chemistry

by

Richard Schubert-Rowles

PRIFYSGOL
BANGOR
UNIVERSITY

Prifysgol Bangor • Bangor University

This thesis contains confidential information

Contents

Acknowledgements

Firstly, I would like to thank Professor Mark Baird for giving me the opportunity to carry out a PhD under his supervision. I would also like to thank Dr Juma'a Al-Dulayymi for his guidance and teachings in the laboratory.

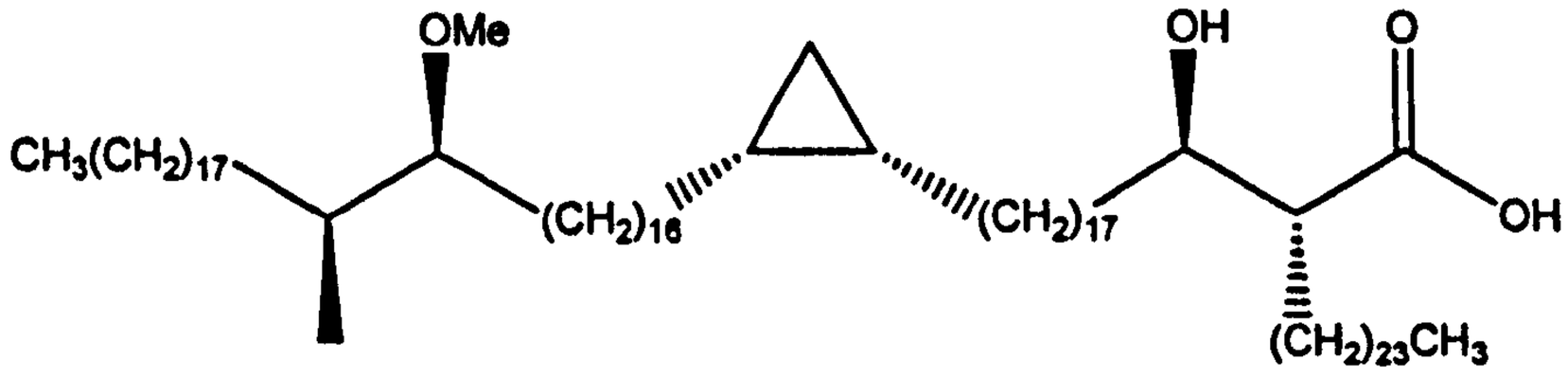
Secondly I would like to thank all the members of the Prof Baird research group, past and present, for their help and "the laughs". In addition I would like to thank all the members of secretarial and technical staff at the School of Chemistry, Bangor. Also thanks go to members of the 7th floor Biocomposites Centre, particularly Slava and Gwynn.

Thirdly, I would also like to thank members of the Professor Johan Grooten research group at Ghent University, Belgium and the Professor Jan Verschoor research group at the University of Pretoria, South Africa, for the testing of samples.

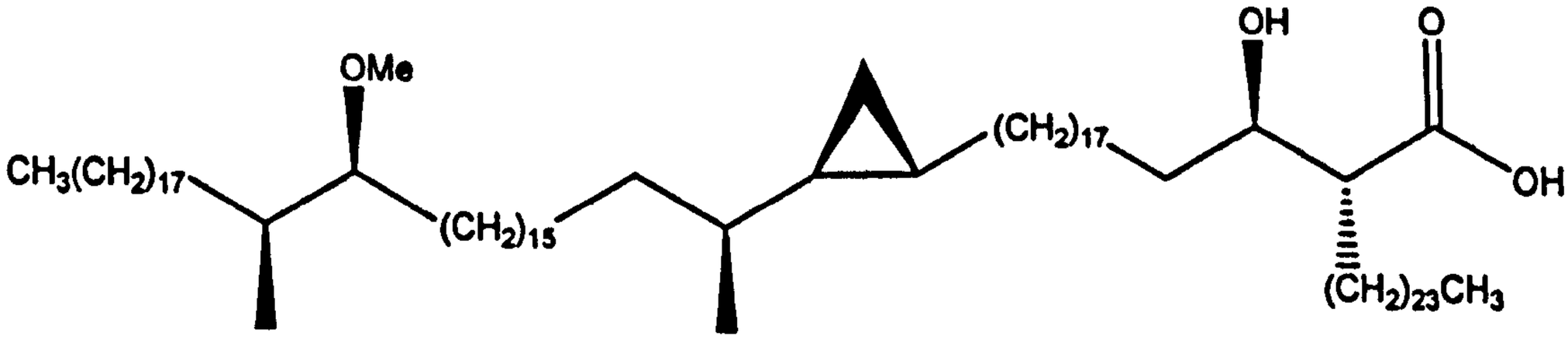
Finally, I would also like to thank every member of my family. Special thanks go to my wife, Heidi, for her support and encouragement.

Abstract

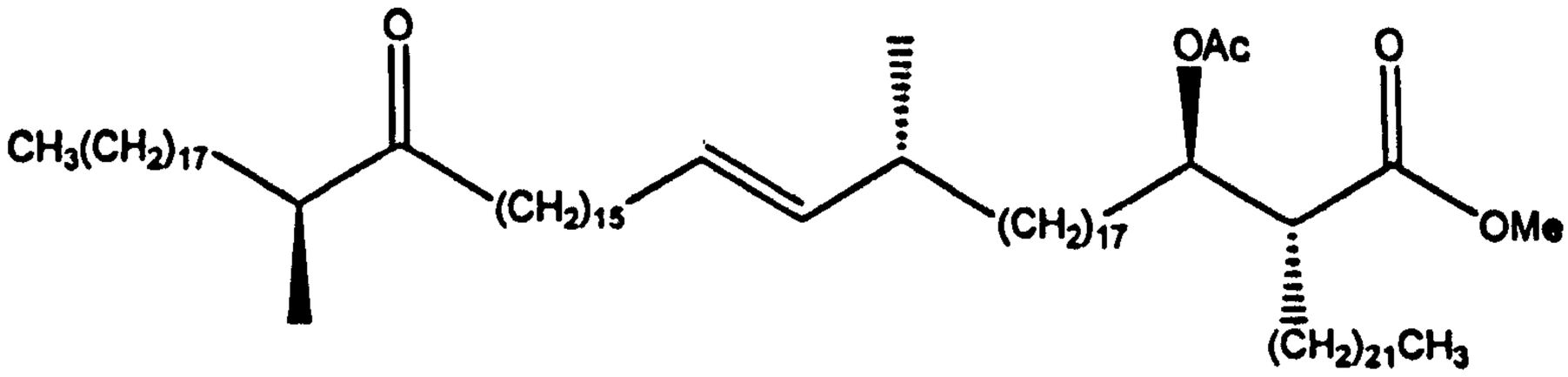
The total synthesis of six naturally occuring enantiomerically pure oxygen containing mycolic acids was achieved. The specific mycolic acids synthesized were *cis*-cyclopropane methoxy-mycolic acid **(I)** and *α*-methyl *trans*-cyclopropane methoxy-mycolic acid **(II)** of *Mycobacterium tuberculosis*; and the protected *S*-*α*-methyl *trans*-alkene keto-mycolic acid **(III)**, *R*-*α*-methyl *trans*-alkene keto-mycolic acid **(IV)**, *R,R* *α*-methyl *trans*-alkene hydroxy-mycolic acid **(V)** and *S,S* *α*-methyl *trans*-alkene hydroxy-mycolic acid **(VI)** of *Mycobacterium marinum*. Novel routes in the preparation of the first synthetic *α*-methyl *trans*-alkene mycolic acids are discussed, utilising methods such as the modified Julia-Kocienski olefination, the Horner-Wadsworth-Emmons reaction and the Michael addition.



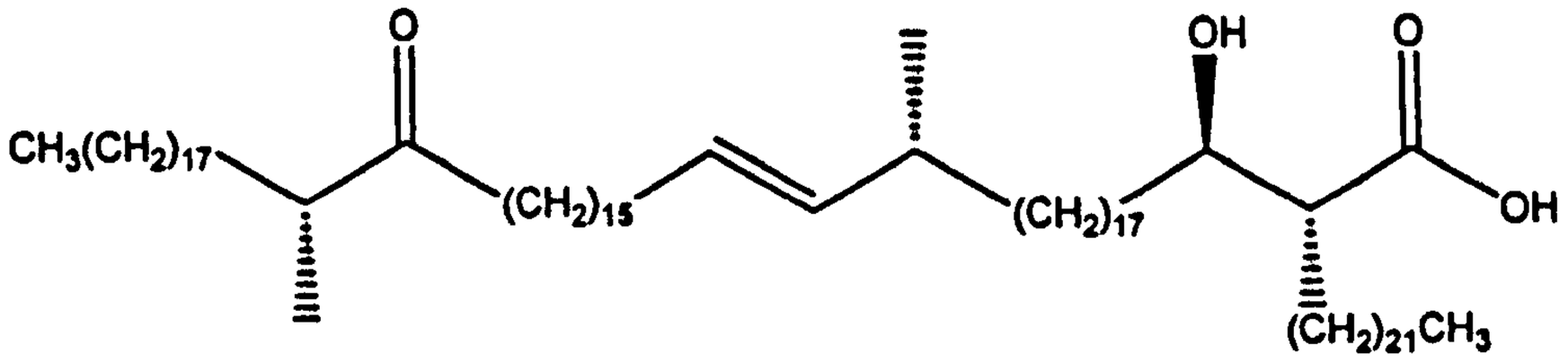
cis-cyclopropane methoxymycolic acid **(I)**



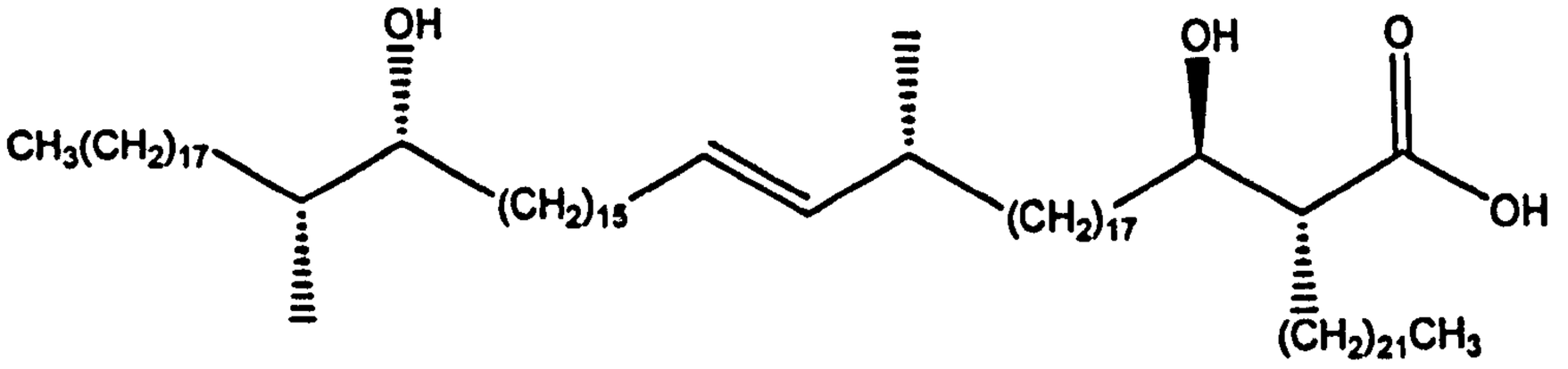
α-methyl *trans*-cyclopropane methoxymycolic acid **(II)**



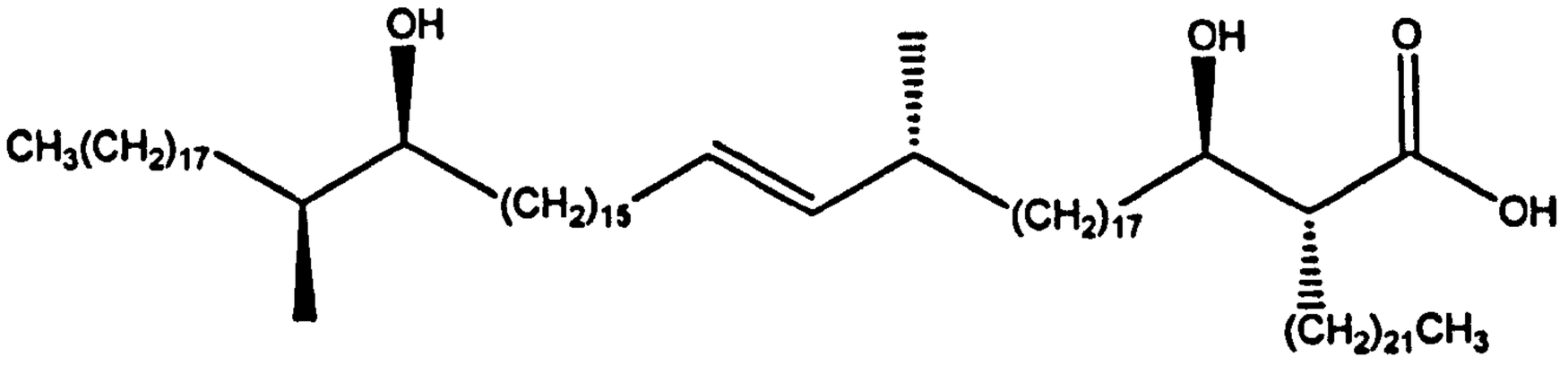
Protected *S*-α-methyl *trans*-alkene keto-mycolic acid **(III)**



R-α-methyl *trans*-alkene keto-mycolic acid **(IV)**



R,R α-methyl *trans*-alkene hydroxy-mycolic acid **(V)**



S,S α-methyl *trans*-alkene hydroxy-mycolic acid **(VI)**

Abbreviations

°C - degrees celsius

aq. – aqueous

b – broad

BAL - broncho-alveolar lavage

BCG - Bacillus Calmette-Guérin

CID-MS - collision-induced dissociation mass spectrometry

d – doublet

d.p. – decimal places

dil. – dilute

DMAP – 4-dimethylaminopyridine

DMF – dimethylformamide

E – entgegen

ELISA - enzyme-linked immunosorbent assay

ether - diethyl ether

GC – gas chromatography

HIV – human immunodeficiency virus

HPLC – high performance liquid chromatography

hrs – hours

Hz - hertz

IMS – industrial methylated spirits

IR – infra-red

J - coupling constant

m - meta-

m - multiplet

M. Marinum – Mycobacterium marinum

m.p. - melting point

M.Tb – Mycobacterium tuberculosis

MALDI - Matrix-assisted laser desorption/ionization

m-CPBA - *m*-chloroperoxybenzoic acid

min – minute

MIRC – Michael Induced Ring Cyclisation

mmol - millimols

mol. equiv. – molecular equivalents

MS – mass spectrometry

NBS - *N*-bromosuccinimide

NMR – nuclear magnetic resonance

PCC - pyridinium chlorochromate

Petrol - petroleum spirit (boiling point 40 to 60°C)

ppm - parts per million

PPTS - pyridinium *p*-toluenesulfonate

PTSA - *p*-toluenesulfonic acid monohydrate

q - quartet

R – rectus

RT – room temperature

s - singlet

S – sinister

sat. – saturated

t - triplet

TB – tuberculosis

TDM – trehalose dimycolate

THF – tetrahydrofuran

THP - tetrahydropyran

TLC – thin layer chromatography

TPBSH - 2,4,6-tri-isopropylbenzenesulphonyl hydrazide

UNICEF - United Nations Children's Fund (Previously United Nations International Children's Emergency Fund)

v.br. - very broad

WHO – world health organisation

Z – zusammen

Chapter 1 - Introduction

1.1 – Tuberculosis

1.1.1 - History

Tuberculosis (TB) is a disease which kills around 3 million people annually, and is thought to have killed more people than any other microbial pathogen.[1, 2] TB is caused by *Mycobacterium tuberculosis* (*M.Tb*), also there are variants originating from Africa including *Mycobacterium africanium*, *Mycobacterium canettii* and *Mycobacterium bovis*, which are all thought to have an ancestral link.[3-5] The genus *Mycobacterium* is estimated at being more than one hundred and fifty million years old and *M.Tb,* three million years old.[3, 6]

It is believed that TB first infected hominoids in East Africa, but there is very little archaeological evidence from this period. Early Africa hominoids began to leave Africa around 1.7 million years ago and it is thought that they took their diseases, including TB, with them. Analysis of Egyptian mummies more than 5000 years old shows evidence of the disease and, in early Egyptian art, the depiction of deformities in figures as a result of contracting TB also supports this theory.[7] There is evidence which shows the presence of TB in India 3300 years ago and China 2300 years ago.[8, 9] It is thought that TB was established in the Americas before the arrival of European explorers, with similar evidence to that found in Egypt.[10, 11]

TB was also well documented in ancient Greece, where it was called phthisis and a treatment was devised by physician Clarissimus Galen of fresh air, milk and sea voyages.[2] Throughout the middle ages there is widespread archaeological evidence of TB.[11] TB was responsible for many deaths throughout the ages and it was not until 1819 when a group of French physicians, the most notable being Rene Theophile Hyacinthe Laennec who wrote *D'Ausculation Mediate,* outlined the physical signs of TB and its pathology.[12] During Laennec's era death rates in many major European and American cities had reached 800-1000 in 100,000 deaths per year.[13, 14]

Over the next 50 years there were no advances in the knowledge of TB, until 1882 when Hermann Heinrich and Robert Koch gave their famous presentation on the tubercle bacillus and on their work to postulate the link between a microbe and disease. More commonly known as the Koch-Henle postulates, to this day they are

still the standard explanation for how an infectious disease arises.[15] Koch was awarded the 1905 Nobel Prize in Medicine and Physiology for his work with TB. In 1890 Koch isolated a substance from tubercle bacilli which rendered the bacteria harmless. He named it tuberculin and in a demonstration he injected himself with tuberculin. He developed a fever and his body temperature was recorded at 39.6 °C, though he never developed TB.[16] After several years of research it was concluded that a positive reaction to tuberculin was caused by latent TB. Over the next 50 years more extensive research into tuberculin and latent TB showed that it was common for a large number of the population to be carriers of latent TB.[17, 18] Since Koch's work a reliable vaccine has never been developed because TB immunity differs from immunity to many more common microbial diseases. Artificial TB immunity is regarded as passive immunity, where it is acquired either via the transfer of antibodies or activated T-cells, with the affects lasting only a few months, whereas the counter is active immunity, which can be life-long immunity.

It was not until 1921 that Albert Calmette and Camelle Guérin developed a vaccine from *Mycobacterium bovis* called Bacillus Calmette-Guérin (BCG).[19] Acceptance of the vaccine was slow as many people did not believe it to be safe as it was based on live TB bacteria. The use of the vaccine took a significant blow in 1930, when there was a case in Lübeck, Germany where 240 infants under 10 days old were vaccinated and almost all developed TB, with 76 recorded deaths.[20-22] It was later discovered that the BCG sample used had been contaminated with a virulent strain, however this dealt massive damage to the acceptance of the vaccine and legal action was taken against the developers.[23] In the 1940's BCG had become more widespread being used in Scandinavia, France, Spain, Russia, Latin America and some Eastern European countries.[24, 25] Swedish Professor Arvid Wallgren was one of the biggest supporters of the vaccine, and argued the case for the vaccine's use in Germany. After the incidents in Lübeck, the BCG vaccine had been banned in Germany and Wallgren highlighted the fact that prior to the tragedy regular vaccinations of infants in Gothenburg had reduced the mortality rate from 4 in 1000 to 1 in 1000.[26] However, in spite of the efforts of Wallgren and others, the vaccine was not accepted in Britain and the United States, with the Director of the Public Health Laboratory Service, Sir Graham Wilson, pointing out flaws in the evidence backing BCG in 1947.[27] In 1950 the opinion remained in the US that BCG was only needed in highly exposed or war-torn countries and as an emergency measure.[21] The

decision whether or not to use to BCG vaccination was dependent upon how the evidence was interpreted as the data was insufficiently clear to fully accept or reject the vaccine.[20] However outside of Western Europe and North America, the largest vaccination effort ever was undertaken by the WHO and UNICEF, who decided to support BCG.[28] During the ninth UNICEF-WHO meeting in 1956 it was discussed whether the BCG vaccination efforts were actually effective and the chairman Professor Debré requested studies of the efficacy of BCG in underdeveloped areas.[29] By 1959 a report was readied which indicated that the value of the BCG vaccination was not clear. This may be interpreted as an admission that the degree of protection provided by the vaccination may not have justified such a large scale application of BCG.[30] The report included evidence from the first trials conducted by Aronson and Palmer on a population of 3000 North American Indians in 1936 which showed a reduction in mortality of 75 % over 15 years. Another study carried out in Britain on 50,000 infants showed similar success, although only in its fourth year in 1959. In contrast there were results from two trials carried out by the US Public Health Services on 200,000 Puerto Rican under 19's and on 65,000 individuals from two US communities in Georgia and Alabama, with protective success rates of 31 % and 36 %, respectively. The WHO suggested that the vaccine used in the trials which showed a low protective rate may have lacked potency and also that some individuals included in the survey may have had a low sensitivity to the vaccine. It was suggested that people with a low sensitivity were "associated with a considerable resistance to tuberculosis" and hence obscured the true protective properties of the vaccine.[30] Several more reports were carried out over the next five years to justify the use of BCG, however in 1963 the WHO proposed a large-scale trial in India with the justification that, although there were many anti-TB drugs available, none had been able to establish themselves and that BCG offered a cost effective method of dealing with TB.[31] In 1968 the trial in India, being carried out by the Indian Council of Medical Research in conjunction with the US Public Health Services and WHO, began. The trial was conducted over nine years on 360,000 people in the Chingleput district of southern India. The results were published in 1977 and to the surprise of the WHO they indicated that BCG showed no protective effects.[32] However the WHO did not abandon its policies regarding the BCG vaccine, but stated that several factors must be considered including the environmental and immunological characteristics of the population studied.[33] After

the Chingleput trial the WHO continued to back the use of BCG as a vaccine stating that "although BCG was not very effective from the epidemiological point of view, it was invaluable from the clinical standpoint for the prevention of severe and fatal forms of TB in children".[34]

Like many other infectious diseases TB has surged in great epidemics and then receded, where epidemics have been recorded during the 18th and 19th centuries in Europe and North America.[2] During the early to mid 19th century there was a noticeable decline in TB cases in Europe and North America; there are many hypotheses for this but none of them can fully explain the decline. However elsewhere the TB epidemic continued, particularly in areas where AIDS is prominent.[35] In the late 19th and early 20th centuries physicians were using a technique called surgical collapse therapy to treat TB, where the infected lung of a patient was collapsed and then sterilized, with a fairly good success rate.[2, 36] It was thought that around 30 years ago that it was no longer a threat and that it was close to eradication, however between the early 1980's and late 1990's TB resurfaced. In 1995 more people died from TB than in any other year.[37] The reoccurrence of the disease is believed to be due to numerous factors, which include the emergence of drug-resistant strains of mycobacteria, poor drug compliance and the ease of infection of victims of the AIDS epidemic.[38]

1.1.2 - Detection

TB was first detected in the 1930's via radiographic fluoroscopy, where initially a patient stood so that an X-ray image was transmitted onto a fluorescent screen without the use of film. As this method is very dangerous the use of film and then later tomography was implemented.[39] At the end of the century computerized axial tomography and nuclear magnetic resonance tomography was being used to distinguish between TB and cancer lesions in the chest. However as pulmonary lesions develop very quickly such screening methods would be needed to be carried out more frequently than would be considered practical.[40]

Until the 1950's diagnosis via bacteriology was carried out using a direct smear Ziehl-Neelsen method. Although a direct smear method is rapid and highly specific it has a low sensitivity. Fluorescent microscopy slowly replaced the direct smear method as it is five times more rapid and more sensetive.[41, 42] From the 1950's onwards many bacteriologists adopted methods using cultures, with many favouring

a Lowenstein-Jensen medium; however a lyophilized liquid medium was later favoured over the Lowerstein-Jensen medium, as it was simpler to prepare and gave better results.[43] In 1977 an automated method using a liquid medium containing radioactive palmitic acid was adopted, although it was deemed too expensive for developing countries and other methods using non-radioactive compounds are now preferred.[44]

1.1.3 – Current Treatments

Mycobacteria are problematic as they are resilient to most commonly used antibiotics and chemotherapeutic agents. The two classes of antibiotics which are most effective against TB are aminoglycosides, comprised of sugar and amino groups, and rifampcins, which are obtained either naturally from the bacterium *Amycolatopsis mediterranei*, or produced artificially. The first antibiotic to be used was streptomycin in 1946 and by 1955 many Western doctors had adopted the strategy of combining streptomycin **(1)** with *p*-aminosalicylic acid **(2)** and isoniazid **(3)** (See Fig. 1).[45]

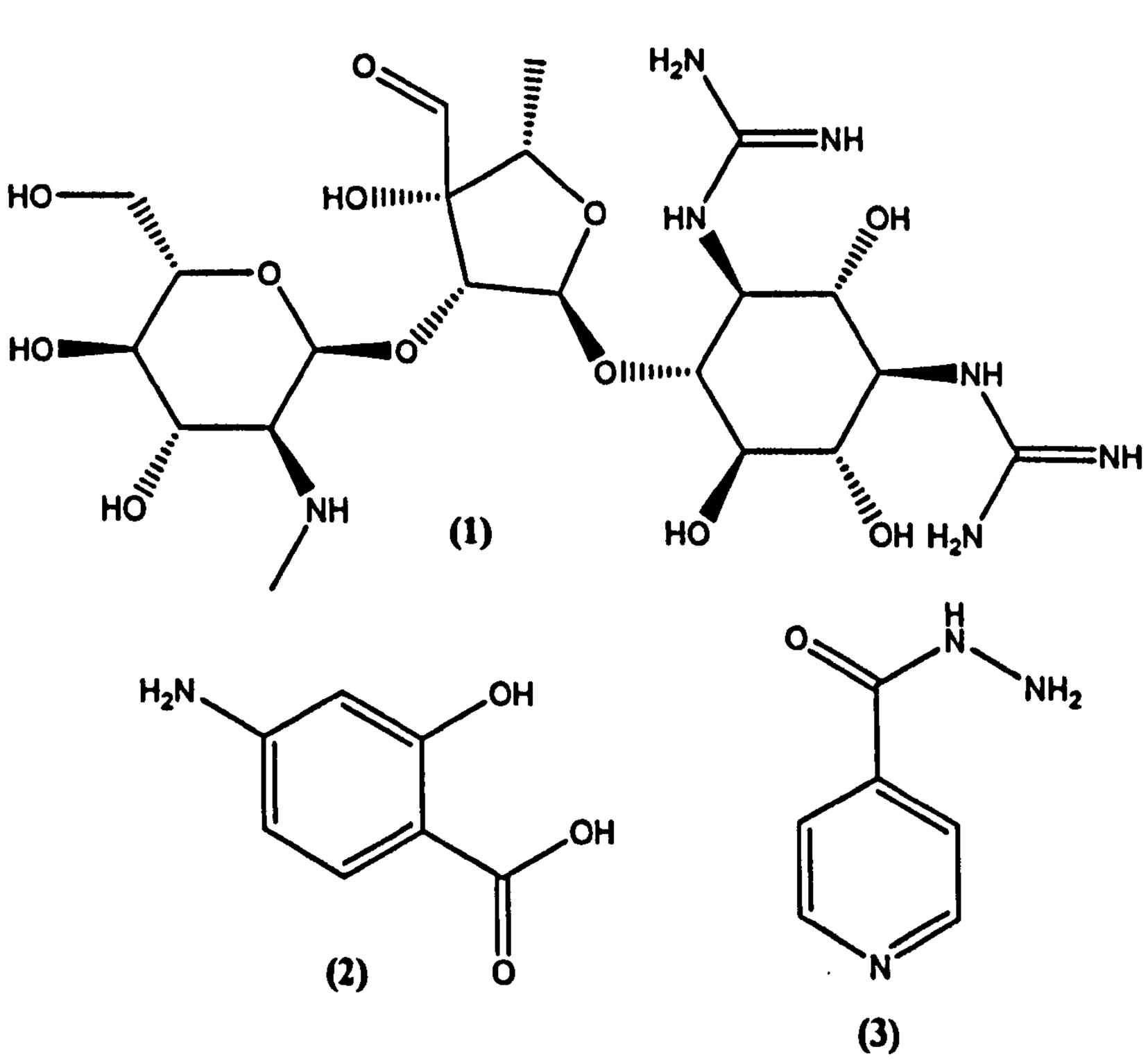


Fig 1: Streptomycin (1), *p*-aminosalicylic acid (2) and isoniazid (3).

Mycobacteria are also resilient to many chemical disinfectants, which makes prevention of transmission in a high density population area very difficult.[1] The use of chemotherapy to treat TB is highly effective and as a result the number of cases of TB has dropped in developed countries. However in underdeveloped countries, where the disease is most prominent, there is poor compliance with the six-month, multi-drug course.[46] In 1994 the WHO developed a short-course chemotherapy (SCC) treatment for TB, now called Directly Observed Therapy, Short-course (DOTS) where a patient is put into a programme where the administration of medication is supervised.[47] The drugs used in this scheme firstly involve a two month course of either: streptomycin **(1)**, isoniazid **(3)**, rifampcin **(4)** and pyrazinamide **(5)** or: isoniazid **(3)**, rifampcin **(4)**, pyrazinamide **(5)** and ethambutol **(6)** (See Fig. 2). This is followed by a four month course of isoniazid **(3)** and rifampcin **(4)**.[45]

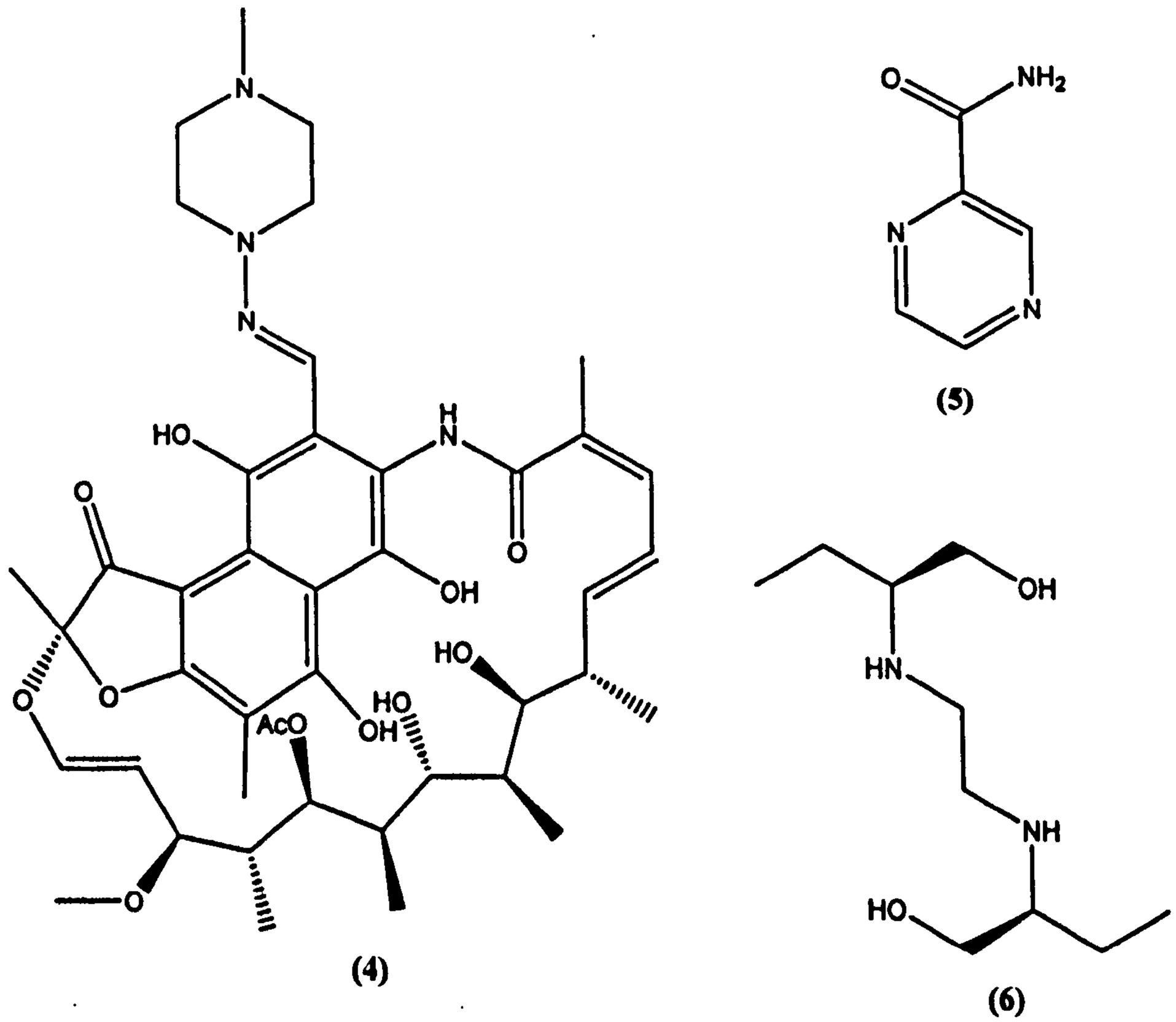


Fig. 2: Rifampcin (4), pyrazinamide (5) and ethambutol (6).[45]

However it has been reported that some patients who follow this regimen have suffered side effects, the most serious of which is hepatotoxicity, chemical-induced liver damage, to isoniazid recorded at an average of 0.92 %, with a fatality rate recorded at 4.7 %, with occurrence higher in older patients. Other serious adverse effects include dermatological, gastrointestinal, hypersensitivity, neurological, haematological and renal reactions.[48] For these reasons some patients do not finish the regimen and as a result the slow growing bacteria are not eradicated; this is problematic as the patient may not be fully cured.

In 1993 the WHO announced a global health emergency with regards to TB. The WHO have developed the Stop TB Partnership which aims to target the areas where current TB rates are worsening and to halve the mortality and prevalence rate by 2015, the result of this would be to save an estimated 14 million lives.[49]

1.1.4 – Immunology and HIV co-infection

M.Tb is known to remain within the granuloma of the host's organs. Granuloma are a collection of cells which are responsible for immunity, including macrophages and lymphocytes. T cells are the lymphocytes which play a primary role in immunity within a host.[50] Another type of immune cell, which plays a role in the modulation of immunity, are the dendritic cell, which are responsible for capturing and processing antigens.[51] The method of processing antigens that the dendritic cell undertakes is to produce T cells, which in turn induces production of a cytokine called Interleukin-12.[51] Interleukin-12 is key in controlling the infection caused by *M.Tb*, where evidence of this has been observed in mice.[52]

With regards to a co-infection of HIV, mainly in Africa and Asia, there has been an increase in the number of cases where HIV-infected patients are developing TB.[53] HIV is a retrovirus that infects the immune system of humans, where it impairs the host's ability to combat disease.[54] In relation to TB infection, HIV decreases the production of T-lymphocytes, which leads to a reduction in Interleukin-12 and hence a high risk of infection by *M.Tb*.[55, 56] It has also been documented that patients who have contracted TB have an increased susceptibility to HIV, which is associated with proinflammatory cytokine production by TB granulomas.[57]

1.1.5 – Multi-drug resistance

The two most important drugs in the DOTS strategy are isoniazid and rifampcin, and when a patient becomes resistant to them they are known as having multidrug-resistant tuberculosis (MDR-TB).[45, 58] MDR-TB can occur during the treatment for fully sensitive TB, if a patient misses a dose, a patient does not complete the course or if the doctor administers the wrong treatment.[58] MDR-TB positive patients are unlikely to transmit MDR-TB to a healthy person, however an immunosuppressed patient is at an increased risk of contracting MDR-TB. [59] The treatment of MDR-TB is a long course of second-line drugs, which are more expensive and have far more severe side effects in comparison to first-line drugs.[60]

If a patient with MDR-TB becomes resistant to second-line drugs, then they are known to have developed extensively drug-resistant tuberculosis (XDR-TB). XDR-TB is very similar to MDR-TB in that transmission between patients is the same and that it arises in a similar fashion. However two differences are in the treatment and mortality rate.[61] The treatment of XDR-TB is an extensive two year course of chemotherapy, however like MDR-TB treatments can vary from patient to patient, depending on which drugs they have become resistant to. The mortality rate for patients having XDR-TB in comparison to MDR-TB is much higher. In 2006, 53 South African patients were confirmed to have XDR-TB where 52 died.[62]

1.1.6 – Symptoms

TB is similar to a common cold in that it is transferred through the air, where an infectious person will propel TB bacilli through the air in a cough, their spit or a sneeze, where only a small number of bacilli need to be inhaled by a person for them to contract an infection.[63] A. Baydur documented the symptoms of tuberculosis, including chronic coughing, fever, weight loss and hemoptysis (expectoration of blood), in a study of 62 patients.[64] It has been reported by C.B. Holmes *et al.* that there are more men infected with TB, but more women sufferers of TB and as a results more deaths in women.[65] A study conducted by N.H. Long *et al.* shows that men are more at risk of experiencing symptoms of TB. Cough (women 90.7 %, men 94.7 %), sputum expectoration (women 83.6 %, men 89.9 %) and hemoptysis (women 27.8%, men 34.9 %) are all more prevalent in men.[66] A reasonable explanation for this is that the development of lung lesions in women is less

advanced in comparison to men, and that men have a stronger hypersensitivity to mycobacteria than women.[66-68]

1.2 – Mycobacteria

1.2.1 – Mycobacterium tuberculosis

M.Tb occurs as a rod-shaped bacillus, roughly 1-4 x 0.3-0.6 μm in size (See Fig. 3), which divides aerobically every 16 to 20 hours.[1] This rate of division is very slow in comparison to other bacteria, such as *Escherichia coli* where division occurs in minutes.[69]

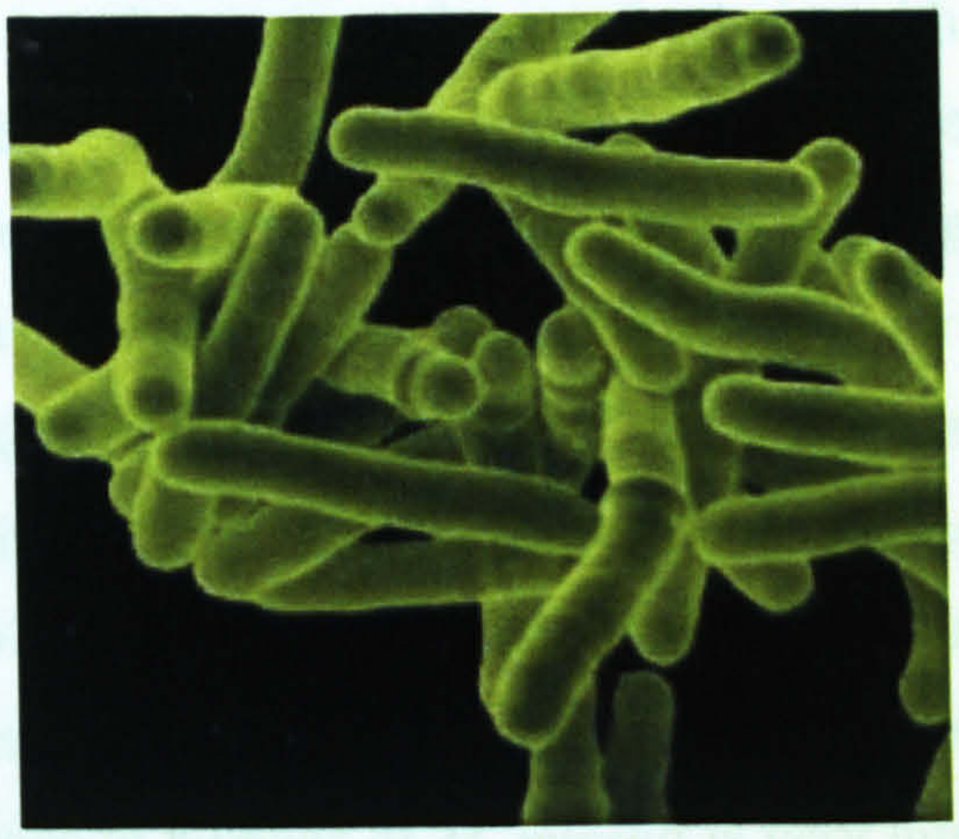


Fig. 3: Scanning electron micrograph of *Mycobacterium tuberculosis*[70]

M.Tb is classified as a Gram-positive bacterium, due to the high lipid content in its cell wall, *M.Tb* only gives very weak results in a Gram stain test.[41] *M.Tb* is empirically classified as an acid-fast Gram positive bacterium, where they are resistant to decolourisation by acids in staining procedures. Mycoacteria have a cell wall high in lipid content, and as a result they have poor absorption and high retention of standard Gram stains, therefore alternative methods must be used.[71] *M.Tb* can be observed using a fluorescent microscope with a Ziehl-Neelsen stain (See Fig. 4). *M.Tb* is usually found within moist conditions, however it can also be classified as a Xerophile, as it can survive for several weeks in a dry state.

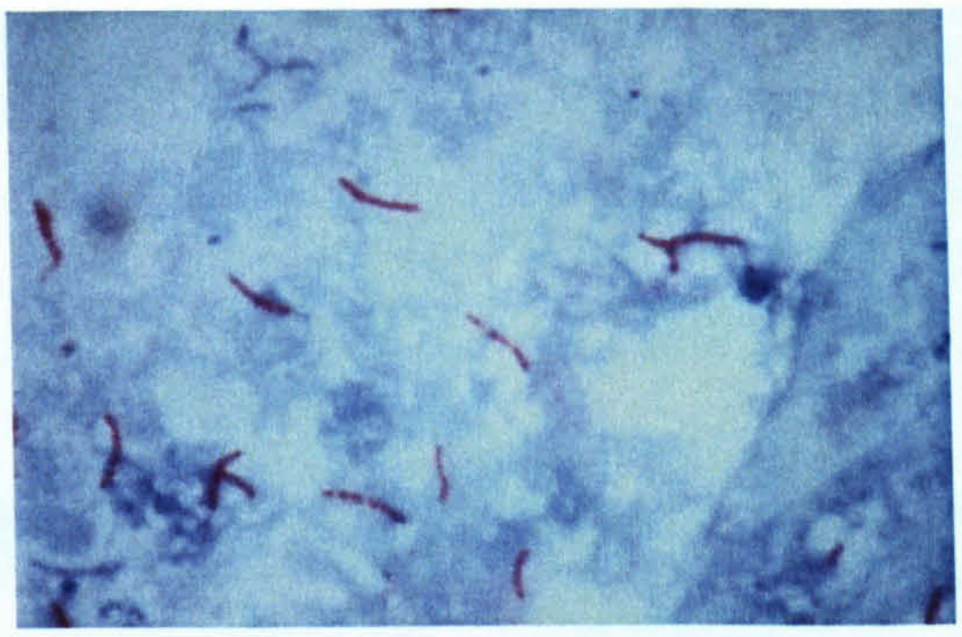


Fig. 4: Red tubercle bacillus in a sputum sample[72]

1.2.2 – Other relevant mycobacteria

M.Tb is related to other pathogenic mycobacteria, with some responsible for causing tuberculosis in other species of animal and some causing other non-tuberculosis diseases in humans.[73] Of the tuberculosis causing mycobacteria one of the more well known is *Mycobacterium bovis*, responsible for tuberculosis in cattle, which is of concern as there is evidence that *M. bovis* is transferable to humans via an infected cow's milk or via an aerosol.[74] *Mycobacterium africanum* is most commonly found in West Africa, with similar symptoms to *M.Tb* being observed.[75, 76] *Mycobacterium microti* is the causative agent behind tuberculosis in small mice and voles, but occurrence in humans of *M. microti* is very limited.[77] *Mycobacterium marinum* infects around 150 species of fish with tuberculosis; J.D. Aronson first isolated the mycobacteria in 1926 from a salt water fish. Tuberculosis is rarely caused by *M. marinum* in humans, however some skin infections occurring after contact with contaminated water sources.[78] *Mycobacterium avium* is responsible for tuberculosis in birds, however it has been reported to cause inflammation of the lymph nodes, lymphadenitis, in children.[79] Of the non-tuberculosis mycobacteria, *Mycobacterium leprae* is responsible for the infamous disease leprosy (a.k.a Hansen's disease).[80] *Mycobacterium scrofulaceum* and *Mycobacterium canettii* also cause lymphadenitis in children.[1, 81] *Mycobacterium kansasii* is considered not to be dangerous to healthy individuals, however it has been found in immunocompromised patients.[1] Another non-tuberculosis mycobacterium of particular interest is *Mycobacterium ulcerans*, responsible for causing skin diseases associated with the Buruli ulcer (See Fig. 5).[82]

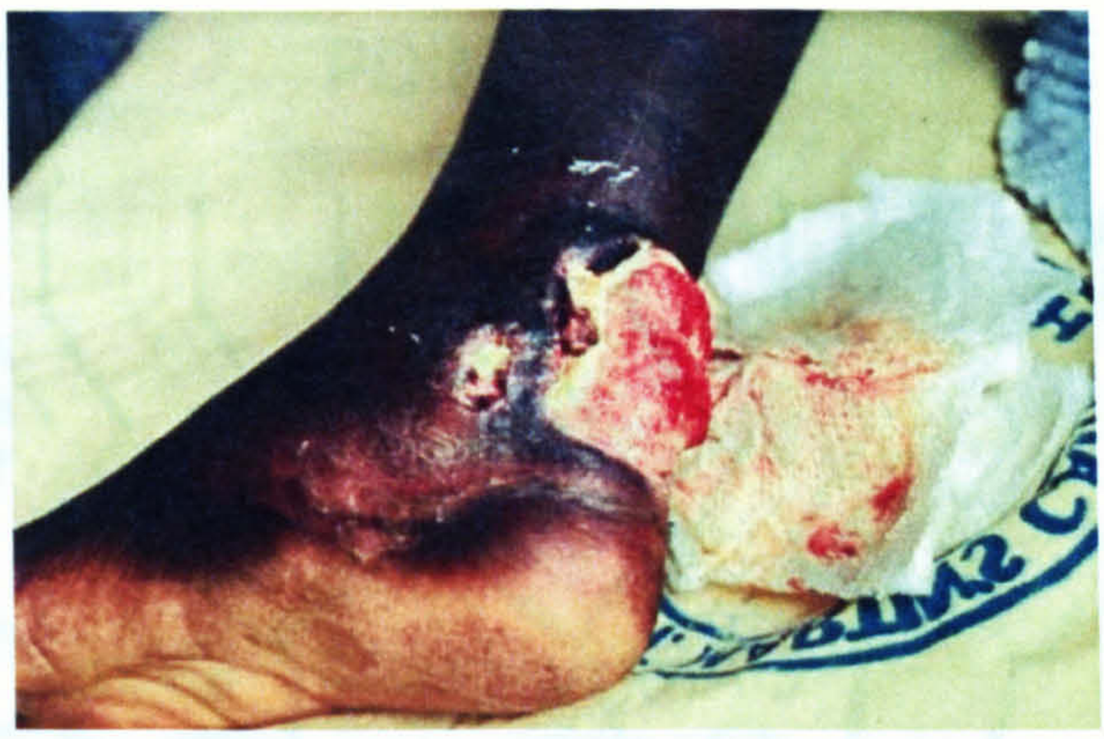


Fig. 5: Buruli ulcer[83]

1.2.3 – Mycobacterial cell envelopes

M.Tb can withstand mild disinfectants, hence prevention of transmission becomes very problematic.[1] This resilience to disinfectants is believed to be due to *M.Tb* having an impermeable, hydrophobic cell wall.[1, 84] The hydrophobicity is due to the complex structure of the cell envelope of mycobacteria, which can be broken down into three main units, the plasma membrane, the cell wall and the capsule layer.[1, 85] The plasma membrane of a *Mycobacterium* is consistent with other organisms, however the cell wall, the capsule layer and its linkages differ. The cell wall is comprised of an *N*-glycolated peptidoglycan linked to a polysaccharide side chain, *D*-arabino-*D*-galactan, which is esterified with mycolic acids, giving the mycolyl-arabinogalactan-peptidoglycan complex. The capsule contains noteworthy quantities of genus/species specific lipids, which form an outer membrane (See Fig. 6).[1, 85-87]

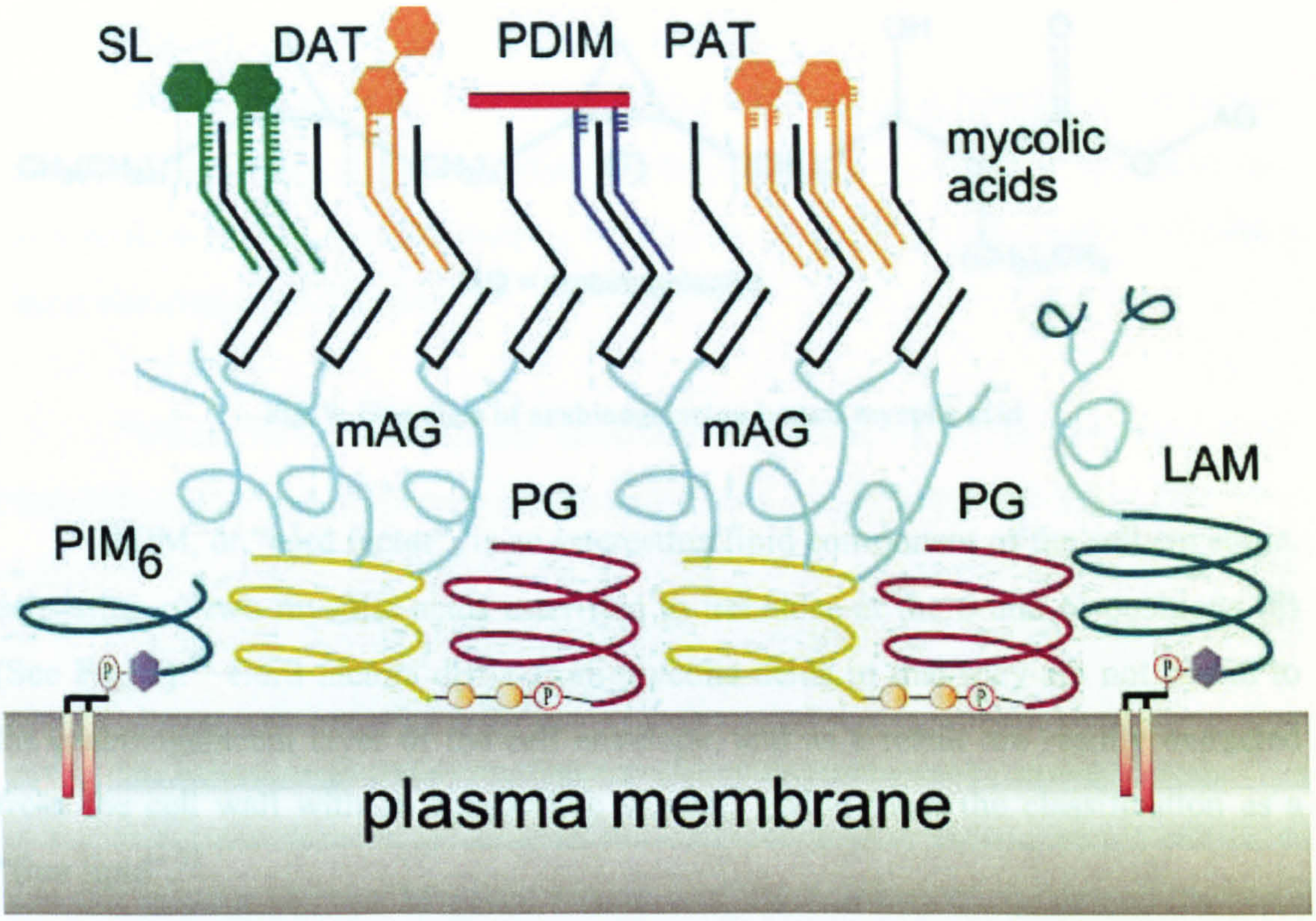


Fig. 6: Cell envelope of *M.Tb*[38]

Mycobacteria are documented as having a thick waxy coating, which is due to a high content of lipid unique to mycobacteria. It was originally believed that this high lipid content was responsible for the low permeability of mycobacteria.[88] Although the arrangement of the major constituents to the cell envelope is not fully understood, P. Draper noted that the capsule layer distinctly separates the pathogenic *Mycobacterium* from any external organisms.[89]

The cell envelope's high lipid content is well documented and unique to mycobacteria, occurring in the form of methyl branched fatty acids, trehalose dimycolate (TDM), as well as many others.[1, 38, 89-91] The mycolic acids are bound directly to the arabinogalactan layer of the cell envelope via the carboxylic acid functional group **(7)** (See Fig. 7), arranging to form a mono-layer which contributes to the protection and hydrophobicity of mycobacteria.[88, 89] These mycolic acids can contribute around 40% of the overall weight of the mycobacterial cell envelope.[1]

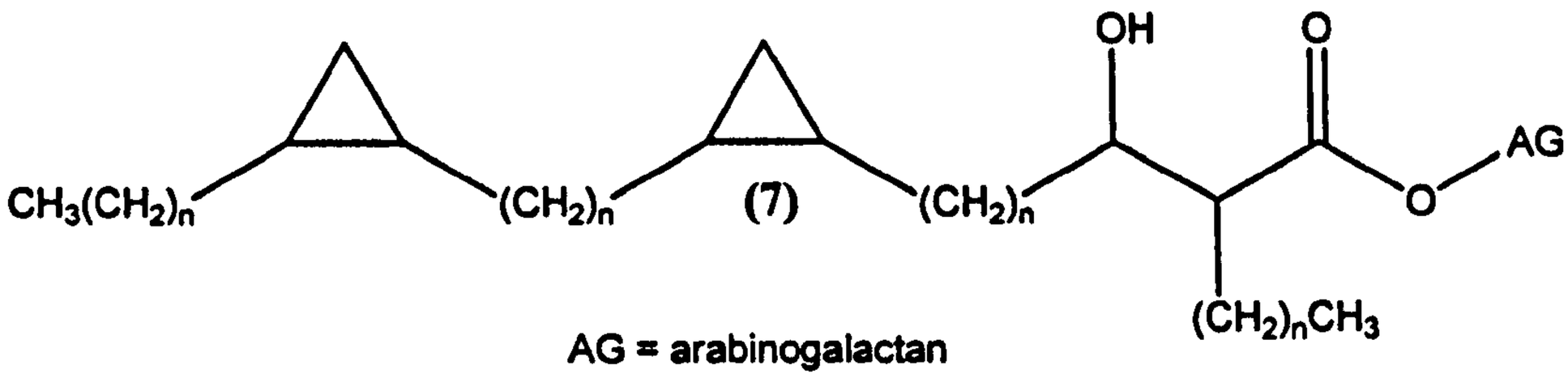


Fig. 7: One class of arabinogalactan bound mycolic acid

TDM, or "cord factor", is an interesting lipid component of the cell envelope, consisting of two mycolic acids esterified to trehalose at the 6 and 6' positions **(8)** (See Fig. 8).[92] Cord factors differ from mycolic acids in that they are not bound to the arabinogalactan layer of the cell envelope, and as a result are readily extracted from the cell wall with an appropriate solvent, giving them the classification as a "free lipid".[92]

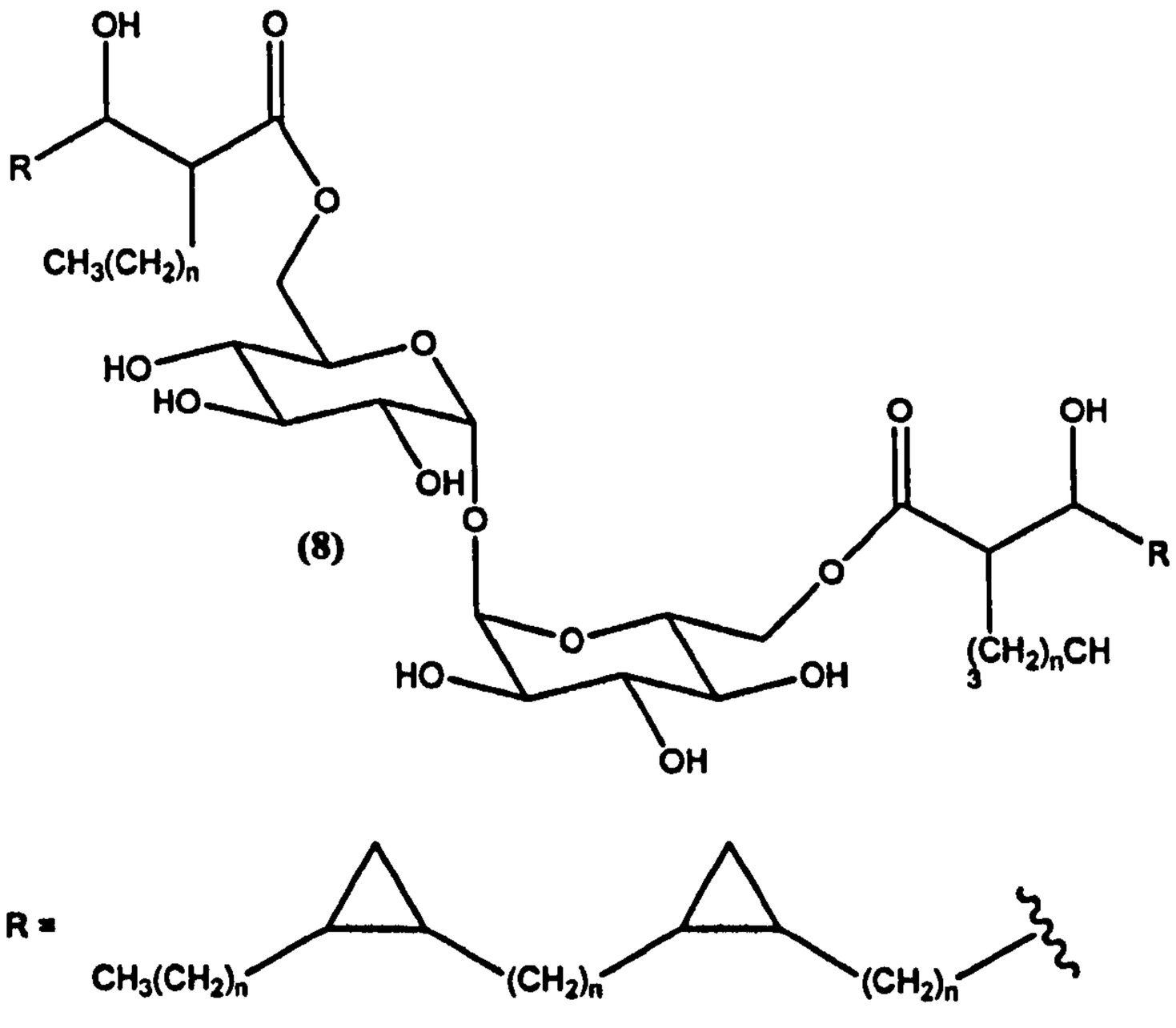


Fig. 8: A typical cord factor

Cord factors have shown to exhibit properties that increase resistance to the influenza virus, *Salmonella typhi* (typhoid) and *Salmonella typhimurium* (gastroenteritis).[93-95] Synthetic cord factors prepared by M. Maza-Iglesias *et al.* have shown optimised performance compared to an *M.Tb* extract.[95, 96]

1.3 – Asthma

Asthma is a chronic disorder of the airway, where narrowing of the airway occurs and breathing becomes extremely difficult. It is recorded in literature that dates back to the time of the ancient Greeks, around 100 A.D..[97] In 1698 Sir John Floyer described the disorder:

"When the Muscles labour much for Inspiration and Expiration thro' some Obstruction, or Compression of the Bronchia, etc. we properly call this a Difficulty of Breath: but if this Difficulty be by the Constriction of the Bronchia, 'tis properly the Periodic Asthma: And if the Constriction be great, it is with Wheezing; but if less, the Wheezing is not so evident." [98, 99]

His explanation is consistent with the symptoms of an asthma attack as recognised today. The main symptoms used today to judge the severity of an attack include alertness, breathlessness and wheezing.[100, 101] The origins of an "attack" are from one or more triggers, including exertion, change in air temperature, environmental allergens such as dust, or stress. Attacks are more common in children and can be brought on by a related infection, such as a common cold.[102, 103] During an asthma attack an environmental stimulant will react with the airway, leading to an immune response in the airway, restricting the airway and producing excess mucus. Hence an asthma attack is a result of the body's immune response, trying to prevent an external organism entering the body. This immune response is triggered because the patient has an inflamed airway and is hypersensitive to specific environmental triggers.[104] The cause of asthma is believe to be due to genetic and environmental factors.[103] Exposure to tobacco smoke, air pollution from automobiles and high levels of ozone are all factors that have been suggested to increase asthma prevalence.[105] It has been suggested that there are 25 genes which are associated with asthma,[106] however research in these areas is ongoing.

Asthma symptoms are not cured, but are relieved; in 1905 this was done using an alkaloid known as daturine, from Jimson Weed (*Datura stramonium*). A tincture of Jimson Weed leaf was made and taken orally, the result of the alkaloid was smoothening of the muscles in the airway.[107] Today similar alkaloids, salbutamol, levosalbutamol and terbutaline, are administered via a metered-dose

inhaler (MDI). The biggest selling asthma therapeutic sold today is fluticasone propionate, available commercially as either Advair or Seretide.[107, 108] Several treatments for asthma have been used during the history of medicine, one of the more interesting methods was the use of asthma cigarettes (See Fig. 9), which were cigarettes comprised of dried Jimson Weed which the patient smoked either by itself or mixed with conventional tobacco. They were commercially available, however, at a time when smoking tobacco was considered of benefit, the possible side-effects may not have been fully understood.[107, 109, 110]

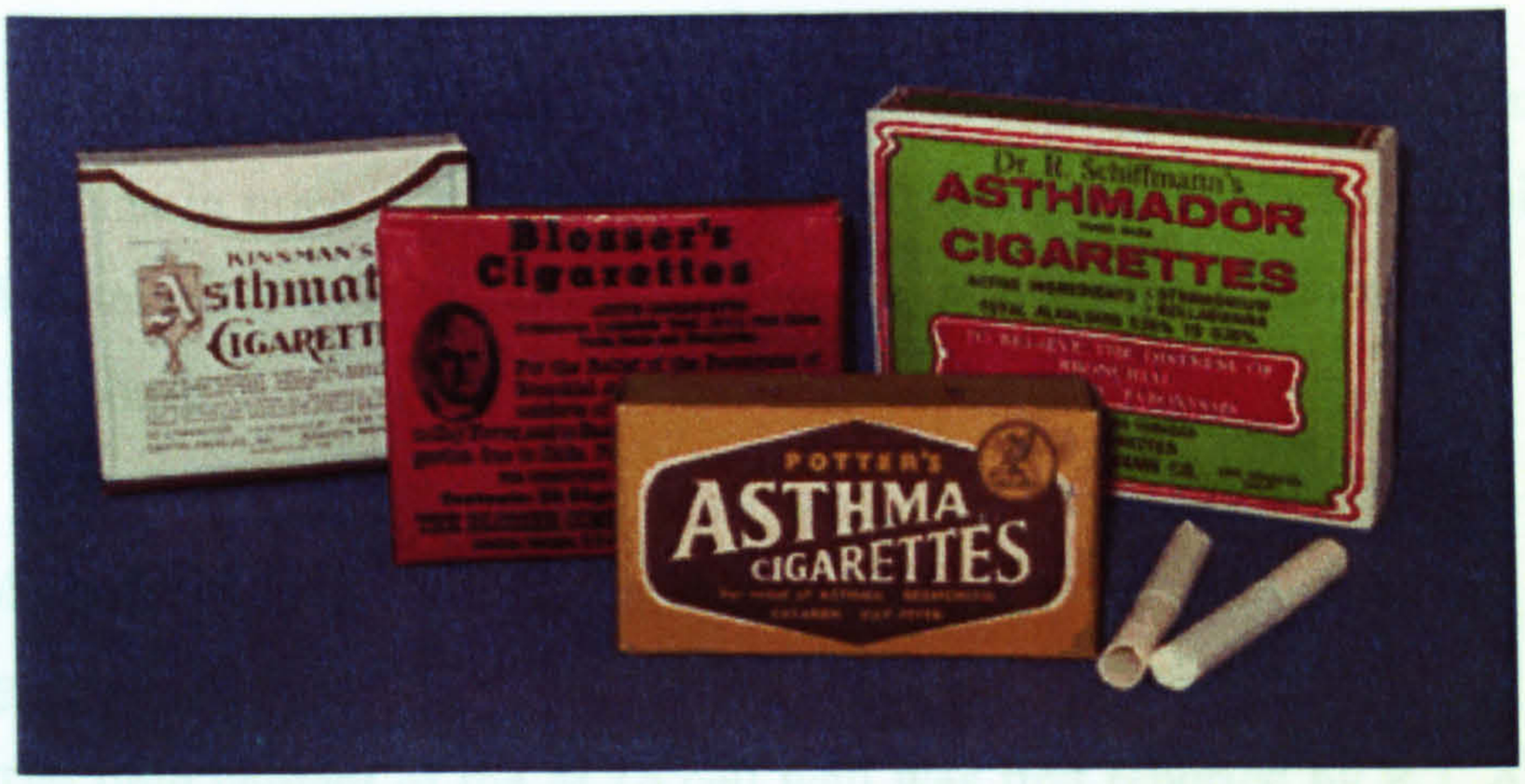


Fig. 9: Asthma cigarettes[111]

J. Korf *et al.* documented evidence that a mycobacterial cell envelope extract provides therapeutic affects toward asthma.[112, 113] They discussed the immune response to a direct application of mycolic acid, incorporated into a liposome to overcome hydrophobicity issues, and TDM to the airway of a mouse. The study shows the result of application of a lipsome control (L), mycolic acid-liposome (MA-L), TDM-liposome (TDM-L) and heat-killed *M.Tb*. They show that the MA-L, TDM-L and *M.Tb* all elicit an immune response in the form of cholesterol rich-foamy macrophages, in varying degrees (See Fig. 10).[113]

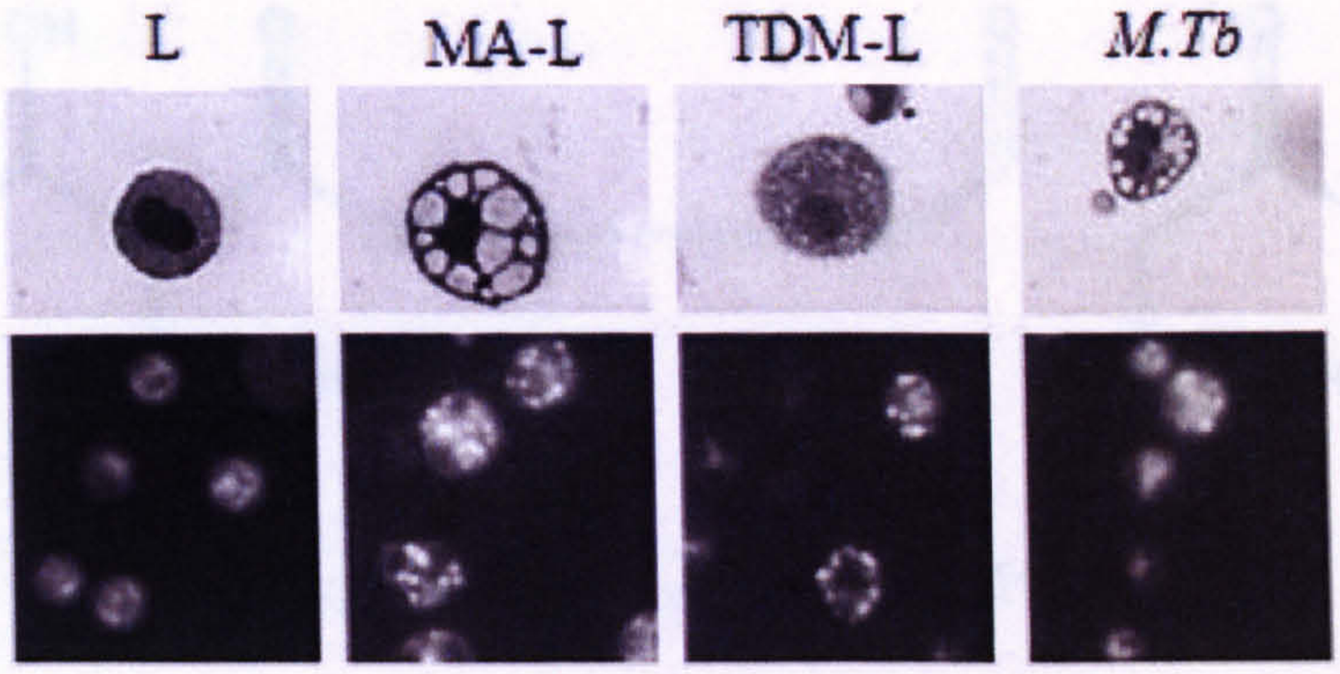


Fig. 10: Foamy macrophages[113]

The foamy cells produced have shown the potential to prevent experimentally induced asthma in mice.[114, 115] Therefore the preparation of synthetic mycolic acids and cord factors (TDM) is of particular interest to provide more information into this immune response.

1.4 – Mycolic Acids

1.4.1 - Background

The lipid content of the *M.Tb* cell wall was first reported by R.J. Anderson, where he discovered that the cell wall of the tubercle bacilli had a high lipid content of around 40 %.[116, 117] Anderson *et al.* characterised the lipids they found as being "hydroxyl acid of very high molecular weight" and they contained complex mixtures with different compositions. They proposed the name of "Mycolic Acid" and calculated empirical formulae from its composition as $C_{88}H_{172}O_4$ or $C_{88}H_{176}O_4$. They also described the methods used to isolate "Mycolic Acid", via saponification of the wax extract from the cell. Anderson *et al.* also elucidated structural details, reporting one COOH, one OH and OMe, and that pyrolysis under vacuum at 300 °C yields hexacosanoic aicd.[118] Mycolic acids were also found in other mycobacterial strains including bovine and avian, showing similar characteristics with regards to the hydroxyl group and pyrolysis products.[119-122] Later work carried out by J. Asselineau and E. Lederer confirmed the position of the hydroxyl group first reported by Anderson *et al.* to be in the β-position to the carboxylic acid, with a pyrolysis reaction of mycolic acid (See Fig. 11).[123]

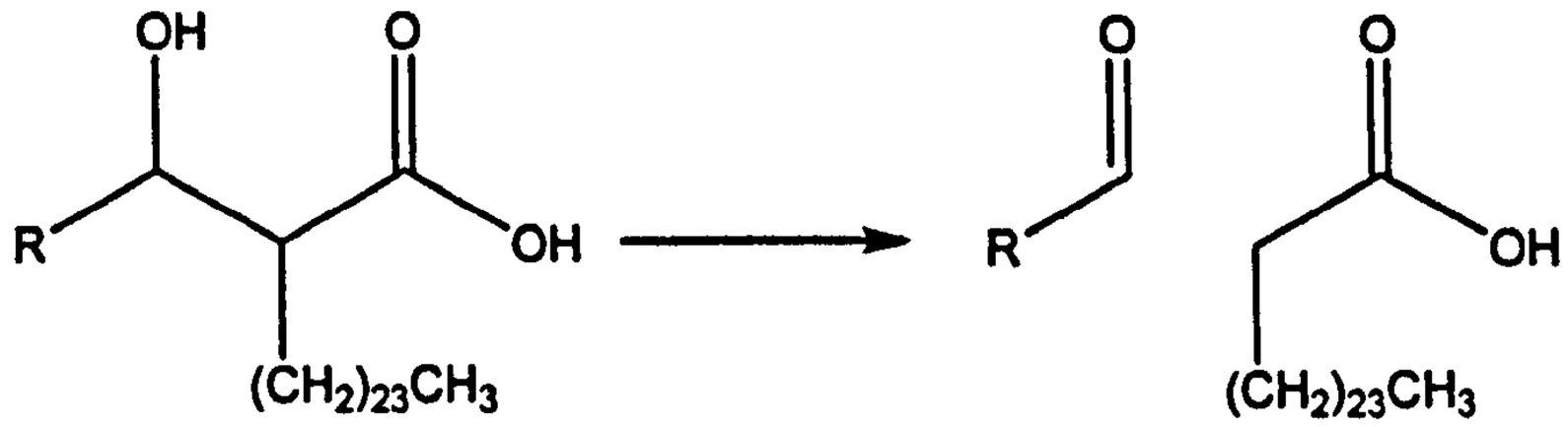


Fig. 11: Pyrolysis of a mycolic acid[123]

However when Asselineau and Lederer carried out this pyrolysis they reported no aldehyde formation, but a mixture of methoxy-free compounds. They then set about confirming the above structure, heating mycolic acid with acetic anhydride and 10 % potassium hydrogen sulphate to hydrolyse the β-hydroxyl group to give the α,β-unsaturated anhydro-mycolic acid **(9)**. Ozonolysis of **(9)** results in oxo-hexacosanoic acid, and further oxidation gives pentacosanoic acid **(10)** (See Fig. 12).[123]

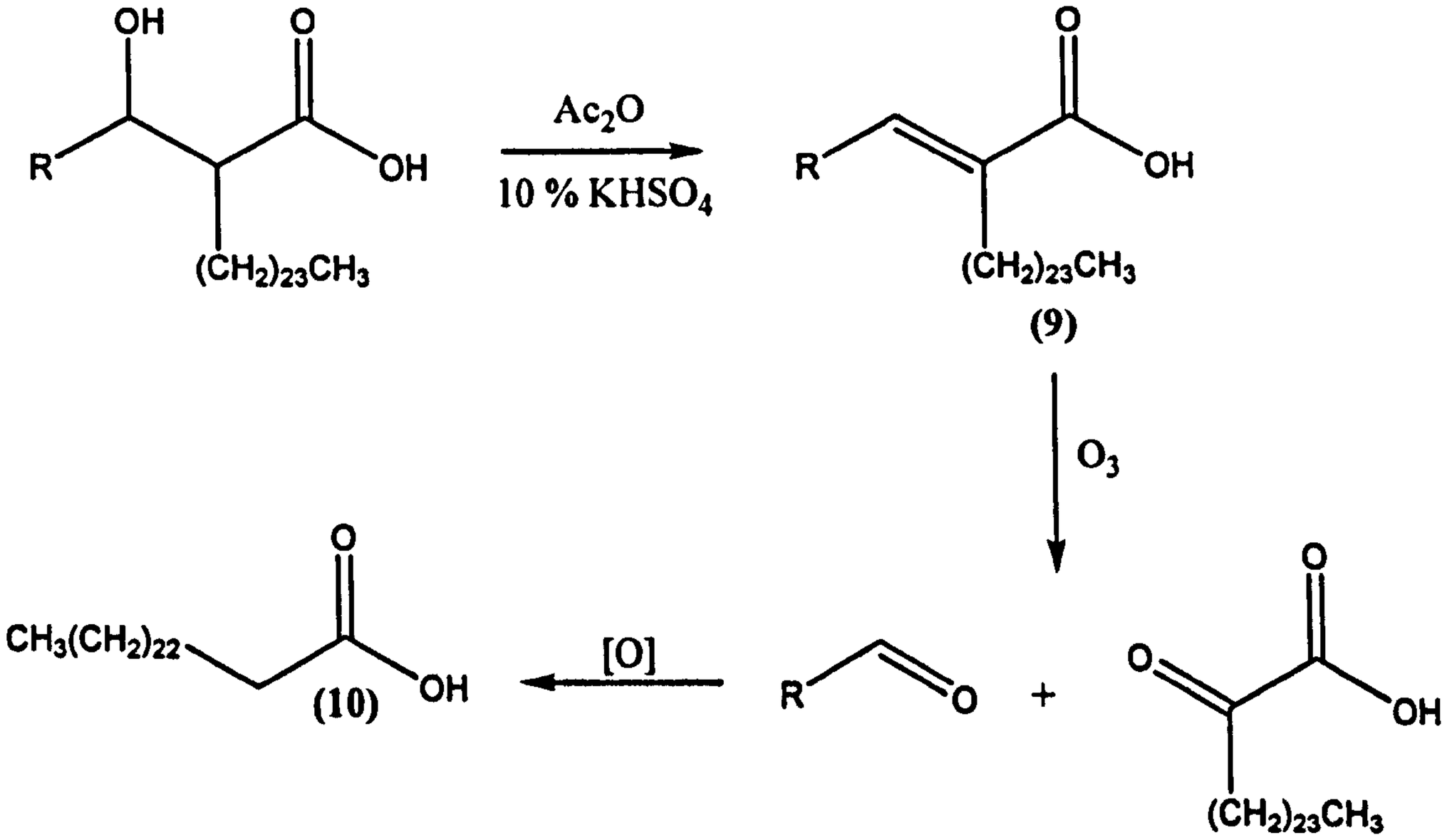


Fig. 12: Asselineau and Lederer's determination of mycolic acids[123]

The work carried out by J. Asselineau and E. Lederer enabled them to define mycolic acids as high-molecular weight β-hydroxy fatty acids with a long chain in the α-position.[123]

With the use of advanced spectroscopic techniques, in the 1960's D.E. Minnikin and N. Polgar worked on the structural determination of mycolic acids.[124-128] Using analytical techniques such as chromatography, to separate the mycobacterial extract, infra-red, ^{1}H NMR, optical rotation, ultra-violet and low-resolution mass spectrometry they were able to derive four main functionalities. With this addition to the α-alkyl β-hydroxy fatty acid functionality discovered by Asselineau and Lederer, full structures of mycolic acids were hypothesised. The four functional groups proposed were keto, methoxy, *cis*-cyclopropane and *trans*-cyclopropane groups, occurring in various configurations at distal and proximal positions.[124, 127] Work has since been carried out confirming these findings and also classifying further functionalities, including *cis* and *trans* olefins and epoxy groups.[129] The structure of a mycolic acid can be separated into two main fragments, the branched hydroxy acid and the meromycolate. The branched hydroxy acid contains the α-alkyl β-hydroxy fatty acid functionality and the other functionality discussed by Minnikin and Polgar is found within meromycolate (See Fig. 13).

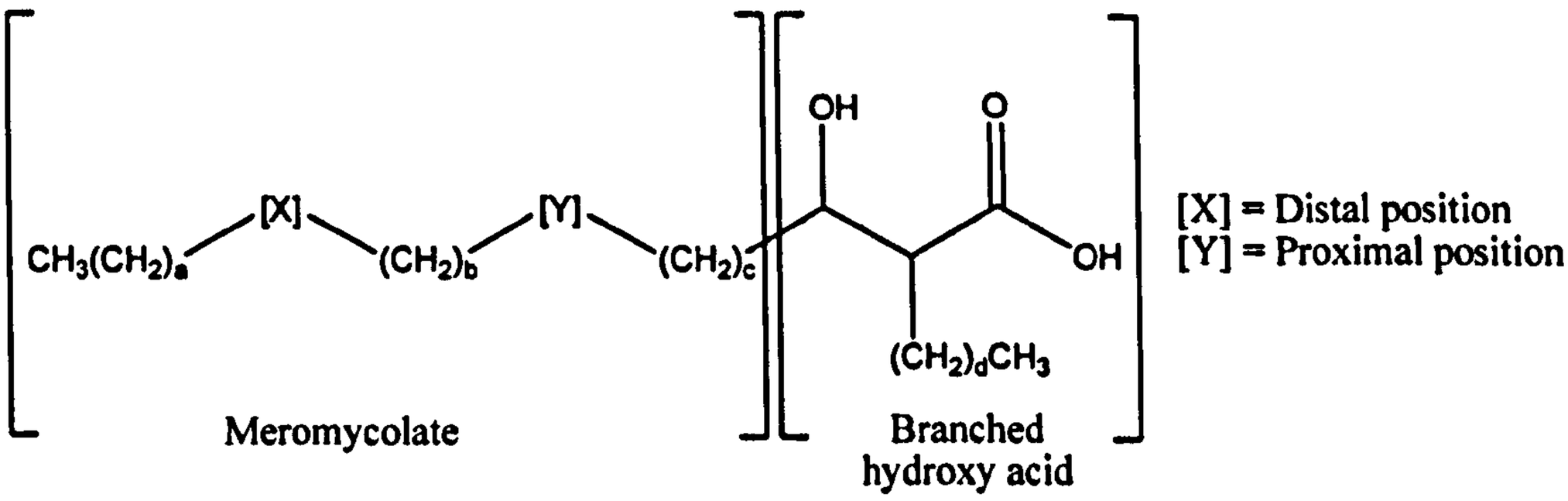


Fig. 13: Generic mycolic acid

It has been reported that mycolic acids are esterified to the arabinogalactan within the mycobacterial cell envelope,[88, 89] and that the meromycolate chains are aligned parallel to each other, with the methyl groups arranged so that they are towards the surface.[1, 38, 89] The stacking of the long hydrocarbon chains is affected by the functional group present at the distal and proximal position, as they vary in type, stereochemistry and spacing.[129] As the functionality of these positions appears to play an important role in the mycobacterial cell envelope, the resultant mycolic acids

have been catalogued by their functionality. Type-1 mycolates contain no olefin, Type-2 include all *trans* olefin mycolates and Type-3 have only *cis* olefins (See Fig. 14).[129, 130]

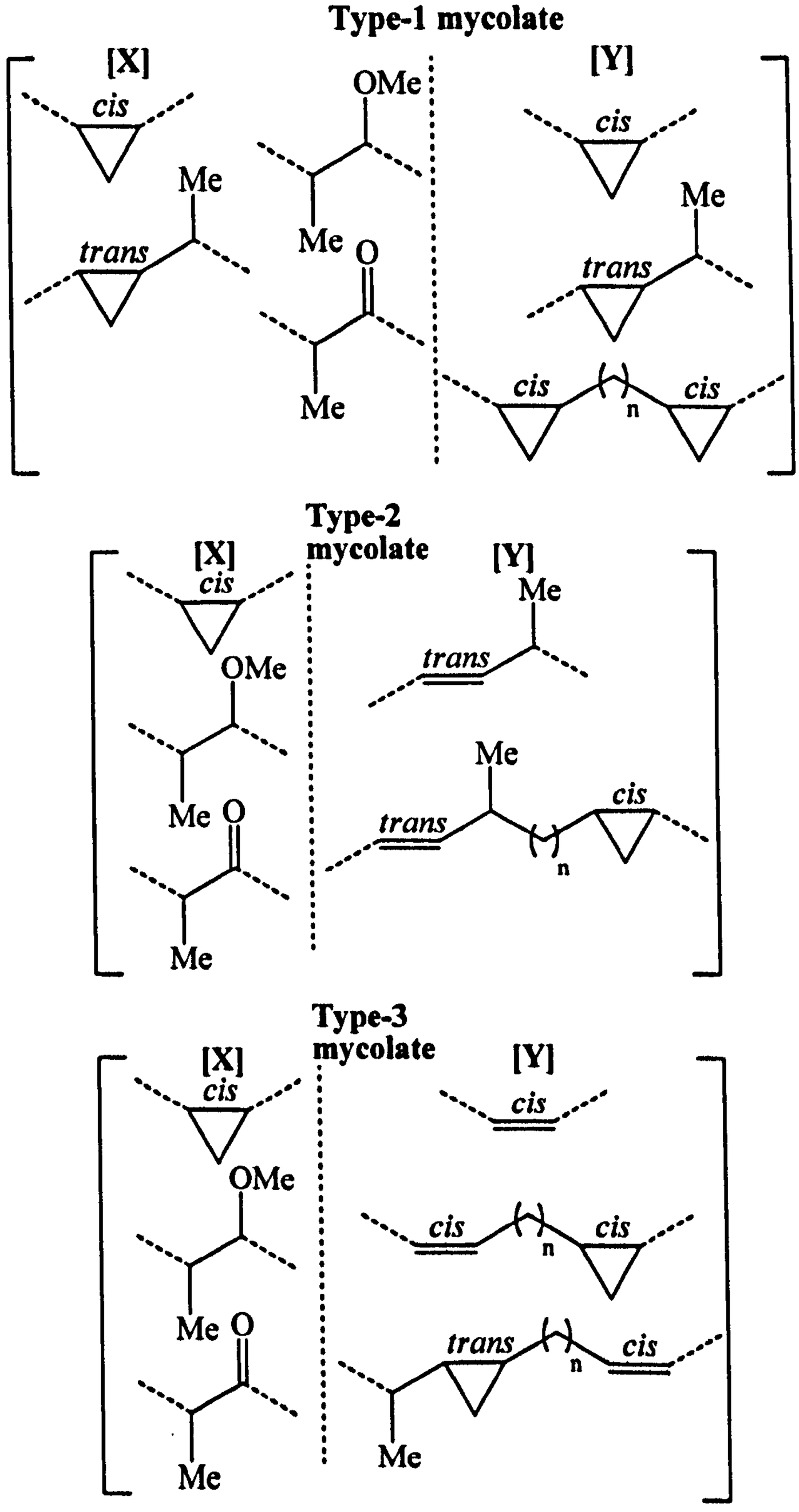


Fig. 14: General functionality of the major mycobacterial mycolic acids[129]

Other oxygenated mycolic acids have been discovered in mycobacteria, where they vary at the distal position [X]. They are, e.g., epoxy-mycolic acids of *Mycobacterium fortuitum*, wax ester-mycolic acids of *Mycobacterium aurum*, hydroxy-mycolic acids of *Mycobacterium bovis*, ω-carboxy-mycolic acids of *Mycobacterium phlei* and ω-1-methoxy-mycolic acids of *Mycobacterium alvei* (See Fig. 15).[131-136]

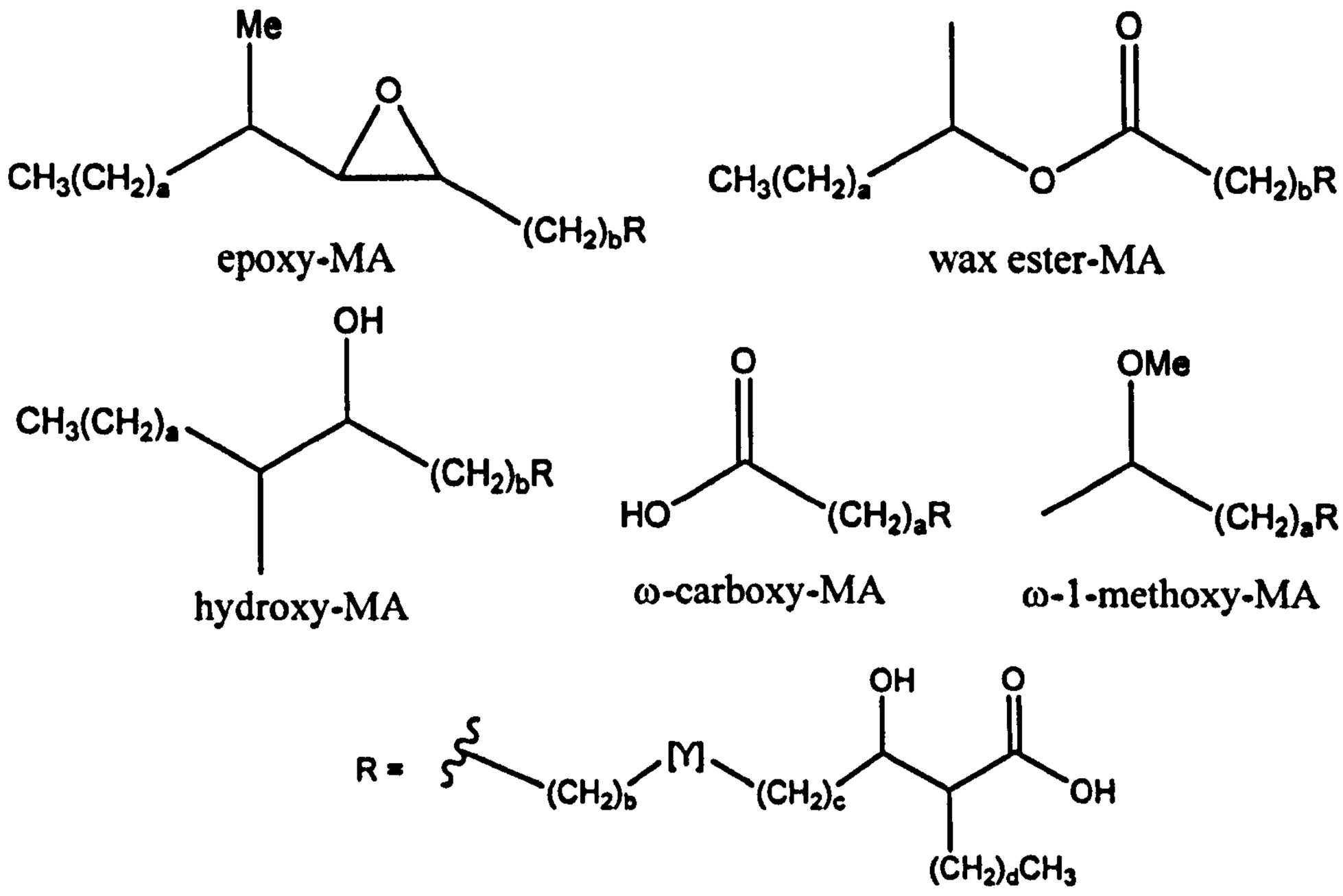


Fig. 15: Other oxygenated mycolic acids

Mixtures in the chain lengths in mycolic acids also occur, as a result this gives rise to a far greater quantity of different mycolic acids. In the *M.Tb* cell envelope there are believed to be over 500 different mycolic acids present.[137] Separation of individual mycolic acids is highly problematic due to the variation in chain lengths, however M. Watanabe *et al.* carried out column chromatography to separate the α-mycolate, methoxymycolate and ketomycolate cell envelope components, eluting with diethyl ether/hexane (6:100). This enabled them to compile a catalogue of mycobacteria with ratios of the different mycolic acids present. Watanabe *et al.* utilised ^{1}H NMR and MALDI-TOF spectroscopy to catalogue the different ratios of functional classes in each *Mycobacterium* and collision-induced dissociation mass spectrometry (CID-MS) to determine the exact positioning of the

functional groups in each meromycolate chain.[129, 130] They discussed the separation and characterisation of meromycolic acids prepared from methyl mycolates as described by J. Krembel and A.H. Etémadi.[138] They also used the pyrolysis of mycolic acid methyl esters, at around 300 °C, to give meromycolaldehydes and carboxylic acid methyl esters, and subsequent oxidation of the meromycolaldehydes with silver oxide to give the corresponding meromycolic acids (See Fig. 16).

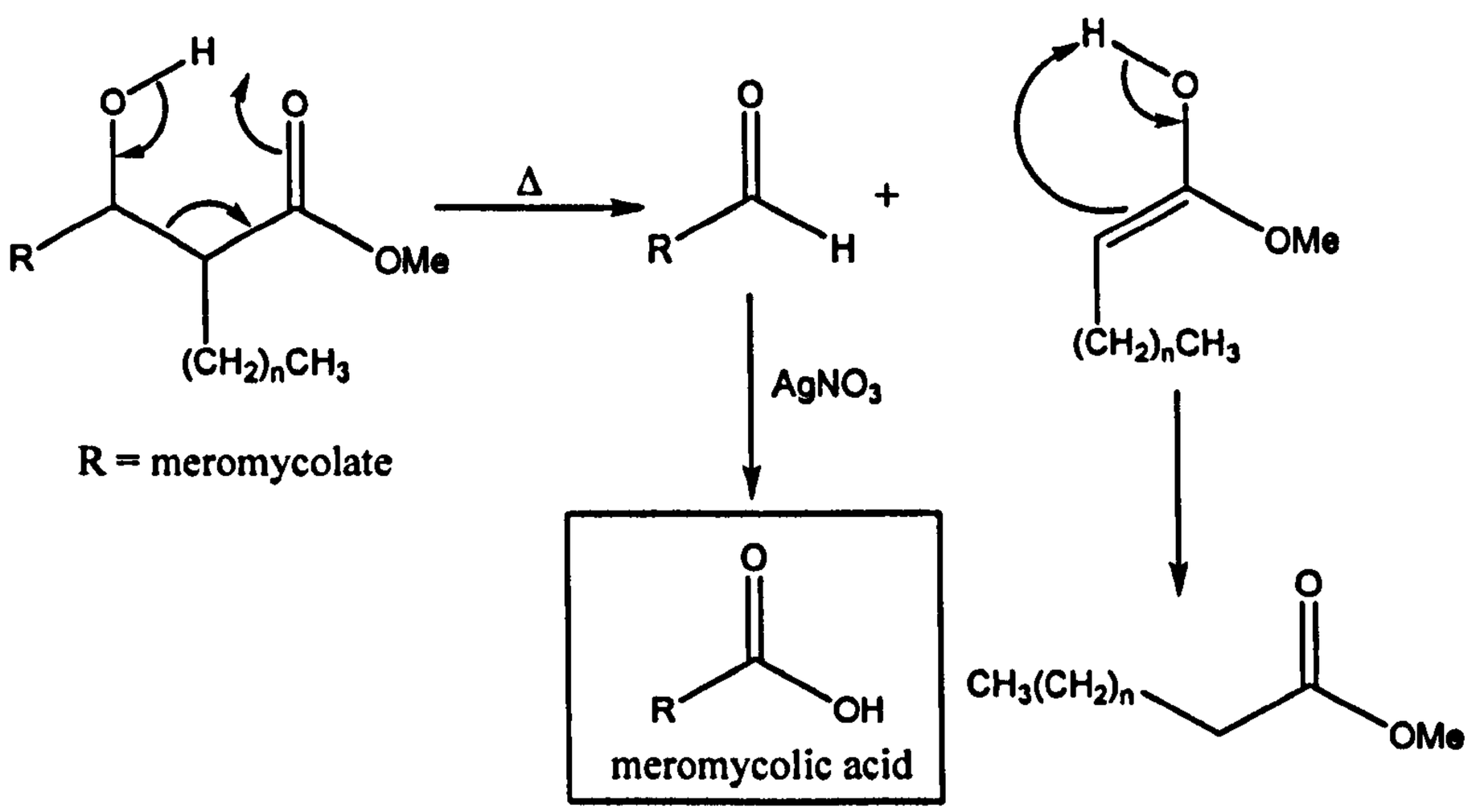
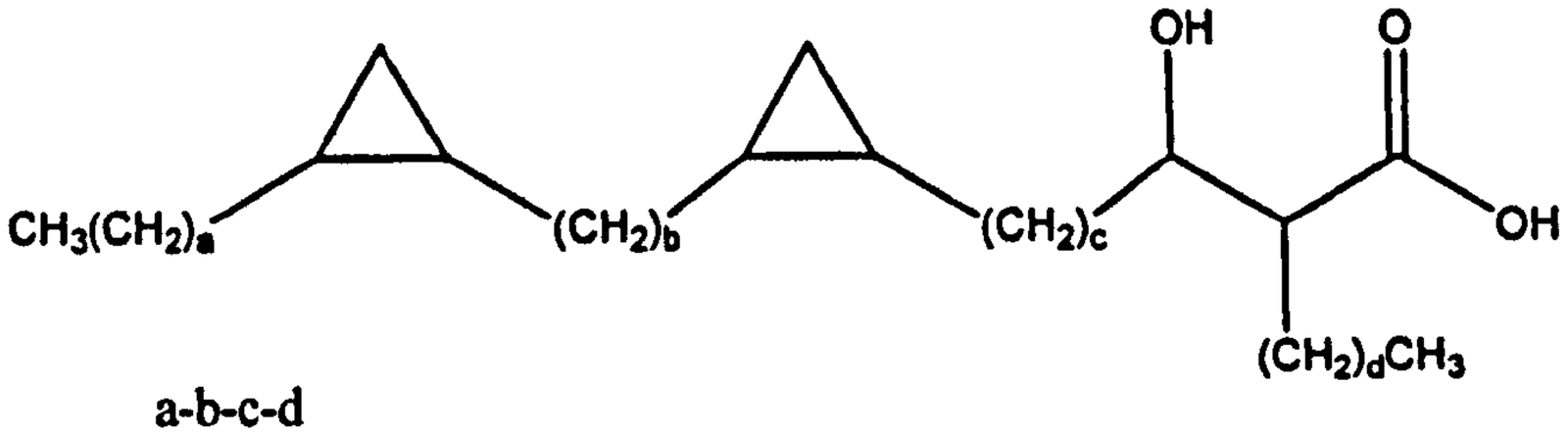
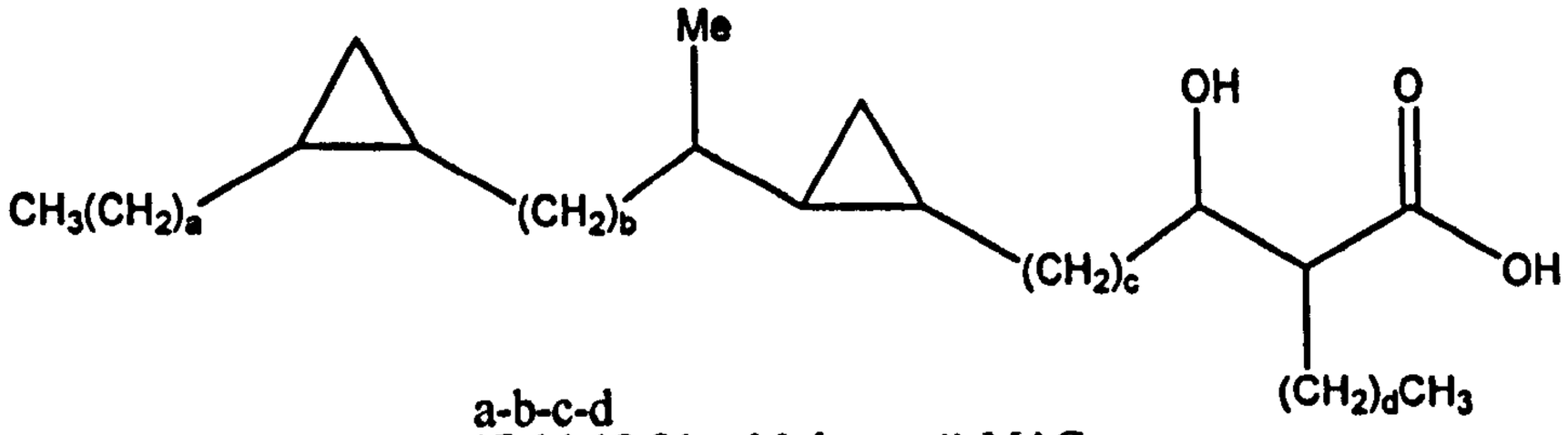


Fig. 16: Meromycolic acid preparation

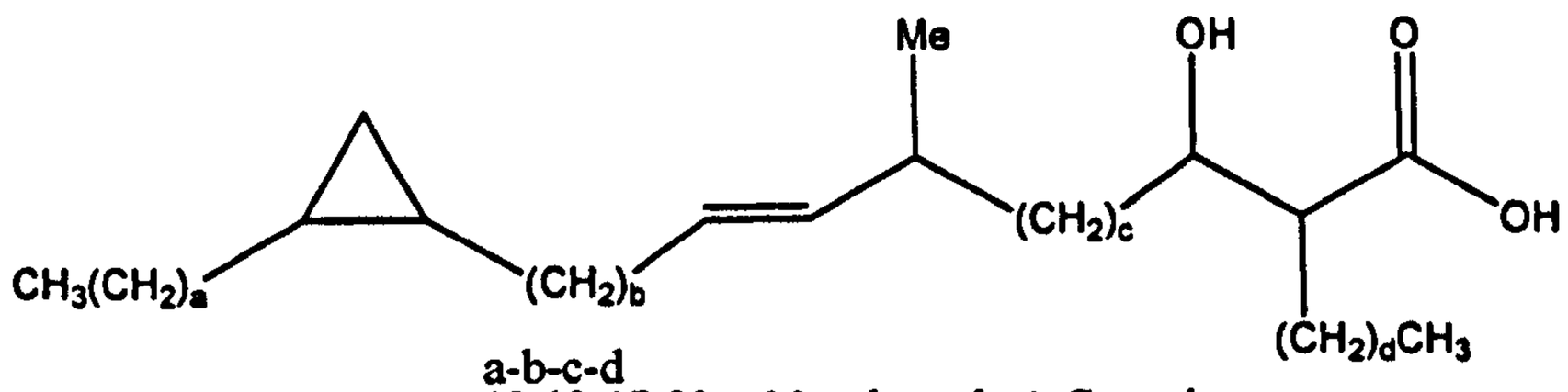
Analysis of the meromycolic acid was carried out by Watanabe *et al.*, utilised CID-MS as meromycolic acids have a stable charged region. This is due to a carboxylate anion being at the end of the meromycolate chain, which enabled Watanabe *et al.* to gather information on the positions of the meromycolate functional groups, hence allowing them to determine a series of chain lengths for each mycolate present.[130] Of the ten mycobacteria that Watanabe *et al.* studied they catalogued over 50 different mycolic acids present, including α-mycolates (See Fig. 17), methoxymycolates (See Fig. 18) and ketomycolates (See Fig.19).



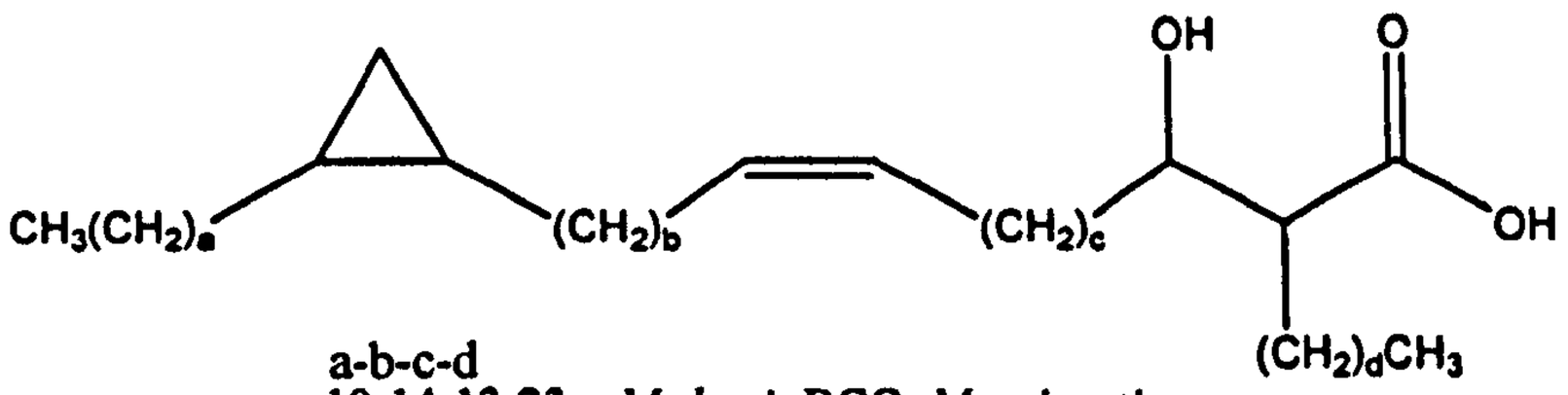
a-b-c-d
19-14-11(13)-23 *M. tuberculosis, M. bovis, M. bovis* BCG, *M. microti*
17-14-17-21 *M. kansasii*, MAC, *M. scrofulacem*
17-14-13-21 *M. marinum*



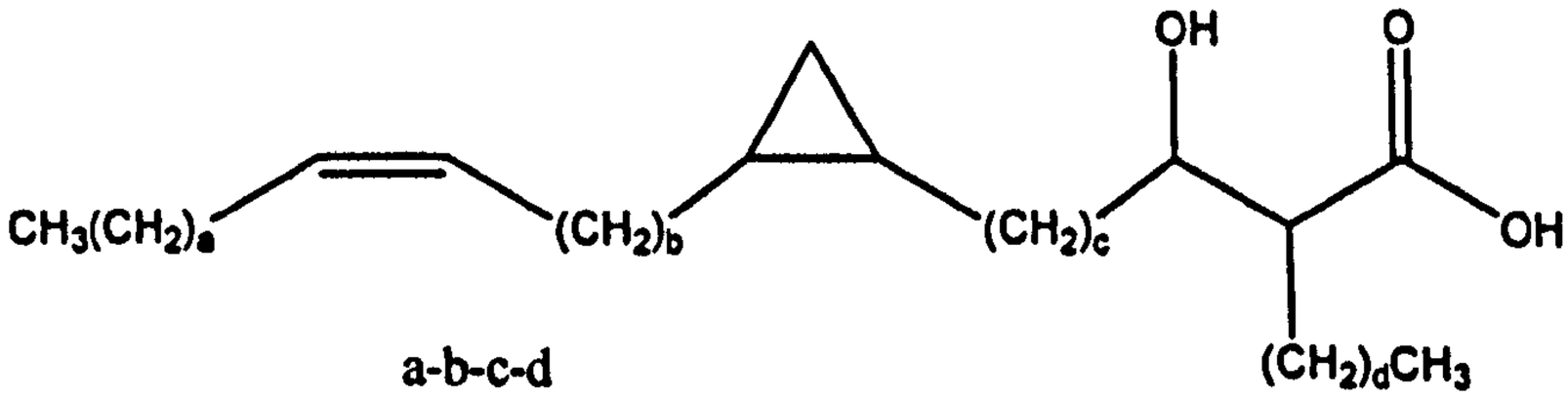
a-b-c-d
17-14-18-21 *M. kansasii*, MAC



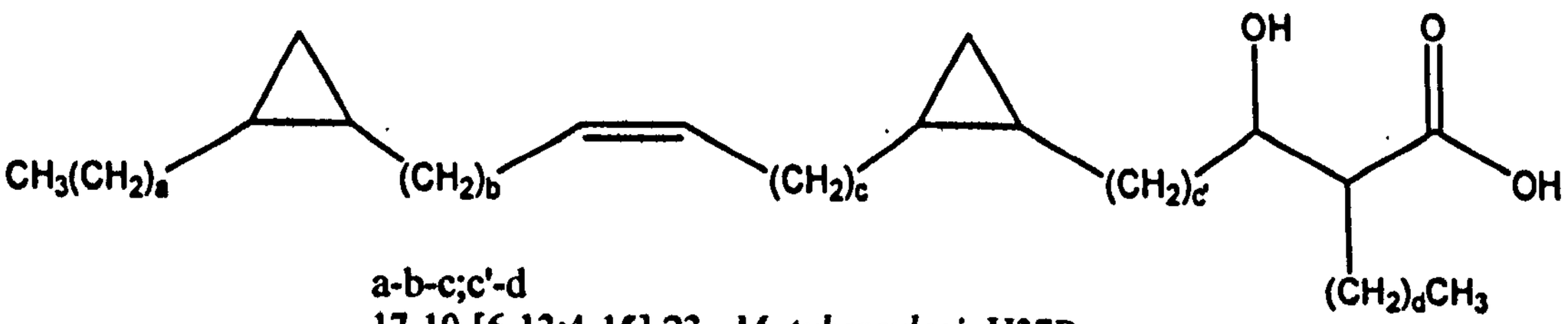
a-b-c-d
19-13-17-23 *M. tuberculosis* Canetti
17-15-17-21 MAC
17-13-17-21 *M. scrofulaceum*



a-b-c-d
19-14-13-23 *M. bovis* BCG, *M. microti*

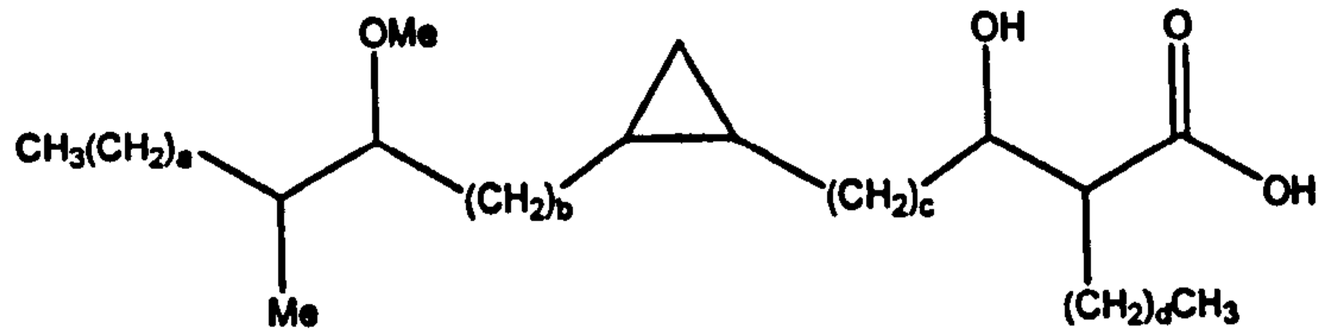


a-b-c-d
19-14-13(11)-23 *M. tuberculosis*
17-14-17-21 MAC
17-14-15-21 *M. marinum*

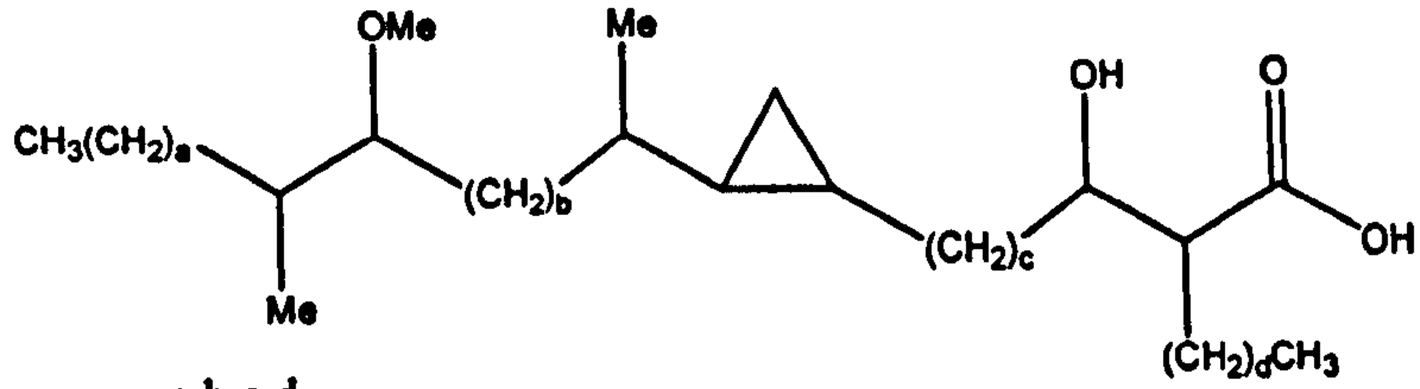


a-b-c;c'-d
17-10-[6-13;4-15]-23 *M. tuberculosis* H37Ra

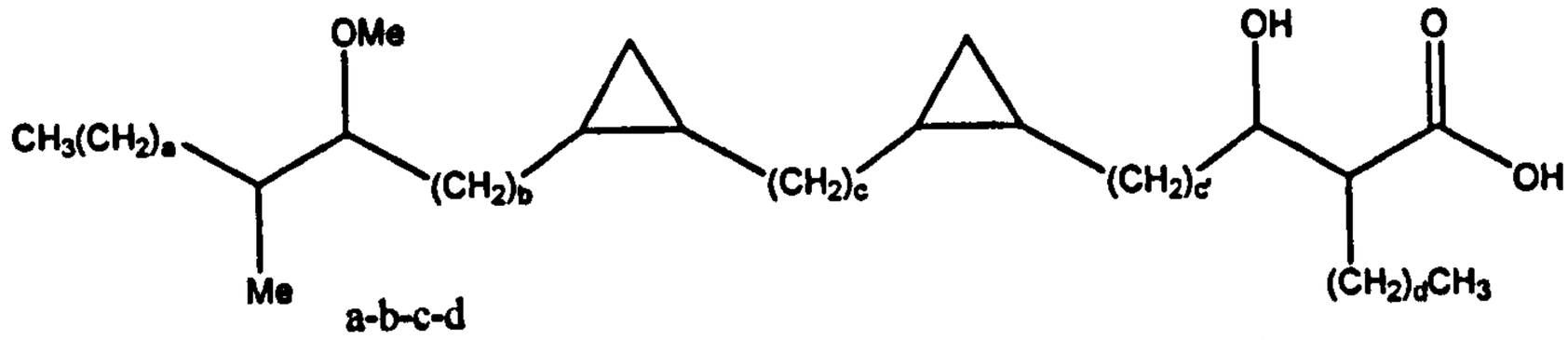
Fig.17: Major α-mycolates[130]



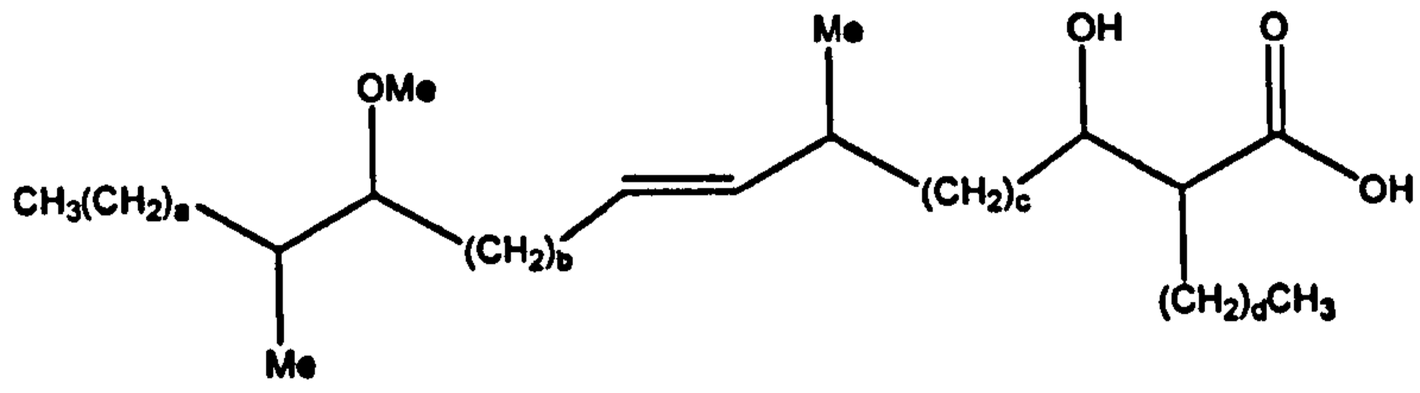
a-b-c-d
17-16-17-23 *M. tuberculosis*, *M. bovis* BCG, *M. microti*
15-14-19-23 *M. tuberculosis* H37Ra
17-16-19(17)-21 *M. kansasii*



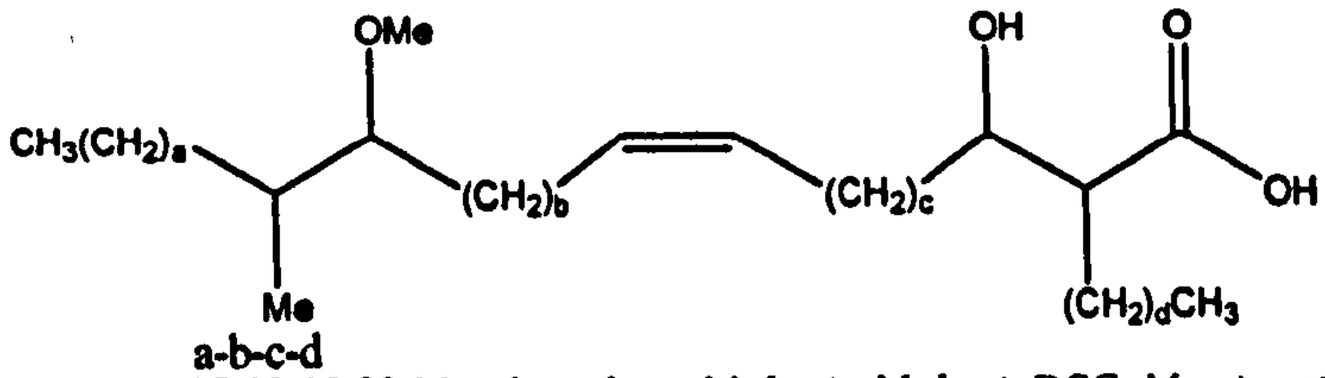
a-b-c-d
17-16-18-23 *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. microti*
17-16-22(20)-23 *M. tuberculosis*, Canetti
17-16-18(20)-21 *M. kansasii*



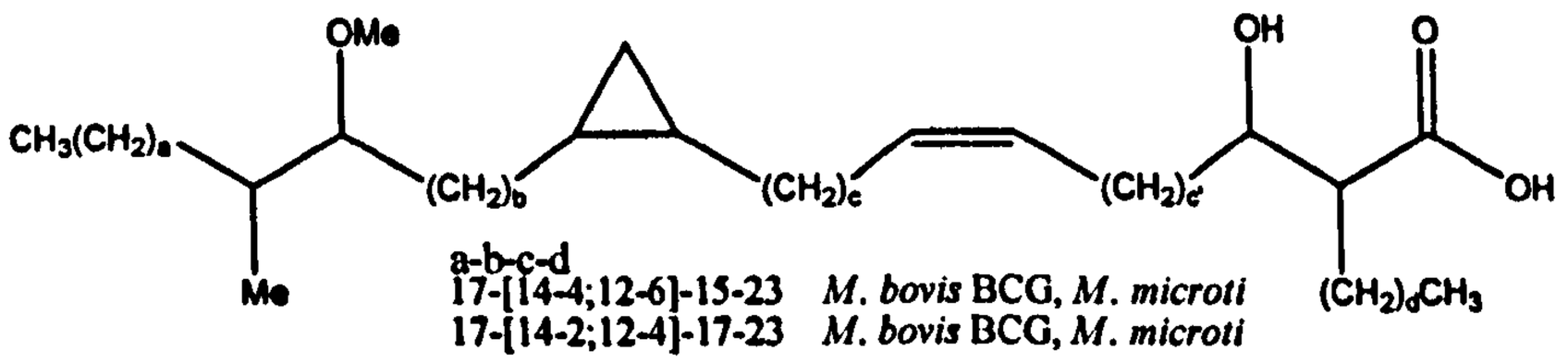
a-b-c-d
[15-10;17-8]-[12-11;10-13;8-15]-23 *M. tuberculosis* H37Ra
[15-12;17-10]-[10-11;8-13;6-15]-23 *M. tuberculosis* H37Ra



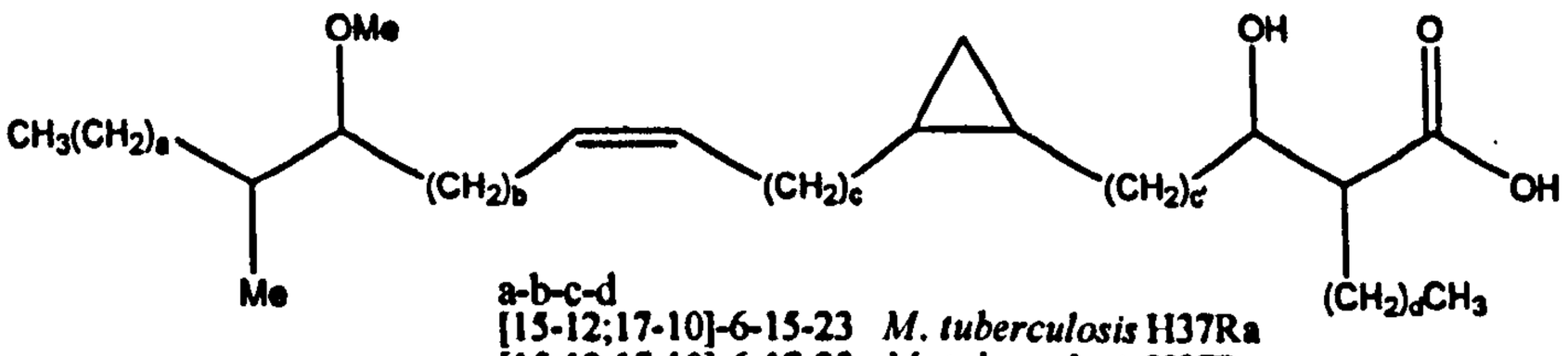
a-b-c-d
17-19-13-23 *M. tuberculosis*, *M. bovis*, *M. bovis* BCG
17-19-15-23 *M. tuberculosis* Canetti
17-15-17-21 *M. marinum*



a-b-c-d
17-18-15-23 *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. microti*

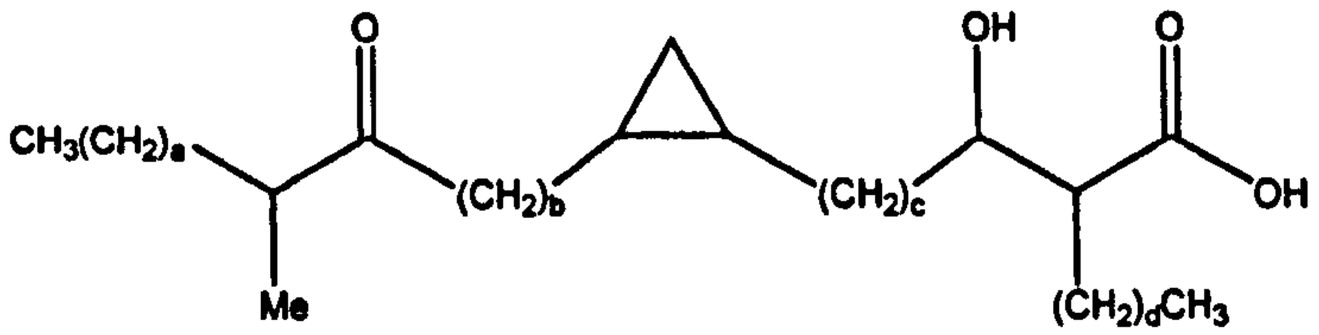


a-b-c-d
17-[14-4;12-6]-15-23 *M. bovis* BCG, *M. microti*
17-[14-2;12-4]-17-23 *M. bovis* BCG, *M. microti*

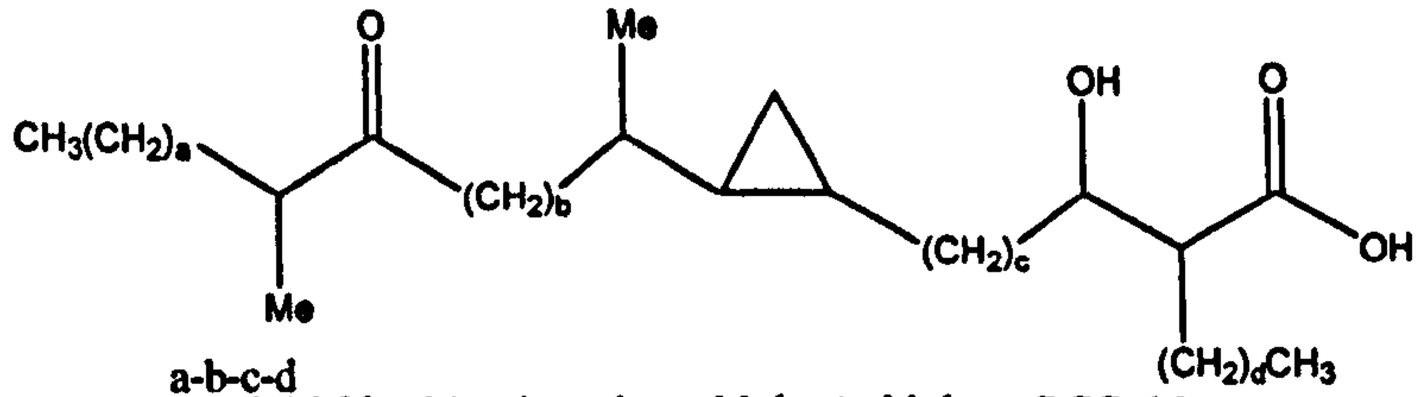


a-b-c-d
[15-12;17-10]-6-15-23 *M. tuberculosis* H37Ra
[15-12;17-10]-6-17-23 *M. tuberculosis* H37Ra

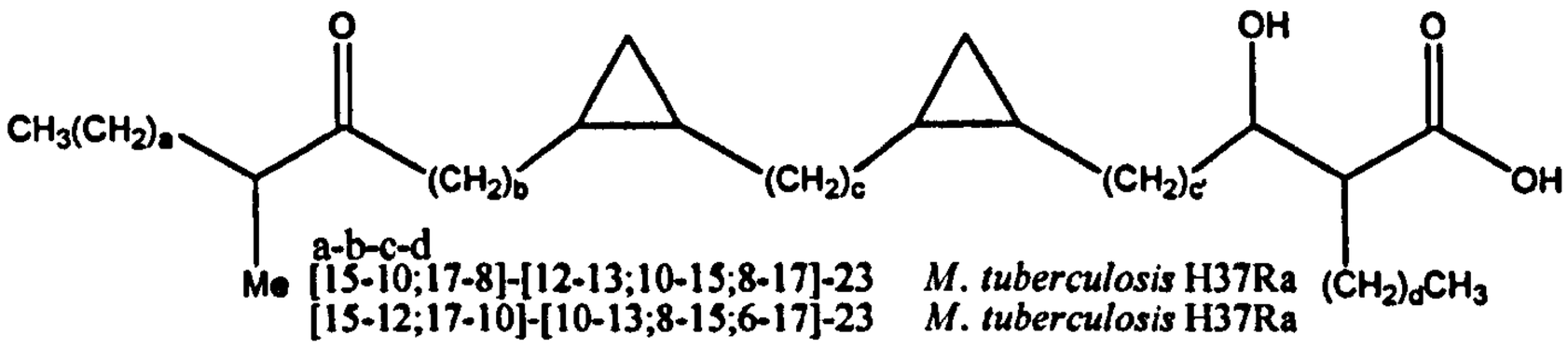
Fig. 18: Major methoxymycolates[130]



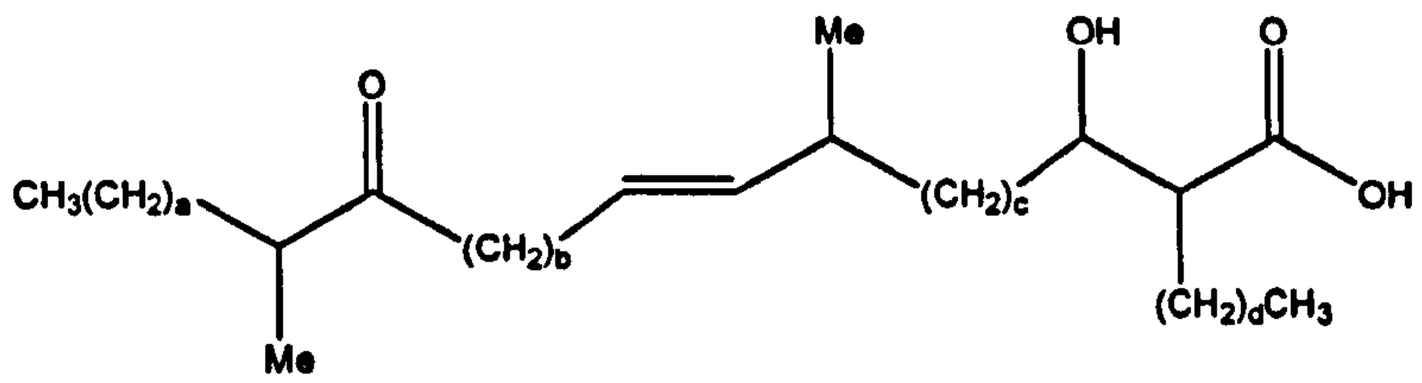
a-b-c-d
17-18-15-23 *M. tuberculosis, M. bovis, M. bovis* BCG, *M. microti*
15-16-19-23 *M. tuberculosis* H37Ra
17-20-17-21 *M. kansasii*
15-18-17-21 MAC
15-16-19-21 *M. scrofulaceum*



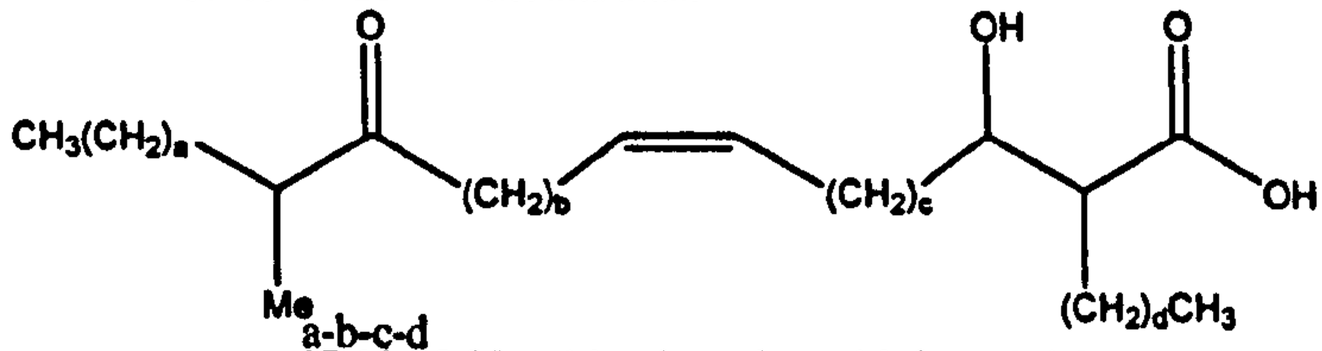
a-b-c-d
17-18-16-23 *M. tuberculosis, M. bovis, M. bovis* BCG, *M. microti*
15-18-18-21 *M. kansasii*
17-16-18-21 MAC
15-16-18-21 *M. scrofulaceum*



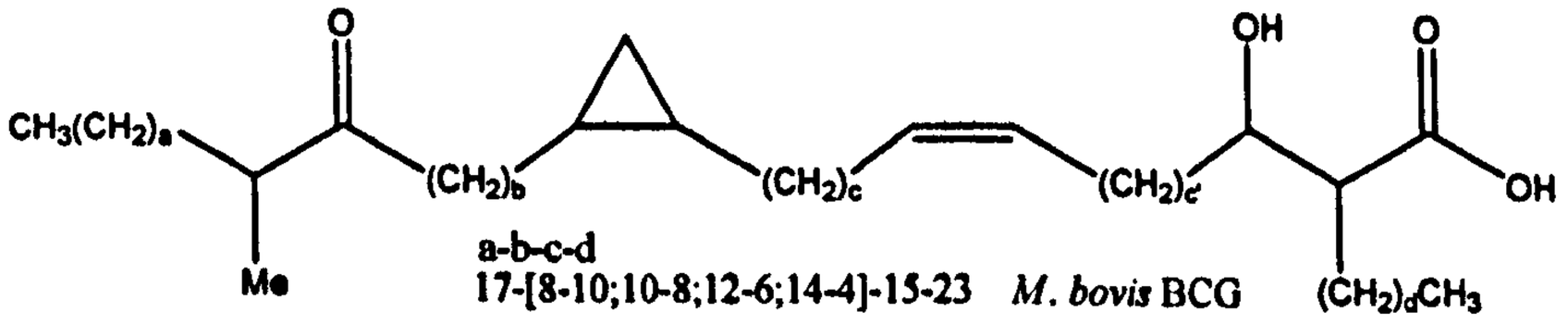
a-b-c-d
[15-10;17-8]-[12-13;10-15;8-17]-23 *M. tuberculosis* H37Ra
[15-12;17-10]-[10-13;8-15;6-17]-23 *M. tuberculosis* H37Ra



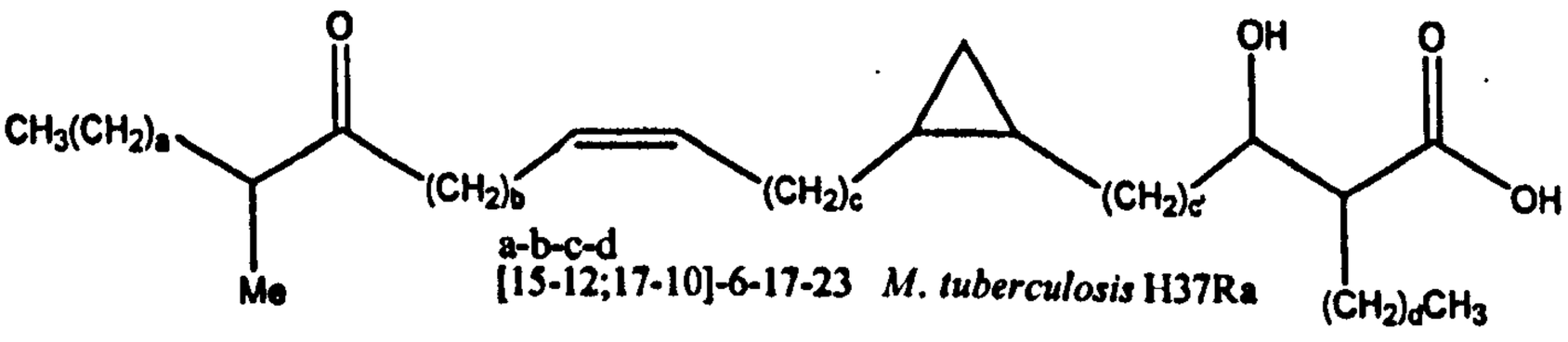
a-b-c-d
17-19-15(13)-23 *M. tuberculosis, M. bovis, M. bovis* BCG, *M. microti*
17-17-17-23 *M. tuberculosis,* Canetti
17-17-17-21 MAC
15-17-17-21 *M. scrofulaceum*
17-15-17-21 *M. marinum*



a-b-c-d
17-18-15-23 *M. tuberculosis, M. bovis* BCG
17-16-19-21 MAC



a-b-c-d
17-[8-10;10-8;12-6;14-4]-15-23 *M. bovis* BCG



a-b-c-d
[15-12;17-10]-6-17-23 *M. tuberculosis* H37Ra

Fig. 19: Major ketomycolates[130]

Mycolic acids occur within all mycobacteria, in varying combinations of functionality type and chain length.[129, 139] Therefore during diagnosis of mycobacterial disease mycolic acids can be useful in predicting if the disease is tubercular or non-tubercular. Methods have been developed using HPLC to identify characteristic mycolic acids specific to one *Mycobacterium*.[140, 141]

Minnikin[88] and Draper[89] both noted that mycolic acids are directly esterified to the arabinogalactan layer with the cell envelope, with the meromycolate chains possibly in a linear conformation facing outward toward the outer surface of the cell. The configuration of the mycolic acid chains are now believed to be able to assume a folded form, with the four alkyl chains occurring parallel to each other (See Fig. 20).[142-145]

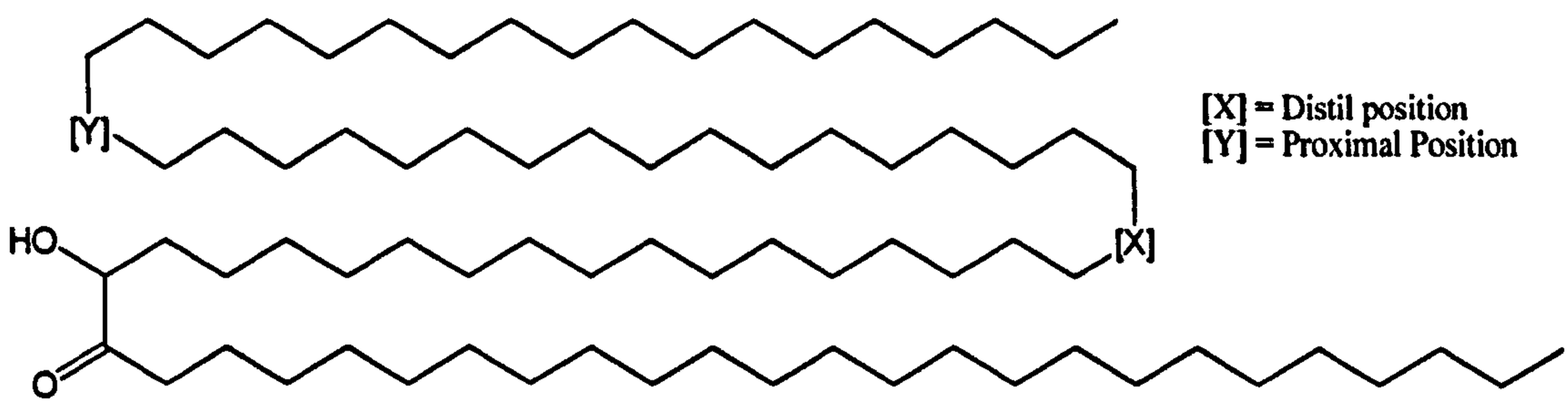


Fig. 20: Mycolic acid folding conformation

Villeneuve *et al.* and Hasegawa *et al.* both discussed the changes which occur in configuration of the alkyl chains when put under varying temperatures and pressure.[142-145] This suggests that at low temperature and pressure the folding configuration observed above (Fig. 20) is retained, however as temperature and pressure is increased this linearity is lost. E.P. Grant *et al.* also suggested that the alkyl chains of oxygenated mycolic acids line up in a linear fashion.[146] The rationale behind this is that this folding configuration would cause the oxygenated groups to form an epitope, acting as a site for recognition within the immune system.

Significant work has been carried out to determine the absolute stereochemistry in mycolic acids, however this work is ongoing. It has been determined that the alkyl chain and hydroxy group, found in the α and β-positions to the carboxylic acid, are found to both be in an *R*-configuration in all mycolic acids which have been investigated (See Fig. 21).[124, 126-128, 147, 148]

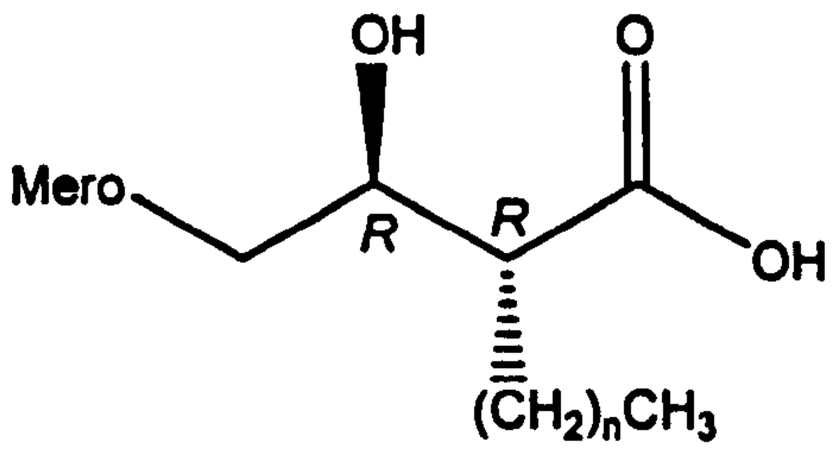


Fig. 21: Configuration of the α-branched β-hydroxy acid unit

The configuration at these two chiral centres is believed to play an important role in T cell recognition.[149] It is believed that the oxygenated mycolic acids of the *M.Tb* complex exhibit distal methyl branches and distal oxygenated functionalities in the *S*-configuration (See Fig. 22), which provides us with a biosynthetic link between the three types.[135, 150]

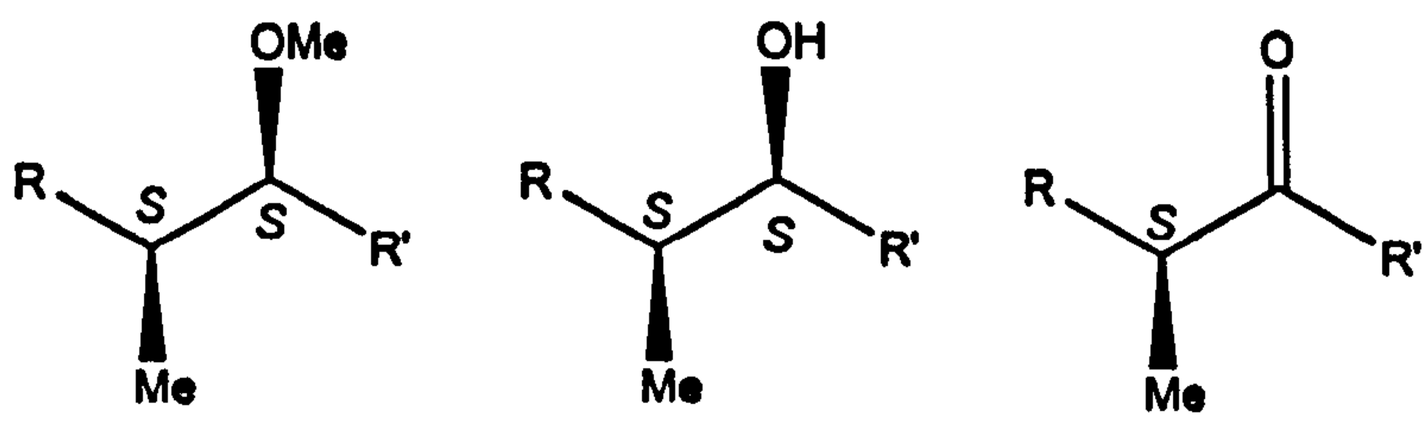


Fig. 22: Oxygenated mycolic acid configurations

Other distal functionalities have known stereochemistries, with the distal epoxy group, which C. Lacave *et al.* report showing *R*-configurations for all three chiral centres. Further inspection of this report shows that they have actually misinterpreted their own evidence, due to improper use of the Cahn-Ingold-Prelog priority rules, and the epoxy group is actually occurring in the *R,S,S* configuration. A recent report by M. S. Baird *et al.* gives a synthetic strategy to obtain the epoxy group in the correct configuration.[151, 152] The wax ester occurs with the methyl group in the *S*-configuration (See Fig. 23), the formation of the wax ester is also believed to be via an enzymatic oxidation of the *S*-keto mycolic acid.[118, 150, 152]

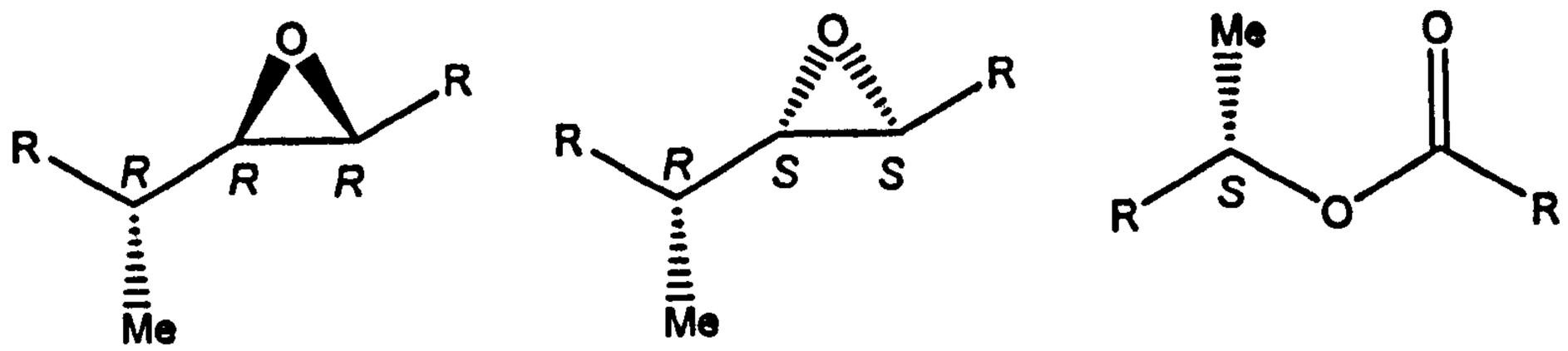


Fig. 23: Epoxy and wax ester mycolic acid configurations

Little is known regarding the non-oxygenated functionalities of mycolic acids, particularly in the case of *cis*-cyclopropanes. The α-methyl of the *trans*-alkene unit, present in the mycolic acids of the *M.Tb* complex, is known to be in the *R*-configuration (See Fig. 24).[130, 152]

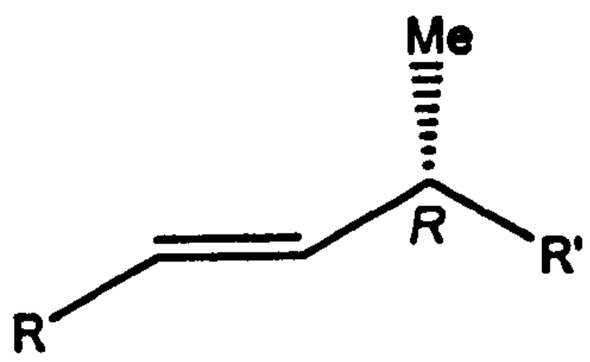


Fig. 24: α-Methyl-*trans*-alkene mycolic acid configuration

However a report discussed a synthetic strategy targeting multiple diastereomers of the α-methyl-*trans*-cyclopropane unit. After analysis of the optical rotation, ^{1}H NMR and ^{13}C NMR, in comparison with work carried out by Anderson *et al.*, M.S. Baird *et al.* deduced that the α-methyl-*trans*-cyclopropane unit is found in the *S,R,S*-configuration (See Fig. 25).[153, 154]

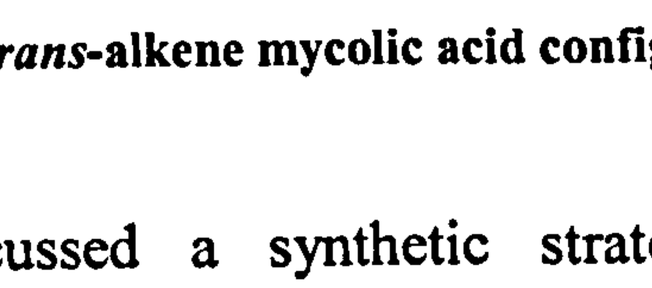


Fig. 25: α-Methyl-*trans*-cyclopropane mycolic acid configuration

The configurations discussed above were deduced using measured molecular rotations (M_D), where M_D can be calculated from measured optical rotation and molecular weight of a compound. Interestingly, it is possible to calculate a

theoretical value for a complete mycolic acid by adding the contributions from each chiral centre.[135]

It has been discovered that the functionality present in the mycolate plays a role in the fluidity of the cell wall and permeability.[155, 156] It is also known that α-mycolates occur in higher amounts than any other subclass of mycolate in *M.Tb*, and that the presence of a cyclopropane ring plays a significant role in its pathogenesis.[129, 157] The cyclopropanes of mycolic acids are able to withstand ozonolysis and other oxidative environments better than their olefin counterparts, which would explain why they are in high abundance within mycobacteria.[158] In the biosynthetic growth of *trans*-cyclopropane containing mycolates a higher proportion of oxygenated mycolates are recorded, which provides evidence to suggest that *trans*-cyclopropanes and oxygenated functionalities are biosynthetically related.[159]

Keto-mycolates have been shown to play key roles in the virulence and in regulating the fluidity of the cell wall of *M.Tb.*[160, 161] It is also known that slow-growing pathogens, such as *M.Tb*, are able to manipulate the proportions of methoxy and keto-mycolates. This enables the pathogen to control the permeability properties of the *Mycobacterium's* cell wall; it is believed that the pathogen does this so that it is better adapted to its environment.[161, 162] It is also known that keto-mycolates behave differently to α-mycolates when subjected to changes in pressure in monolayers; where the α-mycolates are seen to be fully extended at high pressure, keto-mycolates do not exhibit extension to the same extent.[143-145] Therefore it is believed that keto-mycolates play an important role in permeability of the cell wall.[144] It has been documented that loss of the methoxy-mycolates does not have an adverse affect on the pathogen's permeability and hence its resistance to therapeutic agents.[163]

The presence of *cis*-olefins in the meromycolate chain has an effect on the packing of the mycolates in the cell wall, causing "a permanent elbow of about 120°". Whereas a *trans*-olefin would not cause as much disruption in the chain, however due to the presence of a methyl group adjacent to the olefin there is some distruption in the chain, with packing tighter than that of *cis*-olefin containing mycolic acids.[137] It has also been recorded that a higher content of *trans*-olefins is seen if the mycobacterium is grown at increased temperatures; this also suggests that the mycobacterium regulates the production of olefins to favour a more rigid cell wall for additional protection from the external environment.[156]

Studies on the function of mycolic acids are ongoing, however further understanding of the effects that functionality have on the fluidity and permeability of the cell wall may give an insight into possible anti-mycobacterial agents.

1.4.2 – Biosynthesis

The biosynthesis of mycolic acids has been investigated in great detail by J. Krembel and A.H. Etémadi, Y. Yuan *et al.*, E. Dubnau *et al.*, C. Asselineau *et al.*, K. George *et al.* and M. Daffé *et al.*.[137, 158, 159, 164-166] The work carried out by these groups identified that most major functionalities in the meromycolate are derived from a common intermediate. This intermediate is generated using *S*-adenosyl-*L*-methionine (SAM) **(11)**. Methylation of a *cis*-olefin **(12)**, using SAM, gives the carbocation intermediate **(13)** (See Fig.26).

