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Synthesis of mixed cord factors and related compounds

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Synthesis of mixed cord factors and related compounds

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By

Klarah Sherzad Baols

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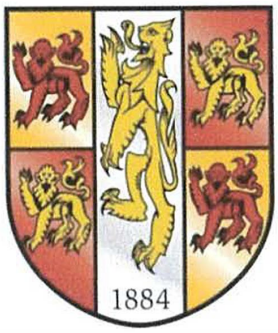

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Abbreviations and acronyms

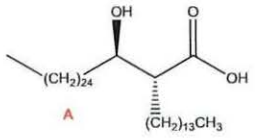
AIDS	Acquired Immuno Deficiency Virus
ATP	Adenosine Tri Phosphate
BC	Before Christ
BCG	Bacillus Calmette-Guerin
br	broad
CID	Collision Induced Dissociation
d	doublet
DAT	Di Acyl Trehaloses
DIAD	DI-Isopropyl Aso Dicarboxylate
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DNA	Deoxyribose Nucleic Acid
DPNase	Di Phospho Pyridine Nucleotidase
EDCI	1-Ethyl-3-3-dimethylaminopropylcarbodiimidhydrochloride
EI	Electron Impact
EIMS	Electron Impact Mass Spectrometry
ELISA	Enzyme-linked Immunosorbent Assay
Ether	Diethyl ether
GC	Gas Chromatography
GMM	Glucose Mono Mycolate
h	hours
HIV	Human Immuno deficiency Virus
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IMS	Industrial methylated spirit
INH	Isoniazid
IR	Infra-Red
J	Coupling constant
LAM	Lipo Arabino Mannan
LDA	Lithium *N,N*-diisopropylamide

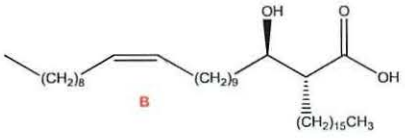
$LiAlH_4$	Lithium aluminium hydride
LiHMDS	Lithium *bis*(trimethylsilyl)amide
m	multiplet
M. tb	*Mycobacterium tuberculosis*
m.p.	melting point
MA	Mycolic Acid
MAC	*Mycobacterium avium Complex*
mAG	mycolyl Arabino Galactan
MALDI	Matrix-assisted laser Desorption/Ionization
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time Of Flight
MAR	Multi-Drug Resistant
MAT	6-Mycolyl-6'-Acetyl Trehalose
m-CPBA	*m*-Chloroperbenzoic acid
MDR-TB	Multiple Drug Resistant tuberculosis
Me	Methyl
MeLi	Methyl Lithium
MHz	MegaHertz
Min	Minutes
mL	milliters
mmol	millimols
mol eq.	molar equivalents
MS	Mass Spectroscopy
NADPH	Nicotinamide Adenine Dinuclotide Phophate Oxidase
NBS	*N*-Bromosuccinimide
*n*BuLi	*n*-Butyllithium
NMR	Nuclear Magnetic Resonance
NTM	Non Tuberculosis Mycobacteria
^oC	Degrees Celsius
PAS	*p*-Aminosalicylic acid
PAT	Penta Acyl Trehalose
PBS	Phosphate Buffered Saline
PCC	Pyridinium Chlorochromate
PDIM	Phthiocerol DI Mycocerosate
PE	Petrol Ether

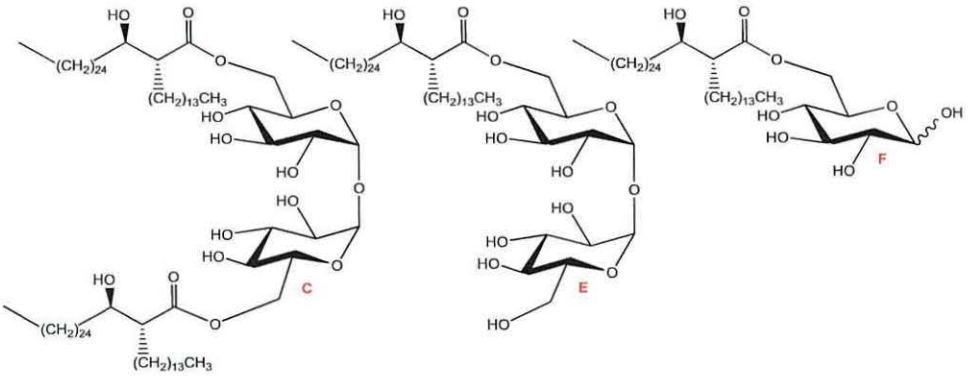
PFGE	Pulse Field Electrophoresis
PG	Peptido Glycan
Ph	Phenyl
PIM	Phosphatidyl Inositol penta Mannoside
ppm	parts per million
PPTS	Pyridinium *p*-toluenesulfonate
PTSA	*p*-Toluenesulfonic acid monohydrate
p-TsCl	*p*-Toluenesulfonyl chloride
p-TsOH	*p*-Toluenesulfonic acid
q	quartet
R.T.	Room Temperature
R_f	Retention factor
RFLP	Restriction Fragment Length Polymorphism
s	singlet
SAM	*S*-Adenosyl-*L*-methoine
sat.	Saturated
SL	Sulfated tetra-acyl Trehalose
SOD	Super Oxide Dismutase
t	triplet
TB	Tuberculosis
TBAF	Tetra-*n*-Butylammonium fluoride
TBDMSCl	*tert*-Butyldimethylsilyl chloride
TBDPSCl	*tert*-Butyldiphenylsilyl chloride
T-cells	T Lymphocites
TDM	Trehalose Di-Mycolate
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin-Layer Chromatography
TMM	Trehalose Mono-Mycolate
TPP	Tri-Phenyl Phosphine
VAP	Ventilator Associated Pneumonia
VLA	Veterinary Laboratories Agency
WHO	World Health Organisation
XDR-TB	Extensively Drug Resistant Tuberculosis

Abstract

Mycobacteria are found in many environments. They have complex mixtures of mycolic acids and lipids present in their cells wall. Mycolic acids are high molecular weight α-alkyl-branched β-hydroxy long-chain fatty acids. They have 60-90 carbon atoms. Different groups of mycolic acids are made by different species of mycobacteria. Sugar esters of mycolic acids which are associated with the cell wall have very interesting toxic and immunological properties. They also have the potential to assist in TB detection for development of sensors, which could be used for the control and treatment of mycobacterial infection. This research involved the synthesis of mycolic acids and trehalose esters. The biological activities were studied, as well as their suitability as antigens to detect mycobacterial infections. There were four objectives: The first part of this project involved the synthesis of saturated **(A)** and unsaturated **(B)** mycolic acids as occurring in *Rhodococcus equi* were achieved, and the synthesis of trehalose dimycolate **(C)**, trehalose monomycolate **(D)** and glucose monomycolate **(E)** present in *R. equi* were also achieved. This was to study whether or not the chain length has any effect on the biological activities in phagosomes-lysosome fusion assays.

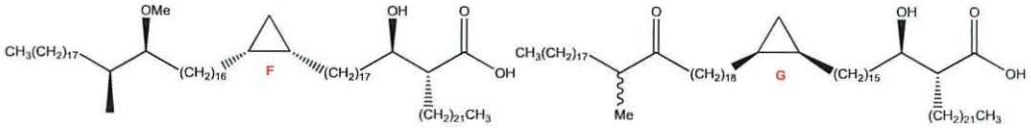


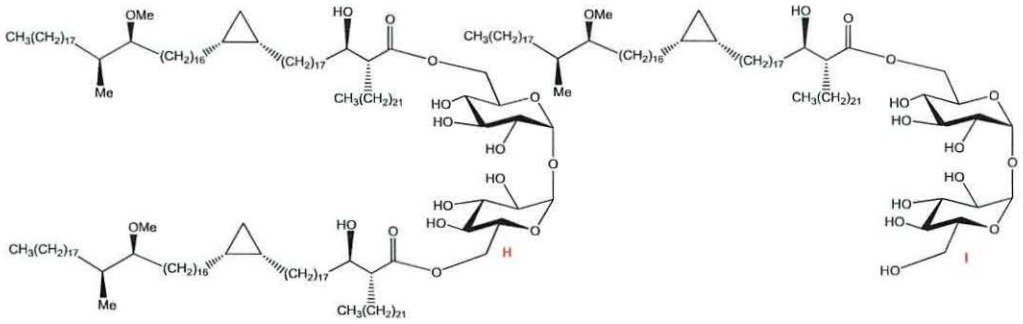




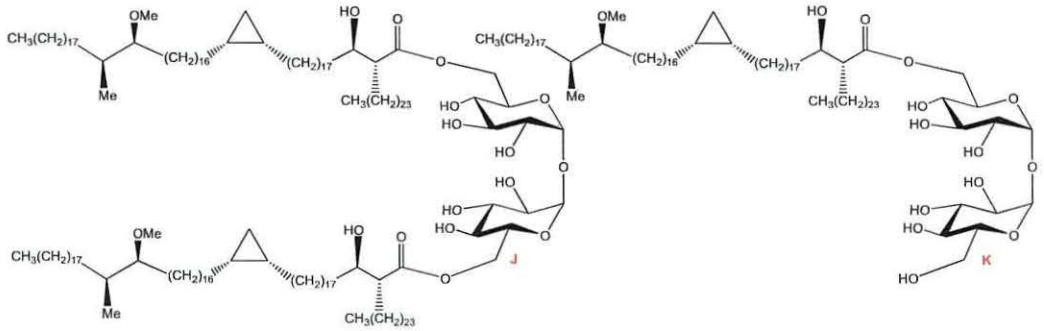
The second part of this project involved the synthesis of two stereoisomers of the key homologue of the methoxy **(F)** and keto **(G)** mycolic acids present in *Mycobacterium kansasii were* achieved successfully, which were then coupled to trehalose to generate the corresponding synthetic trehalose dimycolate **(H)** and trehalose monomycolate **(I)**.

These methoxy mycolic acid and their corresponding trehalose esters would be used as a specific antigens to distinguish the *Mycobacterium kansasii* from *Mycobacterium tuberculosis* specifically in serodiagnostic assays (Enzyme-linked Immunosorbent Assay).



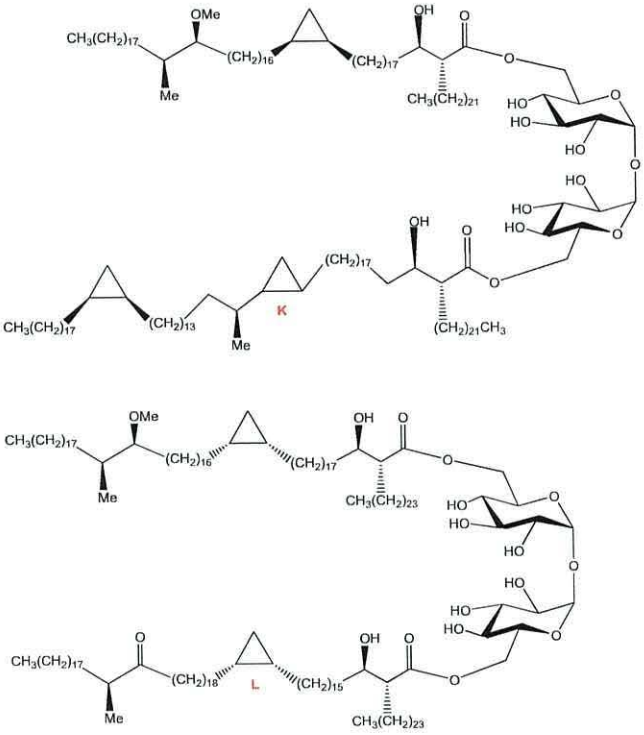


The third part of this study involved the synthesis of sugar ester of the *cis*-cyclopropane methoxy mycolic acid of *Mycobacterium tuberculosis* with identical absolute stereo-chemistry to the natural compound, which were then coupled to trehalose to generate the corresponding synthetic trehalose dimycolate **(J)** and trehalose monomycolate **(K)**. These trehalose esters would be used as a specific antigens in serodiagnostic assays (Enzyme-linked Immunosorbent Assay) for the detection of TB, will allow the effect of the stereochemistry of *cis*-cyclopropane on the assay to be investigated.



The fourth part of this study involved the synthesis of mixed sugar ester **(K, L)** including two different classes of mycolic acids present in *M. kansasii* and *M. tuberculosis* were achieved successfully. Natural TDM consists of a very complex

mixture of different molecules made in a biological system where there are many different types and homologues of MA present; unless there is a specific biological control system that introduces the same MA on both sugar rings of the TDM, it is therefore very unlikely that natural molecules contain two identical MAs. Therefore it is important to prepare compounds of containing two different MA in order to investigate their biological activity and compare them to the TDMs that contain the same MA at both positions.



1. Introduction

Mycolic acids and their sugar esters are components of *Mycobacterim tuberculosis* cells. They are strong signalling agents in the immune system. Natural mycolic acids are not a single molecular species but are present as very complex mixtures in the cell wall. This thesis reports the synthesis of individual mycolic acids and their sugar esters, which allows their specific effects to be determined. Consequently, the introduction below will provide some background information on tuberculosis, mycobacterial characteristics, mycolic acids, sugar esters, biosynthesis, synthesis, and ending with a discussion of the biological assays.

1.1 Tuberculosis

1.1.1 Background

It is estimated that each year there are 9.4 million new cases of TB, and that it is responsible for 1.6 million deaths.[1,2] Thus, it is still one of the greatest causes of death and illness in the world today,[3,4] despite a drop in TB incidence since a peak in 2004. Indeed, following a number of strategies recommended by the World Health Organisation, TB incidence has been slowly decreasing.[5,6] However, as this disease has afflicted mankind since the beginning of history, dating as far back as 9000 before Christ (BC), complacency would be most unwise and it remains to the present day a real scourge to human beings.[7] The Nobel prize winner, Koch, first identified the causative agent of TB when he discovered a stain which enabled the bacilli of *M. tb* to be seen.[5, 8] However, it was Marten who first suggested, in 1720, that TB was caused by a microscopic air-bound organism.[9] Regrettably, his findings were ignored until 1865 when Villemin demonstrated TB transmission from humans to cattle to rabbits, and so confirmed the theory proposed by Marten that TB was contagious.[10]

Many factors have contributed to the decrease in the disease since the end of the 19th century.[8,11] These include isolation hospitals, improved living and working conditions, the Bacillus Calmette Guérin (BCG) vaccine, the pasteurisation of milk, the discovery of X-rays, the tuberculin skin test and the discovery of anti-TB drugs.[5,12] However, the disease remains far from being totally eradicated. It was in the 1940s when the first effective drugs appeared.[8] Streptomycin was discovered by a team led by Waksman,

followed by *p*-aminosalicylic acid (PAS) in 1946 by Lehmann.[5,8] In 1952 isoniazid appeared, as did e.g., ethambutol, rifampicin and pyrazinamide.[5,13] With the exception of PAS, these drugs are in use to this day in the fight against TB. Complicating factors such as HIV/AIDS and drug resistance aside, today's treatment length has been reduced from twelve to six months as more effective chemotherapeutics have been developed. This shorter time span, in itself, helps in the battle against drug resistance and greatly assists drug compliance.[5,14]

The BCG vaccine is the most widely used vaccine in the world. It was developed in the early 19th century by Calmette and Guerin.[15] It is made up of the attenuated strain of *Mycobacterim. bovis* and remains in use.[16] The protection against disease by the vaccine does vary and overall is approximately 50%. For example, that against disseminated and meningeal disease in children is higher than that against pulmonary disease in adults.[15,16] Importantly, it is not known how long and to what extent the protective effect lasts, although giving the vaccine to new-borns (who are mycobacterially naïve) increases its efficiency.[17] Although the reactivation of pulmonary TB or HIV cannot be protected against by routine vaccination, it does, however, reduce mortality and infection from TB in infants.[18,19] Thus the development of a more effective vaccine would help enormously in the fight against TB. A new vaccine would also assist against the scepticism surrounding the BCG vaccine, causing some countries not to routinely vaccinate infants. Currently just 5% of deaths are prevented by the BCG vaccine.[20] This percentage needs to be increased. One advantage of the BCG vaccine is that it also protects against Buruli ulcer, leprosy and helminth infection.[21] In addition, it reduces the risk of superficial bladder cancer progression, and it may help against atopic diseases such as asthma.[22] Recent studies show a 37% reduction in asthma in those who received a neonatal BCG vaccination.[2]

1.1.2 Global illness

The WHO has reported that TB infects one person every second, of whom 5-10% die as a direct result.[6] Indeed, the WHO estimates that up to 50 million people worldwide may be suffering from drug-resistant forms of TB and for this reason they declared a global health emergency in 1993 in an attempt to save 14 million lives between 2006 and 2015.

In 2012, there were an estimated 8.6 million cases of TB globally, corresponding to 122 cases per 100,000 population. The highest number of cases occurred in Asia 58% and the African Region 27%. Smaller proportions occurred in the Eastern Mediterranean 8%, the Europe 4% and the Americas 3%. The cases included 1.0-1.2 million 12-14% among people co-infected with HIV. The proportion of TB cases co-infected with HIV was highest in the African Region (**Figure 1**). In General, 37% of TB cases in this region were estimated to be co-infected with HIV, which accounted for 75% of TB cases amid people living with HIV worldwide. In parts of southern Africa, more than 50% of TB cases were co-infected with HIV (**Figure 1**).[24]

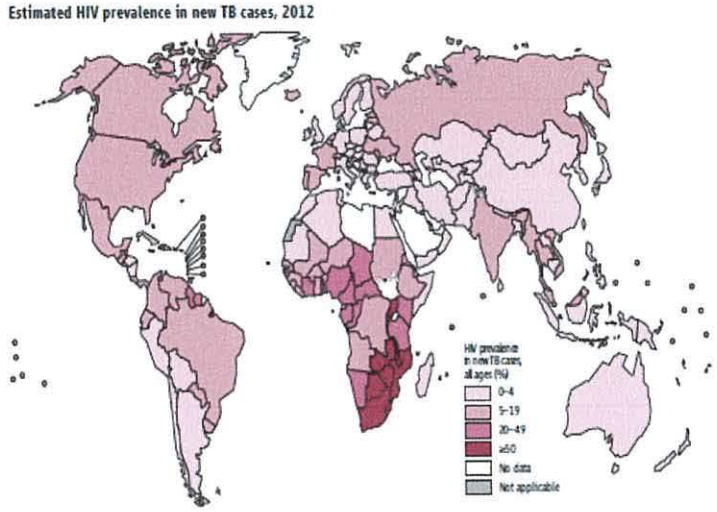


Figure 1: Estimated HIV prevalence in new TB cases in the world. Data from WHO 2013[24]

Thus, the pathogenesis of the HIV/AIDS-TB pandemic presents an urgent challenge to the scientific community due to the terrible effects it has on the world's most vulnerable communities.[25] The new WHO statistics on TB suggest that HIV co-infection causes about half of all TB deaths. Indeed, HIV infection increases the probability of latent TB infection developing into active TB disease. A new TB infection progresses considerably more rapidly to a fully-active disease in immune-compromised AIDS patients. Therefore, TB is the main killer of AIDS patients and often the first disease to develop after the immune system submits to HIV infection.

1.1.3 The emergence of drug resistance

This problem became apparent soon after the first anti-TB drugs were used. TB germs were found to be still growing in the sputum of a patient who had completed a course of streptomycin treatment, and a new deadly form of TB appeared in patients who had seemingly been treated with success.[8] This resistance has become more and more frequent and is most alarming as it is becoming apparent in the case of both first-line and second-line drugs.[8]

Multidrug Drug Resistant (MDR) is the term used for TB strains which are resistant to the main first-line drugs, with resistance to any fluoroquinolone drug or at least one of the three injectable second-line drugs, kanamycin, capreomycin and amikacin. In recent years, MDR-TB has risen in patients because of initial infection by drug resistant strains of TB. This is most common in those injecting drugs using contaminated needles and in HIV sufferers.[13,26] As streptomycin and front-line drugs are ineffective in preventing resistance there is little to prevent its progression to second-line drugs.[5] Soon after it was discovered that using one drug was not effective in the battle against TB, a combination of streptomycin and PAS was used for a two year treatment period. Isoniazid (INH) was added to the combination in 1952 when PAS was replaced by the more effective drug ethambutol. The treatment time was reduced from 24 to 18 months.[14] Combined drug therapy helps prevent resistance from developing as bacterial mutations are random and their incidence will be consequently reduce with the more rapid and comprehensive destruction of the pathogenic population. In addition, as the mutations are not linked the likelihood of the TB microbe developing resistance to two drugs is 1 in 10.[27,28] Treating patients with MDR-TB is difficult even where state-of-the-art facilities and drugs are available.[29] Thus, new non-resistant chemotherapeutics are desperately needed in the battle against TB.[30]

1.2 Mycobacteria

1.2.1 *Mycobacterium tuberculosis*

M. tb is a small rod shaped bacterium which is classified as acid fast, since it retains certain stains after treatment with acidic solutions.[31,32,33] The mycobacterial bacilli are

resistant to weak disinfectants and 1M sodium hydroxide. In fact, they are able to survive under a variety of adverse conditions, including the ability to exist in a dry state for several days.[34,35] 86% of *M. tb* infection is spread by people who have pulmonary TB.[36] Coughing and sneezing propels droplets containing TB bacilli into the air which is then inhaled by others. However, fresh infection will only occur if the inhaled bacilli reach the alveoli where the host's macrophages and other mononuclear phagocytes will be invaded.[36] **Figure 2** illustrates the ingestion of TB bacilli by host macrophages using scanning electron microscopy.[37]

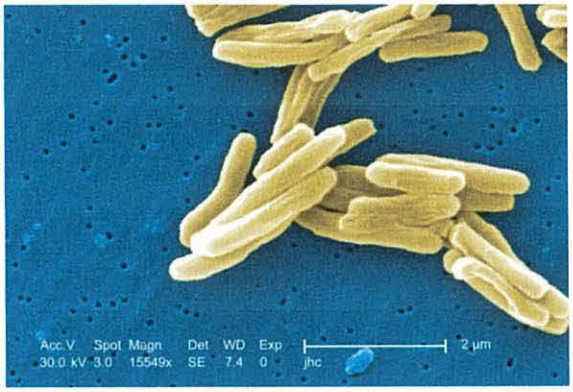


Figure 2: A scanning electron micrograph of *Mycobacterium tuberculosis*[37]

Mycobacteria include both non-pathogenic and pathogenic species.[38,39] Species of pathogenic mycobacteria causing TB in mammals are *M. tb*, *M. bovis*, (which is responsible for causing bovine TB), *Mycobacterium africanum* (a heterogenous group of strains isolated from equatorial Africa inhabitants) and *Mycobacterium microti* (a rodent pathogen).[40] Other mycobacterial species causing disease in man are *Mycobacterium leprae* (which causes leprosy), *Mycobacterium ulcerans* (which is responsible for the dangerous and potentially fatal Buruli ulcer, a skin and sometimes bone affection).[41,42] Other pathogens include *Mycobacterium marinum* (which causes disease in fish and skin infections in humans) and *Mycobacterium avium*, (an illness of poultry first discovered in 1890, and is also known as 'Battery' bacillus).[43,44]

M. bovis has the most diverse range of hosts, as it is found not just in bovine animals but also in man, dogs, cats, pigs, goats and wild animals such as badger and deer.[40] It is economically important to the farming industry and it is reckoned to have cost the UK tax payer £87 m in 2009-2010, a figure expected to soar to £ 1 b in the next

decade.[45,46] It is controlled by routine tuberculin testing of whole herds every 1-4 years depending on how prevalent TB is in the area.[47] It is spread by moving cattle from farm to farm, at cattle auctions and through the badger populations which live on farms where *M. bovis* exists. The proposed cull of badgers in order to remove the reservoir of infection has caused a lot of controversy with animal rights campaigners for many years.[48] This is illustrated in **Figure 3**.[49]

Figure 3: Campaigners against badger culls[49]

Nevertheless, unlike *M. tb*, *M. bovis, M. microti, M. kansasii, M. smegmatis*, a large number of other mycobacterial strains only affect individuals whose immune system is suppressed, for example HIV/AIDS sufferers or transplant patients.[43,44]

1.2.3 *Mycobacterium kansasii*

It is probable that *M. kansasii* is the easiest non-tuberculosis mycobacterial (NTM) pathogen to treat successfully. This is because *M. kansasii* and *M. tb* are similar and because antituberculosis drugs work well against *M. kansasii*. Indeed, more information exists showing how effective antituberculosis drugs are for treating *M. kansasii* than all the other NTM infections. Also, because it is similar to *M. tb*, mycobacterial isolates may be linked to lung disease which can be both aggressive and destructive (**Figure 4**).[50] If it is not treated correctly, it can lead to lung destruction or the mycobacterial isolates becoming drug resistant, or both. The importance of early diagnosis before the disease is able to develop must be emphasised. In addition, it is important that effective overall treatment strategies are used and that patients are given the correct medications for a long time period.[50]

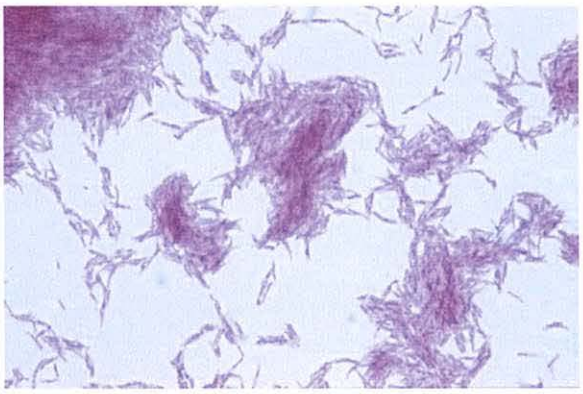


Figure 4: *Mycobacterium kansasii* (coloration de Ziehl)[50]

1.2.3.1 Pathogenesis and epidemiology

It is likely that infection by *M. kansasii* happens by an aerosol route. It is probable that tap water is a major reservoir, causing infection in humans. It has been isolated in tap water in places where patients with the disease have been identified.[51,52] Isolates which have the same phage type as those found in patients have also been found in the drinking water systems in the Netherlands.[53,54] Environmental isolates of the same genotype, which have been determined by pulse field electrophoresis (PFGE) to be clinical isolates, have been found in Paris.[55] Isolating *M. kansasii* from tap water is at times intermittent. This could explain why investigations have failed to uncover it. No other environmental source (water or soil) source of this mycobacterium has been found. It is not yet known why it is not possible to isolate mycobacteria from other environmental sources.