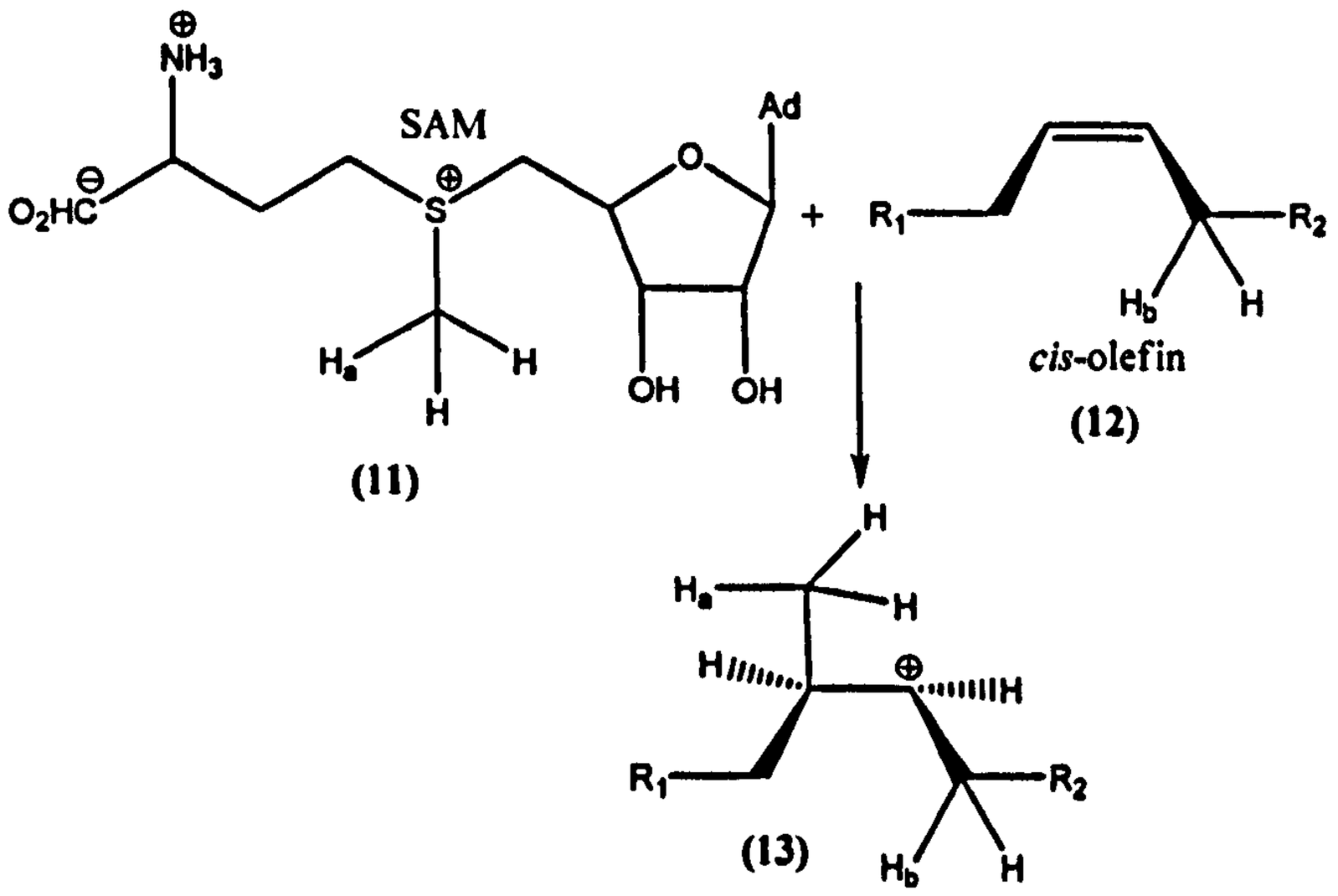


Fig.26: Formation of carbocation intermediate (13) in biological methylations

Further work has been carried out where labelled SAM has been used so that the methylation and any subsequent reactions can be tracked. This has shown that the methyl group introduced via SAM occurs as a bridging methylene in *cis*-cyclopropanes, as methyl branches of *trans*-olefins and cyclopropanes, and the α-methyl branches of hydroxy, keto and methoxy-mycolates.[152, 167-169]

Deprotonation of the carbocation intermediate **(13)** may occur at two different positions, yielding two different products. If deprotonation of H_a occurs, a

cis-cyclopropane **(14)** will result; conversely if deprotonation of H_b occurs then the *trans*-olefin **(15)** is yielded. Further methylation of the *trans*-olefin, involving SAM, gives the *trans*-cyclopropane **(16)**. If the carbocation intermediate **(13)** undergoes a hydration reaction the hydroxy-mycolate **(17)** is formed, which is a precursor for the biosynthesis of the corresponding keto **(18)** and methoxy **(19)** mycolates (See Fig. 27).[137, 162]

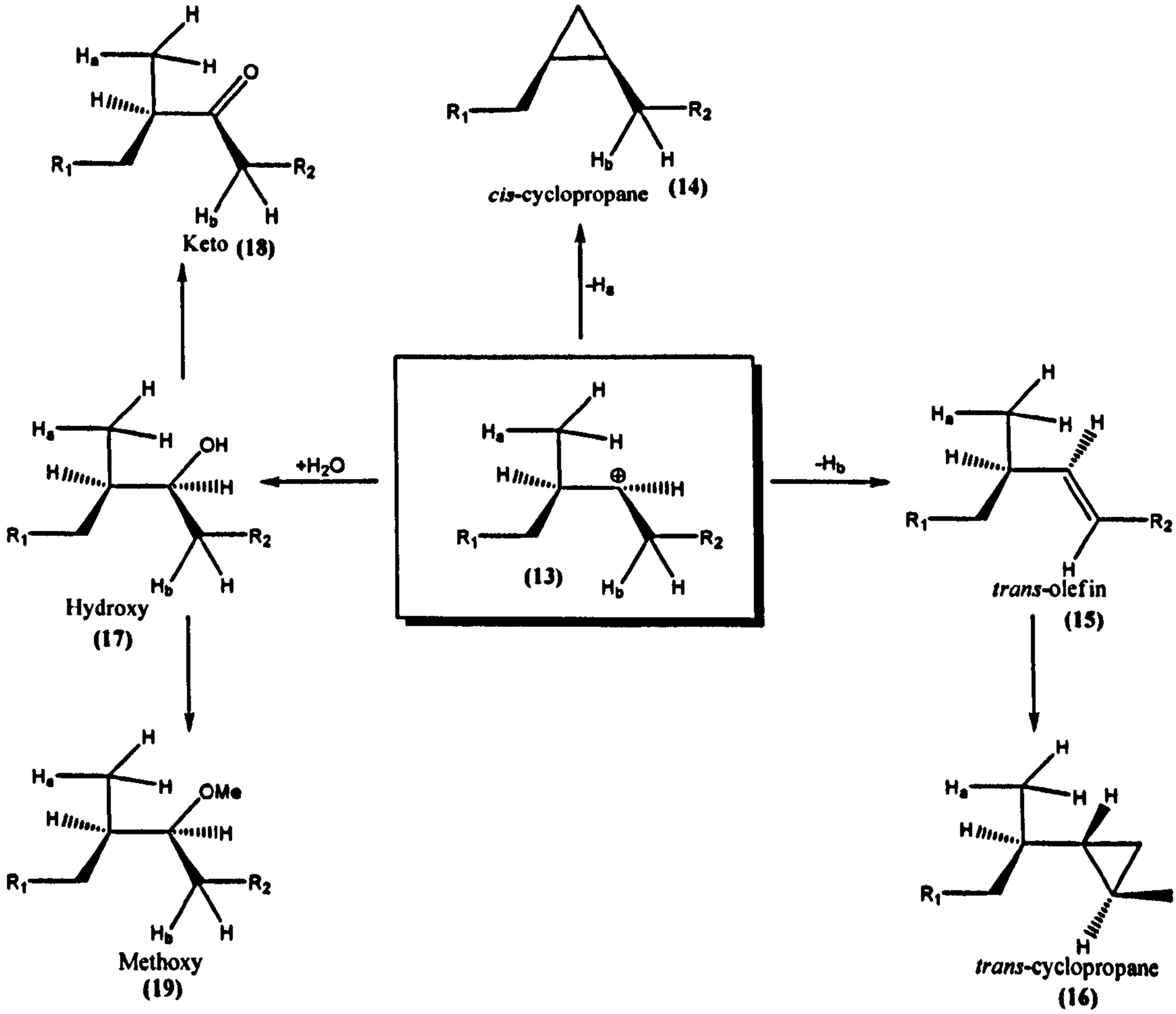


Fig. 27: Biosynthesis of meromycolate functionality

It is interesting that in *M.Tb*, the gene which is required for the biosynthesis of *trans*-cyclopropyl mycolates is active in the production of both keto *trans*-cyclopropyl mycolates and methoxy *trans*-cyclopropyl mycolates.[170] This is of particular importance as it confirms the hypothesized biosynthetic link between the different functionalities. As previously discussed, understanding the impact of individual functionality on virulence, cell wall fluidity and permeability, and how the

different mycolates are naturally prepared is important as it enables us to determine how to manipulate the cell wall of the pathogen, and thus provide us with a tool for which to combat disease.[170]

1.4.3 – Mycolic acid synthesis

Mycolic acids have been of interest to a variety of research groups, particularly from a synthetic point of view. Synthesizing single enantiomers of mycolic acid can help in the determination of stereochemistries of naturally occurring mycolic acids. Preparing mycolic acids with known chiral centres in the meromycolate chain can also help to gain a further understanding of the biosynthesis of mycolic acids. It has previously been shown that the length of the meromycolate chain and the functionalities present play an important role in the functions of the cell wall, so a better understanding of the affects of the mycolic acid content may lead to a new approach in the therapy of mycobacterial disease.[171]

An important application of synthetic mycolic acids is in TB therapy. As discussed earlier, the recurrence of TB and the co-infection with HIV has become a major cause for concern in developing countries. One application of synthetic mycolic acids, which might help alleviate these problems, is in detection of TB causing mycobacteria. It is believed that there are individual mycolic acids which are exclusive to one *Mycobacterium*; therefore, if the structure of these mycolic acids were to be known an enantiomerically pure synthetic compound may be produced and compared to a sample of the mycolic acids obtained from the mycobacteria infecting the patient. Ultimately, every exclusive mycolic acid may be prepared and used in this way to give a definitive diagnosis technique, which would enable medical practitioners to treat mycobacterial disease more efficiently. Another application of mycolic acids is as an adjuvant. An immunologist called J.T. Freund used an extract of the cell envelope of *M.Tb* as an immunologic adjuvant, which promotes dendritic cell response within the body. Experimentally it was believed that Freund's adjuvant could prevent the development of diabetes in children. However many patients developed symptoms of TB and as a result Freund's adjuvant was banned.[172, 173] Mycolic acids have been shown to produce an antibody response similar to that of a TB infection, therefore it may be possible to replace Freund's adjuvant with mycolic acids.[149]

Another area of interest is asthma, where mycolic acids have been shown to produce a positive immune response when directly applied to the airway of a mouse.[113, 114]

Mycolic acids are generically considered to be made up of a meromycolate and a branched hydroxy acid, where the meromycolate contains two major functionalities. When synthesising a mycolic acid it is sensible to address each motif separately.

One of the first syntheses was conducted by W.J. Gensler *et al.* in 1977.[174] They cyclopropanated 1,4-cyclohexadiene **(20)** giving noracarene **(21)**; ozonolysis, followed by a reduction of **(21)** gave *cis*-1,2-cyclopropanediol **(22)**.[175] Protection of the diol **(22)** with a tetrahydropyranyl group, followed by bromination of the resultant alcohol gave **(23)**. Chain extension of the bromide **(23)** was carried out using 2-pentadecyl-1,3-dithiane **(24)**, which was obtained via alkylation of the lithio derivative of 1,3-dithiane with pentadecylbromide, giving the 2,2-disubstituted dithane **(25)**. Desulfurization[176] of **(25)**, followed by hydrolysis, then bromination gave the corresponding bromide **(26)**. Further chain extension using the *bis*-dithiane **(27)** gave **(28)**, which Gensler *et al.* considered the first major component, they called the "Methyl End", of their target meromycolate (See Fig. 28).[174]

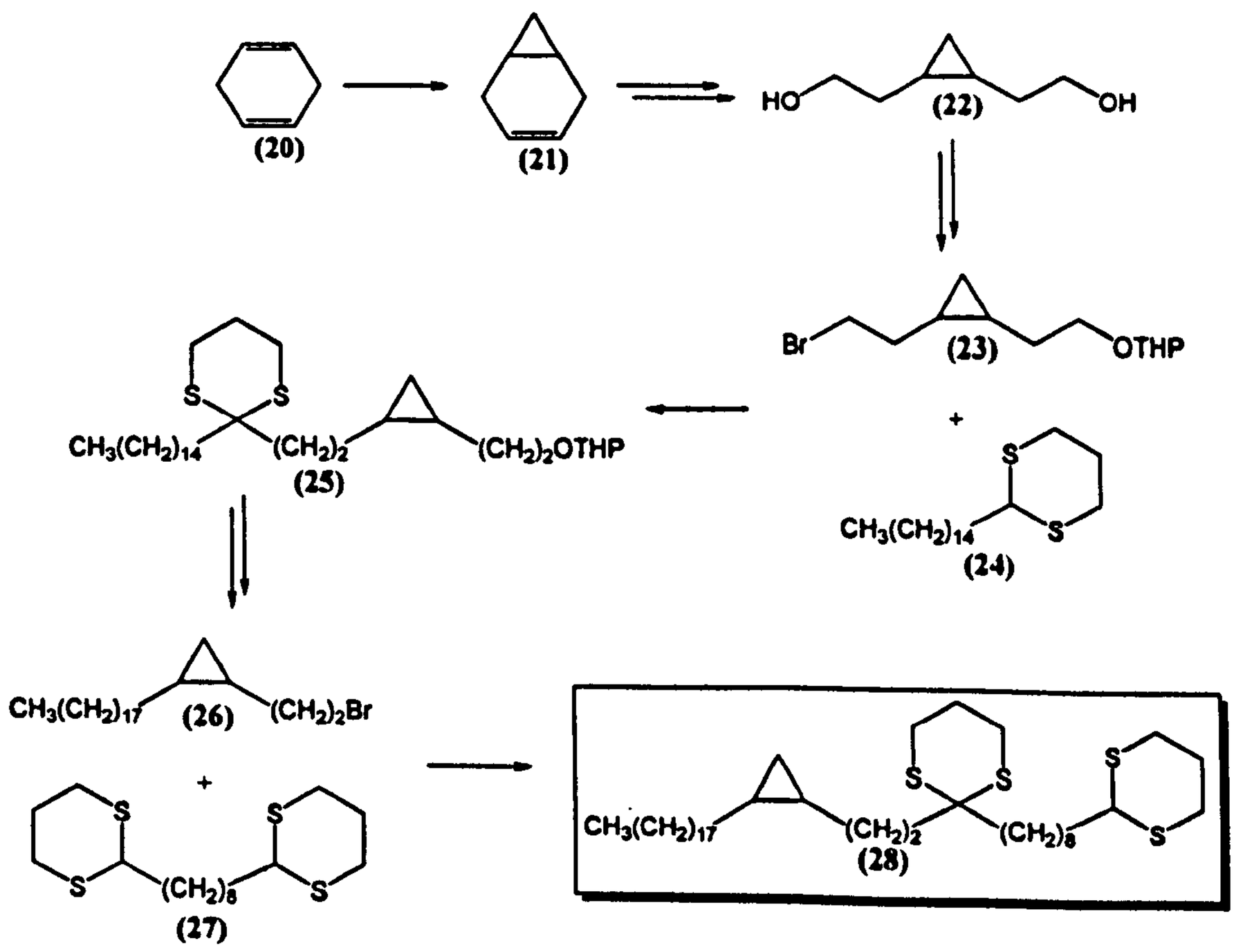


Fig. 28: Gensler *et al.*'s "Methyl End"

The second major constituent of Gensler *et al.*'s meromycolate, called the "Carboxyl End", was prepared starting with ozonoylsis of 10-undecenol **(29)**; conversion of the corresponding alcohol into the acetal was then carried out, followed by bromination to give **(30)**. Chain extension by six carbons gave **(32)**, then coupling of this to the previously prepared bromide **(23)**, followed by desulfurization, deprotection of the tetrahydropyranyl group **(33)** and bromination gave **(34)**. The acetal **(34)** was Gensler *et al.*'s "Carboxyl End" intermediate (See Fig. 29). Coupling of the lithio derivative of *bis*-dithiane intermediate **(28)** with alkyl bromide **(33)**, followed by desulfurization, via hydrogen and Raney nickel, gave the expected product **(33)**. Ozonolysis of **(34)** gave the corresponding hydroxyether meromycolate ester, and a direct base-catalyzed ester interchange yielded the methyl meromycolate **(35)** (See Fig. 29).[174]

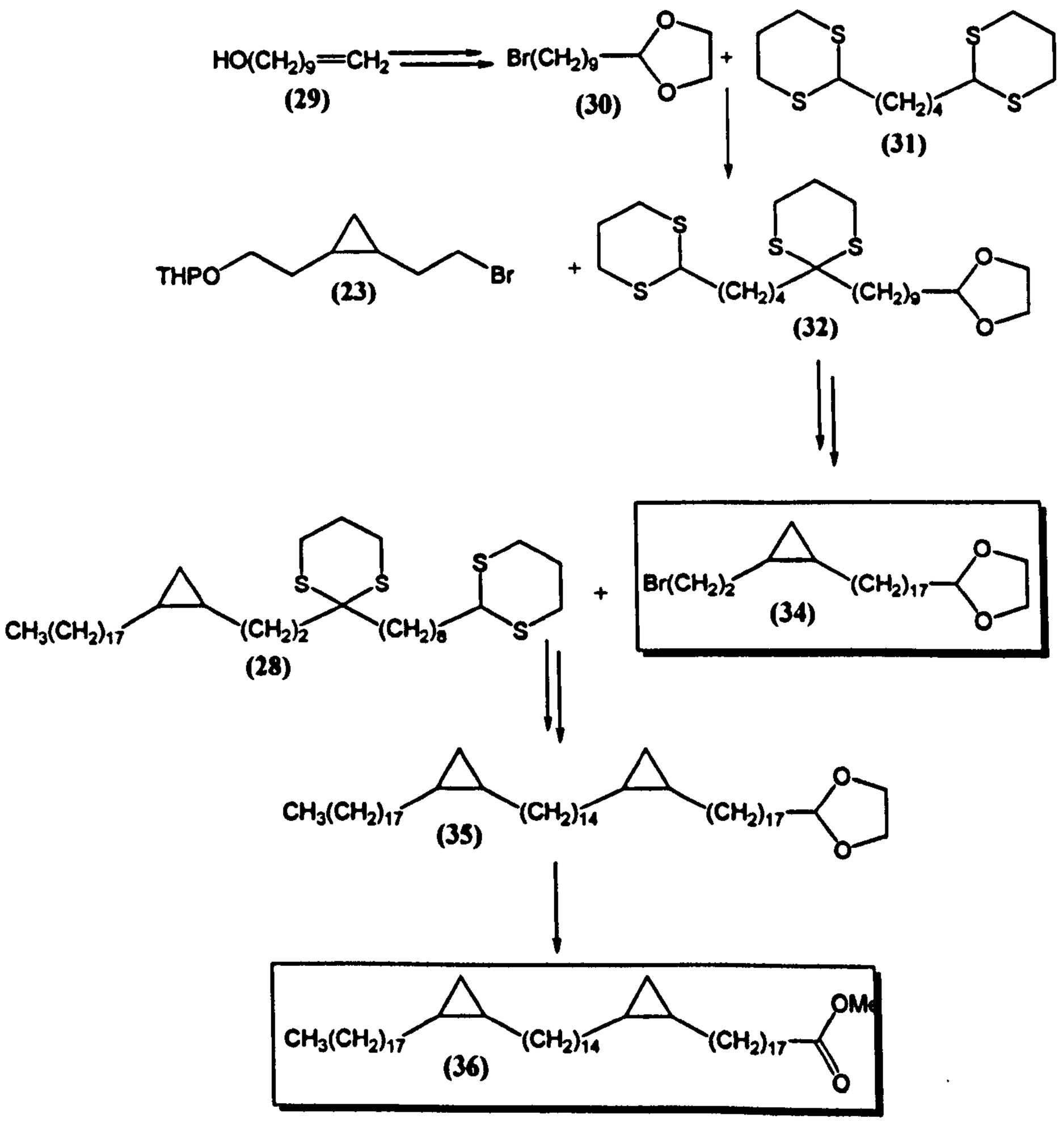
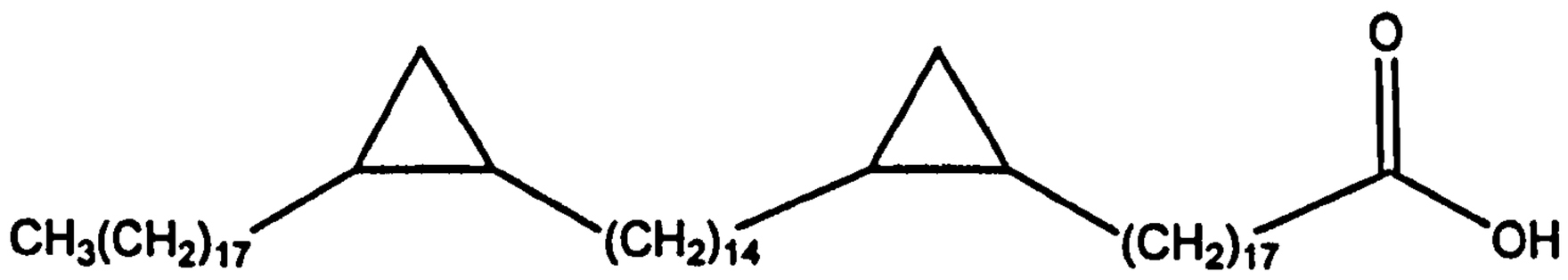


Fig. 29: Gensler *et al.*'s "Carboxyl End" (34) and "Methyl Meromycolate" (36)

Further work by Gensler *et al.*, two years later discussed improved methods in the preparation of a meromycolic acid **(36)** (See Fig. 30), where they utilised Grignard reactions to couple intermediates.[177] Gensler *et al.* were successful in preparing the first meromycolic acid, however the two *cis*-cyclopropane groups were not in a definitive stereochemistry, therefore they were present as a mixture of four stereoisomers.



(36)

Fig. 30: Gensler *et al.*'s "Meromycolic acid"

The first enantiomerically pure meromycolic acid was prepared by M.S. Baird *et al.* in 2000, where they set about preparing single enantiomers of cyclopropane intermediates and then successfully coupled the intermediates together with no loss of stereochemistry.[178] Coxon *et al.* had discussed the synthesis of single enantiomers of cyclopropane intermediates earlier in 1999, and Baird *et al.* exploited this to obtain the first enantiomerically pure meromycolic acid (See Fig. 31).[179] The work by Baird *et al.* pioneered the synthesis of other enantiomerically pure meromycolates.[180]

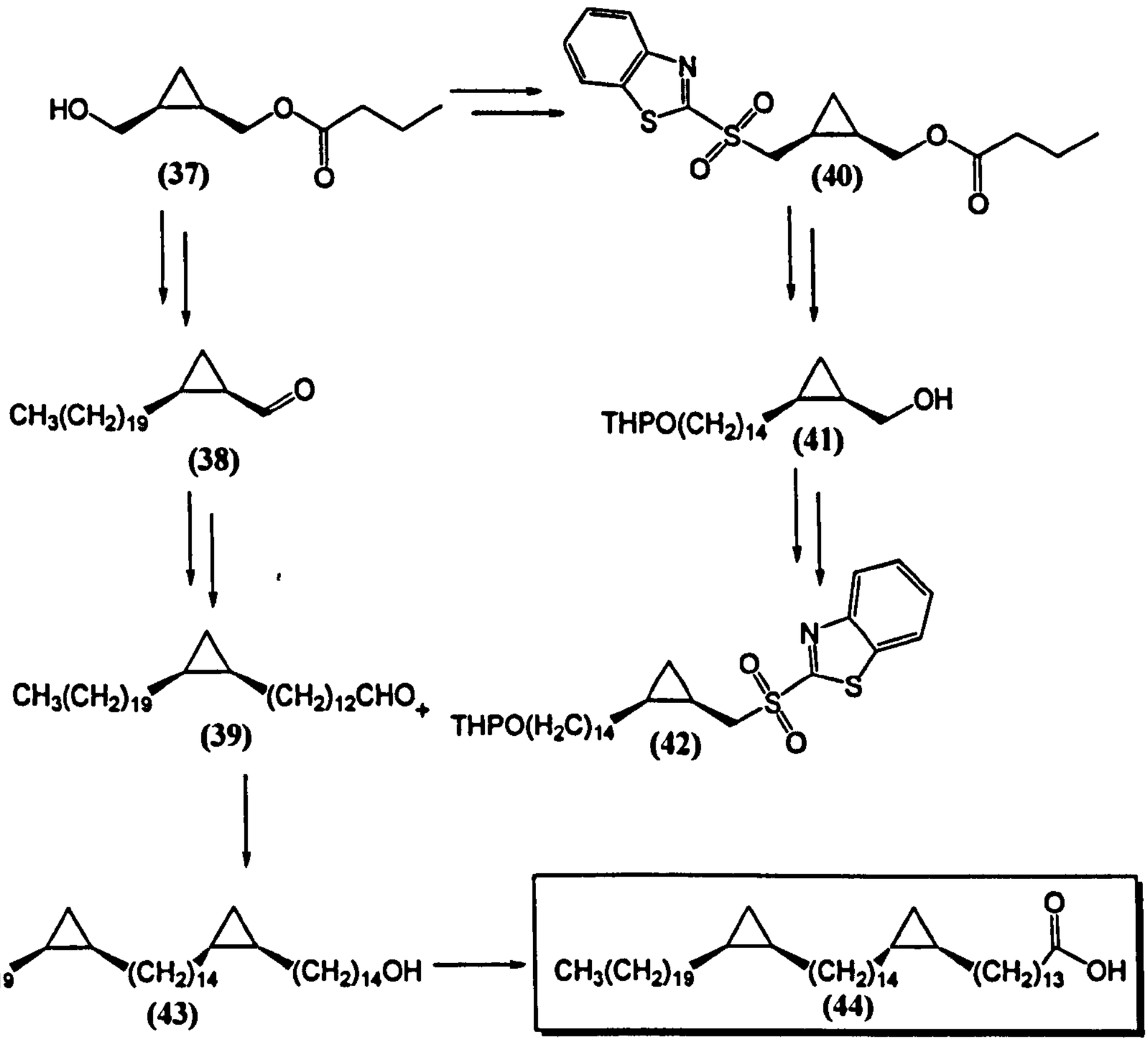


Fig. 31: Baird *et al.*'s synthesis of an enantiomerically pure meromycolic acid.

Baird *et al.* successfully synthesized a single enantiomer of α-mycolic acid, containing two *cis*-cyclopropane rings.[181] Baird *et al.* went about preparing a branched hydroxy acid aldehyde, so that they could then couple this to a meromycolate sulfone using a modified Julia-Kocienski olefination that they utilised during the preparation of their meromycolic acid.[178] The branched hydroxy acid was prepared by a Grignard ring opening of the epoxide **(45)** with 9-bromononan-1-ol tetrahydropyranyl ether to give the secondary alcohol **(46)**. This was then transformed into **(47)**, an alkylation of **(47)** and protection of the primary alcohol to the *tert*-butyl diphenyl silyl ether giving **(48)**. Protection of the secondary alcohol to the acetate, then deprotection of the *tert*-butyl diphenyl silyl ether and oxidation yielded the branched hydroxy acid aldehyde **(49)** (See Fig. 32).[181]

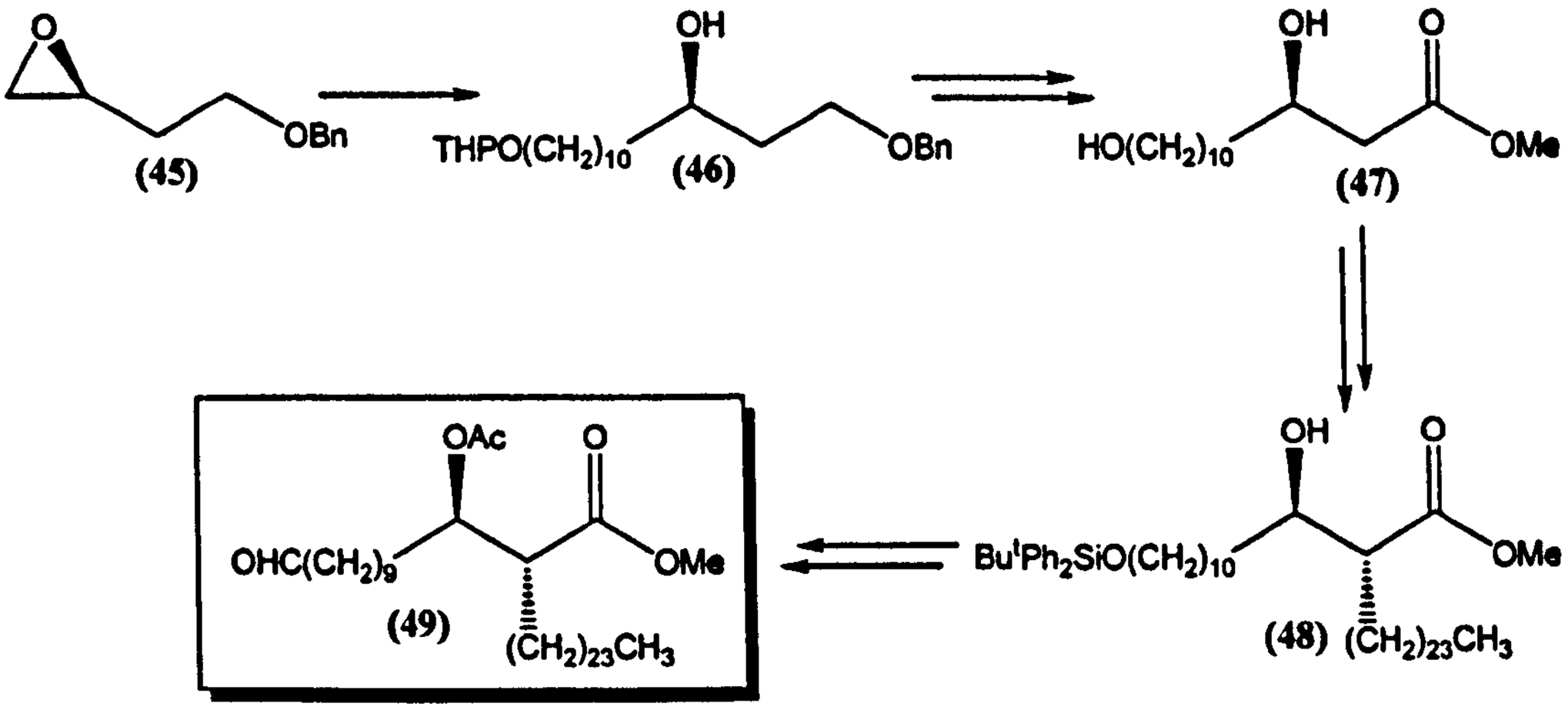


Fig. 32: Baird *et al.*'s branched hydroxy acid aldehyde

Using similar procedures to those used to obtain their first meromycolic acid, Baird *et al.* prepared the meromycolate sulfone **(50)**. Coupling of this sulfone **(50)** to the branched hydroxy acid aldehyde **(49)**, and mild hydrogenation with potassium azodicarboxylate gave the first enantiomerically pure α-mycolic acid **(51)** (See Fig. 33).[178, 181]

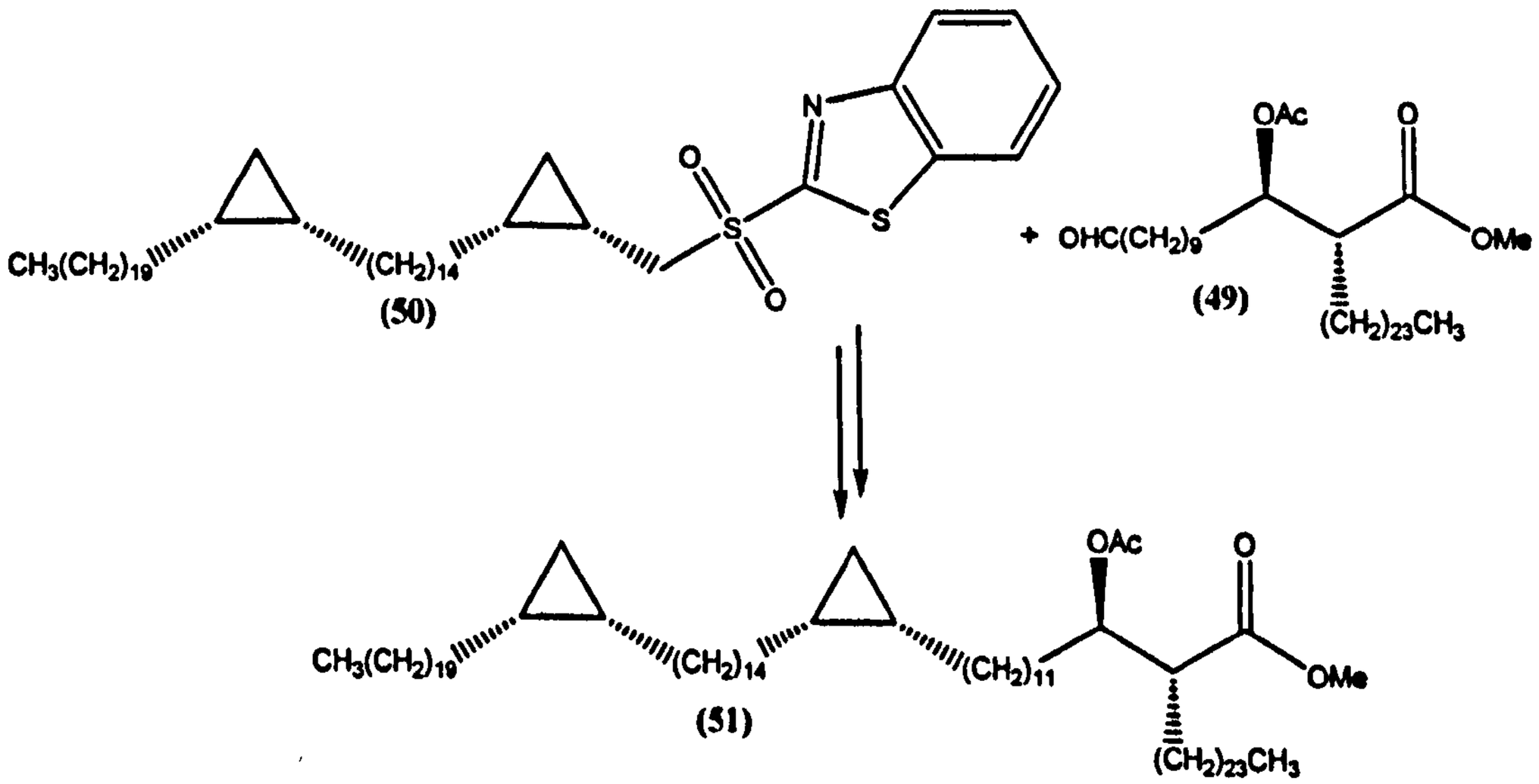
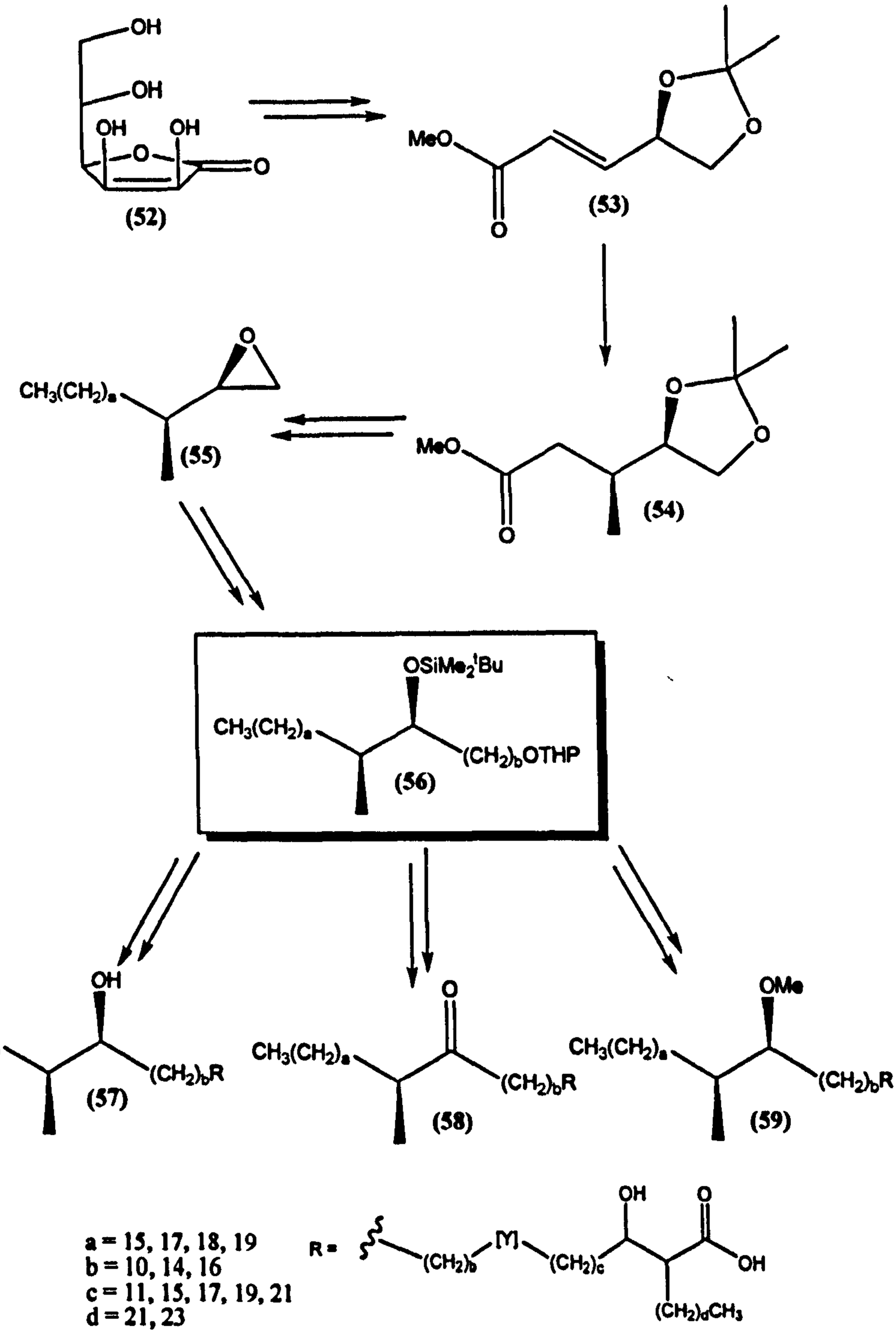


Fig. 33: Baird *et al.*'s first α-mycolic acid

Baird *et al.* were able to use these techniques to develop methods for other mycolates, and they later discussed the synthesis of *S,S*-oxygenated mycolates, obtainable from *L*-ascorbic acid **(52)**.[182-184] The procedure reported is a multi-stage

synthesis to obtain the *key intermediate* **(56)**, key stages in the synthesis are the Michael addition of the methyl across the double bond of **(53)**, to give **(54)**; and the formation of the epoxide **(55)**. It is possible to prepare hydroxy **(57)**, keto **(58)** and methoxy **(59)** mycolic acids from the *tert*-butyl dimethyl silyl ether **(56)** (See Fig. 34).



a = 15, 17, 18, 19
b = 10, 14, 16
c = 11, 15, 17, 19, 21
d = 21, 23

R =

Fig. 34: Baird *et al.*'s preparation of *S,S*-oxygenated mycolates

They also report the preparation of *R,R*-oxygenated mycolates in a similar fashion, following intermediates **(61)**, **(62)** and **(63)**, however starting from *D*-mannitol **(60)**. It is again possible to retain the configuration of the distal position of **(64)** in the preparation of mycolic acids containing **(65)**, **(66)** and **(67)** (See Fig. 35).[182, 184]

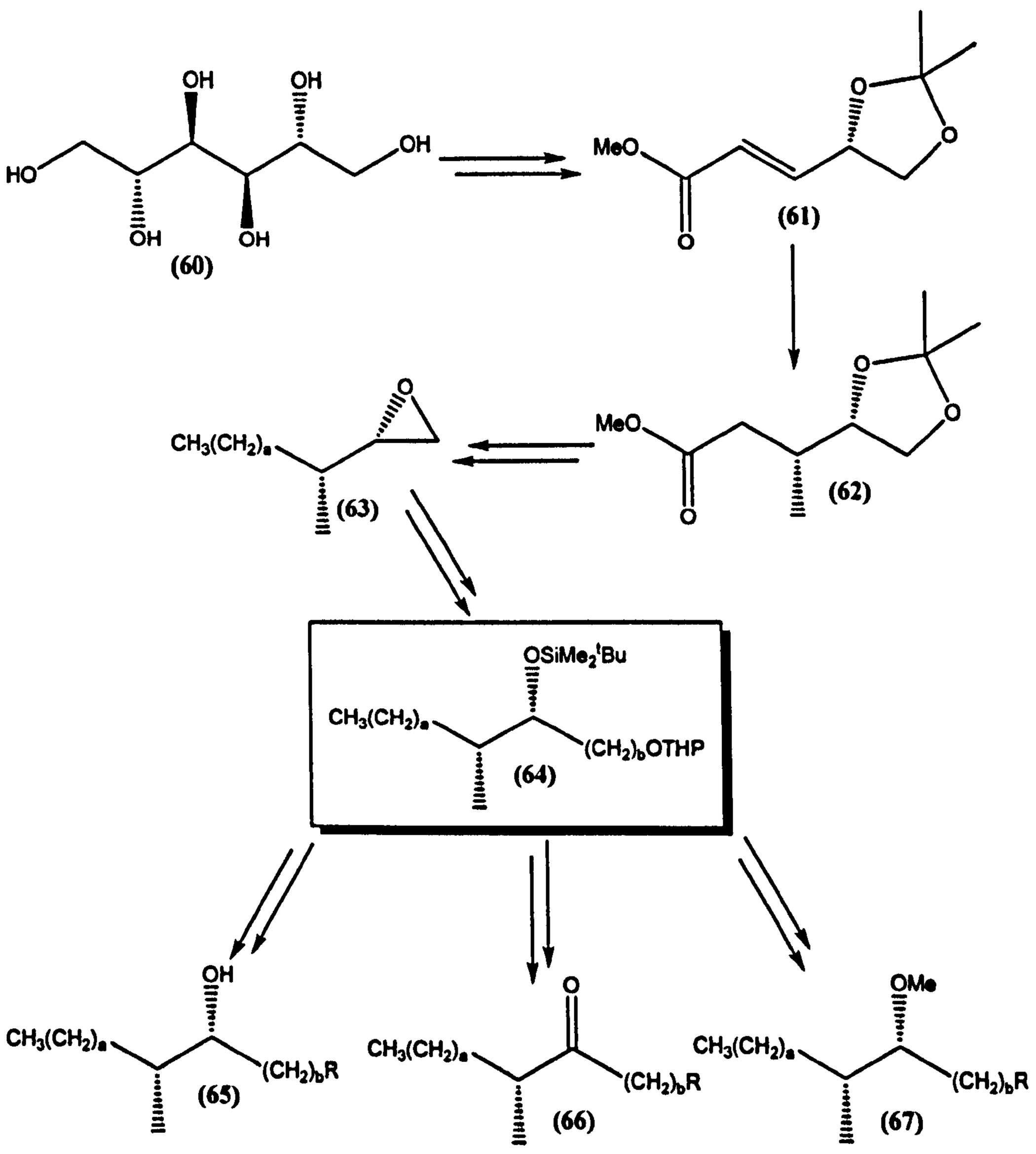


Fig. 35: Baird *et al.*'s preparation of *R,R*-oxygenated mycolates

Baird *et al.* also conducted an improved synthesis of the α-alkyl β-hydroxy unit **(82)**, where they were able to start from very simple materials to prepare the *cis*-olefin **(75)**. It is possible to obtain the aldehyde **(80)** in a multi-stage synthesis via the diol **(76)**, sulfone **(77)**, methyl ester **(78)** and olefin **(79)** (See Fig. 37).[186]

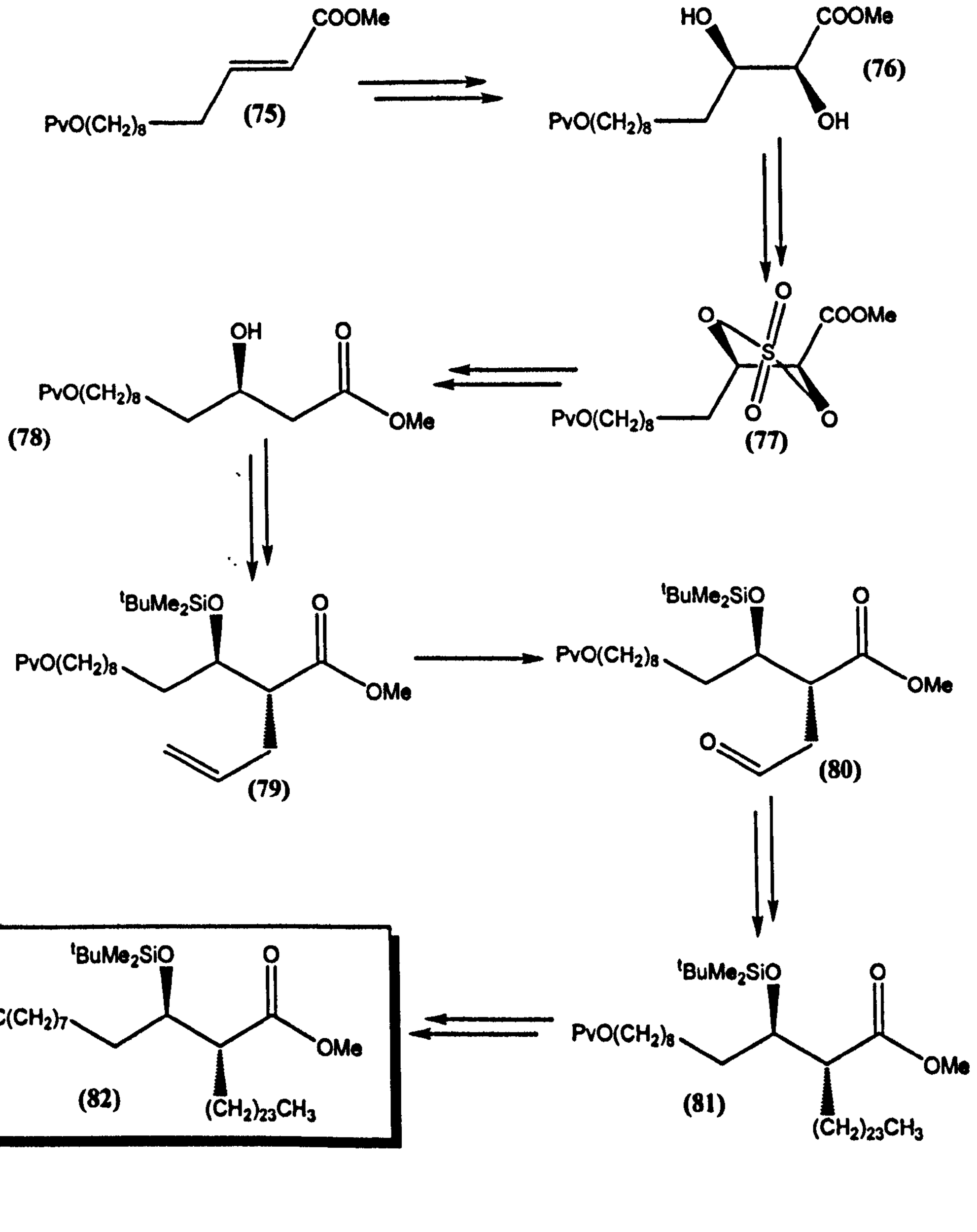


Fig. 37: Baird *et al.*'s improved synthesis of the α-alkyl β-hydroxy unit

The α-alkyl branch is twenty four carbons long in **(81)** and **(82)**. However in this new strategy the aldehyde **(80)** generated allows the introduction of any chain length desired at the α-position, via a modified Julia-Kocienski olefination. Baird *et al.* suggested that one would be able to add a labelled group at this position; this would possibly help gain a further understanding into the folding/conformational behaviour of mycolic acids.

Baird *et al.* have made a considerable contribution to the area of complete synthesis of mycolic acids, where they have published several routes to obtain enantiomerically pure mycolic acids.[96, 153, 178, 180-184, 187, 188]

Chapter 2 - Results and Discussion

2.1 – Project aims

This project is comprised of two main areas, the synthesis of two methoxy-mycolic acids of *Mycobacterium tuberculosis* and hydroxy and keto-mycolic acids of *Mycobacterium marinum*. The target molecules from *Mycobacterium tuberculosis* are *cis*-cyclopropane methoxy-mycolic acid **(83)** and α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)** (See Fig. 38).

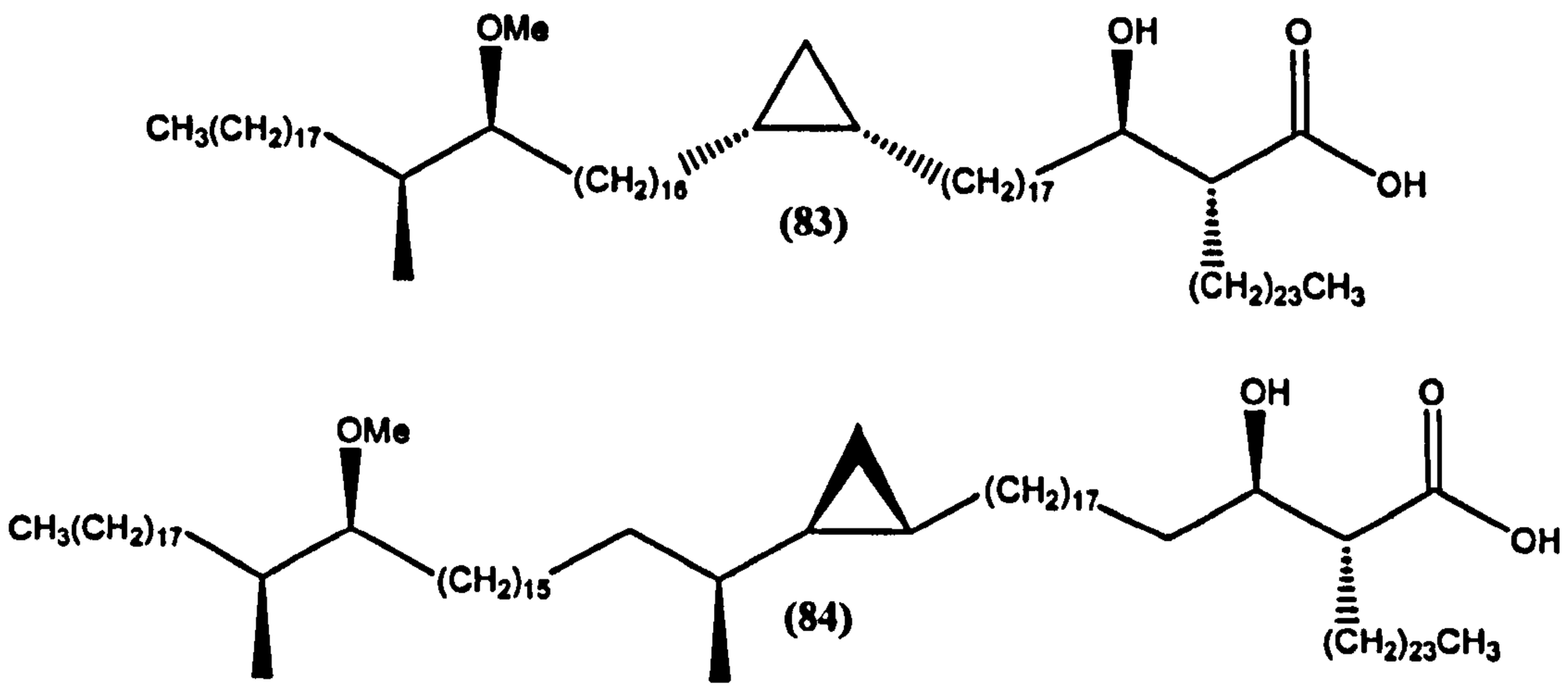
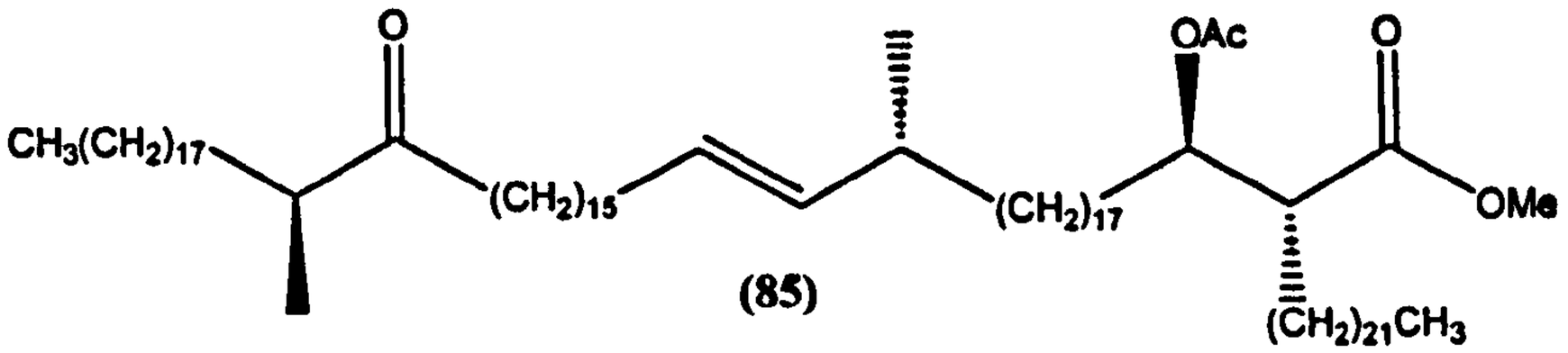


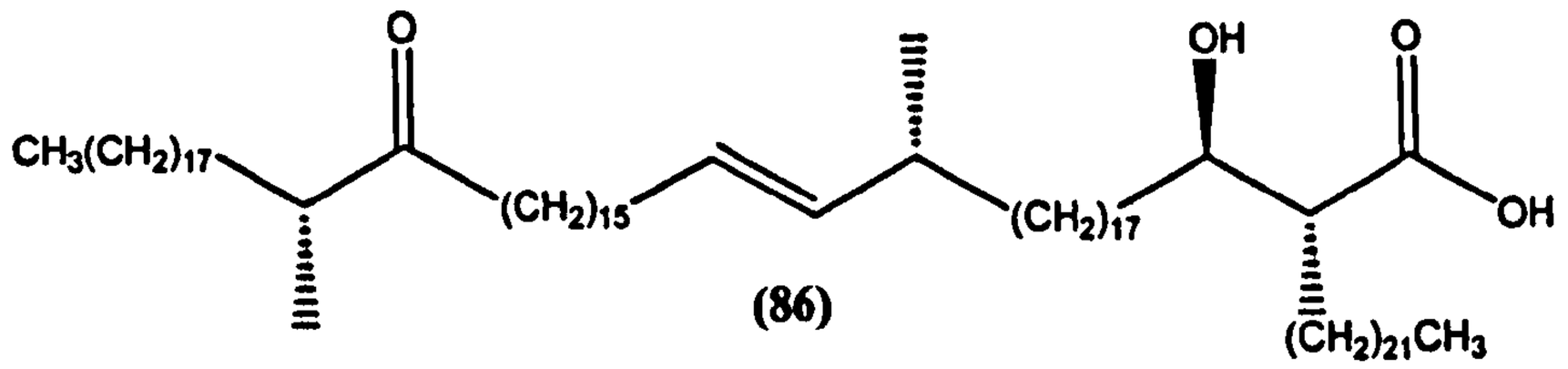
Fig. 38: Targeted mycolic acids of *M.Tb*

Protected α-methyl *trans*-alkene keto **(85)**, *R*-α-methyl *trans*-alkene keto-mycolic acid **(86)** and both enantiomers of α-methyl *trans*-alkene hydroxy **(87)** and **(88)** (See Fig. 39) from *Mycobacterium marinum* were also selected as molecules for study.

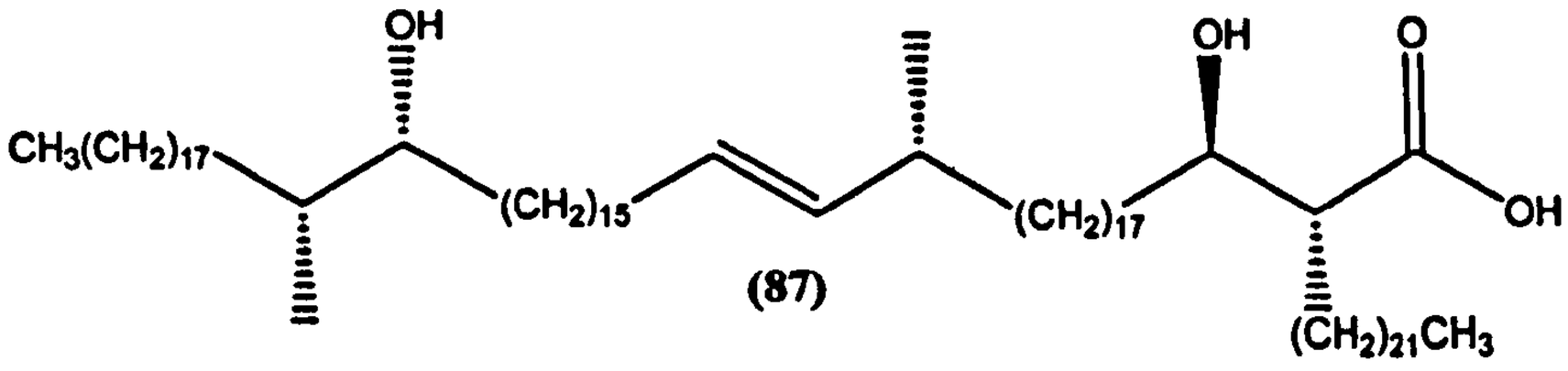
The mycolic acids synthesised in this project will help towards development of detection methods for tuberculosis, discovery of a replacement for Freund's adjuvant, possible therapies in treating asthma and to gain a better understanding of the impact of function on mycobacteria.



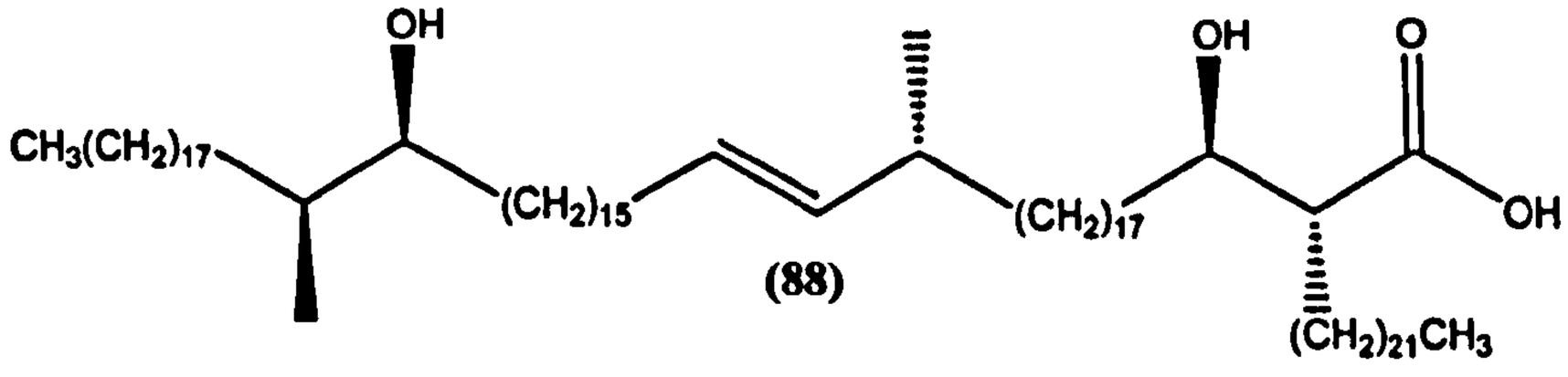
(85)



(86)



(87)



(88)

Fig. 39: Targeted mycolic acids of *M. Marinum*

2.2 – Synthesis of *cis*-cyclopropane methoxy-mycolic acid (83)

Work previously carried out by J.R. Al-Dulayymi *et al.* described the complete syntheses of three out of four of the major methoxy-mycolic acids of *M.Tb.*[182] It was decided to undertake the synthesis of the final methoxy-mycolic acid missing from this set of isomers. The three completed methoxy-mycolic acids contain cyclopropane rings with both *S,R* and *R,S* configurations in the proximal position, and with the methoxy and methyl of the distal position in *S,S* and *R,R* configurations. (See Fig. 40).

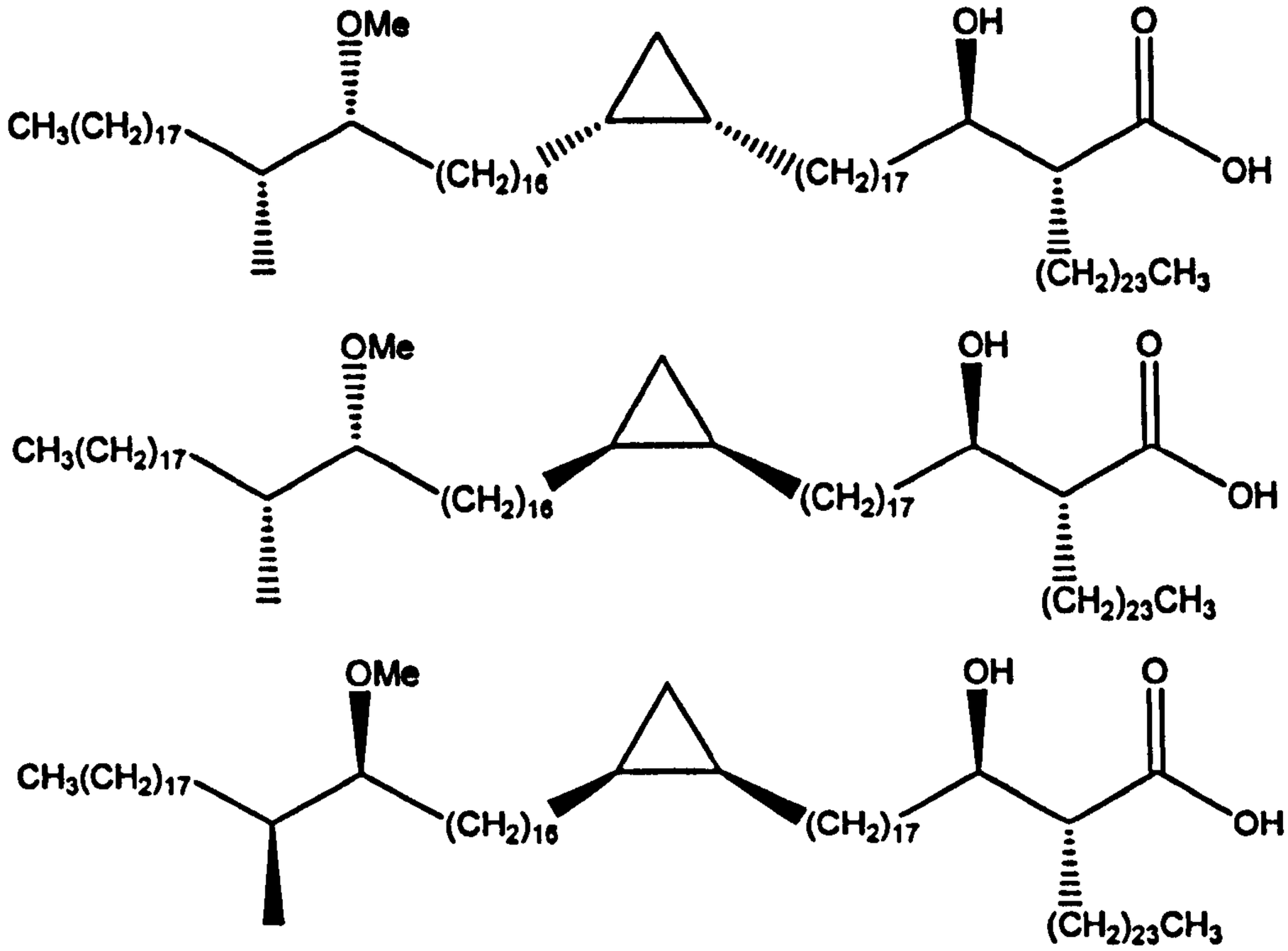


Fig. 40: Synthesised methoxy-mycolic acids of *M.Tb*[182]

2.2.1 – Retrosynthesis of cis-cyclopropane methoxy-mycolic acid (84)

Following the general methods of mycolic acid synthesis developed by J.R. Al-Dulayymi *et al.* retrosynthesis suggests synthesising a meromycolate moiety **(89)** and branched hydroxy acid **(90)** separately (See Fig. 41) as the two main coupling fragments.

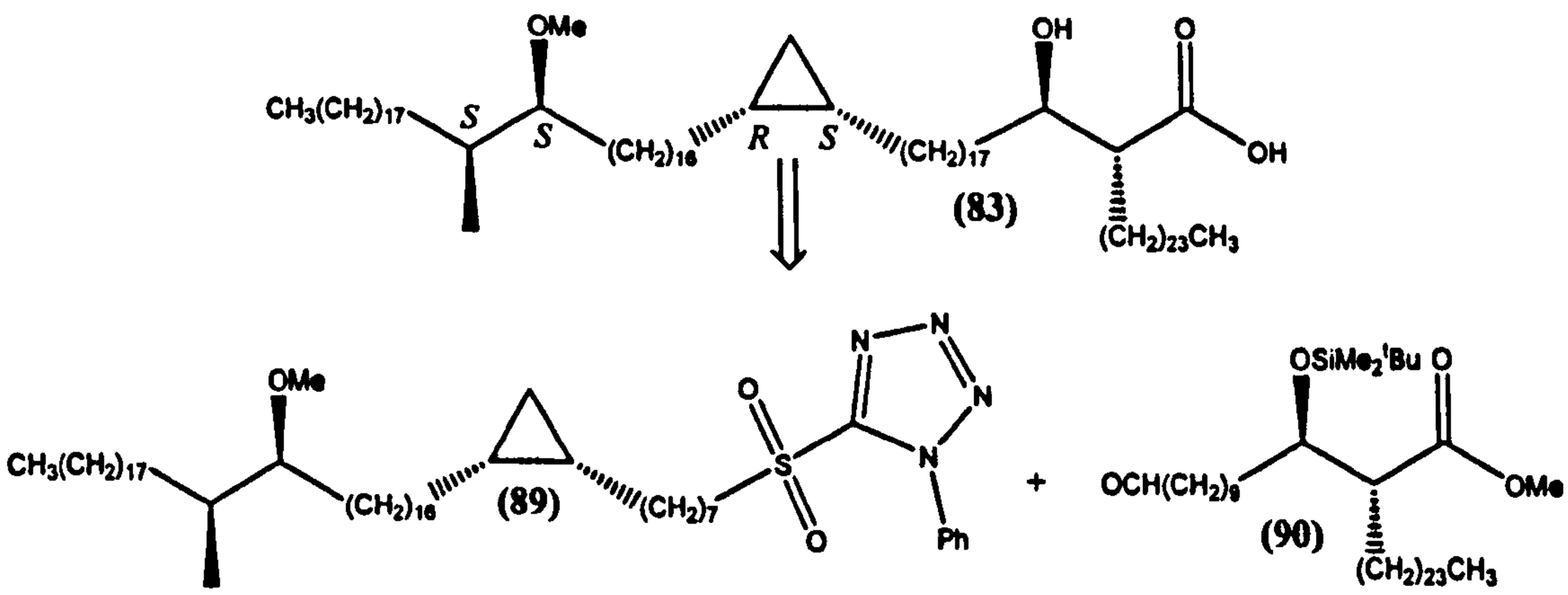


Fig. 41: Meromycolate moiety and branched hydroxy acid disconnection

The meromycolate moiety **(89)** can be separated further into two known fragments as published by J.R. Al-Dulayymi *et al.*, accounting for the distal and proximal positions (See Fig. 42) giving a *S,S* α-methyl methoxy unit **(91)** and a *R,S* *cis*-cyclopropane ring **(92)**.[182]

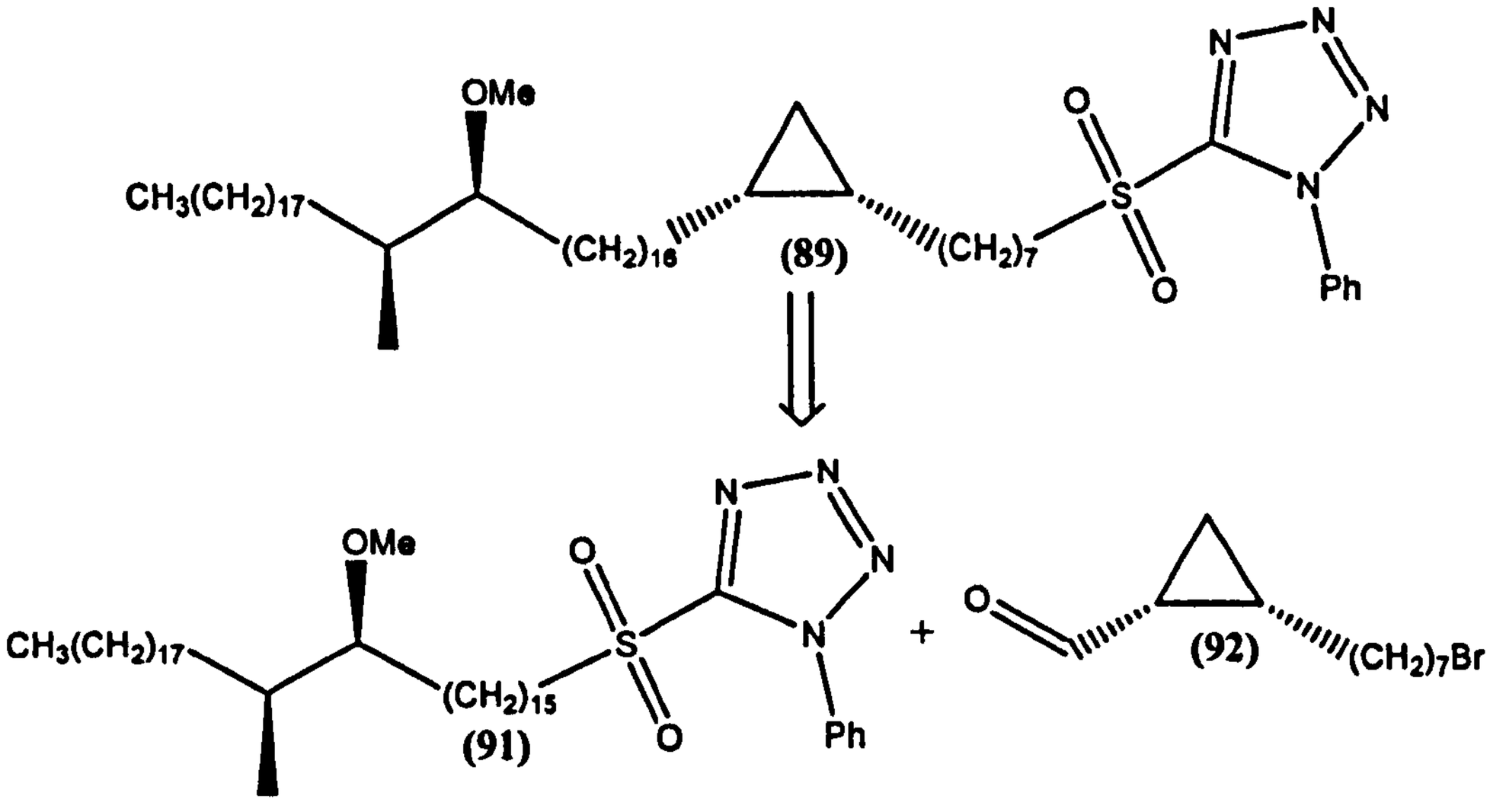


Fig. 42: Meromycolate disconnection

Preparation of the branched hydroxy acid fragment **(90)** could be carried out by the following method (See Fig. 43).

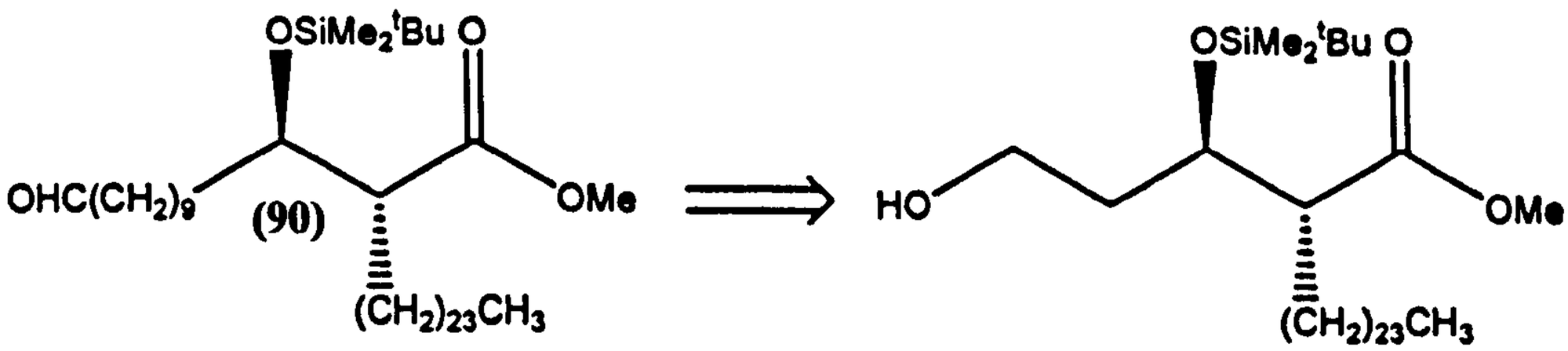


Fig. 43: Branched hydroxy acid retrosynthesis

2.2.2 – Preparation of the distal S,S α-methyl methoxy unit

The *S,S* α-methyl methoxy unit intermediate **(93)** used in this project was previously synthesised by the author. Intermediate **(93)** can be achieved in multi-gram quantities, following literature methods discussed previously in section 1.4.3 (See page 37).[182, 189]

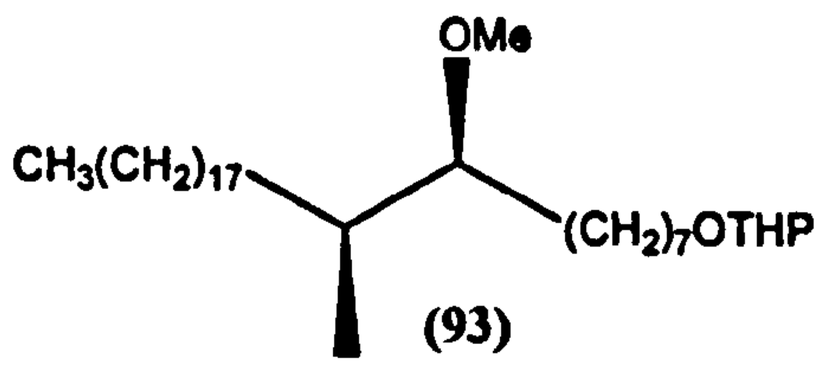


Fig. 44: 2-((8*S*,9*S*)-8-Methoxy-9-methylheptacosyloxy)tetrahydropyran (93)

The tetrahydropyranyl acetal **(93)** can be readily converted into sulfone **(91)**, via intermediates **(94) – (100)**, following a literature method as described by J.R. Al-Dulayymi *et al.*.[182] (See Fig. 45). The data obtained whilst conducting these methods can be seen in Appendix 1 to Appendix 11.

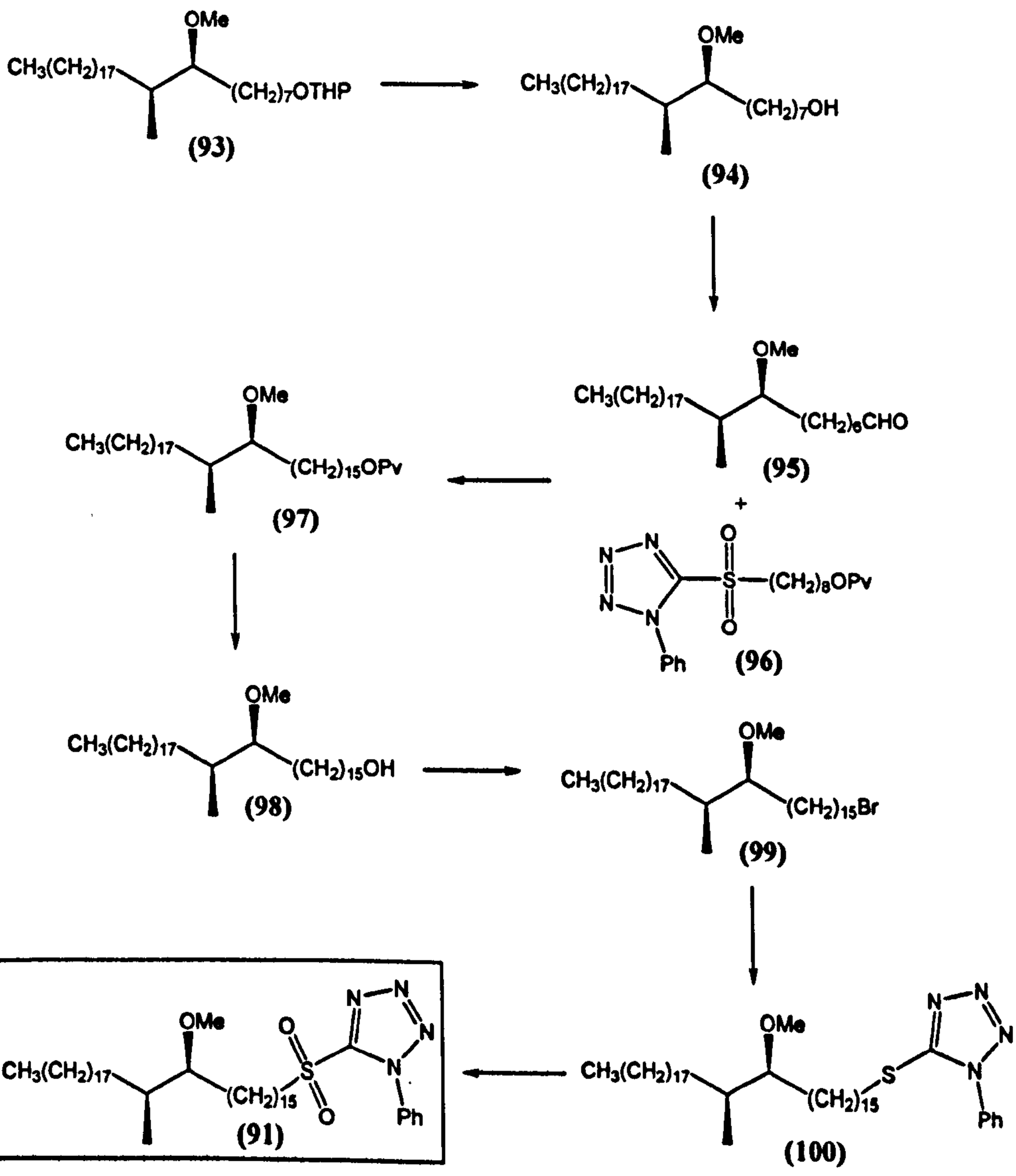


Fig. 45: Preparation of sulfone (91)

To obtain the correct chain length between distal and proximal positions, it was decided that a series of modified Julia-Kocienski reactions could be used to connect distal and proximal positions.

The tetrahydropyranyloxy group of the *S,S* α-methyl methoxy unit **(93)** was then deprotected with *p*-toluenesulfonic acid to give the primary alcohol **(94)**, which was then oxidised to the corresponding aldehyde **(95)**, using pyridinium chlorochromate (See Fig. 46).

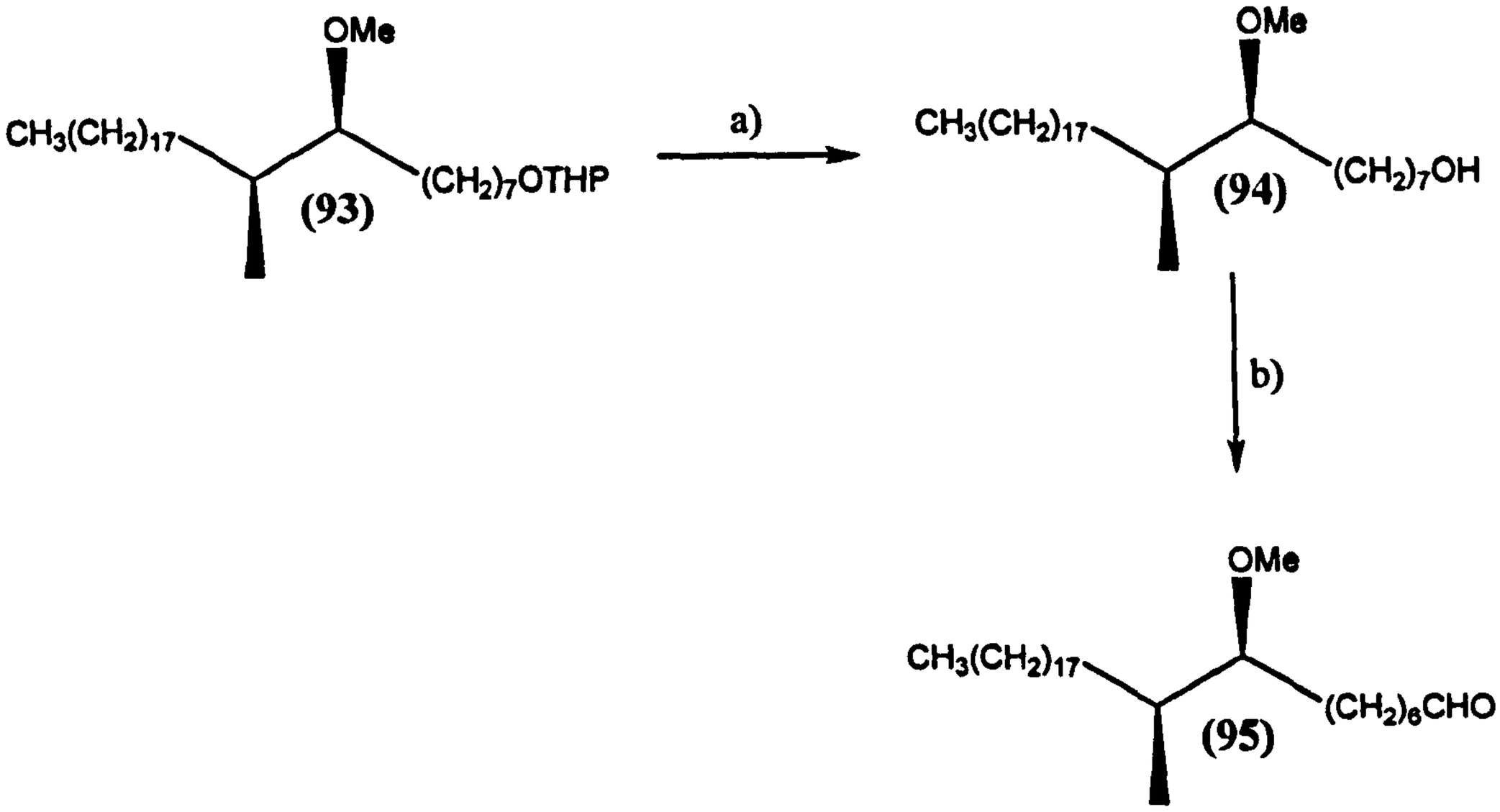


Fig. 46: a) PTSA, (77 %); b) PCC, (77 %)

The conversion of the THP protected compound **(93)** through to the aldehyde **(94)** was followed by ^1H NMR spectroscopy. The ^1H NMR of the tetrahydropyranyloxy compound **(93)** showed characteristic signals for the protecting group, including a triplet at δ 4.58 for the acetal proton, a multiplet between δ 3.93 and 3.86 corresponding to the CH_2 adjacent to the oxygen of the ring, and a doublet of triplets at δ 3.75.

Upon deprotection of the THP group, these signals, as expected were lost and a broad singlet at δ 1.52 was observed for the hydroxyl group. After oxidation a triplet at δ 9.82 (J 1.90 H_z) corresponding to the aldehyde proton and a doublet of triplets for the CH_2 adjacent (J 1.90, 7.30 H_z) were observed.

Following the disconnection route discussed earlier, an eight carbon sulfone with a protected hydroxyl group was required to connect the distal and proximal

groups. This was done by taking commercially available 1,8-octanediol **(101)** and carrying out a bromination of one hydroxyl group using hydrobromic acid at reflux, to give the mono-bromo alcohol **(102)**. A protection of the hydroxyl group was then carried out using trimethylacetyl chloride to give the *tert*-butyl ester bromide **(103)**. This was then converted into the corresponding sulfide **(104)**, using 1-phenyl-1H-tetrazole-5-thiol and potassium carbonate. Oxidation of the sulfide with ammonium molybdate (VI) tetrahydrate and hydrogen peroxide yielded the sulfone **(96)** (See Fig. 47).

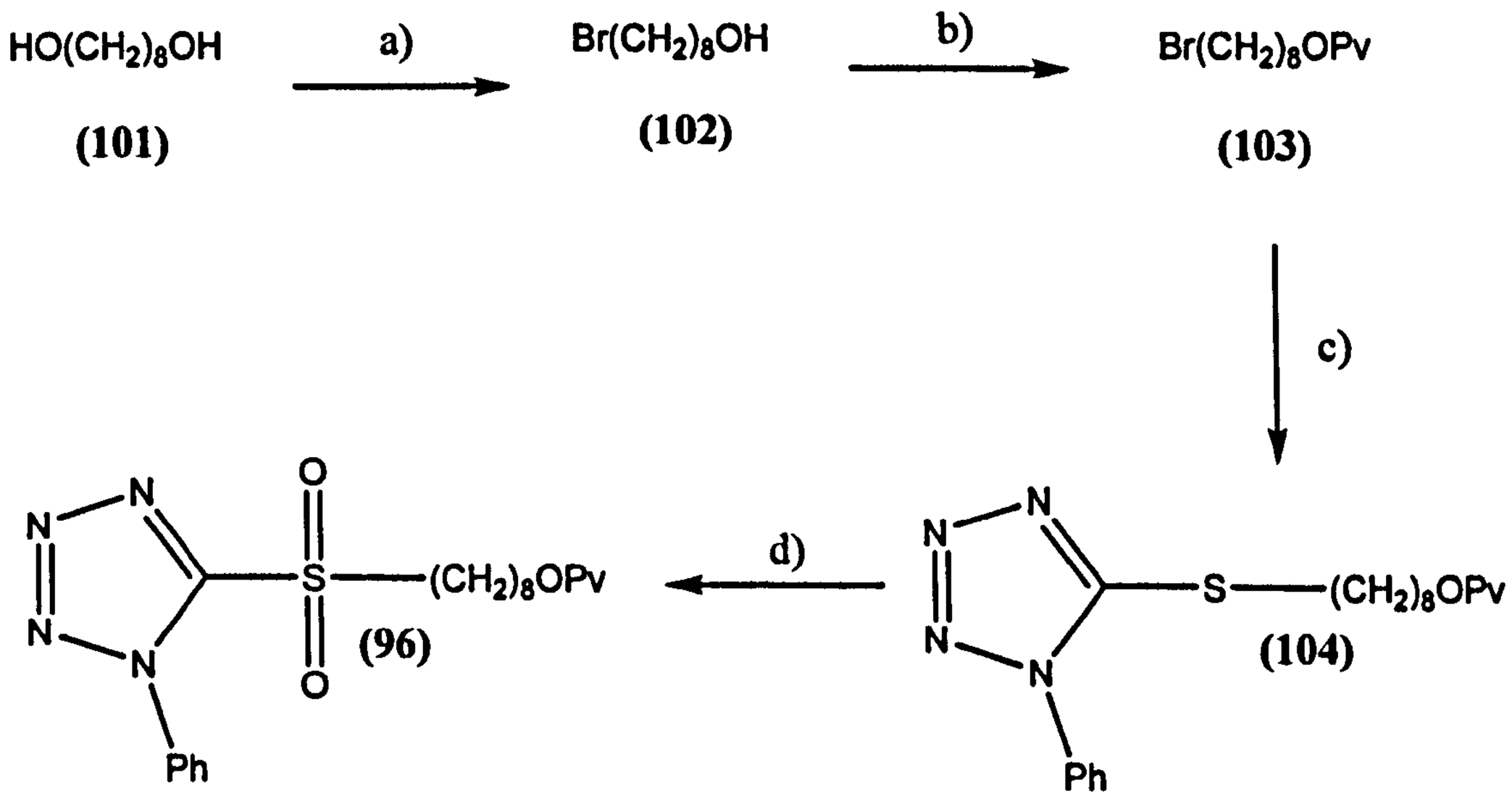


Fig. 47: a) HBr, (81 %); b) $(CH_3)_3CCOCl$, (85 %); c) 1-phenyl-1H-tetrazole-5-thiol, K_2CO_3, (74 %); d) ammonium molybdate (VI) tetrahydrate, H_2O_2, (96 %)

The ^1H NMR spectrum of **(96)** showed a multiplet between δ 7.61 and 7.50 corresponding to the 5 aromatic protons, and a triplet at δ 3.40 for the CH_2 adjacent to the sulfur.

With the sulfone and aldehyde now prepared it was possible to carry out a modified Julia-Kocienski reaction. The procedure is based on a method derived by M. Julia *et al.*, called the Julia olefination.[190] Work carried out by P.J. Kocienski *et al.* on the modification of this method has led it to becoming an important reaction in organic synthesis.[191] The method goes via metallation of the phenylsulfone, acylation of the aldehyde to give a β-alkoxysulfone, followed by a reductive elimination to give a mixture of alkene products (See Fig. 48).

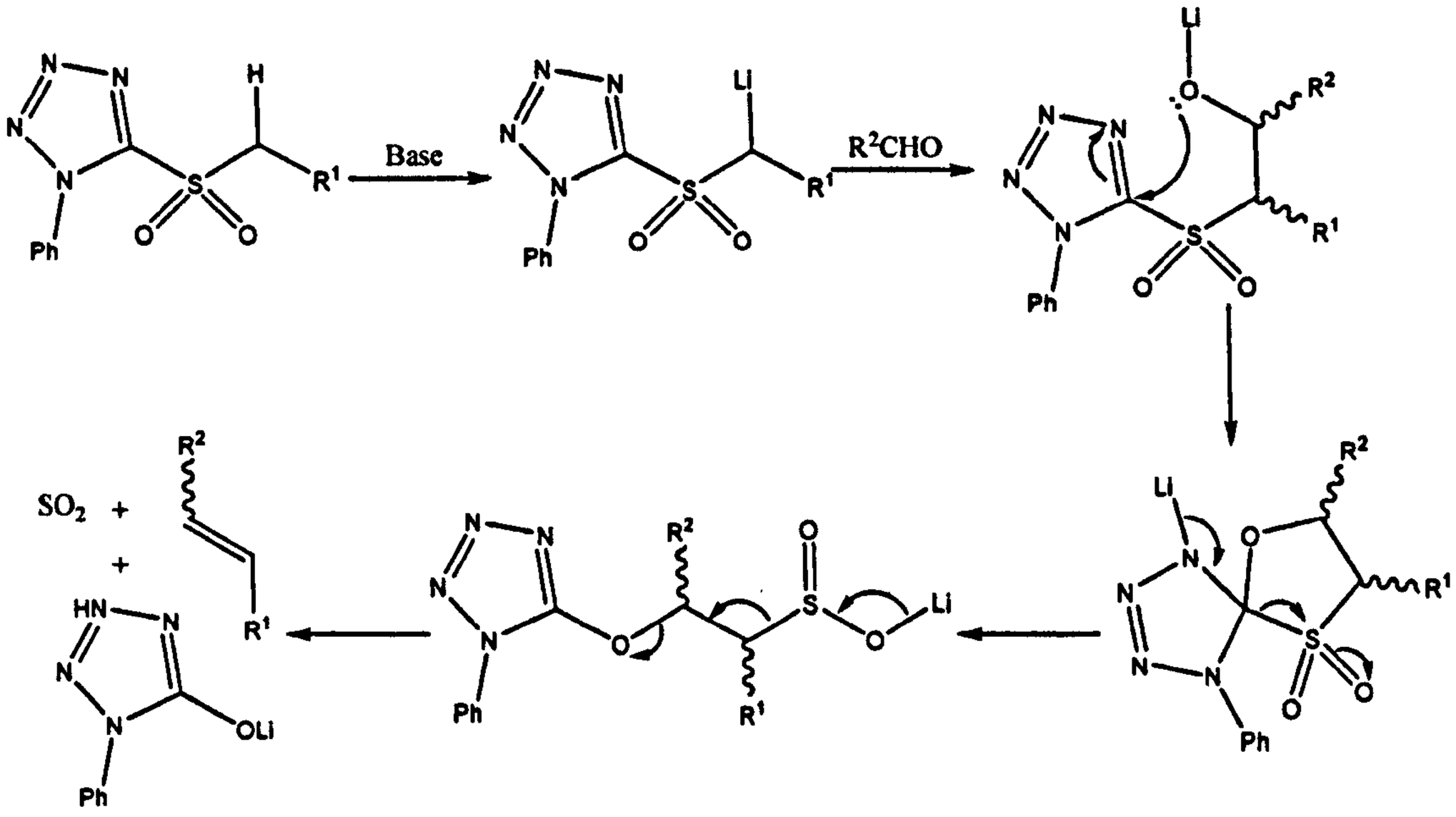


Fig. 48: Modified Julia-Kocienski olefination

From the modified Julia-Kocienski olefination of sulfone **(96)** and aldehyde **(95)**, a mixture of alkenes **(105)** was obtained using lithium *bis*(trimethylsilyl) amide as a base. The alkenes were then hydrogenated at atmospheric pressure using palladium on charcoal (10 %) to give **(106)** (See Fig. 49).

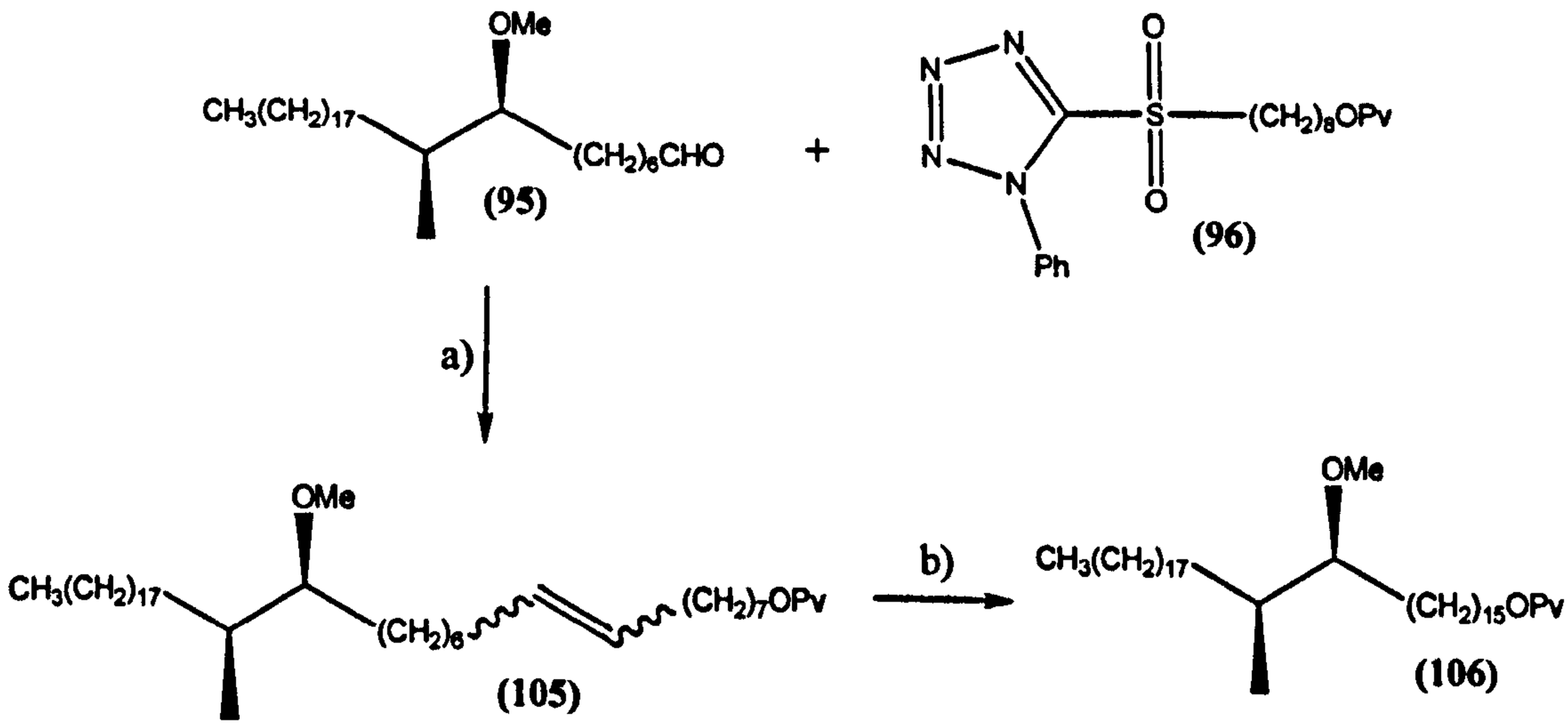


Fig. 49: a) LiHMDS, (86 %); b) H_2, Pd. cat., (99 %)

The olefination was confirmed by ^1H NMR spectroscopy of **(105)**, with the product showing all expected signals, most notably 2 multiplets present in the olefinic region. Upon hydrogenation these signals were no longer present in **(106)**.

The *tert*-butyl ester **(106)** was reduced using $LiAlH_4$ to give the hydroxy compound **(107)**, where potassium hydroxide was used in the original literature. However, although the reaction was a success, there was no significant benefit from using this method as the recorded yield was lower, hence the method from the literature should be preferred for reduction of the ester **(107)**.[182] Compound **(107)** was brominated using N-bromosuccinimide in the presence of sodium hydrogen carbonate to give the bromide **(108)**. The corresponding sulfone **(109)** was then prepared using the same method that was used to prepare the C_8 sulfone **(91)** (See Fig. 50).

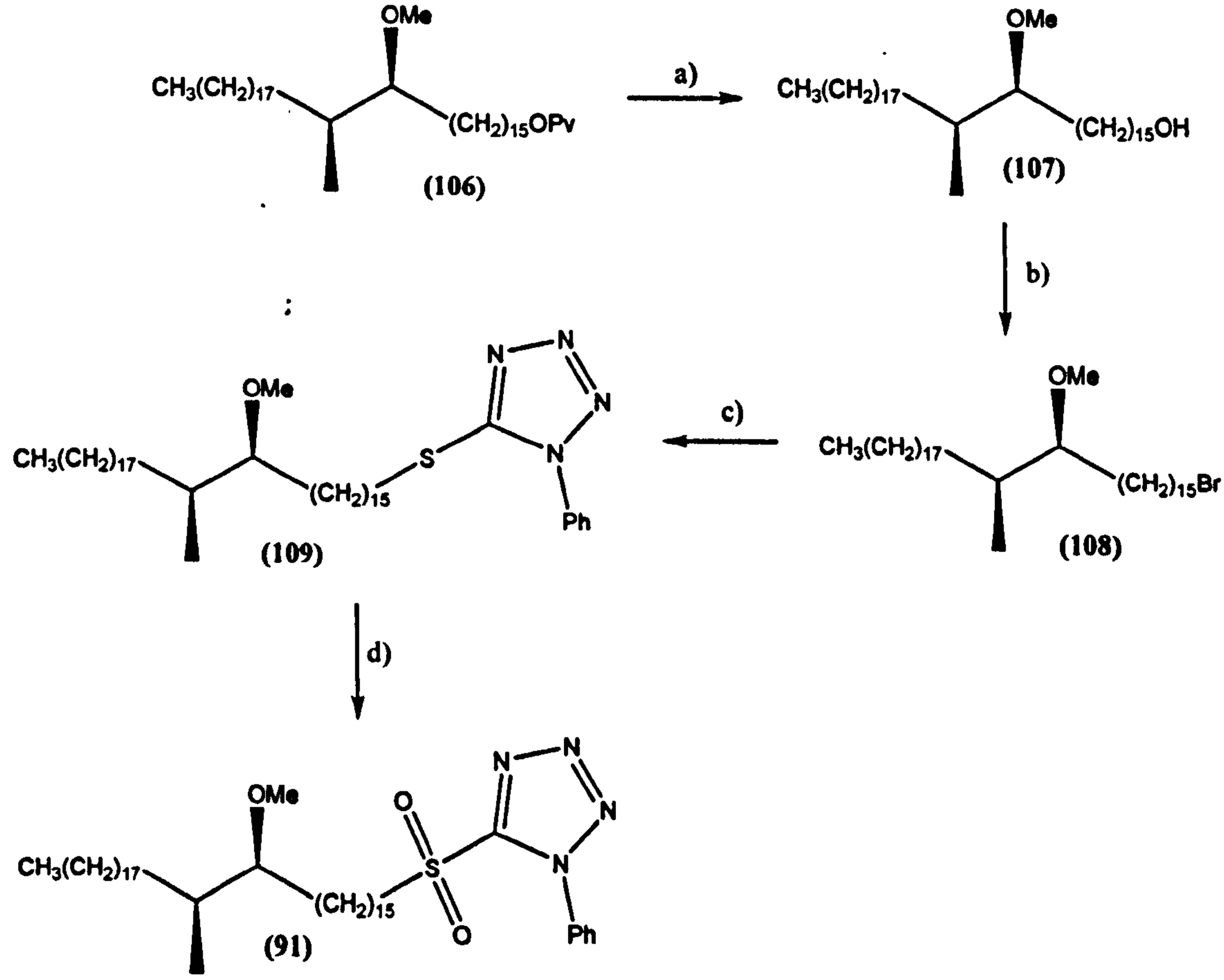


Fig. 50: a) $LiAlH_4$, (79 %); b) NBS, PPh_3, NaHCOs, (84 %); c) 1-phenyl-1H-tetrazole-5-thiol, K_2CO_3, (93 %); d) ammonium molybdate (VI) tetrahydrate, H_2O_2, (87 %)

Bromination of the primary alcohol **(107)** was confirmed via ^1H NMR, which showed a change in chemical shift of the triplet corresponding to the CH_2 adjacent to the alcohol. It is observed that the triplet of the CH_2 adjacent to the alcohol **(107)** at δ 3.72 (J 6.65 H_Z) is shifted upfield to δ 3.44 (J 7 H_Z); in addition the broad singlet at δ 1.48 for the hydroxyl group of the alcohol is no longer present after bromination, further confirming completion of the reaction. Preparation of sulfide **(109)** and sulfone **(91)** was confirmed as before with compounds **(104)** and **(96)**.

2.2.3 – Preparation of the proximal cis-cyclopropane ring

To prepare the *cis*-cyclopropane ring required the following procedure was derived from the disconnection route discussed on page 45.

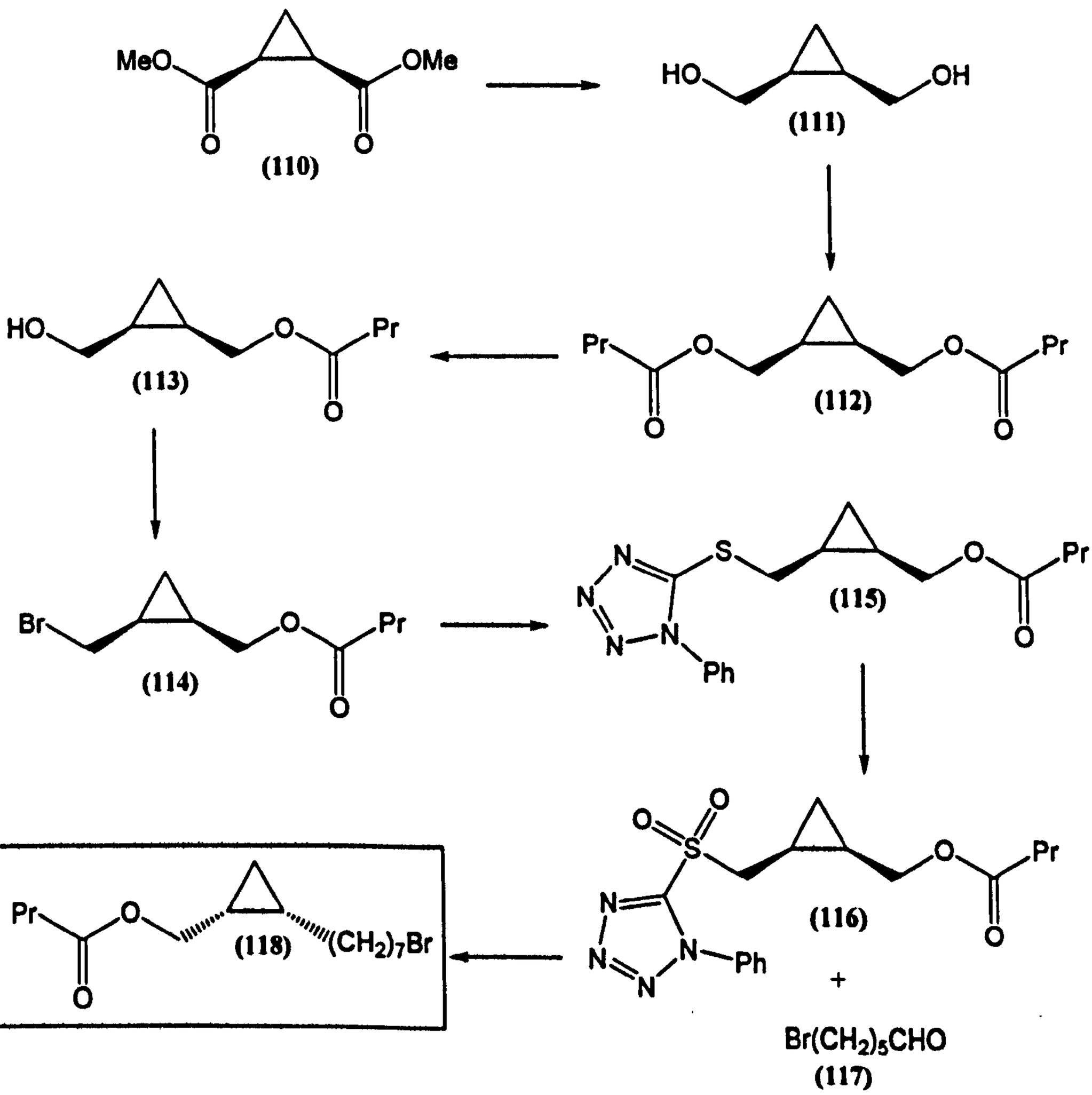


Fig. 51: Preparation of *cis*-cyclopropane intermediate (118)

Having successfully synthesised the distal *S,S* motif, it was required to prepare a *cis*-cyclopropane ring in the *R,S* configuration. This was prepared by a standard method starting from methyl acrylate, methyl chloroacetate and sodium methoxide yielding the diester **(110)** in a Michael Induced Ring Cyclisation.[192] The mechanism of the cyclisation is initiated by the deprotonation of methyl chloroacetate using a base, then the carbanion undergoes a Michael addition across the double bond of the methyl acrylate, which then spontaneously ring closes (See Fig. 52).

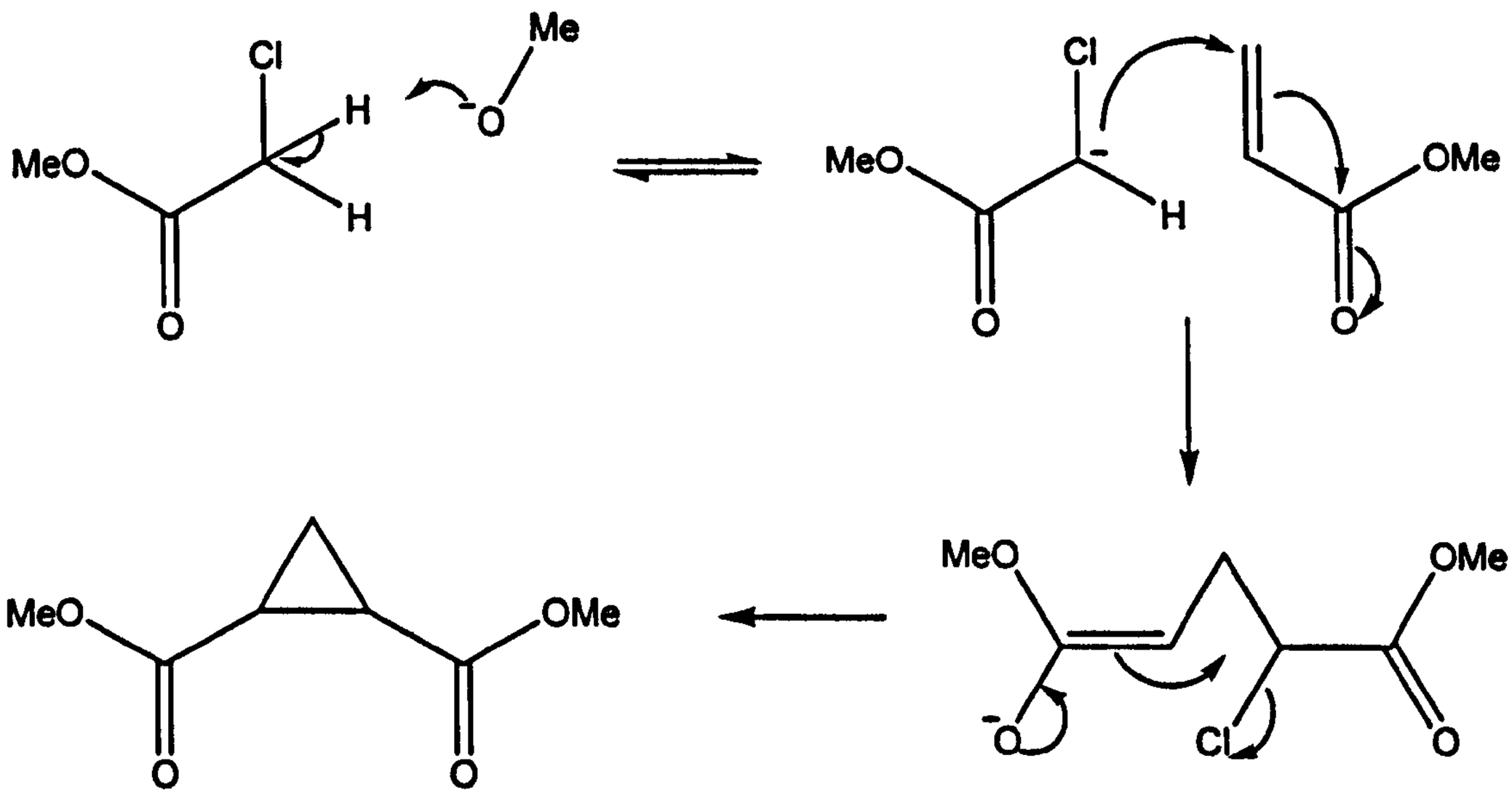


Fig. 52: Michael Induced Ring Cyclisation

The diester **(110)** was then reduced using lithium aluminium hydride, following the same mechanistic route as compound **(106)**, to yield to diol **(111)**. This was in turn protected with butyric anhydride to give the dibutyrate **(112)** (See Fig. 53).

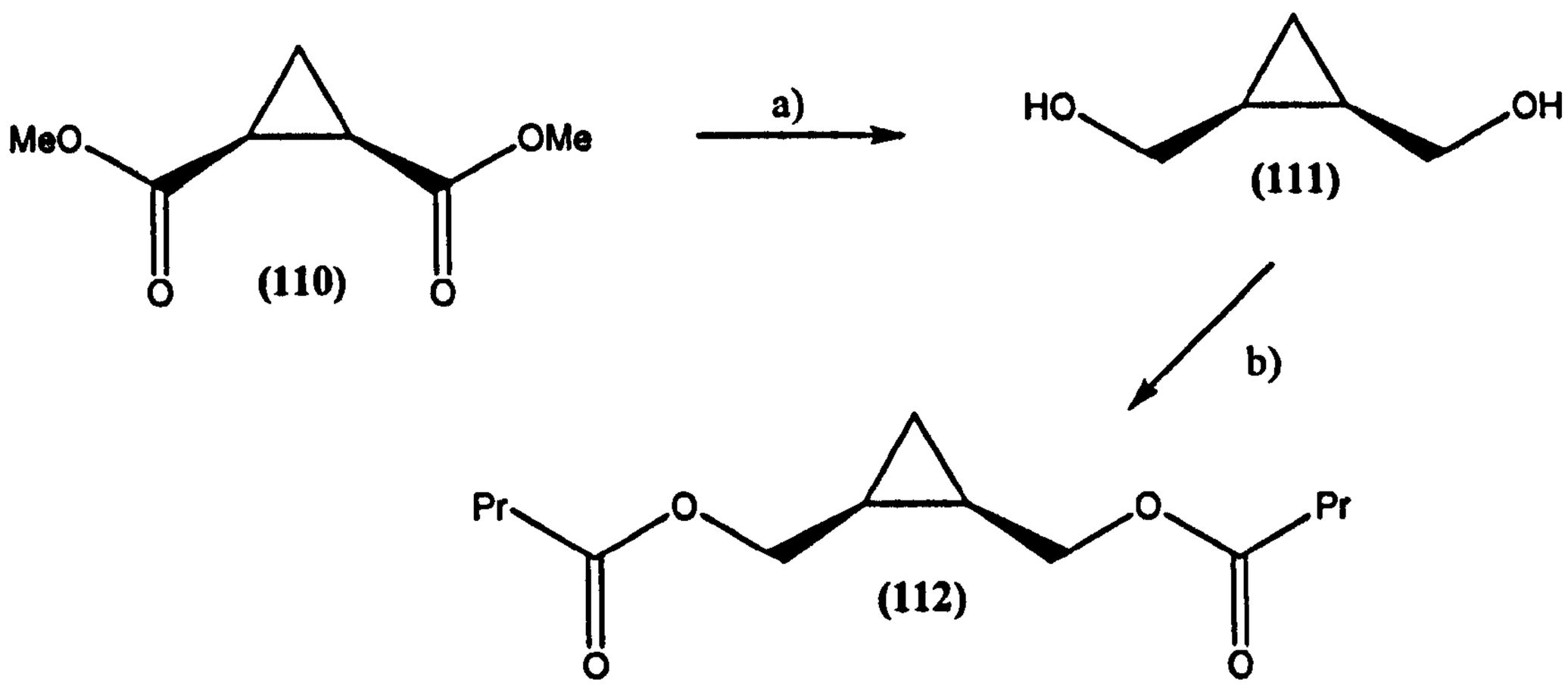


Fig. 53: a) $LiAlH_4$, (80 %); b) $(CH_3CH_2CH_2CO)_2O$, (82 %)

It was then necessary to prepare a monoester from the diester **(112)**. This was carried out using a method reported by Grandjean *et al.*[193] The method gives an enantiomerically pure *cis*-cyclopropane monoester **(113)** using crude lipase extracted from pig pancreas (See Fig. 54). The reaction was carried out under pH control, as a side product of the hydrolysis is butyric acid, which would lead to full hydrolysis giving the diol **(111)**. Therefore to prevent this, pH was maintained *via* addition of 1M aq. sodium hydroxide at 6.5. The optical purity of the monoester, (1*R*,2*S*)-2-hydroxymethyl-cyclopropylmethyl ester **(113)** obtained was confirmed by its optical rotation giving a value of +17.4, which was in accordance with the literature value of +18.2.[193]

Fig. 54: Lipase (PPL, type II, sigma cat. no. L3126), $(CH_2OH)_2$, pH 6.5, (82 %)

The monoester **(113)** was also characterised by its ^{1}H NMR spectra. Where the splitting pattern observed for the *cis*-cyclopropane ring is in accordance with that reported in the literature.

The alcohol group of **(113)** was then brominated using N-bromosuccinimide and triphenylphosphine to give the bromoester **(114)**, in good yield (90 %). The ^{1}H NMR spectrum of butyric acid (1*R*,2*S*)-2-bromomethyl-cyclopropylmethyl ester **(114)** confirmed the success of the reaction, with the two doublet of doublets corresponding to the CH_2 adjacent to the hydroxyl group of the starting material no longer observed, with the two doublet of doublets at δ 3.52 and δ 3.40, corresponding to the CH_2 adjacent to the bromide, now present. The bromide **(114)** was then converted to the corresponding sulfide **(115)** and then sulfone **(116)** in similar procedures as previously discussed with compound **(91)** (See Fig. 55).

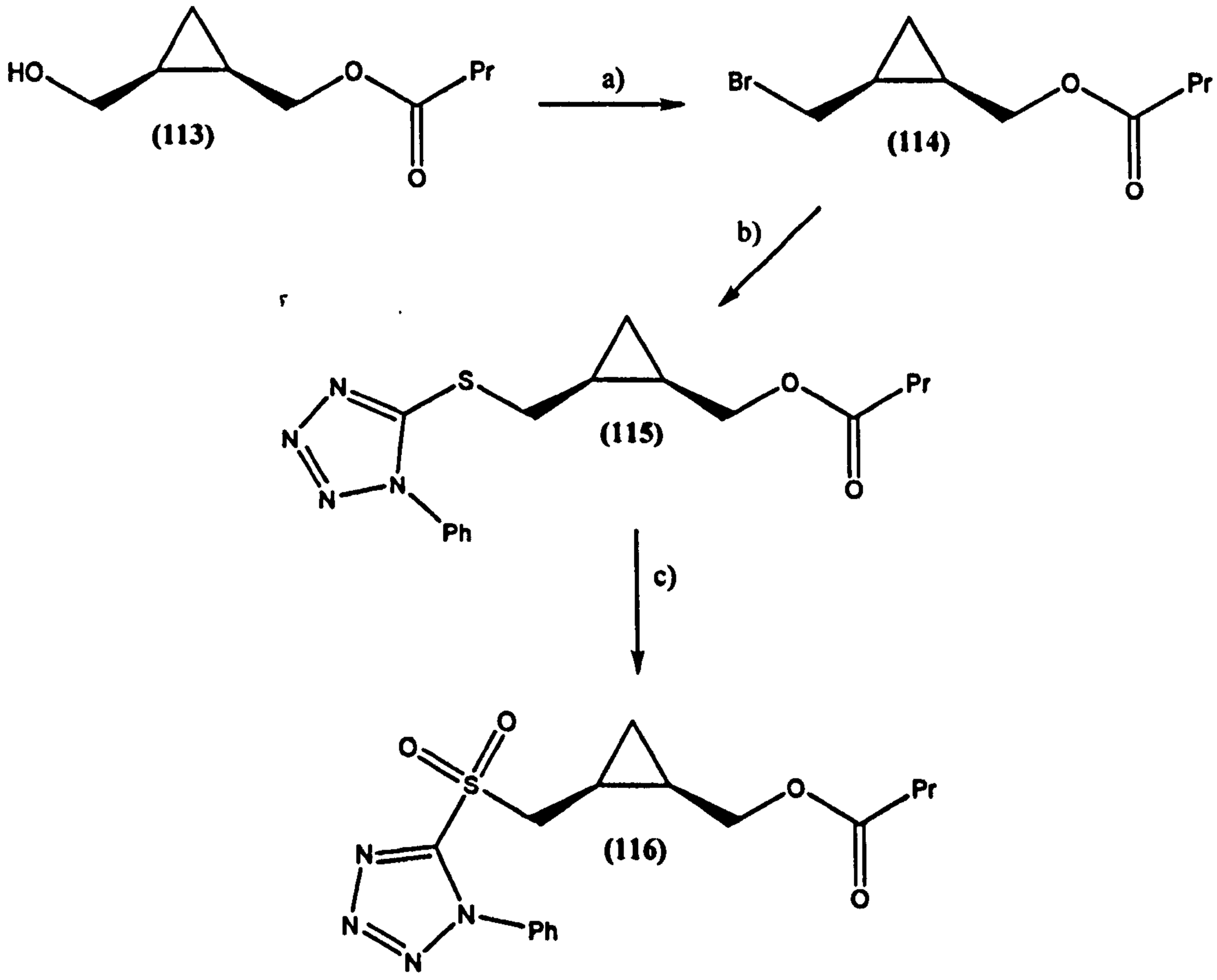


Fig. 55: a) NBS, Ph_3P, $NaHCO_3$, (90 %); b) 1-phenyl-1H-tetrazole-5-thiol, K_2CO_3, (95 %); c) ammonium molybdate (VI) tetrahydrate, H_2O_2, (97 %)

To give the correct stereochemistry of the cyclopropane in the final mycolic acid it was necessary to chain extend from the sulfone **(116)** and then deprotect the ester, oxidise the resultant alcohol and couple to the sulfone **(91)**. Hydrogenation of the resultant olefins gives the previously unsynthesized **(119)** (See Fig. 56).

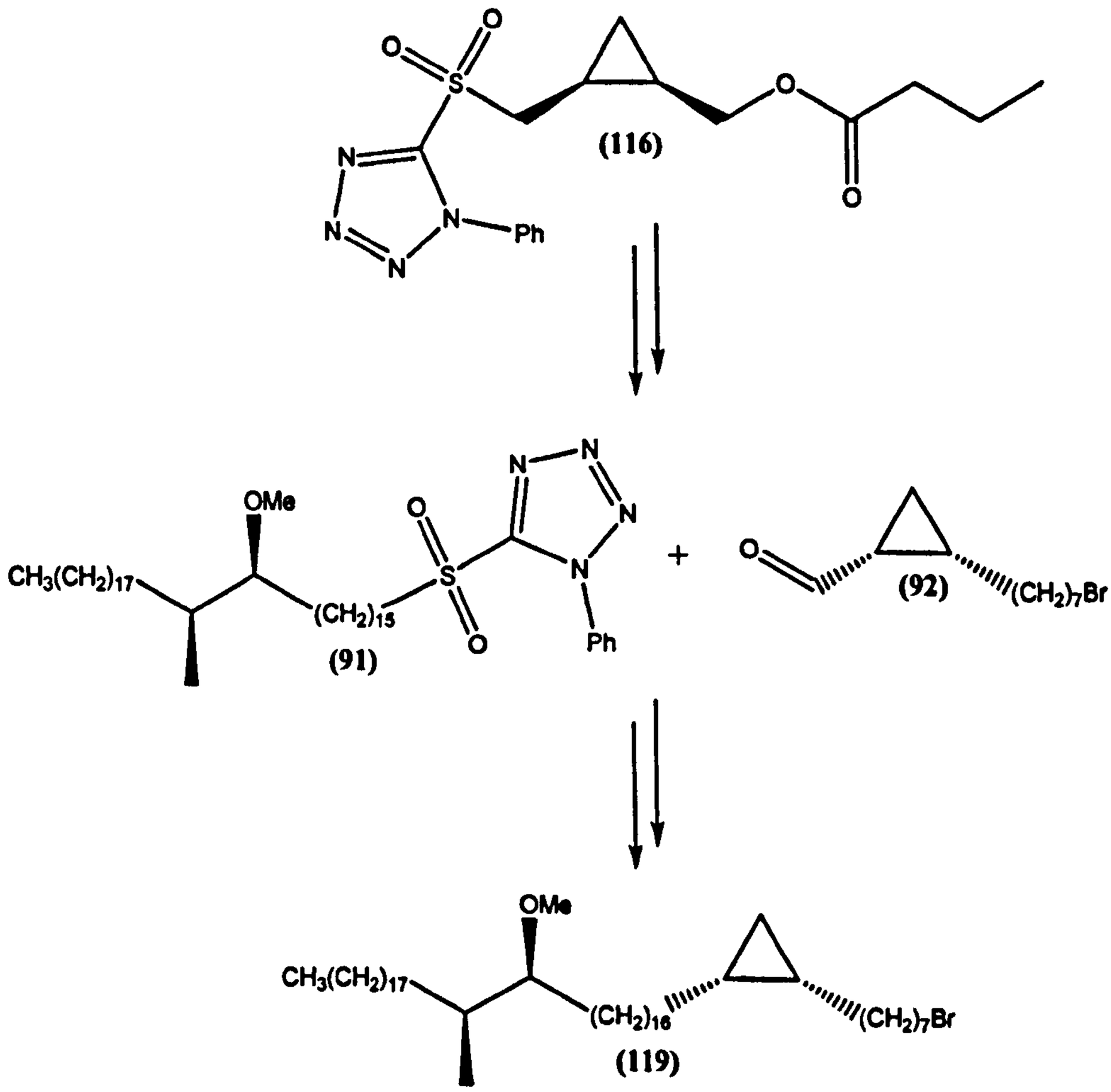


Fig. 56: Generation of *R,S cis*-cyclopropane stereochemistry

To prepare the *cis*-cyclopropane bromide **(92)** it was necessary to synthesise an aldehyde to couple to sulfone **(116)**. 1,6-Hexanediol **(120)** was brominated using hydrobromic acid to give 6-bromohexan-1-ol **(121)**, which was then oxidised to the aldehyde **(117)**, using PCC (See Fig. 57).[182]

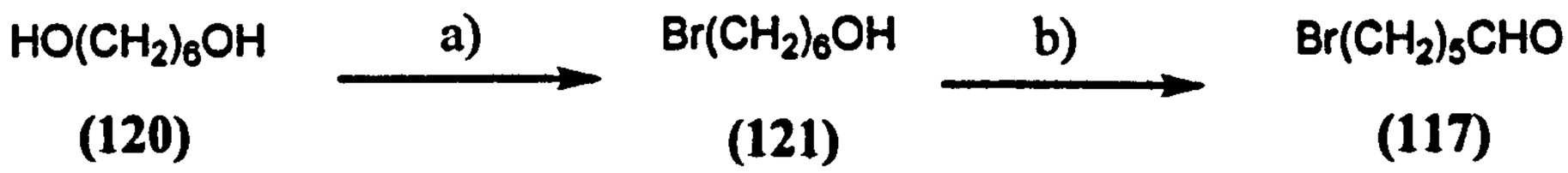


Fig. 57: aq. HBr, (57 %); b) PCC, (68 %)

This aldehyde **(117)** was then coupled to the previously prepared *cis*-cyclopropane sulfone **(116)** using lithium *bis*(trimethylsilyl)amide as a base, using the modified Julia-Kocienski reaction to give the olefin product **(122)** as an *E/Z* mixture. The olefin **(122)** was then hydrogenated; usually this is done using a palladium catalyst, however, in this case hydrogenation using a palladium catalyst would cause ring opening of the cyclopropane. Therefore hydrogenation was carried out using 2,4,6-triisopropyl-benzenesulfonyl hydrazide, so that the cyclopropane ring was maintained, giving butyric acid (1*R*,2*S*)-2-(7-bromoheptyl)cyclopropylmethyl ester **(118)** (See Fig. 58).[194]

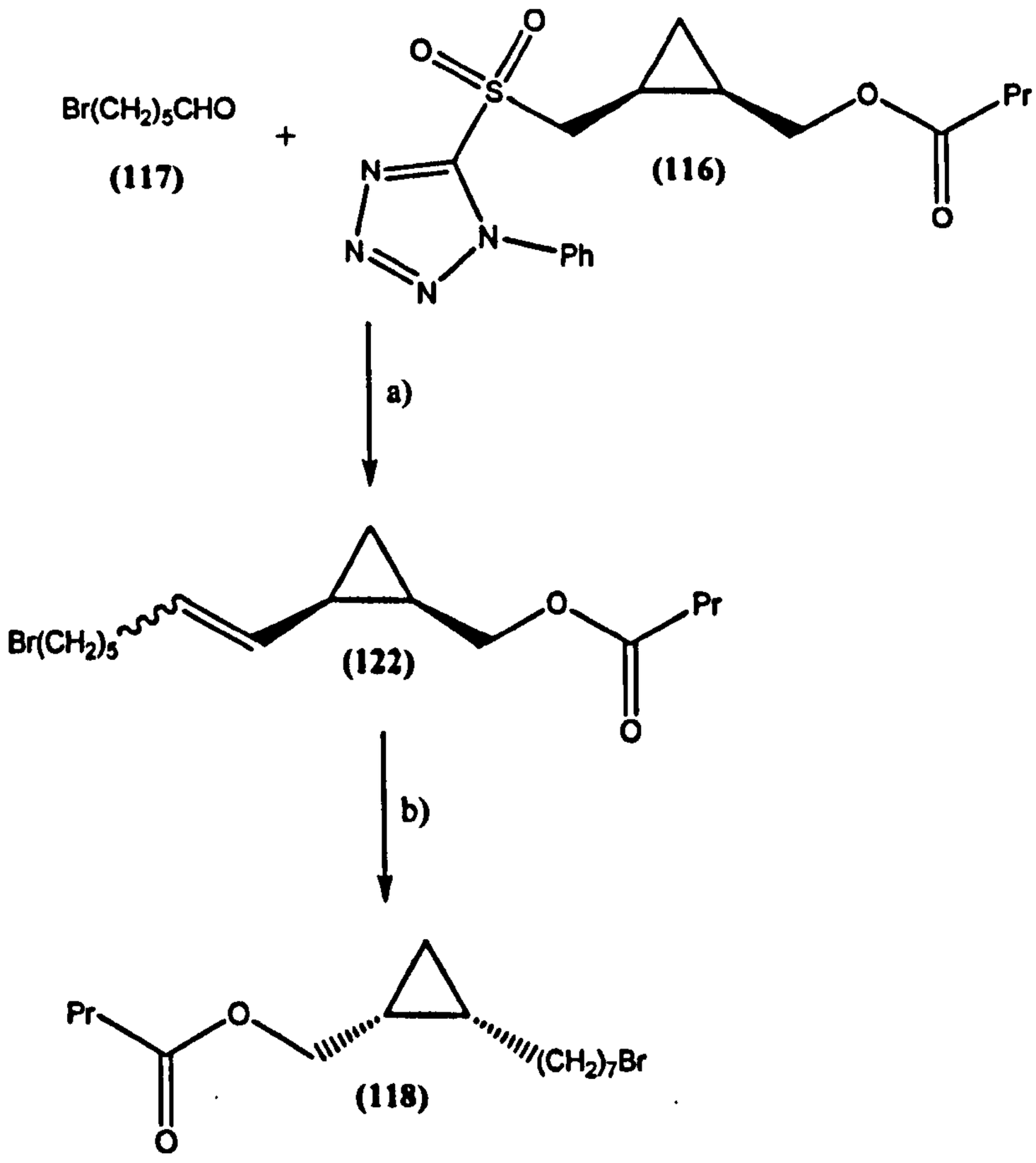


Fig. 58: a) LHMDS, (84 %); b) 2,4,6-triisopropyl-benzenesulfonyl hydrazide, (59 %)

2,4,6-Triisopropyl-benzenesulfonyl hydrazide was prepared from 2,4,6-triisopropyl-benzenesulfonyl chloride and hydrazine hydrate, following a method discussed by L. Friedmann (See Fig. 59).[194] Hydrogenation of an alkene using di-imide goes via the following mechanism (See Fig. 60).

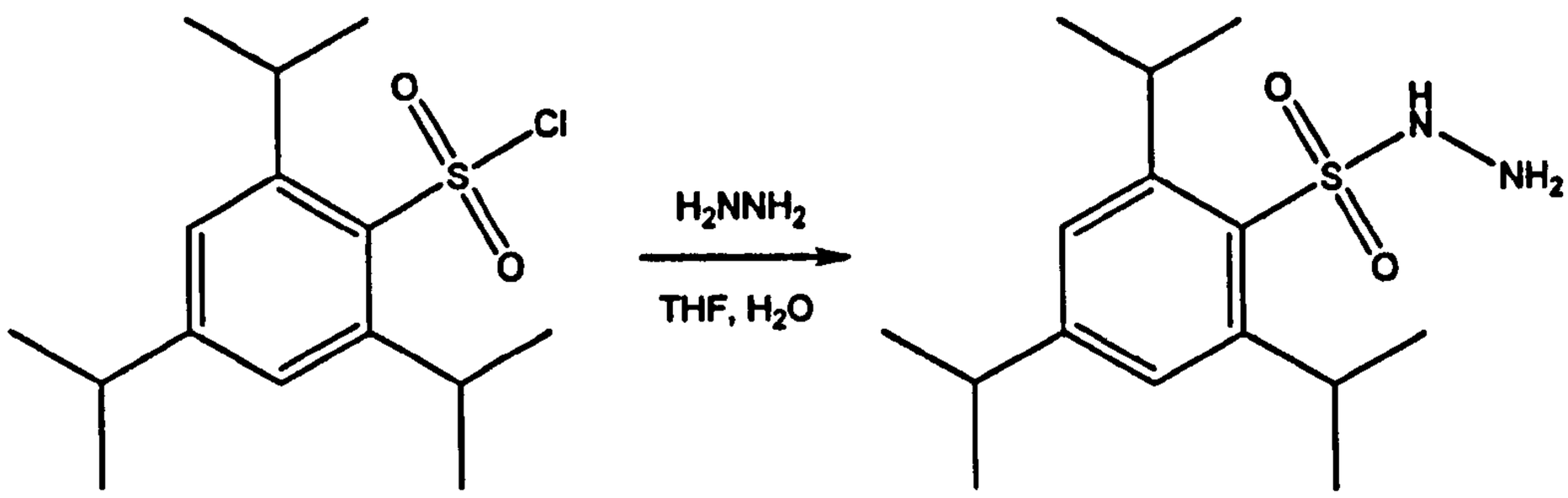


Fig. 59: Preparation of 2,4,6-triisopropyl-benzenesulfonyl hydrazide

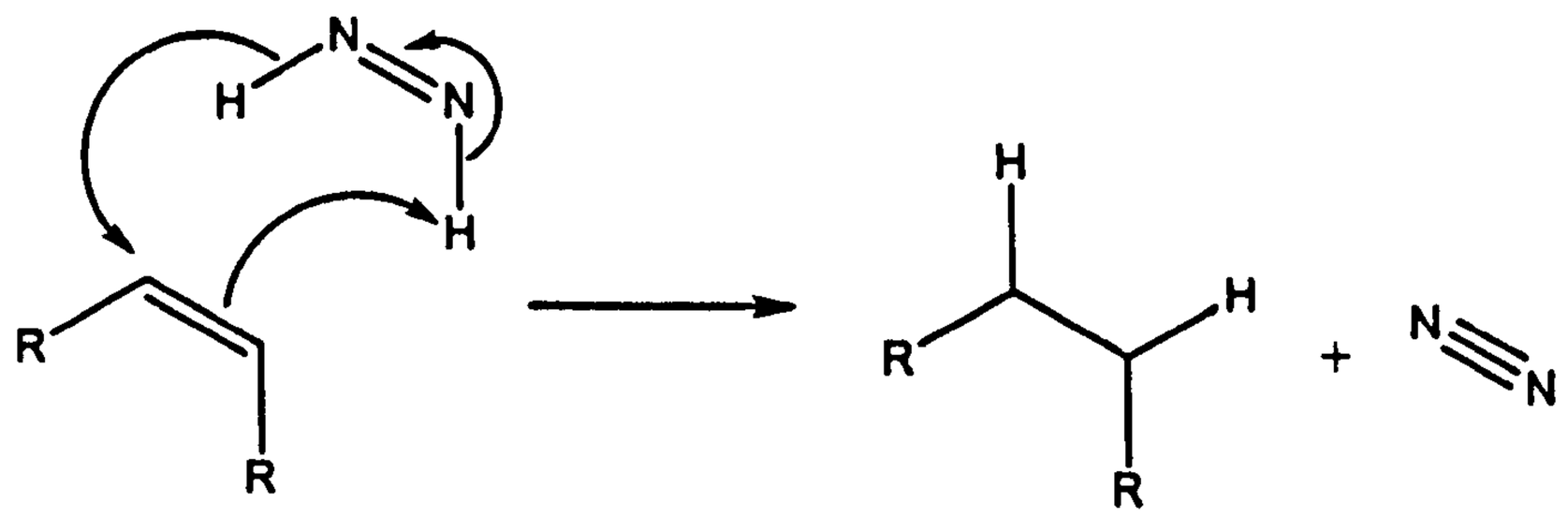


Fig. 60: Hydrogenation of an alkene with di-imide mechanism

The preparation of the six carbon aldehyde **(117)** and the *cis*-cyclopropane bromide **(118)** was monitored using ^{1}H NMR spectroscopy. 6-Bromohexan-1-ol **(121)** gave triplets at δ 3.67 and δ 3.43, corresponding to the CH_2's adjacent to the bromide and hydroxyl groups, respectively, with three pentets occurring upfield corresponding to the 3 other CH_2's. Upon oxidation of the alcohol group of **(121)**, a triplet is observed at δ 9.78 corresponding to the aldehyde proton of **(117)**. The CH_2 adjacent to the aldehyde shows a distorted triplet at δ 2.47; this is because the CH_2 is being split by the CH_2 adjacent to it and the aldehyde proton.

2.2.4 – Preparation of the meromycolate moiety

The butyl ester **(117)** was then removed using potassium carbonate to give (1*R*,2*S*)-2-(7-bromoheptyl)cyclopropyl methanol **(123)**, which was then oxidised

using PCC to give the aldehyde **(92)**. Compound **(92)** was then coupled to the previously prepared sulfone **(92)** in a modified Julia-Kocienski olefination, followed by hydrogenation using dipotassium azodicarboxylate to give the bromide **(119)**, (1*S*,2*R*)-1-(7-bromoheptyl)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl) cyclopropane (See Fig. 61).

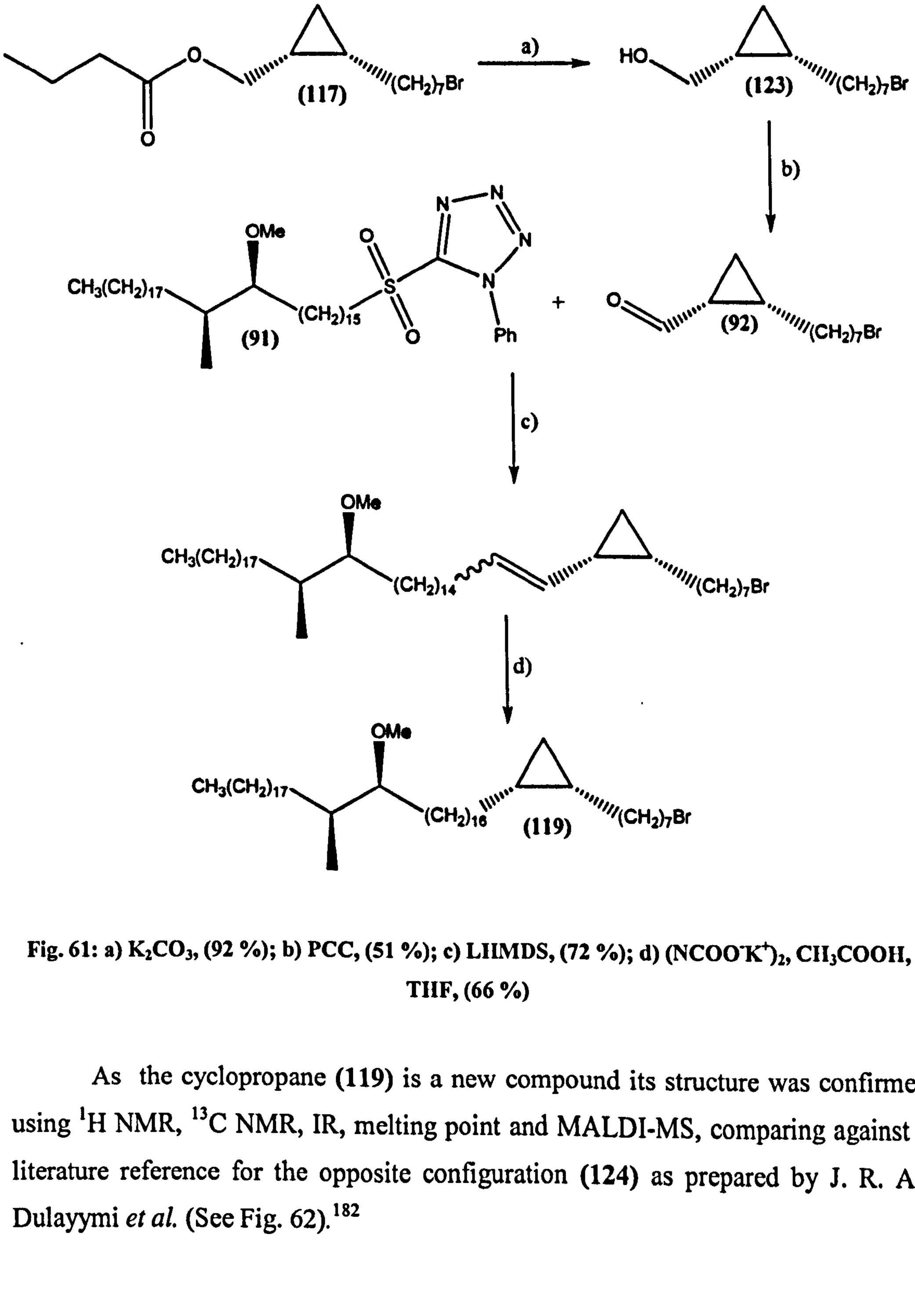
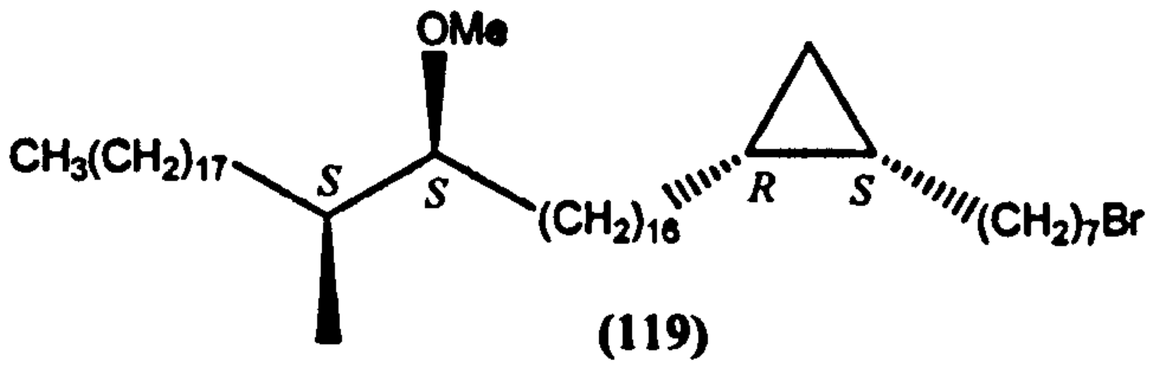
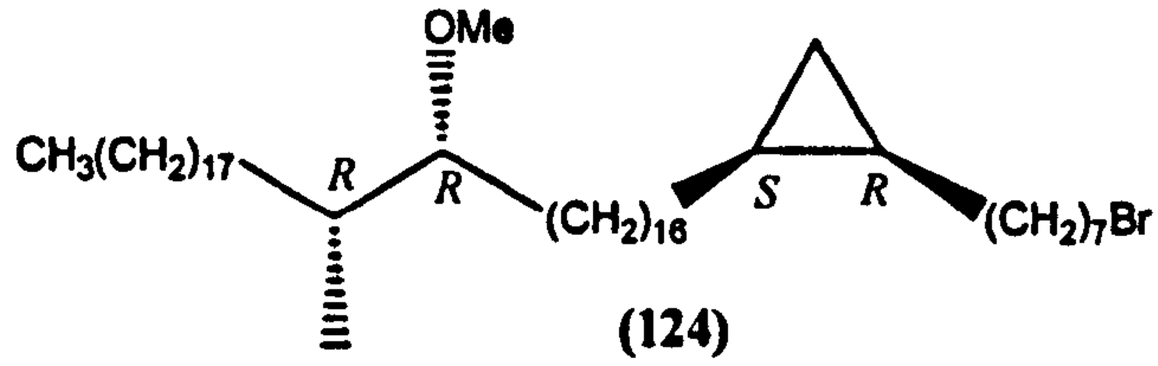


Fig. 61: a) K_2CO_3, (92 %); b) PCC, (51 %); c) LIIMDS, (72 %); d) $(NCOO^-K^+)_2$, CH_3COOH, THF, (66 %)

As the cyclopropane **(119)** is a new compound its structure was confirmed using ^1H NMR, ^{13}C NMR, IR, melting point and MALDI-MS, comparing against a literature reference for the opposite configuration **(124)** as prepared by J. R. Al-Dulayymi *et al.* (See Fig. 62).[182]



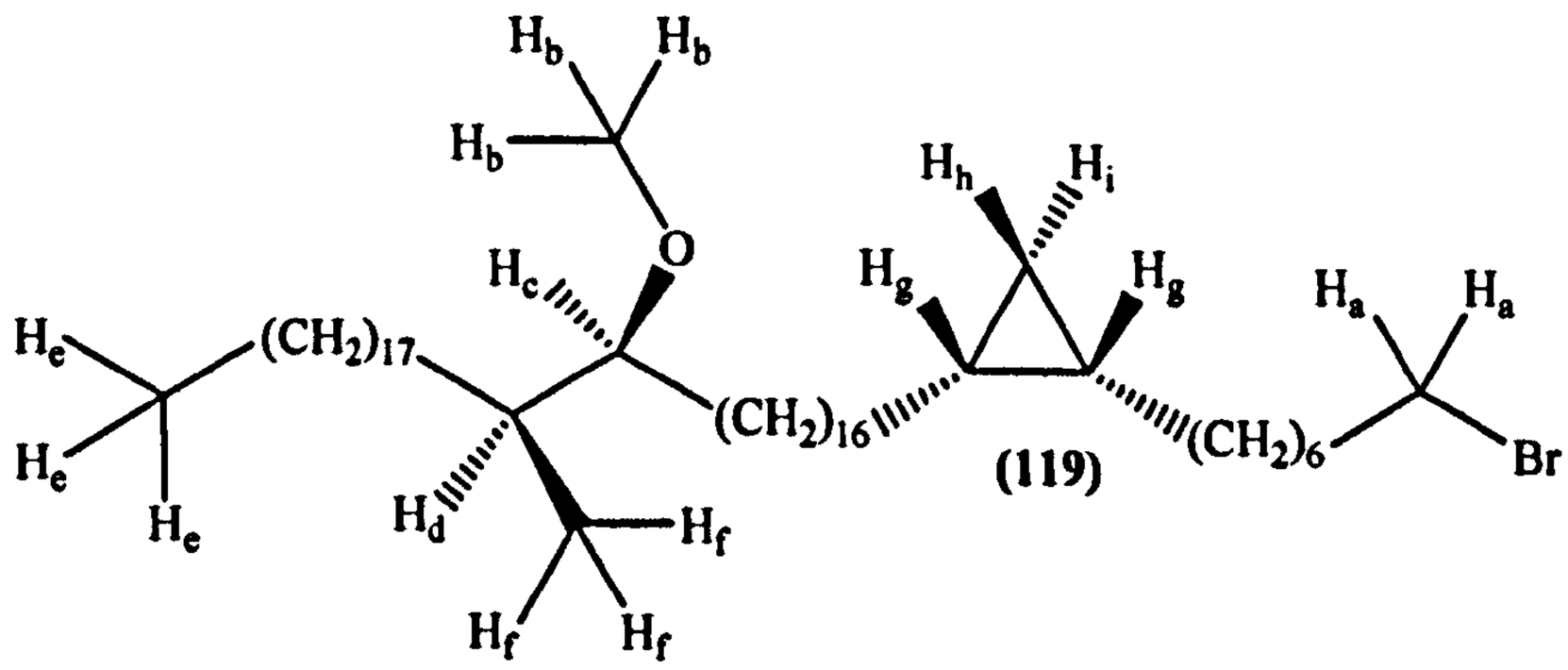
(119)



(124)

Fig. 62: *S,S-R,S* isomer (120) and *R,R-S,R* isomer (124)

The ^{1}H NMR of **(119)** was analysed to contribute to the confirmation of its structure (See Fig. 63).



(119)

H_x	δ	Multiplicity	Integration	J (H_z)
H_a	3.42	t	2	7
H_b	3.34	s	3	-
H_c	2.99-2.93	m	1	-
H_d	1.66-1.61	m	1	-
H_e	0.9	t	3	7
H_f	0.88	d	3	7
H_g	0.75-0.65	m	2	-
H_h	0.61	dt	1	4, 8
H_i	-0.33	q	1	4

Fig. 63: ^{1}H NMR data of methoxy-mycolic acid intermediate (119)

The ^{1}H NMR data of the *S,S-R,S* isomer **(119)** matched that of the *R,R-S,R* isomer **(124)**.[182]

The two proton multiplet shown between δ 0.75 and δ 0.65 arises from the two CH groups of the cyclopropane ring and the doublet of triplets and quartet correspond to the CH_2 within the cyclopropane ring at δ 0.61 (*cis* of the CH_2) and δ -0.33 (*trans* of the CH_2) (See Fig. 64).

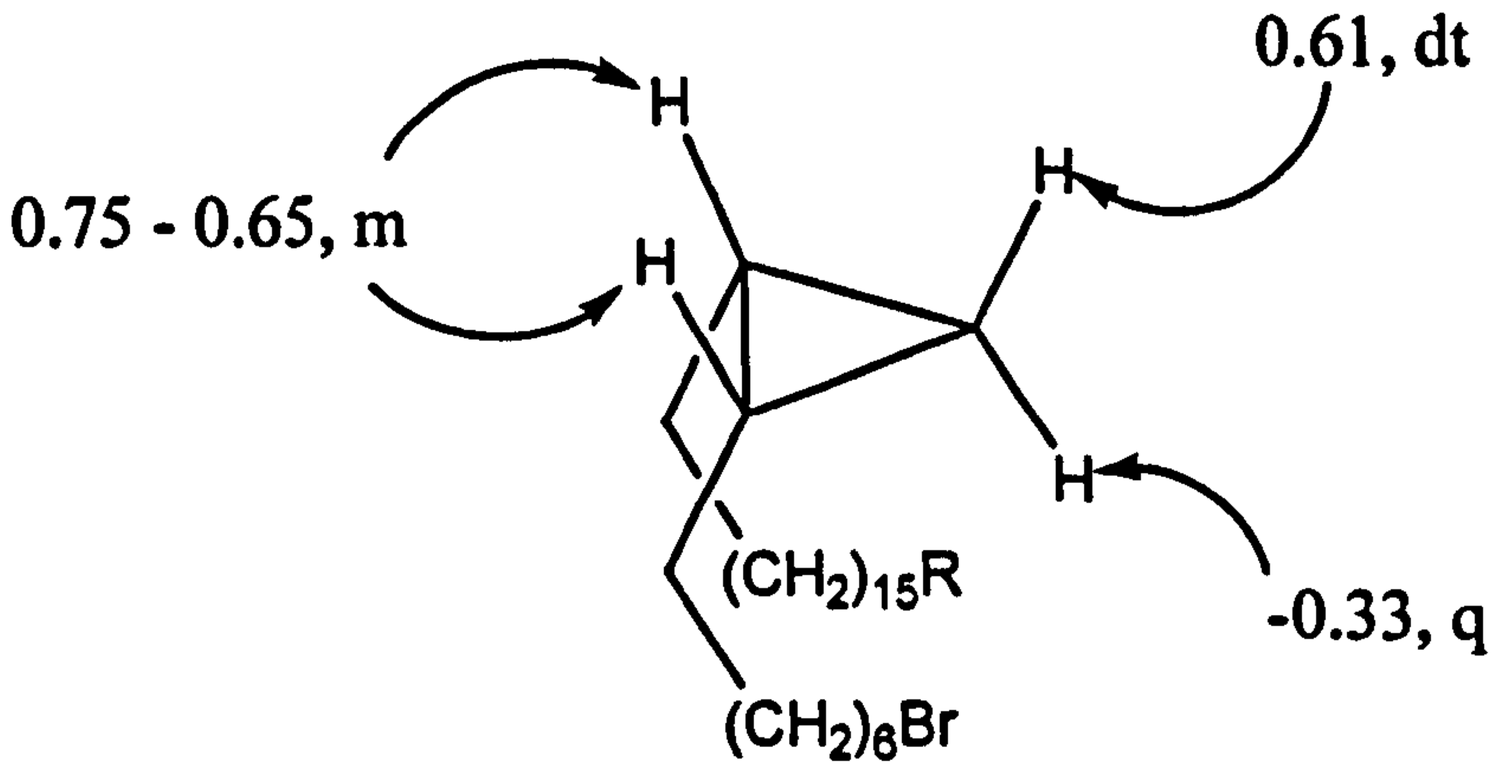


Fig. 64: Cyclopropane ^{1}H NMR signals

Also, as expected, the ^{13}C NMR, IR, MALDI-MS and melting point matched the literature for the opposite isomer **(124)**,[182] hence confirming the structure of **(119)**. The optical rotation confirmed that the predicted stereochemistry of **(119)**, -3.4, was correct, with the literature value for the opposite enantiomer **(124)** being +5.5.[182]

The bromide **(119)** was then converted into the corresponding sulfide **(125)** using 1-phenyl-1H-tetrazol-5-thiol and potassium carbonate, and the sulfide **(125)** was oxidised using *m*-chloroperoxybenzoic acid to give the tetrazole sulfone **(89)** (See Fig. 65).

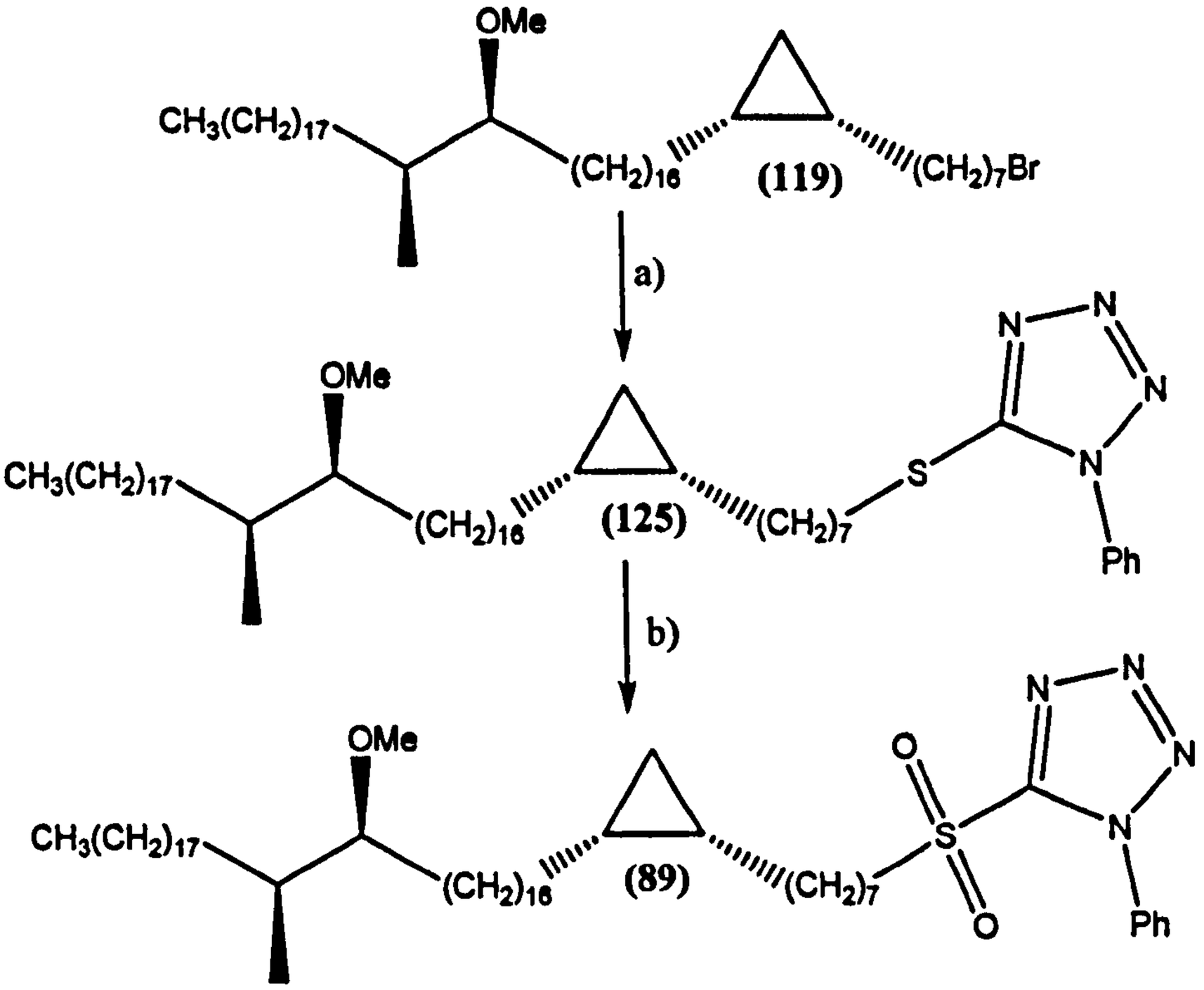


Fig. 65: a) 1-phenyl-1H-tetrazole-5-thiol, K_2CO_3, (67 %); b) m-CPBA, $NaHCO_3$, (65 %)

The preparation of sulfone **(89)** was followed primarily using ^1H NMR, with all relevant data also being collected to fully characterise the compounds. The ^1H NMR of **(89)** as expected exhibited similar characteristics to those of the previously prepared sulfones. It is worth noting that when the sulfide was oxidised to the sulfone, the CH_2 adjacent to the sulfur showed a unique splitting pattern. The signal observed is a characteristic AA'BB' system. This occurs when the two CH_2's adjacent to a substituent have non-magnetically equivalent protons. This can be shown by a Newman Projection, where H_A will show *cis*- splitting to H_B and *trans*- to $H_{B'}$ (See Fig. 66 and 67).

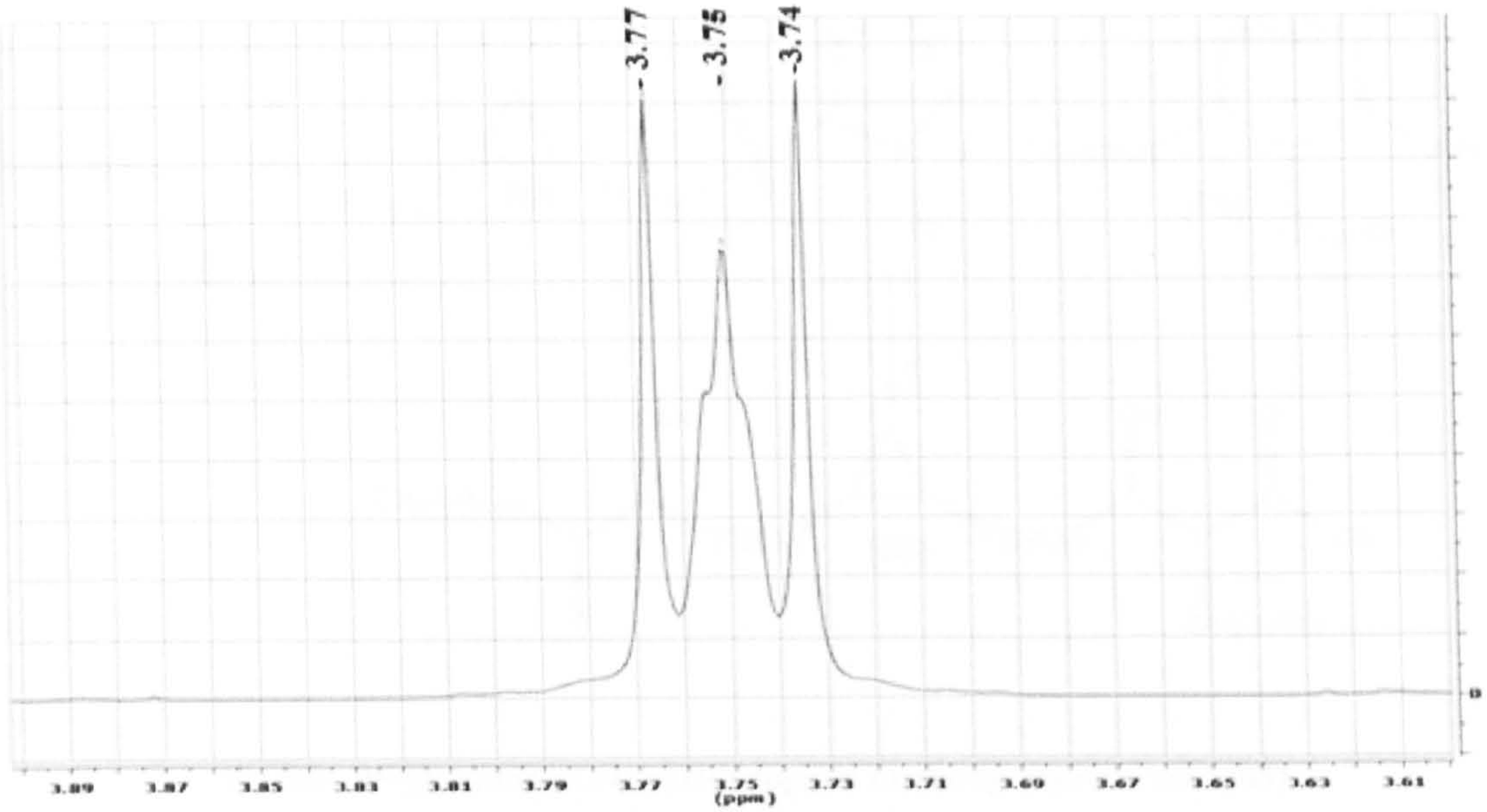


Fig. 66: AA'BB' system ^{1}H NMR

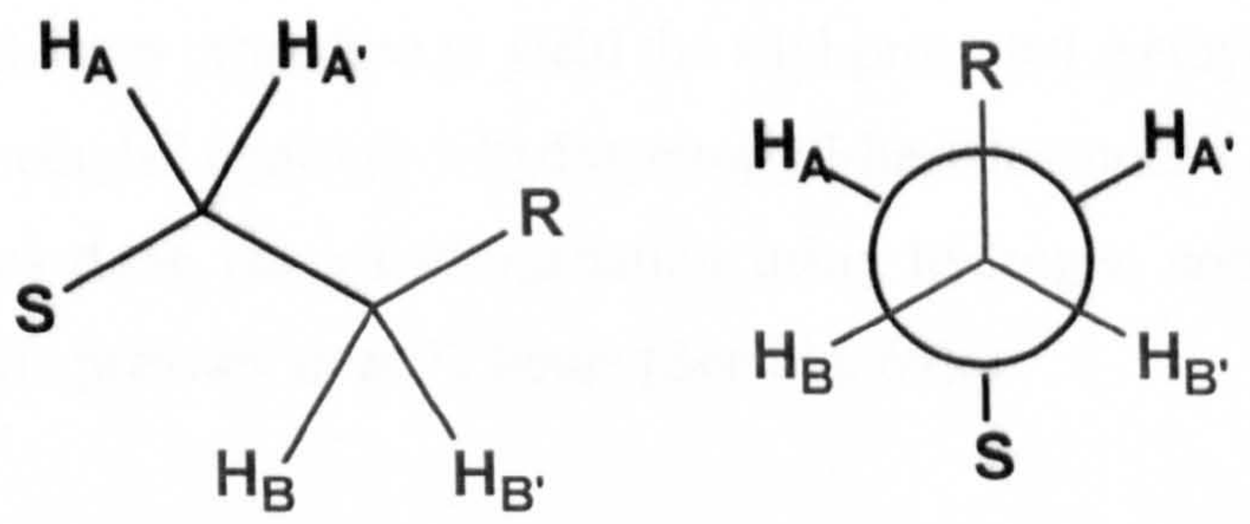


Fig. 67: AA'BB' system Newman projection

2.2.5 – Preparation of the branched hydroxy acid

To obtain the *cis*-cyclopropane methoxy-mycolic acid **(83),** it was necessary to couple a branched hydroxy acid to the meromycolate sulfone intermediate **(89)**. The branched hydroxy acid aldehyde intermediate **(90)** was obtained following the disconnection route previous discussed on page 45 (See Fig. 68).

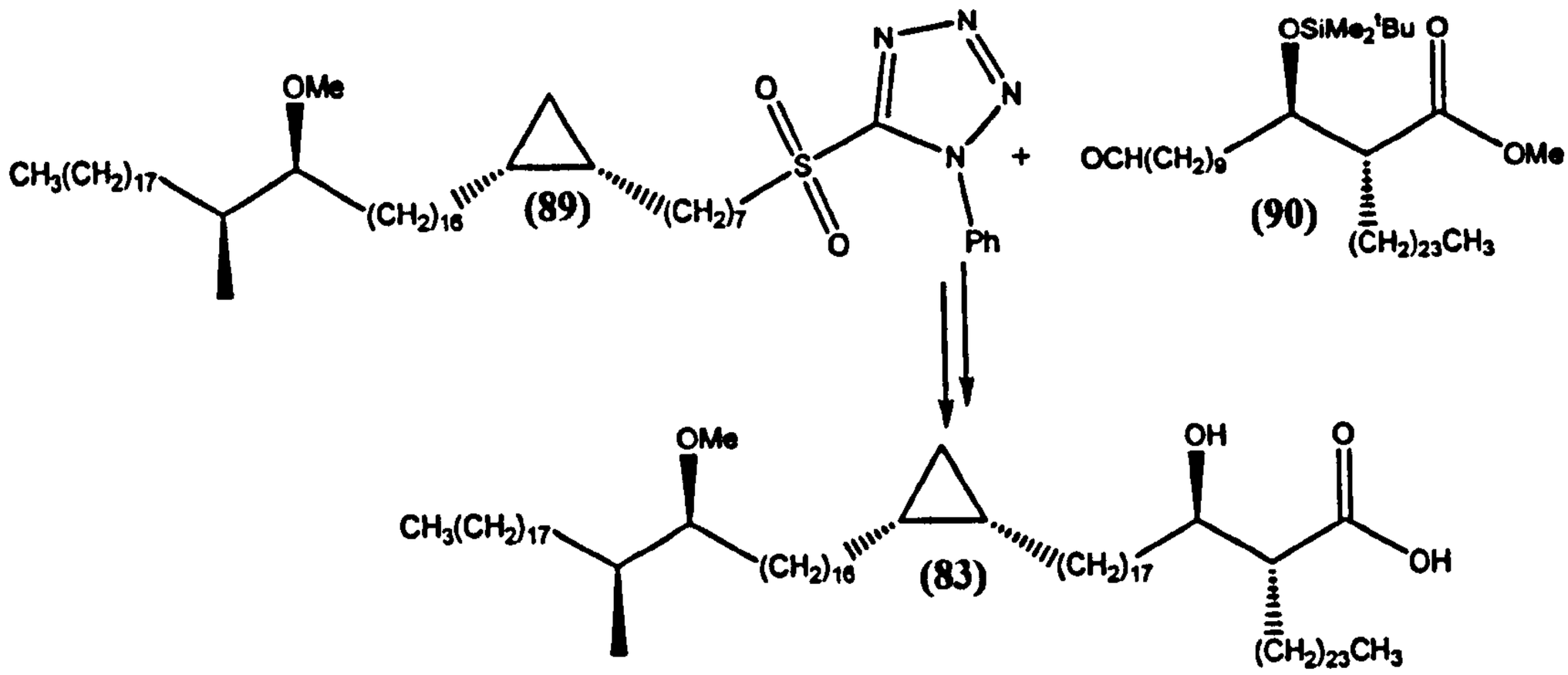


Fig. 68: Preparation of *cis*-cyclopropane methoxy-mycolic acid (83)

The aldehyde **(90)** was prepared using the intermediate **(126)** contributed by Cornelia Theunissen.[195] The benzyl protected alkene **(126)** was hydrogenated and deprotected in a single step procedure to yield the silyl protected methyl ester, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-hydoxypropyl]-hexacosanoic acid methyl ester **(127)**. This was done *via* a hydrogenation using hydrogen and a palladium catalyst at atmospheric pressure over 72 hours (See Fig. 69).

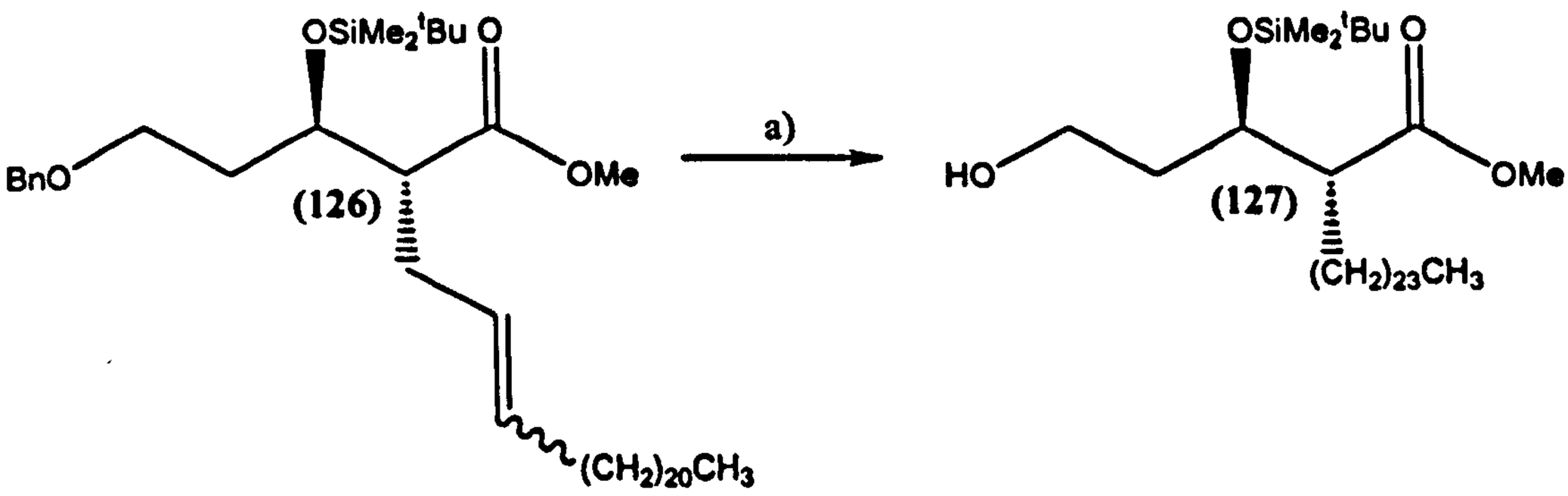


Fig. 69: a) H_2, Pd cat., (89 %)

The structure of compound **(127)** was confirmed primarily using ^{1}H NMR, with the product no longer showing signals for the benzyl group and the olefin in its spectrum. The primary alcohol group was then oxidised using PCC to give the corresponding aldehyde **(128)** using the methods previously described by J.R. Al-Dulayymi *et al.* (See Fig. 70).[182]

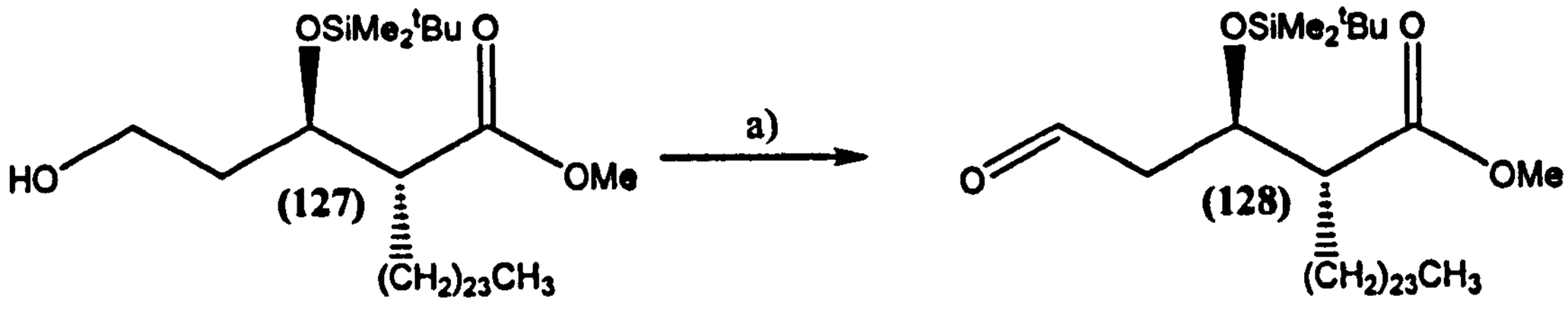


Fig. 70: PCC, (54 %)

Again the structure of the product was confirmed using ^{1}H NMR, with a triplet observed at δ 9.81 corresponding to the aldehyde proton. To give the correct chain length between the meromycolate sulfone intermediate **(89)** and the hypothesized aldehyde branched hydroxy acid intermediate **(90)**, an eight carbon chain was required. The previously discussed 2,2-dimethyl-propionic acid 8-(1-phenyl-1H-tetrazole-5-sulfonyl)-octyl ester **(96)** was used in a modified Julia-Kocienski olefination to give the previously unsynthesized alkene mixture **(129)**, which was hydrogenated at atmospheric pressure using a palladium catalyst to give the *tert*-butyl ester **(130)** (See Fig. 71). The structure of the ester **(130)** was confirmed primarily using ^{1}H NMR (See Fig. 72).

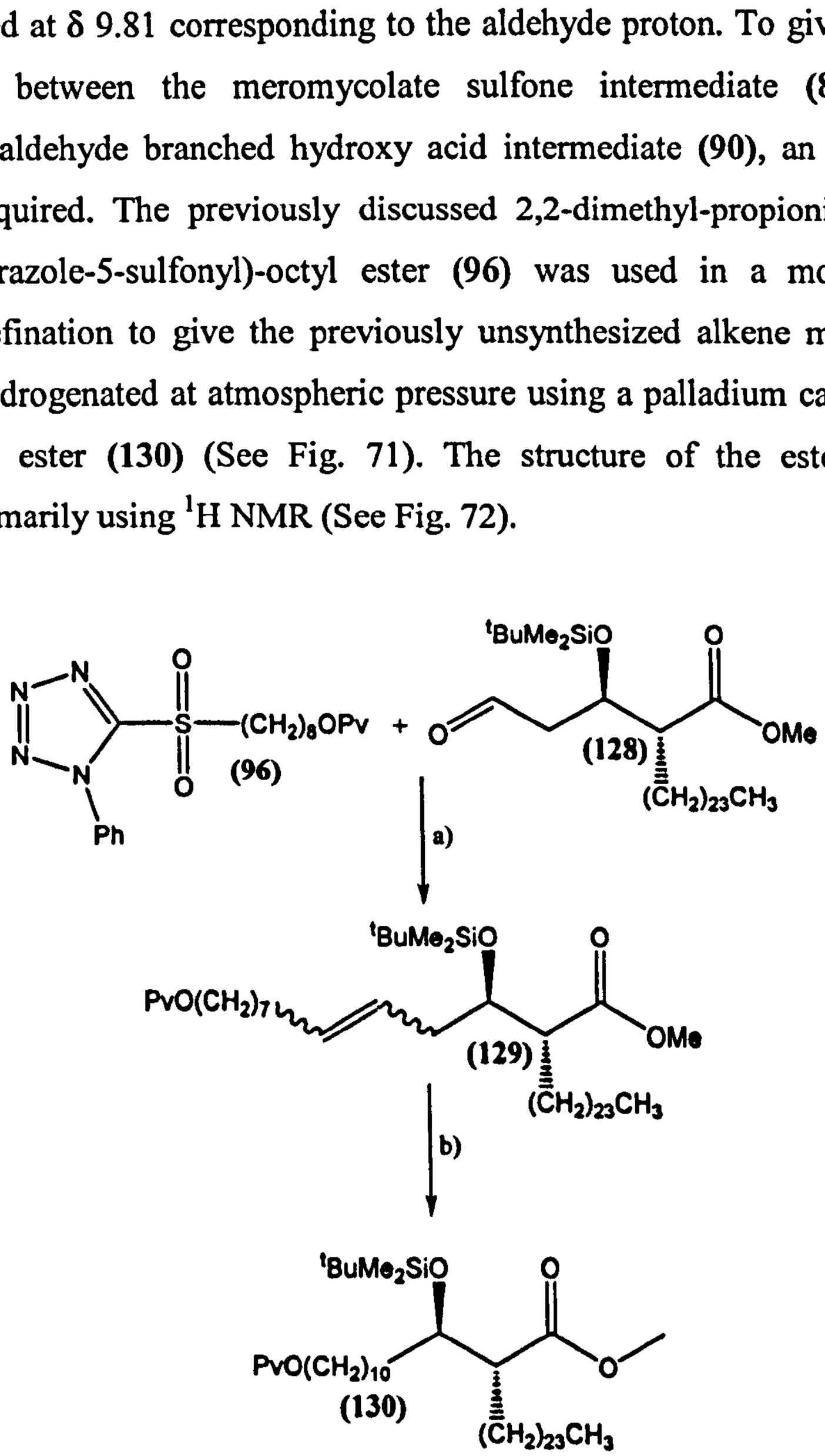
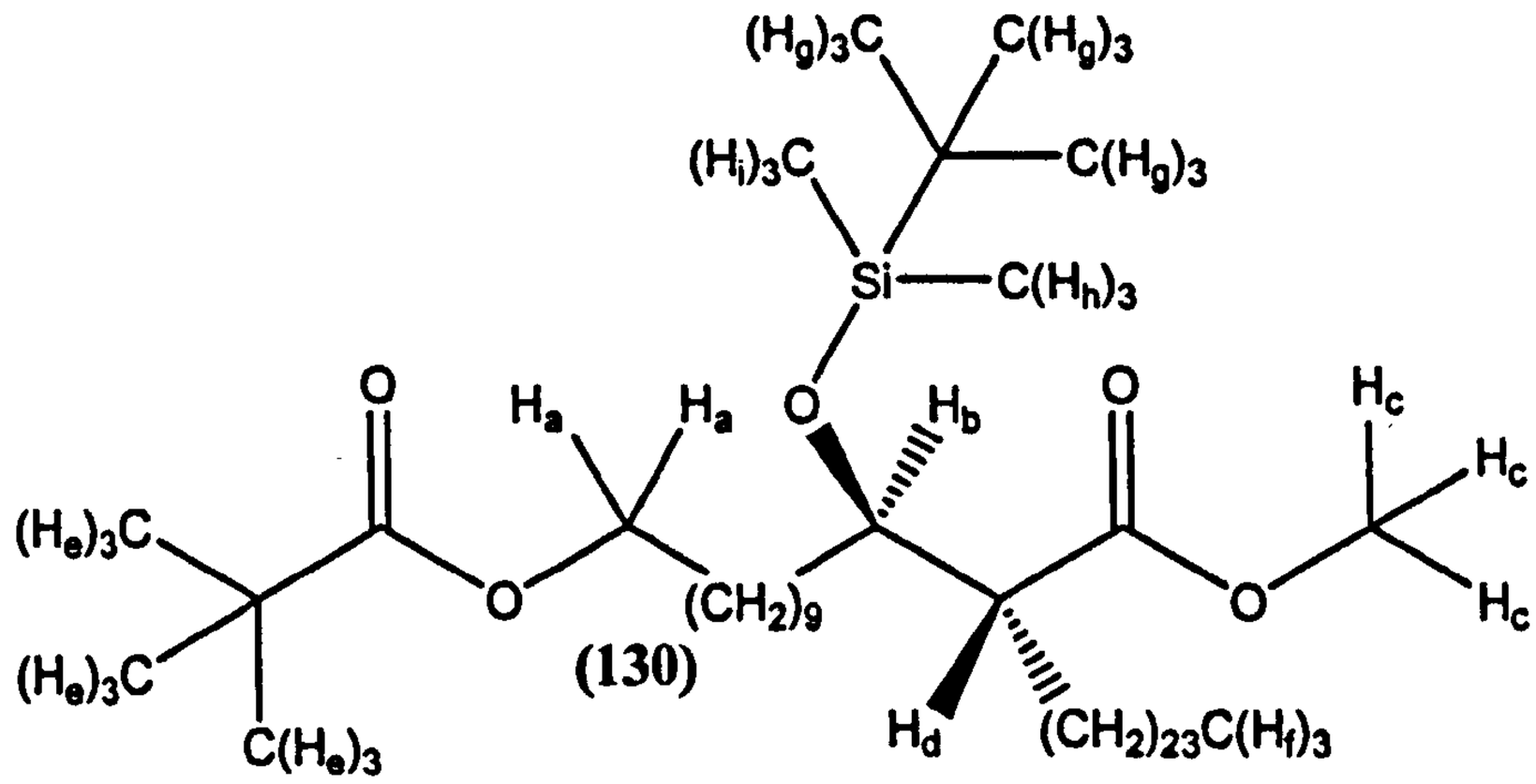


Fig. 71: a) LHMDS, (70 %); b) H_2, Pd cat., (99.9 %)



H_x	δ	Multiplicity	Integration	J (H_z)
H_a	4.06	t	2	6.5
H_b	3.94-3.87	m	1	-
H_c	3.66	s	3	-
H_d	2.55-2.50	m	1	-
H_e	1.21	s	9	-
H_f	0.88	t	3	7
H_g	0.86	s	9	-
H_h	0.05	s	3	-
H_i	0.03	s	3	-

Fig. 72: ^{1}H NMR analysis of (*R*)-2-[(*R*)-1-(*tert*)-butyldimethylsilanyloxy)-11-(2,2-dimethylpropionyloxy)undecyl] hexacosanoic acid methyl ester (130)

The aldehyde **(90)** was prepared by first deprotecting the *tert*-butyl ester **(130)** using potassium hydroxide in THF, methanol and water at reflux. The oxidation of the resultant primary alcohol **(131)** was carried out using PCC (See Fig. 73).

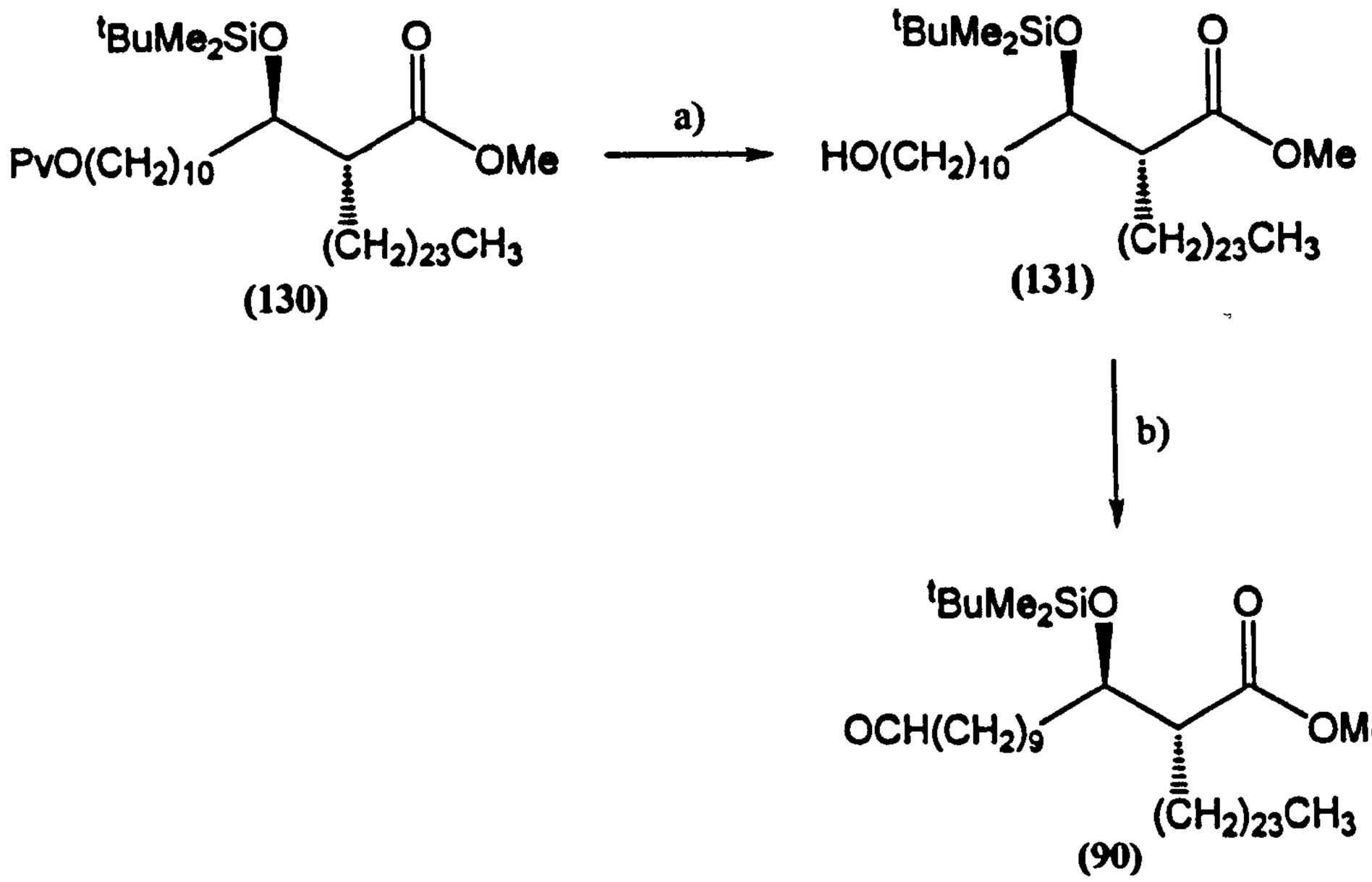


Fig. 73: a) KOH, THF/MeOH/H_2O, (63 %); b) PCC, (99.7 %)

2.2.6 – Coupling the meromycolate moiety and branched hydroxy acid

A modified Julia-Kocienski olefination was carried out on the meromycolate sulfone intermediate **(89)** and the branched hydroxy acid aldehyde **(90)** using lithium *bis*(trimethylsilyl)amide as the base to give the alkene mixture **(132)**, which was hydrogenated using dipotassium azocarboxylate to give the protected *cis*-cyclopropane methoxy-mycolic acid **(133)** (See Fig. 74).

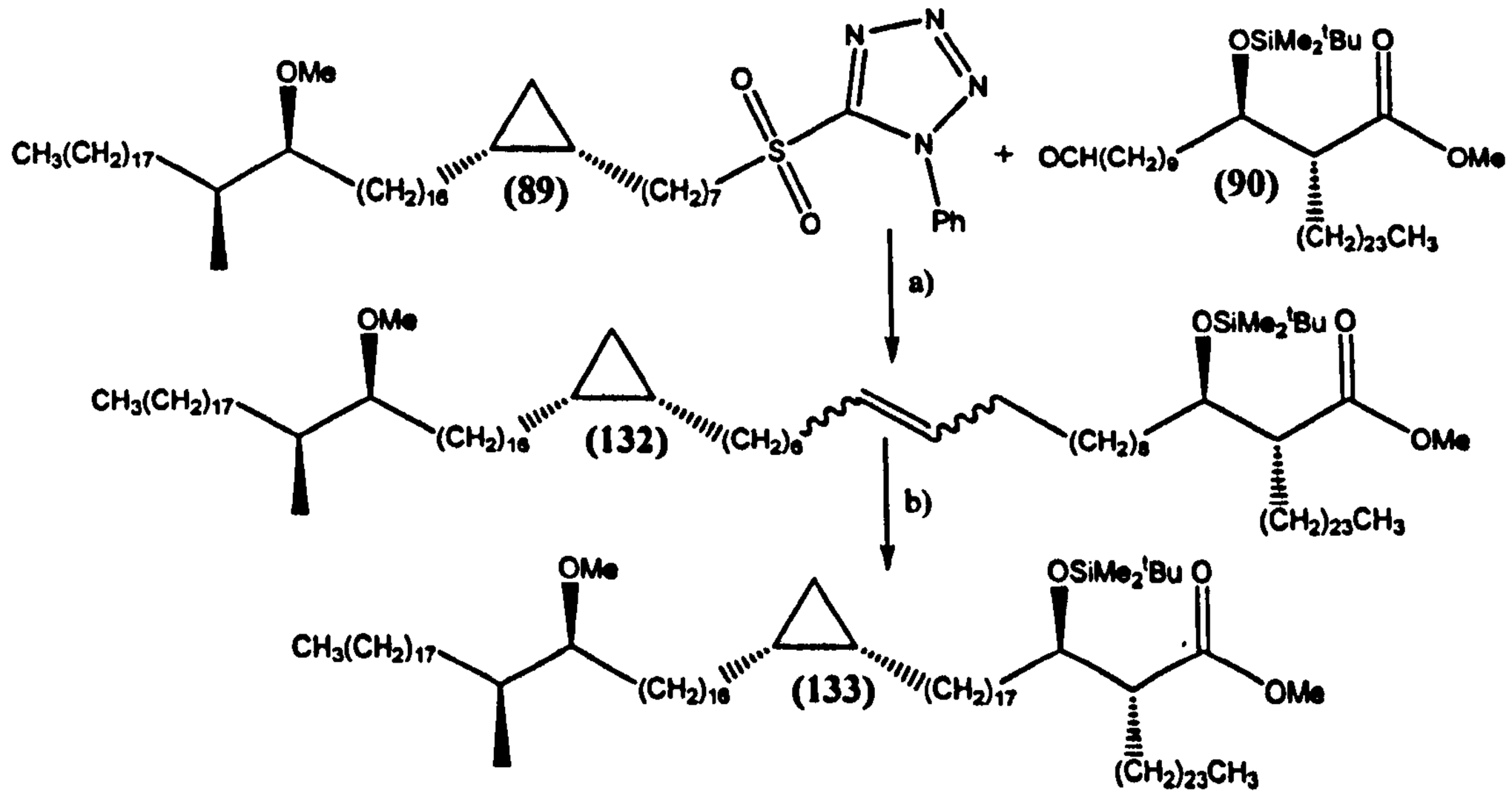


Fig. 74: a) LHMDS, (65 %); b) $(NCOO^-K^+)_2$, CH_3COOH, THF, (86 %)

The protected *cis*-cyclopropane methoxy-mycolic acid **(133)** was characterised using ^{1}H and ^{13}C NMR, IR, optical rotation and MALDI-MS. MALDI-MS was not recorded for J.R. AL-Dulayymi *et al.*'s diastereoisomer **(134)** (See Fig. 75), however the value recorded for **(133)** was correct when compared to the expected theoretical value. The IR and $[\alpha]_D$ were in accordance with the expected values in comparison to **(133)**'s different configuration to **(134)** (See Fig. 76).[182] The ^{1}H and ^{13}C NMR data of ester **(133)** was analysed as follows (See Fig. 77 and 78).

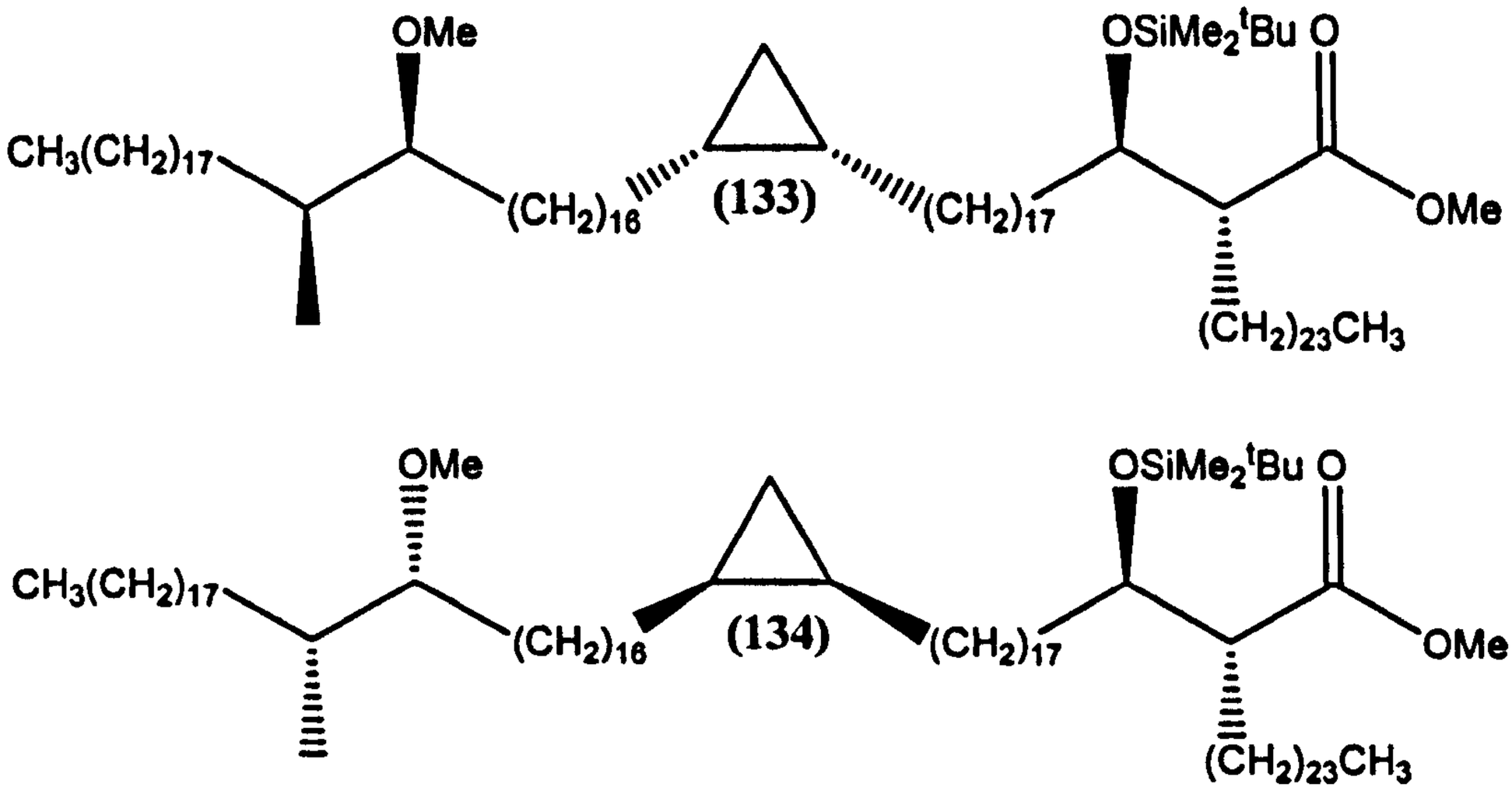
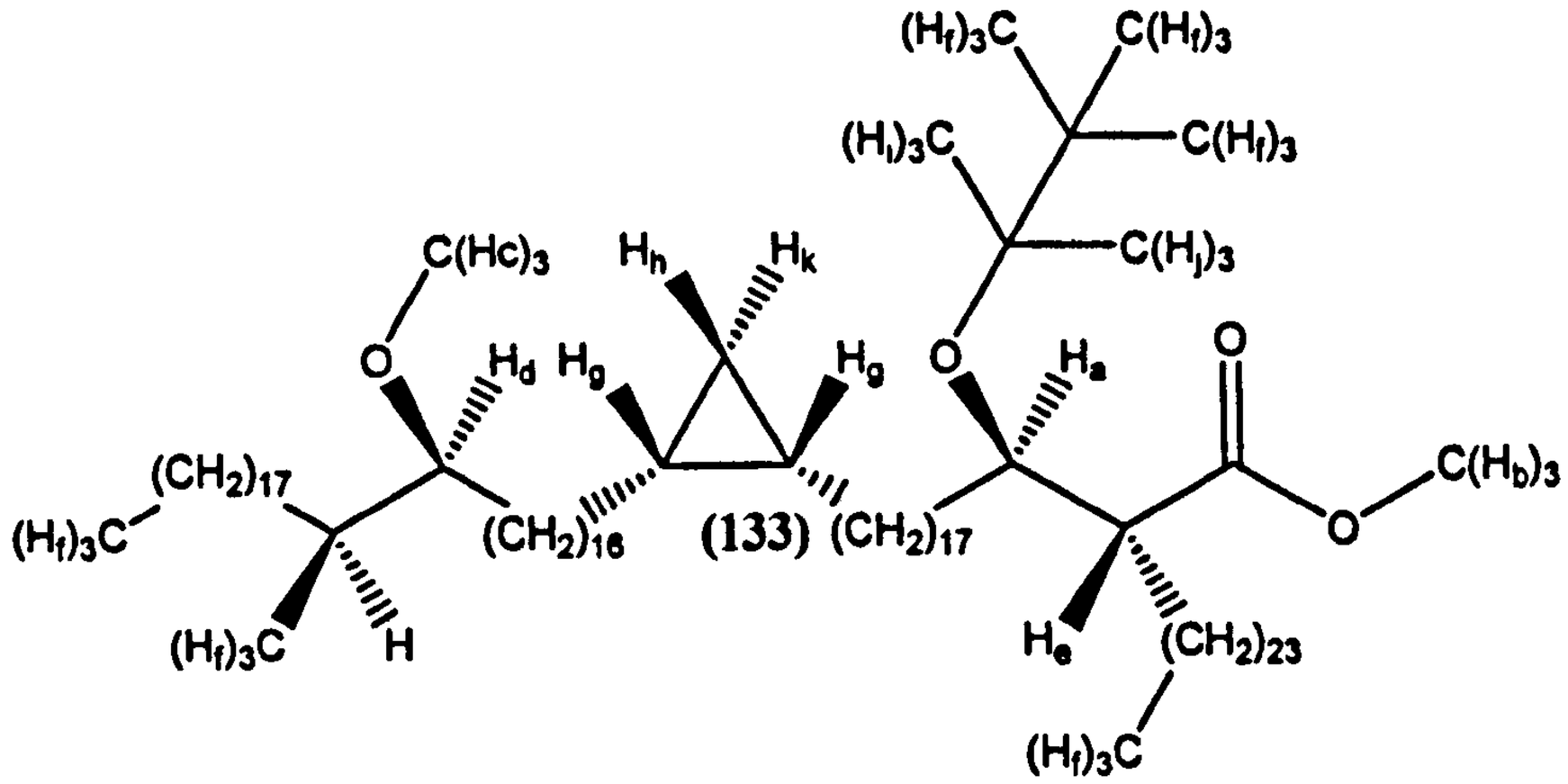


Fig. 75: *cis*-Cyclopropane methoxy-mycolic acids (133) and (134)

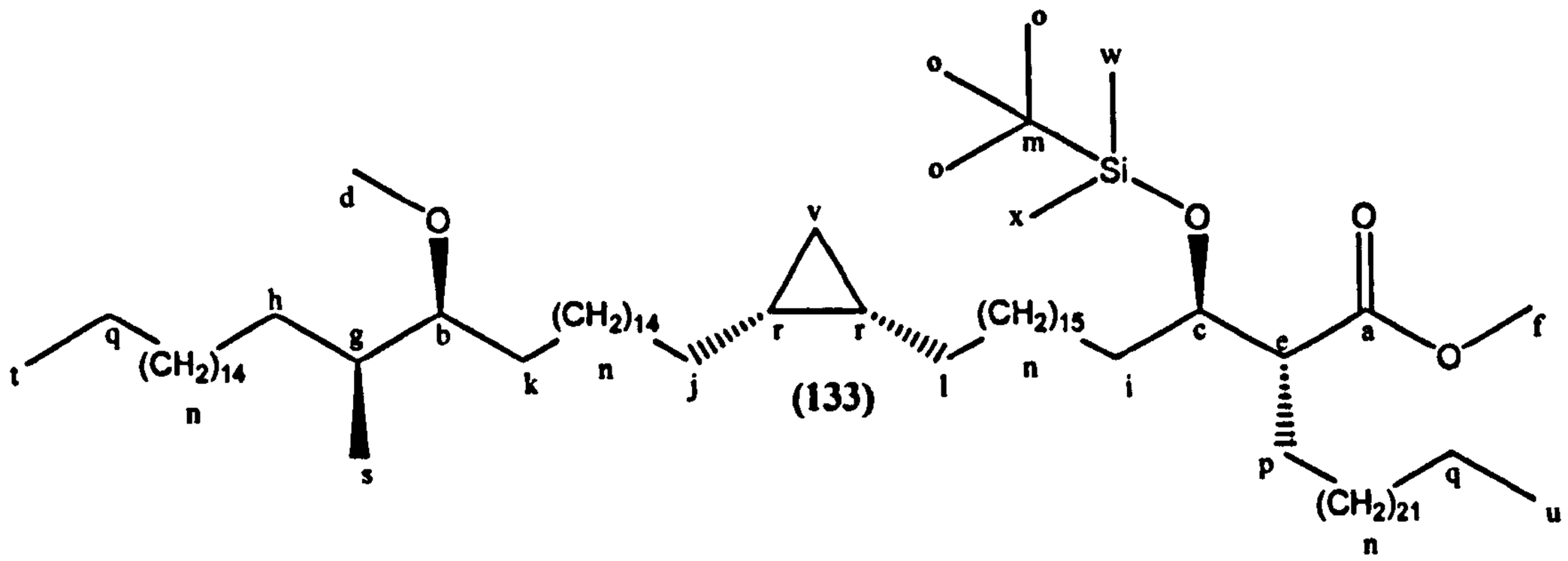
	(133)	(134)
IR	2921, 2876, 1763, 1465, 1102	2922, 2852, 1740, 1465, 1099
$[\alpha]_D$	6.16	-4.4
MALDI-MS ($M+Na^+$)	1404.25 (requires: 1404.39)	not measured

Fig. 76: IR, $[\alpha]_D$ and MALDI-MS data comparison for (133) and (134)



H_x	δ	Multiplicity	Integration	J (H_z)
H_a	3.95-3.89	m	1	-
H_b	3.65	s	3	-
H_c	3.36	s	3	-
H_d	3.00-2.96	m	1	-
H_e	2.55	ddd	1	4, 7, 11
H_f	0.96-0.84	m	18	-
H_g	0.71-0.67	m	2	-
H_h	0.60	dt	1	3.78, 7.65
H_i	0.05	s	3	-
H_j	0.03	s	3	-
H_k	-0.32	q	1	5

Fig. 77: ^{1}H NMR data analysis of protected *cis*-cyclopropane methoxy-mycolic acid (133)



C_x	δ	C_x	δ	C_x	δ
C_a	175.1	C_i	32.4	C_q	22.7
C_b	85.5	C_j	31.9	C_r	18
C_c	73.2	C_k	30.5	C_s	15.8
C_d	57.7	C_l	30.2	C_t	14.9
C_e	51.6	C_m	30.0	C_u	14.1
C_f	51.2	C_n	29.9-26.2	C_v	10.9
C_g	35.4	C_o	25.8	C_w	-4.4
C_h	33.7	C_p	23.7	C_x	-4.9

Fig. 78: ^{13}C NMR data analysis of protected *cis*-cyclopropane methoxy-mycolic acid (133)

Deprotection of the *tert*-butyl dimethylsilyl group in **(133)** was done using hydrofluoric acid in pyridine, to give the *cis*-cyclopropane methoxy-mycolic acid methyl ester, **(135)**. The methyl ester was then hydrolysed using lithium hydroxide in THF, methanol and water to give the acid **(83)** (See Fig. 79).

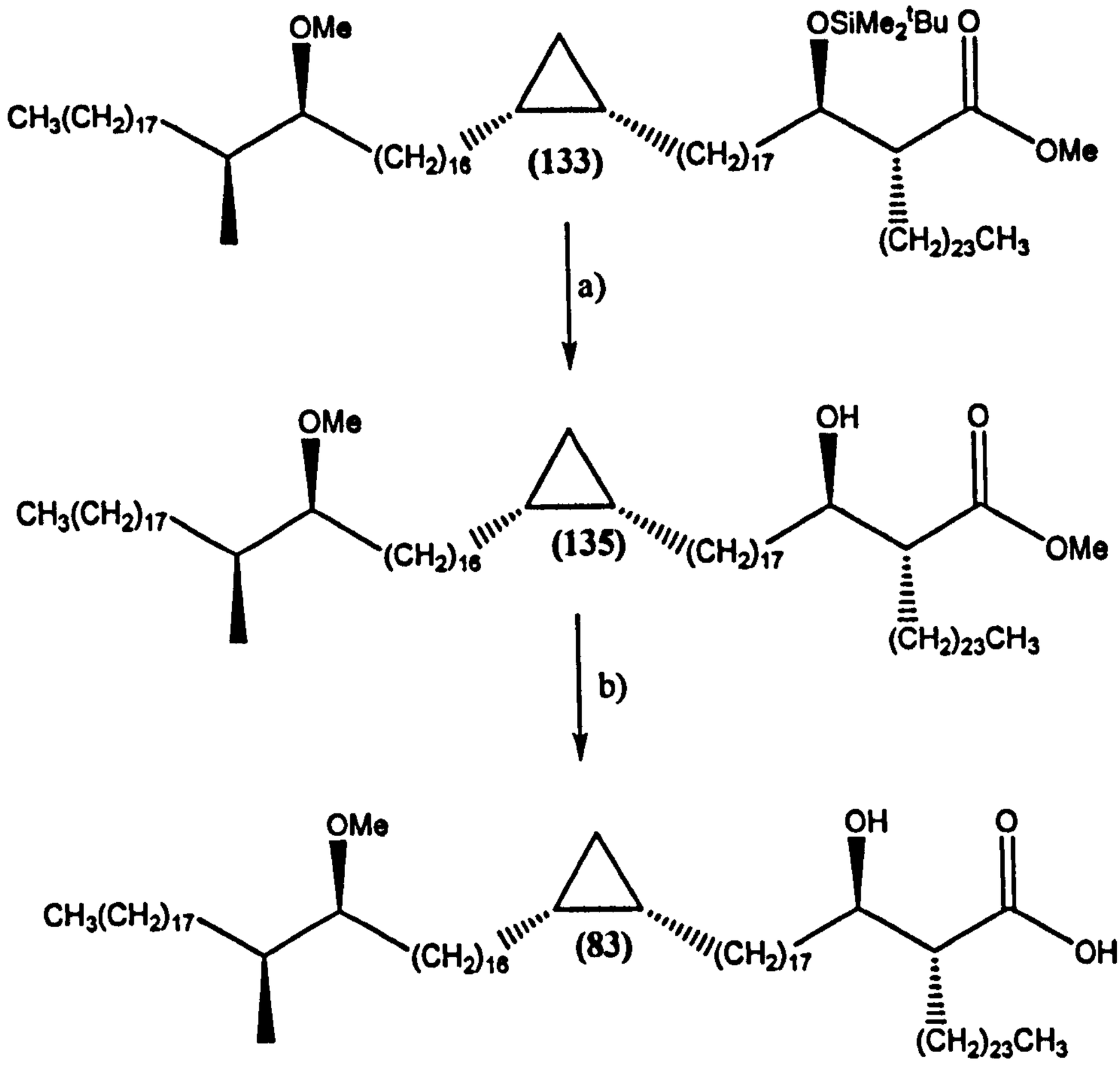


Fig. 79: a) HF. pyr, pyridine, (79 %); b) LiOH, THF/MeOH/H_2O, (63 %)

The successful formation of mycolic acid **(83)** was confirmed primarily using ^{1}H NMR, with the corresponding signals no longer being observed for the *tert*-butyl dimethylsilyl group (H_f, H_i and H_j) and methyl ester (H_b). Also, in the ^{13}C NMR spectrum, the signals corresponding to the *tert*-butyl dimethylsilyl group (C_o, C_m, C_w and C_x) and methyl ester (C_f) were no longer present. The MALDI-MS of the final compound **(83)** was found to be correct, M-Na^+: 1277.23 for $C_{85}H_{168}O_4Na$ (requires: 1277.27). In addition the specific rotation of **(83)** was measured, giving a value of +1.17. The specific rotation can be converted into a molecular rotation, M_D. M_D is calculated from the measured specific rotation (α_D) and the molecular weight of the sample, where:[135, 152]

$$M_D = \alpha_D \times (\text{Mol. Wt}/100)$$

When the chiral group is present in the centre of two very long chains, the molecular rotation for a particular absolute stereochemistry is largely independent of the chain length and so provides an incremental value that can be used to predict M_D and therefore α_D for a molecule containing several such chiral groups. Calculating theoretical values for M_D is only plausible when the chiral centre is present in the centre of two long carbon chains as the overall effects on molecular rotation remain similar to the complete mycolic acid, following the Cahn-Ingold-Prelog priority rules. Hence this method is not plausible in small molecules as the effect on molecular rotation will be considerably different to the complete compound.

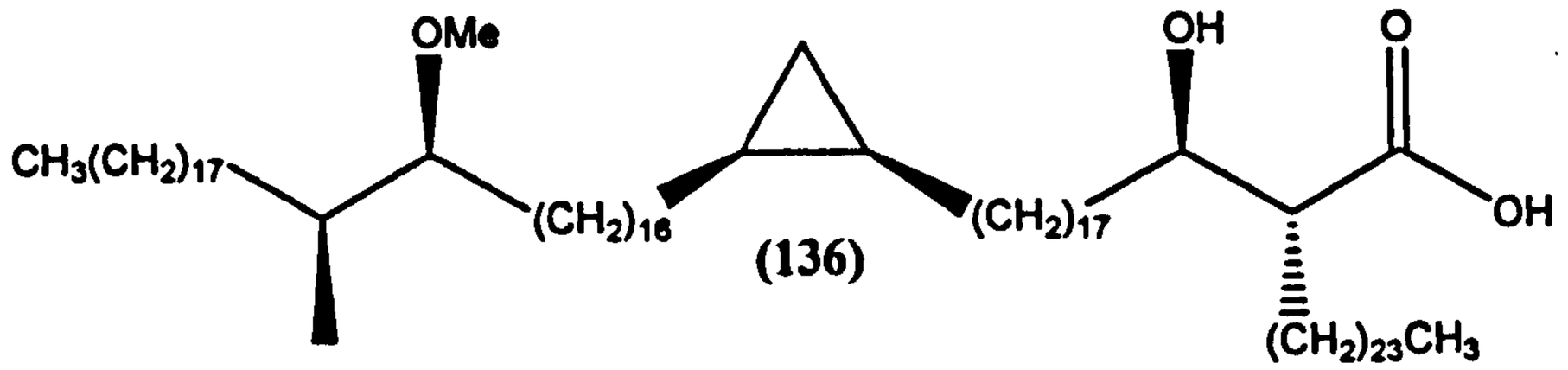
M_D for **(83)** was calculated at +14.67, Al-Dulayymi *et al.* recorded the optical rotations for **(136)** and **(137)** where M_D's are calculated at -13.42 and +87.17 respectively.[182]

$$M_D\,\mathbf{(136)} = -1.07 \times (1254.24/100) = -13.42$$

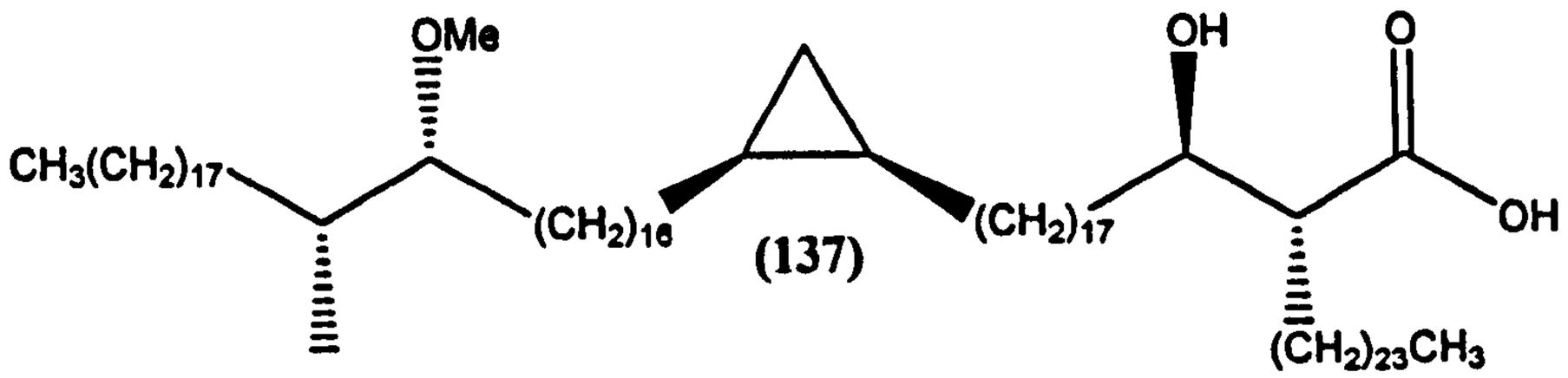
$$M_D\,\mathbf{(137)} = +6.95 \times (1254.24/100) = +87.17$$

Therefore it is possible to determine the contribution of each chiral centre, where the difference between **(83)** and **(136)** is +28.09, which accounts for the effect of the two different configurations of the *cis*-cyclopropane. The difference between **(136)** and **(137)** is +100.59, caused by the distal methyl and methoxy groups. Unfortunately Al-Dulayymi *et al.* did not discuss the hydrolysis of **(138)**; however, theoretically, it would now be possible to calculate the expected M_D and α_D for the 4th configuration, where M_D would be expected at +115.26. Al-Dulayymi *et al.* did give specific rotations for compound **(138)** where calculated M_D is +100.76.

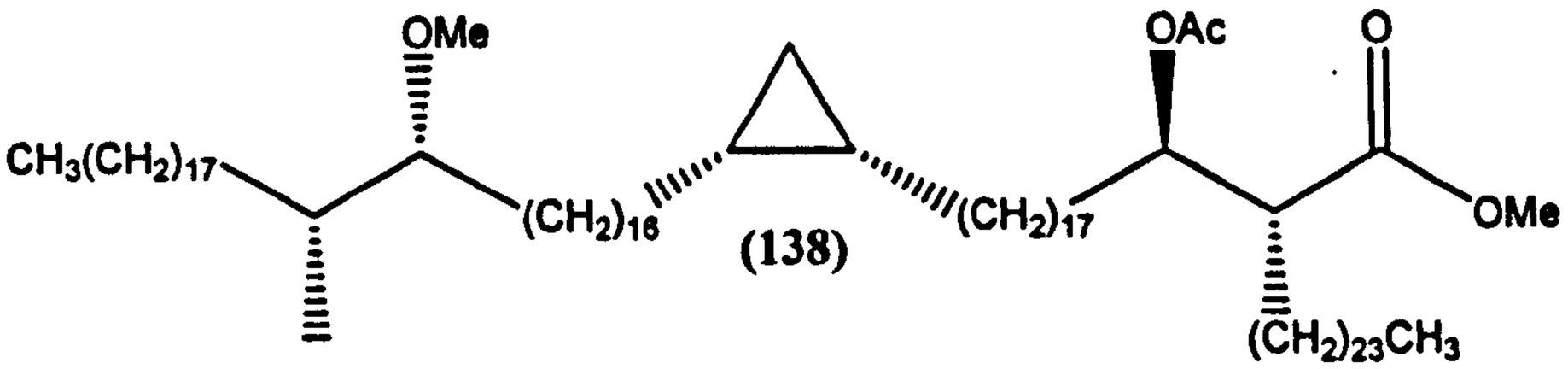
$$M_D\,\mathbf{(138)} = +7.69 \times (1310.30/100) = +100.76$$



(136)



(137)



(138)

The theoretical values calculated are from Al-Dulayymi *et al.* and the author's synthetic compounds show us that it is possible to estimate expected rotations for synthetic compounds.

Quémard *et al.* discussed the use of mycolic acid fragments in determining the overall molecular rotation of the complete mycolic acid, and hence an expected optical rotation for the compound. They calculated individual contributions from each chiral centre present and the sum of this gave the expected molecular rotation (See Fig. 80).[135]

Molecule	M_D	Configuration of the chiral centre carrying the				
		distal Me branch	oxygenated function	proximal Me branch	C_3	C_2
α-mycolate						
BCG	+37	-	-	-	*R*	*R*
smegmatis 155	+12	-	-	*R*	*R*	*R*
Ketomycolate						
BCG						
epimerized	+42	-	-	-	*R*	*R*
non-epimerized	+77	*S*	-	-	*R*	*R*
smegmatis MJ95 (epimerized)	+19	-	-	*R*	*R*	*R*
Hydroxymycolate						
BCG	0	*S*	*S*	-	*R*	*R*
smegmatis MJ95	-16	*S*	*S*	*R*	*R*	*R*
Branched hydroxy acid	+40	-	-	-	*R*	*R*
15-Me hetriaconta -16-one	+44	*S*	-	-	-	-
15 Me hetricontan -16-ol	-43	*S*	*S*	-	-	-

Fig. 80: Quémard *et al.*'s molecular rotation's[135]

Full ^{1}H NMR expansions of *cis*-cyclopropane methoxy-mycolic acid **(83)** can be seen as follows (See Fig. 81a, 81b, 81c and 81d).

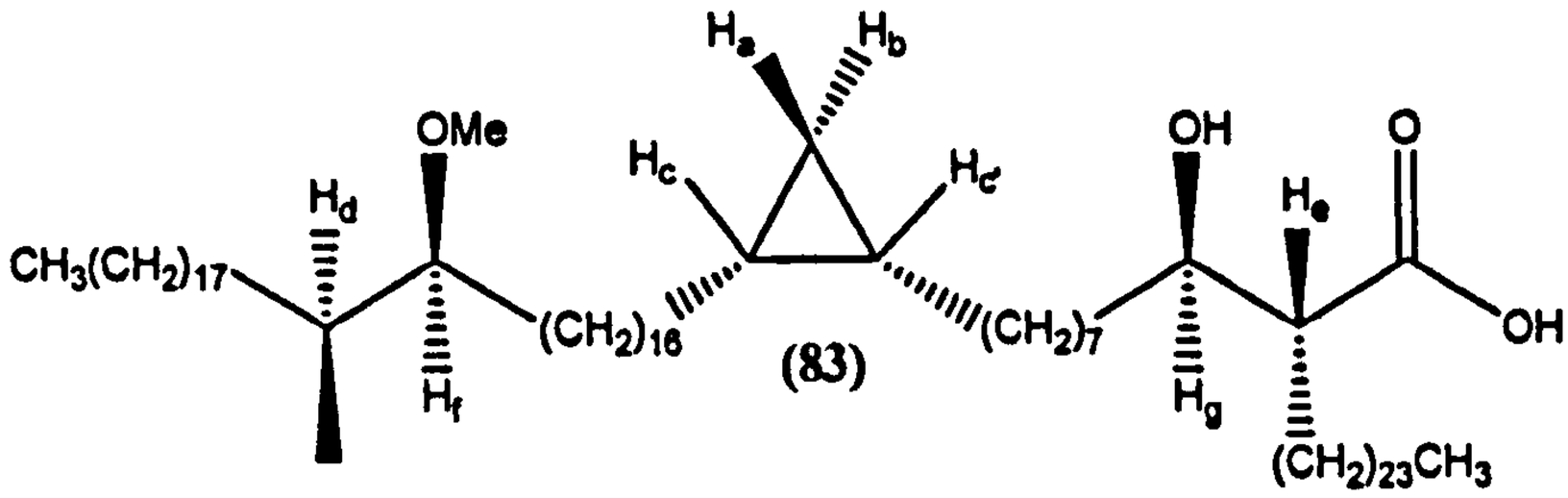


Fig. 81a: *cis*-Cyclopropane methoxy-mycolic acid (83)

The regions of the ^1H NMR spectrum of **(83)** which are of most interest are between δ 0.66 to -0.34, which correspond to the four protons of the *cis*-cyclopropane ring, and δ 3.77-1.71, which includes a three hydrogen singlet at δ 3.35 for the methoxy group. The signal for H_a was a broad doublet of triplets; the broadness of this signal shown at δ -0.31 to -0.34 is possibly because H_c and $H_{c'}$ are not magnetically identical and the signal observed is actually a double doublet of doublets, but due to the signals being at a near identical chemical shift it appears as a doublet of triplets. H_b, theoretically should show a doublet of triplets; however, due to the difference in magnetism of H_c and $H_{c'}$ the signal at δ 0.58-0.54 is distorted. H_c and $H_{c'}$ again showed a distorted multiplet at δ 0.66-0.64 for the same reason, where theoretically they should show a double doublet of triplets.

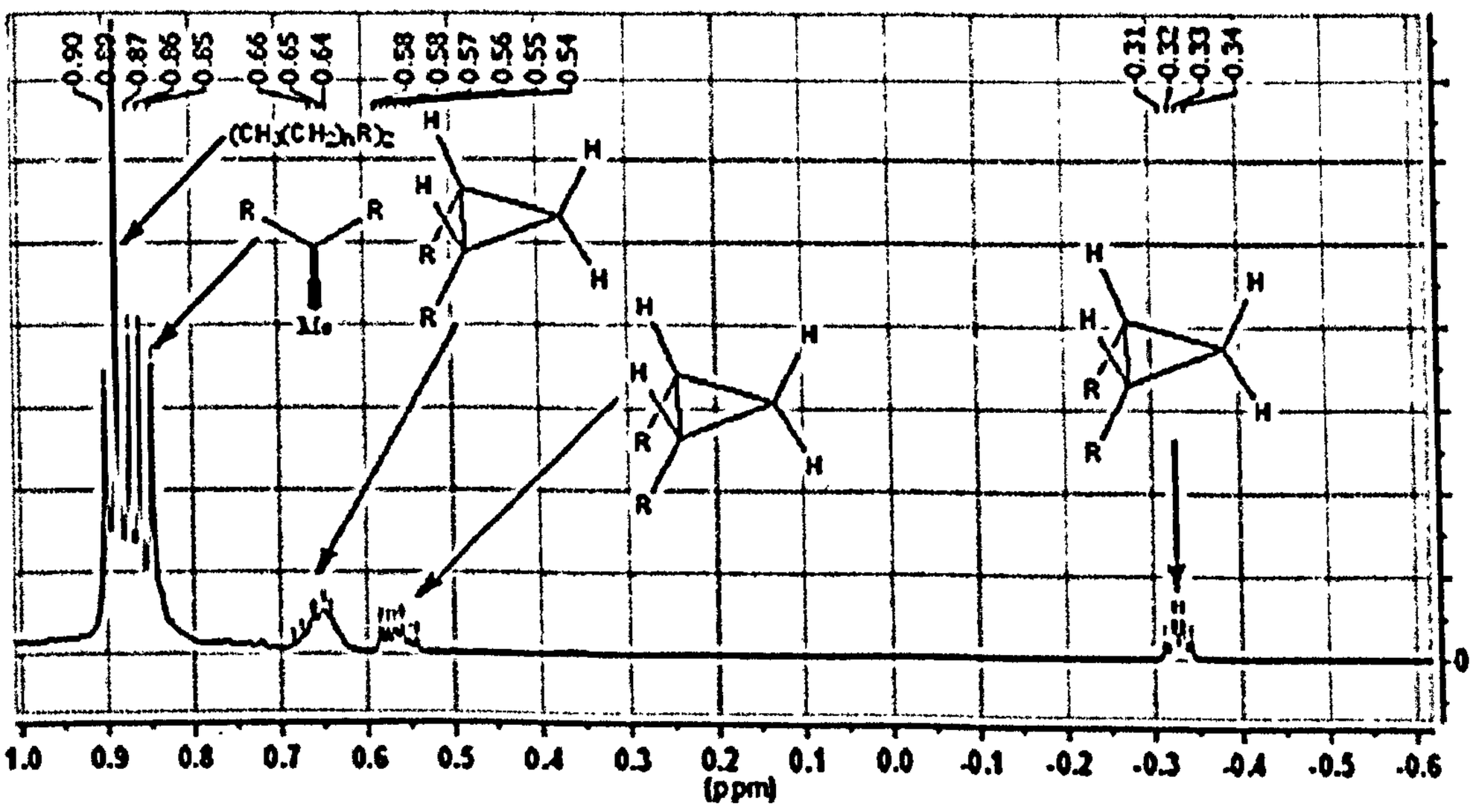


Fig 81b: ^1H NMR spectrum of (83) δ 1.0 to δ -0.6

H_d should show a doublet of triplets, however the signal at δ 1.75-1.71 is distorted. H_e shows to exhibit a doublet of triplets as seen at δ 2.48-2.44, which is consistent with the expected theoretical splitting pattern. H_f and H_g are seen at δ 2.99-2.96 and δ 3.77-3.69, due to their adjacency to the β-hydroxy and methoxy respectively. As H_f and H_g are diastereotopic protons, the splitting patterns observed are slightly distorted due to differing magnetic properties of the protons which are adjacent to them.

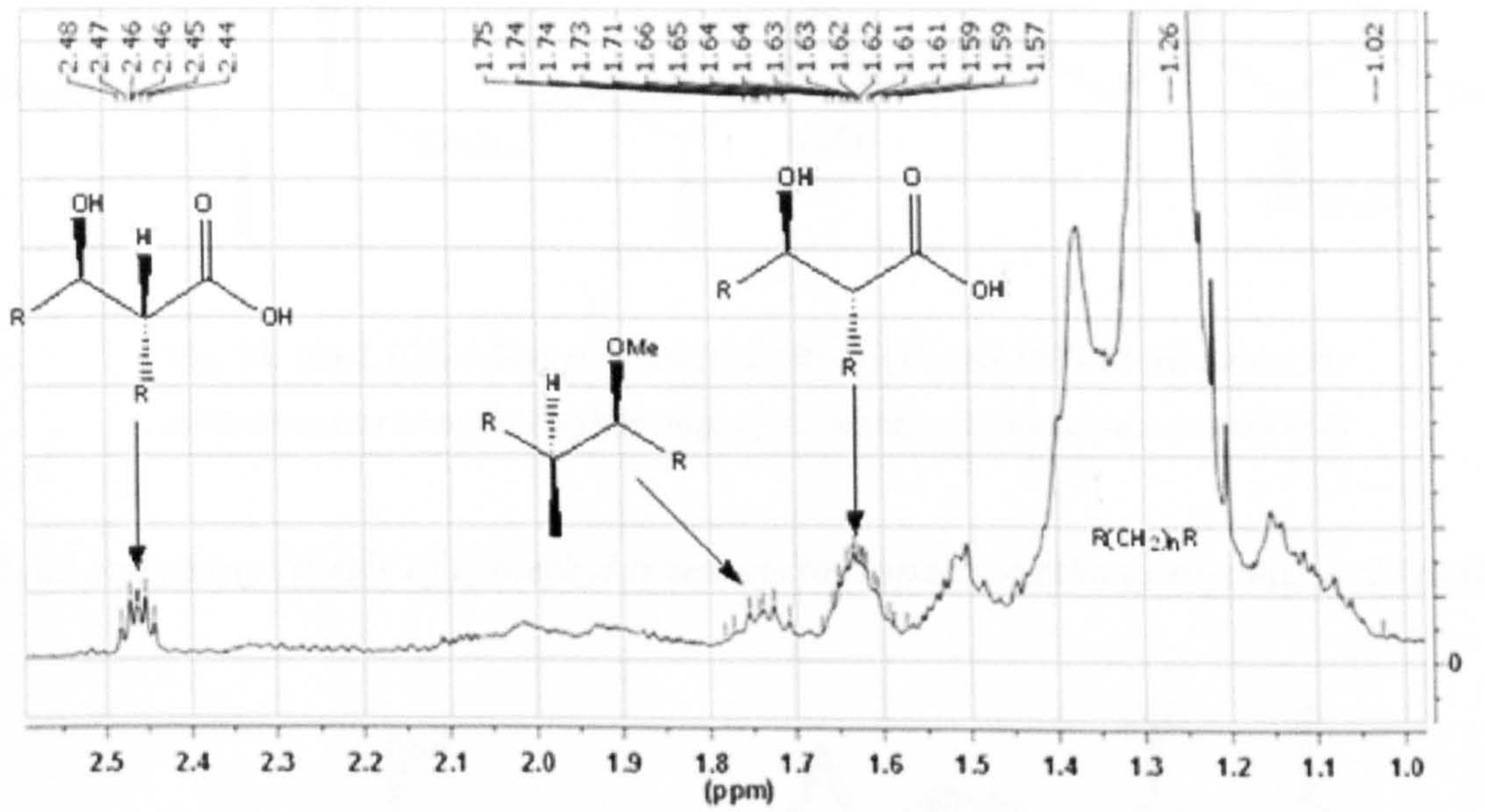


Fig 81c: ^{1}H NMR spectrum of (83) δ 2.6 to δ 1.0

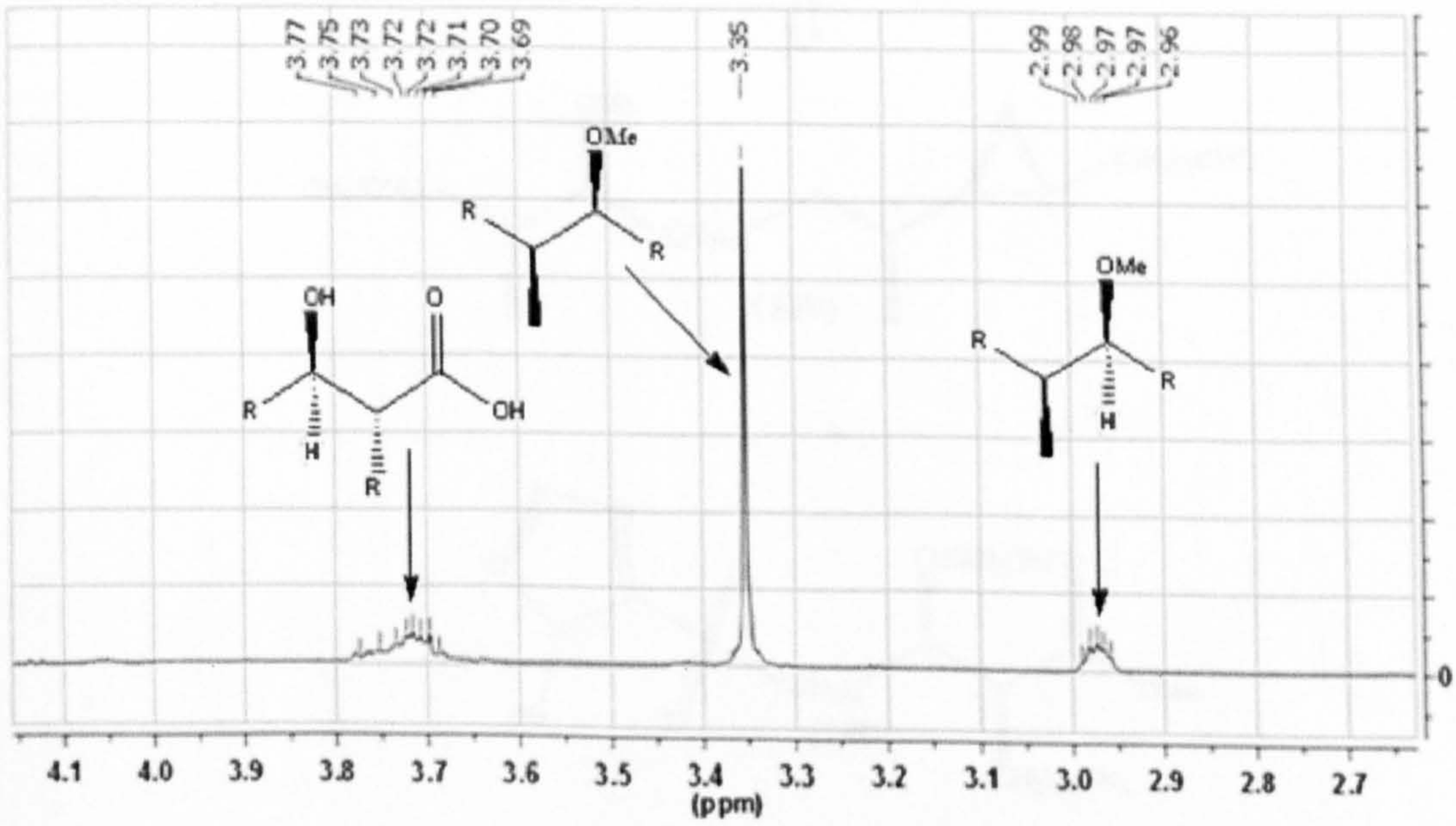


Fig 81d: ^{1}H NMR spectrum of (83) δ 4.2 to δ 2.6

2.3 – Synthesis of α-methyl *trans*-cyclopropane methoxy-mycolic acid (84)

The preparation of α-methyl *trans*-cyclopropane mycolic acids has been previously discussed. [153, 180, 196, 197] Using the methods described in the literature along with methods discussed in this thesis it was possible to prepare α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)** (See Fig. 82).

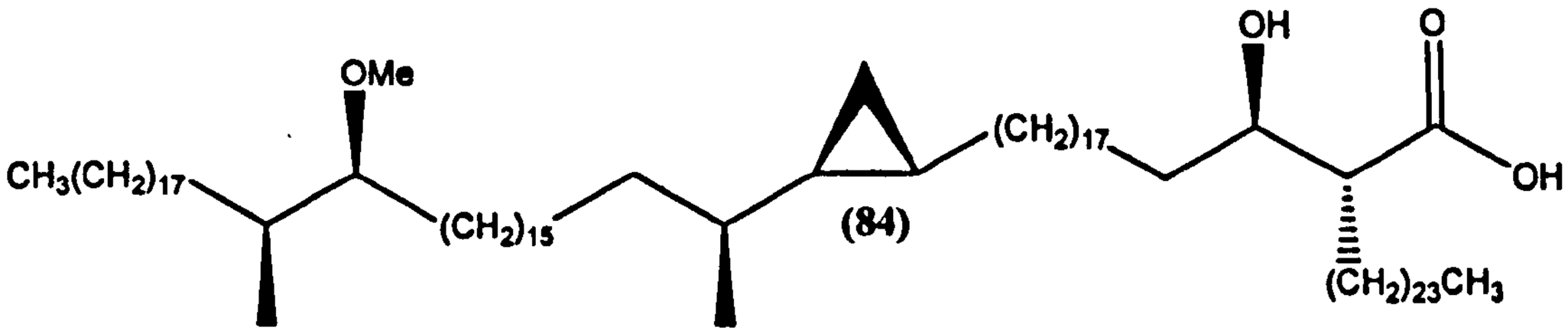


Fig. 82: (*R*)-2-((*R*)-1-Hydroxy-19-((1*S*,2*R*)-2-((2*S*,18*S*,19*S*)-18-methoxy-19-methylheptatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoic acid (84)

2.3.1 – Retrosynthesis of α-methyl trans-cyclopropane methoxy-mycolic acid (84)

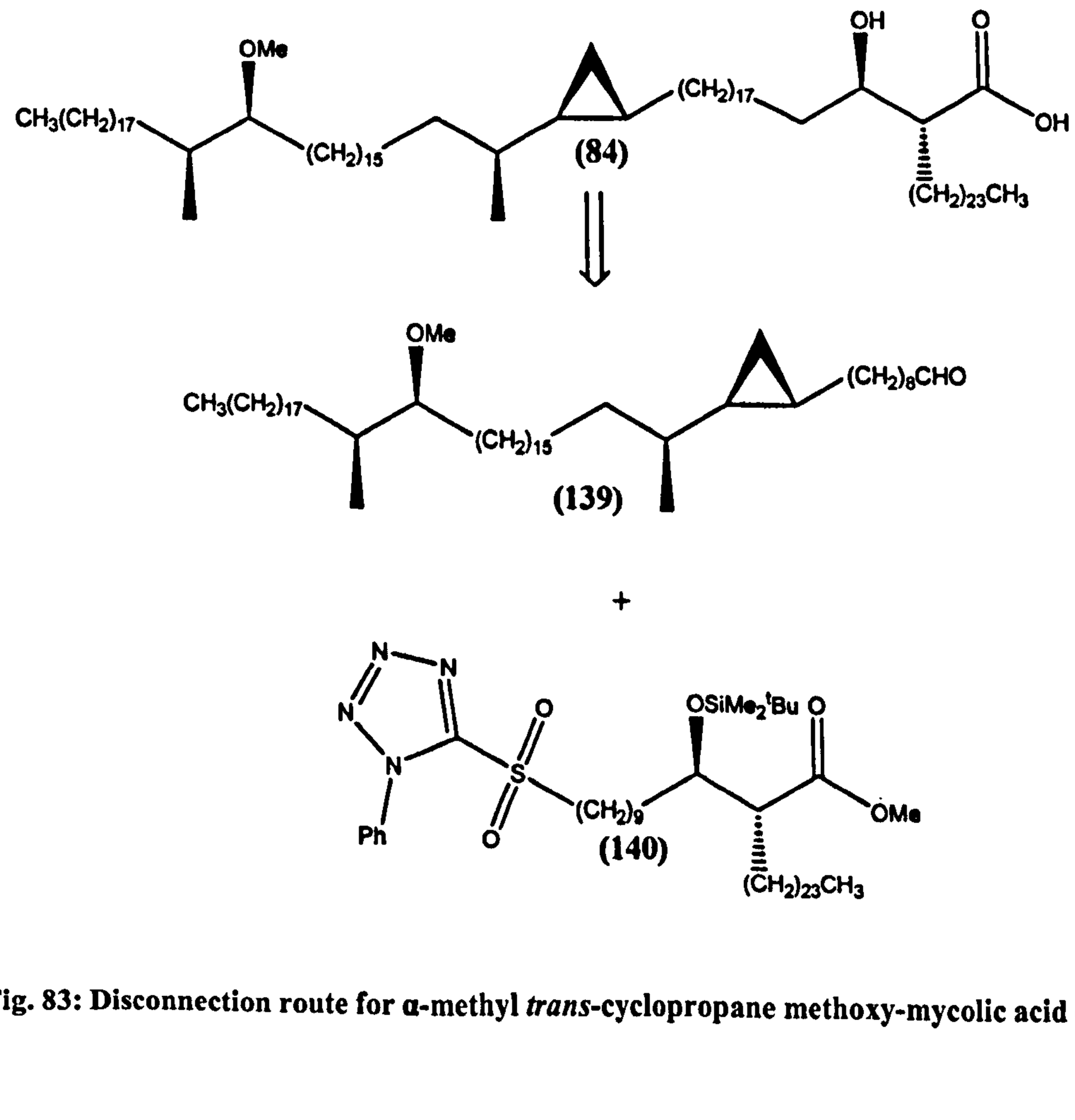


Fig. 83: Disconnection route for α-methyl *trans*-cyclopropane methoxy-mycolic acid (84)

The α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)** can be prepared via a similar disconnection method to the *cis*-cyclopropane methoxy-mycolic acid **(83)** (See Fig. 83) The meromycolate aldehyde **(139)** was separated into its proximal and distal moieties, and branched hydroxy acid sulfone **(140)** (See Fig. 84). The distal *S,S* α-methyl methoxy unit **(141)** was prepared as before, however the α-methyl *trans*-cyclopropane unit **(142)** was prepared, *via* intermediate **(143) – (145)**, following the retrosynthesis in Fig. 85.

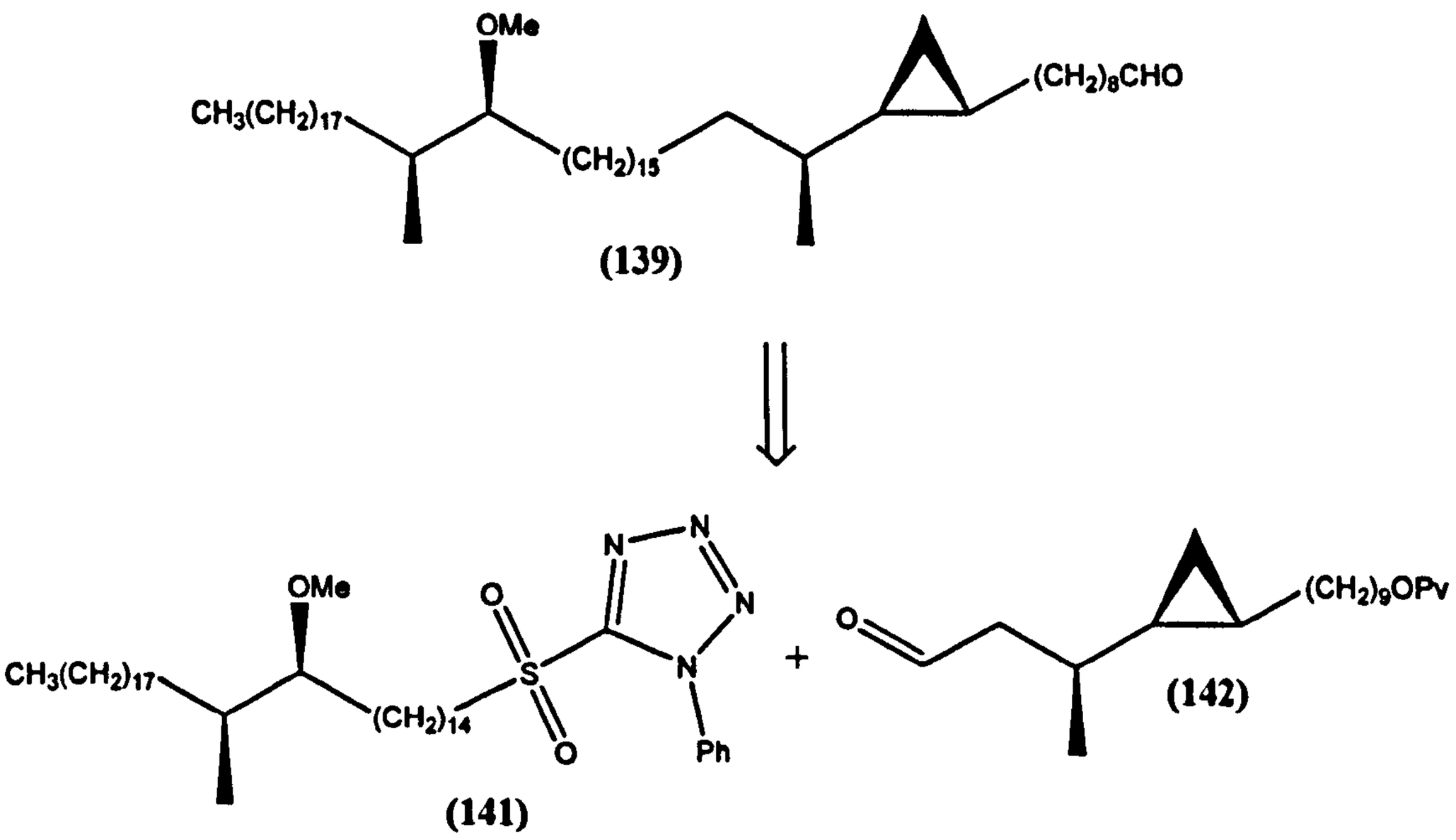


Fig. 84: Disconnection route for meromycolate aldehyde (139)

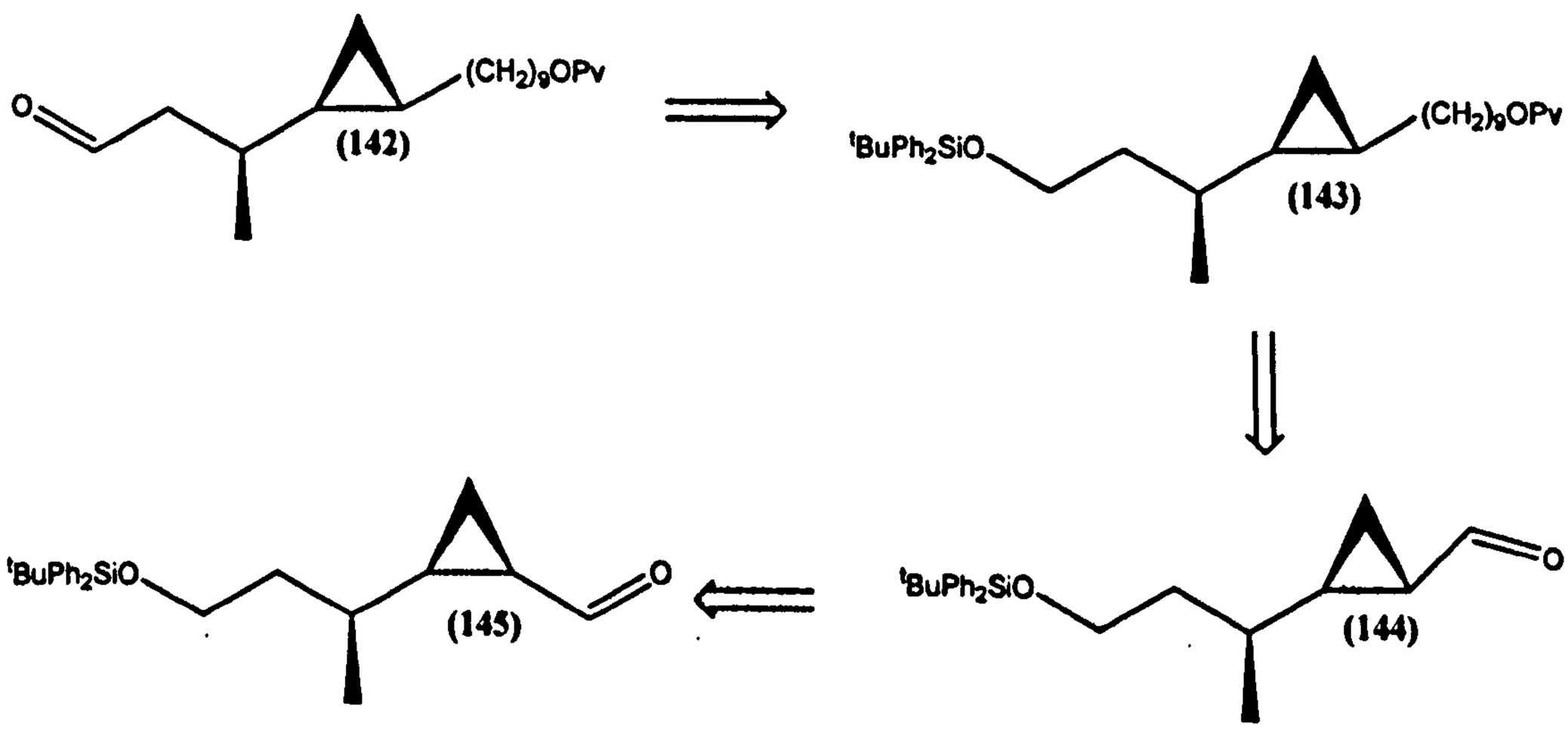


Fig. 85: Disconnection route for α-methyl *trans*-cyclopropane unit (142)

2.3.2. – Preparation of the distal S,S α-methyl methoxy unit (140)

The α-methyl methoxy sulfone **(140)** was prepared using methods similar to those used when preparing **(92)**; however, to obtain the 14 carbon chain, required to connect to the α-methyl *trans*-cyclopropane unit **(141)**, a seven carbon sulfone was used rather than an eight carbon sulfone (See Fig. 86).

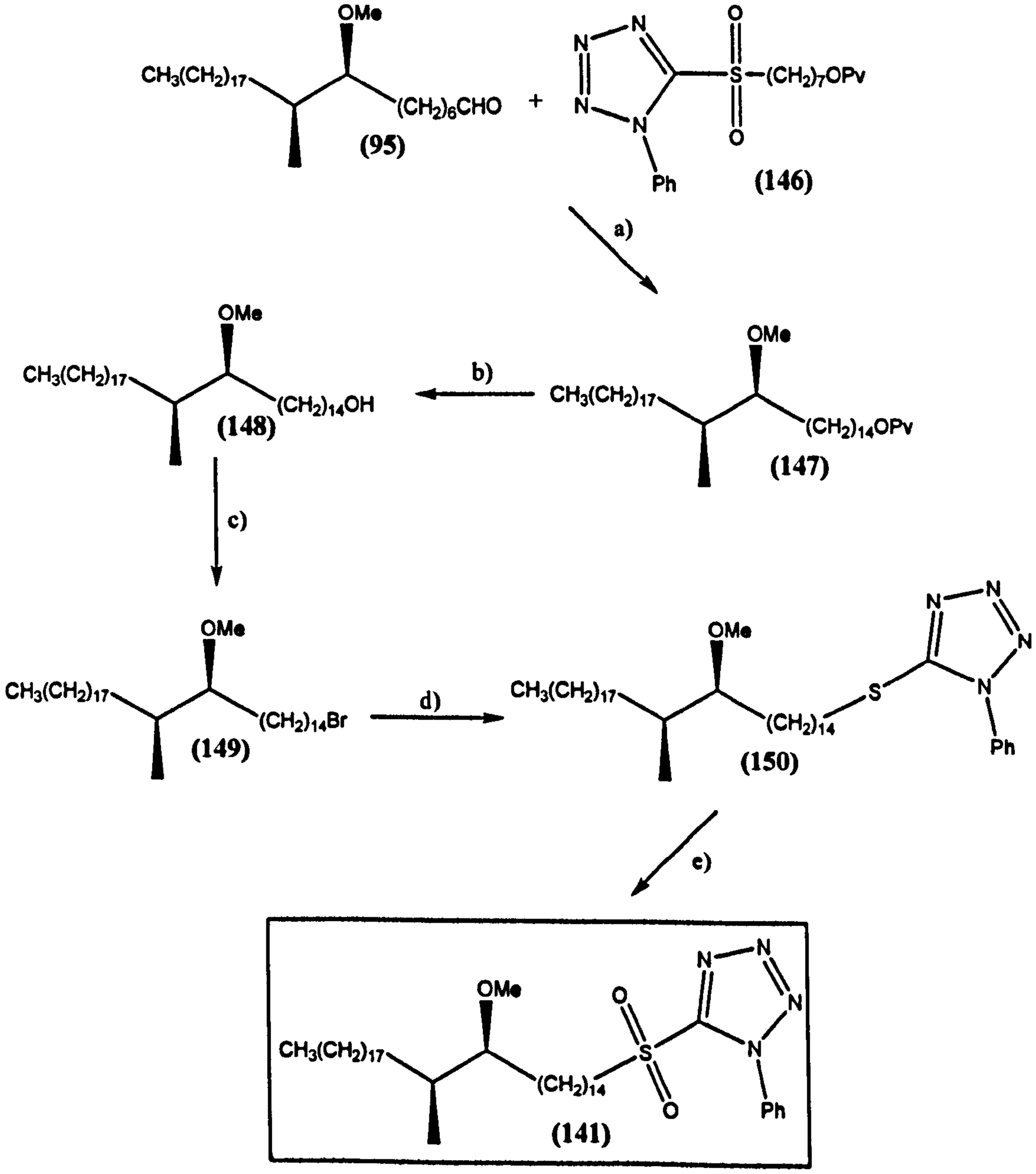
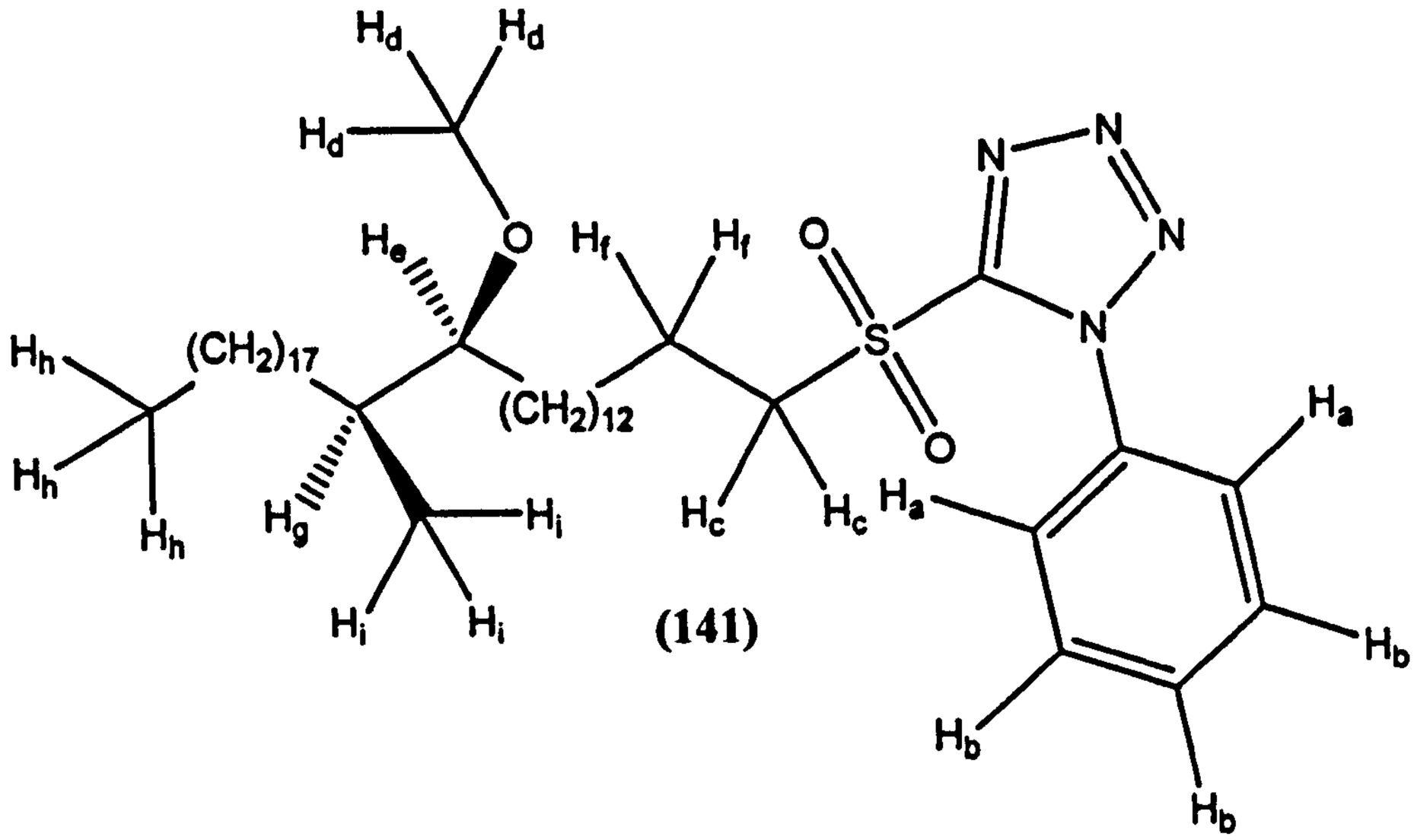


Fig. 86: a) i) LHMDS, (81 %); ii) H_2, Pd. cat., (88 %); b) $LiAlH_4$, (79 %); c) NBS, PPh_3, $NaHCO_3$, (83 %); d) 1-phenyl-1H-tetrazole-5-thiol, K_2CO_3, (93 %); e) *m*-CPBA, $NaHCO_3$, (89 %)

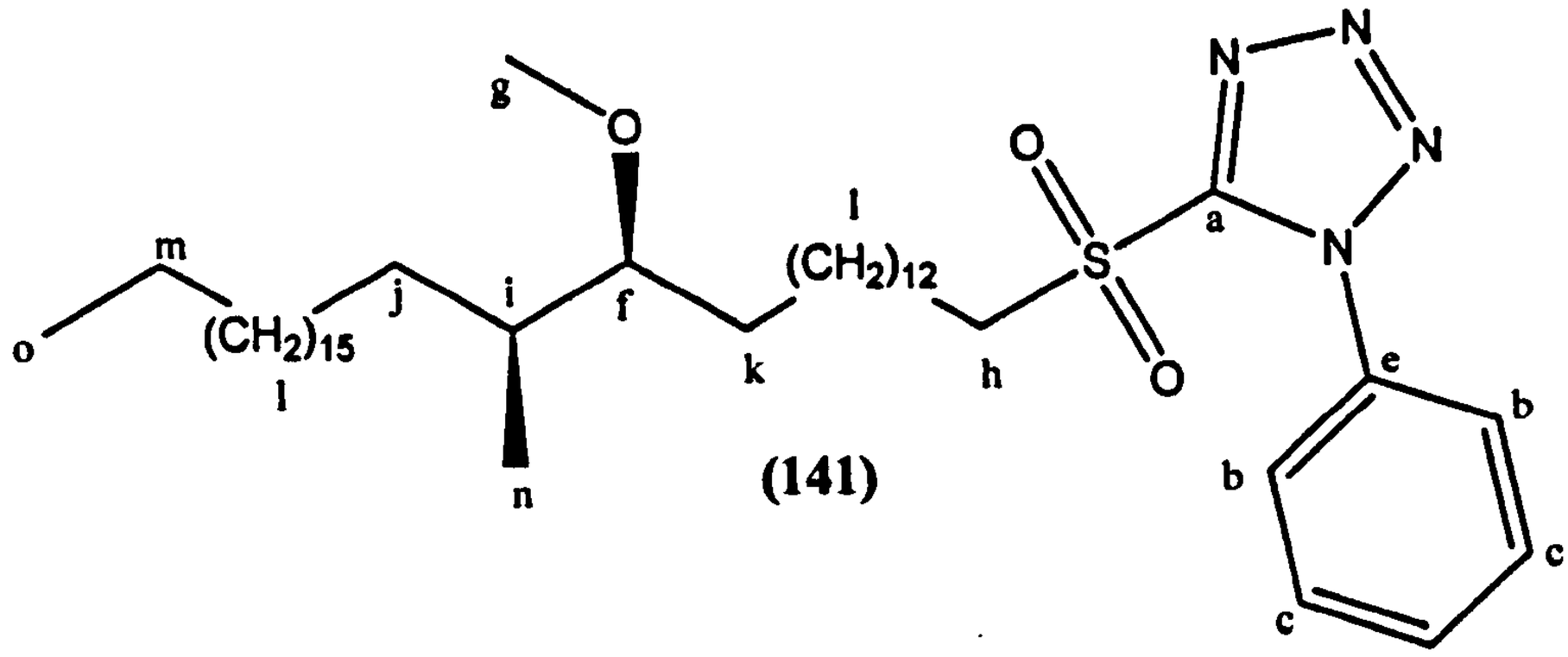
The structure and stereochemistries of the sulfone **(141)** were confirmed using ^{1}H NMR (See Fig. 87), ^{13}C NMR (See Fig. 88), infra-red and optical rotation.



(141)

H_x	δ	Multiplicity	Integration	J (H_z)
H_a	7.71-7.70	m	2	-
H_b	7.69-7.61	m	3	-
H_c	3.74	t	2	7.9
H_d	3.34	s	3	-
H_e	2.97-2.95	m	1	-
H_f	1.96	pent	2	7.9
H_g	1.63-1.58	m	1	-
H_h	0.89	t	3	6.6
H_i	1.60	d	3	6.7

Fig. 87: ^{1}H NMR data analysis of 5-((15*S*,16*S*)-15-methoxy-16-methyltetratriacontyl-1-sulfonyl)-1-phenyl-1H-tetrazole (141)

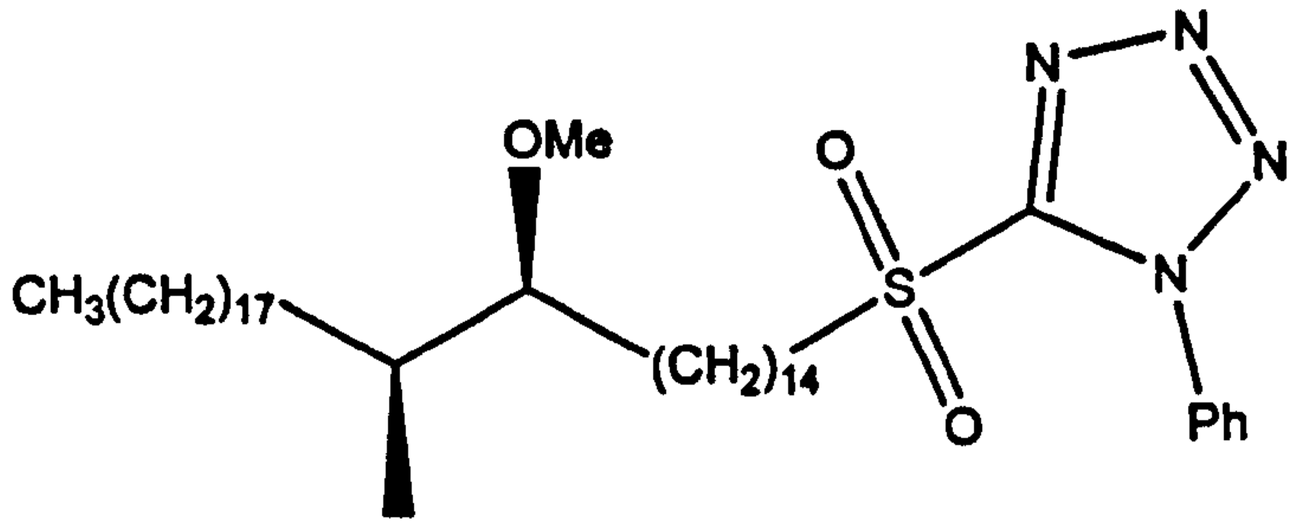


(141)

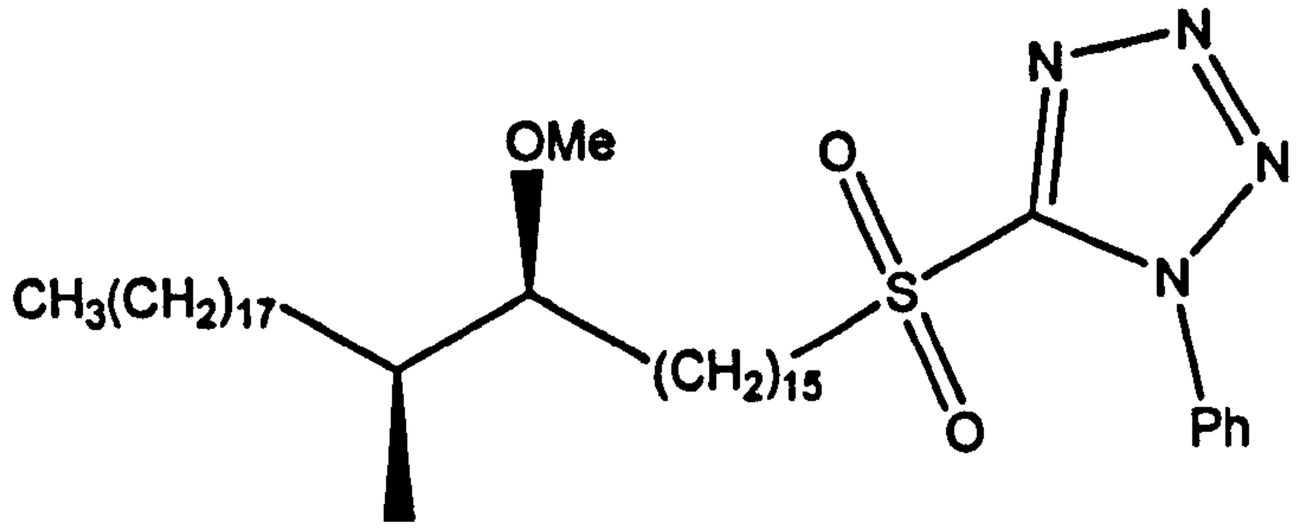
C_x	Δ	C_x	δ
C_a	153.5	C_i	35.3
C_b	133.1	C_j	32.4
C_c	131.5	C_k	31.9
C_d	130.3	C_l	30.5-22.8
C_e	125.1	Cm	22
C_f	85.5	C_n	15.1
C_g	57.7	C_o	14.4
C_h	56.0		

Fig. 88: ^{13}C NMR data analysis of 5-((15*S*,16*S*)-15-methoxy-16-methyltetratriacontyl-1-sulfonyl)-1-phenyl-1H-tetrazole (141)

The infra-red spectrum was identical to that of the previously prepared sulfone **(141)**, as expected due to a variance of only one carbon in chain length. Also the optical rotation of the two molecules was very similar, that for sulfone **(141)** being -6.28 and for sulfone **(91)** -6.32; this was also expected due to the similarity of the two compounds (See Fig. 90). The molecular rotations for both compounds were calculated giving M_D= -45.92 for **(141)** and M_D= -47.10 for **(91)**, as expected the two values are very similar as the additional CH_2 group of **(91)** has no effect on M_D.



(141)



(91)

Fig. 89: *S,S* methoxymycolate intermediates (141) and (91)

2.3.3 – Preparation of the α-methyl trans-cyclopropane unit (142)

To successfully prepare the α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)**, it was necessary to synthesise a meromycolate moiety with an α-methyl *trans*-cyclopropane. To obtain the desired 16 carbon chain bridging the proximal and distal functionalities, the α-methyl *trans*-cyclopropane unit required a two carbon chain with an aldehyde functional group adjacent to the methyl substituent. It was also decided to introduce a chain to the other side of the cyclopropane to contribute to the 18 carbon chain connecting the meromycolate moiety and the branched hydroxy acid as shown previously in Fig. 84. The α-methyl *cis*-cyclopropane unit **(145)** was contributed by Matthew Love, following methods as described by J. R. Al-Dulayymi *et al.*,[153, 196] and the following procedures were followed to obtain the desired compound **(142)** (See Fig. 90).

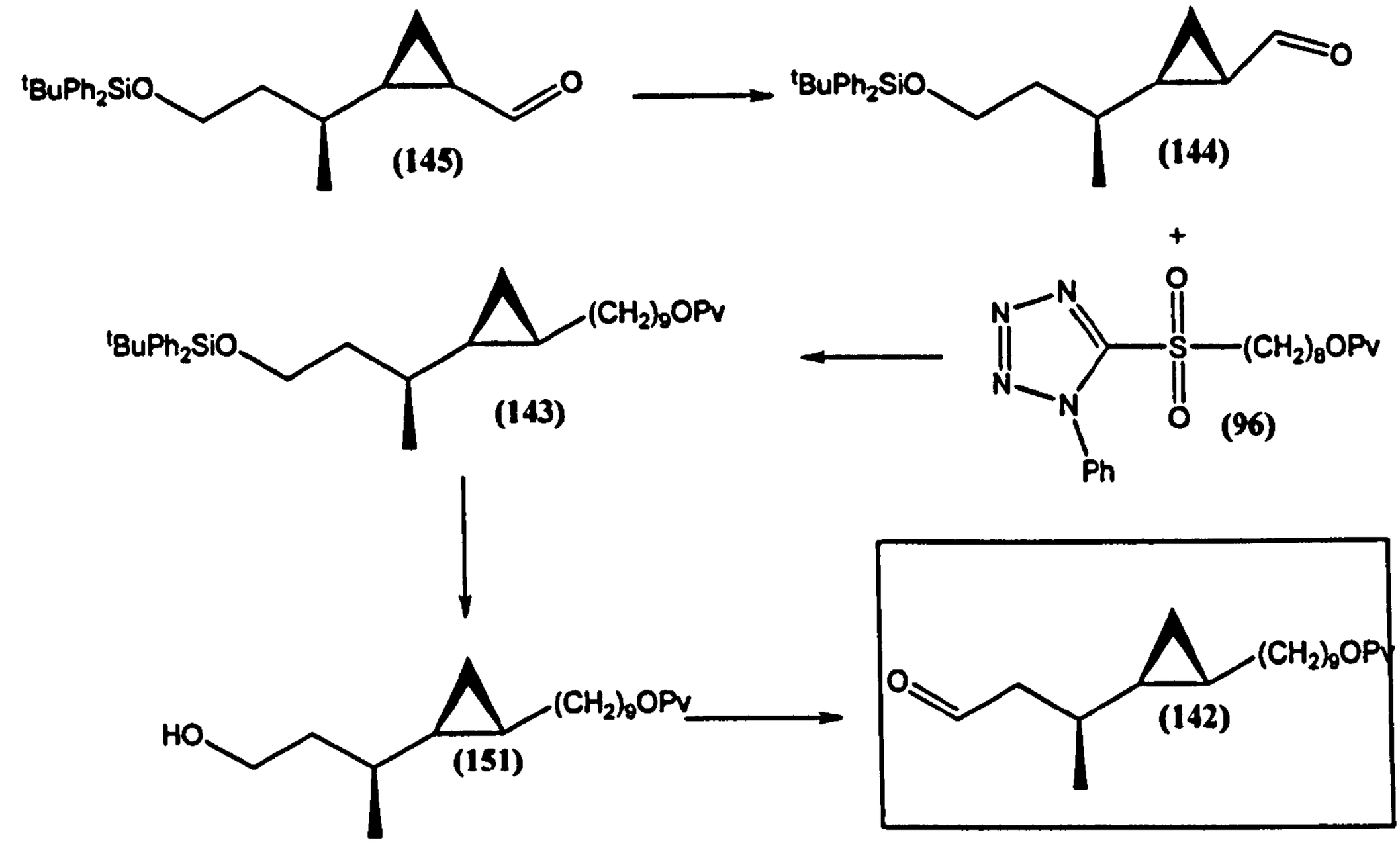


Fig. 90: Preparation of *trans*-cyclopropane intermediate (142)

The α-methyl *cis*-cyclopropane unit **(145)** was converted into the *trans*-configuration **(144)** using sodium methoxide in refluxing methanol over a period of 56 hrs. This was carried out on 8.93 g of the *cis*-compound, giving 4.90 g of the *trans*-product and 0.93 g of the desilyated *trans*-compound **(152)**. The desilyated compound was re-protected using *tert*-butyldiphenylsilylchloride to give 2.30 g of the α-methyl *trans*-cyclopropane aldehyde **(144),** in an overall 81 % yield (See Fig. 91).

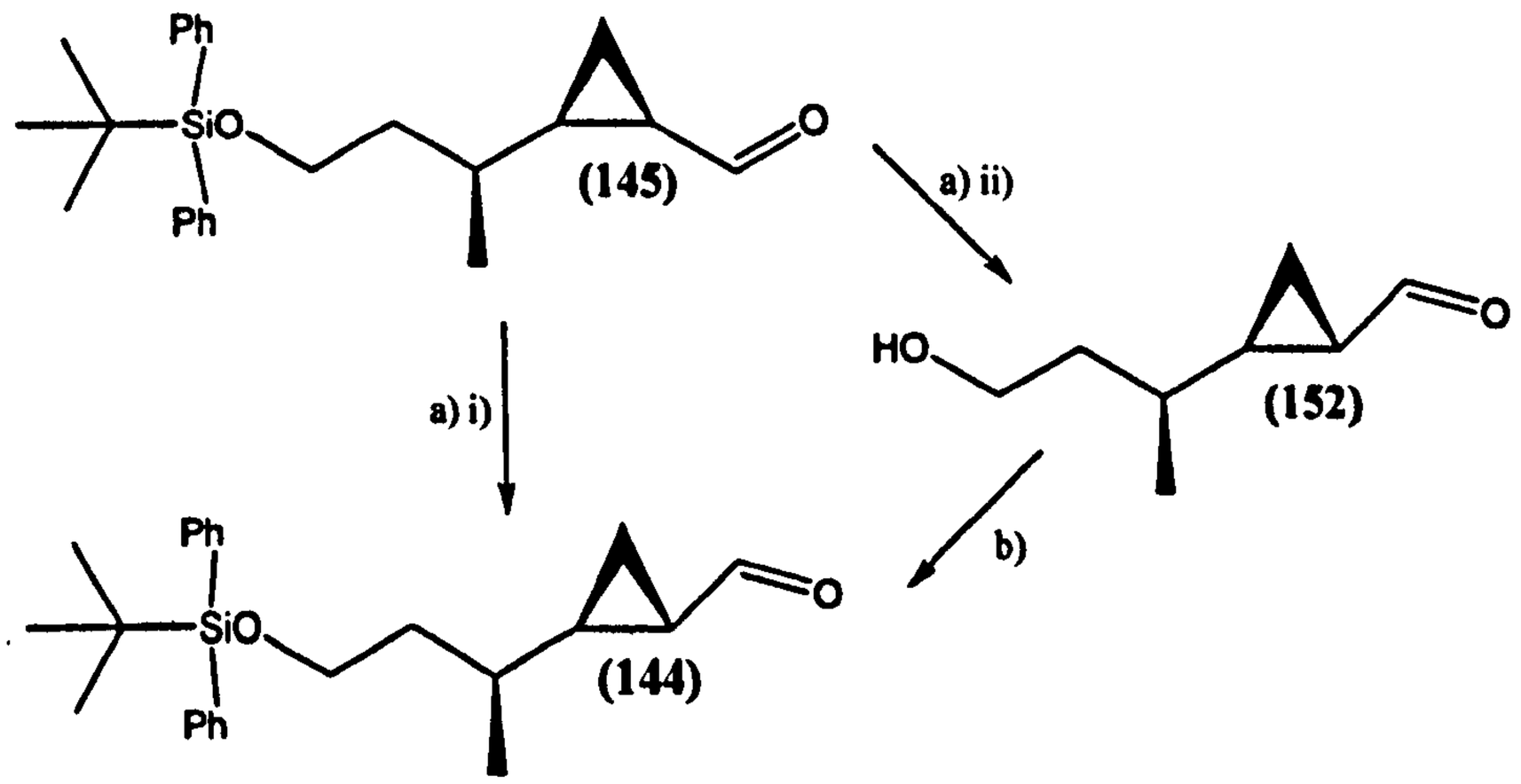


Fig. 91: a) NaOMe, i) (55 %), ii) (30 %); b) *t*BDPSCl, (86 %)

The success of the epimerisation reaction was confirmed primarily by measuring the product's optical rotation, with the *cis*-aldehyde **(145)** showing a rotation of + 3.54 and the *trans*-aldehyde **(144)** showing +21.5. Also, the ^{1}H NMR spectrum shows the proton corresponding to the aldehyde shifting upfield, from δ 9.32 to δ 9.00; it is also shown in the literature that the proton in the α-position to the aldehyde also shifts from δ 1.90-1.86 to δ 0.96-0.95, as a result of this epimerisation reaction.

The α-methyl *trans*-cyclopropane unit is distinctly different to the *cis*-cyclopropane unit discussed earlier. The *cis*-cyclopropane is found not to contain a methyl group in the α-position, unlike the *trans*-cyclopropane, as a result this gives rise to differences in the spectroscopic data for a compound containing these groups. The difference in stereochemistry of these two functionalities also has an effect on the folding of the long carbon chains in a mycolic acid.[130, 143-145, 147]

A modified Julia-Kocienski olefination was then carried out in a similar fashion as previously discussed. This reaction was carried out between *trans*-aldehyde **(144)** and 2,2-dimethyl-propionic acid 8-(1-phenyl-1H-tetrazole-5-sulfonyl)-octyl ester **(96)** using lithium *bis*(trimethylsilyl)amide as a base to give the alkene product **(153)**. A mild hydrogenation using a di-imide source, dipotassium azodicarboxylate and acetic acid was then carried out to yield 2,2-dimethyl-propionic acid 9-(2-[3-(*tert*-butyl-diphenyl-silanyloxy)-1-methyl]-cyclopropyl)-nonyl ester **(143)** (See Fig. 92).

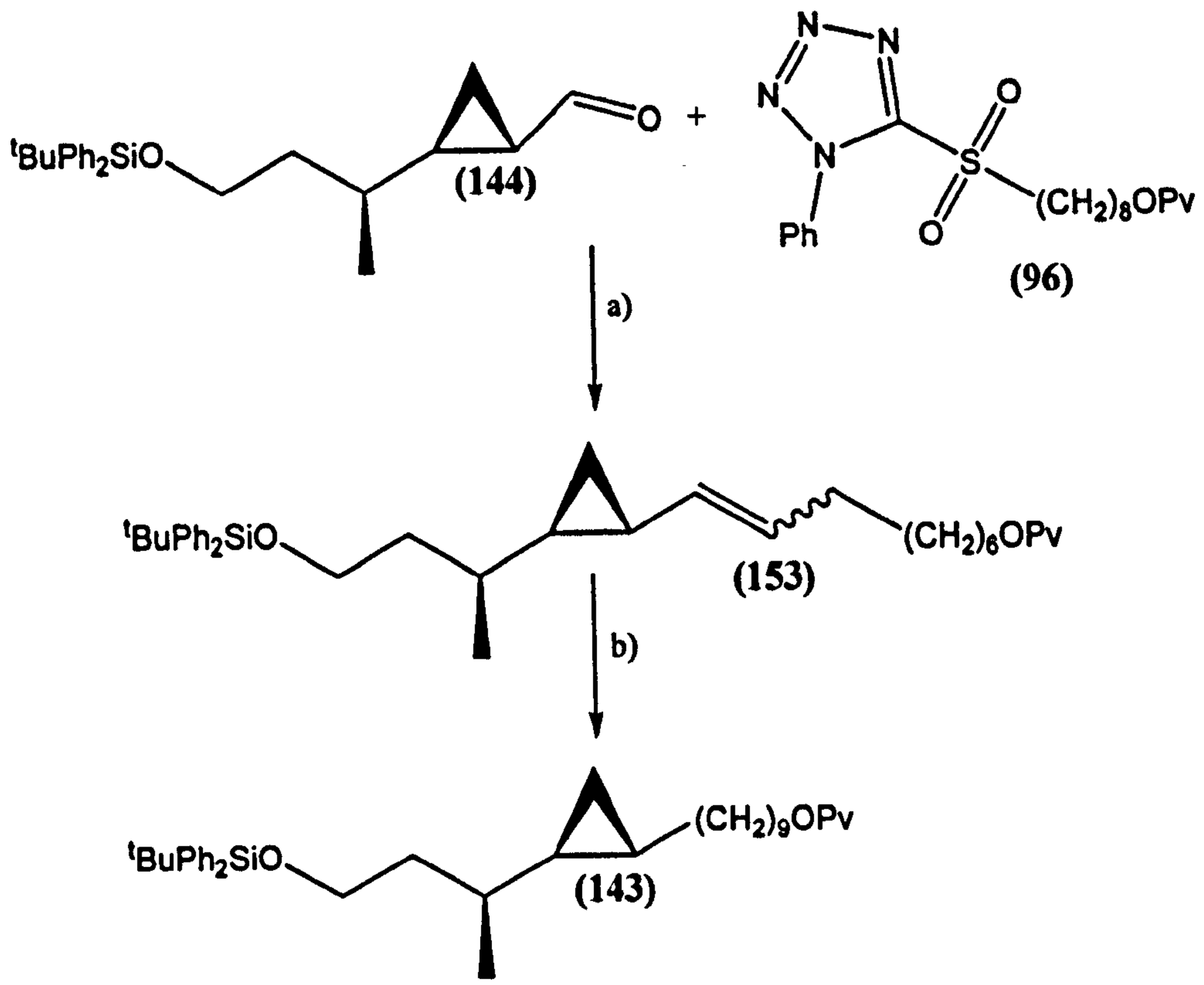


Fig. 92: a) LHMDS, (75 %); b) $(NCOO^{-}K^{+})_2$, CH_3COOH, THF, (93 %)

The *tert*-butyl ester group of **(143)** was removed using hydrofluoric acid in pyridine to give the corresponding alcohol **(151)**, which was then oxidised using PCC to yield the aldehyde **(142)** (See Fig. 93).

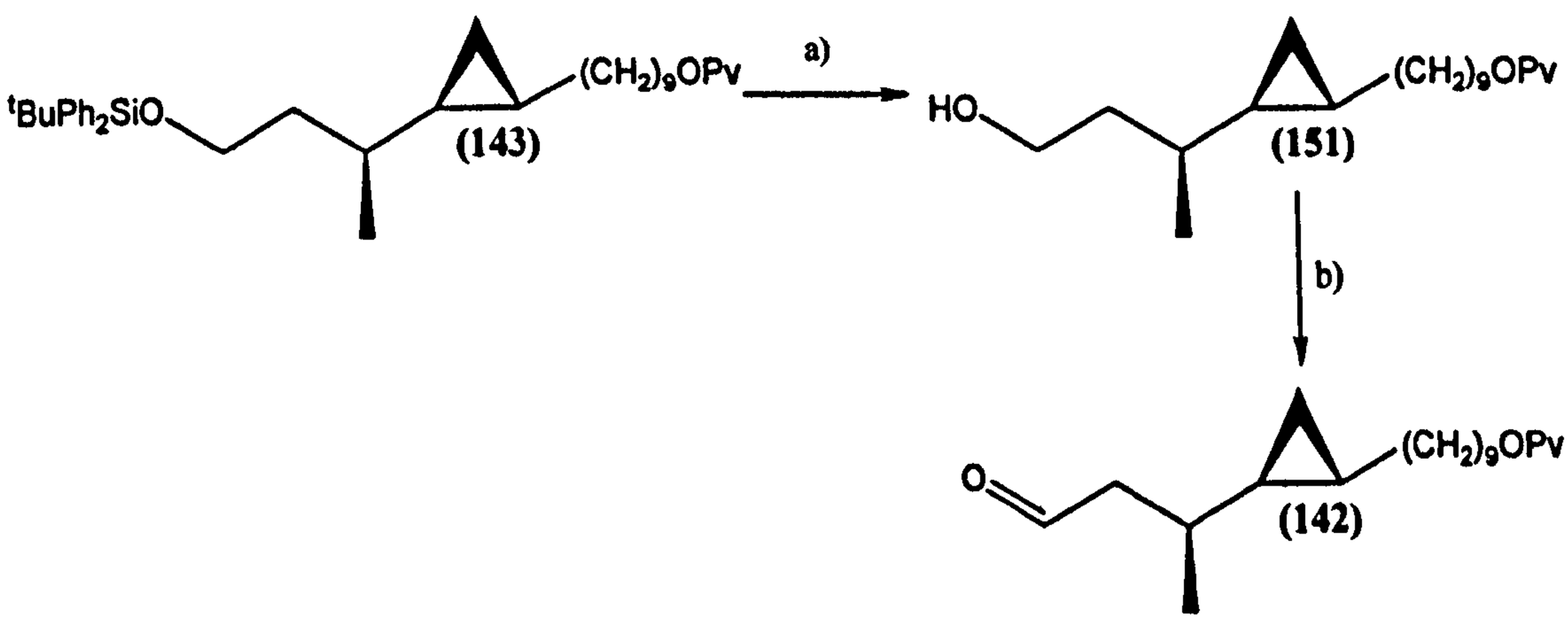


Fig. 93: a) HF.Pyr, pyridine, (79 %); b) PCC, (93 %)

^{1}H NMR spectroscopy was primarily used to confirm the completion of the above reactions. The spectrum for compound **(143)** included a nine hydrogen singlet at δ 1.04 corresponding to the *tert*-butyl group of the silyl ether, a four hydrogen multiplet between δ 7.67-7.65 and a six hydrogen multiplet between δ 7.40-7.37 corresponding to the two phenyl groups. Upon deprotection of the silyl ether, these signals were not observed in the spectrum of **(151)**. Oxidation of **(151)** to the aldehyde **(142)** led to the distorted one hydrogen singlet at δ 9.78 corresponding to the aldehyde proton of **(142)**.

2.3.4 – Preparation of the meromycolate moiety

A key intermediate in the synthesis of any mycolic acid is the meromycolate moiety; in the case of the α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)**, the meromycolate prepared was the aldehyde, 9-((1*R*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonanal **(138)**. The coupling of aldehyde **(141)** and sulfone **(140)** was done using lithium *bis*(trimethylsilyl)amide, which yielded the product **(159)** as a mixture of alkenes The alkenes were hydrogenated using dipotassium azodicarboxylate to give the novel meromycolate moiety intermediate, the pivalate **(157)**. The aldehyde **(138)** was prepared via a deprotection of the *tert*-butyl ester group of **(157)**, then oxidising the resultant primary alcohol **(158)** to give the desired aldehyde. This was carried out using lithium aluminium hydride to first reduce the ester to the alcohol, **(157)**. The aldehyde, **(138)** was then obtained by oxidising the primary alcohol **(158)** with PCC (See Fig. 94).

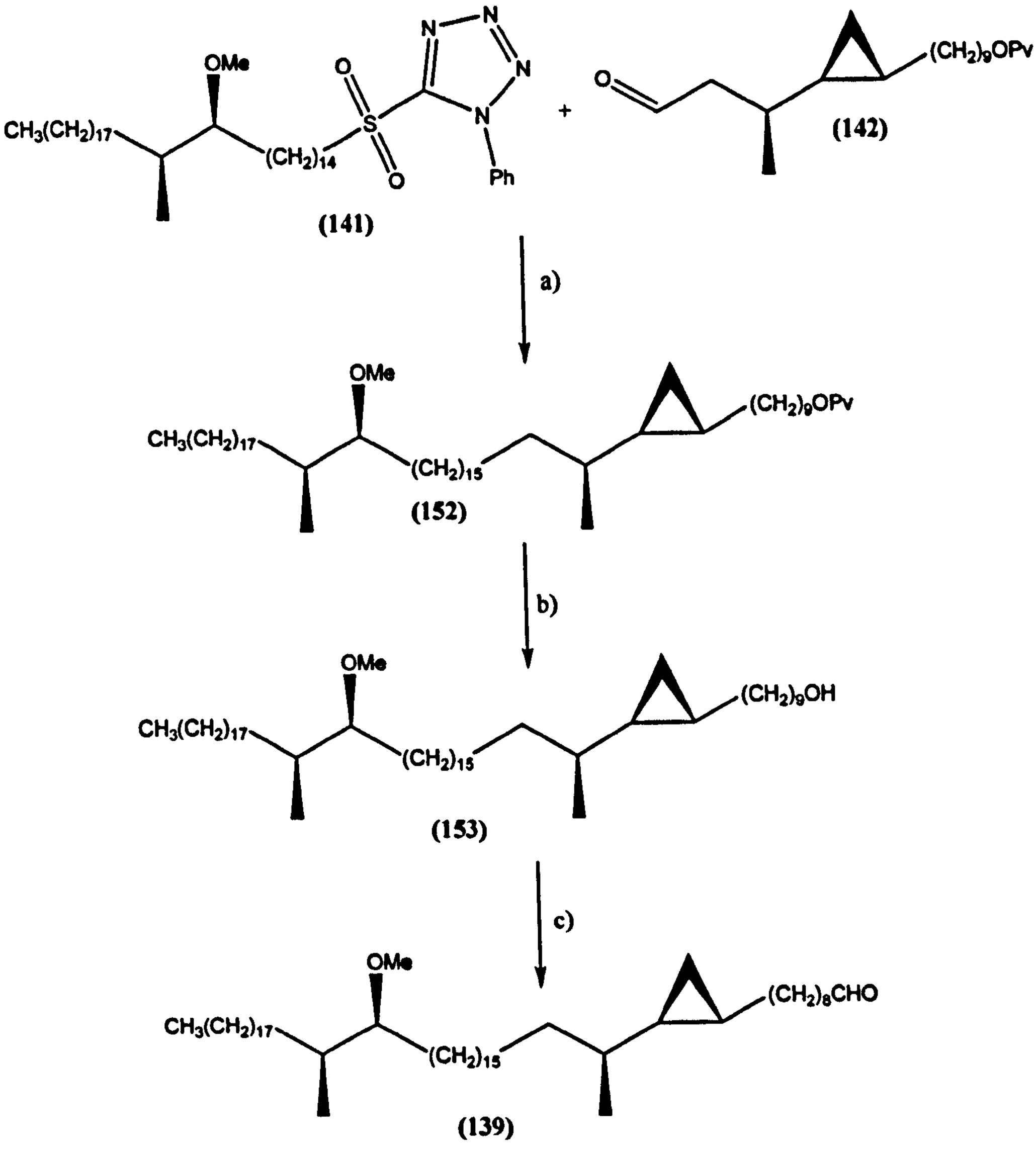
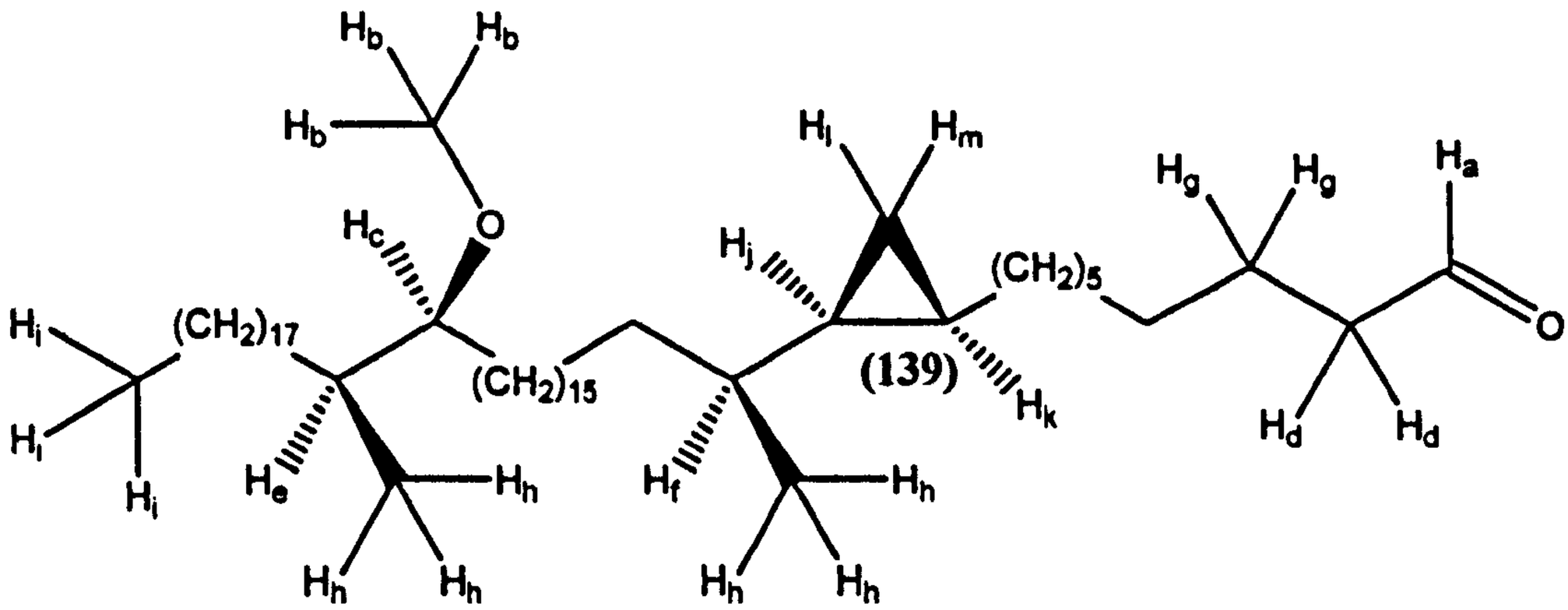


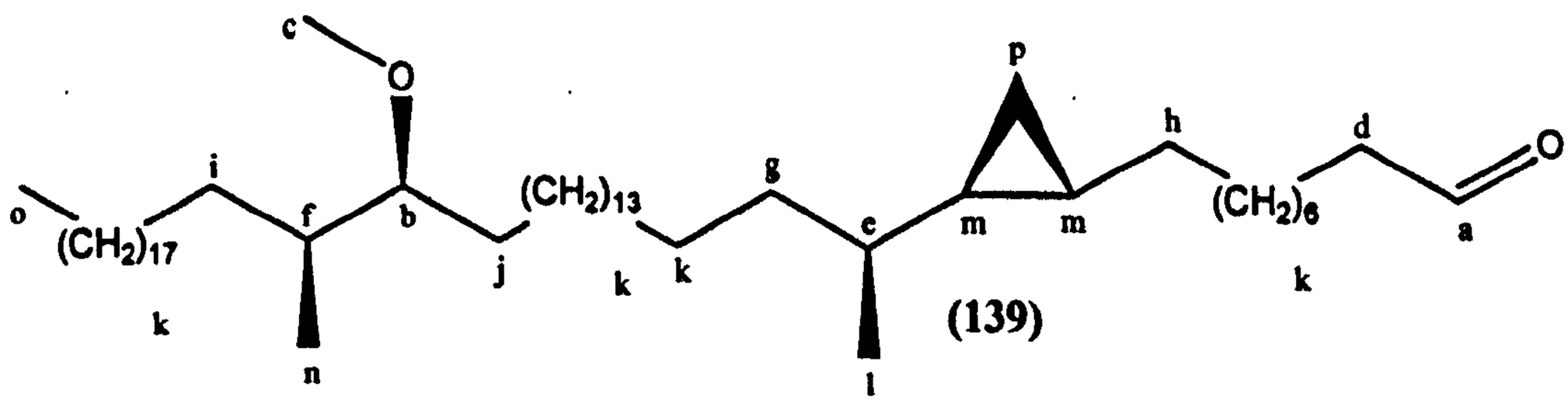
Fig. 94: a) i) LHMDS, (54 %); ii) $(NCOO^{-}K^{+})_2$, CH_3COOH, THF, (97 %); b) $LiAlH_4$, (72 %); c) PCC, (96 %)

The aldehyde **(139)** was characterised via ^{1}H NMR, ^{13}C NMR, IR and optical rotation. The IR spectrum showed C-H stretching, 2984 and 2875 cm^{-1} and C=O stretching, 1724 cm^{-1}. The specific rotation of the aldehyde **(139)** was measured at -3.45. The ^{1}H and ^{13}C NMR were analysed as follows (See Fig. 95 and Fig. 96).



H_x	δ	Multiplicity	Integration	J (H_z)
H_a	9.77	br. t	1	1.85
H_b	3.38	s	3	-
H_c	2.97-2.95	m	1	-
H_d	2.43	dt	2	1.85, 7.55
H_e	1.99-1.97	m	1	-
H_f	1.65-1.61	m	1	-
H_g	1.56	m	2	-
H_h	0.89	dt	6	2.85, 6.6
H_i	0.85	t	3	6.6
H_j	0.48-0.42	m	1	-
H_k	0.22-0.18	m	1	-
H_l	0.17-0.13	m	1	-
H_m	0.12-0.09	m	1	-

Fig. 95: ^{1}H NMR data analysis of (139)



C_x	δ	C_x	δ
C_a	205.1	C_i	31.9
C_b	85.5	C_j	30.5
C_c	57.7	C_k	30.0-22.7
C_d	43.9	C_l	19.7
C_e	38.1	Cm	18.6
C_f	37.4	C_n	16.5
C_g	35.4	C_o	14.3
C_h	32.8	C_p	10.5

Fig. 96: ^{13}C NMR data analysis of (139)

2.3.5 – Preparation of the branched hydroxy acid

It was necessary to prepare a branched hydroxy acid which would give the desired chain length in the final product. With the previously prepared α-methyl-*trans*-cyclopropane meromycolate moiety **(139)** having a nine carbon chain, a sulfone with a nine carbon chain **(140)** was required to obtain the α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)** (See Fig. 97).

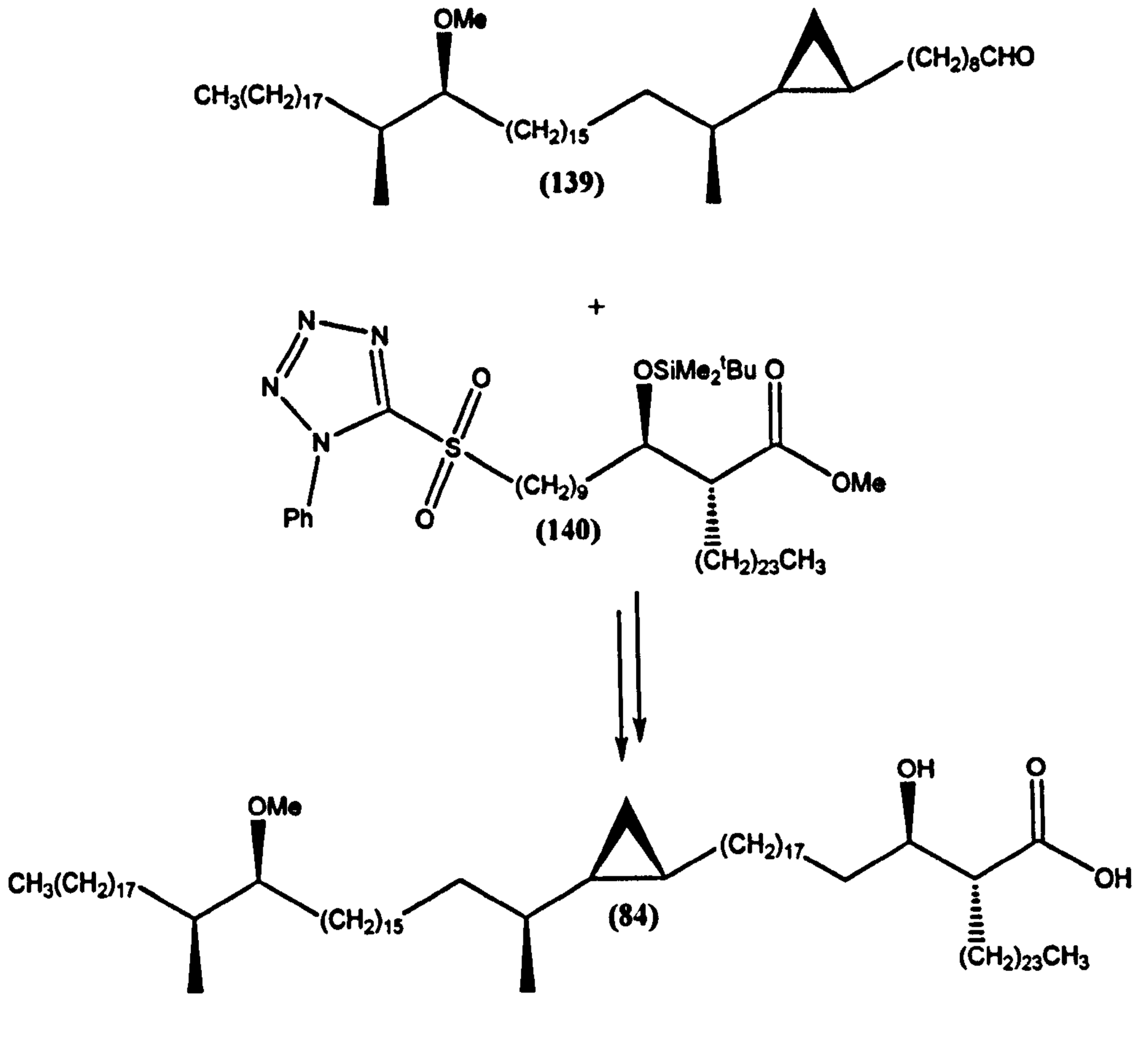


Fig. 97: Preparation of the *trans*-cyclopropane methoxy-mycolic acid (84)

The sulfone **(140)** was prepared starting from the branched hydroxy acid aldehyde **(128)** and the seven carbon chain sulfone, 7-(1-phenyl-1H-tetrazol-5-ylsulfonyl) heptyl pivalate **(154)**. A modified Julia-Kocienski olefination was carried out to give the alkene mixture **(155)**, and this was then hydrogenated with a palladium catalyst to give methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(pivaloyloxy)decyl)hexacosanoate **(156)** (See Fig. 98).