Studies have shown that the *Mycobacterium* has genetic diversity, using isolates from around the world. Restriction Fragment Length Polymorphism (RFLP) analysis of chromosomal DNA is a frequently used technique. This requires endonuclease digestion of whole DNA, giving many variably sized fragments which are separated by Pulsed Field Gel Electrophoresis (PFGE). The DNA fragment patterns can be compared visually or they can be scanned into a computer data base. A recent DNA based study which used PFGE discovered the presence of five taxonomic groups in both human and environmental isolates.[55] A predominant PFGE pattern was found in patients with *M. kansasii* disease.[55] 51 clinical isolates of mycobacteria from the USA

were recently evaluated using PFGE by Zhang *et al.*.[56] Half the isolates had the same PFGE pattern and this was the same predominant pattern reported by Picardeau.[55] Due to the close relationship of most clinical *M. kansasii* isolates by PFGE, epidemiologic studies of strain relatedness of *M. kansasii,* in suspected outbreaks, will not be easy.

Lung disease caused by *M. kansasii* happens in clusters geographically. Studies revealed that disease happened more often than *M. avium* complex (MAC) in southeast England and in Wales.[57,58] Only estimates are available for its prevalence because this is not a public health problem and so is not reported. *M. kansasii* is the second most common cause of NTM lung disease in the USA. It occurs predominantly in the central and southern states . In a recent study by Bittner *et al.*, it was reported that *M. kansasii* was the most common mycobacterial pathogen isolated at Veterans Affairs Hospital in Omaha, Nebraska. This was over a period of 20 years, spanning from 1971 to 1990.[59] The number of *M. tb* isolates declined in this study over 20 years, however the *M. kansasii* isolates stayed relatively stable over the same time period.

The result was that the total number of *M. kansasii* isolates exceeded the *M. tb* isolates. In a demographic study of NTM pulmonary disease in Texas, *M. kansasii* was second only to MAC as a cause of NTM lung disease. This study also reported that *M. kansasii* was much more likely to originate for urban than rural areas. This is consistent with the current understanding of *M. kansasii* reservoirs, as noted above.[60] Where HIV infection is common, the prevalence of *M. kansasii* is likely to also be very high, because of the greater susceptibility of the host population to infection, rather than factors relating to the virulence of the organisms or the presence of the organisms in the environment.[61]

1.2.4 *Rhodococcus equi*

Rhodococcus equi is a nocardioform Gram-positive coccobacillus. It can cause severe pyogranulomatous pneumonia in young horses (**Figure 5**). It causes TB like symptoms and histopathology in AIDS patients. *R. equi* is a facultative intracellular bacterium, able to survive and multiply in macrophages *in vitro* and *in vivo.*[62]

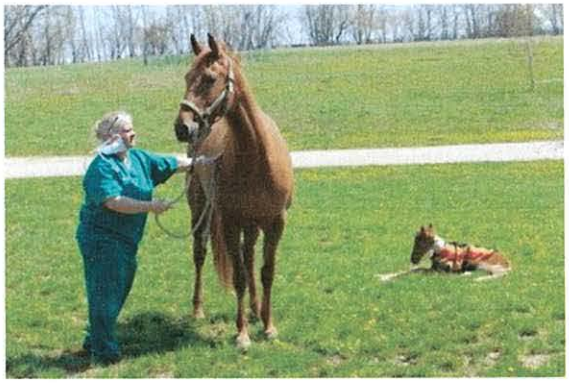


Figure 5: *R. equi* causes a lethal form of equine pneumonia in foals[63]

In macrophages, phagosomes which contain the virulent form (Ventilator Associated Pneumonia (VAP) positive) *R. equi* are arrested early in their maturation. They do not acquire the proton-pumping vacuolar (Adenosine Tri Phosphate (ATPase) complex. Macrophages die by necrosis after 1-3 days of infection, by which time intracellular multiplication has happened. These eventually mature into phagolysosomes where bacteria are slowly killed.[62]

1.2.5 The cell envelope

Fundamental to the pathogenesis of *M. tb* is the organisation and structure of the cell envelope. For this reason, it is important to have an understanding of these factors in the fight against TB and other related diseases.[35] The complex cell envelope of mycobacteria has a high percentage of lipids and it is unusually thick.[35,65] It consists of three different parts, a plasma membrane, a wall and a capsule.[34] The core of the cell wall exists above the plasma membrane and is made up of interaction of methyl branched long chain components with MA matrix. Mycolyl arabinogalactan (mAG) is connected by a phosphoryl linker unit to Peptido Glycan (PG). Complex free lipids (Sulfated tetra-acyl Trehalose (SL), Di Acyl Trehaloses (DAT), Phthiocerol DI Mycocerosate (PDIM), Penta Acyl Trehalose (PAT)) interact with (mAG). Lipoarabinomannan (LAM) and Phosphat Idylinositol Penta Mannoside (PIM) are shown anchored in the plasma membrane. The mAG galactan is shown in yellow, and the LAM mannose components are in dark green; the arabinan of both these polysaccharides is represented in light blue, illustrated in **Figure 6**.[71] These will be discussed in detail in **Section 1.3**.[31,33,67]

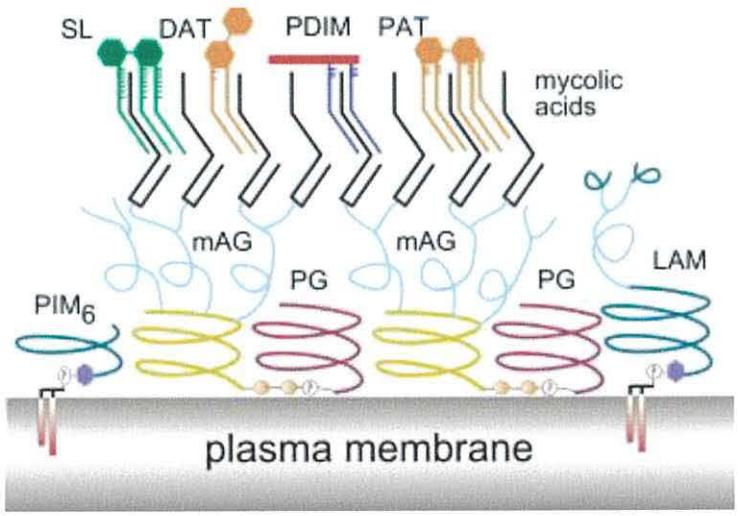


Figure 6: Schematic representation of mycobacterial cell envelope[71]

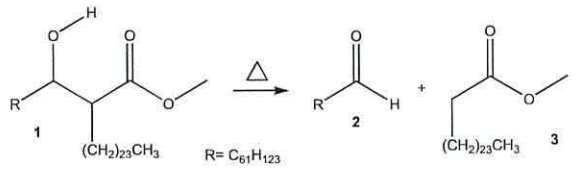
Disruption of the mycobacterial cell wall, followed by extraction using a variety of solvents, results in lipids, lipoglycans and proteins, while the cell wall core remains as a residue which is insoluble. The substances which can be extracted can be considered as signalling effector molecules, while the cell wall core which is made up of arabinogalactan-mycolic acid complex, is essential for the viability of the cell. Therefore, it is very important in terms of effective drug development.[66,68] In order for pathogenic mycobacteria to prosper within host macrophages, a hostile environment, their outer capsule needs to protect the inner parts of the bacterium from the host's defences. The capsule does this by stopping any harmful substances from the host entering the inner parts of the cell envelope.[68] These bacteria are also able to inhibit fusion between phagosomes and lysosomes, which further defends them against degradation by the host macrophages defences.[69] In addition, it is believed that the capsule interferes with the host's defences by rendering inactive small molecules which the phocytic cells use to kill micro-organisms. An example of this would be reactive oxygen derivatives such as hydroxyl, hydrogen peroxide, nitric oxide and superoxide. These species are able to diffuse through the capsule's barrier. However, they are made inactive by *M. tb's* superoxide dismutase (SOD) and its catalase/peroxidase.[68] Thus mutants of *M. tb* defective in catalase had a reduced virulence in guinea pigs. Also, the virulence was restored by the integration of the gene that codes for catalase-peroxidase into a catalase negative mutant.[68]

In the cell wall of the mycobacteria there are mycolic acids, sugar esters and other lipids.[70,71] These include methyl branched fatty acids, phthiocerols, lipomannan, sulpholipids, lipoarabinomannans and many others.[72,73] The mycobacterial lipids make up 40% of the cell envelope's weight. They have been studied in depth to establish their structure, function, biosynthetic pathway and their role in mycobacterial pathogenesis.[74,75] MAs bound to trehalose are extractable lipids also found in the cell wall. These are known as 'cord factor' and are bio-medically important glycolipids. They are made up of a trehalose which is esterified at either one or both primary alcohol groups to form either a trehalose 6,6'–monomycolate (TMM) or trehalose 6,6'–dimycolate (TDM).[71,76] In biosynthesis, TMM transfers newly synthesised mycolic acids to the cell wall. It remains unproved that this is the case for TDM.[71,77,78] The toxic properties and the adjuvant effects of cord factor in mice were discovered early in the study of mycobacterial lipids.[79] In rats, cord factor has good adjuvant effects, inducing delayed hyposensitivity and encouraging antibody production.[80] When mice were injected, it had the same effect as infection with live BCG.[81] TDM has also been seen to have tumour regressive properties with a variety of cancers in guinea pigs.[82,83] TDM and TMM display a range of immune activities and are useful for a wide range of biomedical applications.[84,85]

1.3 Mycolic acids

MAs are a major element of the cell envelope of *M. tb*. They are major components of the waxy portion.[86] They are characteristic of mycobacteria and give unique properties to the cell wall structure, making it impervious to practically all of the host's defences and other chemotherapeutic agents which would otherwise damage or render unviable normal bacterial cells.[76,87] In 1927 Anderson *et al.* conducted the first large scale tubercle bacilli extraction.[88] It was then that the term MA was first used for the 'non saponifiable wax' that was found during this extraction process. The name was first used during subsequent investigations into the hydroxy acid found in the ether fraction.[89,90] Anderson discovered that the lipids of the tubercle bacilli appeared to have more than just the ordinary biological and chemical importance from the point of view of the bacillus having resistance to the host's destructive influences. He also found that some of the components appeared to cause abnormal cell development in animals.[88] The total characteristics of MA were documented in later studies and

Anderson correctly found their formula as being either $C_{88}H_{172}O_4$ or $C_{88}H_{176}O_4$. He also found they have a 'ring structure', a molecular mass of 1284 and that their melting point is 54-56 °C.[91] He described the resulting decomposition of mycolic acids and their esters by a retro-aldol reaction if they are heated to 300-350 °C. This releases a methyl ester of hexacosanoic acid and a non-volatile 'meroaldehyde' (**Scheme 1**).[81] Fungal and avian strains of mycobacteria have a C_{22} side chain and yield tetracosanoic acid. Polgar named this acidic product of decomposition meromycolic acid.[81]



Scheme 1: Products of the pyrolysis of a typical methyl mycolate ($C_{89}H_{178}O_3$) from a human strain of *M. tb*[81]

It has been noted that there are more than 500 types of mycolic acids in *M. tb*, each having intimately related chemical structures.[92] They have been studied since their discovery and research continues because they are unique to mycobacteria and because they are important to their virulence. Although much has been learnt about their structure and function, a lot remains to be learnt. New instrumentation has led to advances in what is known about their structure.[92] Recently a lot of research has been done on MA using electron impact (EI) mass spectroscopy, to understand the location and chain lengths of the functional groups.[86,93,94,95]

This method successfully located the position of cyclopropane rings in the mycolates.[96,97] However, with non-derivatised homologous mixtures of MA, this method is unable to provide full structural elucidation. This is because it is not able to reliably locate certain functional groups within MA. Matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) was introduced. It provided a quick and highly sensitive method for elucidating the structures of MA and other lipids.[98,99,100] Using a combination of IR, NMR and mass spectrometry, the structures of mycolic acids were largely elucidated.[81,101] They are long chain β-hydroxy-α-alkyl fatty acids, 60-90 carbons long and found in all mycobacteria and related micro-organisms.[65,102,103] MAs have been found as free lipids also, linked with mycobacterial

biofilm formation. It is thought that this helps the bacteria resist chemotherapy when in an inactive state.[104] Computer models of the mycobacterial cell envelope have provided evidence to support this hypothesis.[105,106] **Figure 7** illustrates a generalised structure for MAs. In green, the motifmycolate of the mycolic acid is shown. The black depicts the meromycolate part.[107,108]

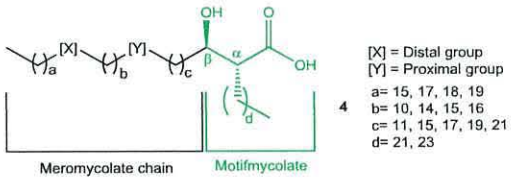


Figure 7: The general structure of mycolic acids[107,108]

Common to all MA is the structure of the motifmycolate. The only difference is the chain length of the branch in the α-position with respect to the carboxylic acid. The meromycolate part of the MA has wider structural diversity. It varies in the substitution of the proximal and distal positions in respect to the carboxylic acid.[65, 98] Based on the substitutions at the proximal and distal positions MA can be divided into three categories. Firstly, type 1 mycolates, containing no double bonds. Secondly, type 2 mycolates, containing a *trans*-double bond. Finally, type 3 mycolates containing a *cis*-double bond (**Figure 8**).[65,98] The functional groups of the meromycolate chain can be any of the following: cyclopropanes, double bonds, a carbonyl group, an epoxy group, a methyl group or a methoxy group.[109] Research using techniques such as 2-D TLC,[110] HPLC[109] and GC[111] with mass spectrometry, NMR and IR has identified several types of mycolic acids in each *Mycobacterium*. To determine the species of mycobacteria, HPLC patterns have been utilised as a quick diagnostic method since these are found for every *Mycobacterium*.[112]

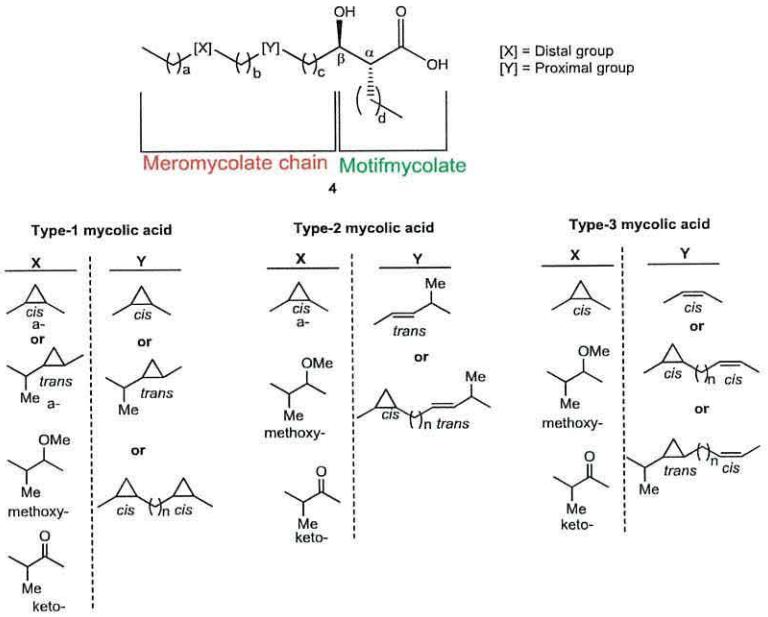


Figure 8: Generalized structures of major mycobacterial mycolic acids[65,98]

The three main types of MA synthesised by *M. tb* and other pathogenic myco-bacteria are α-MA (these are substituted at the proximal and distal positions with cyclopropane rings), keto and methoxy MA (these have oxygenated functional groups in the distal positions) (**Figure 8**).[113]

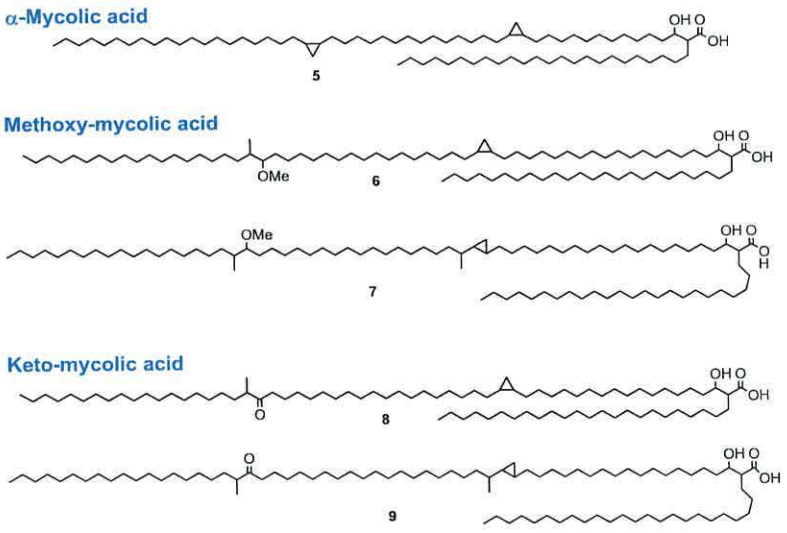


Figure 9: Major types of mycolic acids from *M. tb*[65,98]

Figure 9 illustrates the following:

(1): The cyclopropane rings of the α-MA **(5)** are generally in the *cis*-configuration.

(2): The methoxy-MA **(6,7)** are substituted in the distal position by a MeO-group.

(3): Keto-MA **(8,9)** are substituted with a carbonyl group.[65,98]

With respect to the oxygenated functional group, keto and methoxy MA both have a methyl branch in the α-position. They are substituted at the proximal position in natural mixtures with either a *cis*- or α-methyl *trans*-cyclopropane ring. Watanabe *et al.* carried out extensive research on the nature and position of the functional groups of the meromycolate chains of MA of representative mycobacteria using MS and NMR.[65,98] Other types of mycobacteria contain different mixtures of MA with more variety in the meromycolate chain. Illustrated in **Figure 10** are the MA of saprophytic mycobacteria: *M. smegmatis* α' and α-MA **(10)** and **(11)**, which contain either one or two double bonds in either *cis* or *trans* configuration.[114] The MA of *Mycobacterim. fortuitum* contain an epoxide ring **(12)**.[115,116] In *Mycobacterim. phlei* are found ω-carboxy-MA **(13)**[117] while ω-methoxy-MA have been found in *Mycobacterim. alvei* **(14)**.[118]

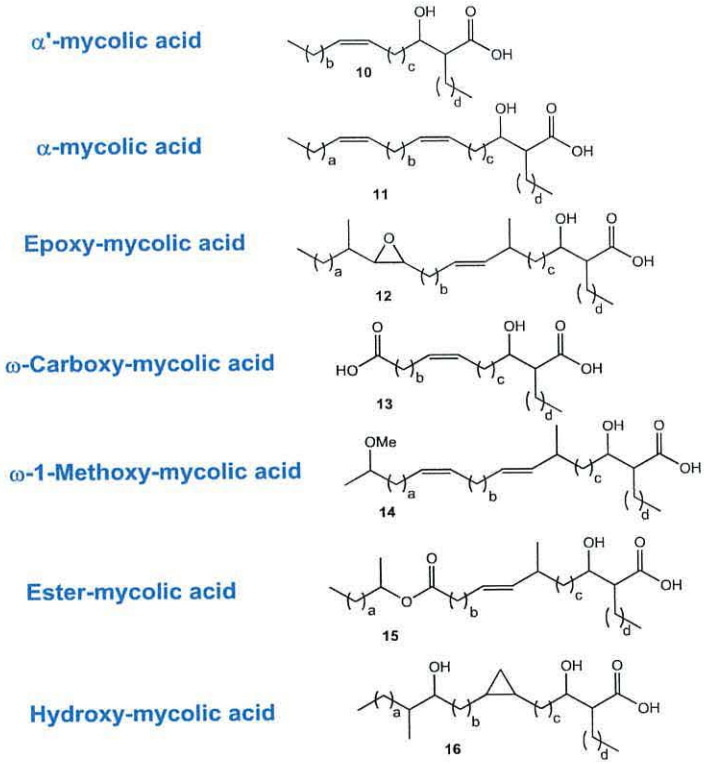
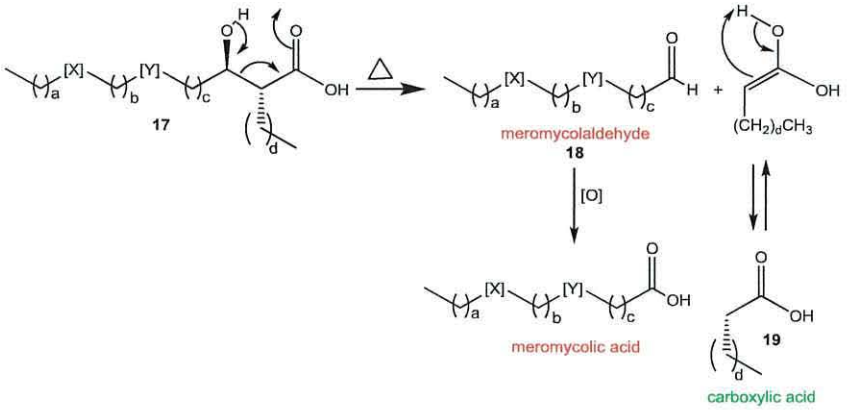


Figure 10: Some of the mycolic acids present in different mycobacteria

Wax ester **(15)** (**Figure 10**), is another variant of MA, found in *M. aurum*. It is made by a Baeyer-Villiger type reaction on the carbonyl group of a keto mycolic acid.[119] MA (both major and minor) present in other mycobacteria, have different variations at the proximal and distal positions. Following a careful examination of all their mycolic-like fatty acids, small amounts of hydroxy MA **(16)** have been found in *M. bovis* BCG and also *M. tb*. It was found that a gene cluster isolated from *M. bovis* (BCG) conferred upon *M. smegmatis* the ability to synthesise hydroxy MA with cyclopropanated keto MAs.[93,94] Through analysis of the mycolic acids of *M. tb* H37Ra, H37Rv and R1, Asselineau found the presence of a MA with two hydroxyl groups. However, the complete structure of this compound was not fully established. From this, it would appear that hydroxy MA are present in all species of the *M. tb* complex.[65]

1.3.1 Chain length of mycolic acids

Lava *et al*. and Watanabe *et al.* were the first to analyse mycolic acids using MALDI-TOF MS in 2001.[65,100] Lava *et al*. studied the length of all the carbon chain of the main types of MA from the fast-growing non-pathogenic mycobacteria to the slow-growing pathogenic mycobacteria. They came to the conclusion that the lengths of the chains were linked to the growth rate of the mycobacterial strains.[100] It was found that pathogenic strains of mycobacteria synthesised chains containing even numbers of carbons (C74-C82). Non-pathogenic strains produced chains with both odd and even numbers of carbons. Also discovered was a similarity between the masses of oxygenated MA in rapid growing mycobacterial species and in the α-MA. This suggested a biogenetic relationship between these two types of MA. This was not the case between α-MA and oxygenated MA of slow-growing pathogenic species (e.g. *M. tb*) and no relationship was discovered.[100] In fact, the chains lengths of the oxygenated MA were between four and six carbons longer than those of the α-MA in the slow-growing pathogenic species.[119,120] To determine the size of meroaldehyde **(18)** and the saturated α-chain **(19)**, pyrolysis of MA **(17)** was carried out, followed by mass spectrometry. This gives information on the internal chain lengths as illustrated in **Scheme 2**.[98,121] It was revealed that the mycolate chains vary between 60 and 90 carbon units and that the α-chain varies between 22 and 26 carbons in length.[92,122]



Scheme 2: Pyrolytic cleavage of a mycolic acid, followed by oxidation with Ag_2O to prepare meromycolic acids for analysis[98,121]

Many techniques have been used to determine the other internal chain lengths (a, b, c, d, in **Scheme 2**) of different MA. The results have been ambiguous.[97,123,124,125] Watanabe *et al.* used a combination of collision-induced dissociation (CID) MS and MALDI-TOF to work out the positions of the functional groups found in both minor and major MA in different strains of the *M. tb* complex.[73,98] The a-b-c values for the MA were elucidated as shown in **Figures 11, 12** and **13**.