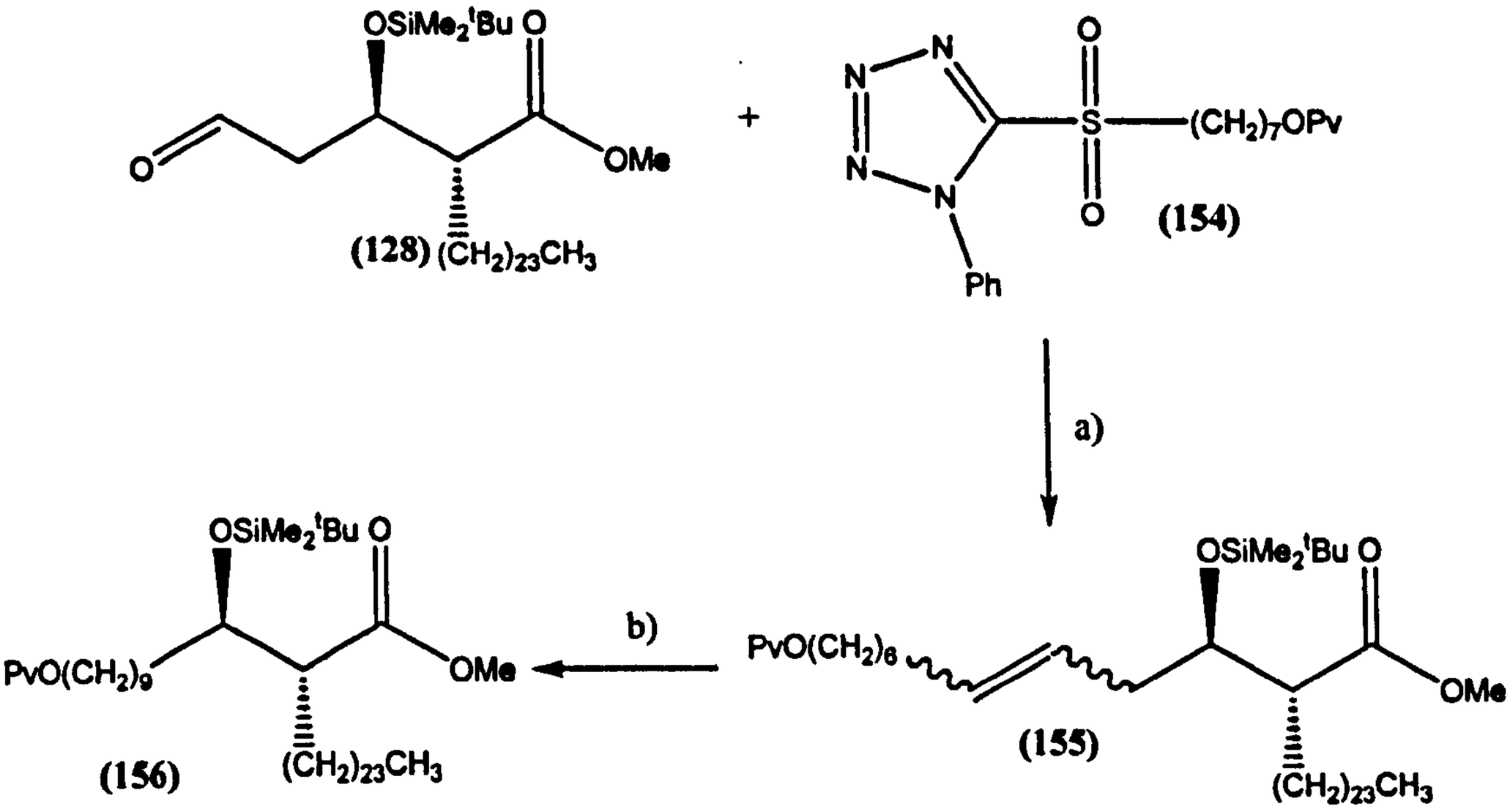


Fig. 98: a) LHMDS, (72 %); b) H_2, Pd cat., (93 %)

The ^1H NMR, ^{13}C NMR and IR data for the *tert*-butyl ester **(156)** were almost identical to that of the previously prepared ester **(130)**, the only difference being for the additional CH_2 group, which was measured using MALDI-MS. Also the optical rotation measured for the two compounds was similar, with that for **(156)** being measured at -3.64 and that for **(130)** at -3.21 (See Fig. 99).

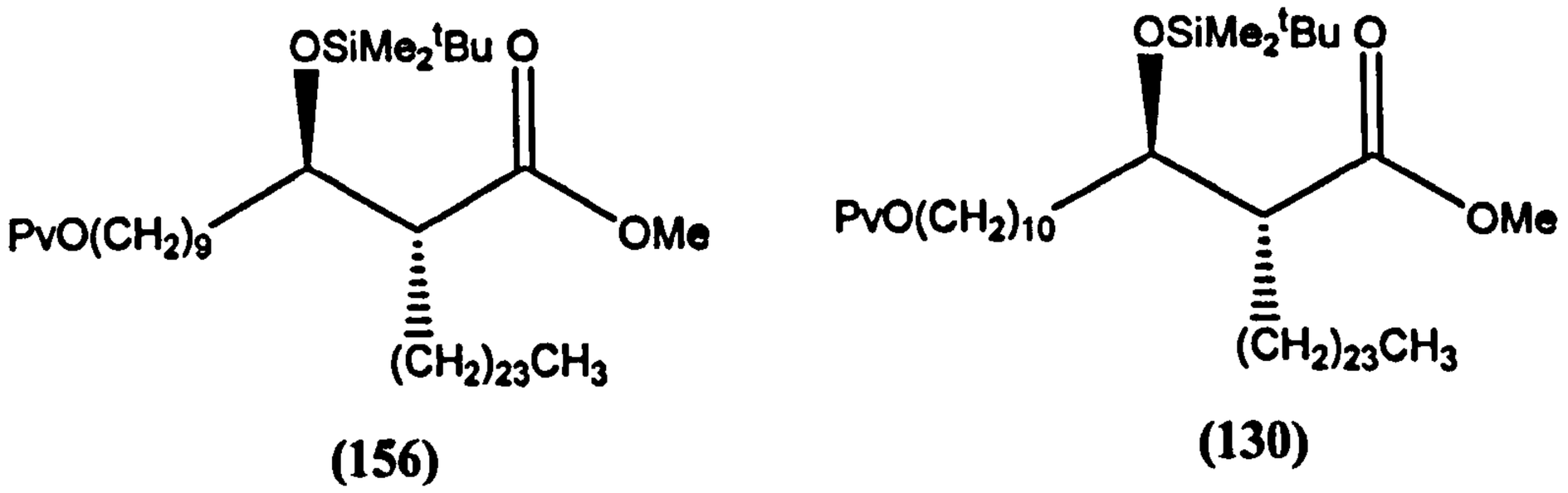


Fig. 99: Branched hydroxy acids (156) and (130)

The *tert*-butyl ester **(156)** was hydrolysed with potassium hydroxide to give the corresponding primary alcohol **(157)**, which was brominated with N-bromosuccinimide and triphenylphosphine yielding the primary bromide **(158)**. The sulfide **(159)** was prepared following the same procedure as previously discussed, by reaction with 1-phenyl-1H-tetrazole-5-thiol and potassium carbonate, and was then

oxidised using *m*-CPBA to give the desired branched hydroxy acid sulfone intermediate **(140)** (See Fig. 100).

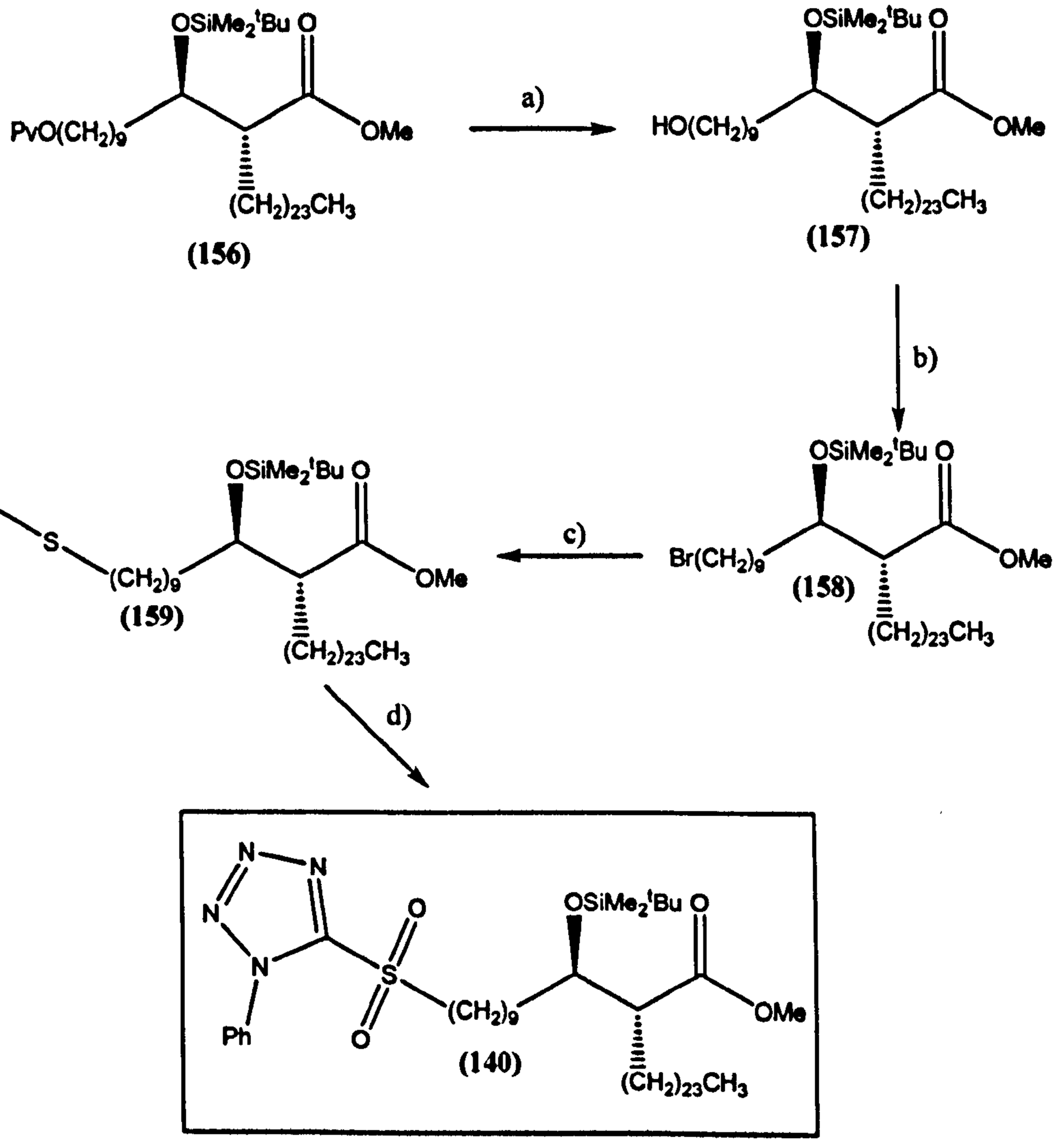
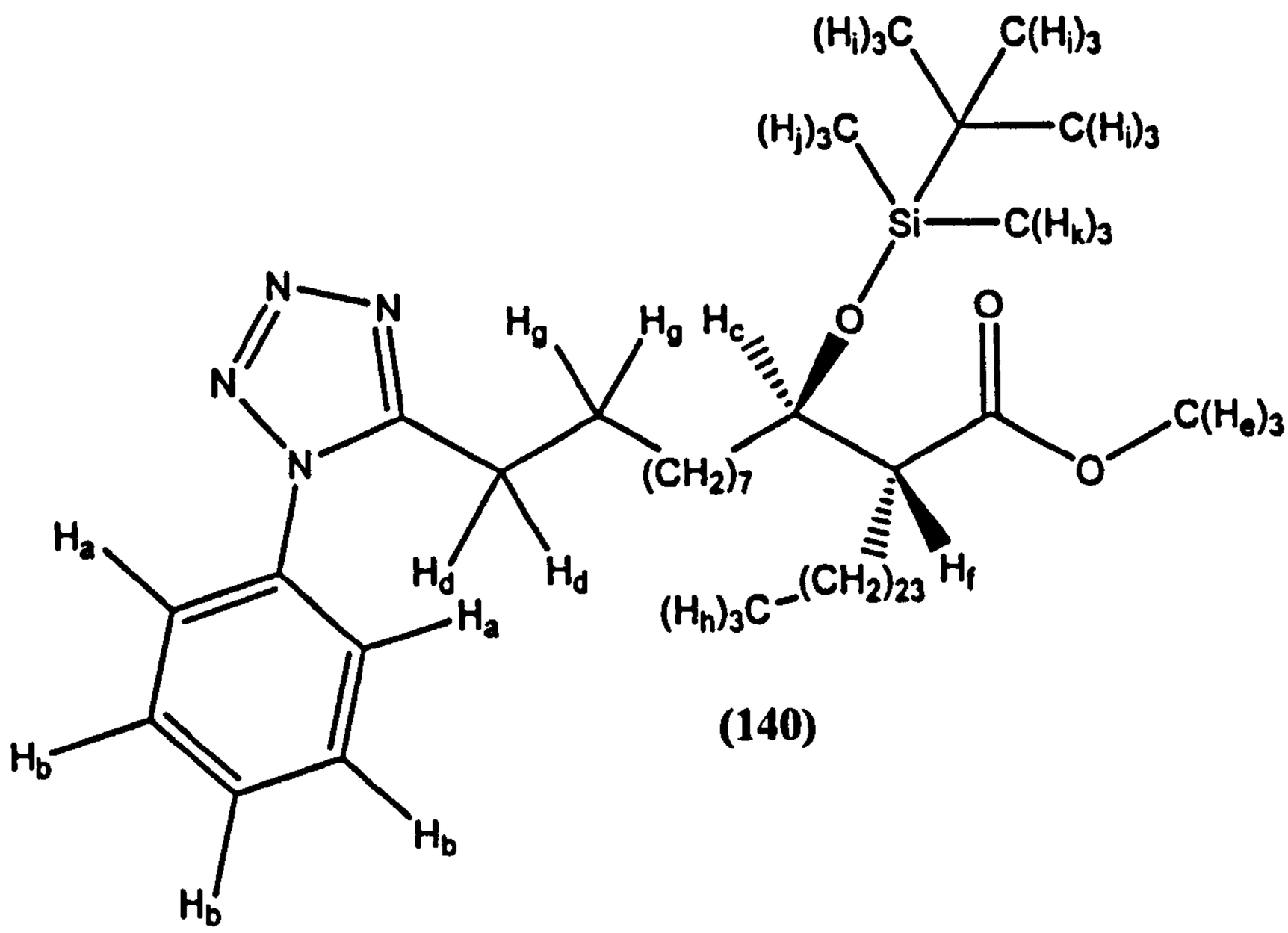


Fig. 100: a) KOH, (67 %); b) NBS, Ph_3P, $NaHCO_3$, (76 %); c) 1-phenyl-1H-tetrazole-5-thiol, K_2CO_3, (73 %); d) *m*-CPBA, (75 %)

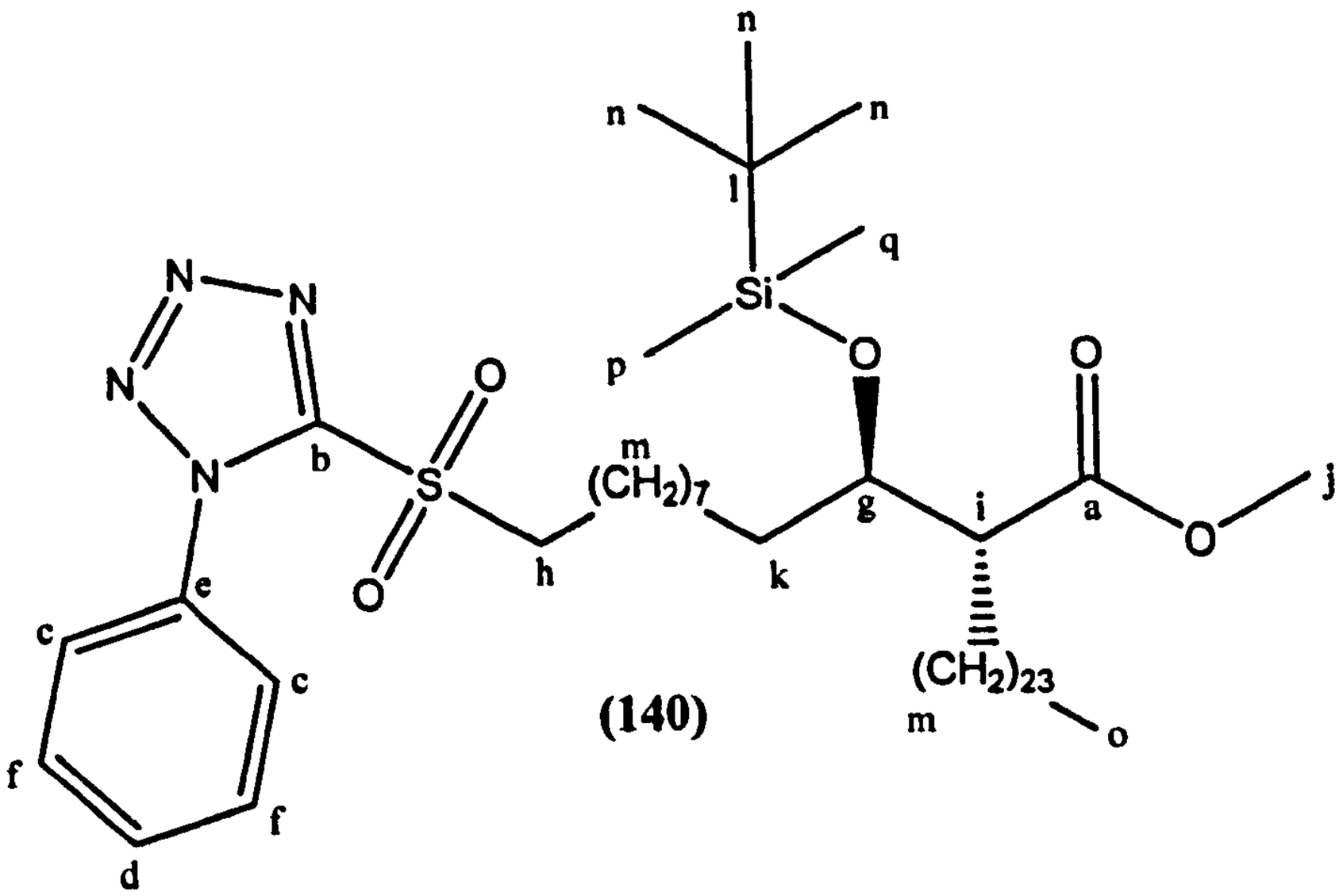
The specific rotation of the sulfone **(140)** was -4.85 and the IR confirmed some of the compound's functionality. Shifts at 2919 and 2848 cm^{-1} corresponding to C-H stretching, 1721 cm^{-1} for C=O stretching and 1484 cm^{-1} for O-CH_3. The ^1H and ^{13}C NMR spectra were analysed as follows (See Fig. 101 and Fig. 102).



(140)

H_x	δ	Multiplicity	Integration	J (H_z)
H_a	7.71-7.70	m	2	-
H_b	7.64-7.60	m	3	-
H_c	3.92-3.89	m	1	-
H_d	3.74	t	2	8.2
H_e	3.66	s	3	-
H_f	2.53	ddd	1	3.8, 6.95, 11.05
H_g	1.96	dist. pent.	2	7.9
H_h	0.89	t	3	6.6
H_i	0.87	s	9	-
H_j	0.05	s	3	-
H_k	0.02	s	3	-

Fig. 101: ^{1}H NMR data analysis of (140)



(140)

C_x	δ	C_x	δ
C_a	175.1	C_j	51.2
C_b	154.5	C_k	33.7
C_c	133.8	C_l	33.4
C_d	130	C_m	31.9-22.7
C_e	129.8	C_n	18
C_f	123.9	C_o	14.1
C_g	73.2	C_p	-4.4
C_h	61.8	C_q	-4.9
C_i	51.6		

Fig. 102: ^{13}C NMR data analysis of (140)

2.3.6 – Coupling the meromycolate moiety and branched hydroxy acid

With the desired meromycolate aldehyde **(139)** and branched hydroxy acid sulfone **(140)** synthesised it was then possible to carry out a modified Julia-Kocienski olefination, a hydrogenation of the resultant alkene and then two deprotection steps to give the α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)** (See Fig. 103).

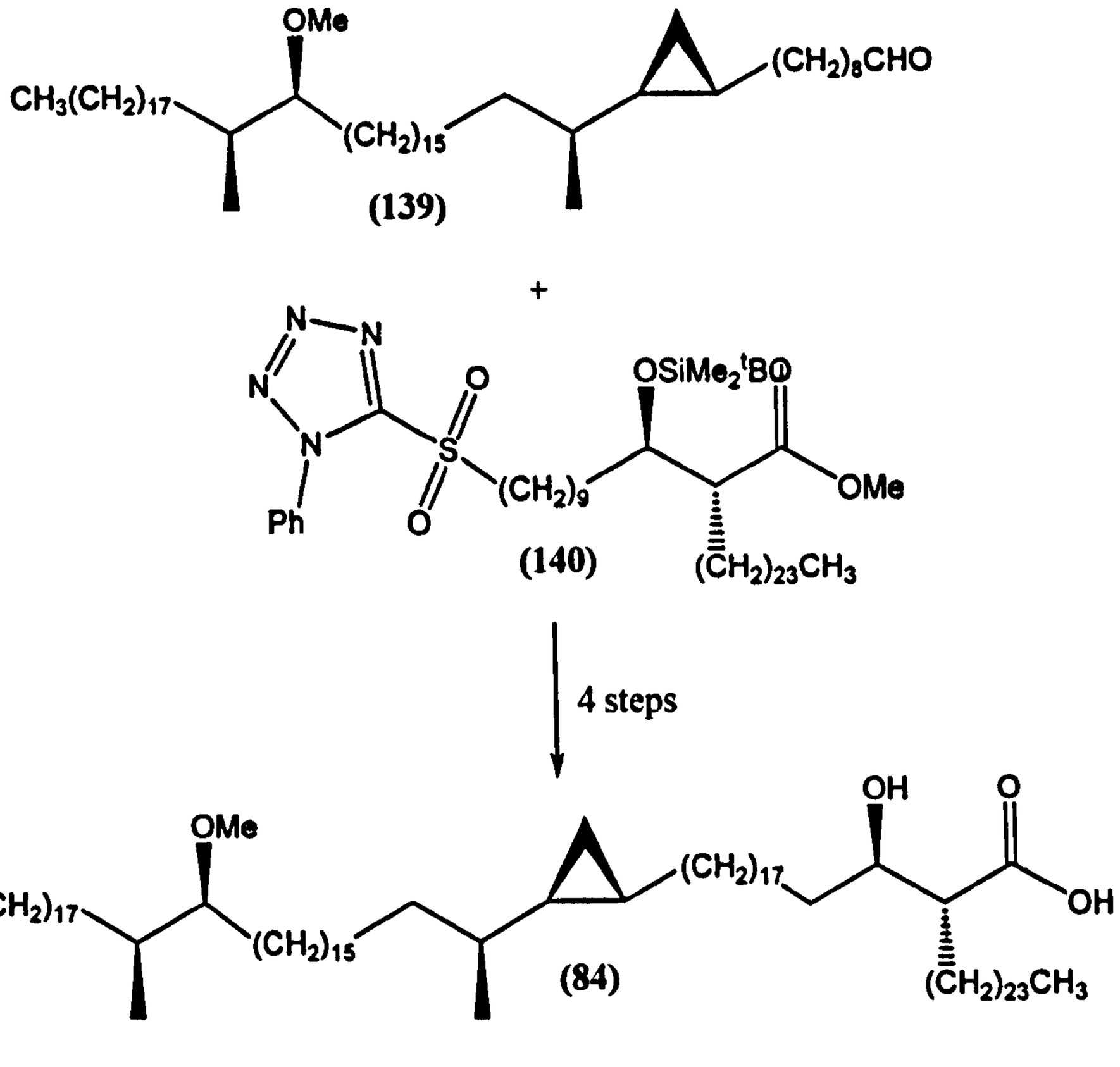


Fig. 103: Preparation of *trans*-cyclopropane methoxy-mycolic acid (84)

A modified Julia-Kocienski olefination of the sulfone **(139)** and aldehyde **(140)**, using lithium *bis(*trimethylsilyl)amide as a base gave the isomeric alkenes **(160)**. The alkenes were mildly hydrogenated using dipotassium azodicarboxylate and acetic acid to give the protected α-methyl *trans*-cyclopropane methoxy-mycolic acid **(161)** (See Fig. 104).

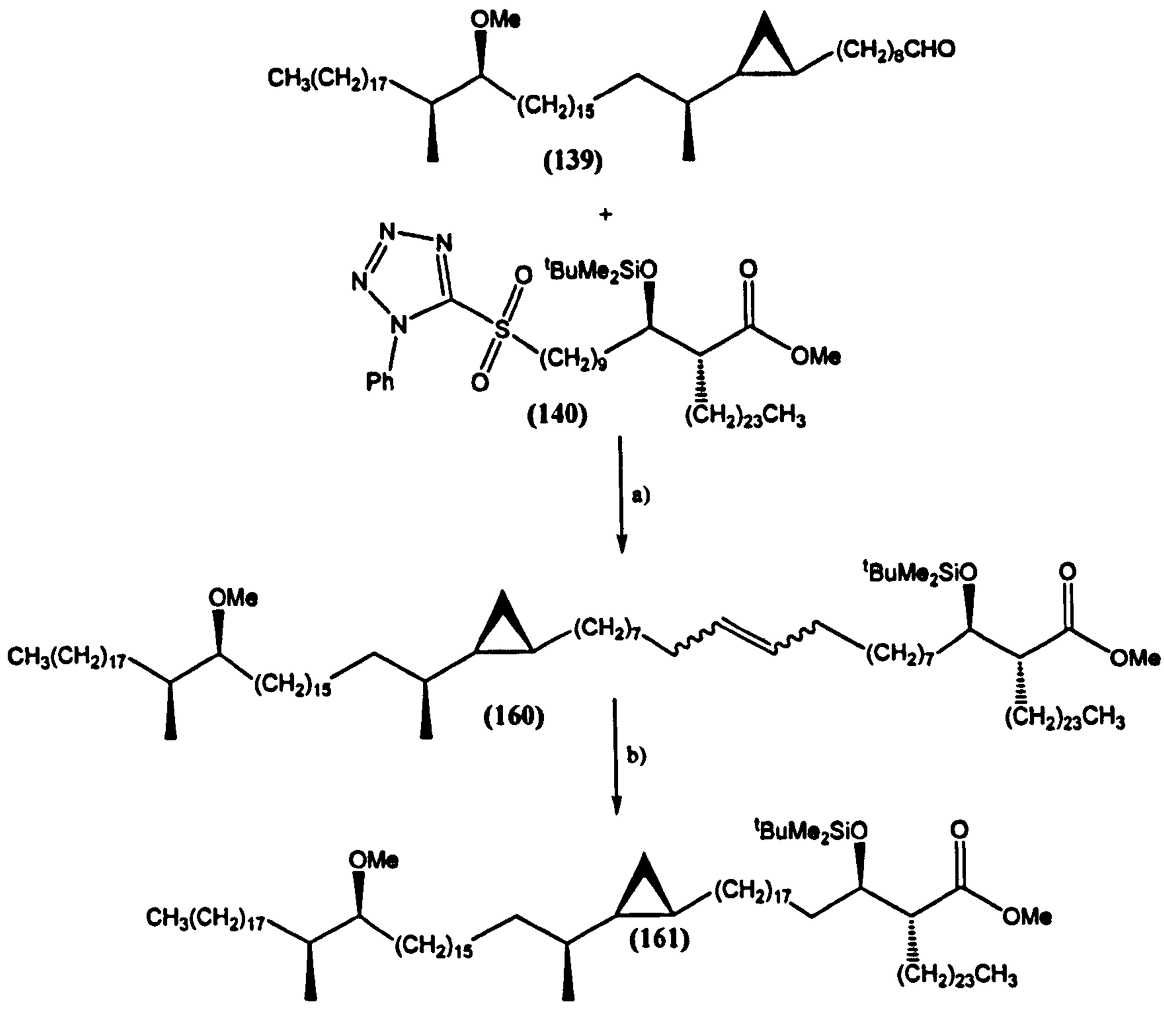
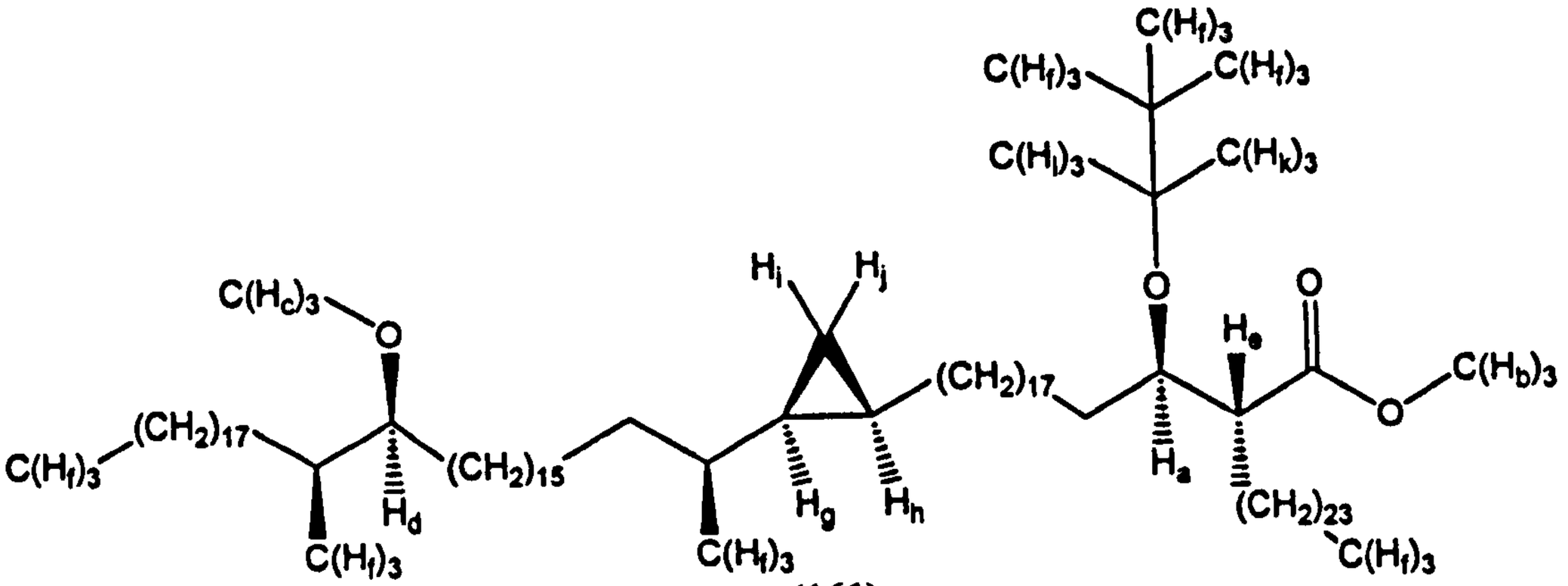


Fig. 104: a) LHMDS, (28 %); b) $(NCOO^{-}K^{+})_2$, CH_3COOH, THF, (94 %)

The olefination gave an unusually low yield in this case; this may have been due to the bulky sulfone substituent. The formation of the sulfone-lithium complex may have been hindered sterically; the reason for this is because the α-methyl *trans*-cyclopropane unit may cause the long chains to fold in a different configuration to that of a *cis*-cyclopropane containing compound.[130, 143-145, 147] As a result, during workup some of the sulfone starting material was recovered and, if the reaction were to be repeated, then addition of a higher equivalence of base would be the most logical course of action.

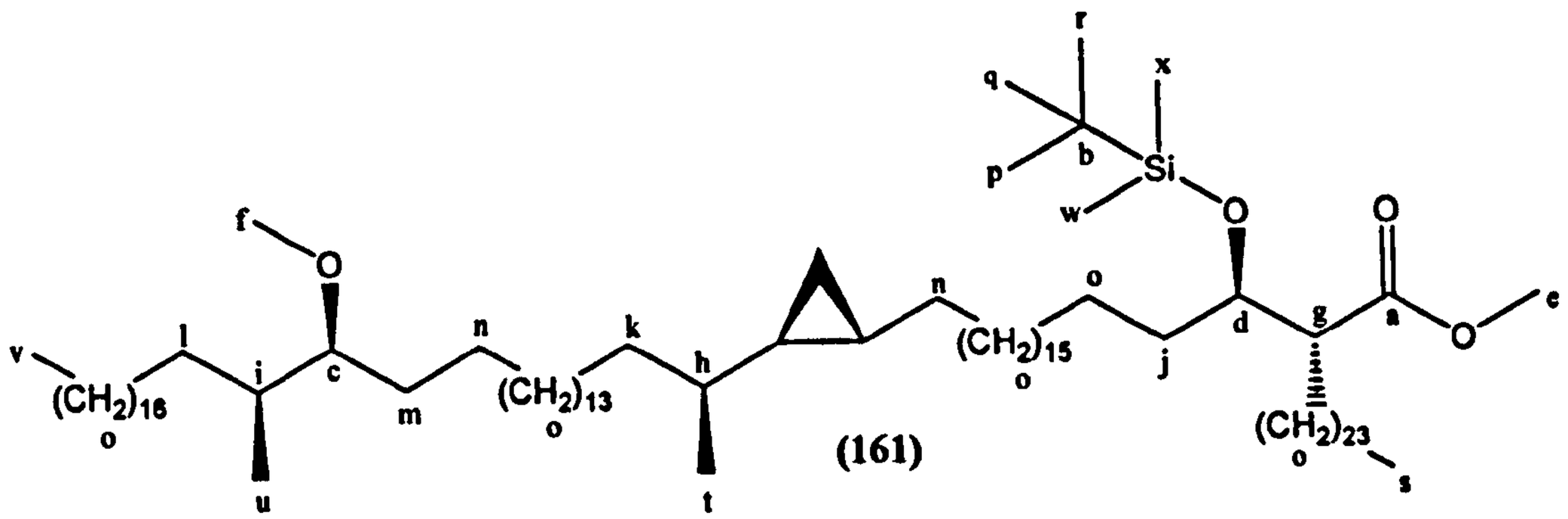
The synthesis of the protected α-methyl *trans*-cyclopropane methoxy-mycolic acid **(161)** was monitored using ^{1}H NMR, ^{13}C NMR, IR, optical rotation and MALDI-MS. The 1H and 13C NMR spectra of **(161)** were analysed as follows. (See Fig. 105 and Fig. 106).



(161)

H_x	δ	Multiplicity	Integration	J (H_z)
H_a	3.92-3.90	m	1	-
H_b	3.66	s	3	-
H_c	3.35	s	3	-
H_d	2.97-2.95	m	1	-
H_e	2.54	ddd	1	11
H_f	0.91-0.85	m	21	-
H_g	0.48-0.43	m	1	-
H_h	0.22-0.18	m	1	-
H_i	0.17-0.14	m	1	-
H_j	0.13-0.09	m	1	-
H_k	0.05	s	3	-
H_l	0.03	s	3	-

Fig. 105: ^{1}H NMR data analysis of (161)



(161)

C_x	δ	C_x	δ	C_x	δ
C_a	175.1	C_i	37.4	C_q	19.7
C_b	125.5	C_j	37.1	C_r	18.6
C_c	85.5	C_k	35.8	C_s	18
C_d	73.2	C_l	35.4	C_t	14.9
C_e	65.9	C_m	34.5	C_u	14.1
C_f	57.7	C_n	33.7	C_v	10.5
C_g	51.6	C_o	32.8-23.7	C_w	-4.4
C_h	38.1	C_p	22.7	C_x	-4.9

Fig. 106: ^{13}C NMR data analysis of (161)

The MALDI-MS of **(161)** also confirmed that the coupling of the two motifs **(139)** and **(140)** was successful as the value obtained was 1446.21, which is in agreement with the expected exact mass for **(161)**, ($C_{95}H_{190}NaO_4Si$ requires: 1446.43).

The silyl group was removed using hydrofluoric acid in pyridine to give the α-methyl *trans*-cyclopropane methoxy-mycolic methyl ester **(162)**. Hydrolysis of the methyl ester using lithium hydroxide gave the α-methyl *trans*-cyclopropane methoxy-mycolic acid, methyl 2-((1*R*,2*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,18*S*,19*S*)-18-methoxy-19-methylheptatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate **(84)** (See Fig. 107).

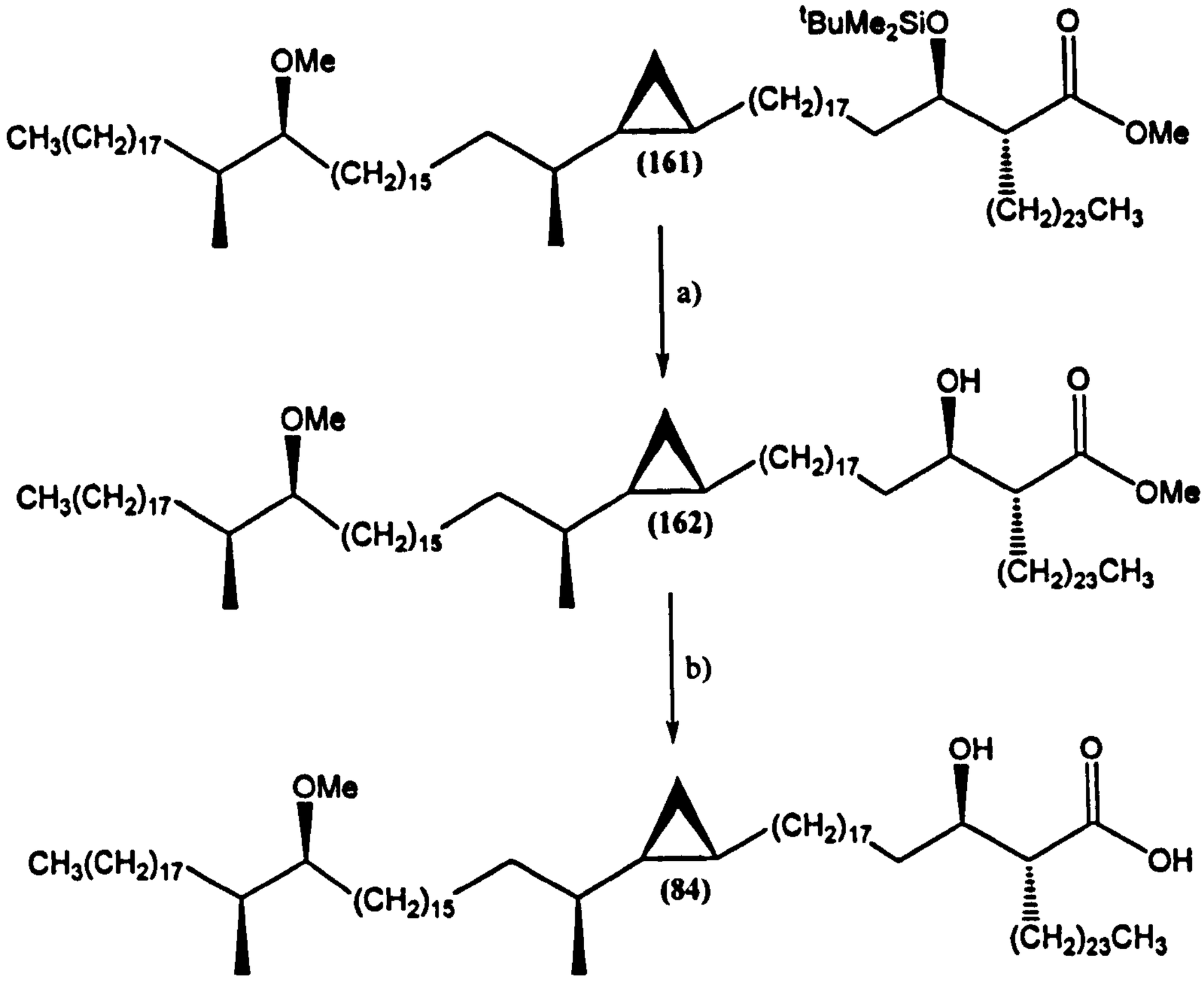


Fig. 107: a) HF.Pyr, pyridine, (54 %); b) LiOH, THF/MeOH/H_2O, (69 %)

The ^1H NMR, ^{13}C NMR, IR, optical rotation and MALDI-MS were used to confirm the structure of **(84)**. Expansions of the various regions of the ^1H NMR spectrum of α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)** are shown in Fig. 108a, 108b, 108c and 108d, with the R group labels corresponding to the bridging carbon chain lengths in each case.

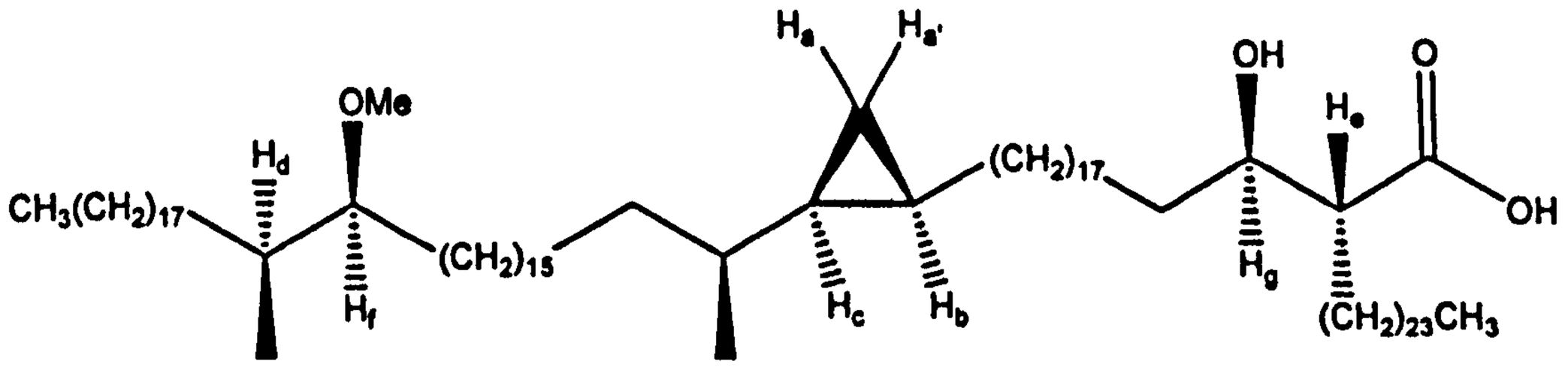


Fig 108a: *trans*-Cyclopropane methoxy-mycolic acid (84)

An area of the spectrum which is of particular interest is that between δ 0.50 and δ 0.00, as this is the region of the spectrum which corresponds to the four

protons directly bonded to the *trans*-cyclopropane ring. The region between δ 0.22-0.09 appears to contain signals for three different hydrogens. This is because H_a and $H_{a'}$ are non-equivalent and each has three couplings, each signal splitting to give a double doublet of doublets (8 lines), which leads to 16 lines. H_b should give a double double double doublet of doublets (32 lines) as it is coupled to five non-equivalent protons; however, due to overlap with the signals for H_a and $H_{a'}$, H_b cannot be resolved fully at δ 0.22-0.18. H_c, represented by the broad multiplet at δ 0.48-0.43, should split to give a doublet double doublet of doublets (16 lines). However, a complex broad multiplet is observed due to the presence of four similar coupling constants leading to overlapping of peaks (See Fig. 108b). H_d and H_e are shown to exhibit doublet of triplets as seen at δ 1.79-1.72 and δ 2.49-2.45, which is consistent with the expected theoretical splitting patterns (See Fig. 108c). H_f and H_g are both shifted downfield, to δ 2.99-2.96 and δ 3.74-3.70, due to their adjacency to the β-hydroxy and methoxy respectively. They both exhibit one hydrogen pentuplets on the spectrum shown below, which is consistent with the expected theoretical splitting pattern (See Fig. 108d).

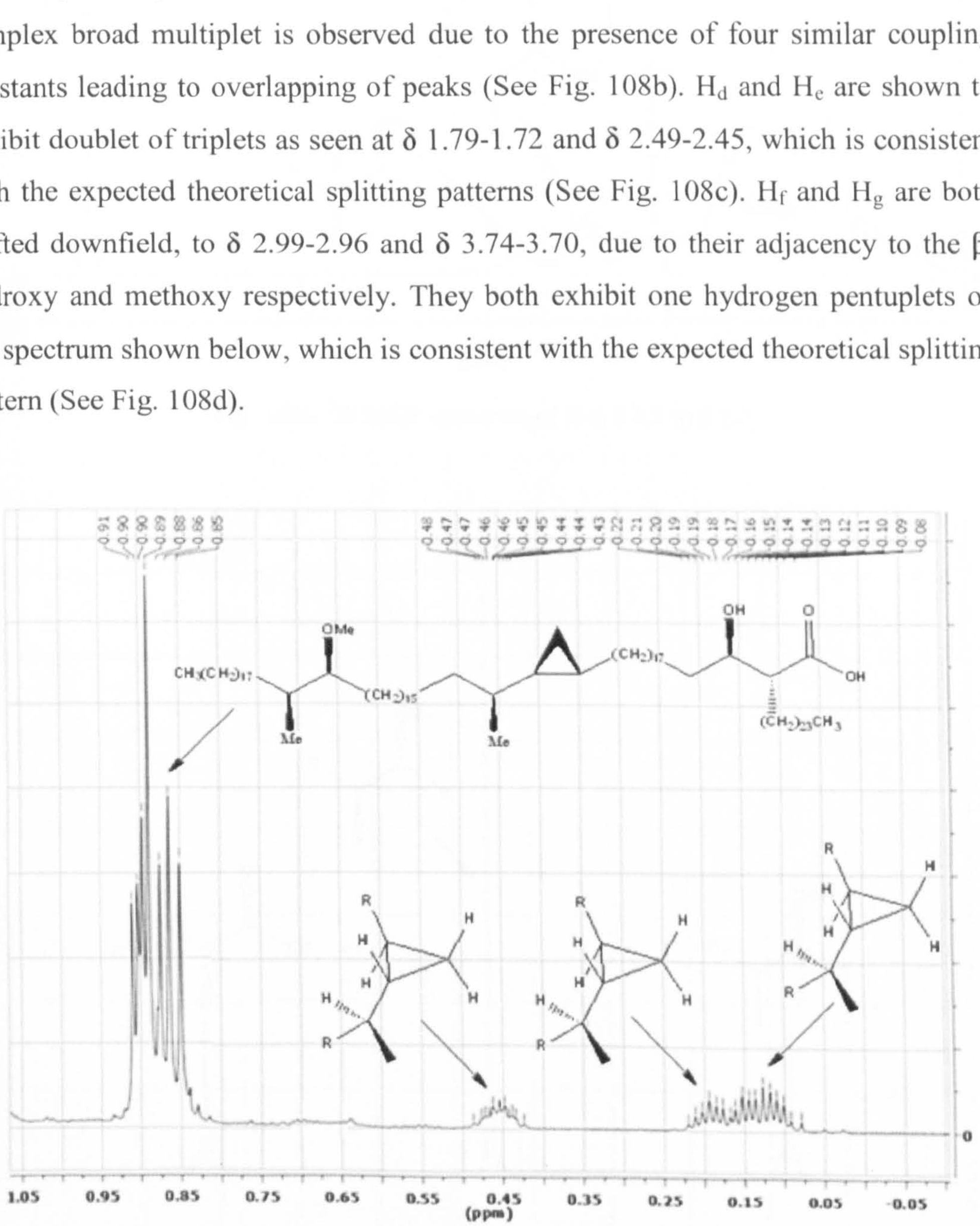


Fig. 108b: ^{1}H NMR spectrum of (84) δ 1.1 to δ -0.1

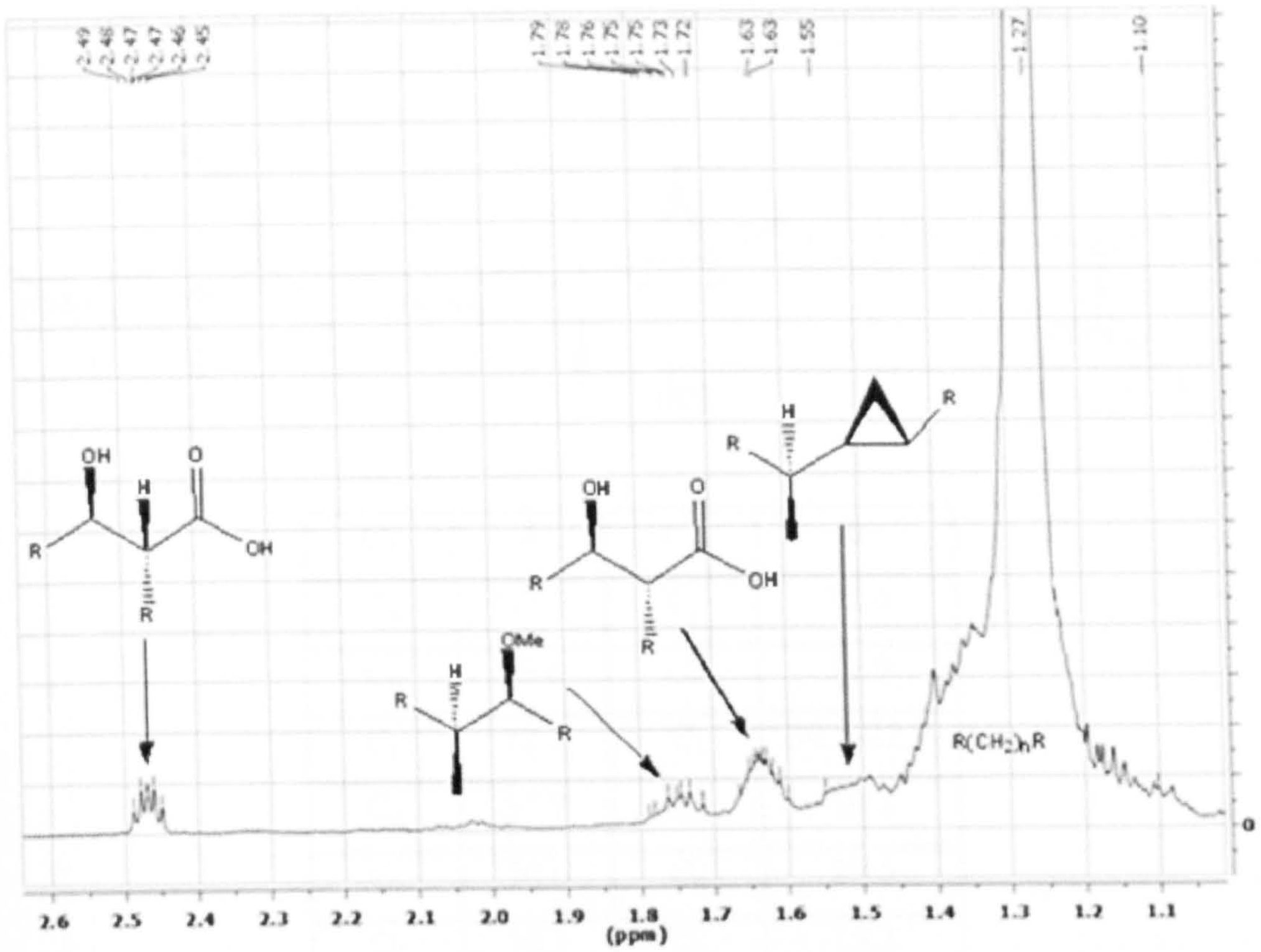


Fig. 108c: ^{1}H NMR spectrum of (84) δ 2.7 to δ 1.0

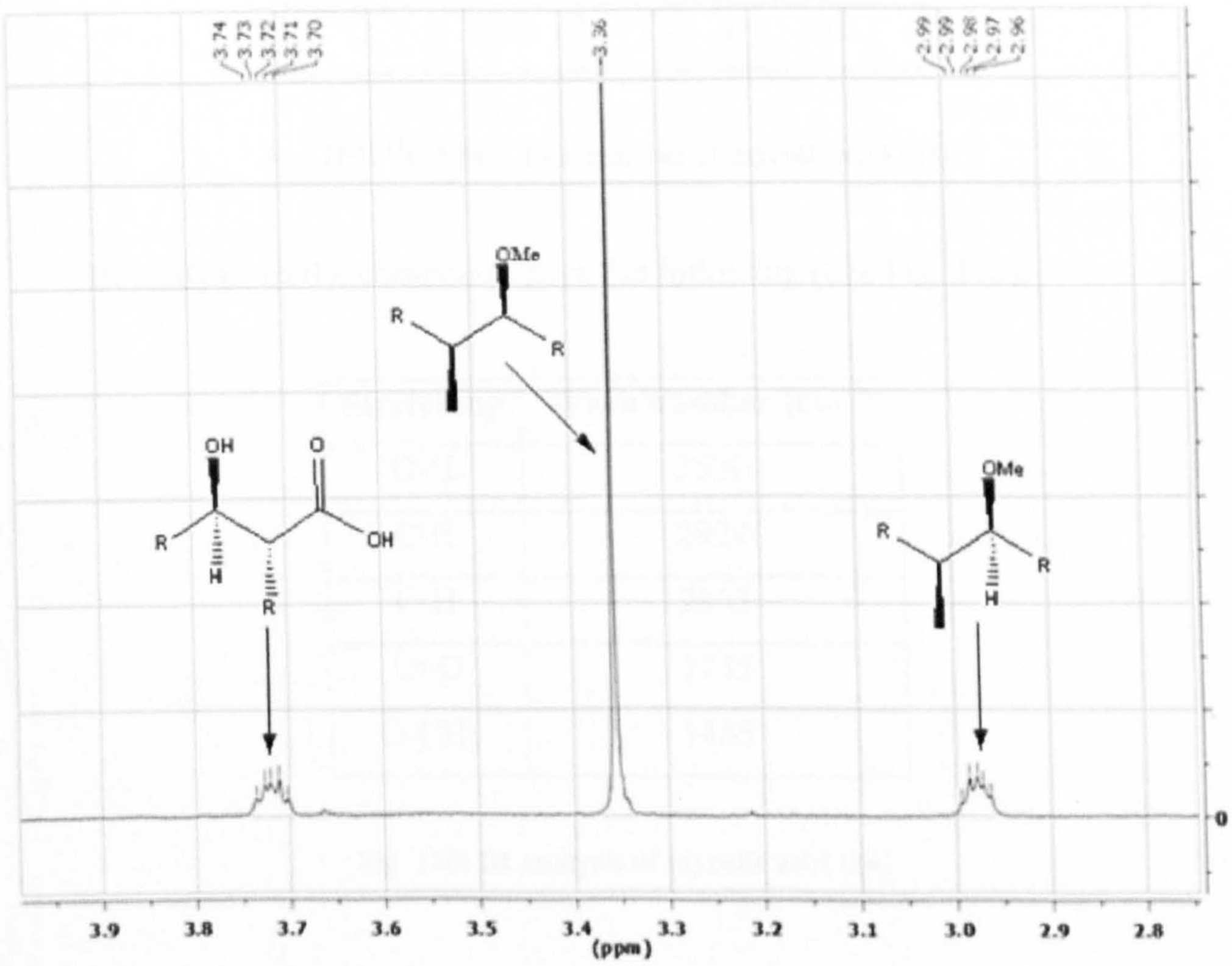
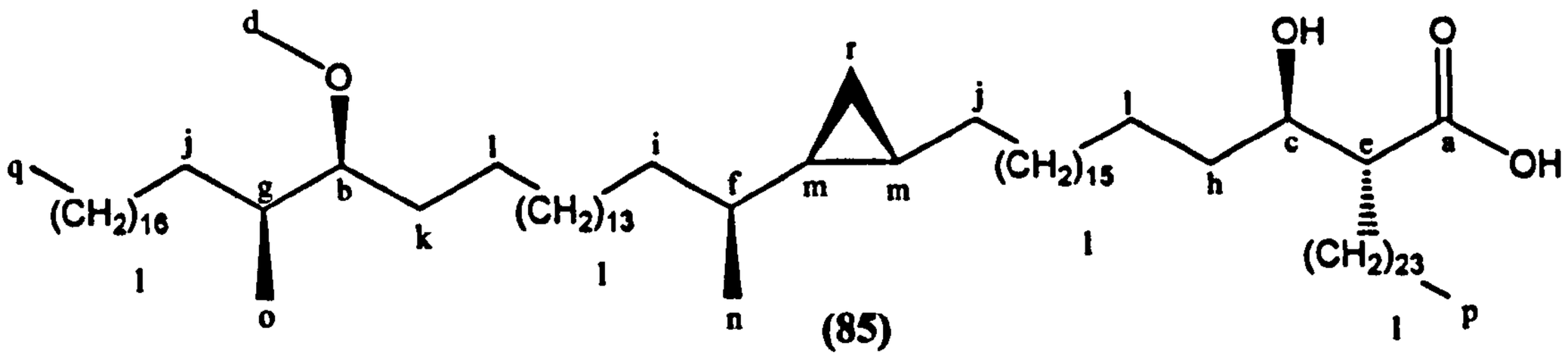


Fig. 108d: ^{1}H NMR spectrum of (84) δ 4.0 to δ 2.6

The ^{13}C NMR spectrum was analysed as follows (See Fig. 109).



(85)

C_x	δ	C_x	δ
C_a	178.7	C_j	32.4
C_b	85.6	C_k	31.9
C_c	72.1	C_l	30.5-22.7
C_d	57.7	C_m	19.7
C_e	50.6	C_n	18.6
C_f	38.2	C_o	15.3
C_g	37.4	C_p	14.9
C_h	35.6	C_q	14.1
C_i	35.3	C_r	10.5

Fig. 109: ^{13}C NMR data analysis of mycolic acid (84)

IR analysis of the compound gave the following (See Fig. 110).

Stretching	Wave number (cm^{-1})
O-H	3520
C-H	2924
C-H	2853
C=O	1735
$O-CH_3$	1465

Fig. 110: IR analysis of mycolic acid (84)

The MALDI-MS gave an ion at 1318.24 ($[M+Na]^+$ for $C_{88}H_{174}NaO_4$ requires: 1318.33). The specific rotation of the compound was found to be $[\alpha]^{22}_D$ = -0.97 ($CHCl_3$, 0.468 μmol). This showed that epimerisation of the compound had not occurred as the optical rotation measured for **(163)** was $[\alpha]^{23}_D$ = -1.12 ($CHCl_3$, 0.993 μmol). The molecular rotation of **(84)** was measured as -12.57, which is compliant with the theoretical value calculated from its intermediate fragments, distal -44.16, proximal +69.30 and branched hydroxy acid -43.14, which give M_D= -18.

2.4 – Synthesis of the protected *S*-α-methyl *trans*-alkene keto-mycolic acid (85)

Work previously carried out by G. Koza reported the synthesis of a protected *R*-α-methyl *trans*-alkene keto-mycolic acid **(163)**. Therefore it was hypothesized that the preparation of the opposite diastereoisomers, which has the expected natural stereochemistry adjacent to the ketone and the shorter α-alkyl chain of mycolic acids present in *M. marinum*, would also be successful. Following a similar method to that of Koza, it was possible to prepare the protected *S*-α-methyl *trans*-alkene keto-mycolic acid **(85)** (See Fig. 111).[183]

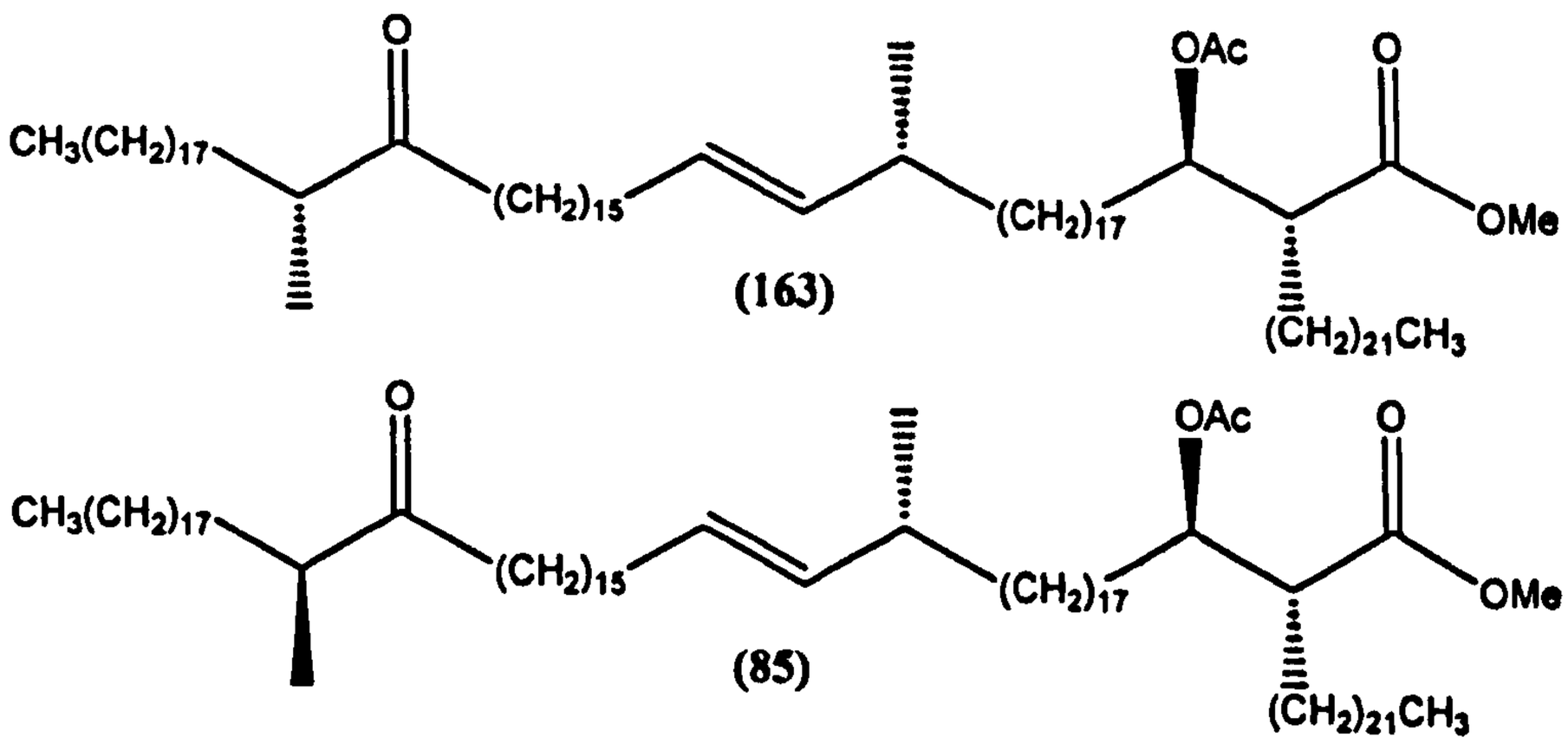


Fig. 111: α-Methyl *trans*-alkene keto-mycolic acids (163) and (85)

2.4.1 - Retrosynthesis of protected S-α-methyl trans-alkene keto-mycolic acid (85)

Following a similar disconnection route as discussed by G. Koza, the major fragments shown below were derived (See Fig. 112).[183]

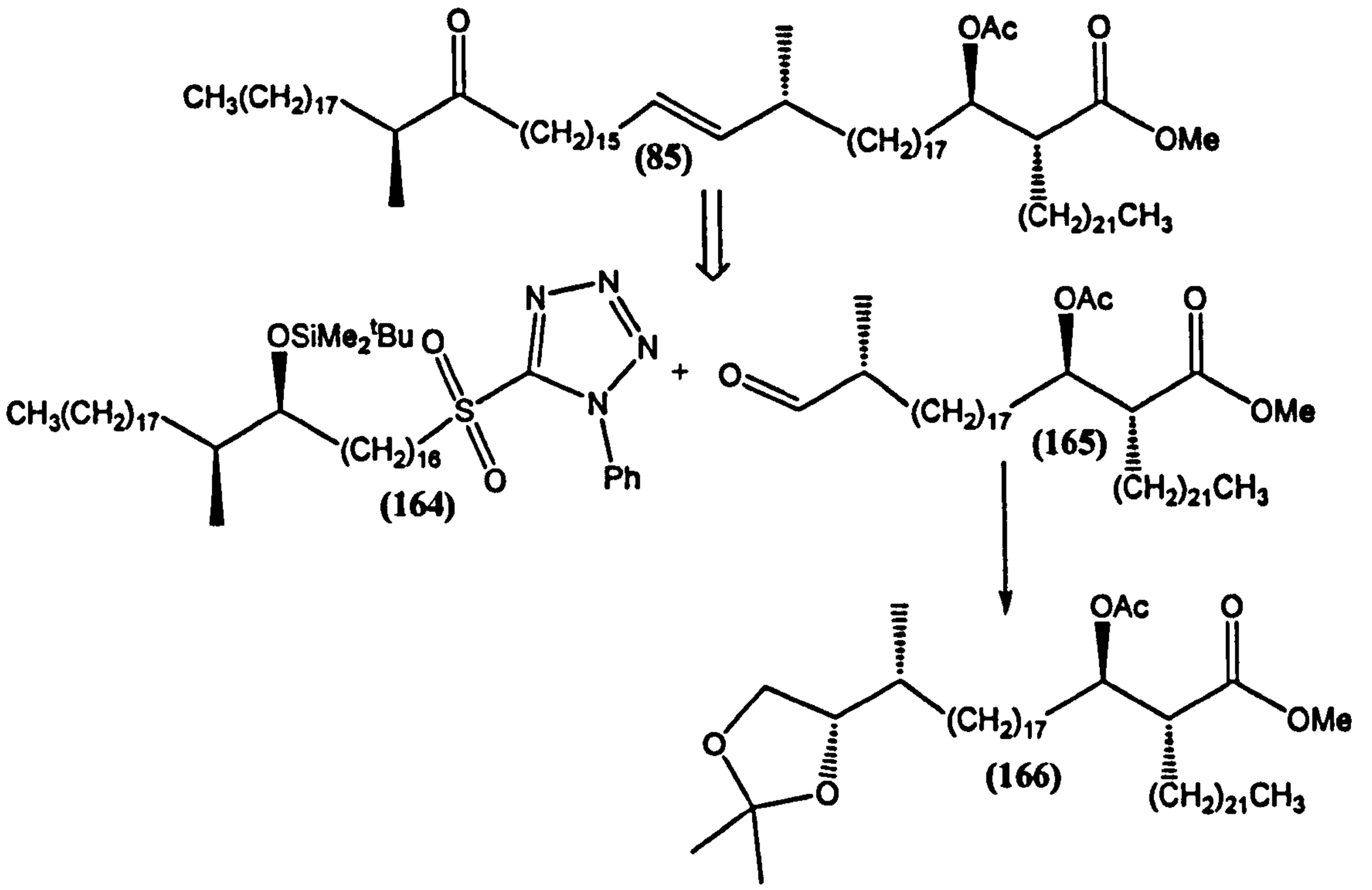


Fig. 112: Protected *S*-α-methyl *trans*-alkene keto-mycolic acid (86) disconnection

The *S,S* α-methyl *tert*-butyl dimethyloxy silane fragment **(164)** can be prepared, following similar methods to those discussed previously, from the secondary alcohol **(167)** (See Fig. 113).

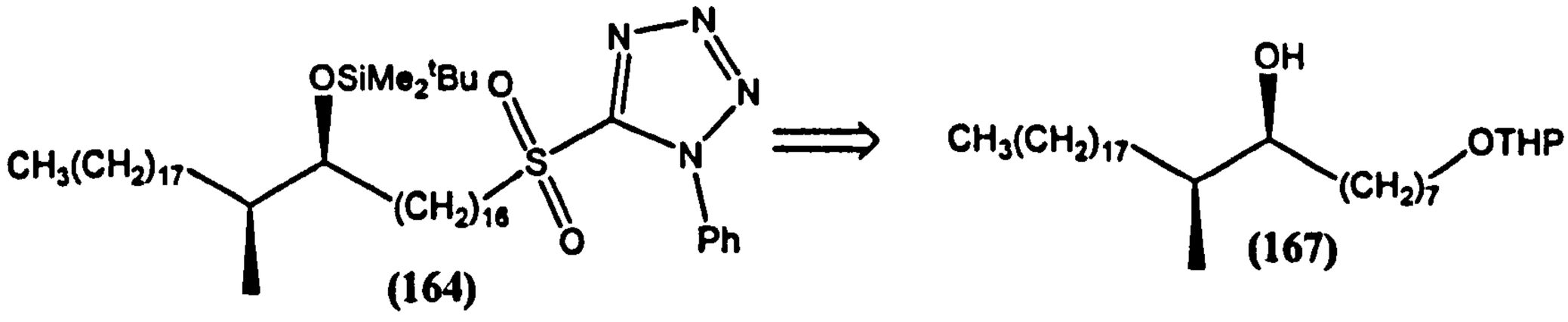


Fig. 113: Retrosynthesis of sulfone (164)

The branched hydroxy acid **(166)** can be prepared from *D*-mannitol **(60)** and *L*-aspartic **(168)** acid following the disconnection route discussed by G. Koza (See Fig. 114).[183]

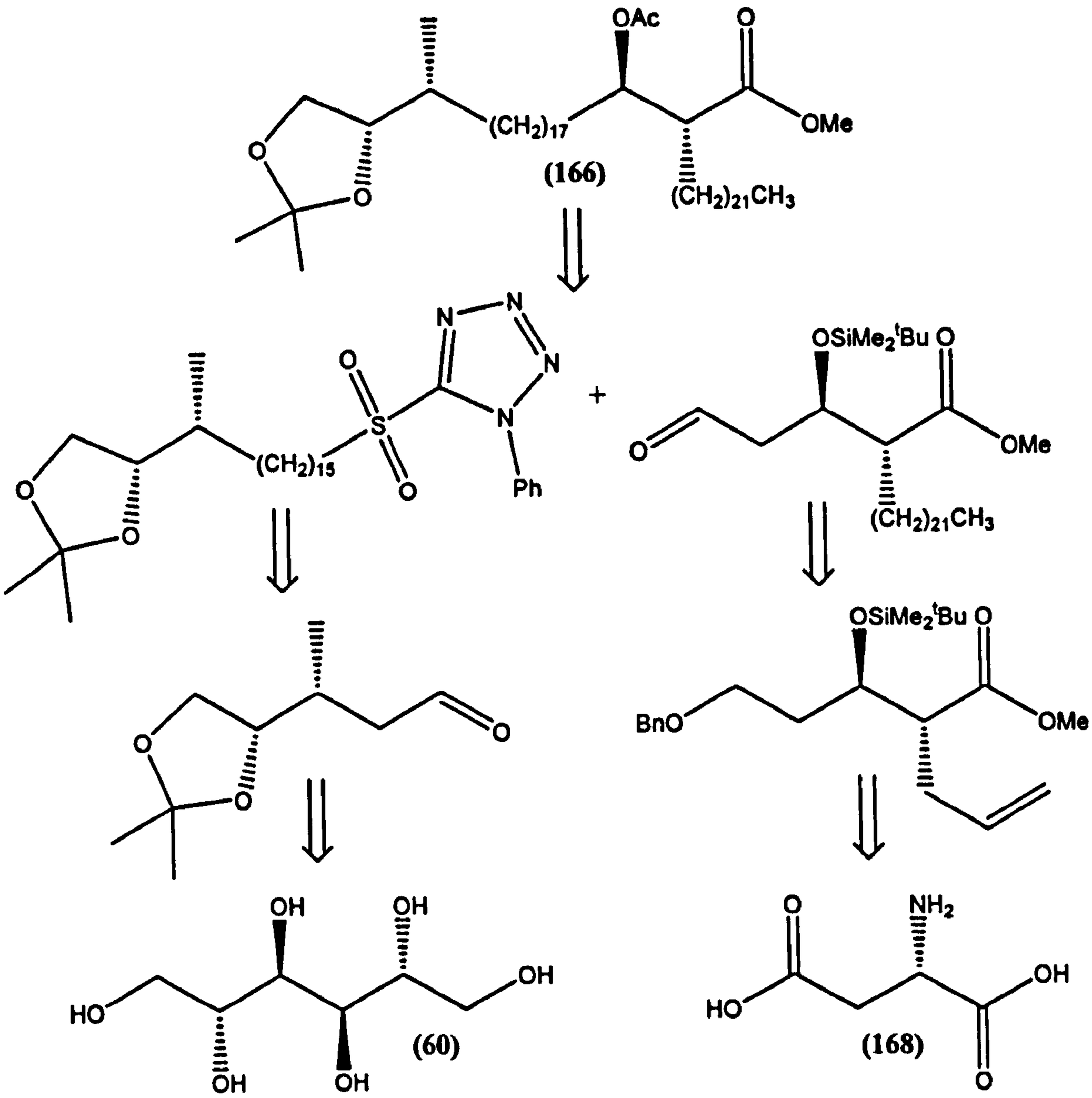


Fig. 114: Retrosynthesis of branched hydroxy acid (166)

2.4.2 – Synthesis of the S,S α-methyl tert-butyl dimethyloxy silane sulfone (164)

The *S,S* α-methyl *tert*-butyl dimethyloxy silane fragment **(164)** was prepared from the previously synthesised secondary alcohol, (8*S*,9*S*)-9-methyl-1-(tetrahydro-2H-pyran-2-yloxy)heptacosan-8-ol **(167)**.[189] The secondary alcohol was protected with a *tert*-butyl dimethylsilyl chloride to yield **(169)**, this was then converted into the primary alcohol **(170)** via a deprotection of the THP group using pyridinium-*p*-toluene sulfonate. Oxidation of the alcohol with PCC gave the aldehyde, (8*S*,9*S*)-8-(*tert*-butyldimethylsilyloxy)-9-methylheptacosanal **(171)** (See Fig. 115).

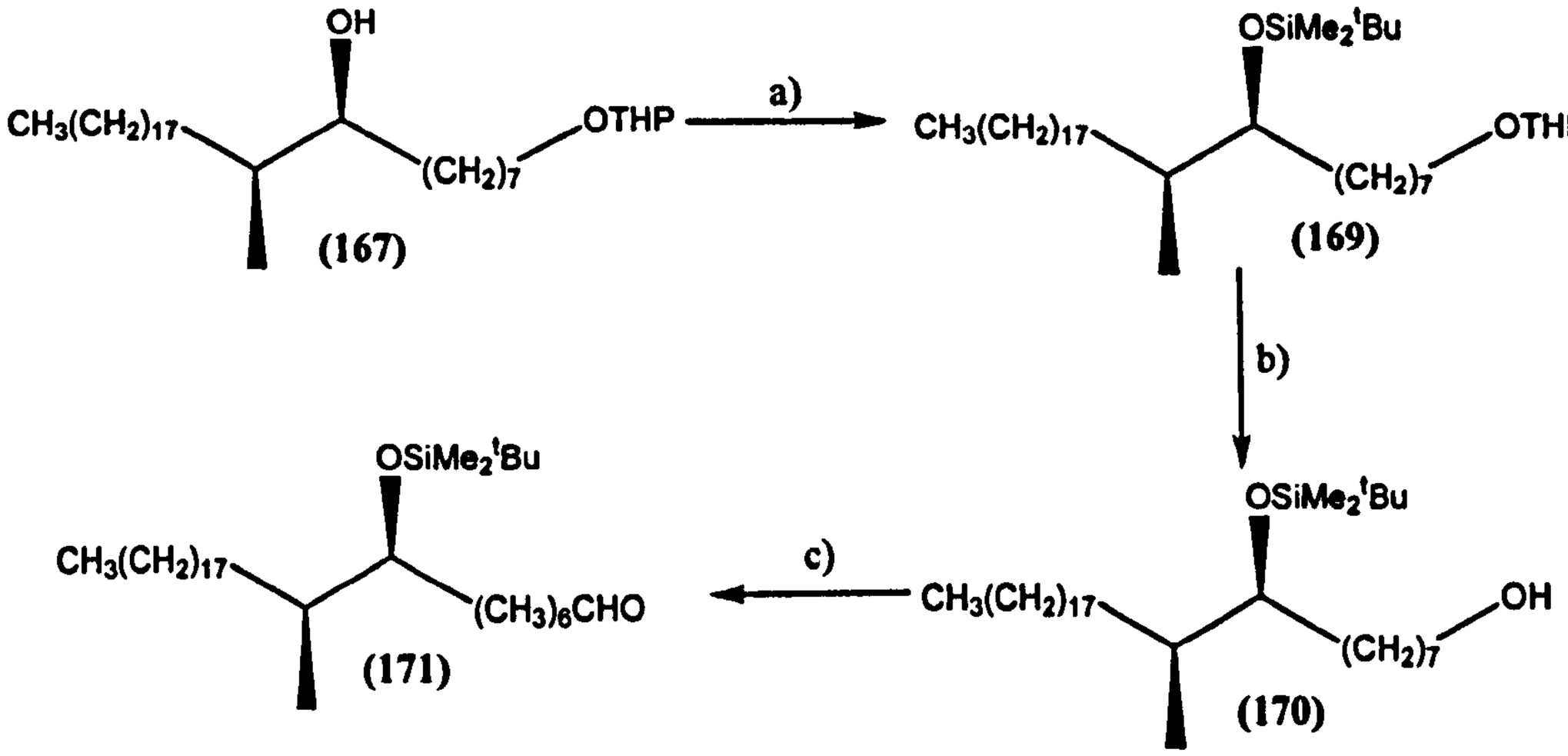


Fig. 115: a) tBDMSCl, $C_3H_4N_2$, dry DMF, (91 %); b) PPTS, (80 %); c) PCC, (94 %)

Confirmation of the above structures was done primarily using ^{1}H NMR. Three singlets were observed in the ^{1}H NMR spectrum of **(169)** at 0.89 (9H), 0.03 (3H) and 0.02 (3H), corresponding to the *tert*-butyl and two methyls of the protecting group. Removal of the THP group gave rise to a less complicated ^{1}H NMR spectrum for **(170)**, with the absence of signals at δ 4.58, δ 3.90-3.86, δ 3.40, δ 1.86-1.82 and δ 1.75-1.70 being observed. In addition the signal corresponding to the CH_2 adjacent to the primary alcohol was shown to have shifted downfield from δ 3.53-3.48 to δ 3.65. Oxidation to the aldehyde **(171)** was confirmed, the NMR spectrum of this showing a one hydrogen triplet at δ 9.77, corresponding to the aldehyde proton.

In a similar method as discussed before, the aldehyde **(171)** could be chain extended to give the sulfone **(164)** with the desired 16 carbon chain (See Fig. 116).

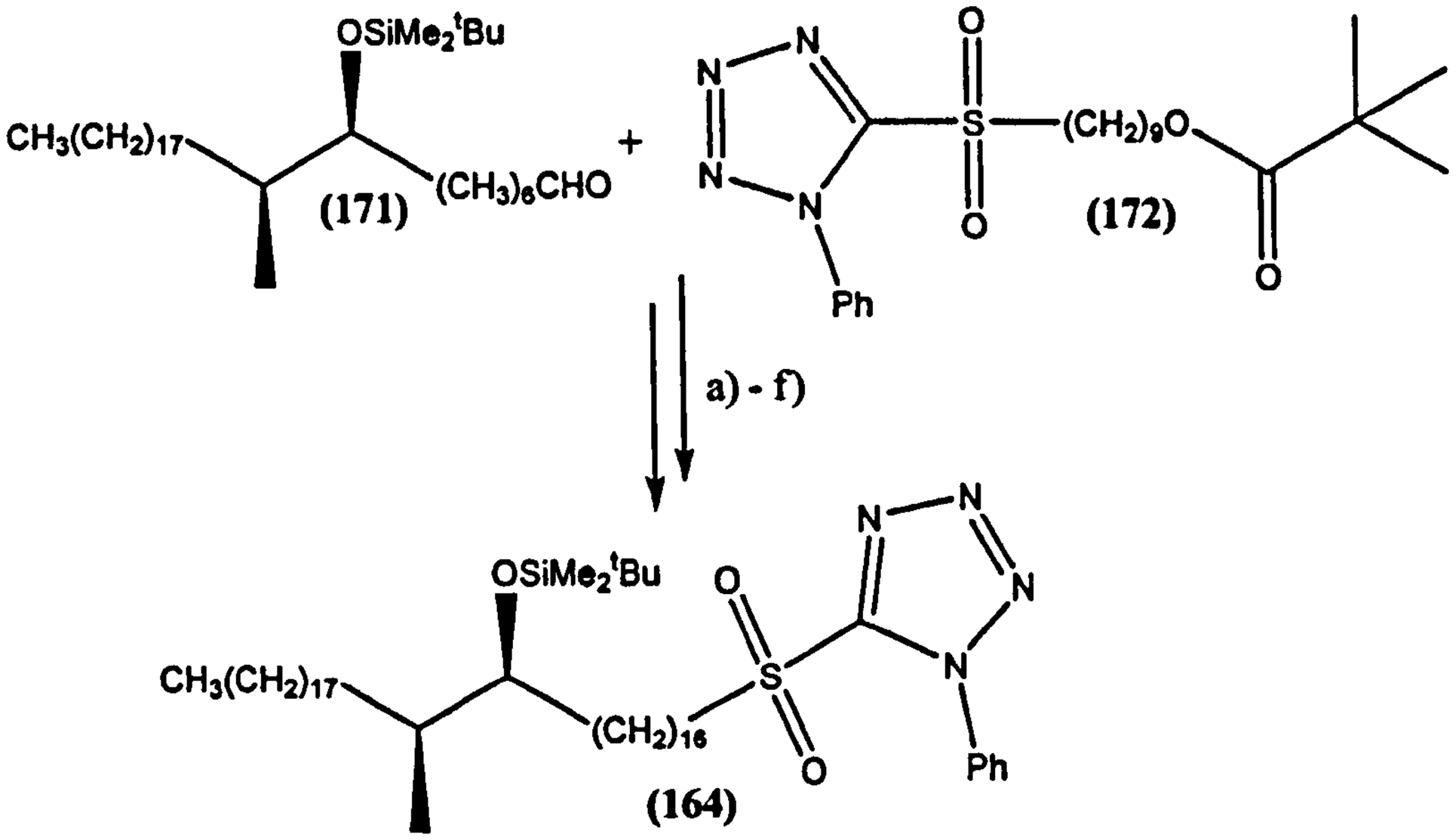


Fig. 116: a) LHMDS, (76 %); b) H_2, Pd cat., (70 %); c) $LiAlH_4$, (90 %); d) NBS, PPh_3, $NaHCO_3$, (81 %); e) 1-phenyl-1H-tetrazole-5-thiol, K_2CO_3, (99 %); f) H_2O_2, $(NH_4)_6Mo_7O_{24}.4H_2O$, (65 %)

2.4.3 – Synthesis of the branched hydroxy acid (171)

With the *S,S* α-methyl *tert*-butyldimethyloxysilyloxy sulfone **(164)** successfully prepared the previously discussed acetal, it was then possible to prepare methyl 2-((1*R*,2*R*,19*R*)-1-acetoxy-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl) tetracosanoate **(166)** (See Fig. 117).

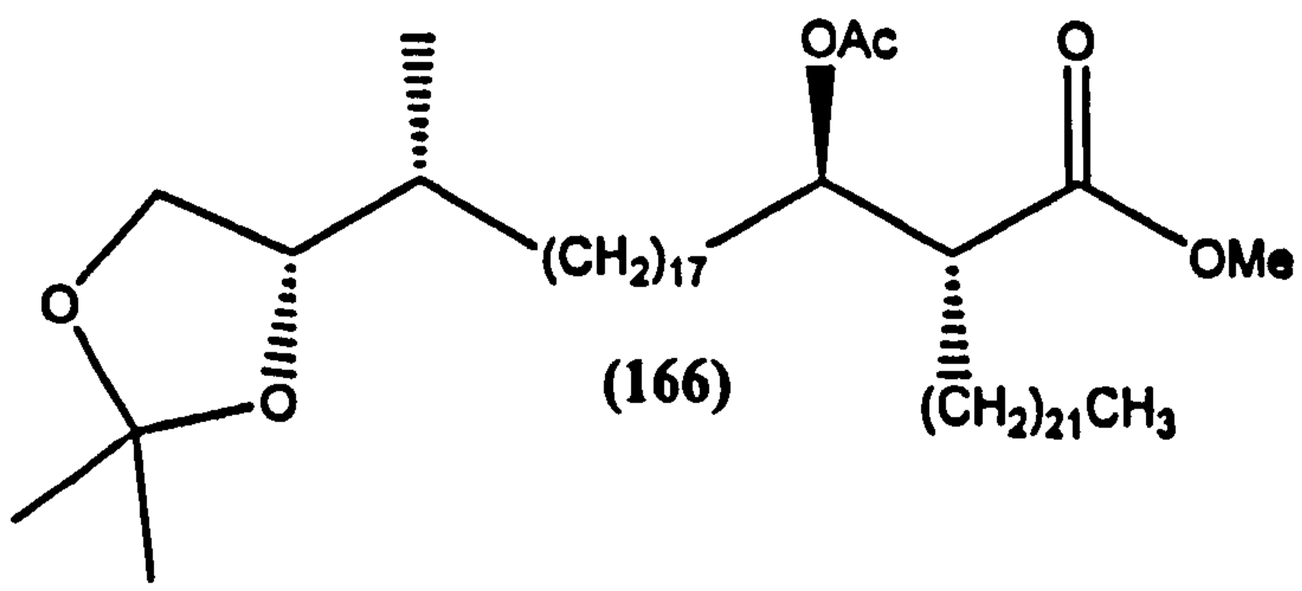
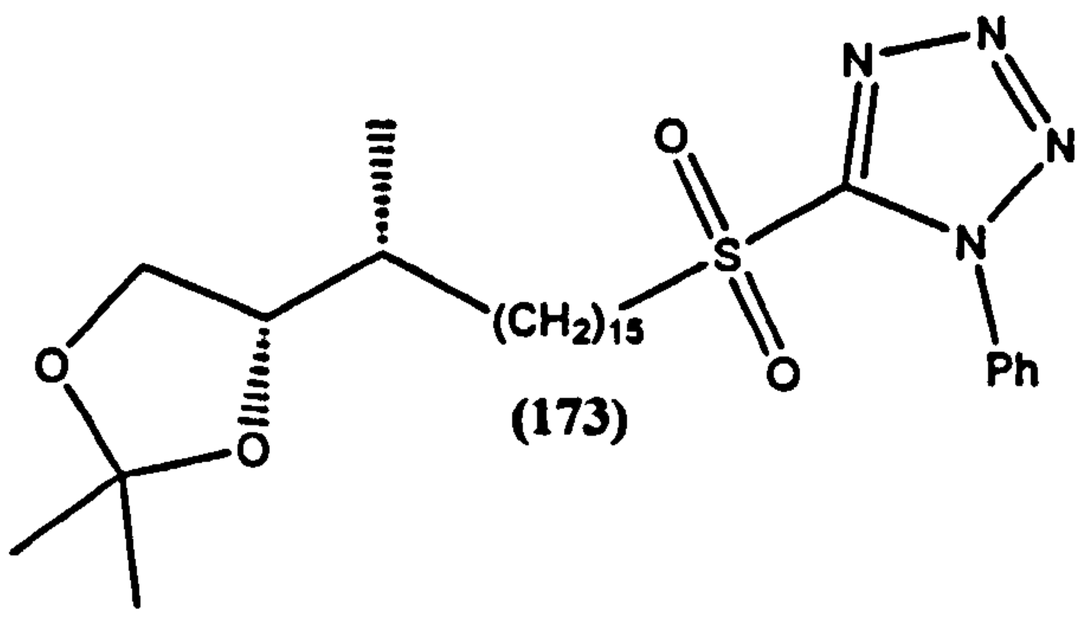
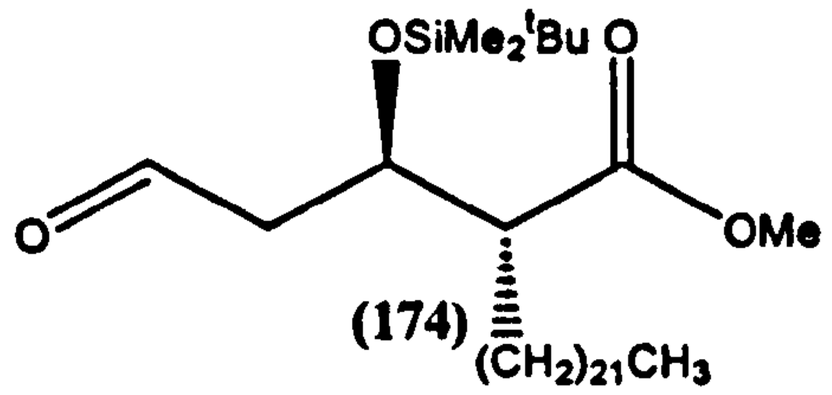


Fig. 117: Methyl 2-((1*R*,2*R*,19*R*)-1-acetoxy-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl)tetracosanoate (166)

The preparation of acetal **(166)** required two major fragments, the acetal sulfone **(173)**, which could be prepared from *D*-mannitol **(60)**, and the known branched hydroxy acid **(174)**, obtainable from *L*-aspartic acid (See Fig. 118).



(173)



(174)

Fig. 118: Branched hydroxy acid intermediates

To obtain the α-methyl acetal **(166)**, *D*-mannitol **(60)** was protected using zinc chloride and acetone to give 1,2:5,6-*O*-isopropylidene-*D*-mannitol **(175)**. Oxidative cleavage of **(175)** with sodium *m*-periodate to yield the glyceraldehyde acetonide **(176)**. Due to the instability of the aldehyde **(176)** it was treated immediately with methyl diisopropoxyphosphinyl acetate, to give the *trans*-alkene **(61)** via a Horner-Wadsworth-Emmons reaction (See Fig. 119).[198, 199]

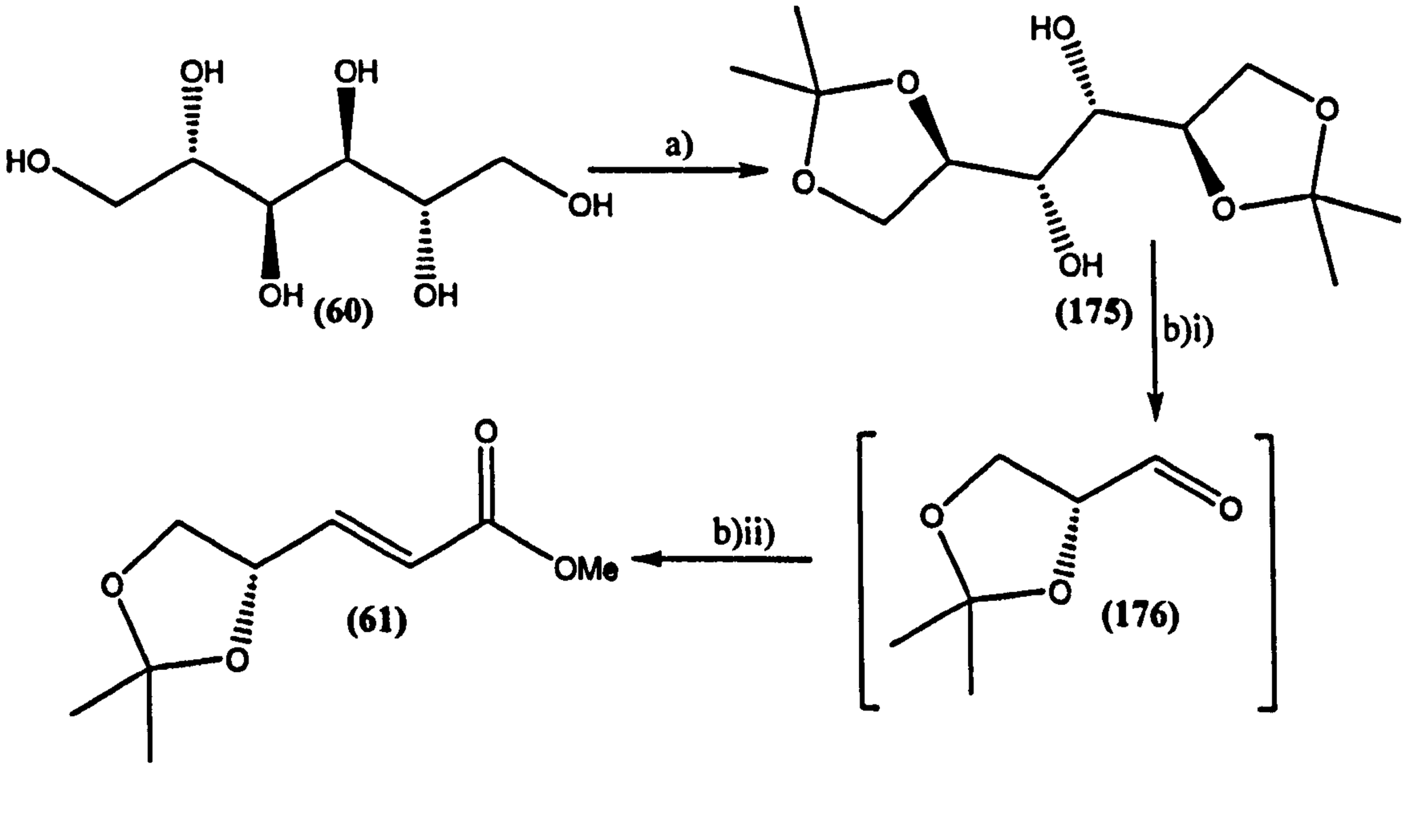


Fig. 119: a) ZnCl, acetone, (53 %); b) i) $NaIO_4$, $NaHCO_3$; ii) methyl diisopropoxyphosphinyl acetate, K_2CO_3, (55 %)

The oxidative cleavage of the diol **(175)** is believed to go via the following mechanism (See Fig. 120).

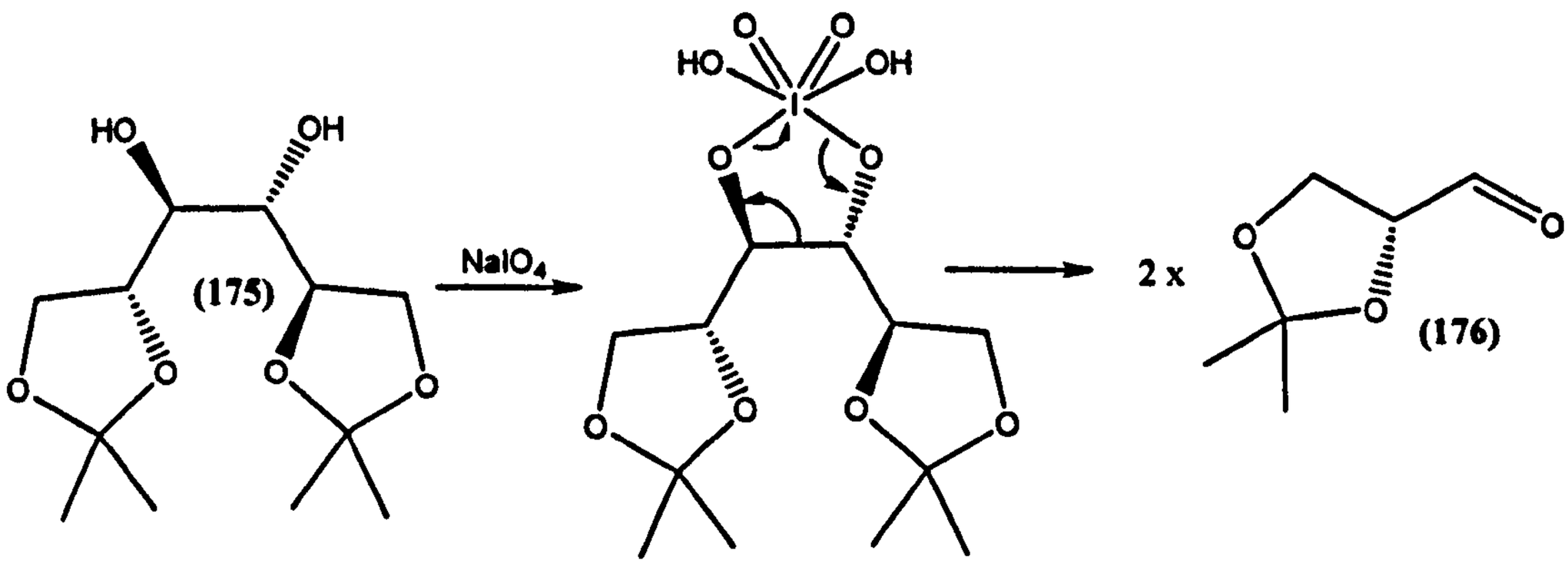


Fig. 120: Oxidative cleavage mechanism of a diol

The *trans*-alkene product **(61)** from the Horner-Wadsworth-Emmons reaction showed two doublets of doublets in the olefin region of the ^{1}H NMR spectrum. The signal at δ 6.90 showed coupling constants corresponding to the vicinal (J 5.7 Hz) and *trans* (J 16.1 Hz) coupling, and the signal at δ 6.08 showed allylic (J 1.6 Hz) and *trans* (J 16.1 Hz) coupling.

A Michael addition was then carried out on the *trans*-alkene **(61)**, giving the methyl ester, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)butyric acid methyl ester **(62)**. This was then reduced with lithium aluminium hydride and the resultant primary alcohol **(177)** was then oxidised to the corresponding aldehyde, (*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)butanal **(178)** (See Fig. 121).

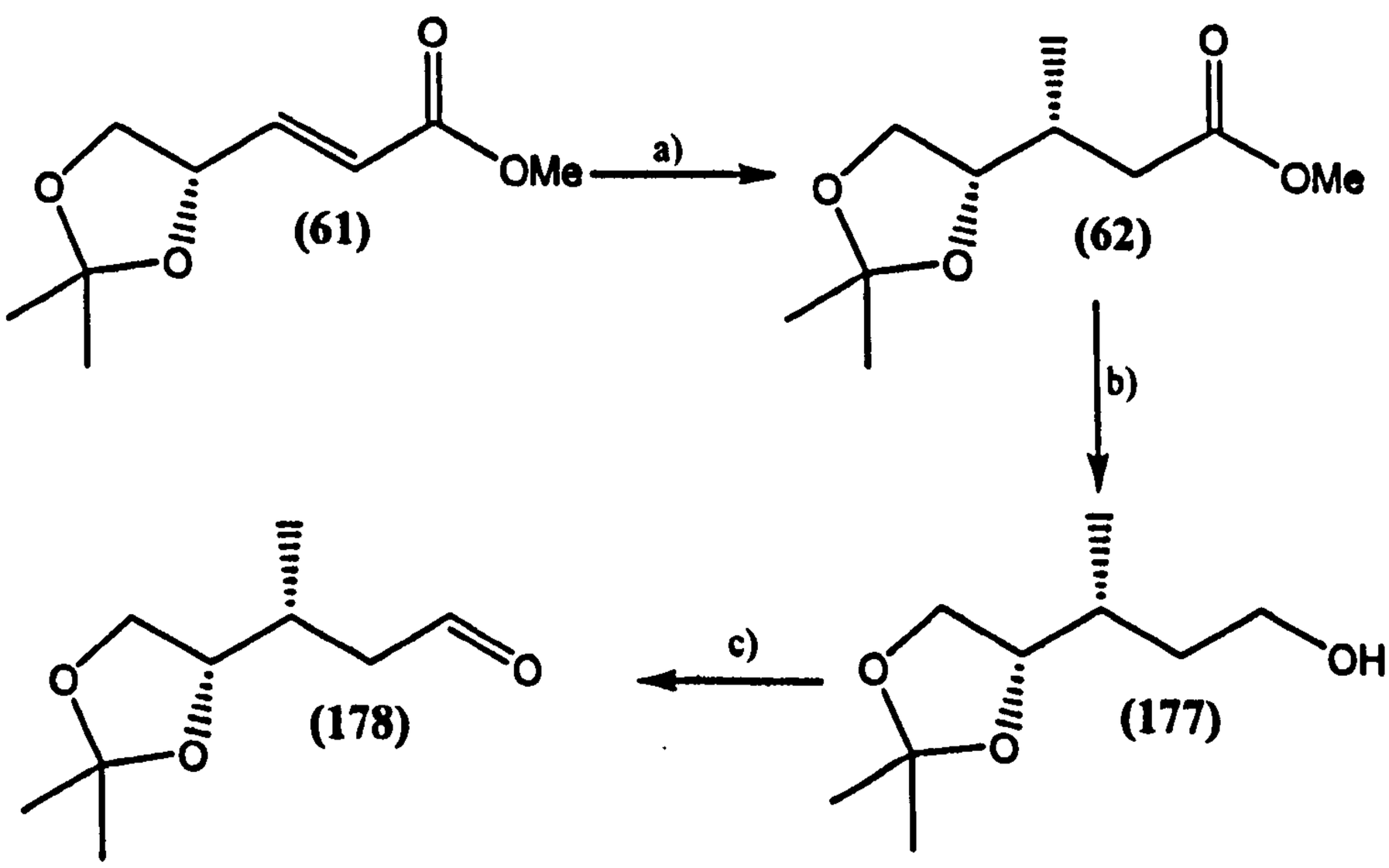


Fig. 121: MeLi, -78 °C, (58 %); b) $LiAlH_4$, (73 %); c) PCC, (77 %)

1,10-Decanediol **(179)** was brominated using refluxing hydrobromic acid, to give 10-bromodecan-1-ol **(180)**. Protection of the alcohol **(180)** was carried out using a tetrahydro-2H-pyran group, to give **(181)**. Iodination of the bromide **(181)** was then carried out as iodide performs better as a leaving group than bromide (See Fig. 122).[200] The structure of the iodide **(182)** was confirmed via ^{13}C NMR, with the carbon adjacent to the halide shifting upfield to δ 6.8 upon iodination.

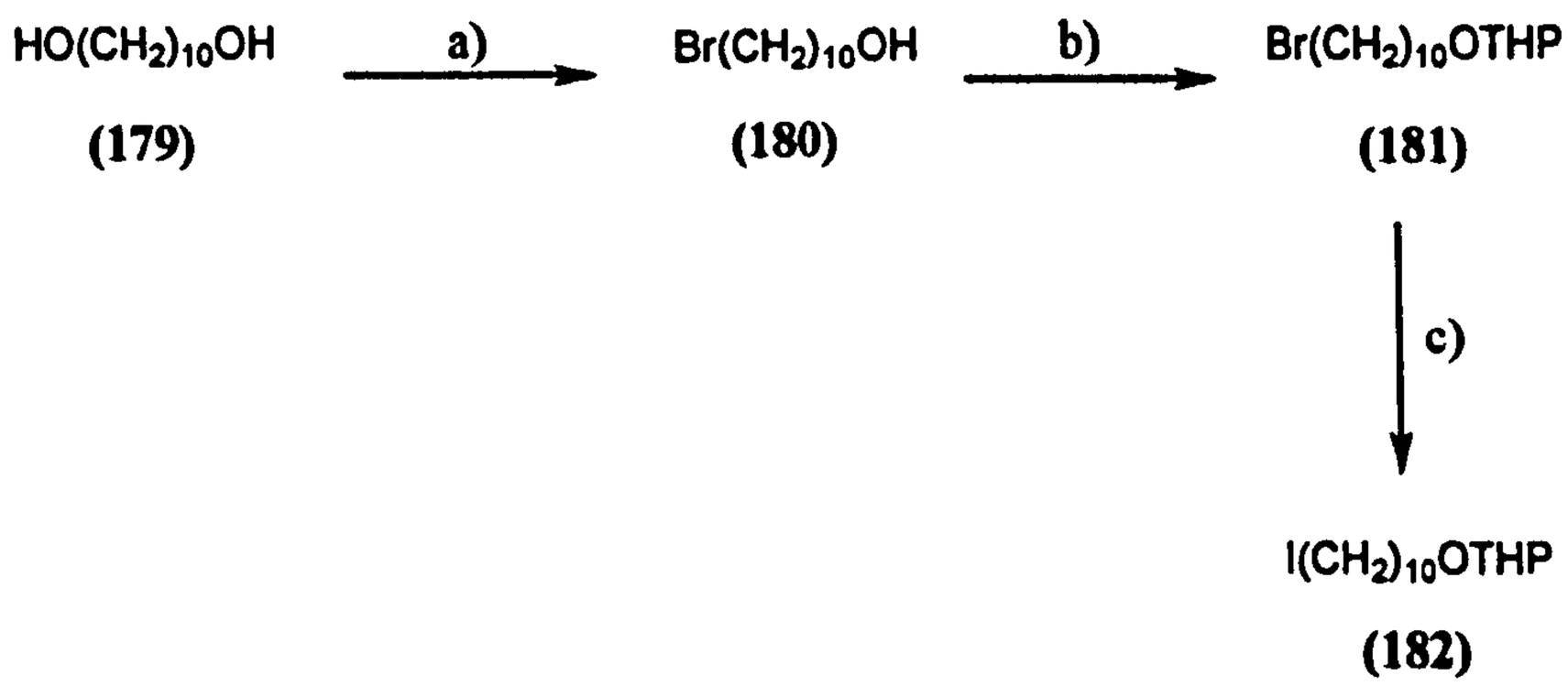


Fig. 122: a) HBr, (90 %); b) 2,3-Dihydropyran, PPTS, (84 %); c) NaI, $NaHCO_3$, (89 %)

2-(10-Iododecyloxy)tetrahydro-2H-pyran **(182)** was coupled to prop-2-yn-1-ol **(183)** following a modification of the literature method reported by T.H. Vaughn *et al.*, as reported by J.R Al-Dulayymi *et al.*[187, 201] The resultant alkyne **(184)** was then hydrogenated at atmospheric pressure using palladium on charcoal as a catalyst to give **(185)** (See Fig. 123).

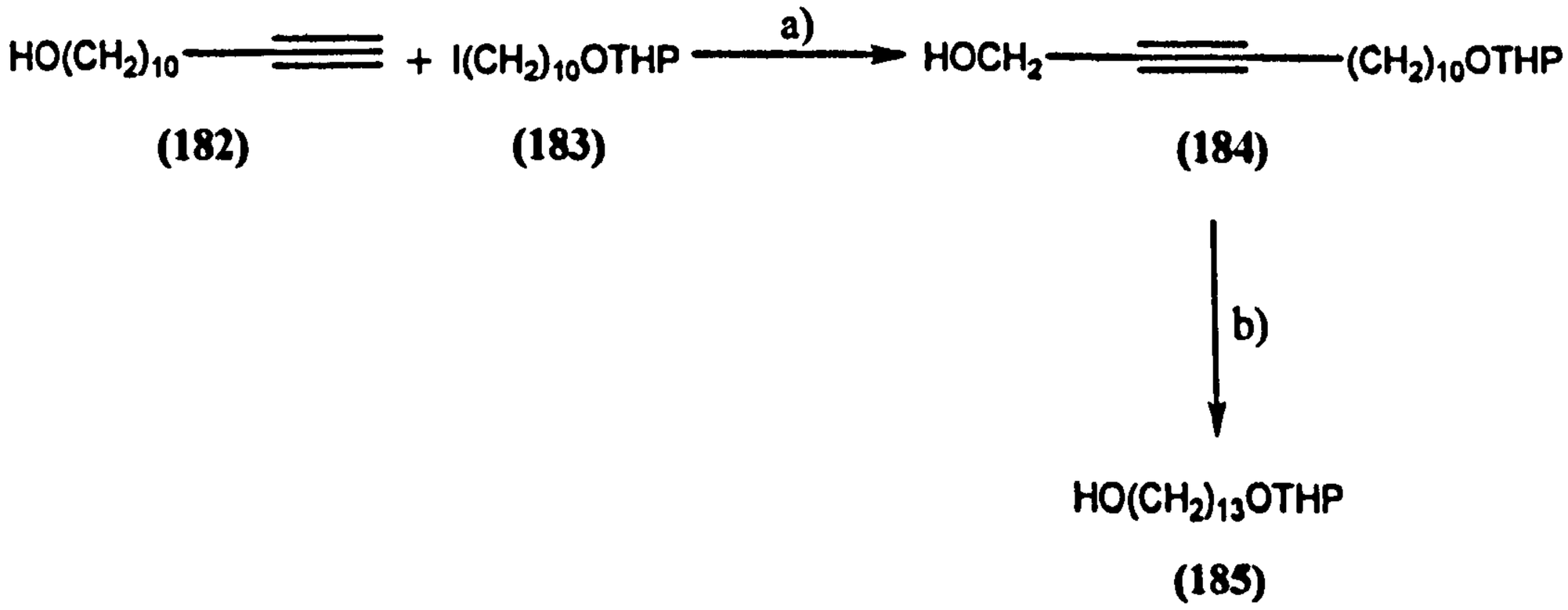


Fig. 123: a) NH_3 (l), Li, $Fe(NO_3)_3$, (53 %); b) H_2, Pd cat., (72 %)

^{13}C NMR spectroscopy was again used to confirm the structure of the alkyne, 13-(tetrahydro-2H-pyran-2-yloxy)tridec-2-yn-1-ol **(184)** with signals observed in its spectrum corresponding to the quaternary carbons of the triple bond at δ 86.0 and δ 78.5, while upon hydrogenation these signals were no longer observed in the ^{13}C NMR spectrum of **(185)**.

The sulfone, 1-phenyl-5-(13-(tetrahydro-2H-pyran-2-yloxy) tridecylsulfonyl)-1H-tetrazole **(188)** was obtained using the same procedure as discussed earlier (See page 48) (See Fig. 124).

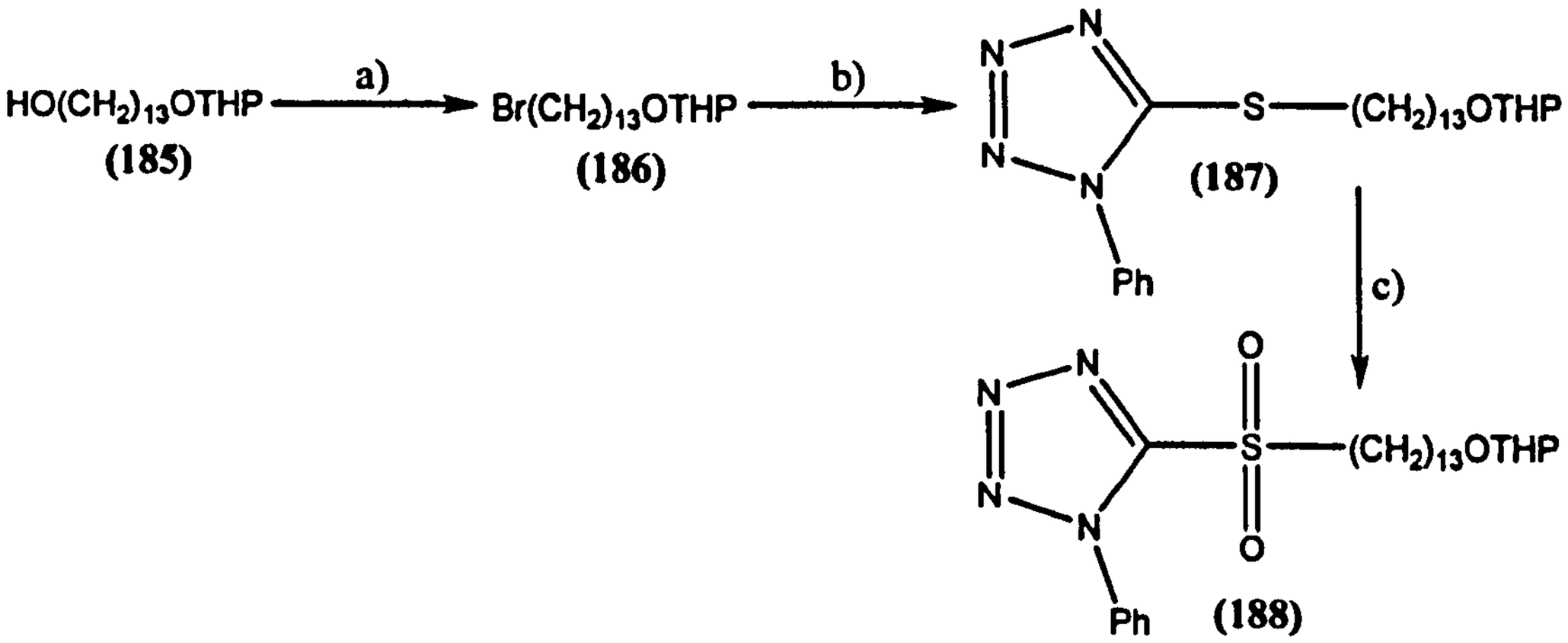


Fig 124: a) NBS, PPh_3, $NaHCO_3$, (81 %); b) 1-phenyl-1H-tetrazole-5-thiol, K_2CO_3, (61 %); c) ammonium molybdate (VI) tetrahydrate, H_2O_2, (71 %)

Using the modified Julia-Kocienski olefination, (*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)butanal **(178)** and 1-phenyl-5-(13-(tetrahydro-2H-pyran-2-yloxy)tridecylsulfonyl)-1H-tetrazole **(188)** were coupled to give the mixture of alkenes **(189)** (See Fig. 125).

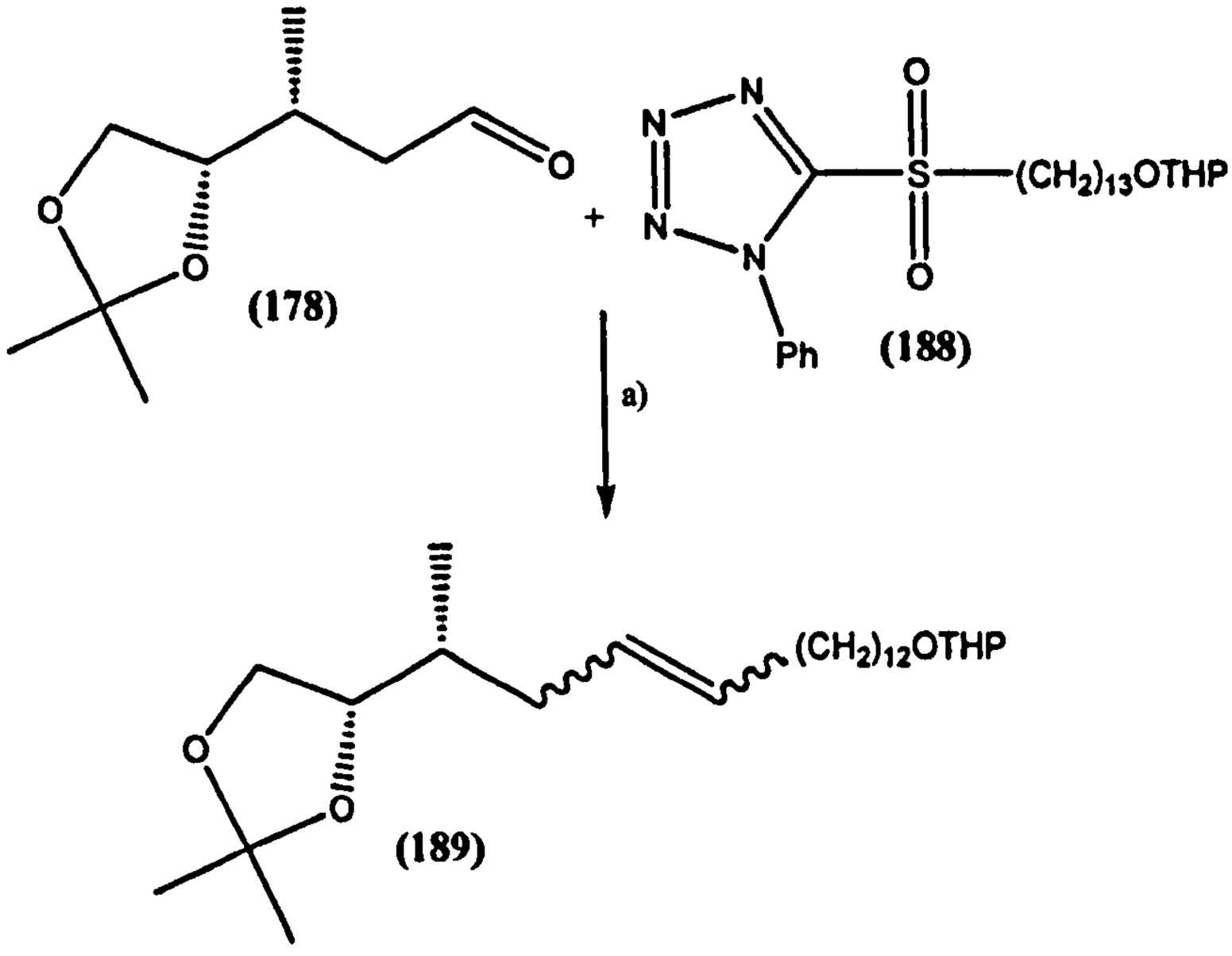


Fig. 125: a) LHMDS, (89 %)

The alkenes **(189)** were hydrogenated at atmospheric pressure using a palladium on charcoal catalyst, giving the tetrahydropyranyl ether **(190)**. Deprotection of the tetrahydropyran protecting group was then carried out using pyridinium-*p*-toluene sulfonate to give the primary alcohol, (*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecan-1-ol **(191)** (See Fig. 126).

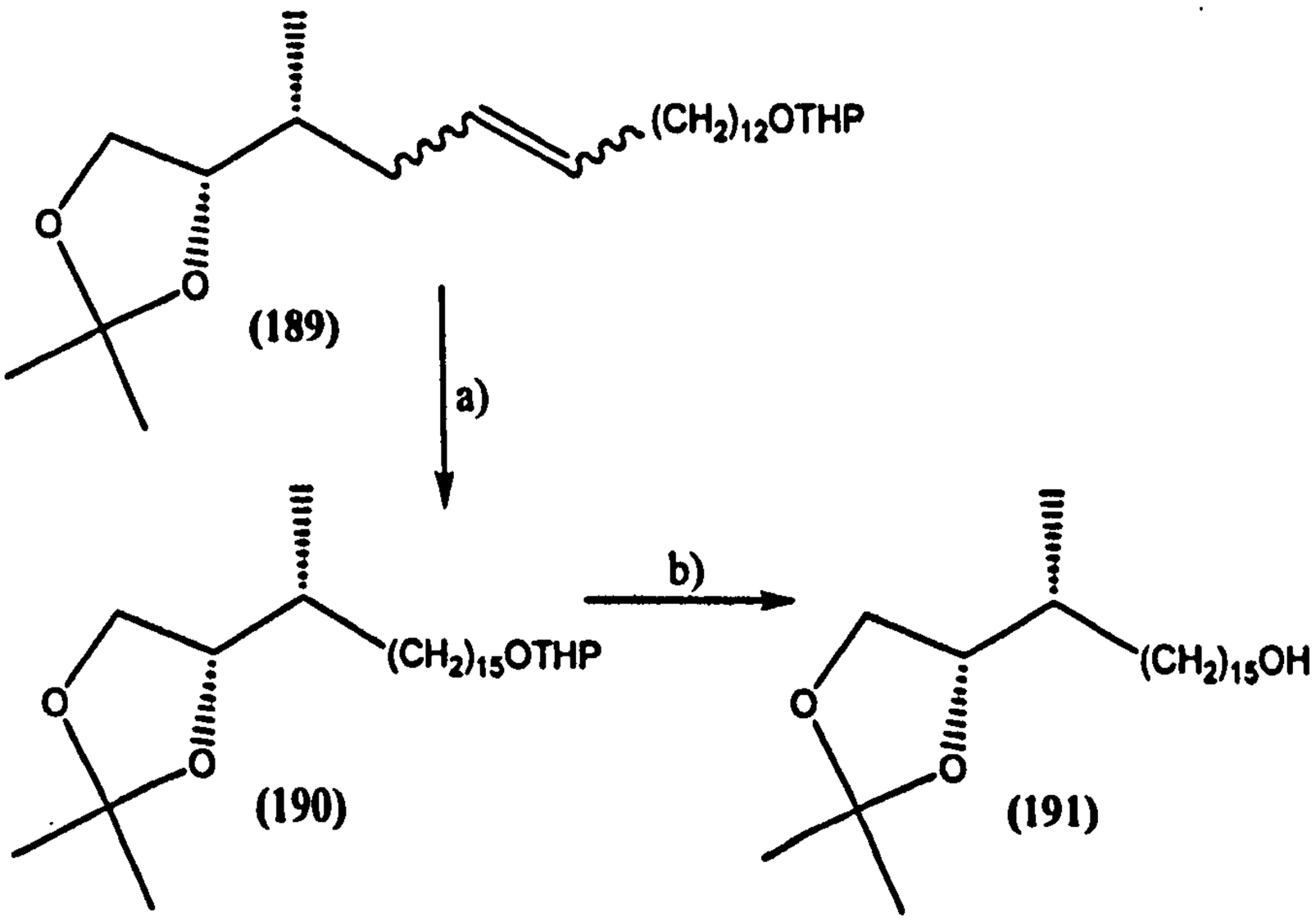


Fig. 126: a) H_2, Pd cat., (90 %); b) PPTS, (43 %)

The completion of the hydrogenation and deprotection were confirmed via ^1H NMR spectroscopy, with signals no longer observed in the olefinic region of the ^1H NMR spectrum of **(201)**, and deprotection of the tetrahydropyran group shown by the lack of signals at δ 4.58, δ 4.00 and δ 3.60 in the spectrum for **(202)**.

The sulfone, 5-((*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecyl sulfonyl)-1-phenyl-1H-tetrazole **(205)** was obtained using the same procedure as discussed earlier, *via* intermediates **(192)** and **(193)** (See page 48) (See Fig. 127).

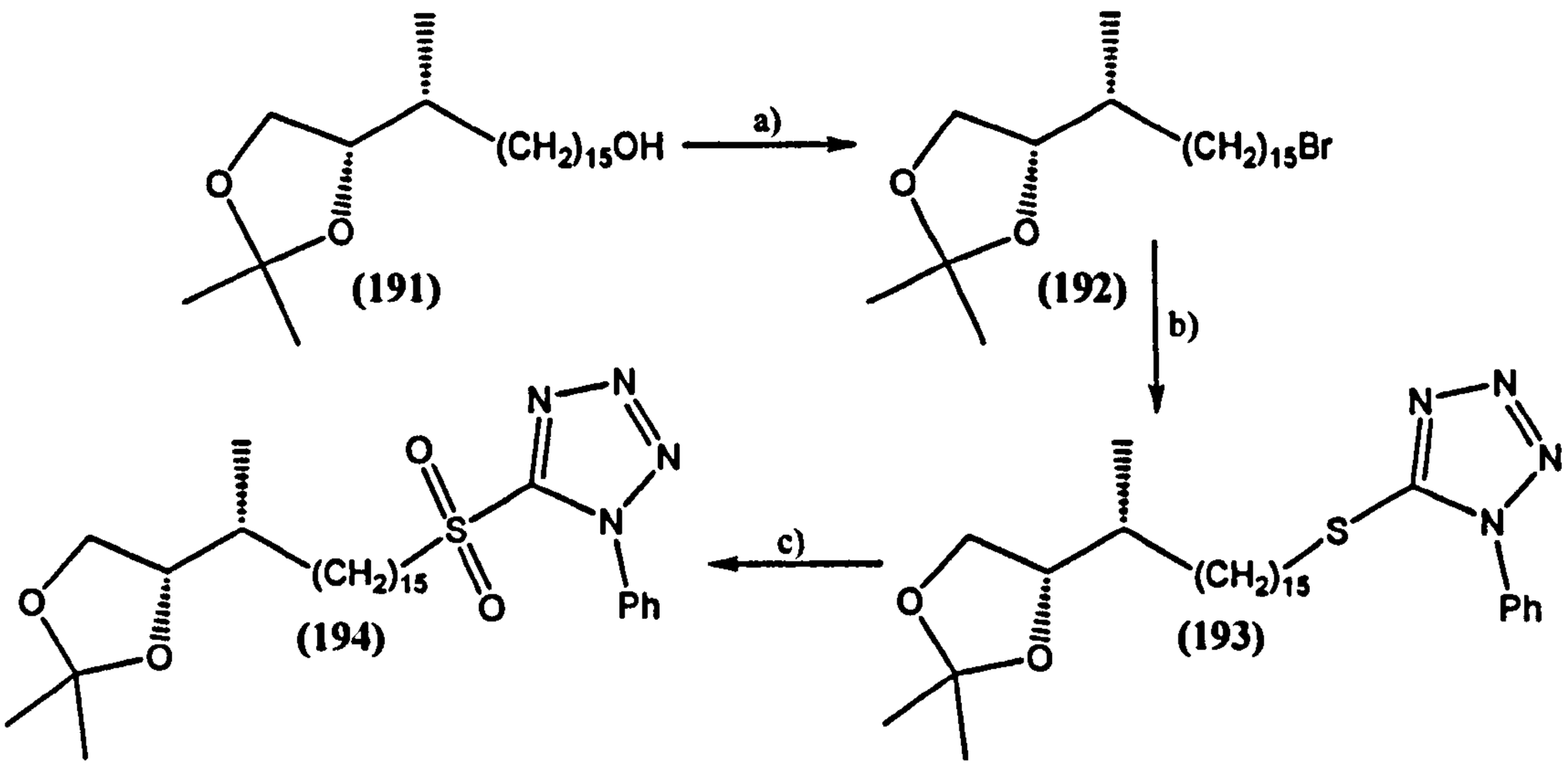


Fig. 127: a) NBS, PPh_3, $NaHCO_3$, (93 %); b) 1-phenyl-1H-tetrazole-5-thiol, K_2CO_3, (92 %); c) ammonium molybdate (VI) tetrahydrate, H_2O_2, (73 %)

To obtain the required aldehyde to complete the branched hydroxy acid, the disconnection route discussed earlier (See page 104), starting from methyl 2-((*R*)-3-(benzyloxy)-1-(tert-butyldimethylsilyloxy) propyl)pent-4-enoate **(195)** was used as in the scheme overleaf (See Fig. 128).

Following the procedure as discussed by W. Yu *et al.*, an improved oxidative cleavage of the olefin **(195)** was carried out to give the aldehyde **(196)**. A modified Julia-Kocienski olefination of the resultant aldehyde **(196)** and 20 carbon sulfone **(197)** resulted in the alkenes **(198)** (See Fig. 128).[202]

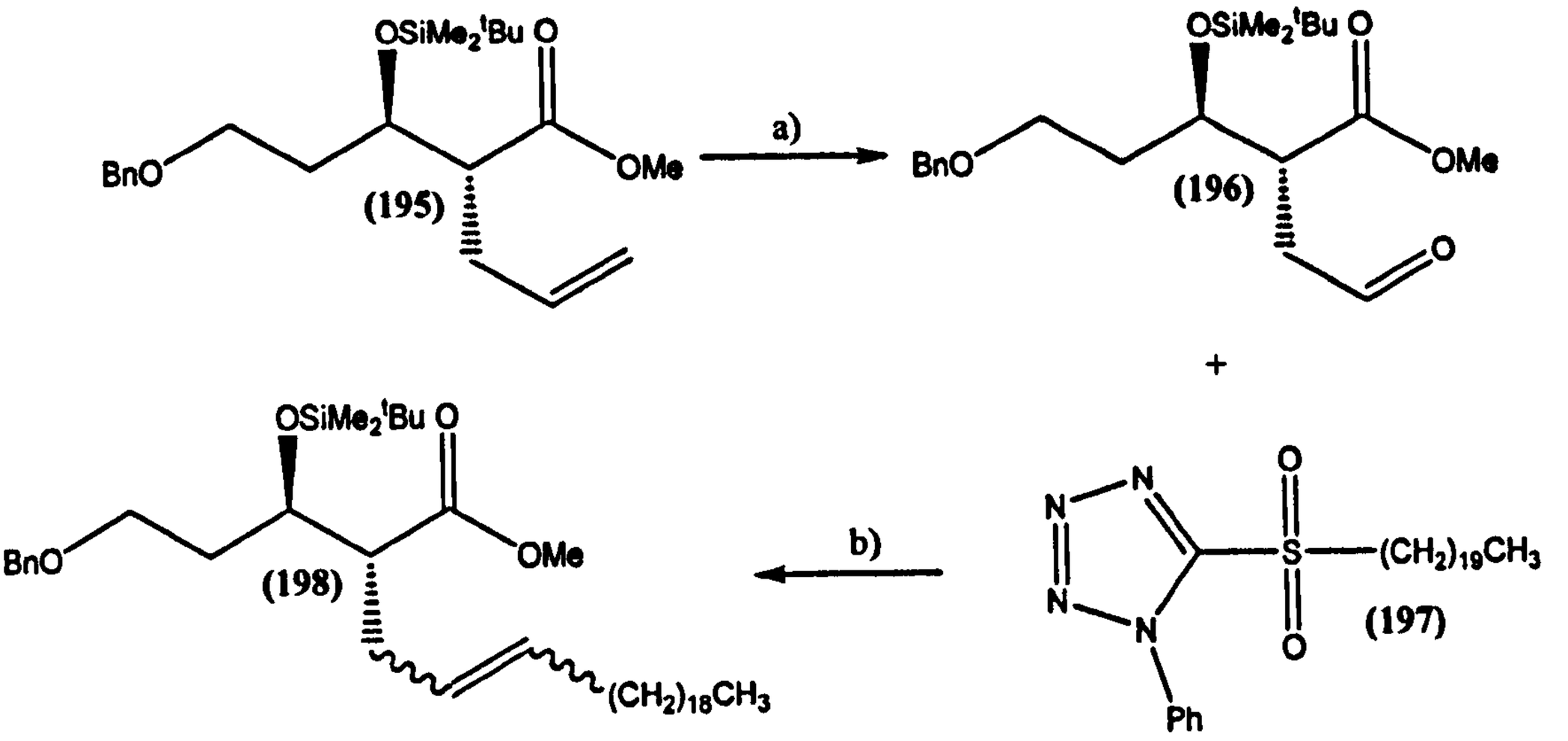


Fig. 128: a) OsO_4, 2,6-lutidine, $NaIO_4$, dioxane/water, (96 %); b) LHMDS, (63 %)

The mechanism of the oxidative cleavage is similar to the oxidative cleavage of 1,2:5,6-*O*-isopropylidene-*D*-mannitol **(175)**; however in this case osmium tetroxide is used to convert the double bond into a diol (See Fig. 129). Once the diol has been generated it is cleaved using sodium *m*-periodate to give the aldehyde.

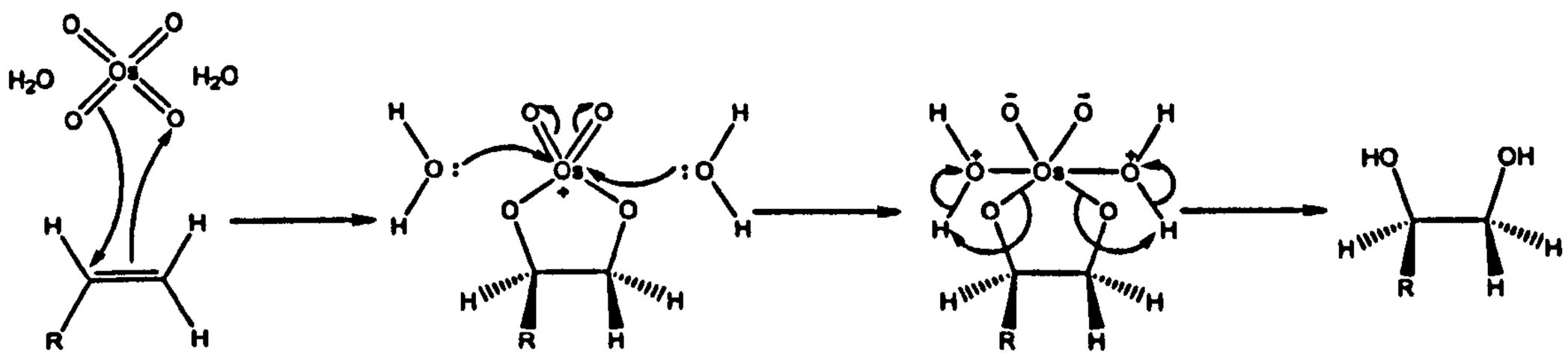


Fig. 129: OsO_4 diol formation

Hydrogenation and debenzylation of the unsaturated compound **(198)** was carried out at atmospheric pressure using a palladium on charcoal catalyst to give the primary alcohol **(199)**, which was then oxidised to give the required aldehyde, **(200)** (See Fig. 130).

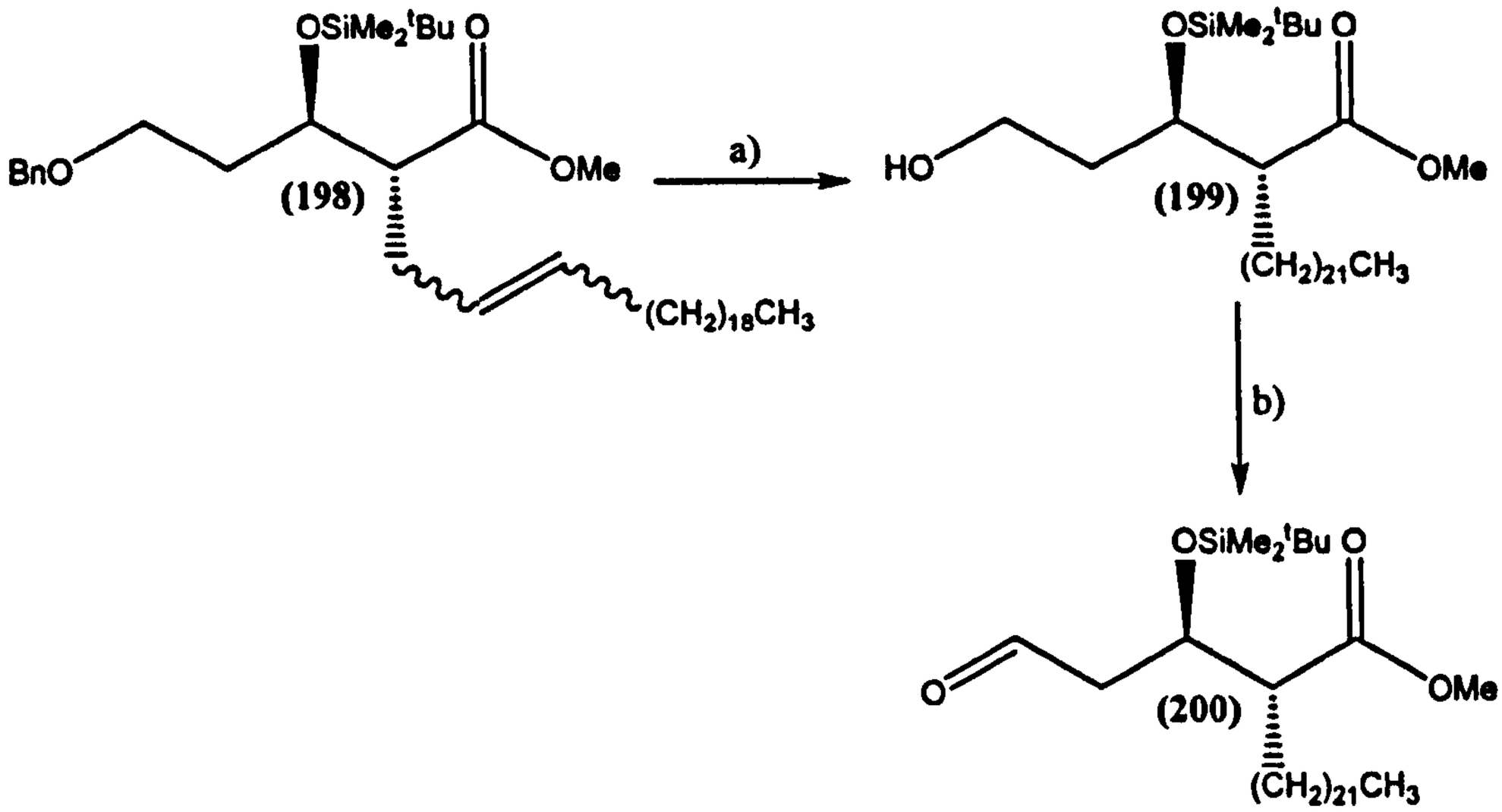


Fig. 130: a) H_2, Pd cat., (80 %); b) PCC, (96 %)

Methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-3-oxopropyl)tetracosanoate **(200)** and 5-((*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecylsulfonyl)-1-phenyl-1H-tetrazole **(194)** were coupled using a modified Julia-Kocienski olefination, followed by a hydrogenation gave the intermediate branched hydroxy acid, methyl 2-((1*R*,2*R*,19*R*)-1-(tert-butyldimethylsilyloxy)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl)tetracosanoate **(201)** (See Fig. 131). ^1H NMR data analysis of **(201)** can be seen overleaf (See Fig. 132).

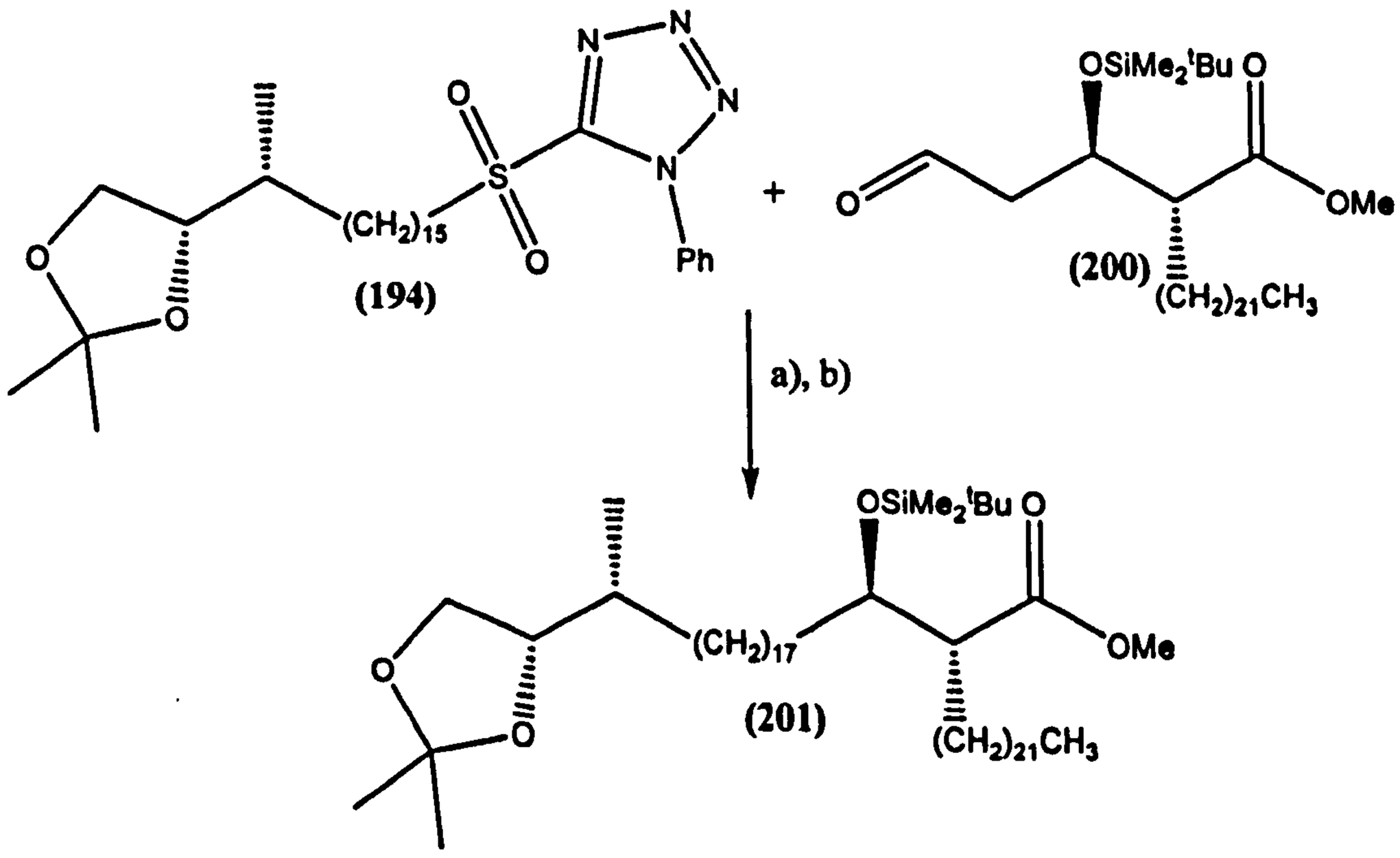
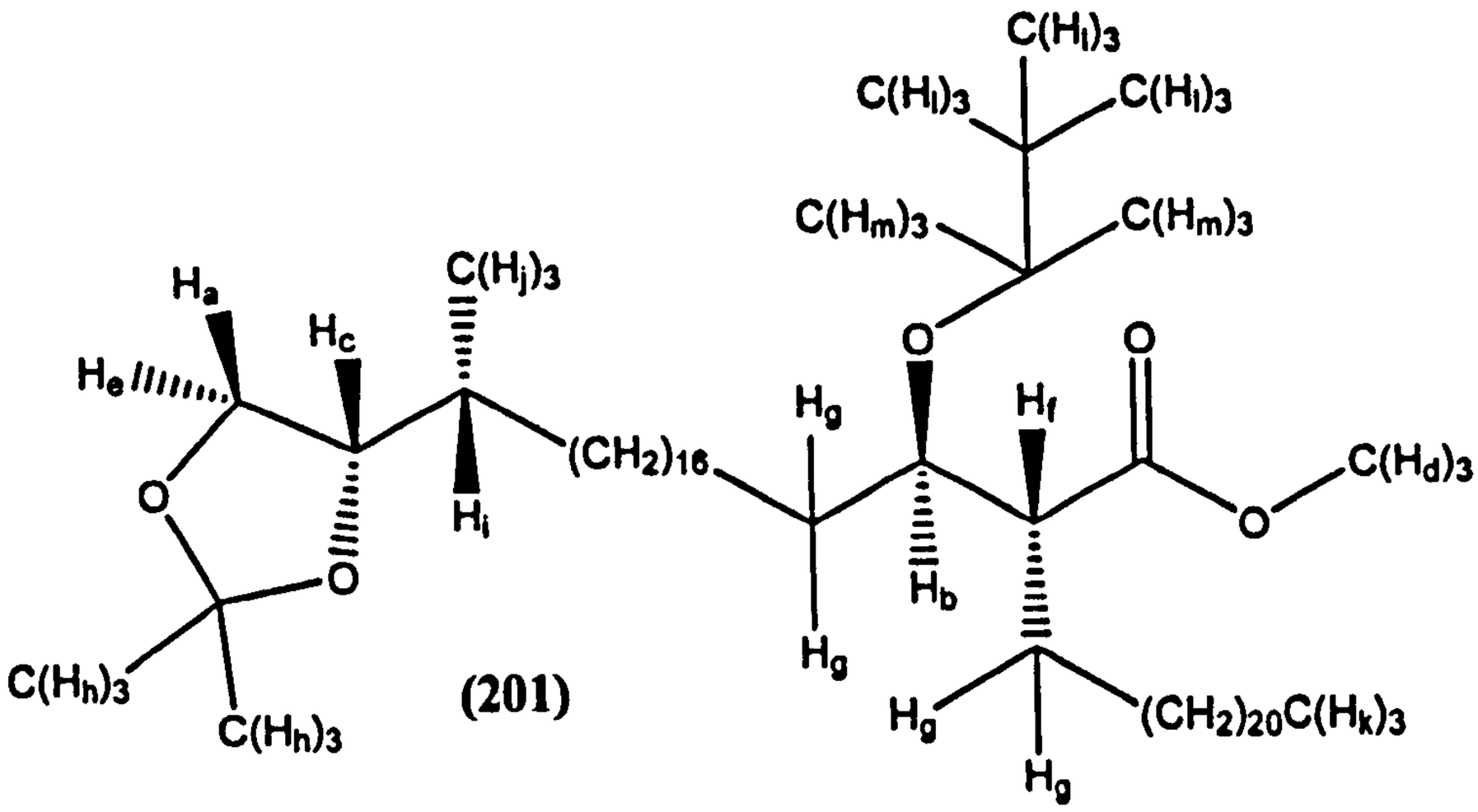


Fig. 131: a) LHMDS, (39 %); b) H_2, Pd cat., (99%)



(201)

H_x	δ	Multiplicity	Integration	J (H_z)
H_a	4.00	dd	1	6.2, 8
H_b	3.95-3.90	m	1	-
H_c	3.88	q	1	7
H_d	3.66	s	3	-
H_e	3.60	t	1	8
H_f	2.54	ddd	1	3.8, 7.2, 11
H_g	1.62-1.51	m	4	-
H_h	1.41, 1.36	s	2 x 3H	-
H_i	1.11-1.07	m	1	-
H_j	0.97	d	3	6.65
H_k	0.89	t	3	7
H_l	0.87	s	9	-
H_m	0.05, 0.03	s	2 x 3H	-

Fig. 132: ^{1}H NMR data analysis of methyl 2-((*R*)-1-(tert-butyldimethylsilyloxy)-3-hydroxypropyl) tetracosanoate (201)

To avoid the occurrence of two identical protecting groups after coupling the silyl ether was deprotected and replaced with an acetate group. This was done because if identical protecting groups were present then it would not be possible to selectively deprotect one and not the other. (See Fig. 133).

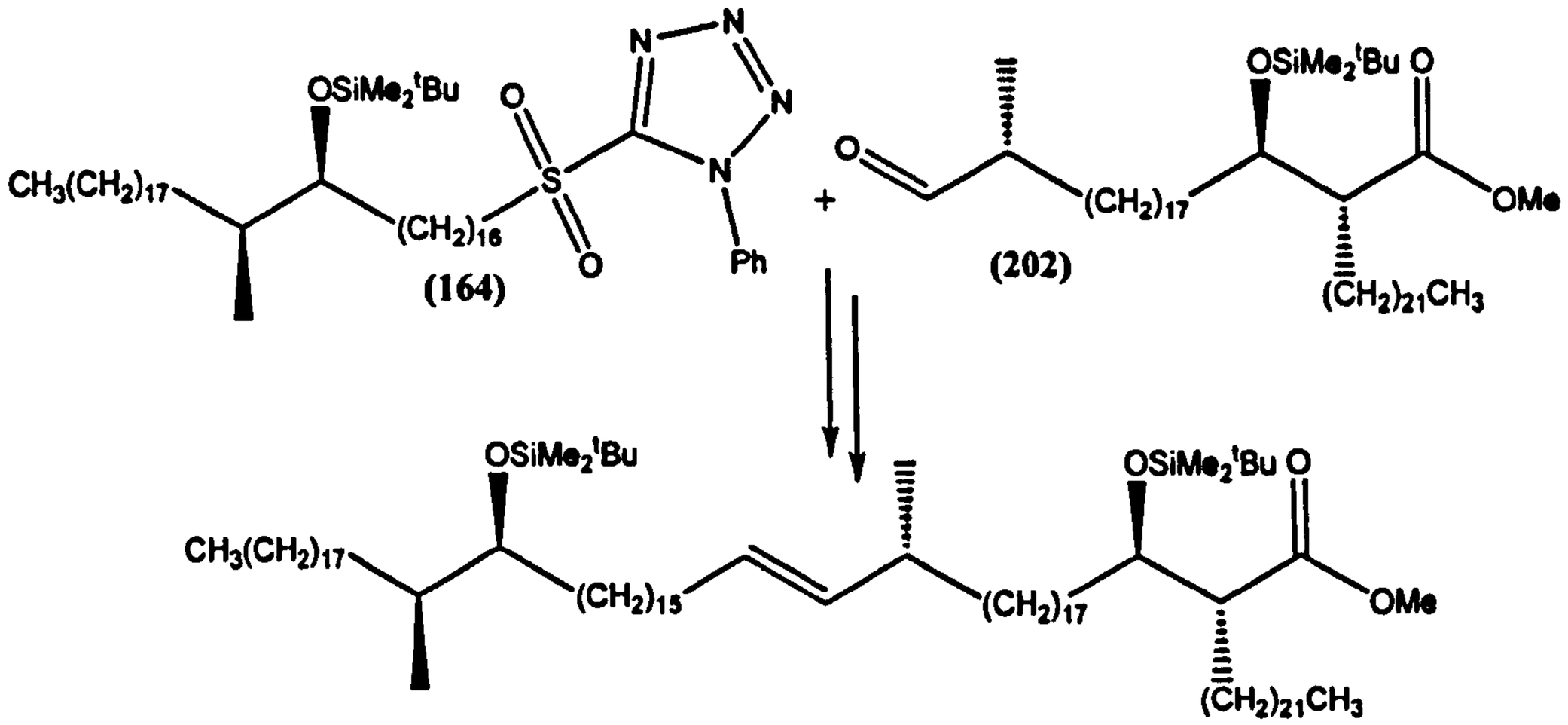


Fig. 133: Undesired *trans*-alkene mycolic acid, with two silyl ether protecting groups

Therefore the silyl ether was removed from **(201)** using hydrofluoric acid/pyridine complex, to give the secondary alcohol **(203)**, which was then re-protected using acetic anhydride to give the corresponding acetate, methyl 2-((1*R*,2*R*,19*R*)-1-acetoxy-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl)tetracosan-oate **(166)** (See Fig. 134).