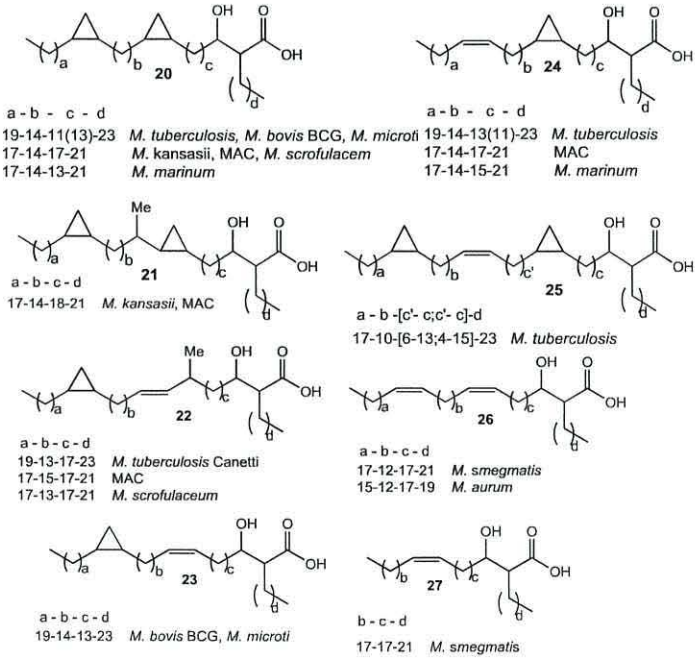


Figure 11: Structures and distribution of major and minor α-mycolic acids[98]

Watanabe discovered that the methyl branch next to the oxygenated function and the *trans*-cyclopropane ring in the keto and methoxy MA (for example, **28, 29, 35** and **36)** was positioned nearest to the ω end with respect to the functional group. It was found that the methyl branch next to the *trans*-double bond, as in **(22, 31** and **38)** was located nearest to the carboxylic end of the mycolic acid. In addition, they discovered a close structural relationship between the keto and the methoxy MA. This was put forward after studying matching keto **(8** and **9)** and methoxy **(6** and **7)** MA. It was found that their a-b-c values generally matched, as shown in **(28, 29, 35** and **36)** in **Figures 12** and **13**.[98]

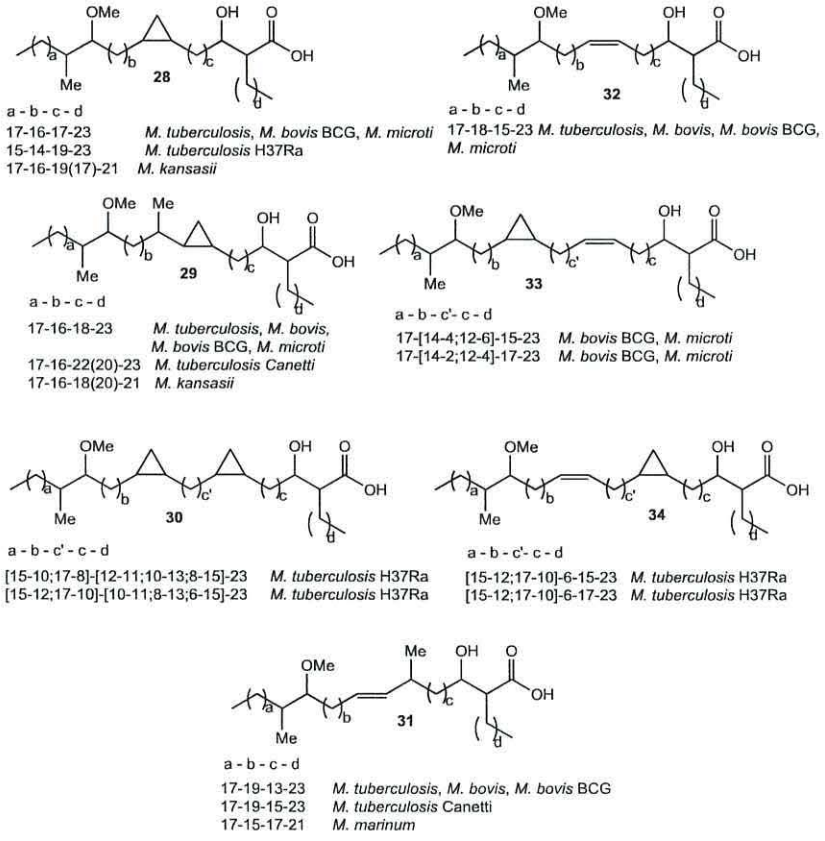


Figure 12: Structures and distribution of major and minor methoxy-mycolic acids[98]

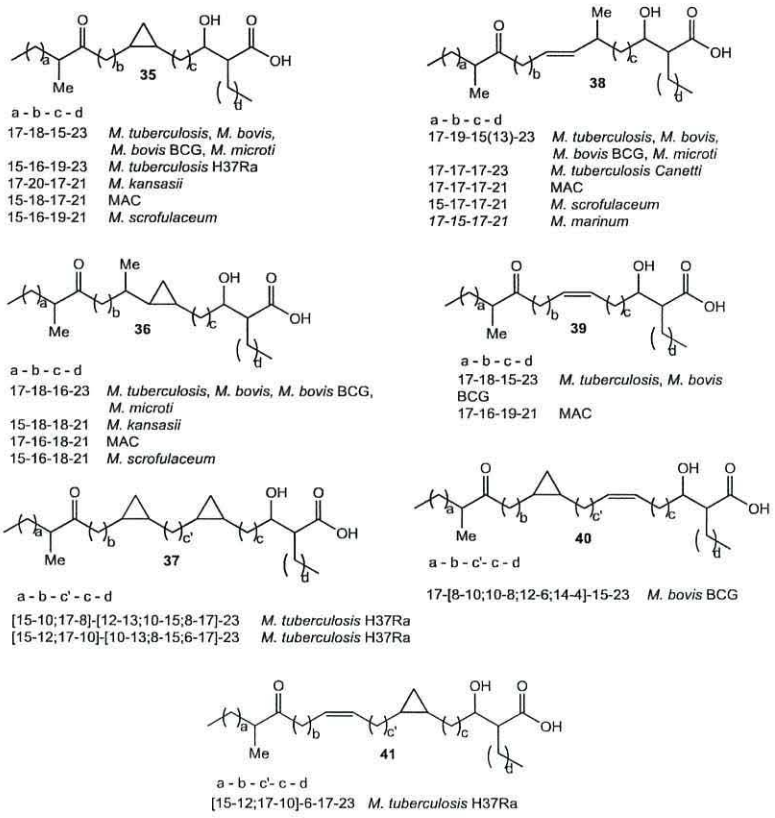


Figure 13: Structures and distribution of keto-mycolic acids[98]

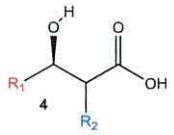
1.3.2 Mycolic acids of *Rhodococcus equi*

Typically these MA contain 34-52 carbons. Klatte *et al.*[126] noted that this range has been extended from 30-54 carbons. The distinction between the alkyl side chain and the meromycolate main chain is an important feature of the structure of MA. Typically the alkyl branch is fully saturated and is 10-14 carbons long.[126] The meromycolate side chain is longer (C28 chain in a C42 mycolic acid with a C14 alkyl side chain) and it might carry up to four double bonds as observed by Alshamaony *et al.*[127] It seems likely that the positions of the unsaturated bonds are located in the distal regions of the meromycolate chain, such as in mycobacterial MA and those of *Nocardia asteroides*.[128] As a result, the region of the meromycolate chain next to the ester-linked terminus is, in effect, a saturated chain.[129] It would seem that the MA in the free lipid fraction are similar in size to those in the bound lipid fraction as found by Asselineau *et al.*[130]

[M-H]⁻ (m/z)	Number of Carbons: Number of unsaturated bonds	Mycolic acids (meromycolate chain/α-branch)	
		Major structure	Other isomers
467	30:0	18:0/12:0; 16:0/14:0	16:0/14:0; 20:0/10:0
481	31:0	17:0/14:0	18:0/13:0; 19:0/12:0; 20:0/11:0
493	32:1	20:1/12:0; 18:1/14:0; 18:0/14:1	20:0/12:1; 16:0/16:1; 16:1/16:0
495	32:0	18:0/14:0	20:0/12:0; 16:0/16:0
509	33:0	19:0/14:0; 18:0/15:0	17:0/16:0; 20:0/13:0; 21:0/12:0;
521	34:1	20:1/14:0	18:0/16:1; 18:1/16:0; 20:0/14:1; 22:1/12:0
523	34:0	20:0/14:0; 18:0/16:0	22:0/12:0
535	35:1	21:1/14:0; 20:1/15:0; 19:1/16:0	19:0/16:1; 18:0/17:1; 20:0/15:1; 23:1/12:1; 22:1/13:0
537	35:0	19:0/16:0; 20:0/15:0	21:0/14:0;18:0/17:0; 22:0/13:0; 23:0/12:0
547	36:2	20:1/16:1	22:1/14:1; 20:2/16:0; 19:2/17:0; 18:2/18:0; 22:2/14:0; 24:1/12:1; 21:2/15:0; 24:2/12:0
549	36:1	20:1/16:0; 22:1/14:0	20:0/16:1; 24:1/12:0
551	36:0	20:0/16:0	22:0/14:0; 24:0/12:0; 18:/18:0
563	37:1	21:1/16:0; 23:1/14:0	22:1/15:0; 20:0/17:1; 20:1/17:0; 21:0/16:1; 19:0/18:1; 19:1/18:0; 25:1/12:0; 22:0/15:1; 26:1/11:0, 27:1/10:0
565	37:0	21:0/16:0	20:0/17:0; 23:0/14:0; 24:0/13:0; 25:0/12:0; 19:0/18:0; 18:0/19:0
575	38:2	22:1/16:1	24:1/14:1; 26:2/12:0; 19:1/19:0; 20:1/18:0; 24:2/14:0; 26:1/12:1; 22:2/16:0; 20:1/18:1
577	38:1	22:1/16:0; 24:1/14:0	26:1/12:0; 22:0/16:1; 20:0/18:1; 24:0/14:1
579	38:0	22:0/16:0	24:0/14:0; 20:0/18:0; 26:0/12:0
591	39:1	23:1/16:0; 25:1/14:0	24:1/15:0; 22:1/17:0; 26:1/13:0; 27:1/12:0; 23:0/16:1; 22:0/17:1; 21:0/18:1
593	39:0	23:0/16:0; 25:0/14:0	22:0/17:0; 24:0/15:0; 27:0/12:0; 26:0/13:0
603	40:2	24:1/16:1; 26:1/14:1; 26:2/14:0	28:2/12:0; 21:2/19:0; 22:2/18:0; 24:2/16:0; 22:1/18:1; 28:1/12:1
605	40:1	26:1/14:0; 28:1/12:0	30:1/10:0; 26:0/14:1
607	40:0	24:0/16:0; 26:0/14:0	28:0/12:0
619	41:1	27:1/14:0; 25:1/16:0	26:1/15:0; 29:1/12:0; 28:1/13:0; 24:1/17:0; 30:1/11:0
631	42:2	28:2/14:0; 26:1/16:1; 26:2/16:0	28:2/14:0; 28:1/14:1; 24:1/18:1; 30:1/12:1
633	42:1	26:1/16:0	28:1/14:0; 30:1/12:0; 26:0/16:1; 24:1/18:0
635	42:0	26:0/16:0	28:1/14:0
645	43:2	29:2/14:0	27:2/16:0; 28:2/15:0; 27:1/16:1; 30:2/13:0; 31:2/12:0; 26:1/17:1;29:1/14:1; 28:1/ 15:1; 26:2/17:0
647	43:1	29:1/14:0; 27:1/16:0	28:1/15:0; 26:1/17:0; 30:1/13:0; 31:1/12:0
659	44:2	30:2/14:0; 28:2/16:0	28:1/16:1; 30:2/14:0; 32:1/12:1; 26:1/18:1
661	44:1	28:1/16:0	30:1/14:0; 26:1/18:0
673	45:2	31:2/14:0; 29:2/16:0	30:2/15:0; 26:2/19:0; 29:1/16:1; 32:2/13:0; 33:2/12:0; 28:2/17:0; 28:1/17:1; 27:2/18:0
675	45:1	29:1/16:0; 31:1/14:0	30:1/15:0; 28:1/17:0; 27:1/18:0
677	45:0	30:0/15:0	29:0/16:0; 31:0/14:0; 28:0/17:0
687	46:2	30:2/16:0; 32:2/14:0	30:1/16:1; 28:1/18:1
689	46:1	30:1/16:0	32:1/14:0
701	47:2	31:2/16:0	33:2/14:0; 32:2/15:0; 30:2/17:0; 31:1/16:1; 32:1/17:1
703	47:1	31:1/16:0	33:1/14:0; 30:1/17:0; 32:1/15:0; 29:1/18:0
715	48:2	32:2/16:0	34:2/14:0; 32:1/16:1; 30:1/18:1; 30:2/18:0
717	48:1	32:1/16:0	30:1/18:0; 34:1/14:0; 29:0/19:1
743	50:2	34:2/16:0	32:2/18:0; 32:1/18:1; 36:2/14:0

Table 1: Mycolic acid composition from *R. equi* strain 103 by Haas *et al.*[131,132]

MA (R_1-(CH(OH)-CH(R_2)-COOH) (**Table 1**) consist of a meromycolic chain (R_1CH) and an α-branch (R_2CH). For example, 2-tetradecyl-3-hydroxy-eicosanioc acid, which contains a C18 meromycolate chain and a C16 α-branch ($R_1 = C_{17}H_{35}, R_2 = C_{14}H_{29}$), is desigated as 18:0/16:0. The MA (18:1/16:0) signifies that the molecule contains an unsaturated bond 18:1 chain and a α- $C_{14}H_{29}$ group ($R_1 = C_{17}H_{33}$, $R_2 = C_{14}H_{29}$) by Haas *et al.*[131,132]



R_1= meromycolate chain
R_2= α branch

Figure 14: Determination of chain lengths of meromycolate and α-branch in MA by Haas *et al.*[131,132]

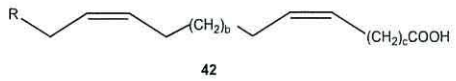
1.3.3 Biosynthesis of mycolic acids

Because MA play an essential role in the survival of TB bacteria, an important area of study is their biosynthesis. It is also the target of several chemotherapeutics, including isoniazid, which is the major front line drug.[98,133,134,135] The production of agents which target other aspects of cell wall biosynthesis would be helpful in the fight against TB.[136,137] The H37Rv strain of *M. tb* contains around 250 genes involved in fatty acid synthesis, compared to just 50 in *E. coli*, which is a similar sized organism, highlighting the importance of MA to the survival of this bacterium.[138,139] The inhibition of the biosynthesis of MA results in the death of mycobacteria.[140] Even though the biosynthesis of MA is not fully understood, research has provided a number of different hypotheses. It is possible to divide mycolic acid biosyntheses into five steps:[141,142,143]

(1): The synthesis of straight chain fatty acids up to C26 to provide the α- alkyl branch.
(2): The synthesis of C18-C50 fatty acids to provide the main meromycolate backbone.
(3): The modification of the meromycolate chain for the introduction of the functional groups which are present.
(4): The Claisen-type condensation step, followed by reduction in order to generate the MA.
(5): Transfer of the mycolic acid to form cellular glycolipids.

The diverse structure of MA of any type of *Mycobacterium* is caused by the action of a group of methyl transferase enzymes, which work on unsaturated precursors to make the array of chemical moieties which are found in MA.[87] It is not known for certain

when in the biosynthesis of MA these changes to the mero-mycolate backbone occur. However, it seems that the differentiation of the functional groups occurs prior to the terminal condensation reaction.[144] It is thought that the changes to the meromycolate backbone occur with the desaturation of the saturated alkyl chain to provide a *cis*-double bond in the proximal position and another in the distal position. This gives an unsaturated meromycolate **(42)**, **Figure 15**.[74,92]



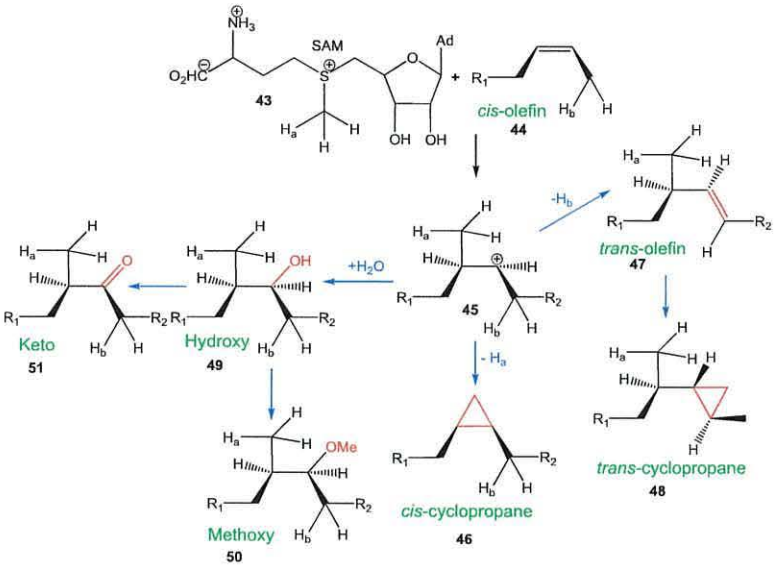
42

Figure 15: The unsaturated meromycolate backbone prior to the different of the functional groups[74]

A common intermediate for the introduction of the different functional groups in the meromycolate chain was proposed by Jaureguiberry *et al.*[144] This included cyclopropanes, oxygenated functional groups and methyl branches in the meromycolate chain.

Further research has shown this common precursor to be generated from a *Z*-alkene, facilitated by *S*-adenosyl-*L*-methionine (SAM) (**Scheme 3**).[145,146,147] The methylation of *cis*-olefin **(44)** by SAM causes a carbocation intermediate **(45)** to form. This may be stabilised through π-cation interactions with aromatic residues in the active site.

Cyclopropane rings have been shown to maintain their substrate's configuration. Therefore, the generation of the intermediate **(45)** must be a slow step which is followed by the quick transformation of the unstable intermediate to yield the functional groups.[74,148]



Scheme 3: The mechanism of the formation of functional groups in the meromycolate by SAM[147]

The deprotonation of the carbocation intermediate **(45)** occurs at Ha or Hb as shown in **Scheme 3**. When it happens at Ha, a *cis*-cyclopropane **(46)** is made. When it happens at Hb a *trans*-olefin **(47)** occurs. This undergoes more SAM mediated methylation to give a *trans*-cyclopropane **(48)** (**Scheme 3**). If hydration of intermediate **(45)** happens, the formation of the hydroxyl mycolate **(49)** is the result. It is thought that this is the precursor to the methoxy **(50)** and keto mycolates **(51)**.[93,98,149,150,151] The origin of the methyl branch of *trans*-double bonds is from SAM. Since the methyl branch of both methoxy and keto MA is believed to be in an *S*-configuration, studies were undertaken with labelled SAM in order to find the origin of these methyl branches.[152] These revealed that not only are the methyl branches supplied by SAM, but the methoxy carbons of the methoxy MA are as well. The methylene carbon which bridges in *cis*-cyclopropane rings and the methyl branch of *trans*-cyclopropane rings have also been proved to come from SAM.[116,151,152,153,154,155] SAM is an excellent target for a drug because it is needed for polyamine synthesis, which is needed for the division of cells during the active stage of the disease. Also, it is required for cyclopropanation and methylation of MA, which are essential for the survival of the organism during the stage where the disease is latent.[156] To cure TB, a drug is likely to need to be able to penetrate the macrophage and also the waxy coat of the bacteria. It also needs to target both the active and inactive stages of the organism.[156]

1.4 Trehalose esters of mycolic acids: 'Cord Factors'

Working on tubercle bacilli in 1884, Koch discovered that they formed cords or filaments. These were made up of MA which was esterified to trehalose to form a glycolipid. Bloch reported the toxic behaviour of these cords when he extracted four different strains with petrol. This extract was tested on mice.[157,158] TDM gets its name because of the 'serpentine cord' which appears in *M. tb* colonies (**Figure 16**).[159,160]

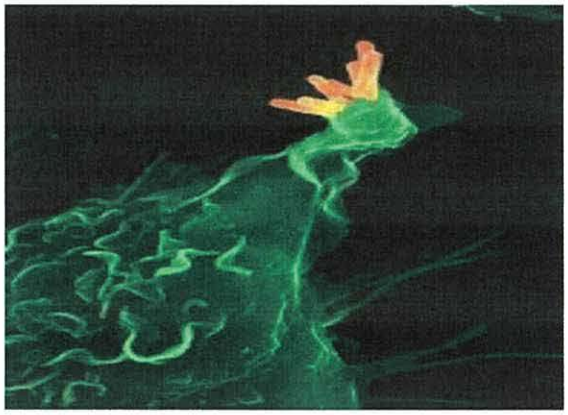


Figure 16: The 'serpentine cord' which appears in *M. tb* colonies[160]

This cord only appears in virulent tubercle bacilli colonies which are arranged in parallel bundles. Avirulent bacilli have an orientation which is random.[157,158] In addition, it has been observed that filament forming strains of mycobacteria absorb and fix neutral red dyes. This led to the hypothesis that substances in the outer part of the virulent tubercle bacilli cell are implicated in these two phenomena and also in the virulence of mycobacteria. Thus research was carried out to find the substances which caused these effects, and this led to the discovery of TDM (6,6'–dimycoloyl trehalose) and mycobacterial sulfolipids (multi-acyltrehalose-2-sulfates).[161] It was thought that the sulfolipids caused the dye to be absorbed, but research dispelled this theory.[162] The work of Noll *et al.*[163,164] confirmed the structure of cord factor. TDM **(52)** was isolated from *M. tb* and then hydrolysed with alkali. This gave two parts, MA and a non-reducing carbohydrate moiety. After acid hydrolysis, the carbohydrate moiety yielded D-glucose. To further confirm the structure of the sugar, it was converted into a crystalline acetate. This was identified as α,α-trehalose octa-acetate. The position of the MA attached to the trehalose was clarified firstly by methylation and then by saponification of the TDM. The result was hexa-methyltrehalose. Acid hydrolysis gave

trimethylgluscose **(53)**. This showed that the MA is attached to the trehalose sugar at the 6,6' positions respectively (**Figure 17**).[163]

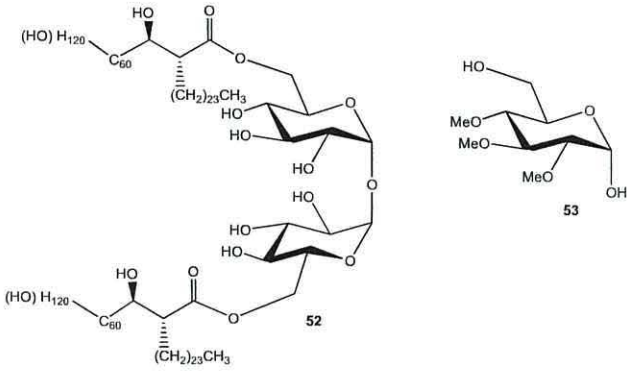


Figure 17: Structure of the cord factor as proposed by Noll *et al.*[163]

Other research has confirmed that the general structure of cord factor is an ester of a trehalose and two MA. The ratio of MA to trehalose is 2:1, which corresponds to the 6,6'–dimycolate.[158,161,163,165,166] Different analytical tools were used to analyse samples, which had been extracted from different mycobacteria, in order to elucidate the structure of cord factor. All cord factors are made up of MA which are bound to trehalose. However, the MA moiety of cord factor varies a great deal, even in the same cell wall.[163] To determine the structure of the MA which is found in each cord factor, mass spectrometry is employed. An analysis of the fragment pattern of the mass spectrum will show the fragmentation of the mycolic acid. It will also give an idea of what functional groups are contained in the molecule. This is particularly so for the meromycolic moiety. TMM and TDM are among the most characteristic components of the cell wall in mycobacteria.[157,163,167] MALDI-TOF mass spectrometry was used by Fujita *et al.* in order to analyse the cell wall components of several types of mycobacteria. The structure of TMM and TDM were thus confirmed, (**Figure 18**).[168,169] TMM, TDM, arabinogalactan and mycolate all include long alkyl chains. They provide the cell wall with a surface which is extremely hydrophobic.