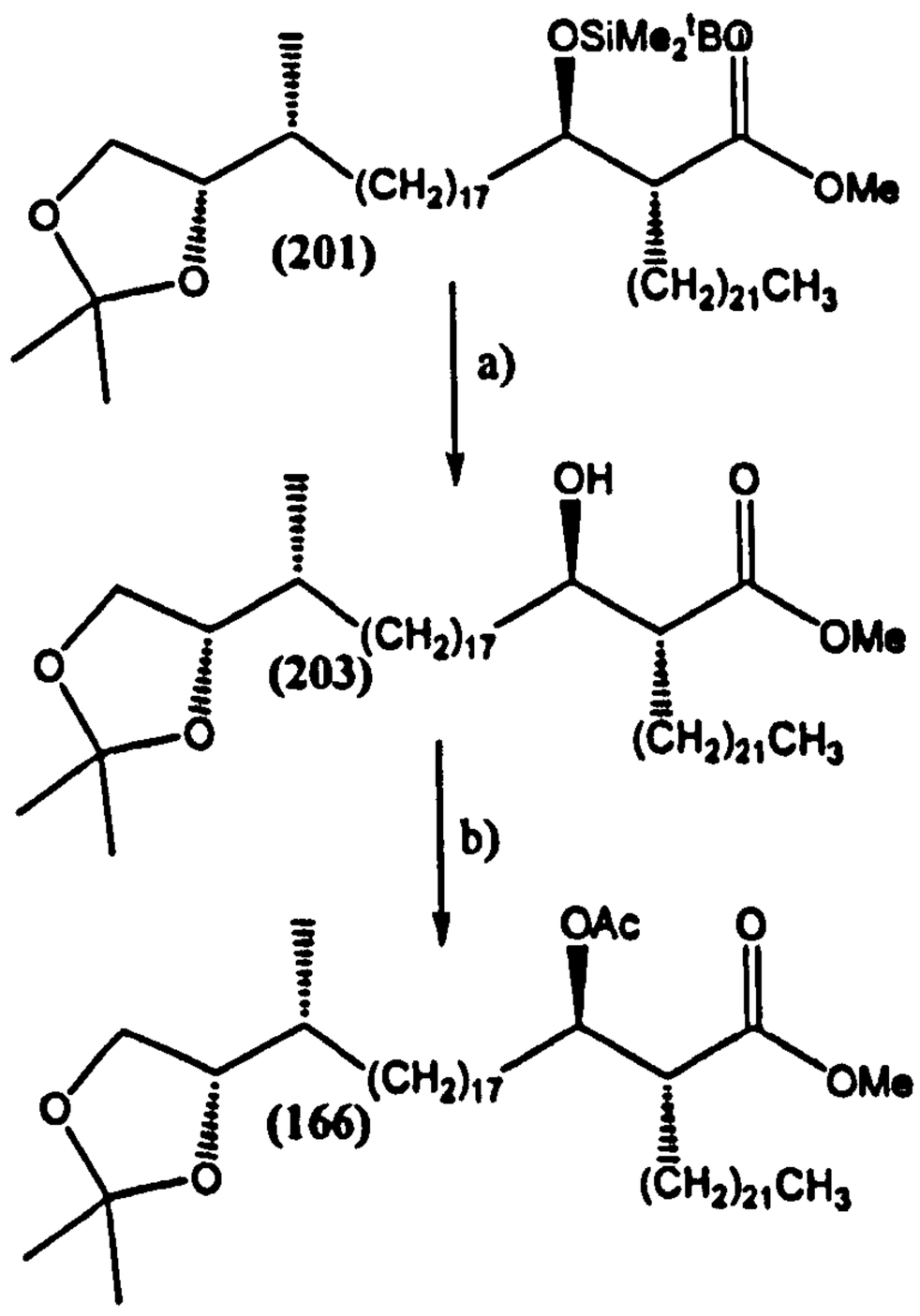


Fig 134: a) HF.Pyr, pyridine, (71 %); b) Ac_2O, dry toluene, (89 %)

2.4.4 - Coupling the meromycolate moiety and branched hydroxy acid

Oxidation of the branched hydroxy acid acetal **(166)** with periodic acid gave methyl 2-((1*R*,19*R*)-1-acetoxy-19-methyl-20-oxoicosyl)tetracosanoate **(165)** (See Fig. 135).

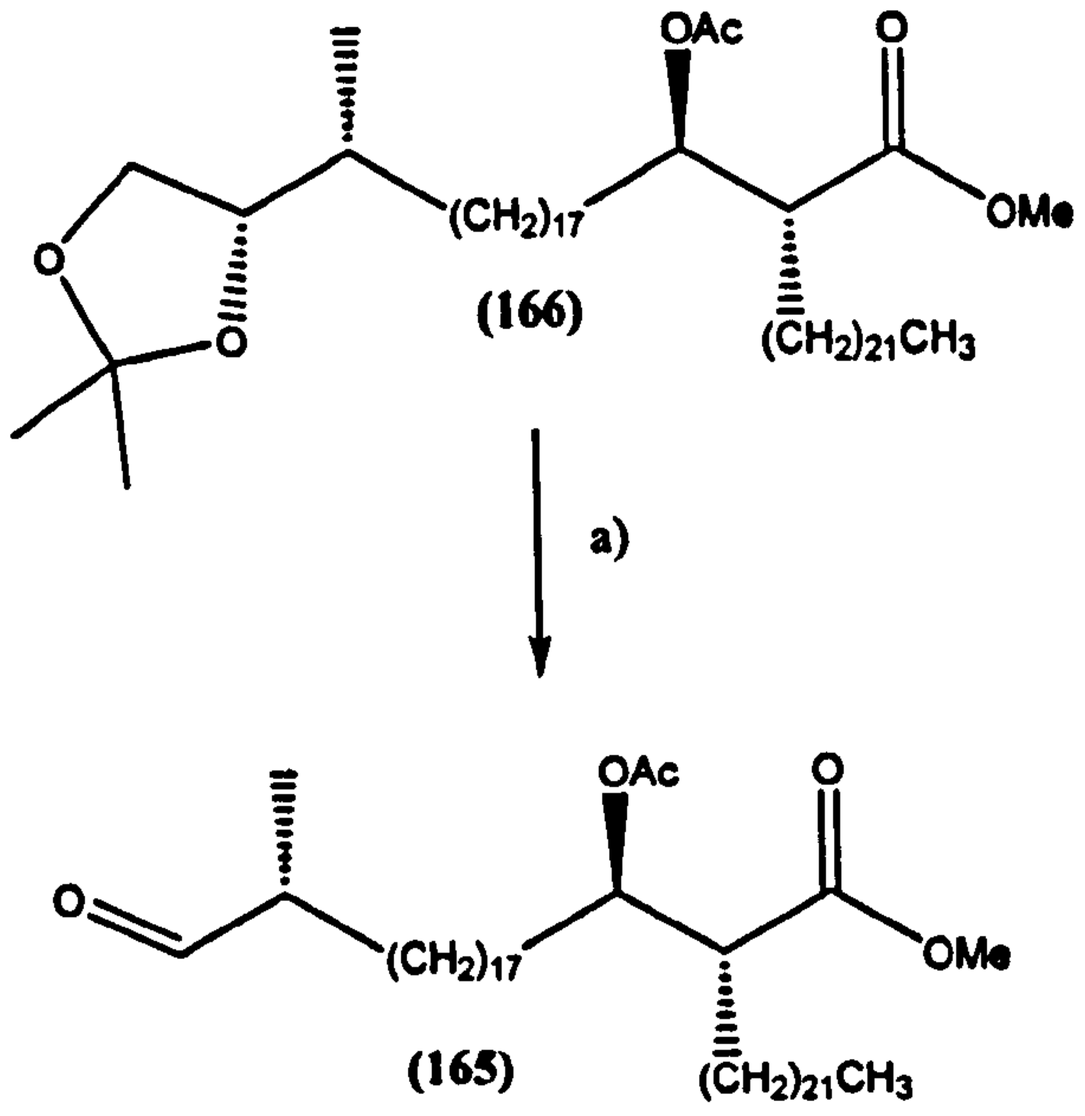


Fig. 135: a) HIO_4, (60 %)

A typical modified Julia-Kocienski olefination using lithium *bis(*trimethylsilyl) amide would give the olefin product as a mixture of *cis*- and *trans*- stereoisomers (See Fig. 136).

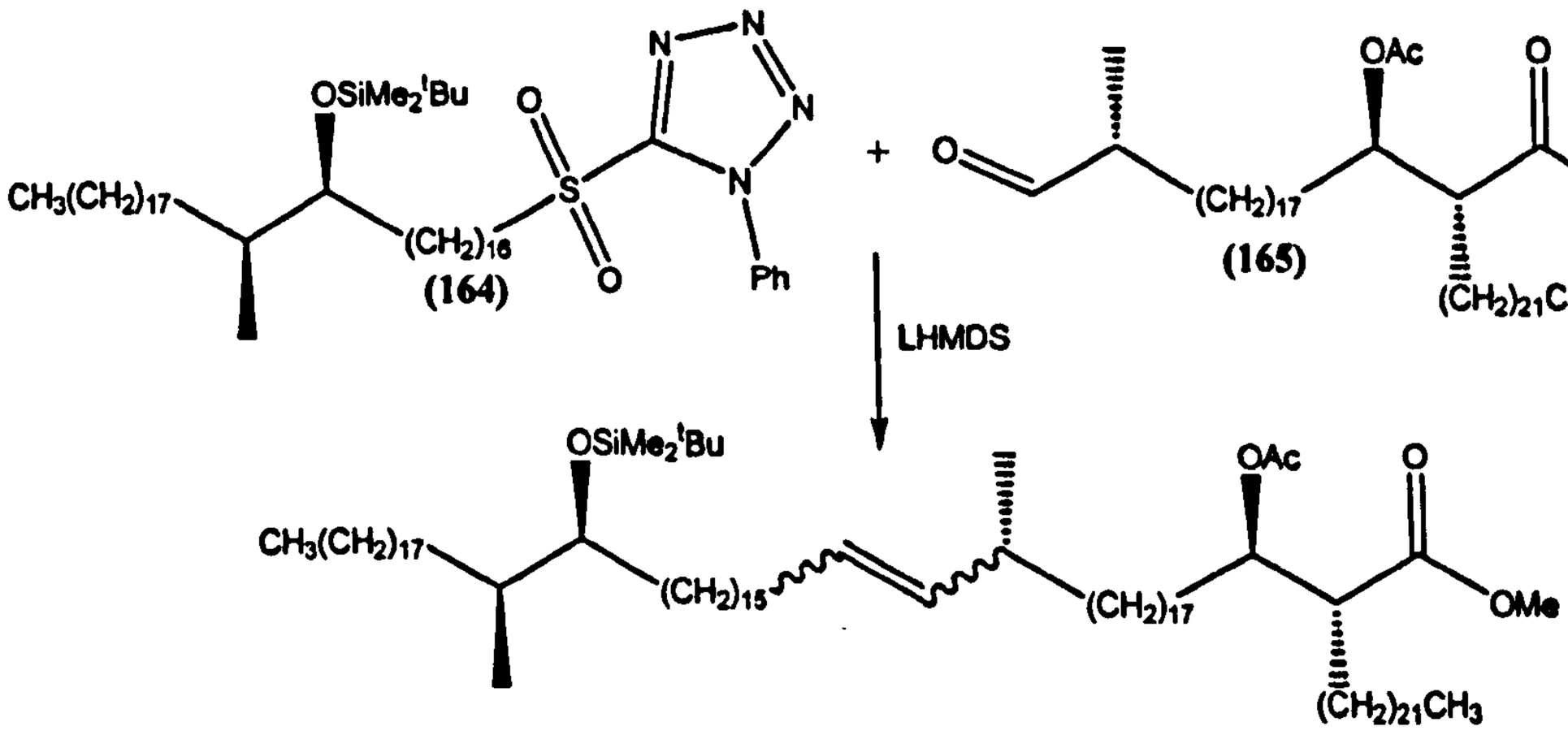


Fig. 136: Undesired alkene mycolic acids, mixure of *cis* and *trans* isomers

Therefore, as isolation of the *trans*-olefin would be inconvenient, lithium *bis(*trimethylsilyl) amide was not used as the base. Kocienski *et al.* and Pospíšil *et al.* both discussed the use of different bases, most significantly potassium *bis*(trimethylsilyl)amide, to increase stereoselectivity in favour of the *trans*-isomer.[203, 204] Koceinski *et al.* discovered that changing the base used increases stereoselectivity in the order Li<Na<K, and Pospíšil *et al.* give evidence that increases in the size of the alkyl chain of the aldehyde lead to higher stereoselectivity. The mechanism of the olefination is the same as previously discussed in section 2.2.2, but leading to the *trans*-product.

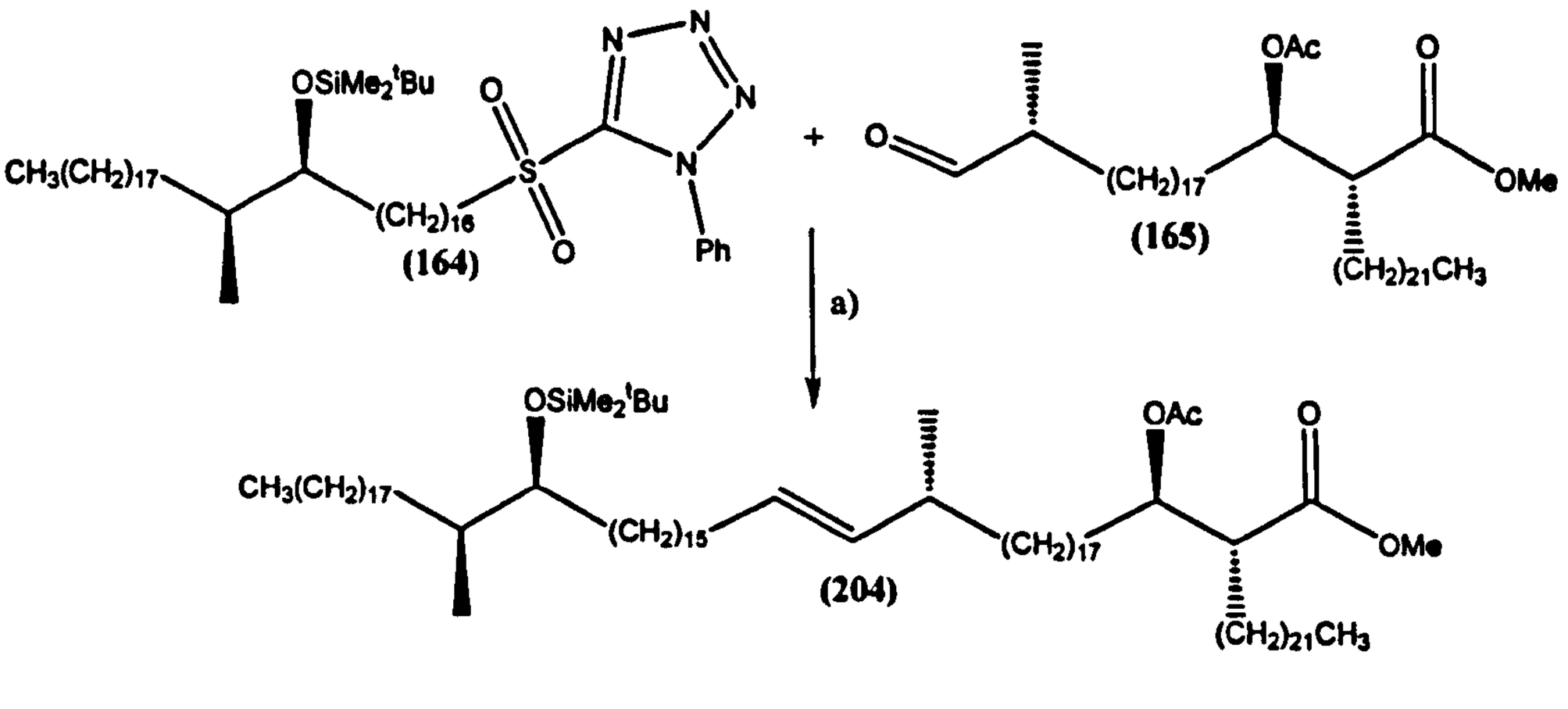


Fig. 137: a) KHMDS, DME, (34 %)

The stereoselective formation of the *trans*-isomer was confirmed with NMR spectroscopy, the spectrum showing two olefinic protons at δ 5.34 and δ 5.23 both with a coupling constant of 15 Hz corresponding to the two *trans*-protons of the double bond. The ^{13}C NMR spectrum of **(204)** showed signals at δ 136.5 and δ 128.4, corresponding to just two olefinic carbons. The molecular rotation of **(204)** was calculated, $M_D = +43.96$.

The silyl ether was removed using hydrofluoric acid/pyridine complex to give the protected *S,S trans*-alkene hydroxy-mycolic acid **(205)**. Oxidation with PCC followed giving the target molecule, methyl (2*R*,3*R*,21*R*,40*S*,*E*)-3-acetoxy-2-docosyl-21,40-dimethyl-39-oxooctapentacont-22-enoate **(85)** (See Fig. 138).

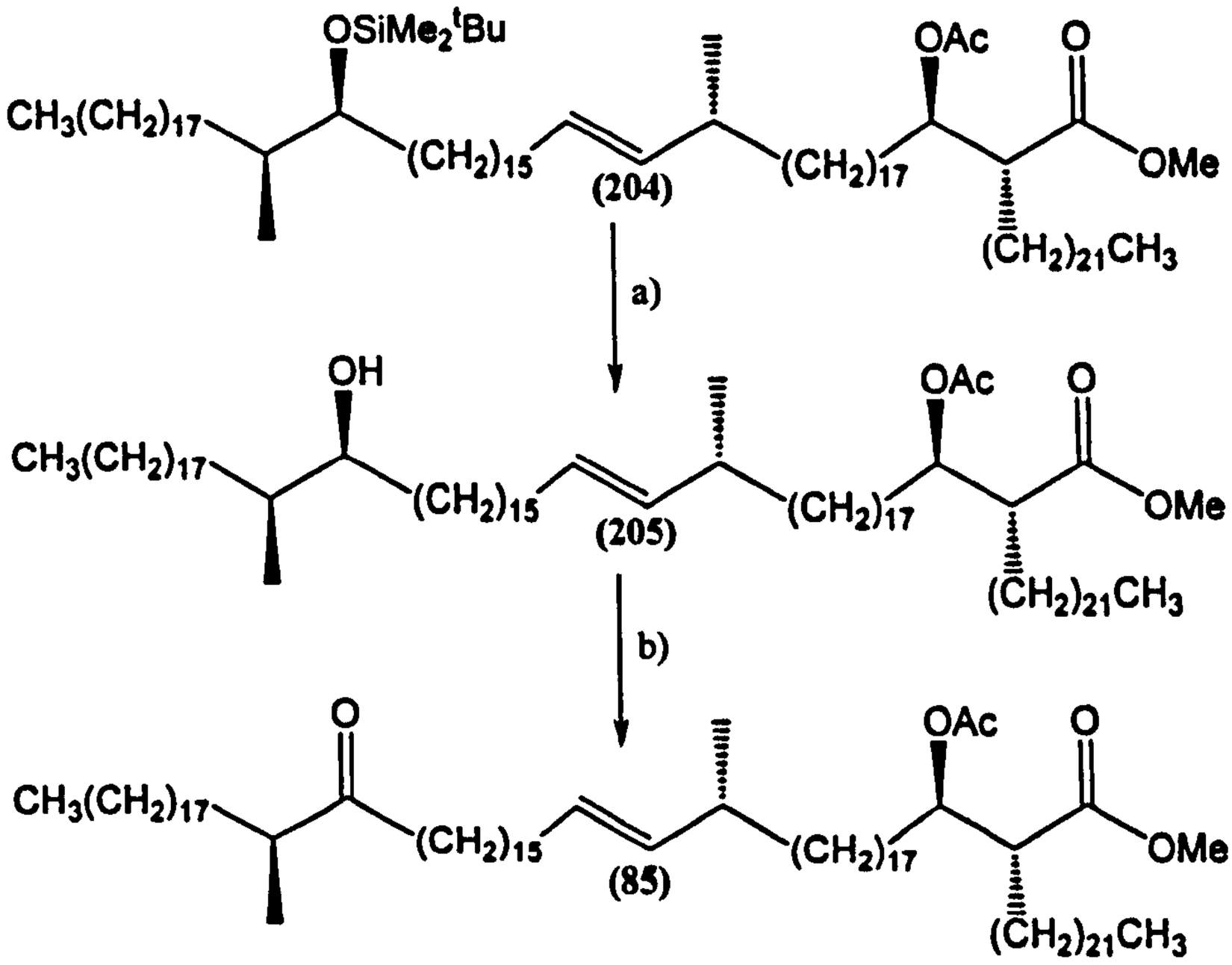


Fig. 138: a) HF.Pyr, pyridine, (50 %); b) PCC, (80 %)

Full ^{1}H NMR expansions of *S*-α-methyl *trans*-alkene keto-mycolic acid **(85)** can be seen as follows (See Fig. 139a, 139b, 139c, 139d and 139e). H_a and $H_{a'}$ are shown to give a doublet of triplets splitting pattern in the ^{1}H NMR spectrum of **(86)**. This shows that H_a and $H_{a'}$ are not magnetically equivalent, due to the effect of the chrial centre on the opposite side of the ketone. Proton H_b shows a sextet, and H_c a doublet of triplets, consistent with the theoretical expectation (See Fig. 139b). Signals for H_e and H_f are observed in the olefinic region, H_e giving a doublet of doublets and H_f a doublet of triplets. The configuration of the double bond was confirmed to be *trans* as the signals observed at δ 5.33 and δ 5.24, corresponding to the olefinic protons H_f and H_e, both exhibiting a coupling constant of 15.1 H_z. This is again consistent with the theoretical splitting pattern for these protons (See Fig. 139e).

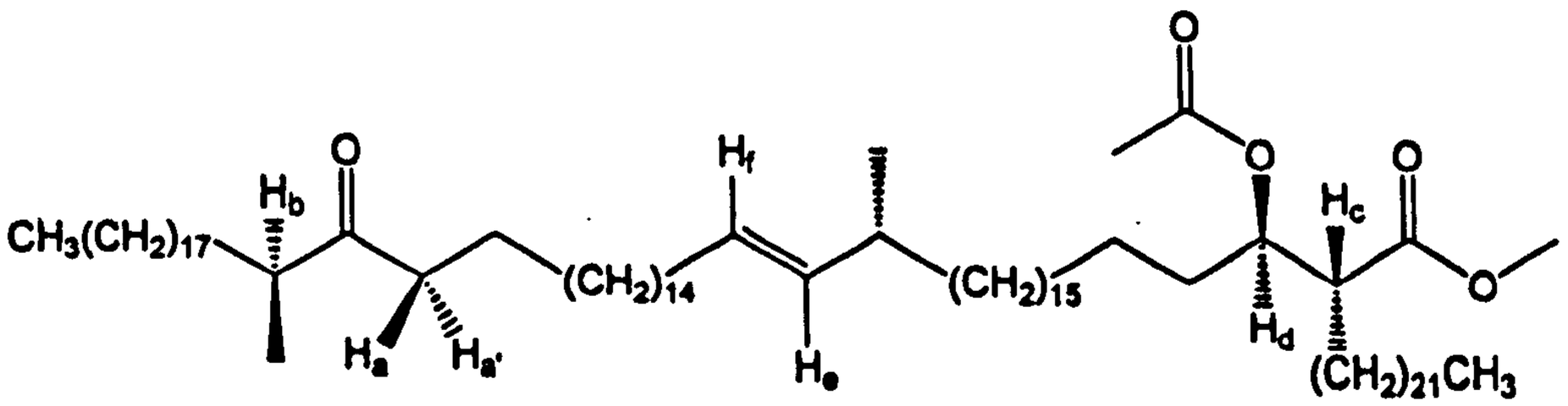


Fig. 139a: *S*-α-Methyl *trans*-alkene keto-mycolic acid (85)

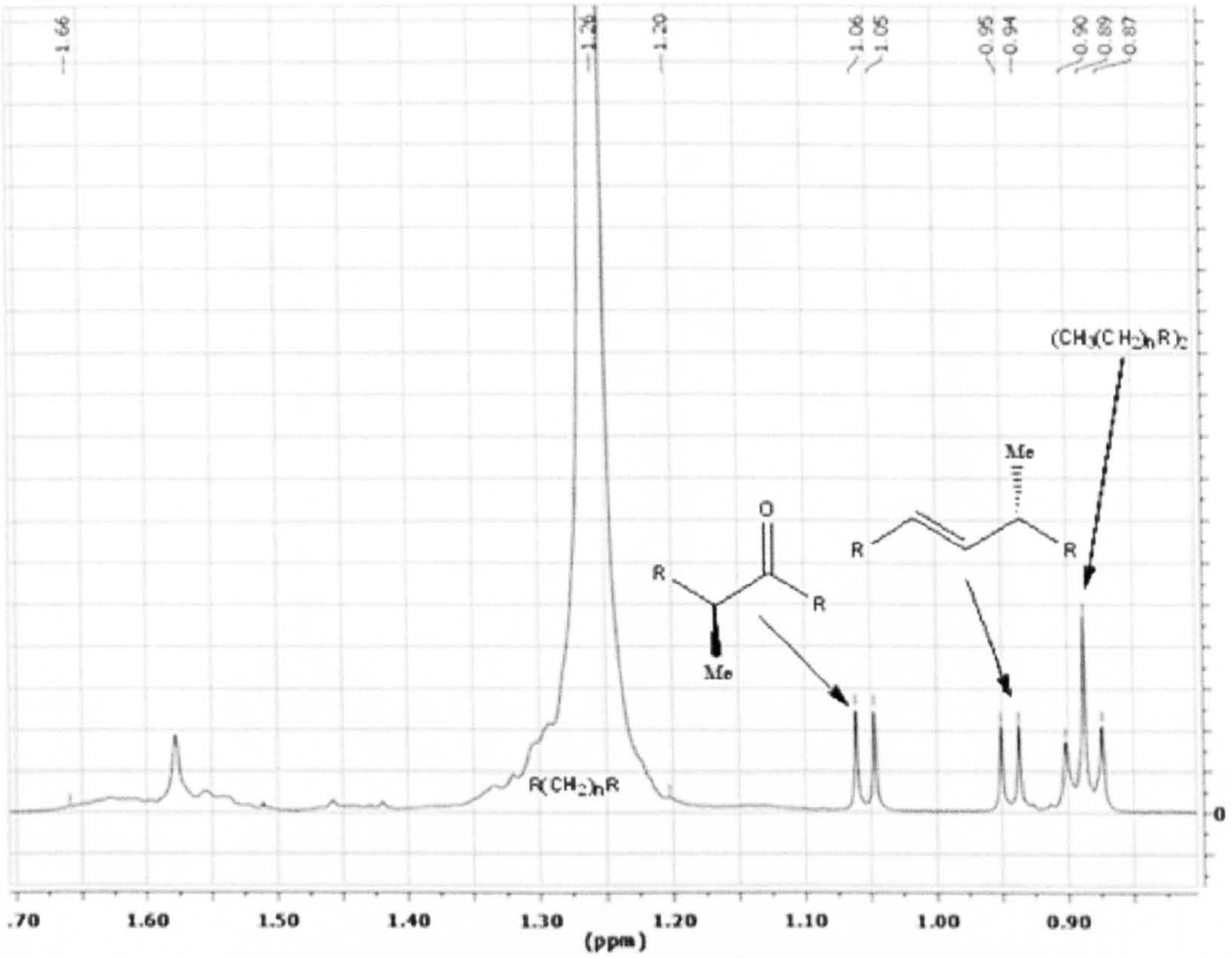


Fig. 139b: ^{1}H NMR spectrum of (85) δ 1.7 to δ 0.8

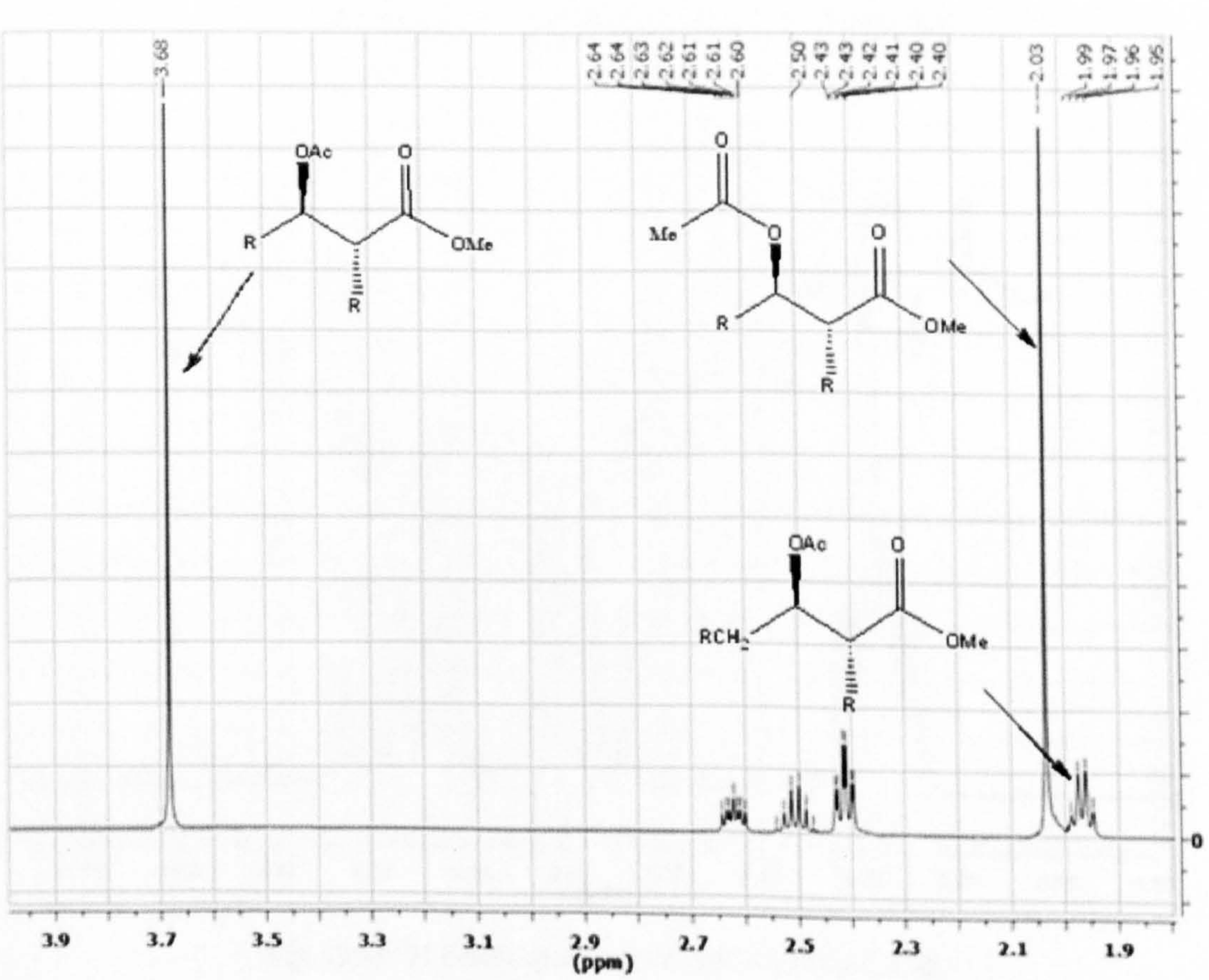


Fig. 139c: ^{1}H NMR spectrum of (85) δ 4.0 to δ 1.8

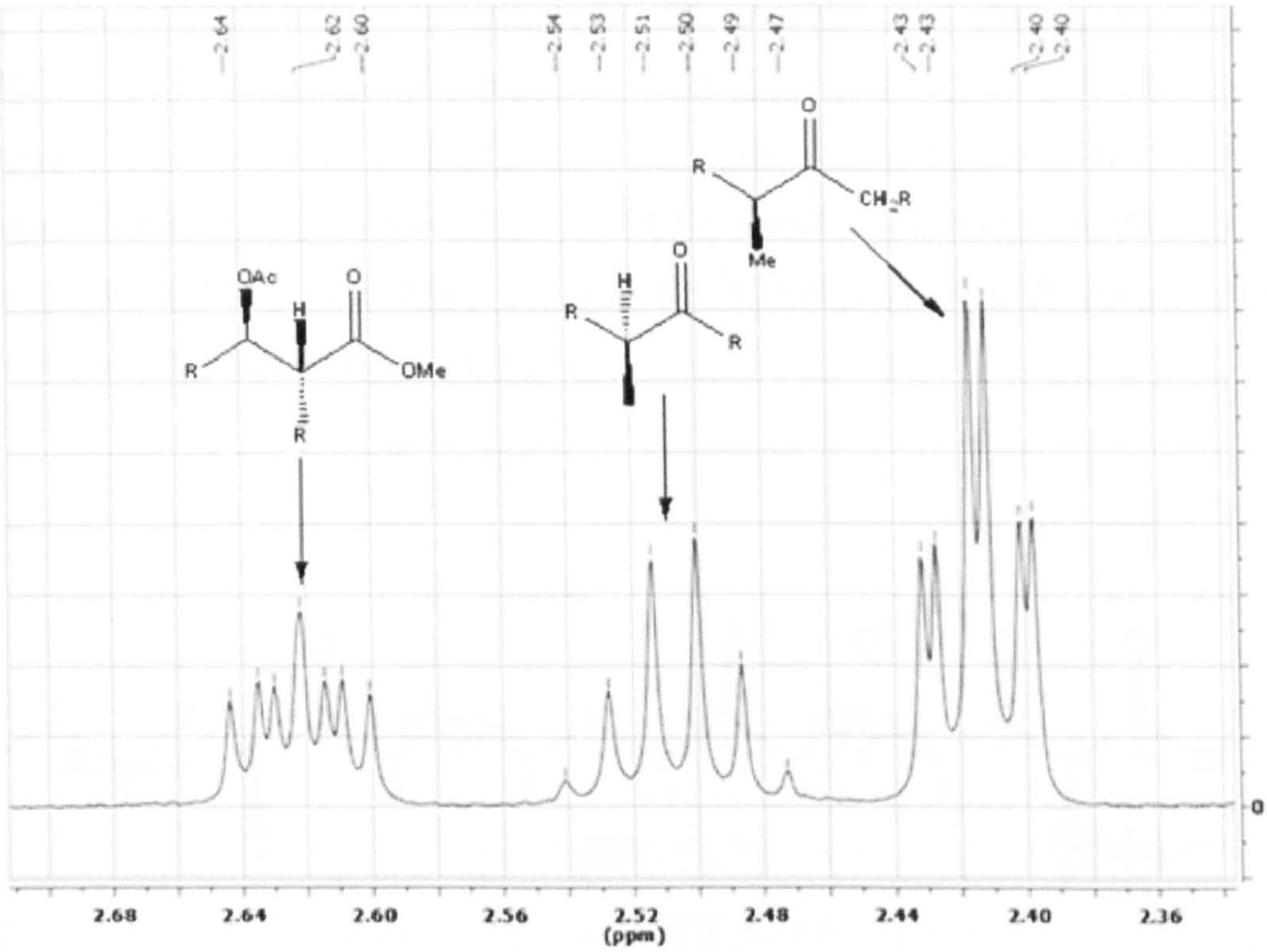


Fig. 139d: ^{1}H NMR spectrum of (85) δ 2.70 to δ 2.34

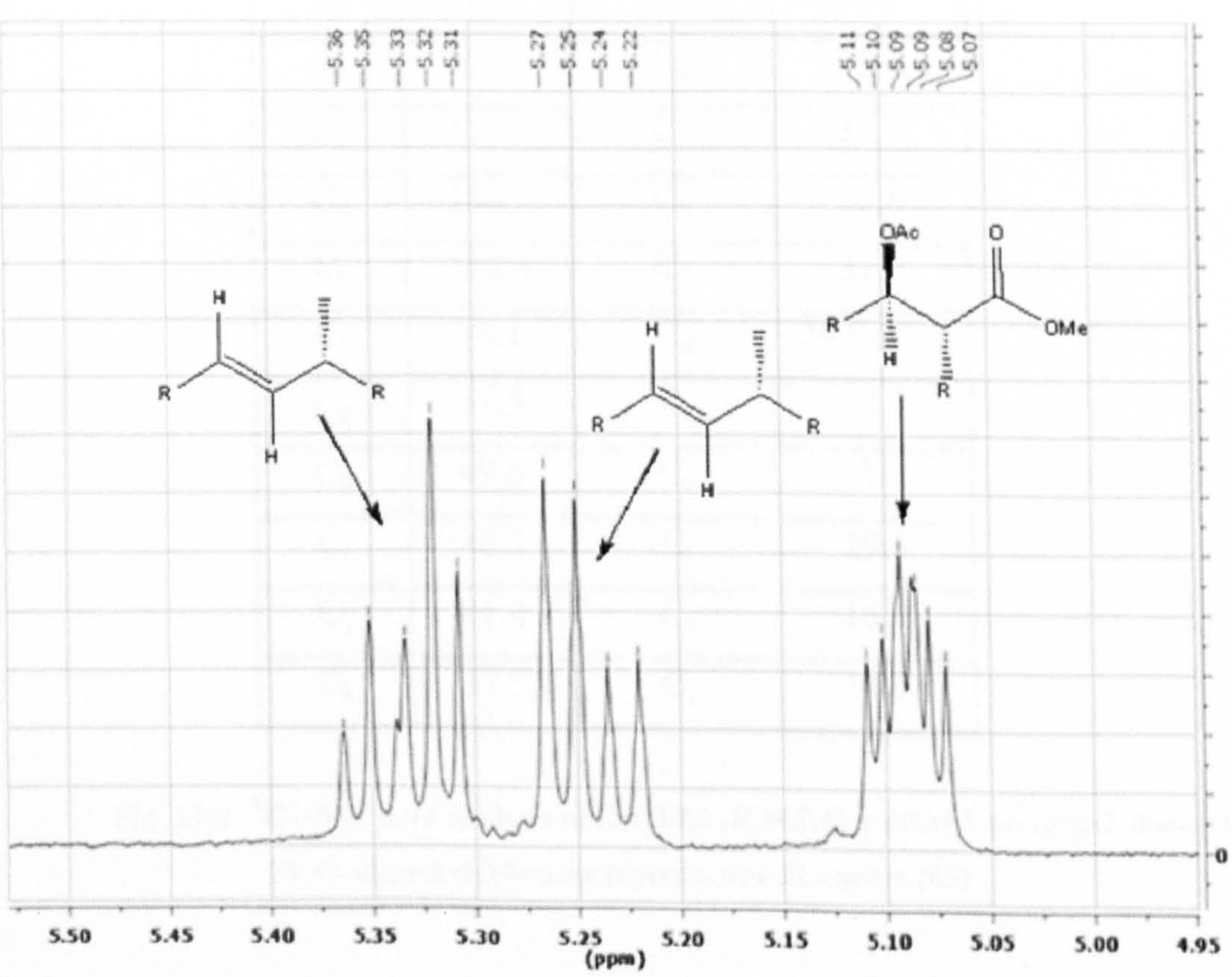
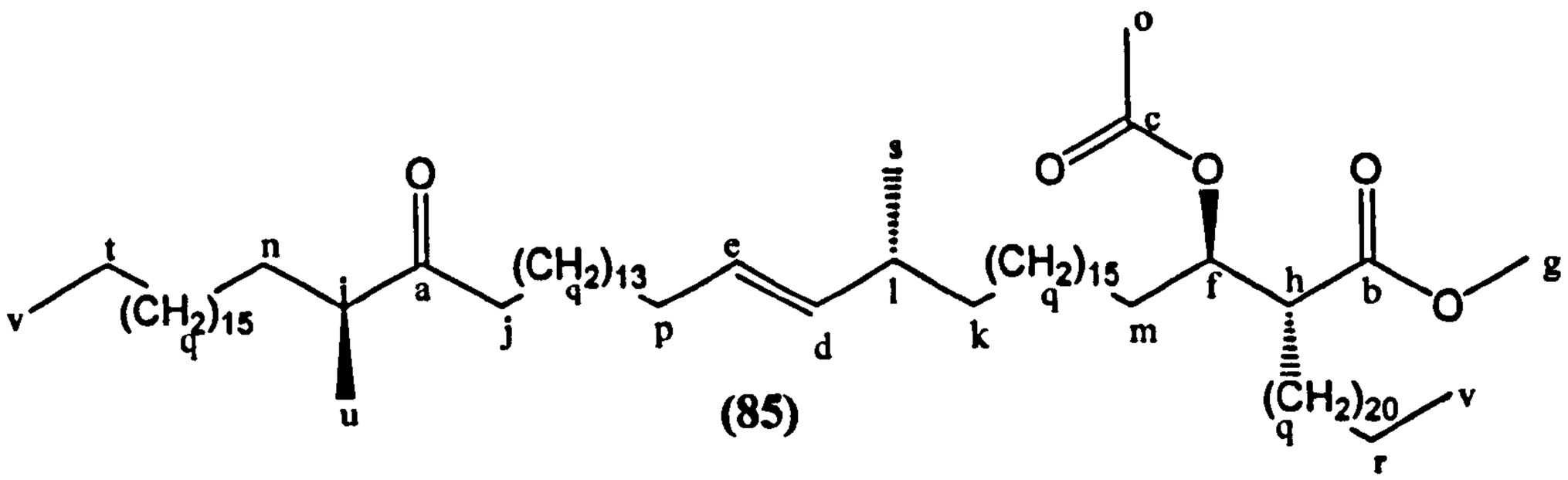


Fig. 139e: ^{1}H NMR spectrum of (85) δ 5.55 to δ 4.95

The MALDI-MS of the protected keto-mycolic acid **(86)** gave an ion at $M+Na^+$: 1288.22 [$C_{85}H_{164}NaO_5$ requires: 1288.25]. The specific rotation of the compound was found to be $[\alpha]^{22}_D$ = +3.52 ($CHCl_3$, 1.094 μmol), the molecular rotation of **(86)** was calculated, M_D = +44.57, which confirmed that **(86)** had not epimerised as **(215)** gave an M_D = +43.96. The ^{13}C NMR data and IR analysis was as follows (See Fig. 140 and Fig. 141).



(85)

C_x	δ	C_x	δ
C_a	215.2	C_l	36.7
C_b	173.7	C_m	35.6
C_c	170.3	C_n	33.1
C_d	136.5	C_o	32.6
C_e	128.4	C_p	31.9
C_f	74.1	C_q	29.8-23.7
C_g	51.5	C_r	22.7
C_h	49.6	C_s	21
C_i	46.3	C_t	19.5
C_j	41.2	C_u	16.4
C_k	37.3	C_v	14.1

Fig. 140: ^{13}C NMR data analysis of (2*R*,3*R*,21*R*,40*S*,*E*) methyl 3-acetoxy-2-docosyl-21,40-dimethyl-39-oxooctapentacont-22-enoate (85)

Stretching	Wave number (cm^{-1})
O-H	3420
C=C-H	3019
COOH	2926
COOH	2855
C=C	1521
CH2C=O	1420
C-H	1215

Fig. 141: IR data analysis of methyl (2*R*,3*R*,21*R*,40*S*,*E*)-3-acetoxy-2-docosyl-21,40-dimethyl-39-oxooctapentacont-22-enoate (86)

2.5 – Synthesis of *R*-α-methyl *trans*-alkene keto-mycolic acid (86) and of *R*,*R* and *S*,*S* α-methyl *trans*-alkene hydroxy-mycolic acid (87) and (88)

2.5.1 – Synthesis of R-α-methyl trans-alkene keto-mycolic acid (86)

Work previously by G. Koza synthesised a protected α-methyl *trans*-alkene keto-mycolic acid **(163)**.[183] However hydrolysis of this compound was not successfully carried out as epimerisation of the methyl group adjacent to the keto group occurred (See Fig. 142).

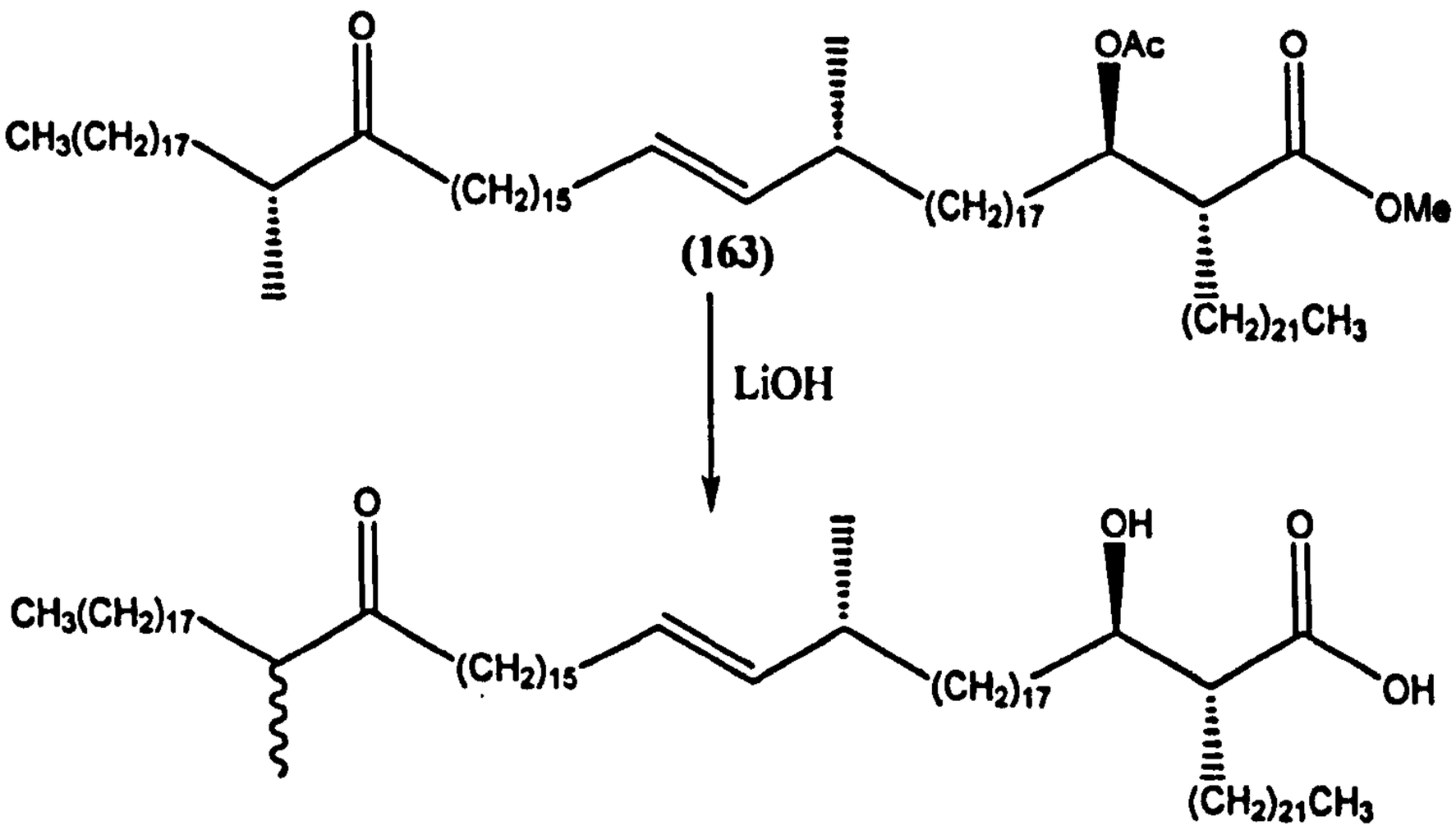


Fig. 142: Racemisation of α-methyl *trans*-alkene keto-mycolic acid

It is necessary to have single enantiomers of mycolic acids for biological activity testing in order to determine whether or not the stereochemistry adjacent to the ketone is important. Therefore successfully synthesising the unprotected *R*-α-methyl *trans*-alkene keto-mycolic acid **(86)** was a significant goal of this project. The successful synthesis of the *R*-α-methyl *trans*-alkene keto-mycolic acid **(86)** was completed following the scheme below (See Fig. 143). The protected *R,R* α-methyl *trans*-alkene hydroxy-mycolic acid **(206)** was previously synthesised by G. Koza during his preparation of the protected keto-mycolic acid **(163)**.[183]

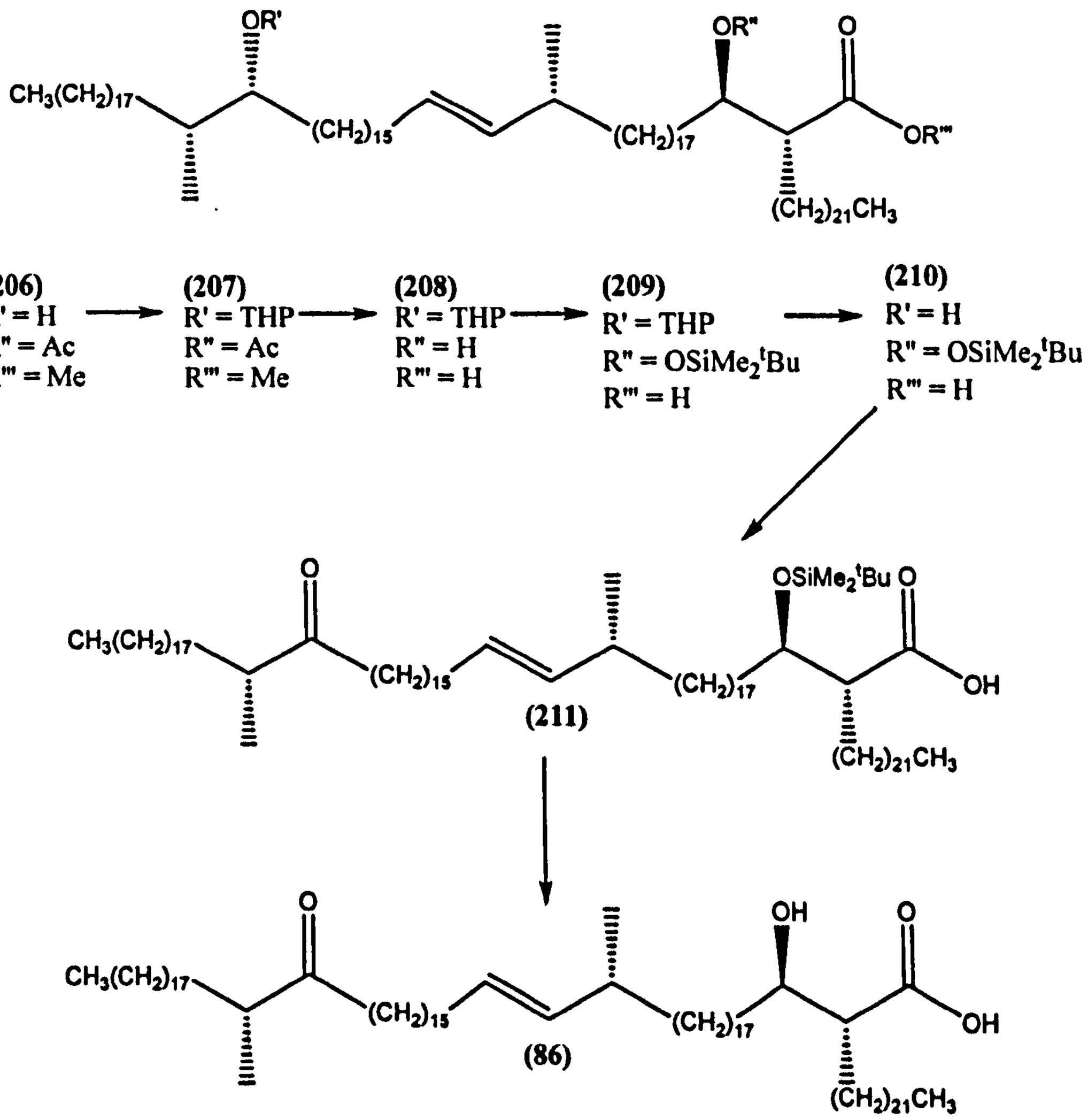


Fig. 143: Deprotection scheme for *R*-α-methyl *trans*-alkene keto-mycolic acid (86)

The secondary alcohol of the protected α-methyl *trans*-alkene hydroxy-mycolic acid **(206)** was protected with a tetrahydropyran group so that hydrolysis of

the methyl ester and acetate could be then carried out without racemisation. This was done by first reacting with 2,3-dihydropyran and pyridinium-*p*-toluene sulfonate to yield **(207)**. Hydrolysis was then carried out using lithium hydroxide with THF, methanol and water as solvents to give **(208)** (See Fig. 144).

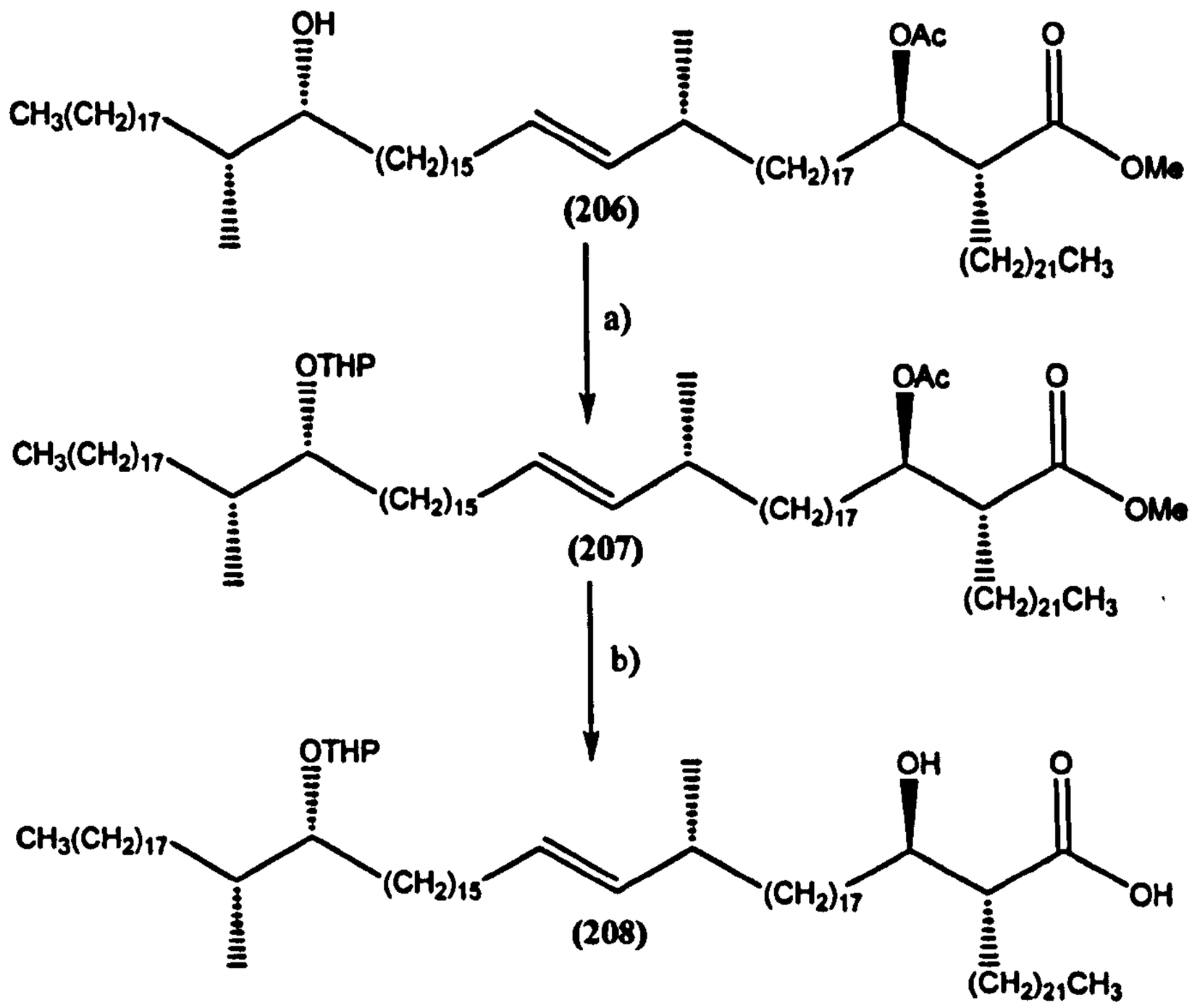


Fig. 144: a) 2,3-Dihydropyran, PPTS, (84 %); b) LiOH, THF/MeOH/H_2O, (72 %)

The above compounds were characterised using NMR, signals corresponding to the tetrahydropyran group being observed at δ 4.68-4.61 and δ 3.96-3.89 in the ^1H NMR spectrum of **(207)**. Hydrolysis of the esters was confirmed by the loss of both three hydrogen singlets at δ 3.68 and δ 2.03 in the spectrum of **(208)**. The molecular rotations of **(207)** and **(208)** confirmed that the methyl group in the distal position had not epimerised, as **(207)** M_D = +26.91 and **(208)** M_D = +22.68.

The β-hydroxy group was then silyated, so that the tetrahydropyran group could be removed and the resultant secondary alcohol oxidised, to give the silyated keto-mycolic acid **(209)**. The THP group was removed using pyridinium-*p*-toluene sulfonate, using THF, methanol and water as a solvent mixture, giving **(210)**. Oxidation was carried out using PCC to give the silyated keto-mycolic acid,

(2*R*,3*R*,19*R*,38*R*,*E*)-3-(*tert*-butyldimethylsilyloxy)-2-docosyl-19,38-dimethyl-37-oxohexapentacont-20-enoic acid **(211)** (See Fig. 145).

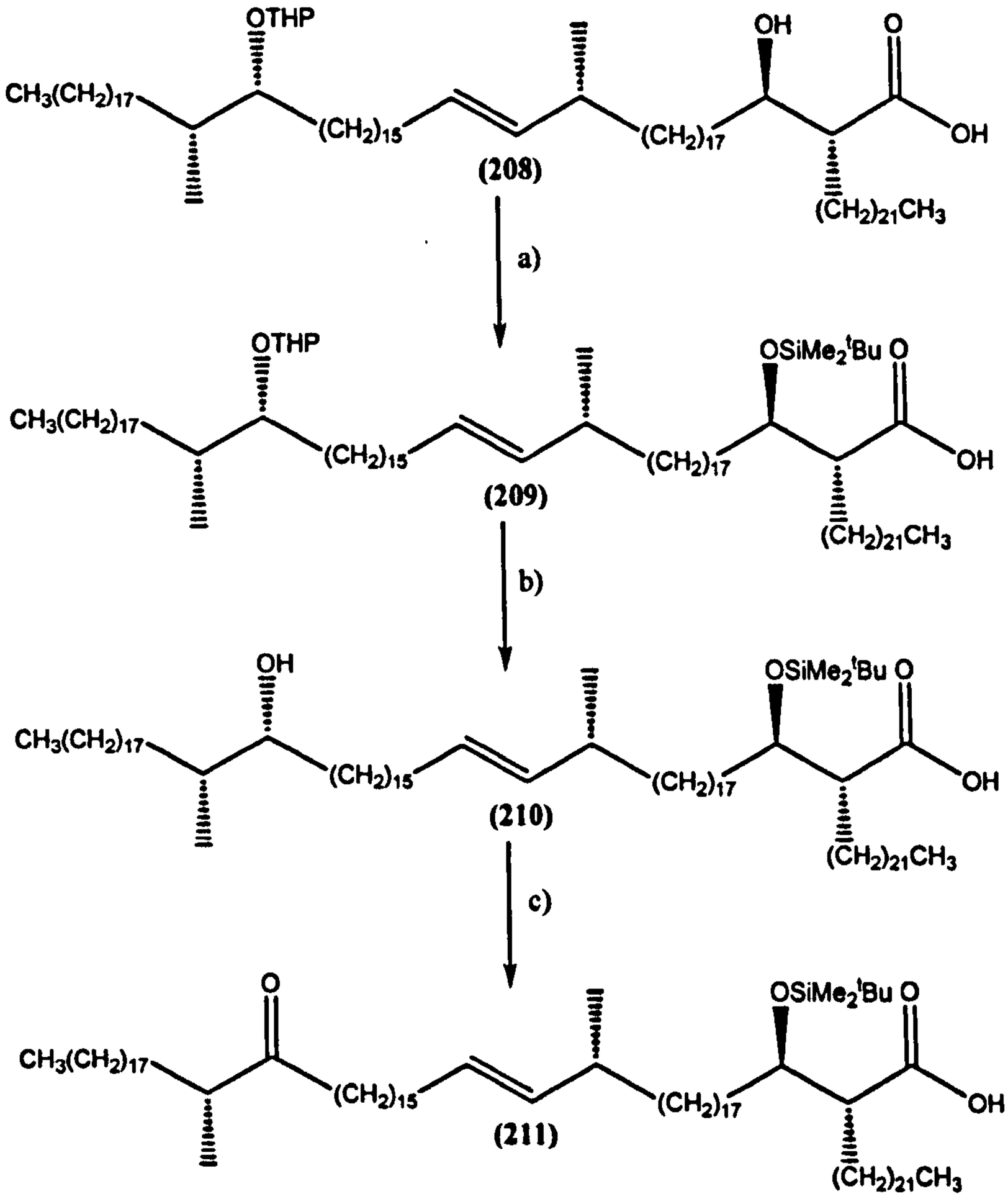


Fig. 145: a) $^{t}BuMe_2SiCl$, $C_3H_4N_2$, DMAP, (70 %); b) PPTS, THF/MeOH/H_2O, (73 %); c) PCC, (89 %)

The introduction of the silyl ester protecting group was observed in the ^{1}H NMR spectrum of **(209)** as two three hydrogen singlets at δ 0.15 and δ 0.14 corresponding to the two methyl groups, and the nine hydrogen singlet at δ 0.93 corresponding to the *tert*-butyl group. Deprotection of the tetrahydropyran group caused loss of multiplets at δ 4.67-4.62 and δ 3.93-3.89. Oxidation of the primary alcohol **(210)** was observed via ^{13}C NMR, with a signal corresponding to the quaternary carbon of the keto group at δ 215.3 in the spectrum of **(211)**.

The deprotection of the silyl group was then carried out using hydrofluoric acid in pyridine to yield the *R*-α-methyl *trans*-alkene keto-mycolic acid **(86)** (See Fig. 146).

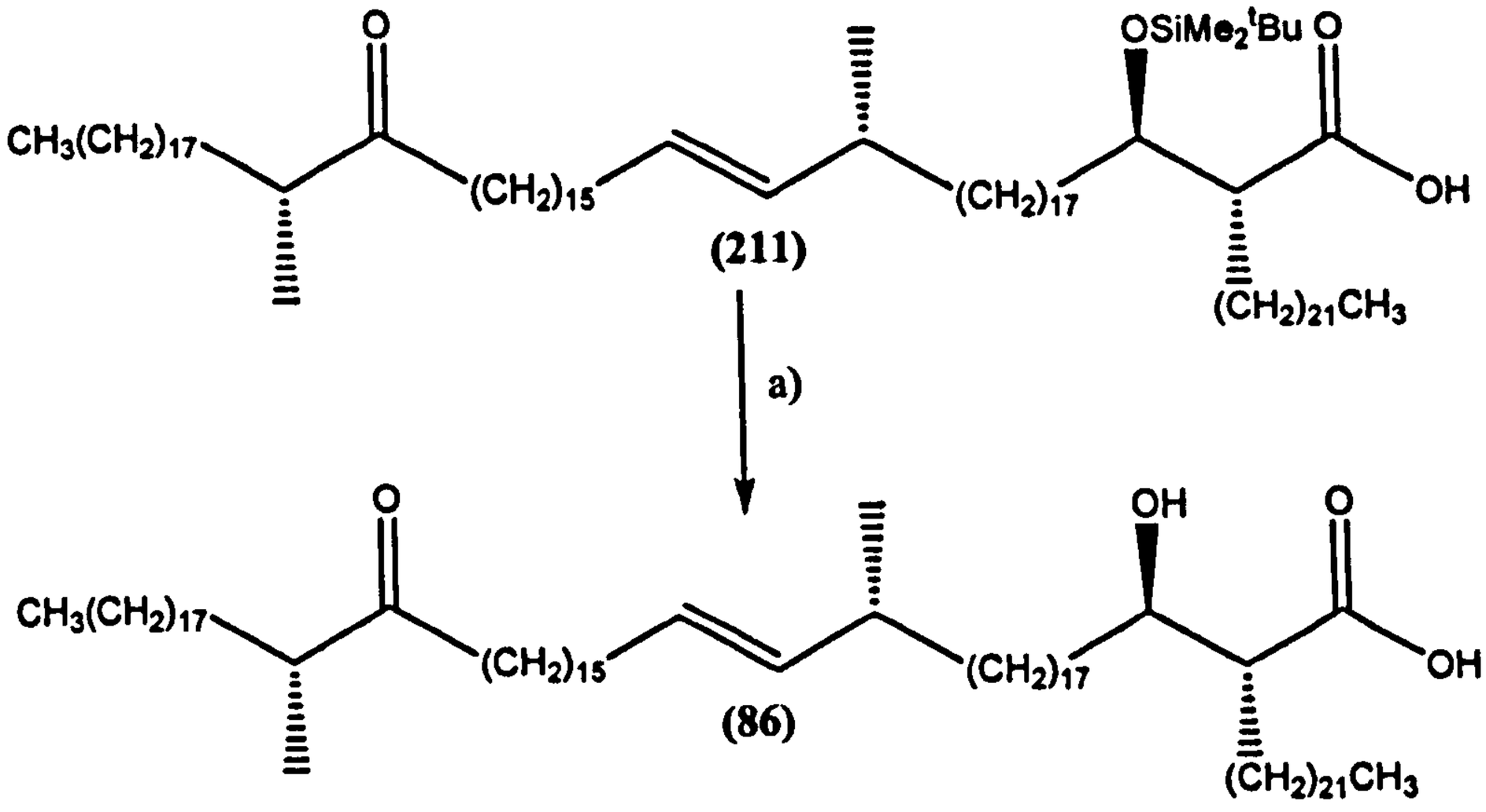
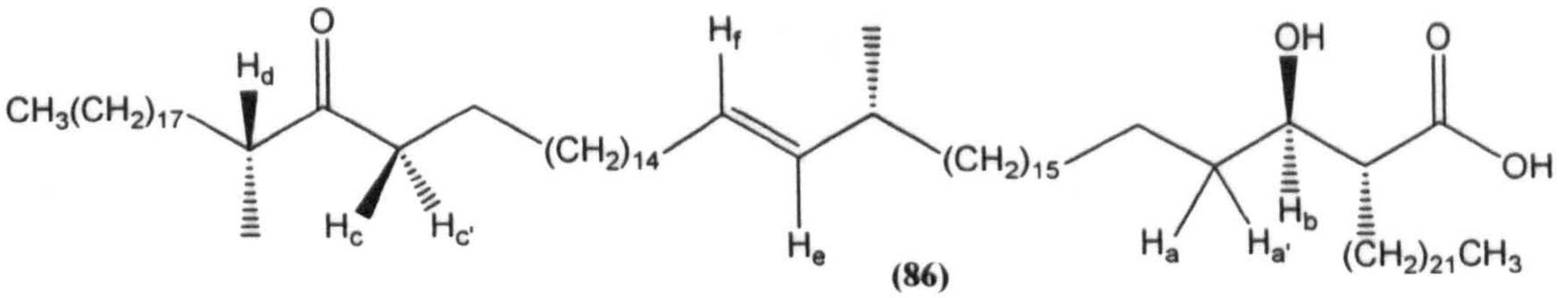


Fig. 146: a) HF.pyr, pyridine, (44 %)

Full ^{1}H NMR expansions of *R*-α-methyl *trans*-alkene keto-mycolic acid **(86)** can be seen as follows (See Fig. 147a, 147b, 147c, 147d and 147e). H_a and $H_{a'}$ is observed to give a broad quartet in the ^{1}H NMR spectrum; theoretically a doublet of triplets would be expected, however the broad quartet observed is possibly due to the three protons adjacent having similar coupling constants. The broadness of this signal is caused due to the fact that H_a and $H_{a'}$ are not magnetically equivalent. The signal corresponding to H_b is a sextet, agreeing with the expected theoretical splitting. H_c and $H_{c'}$ are observed to give two triplets, because the two are non-magnetically equivalent. In theory H_d should show a double doublet of quartets splitting pattern (16 lines), but a broad signal is observed due to similar coupling constants with neighbouring protons (See Fig. 147c). H_e and H_f are observed as protons in the olefinic region, with H_e splitting to give a doublet of doublets and H_f a doublet of triplets. This is consistent with the theoretical splitting pattern for these protons (See Fig. 147e).



(86)

Fig. 147a: *R*-α-methyl *trans*-alkene keto-mycolic acid (86)

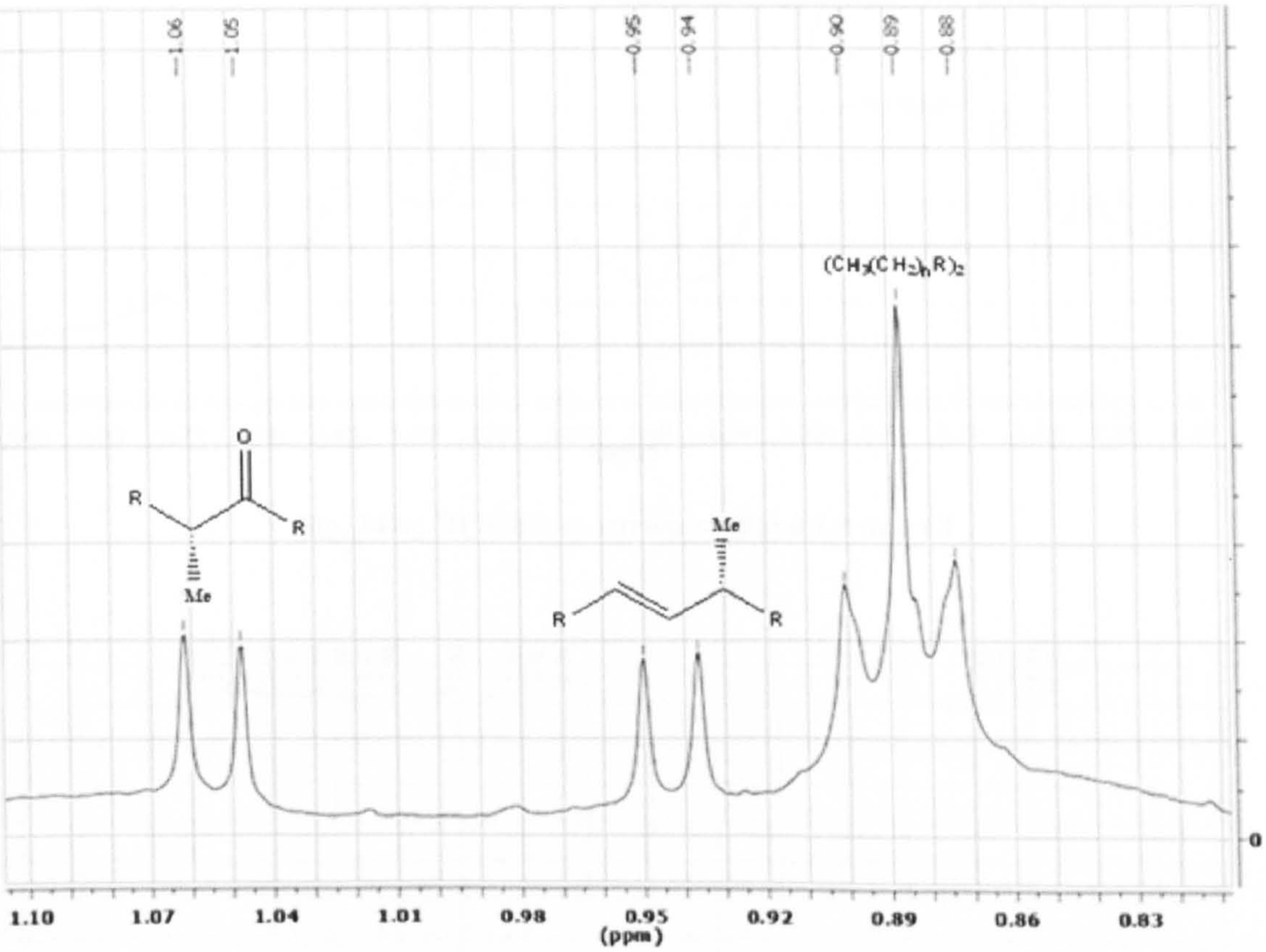


Fig. 147b: ^{1}H NMR spectrum of (86) δ 1.1 to δ 0.8

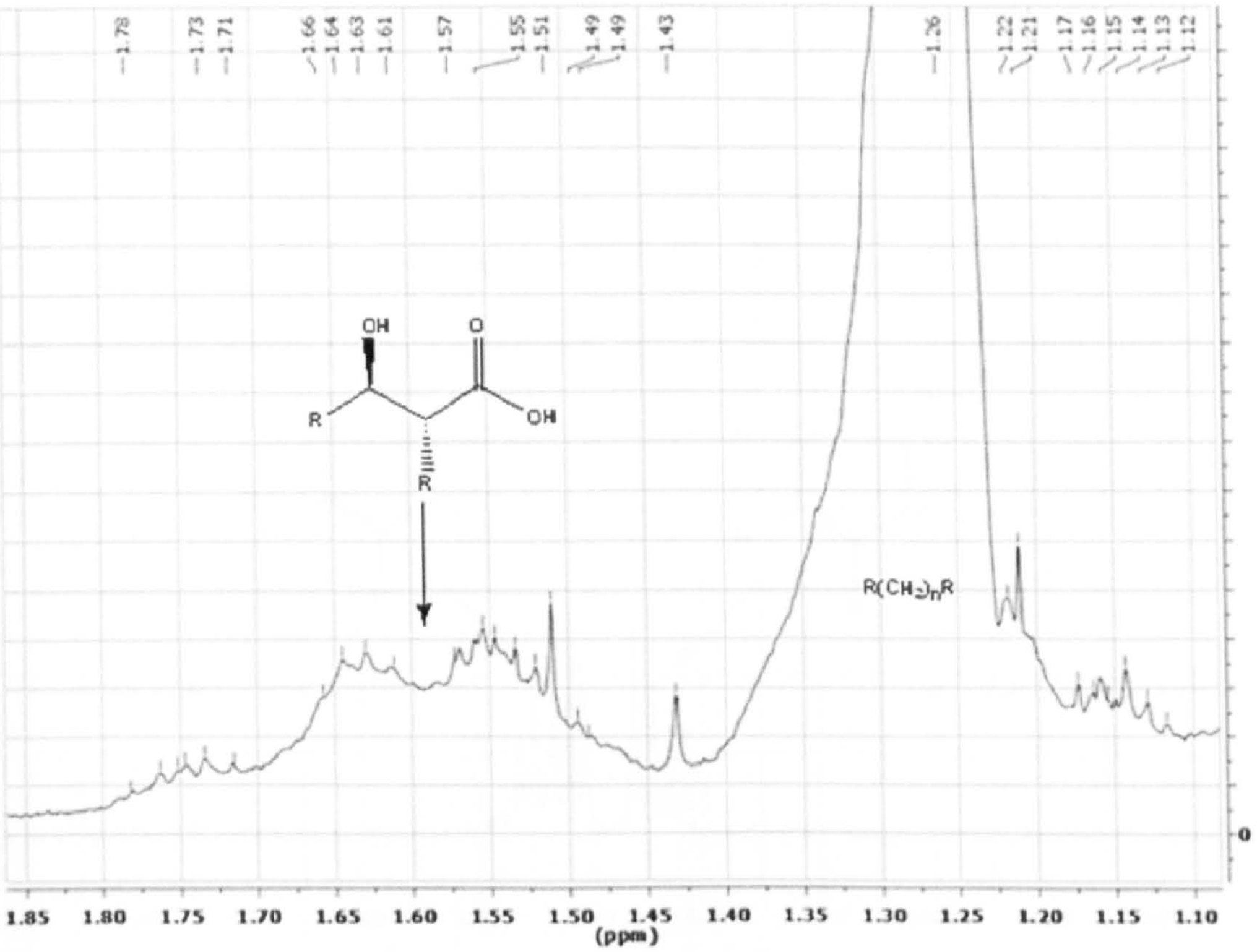


Fig. 147c: ^{1}H NMR spectrum of (86) δ 1.9 to δ 1.1

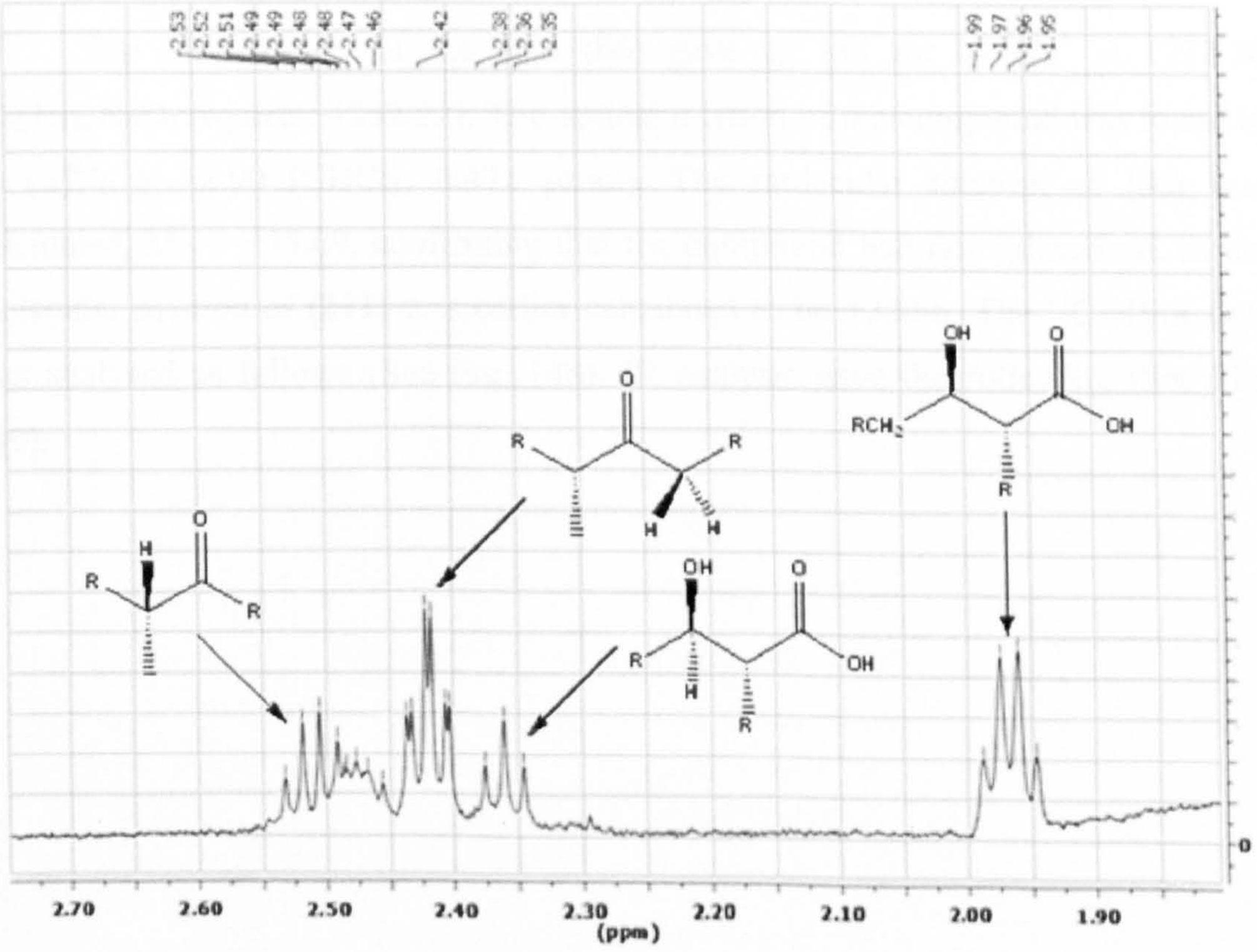


Fig. 147d: ^{1}H NMR spectrum of (86) δ 2.8 to δ 1.8

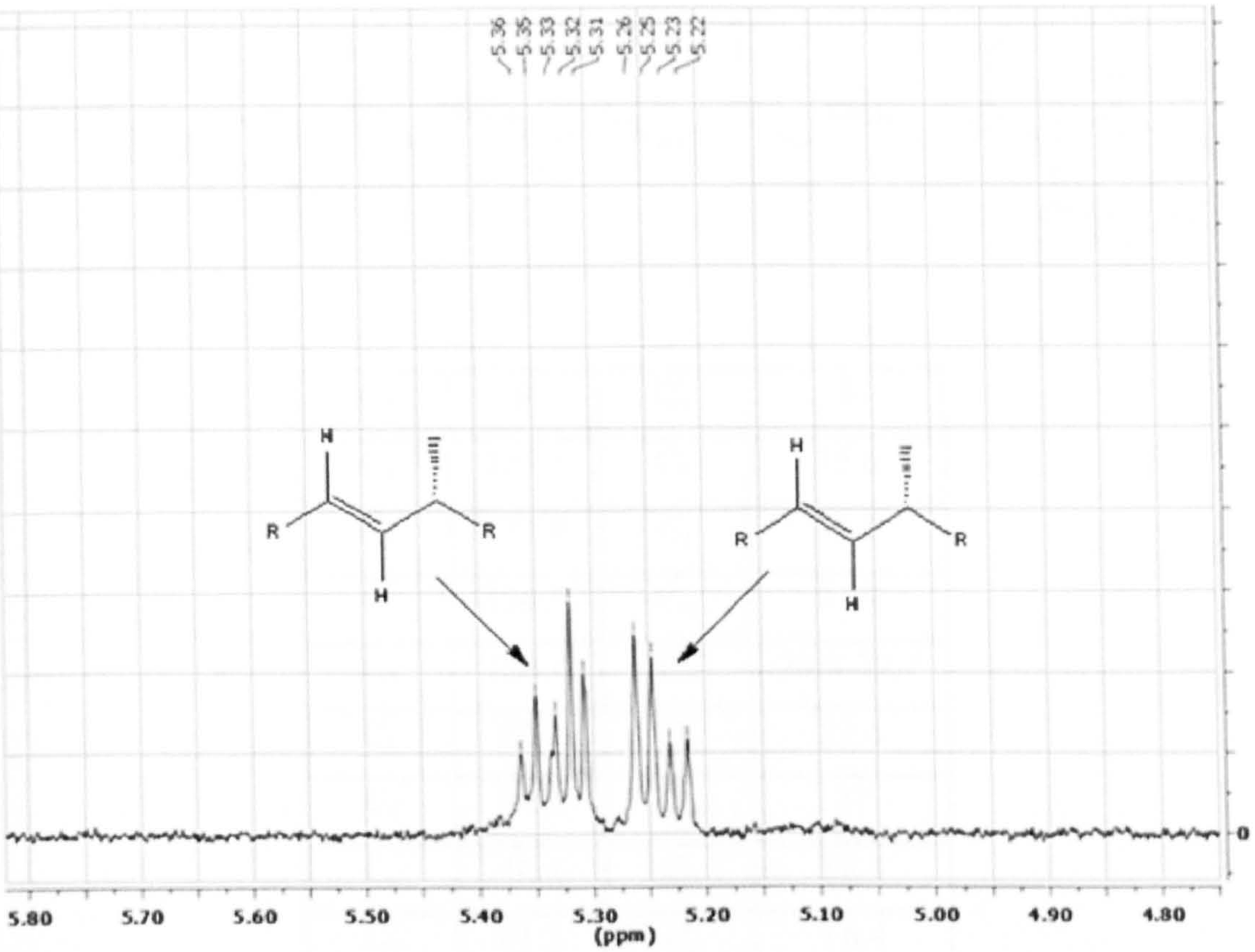
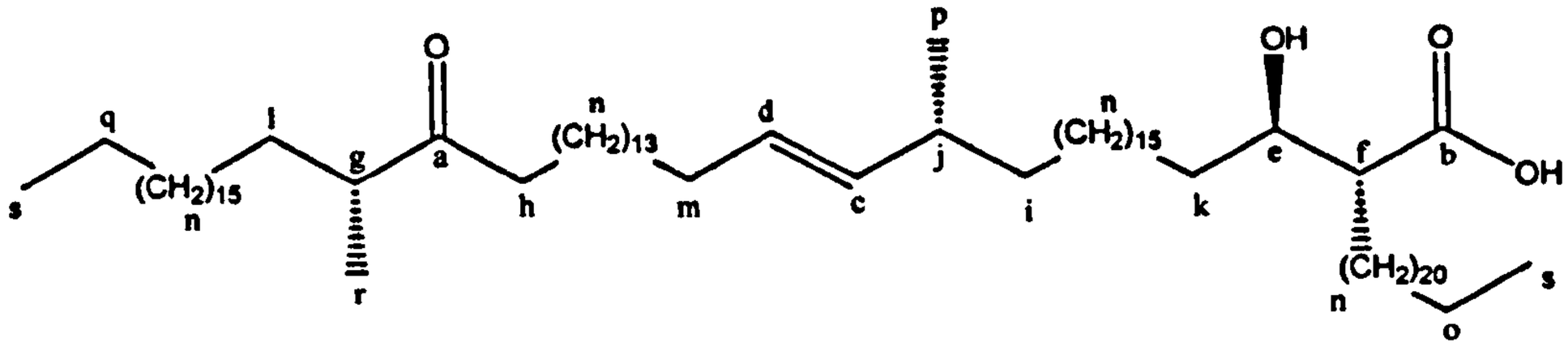


Fig. 147e: ^{1}H NMR spectrum of (86) δ 5.9 to δ 4.7

The MALDI-MS of the acid **(86)** gave an ion for $M+Na^{+}$ at 1232.36 [$C_{82}H_{160}NaO_4$ requires: 1232.22]. The optical rotation of the compound was found to be $[\alpha]^{23}_{D}$ = +2.90 ($CHCl_3$, 0.471 μmol). The molecular rotation of **(86)** was calculated, M_D = +35.09, confirming that the compound had not epimerised, as the molecular rotation of **(211)** was earlier calculated to be +22.68. The ^{13}C NMR data was analysed as follows (See Fig. 148). IR analysis gave the following (See Fig. 149).



C_x	δ	C_x	δ
C_a	215.5	C_k	35.6
C_b	177.9	C_l	33.1
C_c	136.5	C_m	31.9
C_d	128.4	C_n	29.8-22.7
C_e	72.2	C_o	22.6
C_f	50.6	C_p	21
C_g	46.4	C_q	19.4
C_h	41.2	C_r	16.4
C_i	37.3	C_s	14.1
C_j	36.7		

Fig. 148: ^{13}C NMR data analysis of (2*R*,3*R*,19*R*,38*R*,*E*)-2-docosyl-3-hydroxy-19,38-dimethyl-37-oxohexapentacont-20-enoic acid (86)

Stretching	Wave number (cm^{-1})
O-H	3420
C=C-H	3019
COOH	2926
COOH	2855
C=C	1521
CH2C=O	1420
C-H	1215

Fig. 149: IR data analysis of (2*R*,3*R*,19*R*,38*R*,*E*)-2-docosyl-3-hydroxy-19,38-dimethyl-37-oxohexapentacont-20-enoic acid (86)

2.5.2 – Synthesis of R,R and S,S α-methyl trans-alkene hydroxy-mycolic acid (87) and (89)

From the Koza protected *R,R* α-methyl *trans*-alkene hydroxy-mycolic acid **(163)** it was possible to prepare the corresponding free hydroxy acid **(87)** via hydrolysis. This was carried out using lithium hydroxide with THF, methanol and water as a solvent mixture to give the *R,R* α-methyl *trans*-alkene hydroxy-mycolic acid **(87)** (See Fig. 150). The diastereomer **(88)** was prepared using the protected *S,S* α-methyl *trans*-alkene hydroxy-mycolic acid intermediate **(205)** as previously discussed in section 2.4.4. Again hydrolysis with lithium hydroxide with THF, methanol and water as a solvent mixture gave the free hydroxy acid **(88)** (See Fig. 151).

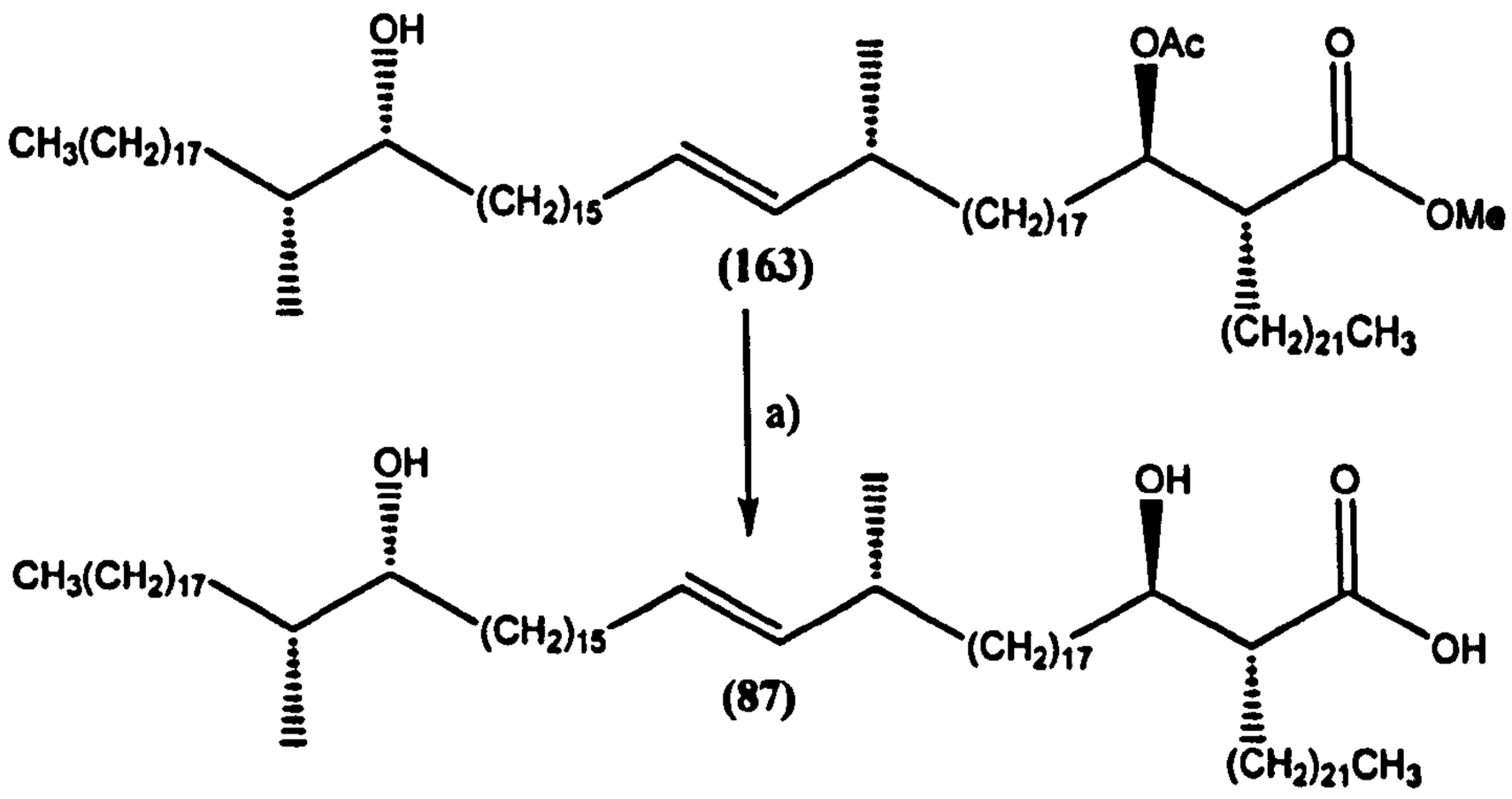


Fig. 150: a) LiOH, THF/MeOH/H_2O, (59 %)

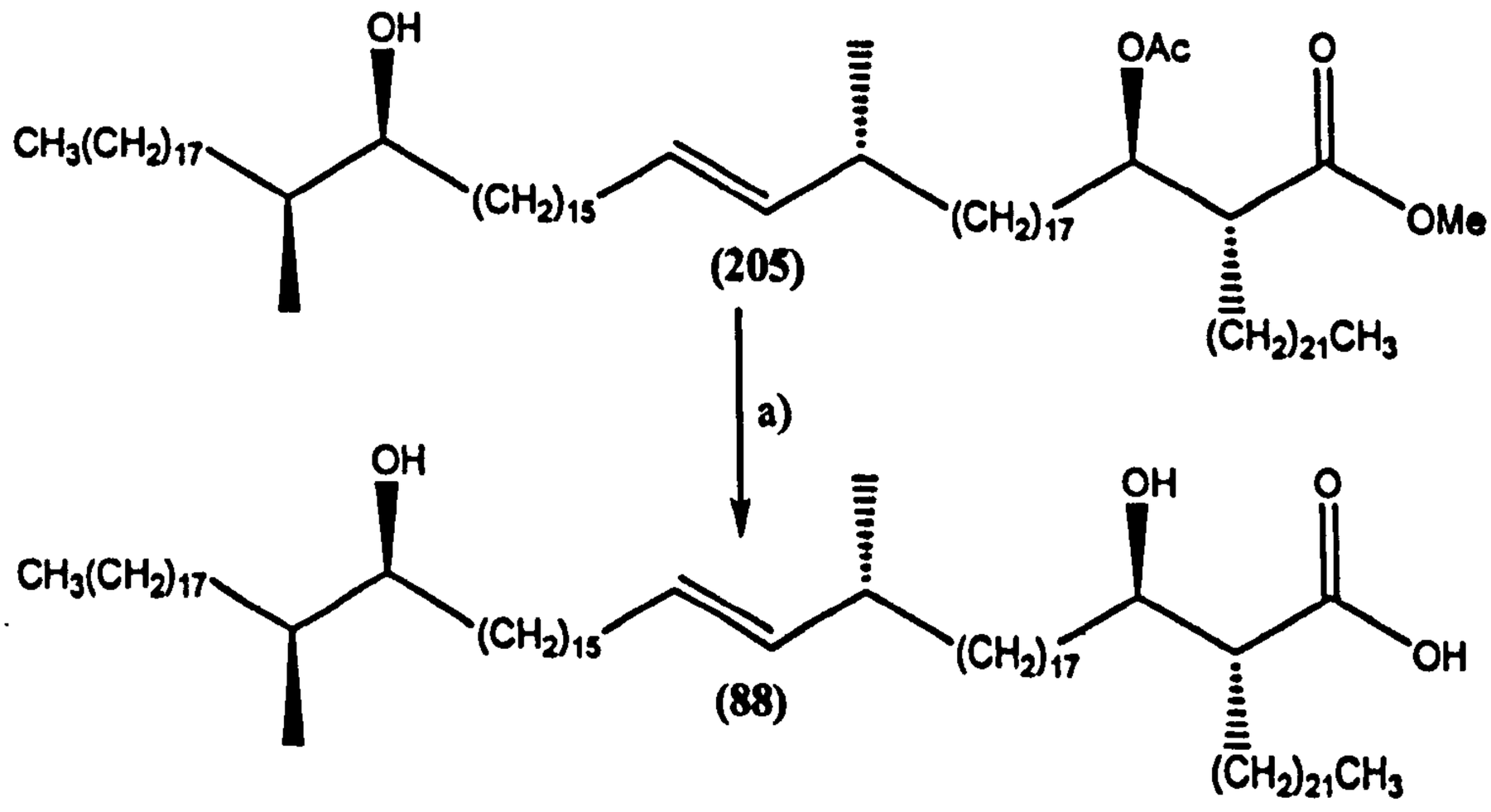


Fig. 151: a) LiOH, THF/MeOH/H_2O, (65 %)

Full ^{1}H NMR expansions of *R,R* and *S,S* α-methyl *trans*-alkene hydroxy-mycolic acid **(87)** and **(88)** can be seen as follows (See Fig. 152a, 152b, 152c, 152d and 152e).

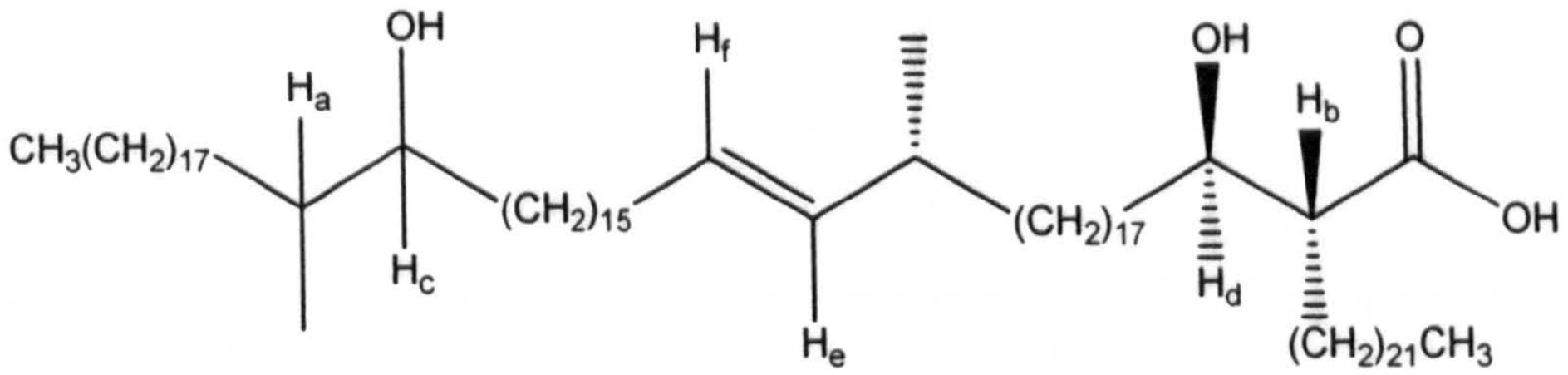


Fig. 152a: α-Methyl *trans*-alkene hydroxy-mycolic acid (87) and (88)

The splitting patterns observed for the oxygenated regions, H_a, H_b, H_c and H_d, for the *R,R* and *S,S* α-methyl *trans*-alkene hydroxy-mycolic acid **(88)** and **(89)** are the same as that for the *cis*-cyclopropane methoxy-mycolic acid **(84)**. They only differ in chemical shift because of the hydroxyl group at the C_{39} position (See Fig. 152b, 152c and 152d). H_e and H_f are observed as protons in the olefinic region, with H_e splitting to give a doublet of doublets and H_f a doublet of triplets. This is consistent with the theoretical splitting pattern for these protons (See Fig. 152e).

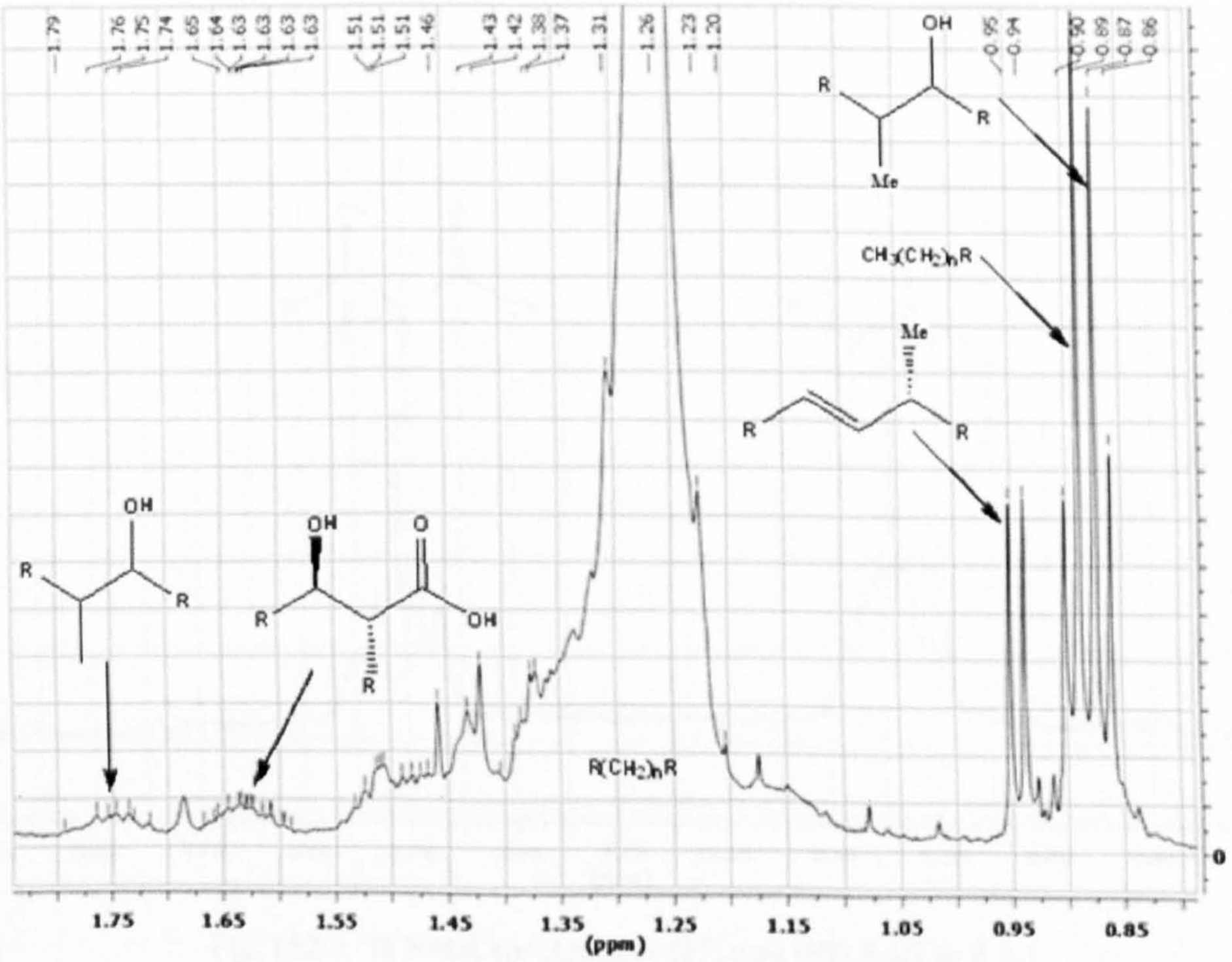


Fig. 152b: ^{1}H NMR spectrum of (87) and (88) δ 1.8 to δ 0.8

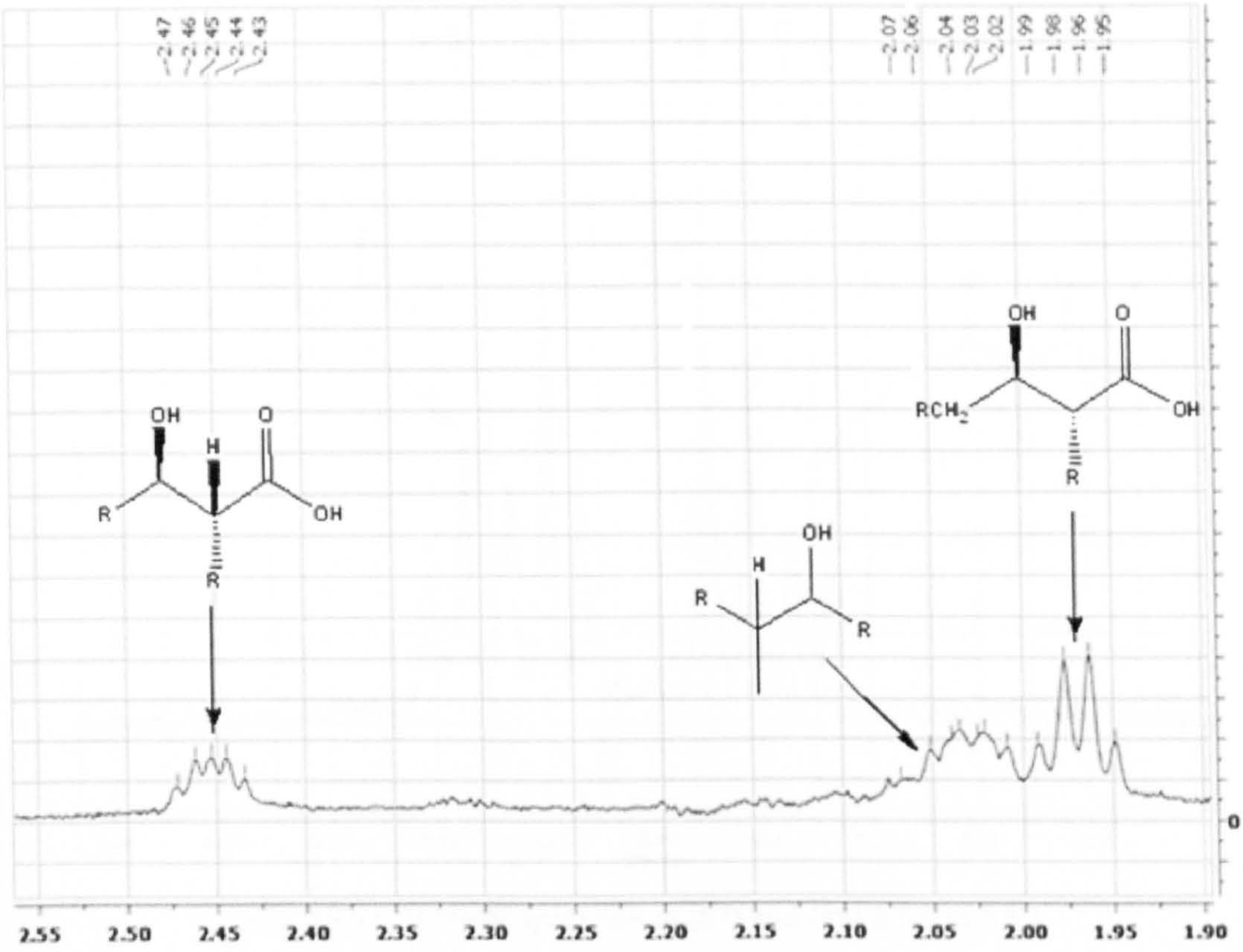


Fig. 152c: ^{1}H NMR spectrum of (87) and (88) δ 2.6 to δ 1.9

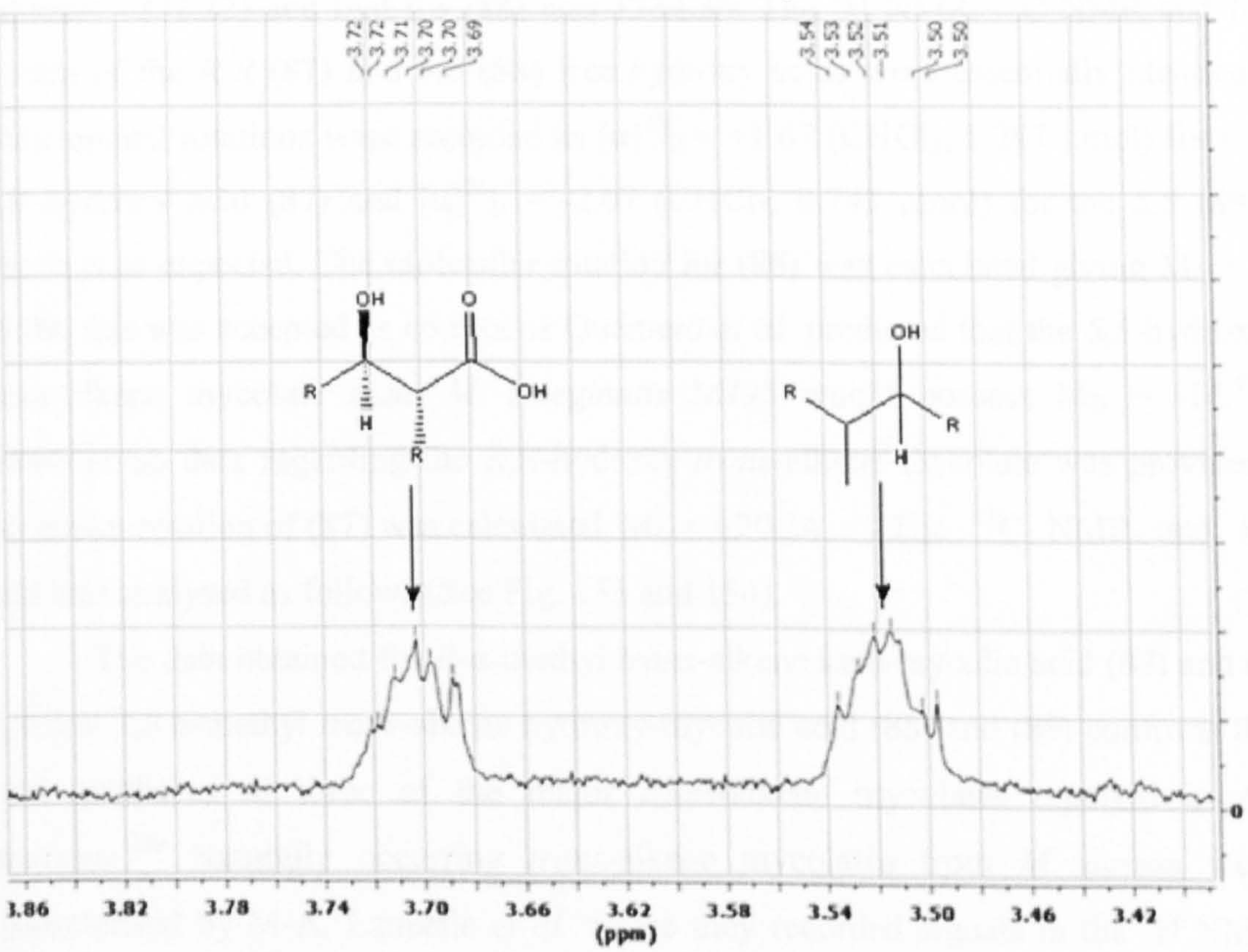


Fig. 152d: ^{1}H NMR spectrum of (87) and (88) δ 3.9 to δ 3.4

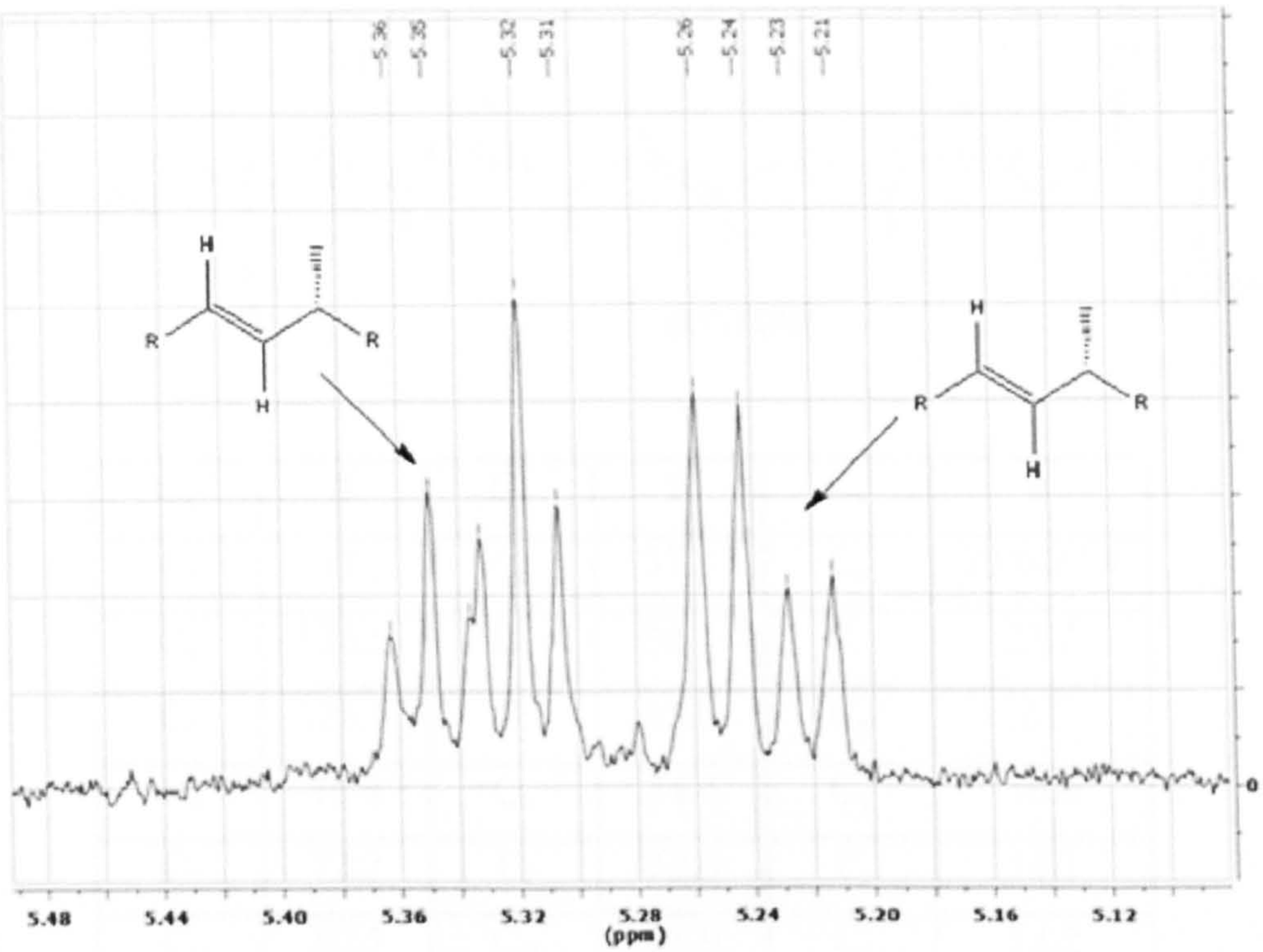
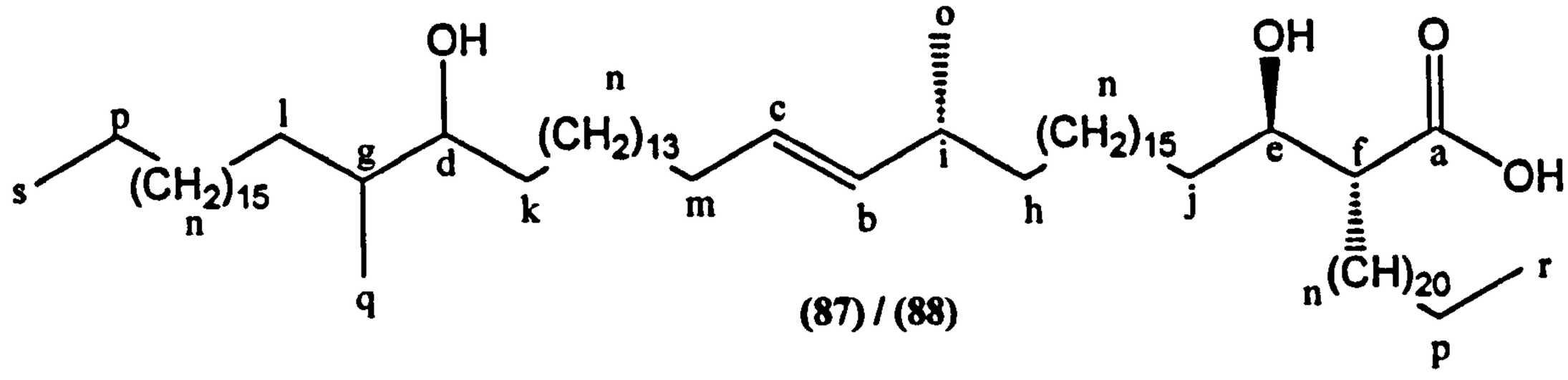


Fig. 152e: ^{1}H NMR spectrum of (87) and (88) δ 5.5 to δ 5.1

The MALDI-MS measured for **(87)** was M+Na^{+} 1232.47 [$C_{82}H_{160}NaO_4$ requires: 1232.22] and that for **(88)** was 1232.36. The ^{1}H NMR, ^{13}C NMR and IR spectra of the *R,R* **(87)** and *S,S* **(88)** free hydroxy acids were essentially identical. Their optical rotations were recorded as $[\alpha]^{20}_{D}$ = +1.67 ($CHCl_3$, 1.287 μmol) for the *R,R* hydroxy acid **(87)** and $[\alpha]^{21}_{D}$ = -2.07 ($CHCl_3$, 0.743 μmol) for the *S,S* **(88)**, which is as expected. The molecular rotation for **(88)** was calculated giving M_D = -25.09, this was accepted as correct as Quémard *et al.* predicted that the *S,S*-hydroxy *trans*-alkene mycolate from *M. smegmatis* MJ95 would possess M_D = -16.[135] However no data regarding the *R,R*-hydroxy *trans*-alkene mycolate was provided, molecular rotation of **(87)** was calculated, M_D = +20.24. The ^{13}C NMR and IR data was analysed as follows (See Fig. 153 and 154).

The data obtained for *R*-α-methyl *trans*-alkene keto-mycolic acid **(87)** and of *R,R* and *S,S* α-methyl *trans*-alkene hydroxy-mycolic acid **(88)** and **(89)** confirms the total syntheses of three of the major *trans*-alkene mycolates reported in *M. marinum*.[130] Naturally occurring *trans*-alkene mycolates from *M. aurum* were characterised by M-A. Lanéelle *et al.* where they recorded signals in the ^{1}H NMR which are consistent with the author's results, where signals were recorded at δ 5.34 and δ 0.94, corresponding to the double bond and the adjacent methyl group.[205]



(87) / (88)

C_x	δ	C_x	δ	C_x	δ
C_a	177.3	C_h	37.3	C_n	30.0-27.4
C_b	136.5	C_i	36.7	C_o	22.7
C_c	128.5	C_j	35.6	C_p	21
C_d	75.4	C_k	34.4	C_q	16.6
C_e	72.2	C_l	32.6	C_r	14.1
C_f	50.5	C_m	31.9	C_s	13.2
C_g	41.4				

Fig. 153: ^{13}C NMR data analysis of *R,R* and *S,S* α-methyl *trans*-alkene hydroxy-mycolic acid (87) and (88)

Stretching	Wave number (cm^{-1})
O-H	3534
COOH	2922
COOH	2854
C=O	1751
C-C	1466

Fig. 154: IR data analysis of both *R,R* and *S,S* α-methyl *trans*-alkene hydroxy-mycolic acid (87) and (88)

2.6 – Biological activity

All six compounds prepared during this study were sent to the Professor Jan Verschoor research group of the University of Pretoria, South Africa and the Professor Johan Grooten research group of Ghent University, Belgium for testing of their biological activity.

Thus far data on the biological activities has been generated for two of the six compounds prepared during this study.

2.6.1 – Tuberculosis antibody activity

The tests carried out in South Africa studied the recognition of the mycolic acids by lipid antibodies present in the serum of patients known to be infected with TB, where the response is measured using enzyme-linked immunosorbent assay (ELISA). In ELISA, an antigen impregnated surface is washed with a specific antibody which is linked to an enzyme. The final stage of the preparation process is the addition of a substrate which then gives a detectable signal, e.g. fluorescence. Hence the number of antigen-antibody interactions can be measured and as a result the efficiency of an immunological agent. The test is carried out on both TB positive and TB negative sera so that the response measured is not masked by the presence of *M.Tb*.

The two compounds tested at the time of going to print were *cis*-cyclopropane methoxy-mycolic acid **(83)** and α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)**.

With respect to the *cis*-cyclopropane methoxy-mycolic acid **(83)**, the results indicate that the recognition of this sample by TB anti-bodies is relatively low. However, as previously discussed, Al-Dulayymi *et al.* successfully prepared three other *cis*-cyclopropane methoxy-mycolic acids, **(212) – (214)** with the alternative stereochemistries in the mero-chain.[182, 206] It is interesting that there are definite differences in activity shown with the four synthetic methoxy-mycolic acid variants tested, i.e. stereochemistry does affect recognition.

The data provided by the Verschoor group showed the following activity, where 'ma mix' is the total mycobacterial mycolic acid extract for comparison (See Fig. 155).

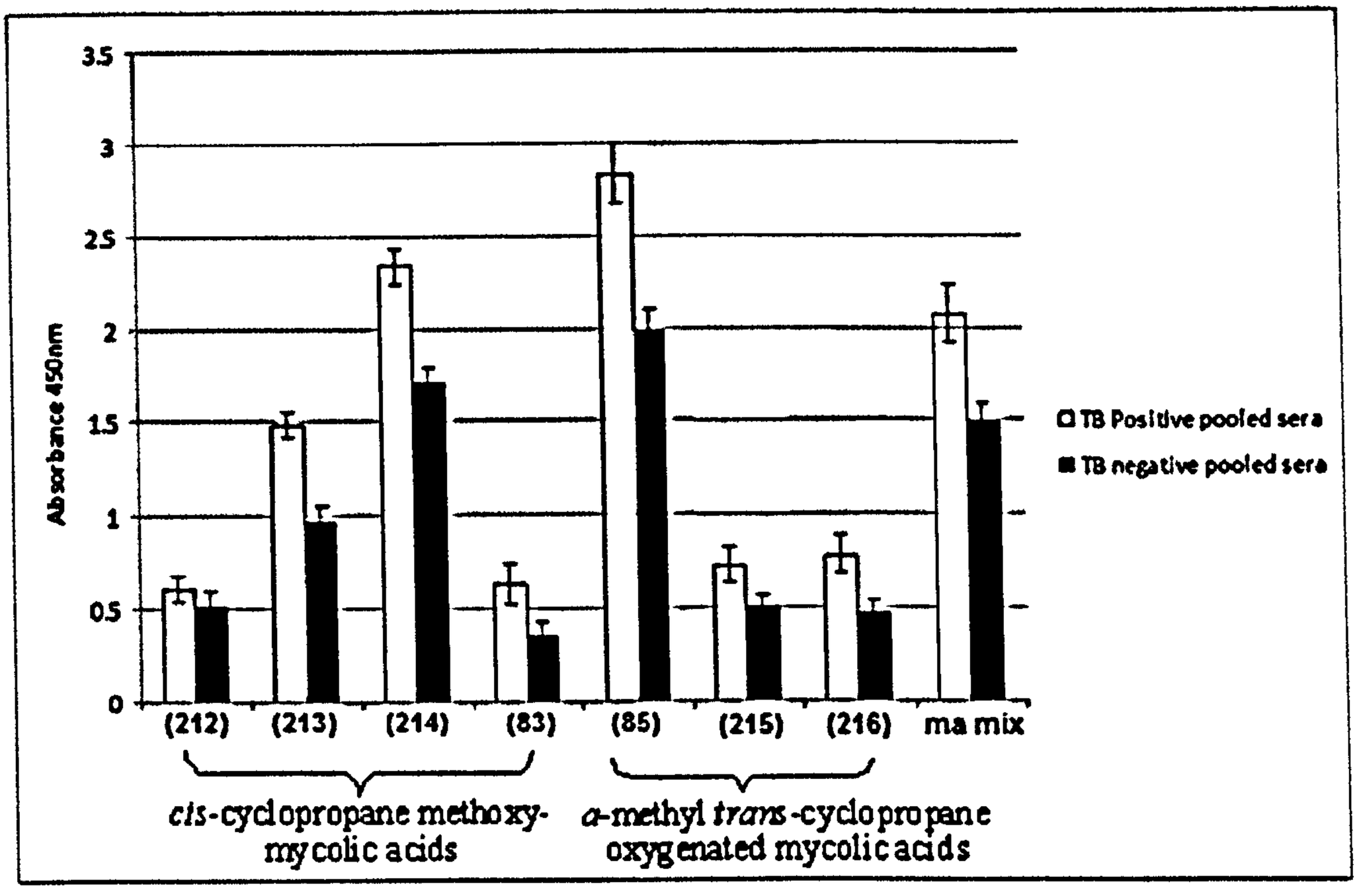
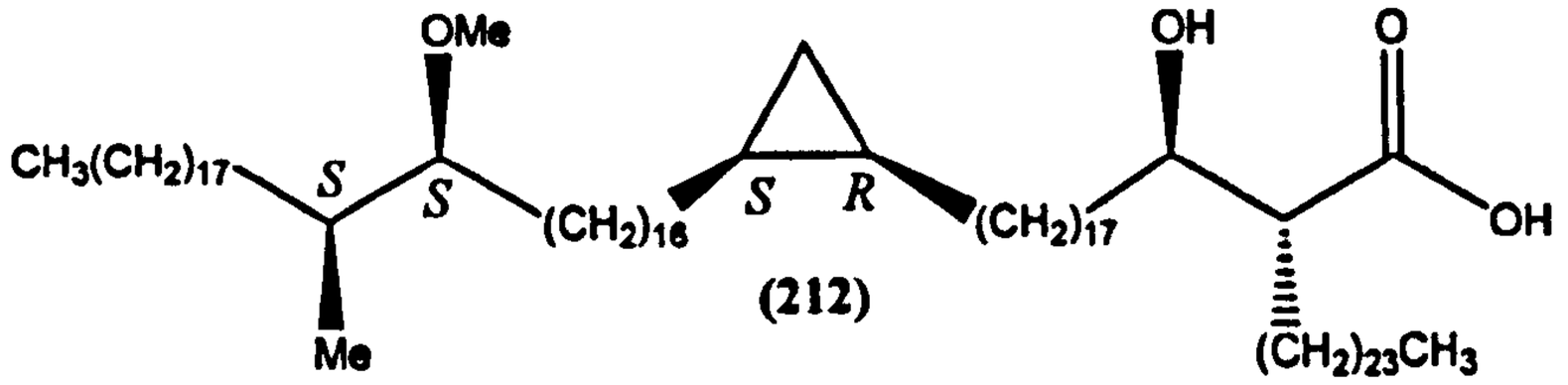
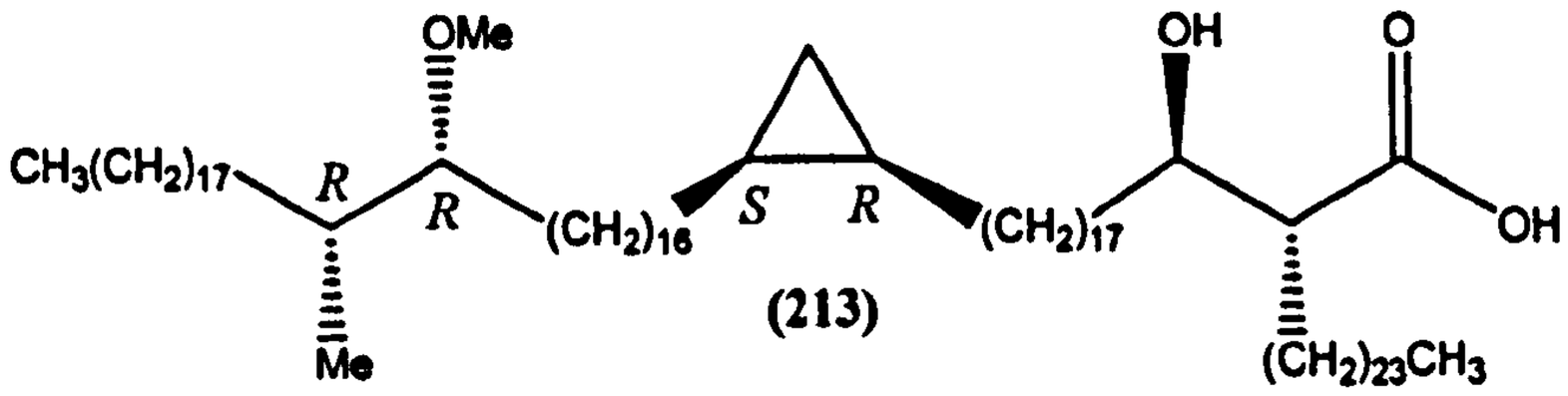


Fig 155: Synthetic mycolic acid ELISA assay results[206]

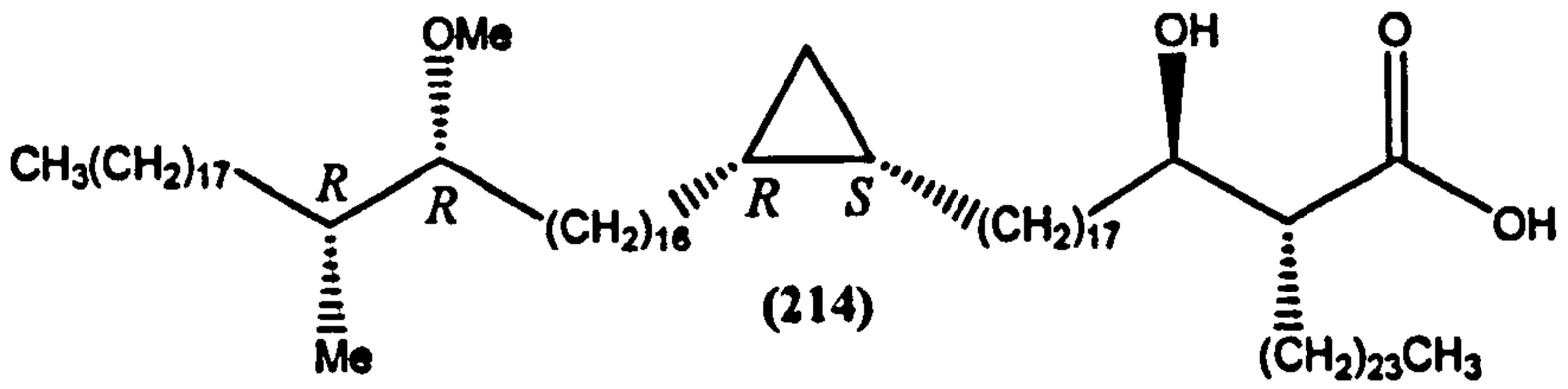
The activities of the four compounds, shown in Fig. 156 fall into the following trend **(214)** > **(213)** > **(212)** ≈ **(8)**. What is of particular interest is the distinct difference between compounds **(214)** and **(83)**, where the only structural variance is in the distal position. These preliminary results indicate that the presence of a methoxy-mycolate in the *R,R* configuration yields improved recognition by TB anti-bodies. Another interesting point is the relationship activity between compounds **(213)** and **(214)**, where the only structural variance is in the proximal *cis*-cyclopropane ring. The results show that there is a distinct increase in recognition when the *cis*-cyclopropane ring is found in the *R,S*-configuration.[207]



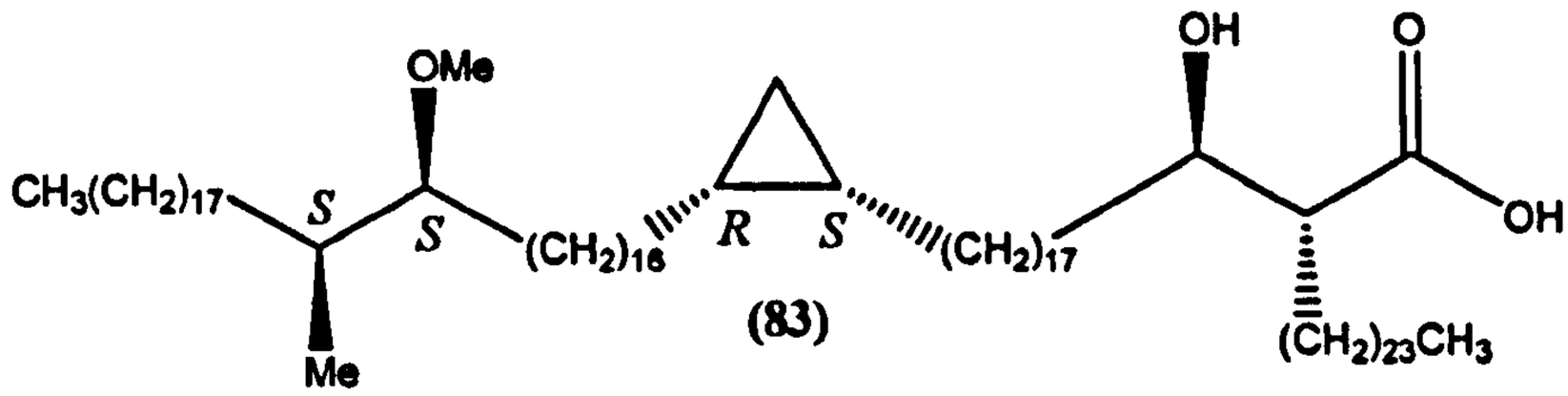
(212)



(213)



(214)



(83)

Fig. 156: *cis*-Cyclopropane methoxy-mycolic acids

The results indicated that the α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)** (See Fig. 157) showed excellent activity when compared to other mycolates tested. However as **(84)** was the first enantiomerically pure α-methyl *trans*-cyclopropane methoxy-mycolic acid prepared it is difficult to draw comparisons, as there are presently no diastereoisomers to directly compare to. However, two other related samples were prepared and tested, a α-methyl *trans*-cyclopropane hydroxy-mycolic acid **(215)**, in the same configuration as **(84)**, and a racemic mixture of α-methyl *trans*-cyclopropane keto-mycolic acid **(216)** (See Fig. 157). Although the chain lengths of the samples tested are not identical an interesting trend occurred in the results where recognition by TB antibodies was in the order methoxy > hydroxy ≈ keto.[206]

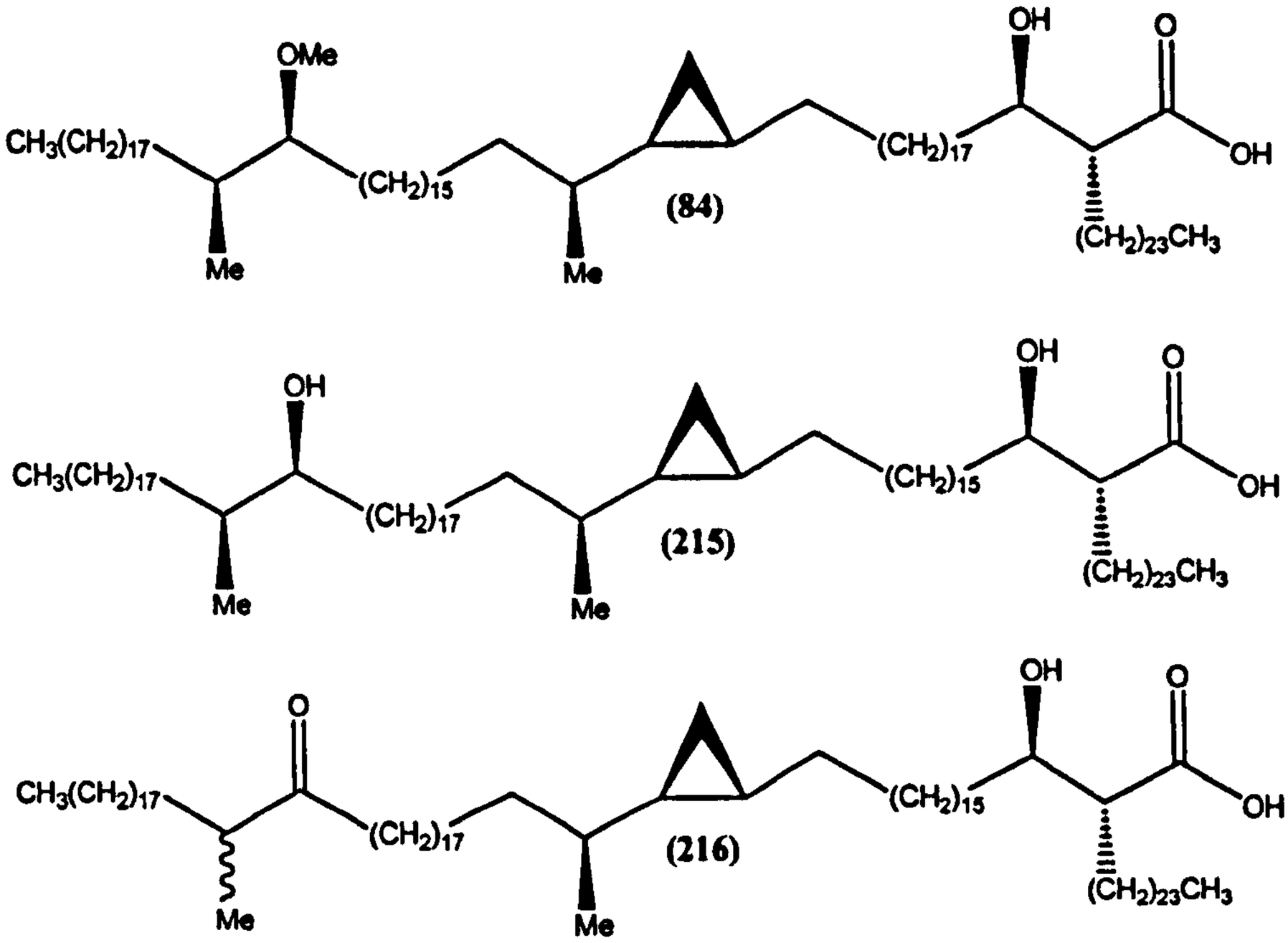


Fig. 157: α-Methyl *trans*-cyclopropane mycolic acids

Also another interesting point is that when comparing *cis*- and *trans*-isomers there is a distinct difference. The *trans*-isomer **(84)** shows a five-fold increase in recognition in comparison to its *cis*-counterpart **(212)**.[206] However, this comparison cannot be regarded as being direct, as the trans-isomer **(84)** has a methyl group adjacent to cyclopropane ring and the *cis*-isomer **(212)** does not posses a methyl group in this position. This information is important as it provides evidence that the stereochemistry of the cyclopropane rings of a mycolic acid play an important role in the recognition of MA by *M.Tb* antibodies. It is believed that the methyl branch adjacent to the *trans*-cyclopropane ring leads to a different packing of mycolic acids, in comparison to *cis*-isomers.[137] The increase in activity shown by the trans-isomer may be due to the conformation of the compound caused by the methyl group adjacent to the cyclopropane, as the packing of the mycolic acid may be more preferential. This may provide the key to increasing the sensitivity and selectivity of sensors to detect the disease in the presence of HIV/AIDS.

2.6.2 – Asthma related immunological activity

Work carried out by the Prof. Johan Grooten research group has yielded additional interesting results.[207, 208] The data provided to the author shows results for compound **(84)** compared to a range of different synthetic mycolic acids; only selected data are reported below. The data confirms that the stereochemistry of the proximal position does have a significant effect in a series of bioassays. The compounds chosen from this set of results to illustrate this hypothesis are shown in Fig. 158.

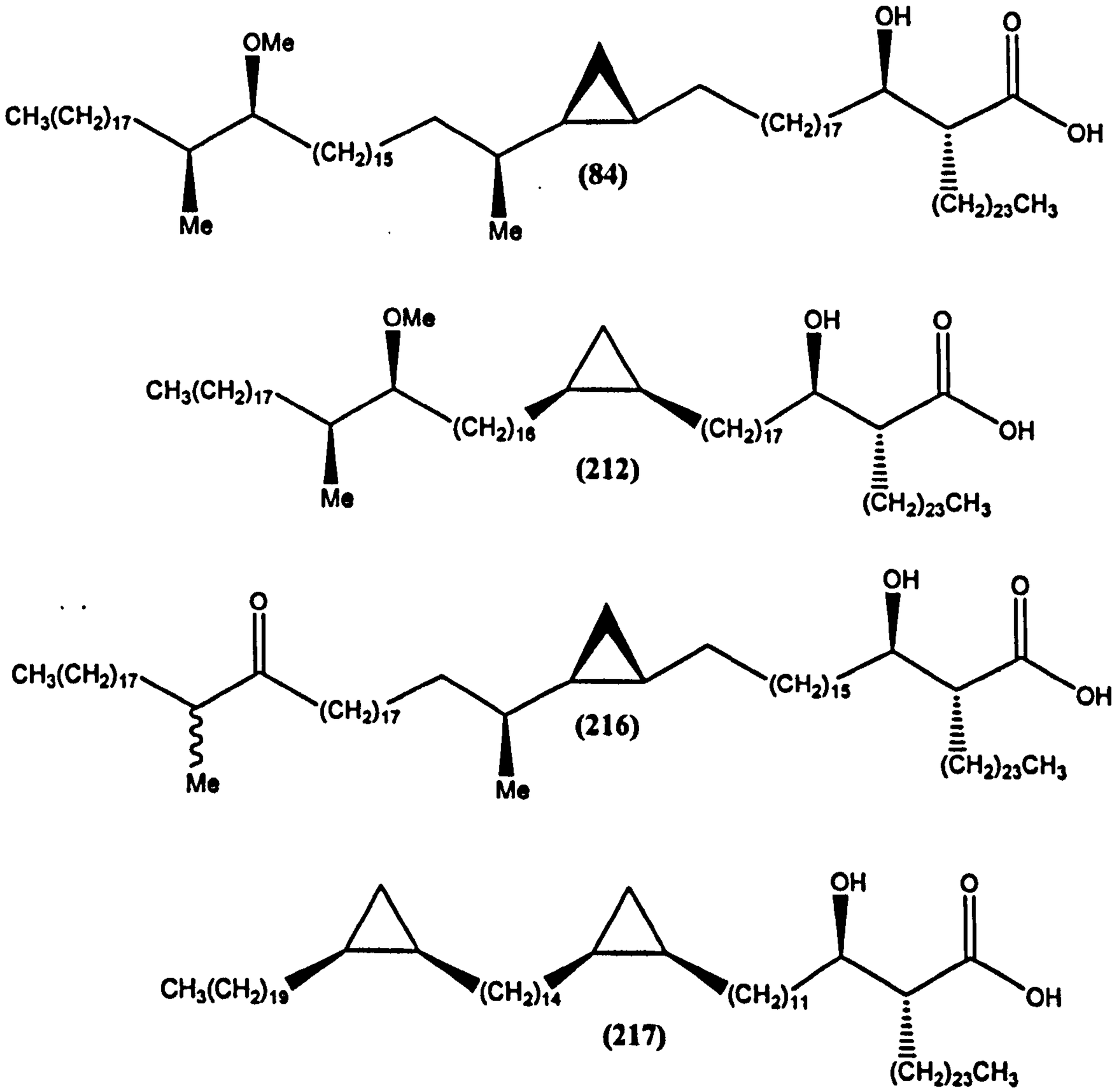


Fig. 158: Mycolic acid samples

Firstly, the brocheo-alveolar inflammatory cell infiltrate was measured. The results show that mycolic acids from *M.Tb* elicit an innate immune airway response,

where the number of neutrophils, white blood cells, represented the majority and were significantly increased by the process. The numbers were recorded using a broncho-alveolar lavage (BAL) to recover cells from the airway. Fig. 159 shows the total number of cells collected as a result of the response, where the broken line indicates the mean number of cells per ml in the BAL-fluid.

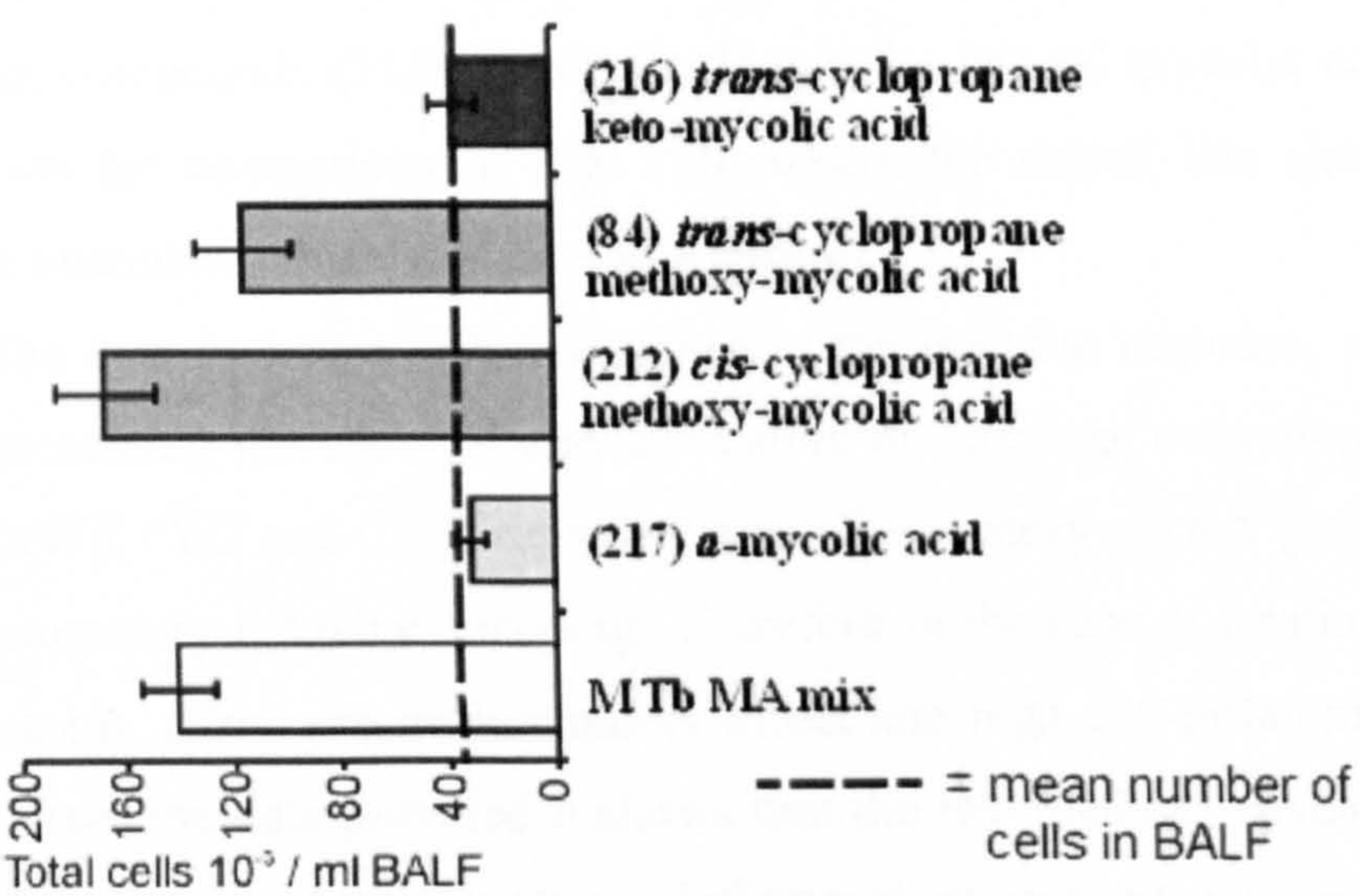


Fig. 159: Broncho-alveolar inflammatory cell count[207, 208]

The results indicated that the α-methyl *trans*-cyclopropane methoxy mycolic acid **(84)** prepared by the author showed lower activity in comparison to the natural mycolic acid mixture, and significantly lower than the *cis*-cyclopropane methoxy-mycolic acid **(212)**. This provides important information into the activity of different subclasses of mycolic acids. It is clear to see that there is a definite difference between the methoxy and keto variations of the α-methyl *trans*-cyclopropane containing mycolic acids, with the methoxy performing better than the keto, also both methoxy mycolates tested, **(84)** and **(212)**, show differing results. This is interesting as it is now apparent from this data that the *cis*-cyclopropane containing compound **(212)** is more active that the α-methyl *trans*-cyclopropane containing compound **(84)**. The data collected shows two definite trends in reduction of imflammation brought on by mycolic acids, where *trans*-cyclopropane containing mycolic acids elicit more of a reduction that *cis*-cyclopropanes. Also alpha-mycolates show the best response, followed by keto and then methoxy, alpha > keto

> methoxy. As alpha show the best response with regard to neutrophillic inflammation, therefore it would be sensible to investigate other configurations of alpha-mycolic acids.

During this study the α-methyl *trans*-cyclopropane methoxy mycolic acid **(84)** was also tested for its immunological response, particularly alveolar macrophage transcription activity. These tests were carried out using liposome carriers to transmit the mycolic acid isomers to their target, alveolar macrophages. Data from compounds **(212)**, **(216)**, **(217)** and the natural mycolic acid mixture is also shown for comparison in Fig. 160, where the dotted line shows an empty liposome treatment, which was used as a control.

The data indicates different stages in the immune response, with *Nos2* and *Arg1* representing the classical and alternative macrophage activation markers. *Il6*, *Il12b*, *Cxcl10*, *Ccl2* and *Cxcl1* represent pro-inflammatory mRNA genes and *Il10* for anti-inflammatory cytokine encoding. Therefore in the case of treating asthma with mycolic acids, a low pro-imflammatory effect and high anti-inflammatory effect is desired. From the data provided it shows that the two methoxy-mycolates, **(84)** and **(212)**, elicit an increase in both pro-inflammatory and anti-inflammatory effects, whereas alpha-mycolates show little or no response, and keto-mycolates showed a slight response. Therefore the conclusion can be drawn that mycolic acids containing an oxygenated functionaliy in the distal position elicit inflammatory responses.

What is also interesting about these results is that the two methoxy mycolates, **(84)** and **(212)**, only significantly differed in activity in the case of *Cxcl1* expression; *Cxcl1* translates to the most important neutrophil chemoattracting and activating chemokine CXCL1.

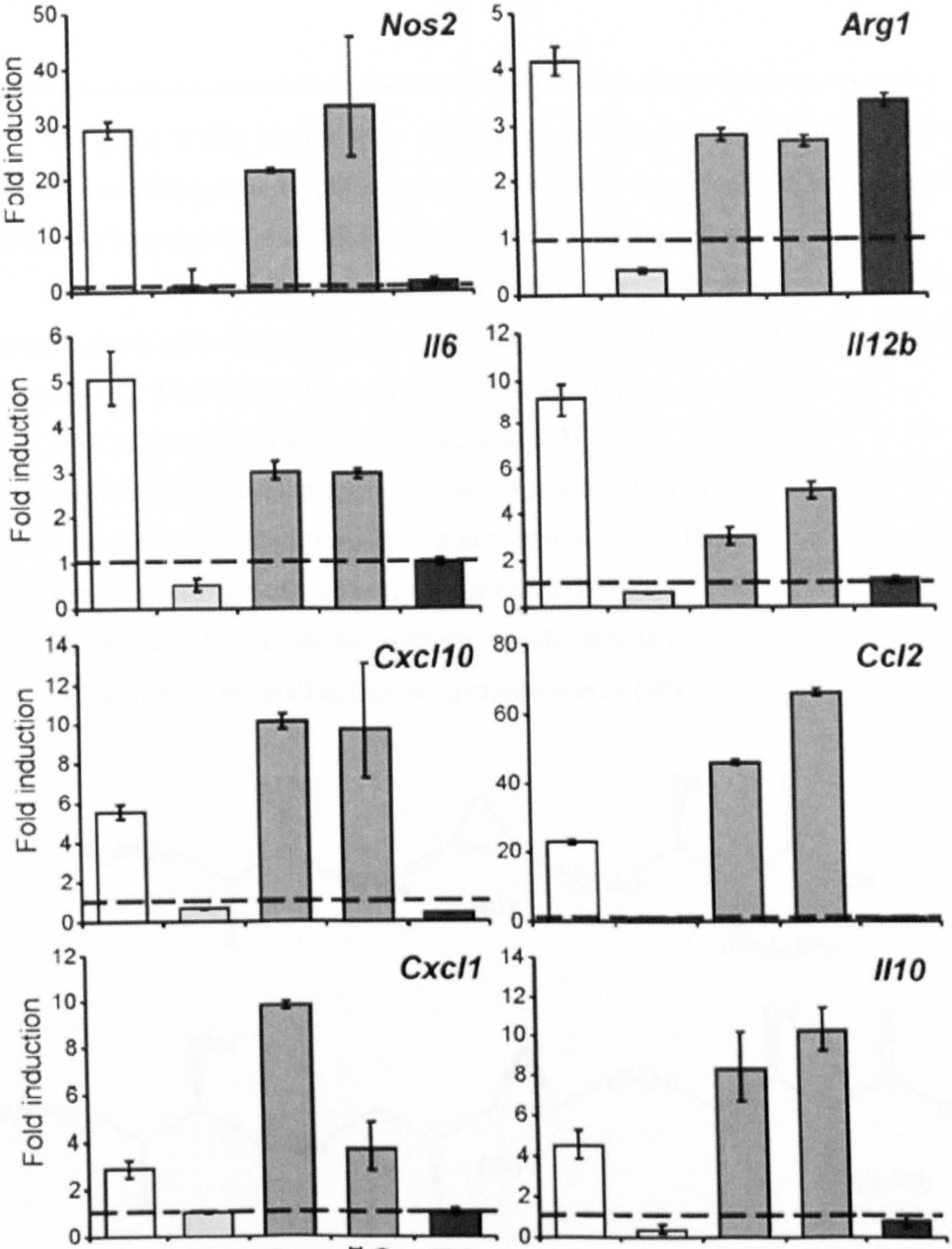


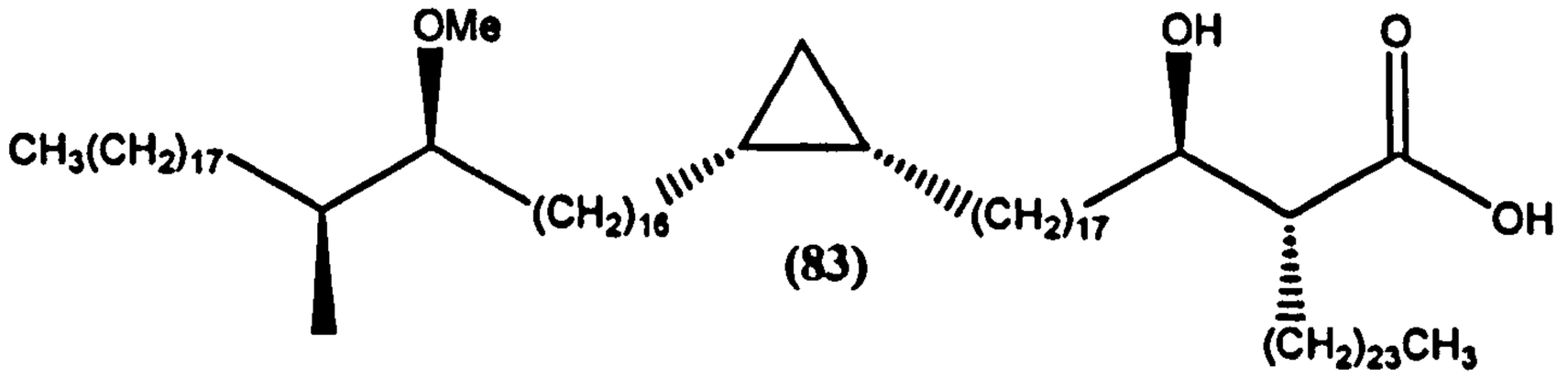
Fig. 160: Alveolar macrophage inflammatory gene transcription[207, 208]

Chapter 3 - Conclusions

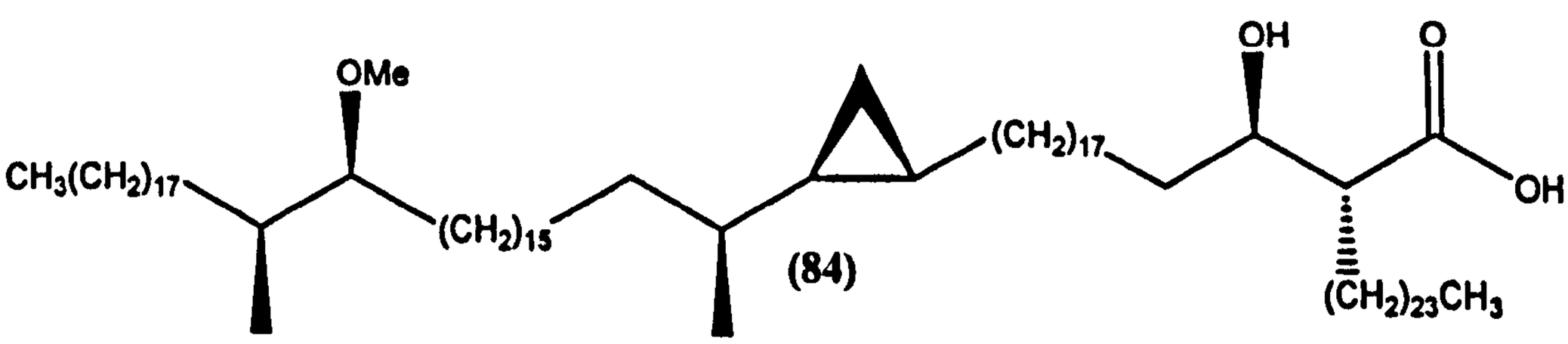
The goals of this project were to synthesise a range of oxygenated mycolic acids that are components of pathogenic mycobacteria; the mycolic acids prepared were then to be tested for their biological activity toward TB and asthma.

Six naturally occuing mycolic acids were successfully synthesised, for the first time, along with several key intermediates, which will provide building blocks for future work. The six mycolic acids synthesised were;

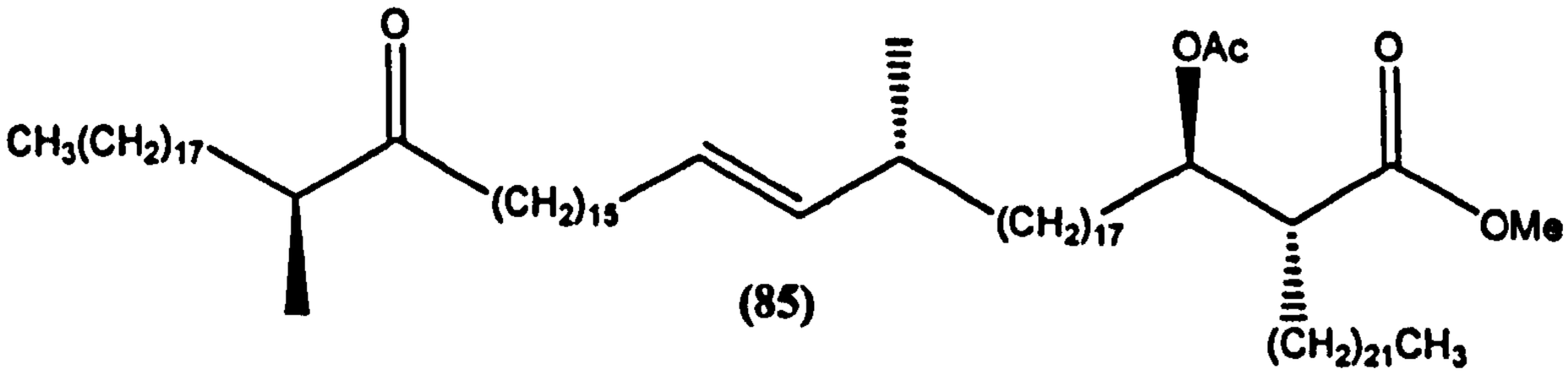
1. *cis*-Cyclopropane methoxy-mycolic acid **(83)**
2. α-Methyl *trans*-cyclopropane methoxy-mycolic acid **(84)**
3. Protected *S* α-methyl *trans*-alkene keto-mycolic acid **(85)**
4. *R*-α-Methyl *trans*-alkene keto-mycolic acid **(86)**
5. *R,R* α-Methyl *trans*-alkene hydroxy-mycolic acid **(87)**
6. *S,S* α-Methyl *trans*-alkene hydroxy-mycolic acid **(88)**



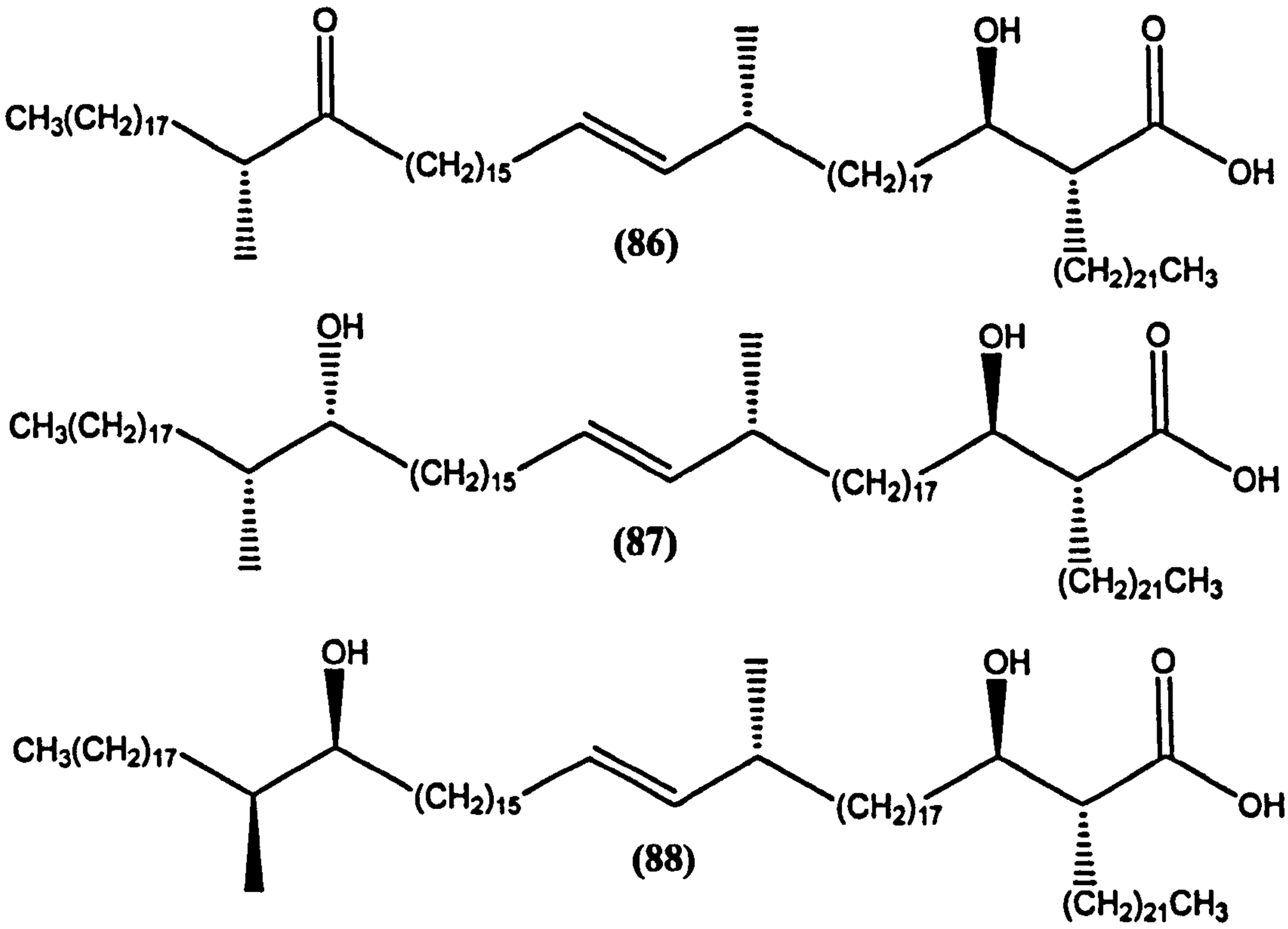
(83)



(84)



(85)



(86)

(87)

(88)

The *cis*-cyclopropane methoxy-mycolic acid **(83)** was selected as a target molecule for this project as J. R. Al-Dulayymi *et al.* had completed the synthesis of three *cis*-cyclopropane methoxy-mycolic acids with differing configurations. Therefore the preparation of **(83)** was carried out to complete the set of major isomer variants from *M.Tb*, enabling exploration of the effects of differing chiral centres on biological activity.

The synthesis of α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)** was completed, this being the first of its sub-class, present in *M.Tb,* to be prepared. Biological data collected for the α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)** and the four *cis*-cyclopropane methoxy mycolic acids prepared by the author and J.R. Al Dulayymi *et al.*[182, 183] showed that the configuration of the cyclopropane ring in the proximal position has an effect upon the performance of the compound on a biological level.

The four *trans*-alkene mycolic acids **(85-88)** were prepared during the course of this work. Preparation of the *S* configuration of α-methyl *trans*-alkene keto-mycolic acid ester **(85)** and the *S,S* α-methyl *trans*-alkene hydroxy-mycolic acid **(88)** was completed without epimerisation of the chiral centres. G. Koza discussed the synthesis of the *R* configuration, however he was unable to hydrolyse the acetate and

methyl ester groups.[183] The hydrolysis of the protected precurser described by G. Koza, was completed to produce *R*-α-methyl *trans*-alkene keto-mycolic acid **(87)**, and the corresponding hydroxy-mycolic acid, *R,R* α-methyl *trans*-alkene hydroxy-mycolic acid **(87)** was also prepared to complete the synthesis of all four *trans*-alkene mycolic acids. In this report the author discusses the preparation of the first α-methyl *trans*-alkene hydroxy-mycolic acids and also the first deprotected α-methyl *trans*-alkene keto-mycolic acid, which are naturally occurring in *M. marinum*.

The work carried out provides a synthetic route for the preparation of α-methyl *trans*-alkene mycolic acids, without epimerisation of any chiral centres. This was important as methods previously discussed by G. Koza[183] gave incomplete synthesis of α-methyl *trans*-alkene mycolic acids. The author's work now confirms that the procedure developed by G. Koza *et al.* can be used to synthesize α-methyl *trans*-alkene keto and hydroxy mycolic acids, without epimerisation of any chrial centres.[188] Should the immunological reports on these compounds be positive, this would provide further validity to continue work in the preparation of other α-methyl *trans*-alkene mycolic acids. Another area of future work could be in the preparation of the S-α-methyl *trans*-alkene keto-mycolic acid; an attempt was made to prepare this compound, however due to time restraints and the small quantity of intermediates, this was not completed.

The current synthetic strategy for mycolic acids is very reliable and flexible; in particular the use of the modified Julia-Kocienski olefination, followed by a hydrogenation is an excellent method for chain extension. Any stereochemistry present, in either aldehyde or sulfone used, is retained and a simple hydrogenation at atmospheric pressure and room temperature, or use of a di-imide completes the synthesis. The modified Julia-Kocienski olefination was also used in the preparation of *trans*-alkene mycolic acids, again with retention of stereochemistry in the adjacent methyl group. With optimisation of the *trans*-alkene olefination, this reaction would prove to be highly practical.

L-Ascorbic acid **(52)** and *D*-mannitol **(60)** were used as starting materials in the preparation of mycolic acids as they are low in cost and readily available. One issue when using L-ascorbic acid **(52)** is that hydrogenation at high pressure is required to obtain the corresponding lactone required; some problems were presented in obtaining the use of an adequate reactor. However there may be a possible method which could be exploited that would provide an alternative route, D-mannitol **(60)**

would be used as a starting material in the preparation of such intermediates, where an **(61)** can be obtained from methods discussed in this thesis, reduction of the methyl ester using lithium aluminium hydride to give the hydroxide **(218)**. Epoxidation of the olefin **(218)** and subsequent ring opening of **(219)** yields the diol **(220)**. From this the intermediate **(221)** can be obtained as an alternative to the current methods being used.[209]

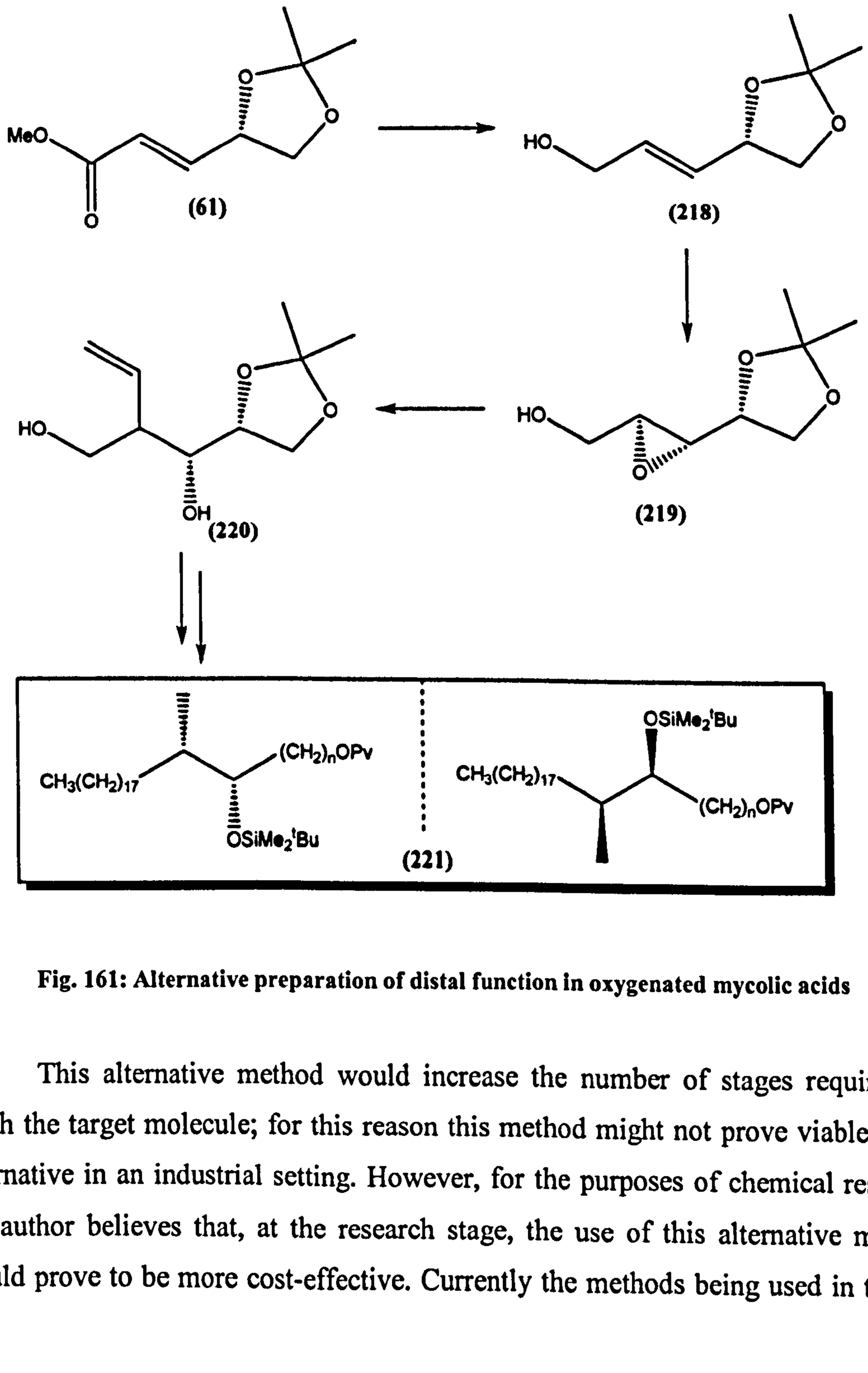


Fig. 161: Alternative preparation of distal function in oxygenated mycolic acids

This alternative method would increase the number of stages required to reach the target molecule; for this reason this method might not prove viable as an alternative in an industrial setting. However, for the purposes of chemical research the author believes that, at the research stage, the use of this alternative method would prove to be more cost-effective. Currently the methods being used in the M.

S. Baird research group would not be too convenient as they rely heavily upon column chromatography as a purification method. Therefore, in addition to reducing the length of the scheme, alternative purification methods, such as recrystallisation or the development of automated chromatography methods, would make mycolic acid synthesis more commercially viable.

The results of biochemical assays, which have been received, are interesting as it was previously believed that the stereochemistry of the cyclopropane ring had little effect on the virulence of the mycobacteria.[157] However, although the ELISA results are preliminary, they indicate that the stereochemistry of the both proximal and distal functionalities have a significant influence on the detection of the mycobacteria, as they show a differential antibody recognition in an ELISA assay. Following the results of the biological activity testing another viable area of future study is in the preparation of the remaining α-methyl *trans*-cyclopropane methoxy-mycolic acids. The data for the *cis*-cyclopropane methoxy-mycolic acids has shown that the configuration of the cyclopropane ring, the presence of a methyl branch adjacent to the cyclopropane ring and the configuration of the distal α-methyl methoxy functionalities both affect antibody production. Therefore with the promising data received for α-methyl *trans*-cyclopropane methoxy-mycolic acid **(84)**, exploring the activity of the other configurations may provide some interesting results.

With regard to the studies carried out by the Johan Grooten research group, they provided results which show that mycolic acids containing an oxygenated distal position elicit a higher inflammatory response. The results also indicate that this response can be both pro-inflammatory and anti-inflammatory; hence a viable area of future study could be to explore these results further and to prepare relevant compounds which could help give a higher anti-inflammatory response.

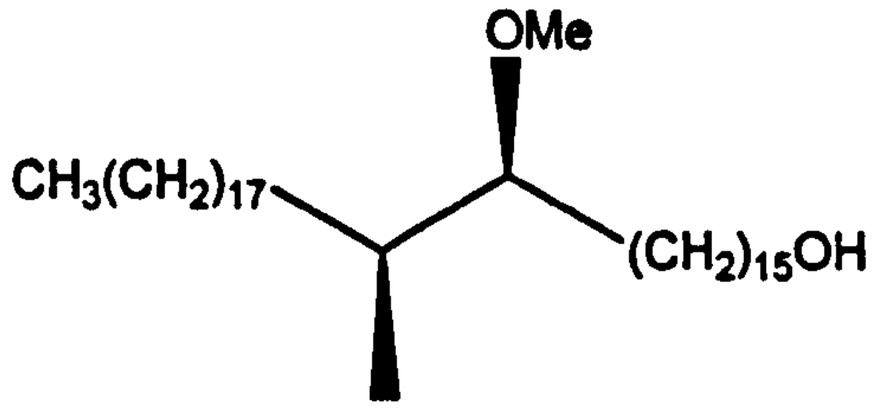
Chapter 4 - Experimental

Chemicals used were obtained from commercial suppliers or prepared from them by the methods described. Diethyl ether and tetrahydrofuran were dried over sodium wire using benzophenone as an indicator; dichloromethane was dried over calcium hydride powder. Petrol used was of boiling point 40-60°C. Reactions carried out under inert conditions were under a slow stream of nitrogen. Those carried out at low temperatures were cooled using a bath of methylated spirits and liquid nitrogen. All reagents and solvents used were of reagent grade unless otherwise stated. Anhydrous magnesium sulfate was used as a drying agent. Aldrich provided silica gel and silica gel plates used for column chromatography and thin layer chromatography respectively. Anhydrous magnesium sulfate was used to dry organic solutions. IR spectra were carried out on a Perkin-Elmer 1600 F.T.I.R. spectrometer as liquid films. NMR spectra were carried out on a Bruker AC250 or Advance 500 spectrometer; for ^{1}H spectra the machine was ran at 500MHz, for ^{13}C spectra the machine was ran at 125MHz, (+ = CH_2, - = CH, CH_3). $[\alpha]_D$ values were recorded in $CHCl_3$ on a POLAAR 2001 Optical Activity polarimeter. Mass spectra were recorded on a Bruker Microtof. Matrix-assisted laser desorption/ionization mass spectrometer values are given plus sodium to an accuracy of 2 d.p..

The preparation of known intermediates used in this project was carried out using literature methods, information regarding these procedures can be found in the accompanying appendices on Page 246.

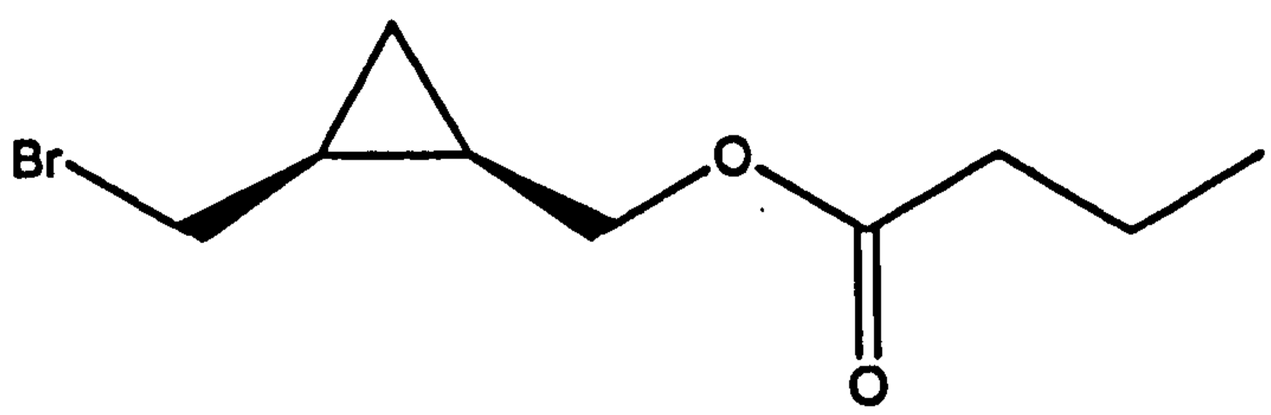
4.1 - Synthesis of *cis*-cyclopropane methoxy-mycolic acid (83)

Experiment 1: (16*S*,17*S*)-16-Methoxy-17-methyl-pentatriacontan-1-ol (107)



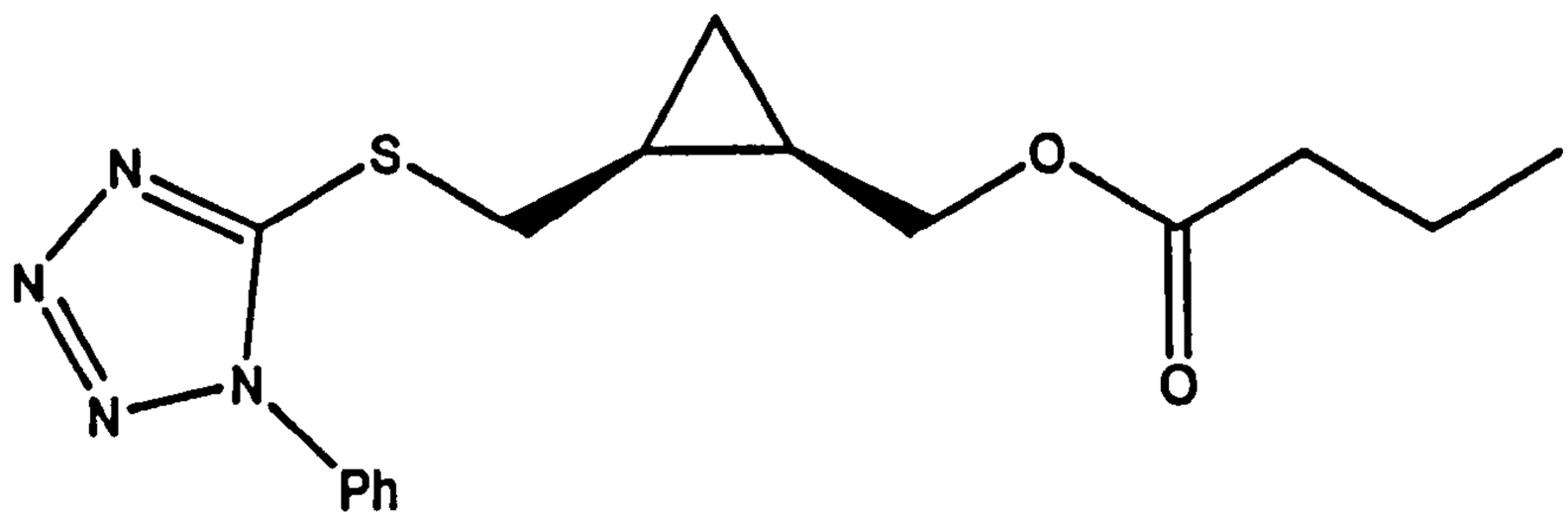
Lithium aluminium hydride (0.56 g, 16.10 mmol, 2.5 mol. equiv.) was added portionwise to a stirred THF (100 ml, HPLC grade) at -20 °C, where vigorous evolution of hydrogen was observed. A solution of (16*S*,17*S*)-16-methoxy-17-methyl-pentatriacontyl ester **(106)** (4.10 g, 164.0 mmol) in THF (50 ml) was added dropwise to the above suspension at -20 °C and then the reaction mixture was refluxed for 2 hrs. When TLC analysis indicated completion of the reaction a freshly prepared solution of sat. aq. sodium sulfate (40 ml) was added at -20 °C, where formation of a white precipitate was observed, and the reaction mixture stirred at RT for 2 hrs. The solution was filtered through a bed of silica gel and the solvent evaporated. The resulting solution was taken up in dichloromethane (100 ml) and washed with water (25 ml) and then dried. The solvent was evaporated and the crude product was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a white solid, (16S,17S)-16-methoxy-17-methyl-pentatriacontan-1-ol **(107)** (2.64 g, 79 %), which showed δ_H (500 MHz, $CDCl_3$): 3.72 (2H, t, J 6.65 Hz), 3.35 (3H, s), 3.00-2.95 (1H, m), 1.68-1.65 (1H, m), 1.60 (2H, pent, J 6.65 Hz), 1.48 (1H, br.s) 1.46-1.20 (59H, m), 1.16-1.06 (1H, m), 0.89 (3H, t, J 7 Hz) 0.86 (3H, d, J 6.65 Hz); δ_C (125 MHz, $CDCl_3$): 86.0, 63.0, 57.7, 35.4, 32.9, 32.4, 31.9, 30.5, 30.0, 29.9, 29.72, 29.68, 29.6, 29.5, 29.4, 27.6, 26.2, 25.7, 22.8, 15.0, 14.2; ν_{max}: 3380, 2934, 1108, 1082 cm^{-1}; $[\alpha]^{22}_D$ = -8.23 ($CHCl_3$, 1.322 μmol); m.p. 46-48 °C; [Found $M+Na^+$: 575.84; $C_{37}H_{76}NaO_2$ requires: 575.99].

Experiment 2: Butyric acid (1*R*,2*S*)-2-bromomethyl-cyclopropylmethyl ester (114)



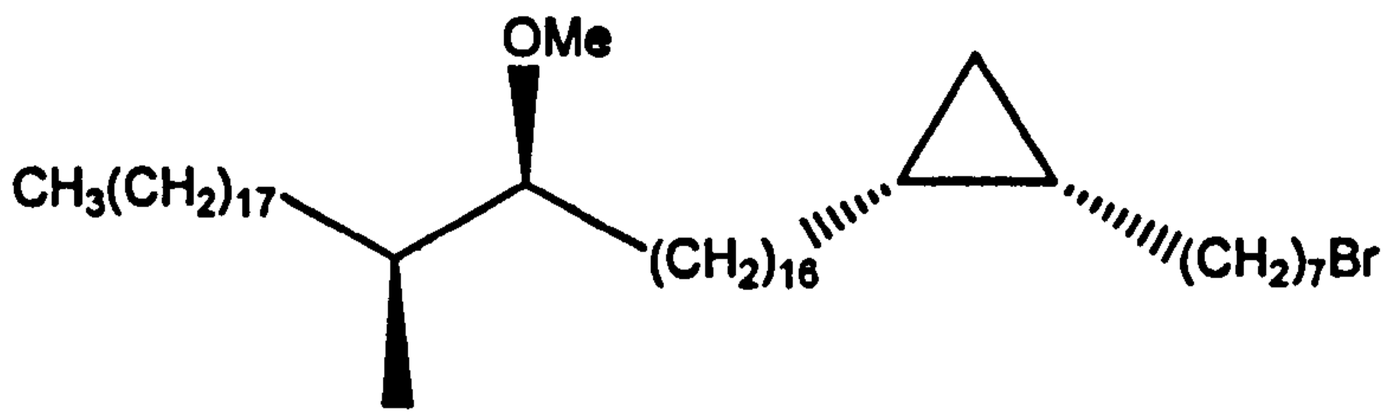
Triphenylphosphine (10.7 g, 40.7 mmol, 1.15 mol. equiv.) was added to a stirred solution of butyric acid (1*R*,2*S*)-2-hydroxymethyl-cyclopropylmethyl ester **(113)** (6.05 g, 35.4 mmol) in dichloromethane (200 ml) and then sodium hydrogen carbonate (1.5 g) was added. The mixture was cooled to 0 °C and N-bromosuccinimide (8.0 g, 44.9 mmol, 1.27 mol. equiv.) was added portionwise over 10 mins at 0 – 4 °C. The reaction mixture was stirred for 1 hr at 0 – 3 °C, when TLC analysis indicated completion of the reaction sat. aq. sodium bisulfite (150 ml) was added and the mixture was extracted. The aqueous layer was re-extracted with dichloromethane (2 x 100 ml) and the combined organic layers washed with water (200 ml) and then dried. The solvent was evaporated and the crude product was taken up in petrol/ether (1:1, 200 ml) and stirred for 30 mins. The triphenylphosphonium oxide was filtered off and washed well with petrol/ether (1:1, 100 ml) and the solvent evaporated. The crude product was purified via column chromatography eluting with petrol/ether (4:1, then 2:1) to give a colourless oil, butyric acid (1*R*,2*S*)-2-bromomethyl-cyclopropylmethyl ester **(114)** (7.42 g, 90 %), which showed δ_H (500 MHz, $CDCl_3$): 4.21 (1H, dd, J 6.9, 11.8 Hz), 4.05 (1H, dd, J 7.9, 11.8 Hz), 3.52 (1H, dd, J 7.5, 10.2 Hz), 3.40 (1H, dd, J 7.9, 11.8 Hz), 2.33 (2H, t, J 7.75 Hz), 1.62 (2H, sext, J 7.75 Hz), 1.52-1.32 (2H, m), 0.98 (1H, dt, J 5.2, 8.5 Hz), 0.92 (3H, t, J 7.6 Hz), 0.37 (1H, q, J 5.2 Hz); δ_C (125 MHz, $CDCl_3$): 174.3, 63.5, 36.6, 34.5, 19.5, 18.7, 18.1, 14.1, 12.9; ν_{max}: 2966, 2887, 1754, 1543, 1323, 1189, 1089 cm^{-1}; $[\alpha]^{22}_D$ = -11.1 ($CHCl_3$, 0.984 μmol) [Found M+ : 235.1203; $C_9H_{15}BrO_2$ requires: 235.1211].

Experiment 3: Butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazol-5-ylsulfanylmethyl) -cyclopropylmethyl ester (115)



Butyric acid (1*R*,2*S*)-2-bromomethyl-cyclopropylmethyl ester **(114)** (7.42 g, 31.7 mmol) was added with vigorous stirring to 1-phenyl-1H-tetrazole-5-thiol (11.30 g, 63.4 mmol, 2 mol. equiv.) and potassium carbonate (8.75 g, 63.4 mmol, 2 mol. equiv.) in acetone (150 ml) and the reaction mixture was stirred for 18 hrs at RT. When TLC analysis indicated completion of the reaction water (1 l) was added and extracted with dichloromethane (4 x 100 ml) and the combined organic layers were washed with brine (2 x 300 ml). The organic extracts were dried and evaporated to give a crude product, which was purified via column chromatography eluting with petrol/ether (2:1, then 1:1) to give a yellow oil, butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazol-5-ylsulfanylmethyl)-cyclopropylmethyl ester **(115)** (10.03 g, 95 %), which showed δ_H (500 MHz, $CDCl_3$): 7.62-7.51 (5H, m), 4.40 (1H, dd, J 6.7, 12.5 Hz), 3.99 (1H, dd J 9.2, 12.2 Hz), 3.56 (1H, dd, J 7.9, 13.2 Hz), 3.41 (1H, dd, J 7.9, 13.2 Hz), 2.30 (2H, t, J 7.5 Hz), 1.67 (2H, sext, J 7.5 Hz), 1.55 (1H, pent, J 5.6, 8.2 Hz), 1.45-1.38 (1H, m), 0.99 (1H, dt, J 5.5, 8.2 Hz), 0.93 (3H, t, J 7.2 Hz), 0.43 (1H, q, J 5.4 Hz); δ_C (125 MHz, $CDCl_3$): 174.5, 154.3, 133.8, 130.1, 129.7, 123.8, 63.7, 36.2, 34.2, 18.4, 16.4, 15.5, 13.6, 11.0; ν_{max}: 2955, 2888, 1754, 1512, 1184, 989, 785 cm^{-1}; $[\alpha]^{23}_D$ = -1.15 ($CHCl_3$, 1.012 μmol) [Found M+ : 332.4213; $C_{16}H_{20}N_4O_2S$ requires: 332.4260].

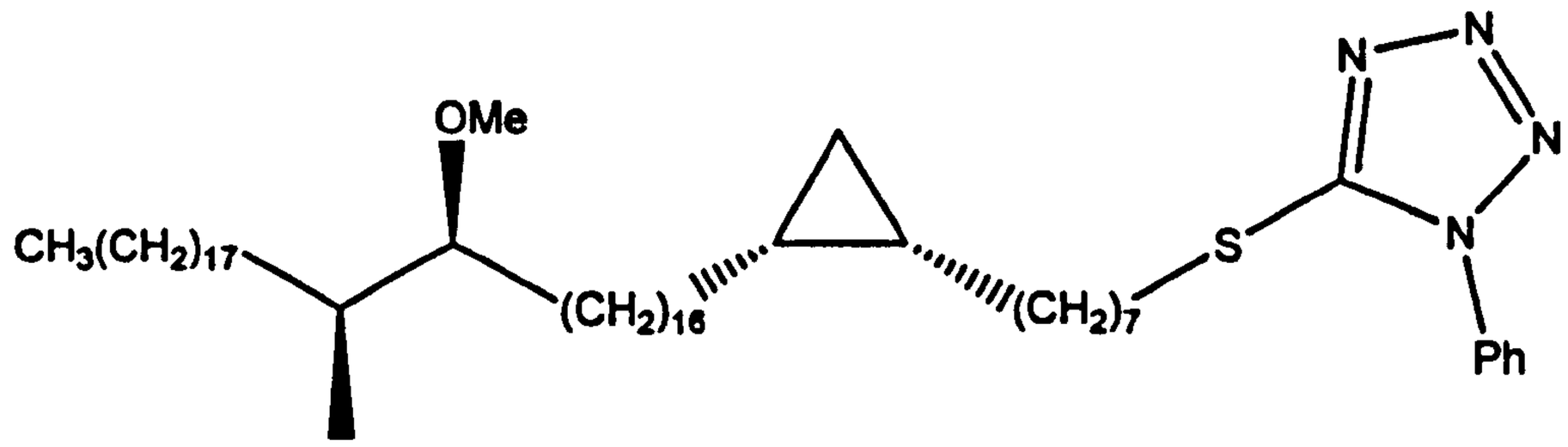
Experiment 4: (1*S*,2*R*)-1-(7-Bromoheptyl)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropane (119)



Lithium *bis*(trimethylsilyl)amide (4.18 ml, 4.44 mmol, 1.06 M) was added dropwise to a stirred solution of (1*R*,2*S*)-2-(7-bromohepytl)cyclopropanecarbaldehyde **(92)** (0.70 g, 2.84 mmol) and 5-((16*S*,17*S*)-16-methoxy-17-methylpentatriacontan-1-sulfonyl)-1-phenyl-1H-tetrazole **(91)** (2.48 g, 3.33 mmol) in dry THF (30 ml) under nitrogen at -15 °C. The mixture was allowed to reach RT and was then stirred for 18 hrs. When TLC analysis indicated completion of the reaction, sat. aq. ammonium chloride (30 ml) and petrol/ether (1:1, 50 ml) were added. The aqueous layer was re-extracted with petrol/ether (1:1, 2 x 25 ml) and the combined organic extracts were washed with brine (25 ml), dried and evaporated. The resultant crude oil was purified via column chromatography eluting with petrol/ether (9:1) to give a viscous oil, (1*S*,2*R*)-1-(7-bromoheptyl)-2-((*E*/*Z*)-(17*S*,18*S*)-17-methoxy-18-methylhexatria-cont-1-enyl)cyclopropane (1.54 g, 72 %). Dipotassium azodicarboxylate (4.97 g, 25.6 mmol) was added to a stirred solution of (1*S*,2*R*)-1-(7-bromoheptyl)-2-((*E*/*Z*)-(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropane (0.64 g, 0.85 mmol) in THF (20 ml) and methanol (10 ml) at 10 °C under nitrogen resulting in a yellow precipitate. A solution of glacial acetic acid (3 ml) and THF (6 ml) was added dropwise over 16 hrs, after which a white precipitate had formed. The mixture was cooled to 0 °C and poured slowly into sat. aq. sodium hydrogen carbonate (10 ml) and the product was extracted with petrol/ether (1:1, 3 x 50 ml). The combined organic extracts were washed with water (30 ml), dried and evaporated to give a thick oil which later solidified. The residue was then purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, (1*S*,2*R*)-1-(7-bromoheptyl)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropane **(119)** (0.42 g, 66 %), which showed δ_H (500 MHz, $CDCl_3$): 3.42 (2H, t, J 7 Hz), 3.34 (3H, s), 2.99-2.93 (1H, m), 1.87 (2H, pent, J 6.7 Hz), 1.66-1.61 (1H, m), 1.50-1.25

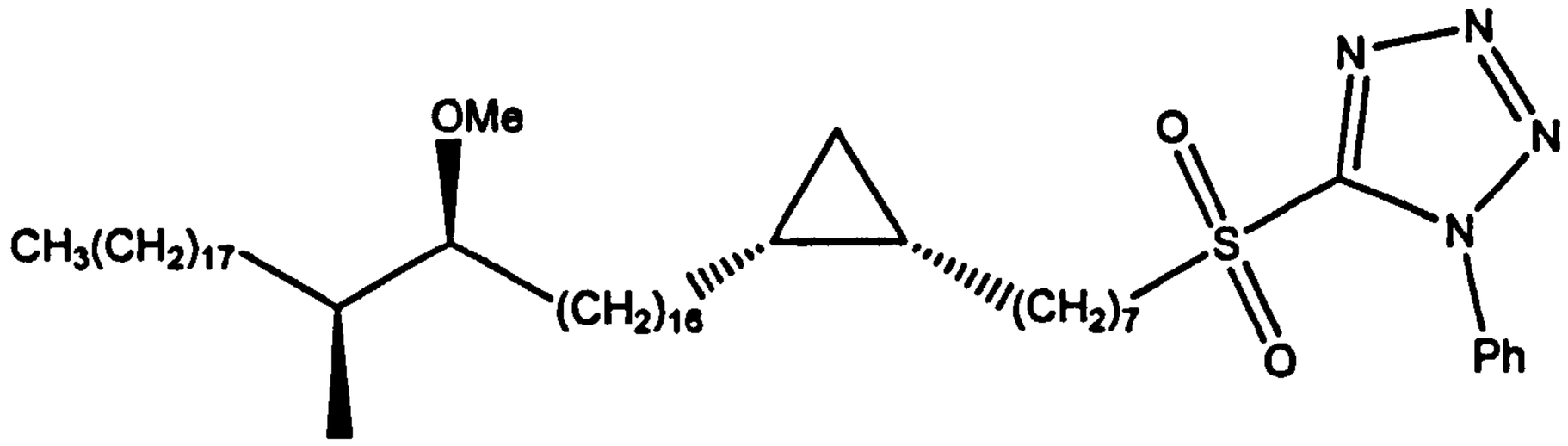
(72H, m), 1.17-1.06 (4H, m), 0.9 (3H, t, J 7 Hz), 0.88 (3H, d, J 7 Hz), 0.75-0.65 (2H, m), 0.61 (1H, dt, J 4, 8 Hz), -0.33 (1H, dt, J 4 Hz); δ_C (125 MHz, $CDCl_3$): 85.4, 57.7, 35.3, 34.0, 32.8, 32.3, 31.9, 30.4, 29.9, 29.7, 29.4, 29.3, 28.8, 28.7, 28.6, 28.2, 27.6, 26.1, 24.1, 22.7, 15.8, 15.7, 14.9, 14.1, 10.9; ν_{max}: 2854, 1465, 1099, 760 cm^{-1}; $[\alpha]^{27}_D$ = -3.40 ($CHCl_3$, 0.786 μmol); m.p. 31-33 °C; [Found M+Na^+: 789.48; $C_{48}H_{95}NaOBr$ requires: 789.65].

Experiment 5: 5-[7-[1*S*,2*R*]-(2-((17*S*,18*S*)-17-Methoxy-18-methyl-hexatriacont-yl)cyclopropyl)heptylsulfany]-1-phenyl-1H-tetrazole (125)



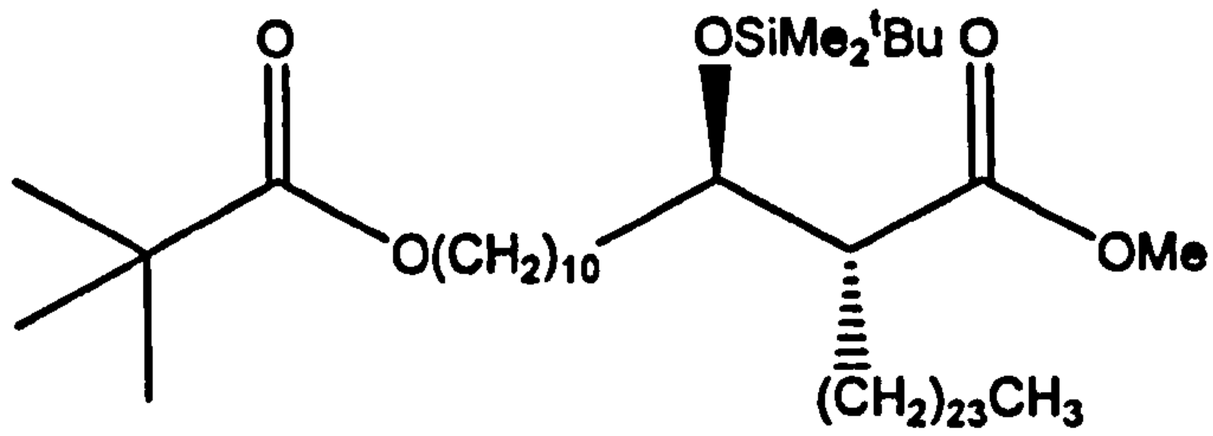
(1*S*,2*R*)-1-(7-Bromoheptyl)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl) cyclopropane **(119)** (0.3 g, 0.39 mmol) in THF (5 ml) was added to a stirred solution of 1-phenyl-1H-tetrazole-5-thiol (0.10 g, 0.58 mmol, 1.5 mol. equiv.) and anhydrous potassium carbonate (0.21 g, 1.52 mmol, 3.9 mol. equiv.) in acetone (15 ml) at RT. The reaction mixture was stirred for 18 hrs; when TLC analysis indicated completion of the reaction, the mixture was diluted with water (20 ml) and dichloromethane (50 ml). The aqueous layer was re-extracted with dichloromethane (2 x 10 ml). The - combined organic extracts were washed with brine (2 x 20 ml), dried and evaporated. The resultant residue was then purified via column chromatography eluting with petrol/ether (5:1) to give a white solid, 5-(7-[1*S*,2*R*]-(2-((17*S*,18*S*)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl)-heptylsulfanyl)-1-phenyl-1H-tetrazole **(125)** (0.23 g, 67 %), which showed δ_H (500 MHz, $CDCl_3$): 7.67-7.58 (5H, m), 3.44 (2H, t, J 7.2 Hz), 3.33 (3H, s), 2.99-2.92 (1H, m), 1.88 (2H, pent, J 7.5 Hz), 1.66-1.62 (1H, m), 1.49-1.23 (72H, m), 1.19-1.08 (4H, m), 0.90 (3H, t, J 7 Hz), 0.87 (3H, d, J 6 Hz) 0.68-0.61 (2H, m), 0.56 (1H, dt, J 4, 7.8 Hz), -0.33 (1H, dt, J 5 Hz); δ_C (125 MHz, $CDCl_3$): 154.3, 134.1, 129.9, 129.7, 123.9, 85.5, 57.7, 35.4, 33.4, 32.4, 31.9, 30.5, 30.2, 30.1, 30.0, 29.9, 29.71, 29.68, 29.6, 29.4, 29.3, 29.12, 29.08, 28.7, 28.6, 27.6, 26.2, 22.7, 15.8, 15.7, 14.9, 14.1, 10.9; ν_{max}: 2934, 2834, 1519, 1443, 1076 cm^{-1} ; $[\alpha]^{21}_D$ = -4.1 ($CHCl_3$, 0.769 µmol); m.p. 36-38 °C; [Found M+Na^+: 887.51; $C_{55}H_{100}N_4NaOS$ requires: 887.75].

Experiment 6: 5-(7-[1*S*,2*R*]-(2-((17*S*,18*S*)-17-Methoxy-18-methyl-hexatria contyl)-cyclopropyl)-heptylsulfonyl)-1-phenyl-1H-tetrazole (89)



m-Chloroperoxybenzoic acid (125.70 mg, 0.73 mmol, 3 mol. equiv) in dichloromethane (2 ml) was added slowly to a stirred solution of 5-(7-[1*S*,2*R*]-(2-((17*S*,18*S*)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl)-heptylsulfanyl)-1-phenyl-1H-tetrazole **(125)** (209.40 mg, 0.24 mmol) and sodium hydrogen carbonate (91.80 mg, 1.09 mmol, 4.5 mol. equiv.) in dichloromethane (2 ml) at 5 °C. The mixture was stirred for 18 hrs at RT, when TLC analysis indicated completion of the reaction the solvent was evaporated. The resultant residue was diluted with ethyl acetate (5 ml) and slowly quenched with sat. aq. sodium metabisulfite (2 ml). The aqueous layer was re-extracted with ethyl acetate (2 x 10 ml) and the combined organic extracts were washed with sat. aq. sodium hydrogen carbonate (10 ml) and then water (20 ml). The organic extract was then dried and evaporated and the resultant yellow oil purified by column chromatography eluting with petrol/ether (1:1) to give a white solid, 5-(7-[1*S*,2*R*]-(2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclo-propyl)-heptylsulfonyl)-1-phenyl-1H-tetrazole **(89)** (140.00 mg, 65 %), which showed δ_H (500 MHz, $CDCl_3$): 7.70-7.68 (2H, dd, J 1.2, 7.8 Hz), 7.63-7.56 (3H, m), 3.75 (2H, t, J 8 Hz), 3.40 (3H, s) 2.95-2.92 (1H, pent, J 4 Hz), 1.99 (2H, pent J 7.5 Hz), 1.66-1.62 (1H, m), 1.55 (2H, pent, J 7.5 Hz), 1.49-1.20 (70H, m), 1.18-1.04 (4H, m), 0.90 (3H, t, J 7 Hz), 0.88 (3H, d, J 7 Hz), 0.70-0.65 (2H, m), 0.59 (1H, dt, J 4, 8 Hz), -0.32 (1H, dt, J 5 Hz); δ_C (125 MHz, $CDCl_3$): 154.7, 133.5, 131.9, 129.8, 125.2, 86.0, 57.8, 56.1, 35.5, 32.5, 32.0, 30.5, 30.2, 30.0, 29.9, 29.72, 29.70, 29.3, 29.2, 28.9, 28.7, 28.6, 28.1, 27.6, 26.2, 22.7, 22.0, 15.8, 15.7, 14.8, 14.1, 10.9; ν_{max}: 2913, 1609, 1541, 1474, 1367, 1183, 1105, 782 cm^{-1}; $[\alpha]^{23}_D$ = -4.67 ($CHCl_3$, 0.983 μmol); m.p. 38-40 °C; [Found $M+Na^+$: 919.63; $C_{55}H_{100}N_4NaO_3S$ requires: 919.74].

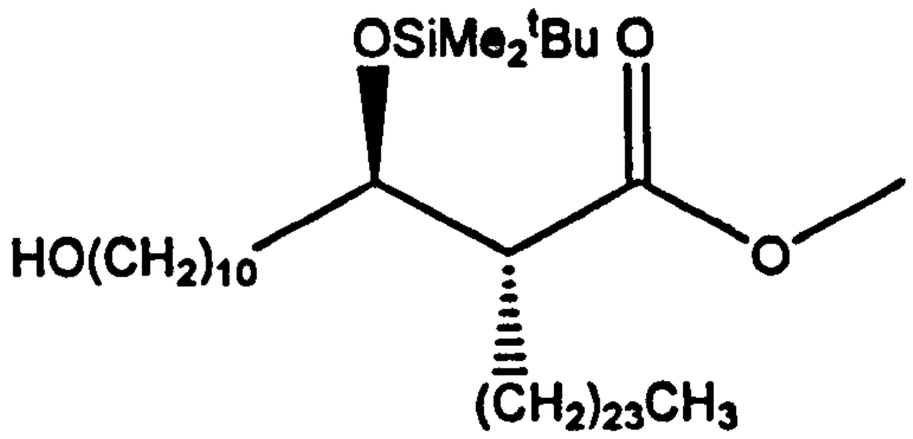
Experiment 7: (*R*)-2-[(*R*)-1-(*tert*)-Butyldimethylsilanyloxy)-11-(2,2-dimethyl-propionyloxy)undecyl]hexacosanoic acid methyl ester (130)



Lithium *bis*(trimethylsilyl)amide (2.41 ml, 2.56 mmol, 1.06 M) was added dropwise to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxo-propyl]-hexacosanoic acid methyl ester **(128)** (1.00 g, 1.64 mmol) and 2,2-dimethyl-propionic acid 8-(1-phenyl-1H-tetrazole-5-sulfonyl)-octyl ester **(96)** (0.83 g, 1,97 mmol, 1.2 mol. equiv.) in dry THF (35 ml) at -15 °C. The mixture was then stirred for 18 hrs at RT; when TLC analysis indicated completion of the reaction, sat. aq. ammonium chloride (25 ml) and petrol/ether (1:1, 50 ml) were added. The aqueous layer was re-extracted with petrol/ether (1:1, 2 x 50 ml) and the combined organic extracts washed with brine (50 ml), dried and evaporated to give a yellow oil. The crude product was purified via column chromatography eluting with petrol/ether (9:1) to give a colourless oil, (*E*/*Z*)-(*R*)-3-(*tert*-butyl-dimethyl-silanyloxy)-13-(2,2-dimethyl-propionyloxy)-2-tetracosyl-tridec-5-enoic acid methyl ester **(129)** (0.92 g, 70 %). Palladium on charcoal (10 %, 0.25 g)was added to a stirred solution of (*E*/*Z*)-(*R*)-3-(*tert*-butyl-dimethyl-silanyloxy)-13-(2,2-dimethyl-propionyloxy)-2-tetracosyl-tridec-5-enoic acid methyl ester **(129)** (0.92 g, 1.03 mmol) in IMS (25 ml) and THF (5 ml). The mixture was stirred while being hydrogenated at atmospheric pressure, when hydrogen absorption was complete the mixture was filtered through a pad of celite and washed with THF (100 ml). The filtrate was evaporated to give a colourless oil, (*R*)-2-[(*R*)-1-(*tert*)-butyldimethylsilanyloxy)-11-(2,2-dimethylpropionyloxy) undecyl]hexacosanoic acid methyl ester **(130)** (0.92, 99.9 %), which showed δ_H (500 MHz, $CDCl_3$): 4.06(2H, t, J 6.5 Hz), 3.94-3.87 (1H, m), 3.66 (3H, s), 2.55-2.50 (1H, m), 1.66-1.24 (64H, m), 1.21 (9H, s), 0.88 (3H, t, J 7 Hz), 0.86 (9H, s) 0.05 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 178.9, 175.1, 73.2, 64.5, 51.6, 51.2, 38.7, 33.7, 31.9, 29.9, 29.7, 29.62, 29.57, 29.51, 29.46, 29.4, 29.3, 29.2, 28.6, 27.8, 27.5, 27.2, 25.9, 25.81, 25.79, 23.7, 22.7, 17.9, 14.1, -4.4, -4.9; v

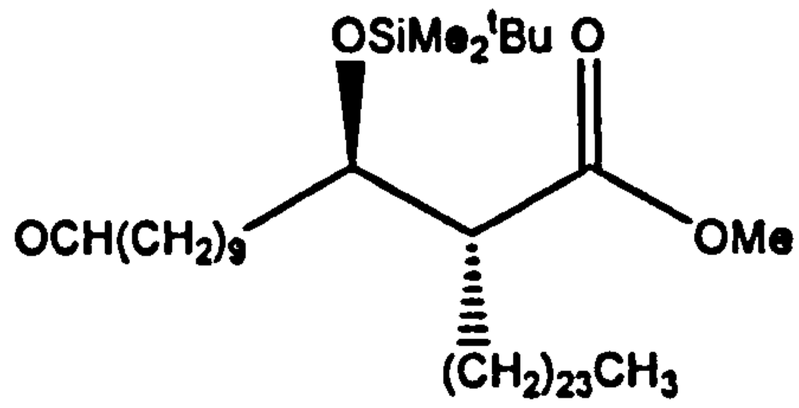
$_{max}$: 2943, 2876, 1743, 1467, 1154 cm^{-1}; $[\alpha]^{22}_{D}$ = -3.21 ($CHCl_3$, 1.323 μmol) [Found M+Na^+: 817.64; $C_{49}H_{98}NaO_5S$ requires: 817.71].

Experiment 8: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-hydroxyundecyl]-hexacosanoic acid methyl ester (131)



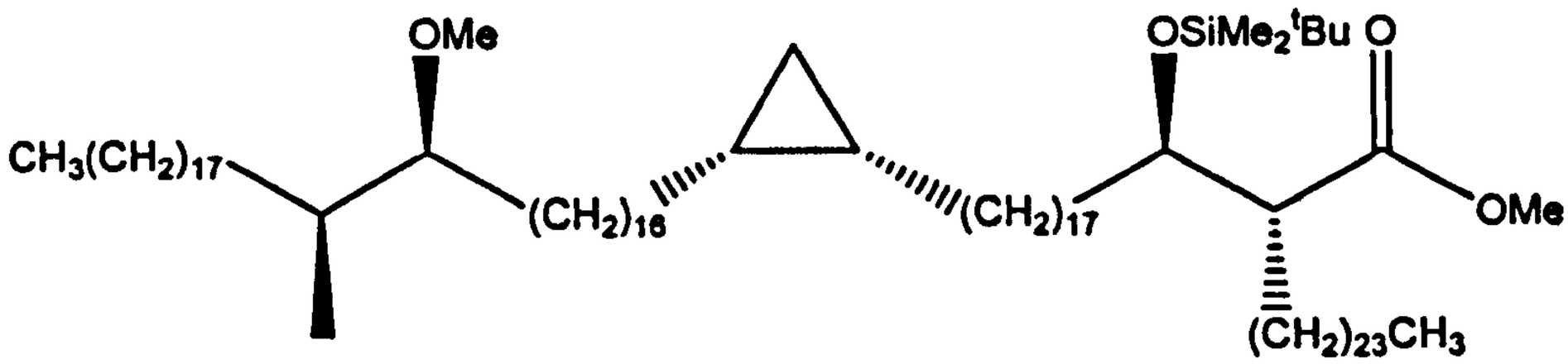
(*R*)-2-[(*R*)-1-(*tert*)-Butyldimethylsilanyloxy)-11-(2,2-dimethylpropionyloxy) undecyl]hexacosanoic acid methyl ester **(130)** (0.74 g, 0.93 mmol) in THF (5 ml) was added to a stirred solution of potassium hydroxide (0.78 g, 14.00 mmol, 15 mol. equiv.) in THF (20 ml), methanol (10 ml) and water (2 ml). The mixture was heated to 70 °C and reflux was maintained for 2 hrs. When TLC analysis indicated completion of the reaction, the mixture was quenched with water (10 ml) and the aqueous layer extracted with ethyl acetate (3 x 40 ml). The combined organic extracts were dried and evaporated and the crude product purified via column chromatography eluting with petrol/ether (5:2) to give a colourless oil, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-11-hydroxyundecyl]hexacosanoic acid methyl ester **(131)** (0.42 g, 63 %), which showed δ_H (500 MHz, $CDCl_3$): 3.92-3.88 (1H, m), 3.65 (3H, s), 3.62 (2H, t, J 6.5 Hz), 2.55 (1H, ddd, J 3.7, 7, 11 Hz), 1.65-1.20 (65H, m), 0.90 (3H, t J 7 Hz), 0.86 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 175.1, 73.2, 63.1, 51.6, 51.2, 33.7, 32.8, 31.9, 29.8, 29.7, 29.62, 29.58, 29.5, 29.42, 29.40, 29.3, 27.9, 27.5, 25.8, 25.7, 23.8, 22.7, 18.0, 14.1, -4.4, -4.9; ν_{max}: 3323, 2943, 2854, 1753, 1434 cm^{-1}; $[\alpha]^{23}_D$ = -5.04 ($CHCl_3$, 1.232 µmol) [Found M+Na^+: 733.60; $C_{44}H_{90}NaO_4Si$ requires: 733.65].

Experiment 9: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-oxoundecyl]-hexa cosanoic acid methyl ester (90)



(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-hydroxyundecyl]hexacosanoic acid methyl ester **(131)** (200.0 mg, 0.28 mmol) in dichloromethane (3 ml) was added to a stirred suspension of pyridinium chlorochromate (152.0 mg, 0.706 mmol, 2.5 mol. equiv.) in dichloromethane (10 ml) at RT. The mixture was stirred vigorously for 2 hrs, when TLC analysis indicated completion of the reaction. The mixture was diluted with ether (50 ml) and filtered through a bed of silica gel. The filtrate was evaporated to give a residue which was purified via column chromatography eluting with petrol/ether (5:2) to give a colourless oil, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-11-oxoundecyl]hexacosanoic acid methyl ester **(90)** (0.2 g, 99.7 %), which showed δ_H (500 MHz, $CDCl_3$): 9.78 (1H, t, J 2 Hz), 3.94-3.90 (1H, m), 3.71 (3H, s), 2.55 (1H, ddd, J 11, 7 , 4Hz), 2.43 (2H, dt, J 2, 7.5 Hz), 1.65 (2H, pent, J 6.5 Hz), 1.54-1.21 (60H, m), 0.90 (3H, t, J 7 Hz), 0.88 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 202.8, 175.1, 73.2, 51.6, 51.2, 43.9, 33.7, 31.9, 29.8, 29.7, 29.63, 29.59, 29.5, 29.42, 29.38, 29.3, 29.2, 27.9, 27.5, 25.8, 23.8, 22.7, 22.1, 18.0, 14.1, -4.4, -4.9; ν_{max}: 2943, 2843, 1784, 1423 cm^{-1}; $[\alpha]^{22}_D$ = -4.21 ($CHCl_3$, 1.184 µmol) [Found $M+Na^+$: 731.61; $C_{44}H_{88}NaO_4Si$ requires: 731.63].

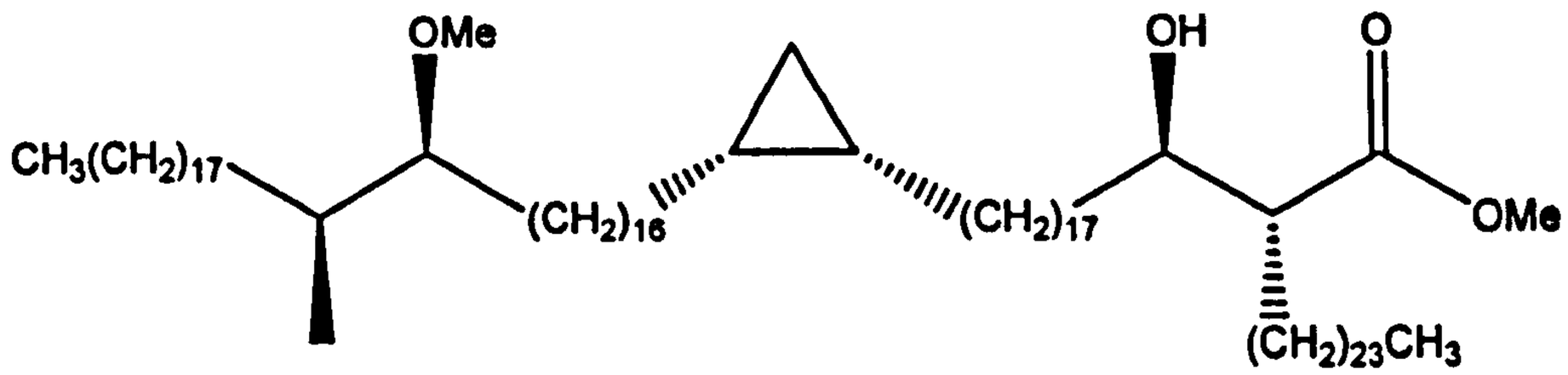
Experiment 10: (*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*S*,2*R*)-2 ((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl)-hexacosanoic acid methyl ester (133)



Lithium *bis*(trimethylsilyl)amide (0.20 ml, 0.2091 mmol, 1.06 M 1.5 mol. equiv.) was added dropwise to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-11-oxoundecyl]hexacosanoic acid methyl ester **(90)** (130 mg, 0.1829 mmol, 1.3 mol. equiv.) and 5-(7-[1*S*,2*R*]-(2-((17*S*,18*S*)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl)-heptylsulfonyl)-1-phenyl-1H-tetrazole **(89)** (125 mg, 0.1393 mmol) in dry THF (4 ml) under nitrogen at -12 °C. The reaction was allowed to reach RT. When TLC analysis indicated completion of the reaction, sat. aq. ammonium chloride (20 ml) was added and the aqueous layer was extracted with petrol/ether (1:1, 3 x 20 ml). The combined organic extracts were dried and evaporated, the crude product was purified via column chromatography eluting with petrol/ether (20:1) to give a colourless oil, (*E*/*Z*)(*R*)-2-((*R*)-1-(*tert*-butyldimethylsilanyloxy)-18-[(1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatria contyl)cyclopropyl]-octadec-11-enyl)hexacosanoic acid methyl ester **(132)** (125 mg, 65 %). Hydrogenation was carried out with dipotassium azocarboxylate as before and the crude product was purified via column chromatography eluting with petrol/ether (20:1) to give a colourless oil, (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilanyloxy)-18-[(1*S*,2*R*)-2((17*S*,18*S*)-17-methoxy-18-methylhexatria-contyl)cyclopropyl]octadecyl)hexacosanoic acid methyl ester **(133)** (108.1 mg, 86 %), which showed δ_H (500 MHz, $CDCl_3$): 3.95-3.89 (1H, m), 3.65 (3H, s), 3.36 (3H, s), 3.00-2.96 (1H, m), 2.55 (1H, ddd, J 4, 7, 11 Hz), 1.67-1.09 (147H, m), 0.96-0.84 (18H, m), 0.71-0.67 (2H, m), 0.60 (1H, dt, J 3.78, 7.65 Hz), 0.05 (3H, s), 0.03 (3H, s), -0.32 (1H, dt, J 5 Hz); δ_C (125 MHz, $CDCl_3$): 175.1, 85.5, 73.2, 57.7, 51.6, 51.2, 35.4, 33.7, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.8, 29.7, 29.62, 29.59, 29.43, 29.40, 28.7, 27.8, 27.6, 27.5, 26.2, 25.8, 23.7, 22.7, 18.0, 15.8, 14.9, 14.1, 10.9, -4.4, -4.9; v

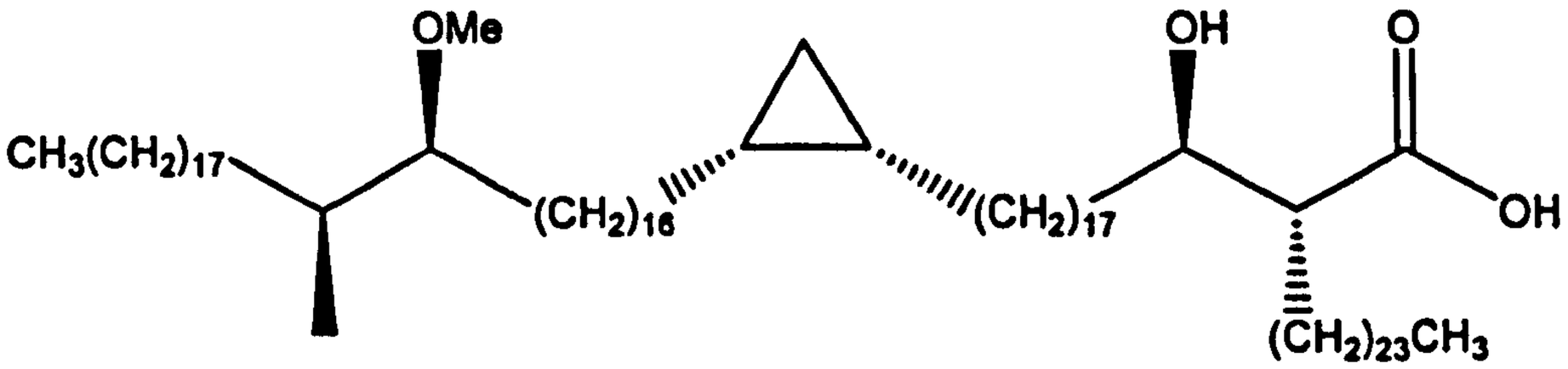
$_{max}$: 2921, 2876, 1763, 1465, 1102 cm^{-1}; $[\alpha]^{27}_{D}$ = +1.16 ($CHCl_3$, 0.897 μmol) [Found M+Na^+: 1404.25; $C_{92}H_{184}NaO_4Si$ requires: 1404.39].

Experiment 11: (*R*)-2-((*R*)-1-Hydroxy-18-[(1*S*,2*R*)-2((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl)-hexacosanoic acid methyl ester (135)



A dry polyethylene vial equipped with a rubber septum was charged with (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilanyloxy)-18-[(1*S*,2*R*)-2((17*S*,18*S*)-17-methoxy-18-methyl hexatriacontyl)cyclopropyl]octadecyl)-hexacosanoic acid methyl ester **(133)** (108.90 mg, 0.0776 mmol) in dry THF (4 ml) under nitrogen at 0 °C. Pyridine (0.3 ml) and hydrogen fluoride-pyridine complex (0.33 ml, 0.2327 mmol, 3 mol. equiv.) were added and the mixture stirred for 18hrs at 43 °C. When TLC analysis indicated completion of the reaction, the mixture was neutralised by slowly pouring the mixture into sat. aq. sodium hydrogen carbonate (10 ml) until no more carbon dioxide was liberated. The product was extracted with petrol/ether (5:2, 3 x 25 ml), dried and evaporated to give a white solid. This was purified via column chromatography eluting with petrol/ether (5:1) to give a white solid, (*R*)-2-((*R*)-1-hydroxy-18-[(1*S*,2*R*)-2((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclo-propyl]octadecyl)hexacosanoic acid methyl ester **(135)** (77.2 mg, 79 %), which showed δ_H (500 MHz, $CDCl_3$): 3.74 (3H, s), 3.66-3.62 (1H, m), 3.34 (3H, s), 2.98-2.95 (1H, m), 2.45 (1H, dt, J 5.5, 9.5 Hz), 1.76-1.03 (148H, m), 0.92-0.83 (9H, m), 0.69-0.62 (2H, m), 0.60 (1H, dt, J 4, 8.1 Hz), -0.31 (1H, dt, J 5 Hz); δ_C (125 MHz, $CDCl_3$): 176.1, 85.4, 72.3, 57.7, 51.5, 50.9, 35.7, 35.4, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.7, 29.64, 29.61, 29.59, 29.57, 29.53, 29.48, 29.42, 29.37, 28.7, 27.6, 27.4, 27.2, 25.7, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}: 3342, 2923, 2843, 1715, 1435 cm^{-1}; $[\alpha]^{26}_D$ = +1.04 ($CHCl_3$, 0.989 μmol); m.p. = 62 - 65 °C [Found M+Na^+: 1290.26; $C_{86}H_{170}NaO_4$ requires: 1290.30].

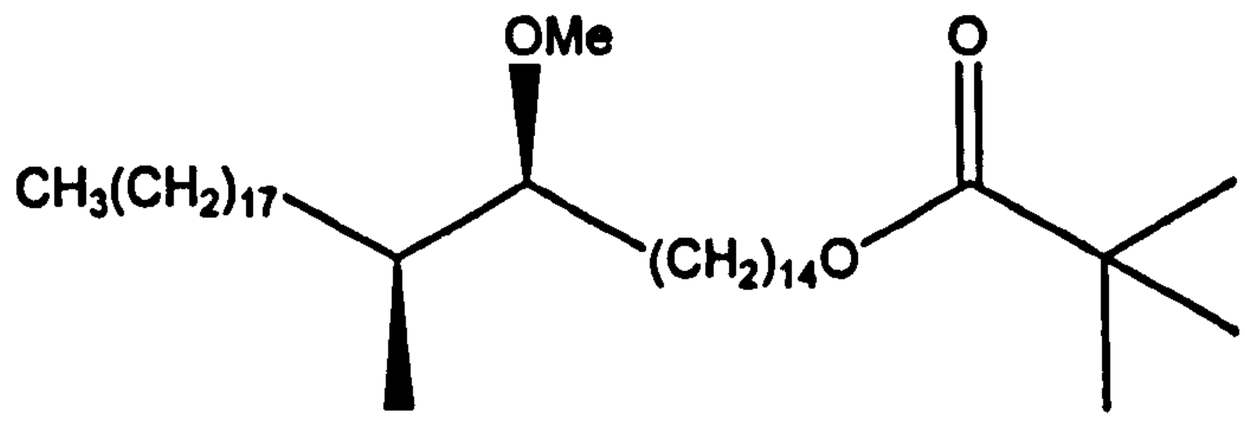
Experiment 12: (*R*)-2-[(*R*)-1-Hydroxy-18-[(1*S*,2*R*)-2((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl]-hexacosanoic acid (83)



Lithium hydroxide monohydrate (38.7 mg, 1.614 mmol, 30 mol. equiv.) was added to a stirred solution of (*R*)-2-((*R*)-1-hydroxy-18-[(1*S*,2*R*)-2((17*S*,18*S*)-17-methyoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl)-hexacosanoic acid methyl ester **(135)** (68.0 mg, 0.0538 mmol) in THF (5 ml), methanol (0.5 ml) and water (0.5 ml) at RT. The mixture was stirred at 43 °C for 18 hrs, when TLC analysis indicated completion of the reaction. The mixture was cooled to RT and acidified with hydrochloric acid (5 %, 2 ml) and the aqueous layer extracted with warm petrol/ether (1:1, 3 x 10 ml). The combined organic extracts were dried and evaporated, and then purified via column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, (*R*)-2-((*R*)-1-hydroxy-18-[(1*S*,2*R*)-2((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl)-hexacosanoic acid **(83)** (24.0 mg, 63 %), which showed δ_H (500 MHz, $CDCl_3$): 3.77-3.69 (1H, m), 3.35 (3H, s), 2.99-2.96 (1H, m), 2.46 (1H, dt, J 5.26, 9.31), 1.78-1.70 (1H, m), 1.68-1.60 (2H, m), 1.56-1.03 (145H,br.m including br.s at 1.26), 0.89 (6H, t, J 6.86 Hz), 0.85 (3H, d, J 6.9 Hz), 0.68-0.62 (2H, m), 0.56 (1H, dt, J 4, 8.15 Hz), -0.32 (1H, dt, J 5 Hz); δ_C (125 MHz, $CDCl_3$): 178.9, 85.6, 72.1, 57.7, 50.7, 35.6, 35.3, 32.4, 31.9, 30.5, 30.3, 30.2, 29.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 28.7, 27.6, 27.3, 26.1, 22.7, 18.0, 15.8, 14.9, 14.1, 10.9; ν_{max}: 3521, 2923, 2854, 1713, 1459 cm^{-1}; $[\alpha]^{21}_D$ = +1.17 ($CHCl_3$, 0.496 µmol): m.p. 65-67 °C; [Found $M+Na^+$: 1276.12; $C_{85}H_{168}NaO_4$ requires: 1276.28].

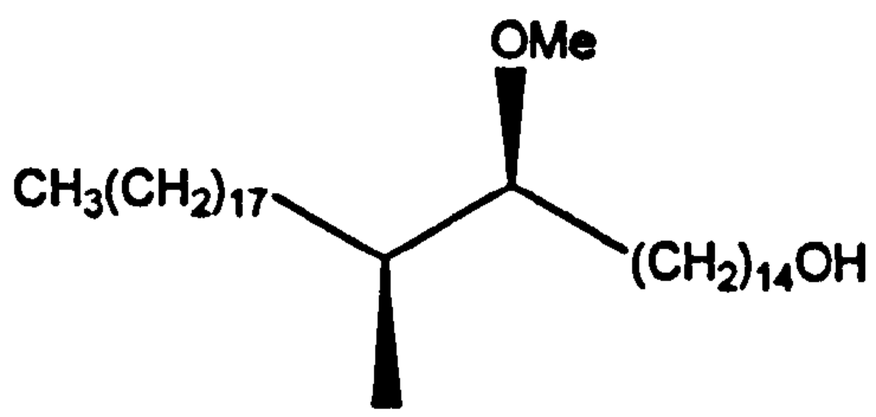
4.2 – Synthesis of α-methyl trans-cyclopropane methoxy-mycolic acid (84)

Experiment 13: (15*S*,16*S*)-15-Methoxy-16-methyltetratriacontyl pivalate (147)



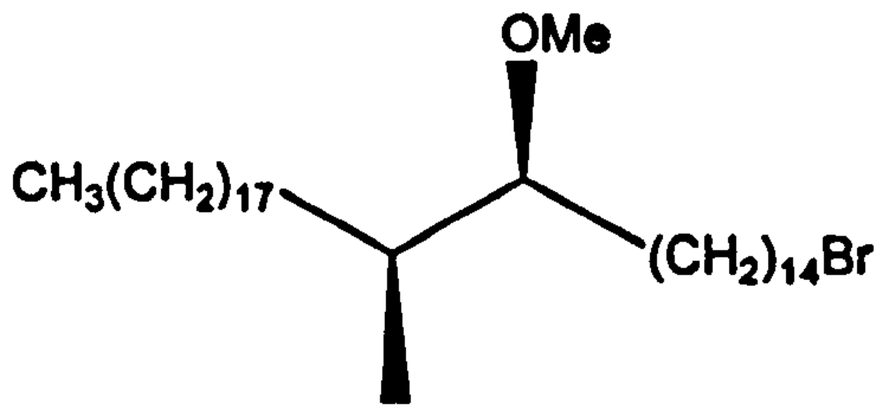
A solution of (8*S*,9*S*)-8-methoxy-9-methylheptacosanal **(95)** (1,56 g, 3.56 mmol) and 2,2-dimethylpropionic acid-7-(1-phenyl-1H-tetrazol-5-ylsulfonyl)-heptyl ester **(146)** (1.74 g, 4.27 mmol) in dry THF (50 ml) was stirred under nitrogen at -10 °C. Lithium *bis*(trimethylsilyl)amide (5.24 ml, 5.56 mmol, 1.06 M) was added dropwise between -12 °C and -5 °C, the solution was stirred for 18 hrs. When TLC showed no starting material was left, dichloromethane (50 ml) and sat. aq. ammonium chloride (50 ml) were added. The aqueous layer was re-extracted with further dichloromethane (2 x 100 ml) and the combined organic layers were dried and evaporated to give a crude product. This was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, (*E*/*Z*)-2,2-dimethyl-propionic acid 15-methoxy-16-methyl-tetratriacont-7-enyl ester (1.78 g, 81 %). Palladium on charcoal (0.2 g, 10%) was added to a stirred solution of the above product (1.78 g, 2.87 mmol) in THF (5 ml) and IMS (40 ml). The mixture was stirred while being hydrogenated at atmospheric pressure. When no more hydrogen was being absorbed the catalyst was removed via suction filtration through a pad of celite and was washed with THF (50 ml). The filtrate was evaporated to give a colourless oil, (15*S*,16*S*)-15-methoxy-16-methyltetratriacontyl pivalate **(147)** (1.57 g, 88 %), which showed δ_H (500 MHz, $CDCl_3$): 4.04 (2H, t, J 6.6 Hz), 3.34 (3H, s), 3.02-2.96 (1H, m), 1.66 (2H, pent, J 6.6 Hz), 1.55-1.28 (58H, m), 1.24 (9H, s), 1.14-1.03 (1H, m), 0.93 (3H, t, J 6.6 Hz), 0.90 (3H, d, J 7.0 Hz); δ_C (125 MHz, $CDCl_3$): 178.3, 85.5, 64.5, 57.7, 35.4, 32.4 31.9, 30.9, 30.5, 29.92, 29.89, 29.7, 29.6, 29.5, 29.4, 29.3, 28.6, 27.6, 26.1, 26.0, 22.4, 14.8, 14.1; ν_{max}: 2935, 1749, 1134 cm^{-1}; $[\alpha]^{22}_D = -6.7$ ($CHCl_3$, 1.245 μmol) [Found $M+Na^+$: 645.49; $C_{41}H_{82}NaO_3$ requires: 645.62].

Experiment 14: (15*S*,16*S*)-15-Methoxy-16-methyl-tetratriacontan-1-ol (148)



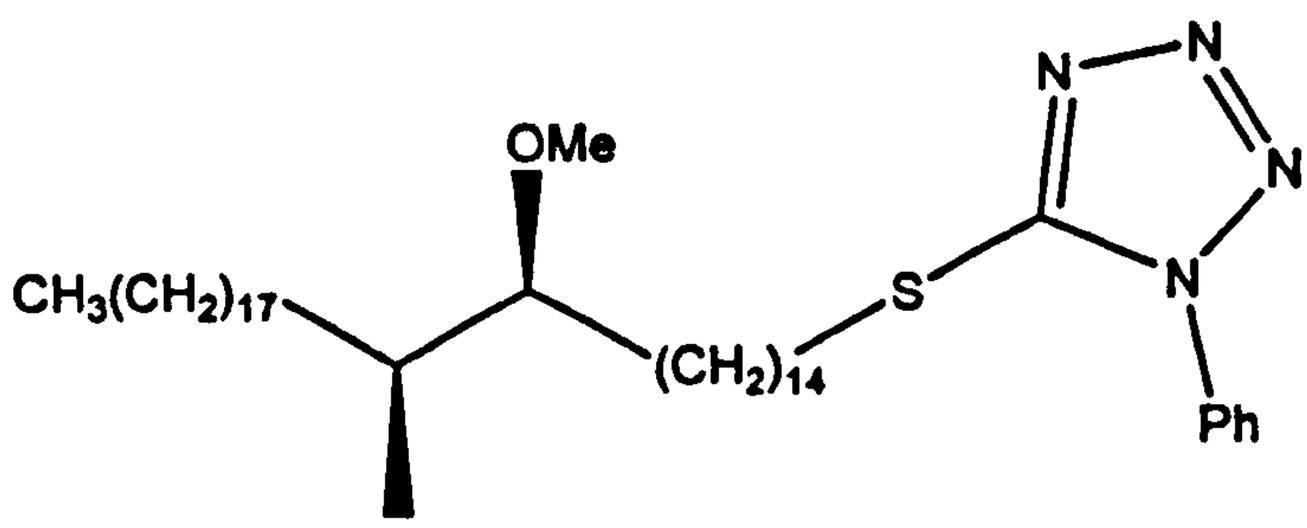
Lithium aluminium hydride (0.14 g, 3.79 mmol) was added to THF (20 ml) stirred at -20 °C under nitrogen. A solution of (15*S*,16*S*)-15-methoxy-16-methyltetratriacontyl pivalate **(147)** (1.57 g, 2.52 mmol) in THF (10 ml) was added slowly and allowed to reach RT, then refluxed for 1 hr. When TLC showed no starting material was left the reaction was cooled to -20 °C and was quenched with sat. aq. sodium sulfate until a white precipitate formed. THF (30 ml) was added and the mixture was stirred for 30 mins, filtered through a bed of silica gel and the solvent evaporated. The resulting solution was taken up in dichloromethane (50 ml) and washed with water (10 ml) and then dried. The solvent was evaporated and the crude product was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a white solid, (15*S*,16*S*)-15-methoxy-16-methyl-tetratriacontan-1-ol **(148)** (1.04 g, 79 %), which showed δ_H (500 MHz, $CDCl_3$): 3.70 (2H, t, J 6.6 Hz), 3.33 (3H, s), 3.01-2.96 (1H, m), 1.66-1.64 (1H, m), 1.61 (2H, pent, J 6.6 Hz), 1.47 (1H, br.s) 1.47-1.20 (56H, m), 1.16-1.04 (1H, m), 0.90 (3H, t, J 7 Hz) 0.88 (3H, d, J 6.6 Hz); δ_C (125 MHz, $CDCl_3$): 86.2, 63.1, 57.8, 35.6, 32.8, 32.6, 32.0, 30.7, 30.2, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 27.5, 26.3, 25.8, 22.9, 14.9, 14.1; ν_{max}: 3376, 2929, 1103, 1080 cm^{-1}; $[\alpha]^{20}_D$ = -8.97 ($CHCl_3$, 1.217 μmol); m.p. 46-48 °C; [Found $M+Na^+$: 561.43; $C_{36}H_{74}NaO_2$ requires: 561.56].

Experiment 15: (15*S*,16*S*)-1-Bromo-15-methoxy-16-methyl-tetratriacontane (149)



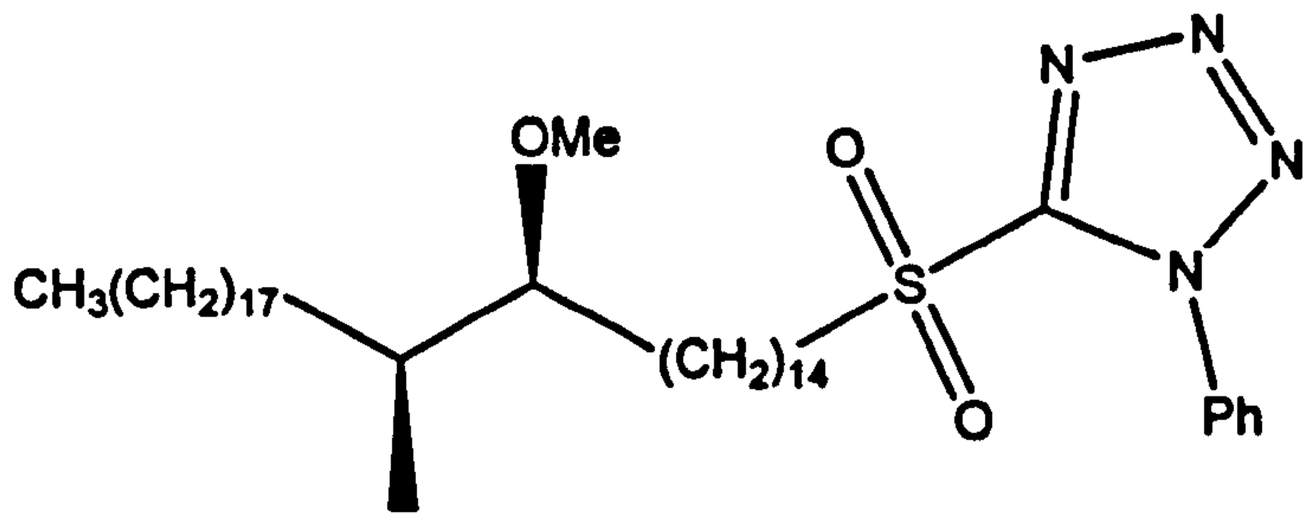
N-Bromosuccinimide (0.44 g, 2.46 mmol, 1.3 mol. equiv.) was added in portions over 15 mins to a stirred solution of (15*S*,16*S*)-15-methoxy-16-methyl-tetratriacontan-1-ol **(148)** (1.04 g, 1.89 mmol) and triphenylphosphine (0.56 g, 2.14 mmol, 1.13 equiv) in dichloromethane (20 ml) at 0 °C. The mixture was stirred at RT for 1 hr, when TLC analysis indicated completion of the reaction. It was quenched with sat. aq. sodium meta-bisulfite (25 ml) then the aqueous layer was re-extracted with dichloromethane (2 x 20 ml) and the combined organic extracts washed with water (50 ml), dried and evaporated to give a residue. This residue was treated with petrol/ether (1:1, 50 ml), refluxed for 30 mins and then filtered and washed with petrol/ether (1:1, 25 ml). The filtrate was evaporated and the resultant residue purified via column chromatography eluting with petrol/ether (10:1) to give a white solid, (15*S*,16*S*)-1-bromo-15-methoxy-16-methyl-tetratriacontane **(149)** (0.76 g, 83 %), which showed δ_H (500 MHz, $CDCl_3$): 3.46 (2H, t, J 6.8 Hz), 3.39 (3H, s), 3.03-2.98 (1H, m), 1.91 (2H, pent., J 6.8 Hz), 1.71-1.65 (1H, m), 1.52-1.26 (58H, m), 0.91 (3H, t, J 6.4 Hz), 0.88 (3H, d, J 6.8 Hz); δ_C (125 MHz, $CDCl_3$): 85.8, 57.9, 35.3, 34.2, 32.9, 32.6, 31.9, 30.8, 30.3, 29.9, 29.7, 29.6, 29.52, 29.48, 29.4, 28.9, 28.4, 27.7, 26.3, 22.8, 14.9, 14.4; ν_{max}: 2943, 2868, 1123, 743 cm^{-1}; $[\alpha]^{21}_D$ = -7.74 ($CHCl_3$, 1.254 μmol); m.p. 38-40 °C; [Found $M+Na^+$: 623.28; $C_{36}H_{73}NaOBr$ requires: 623.47].

Experiment 16: 5-((15*S*,16*S*)-15-Methoxy-16-methyltetratriacontyl-1-sulfanyl)-1-phenyl-1H-tetrazole (150)



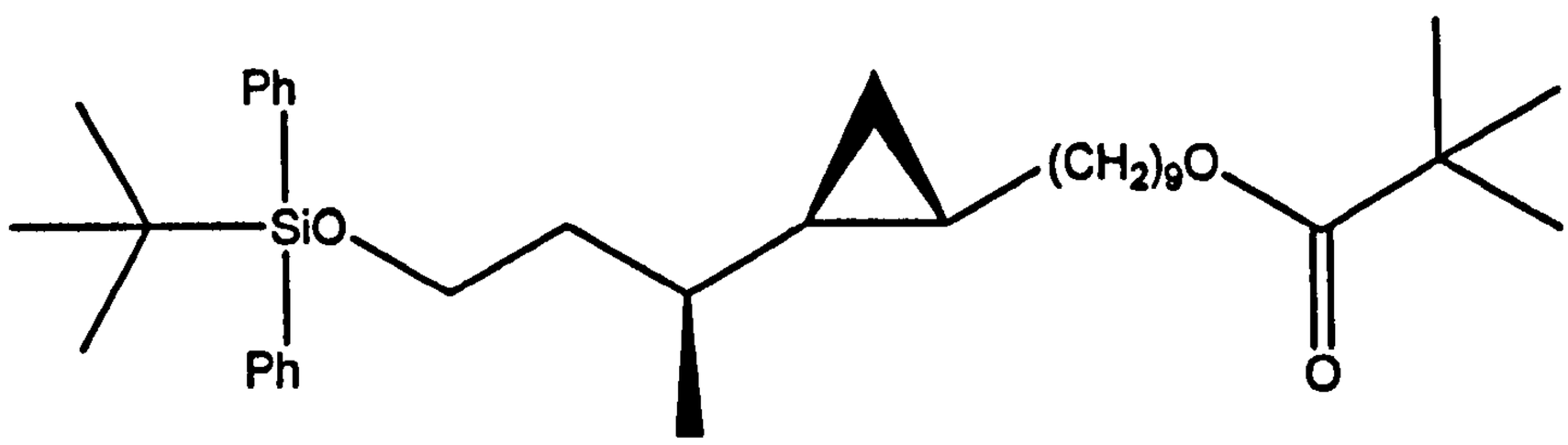
(15*S*,16*S*)-1-Bromo-15-methoxy-16-methyl-tetratriacontane **(149)** (0.70 g, 1.14 mmol) in THF (3 ml) and acetone (3 ml) was added to a stirred solution of 1-phenyl-1H-tetrazole-5-thiol (0.22 g, 1.26 mmol, 1.1 mol. equiv.) and anhydrous potassium carbonate (0.55 g, 4.00 mmol, 3.5 mol. equiv.) in acetone (15 ml) at RT. The mixture was stirred at RT for 18 hrs, then the solvent was evaporated and the residue was diluted with petrol/ether (1:1, 20 ml) and water (20 ml). The aqueous layer was re-extracted with petrol/ether (1:1, 2 x 10 ml). The combined organic extracts were dried and evaporated to give a crude oil which was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, 5-((15*S*,16*S*)-15-methoxy-16-methyltetratriacontyl-1-sulfanyl)-1-phenyl-1H-tetrazole **(150)** (0.76 g, 93 %), which showed δ_H (500 MHz, $CDCl_3$): 7.64-7.51 (5H, m), 3.39 (2H, t, J 7.3 Hz), 3.30 (3H, s), 2.99-2.94 (1H, m), 1.82 (2H, pent, J 7.4 Hz), 1.67-1.60 (1H, m), 1.49-1.20 (58H, m), 0.91 (3H, t, J 6.55 Hz), 0.87 (3H, d, J 6 Hz); δ_C (125 MHz, $CDCl_3$): 154.5, 130.1, 129.8, 123.9, 85.5, 57.7, 35.3, 30.5, 30.0, 29.6, 29.5, 29.1, 28.7, 26.2, 22.7, 14.9, 14.1; ν_{max}: 2926, 2861, 1098 cm^{-1}; $[\alpha]^{21}_D$ = -6.49 ($CHCl_3$, 1.106 μmol) [Found $M+Na^+$: 721.44; $C_{43}H_{78}N_4NaOS$ requires: 721.58].

Experiment 17: 5-((15*S*,16*S*)-15-Methoxy-16-methyltetratriacontyl-1-sulfonyl)-1-phenyl-1H-tetrazole (141)



m-Chloroperoxybenzoic acid (0.52 g, 3.04 mmol, 3 mol. equiv.) in dichloromethane (5 ml) was added slowly to 5-((15*S*,16*S*)-15-methoxy-16-methyltetratriacontyl-1-sulfanyl)-1-phenyl-1H-tetrazole **(150)** (0.72 g, 1.01 mmol) and sodium hydrogen carbonate (0.38 g, 4.56 mmol, 4.5 mol. equiv.) in dichloromethane (5 ml) at 5 °C. The mixture was stirred for 18 hrs at RT, when TLC analysis indicated completion of the reaction. The solvent was evaporated and the resultant residue was diluted with ethyl acetate (5 ml) and slowly quenched with sat. aq. sodium metabisulfite (2 ml). The aqueous layer was re-extracted with ethyl acetate (2 x 10 ml) and the combined organic extracts were washed with sat. aq. sodium hydrogen carbonate (10 ml) and water (20 ml). The organic extract was then dried and evaporated and the resultant yellow oil purified via column chromatography eluting with petrol/ether (1:1) to give a white solid, 5-((15*S*,16*S*)-15-methoxy-16-methyltetratriacontyl-1-sulfonyl)-1-phenyl-1H-tetrazole **(141)** (0.67 g, 89 %), which showed δ_H (500 MHz, $CDCl_3$): 7.71-7.70 (2H, m), 7.69-7.61 (3H, m), 3.74 (2H, t, J 7.9 Hz), 3.34 (3H, s), 2.97-2.95 (1H, m), 1.96 (2H, pent, J 7.9 Hz), 1.63-1.58 (1H, m), 1.50 (2H, pent, J 7.6 Hz), 1.45-1.22 (56H, m), 0.89 (3H, t, J 6.6 Hz), 0.85 (3H, d, J 6.7 Hz); δ_C (125 MHz, $CDCl_3$): 153.5, 133.1, 131.5, 130.3, 125.1, 85.5, 57.7, 56.0, 35.3, 32.4, 31.9, 30.5, 30.0, 29.9, 29.7, 29.7, 29.62, 29.60, 29.5, 29.4, 29.2, 28.9, 26.5, 22.8, 22.0, 15.1, 14.4; ν_{max}: 2947, 2852, 1321, 1164, 1097 cm^{-1}; $[\alpha]^{23}_D$ = -6.28 ($CHCl_3$, 1.024 μmol); m.p. 42-44 °C; [Found $M+Na^+$: 753.50; $C_{43}H_{78}N_4NaO_3S$ requires: 753.57].

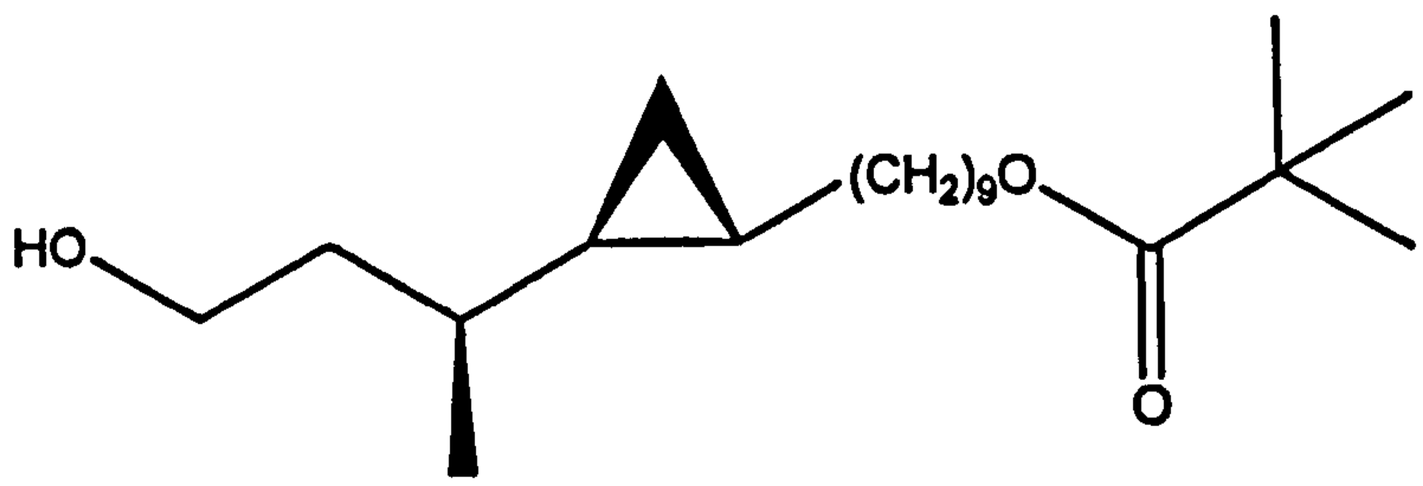
Experiment 18: 9-((1*S*,2*R*)-2-((*S*)-4-(*tert*-Butyldiphenylsilyloxy)butan-2-yl)cyclopropyl)nonyl pivalate (143)



Lithium *bis*(trimethylsilyl)amide (7.76 ml, 8.22 mmol, 1.06M) was added dropwise to a stirred solution of 2,2-dimethyl-propionic acid 8-(1-phenyl-1H-tetrazole-5-sulfonyl)-octyl ester **(96)** (2.67 g, 6.32 mmol) and (1*S*,2*R*)-2-((*S*)-4-(*tert*-butyldiphenylsilyloxy)butan-2-yl)cyclopropanecarbaldehyde **(144)** (1.93 g, 5.27 mmol) in dry THF (50 ml) under nitrogen at -20 °C. The temperature rose to -10 °C during the addition of the base, and a yellow solution resulted. The mixture was allowed to reach RT and was stirred for 2 hrs, when TLC showed no starting material was left and then cooled to 0 °C and quenched with sat. aq. ammonium chloride (100 ml). The product was extracted with petrol/ether (1:1, 3 x 50 ml). The combined organic layers were washed with brine (100 ml), dried and evaporated to give an oil, which was purified via column chromatography eluting with petrol/ether (7:1) to give (*E*/*Z*)-9-((1*R*,2*S*)-2-((*R*)-1-(*tert*-butyldiphenylsilyloxy)propan-2-yl)cyclopropyl)non-8-enyl pivalate **(153)** (2.28 g, 75 %). Dipotassium azodicarboxylate (45.20 g, 232.70 mmol) was added to a stirred solution of **(153)** (4.47 g, 7.76 mmol) in THF (200 ml) and methanol (100 ml) at 10 °C under nitrogen, giving a yellow precipitate. A solution of glacial acetic acid (10 ml) and THF (20 ml) was added dropwise over 48 hrs, after which a white precipitate had formed. The mixture was cooled to 0 °C and poured slowly into sat. aq. sodium hydrogen carbonate (50 ml) and then extracted with petrol/ether (1:1, 3 x 100 ml). The combined organic layers were washed with water (50 ml), dried and evaporated to give a thick oil which slowly solidified. The residue was purified by column chromatography eluting in petrol/ether (10:1) to give a colourless oil, 2,2-dimethyl-propionic acid 9-((1*S*,2*R*)-2-((*S*)-4-(*tert*-butyldiphenylsilyloxy)butan-2-yl)cycloprop-yl)nonyl pivalate **(143)** (4.14 g, 93 %), which showed δ_H (500 MHz, $CDCl_3$): 7.67-7.65 (4H, m), 7.40-7.37 (6H, m), 4.05 (2H, t, J 13.45 Hz), 3.77-3.72 (2H, m), 1.88-

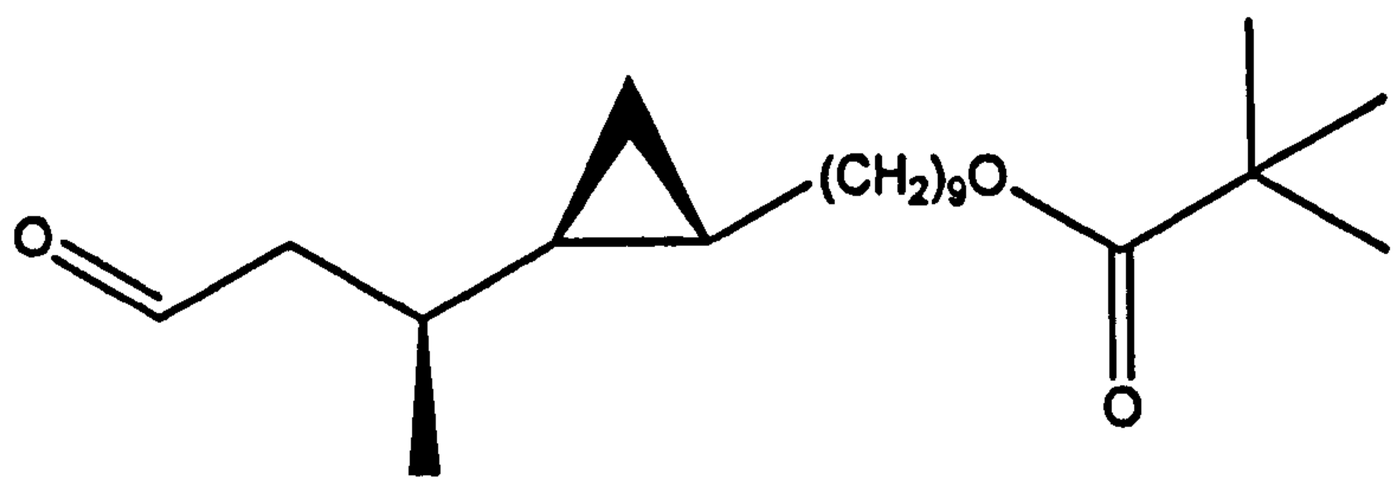
1.79 (1H, m), 1.62-1.59 (2H, m), 1.50-1.44 (1H, m), 1.29-1.22 (16H, m), 1.21 (9H, s), 1.19-1.10 (1H, m), 1.04 (9H, s), 0.88 (3H, br.s), 0.49-0.42 (1H, m), 0.16-0.11 (1H, m); δ_C (125 MHz, $CDCl_3$): 178.7, 135.7, 134.2, 129.5, 127.6, 64.5, 62.4, 40.2, 38.7, 34.8, 34.4, 29.8, 29.62, 29.58, 29.5, 29.2, 28.6, 27.2, 27.0, 25.8, 19.9, 19.3, 18.7, 10.6; ν_{max}: 2930, 2578, 1743 cm^{-1}; $[\alpha]^{20}_D$ = +6.54 ($CHCl_3$, 1.054 μmol) [Found $M+Na^+$: 601.38; $C_{37}H_{58}NaO_3Si$ requires: 601.41].

Experiment 19: 9-((1*S*,2*R*)-2-((*S*)-4-Hydroxybutan-2-yl)cyclopropyl)nonyl pivalate (151)



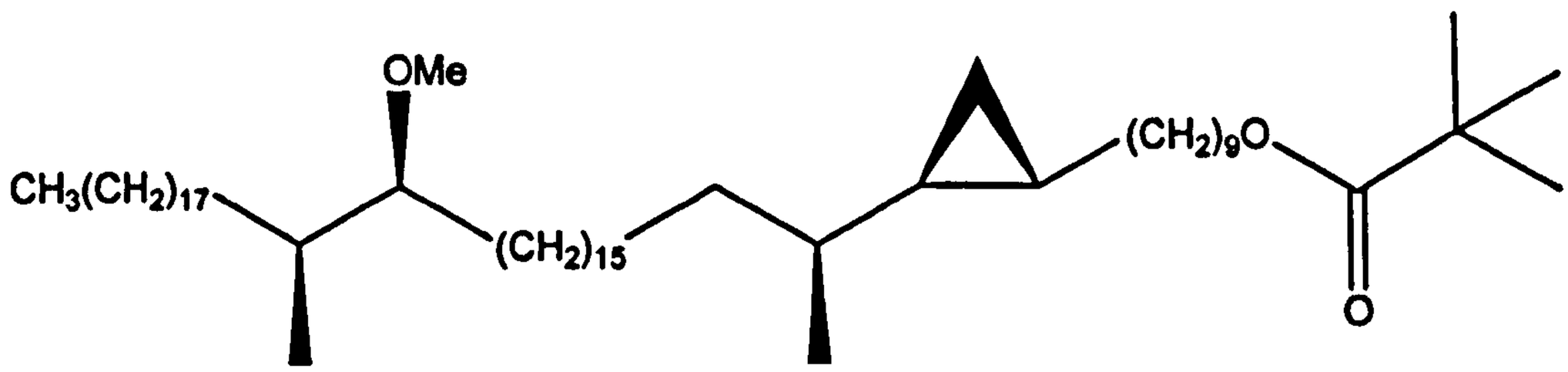
9-((1*S*,2*R*)-2-((*S*)-4-(*tert*-Butyldiphenylsilyloxy)butan-2-yl)cyclopropyl)nonyl pivalate **(143)** (4.14 g, 7.16 mmol) was dissolved in dry THF (20 ml) in a polyethylene vial under nitrogen at RT. Pyridine (2 ml) and HF.Pyridine (10.23 ml, 7.16 mmol) were added and the mixture stirred for 17 hrs at 45 °C, when TLC showed no starting material was left. The mixture was diluted with petrol/ether (1:1, 20 ml) and neutralised with by adding to sat. aq. sodium hydrogen carbonate (25 ml) until no more carbon dioxide was liberated. The compound was extracted with petrol/ether (1:1, 2 x 50 ml) and washed with brine (100 ml), dried and evaporated. The resultant oil was purified via column chromatography eluting with petrol/ether (4:1) to give a colourless oil, 9-((1*S*,2*R*)-2-((*S*)-4-hydroxybutan-2-yl)cyclopropyl)nonyl pivalate **(151)** (1.92 g, 79 %), which showed δ_H (500 MHz, $CDCl_3$): 4.04 (2H, t, J 6.8 Hz), 3.77-3.72 (2H, m), 1.78-1.69 (1H, m), 1.63-1.59 (2H, m), 1.54-1.49 (1H, m), 1.39-1.26 (15H, m), 1.22 (9H, s), 1.17-1.09 (1H, m), 0.99 (3H, d, J 6.8 Hz), 0.90-0.85 (1H, m), 0.52-0.48 (1H, m), 0.31-0.16 (1H, m); δ_C (125 MHz, $CDCl_3$): 178.7, 64.5, 61.4, 40.4, 38.7, 35.0, 34.3, 34.4, 29.8, 29.62, 29.59, 29.5, 29.2, 28.6, 27.2, 27.0, 25.8, 19.9, 19.3, 18.7, 10.5; ν_{max}: 3341, 2931, 2789, 1734, 1426 cm^{-1}; $[\alpha]^{22}_D$ = +13.14 ($CHCl_3$, 1.512 μmol) [Found $M+Na^+$: 363.19; $C_{21}H_{40}NaO_3$ requires: 363.29].

Experiment 20: 9-((1*S*,2*R*)-2-((*S*)-4-Oxobutan-2-yl)cyclopropyl)nonyl pivalate (142)



9-((1*S*,2*R*)-2-((*S*)-4-Hydroxybutan-2-yl)cyclopropyl)nonyl pivalate **(151)** (0.42 g, 1.23 mmol) was added to a stirred suspension of PCC (0.67 g, 3.09 mmol, 2.5 mol. equiv.) in dichloromethane (10 ml). The reaction mixture was stirred for 2 hrs at RT, when TLC analysis confirmed completion of the reaction, and diluted with ether (50 ml). The mixture was filtered through a bed of silica gel and washed with ether (2 x 10 ml), the solvent evaporated and the product was purified via column chromatography eluting with petrol/ether (5:2) to give a colourless oil, 9-((1*S*,2*R*)-2-((*S*)-4-oxobutan-2-yl)cyclopropyl)nonyl pivalate **(142)** (0.39 g, 93 %), which showed δ_H (500 MHz, $CDCl_3$): 9.78 (1H, s), 2.50 (1H, ddd, J 15.75, 6.3, 1.9 Hz), 2.35 (1H, ddd, J 15.75, 7.9, 2.5 Hz), 1.61 (2H, pent, J 6.6 Hz), 1.32-1.13 (26H, m), 1.00 (3H, d, J 6.65 Hz), 0.49 (1H, m), 0.34-0.21 (3H, m); δ_C (125 MHz, $CDCl_3$): 202.9, 178.6, 64.4, 51.4, 38.7, 34.1, 33.9, 29.6, 29.51, 29.48, 29.2, 28.6, 27.2, 25.9, 25.6, 20.0, 18.8, 11.4; ν_{max}: 2924, 2878, 1727 cm^{-1}; $[\alpha]^{22}_D$ = +20.47 ($CHCl_3$, 1.076 µmol) [Found $M+Na^+$: 361.24; $C_{21}H_{38}NaO_3$ requires: 361.27].

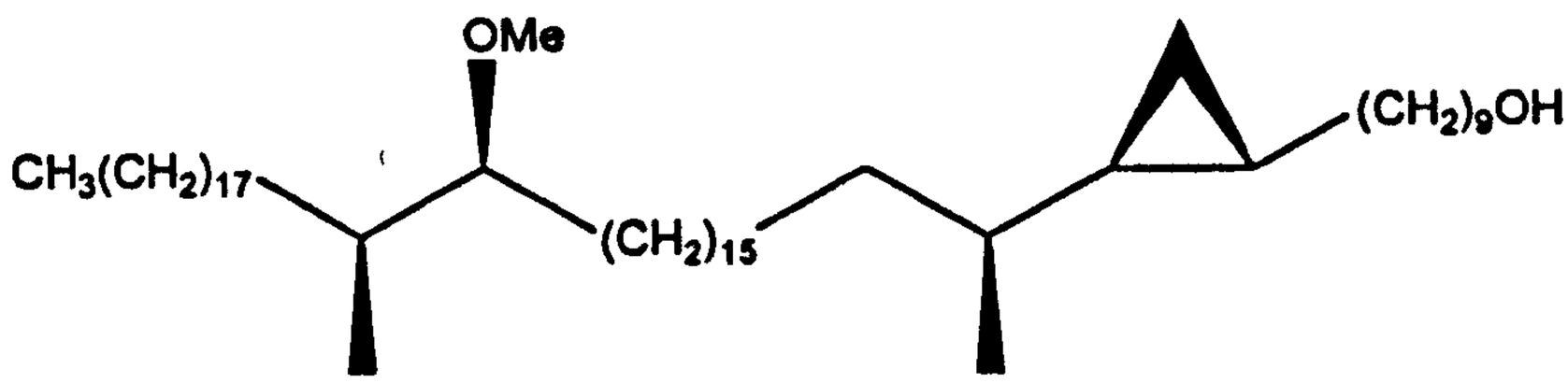
Experiment 21: 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-Methoxy-20-methyloctatria contan-2-yl)cyclopropyl)nonyl pivalate (152)



Lithium *bis*(trimethylsilyl)amide (0.923 ml, 0.978 mmol, 1.06M) was added dropwise to a stirred solution of 5-((15*S*,16*S*)-15-methoxy-16-methyltetratriacontyl-1-sulfonyl)-1-phenyl-1H-tetrazole **(141)** (670 mg, 0.903 mmol) and 9-((1*S*,2*R*)-2-((*S*)-4-oxobutan-2-yl)cyclopropyl)nonyl pivalate **(142)** (255 mg, 0.752 mmol) in dry THF (10 ml) under nitrogen at -20 °C. The reaction mixture rose to -10 °C during the addition of the base, and a yellow solution resulted. The mixture was allowed to reach RT and was stirred for 1 hr, when TLC showed no starting material was left. The reaction mixture was cooled to 0 °C and quenched with sat. aq. ammonium chloride (10 ml). The product was extracted with petrol/ether (1:1, 3 x 10 ml). The combined organic layers were washed with brine (20 ml), dried and evaporated to give an oil, which was purified via column chromatography eluting with petrol/ether (20:1) to give 9-((1*S*,2*R*)-2-[(*E*/*Z*)-(2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacont-4-en-2-yl]cyclopropyl)nonyl pivalate (410 mg, 54 %). Dipotassium azodicarboxylate (2.49 g, 12.83 mmol, 30 mol. equiv.) was added to a stirred solution of the alkenes (410 mg, 0.487 mmol) in THF (20 ml) and methanol (10 ml) at 10 °C under nitrogen, giving a yellow precipitate. A solution of glacial acetic acid (1 ml) and THF (2 ml) was added dropwise over 48 hrs, after which a white precipitate had formed. The mixture was cooled to 0 °C and poured slowly into sat. aq. sodium hydrogen carbonate (5 ml) and then extracted with petrol/ether (1:1, 3 x 25 ml). The combined organic layers were washed with water (10 ml), dried and evaporated to give a thick oil which slowly solidified. The residue was purified via column chromatography eluting in petrol/ether (10:1) to give a colourless oil, 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonyl pivalate **(152)** (400 mg, 97 %), which showed δ_H (500 MHz, $CDCl_3$): 4.05 (2H, t, J 6.65 Hz), 3.70 (1H, m), 3.35 (3H, s), 1.60 (9H, s), 1.44-1.19 (84H, br.m including br.s at 1.26),

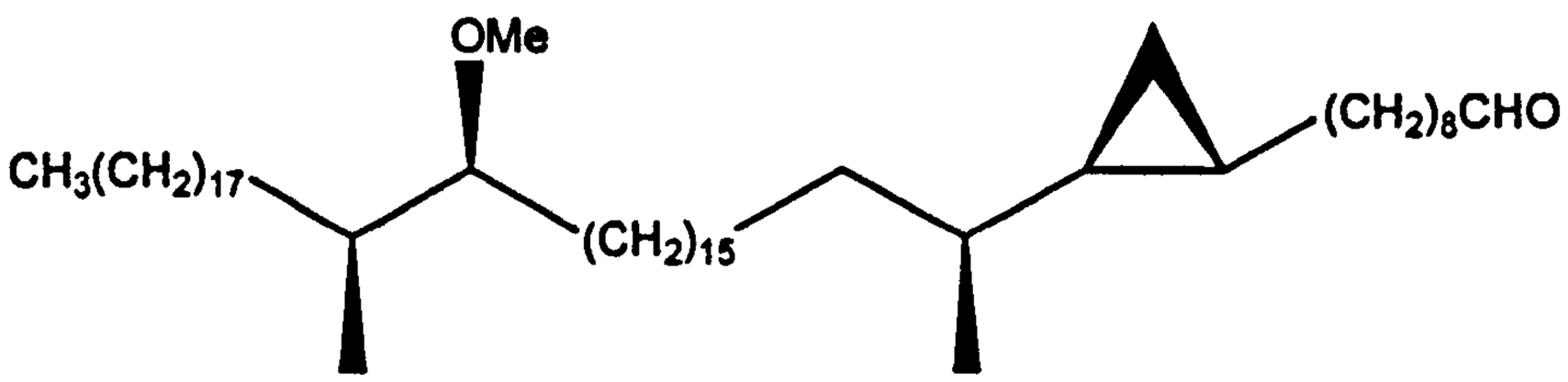
0.92-0.84 (10H, m), 0.47-0.44 (1H, m), 0.22-0.18 (1H, m), 0.17-0.14 (1H, m), 0.13-0.09 (1H, m); δ_C (125 MHz, $CDCl_3$): 85.5, 65.8, 63.1, 57.7, 38.1, 35.4, 34.5, 32.8, 32.4, 31.9, 30.5, 30.1, 30.0, 29.9, 29.7, 29.6, 29.5, 29.4, 27.6, 27.3, 26.2, 26.1, 25.8, 22.7, 19.7, 18.6, 15.3, 14.9, 14.1, 10.5; ν_{max}: 2918, 2857, 1745 cm^{-1}; $[\alpha]^{21}_D$ = -6.85 ($CHCl_3$, 0.865 μmol) [Found M+Na^+: 867.52; $C_{57}H_{112}NaO_3$ requires: 867.85].

Experiment 22: 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-Methoxy-20-methyloctatria contan-2-yl)cyclopropyl)nonan-1-ol (153)



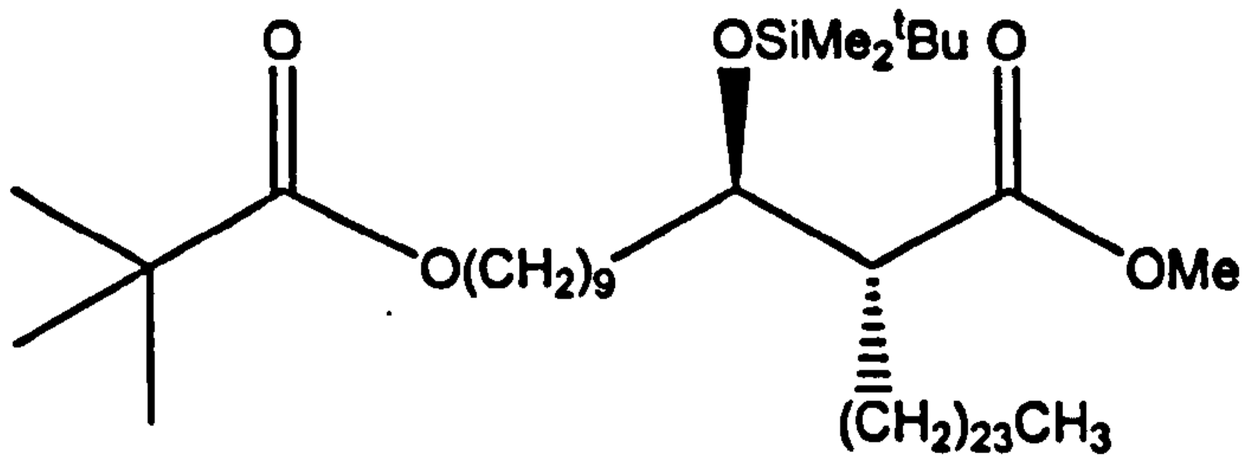
Lithium aluminium hydride (36.0 mg, 0.9479 mmol, 2 mol. equiv.) was added to stirred THF (5ml, HPLC grade) at -20 °C under nitrogen. A solution of 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonyl pivalate **(152)** (400 mg, 0.4737 mmol) in THF (5 ml, HPLC grade) was added slowly and then the reaction was allowed to reach RT, then refluxed for 1 hr. When TLC showed no starting material was left, the reaction mixture was cooled to -20 °C and quenched with sat. aq. sodium sulfate until a white precipitate formed. The resultant mixture was stirred for 30 mins and then filtered through a bed of silica gel and the solvent evaporated. The product was purified via column chromatography eluting with petrol/ether (1:1) to give a colourless oil, 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonan-1-ol **(153)** (260 mg, 72 %), which showed δ_H (500 MHz, $CDCl_3$): 4.03 (2H, t, J 6.65 Hz), 3.69 (1H, m), 3.35 (3H, s), 1.52-1.20 (84H, br.m including br.s at 1.26), 0.90-0.82 (10H, m), 0.46-0.44 (1H, m), 0.22-0.18 (1H, m), 0.17-0.14 (1H, m), 0.13-0.09 (1H, m);δ_C (125 MHz, $CDCl_3$): 85.5, 63.1, 57.7, 38.1, 37.4, 35.4, 34.5, 32.8, 32.4, 31.9, 30.5, 30.0, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 27.6, 27.3, 26.2, 25.8, 22.7, 19.7; ν_{max}:3343, 2975, 2847 cm^{-1}; $[\alpha]^{19}_D$ = -6.11 ($CHCl_3$, 1.075 μmol) [Found $M+Na^+$: 783.64; $C_{52}H_{104}NaO_2$ requires: 783.79].

Experiment 23: 9-((1*R*,2*R*)-2-((2*S*,19*S*,20*S*)-19-Methoxy-20-methyloctatria contan-2-yl)cyclopropyl)nonanal (139)



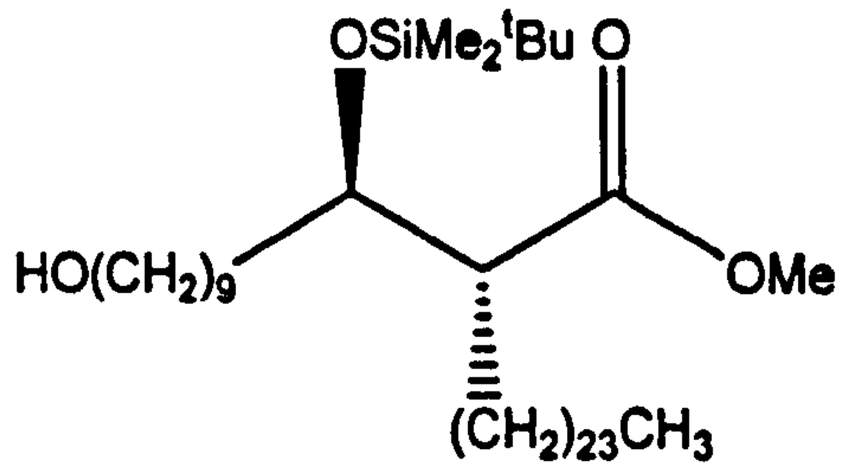
9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-Methoxy-20-methyloctatriacontan-2-yl)cyclopropyl) nonan-1-ol **(153)** (0.26 g, 0.343 mmol) was added to a stirred suspension of PCC (0.22 g, 1.03 mmol, 3 mol. equiv.) in dichloromethane (10 ml). The reaction mixture was stirred for 1 hr at RT, when TLC analysis confirmed completion of the reaction, then diluted with ether (10 ml). The mixture was filtered through a bed of silica gel and washed with ether (2 x 5 ml), the solvent evaporated and the product was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, 9-((1*R*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)-nonanal **(139)** (0.24 g, 96 %), which showed δ_H (500 MHz, $CDCl_3$): 9.77 (1H, br. t, J 1.85 Hz), 3.38 (3H, s), 2.97-2.95 (1H, m), 2.43 (2H, dt, J 1.85, 7.55 Hz), 1.99-1.97 (1H, m), 1.65-1.61 (1H, m), 1.56 (2H, m), 1.40-1.09 (78H, br.m including br.s at 1.27), 0.89 (6H, dt, J 2.85, 6.6 Hz), 0.85 (3H, d, J 6.6 Hz), 0.48-0.43 (1H, m), 0.22-0.18 (1H, m), 0.17-0.14 (1H, m), 0.13-0.09 (1H, m);; δ_C (125 MHz, $CDCl_3$): 205.1, 85.5, 65.6, 57.7, 43.9, 38.1, 37.4, 35.4, 34.4, 32.8, 32.4, 31.9, 30.5, 30.0, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 27.6, 27.3, 26.2, 25.8, 22.7, 19.7, 18.6, 16.5, 14.3, 10.5; ν_{max}: 2984, 2875, 1724 cm^{-1}; $[\alpha]^{19}_D$ = -3.45 ($CHCl_3$, 1.247 µmol) [Found $M+Na^+$: 781.72; $C_{52}H_{102}NaO_2$ requires: 781.78].

Experiment 24: Methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(pivaloyl oxy)decyl)hexacosanoate (156)



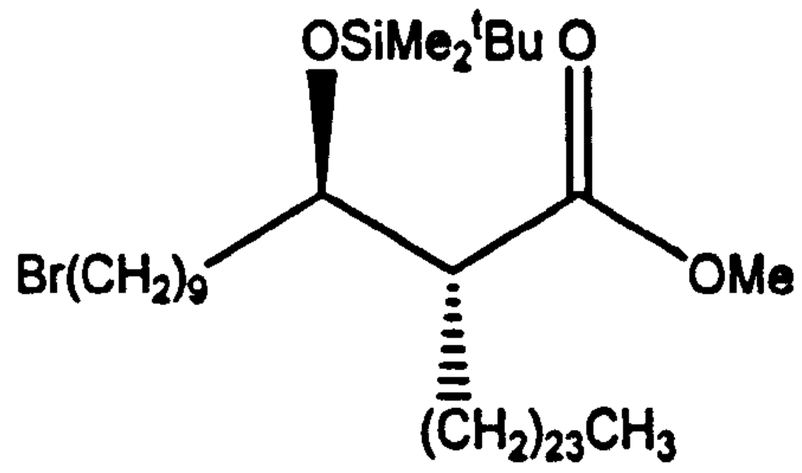
Lithium *bis*(trimethylsilyl)amide (4.14 ml, 4.39 mmol, 1.06 M) was added dropwise to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxo-propyl]-hexacosanoic acid methyl ester **(128)** (1.30 g, 2.25 mmol) and 7-(1-phenyl-1H-tetrazol-5-ylsulfonyl)heptyl pivalate **(154)** (1.20 g, 2.93 mmol, 1.2 mol. equiv.) in dry THF (50 ml) at -15 °C. The mixture was then stirred for 18 hrs at RT; when TLC analysis indicated completion of the reaction, sat. aq. ammonium chloride (20 ml) and petrol/ether (1:1, 50 ml) were added. The aqueous layer was re-extracted with petrol/ether (1:1, 3 x 50 ml) and the combined organic extracts washed with brine (50 ml), dried and evaporated to give a yellow oil. The crude product was purified by column chromatography eluting with petrol/ether (20:1) to give a colourless oil, methyl 2-((*R*-(*E*,*Z*)-1-(*tert*-butyldimethylsilyloxy)-10-(pivaloyloxy)dec-3-nyl)hexa cosanoate **(155)** (1.23 g, 72 %). Palladium on charcoal (10 %, 0.5 g) was added to a stirred solution of methyl 2-((*R*-(*E*,*Z*)-1-(*tert*-butyldimethylsilyloxy)-10-(pivaloyloxy)dec-3-enyl)hexacosanoate **(155)** (1.23 g, 1.54 mmol) in ethanol (20 ml) and THF (20 ml). The mixture was stirred while being hydrogenated at atmospheric pressure, and when hydrogen absorption was complete was filtered through a pad of celite and washed with ethyl acetate (100 ml). The filtrate was evaporated to give a colourless oil, methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(pivaloyloxy)decyl)hexacosan-oate **(156)** (1.12, 93 %), which showed δ_H (500 MHz, $CDCl_3$): 4.06 (2H, t, J 6.65 Hz), 3.92-3.89 (1H, m), 3.66 (3H, s), 2.53 (1H, ddd, J 3.75, 6.9, 10.7), 1.62 (2H, pent, J 6.6 Hz), 1.40-1.23 (60H, br.m including br.s at 1.26), 1.20 (9H, s), 0.89 (3H, t, J 6.95 Hz), 0.87 (9H, s) 0.05 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 178.6, 175.1, 73.2, 64.4, 51.6, 51.2, 38.7, 33.7, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.6, 27.9, 27.5, 27.2, 25.9, 25.8, 23.8, 22.7, 18.0, 14.1, -4.4, -4.9; ν_{max}: 2937, 2866, 1733, 1470 cm^{-1}; $[\alpha]^{20}_D$ = -3.64 ($CHCl_3$, 1.047 µmol) [Found M+Na^+: 803.45; $C_{48}H_{96}NaO_5Si$ requires: 803.69].

Experiment 25: Methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-hydroxy decyl)hexacosanoate (157)



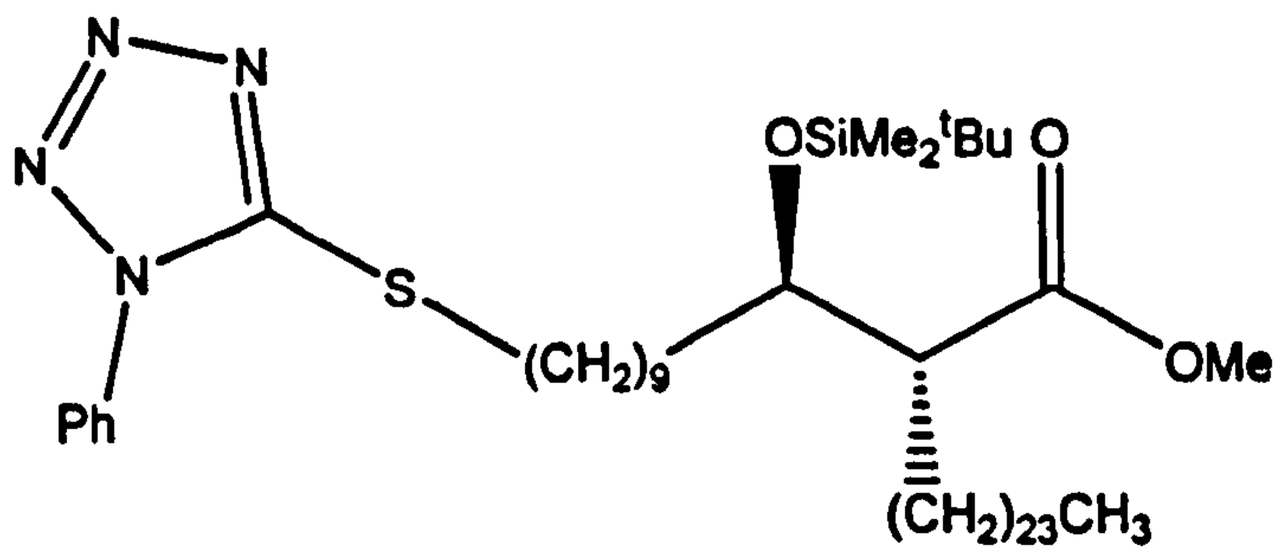
Methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(pivaloyloxy)decyl) hexacos anoate **(156)** (1.10 g, 1.14 mmol) in THF (10 ml) was added to a stirred solution of potassium hydroxide (1.19 g, 21.18 mmol, 15 mol. equiv.) in THF (20 ml), methanol (20 ml) and water (2 ml). The mixture was heated to 70 °C and reflux was maintained for 2 hrs. When TLC analysis indicated completion of the reaction, the mixture was quenched with water (10 ml) and the aqueous layer extracted with ethyl acetate (3 x 50 ml). The combined organic extracts were dried and evaporated and the crude product purified via column chromatography eluting with petrol/ether (5:2) to give a colourless oil, methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-hydroxydecyl)hexacosanoate **(157)** (0.66 g, 67 %), which showed δ_H (500 MHz, $CDCl_3$): 3.92-3.89 (1H, m), 3.66 (3H, s), 3.64 (2H, t, J 6.6 Hz), 2.53 (1H, ddd, J 3.8, 6.95, 10.7 Hz), 1.57 (2H, pent, J 6.3 Hz), 1.36-1.23 (60H, br.m including br.s at 1.26), 0.90 (3H, t J 6.9 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.02 (3H, s); δ_C (125 MHz, $CDCl_3$): 175.1, 73.2, 65.8, 63.0, 51.6, 51.2, 33.7, 32.8, 31.9, 29.8, 29.7, 29.64, 29.59, 29.5, 29.43, 29.40, 29.3, 27.9, 27.4, 27.1, 25.8, 23.8, 22.7, 18.0, 15.2, 14.1, -4.4, -4.9; ν_{max}: 3334, 2950, 2847, 1757, 1457 cm^{-1}; $[\alpha]^{20}_D$ = -5.31 ($CHCl_3$, 1.257 µmol) [Found $M+Na^+$: 719.40; $C_{43}H_{88}NaO_4Si$ requires: 719.63].

Experiment 26: Methyl 2-((1*R*,2*R*)-10-bromo-1-(*tert*-butyldimethylsilyloxy) decyl)hexacosanoate (158)



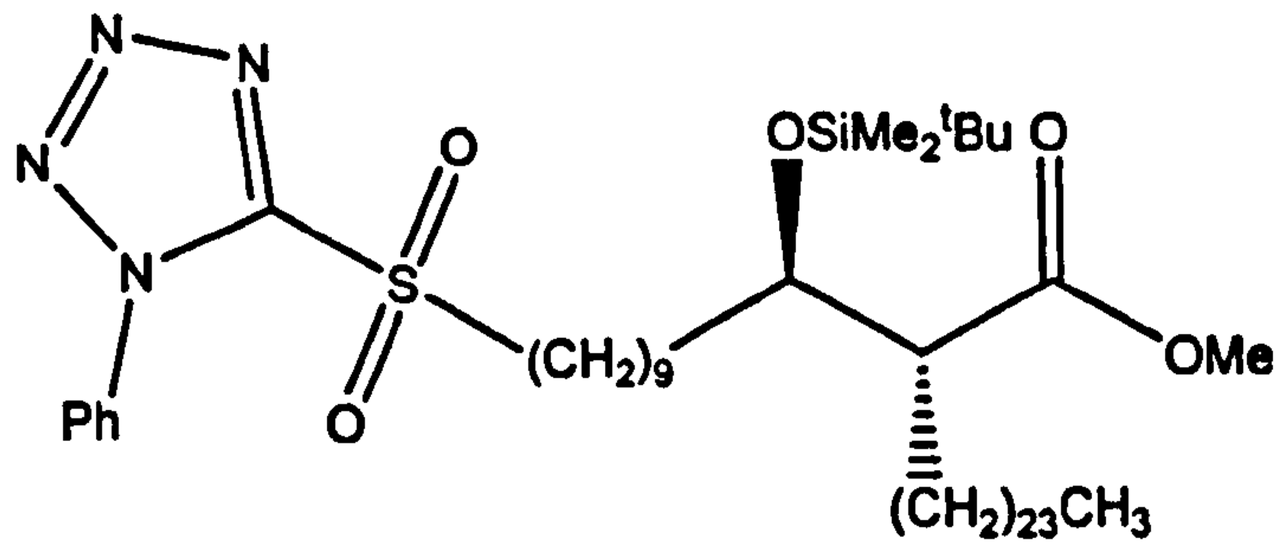
Triphenyl phosphine (0.29 g, 1.12 mmol, 1.2 mol. equiv.) was added to a stirred solution of methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-hydroxydecyl)hexa cosanoate **(157)** (0.65 g, 0.935 mmol) in dry dichloromethane (20 ml) and then sodium hydrogen carbonate (0.10 g) was added. The mixture was cooled to 0 °C and N-bromosuccinimide (0.22 g, 1.22 mmol, 1.3 mol. equiv.) was added portion wise over 10 mins at 0-4 °C. The reaction was stirred for 1 hr at 0-3 °C, when TLC analysis showed completion of the reaction, sat. aq. sodium bisulfate (10 ml). The aqueous layer was re-extracted with dichloromethane (2 x 20 ml) and the combined organic layers were washed with water (20 ml), dried and the solvent evaporated. The resultant crude product was taken up in petrol/ether (1:1, 40 ml) and the mixture stirred for 30 mins, then the triphenylphosphonium oxide was filtered was washed with petrol/ether (1:1, 20 ml). The solvent was evaporated and the crude product purified via column chromatography eluting with petrol/ ether (20:1) to give a colourless oil, methyl 2-((1*R*,2*R*)-10-bromo-1-(*tert*-butyldimethylsilyloxy)decyl) hexacos anoate **(158)** (0.54 g, 76 %), which showed δ_H (500 MHz, $CDCl_3$): 3.93-3.90 (1H, m), 3.66 (3H, s), 3.41 (2H, t, J 6.95 Hz), 2.53 (1H, ddd, J 3.75, 7.25, 11 Hz), 1.58 (2H, pent, J 6.95 Hz), 1.58-1.23 (60H, br.m including br.s at 1.26), 0.89 (3H, t J 6.95 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 175.1, 73.2, 65.8, 51.6, 51.2, 33.9, 33.7, 32.8, 31.9, 31.6, 29.8, 29.7, 29.64, 29.60, 29.42, 29.38, 29.1, 28.7, 28.2, 27.9, 27.7, 27.1, 25.8, 23.8, 22.7, 18.0, 15.2, 14.1, -4.4, -4.9; ν_{max}: 2934, 2857, 1742, 1467 cm^{-1}; $[\alpha]^{21}_D$ = -5.68 ($CHCl_3$, 0.987 µmol) [Found M+Na^+: 781.28; $C_{43}H_{87}NaO_3SiBr$ requires: 781.55].

Experiment 27: Methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(1-phenyl-1H-tetrazol-5-ylthio)decyl)hexacosanoate (159)



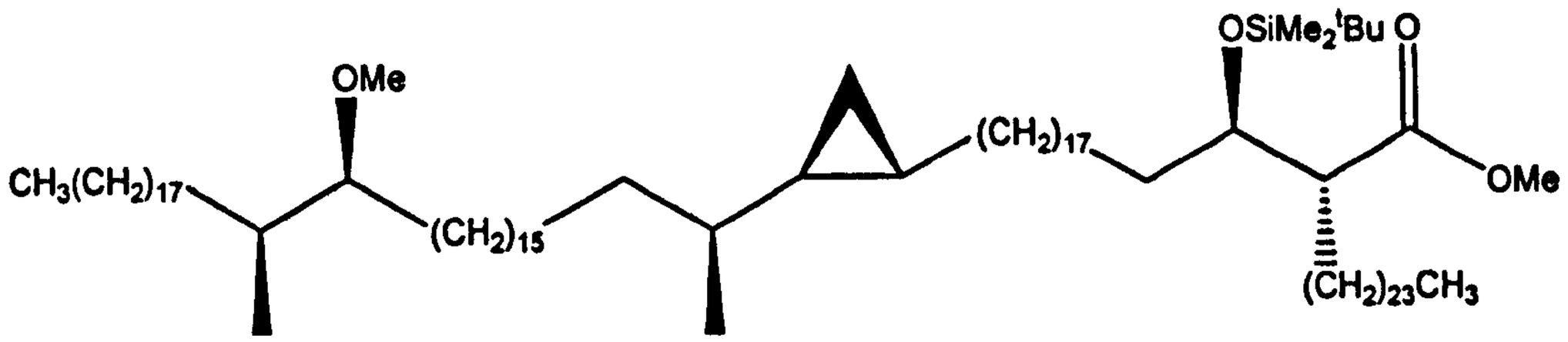
Methyl 2-((1*R*,2*R*)-10-bromo-1-(*tert*-butyldimethylsilyloxy)decyl)hexacosanoate **(158)** (0.54 g, 0.71 mmol) in THF (1.5 ml) and acetone (1.5 ml) was added to a stirred solution of 1-phenyl-1H-tetrazole-5-thiol (0.15 g, 0.857 mmol, 1.2 mol. equiv.) and anhydrous potassium carbonate (0.29 g, 2.14 mmol, 3 mol. equiv.) in acetone (10 ml, HPLC grade) at RT. The mixture was stirred at RT for 18 hrs; the solvent was evaporated and the residue was diluted with petrol/ether (1:1, 20 ml) and water (20 ml). The aqueous layer was re-extracted with petrol/ether (1:1, 3 x 10 ml). The combined organic extracts were dried and evaporated to give a crude oil which was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(1-phenyl-1H-tetrazol-5-ylthio)decyl)hexacosanoate **(159)** (0.45 g, 73 %), which showed δ_H (500 MHz, $CDCl_3$): 7.57-7.53 (5H, m), 3.92-3.89 (1H, m), 3.66 (3H, s), 3.40 (2H, t, J 7.25 Hz), 2.53 (1H, ddd, J 3.8, 7.25, 11.05 Hz), 1.82 (2H, pent, J 7.55 Hz), 1.56-1.20 (60H, br.m including br.s at 1.26), 0.89 (3H, t J 6.6 Hz), 0.87 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C (125 MHz, $CDCl_3$): 175.1, 154.5, 133.8, 130.0, 129.8, 123.9, 73.2, 65.8, 51.6, 51.2, 33.7, 33.4, 31.9, 29.8, 29.7, 29.64, 29.60, 29.43, 29.38, 29.1, 29.0, 28.7, 27.9, 27.7, 27.1, 25.8, 23.8, 22.7, 18.0, 15.2, 14.1, -4.4, -4.9 ; ν_{max}: 2929, 2854, 1741 1465 cm^{-1}; $[\alpha]^{21}_D$ = -5.14 ($CHCl_3$, 1.452 µmol) [Found $M+Na^+$: 879.49; $C_{50}H_{92}NaO_3SiN_4S$ requires: 879.66].

Experiment 28: Methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(1-phenyl-1H-tetrazol-5-ylsulfonyl)decyl)hexacosanoate (140)



m-Chloroperoxybenzoic acid (0.39 g, 1.58 mmol, 3 mol. equiv) in dichloromethane (2 ml) was added slowly to a stirred solution of methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(1-phenyl-1H-tetrazol-5-ylthio)decyl)hexacosanoate **(159)** (0.45 g, 0.53 mmol) and sodium hydrogen carbonate (0.20 g, 2.37 mmol, 4.5 mol. equiv.) in dichloromethane (5 ml) at 5 °C. The mixture was stirred for 18 hrs at RT; when TLC analysis indicated completion of the reaction, the solvent was evaporated. The resultant residue was diluted with ethyl acetate (5 ml) and slowly quenched with sat. aq. sodium metabisulfite (2 ml). The aqueous layer was re-extracted with ethyl acetate (2 x 10 ml) and the combined organic extracts were washed with sat. aq. sodium hydrogen carbonate (10 ml) and then water (20 ml). The organic extract was then dried and evaporated and the resultant yellow oil purified via column chromatography eluting with petrol/ether (1:1) to give a colourless oil, methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(1-phenyl-1H-tetrazol-5-ylsulfonyl)decyl)hexacosanoate **(140)** (0.35 g, 75 %), which showed δ_H (500 MHz, $CDCl_3$): 7.71-7.70 (2H, m), 7.64-7.60 (3H, m), 3.92-3.89 (1H, m), 3.74 (2H, t, J 8.2 Hz), 3.66 (3H, s), 2.53 (1H, ddd, J 3.8, 6.95, 11.05 Hz), 1.96 (2H, dist. pent, J 7.9 Hz), 1.51-1.20 (60H, br.m including br.s at 1.26), 0.89 (3H, t J 6.6 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.02 (3H, s); δ_C (125 MHz, $CDCl_3$): 175.1, 154.5, 133.8, 130.0, 129.8, 123.9, 73.2, 61.8, 51.6, 51.2, 33.7, 33.4, 31.9, 29.8, 29.7, 29.6, 29.6, 29.4, 29.4, 29.1, 29.0, 28.7, 27.9, 27.7, 27.1, 25.8, 23.8, 22.7, 18.0, 15.2, 14.1, -4.4, -4.9 ; ν_{max}: 2919, 2848, 1721 1464 cm^{-1}; $[\alpha]^{21}_D$ = -4.85 ($CHCl_3$, 1.201 µmol); [Found M+Na^+: 911.52; $C_{50}H_{92}NaO_5SiN_4S$ requires: 911.65].

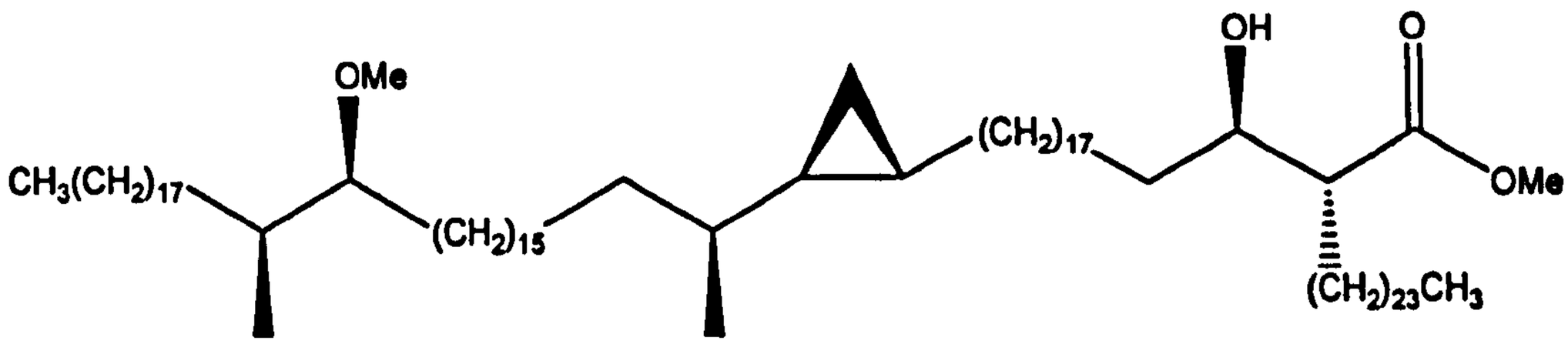
Experiment 29: Methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,18*S*,19*S*)-18-methoxy-19-methylheptatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate (161)



Lithium *bis*(trimethylsilyl)amide (0.2920 ml, 0.309 mmol, 1.06M) was added dropwise to a stirred solution of methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(1-phenyl-1H-tetrazol-5-ylsulfonyl)decyl)hexacosanoate **(140)** (183 mg, 0.206 mmol) and 9-((1*R*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl) cyclopropyl) nonanal **(139)** (172 mg, 0.227 mmol) in dry THF (10 ml) under nitrogen at -20 °C. The temperature rose to -10 °C during the addition of the base, and a yellow solution resulted. The mixture was allowed to reach RT, stirred for 1 hr, when TLC showed no starting material was left; then cooled to 0 °C and quenched with sat. aq. ammonium chloride (10 ml). The product was extracted with petrol/ether (1:1, 3 x 10 ml). The combined organic layers were washed with brine (20 ml), dried and evaporated to give an oil, which was purified by column chromatography eluting with petrol/ether (20:1) to give methyl 2-((*R*)-(*E*/*Z*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,18*S*,19*S*)-18-methoxy-19-methyl heptatriacontan-2-yl)cyclopropyl)nonadec-10-enyl)hexacosanoate **(160)** (80.6 mg, 28 %). Dipotassium azodicarboxylate (0.33 g, 1.708 mmol, 30 mol. equiv.) was added to a stirred solution of compound **(160)** (80.6 mg, 0.057 mmol) in THF (5 ml) and methanol (5 ml) at 10 °C under nitrogen, giving a yellow precipitate. A solution of glacial acetic acid (1 ml) and THF (2 ml) was added dropwise over 48 hrs, after which a white precipitate had formed. The mixture was cooled to 0 °C and poured slowly into sat. aq. sodium hydrogen carbonate (5 ml) and then extracted with petrol/ether (1:1, 3 x 10 ml). The combined organic layers were washed with water (10 ml), dried and evaporated to give a thick oil which slowly solidified. The residue was purified via column chromatography eluting in petrol/ether (10:1) to give a white solid, methyl 2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,18*S*,

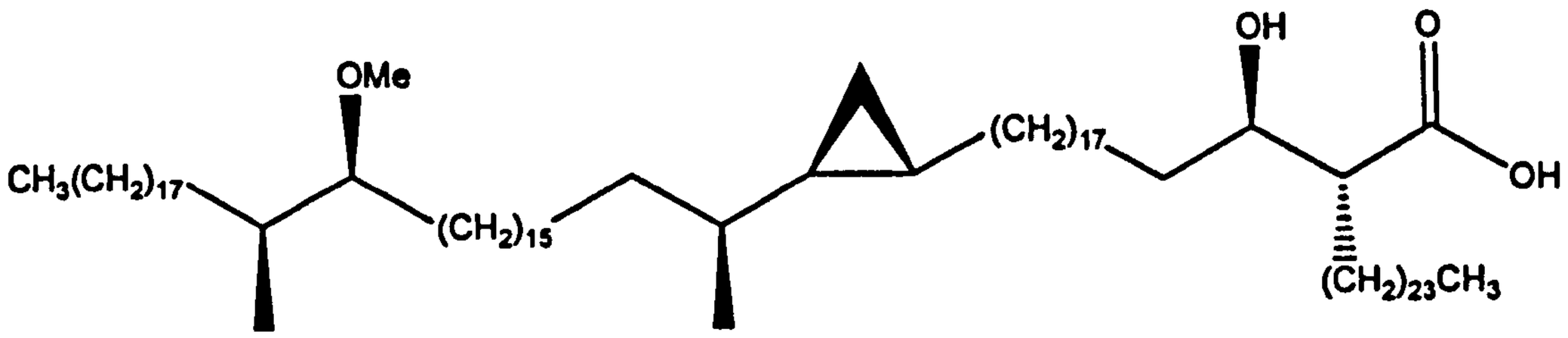
19*S*)-18-methoxy-19-methylheptatriacontan-2-yl)cyclopropyl)nonadecyl) hexacosan -oate **(161)** (75.7 mg, 94 %), which showed δ_H (500 MHz, $CDCl_3$): 3.92-3.90 (1H, m), 3.66 (3H, s), 3.35 (3H, s), 2.97-2.95 (1H, m), 2.54 (1H, ddd, J 3.75, 7.25, 11 Hz), 1.58-1.18 (150H, br.m including br.s at 1.27), 0.91-0.85 (21H, m), 0.48-0.44 (1H, m), 0.22-0.18 (1H, m), 0.17-0.14 (1H, m), 0.13-0.09 (1H, m), 0.05 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 175.1, 125.5, 85.5, 73.2, 65.9, 57.7, 51.6, 38.1, 37.4, 37.1, 35.8, 35.4, 34.5, 33.7, 32.8, 32.4, 31.9, 31.1, 30.5, 30.3, 30.1, 30.0, 29.9, 29.8, 29.7, 29.64, 29.60, 29.52, 29.49, 29.4, 27.8, 27.6, 27.5, 27.3, 26.2, 26.1, 25.8, 23.7, 22.7, 19.7, 18.6, 18.0, 14.9, 14.1, 10.5, -4.4, -4.9; ν_{max}: 2923, 2852, 1741, 1465cm^{-1}; $[\alpha]^{24}_D$ = -1.45 ($CHCl_3$, 0.856 μmol); m.p. 61-63 °C; [Found $M+Na^+$: 1446.21; $C_{95}H_{190}NaO_4Si$ requires: 1446.43].