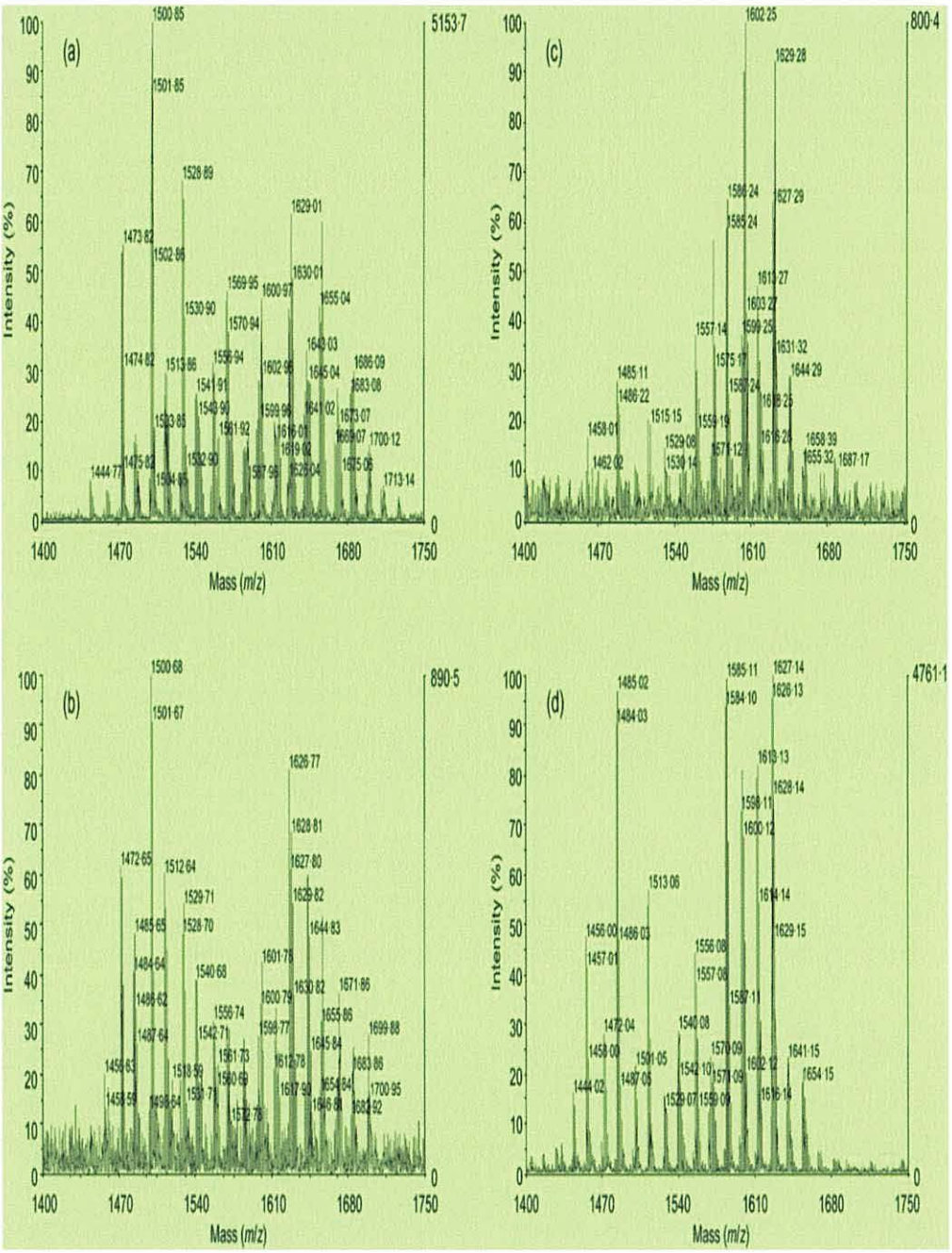


Figure 18: MALDI-TOF spectrum of TMM from *M. tb* (a), *M. tb* Aoyama (b) BCG Tokyo (c) and *M. bovis* BCG Connaught (d)[168]

Having been isolated from *Corynebacterium diphtheria,*[170] TDM was purified and prepared as a trimethylsilyl derivative. This was then used in order to determine the structure of the cell wall compounds, by using electron-impact mass spectrometry (EIMS). Three different compounds were reported from this study by Puzo *et al.*;[171]

firstly, true coryno-cord factor, then two 3-oxoacyl containing trehaloses, the only difference between them being the degree of saturation. Another effective tool for identifying cord factors is NMR. Datta *et al.*[172] made an attempt to isolate cord factor from *Corynebacterium matruchotii* and to protect it with trimethyl silyl groups, using the same methods as used by Puzo *et al.*[171] The synthetic TDM[172] gave an NMR spectrum which was very similar to that of natural cord factor, (**Figure 19**).[171,173]

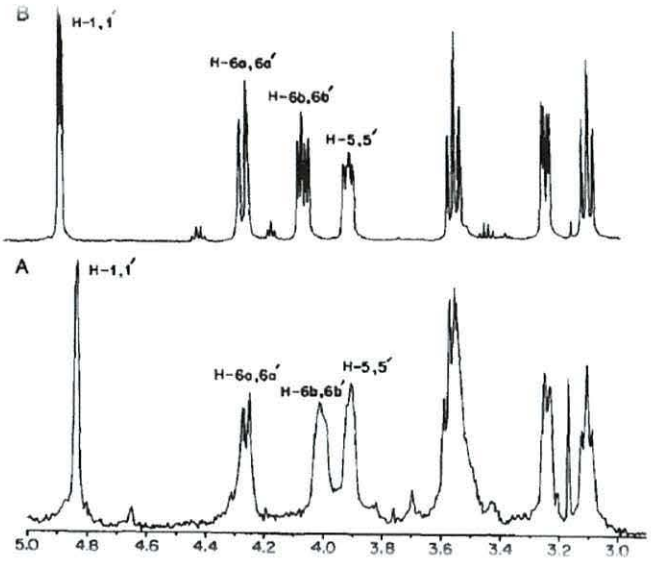


Figure 19: The difference in the ^{1}H NMR between: A) purified cord factor from *C. matruchotii* and B) synthetic cord factor[173]

1.4.1 Biosynthesis of cord factor

Takayama *et al.* put forward a hypothesis for the biosynthesis of the TDM of the H37Ra strain of *M. tb* (**Figure 20**).[174,175] This was based on the discovery of a mycolic acid attached to glycolipid, 6-mycolyl-6'-acetyltrehalose (MAT).

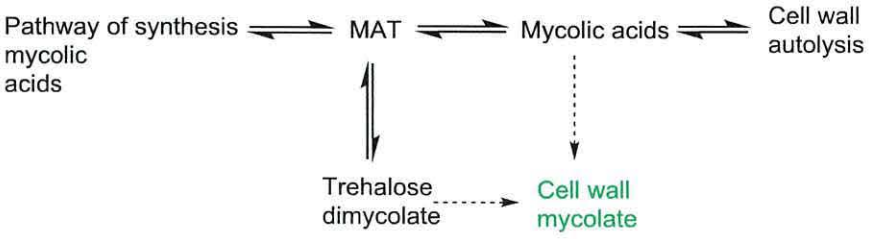


Figure 20: Suggested biosynthesis of cord factors[174]

MAT is the main mycolate containing free acid which is responsible for transferring newly synthesised MA to the cell wall. GC was used to detect the substrate of growing H37Ra strain *M. tb*. The use of NMR and mass spectrometry led to the identification of the structure of MAT **(54)** (**Figure 21**), thus confirming that TMM is the first step to prepare TDM.[175]

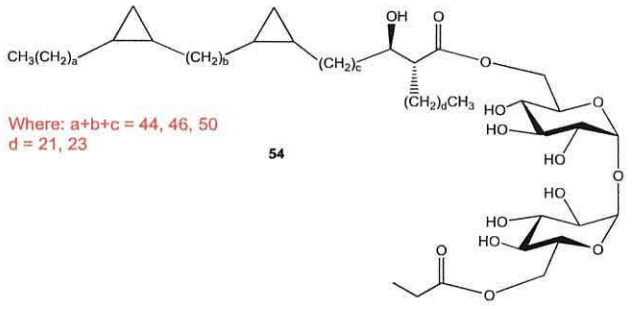


Figure 21: The structure of MAT[175]

1.4.2 Biological effects of cord factor

An increase in antibody responses is caused by TDM.[176] It also causes a sharp increase in DPNase activity in the liver, lungs and spleen.[177] Research also reported that TDM was responsible for an inhibition of the phosphorylation of NADPH, and that it caused a loss of respiratory control in mouse liver because it affected the mitochondrial membranes.[178] TDM coated *B. subtilis* caused the inhibition of the immigration of blood leukocytes, as reported by Rastogi *et al.*[179] If TDM is purified and injected into mice, they die.[179] Another study showed that mice injected with TDM from different mycobacteria showed a lower humoral response than with TDM from BCG.[180] This did not give protection against TB; however, it did display anti-tumour properties.[181,182] All of these biological properties of natural TDMs are the combination of the effects of many different MA bound to trehalose. Since a natural TDM containing just one or two different MA would be almost impossible to obtain, so a synthetic TDM which has a completely defined structure is needed for testing its biological properties.

1.4.3 ELISA and tuberculosis diagnosis

Enzyme-Linked Immunosorbent Assay (ELISA) is an inexpensive, relatively simple to use, quick immuno-diagnostic test which used in clinical medicine. Developed in the early 1970s it has become widely accepted as a high throughput screening protocol for many different diseases, though not results have been reliable for all diseases. An ELISA plate is made of tiny wells, and when the assay is used to detect the presence of antibodies in samples, these wells hold antigens. In this work, where the assay is used to detect antibodies to TB in serum samples, the wells are coated with antigens, such as TDM or MA. A reagent, casein/PBS, is then added to the wells so that any free non-specific binding sites are blocked. The next step involves incubation of the serum under investigation with the antigens in the wells of the ELISA plate. Excess antibodies are then washed away, leaving only antibodies which have bound onto the antigens in the wells. Next, a secondary antibody conjugate is added to the well. This then binds to the primary antibodies (the antibodies from the serum samples). This secondary antibody conjugate also contains an enzyme which is required to quantify the amount of antibodies present later in the assay. Washing follows in order to remove any excess secondary antibody. Then, the coated plate is treated with a reagent which interacts with the enzyme giving a colour. The intensity of the colour is quantified to infer the relative amount of antibodies binding to the antigen.[183,184]

M. tb possesses many antigens which have been used in ELISA tests for TB.[185] Some of the most common antigens used for such ELISA tests are A60 or 38 KDa antigens. However, like so many other antigens which are used to detect TB these suffer from low specificity and sensitivity.[185] The group led by Yano demonstrated identified cord factors as antigens as surrogate markers of TB infection using ELISA in the 1990s.[186,187,188] These ELISA tests gave excellent specificity and sensitivity. They were also reproducible. Nevertheless, these results were challenged when they were performed on patients who may have been subjected to sub-clinical levels of *M. tb* in high burden countries or who were also infected with HIV[189] and so the reliability of the test was ruled out in HIV-TB co-infection countries. One reason for this loss of specificity could be the presence of cross-reactive anti-cholesterol antibodies in the blood of such patients.[190] A solution to this problem may be anti-mycolic monoclonal antibodies. A thorough investigation of the antigenicity of the mycolic acid subclasses

may improve the sensitivity. Significant differences in the antigenicity of the different subclasses have been identified. In order to determine their biological activities and their application to diagnosis, research continues to test MA and TDMs. They are both considered to be very potent signalling agents in the serodiagnosis of TB.[141,191,192] Despite the selectivity not being high enough for application, it has been discovered that it is possible to use natural MA on an ELISA plate assay for the serodiagnosis of TB.[188,190] A range of ELISA tests done on a natural *M. tb* mycolic acid and unique synthetic MA which represent the three main functional classes in *M. tb* were reported by Beukes *et al.* in 2011.[193] The synthetic MA were α-, keto-, hydroxy- and methoxy-MA. It was confirmed by these tests that antibodies recognise free MA. Also confirmed was that individual synthetic MA displayed varying antigenic activities against human TB sera. This reflects the differences in their functional and stereochemistries. Indeed, the oxygenated MA were more antigenic than α-mycolic acid. Methoxy-MA was discovered to be the most antigenic, next were hydroxyl-, then keto- and lastly α-MA which showed the least recognition.[193] In contrast, natural TDMs which contain complex mixtures of isomers give a higher sensitivity and selectivity.[92,194] Nevertheless, analysis with natural TDM is highly complicated due to the many combinations of MA that can be bonded to the trehalose, so increasing the number of potential structures which are thought to contribute to the variation of immune related effects.[187,195,196,197] Using ELISA in the diagnosis of TB has not been as successful as it has for other diseases. Given the enormous number of new cases and how highly contagious this disease is, it is hoped that the ELISA test can be developed into a major weapon against TB.