Experiment 30: Methyl 2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,18*S*,19*S*)-18-methoxy-19-methylheptatriacontan-2-yl)cyclopropyl)nonadecyl) hexacosanoate (162)



A dry polyethylene vial equipped with a rubber septum was charged with methyl 2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,18*S*,19*S*)-18-methoxy-19-methylheptatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate **(161)** (70 mg, 0.0494 mmol) in dry THF (4 ml) under nitrogen at 0 °C. Pyridine (0.2 ml) and hydrogen fluoride-pyridine complex (0.2 ml, 0.140 mmol, 208 mol. equiv.) were added and the mixture stirred for 32hrs at 43 °C. When TLC analysis indicated completion of the reaction, the mixture was neutralised by slowly pouring the mixture into sat. aq. sodium hydrogen carbonate (10 ml) until no more carbon dioxide was liberated. The product was extracted with petrol/ether (1:1, 3 x 50 ml), dried and evaporated to give a white solid. This was purified via column chromatography eluting with petrol/ether (4:1) to give a white solid, methyl 2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,18*S*,19*S*)-18-methoxy-19-methylheptatriacontan-2-yl)cyclopropyl)nonadecyl) hexacosanoate **(162)** (35 mg, 54 %), which showed δ_H (500 MHz, $CDCl_3$): 3.72 (3H, s), 3.69-3.64 (1H, m), 3.35 (3H, s), 2.97-2.95 (1H, m), 2.44 (1H, dt, J 5.35, 9.15 Hz), 1.63-1.13 (150H, br.m including br.s at 1.26), 0.91-0.85 (12H, m), 0.47-0.43 (1H, m), 0.22-0.18 (1H, m), 0.17-0.14 (1H, m), 0.13-0.09 (1H, m); δ_C (125 MHz, $CDCl_3$): 176.2, 85.5, 72.3, 57.7, 51.5, 50.9, 38.1, 37.4, 35.7, 35.3, 32.4, 31.9, 31.6, 30.5, 30.3, 30.1, 29.9, 29.7, 29.64, 29.62, 29.60, 29.58, 29.53, 29.49, 29.42, 29.38, 27.6, 27.4, 27.3, 26.2, 26.1, 25.7, 22.7, 19.7, 18.6, 15.3, 14.9, 14.1, 10.5; ν_{max}: 3509, 2916, 2848, 2360, 2341, 1727, 1714, 1468 cm^{-1}; $[\alpha]^{23}_D$ = -1.12 ($CHCl_3$, 0.993 μmol); m.p. = 63 - 65 °C [Found $M+Na^+$: 1332.19; $C_{89}H_{176}NaO_4$ requires: 1332.35].

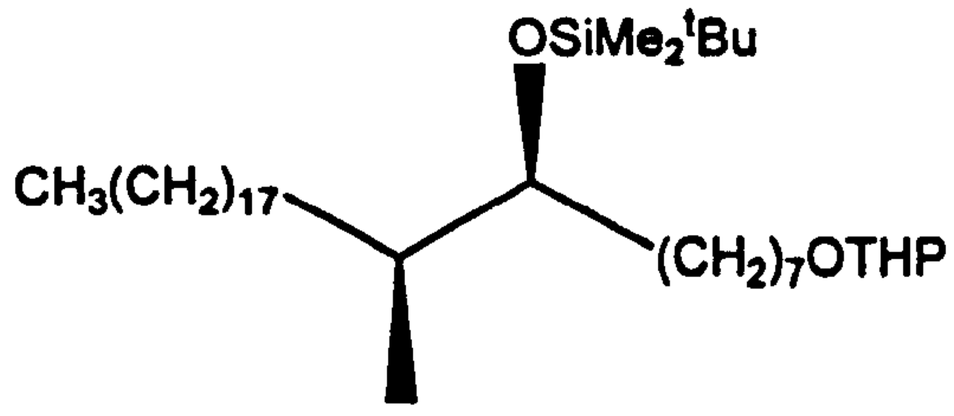
Experiment 31: (*R*)-2-((*R*)-1-Hydroxy-19-((1*S*,2*R*)-2-((2*S*,18*S*,19*S*)-18-methoxy-19-methylheptatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoic acid (84)



Lithium hydroxide monohydrate (20 mg, 0.835 mmol, 30 mol. equiv.) was added to a stirred solution of methyl 2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,18*S*,19*S*)-18-methoxy-19-methylheptatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate **(162)** (35 mg, 0.0269 mmol) in THF (2.5 ml), methanol (0.3 ml) and water (0.3 ml) at RT. The mixture was stirred at 43 °C for 18 hrs; when TLC analysis indicated completion of the reaction, the reaction was then cooled to RT and acidified with hydrochloric acid (5 %, 1 ml) and the aqueous layer extracted with warm petrol/ether (1:1, 3 x 10 ml). The combined organic extracts were dried and evaporated, and then purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,18*S*,19*S*)-18-methoxy-19-methylheptatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoic acid **(84)** (24.0 mg, 69 %), which showed δ_H (500 MHz, $CDCl_3$): 3.74-3.70 (1H, m), 3.36 (3H, s), 2.99-2.96 (1H, m), 2.49-2.45 (1H, m), 1.79-1.72 (1H, m), 1.69-1.61 (2H, br.m), 1.55-1.10 (149H, br.m including br.s at 1.27), 0.91-0.85 (12H, br.m including t, J 6.95 Hz), 0.48-0.43 (1H, m), 0.22-0.18 (1H, m), 0.17-0.14 (1H, m), 0.13-0.09 (1H, m); δ_C (125 MHz, $CDCl_3$): 178.7, 85.6, 72.1, 65.9, 57.7, 50.6, 38.2, 37.4, 35.6, 35.3, 34.5, 32.4, 31.9, 30.5, 30.1, 30.0, 29.9, 29.72, 29.69, 29.64, 29.59, 29.5, 29.43, 29.39, 27.6, 27.3, 27.2, 26.2, 25.8, 22.7, 19.7, 18.6, 15.3, 14.9, 14.1, 10.5; ν_{max}: 3520, 2924, 2853, 1735, 1465 cm^{-1}; $[\alpha]^{22}_D$ = -0.97 ($CHCl_3$, 0.468 µmol); m.p. 70-72 °C; [Found $M+Na^+$: 1318.24; $C_{88}H_{174}NaO_4$ requires: 1318.33].

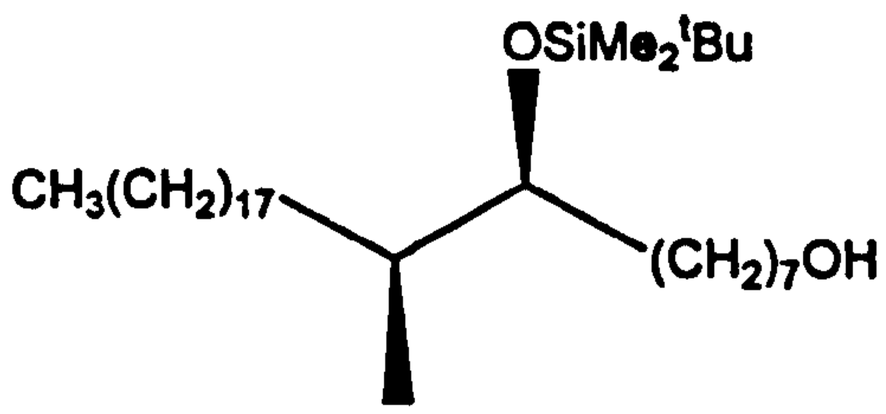
4.3 – Synthesis of protected α-methyl trans-alkene keto-mycolic acid (85)

Experiment 32: *tert*-Butyldimethyl((8*S*,9*S*)-9-methyl-1-(tetrahydro-2H-pyran-2-yloxy)heptacosan-8-yloxy)silane (169)



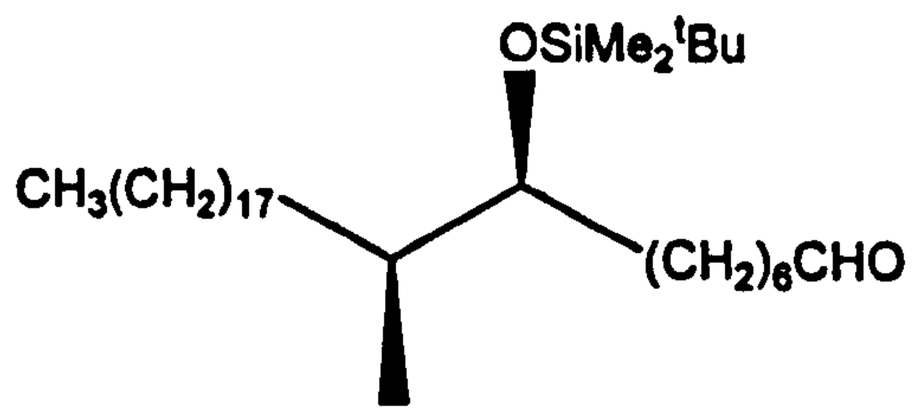
Imidazole (5.0 g, 73.5 mmol, 2.5 mol. equiv.) was added to a stirred solution of (8*S*,9*S*)-9-methyl-1-(tetrahydro-2H-pyran-2-yloxy)heptacosan-8-ol **(167)** (15.0 g, 29.4 mmol) in dry DMF (100 ml) at RT followed by addition of *tert*-butyl dimethylsilyl chloride (5.76 g, 38.2 mmol, 1.3 mol. equiv.). The mixture was heated to 50 °C for 18 hrs. When TLC showed no starting material was left. the mixture was quenched with water (200 ml) and extracted with dichloromethane (3 x 200 ml). The combined organic extracts were washed with water (100 ml), dried and the solvent evaporated; the crude product was purified via column chromatography eluting with petrol/ether (5:1) to give a colourless oil, *tert*-butyldimethyl((8*S*,9*S*)-9-methyl-1-(tetrahydro-2H-pyran-2-yloxy)heptacosan-8-yloxy)silane **(169)** (16.71 g, 91 %), which showed δ_H (500 MHz, $CDCl_3$): 4.58 (1H, t, J 2.8 Hz), 3.90-3.86 (1H, m), 3.74 (1H, dt, J 6.95, 9.8 Hz), 3.53-3.48 (2H, m), 3.40 (1H, dt, J 6.95, 9.8 Hz), 1.86-1.82 (1H, m), 1.75-1.70 (1H, m), 1.62-1.17 (50H, br.m including br.s at 1.27), 0.89 (9H, s), 0.88 (3H, t, J 7.0 Hz), 0.80 (3H, d, J 6.95 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C (125 MHz, $CDCl_3$): 98.8, 75.9, 67.7, 62.3, 37.8, 33.5, 32.5, 31.9, 30.8, 30.0, 29.80, 29.76, 29.7, 29.6, 29.5, 29.4, 29.1, 27.73, 27.68, 26.2, 26.0, 25.9, 25.5, 22.7, 19.7, 18.2, 14.4, 14.3, 14.1, -4.2, -4.4; ν_{max}: 2923, 2854, 1464 cm^{-1}; $[\alpha]^{22}_D$ = -6.41 ($CHCl_3$, 1.088 μmol) [Found $M+Na^+$: 647.39; $C_{39}H_{80}NaO_3Si$ requires: 647.58].

Experiment 33: (8*S*,9*S*)-8-(*tert*-Butyldimethylsilyloxy)-9-methylheptacosan-1-ol (170)



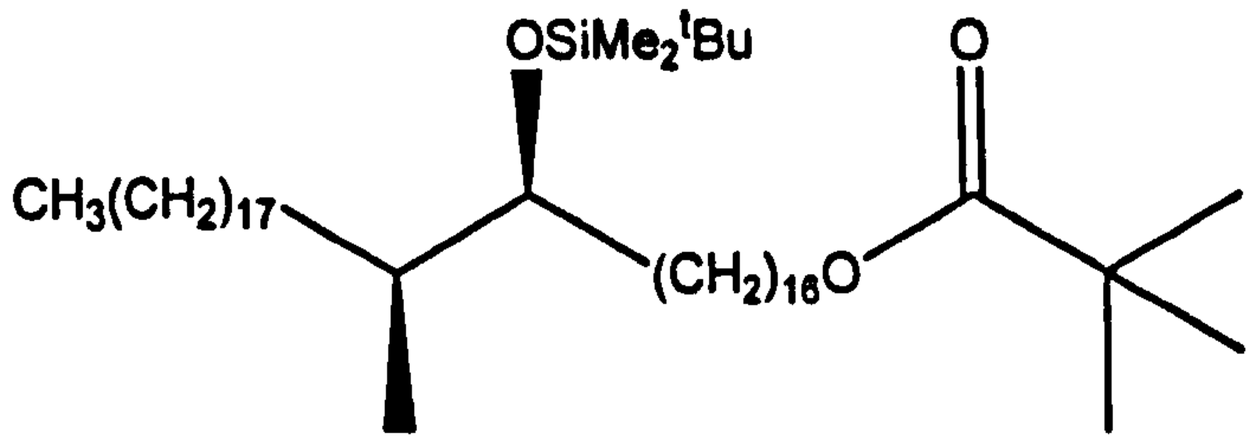
A solution pyridinium-*p*-toluene sulfonate (3.36 g, 13.4 mmol) in methanol (150 ml)was added to a stirred solution of *tert*-butyldimethyl((8*S*,9*S*)-9-methyl-1-(tetrahydro-2H-pyran-2-yloxy)heptacosan-8-yloxy)silane **(169)** (16.71 g, 26.8 mmol) in THF (150 ml) and refluxed for 2.5 hrs, when TLC showed no starting material was left. Sat. aq. sodium hydrogen carbonate (50 ml) and water (100 ml) were added and the product extracted with ether (3 x 100 ml). The combined organic layers were dried and the solvent evaporated to give a crude oil, which was purified via column chromatography eluting with petrol/ether (5:1) then petrol/ether (1:1) to give a colourless oil, *tert*-butyldimethyl((8*S*,9*S*)-9-methyl-1-(tetrahydro-2H-pyran-2-yloxy)heptacosan-8-yloxy)silane **(170)** (11 g, 80 %), which showed δ_H (500 MHz, $CDCl_3$): 3.65 (2H, t, J 6.5 Hz), 3.50 (1H, dt, J 3.5, 6.0 Hz), 1.58 (2H, pent, J 7.3 Hz), 1.50-1.13 (45H, br.m including br.s at 1.27), 1.09-1.02 (1H, m), 0.89 (9H, s), 0.88 (3H, t, J 6.95 Hz), 0.80 (3H, d, J 6.6 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C (125 MHz, $CDCl_3$): 75.9, 63.1, 37.8, 33.5, 32.8, 32.4, 31.9, 30.0, 29.9, 29.73, 29.68, 29.5, 29.4, 27.7, 26.0, 25.9, 25.7, 22.7, 18.2, 14.5, 14.1, -4.2, -4.4; ν_{max}: 3321, 2942, 2845, 1464 cm^{-1}; $[\alpha]^{22}_D$ = -12.7 ($CHCl_3$, 1.245 μmol) [Found $M+Na^+$: 563.47; $C_{34}H_{72}NaO_2Si$ requires: 563.52].

Experiment 34: (8*S*,9*S*)-8-(*tert*-butyldimethylsilyloxy)-9-methylheptacosanal (171)



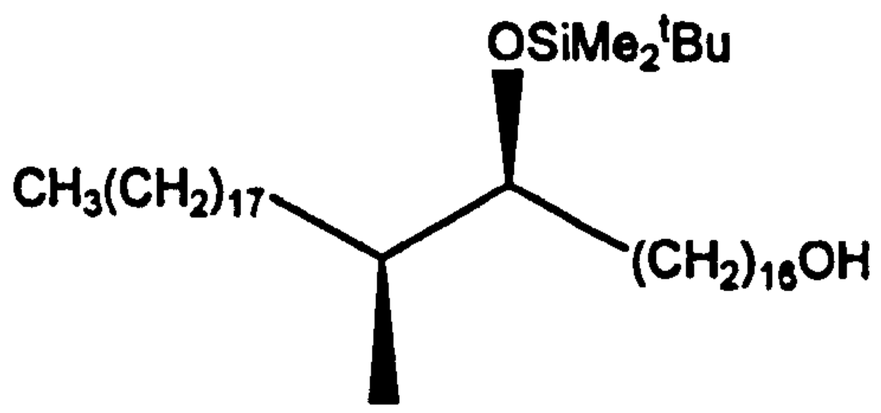
(8*S*,9*S*)-8-(*tert*-Butyldimethylsilyloxy)-9-methylheptacosan-1-ol **(170)** (0.60 g, 1.11 mmol) was added to a stirred suspension of PCC (0.60 g, 2.78 mmol, 2.5 mol. equiv.) in dichloromethane (25 ml). The reaction was stirred for 2 hrs at RT, when TLC showed no starting material was left, then diluted with ether (100 ml), filtered through a bed of silica gel and the solvent evaporated. The crude product was purified via column chromatography eluting with petrol/ether (5:2) to give a yellow oil, (8*S*,9*S*)-8-(*tert*-butyldimethylsilyloxy)-9-methylheptacosanal **(171)** (0.56 g, 94 %), which showed δ_H (500 MHz, $CDCl_3$): 9.77 (1H, t, J 1.5 Hz), 3.50-3.47 (1H, m), 2.44 (2H, dt, J 1.5, 7.2 Hz), 1.63 (2H, pent, J 7.3 Hz), 1.48-1.18 (45H, br.m including br.s at 1.27), 1.09-1.02 (1H, m), 0.89 (9H, s), 0.87 (3H, t, J 6.95 Hz), 0.80 (3H, d, J 6.6 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C (125 MHz, $CDCl_3$): 202.9, 75.9, 37.8, 33.5, 33.8, 32.4, 31.9, 30.0, 29.9, 29.73, 29.68, 29.5, 29.4, 27.7, 26.0, 25.9, 25.7, 22.7, 20.9, 18.2, 14.5, 14.1, -4.2, -4.4; ν_{max}: 2924, 2855, 1730, 1464 cm^{-1}; $[\alpha]^{21}_D$ = +7.7 ($CHCl_3$, 1.047 μmol) [Found $M+Na^+$: 561.44; $C_{34}H_{70}NaO_2Si$ requires: 561.50].

Experiment 35: (17*S*,18*S*)-17-(*tert*-Butyldimethylsilyloxy)-18-methylhexatria contyl pivalate



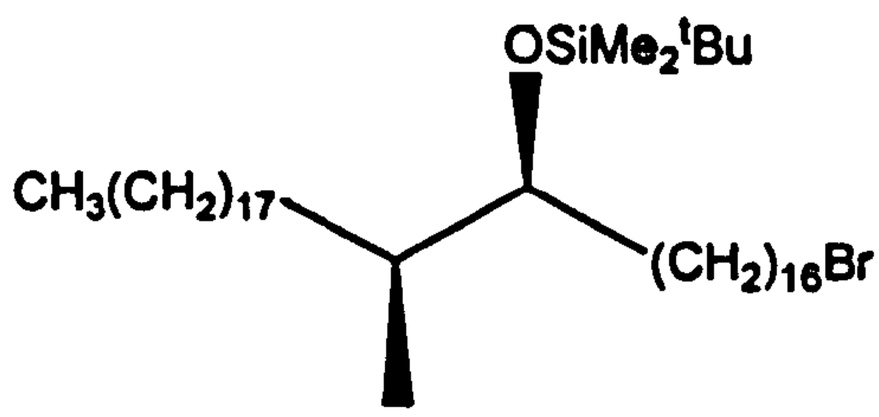
A solution of (8*S*,9*S*)-8-(*tert*-butyldimethylsilyloxy)-9-methylheptacosanal **(171)** (1.43 g, 3.26 mmol) and 9-(1-phenyl-1H-tetrazol-5-ylsulfonyl)nonyl pivalate **(172)** (1.711 g, 3.91 mmol, 1.2 mol. equiv.) in dry THF (50 ml) was stirred under nitrogen at -15 °C. Lithium *bis*(trimethylsilyl)amide (4.80 ml, 5.09 mmol, 1.06 M) was added dropwise between -12 °C and -5 °C, the solution was stirred for 18 hrs. When TLC showed no starting material was left, sat. aq. ammonium chloride (50 ml) and petrol/ether (100 ml, 1:1) were added. The aqueous layer was re-extracted with further petrol/ether (2 x 50 ml, 1:1) and the combined organic layers were dried and evaporated to give a crude product. This was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, (17*S*,18*S*)-(*E*/*Z*)-17-(*tert*-butyldimethylsilyloxy)-18-methylhexatriacont-9-enyl pivalate **(232)** (1.85 g, 76 %). Palladium on charcoal (0.25 g, 10%) was added to a stirred solution of the above product (1.83 g, 2.44 mmol) in THF (5 ml) and IMS (50 ml). The mixture was stirred while being hydrogenated at atmospheric pressure. When no more hydrogen was being absorbed the catalyst was removed via suction filtration on a pad of celite and was washed with THF (100 ml). The filtrate was evaporated to give a colourless oil, (17*S*,18*S*)-17-(*tert*-butyldimethylsilyloxy)-18-methylhexatriacontyl pivalate **(233)** (1.28 g, 70 %), which showed δ_H (500 MHz, $CDCl_3$): 4.04 (2H, t, J 6.6 Hz), 3.51-3.49 (1H, m), 1.66-1.60 (2H, m), 1.51-1.25 (62H, br.m including br.s at 1.27), 1.21 (9H, s), 1.07-1.03 (1H, m), 0.89 (9H, s), 0.87 (3H, t, J 7.2 Hz), 0.80 (3H, d, J 7.0 Hz), 0.05 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 178.6, 75.8, 64.5, 38.7, 37.7, 33.5, 32.5, 31.9, 30.0, 29.9, 29.73, 29.68, 29.6, 29.5, 29.4, 29.3, 28.6, 27.7, 27.2, 26.0, 25.9, 22.7, 18.2, 14.4, 14.1, -4.2, -4.4; ν_{max}: 2925, 2855, 1745, 1464 cm^{-1}; $[\alpha]^{24}_D$ = -5.98 ($CHCl_3$, 1.078 μmol) [Found $M+Na^+$: 773.66; $C_{48}H_{98}NaO_3Si$ requires: 773.72].

Experiment 36: (17*S*,18*S*)-17-(*tert*-Butyldimethylsilyloxy)-18-methylhexa triacontan-1-ol



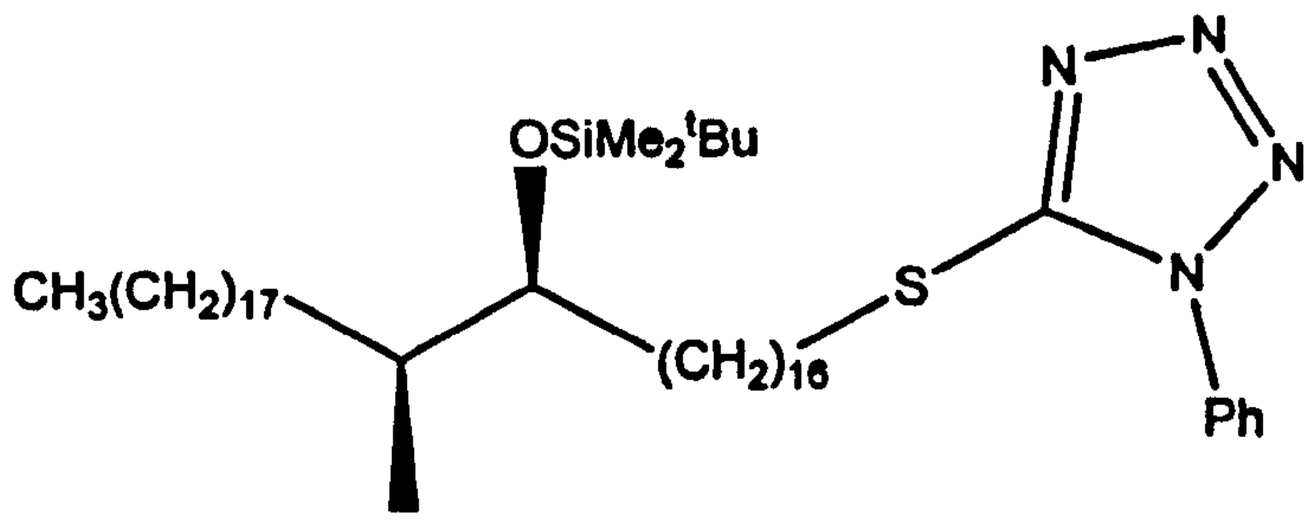
Lithium aluminium hydride (0.10 g, 2.56 mmol) was added to stirring THF (20 ml) at -20 °C under nitrogen. A solution of (17*S*,18*S*)-17-(*tert*-butyldimethylsilyloxy)-18-methylhexatriacontyl pivalate (1.28 g, 1.71 mmol) in THF (10 ml) was added slowly and allowed to reach RT, then refluxed for 1 hr. When TLC showed no starting material was left the reaction was cooled to -20 °C and quenched with sat. aq. sodium sulfate until a white precipitate formed. THF (50 ml) was added and the mixture was stirred for 30 mins and then filtered on a bed of silica gel and the solvent evaporated. The residue was taken up in dichloromethane (50 ml) and washed with water (10 ml) and then dried. The solvent was evaporated and the crude product was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a colourless oil, (17*S*,18*S*)-17-(*tert*-butyldimethylsilyloxy)-18-methylhexatriacontan-1-ol (1.02 g, 90 %), which showed δ_H (500 MHz, $CDCl_3$): 3.65 (2H, t, J 6.5 Hz), 3.52-3.49 (1H, m), 1.60 (2H, pent, J 7.25 Hz), 1.51-1.22 (62H, br.m including br.s at 1.27), 1.09-1.01 (1H, m), 0.89 (9H, s), 0.87 (3H, t, J 6.95 Hz), 0.80 (3H, d, J 6.6 Hz), 0.04 (3H, s), 0.02 (3H, s); δ_C (125 MHz, $CDCl_3$): 75.9, 63.1, 37.8, 33.6, 32.9, 32.6, 31.9, 30.0, 29.9, 29.73, 29.68, 29.64, 29.61, 29.57, 29.5, 29.4, 27.7, 26.0, 25.9, 25.8, 22.7, 18.2, 14.4, 14.1, -4.2, -4.4; ν_{max}: 3335, 2924, 2860, 1464 cm^{-1}; $[\alpha]^{20}_D$ = -5.47 ($CHCl_3$, 1.145 μmol) [Found $M+Na^+$: 689.57; $C_{43}H_{90}NaO_2Si$ requires: 689.66].

Experiment 37: ((17*S*,18*S*)-1-Bromo-18-methylhexatriacontan-17-yloxy)(*tert*-butyl)dimethylsilane



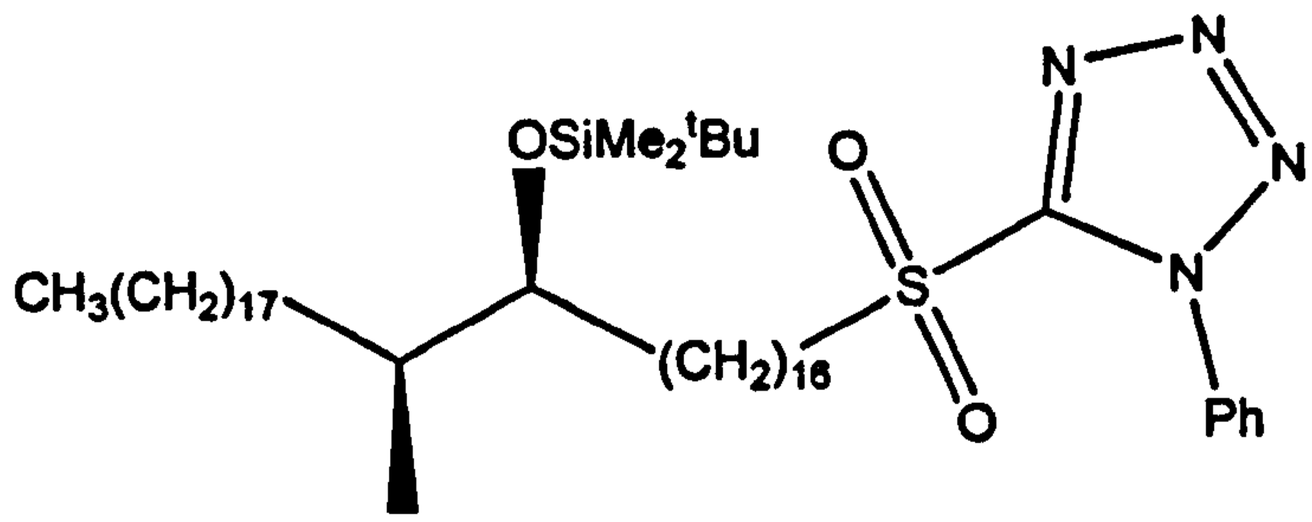
N-Bromosuccinimide (0.32 g, 1.82 mmol, 1.3 mol. equiv.) was added in portions over 15 mins to a stirred solution of (17*S*,18*S*)-17-(*tert*-butyldimethylsilyloxy)-18-methylhexatriacontan-1-ol (1.02 g, 1.53 mmol) and triphenyl phosphine (0.46 g, 1.76 mmol, 1.15 equiv) in dichloromethane (30 ml) at 0 °C. The mixture was stirred at RT for 1 hr. When TLC analysis indicated completion of the reaction, it was quenched with sat. aq. sodium meta-bisulfite (25 ml). The aqueous layer was re-extracted with dichloromethane (2 x 20 ml) and the combined organic extracts washed with water (20 ml), dried and evaporated to give a residue. This residue was treated with petrol/ether (1:1, 50 ml), refluxed for 30 mins and then filtered and washed with petrol/ether (1:1, 25 ml). The filtrate was evaporated and the resultant residue purified via column chromatography eluting with petrol/ether (4:1) then (2:1) to give a yellow oil, ((17*S*,18*S*)-1-bromo-18-methylhexatriacontan-17-yloxy)(*tert*-butyl)dimethylsilane (0.90 g, 81 %), which showed δ_H (500 MHz, $CDCl_3$): 3.53-3.57 (1H, m), 3.42 (2H, t, J 6.7 Hz), 1.87 (2H, pent, J 7.0 Hz), 1.56-1.12 (62H, br.m including br.s at 1.27), 1.10-1.02 (1H, m), 0.91 (9H, s), 0.88 (3H, t, J 7.2 Hz), 0.81 (3H, d, J 7.0 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 75.9, 37.7, 34.0, 33.5, 32.5, 31.9, 30.0, 29.9, 29.7, 29.6, 29.5, 29.4, 28.8, 28.2, 27.7, 26.0, 25.9, 22.7, 18.2, 14.4, 14.1, -4.2, -4.4; ν_{max}: 2922, 2855, 1464 cm^{-1}; $[\alpha]^{21}_D$ = +4.44 ($CHCl_3$, 1.357 μmol) [Found $M+Na^+$: 751.49; $C_{43}H_{89}BrNaOSi$ requires: 751.58].

Experiment 38: 5-((17*S*,18*S*)-17-(*tert*-Butyldimethylsilyloxy)-18-methylhexa triacontylthio)-1-phenyl-1H-tetrazole



((17*S*,18*S*)-1-Bromo-18-methylhexatriacontan-17-yloxy)(*tert*-butyl)dimethylsilane (0.82 g, 1.13 mmol) was added to a stirred solution of 1-phenyl-1H-tetrazole-5-thiol (0.22 g, 1.24 mmol, 1.1 mol. equiv.) and anhydrous potassium carbonate (0.46 g, 3.29 mmol, 3 mol. equiv.) in acetone (20 ml) at RT. The mixture was stirred at RT for 18 hrs, then the solvent was evaporated and the residue was diluted with petrol/ether (1:1, 50 ml) and water (25 ml). The aqueous layer was re-extracted with petrol/ether (1:1, 2 x 25 ml). The combined organic extracts were dried and evaporated to give a crude oil, which was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, 5-((17*S*,18*S*)-17-(*tert*-butyldimethylsilyloxy)-18-methylhexatriacontylthio)-1-phenyl-1H-tetrazole (0.92 g, 99 %), which showed δ_H (500 MHz, $CDCl_3$): 7.66-7.58 (5H, m), 3.55-3.45 (1H, m), 3.40 (2H, t, J 7.4 Hz), 1.83 (2H, pent, J 7.4 Hz), 1.60-1.10 (62H, br.m including br.s at 1.27), 1.10-1.02 (1H, m), 0.91 (9H, s), 0.88 (3H, t, J 7.2 Hz), 0.81 (3H, d, J 7.0 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 154.5, 130.0, 129.7, 123.8, 75.9, 37.7, 33.4, 32.5, 31.9, 29.9, 29.7, 29.4, 29.3, 29.1, 29.0, 28.6, 27.7, 25.9, 22.7, 14.9, 14.1, -4.2, -4.4; ν_{max}: 2926, 2854, 1598, 1499, 1464 cm^{-1}; $[\alpha]^{22}_D$ = -3.20 ($CHCl_3$, 1.324 µmol) [Found M+Na^+: 849.53; $C_{50}H_{94}N_4NaOSSi$ requires: 849.68].

Experiment 39: 5-((17*S*,18*S*)-17-(*tert*-Butyldimethylsilyloxy)-18-methylhexa triacontylsulfonyl)-1-phenyl-1H-tetrazole (164)



A solution of ammonium molybdate (VI) tetrahydrate (1.33 g, 1.08 mmol, 0.5 mol. equiv.) in -10 °C hydrogen peroxide (5 ml, 35 % w/w) was added and to a stirred solution of 5-((17*S*,18*S*)-17-(*tert*-butyldimethylsilyloxy)-18-methylhexatria contylthio)-1-phenyl-1H-tetrazole pivalate (0.89 g, 1.08 mmol) in THF (10 ml) and IMS (35 ml) at 12 °C and stirred at 15-20 °C for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (0.67 g, 0.54 mmol, 0.2 mol. equiv.) in -10 °C hydrogen peroxide (2 ml, 35 % w/w) was added and the reaction mixture stirred for 18 hrs. The mixture was then poured into water (100 ml) and extracted with dichloromethane (3 x 15 ml), washed with water (50 ml), dried and evaporated. The crude product was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, 5-((17*S*,18*S*)-17-(*tert*-butyldimethylsilyloxy)-18-methylhexatriacontylsulfonyl)-1-phenyl-1H-tetrazole **(164)** (0.79 g, 85 %), which showed δ_H (500 MHz, $CDCl_3$): 7.72-7.70 (2H, m), 7.65-7.58 (3H, m), 3.74 (2H, t, J 7.9 Hz), 1.97 (1H, dist. pent, J 7.9 Hz), 1.59-1.11 (62H, br.m including br.s at 1.27), 1.10-1.02 (1H, m), 0.92 (9H, s), 0.88 (3H, t, J 7.25 Hz), 0.81 (3H, d, J 7.0 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 153.5, 133.1, 131.4, 129.7, 125.1, 75.9, 56.0, 37.7, 33.5, 32.5, 31.9, 30.0, 29.9, 29.73, 29.68, 29.6, 29.5, 29.4, 29.2, 28.9, 28.1, 27.7, 26.0, 25.9, 22.7, 21.9, 18.2, 14.4, 14.1, -4.2, -4.4; ν_{max}: 2929, 2854, 1599, 1499 cm^{-1}; $[\alpha]^{21}_D$ = -4.85 ($CHCl_3$, 1.164 µmol) [Found $M+Na^+$: 881.57; $C_{50}H_{94}N_4NaO_3SSi$ requires: 881.67].

Experiment 40: 2-(10-Bromodecyloxy)tetrahydro-2H-pyran (181)

$Br(CH_2)_{10}OTHP$

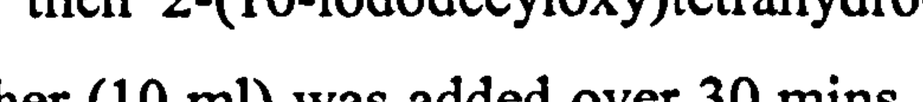
2,3-Dihydropyran (16.77 g, 199.4 mmol, 2.1 mol. equiv., d = 0.928, 18.07 ml) and pyridinium-*p*-toluene sulfonate (2.5 g) were added to a stirred solution of 10-bromodecan-1-ol **(180)** (22.50 g, 94.94 mmol) in dry dichloromethane (200 ml) under nitrogen at RT. The mixture was stirred for 3 hrs; when TLC indicated the reaction was complete, the mixture was quenched with sat. aq. sodium hydrogen carbonate (100 ml) and extracted with dichloromethane (2 x 100 ml). The combined organic layers were washed with water (100 ml), dried and evaporated to give a crude oil which was purified via flash distillation (120 °C) to give a colourless oil, 2-(10-bromodecyloxy)tetrahydro-2H-pyran **(181)** (25.54 g, 84 %), which showed δ_H (500 MHz, $CDCl_3$): 4.60-4.57 (1H, m), 3.89-3.85 (1H, m), 3.75 (1H, dt, J 6.5, 9.5 Hz), 3.55-3.50 (1H, m), 3.45 (2H, t, J 6.5 Hz), 3.40 (1H, dt, J 6.5, 9.5 Hz), 1.91-1.80 (3H, m), 1.76-1.72 (1H, m), 1.68-1.40 (18H, m); δ_C (125 MHz, $CDCl_3$): 98.8, 67.6, 62.3, 33.9, 30.8, 29.9, 29.7, 29.5, 29.4, 29.0, 28.7, 28.6, 26.2, 25.5, 19.7; ν_{max}: 2939, 2865, 1440 cm^{-1} [Found M+: 321.2914; $C_{15}H_{29}BrO_2$ requires: 321.2990].

Experiment 41: 2-(10-Iododecyloxy)tetrahydro-2H-pyran (182)

$I(CH_2)_{10}OTHP$

2-(10-Bromodecyloxy)tetrahydro-2H-pyran **(181)** (25.54 g, 79.78 mmol) was added to a stirred suspension of sodium iodide (35.87 g, 239.33 mmol, 3 mol. equiv.) in acetone (400 ml) at RT. After the addition of sodium hydrogen carbonate (7.04 g, 83.77 mmol, 1.05 mol. equiv.), the reaction mixture was refluxed for 3 hrs and stirred at RT for 18 hrs. The solvent was then evaporated and the residue was dissolved in dichloromethane (500 ml) and washed with water (200 ml). The aqueous layer was extracted with dichloromethane (3 x 50 ml) and the combined organic layers were dried and the solvent evaporated. The resultant crude product was purified via column chromatography eluting with petrol/ethyl acetate (10:1) to give an oil which slowly turned brown over time, 2-(10-iododecyloxy)tetrahydro-2H-pyran **(182)** (26.24, 89 %), which showed δ_H (500 MHz, $CDCl_3$): 4.60-4.57 (1H, m), 3.89-3.85 (1H, m), 3.75 (1H, dt, J 6.5, 9.5 Hz), 3.55-3.50 (1H, m), 3.40 (1H, dt, J 6.5, 9.5 Hz), 3.18 (2H, t, J 6.5 Hz), 1.91-1.80 (3H, m), 1.76-1.72 (1H, m), 1.68-1.40 (18H, m); δ_C (125 MHz, $CDCl_3$): 98.8, 67.6, 62.3, 30.8, 29.9, 29.7, 29.5, 29.4, 29.0, 28.7, 28.6, 26.2, 25.5, 19.7, 6.8; ν_{max}: 2939, 2865, 1440 cm^{-1} [Found M+: 368.2912; $C_{15}H_{29}IO_2$ requires: 368.2999].

Experiment 42: 13-(Tetrahydro-2H-pyran-2-yloxy)tridec-2-yn-1-ol (184)



Liquid ammonia (200 ml) was decanted into a 3 neck 500 ml round-bottomed flask surrounded with cotton wool and fitted with a liquid nitrogen/IMS condenser, protected with a soda lime tube. Lithium wire (1.18 g, 170.7 mmol, 2.2 mol. equiv) was washed with petrol and added in 1 cm portions over 30 mins, with a deep blue colour being observed. Iron (III) nitrate (0.2 g) was then added and the solution stirred with a mechanical stirrer for 30 mins, prop-2-yn-1-ol **(183)** (4.35 g, 77.66 mmol) in dry ether (10 ml) was then added over 30 mins. The resultant mixture was then stirred for 1 hr, then 2-(10-iododecyloxy)tetrahydro-2H-pyran **(182)** (20 g, 54.31 mmol) in dry ether (10 ml) was added over 30 mins. The reaction was stirred for 3 hrs, maintaining the temperature of the condensers; the reaction was then left without stirring for 18 hrs to allow the ammonia to evaporate. The reaction mixture was then diluted with ethyl acetate (250 ml) and the mixture quenched with 10 % sulfuric acid (50 ml); the aqueous layer was re-extracted with ethyl acetate (3 x 50 ml). The combined organic extracts were dried and the solvent evaporated to give a crude dark brown oil, which was purified via column chromatography eluting with petrol/ethyl acetate (5:1) to give a yellow oil, 13-(tetrahydro-2H-pyran-2-yloxy)tridec-2-yn-1-ol **(184)** (8.54 g, 53 %), which showed δ_H (500 MHz, $CDCl_3$): 4.52 (1H, t, J 3.75), 4.20-4.17 (2H, m), 3.83-3.79 (1H, m), 3.67 (1H, dt, J 6.95, 9.45 Hz), 3.46-3.42 (1H, m), 3.32 (1H, dt, J 6.65, 9.45 Hz), 2.52 (1H, br.s), 2.15-2.13 (2H, m), 1.80-1.75 (1H, m), 1.68-1.63 (1H, m), 1.54-1.41 (8H, m), 1.30-1.18 (12H, br.m including br.s at 1.22); δ_C (125 MHz, $CDCl_3$): 98.7, 86.0, 78.5, 62.2, 51.0, 30.7, 30.3, 29.7, 29.42, 29.40, 29.0, 28.8, 28.6, 25.4, 20.9, 19.6; ν_{max}: 3333, 2925, 2864, 2238, 1442 cm^{-1} [Found M+: 296.4424; $C_{18}H_{32}O_3$ requires: 296.4518].

Experiment 43: 13-(Tetrahydro-2H-pyran-2-yloxy)tridecan-1-ol (185)

$HO(CH_2)_{13}OTHP$

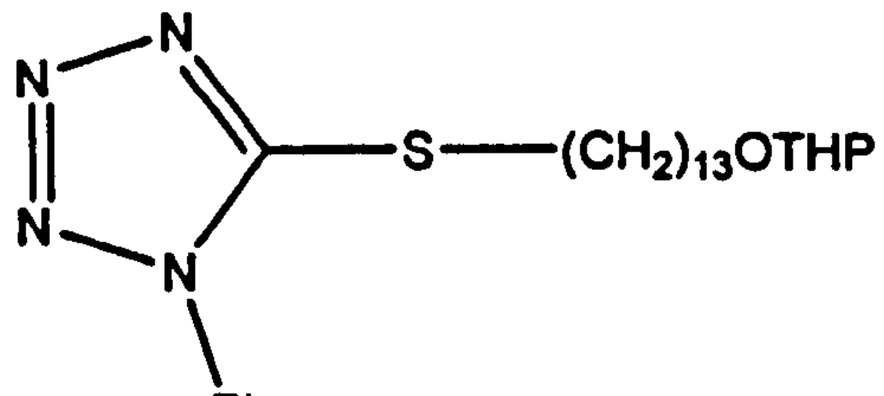
Palladium on charcoal (10 %, 1 g) was added to a stirred solution of 13-(tetrahydro-2H-pyran-2-yloxy)tridec-2-yn-1-ol **(184)** (8.54 g, 28.67 mmol) in IMS (150 ml). The mixture was stirred while being hydrogenated at atmospheric pressure; when hydrogen absorption was complete the mixture was filtered through a pad of celite and washed with THF (100 ml). The filtrate was evaporated to give a colourless oil, 13-(tetrahydro-2H-pyran-2-yloxy)tridecan-1-ol **(185)** (6.24 g, 72 %), which showed δ_H (500 MHz, $CDCl_3$): 4.57 (1H, t, J 2.85 Hz), 3.88-3.84 (1H, m), 3.74-3.68 (2H, m), 3.62 (1H, dt, J 2.85, 6.6 Hz), 3.50-3.48 (1H, m), 3.37 (1H, dt, J 6.6, 8.5 Hz), 1.85-1.80 (1H, m), 1.72-1.68 (1H, m), 1.63-1.50 (8H, m), 1.39-1.22 (18H, br.m including br.s at 1.25), 0.87 (1H, t, J 6.65 Hz); δ_C (125 MHz, $CDCl_3$): 98.8, 67.7, 62.3, 32.8, 30.7, 30.2, 29.6, 29.0, 26.1, 25.4, 20.9; ν_{max}: 3333, 2925, 2864, 1442 cm^{-1} [Found M+: 300.4827; $C_{18}H_{36}O_3$ requires: 300.4837].

Experiment 44: 2-(13-Bromotridecyloxy)tetrahydro-2H-pyran (186)

$Br(CH_2)_{13}OTHP$

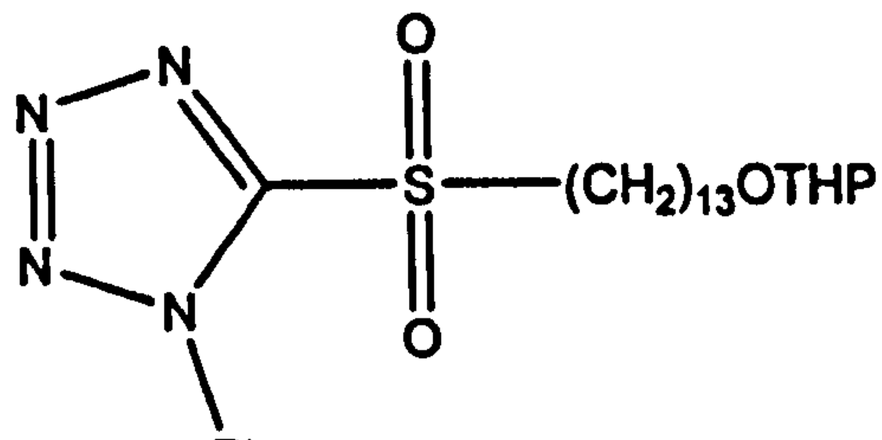
N-Bromosuccinimide (4.81 g, 27.00 mmol, 1.3 mol. equiv.) was added in portions over 15 mins to a stirred solution of 13-(tetrahydro-2H-pyran-2-yloxy)tridecan-1-ol **(185)** (6.24 g, 20.77 mmol) and triphenyl phosphine (6.26 g, 23.88 mmol, 1.15 equiv) and sodium hydrogen carbonate (0.5 g) in dichloromethane (100 ml) at 0 °C. The mixture was stirred at RT for 1 hr; when TLC analysis indicated completion of the reaction it was quenched with sat. aq. sodium meta-bisulfite (100 ml). The aqueous layer was re-extracted with dichloromethane (2 x 100 ml) and the combined organic extracts washed with water (50 ml), dried and evaporated to give a residue. This residue was treated with petrol/ether (1:1, 200 ml), under reflux for 30 mins, filtered and washed with petrol/ether (1:1, 200 ml). The filtrate was evaporated and the resultant residue purified via column chromatography eluting with petrol/ether (4:1) then (2:1) to give a yellow oil, 2-(13-bromotridecyloxy)tetrahydro-2H-pyran **(186)** (6.13 g, 81 %), which showed δ_H (500 MHz, $CDCl_3$): 4.58 (1H, t, J 2.8 Hz), 3.90-3.85 (1H, m), 3.73 (1H, dt, J 6.95, 9.8 Hz), 3.52-3.47 (1H, m), 3.38 (1H, dt, J 6.6, 9.45 Hz), 1.85 (2H, pent, J 7.25 Hz), 1.83-1.80 (1H, m), 1.74-1.69 (1H, m), 1.64-1.51 (8H, m), 1.45-1.39 (2H, m), 1.38-1.22 (15H, br.m including br.s at 1.34), 0.88 (1H, t, J 6.65 Hz); δ_C (125 MHz, $CDCl_3$): 98.8, 63.4, 62.3, 34.0, 32.9, 30.8, 29.8, 29.6, 29.2, 26.2, 25.5, 20.9; ν_{max}; 2943, 2864, 1442 cm^{-1} [Found M+: 363.3718; $C_{18}H_{35}BrO_2$ requires: 363.3800].

Experiment 45: 1-Phenyl-5-(13-(tetrahydro-2H-pyran-2-yloxy)tridecylthio)-1H-tetrazole (187)



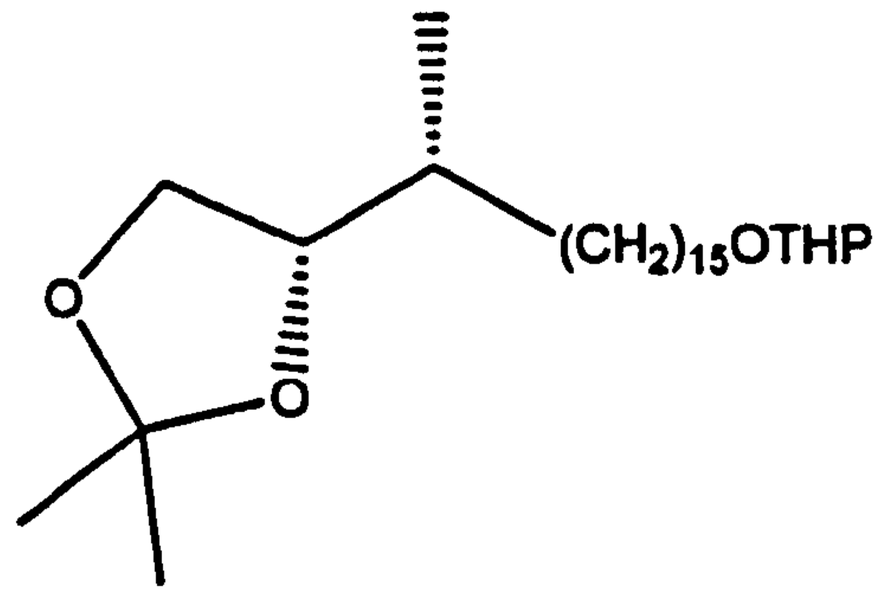
2-(13-Bromotridecyloxy)tetrahydro-2H-pyran **(186)** (3.00 g, 8.26 mmol) was added, with vigorous stirring, to 1-phenyl-1H-tetrazole-5-thiol (1.77 g, 9.91 mmol, 1.2 mol. equiv.) and anhydrous potassium carbonate (3.42 g, 24.77 mmol, 3 mol. equiv.) in acetone (100 ml, HPLC grade). The reaction was refluxed for 18 hrs; when TLC analysis indicated completion of the reaction, the inorganic solids were filtered off and washed with acetone. The organic filtrate was evaporated and extracted with dichloromethane (100 ml) and water (150 ml). The aqueous layer was re-extracted with further dichloromethane (3 x 50 ml) and the combined organic layers were dried and evaporated. The crude yellow oil was purified via column chromatography eluting with petrol/ethyl acetate (4:1 then 2:1) to give a colourless oil, 1-phenyl-5-(13-(tetrahydro-2H-pyran-2-yloxy)tridecylthio)-1H-tetrazole **(187)** (2.31 g, 61 %), which showed δ_H (500 MHz, $CDCl_3$): 7.60-7.51 (5H, m), 4.56 (1H, t, J 2.85 Hz), 3.88-3.84 (1H, m), 3.72 (1H, dt, J 6.9, 9.45 Hz), 3.51-3.47 (1H, m), 3.36 (1H, dt, J 6.6, 13.2 Hz), 1.80 (4H, pent, J 7.55 Hz), 1.73-1.68 (1H, m), 1.60-1.52 (8H, m), 1.43 (2H, pent, J, 7.55 Hz), 1.34-1.23 (15H, br.m including br.s at 1.25); δ_C (125 MHz, $CDCl_3$): 154.5, 133.8, 130.0, 129.8, 123.9, 98.9, 64.4, 62.3, 60.4, 33.4, 30.8, 30.3, 30.2, 29.8, 29.6, 29.2, 26.2, 25.7, 25.5, 20.9; ν_{max}: 2943, 2864, 1497, 1442 cm^{-1} [Found M+: 460.6829; $C_{25}H_{40}N_4O_2S$ requires: 460.6849].

Experiment 46: 1-Phenyl-5-(13-(tetrahydro-2H-pyran-2-yloxy)tridecylsulfonyl)-1H-tetrazole (188)



A solution of ammonium molybdate (VI) tetrahydrate (3.10 g, 2.51 mmol, 0.5 mol. equiv.) in -10 °C hydrogen peroxide (8 ml, 35 % w/w), was added to a stirred solution of 1-phenyl-5-(13-(tetrahydro-2H-pyran-2-yloxy)tridecylthio)-1H-tetrazole **(187)** (2.31 g, 5.01 mmol) in THF (20 ml) and IMS (40 ml) at 12 °C and stirred the mixture at 15-20 °C for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (1.24 g, mmol, 0.2 mol. equiv.) in -10 °C hydrogen peroxide (4 ml, 35 % w/w) was added and the reaction mixture stirred for 18 hrs at RT. The mixture was poured into water (500 ml) and extracted with dichloromethane (3 x 200 ml); the combined organic extracts were dried and the solvent evaporated. The crude product was purified via column chromatography eluting with petrol/ether (4:1) to give a colourless oil, 1-phenyl-5-(13-(tetrahydro-2H-pyran-2-yloxy)tridecylsulfonyl)-1H-tetrazole **(188)** (1.75 g, 71 %), which showed δ_H (500 MHz, $CDCl_3$): 7.71-7.68 (2H, m), 7.62-7.59 (3H, m), 4.56 (1H, t, J 2.85 Hz), 3.90-3.83 (1H, m), 3.70 (1H, dist. t, J 7.6 Hz), 3.53-3.47 (1H, m), 3.37 (1H, dt, J 6.75, 9.45 Hz), 1.89 (2H, pent, J 7.9 Hz), 1.83-1.68 (1H, m), 1.60-1.52 (8H, m), 1.50-1.23 (19H, br.m including br.s at 1.28); δ_C (125 MHz, $CDCl_3$): 153.5, 133.1, 131.4, 129.7, 125.1, 98.9, 64.4, 62.3, 60.4, 42.8, 30.8, 30.3, 30.2, 29.8, 29.6, 29.2, 26.2, 25.7, 25.5, 20.9; ν_{max}: 2943, 2864, 1497, 1442 cm^{-1} [Found M+: 492.6728; $C_{25}H_{40}N_4O_4S$ requires: 492.6835].

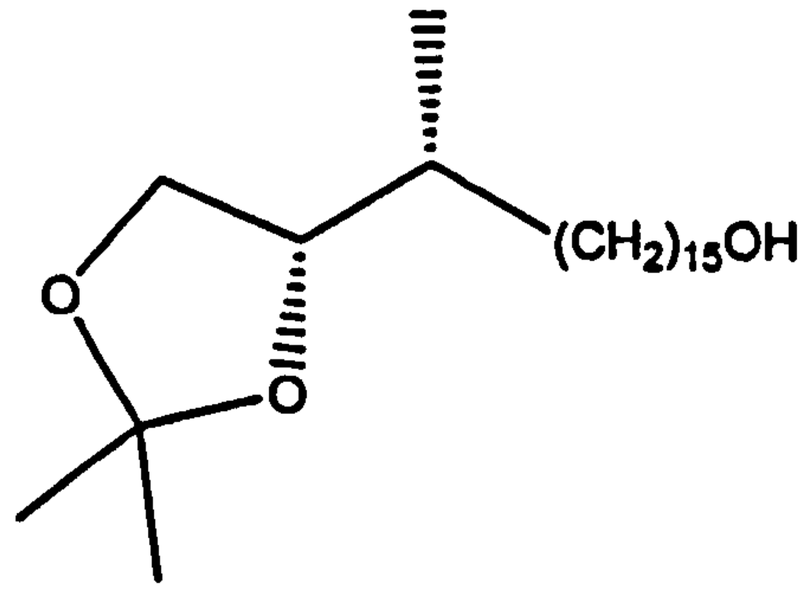
Experiment 47: 2-((*R*)-16-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)heptadecyloxy) tetrahydro-2H-pyran (190)



A solution of (*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)butanal **(178)** (0.80 g, 4.62 mmol, 1.3 mol. equiv.) and 1-phenyl-5-(13-(tetrahydro-2H-pyran-2-yloxy)tridecylsulfonyl)-1H-tetrazole **(188)** (1.75 g, 3.55 mmol) in dry THF (50 ml), was stirred under nitrogen at -15 °C. Lithium *bis*(trimethylsilyl)amide (5.03 ml, 5.33 mmol, 1.06 M) was added dropwise between -12 °C and -5 °C and the solution was stirred for 18 hrs. When TLC showed no starting material was left, sat. aq. ammonium chloride (50 ml) and petrol/ethyl acetate (50 ml, 1:1) were added. The aqueous layer was re-extracted with further petrol/ether (2 x 50 ml, 1:1) and the combined organic layers were dried and evaporated to give a crude product. This product was purified via column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil, 2-((*R*)-(*E*/*Z*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadec-13-enyloxy)tetrahydro-2H-pyran **(189)** (1.39 g, 89 %). Palladium on charcoal (0.5 g, 10%) was added to a stirred solution of the above product (1.39 g, 3.16 mmol) in IMS (30 ml). The mixture was stirred, while being hydrogenated at atmospheric pressure. When no more hydrogen was being absorbed, the catalyst was removed via suction filtration on a pad of celite and was washed with THF (25 ml). The filtrate was evaporated to give a colourless oil, 2-((*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecyloxy)tetrahydro-2H-pyran **(190)** (1.27 g, 90 %), which showed δ_H (500 MHz, $CDCl_3$): 4.58 (1H, dd, J 2.85, 4.4 Hz), 4.00 (1H, dd, J 6.3, 7.9 Hz), 3.89-3.85 (2H, m), 3.76-3.70 (1H, m), 3.60 (1H, t, 7.55 Hz), 3.52-3.48 (1H, m), 3.39 (1H, dt, J 6.6, 9.45 Hz), 1.86-1.80 (1H, m), 1.75-1.69 (1H, m), 1.61-1.51 (9H, m), 1.41 (3H, s), 1.36 (3H, s), 1.34-1.21 (23H, br.m including br.s at 1.26), 1.11-0.97 (1H, m), 0.96 (3H, d, J 6.6 Hz); δ_C (125 MHz, $CDCl_3$): 108.5, 98.9, 80.4, 67.8, 67.7, 62.3, 36.5, 32.7, 30.8, 29.9, 29.8, 29.7, 29.6, 29.5, 26.9, 26.6, 26.3, 25.6, 25.5, 19.7,

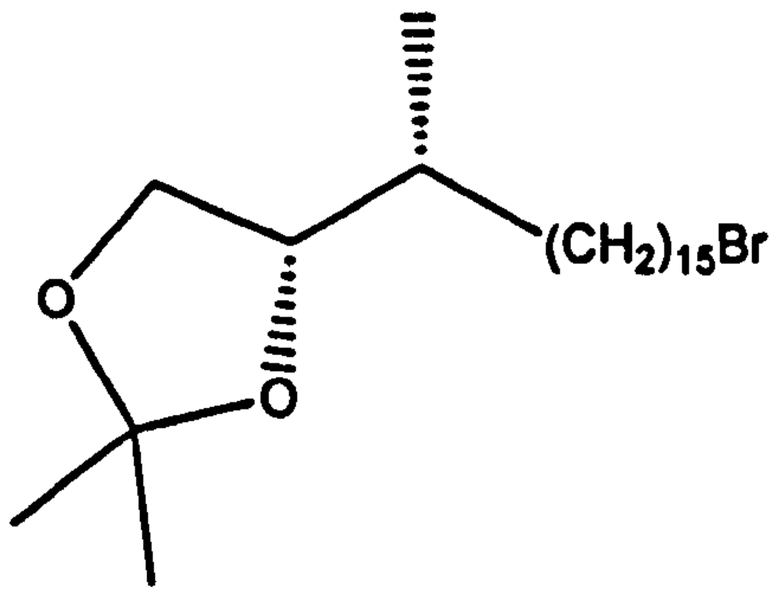
15.6; ν_{max}: 2941, 2843, 1464 cm^{-1}; $[\alpha]^{22}_{D}$ = +15.78 ($CHCl_3$, 1.098 μmol) [Found M+Na^+: 463.41; $C_{27}H_{52}NaO_4$ requires: 463.38].

Experiment 48: (*R*)-16-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)heptadecan-1-ol (191)



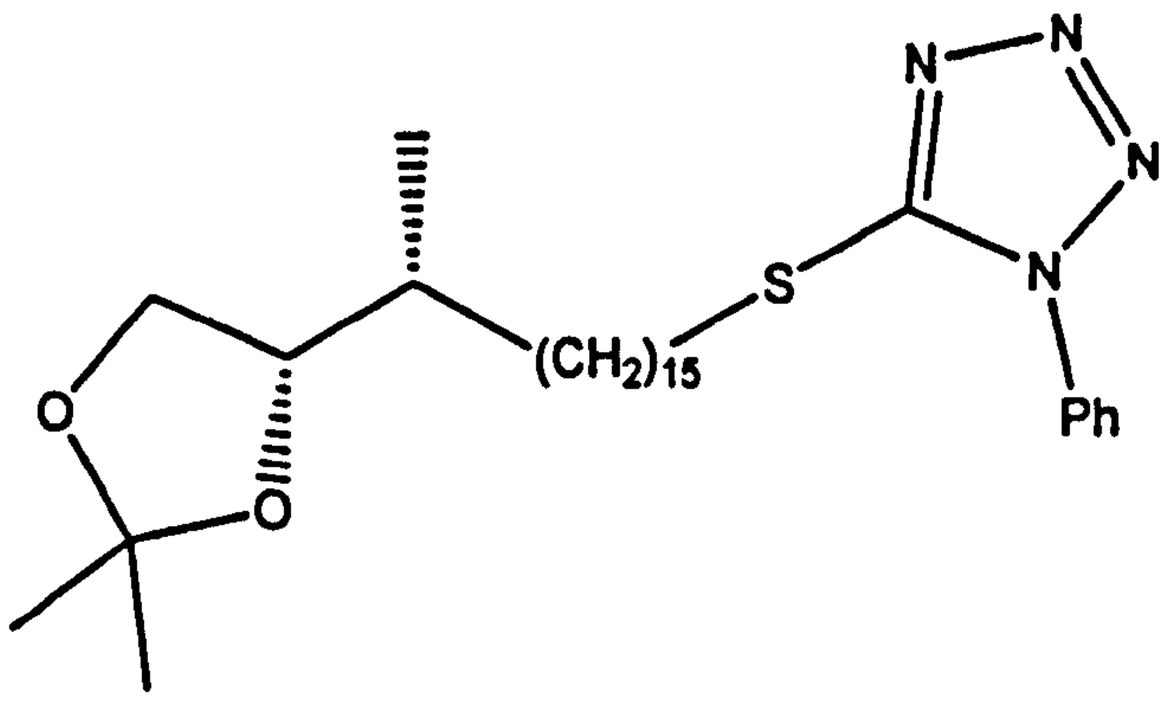
A solution of pyridinium-*p*-toluene sulfonate (6.67 g, 26.55 mmol, 10 mol. equiv.) in methanol (5 ml) was added to a stirred solution of 2-((*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecyloxy)tetrahydro-2H-pyran **(190)** (1.17 g, 2.65 mmol) in THF (50 ml) and the solution refluxed for 2.5 hrs. When TLC showed no starting material was left, sat. aq. sodium hydrogen carbonate (20 ml) and water (50 ml) were added and the product extracted with ethyl acetate (3 x 50 ml). The combined organic layers were dried and the solvent evaporated to give a crude oil, which was purified via column chromatography eluting with petrol/ethyl acetate (5:1) then petrol/ethyl acetate (1:1) to give a white solid, (*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecan-1-ol **(191)** (0.40 g, 43 %), which showed δ_H (500 MHz, $CDCl_3$): 3.85 (1H, dt, J 6.2, 7.75 Hz), 3.77 (1H, q, J 7 Hz), 3.49 (1H, t, J 7.75 Hz), 3.41 (2H, t, J 6.6 Hz), 1.52-1.48 (1H, m), 1.44 (3H, s), 1.43-1.39 (1H, m), 1.35 (3H, s), 1.36-1.12 (28H, m), 1.01 (3H, d, J 7 Hz); δ_C (125 MHz, $CDCl_3$): 108.7, 80.6, 68.2, 62.7, 37.0, 33.3, 33.2, 30.4, 30.2, 30.2, 30.14, 30.11, 30.08, 30.0, 27.4, 27.0, 26.3, 25.9, 15.9; ν_{max}: 3399, 2925, 2854, 1464 cm^{-1}; $[\alpha]^{22}_D$ = +21.54 ($CHCl_3$, 1.078 µmol); m.p. 55-56 °C; [Found M+Na^+: 379.31; $C_{22}H_{44}NaO_3$ requires: 379.32].

Experiment 49: (*S*)-4-((*R*)-17-Bromoheptadecan-2-yl)-2,2-dimethyl-1,3-dioxo lane (192)



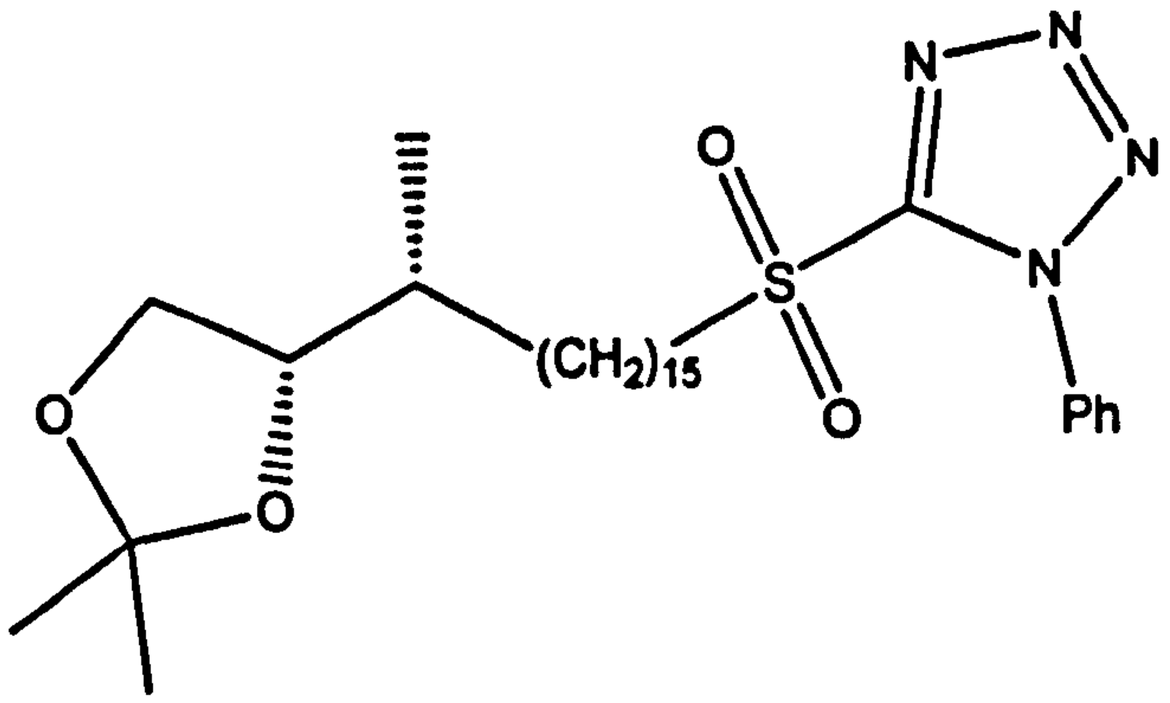
N-Bromosuccinimide (0.26 g, 1.46 mmol, 1.3 mol. equiv.) was added in portions, over 15 mins, to a stirred solution of (*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecan-1-ol **(191)** (0.40 g, 1.12 mmol) and triphenyl phosphine (0.59 g, 2.25 mmol, 2 equiv) and sodium hydrogen carbonate (0.1 g) in dichloromethane (20 ml) at 0 °C. The mixture was stirred at RT for 1 hr; when TLC analysis indicated completion of the reaction, it was quenched with sat. aq. sodium meta-bisulfite (10 ml). The aqueous layer was re-extracted with dichloromethane (2 x 25 ml) and the combined organic extracts washed with water (25 ml), dried and evaporated to give a residue. This was refluxed for 30 mins with petrol/ethyl acetate (1:1, 50 ml), filtered and washed with petrol/ethyl acetate (1:1, 50 ml). The filtrate was evaporated and the resultant residue purified via column chromatography eluting with petrol/ethyl acetate (5:2) to give a colourless oil, (*S*)-4-((*R*)-17-bromoheptadecan-2-yl)-2,2-dimethyl-1,3-dioxolane **(192)** (0.44 g, 93 %), which showed δ_H (500 MHz, $CDCl_3$): 3.99 (1H, dd, J 6.2, 7.9 Hz), 3.86 (1H, q, J 7 Hz), 3.59 (1H, t, J 7.8 Hz), 3.41 (2H, t, J 7 Hz), 1.85 (2H, pent, J 8 Hz), 1.60-1.52 (1H, m), 1.44-1.40 (2H, m), 1.42 (3H, s), 1.36 (3H, s), 1.3*2-1.19 (23H, m), 1.11-1.06 (1H, m), 0.96 (3H, d, J 6.6 Hz); δ_C (125 MHz, $CDCl_3$): 108.5, 80.4, 67.8, 36.5, 34.0, 32.7, 29.9, 19.6, 29.6, 29.5, 29.4, 28.8, 28.2, 27.0, 26.6, 25.5, 15.6; ν_{max}: 2925, 2855, 1464 cm^{-1}; $[\alpha]^{22}_D$ = +15.85 ($CHCl_3$, 1.051 μmol) [Found $M+Na^+$: 441.19; $C_{22}H_{43}BrNaO_2$ requires: 441.23].

Experiment 50: 5-((*R*)-16-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)heptadecylthio)-1-phenyl-1H-tetrazole (193)



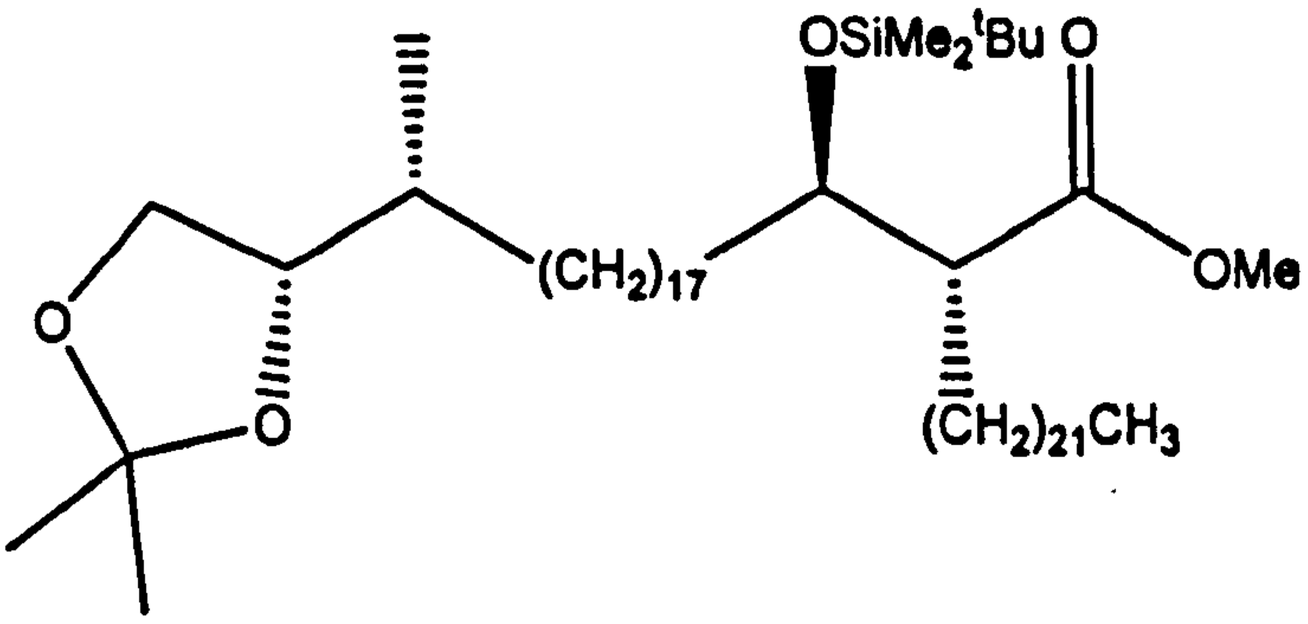
(*S*)-4-((*R*)-17-Bromoheptadecan-2-yl)-2,2-dimethyl-1,3-dioxolane **(192)** (0.44 g, 1.05 mmol) was added, with vigorous stirring, to 1-phenyl-1H-tetrazole-5-thiol (0.28 g, 1.55 mmol) and anhydrous potassium carbonate (0.53 g, 3.87 mmol, 3 mol. equiv.) in acetone (30 ml). The reaction was stirred for 18 hrs, then the solvent was evaporated and the resultant residue taken up in petrol/ethyl acetate (1:1, 50 ml). The resultant solution was washed with water (50 ml), which was then back-extracted with petrol/ethyl acetate (1:1, 2 x 50 ml) and the combined organic layers were then dried and evaporated. The crude product was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, 5-((*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecylthio)-1-phenyl-1H-tetrazole **(193)** (0.49 g, 92 %), which showed δ_H (500 MHz, $CDCl_3$): 7.60-7.54 (5H, m), 4.00 (1H, dd, J 6.1, 7.6 Hz), 3.87 (1H, q, J 6.7 Hz), 3.60 (1H, t, J 7.6 Hz), 3.39 (2H, t, J 7.3 Hz), 1.82 (2H, pent, J 7.3 Hz), 1.58-1.53 (1H, m), 1.46-1.41 (2H, m), 1.40 (3H, s), 1.35 (3H, s), 1.33-1.25 (23H, m), 1.11-1.04 (1H, m), 0.96 (3H, d, J 6.6 Hz); δ_C (125 MHz, $CDCl_3$): 154.5, 133.8, 130.0, 129.7, 123.8, 108.4, 80.4, 67.8, 36.5, 33.3, 32.7, 29.8, 29.61, 29.57, 29.5, 29.4, 29.1, 29.0, 28.6, 27.0, 26.6, 25.5, 15.6; ν_{max}: 2925, 2855, 1598, 1499, 1464 cm^{-1}; $[\alpha]^{23}_D$ = +13.48 ($CHCl_3$, 0.987 µmol) [Found $M+Na^+$: 539.31; $C_{29}H_{48}N_4NaO_2S$ requires: 539.34].

Experiment 51: 5-((*R*)-16-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)heptadecyl sulfonyl)-1-phenyl-1H-tetrazole (194)



A solution of ammonium molybdate (VI) tetrahydrate (0.5873 g, 0.4752 mmol, 0.5 mol. equiv.) in -10 °C hydrogen peroxide (5 ml, 35 % w/w) was added to a stirred solution of 5-((*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecylthio)-1-phenyl-1H-tetrazole **(193)** (0.4906 g, 0.9504 mmol) in THF (5 ml) and IMS (10 ml) at 12 °C and stirred at 15-20 °C for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (0.2349 g, 0.1901 mmol, 0.2 mol. equiv.) in -10 °C hydrogen peroxide (2 ml, 35 % w/w) was added and the reaction mixture stirred for 18 hrs. The mixture was then poured into water (50 ml) and extracted with dichloromethane (3 x 50 ml), dried and evaporated. The crude product was purified via column chromatography eluting with petrol/ethyl acetate (5:2) to give a white solid, 5-((*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecylsulfonyl)-1-phenyl-1H-tetrazole **(194)** (0.3797 g, 73 %), which showed δ_H (500 MHz, $CDCl_3$): 7.70-7.68 (2H, m), 7.65-7.59 (3H, m), 4.00 (1H, dd, J 6.3, 7.9 Hz), 3.87 (1H, q, J 7 Hz), 3.73 (2H, dist t), 3.60 (1H, t, J 7.9 Hz), 1.93 (2H, pent, J 7.2 Hz), 1.53-1.48 (2H, m), 1.41 (3H, s), 1.35 (3H, s), 1.33-1.25 (24H, m), 1.11-1.04 (1H, m), 0.96 (3H, d, J 6.6 Hz); δ_C (125 MHz, $CDCl_3$): 153.5, 133.0, 131.4, 129.7, 125.0, 108.5, 80.4, 67.8, 56.0, 36.5, 32.7, 29.9, 29.7, 29.61, 29.57, 29.5, 29.2, 28.9, 28.2, 27.0, 26.6, 25.6, 15.6; ν_{max}: 3015, 2918, 2855, 1598, 1499, 1464 cm^{-1}; $[\alpha]^{23}_D$ = +12.87 ($CHCl_3$, 1.574 µmol); m.p. 51-53 °C; [Found $M+Na^+$: 571.30; $C_{29}H_{48}N_4NaO_4S$ requires: 571.33].

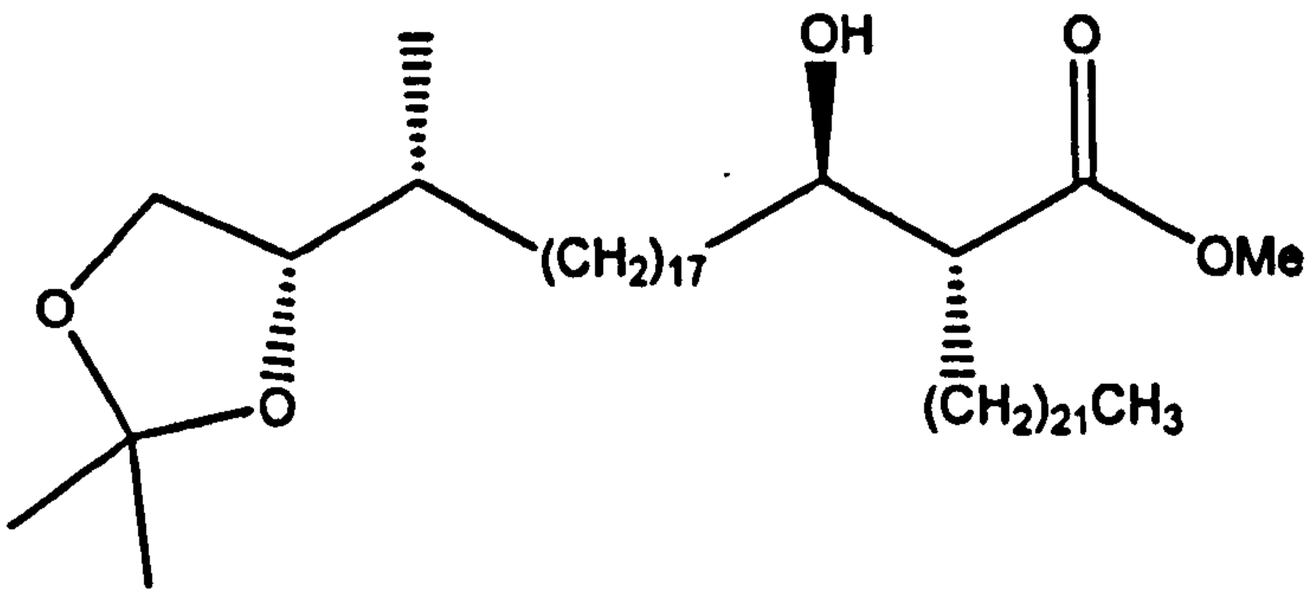
Experiment 52: Methyl 2-((1*R*,2*R*,19*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)eicosyl)tetracosanoate (201)



Lithium *bis*(trimethylsilyl)amide (0.979 ml, 1.038 mmol, 1.06 M) was added dropwise to a stirred solution of methyl 2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-3-oxopropyl)tetracosanoate **(200)** (0.3936 g, 0.617 mmol) and 5-((*R*)-16-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)heptadecylsulfonyl)-1-phenyl-1H-tetrazole **(194)** (0.3797 g, 0.6917 mmol) in dry THF (30 ml) at -15 °C. The mixture was stirred for 18 hrs at RT, when TLC analysis indicated completion of the reaction. Sat. aq. ammonium chloride (20 ml) and petrol/ethyl acetate (1:1, 20 ml) were added. The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 x 20 ml) and the combined organic extracts washed with brine (20 ml), dried and evaporated to give a yellow oil. The crude product was purified via column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, methyl 2-((1*R*,2*R*,19*R*)-(*E*/*Z*)-1-(*tert*-butyldimethylsilyloxy)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)eicos-3-enyl)tetracosanoate (0.2330 g, 39 %). Palladium on charcoal (10 %, 0.1 g) was added to a stirred solution of the above alkenes (0.3517 g, 0.3945 mmol) in ethyl acetate (15 ml). The mixture was stirred, while being hydrogenated at atmospheric pressure; when hydrogen absorption was complete, the mixture was filtered through a pad of celite and washed with ethyl acetate (15 ml). The filtrate was evaporated to give a colourless oil, methyl 2-((1*R*,2*R*,19*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl)tetracosanoate **(201)** (0.2314 g, 99 %), which showed δ_H (500 MHz, $CDCl_3$): 4.00 (1H, dd, J 6.2, 8 Hz), 3.95-3.90 (1H, m), 3.88 (1H, q, J 7 Hz), 3.66 (3H, s), 3.60 (1H, t, J 8 Hz), 2.54 (1H, ddd, J 3.8, 7.2, 11 Hz), 1.62-1.51 (4H, m), 1.41 (3H, s), 1.36 (3H, s), 1.35-1.25 (72H, br.m including br.s at 1.26), 1.11-1.07 (1H, m), 0.97 (3H, d, J 6.65 Hz), 0.89 (3H, t, J 7 Hz), 0.87 (9H, s),

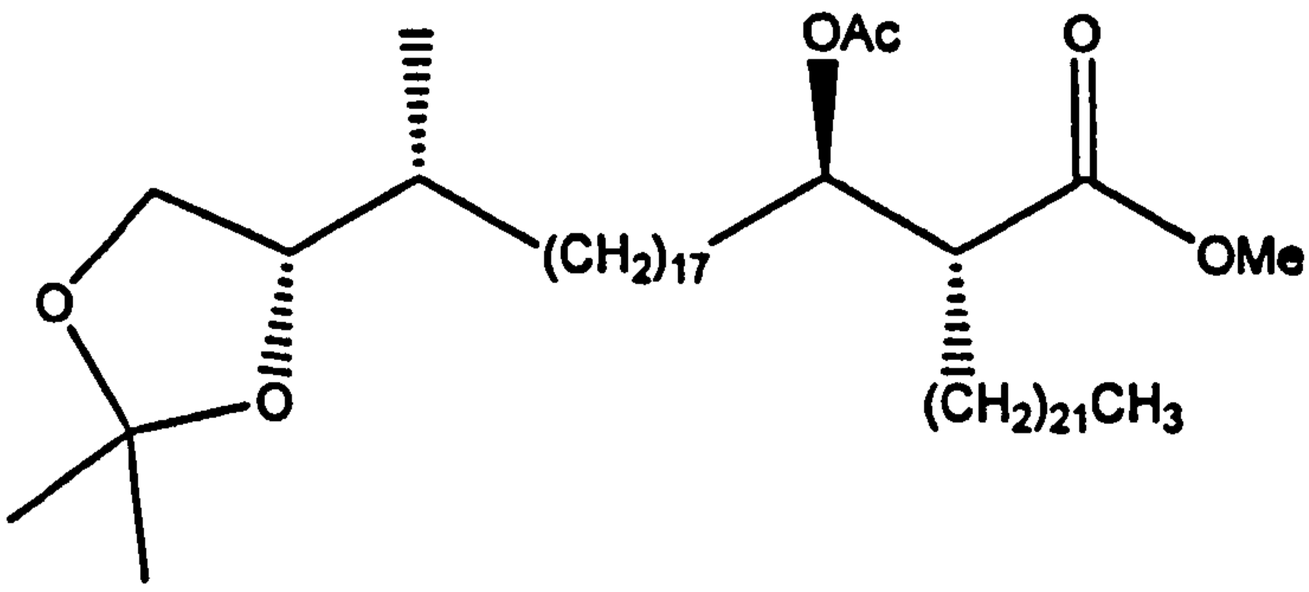
0.05 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 175.1, 108.5, 80.5, 76.8, 73.2, 51.5, 51.2, 36.5, 33.7, 31.9, 29.9, 29.8, 29.72, 29.68, 29.59, 29.55, 29.5, 29.4, 29.3, 27.8, 27.5, 27.0, 26.6, 25.7, 25.5, 23.7, 22.7, 18.0, 15.6, 14.1, -4.4, -4.9; ν_{max}: 2946, 2855, 1740, 1464 cm^{-1}; $[\alpha]^{24}_D$ = +3.78 ($CHCl_3$, 0.978 μmol) [Found M+Na^+: 915.73; $C_{56}H_{112}NaO_5Si$ requires: 915.82].

Experiment 53: Methyl 2-((1*R*,2*R*,19*R*)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-hydroxyicosyl)tetracosanoate (203)



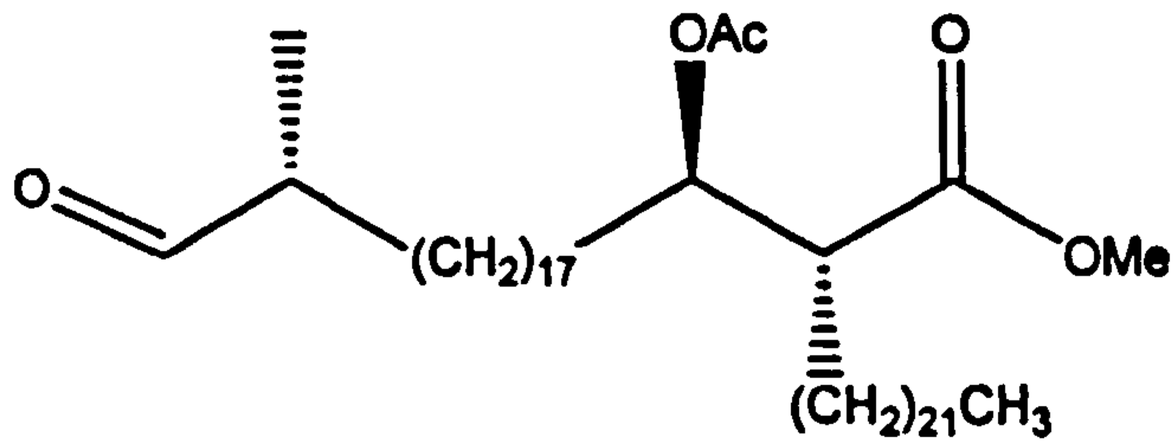
A dry polyethylene vial, equipped with a rubber septum, was charged with methyl 2-((1*R*,2*R*,19*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl)tetracosanoate **(201)** (0.2314 g, 0.2584 mmol) in dry THF (10 ml) under nitrogen at 0 °C. Pyridine (2.5 ml) and hydrogen fluoride-pyridine complex (1.1 ml, 0.7751 mmol, 3 mol. equiv.) were added and the mixture stirred for 18hrs at 43 °C. When TLC analysis indicated completion of the reaction the mixture was neutralised by slowly pouring it into sat. aq. sodium hydrogen carbonate (20 ml) until no more carbon dioxide was liberated. The product was extracted with petrol/ethyl acetate (1:1, 3 x 10 ml), dried and evaporated to give a white solid. This was purified via column chromatography eluting with petrol/ethyl acetate (4:1) to give a white solid, methyl 2-((1*R*,2*R*,19*R*)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-hydroxyicosyl) tetracosan- oate **(203)** (0.1425 g, 71 %), which showed δ_H (500 MHz, $CDCl_3$): 4.00 (1H, dd, J 6.2, 8 Hz), 3.88 (1H, q, J 7 Hz), 3.71 (3H, s), 3.68-3.63 (1H, m), 3.60 (1H, t, J 7.7 Hz), 2.45 (1H, dt, J 5.5, 10.8 Hz), 1.41 (3H, s), 1.36 (3H, s), 1.35-1.19 (69H, br.m including br.s at 1.26), 1.11-1.07 (1H, m), 0.96 (3H, d, J 7 Hz), 0.88 (3H, t, J 7 Hz); δ_C (125 MHz, $CDCl_3$): 176.2, 108.5, 80.5, 72.3, 67.8, 51.5, 50.9, 36.5, 35.7, 32.7, 31.9, 29.9, 29.69, 29.66, 29.64, 29.60, 29.57, 29.52, 29.50, 29.47, 29.4, 29.3, 27.4, 27.0, 26.6, 25.7, 25.5, 22.7, 15.6, 14.1; ν_{max}: 3489, 2925, 2855, 1715, 1464 cm^{-1}; $[\alpha]^{24}_D$ = +15.21 ($CHCl_3$, 1.156 μmol); m.p. 67-68 °C; [Found $M+Na^+$: 801.71; $C_{50}H_{90}NaO_5$ requires: 801.73].

Experiment 54: Methyl 2-((1*R*,2*R*,19*R*)-1-acetoxy-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl)tetracosanoate (166)



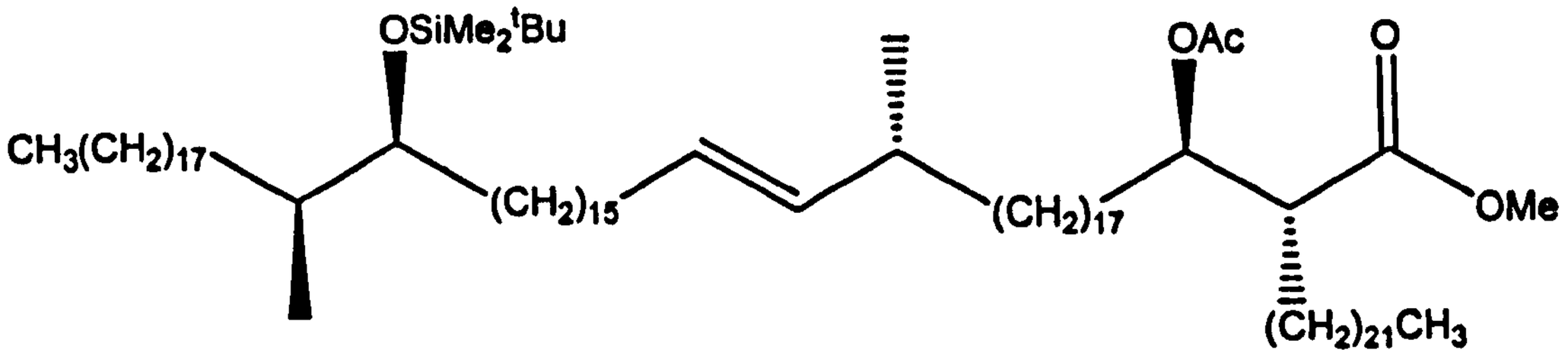
A mixture of acetic anhydride (3 ml) and anhydrous pyridine (3 ml) was added to a stirred solution of methyl 2-((1*R*,2*R*,19*R*)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-hydroxyicosyl)tetracosanoate **(203)** (0.1425 g, 0.1829 mmol) in dry toluene (10 ml). The mixture was stirred for 16 hrs at RT, then diluted with toluene (25 ml); the solvent was removed under reduced pressure to give a crude oil. This was purified via column chromatography eluting with petrol/ethyl acetate (10:1) to give a white solid, methyl 2-((1*R*,2*R*,19*R*)-1-acetoxy-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl)tetracosanoate **(166)** (0.1337 g, 89 %), which showed δ_H (500 MHz, $CDCl_3$): 5.11-5.00 (1H, m), 4.00 (1H, dd, J 6, 7.9 Hz), 3.88 (1H, q, J 6.9 Hz), 3.70 (3H, s), 3.61 (1H, t, J 737 Hz), 2.63 (1H, ddd, J 4.5, 6.9, 10.8 Hz), 2.04 (3H, s), 1.41 (3H, s), 1.36 (3H, s), 1.65-1.23 (76H, br.m including br.s at 1.26), 1.12-1.07 (1H, m), 0.96 (3H, d, J 7 Hz), 0.88 (3H, t, J 7 Hz); δ_C (125 MHz, $CDCl_3$): 173.6, 170.5, 108.5, 80.5, 74.1, 67.8, 51.5, 49.6, 36.5, 33.7, 32.7, 31.9, 31.7, 29.9, 29.7, 29.62, 29.59, 29.53, 29.51, 29.39, 29.35, 29.3, 28.1, 27.5, 27.0, 26.6, 25.5, 25.0, 22.7, 21.0, 21.0, 15.6, 14.1; ν_{max}: 2925, 2855, 1745, 1464 cm^{-1}; $[\alpha]^{24}_D$ = +15.89 ($CHCl_3$, 1.245 µmol); m.p. 37-38 °C; [Found M+Na^+: 843.68; $C_{52}H_{100}NaO_6$ requires: 843.74].

Experiment 55: Methyl 2-((1*R*,2*R*,19*R*)-1-acetoxy-19-methyl-20-oxoicosyl) tetracosanoate (165)



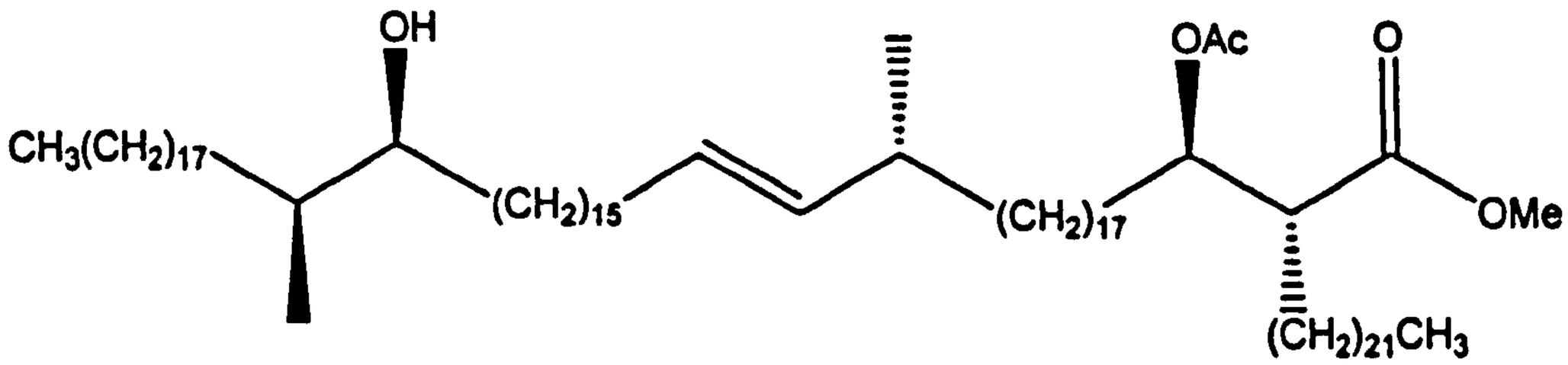
Periodic acid (0.41 g, 1.83 mmol, 3 mol. equiv.) was added to a stirred solution of methyl 2-((1*R*,2*R*,19*R*)-1-acetoxy-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl) tetracosanoate **(166)** (0.5 g, 0.61 mmol) in dry ether (20 ml), at RT under nitrogen, and the reaction mixture was stirred for 18 hrs. The mixture was filtered through a bed of celite and washed with ether (20 ml). The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (4:1) to give a white solid, methyl 2-((1*R*,2*R*,19*R*)-1-acetoxy-19-methyl-20-oxoicosyl)tetracosanoate **(165)** (0.27 g, 60 %), which showed δ_H (500 MHz, $CDCl_3$): 9.61 (1H, t, J 1.9Hz), 5.11-5.07 (1H, m), 3.68 (3H, s), 2.62 (1H, ddd, J 4.0, 7.0, 10.5 Hz), 2.32 (1H, sext, J 2.0, 7.0 Hz), 2.05 (3H, s), 1.75-1.19 (76H, br.m including br.s at 1.26), 1.10 (3H, d, J 7.0 Hz), 0.89 (3H, t, J 6.7 Hz); δ_C (125 MHz, $CDCl_3$): 205.4, 173.7, 170.3, 74.1, 51.5, 49.6, 46.3, 31.9, 31.7, 30.5, 29.7, 29.63, 29.60, 29.51, 29.48, 29.39, 29.35, 29.3, 28.1, 27.5, 26.9, 25.0, 22.7, 21.0, 14.1, 13.3; ν_{max}: 2925, 2855, 1745, 1468 cm^{-1}; $[\alpha]^{26}_D$ = +4.73 ($CHCl_3$, 1.245 μmol); m.p. = 32 - 33 °C [Found $M+Na^+$: 771.58; $C_{48}H_{92}NaO_5$ requires: 771.68].

Experiment 56: Methyl (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-3-acetoxy-39-(*tert*-butyldimethylsilyloxy)-2-docosyl-21,40-dimethyloctapentacont-22-enoate (204)



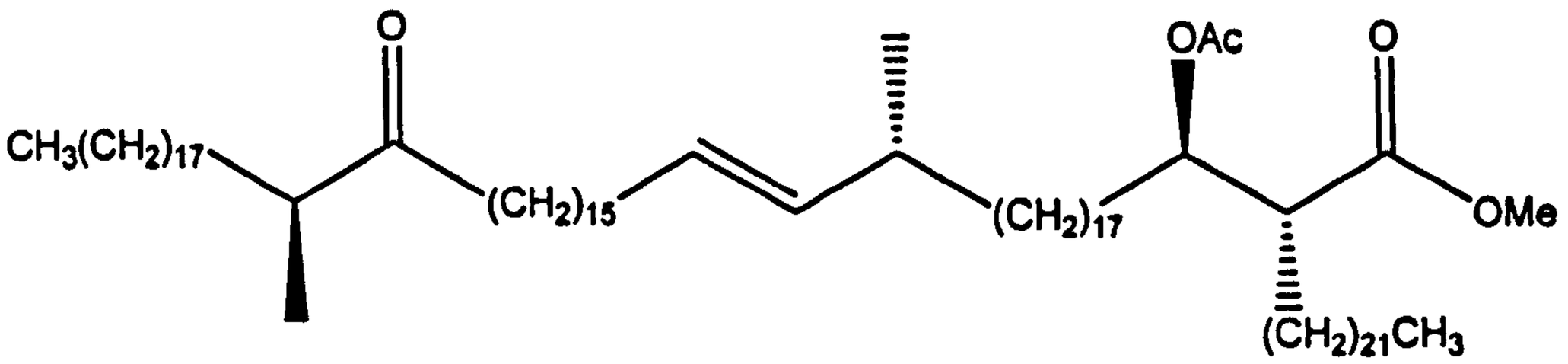
Methyl 2-((1*R*,2*R*,19*R*)-1-acetoxy-19-methyl-20-oxoicosyl)tetracosanoate **(165)** (0.24 g, 0.32 mmol) in dry 1,2-dimethoxyethane (10 ml) was added to a stirred solution of 5-((17*S*,18*S*)-17-(*tert*-butyldimethylsilyloxy)-18-methylhexatriacontyl sulfonyl)-1-phenyl-1H-tetrazole **(164)** (0.34 g, 0.40 mmol, 1.25 mol. equiv.) in dry 1,2-dimethoxyethane (20 ml) under nitrogen at RT. The mixture was cooled to -20° C and potassium *bis*(trimethylsilyl)amide (1.04 ml, 0.52 mmol, 0.5 M in toluene) was added and the mixture was allowed to reach RT and then stirred for 1.5 hrs. When TLC analysis indicated completion of the reaction, sat. aq. ammonium chloride (25 ml) and petrol/ether (1:1, 50 ml) were added. The aqueous layer was re-extracted with petrol/ether (1:1, 2 x 40 ml) and the combined organic layers were dried and the solvent evaporated. The crude compound was purified via column chromatography eluting with petrol/ether (18:1) to give a white solid, methyl (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-3-acetoxy-39-(*tert*-butyldimethylsilyloxy)-2-docosyl-21,40-dimethyloctapentacont-22-enoate **(204)** (0.15 g, 34 %), which showed δ_H (500 MHz, $CDCl_3$): 5.34 (1H, td, J 6.65, 15 Hz), 5.23 (1H, dd, J 7.5, 15 Hz), 5.09 (1H, ddd, J 4.1, 7.25, 8.2 Hz), 3.68 (3H, s), 3.49 (1H, dt, J 3.5, 6.3 Hz), 2.62 (1H, ddd, J 4.4, 7, 11 Hz), 2.05 (3H, s), 1.99 (2H, q, J 7 Hz), 1.70-1.20 (139H, br.m including br.s at 1.27), 1.08-1.00 (1H, m), 0.93 (3H, d, J 7 Hz), 0.89 (6H, t, J 6.65 Hz), 0.88 (9H, s), 0.80 (3H, d, J 7 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C (125 MHz, $CDCl_3$): 173.6, 170.3, 136.5, 128.4, 75.9, 74.1, 51.5, 49.6, 37.7, 37.2, 36.7, 33.5, 32.6, 32.5, 31.9, 31.7, 30.0, 29.9, 29.8, 29.70, 29.67, 29.62, 29.58, 29.54, 29.52, 29.43, 29.41, 29.38, 29.1, 28.1, 27.7, 27.5, 27.4, 26.0, 25.9, 25.0, 22.7, 21.0, 20.9, 18.2, 14.4, 14.1, -4.2, -4.4; ν_{max}: 2923, 2853, 1745, 1464 cm^{-1}; $[\alpha]^{22}_D$ = -3.18 ($CHCl_3$, 0.875 μmol); m.p. = 25-27 °C [Found M+Na^+: 1404.25; $C_{91}H_{180}NaO_5Si$ requires: 1404.35].

Experiment 57: Methyl (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-3-acetoxy-2-docosyl-39-hydroxy-21,40-dimethyloctapentacont-22-enoate (205)



A dry polyethylene vial, equipped with a rubber septum, was charged with methyl (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-3-acetoxy-39-(*tert*-butyldimethylsilyloxy)-2-docosyl-21,40-dimethyloctapentacont-22-enoate **(204)** (120 mg, 0.0869 mmol) in dry THF (5 ml) under nitrogen at 0 °C. Pyridine (0.5 ml) and hydrogen fluoride-pyridine complex (0.5 ml, 0.2607 mmol, 3 mol. equiv.) were added and the mixture stirred for 18hrs at 43 °C. When TLC analysis indicated completion of the reaction, the mixture was neutralised by slowly pouring it into sat. aq. sodium hydrogen carbonate (10 ml) until no more carbon dioxide was liberated. The product was extracted with petrol/ether (1:1, 3 x 25 ml), dried and evaporated to give a white solid. This was purified via column chromatography eluting with petrol/ether (5:1) to give a white solid, methyl (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-3-acetoxy-2-docosyl-39-hydroxy-21,40-dimethyloctapentacont-22-enoate **(205)** (60 mg, 55 %), which showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, td, J 6.6, 15.5 Hz), 5.24 (1H, dd, J 7.6, 15.5 Hz), 5.09 (1H, dt, J 3.9, J 8 Hz), 3.69 (3H, s), 3.53-3.49 (1H, m), 2.62 (1H, ddd, J 4.4, 7, 10.9 Hz), 2.04 (3H, s), 1.98 (2H, q, J 7 Hz), 1.63-1.20 (140H, br.m including br.s at 1.27), 1.18-1.11 (1H, m), 0.94 (3H, d, J 6.6 Hz), 0.89 (6H, t, J 6.7 Hz), 0.86 (3H, d, J 7 Hz); δ_C (125 MHz, $CDCl_3$): 173.6, 170.3, 136.5, 128.4, 75.3, 74.1, 51.5, 49.6, 38.3, 37.2, 36.7, 34.5, 33.5, 32.6, 31.9, 31.7, 30.0, 29.9, 29.8, 29.72, 29.68, 29.63, 29.60, 29.48, 29.45, 29.44, 29.42, 29.39, 29.1, 28.1, 27.7, 27.5, 27.4, 27.3, 26.2, 25.0, 22.7, 21.0, 20.9, 18.2, 14.4, 13.6; ν_{max}: 3542, 2923, 2843, 1745, 1464 cm^{-1}; $[\alpha]^{22}_D$ = -3.59 ($CHCl_3$, 1.045 μmol); m.p. = 36-38 °C [Found $M+Na^+$: 1290.22; $C_{85}H_{166}NaO_5$ requires: 1290.26].

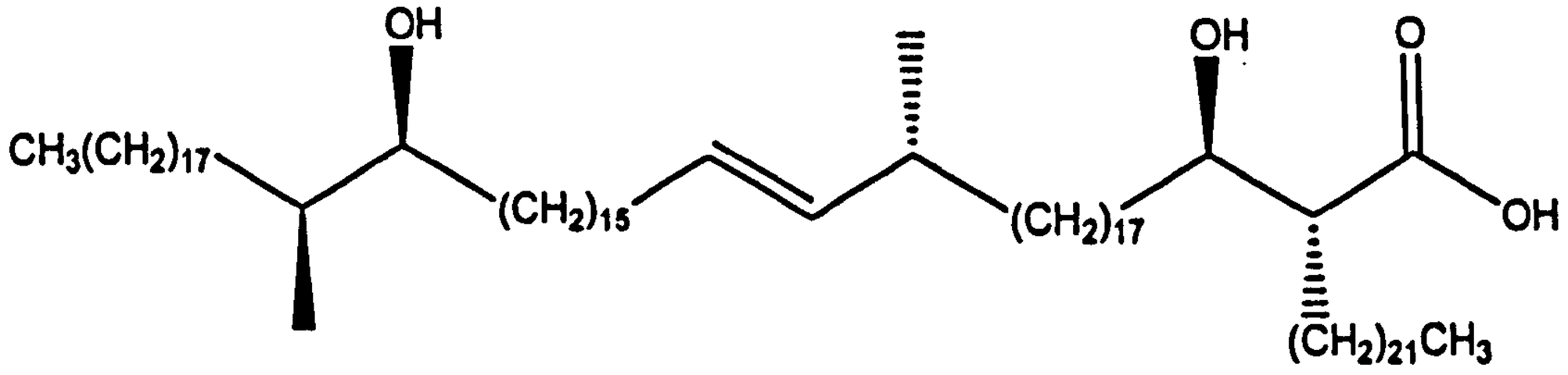
Experiment 58: Methyl (2*R*,3*R*,21*R*,40*S*,*E*)-3-acetoxy-2-docosyl-21,40-dimethyl-39-oxooctapentacont-22-enoate (85)



Methyl (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-3-acetoxy-2-docosyl-39-hydroxy-21,40-dimethyl octapentacont-22-enoate **(205)** (25.0 mg, 0.0237 mmol) in dichloromethane (1 ml) was added to a stirred suspension of pyridinium chlorochromate (10 mg, 0.0701 mmol, 3 mol. equiv.) in dichloromethane (3 ml). The resultant mixture was stirred at RT for 2 hrs and when TLC analysis indicated completion of the reaction the mixture was diluted with ether (1 ml) and then filtered on a bed of silica gel. The filtrate was evaporated and the crude product purified via column chromatography eluting with petrol/ether (5:2) to give a white solid, (2*R*,3*R*,21*R*,40*S*,*E*) methyl 3-acetoxy-2-docosyl-21,40-dimethyl-39-oxooctapentacont-22-enoate **(85)** (20 mg, 80 %), which showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, J 6.6, 15.1 Hz), 5.24 (1H, dd, J 7.25, 15.1 Hz), 5.09 (1H, dt, J 4.1, 8.15 Hz), 3.68 (3H, s), 2.62 (1H, ddd, J 4.45, 6.95, 11.05 Hz), 2.50 (1H, sext, J 6.95 Hz), 2.41 (2H, dt, J 2.25, 7.25 Hz), 2.03 (3H, s), 1.97 (2H, q, J 6.6 Hz), 1.63-1.18 (137H, m, including s at 1.26), 1.05 (3H, d, J 6.6 Hz), 0.94 (3H, d, J 6.6 Hz), 0.89 (6H, t, J 6.95 Hz); δ_C: 215.2, 173.7, 170.3, 136.5, 128.4, 74.1, 51.5, 49.6, 46.3, 41.2, 37.3, 36.7, 35.6, 33.1, 32.6, 31.9, 29.8, 29.7, 29.64, 29.59, 29.54, 29.51, 29.48, 29.43, 29.39, 29.2, 28.1, 27.5, 27.4, 27.3, 25.0, 23.7, 22.7, 21.0, 19.5, 16.4, 14.1; ν_{max}: 2932, 2853, 1748, 1711, 1466 cm^{-1}; $[\alpha]^{22}_D$ = +3.52 ($CHCl_3$, 1.094 μmol); [Found $M+Na^+$: 1288.22; $C_{85}H_{164}NaO_5$ requires: 1288.25].

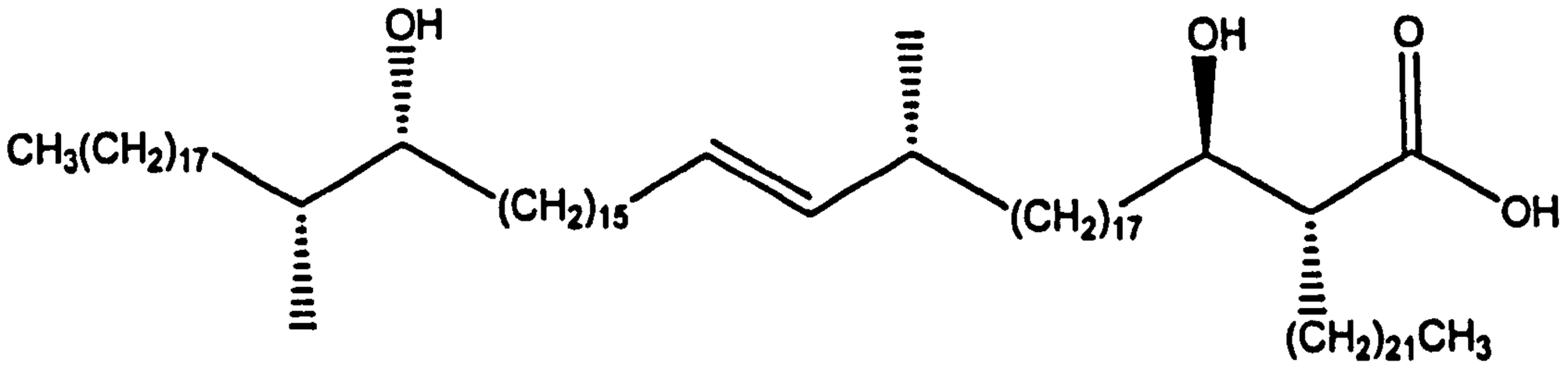
4.4 - Synthesis of *trans*-alkene hydroxy and keto mycolic acids (86), (87) and (88).

Experiment 59: (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-2-Docosyl-3,39-dihydroxy-21,40-dimethyloctapentacont-22-enoic acid (88)



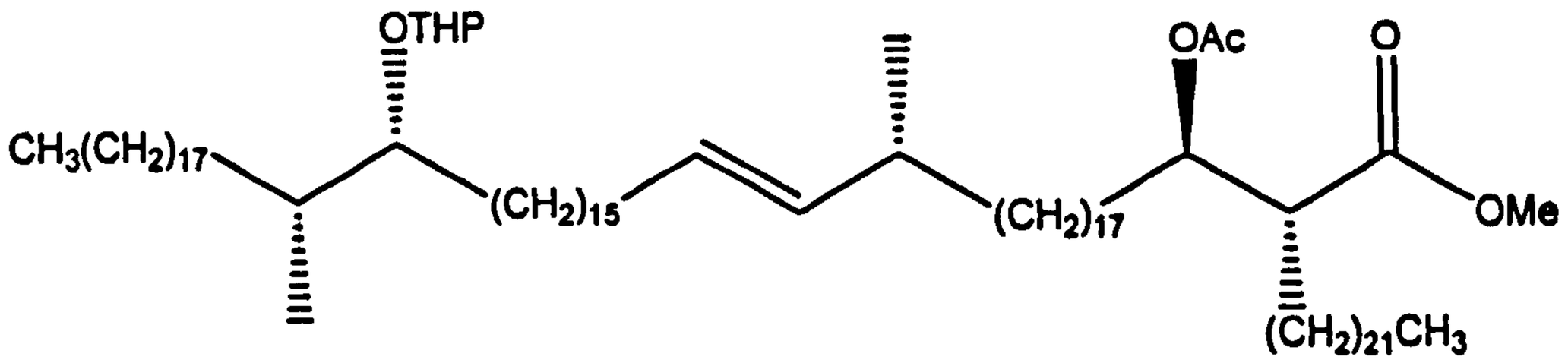
Lithium hydroxide monohydrate (17.04 mg, 0.7102 mmol, 30 mol. equiv.) was added to a stirred solution of methyl (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-3-acetoxy-2-docosyl-39-hydroxy-21,40-dimethyloctapentacont-22-enoate **(205)** (30.0 mg, 0.0237 mmol) in THF (3 ml), methanol (0.5 ml) and water (0.5 ml) at RT. The mixture was stirred at 43 °C for 18 hrs, when TLC analysis indicated completion of the reaction. The mixture was cooled to RT and acidified with hydrochloric acid (5 %, 2 ml) and the aqueous layer extracted with warm petrol/ether (1:1, 3 x 10 ml). The combined organic extracts were dried and evaporated, and then purified via column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-2-docosyl-3,39-dihydroxy-21,40-dimethyloctapentacont-22-enoic acid **(88)** (18.6 mg, 65 %), which showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, J 6.6, 15.45 Hz), 5.23 (1H, dd, J 7.55, 15.45 Hz), 3.70-3.69 (1H, m), 3.52-3.51 (1H, m), 2.45 (1H, br. pent, J 4.7 Hz), 2.05-2.00 (1H, m), 1.97 (2H, q, J 6.9 Hz), 1.79-1.71 (1H, m), 1.66-1.59 (2H, m), 1.64-1.23 (139H, br. m, including br. s at 1.26), 0.94 (3H, d, J 6.6 Hz), 0.89 (6H, t, J 6.95 Hz), 0.86 (3H, d, J 7.25 Hz); δ_C: 177.3, 136.5, 128.5, 75.4, 72.2, 50.5, 37.3, 36.7, 34.4, 33.4, 32.6, 31.9, 30.0, 29.7, 29.6, 29.52, 29.46, 29.4, 29.1, 27.4, 22.7, 21.0, 16.6, 14.1; ν_{max}: 3534, 2922, 2854, 1751, 1466cm^{-1}; $[\alpha]^{21}_D$ = -2.07 ($CHCl_3$, 0.743 μmol) [Found $M+Na^+$: 1234.39; requires: 1234.24].

Experiment 60: (2*R*,3*R*,21*R*,39*R*,40*R*,*E*)-2-Docosyl-3,39-dihydroxy-21,40-dimethyloctapentacont-22-enoic acid (87)



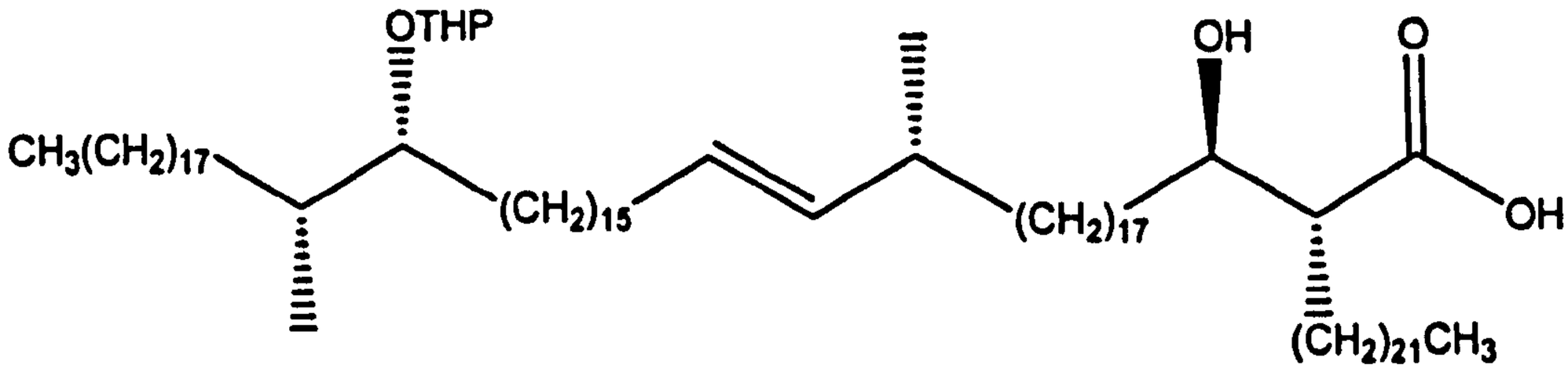
Lithium hydroxide monohydrate (12.0 mg, 0.3551 mmol, 30 mol. equiv.) was added to a stirred solution of (2*R*,3*R*,21*R*,39*R*,40*R*,*E*) methyl 3-acetoxy-2-docosyl-39-hydroxy-21,40-dimethyloctapentacont-22-enoate **(163)** (15.0 mg, 0.01184 mmol) in THF (2 ml), methanol (0.2 ml) and water (0.2 ml) at RT. The mixture was stirred at 43 °C for 18 hrs, when TLC analysis indicated completion of the reaction. The mixture was cooled to RT and acidified with hydrochloric acid (5 %, 2 ml) and the aqueous layer extracted with warm petrol/ether (1:1, 3 x 10 ml). The combined organic extracts were dried and evaporated, and purified via column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, (2*R*,3*R*,21*R*,39*R*,40*R*,*E*)-2-docosyl-3,39-dihydroxy-21,40-dimethyloctapentacont-22-enoic acid **(87)** (8.6 mg, 59 %), which showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, J 6.6, 15.45 Hz), 5.23 (1H, dd, J 7.55, 15.45 Hz), 3.70-3.69 (1H, m), 3.52-3.51 (1H, m), 2.45 (1H, br. pent, J 4.7 Hz), 2.05-2.00 (1H, m), 1.97 (2H, q, J 6.9 Hz), 1.79-1.71 (1H, m), 1.66-1.59 (2H, m), 1.64-1.23 (139H, br. m, including br. s at 1.26), 0.94 (3H, d, J 6.6 Hz), 0.89 (6H, t, J 6.95 Hz), 0.86 (3H, d, J 7.25 Hz); δ_C: 177.3, 136.5, 128.5, 75.4, 72.2, 50.5, 37.3, 36.7, 34.4, 33.4, 32.6, 31.9, 30.0, 29.7, 29.6, 29.52, 29.46, 29.4, 29.1, 27.4, 22.7, 21.0, 16.6, 14.1; ν_{max}: 3534, 2922, 2854, 1751, 1466cm^{-1}; $[\alpha]^{20}_D$ = +1.67 ($CHCl_3$, 1.287 μmol) [Found $M+Na^+$: 1234.47; $C_{82}H_{162}NaO_4$ requires: 1234.24].

Experiment 61: (2*R*,3*R*,19*R*,37*R*,38*R*,*E*) Methyl 3-acetoxy-2-docosyl-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoate (207)



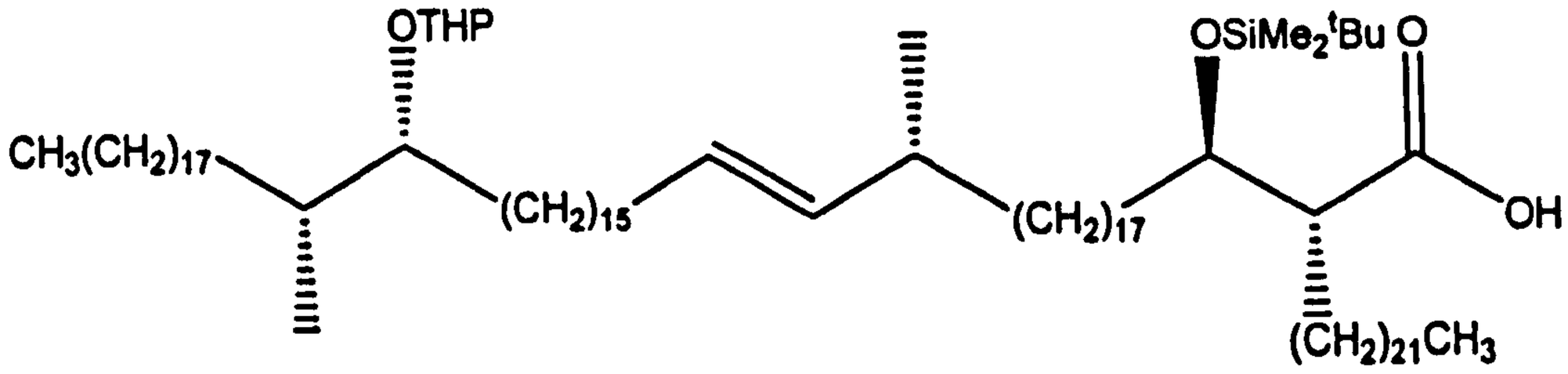
2,3-Dihydropyran (92.8 mg, 1.103 mmol, 20 mol. equiv., d = 0.928, 0.1 ml) and pyridinium-*p*-toluene sulfonate (7 mg, 0.02762 mmol, 0.5 mol. equiv.) were added to a stirred solution of (2*R*,3*R*,21*R*,39*R*,40*R*,*E*) methyl 3-acetoxy-2-docosyl-39-hydroxy-21,40-dimethyloctapentacont-22-enoate **(206)** (70 mg, 0.05524 mmol) in dry dichloromethane (0.5 ml) under nitrogen at RT. The mixture was stirred for 1 hr; when TLC indicated the reaction was complete, the reaction was quenched with sat. aq. sodium hydrogen carbonate (3 ml) and extracted with dichloromethane (2 x 15 ml). The combined organic layers were washed with water (5 ml), dried and evaporated to give a crude oil, which was purified via column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil, (2*R*,3*R*,19*R*,37*R*,38*R*,*E*) methyl 3-acetoxy-2-docosyl-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoate **(207)** (72.4 g, 84 %), which showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, J 6.65, 8.5 Hz), 5.24 (1H, dd, J 7.55, 15.15 Hz), 5.11-5.07 (1H, m), 4.68-4.61 (1H, m), 3.96-3.89 (2H, m), 3.68 (3H, s), 3.50-3.44 (1H, m), 2.64-2.60 (1H, m), 2.03 (3H, s), 1.97 (2H, q, J 6.6 Hz), 1.86-1.82 (1H, m), 1.59-1.14 (146H, br.m including br.s at 1.26), 0.94 (3H, d, J 6.65 Hz), 0.89 (6H, t, J 6.6 Hz), 0.84 (3H, d, J 6.65 Hz); δ_C (125 MHz, $CDCl_3$): 173.7, 170.3, 136.5, 128.4, 98.5, 81.4, 74.1, 62.8, 51.5, 49.6, 38.3, 37.2, 36.7, 34.5, 33.5, 32.6, 31.9, 31.7, 30.0, 29.9, 29.8, 29.71, 29.65, 29.61, 29.57, 29.52, 29.49, 29.44, 29.42, 29.38, 29.1, 28.1, 27.7, 27.5, 27.4, 27.3, 26.2, 25.0, 22.7, 21.0, 20.9, 18.2, 14.4, 13.6; ν_{max}: 2924, 2853m 2360m 2341m 1464 cm^{-1}; $[\alpha]^{23}_D$ = +1.99 ($CHCl_3$, 1.487 μmol) [Found $M+Na^+$: 1374.39; requires: 1374.32].

Experiment 62: (2*R*,3*R*,19*R*,37*R*,38*R*,*E*)-2-Docosyl-3-hydroxy-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoic acid (208)



Lithium hydroxide monohydrate (52.8 mg, 1.554 mmol, 30 mol. equiv.) was added to a stirred solution of (2*R*,3*R*,19*R*,37*R*,38*R*,*E*) methyl 3-acetoxy-2-docosyl-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoate **(207)** (70 mg, 0.05180 mmol) in THF (5 ml), methanol (0.5 ml) and water (0.5 ml) at RT. The mixture was stirred at 43 °C for 18 hrs; when TLC analysis indicated completion of the reaction, it was cooled to RT and acidified with hydrochloric acid (5 %, 2 ml) and the aqueous layer extracted with warm petrol/ether (1:1, 3 x 10 ml). The combined organic extracts were dried and evaporated, and purified via column chromatography eluting with petrol/ethyl acetate (5:2) to give a white solid, (2*R*,3*R*,19*R*,37*R*,38*R*,*E*)-2-docosyl-3-hydroxy-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoic acid **(208)** (46.4 mg, 72 %), which showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, J 6.65, 8.5 Hz), 5.24 (1H, dd, J 7.55, 15.15 Hz), 4.68-4.62 (1H, m), 3.96-3.89 (1H, m), 3.50-3.44 (1H, m), 2.49-2.42 (1H, m), 1.97 (2H, q, J 7.25 Hz), 1.88-1.82 (1H, m), 1.63-1.21 (149H, br.m including br.s at 1.26), 0.94 (3H, d, J 6.65 Hz), 0.89 (6H, t, J 6.6 Hz), 0.84 (3H, d, J 6.65 Hz); δ_C (125 MHz, $CDCl_3$): 177.5, 145.5, 136.5, 72.2, 67.4, 60.4, 50.5, 37.3, 36.7, 35.6, 34.5, 32.6, 31.9, 30.9, 30.3, 30.1, 30.0, 29.9, 29.7, 29.6, 29.5, 29.42, 29.37, 29.3, 29.12, 29.08, 28.9, 28.1, 27.3, 26.1, 25.8, 22.7, 22.6, 22.3, 21.1, 21.0, 20.4, 19.4, 14.2, 14.11, 14.06; ν_{max}: 3424, 2922, 2852, 2361, 1646 cm^{-1}; $[\alpha]^{22}_D$ = + 1.75 ($CHCl_3$, 1.188 µmol) [Found $M+Na^+$: 1318.14; requires: 1318.26].

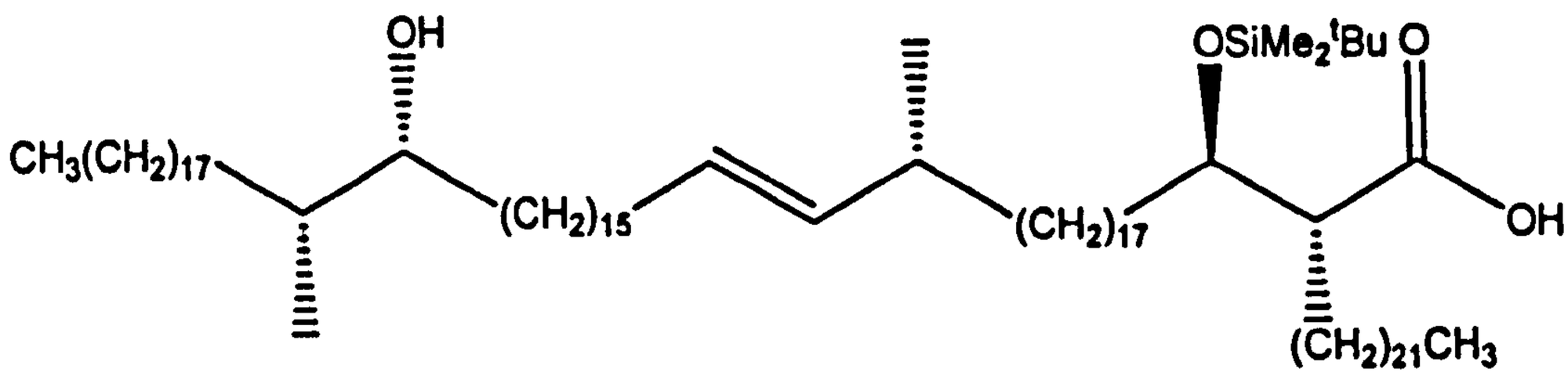
Experiment 63: (2*R*,3*R*,19*R*,37*R*,38*R*,*E*)-3-(*tert*-Butyldimethylsilyloxy)-2-docosyl-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoic acid (209)



Imidazole (22.7 mg, 0.3330 mmol, 10 mol. equiv.) was added to a stirred solution of (2*R*,3*R*,19*R*,37*R*,38*R*,*E*)-2-docosyl-3-hydroxy-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoic acid **(208)** (45 mg, 0.03330 mmol) in dry DMF (0.2 ml) at RT followed by addition of *tert*-butyl dimethylsilylchloride (50.0 mg, 0.3330 mmol, 10 mol. equiv.) and 4-dimethylaminopyridine (4.01 mg, 0.03330 mmol). The mixture was heated to 70 °C for 18 hrs; when TLC showed no starting material was left, it was diluted with petrol/ethyl acetate (1:1, 15 ml) and sat. aq. sodium hydrogen carbonate (3 ml). The aqueous layer was re-extracted with petrol/ethyl acetate (3 x 15 ml), dried and the solvent evaporated. The residue was dissolved in THF (5 ml), methanol (0.5 ml) and water (0.5 ml); to this, potassium carbonate (100 mg) was added and the mixture stirred at 45 °C for 6 hrs. The mixture was diluted with petrol/ethyl acetate (1:1, 10 ml) and water (1 ml) and acidified with potassium hydrogen sulfate to pH 2. The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 x 10 ml), dried and the solvent evaporated. The crude product was purified via column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, (2*R*,3*R*,19*R*,37*R*,38*R*,*E*)-3-(*tert*-butyldimethylsilyloxy)-2-docosyl-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoic acid **(209)** (34.2 g, 70 %), which showed δ_H (500 MHz, $CDCl_3$): 5.34 (1H, dt, J 6.6, 8.85 Hz), 5.24 (1H, dd, J 7.55, 15.1 Hz), 4.67-4.62 (1H, m), 3.93-3.89 (1H, m), 3.50-3.44 (2H, m), 2.55-2.51 (1H, m), 1.97 (2H, q, J 6.95 Hz), 1.88-1.82 (1H, m), 1.70-1. 31 (147H, br.m including br.s at 1.26), 0.94 (3H, d, J 6.95 Hz), 0.93 (9H, s), 0.89 (6H, t, J 6.6 Hz), 0.84 (3H, d, J 6.95 Hz), 0.15 (3H, s), 0.14 (3H, s); δ_C (125 MHz, $CDCl_3$): 177.4, 136.6, 128.3, 98.5, 75.3, 73.7, 67.4, 49.9, 41.7, 39.7, 37.6, 36.7, 35.8, 34.8, 33.3, 32.6, 31.9, 31.6, 30.1, 30.0, 29.82,

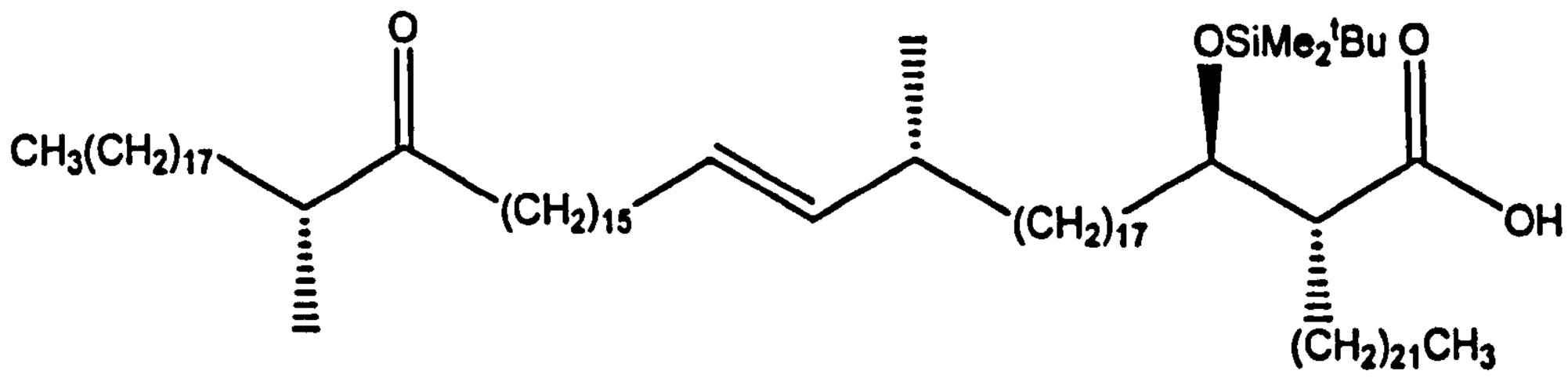
29.81, 29.73, 29.70, 29.61, 29.57, 29.52, 29.48, 29.42, 29.37, 29.1, 29.0, 27.7, 27.41, 27.38, 26.3, 25.8, 25.7, 25.2, 22.73, 22.69, 22.6, 21.1, 20.9, 20.4, 19.4, 18.8, 17.8, 14.3, 14.1, 13.6, 11.4, -4.3, -4.9; v_{max}: 3425, 2924, 2853, 2362, 1702, 1464 cm^{-1}; $[\alpha]^{23}_{D}$ = +1.58 ($CHCl_3$, 0.436 μmol) [Found M+Na^+: 1432.32; $C_{93}H_{184}NaO_5Si$ requires: 1432.38].

Experiment 64: (2*R*,3*R*,19*R*,37*R*,38*R*,*E*)-3-(*tert*-Butyldimethylsilyloxy)-2-docosyl-37-hydroxy-19,38-dimethylhexapentacont-20-enoic acid (210)



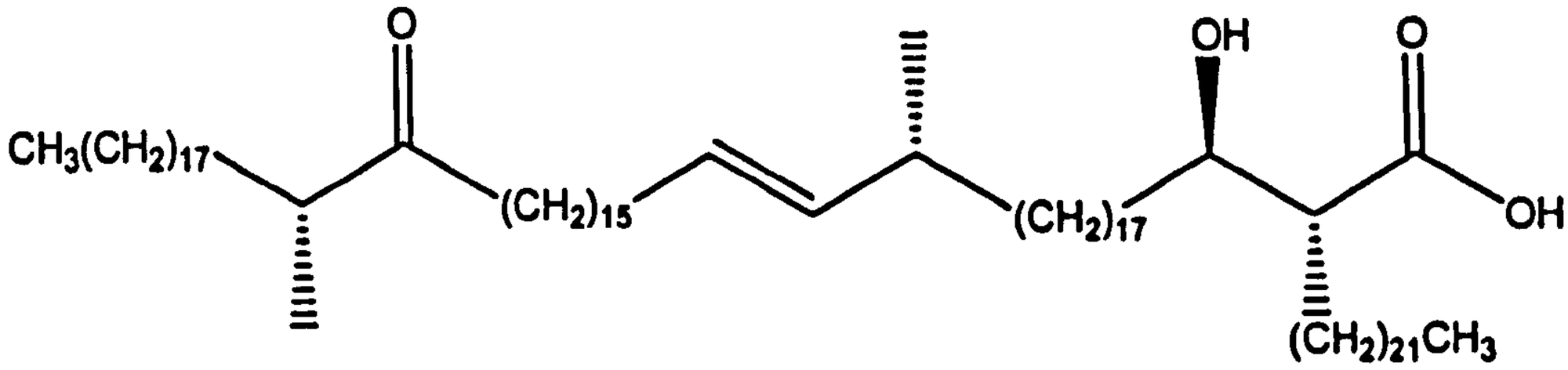
Pyridinium-*p*-toluene sulfonate (29.67 mg, 0.1181 mmol) was added to a stirred solution of (2*R*,3*R*,19*R*,37*R*,38*R*,*E*)-3-(*tert*-butyldimethylsilyloxy)-2-docosyl-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoic acid **(209)** (17.3 mg, 0.01181 mmol) in THF (1 ml) methanol (0.1 ml) and water (0.1 ml) and the mixture refluxed for 2.5 hrs followed by stirring at 47 °C for 24 hrs, when TLC showed no starting material was left, sat. aq. sodium hydrogen carbonate (0.2 ml) was added and the product extracted with petrol/ethyl acetate (1:1, 3 x 5 ml). The combined organic layers were dried and the solvent evaporated to give a crude oil, which was purified via column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil, (2*R*,3*R*,19*R*,37*R*,38*R*,*E*)-3-(*tert*-butyldimethylsilyloxy)-2-docosyl-37-hydroxy-19,38-dimethylhexapentacont-20-enoic acid **(210)** (11.9 mg, 73 %), which showed δ_H (500 MHz, $CDCl_3$): 5.31 (1H, dt, J 7.55 Hz), 5.24 (1H, dd, J 7.55, 7.6 Hz), 3.83 (1H, dist. pent, J 2.85 Hz), 3.52-3.49 (1H, m), 2.55-2.51 (1H, m), 1.97 (2H, q, J 6.6 Hz), 1.63-1.22 (142H, br.m including br.s at 1.26), 0.97 (3H, d, J 6.65 Hz), 0.93 (9H, s), 0.89 (3H, d, J 7.25 Hz), 0.88 (6H, t, J 6.6 Hz), 0.15 (3H, s), 0.14 (3H, s); δ_C (125 MHz, $CDCl_3$): 177.7, 136.5, 128.4, 75.3, 73.7, 50.0, 41.4, 38.2, 37.3, 36.7, 35.8, 34.5, 33.4, 32.6, 31.9, 31.6, 30.1, 30.0, 29.82, 29.79, 29.73, 29.65, 29.64, 29.59, 29.52, 29.50, 29.44, 29.42, 29.1, 29.0, 27.7, 27.40, 27.37, 26.3, 25.8, 25.7, 25.2, 22.72, 22.70, 22.6, 21.1, 20.9, 20.4, 19.4, 18.8, 17.9, 14.3, 14.2, 14.1, 13.6, 11.4, -4.2, -4.9; ν_{max}: 3424, 2923, 2852, 1709, 1464 cm^{-1}; $[\alpha]^{27}_D$ = +1.46 ($CHCl_3$, 1.425 μmol) [Found $M+Na^+$: 1348.58; $C_{88}H_{176}NaO_4Si$ requires: 1348.32].

Experiment 65: (2*R*,3*R*,19*R*,38*R*,*E*)-3-(*tert*-Butyldimethylsilyloxy)-2-docosyl-19,38-dimethyl-37-oxohexapentacont-20-enoic acid (211)



(2*R*,3*R*,19*R*,37*R*,38*R*,*E*)-3-(*tert*-Butyldimethylsilyloxy)-2-docosyl-37-hydroxy-19,38-dimethylhexapentacont-20-enoic acid **(210)** (18.1 mg, 0.0131 mmol), in dichloromethane (1 ml), was added to a stirred suspension of pyridinium chlorochromate (8.47 mg, 0.0393 mmol, 3 mol. equiv.) in dichloromethane (2 ml) at RT. The mixture was stirred for 1 hr, when TLC analysis indicated completion of the reaction. The solvent was evaporated and the resultant residue was purified via column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, (2*R*,3*R*,19*R*,38*R*,*E*)-3-(*tert*-butyldimethylsilyloxy)-2-docosyl-19,38-dimethyl-37-oxohexapentacont-20-enoic acid **(211)** (16.1 mg, 89 %), which showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, J 6, 8.5 Hz), 5.24 (1H, dd, J 7.55, 8.2 Hz), 3.85-31 (1H, m), 2.56-2.52 (1H, m), 2.50 (1H, t, J 6.9 Hz), 2.41 (2H, dt, J, 1.9, 7.25 Hz), 2.05-2.00 (1H, m), 1.97 (2H, q, J 6.6 Hz), 1.75-1.68 (2H, m), 1.64-1.21 (135H, br.m including br.s at 1.26), 1.05 (3H, d, J 6.6 Hz), 0.95 (3H, d, J 6.95 Hz), 0.93 (9H, s), 0.89 (6H, t, J 6.6 Hz), 0.15 (3H, s), 0.14 (3H, s); δ_C (125 MHz, $CDCl_3$): 215.3, 178.0, 136.5, 128.4, 73.7, 50.0, 46.3, 41.2, 37.3, 36.7, 35.8, 33.1, 32.6, 31.9, 29.8, 29.72, 29.69, 29.64, 29.61, 29.58, 29.54, 29.50, 29.48, 29.46, 29.44, 29.37, 29.3, 29.2, 29.2, 27.4, 27.32, 27.27, 25.7, 25.2, 23.7, 22.7, 20.1, 17.9, 16.4, 14.1, -4.2, -4.9; ν_{max}: 3419, 2925, 2854, 2360, 2341, 1711, 1464 cm^{-1}; $[\alpha]^{24}_D$ = +3.70 ($CHCl_3$, 0.457 μmol) [Found $M+Na^+$: 1346.47; $C_{88}H_{174}NaO_4Si$ requires: 1346.31].

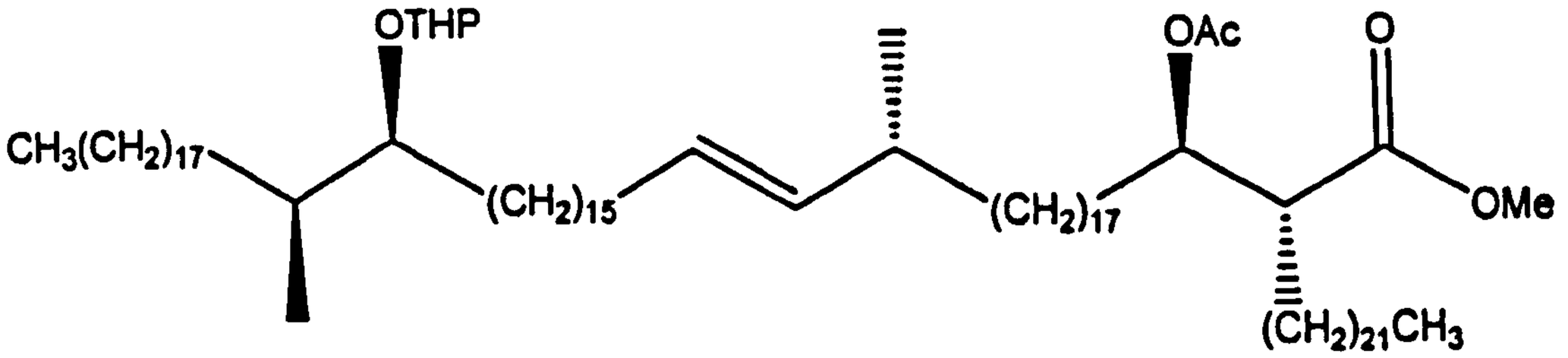
Experiment 66: (2*R*,3*R*,19*R*,38*R*,*E*)-2-Docosyl-3-hydroxy-19,38-dimethyl-37-oxohexapentacont-20-enoic acid (86)



A dry polyethylene vial equipped with a rubber septum, was charged with (2*R*,3*R*,19*R*,38*R*,*E*)-3-(*tert*-butyldimethylsilyloxy)-2-docosyl-19,38-dimethyl-37-oxohexapentacont-20-enoic acid **(211)** (15 mg, 0.01088 mmol) in dry THF (1 ml) under nitrogen at 0 °C. Pyridine (0.1 ml) and hydrogen fluoride-pyridine complex (0.05 ml, 0.03263 mmol, 3 mol. equiv.) were added and the mixture stirred for 18hrs at 43 °C. When TLC analysis indicated completion of the reaction, the mixture was neutralised by slowly pouring the mixture into sat. aq. sodium hydrogen carbonate (3 ml) until no more carbon dioxide was liberated. The product was extracted with petrol/ether (1:1, 3 x10 ml), dried and evaporated to give a white solid. This was purified via column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, (2*R*,3*R*,19*R*,38*R*,*E*)-2-docosyl-3-hydroxy-19,38-dimethyl-37-oxohexa pentacont-20-enoic acid **(86)** (6 mg, 44 %), which showed δ_H (500 MHz, $CDCl_3$): 5.32 (1H, td, J 6.65, 15.45 Hz), 5.24 (1H, dd, J 7.55, 15.45 Hz), 2.50 (1H, sext, J 6.6 Hz), 2.47 (1H, m), 2.42 (2H, dt, J 1.55, 6.95 Hz), 2.36 (1H, t, J 7.55 Hz), 1.97 (2H, q, J 6.95 Hz), 1.65-1.17 (139H, br. m, including br. s at 1.26), 1.05 (3H, d, J 6.95 Hz), 0.95 (3H, d, J 6.95 Hz), 0.89 (6H, t, J 6.65 Hz); δ_C: 215.5, 177.9, 136.5, 128.4, 72.2, 50.6, 46.4, 41.2, 37.3, 36.7, 35.6, 33.1, 32.6, 31.9, 29.8, 29.7, 29.64, 29.59, 29.54, 29.51, 29.48, 29.43, 29.39, 29.3, 29.1, 28.9, 27.4, 27.3, 25.7, 23.7, 22.7, 22.6, 21.0, 19.4, 16.4, 14.1; ν_{max}: 3420, 3019, 2926, 2855, 1521, 1420, 1215 cm^{-1}; $[\alpha]^{23}_D$ = +2.90 ($CHCl_3$, 0.471 µmol) [Found $M+Na^+$: 1232.36; $C_{82}H_{160}NaO_4$ requires: 1233.14].

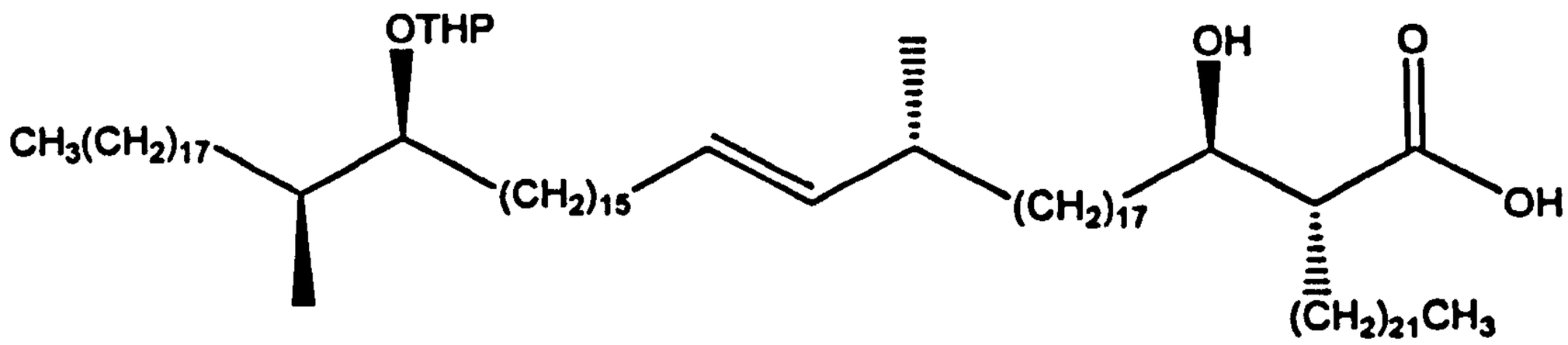
4.5 - Additional Experimental

Experiment 67: (2*R*,3*R*,19*R*,37*S*,38*S*,*E*) Methyl 3-acetoxy-2-docosyl-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoate



2,3-Dihydropyran (0.05 ml, 52.0 mg, 0.6185 mmol, 20 mol. equiv., d = 0.928) and pyridinium-*p*-toluene sulfonate (4 mg, 0.0155 mmol, 0.5 mol. equiv.) were added to a stirred solution of (2*R*,3*R*,21*R*,39*R*,40*R*,*E*) methyl 3-acetoxy-2-docosyl-39-hydroxy-21,40-dimethyloctapentacont-22-enoate **(205)** (31.4 mg, 0.0309 mmol) in dry dichloromethane (0.5 ml) under nitrogen at RT. The mixture was stirred for 1 hr; when TLC indicated the reaction was complete, it was quenched with sat. aq. sodium hydrogen carbonate (3 ml) and extracted with dichloromethane (2 x 15 ml). The combined organic layers were washed wtih water (5 ml), dried and evaporated to give a crude oil, which was purified via column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil, (2*R*,3*R*,21*R*,39*S*,40*S*,*E*) methyl 3-acetoxy-2-docosyl-39-hydroxy-21,40-dimethyloctapentacont-22-enoate (30.0 mg, 90 %), which showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, J 6.65, 8.5 Hz), 5.24 (1H, dd, J 7.55, 15.15 Hz), 5.11-5.07 (1H, m), 4.68-4.61 (1H, m), 3.96-3.89 (1H, m), 3.68 (3H, s), 3.50-3.44 (1H, m), 2.64-2.60 (1H, m), 2.03 (3H, s), 1.97 (2H, q, J 6.6 Hz), 1.86-1.82 (1H, m), 1.59-1.14 (146H, br.m including br.s at 1.26), 0.94 (3H, d, J 6.65 Hz), 0.89 (6H, t, J 6.6 Hz), 0.84 (3H, d, J 6.65 Hz); δ_C (125 MHz, $CDCl_3$): 173.7, 170.3, 136.5, 128.4, 98.5, 81.4, 74.1, 62.8, 51.5, 49.6, 38.3, 37.2, 36.7, 34.5, 33.5, 32.6, 31.9, 31.7, 30.0, 29.9, 29.8, 29.71, 29.65, 29.61, 29.57, 29.52, 29.49, 29.44, 29.42, 29.38, 29.1, 28.1, 27.7, 27.5, 27.4, 27.3, 26.2, 25.0, 22.7, 21.0, 20.9, 18.2, 14.4, 13.6; ν_{max}: 2924, 2853m 2360m 2341m 1464 cm^{-1}; $[\alpha]^{23}_D$ = +1.99 ($CHCl_3$, 1.487 μmol) [Found $M+Na^+$: 1374.21; $C_{90}H_{174}O_6Na$ requires: 1374.32].

Experiment 68: (2*R*,3*R*,19*R*,37*S*,38*S*,*E*)-2-Docosyl-3-hydroxy-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoic acid



Lithium hydroxide monohydrate (23.1 mg, 0.8874 mmol, 30 mol. equiv.) was added to a stirred solution of (2*R*,3*R*,21*R*,39*S*,40*S*,*E*) methyl 3-acetoxy-2-docosyl-39-hydroxy-21,40-dimethyloctapentacont-22-enoate (30 mg, 0.02958 mmol) in THF (4 ml), methanol (0.5 ml) and water (0.5 ml) at RT. The mixture was stirred at 43 °C for 18 hrs; when TLC analysis indicated completion of the reaction, it was cooled to RT and acidified with hydrochloric acid (5 %, 2 ml) and the aqueous layer extracted with warm petrol/ethyl acetate (1:1, 3 x 10 ml). The combined organic extracts were dried and evaporated, and purified via column chromatography eluting with petrol/ethyl acetate (5:2) to give a white solid, (2*R*,3*R*,19*R*,37*S*,38*S*,*E*)-2-docosyl-3-hydroxy-19,38-dimethyl-37-(tetrahydro-2H-pyran-2-yloxy)hexapentacont-20-enoic acid (8.1 mg, 28 %), which showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, J 6.65, 8.5 Hz), 5.24 (1H, dd, J 7.55, 15.15 Hz), 4.68-4.62 (1H, m), 3.96-3.89 (1H, m), 3.50-3.44 (1H, m), 2.49-2.42 (1H, m), 1.97 (2H, q, J 7.25 Hz), 1.88-1.82 (1H, m), 1.63-1.21 (149H, br.m including br.s at 1.26), 0.94 (3H, d, J 6.65 Hz), 0.89 (6H, t, J 6.6 Hz), 0.84 (3H, d, J 6.65 Hz); δ_C (125 MHz, $CDCl_3$): 177.5, 145.5, 136.5, 72.2, 67.4, 60.4, 50.5, 37.3, 36.7, 35.6, 34.5, 32.6, 31.9, 30.9, 30.3, 30.1, 30.0, 29.9, 29.7, 29.6, 29.5, 29.42, 29.37, 29.3, 29.12, 29.08, 28.9, 28.1, 27.3, 26.1, 25.8, 22.7, 22.6, 22.3, 21.1, 21.0, 20.4, 19.4, 14.2, 14.11, 14.06; ν_{max}: 3424, 2922, 2852, 2361, 1646 cm^{-1}; $[\alpha]^{22}_D$ = + 1.75 ($CHCl_3$, 0.617 μmol) [Found $M+Na^+$: 1318.18; $C_{87}H_{170}O_5Na$ requires: 1318.29].

13. Krause, A. K., Tuberculosis and public health. *Am. Rev. Tuberc.* **1928,** 18, 271-322.

14. Grigg, E.R.N., The arcana of tuberculosis with a brief epidemiologic history of the disease in the USA. *Am. Rev. Tuberc. Pulm. Dis.* **1958,** 78, 426-453.

15. Koch, R., Die Aetiologie der Tuberkulose. *Mittheilungen aus dem Kaiserliched Gesundheitsamte* **1884,** 2, 1-88.

16. Koch, R., Weitere mitteilungen uber ein heilmittel gegen tuberculose. *Dtsch. Med. Wscher.* **1891,** 17, 101-102.

17. von Piquet, C., Frequency of tuberculosis in childhood. *J. Am. Med. Assoc.* **1907,** 52, 675-678.

18. Edwards, L. B.; Acquaviva, F. A.; Livesay, V. T.; Cross, F. W.; Palmer, C. E., An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States. *Am. Rev. Respir. Dis.* **1969,** 99, 1-132.

19. Calmette, A., On preventive vaccination of the new-born against tuberculosis by B.C.G. *Br. J. Tuberc.* **1928,** 22, 161-165.

20. Bryder, L., We shall not find salvation in inoculation : BCG vaccination in Scandinavia, Britain and the USA, 1921-1960. *Social Science & Medicine* **1999,** 49, (9), 1157-1167.

21. Feldberg, G. D., Disease and class. Tuberculosis and the shaping of Modern North American Society. *New Brunswick : Rutgers University Press* **1995.**

22. Menut, P., The Lubeck catastrophe and its consequences for anti-tuberculosis BCG-vaccination *Paris: Elsevier* **2001,** 202-210.

23. Rosenthal, S.R., BCG vaccination against tuberculosis *Boston: Litte, Brown & Co.* **1957.**

24. Bonah, C., The "experimental stable" of the BCG vaccine: safety, efficacy, proof and standards, 1921-1933. *Studies in History and Philosophy of Biomedical Sciences* **2005,** 36, (696-721).

25. McDougall, J. B., Tuberculosis. A Global Study in Social Pathology. *Baltimore: Williams and Wilkins* **1949.**

26. Wallgren, A., Zur Frage der Tuberkulose-Schutzimpfung *Beitrage zur Klinik der Tuberkulose und spezifischen Tuberkulose-Forschung* **1949,** 101, 296-315.

27. Wilson, G., The value of BCG vaccination in control of tuberculosis. *British Medical Journal* **1947,** 2, (4534), 855-859.

References

1. Brennan, P. J.; Nikaido, H., The Envelope of Mycobacteria. *Annu. Rev. Biochem* **1995,** 64, 29-63.
2. Daniel, T. M., The history of tuberculosis. *Respiratory Medicine* **2006,** 100, 1862-1870.
3. Gutierrez, M. C.; Brisse, S.; Brosch, R.; Fabre, M.; Omais, B.; Marmiesse, M., Ancient origin and gene mosaicism of the progenitor of *Mycobacterium tuberculosis*. *PloS. Pathog.* **2005,** 1.
4. Kapur, V.; Whittam, T. S.; Musser, J. M., Is *Mycobacterium tuberculosis* 15,000 years old? *J. Infect. Dis.* **1944,** 170, 1348-1349.
5. Brosch, R.; Gordon, S. V.; Marmiesse, M.; Brodin, P.; Buchrieser, C.; Eiglmeier, K., A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc. Natl. Acad. Sci.* **2002,** 99, 3684-3689.
6. Hayman, J., *Mycobacterium ulcerans*: an infection from Jurassic time? *Lancet* **1984,** 2, 1015-6.
7. Cave, A. J. E., The evidence for the incidence of tuberculosis in acient Egypt. *Br. J. Tuberc.* **1939,** 33, 142-152.
8. Brothwell, D.; Sandison, T., Diseases in antiquity. A survey of the diseases, injuries and surgery of early populations *C.C. Thomas*, **1967,** 19, 766.
9. Brown, L., The story of clinical pulmonary tuberculosis. *Williams & Wilkins Company Baltimore,* **1941,** 411.
10. Daniel, T. M., The origins and precolonial epidemiology of tuberculosis in the Americas: can we figure them out? *Int. J. Tuberc. Lung Dis.* **2000,** 4, 395-400.
11. Roberts, C. A.; Buikstra, J. E., The bioarchaeology of tuberculosis. A global view on a reemerging disease. *Gainesville, FL: University of Florida Press* **2003.**
12. Laennec, R.T.H, A treatise on the disease of the chest; translated by Forbes, J. *J. New York, NY: Hafner Publishing* **1962.**

13. Krause, A. K., Tuberculosis and public health. *Am. Rev. Tuberc.* **1928,** 18, 271-322.

14. Grigg, E.R.N., The arcana of tuberculosis with a brief epidemiologic history of the disease in the USA. *Am. Rev. Tuberc. Pulm. Dis.* **1958,** 78, 426-453.

15. Koch, R., Die Aetiologie der Tuberkulose. *Mittheilungen aus dem Kaiserliched Gesundheitsamte* **1884,** 2, 1-88.

16. Koch, R., Weitere mitteilungen uber ein heilmittel gegen tuberculose. *Dtsch. Med. Wscher.* **1891,** 17, 101-102.

17. von Piquet, C., Frequency of tuberculosis in childhood. *J. Am. Med. Assoc.* **1907,** 52, 675-678.

18. Edwards, L. B.; Acquaviva, F. A.; Livesay, V. T.; Cross, F. W.; Palmer, C. E., An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States. *Am. Rev. Respir. Dis.* **1969,** 99, 1-132.

19. Calmette, A., On preventive vaccination of the new-born against tuberculosis by B.C.G. *Br. J. Tuberc.* **1928,** 22, 161-165.

20. Bryder, L., We shall not find salvation in inoculation : BCG vaccination in Scandinavia, Britain and the USA, 1921-1960. *Social Science & Medicine* **1999,** 49, (9), 1157-1167.

21. Feldberg, G. D., Disease and class. Tuberculosis and the shaping of Modern North American Society. *New Brunswick : Rutgers University Press* **1995.**

22. Menut, P., The Lubeck catastrophe and its consequences for anti-tuberculosis BCG-vaccination *Paris: Elsevier* **2001,** 202-210.

23. Rosenthal, S.R., BCG vaccination against tuberculosis *Boston: Litte, Brown & Co.* **1957.**

24. Bonah, C., The "experimental stable" of the BCG vaccine: safety, efficacy, proof and standards, 1921-1933. *Studies in History and Philosophy of Biomedical Sciences* **2005,** 36, (696-721).

25. McDougall, J. B., Tuberculosis. A Global Study in Social Pathology. *Baltimore: Williams and Wilkins* **1949.**

26. Wallgren, A., Zur Frage der Tuberkulose-Schutzimpfung *Beitrage zur Klinik der Tuberkulose und spezifischen Tuberkulose-Forschung* **1949,** 101, 296-315.

27. Wilson, G., The value of BCG vaccination in control of tuberculosis. *British Medical Journal* **1947,** 2, (4534), 855-859.

28. W.H.O., Report of the expert commitee on tuberculosis. *Bull. World Health Organ.* **1948,** 1, (2), 205-212.

29. Archives, U., UNICEF-WHO Joint Commitee on health policy, ninth session *UN Archives JC9/UNICEF-WHO/Min/1* **1956**.

30. W.H.O., Review of BCG Vaccination Programmes. *Official Records of the WHO* **1959,** 12, 91, 96, 99.

31. W.H.O., Justification and design for the undertaking of a large-scale BCG trial in India. *WHO Archives* **1963,** TB/Int./50.

32. WHO, Report on the ICMR Consultative Commitee on the Tuberculosis Prevention Trial. *WHO Archives* **1977,** T9/157/B/5.

33. W.H.O., Vaccination against tuberculosis: report of an ICMR/WHO scientific group. *WHO Technical Report Series* **1980,** 651.

34. W.H.O., The Executive Board. Summary records: Sixteenth meeting. *WHO Archives* **1983,** EB71/1983/REC/2.

35. Davies, R. P. O.; Tocque, K.; Bellis, M. A.; Rimmington, T.; Davies, P. D. O., Historical declines in tuberculoss in England and Wales:improving social conditions or natural selection. *Int. J. Tuberc. Lung Dis.* **1999,** 3, 1051-1054.

36. Thearle, W.H., The Trend of Collapse Therapy in Tuberculosis. *Chest* **1935,** 1, 6-22.

37. W.H.O., TB: Groups at Risk. *WHO Report in the Tuberculosis Epidemic 1996* **1996.**

38. Minnikin, D. E.; Kremer, L.; Dover, L. G.; Besra, G. S., The methyl-branched fortifications of *Mycobacterium tuberculosis. Chemistry & Biology* **2002,** 9, 545-553.

39. Wei, C. J.; Tiu, C. M.; Chen, J. D.; Chou, Y. H.; Chang, C. Y.; Yu, C., Computed tomography features of acute pulmonary tuberculosis. *Am. J. Emerg. Med.* **2004,** 22, 171-174.

40. Seiler, H. E.; Welstead, A. G.; Williamson, J., Report on Edinburgh X-ray campaign. *Tubercle* **1958,** 39, 339-359.

41. Madison, B., Application of stains in clinical microbiology. *Biotech. Histochem.* **2001,** 76, (3), 119-125.

42. Bennedsen, J.; Larsen, S. O., Examination for tubercle bacilli by fluorescence microscopy. *Scand. J. Respir. Dis.* **1966,** 47, 114-120.

43. Sula, L.; Sundaresan, T. K., WHO co-operative studies of a simple culture technique for the isolation of mycobacteria:compatison of the efficacy of lyophilized liquid medium with that of Lowenstein-Jensen (L-J) medium. *Bull. World Health Organ.* **1963,** 29, 607-625.

44. Middlebrook, G.; Reggiardo, Z.; Tigertt, W. D., Automatable radiometric detection of growth of *Mycobacterium tuberculosis* in selective media. *Am. Rev. Respir. Dis.* **1977,** 115, 1066-1069.

45. Janin, Y. L., Antituberculosis drugs: Ten years of research. *Bioorganic & Medicinal Chemsitry* **2007,** 15, 2479-2513.

46. Espinal, M. A., The global situation of MDR-TB. *Tuberculosis* **2003,** 83, 44-51.

47. W.H.O., W.H.O. Tuberculosis Programme: framework for effective tuberculosis control. *WHO/TB/94.179* **1994.**

48. Forget, E. J.; Menzies, D., Adverse reactions to first-line antituberculosis drugs. *Expert Opin. Drug Saf.* **2006,** 5, (2), 231-249.

49. W.H.O., The Stop TB Strategy. *WHO/HTM/TB/2006.368* **2006.**

50. Raychaudhuri, S.; Rock, K. L., Fully mobilizing host defences: building better vaccines. *Nat. Biotechnol.* **1998,** 16, 1025-1031.

51. Banchereau, J.; Briere, F.; Caux, C.; Davoust, J.; Lebecque, S.; Liu, Y. J.; Pulendra, B.; Palucka, K., Immunobiology of dendritic cells. *Annu. Rev. Immunol.* **2000,** 18, 767-811.

52. Flynn, J. L.; Goldstein, M. M.; Triebold, K. J.; Sypek, J.; Wolf, S.; Bloom, B. R., IL-12 inceases resistance of BALB/c mice to *Mycobacterium tuberculosis* infection. *J. Immunol.* **1995,** 155, 2515-2524.

53. Aaron, L.; Saadou, D.; Calatroni, I.; Launay, O.; Mémain, N.; Vincent, V.; Marchal, G.; Dupont, B.; Bouchaud, O.; Valeyre, D.; Lortholary, O., Tuberculosis in HIV-infected paients: a comprehensive review. *Clin. Microbiol. Infect.* **2004,** 10, 388-398.

54. W.H.O., HIV/AIDS prevention, treatment and care in the health sector. *HIV/AIDS Department* **2009,** 1.2.

55. Marchal, G., Pathophysiology and immunology of tuberculosis. *Rev. Mal. Respir* **1997,** 14, 19-26.

56. Havlir, D. V.; Barnes, P. F., Current Concepts. Tuberculosis in patients with human immunodeficiency virus infection. *N. Engl. J. Med.* **1999,** 340, 367-373.

57. Garrait, V.; Cadranel, J.; Esvant, H.; Herry, I.; Morinet, P.; Mayaud, C.; Israel-Biet, D., Tuberculosis Generates a Microenvironment Enhancing the Productive Infection of Local Lymphocytes by HIV. *J. Immunol.* **1997,** 159, (2824-2830).

58. Dye, C.; Espinal, M. A.; Watt, C. J.; Mbiaga, C.; Williams, B. G., Worldwide incidence of multidrug-resistant tuberculosis. *Journal of Infectious Diseases* **2002,** 185, 1197-1202.

59. Pitchenik, A. E.; Burr, J.; Laufer, M.; Miller, G.; Cacciatore, R.; Bigler, W. J.; Witte, J. J.; Cleary, T., Outbreaks of drug-resistant tuberculosis at AIDS centre. *The Lancet* **1990,** 336, 440-441.

60. Hye-Ryoun, K.; Seung Sik, H.; Hyun Ji, K.; Sang Min, L.; Chul-Gyu, Y.; Young Whan, K.; Sung Koo, H.; Young-Soo, S.; Jae-Joon, Y., Impact of Extensive Drug Resistance on Treatment Outcomes in Non-HIV Infected Patients with Multidrug-Resistant Tuberculosis. *Clinical Infectious Diseases* **2007,** 15, 1290-1295.

61. W.H.O., W.H.O. Global Task Force outlines measures to combat XDR-TB worldwide. *W.H.O. Press release* **2006,** 55, 301-305.

62. Gandhi, N. R.; Moll, A.; Sturm, W.; Pawinski, R.; Govender, T.; Lalloo, U.; Zeller, K.; Andrews, J.; Friedland, G., Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *The Lancet* **2006,** 368, 1575-1580.

63. W.H.O., Global and regional incidence. *Tuberculosis Fact Sheet No 104* **2007.**

64. Baydur, A., The spectrum of extrapulmonary tuberculosis. *West. J. Med.* **1977,** 126, 253-262.

65. Holmes, C. B.; Hausler, H.; Nunn, P., A review of sex differences in the epidemiology of tuberculosis. *int. J. Tuberc. Lung Dis.* **1998,** 2, (2), 96-104.

66. Long, N. H.; Diwan, V. K.; Winkvist, A., Difference in symptoms suggesting pulmonary tuberculosis among men and women. *Journal of Clinical Epidemiology* **2002,** 55, 115-120.

67. Matsushita, Y.; Ikeda, N.; Kurasawa, T.; Sato, A.; Nakatani, K.; Inoue, T.; Ikeda, T.; Sakatani, M.; Kobayashi, C.; Ozawa, S.; Kanai, K.; Suruta, N., The characteristics of clinical features of pulmonary tuberculosis in females. *Kekkaku* **1996,** 71, 391-398.

68. Bothamley, G. H., Sex and gender in the pathogenesis of infectious tuberculosis: a perspective from immunology, microbiology and human genetics. *Gothenburg: The Nordic School of Public Health, NHV report* **1998**, 41-53.

69. Fotadar, U.; Zaveloff, P.; Terracio, L., Growth of *Escherichia coli* at elevated temperatures. *J. Basic Microbiol.* **2005,** 45, (5), 403-404.

70. *http://medicineworld.org/images/blogs/11-2007/mycobacterium-tuberculosis-299290.jpg.* (Last accessed 04/06/10)

71. Hall, G.; Shah, M.; McEwan, P. A.; Laughton, C.; Stevens, M.; Westwell, A.; Emsley, J., Structure of *Mycobacterium tuberculosis* thioredoxin C. *Biological Crystallography* **2006,** D62, 1453-1457.

72. *http://bioweb.uwlax.edu/bio203/s2007/millard_ashl/images/Ziehl-Neelsen%20stain.jpg.* (Last accessed 04/06/10)

73. Grange, J. M.; Yates, M. D.; de Kantor, I. N., Guidelines for speciation within the *Mycobacterium tuberculosis* complex. *World Health Organization: Emerging and other Communicable Diseases, Surveillance and Control* **1996,** WHO/EMC/ZOO/96.4.

74. O'Reilly, L. M.; Daborn, C. J., The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. *Tuber. Lung Dis.* **1995,** 76, (Supp. 1), 1-46.

75. Skerman, V.; McGowan, V.; Sneath, P., Approved Lists of Bacterial Names. *Int. J. Syst. Bacteriol.* **1980,** 30, (1), 225-420.

76. Castets, M.; Rist, N.; Boisvert, H., *Medecine D'Afrique Noire* **1969,** 16, 321-322.

77. van Soolingen, D.; van der Zanden, A. G. M.; de Haas, P. E. W.; Noordhoek, G. T.; Kiers, A.; Foudraine, N. A.; Portaels, F.; Kolk, A. H. J.; Kremer, K.; van Embden, J. D. A., Diagnosis of *Mycobacterium microti* Infection among Humans by Using Novel Genetic Markers. *J. Clin. Microbiol.* **1998,** 36, (7), 1840-1845.

78. Aronson, J. D., Spontaneous tuberculosis in salt water fish. *J. Infect. Dis.* **1926,** 39, 314-320.

79. Lindeboom, J. A.; Kuijper, E. J.; Bruijnesteijn, E. S.; van Coppenraet, B.; Lindeboom, R.; Prins, J. M., Surgical Excision versus Antiiotic Treatment for Nontuberculous Mycobacterial Cervicofacial Lymphadenitis in Children: A Multicenter, Randomized, Controlled Trial. *Clin. Infect. Dis.* **2007,** 44, (8), 1057-1064.

80. Sasaki, S.; Takeshita, F.; Okuda, K.; Ishii, N., *Mycobacterium leprae* and Leprosy: A Compendium. *Microbiol. Immunol.* **2001,** 45, (11), 729-736.

81. Ryoo, S. W.; Shin, S.; Shim, M.; Park, Y. S.; Lew, W. J.; Park, S. N.; Park, Y. K.; Kang, S., Spread of nontuberculous mycobacteria from 1993 to 2006 in Koreans. *J. Clin. Lab. Anal.* **2008,** 22, (6), 415-420.

82. van der Werf, T. S.; Stinear, T.; Stienstra, Y.; van der Graaf, W. T. A.; Small, P. L., Mycolactones and *Mycobacterium ulcerans* disease *Lancet* **2003,** 362, (9389), 1062-1064.

83. *http://www.cdc.gov/ncidod/eid/vol8no2/images/01-0119-1b.jpg*. (Last accessed 04/06/10)

84. Heath, R. J.; White, S. W.; Rock, C. O., Inhibitors of fatty acid synthesis as antimicrobial chemotherapeutics. *Appl. Microbiol. Biotechnol* **2002,** 58, 695-703.

85. Rezwan, M.; Lanéelle, M.-A.; Sander, P.; Daffé, M., Breaking down the wall: Fractionation of mycobacteria. *Journal of Microbiological Methods* **2007,** 68, (1), 32-39.

86. Brennan, P. J., Structure, function, and biogenesis of the cell wall of Mycobacterium tuberculosis. *Tuberculosis* **2003,** 83, 91-97.

87. Camacho, L. R.; Constant, P.; Raynaud, C.; Lanéelle, M.-A.; Triccas, J. A.; Gicquel, B.; Daffé, M.; Guilhot, C., Analysis of the Phthiocerol Dimycocerosate Locus of *Mycobacterium tuberculosis. J. Biol. Chem* **2001,** 276, 19845-19854.

88. Minnikin, D. E., Lipids: complex lipids, their chemistry, biosynthesis and roles. . *The biology of mycobacteria C. Ratledge and J. Standford, eds (London Academic Press)* **1982,** 96-184.

89. Draper, P., The outer parts of the mycobacterial envelope as permiability barriers. *Front. Biosci.* **1998,** 3, 1253-1261.

90. Daffé, M.; Lanéelle, M.-A., Distribution of Phthiocerol Diester, Phenolic Mycosides and Related Compounds in Mycobacteria. *J. Gen. Microbiol.* **1988,** 134, 2049-2055.

91. Minnikin, D. E.; Polgar, N. J., Studies relating to phthiocerol. Part V. Phthiocerol A and B. *J. Chem. Soc.,* **1966,** 2107; Structural studies on the mycolic acids. *Chem. Commun.*, **1967,** 312; The mycolic acids from human and avian tubercle bacilli. *Chem. Commun.*, **1967,** 18, 916-918.

92. Noll, H.; Bloch, H.; Asselineau, J.; Lederer, E., The chemical structure of the cord factor of *Mycobacterium tuberculosis*. *Biochim. Biophys. Acta* **1956,** 20, 299-309.

93. Hoq, M.; Suzutani, T.; Toyoda, T.; Horiike, G.; Yoshida, I.; Azume, M., *J. Gen. Virology* **1997,** 78, 1597-1603.

94. Yarkoni, E.; Bekierkunst, A., Nonspecific resistance against infection with Salmonella typhi and Salmonella typhimurium induced in mice by cord factor (trehalose-6,6'-dimycolate) and its analogues. *Infect. Immun.* **1976,** 14, 1125-1129.

95. Hoq, M.; Suzutani, T.; Nakaya, K.; Yoshida, I.; Ogasawara, M.; Takeda, Y.; Shibaki, T.; Itohara, S.; Yamamoto, H.; Azume, M., Role of gamma delta TCR+ lymphocytes in the augmented resistance of trehalose 6,6'-dimycolate-treated mice to influenza virus infection. *Microbiol. Immunol.* **1999,** 43, 491-493.

96. Al Dulayymi, J. R.; Baird, M. S.; Maza-Iglesias, M.; Beken, S. V.; Grooten, J., The first unique synthetic mycobacterial cord factors. *Tetrahedron Letters* **2009,** 50, 3702-3705.

97. Marketos, S. G.; Ballas, C. N., Historical perspectives: bronchial asthma in the medical literature of Greek antiquity. *J. Asthma* **1982,** 19, (4), 263-269.

98. Floyer, J. S., A treatise of the asthma. *Divided into four parts printed for Richard Wilkin at St. Pauls' Churchyard, London* **1698.**

99. Sakula, A., Sir John Floyer's *A Treatise of the Asthma* (1698). *Thorax* **1984,** 39, 248-254.

100. Sistek, D.; Wickens, K.; Armstrong, R.; D'Souza, W.; Town, I.; Crane, J., Predictive value of respiratory symptoms and bronchial hyperresponsiveness to diagnose asthma in New Zealand. *Respiratory Medicine* **2006,** 100, 2107-2111.

101. British Thoracic Society, British Guideline on the Management of Asthma. *NHS SIGN* **2009.**

102. Zhao, J.; Takamura, M.; Yamaoka, A.; Odajima, Y.; Iikura, Y., Altered eosinophil levels as a result of viral infection in asthma exacerbation in childhood. *Pediatr. Allergy Immunol.* **2002,** 13, 47-50.

103. Martinez, F. D., Genes, environments, development and asthma: a reappraisal. *Eur. Respir. J.* **2007,** 29, 179-184.

104. Maddox, L.; Schwartz, D. A., The Pathophysiology of Asthma. *Annu. Rev. Med.* **2002,** 53, 477-498.

105. Gold, D. R.; Wright, R., Population Disparities in Asthma. *Annu. Rev. Public Health* **2005,** 26, 89-113.

106. Ober, C.; Hoffjan, S., Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun.* **2006,** 7, (2), 95-100.

107. Chu, E. K.; Drazen, J. M., Asthma: One Hundred Years of Treatment and Onward. *Am. J. Resp. Med.* **2005,** 171, 1202-1208.

108. Katz, Y.; Lebas, F. X.; Medley, H. V.; Robson, R., Fluticasone Propionate 50 μg BID Versus 100 μg BID in the Treatment of Children with Presistent Asthma. *Clinical Therapeutics.* **1998,** 20, (3), 424-437.

109. Stewart, T. G.; Gibson, G. A., Asthma. *Twentieth century practice: an international encyclopedia of modern medical science by leading authorities of Europe and America; Stedman, T.L.; New York: William Wood and Co.* **1896,** 6, 585-617.

110. Bethel, R. G., Abuse of asthma cigarettes. *Br. Med. J.* **1978,** 2, (6142), 959.

111. *http://4.bp.blogspot.com/_kE4lQ4oqHVc/R35J1MRfXbI/AAAAAAAAAdY/pc1f6UOjii4/s1600-h/200402-257OEf1.gif* (Last accessed 04/06/10)

112. Ordway, D.; Henao-Tamayo, M.; Orme, I. M.; Gonzalez-Juarrero, M., Foamy macrophages within lung granulomas of mice infected with *Mycobacterium tuberculosis* express molecules characteristic of dendritic cells and antiapoptotic markers of the TNF receptor-associated factor family. *J. Immunol.* **2005,** 175, 3873-3881.

113. Korf, J.; Stolz, A.; Verschoor, J.; de Baetselier, P.; Grooten, J., The *Mycobacterium tuberculosis* cell wall component mycolic acid elicits

pathogen-associated host innate immune responses. *Eur. J. Immunol.* **2005,** 35, (890-900).

114. Korf, J.; Pynaert, G.; Tournoy, K.; Boonefaes, T.; Van Oosterhout, A.; Ginneberge, D.; Haegeman, A.; Verschoor, J.; De Baetselier, P.; Grooten, J., Macrophage reprogramming by mycolic acid promotes a tolerogenic response in experimental asthma. *Am. J. Respir. Crit. Care Med.* **2006,** 174, (2), 152-160.

115. Benadie, Y.; Deysel, M.; Gilbert, D.; Siko, R.; Roberts, V. V.; Van Wyngaardt, S.; Thanyani, S. T.; Sekanka, G.; Ten Bokum, A. M. C.; Collett, L. A.; Grooten, J.; Baird, M. S.; Verschoor, J., Cholesteroid nature of free mycolic acids from *M. tuberculosis*. *Chem. Phys. Lipids* **2008,** 152, 95-103.

116. Anderson, R. J., The separation of lipoid fractions from tubercle bacilli. *J. Biol. Chem* **1927,** 74, 525-535.

117. Anderson, R. J., Structural peculiarities of acid-fast bacterial lipids. *Chem. Rev.* **1941,** 29, 225-243.

118. Stodola, F. H.; Lesuk, A.; Anderson, R. J., The chemistry of the lipids of tubercle bacilli: 54. The isolation and properties of mycolic acid. *J. Biol. Chem.* **1938,** 126, 505-513.

119. Cason, J.; Anderson, R. J., The chemistry of the lipids of tuberle bacilli. LVI. The wax of the bovine tubercle bacillus. *J. Biol. Chem.* **1938,** 126, 527-541.

120. Creighton, M. M.; Anderson, R. J., The chemistry of the lipids of tubercle bacilli. LVII. The mycolic acids of the avian tubercule bacillus wax. *J. Biol. Chem.* **1939,** 129, 57-63.

121. Peck, R.; Anderson, R. J., The chemistry of the lipids of tubercle bacilli. LXIV. Concerning phleimycolic acid. *J. Biol. Chem.* **1941,** 140, 89-96.

122. Anderson, R. J.; Crowder, J. A.; Newman, M. S.; Stodola, F. H., The chemistry of the lipids of tubercle bacilli. XLIII. The composition of leprosin. *J. Biol. Chem.* **1936,** 113, 637-647.

123. Asselineau, J.; Lederer, E., Structure of the mycolic acids of mycobacteria. *Nature* **1950,** 4227, 782-783.

124. Minnikin, D. E.; Polgar, N. J., Studies on the Mycolic Acids from Human Tubercle Bacilli. *Tetrahedron Letters* **1966,** 23, 2643-2647.

125. Malani, C.; Polgar, N. J., Studies on the Mycolic Acids. Part II. *J. Chem. Soc., Chem. Commun.* **1963,** 567, 3092-3096.

126. Minnikin, D. E.; Polgar, N. J., The Mycolic Acids from Human and Avian Tubercle Bacilli. *Chem. Commun.* **1967,** 18, 916-918.

127. Minnikin, D. E.; Polgar, N. J., The Methoxymycolic and Ketomycolic Acids from Human Tubercle Bacilli. *Chem. Commun.* **1967,** 1172-1174.

128. Minnikin, D. E.; Polgar, N. J., Structural Studies on the Mycolic Acids. *Chem. Commun.* **1967,** 312-314.

129. Watanabe, M.; Aoyagi, Y.; Ridell, M.; Minnikin, D. E., Separation and characterization of individual mycolic acids in representative mycobacteria. *Microbiology* **2001,** 147, 1825-1837.

130. Watanabe, M.; Aoyagi, Y.; Mitome, H.; Fujita, T.; Naoki, H.; Ridell, M.; Minnikin, D. E., Location of functional groups in mycobacterial meromycolate chains; the recognition of new structural principles in mycolic acids. *Microbiology* **2002,** 148, 1881-1902.

131. Wheeler, P. R.; Besra, G. S.; Minnikin, D. E.; Ratledge, C., Stimulation of mycolic acid biosynthesis by incorporation of cis-tetracos-5-enoic acid in a cell-wall preparation from *Mycobacterium smegmatis. Biochim. Biophys. Acta* **1993,** 1167, 182-188.

132. Minnikin, D. E.; Minnikin, S. E.; Goodfellow, M., The oxygenated mycolic acids of *Mycobacterium fortuitim, M. farcinogenes* and *M. senegalense. Biochim. Biophys. Acta.* **1982,** 712, (3), 616-620.

133. Minnikin, D. E.; Minnikin, S. E.; Parlett, J. H.; Goodfellow, M.; Magnusson, M., Mycolic acid patterns of some species of *Mycobacterium. Arch. Microbiol.* **1984,** 139, 225-231.

134. Lanéelle, M.-A.; Lanéelle, G., Structure d'acides mycoliques et d'un intermediaire dans la biosynthese d'acides mycoliques dicarboxyliques. *Eur. J. Biochem.* **1969,** 12, 296-300.

135. Quémard, A.; Lanéelle, M.-A.; Marrakchi, H.; Promé, D.; Dubnau, E.; Daffé, M., Structure of a hydroxymycolic acid potentially involved in the synthesis of oxygenated mycolic acids of the *Mycobacterium tuberculosis* complex. *Eur. J. Biochem.* **1997,** 250, 758-763.

136. Luquin, M.; Roussel, J.; Lopez-Calahorra, F.; Lanéelle, G.; Ausina, V.; Lanéelle, M.-A., A novel mycolic acid in a *Mycobacterium* sp. from the environment. *Eur. J. Biochem.* **1990,** 192, 753-759.

137. Barry III, C. E.; Lee, R. E.; Mdluli, K.; Sampson, A. E.; Schroeder, B. G.; Slayden, R. A.; Yuan, Y., Mycolic Acids: Structure, Biosynthesis and Physiological Functions. *Prog. Lipid Res.* **1998,** 37, (2/3), 143-179.

138. Krembel, J.; Etémadi, A. H., Sur la structure d'un nouveau type: D'Acides mycoliques de *Mycobacterium smegmatis*. *Tetrahedron* **1966,** 22, (3), 1113-1119.

139. Koh, W.-J.; Kwon, J. O.; Lee, K. S., Diagnosis and Treatment of Nontuberculous Mycobacterial Pulmonary Diseases: A Korean Perspective *J. Korean Med. Sci.* **2005,** 20, (6), 913-925.

140. Rong, D.; Baowen, C.; Lei, G.; Yang, L.; Jiuanwei, X.; Guozhi, W.; Hongbing, Z., Identification of *Mycobacterium* species using reversed-phase high performance liquid chromatographic analysis of mycolic acid. *Chin. J. Chromatogr.* **2008,** 26, (5), 534-539.

141. Butler, W. R.; Guthertz, L. S., Mycolic Acid Analysis by High-Performance Liquid Chromatography for Identification of *Mycobacterium* Species. *Clin. Microbiol. Rev.* **2001,** 14, (4), 704-726.

142. Villeneuve, M.; Kawai, M.; Watanabe, M.; Aoyagi, Y.; Hitotsuyanagi, Y.; Takeya, K.; Gouda, H.; Hirono, S.; Minnikin, D. E.; Nakahara, H., Conformational behavior of oxygenated mycobacterial mycolic acids from *Mycobacterium bovis* BCG. *Biochim. Biophys. Acta* **2007,** 1768, 1717-1726.

143. Hasegawa, T.; Leblanc, R. M., Aggregation properties of mycolic acid molecules in monolayer films: a comparative study of compounds from various acid-fast bacterial species. *Biochim. Biophys. Acta* **2003,** 1617, 89-95.

144. Hasegawa, T.; Amino, S.; Kitamura, S.; Matsumoto, L.; Katada, S.; Nishijo, J., Study of the Molecular Conformation of alpha- and Keto-Mycolic Acid Monolayers by the Langmuir-Blodgett Technique and Fourier Transform Infrared Reflection-Absorption Spectroscopy. *Langmuir* **2003,** 19, 105-109.

145. Hasegawa, T.; Nishijo, J.; Watanabe, M., Conformational Characterization of α-Mycolic Acid in a Monolayer Film by the Langmuir-Blodgett Technique and Atomic Force Microscopy. *Langmuir* **2000,** 16, 7325-7330.

146. Grant, E. P.; Beckman, E. M.; Behar, S. M.; Degano, M.; Frederique, D.; Besra, G. S.; Wilson, I. A.; Porcelli, S. A.; Furlong, S. T.; Brenner, M. B.,

Fine Specificity of TCR Complementarity-Determining Region Residues and Lipid Antigen Hydrophilic Moieties in the Recognition of a CD1-Lipid Complex. *J. Immunol.* **2002,** 168, 3933-3940.

147. Minnikin, D. E.; Polgar, N. J., Stereochemical Studies on the Mycolic Acids. *Chem. Commun.* **1966,** 648-649.

148. Daffé, M.; Lanéelle, M.-A.; Valero Guillen, P. L., Tetraenoic and pentaenoic mycolic acids from *Mycobacterium thamnopheos* Structure, taxonomic and biosynthetic implications. *Eur. J. Biochem.* **1988,** 177, (2), 339-344.

149. Moody, B. D.; Guy, M. R.; Grant, E. P.; Cheng, T.; Brenner, M. B.; Besra, G. S.; Porcelli, S. A., CD1b-mediated T Cell Recognition of a Glycolipid Antigen Generated from Mycobacterial Lipid and Host Carbohydrate during Infection. *J. Exp. Med.* **2000,** 192, (7), 965-976.

150. Dubnau, E.; Lanéelle, M.-A.; Soares, S.; Benichou, A.; Vaz, T.; Promé, D.; Promé, J.; Daffé, M.; Quémard, A., *Mycobacterium bovis* BCG genes involved in the biosynthesis of cyclopropyl keto- and hydroxy-mycolic acids. *Mol. Microbiol.* **1997,** 23, (2), 313-322.

151. Al Kremawi, D. Z.; Al Dulayymi, J. R.; Baird, M. S., The first synthesis of epoxy-mycolic acids. *Tetrahedron* **2010,** 51, 1698-1701.

152. Lacave, C.; Lanéelle, M.-A.; Daffé, M.; Montrozier, H.; Rols, M.; Asselineau, C., Etude structurale et mbtabolique des acides mycoliques de *Mycobacterium fortuitum. Eur. J. Biochem.* **1987,** 163, (2), 369-378.

153. Al Dulayymi, J. R.; Baird, M. S.; Mohammed, H.; Roberts, E.; Clegg, W., The synthesis of one entiomer of the alpha-methyl-trans-cyclopropane unit of mycolic acids. *Tetrahedron* **2006,** 62, (4851-4862).

154. Anderson, R. J.; Creighton, M. M.; Peck, R., The Chemistry of the Lipids of Tubercle Bacilli: LX. Concerning the Firmly Bound Lipids of the Avian Tubercle Bacillus. *J. Biol. Chem.* **1940,** 132, 675-693.

155. Wang, L.; Slayden, R. A.; Barry III, C. E.; Liu, J., Cell Wall Structure of a Mutant of *Mycobacterium smegmatis* Defective in the Biosynthesis of Mycolic Acids. *J. Biol. Chem* **2000,** 275, (10), 7224-7229.

156. Liu, J.; Barry III, C. E.; Besra, G. S.; Nikaido, H., Mycolic Acid Structure Determines the Fluidity of the Mycobacterial Cell Wall. *J. Biol. Chem.* **1996,** 271, (47), 29545-29551.

157. Glickman, M. S.; Cox, J. S.; Jacobs, W. R., A Novel Mycolic Acid Cyclopropane Synthetase is Required for Cording, Persistence, and Virulence of *Mycobacterium tuberculosis*. *Mol. Cell.* **2000,** 5, (4), 717-727.

158. Grogan, D. W.; Cronan Jr., J. E., Cyclopropane Ring Formation in Membrance Lipids of Bacteria. *Microbiol. Mol. Biol. Rev.* **1997,** 61, (4), 429-441.

159. Yuan, Y.; Crane, D. D.; Musser, J. M.; Sreevatsan, S.; Barry III, C. E., MMAS-1, the Branch Point Between *cis*- and *trans*-Cyclopropane containing Oxygenated Mycolates in *Mycobacterium tuberculosis*. *J. Biol. Chem* **1997,** 272, (15), 10041-10049.

160. Dubnau, E.; Chan, J.; Raynaud, C.; Mohan, V. P.; Lanéelle, M.-A.; Yu, K.; Quémard, A.; Smith, I.; Daffé, M., Oxygenated mycolic acids are necessary for virulence of *Mycobacterium tuberculosis* in mice. *Mol. Microbiol.* **2000,** 36, (3), 630-637.

161. Yuan, Y.; Zhu, Y.; Crane, D. D.; Barry III, C. E., The effect of oxygenated mycolic acid composition on cell wall function and macrophage growth in *Mycobacterium tuberculosis*. *Mol. Microbiol.* **1998,** 29, (6), 1449-1458.

162. Yuan, Y.; Barry III, C. E., A common mechanism for the biosynthesis of methoxy and cyclopropyl mycolic acids in *Mycobacterium tuberculosis*. *PNAS* **1996,** 93, (23), 12828-12833.

163. Belley, A.; Alexander, D.; Di Pietrantonio, T.; Girand, M.; Jones, J.; Schurr, E.; Liu, J.; Sherman, D. R.; Behr, M. A., Impact of Methoxymycolic Acid Production by *Mycobacterium bovis* BCG Vaccines *Infect. Immun.* **2004,** 72, (5), 2803-2809.

164. Asselineau, C.; Asselineau, J.; Lanéelle, G.; Lanéelle, M.-A., The biosynthesis of mycolic acids by mycobacteria: current and alternative hypotheses. *Prog. Lipid Res.* **2002,** 41, (6), 501-523.

165. George, K. M.; Yuan, Y.; Sherman, D. R.; Barry III, C. E., The Biosynthesis of Cyclopropanated Mycolic Acids in *Mycobacterium tuberculosis*. *J. Biol. Chem* **1995,** 270, (45), 27292-27298.

166. Yuan, Y.; Mead, D.; Schroeder, B. G.; Zhu, Y.; Barry III, C. E., The Biosynthesis of Mycolic Acids in *Mycobacterium tuberculosis*. *J. Biol. Chem.* **1998,** 273, (33), 21282-21290.

167. Daffé, M.; Lanéelle, M.-A.; Lacave, C., Structure and stereochemistry of mycolic acids of *Mycobacterium marinum* and *Mycobacterium ulcerans*. *Res. Microbiol.* **1991,** 142, (4), 397-403.

168. Danielson, S. J.; Gray, G. R., Structures of the two homologous series of dialkene mycolic acids from *Mycobacterium smegmatis*. *J. Biol. Chem.* **1982,** 257, 12196-12203.

169. Wong, M. Y.; Steck, P. A.; Gray, G. R., The major mycolic acids of *Mycobacterium smegmatis*. Characterization of their homologous series. *J. Biol. Chem.* **1979,** 254, 5734-5740.

170. Glickman, M. S.; Cahill, S. M.; Jacobs Jr, W. R., The *Mycobacterium tuberculosis* cmaA2 Gene Encodes a Mycolic Acid trans-Cyclopropane Synthetase. *J. Biol. Chem.* **2001,** 276, (3), 2228-2233.

171. Ryll, R.; Kumazawa, Y.; Yano, I., Immunological Properties of Trehalose Dimycolate (Cord Factor) and Other Mycolic Acid-Containing Glycolipids - A Review. *Microbiol. Immunol.* **2001,** 45, (12), 801-811.

172. Qin, H.; Sadelain, M. W. J.; Hitchon, C.; Lauzon, J.; Singh, B., Complete Freund's Adjuvant-Induced T Cells Prevent the Development and Adoptive Transfer of Diabetes in Nonobese Diabetic Mice. *J. Immunol.* **1993,** 150, (5), 2072-2080.

173. Gupta, R. K.; Relyveld, E. H.; Lindblad, E. B.; Bizzini, B.; Ben-Efraim, S.; Gupta, C. K., Adjuvants - a balance between toxicity and adjuvanticity. *Vaccine* **1993,** 11, (3), 293-306.

174. Gensler, W. J.; Marshall, J. P.; Langone, J. L.; Chen, J. C., Synthesis of DL-Methyl Meromycolate. *J. Org. Chem.* **1977,** 42, (1), 118-125.

175. Weinstein, S.; Sonnenberg, J., Homoconjugation and Homoaromaticity. III. The 3-Bicyclo [3.1.0]hexyl System. *J. Am. Chem. Soc.* **1961,** 83, 3235.

176. Pettit, G. R.; Van Tamelen, E. E., Desulfurization with Raney Nickel. *Org. React.* **1962,** 12, 356.

177. Gensler, W. J.; Prasad, A. P.; Chaudhuri, I.; Alam, J., New synthesis of meromycolic acid. *J. Org. Chem.* **1979,** 44, (21), 3643-3652.

178. Al Dulayymi, J. R.; Baird, M. S.; Roberts, E., The synthesis of single enantiomers of a meromycolic acid. *Tetrahedron Letters* **2000,** 41, 7107-7110.

179. Coxon, G. D.; Knobl, S.; Roberts, E.; Baird, M. S.; Al Dulayymi, J. R.; Besra, G. S.; Brennan, P. J.; Minnikin, D. E., The synthesis of both enantiomers of lactobacillic acid and mycolic acid analogues. *Tetrahedron Letters* **1999,** 40, 6689-6692.

180. Al Dulayymi, J. R.; Baird, M. S.; Roberts, E.; Minnikin, D. E., The synthesis of single enantiomers of meromycolic acids from mycobacterial wax esters. *Tetrahedron* **2006,** 62, 11867-11880.

181. Al Dulayymi, J. R.; Baird, M. S.; Roberts, E., The synthesis of a single enantiomer of a major α–mycolic acid of *M. tuberculosis*. *Tetrahedron* **2005,** 61, 11939-11951.

182. Al Dulayymi, J. R.; Baird, M. S.; Roberts, E.; Deysel, M.; Verschoor, J., The first syntheses of single enantiomers of the major methoxymycolic acid of *Mycobacterium tuberculosis*. *Tetrahedron* **2007,** 63, 2571-2592.

183. Koza, G., Synthesis of single enantiomers of ketomycolic acids. *PhD Thesis, Bangor University* **2007.**

184. Koza, G.; Rowles, R.; Theunissen, C.; Al Dulayymi, J. R.; Baird, M. S., The synthesis of single enantiomers of trans-alkene-containing mycolic acids. *Tetrahedron Letters* **2009,** 50, 7259-7262.

185. Martin, B. K. D.; Mann, J.; Sageot, O. A., Synthesis of analogues of the marine anti-tumour agent curacin A. *J. Chem. Soc., Perkin Trans. 1* **1999,** (17), 2455-2460.

186. Toschi, G.; Baird, M. S., An improved procedure for the preparation of the β-hydroxy-α–alkyl fatty acid fragment of mycolic acids. *Tetrahedron* **2006, 62,** 3221-3227.

187. Al Dulayymi, J. R.; Baird, M. S.; Simpson, M. J.; Nyman, S.; Port, G. R., Structure Based Interference with Insect Behaviour - Cyclopropene Analogues of Pheromones Containing Z-Alkenes. *Tetrahedron* **1996, 52,** (38), 12509-12520.

188. Koza, G.; Theunissen, C.; Al Dulayymi, J. R.; Baird, M. S., The synthesis of single enantiomers of mycobacterial ketomycolic acids containing *cis*-cyclopropanes *Tetrahedron* **2009,** 65, (49), 10214-10229.

189. Rowles, R., The large-scale synthesis of a single enantiomer of the major methoxymycolic acid of *Mycobacterium tuberculosis. MSc Thesis; Bangor University* **2007.**

190. Julia, M.; Paris, J. M., Syntheses a l'aide de sulphones - methode de syntheses generale de doubles liaisons. *Tetrahedron Letters* **1973,** 49, 4833-4836.

191. Kocienski, P. J.; Lythgoe, B.; Rustonl, S., Scope and stereochemistry of an olefin synthesis from beta-hydroxysulphones. *J. Chem. Soc., Perkin Trans. 1* **1978,** 829-834.

192. McCoy, L. L., Three-membered Rings. The Preparation of Some 1,2-Cyclopropanedicarboxylic Acids. *J. Am. Chem. Soc* **1958,** 80, (24), 6568-6572.

193. Grandjean, D.; Pale, P.; Chuche, J., Enzymatic hydrolysis of cyclopropanes. Total synthesis of optically pure dictyopterenes a and c'. *Tetrahedron* **1991,** 47, (7), 1215-1230.

194. Friedmann, L.; Litle, R. L.; W.R., R., p-toluenesulfonylhydrazide. *Organic Syntheses, Coll.* **1973, 1960,** 5, 40, 1055, 93.

195. Theunissen, C., Unpublished findings / PhD research, Bangor University. **2006-2009.**

196. Love, M. L., Preparation of the α-methyl-*cis*-cyclopropane unit for the use in mycolic acid synthesis. *MSc Thesis; Bangor University* **2007.**

197. Morikawa, T.; Sasaki, H.; Hanai, R.; Shibuya, A.; Taguchi, T., Synthesis of optically active cis- and trans-1,2-disubstituted cyclopropane derivatives by the Simmons-Smith reaction of allyl alcohol derivatives derived from (R)-2,3-O-isopropylideneglyceraldehyde. *J. Org. Chem* **1994,** 59, (1), 97-103.

198. Takano, S.; Kurotaki, A.; Takahashi, M.; Ogasawara, K., Practical Synthesis of Some Versatile Chiral Building Blocks from (D)-Mannitol. *Synthesis* **1986,** 403-406.

199. Villieras, J.; Rambaud, M., Wittig-Horner Reactions in Heterogeneous Media. A Convenient Synthesis of α,β-Unsaturated Esters and Ketones using Weak Bases in Water. *Synthesis* **1983,** 300-303.

200. March, J.; Smith, M. B., *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure* **2001,** 5th edition, 449.

201. Vaughn, T. H.; Hennion, G. F.; Vogt, R. R.; Nieuwland, J. A., The preparation and alkylation of metal acetylides in liquid ammonia. *J. Org. Chem.* **1957,** 2, (1), 1-22.

202. Yu, W.; Mei, Y.; Kang, Y.; Hua, Z.; Jin, Z., Improved procedure for the oxidative cleavage of olefins *via* OsO_4 - $NaIO_4$. *Org. Lett.* **2004,** 6, (19), 3217-3219.

203. Kocienski, P. J.; Blakemore, P. R.; Cole, W. J.; Morley, A., A Stereoselective Synthesis of trans-1,2-Disubstituted Alkenes Based on the Condensation of Aldehydes with Metallated 1-Phenyl-1H-tetrazol-5-yl Sulfones. *Syn. Lett.* **1998,** 1, 26-28.

204. Pospisil, J.; Marko, I. E., Efficient and Stereoselective Synthesis of Allylic Ethers and Alcohols. *Org. Lett.* **2006,** 8, (26), 5983-5986.

205. Lanéelle, M.-A.; Lacave, C.; Daffé, M.; Lanéelle, G., Mycolic acids of *Mycobacterium aurum*. *Eur. J. Biochem.* **1988,** 177, (3), 631-635.

206. Thanks to Y. Lemmer and M. Deysel for providing this information. *University of Pretoria, South Africa.*

207. Beken, S. V.; Al Dulayymi, J. R.; Naessens, T.; Koza, G.; Maza-Iglesias, M.; Rowles, R.; Theunissen, C.; De Medts, J.; Lanckacker, E.; Baird, M. S.; Grooten, J., *Mycobacterium tuberculosis* mycolic acid oxygenation and cyclopropane chemistry determine host airway inflammation and immune responses. *Microbiology* **2010,** In Press.

208. Thanks to S. V. Beken and J. Grooten for providing this information. *Ghent University, Belgium.*

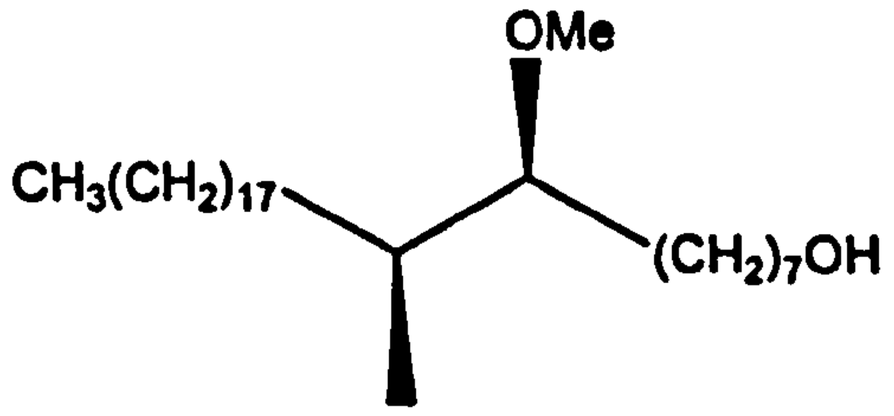
209. Roush, W. R.; Adam, M. A.; Walts, A. E.; Harris.D.J., Stereochemistry of the reactions of substituted allylboronates with chiral aldehydes. Factors influencing aldehyde diastereofacial selectivity. *J. Am. Chem. Soc* **1986,** 108, (12), 3422-3434.

210. Kuszmann, J.; Tomori, E.; Meerwald, I., The synthesis of 1,2:5,6-di-*O*-isopropylidene-D-mannitol: a comparative study. *Carbohydr. Res.* **1984,** 128, 87.

211. Leonard, J.; Mohialdin, D.; Reed, D.; Ryan, G.; Swain, A., Stereoselective conjugate addition of organolithium and organocopper reagents to gamma-oxygenated alpha,beta-unsaturated carbonyl systems dervied from glyceraldehyde acetonide. *Tetrahedron* **1995,** 51, (47), 12843-12858.

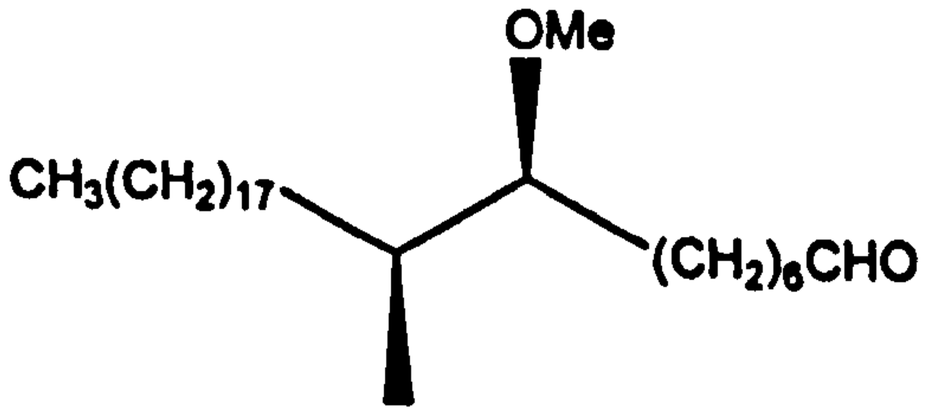
Appendices

Appendix 1: (8*S*,9*S*)-8-Methyoxy-9-methylheptacosan-1-ol (94)[182]



p-Toluene sulfonic acid monohydrate (0.56 g, 2.9 mmol, 0.25 mol. equiv.) was added to a stirred solution of 2-((8*S*,9*S*)-8-methoxy-9-methylheptacosyloxy) tetrahydropyran **(93)** (6.15 g, 11.7 mmol)[189] in THF (20 ml), methanol (70 ml) and water (1 ml) at RT. The mixture was refluxed for 30 mins; when TLC showed no starting material was left, sat. aq. sodium hydrogen carbonate (50 ml) and petrol / ether (1:1, 150 ml) was added. The aqueous layer was re-extracted with petrol / ether (1:1, 2 x 50 ml). The combined organic layers were washed with brine (100 ml), dried and evaporated to give a residue, which was purified via column chromatography on silica gel eluting with petrol / ether (5:2) to give (8*S*,9*S*)-8-methoxy-9-methylheptacosan-1-ol **(94)** (3.97 g, 77 %) as a colourless oil, which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{19}_D = -9.52$ ($CHCl_3$, 0.681 μmol); lit. value $[\alpha]^{22}_D = -10.74$ (*c* 1.20, $CHCl_3$).[182]

Appendix 2: (8*S*,9*S*)-8- Methoxy-9-methylheptacosanal (95)[182]



(8*S*,9*S*)-8-Methoxy-9-methylheptacosan-1-ol **(94)** (3.81 g, 8.66 mmol) in dichloromethane (30 ml) was added to a stirred suspension of pyridinium chlorochromate (4.67 g, 21.65 mmol, 2.5 mol. equiv.) in dichloromethane (200 ml) at RT. The mixture was stirred for 2 hrs; when TLC analysis indicated completion of

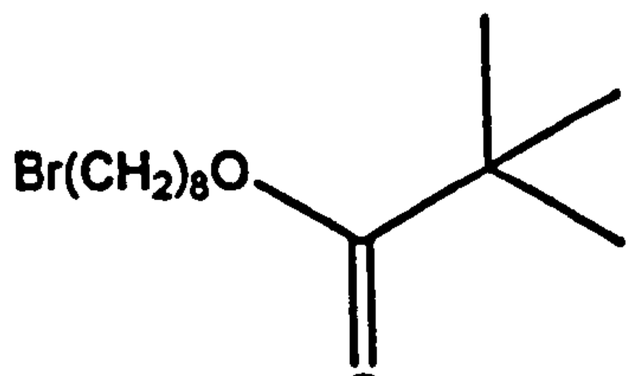
the reaction the mixture was poured into ether (200 ml). The mixture was filtered through a pad of silica gel and washed with ether (100 ml) and the filtrate evaporated. The crude product was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, (8*S*,9*S*)-8-methoxy-9-methylheptacosanal **(95)** (3.39 g, 89 %) which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{19}_D$ = -11.12 ($CHCl_3$, 1.421 μmol); lit. value $[\alpha]^{22}_D$ = -11.3 (*c* 1.50, $CHCl_3$).[182]

Appendix 3: 8-Bromooctan-1-ol (102)[182]

$Br(CH_2)_8OH$

1,8-Octanediol **(101)** (25.00 g, 171.0 mmol) was dissolved in toluene (100 ml) and aqueous hydrobromic acid (30 ml, 44.7 g, 552.4 mmol, d = 1.49) was added and the mixture was refluxed for 18 hrs. When TLC analysis indicated completion of the reaction, the mixture was extracted with water (100 ml) and washed with sat. aq. sodium hydrogen carbonate (3 x 50 ml). The combined organic layers were dried and evaporated to give a crude product, which was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a yellow oil, 8-bromooctan-1-ol **(102)** (29.02 g, 81 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[182]

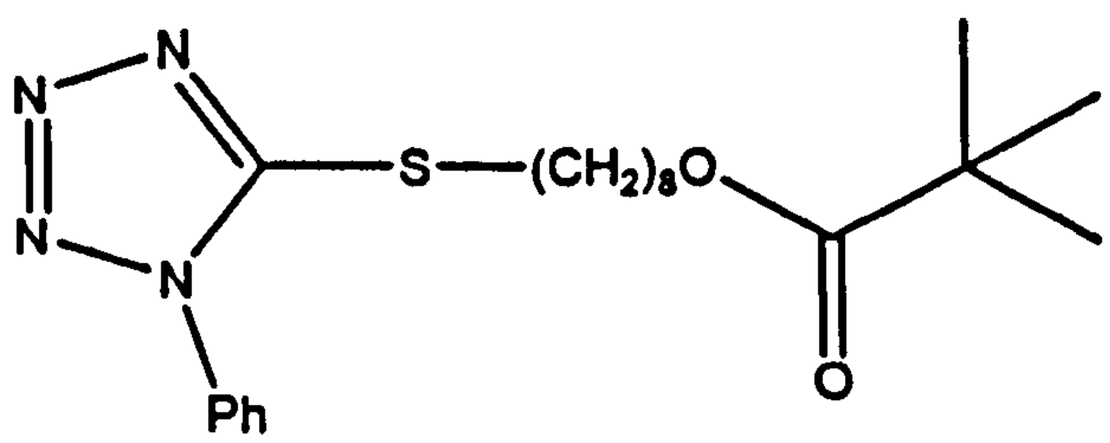
Appendix 4: 2,2-Dimethyl-propionic acid 8-bromo-octyl ester (103)[182]



A solution of trimethyl acetylchloride (20.50 ml, 20.09 g, 166.6 mmol, d = 0.979, 1.2 mol. equiv.) in dichloromethane (150 ml) was added to a stirred solution of 8-bromooctan-1-ol **(102)** (29.02 g, 138.9 mmol) in dichloromethane (200 ml), pyridine (22.5 ml, 21.97 g, 277.7 mmol, d = 0.978, 2 mol. equiv.) and 4-

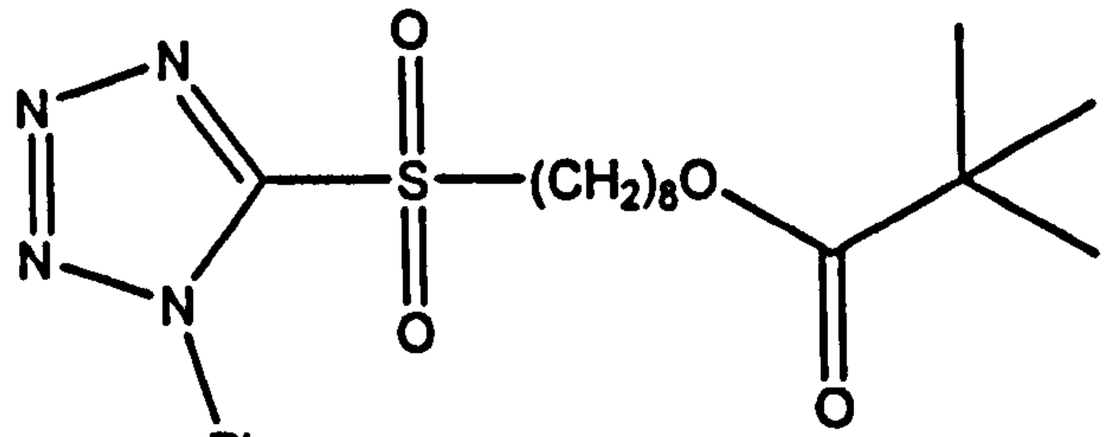
dimethylaminopyridine (0.679 g, 5.55 mmol, 0.04 mol. equiv.) over 15 mins at 5 °C. The mixture was then stirred at RT for 72 hrs; when TLC analysis indicated completion of the reaction, dil. hydrochloric acid (250 ml) was added and the organic layer extracted and washed with further dil. hydrochloric acid (100 ml) and brine (2 x 300 ml). The combined organic extracts were dried and evaporated to give a crude product, which was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a colourless oil, 2,2-dimethyl-propionic acid 8-bromo-octyl ester **(103)** (34.62 g, 85 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[182]

Appendix 5: 2,2-Dimethyl-propionic acid 8-(1H-phenyl-1H-tetrazole-5-yl sulfanyl)-octyl ester (104)[182]



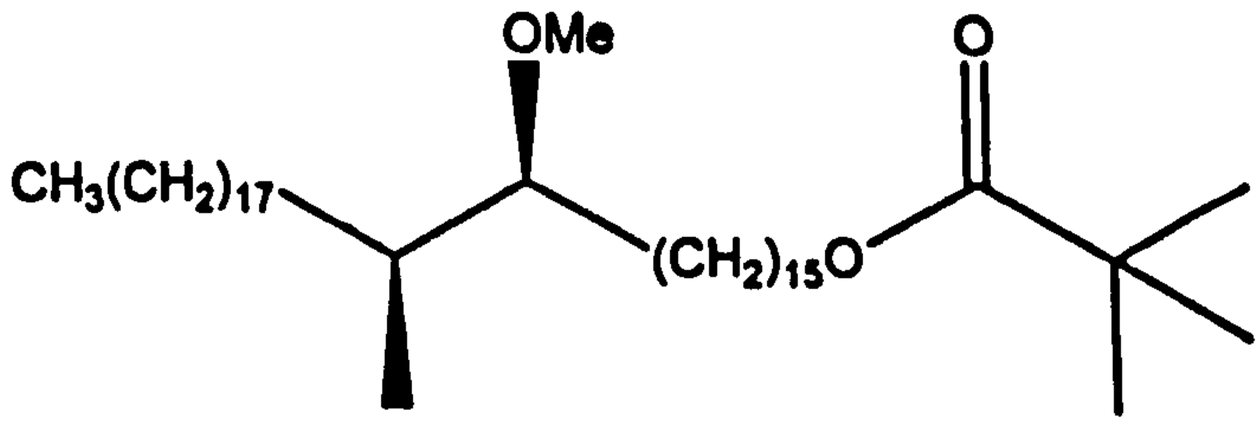
2,2-Dimethyl-propionic acid 8-bromo-octyl ester (34.62 g, 118.2 mmol) was added with vigorous stirring to 1-phenyl-1H-tetrazole-5-thiol **(103)** (21.06 g, 118.2 mmol) and anhydrous potassium carbonate (32.7 g, 236.3 mmol, 2 mol. equiv.) in acetone (300 ml). The reaction was refluxed for 18 hrs; when TLC analysis indicated completion of the reaction, the inorganic solids were filtered off and washed with acetone. The organic filtrate was evaporated and extracted with dichloromethane (200 ml) and water (150 ml). The aqueous layer was re-extracted with further dichloromethane (3 x 100 ml) and the combined organic layers were dried and evaporated. The crude yellow oil was purified via column chromatography eluting with petrol/ether (5:2) to give a colourless oil, 2,2-dimethyl-propionic acid 8-(1H-phenyl-1H-tetrazole-5-yl sulfanyl)-octyl ester **(104)** (34.34 g, 74 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[182]

Appendix 6: 2,2-Dimethyl-propionic acid 8-(1H-phenyl-1H-tetrazole-5-yl sulfonyl)-octyl ester (96)[182]



A solution of ammonium molybdate (VI) tetrahydrate (54.4 g, 44.0 mmol, 0.5 mol. equiv.) in -10 °C hydrogen peroxide (85 ml, 35 % w/w) was added and to a stirred solution of 2,2-dimethyl-propionic acid 8-(1-phenyl-1H-tetrazole-5-yl sulfanyl)-octyl ester **(104)** (34.34 g, 88.0 mmol) in THF (300 ml) and IMS (600 ml) at 12 °C and the mixture stirred at 15-20 °C for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (21.7 g, 17.6 mmol, 0.2 mol. equiv.) in -10 °C hydrogen peroxide (45 ml, 35 % w/w) was added and the reaction mixture stirred for 72 hrs. The mixture was then poured into water (3 l) and extracted with dichloromethane (3 x 250 ml), dried and evaporated. The crude product was purified via column chromatography eluting with petrol/ether (4:1) to give a colourless oil, 2,2-dimethyl-propionic acid 8-(1-phenyl-1H-tetrazole-5-sulfonyl)-octyl ester **(96)** (35.68 g, 96 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[182]

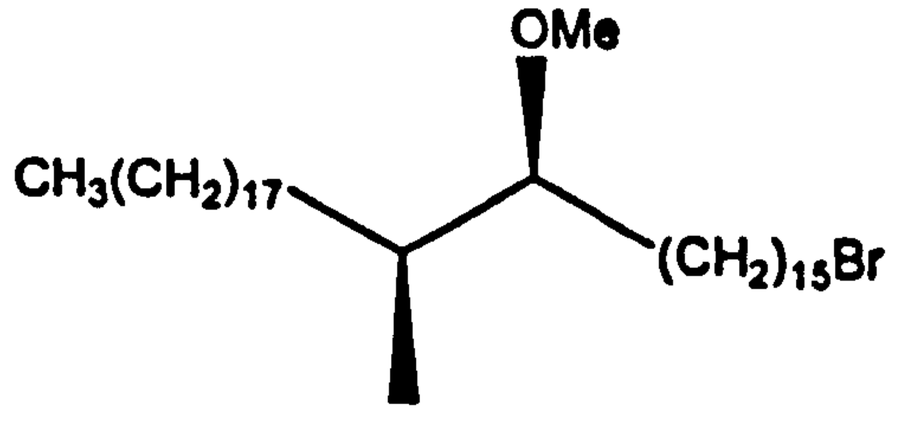
Appendix 7: 2,2-Dimethyl-propionic acid (16*S*,17*S*)-16-methoxy-17-methyl-pentatriacontyl ester (106)[182]



(8*S*,9*S*)-8-Methoxy-9-methylheptacosanal **(95)** (3.39 g, 7.74 mmol) was dissolved in dry THF (30 ml) and added to a stirred solution of 2,2-dimethyl-propionic acid 8-(1-phenyl-1H-tetrazole-5-sulfonyl)-octyl ester **(96)** (3.92 g, 9.29 mmol) in dry THF (40

ml) at RT under nitrogen. The mixture was cooled to -10 °C and lithium *bis*(trimethyl silyl) amide (11.39 ml, 12.07 mmol, 1.06 M) was added dropwise between -12 °C and -5 °C. The solution was stirred for 18 hrs; when TLC analysis indicated completion of the reaction, dichloromethane (100 ml) and sat. aq. ammonium chloride (50 ml) were added and the aqueous layer re-extracted with dichloromethane (2 x 200 ml). The combined organic extracts were dried and evaporated to give a crude product, which was purified via column chromatography eluting with petrol/ether (20:1) to give a colourless oil, (*E*/*Z*)-2,2-dimethyl-propionic acid (16*S*,17*S*)-16-methoxy-17-methyl-pentatriacont-8-enyl ester **(105)** (4.21 g, 86 %). Palladium on charcoal (0.5 g, 10 %) was added to a stirred solution of (*E*/*Z*)-2,2-dimethyl-propionic acid (16*S*,17*S*)-16-methoxy-17-methyl-pentatriacont-8-enyl ester **(105)** (4.09 g, 6.44 mmol) in IMS (150 ml). The mixture was stirred at atmospheric pressure and, when no more hydrogen was being absorbed it was filtered through a pad of celite and washed with IMS (100 ml). The filtrate was evaporated to give a colourless oil, 2,2-dimethyl-propionic acid (16*S*,17*S*)-16-methoxy-17-methyl-pentatriacontyl ester **(106)** (4.10 g, 99.9%), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{21}_D$ = -6.9 ($CHCl_3$, 1.456 μmol); lit. value $[\alpha]^{22}_D$ = -6.5 (*c* 1.503, $CHCl_3$).[182]

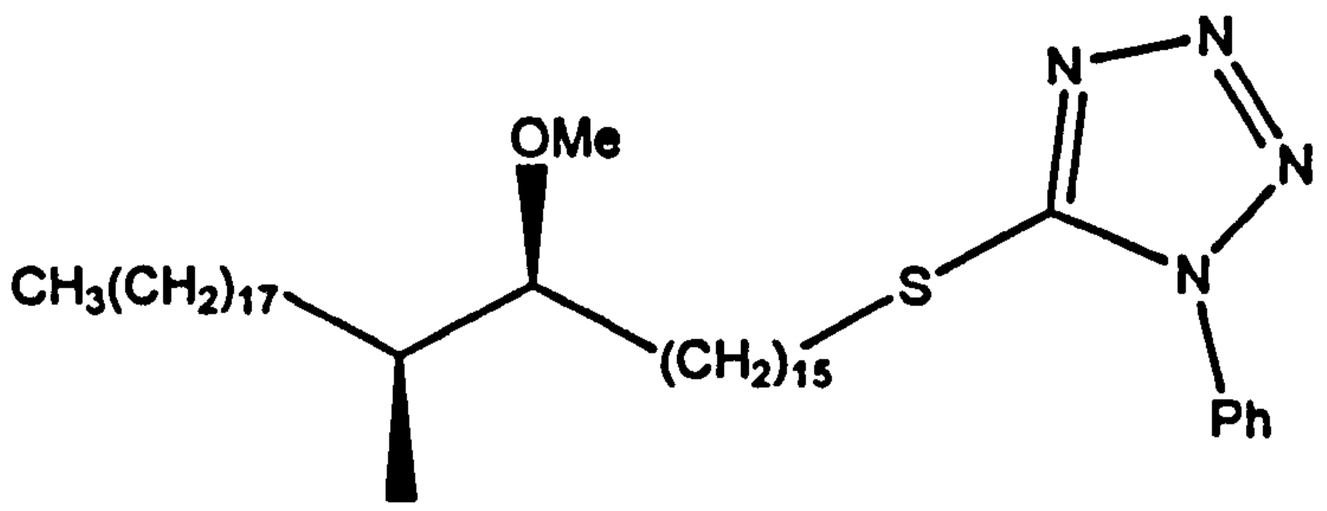
Appendix 8: (16*S*,17*S*)-1-Bromo-16-methoxy-17-methyl-pentatriacontane (108)[182]



N-Bromosuccinimide (1.16 g, 6.56 mmol, 1.3 mol. equiv.) was added portionwise over 15 mins to a stirred solution of (16*S*,17*S*)-16-methoxy-17-methyl-pentatriacontan-1-ol **(107)** (2.64 g, 5.05 mmol) in dichloromethane (40 ml) at 0 °C. The mixture was stirred at RT for 1 hr; when TLC analysis indicated complete reaction, it was quenched with sat. aq. sodium meta-bisulfite (50 ml). The aqueous layer was re-extracted with dichloromethane (2 x 30 ml) and the combined organic

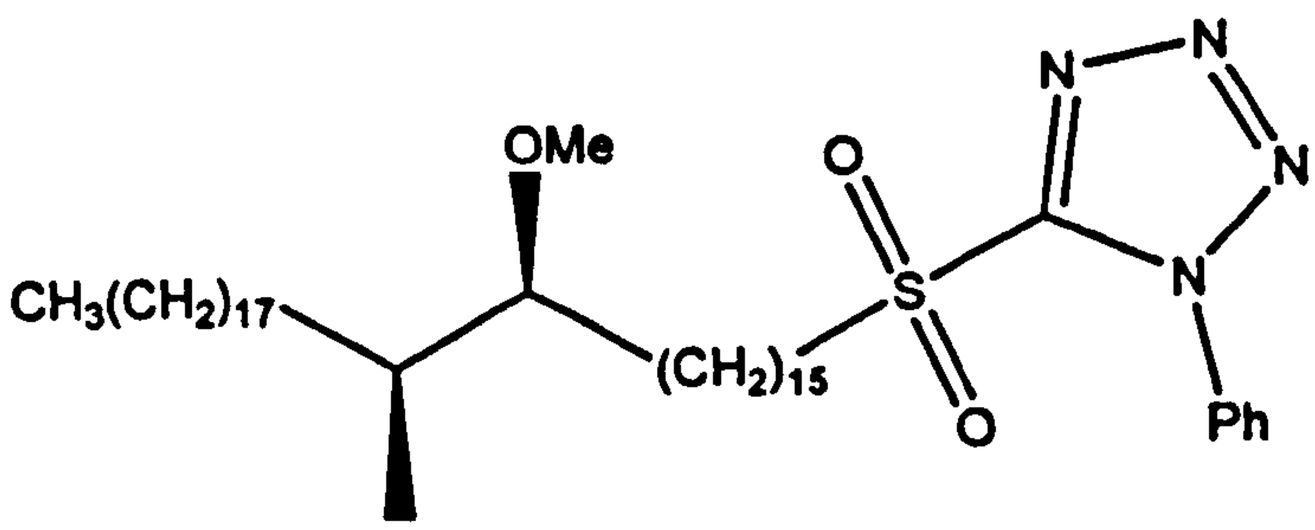
extracts washed with water (100 ml), dried and evaporated to give a residue. This residue was treated with petrol/ether (1:1, 100 ml), refluxed for 30 mins and then filtered and washed with petrol/ether (1:1, 50 ml). The filtrate was evaporated and the resultant residue purified via column chromatography eluting with petrol/ether (50:1) to give a white solid, (16*S*,17*S*)-1-bromo-16-methoxy-17-methyl-pentatriacontane **(108)** (2.60 g, 84 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{23}_D = -7.54$ ($CHCl_3$, 1.087 μmol); lit. value $[\alpha]^{22}_D = -7.35$ (*c* 1.16, $CHCl_3$).[182]

Appendix 9: 5-((16*S*,17*S*)-16-Methoxy-17-methylpentatriacontyl-1-sulfanyl)-1-phenyl-1H-tetrazole (109)[182]



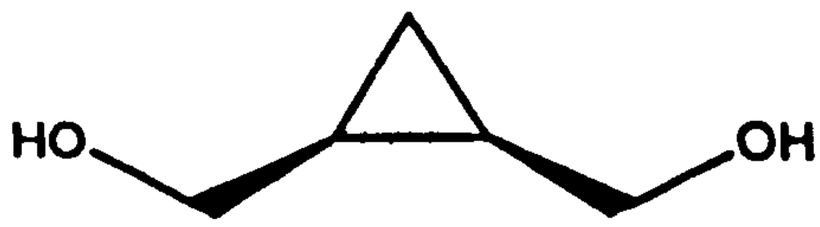
(16*S*,17*S*)-1-Bromo-16-methoxy-17-methyl-pentatriacontane **(108)** (2.60 g, 4.23 mmol) was added to a stirred solution of 1-phenyl-1H-tetrazole-5-thiol (0.83 g, 4.65 mmol, 1.1 mol. equiv.) and anhydrous potassium carbonate (1.75 g, 12.70 mmol, 3 mol. equiv.) in acetone (60 ml) at RT. The mixture was stirred at RT for 18 hrs, the solvent was evaporated and the residue was diluted with petrol/ether (1:1, 100 ml) and water (75 ml). The aqueous layer was re-extracted with petrol/ether (1:1, 2 x 50 ml). The combined organic extracts were dried and evaporated to give a crude oil, which was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, 5-((16*S*,17*S*)-16-methoxy-17-methylpentatriacontyl-1-sulfanyl)-1-phenyl-1H-tetrazole **(109)** (2.80 g, 93 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{22}_D = -6.51$ ($CHCl_3$, 0.981 μmol); lit. value $[\alpha]^{22}_D = -6.5$ (*c* 1.32, $CHCl_3$).[182]

Appendix 10: 5-((16*S*,17*S*)-16-Methoxy-17-methylpentatriacontan-1-sulfonyl)-1-phenyl-1H-tetrazole (91)[182]



To a stirred solution of 5-((16*S*,17*S*)-16-methoxy-17-methylpentatriacontyl-1-sulfanyl)-1-phenyl-1H-tetrazole **(109)** (2.80 g, 3.93 mmol) in a mixture of IMS (70 ml) and THF (30 ml), a solution of ammonium molybdate (VI) tetrahydrate (4.86 g, 3.93 mmol) in -10 °C hydrogen peroxide (10 ml, 35 % w/w) was added at 5 °C. The resulting yellow solution was stirred for 2 hrs at RT, then further ammonium molybdate (VI) tetrahydrate (2.43 g, 1.96 mmol, 0.5 mol. equiv.) in -10 °C hydrogen peroxide (5 ml, 35 % w/w) was added and the reaction mixture was stirred for 18 hrs. The reaction mixture was poured into water (300 ml) and extracted with dichloromethane (3 x 60 ml). The combined organic extracts were washed with water (100 ml), dried and evaporated. The crude product was purified via column chromatography eluting with petrol/ether (10:1) to give a white solid, 5-((16*S*,17*S*)-16-methoxy-17-methylpentatriacontan-1-sulfonyl)-1-phenyl-1H-tetrazole **(91)** (2.56 g, 87 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{23}_D = -6.32$ ($CHCl_3$, 1.311 μmol); lit. value $[\alpha]^{22}_D = -6.19$ (*c* 1.39, $CHCl_3$).[182]

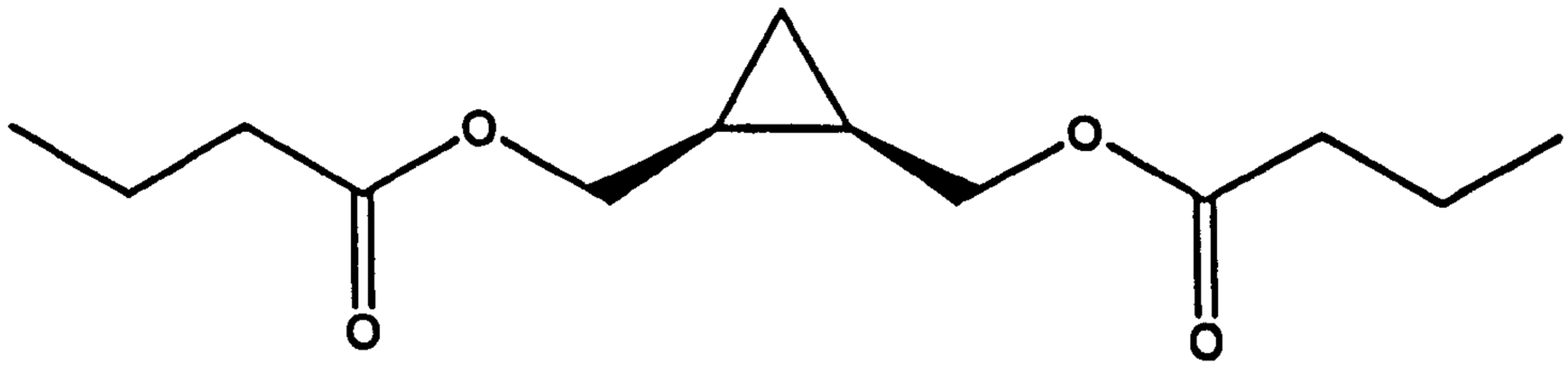
Appendix 11: (*cis*-2-Hydroxymethyl-cyclopropyl)-methanol (111)[193]



Lithium aluminium hydride (18.68 g, 492.2 mmol, 3 mol. equiv.) was added portionwise to stirred THF (350 ml, HPLC grade) at -20 °C, when vigorous evolution of hydrogen was observed. A solution of *cis*-cyclopropane-1,2-

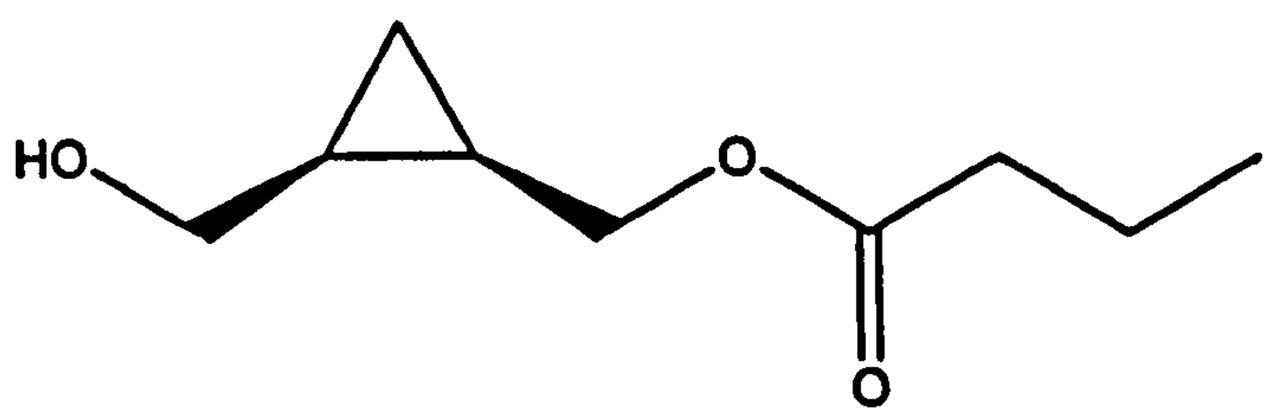
dicarboxylic acid dimethyl ester **(110)** (25.92 g, 164.0 mmol) in THF (50 ml) was added dropwise to the above suspension at -20 °C and the reaction mixture was refluxed for 2 hrs. When TLC analysis indicated completion of the reaction, a freshly prepared solution of sat. aq. sodium sulfate (40 ml) was added at -20 °C. Formation of a white precipitate was observed and the reaction mixture was stirred at RT for 2 hrs. The mixture was filtered through a bed of silica gel and the solvent evaporated. The resulting solution was taken up in dichloromethane (100 ml), washed with water (25 ml) and dried. The solvent was evaporated and the crude product was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a colourless oil, (*cis*-2-hydroxymethyl-cyclopropyl)-methanol **(111)** (12.86 g, 80 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[193]

Appendix 12: Butyric acid *cis*-2-butyryloxymethyl-cyclopropylmethyl ester (112)[193]



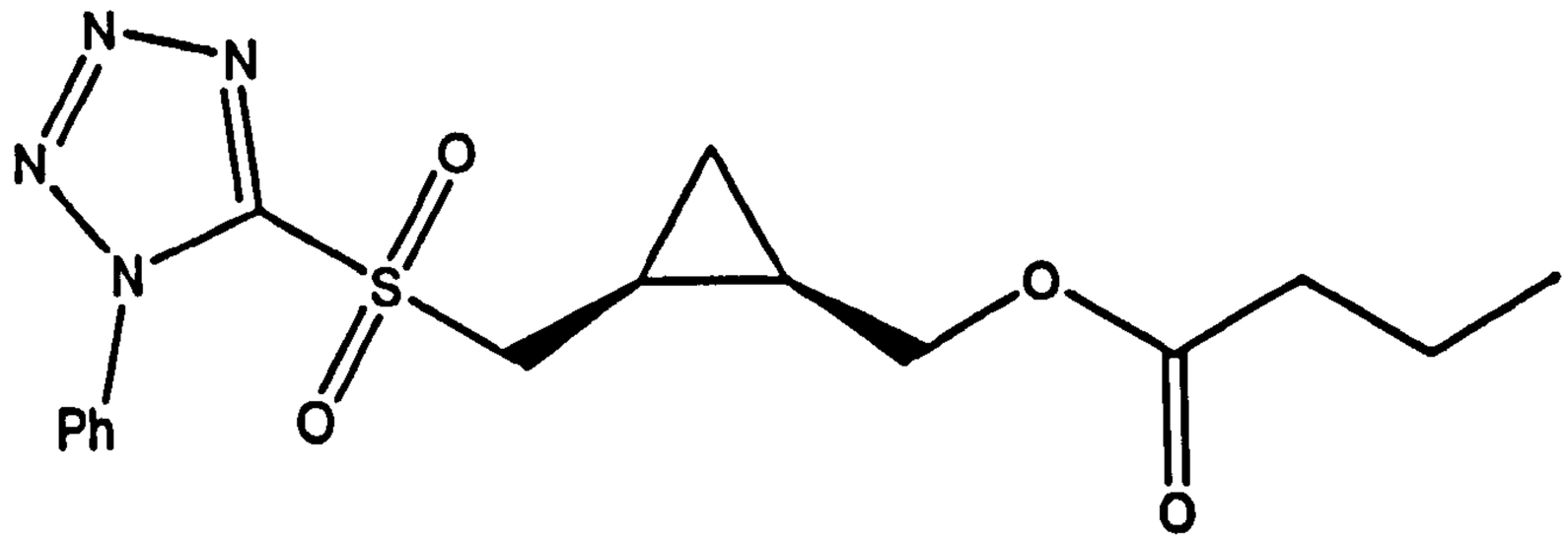
Butyric anhydride (40.58 ml, 39.28 g, 248.3 mmol, 2.2 mol. equiv., d = 0.968) was added to (*cis*-2-hydroxymethyl-cyclopropyl)-methanol **(111)** (11.06 g, 112.9 mmol) and the mixture was refluxed for 18 hrs at 120 °C. The reaction mixture was then cooled to RT and dichloromethane (100 ml) and sodium hydroxide solution (100 ml, 7 %) was added. The aqueous layer was re-extracted with dichloromethane (2 x 25 ml) and the combined organic layers were washed with sat. aq. sodium hydrogen carbonate (50 ml). The solution was dried and the solvent evaporated and the excess butyric anhydride removed via flash distillation at 0.1 – 0.2 mmHg. The crude product was purified via column chromatography eluting with petrol/ether (5:1, then 1:1) to give a colourless oil, butyric acid *cis*-2-butyryloxymethyl-cyclopropylmethyl ester **(112)** (22.37 g, 82 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[193]

Appendix 13: Butyric acid (1*R*,2*S*)-2-hydroxymethyl-cyclopropylmethyl ester (113)[193]



Lipase (1 g, PPL, type II, crude, Sigma cat. no. L3126) was added to a flask fitted with a pH electrode, which has been accurately calibrated, containing a gently stirred solution of ethylene glycol (41 ml) and distilled water (161 ml) at 3 °C under nitrogen. At pH 6.8 butyric acid *cis*-2-butyryloxymethyl-cyclopropylmethyl ester **(112)** (10.5 g, 43.4 mmol) was added. The pH lowered, indicating that hydrolysis had begun, due to the formation for butyric acid. The pH was returned to 6.5 by the careful addition of aq. sodium hydroxide (1 M), maintaining a temperature of 3 °C throughout the process. The reaction mixture was stirred for 1 hr followed by addition of further lipase (0.75 g), and aq. sodium hydroxide (1 M) was added during the reaction to maintain a pH of 6.5. After 5 hrs 30 mins the pH stopped decreasing indicating completion of the reaction; a total of 37 ml of aq. sodium hydroxide (1 M) was required. The mixture was filtered through a bed of celite and washed with water (25 ml), followed by ether (50 ml). The pH was raised to 8.3 by the addition of sat. aq. sodium hydrogen carbonate and the mixture neutralised by the addition of sat. aq. ammonium chloride, pH 7.2. The mixture was extracted with ether (2 x 300 ml) and the combined organic layers were dried and the solvent evaporated. The crude product was purified via column chromatography eluting with petrol/ether (1:1, then 1:3) to give a colourless oil, butyric acid (1*R*,2*S*)-2-hydroxymethyl-cyclopropylmethyl ester **(113)** (6.05 g, 82 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{22}_D = +17.4$ ($CHCl_3$, 1.021 μmol); lit. value $[\alpha]^{22}_D = +18.2$ (*c* 1.58, $CHCl_3$).[193]

Appendix 14: Butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazole-5-sulfonylmethyl)-cyclopropylmethyl ester (116)[182]



A solution of ammonium molybdate (VI) tetrahydrate (18.7 g, 15.1 mmol) in -10 °C hydrogen peroxide (30 ml, 35 % w/w) was added to a stirred solution of butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazol-5-ylsulfanylmethyl)-cyclopropylmethyl ester **(115)** (10.03 g, 30.3 mmol) in THF (100 ml) and IMS (200 ml) at 12 °C and the solution stirred at 15 – 20 °C for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (7.5 g, 6.1 mmol, 0.2 mol. equiv.) in -10 °C hydrogen peroxide (15 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 100 ml), dried and evaporated. The crude product was purified via column chromatography eluting with petrol/ether (4:1, then 1:1) to give a yellow oil, butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazole-5-sulfonylmethyl)-cyclopropylmethyl ester **(116)** (10.68 g, 97 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{21}_D = +47.6$ ($CHCl_3$, 1.287 μmol); lit. value $[\alpha]^{22}_D = +52.7$ (*c* 1.45, $CHCl_3$).[182]

Appendix 15: 6-Bromohexan-1-ol (121)[182]

1,6-Hexanediol **(120)** (25.00 g, 211.5 mmol) was dissolved in toluene (100 ml) and aqueous hydrobromic acid (30 ml, 44.7 g, 552.4 mmol, d = 1.49) was added and the mixture was refluxed for 18 hrs. When TLC analysis indicated completion of the reaction, the mixture was extracted with water (100 ml) and washed with sat. aq. sodium hydrogen carbonate (3 x 50 ml). The organic layer was dried and evaporated

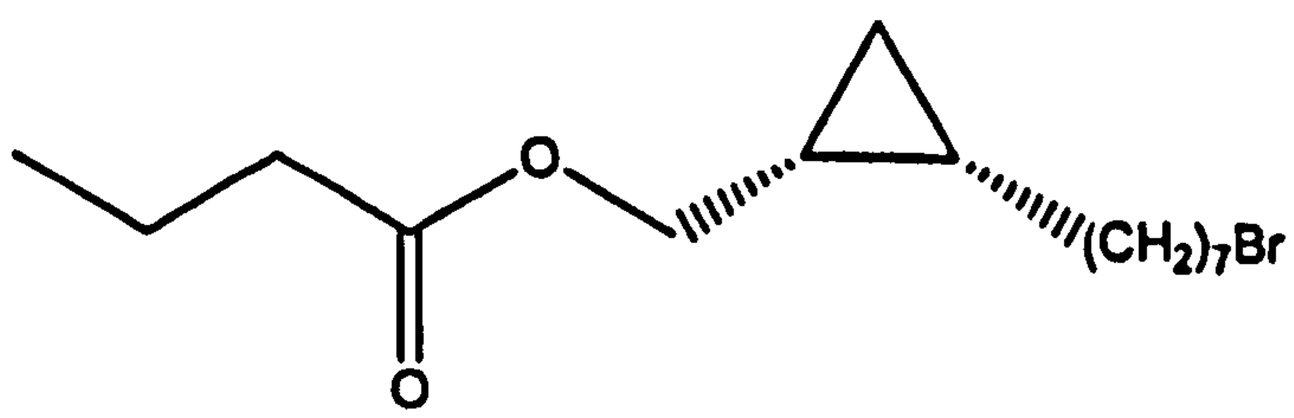
to give a crude product, which was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a yellow oil, 6-bromohexan-1-ol **(121)** (21.64 g, 57 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[182]

Appendix 16: 6-Bromohexanal (117)[182]

$Br(CH_2)_5CHO$

6-Bromohexna-1-ol **(121)** (21.64 g, 119.6 mmol) in dichloromethane (50 ml) was added to a stirred suspension of pyridinium chlorochromate (51.5 g, 239.1 mmol, 2 mol. equiv.) in dichloromethane (500 ml) at RT. The mixture was stirred vigorously for 2 hrs; when TLC analysis indicated completion of the reaction, the mixture was filtered over a silica gel/celite pad and washed well with ether (200 ml). The filtrate was evaporated to give a residue which was purified via column chromatography eluting with petrol/ether (1:1) to give a colourless oil, 6-bromohexanal **(117)** (14.62 g, 68 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[182]

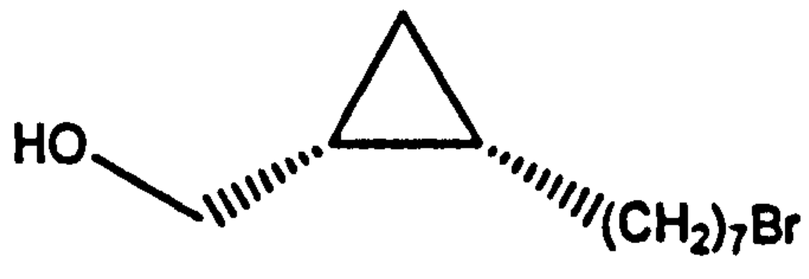
Appendix 17: Butyric acid (1*R*,2*S*)-2-(7-bromoheptyl)cyclopropylmethyl ester (118)[182]



A solution of 6-bromohexanal **(117)** (4.40 g, 24.5 mmol) in dry THF (50 ml) was added to a stirred solution of butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazole-5-sulfonylmethyl)-cyclopropylmethyl ester **(116)** (10.68 g, 29.4 mmol) in dry THF (50 ml) at RT under nitrogen. The mixture was cooled to -10 °C and lithium *bis*(trimethylsilyl)amide (36.1 ml, 38.2 mmol, 1.06 M) was added dropwise between -12 °C and -5 °C. The solution was stirred for 2 hrs; when TLC analysis indicated

completion of the reaction, dichloromethane (200 ml) and sat. aq. ammonium chloride (100 ml) was added. The aqueous layer was re-extracted with further dichloromethane (2 x 200 ml) and the combined organic extracts were dried and evaporated to give a crude yellow oil. This was purified via column chromatography eluting with petrol/ether (20:1) to give a colourless oil, (1*R*,2*S*)-2-((*E*/*Z*)-7-bromohept-1-enyl)cyclopropylmethyl ester **(122)** (6.49 g, 84 %). 2,4,6-Triisopropyl-benzenesulfonyl hydrazide (8.70 g, 29.2 mmol, 2.75 mol. equiv.) was added to a stirred solution of (1*R*,2*S*)-2-((*E*/*Z*)-7-bromohept-1-enyl)cyclopropylmethyl ester **(122)** (3.37 g, 10.6 mmol) in THF (75 ml) and the mixture was stirred for 18 hrs at 50 °C. Further 2,4,6-triisopropyl-benzenesulfonyl hydrazide (3.17 g, 10.6 mmol) was added and the mixture stirred for 24hrs at 50 °C. The reaction mixture was then diluted with petrol/ether (1:1, 200 ml) and aq. sodium hydroxide (2 %, 100 ml). The aqueous layer was re-extracted with petrol/ether (1:1, 2 x 50 ml) and the combined organic extracts were washed with water (100 ml), dried and evaporated. The crude product was purified via column chromatography eluting with petrol/ether (10:1) to give a colourless oil, butyric acid (1*R*,2*S*)-2-(7-bromoheptyl)cyclopropylmethyl ester **(118)** (2.00 g, 59 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{21}_D = +9.76$ ($CHCl_3$, 1.073 μmol); lit. value $[\alpha]^{24}_D = +10.3$ (*c* 1.50, $CHCl_3$).[182]

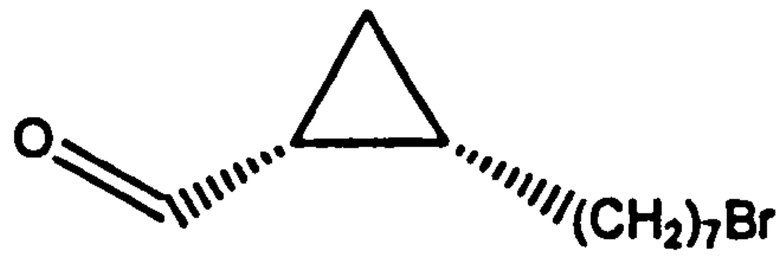
Appendix 18: (1*R*,2*S*)-2-(7-Bromoheptyl)cyclopropyl methanol (123)[182]



Anhydrous potassium carbonate (2.30 g, 16.6 mmol, 2.65 mol. equiv) was added to a stirred solution of butyric acid (1*R*,2*S*)-2-(7-bromoheptyl)cyclopropylmethyl ester **(117)** (2.00 g, 6.27 mmol) in methanol (15 ml) and THF (20 ml) at RT. The reaction mixture was stirred for 18 hrs at 45 °C, when TLC analysis indicated completion of the reaction. The reaction mixture was diluted with water (100 ml) and ether (50 ml). The aqueous layer was re-extracted with ether (2 x 25 ml). The combined organic extracts were washed with brine (50 ml), dried and evaporated to give a residue. The crude product was purified via column chromatography eluting with petrol/ether

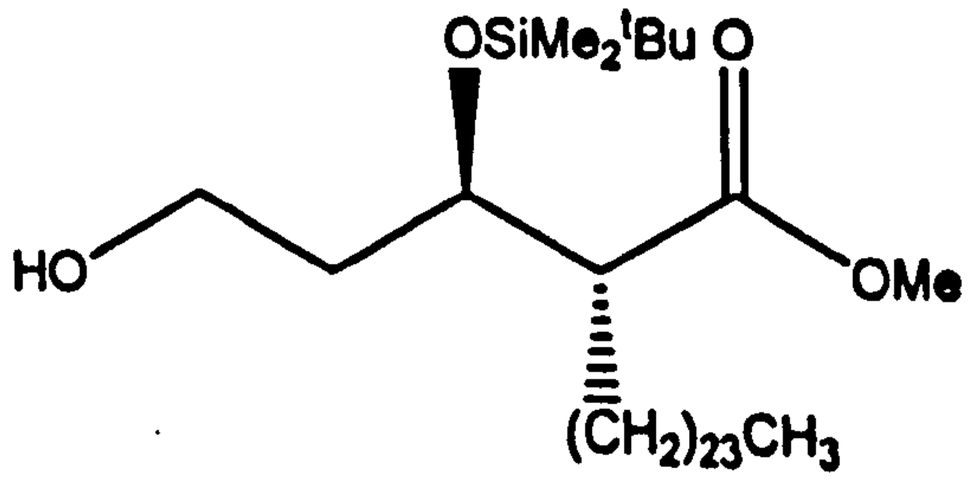
(5:1) to give a yellow oil, (1*R*,2*S*)-2-(7-bromoheptyl)cyclopropyl methanol **(123)** (1.44 g, 92 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{19}_D = +13.1$ ($CHCl_3$, 1.232 μmol); lit. value $[\alpha]^{22}_D = +12.87$ (*c* 1.65, $CHCl_3$).[182]

Appendix 19: (1*R*,2*S*)-2-(7-Bromoheptyl)cyclopropanecarbaldehyde (92)[182]



(1*R*,2*S*)-2-(7-Bromoheptyl)cyclopropylmethanol **(123)** (1.44 g, 5.80 mmol) in dichloromethane (10 ml) was added to a stirred suspension of pyridinium chlorochromate (3.13 g, 14.51 mmol, 2.5 mol. equiv.) in dichloromethane (40 ml) at RT. The mixture was stirred for 3 hrs; when TLC analysis indicated completion of the reaction, the mixture was poured into ether (200 ml), filtered through a pad of silica gel and washed well with ether (100 ml). The filtrate was evaporated and the resultant residue was purified via column chromatography eluting with petrol/ether (5:2) to give a colourless oil, (1*R*,2*S*)-2-(7-bromohepytl) cyclopropanecarbaldehyde **(92)** (0.73 g, 51 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{20}_D = +8.6$ ($CHCl_3$, 1.134 μmol); lit. value $[\alpha]^{22}_D = +8.19$ (*c* 1.28, $CHCl_3$).[182]

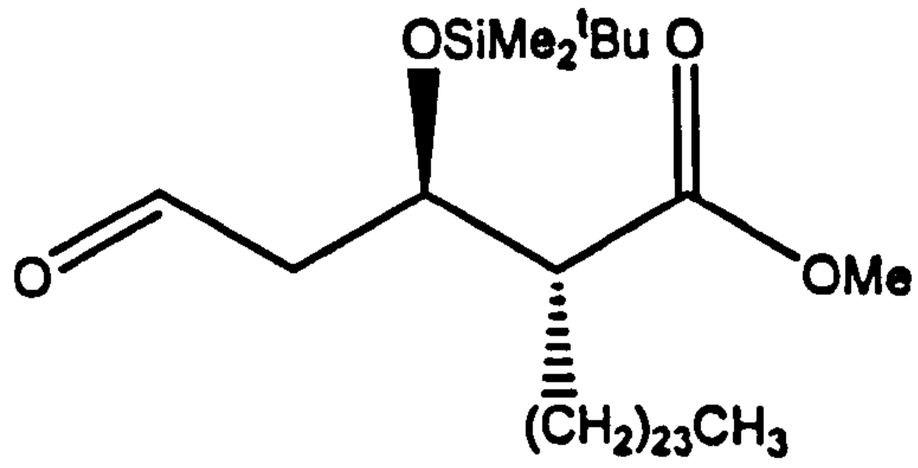
Appendix 20: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-hydoxypropyl]-hexacosanoic acid methyl ester (127)[183]



Palladium on charcoal (0.5 g, 10 %) was added to a stirred solution of (*E/Z*)-(*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyl-dimethyl-silanyloxy-propyl]-hexacos-4-enoic acid

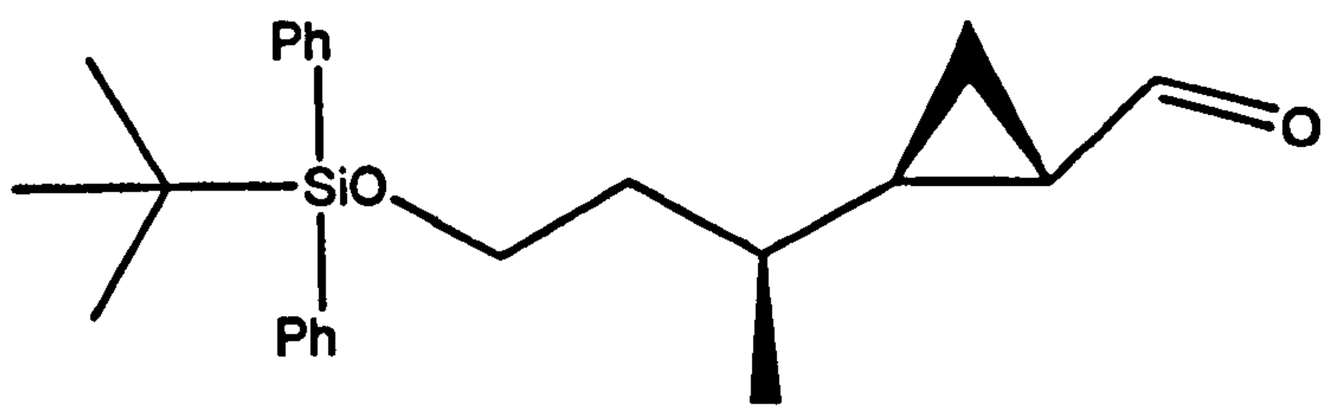
methyl ester **(126)** (2.57 g, 3.74 mmol) in ethanol (25 ml). The mixture was hydrogenated at atmospheric pressure for 3 days and then the catalyst was removed via suction filtration through a bed of celite, washed with ethyl acetate (3 x 10 ml) and the solvent was evaporated. The product was purified via column chromatography eluting with petrol/ethyl acetate (5:1) to give (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-hydoxypropyl]-hexacosanoic acid methyl ester **(127)** (2.00 g, 89 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{19}_D$ = -8.47 ($CHCl_3$, 0.975 μmol); lit. value $[\alpha]^{22}_D$ = – 8.3 (*c* 0.4, C_6H_6).[183]

Appendix 21: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-oxo-propyl]-hexacosanoic acid methyl ester (128)[183]



(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-hydoxypropyl]-hexacosanoic acid methyl ester **(127)** (2.00 g, 3.26 mmol) in dichloromethane (10 ml) was added to a stirred suspension of pyridinium chlorochromate (1.76 g, 8.16 mmol, 2.5 mol. equiv.) in dichloromethane (70 ml). The resultant mixture was stirred at RT for 2 hrs; when TLC analysis indicated completion of the reaction, the mixture was diluted with ether (100 ml) and then filtered on a bed of silica gel. The filtrate was evaporated and the crude product purified via column chromatography eluting with petrol/ether (5:2) to give a white solid, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxo-propyl]-hexacosanoic acid methyl ester **(128)** (1.07 g, 54 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{28}_D$ = -4.42 ($CHCl_3$, 1.290 μmol); lit. value $[\alpha]^{28}_D$ = – 5.0 (*c* 1.23, $CHCl_3$).[183]

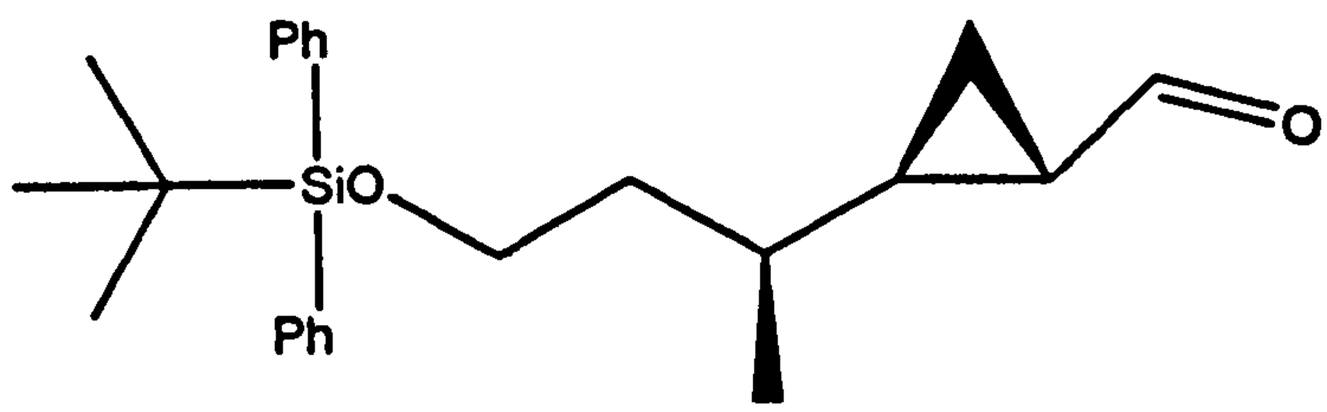
Appendix 22: (1*S*,2*R*)-*trans*-2-((*S*)-4-(*tert*-Butyldiphenylsilyloxy)butan-2-yl)cyclopropanecarbaldehyde (144)[180, 193]



Sodium methoxide (1.45 g, 26.82 mmol, 1.1 mol. equiv.) was added to a stirred solution of (1*R*,2*R*)-*cis*-2-((*S*)-4-(*tert*-butyldiphenylsilyloxy)butan-2-yl)cyclopropane -carbaldehyde **(145)** (8.93 g, 24.39 mmol) in methanol (300 ml) and the mixture refluxed for 56 hrs. The mixture was cooled to RT, quenched with sat. aq. ammonium chloride (250 ml) and extracted with ether (3 x 100 ml). The combined organic layers were dried and evaporated to give a thick oil. This was purified via column chromatography eluting with petrol/ether (20:1) to give (1*S*,2*R*)-*trans*-2-((*S*)-4-(*tert*-butyldiphenylsilyloxy)butan-2-yl)cyclopropanecarbaldehyde **(144)** (4.90 g, 55 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{22}_D$ = +21.5 ($CHCl_3$, 1.544 μmol); lit. value $[\alpha]^{22}_D$ = +22.9 (*c* 1.28, $CHCl_3$).[180]

A second fraction was also obtained eluting with petrol/ether (5:1), (1*S*,2*R*)-*trans*-2-((*S*)-3-hydroxy-1-methyl-propyl)cyclopropanecarbaldehyde **(152)** (0.93 g, 30 %), which showed δ_H (500 MHz, $CDCl_3$): 8.99 (1H, d, J 9.75 Hz), 3.76-3.66 (2H, m), 2.20 (1H, br.s), 1.80-1.63 (1H, m), 1.59-1.53 (1H, m), 1.36-1.30 (1H, m), 1.29-1.25 (1H, m), 1.03-0.95 (4H, m); δ_C (125 MHz, $CDCl_3$): 200.9, 60.5, 39.7, 33.2, 30.1, 29.1, 19.5, 13.2; ν_{max}: 3427 cm^{-1}.

Appendix 23: (1*S*,2*R*)-*trans*-2-((*S*)-4-(*tert*-Butyldiphenylsilyloxy)butan-2-yl)cyclopropanecarbaldehyde (144)[180]



Triethylamine (2.03 ml, 14.56 mmol, 1.47 g, 2 mol. equiv., d=0.7255) was added to a stirred solution of (1*S*,2*R*)-*trans*-2-((*S*)-3-hydroxy-1-methyl-propyl)cyclopropane-carbaldehyde **(152)** (0.93 g, 7.28 mmol) in dry dichloromethane (10 ml). After 10 mins, *tert*-butyldiphenylsilylchloride (2.43 ml, 9.46 mmol, 1.3 mol. equiv., 2.60 g, d=1.072) in dry dichloromethane (5 ml) was added, followed by addition of DMAP (0.05 g). The reaction mixture was stirred for 2 hrs; when TLC showed completion of the reaction, it was quenched with water (20 ml), extracted with dichloromethane (3 x 25 ml), the extract washed with brine (50 ml), dried and evaporated. The resultant oil was purified via column chromatography eluting with petrol/ether (5:1) to give (1*S*,2*R*)-*trans*-2-((*S*)-4-(*tert*-butyldiphenylsilyloxy)butan-2-yl)cyclopropane-carbaldehyde **(144)** (2.3 g, 86 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{22}_D = +21.5$ ($CHCl_3$, 1.544 µmol); lit. value $[\alpha]^{22}_D = +22.9$ (*c* 1.28, $CHCl_3$).[180]

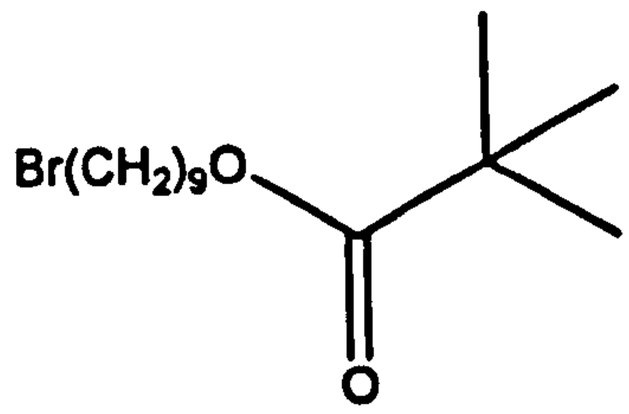
Appendix 24: 9-Bromononan-1-ol[183]

$Br(CH_2)_9OH$

1,9-Nonanediol (25.00 g, 156.0 mmol) was dissolved in toluene (100 ml) and aqueous hydrobromic acid (30 ml, 44.7 g, 552.4 mmol, d = 1.49) was added and the mixture was refluxed for 18 hrs. When TLC analysis indicated completion of the reaction, the mixture was extracted with water (100 ml) and washed with sat. aq. sodium hydrogen carbonate (3 x 50 ml). The combined organic layers were dried and evaporated to give a crude product which was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a yellow oil, 8-bromooctan-1-ol

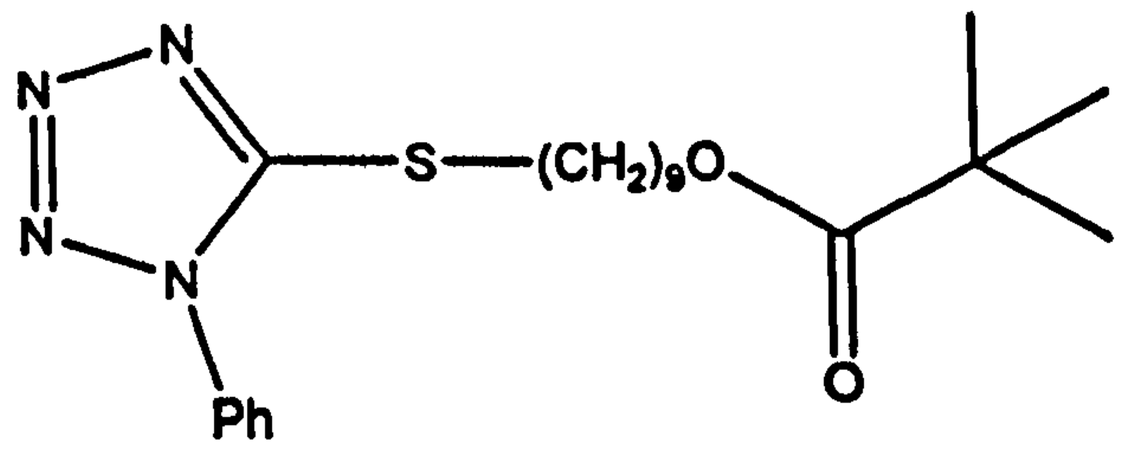
(19.0 g, 55 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[183]

Appendix 25: 9-Bromononyl pivalate[183]



A solution of trimethyl acetylchloride (9.15 ml, 8.96 g, 74.3 mmol, d = 0.979, 1.2 mol. equiv.) in dichloromethane (30 ml) was added to a stirred solution of 9-bromononan-1-ol (19.0 g, 61.9 mmol) in dichloromethane (100 ml), pyridine (10.0 ml, 9.79 g, 123.8 mmol, d = 0.978, 2 mol. equiv.) and 4-dimethylaminopyridine (0.302 g, 2.48 mmol, 0.04 mol. equiv.) over 15 mins at 5 °C. The mixture was then stirred at RT for 18 hrs, when TLC analysis indicated completion of the reaction. Dil. hydrochloric acid (100 ml) was added and the organic layer extracted and washed with further dil. hydrochloric acid (70 ml) and brine (2 x 150 ml). The combined organic extracts were dried and evaporated to give a crude product, which was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a colourless oil, 9-bromononyl pivalate (23.48 g, 90 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[183]

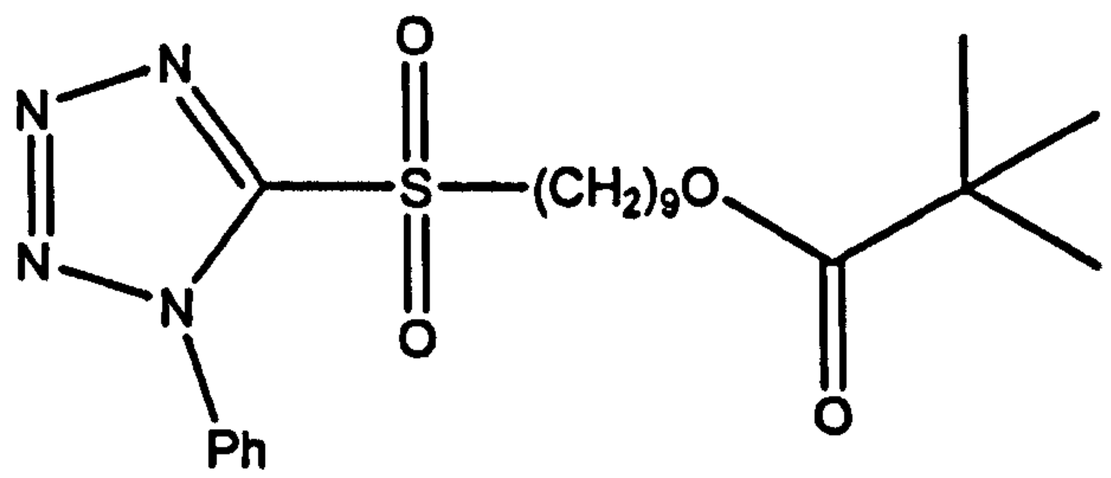
Appendix 26: 9-(1-Phenyl-1H-tetrazol-5-ylthio)nonyl pivalate[183]



9-Bromononyl pivalate (23.48 g, 76.5 mmol) was added with vigorous stirring to 1-phenyl-1H-tetrazole-5-thiol (13.60 g, 76.5 mmol) and anhydrous potassium carbonate (21.10 g, 153.0 mmol, 2 mol. equiv.) in acetone (200 ml). The reaction

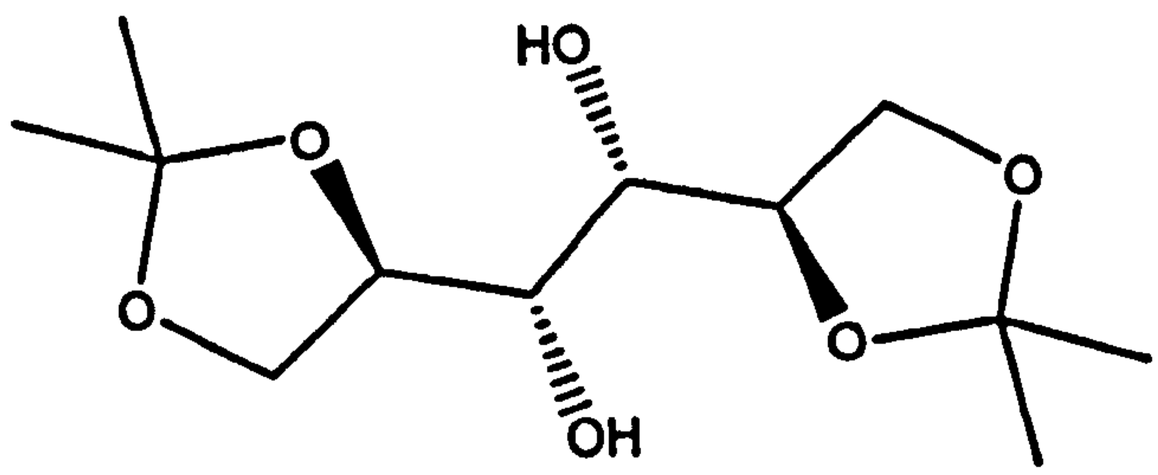
was refluxed for 18 hrs; when TLC analysis indicated completion of the reaction, the inorganic solids were filtered off and washed with acetone (100 ml). The organic filtrate was evaporated and extracted with dichloromethane (150 ml) and water (300 ml). The aqueous layer was re-extracted with further dichloromethane (3 x 100 ml) and the combined organic layers were dried and evaporated. The crude yellow oil was purified via column chromatography eluting with petrol/ether (5:2) to give a colourless oil, 9-(1-phenyl-1H-tetrazol-5-ylthio)nonyl pivalate (29.43 g, 96 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[183]

Appendix 27: 9-(1-Phenyl-1H-tetrazol-5-ylsulfonyl)nonyl pivalate (172)[183]



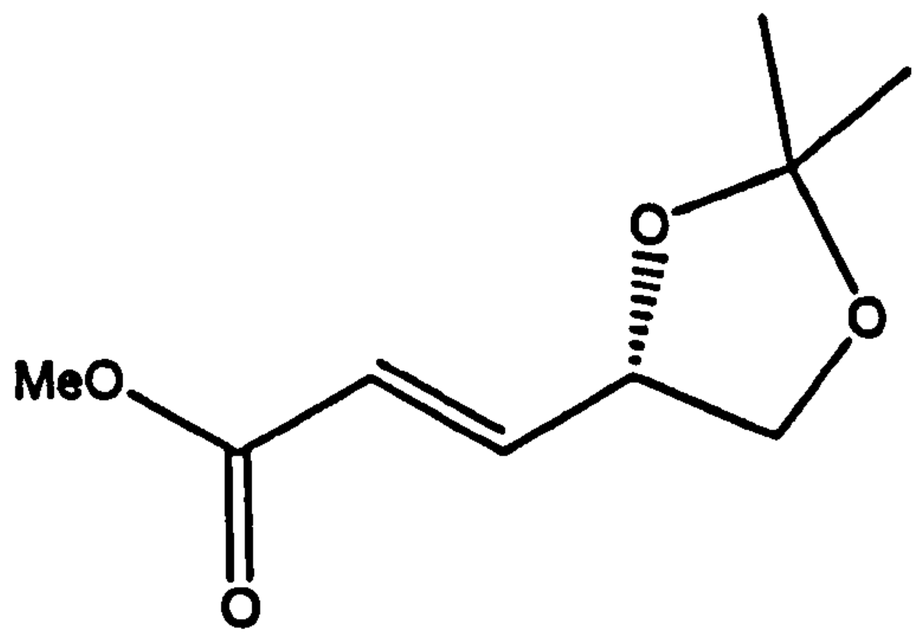
A solution of ammonium molybdate (VI) tetrahydrate (45.0 g, 36.4 mmol, 0.5 mol. equiv.) in -10 °C hydrogen peroxide (90 ml, 35 % w/w) was added to a stirred solution of 9-(1-phenyl-1H-tetrazol-5-ylthio)nonyl pivalate (29.43 g, 72.8 mmol) in THF (250 ml) and IMS (500 ml) at 12 °C and the mixture stirred at 15-20 °C for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (18.0 g, 14.6 mmol, 0.2 mol. equiv.) in -10 °C hydrogen peroxide (37.5 ml, 35 % w/w) was added and the reaction mixture stirred for 18 hrs. The mixture was then poured into water (3 l) and extracted with dichloromethane (3 x 250 ml) and the extract dried and evaporated. The crude product was purified via column chromatography eluting with petrol/ether (4:1) to give a colourless oil, 9-(1-phenyl-1H-tetrazol-5-ylsulfonyl)nonyl pivalate **(172)** (28.38 g, 89 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[183]

Appendix 28: 1,2:5,6-*O*-Isopropylidene-*D*-mannitol (175)[210]



Zinc chloride (161.59 g, 1.19 mmol, 2.7 mol. equiv.) was dissolved in HPLC grade acetone (750 ml) and *D*-mannitol **(60)** (80 g, 0.44 mmol) was added with vigorous stirring at RT. The reaction mixture was stirred at RT for 18 hrs and then K_2CO_3 (100 g) in water (100 ml). The solid inorganics were filtered under suction and washed with dichloromethane (4 x 100 ml) and then the solvent was evaporated to give a crude oil. This was dissolved in dichloromethane (300 ml) and washed with water (2 x 150 ml), dried and evaporated to give a white solid. Recrystallisation from petrol/ethyl acetate (7:1) to give a white solid, 1,2:5,6-*O*-isopropylidene-*D*-mannitol **(175)** (60.46 g, 53 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), v_{max} identical to the literature.[210]

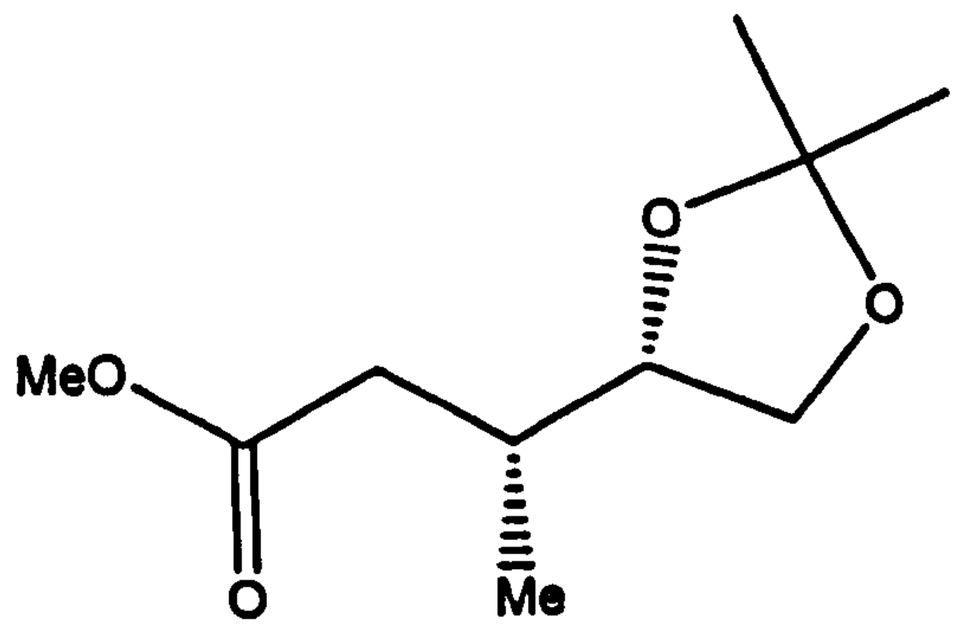
Appendix 29: (*E*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester (61)[183, 211]



A solution of $NaIO_4$ (40.80 g, 190.75 mmol, 2 mol. equiv.) in water (150 ml) was added dropwise to a stirred solution of 1,2:5,6-*O*-isopropylidene-D-mannitol **(175)** (25.00 g, 95.38 mmol) in 5 % $NaHCO_3$ (200 ml) at 0 °C and stirring continued for 1hr at RT. The mixture was cooled to 0 °C and (diisopropoxy phosphoryl)-acetic

acid methyl ester (50.00g, 210.00 mmol, 2.2 mol. equiv.) was added with stirring followed by a 6M solution of aq. K_2CO_3 (65 ml) at 0-4 °C. The reaction was allowed to reach RT and stirred for 18 hrs; the mixture was then extracted with dichloromethane (3 x 300 ml). The combined organic layers were dried and the solvent evaporated to give a crude oil, which was purified via column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil, (*E*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester **(61)** (9.71 g, 55 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{22}_D$ = + 41.7 ($CHCl_3$, 1.540 μmol); lit. value $[\alpha]^{24}_D$ = + 40.4 (*c* 1.09, $CHCl_3$).[183, 211]

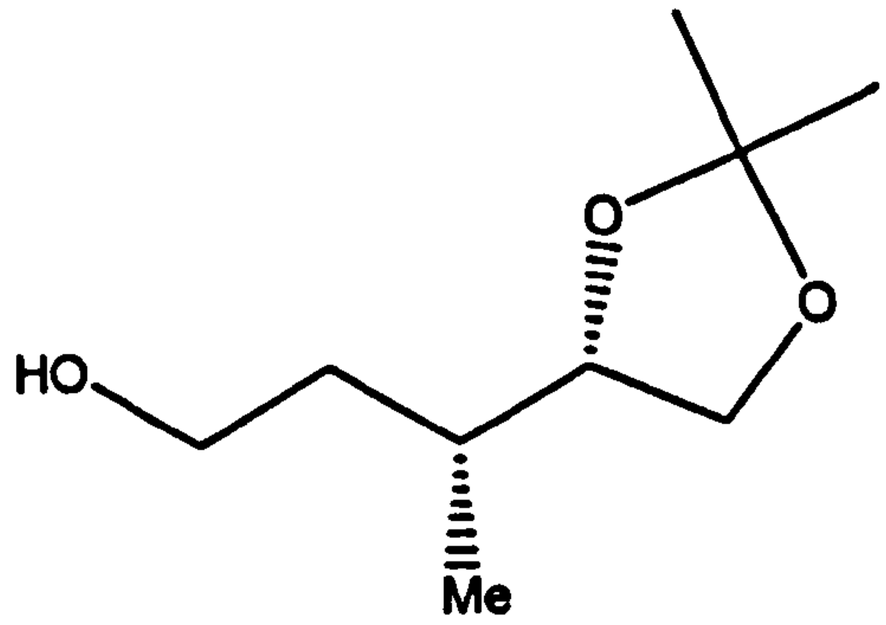
Appendix 30: (*R*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)butyric acid methyl ester (62)[183, 211]



Methyl lithium (61.10 ml, 102.15 mmol, 1.5 M in diethyl ether) was added to a stirred solution of (*E*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester **(61)** (9.5 g, 51.08 mmol) in dry ether (200 ml) at -78 °C under nitrogen. The reaction mixture was stirred at RT for 2.5 hrs, then allowed to reach -60 °C, followed by addition of water (10 ml). After 5 mins, sat. aq. ammonium chloride (30 ml) was added, whereupon the temperature rose to -40 °C. The mixture was allowed to reach 0 °C and was then quenched with water (100 ml). The aqueous layer was extracted with ethyl acetate (3 x 50 ml), and the organic phase washed with sat. aq sodium chloride (2 x 100 ml). The combined organic layers were dried and evaporated to give a crude yellow oil. Chromatography on silica gel eluting with petrol/ethyl acetate (10:1) (TLC visualised with potassium permanganate) gave (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)butyric acid methyl ester **(62)** (5.95 g, 58 %) as a

colourless oil, which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{19}_D = +8.12$ ($CHCl_3$, 1.344 μmol); lit. value $[\alpha]^{24}_D = +8.6$ (*c* 1.05, $CHCl_3$).[183, 211]

Appendix 31: (*R*)-3-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)butan-1-ol (177)[183]



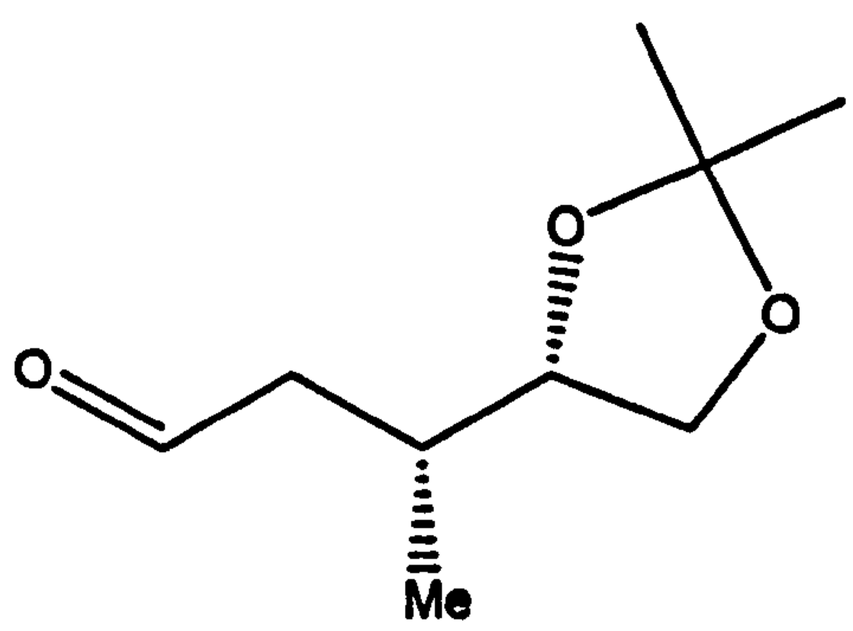
(*R*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)butyric acid methyl ester **(62)** (3.12 g, 15.44 mmol) in THF (20 ml) was added dropwise over 15 mins to a suspension of lithium aluminium hydride (0.82 g, 21.61 mmol, 1.4 mol. equiv.) in THF (100 ml) under nitrogen at -20 °C. The reaction mixture was refluxed for 2 hrs, when TLC showed no starting material was left. The mixture was quenched carefully with freshly prepared sat. aq. sodium sulfate (20 ml) at -20 °C until a white precipitate was formed, followed by addition of magnesium sulfate (5 g). The mixture was stirred vigorously for 1 hr then filtered over a celite pad and washed well with THF (2 x 100 ml). The combined organic layers were evaporated to give a residue. Chromatography on silica gel eluting with petrol/ethyl acetate (5:2) gave (*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)butan-1-ol **(177)** (1.97 g, 73 %) as a colourless oil, which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{21}_D = +19.38$ ($CHCl_3$, 1.223 μmol); lit. value $[\alpha]^{24}_D = +19.2$ (*c* 1.12, $CHCl_3$).[183]

Appendix 32: 10-Bromodecan-1-ol (180)[183]

$Br(CH_2)_{10}OH$

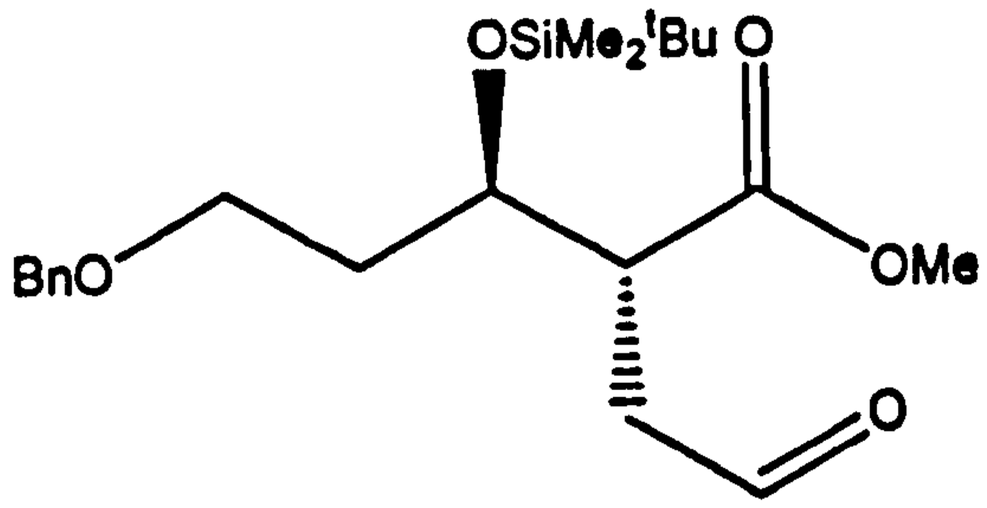
1,10-Decanediol **(179)** (25.00 g, 143 mmol) was dissolved in toluene (100 ml) and aq. hydrobromic acid (30 ml, 552.4 mmol, d = 1.49, 44.7 g) was added and the mixture was refluxed for 18 hrs. When TLC showed no starting material was left, the mixture was extracted with water (100 ml) and sat. aq. sodium hydrogen carbonate (3 x 50 ml). The organic extract was dried and the toluene removed with flash distillation at atmospheric pressure. The crude product was purified via column chromatography eluting with petrol/ether (20:1), then petrol/ether (1:1) to give a colourless oil, 10-bromodecan-1-ol **(180)** (30.79 g, 90 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[183]

Appendix 33: (*R*)-3-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)butanal (178)[182]



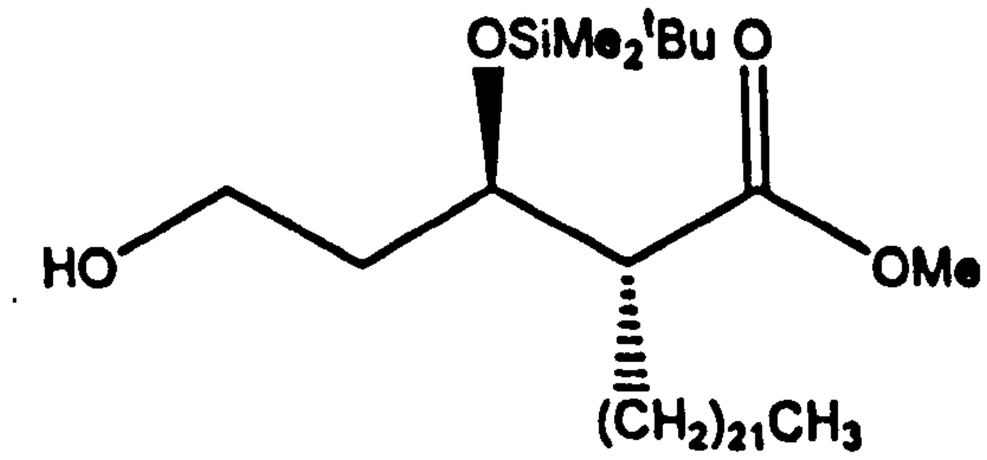
(*R*)-3-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)butan-1-ol **(177)** (0.97 g, 5.57 mmol) in dichloromethane (10 ml) was added to a stirred suspension of pyridinium chlorochromate (3.00 g, 13.92 mmol, 2.5 mol. equiv.) in dichloromethane (40 ml) at RT. The mixture was stirred for 2 hrs, when TLC analysis indicated completion of the reaction. The mixture was poured into ethyl acetate (50 ml) then filtered through a pad of silica gel and washed with ethyl acetate (50 ml) and the filtrate evaporated. The crude product was purified via column chromatography eluting with petrol/ethyl acetate (2:1) to give a colourless oil, (*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)butanal **(178)** (0.74 g, 77 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{19}_D$ = +8.51 ($CHCl_3$, 1.047 μmol); lit. value $[\alpha]^{2}_D$ = +8.27 (*c* 1.44, $CHCl_3$).

Appendix 34: (2*R*,3*R*)-Methyl 5-(benzyloxy)-3-(tert-butyldimethylsilyloxy)-2-(2-oxoethyl)pentanoate (196)[183, 202]



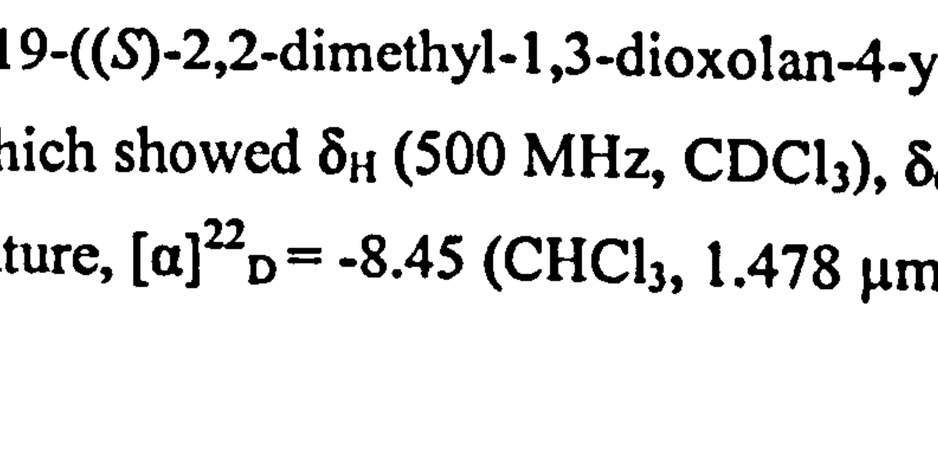
2,6-Lutidine (1.19 ml, 1.09 g, 10.19 mmol, 2 mol. equiv.), OsO_4 2.5 % in 2-methyl-2-propanol (1.28 ml, 0.1019 mmol, 0.02 mol. equiv.) and $NaIO_4$ (4.36 g, 20.38 mmol, 4 mol. equiv.) were added to a stirred solution of methyl 2-((*R*)-3-(benzyloxy)-1-(tert-butyldimethylsilyloxy)propyl)pent-4-enoate **(195)** (2.00 g, 5.09 mmol) in 1,4-dioxane/water (3:1, 80 ml) at RT. The mixture was stirred for 2 hrs at 25 °C, when TLC showed no starting material was left. Water (200 ml) and dichloromethane (200 ml) were added and the organic layer extracted. The aqueous layer was re-extracted with dichloromethane (3 x 50 ml) and the combined organic layers were washed with brine (200 ml), dried and the solvent evaporated. The product was purified via column chromatography eluting with petrol/ethyl acetate (2:1) to give a colourless oil, (2*R*,3*R*)-methyl 5-(benzyloxy)-3-(tert-butyldimethylsilyloxy)-2-(2-oxoethyl)pentanoate **(196)** (1.92 g, 96 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{22}_D$ = -18.75 ($CHCl_3$, 1.05 μmol); lit. value $[\alpha]^{26}_D$ = -18.4 (*c* 0.97, $CHCl_3$).[183]

Appendix 35: Methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-3-hydroxypropyl tetracosanoate (199)[183]



Lithium *bis*(trimethylsilyl)amide (6.9 ml, 7.30 mmol, 1.06 M) was added dropwise to a stirred solution of (2*R*,3*R*)-methyl 5-(benzyloxy)-3-(*tert*-butyldimethylsilyloxy)-2-(2-oxoethyl)pentanoate **(196)** (1.92 g, 4.87 mmol) and 5-(eicosylsulfonyl)-1-phenyl-1H-tetrazole **(197)** (2.86 g, 5.84 mmol, 1.2 mol. equiv.) in dry THF (75 ml) at -15 °C. The mixture was stirred for 18 hrs at RT, when TLC analysis indicated completion of the reaction. Sat. aq. ammonium chloride (50 ml) and petrol/ethyl acetate (1:1, 50 ml) were added. The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 x 50 ml) and the combined organic extracts were dried and evaporated to give an oil. The crude product was purified via column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, (*E*/*Z*)-methyl 2-((*R*)-3-(benzyloxy)-1-(tert-butyldimethylsilyloxy)propyl)tetra-cos-4-enoate **(198)** (2.03 g, 63 %). Palladium on charcoal (10 %, 0.5 g) was added to a stirred solution of (*E*/*Z*)-methyl 2-((*R*)-3-(benzyloxy)-1-(tert-butyldimethylsilyloxy)propyl)tetracos-4-enoate **(198)** (2.03 g, 3.08 mmol) in ethyl acetate (50 ml). The mixture was stirred at atmospheric pressure and when hydrogen absorption was complete, the mixture was filtered through a pad of celite and washed with ethyl acetate (50 ml). The filtrate was evaporated to give a white solid, methyl 2-((1*R*,2*R*,19*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)icosyl)tetracosanoate **(199)** (1.41 g, 80 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{22}_D = -8.45$ ($CHCl_3$, 1.478 μmol); lit. value $[\alpha]^{24}_D = -8.3$ (*c* 0.4, C_6H_6).[183]

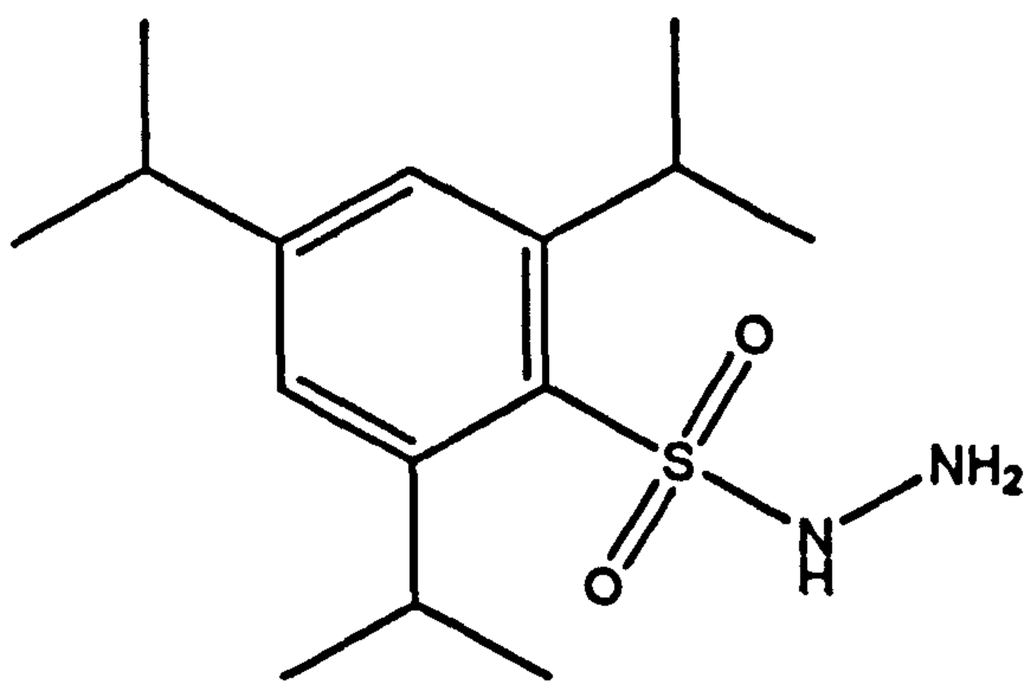
Appendix 36: Methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-3-oxopropyl)tetracosanoate (200)[183]



Methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-3-hydroxypropyl)tetracosanoate **(199)** (0.60 g, 1.05 mmol) in dichloromethane (10 ml) was added to a stirred suspension of pyridinium chlorochromate (0.57 g, 2.63 mmol, 2.5 mol. equiv.) in

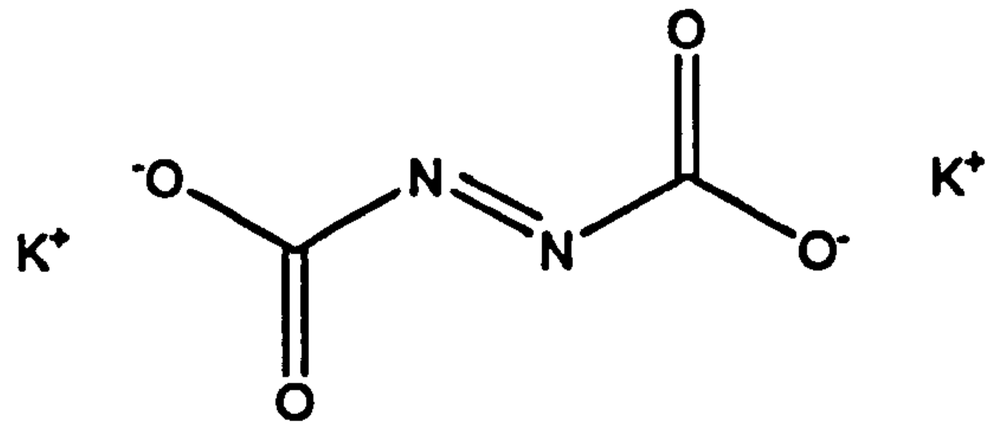
dichloromethane (40 ml). The resultant mixture was stirred at RT for 2 hrs and when TLC analysis indicated completion of the reaction, the mixture was diluted with ethyl acetate (25 ml) and then filtered on a bed of silica gel. The filtrate was evaporated and the crude product purified via column chromatography eluting with petrol/ethyl acetate (10:1) to give a white solid, methyl 2-((1*R*,2*R*)-1-(*tert*-butyldimethylsilyloxy)-3-oxopropyl)tetracosanoate **(200)** (0.57 g, 96 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]^{21}_D$ = -5.04 ($CHCl_3$, 1.234 μmol); lit. value $[\alpha]^{24}_D$ = -5.0 (*c* 1.23, $CHCl_3$).[183]

Appendix 37: 2,4,6-Triisopropyl-benzenesulfonyl hydrazide[194]



2,4,6-Triisopropyl-benzenesulfonyl chloride (30.0 g, 99.05 mmol) was dissolved in THF (75 ml) and cooled to -10 °C. Hydrazine hydrate (9.9 g, 198.1 mmol) was slowly added, whilst maintaining the solution in the temperature range of -4 °C and -10 °C. The mixture was kept in the temperature range for a further 2 hrs. Water (3 ml) was added and the solid dissolved. The mixture was separated into two phases and the organic extract was washed with brine (2 x 20 ml). The organic phase was dried at 0 °C for 30 mins, filtered and washed with ether. The solvent was evaporated at 10 °C to a white solid, 2,4,6-triisopropyl-benzenesulfonyl hydrazide (26.85 g, 91 %), which was stored in the freezer under nitrogen.

Appendix 38: Di-potassium azodicarboxylate[183]



Azodicarbonamide (7.5 g, 64 mmol) was slowly added in small portions to a vigorously stirred solution of potassium hydroxide (15 g, 260 mmol) in distilled water (15 ml) at 0 °C on a salted ice-water bath, maintaining the temperature below 5 °C. The resultant bright yellow solution was stirred at 0-5 °C for 45 mins, during which a bright yellow precipitate formed. The precipitate was filtered on a sintered funnel and washed with ice-cold methanol (60 ml). The yellow precipitate was dissolved in water (40 ml) and then filtered through into pre-cooled IMS (60 ml, - 20 °C) to give a yellow precipitate. The precipitate was then filtered again through a sinter funnel and washed with cold methanol (50 ml, -20 °C), followed by cold petrol (50 ml, -20 °C) and the solid dried under vacuum. The solid was then transferred to a pre-cooled round bottomed flask under nitrogen. The flask was then stored in the freezer.