1.5 The synthesis of mycolic acids

An organic chemist is presented with many challenges by the synthesis of MA. Most importantly, generating enantiomerically pure compounds can be a problem because all MA include at least two chiral centres in the α and β positions relative to the carboxylic acid. The synthesis of a full MA requires the linkage of different functional units in stages. In many cases, this means repetitive cycles of alkylation and chain extension. These are essentially a sequence of reactions such as protection, deprotection, oxidation, olefination and hydrogenation etc. We are able to divide these into the following stages:

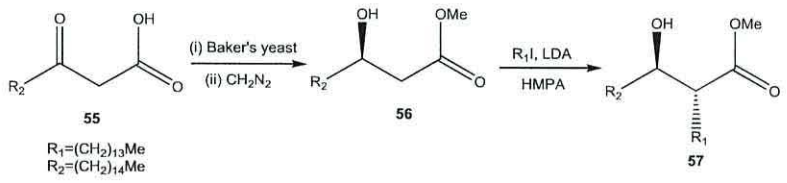
(1) The synthesis of the long-chain carboxylic acid with required substituents at the α and β positions.

(2) The synthesis of the meromycolate unit with different functional group.

(3) The coupling reaction which joins the meromycolate to the mycolic motif.

1.5.1 Synthesis of the mycolic motif

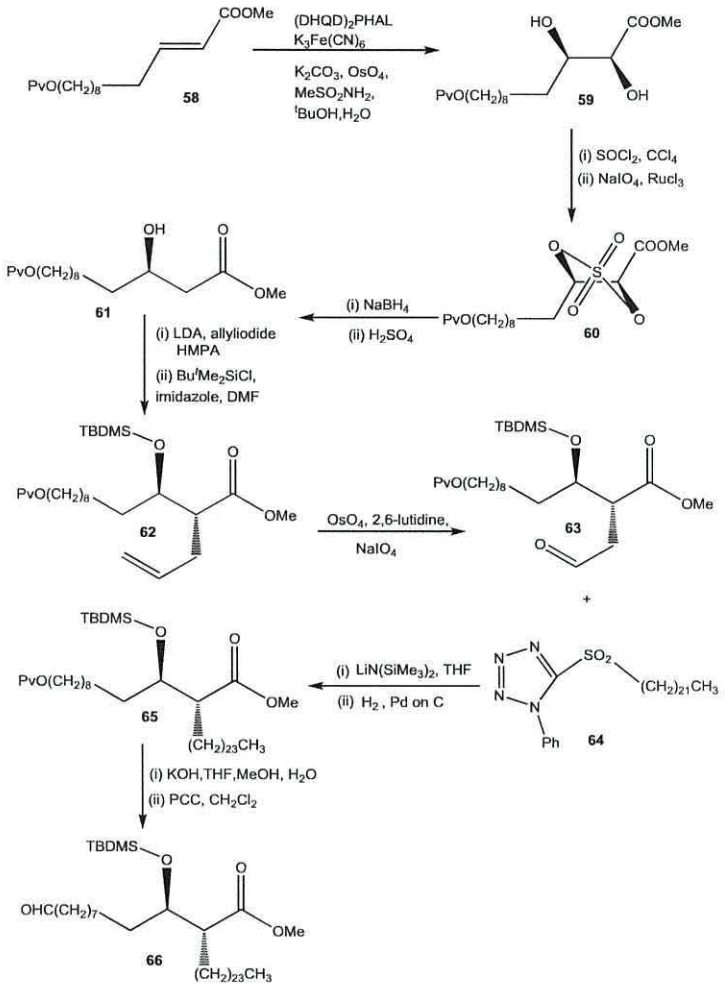
There have been a number of studies on the preparation of the α-alkyl-β-hydroxy unit. In 1952, Lederer *et al.* conducted one of the first trials to synthesise this kind of compound.[198] The same group prepared other corynomycolate analogues using similar methods based on the Claisen condensation, but as a mixture of diastereo-isomers.[199,200] The first enantiomerically pure α-alkyl-β-hydroxy unit was synthesised by Kitano *et al.*[201,202] Utaka *et al.* in 1987,[203,204] introduced the hydroxy group at the β-position by stereoselective reduction of the β-ketoester **(55)** with Baker's yeast (**Scheme 4**). An alkyl chain was then directly introduced to the α-position of a β-hydroxy ester **(56)** in a Fräter reaction.[205] This yielded the α-alkyl-β-hydroxyl carboxylates **(57)** with two chiral centres in the correct configuration and with the α-chain of the correct length.



Scheme 4: Method of Utaka *et al.*[203]

An improved synthesis of the α-alkyl-β-hydroxyl unit was conducted by Baird *et al.*[206] This used 1,10-decanediol to prepare the *trans*-olefin **(58)** in four stages. The olefin was converted into the α,β-dihydroxy compound **(59)**, which was then converted into a cyclic sulfate **(60),** which was subsequently regioselectivity reduced and hydrolysed to form the (3*R*)-hydroxy ester **(61)**. A Fräter allylation[205] with allyl iodide, introduced an allyl chain at the α-position and then the hydroxyl group was protected in order to give a (2*R*,3*R*)-2-allyl-3-hydroxy ester **(62)**. Next the allylated ester **(62)** was oxidised to an aldehyde **(63)**, whereupon the α-chain was extended, using a Julia reaction,

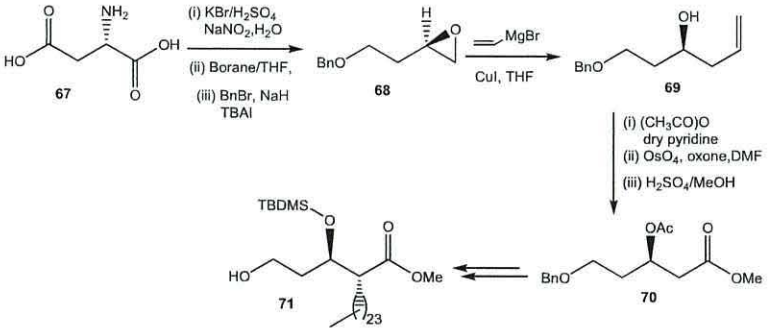
followed by saturation of the alkene intermediate. Lastly, deprotection of the primary alcohol of the diol **(65)**, followed by oxidation with PCC, gave the desired compound **(66)** (**Scheme 5**).[206,207]



Scheme 5: Improved synthesis of the motif unit by Baird *et al.*[206]

What is interesting about this approach is that the generation of aldehyde **(63)** permits the introduction of any chain length which is desired at the α-position, by means of a Julia-Kocienski olefination. Koza *et al.* made a further development by preparing the mycolic motif **(71)**.[207] *L*-Aspartic acid **(67)** was the starting material instead of *R*-aspartic acid so as to get an epoxide intermediate **(68)**.[208] The reason for this was that *L*-aspartic acid was not so expensive as *R*-aspartic acid. The ring opening of the epoxide **(68)** with a Grignard reagent led to the desired compound **(69)**. This was converted into a β-hydroxy ester **(70)**. However, instead of a direct long chain

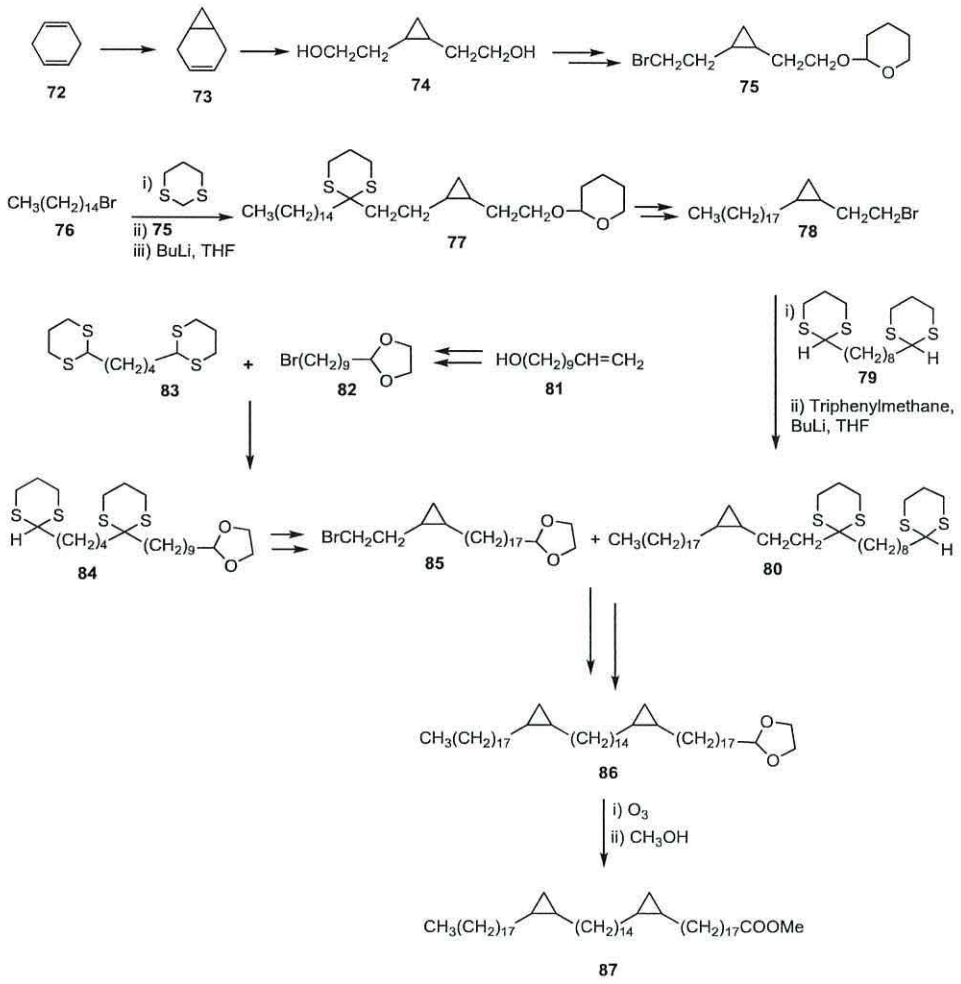
alkylation at the α-position, a short chain Fräter allylation was carried out again. A further chain extension using a Julia-Kocienski reaction created the desired product **(71)** (**Scheme 6**).[207]



Scheme 6: The preparation of the mycolate motif (47) by Koza *et al.*[207]

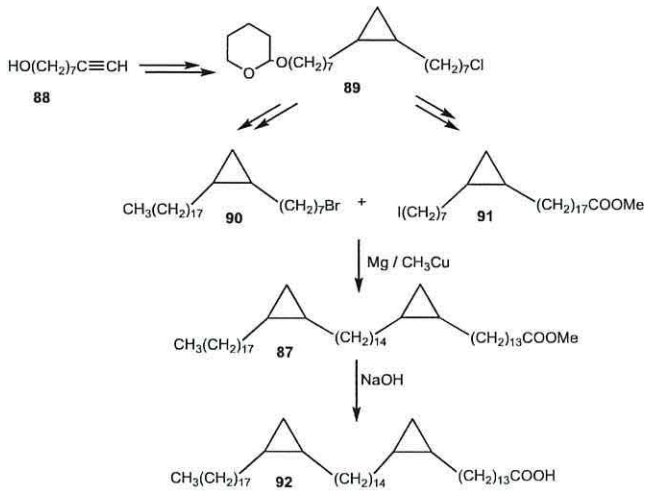
1.5.2 Synthesis of the meromycolate unit

Gensler *et al.* synthesised the first meromycolate acid **(87)** (**Scheme 7**).[209] Using 1,4-cyclohexadiene **(72)**, norcarene **(73)** was prepared. After several stages, the cyclopropane-containing bromide **(75)** was obtained. The cyclopropane **(78)** was made by alkylation of pentadecyl bromide **(76)** with the cyclopropane-containing bromide **(75)**, and also by desulfurisation of the compound **(77)** with Raney nickel.[210] Using the bisdithiane **(79)**, chain extension yielded **(80)**, one of two major components which make up the meromycolate acid product. Next, the synthesis was continued to make the second major component of the meromycolate. To begin with, the ozonoylsis of 10-undecenol **(81)** was undertaken. Then the conversion of the corresponding alcohol into the acetal was performed. This was followed by bromination to yield **(82)**. Chain extension by six carbons gave **(84)**. Coupling this to the bromide **(75)**, desulfurisation, deprotection of the tetrahydropyranyl group and bromination gave **(85)**, which was the second key intermediate. The lithio derivative of the bis-dithiane intermediate **(80)** was coupled with this and, following desulfurization, the desired product **(86)** was obtained. Ozonolysis of the latter gave methyl meromycolate **(87)** which contained two *cis*-cyclopropane rings, (**Scheme 7**).



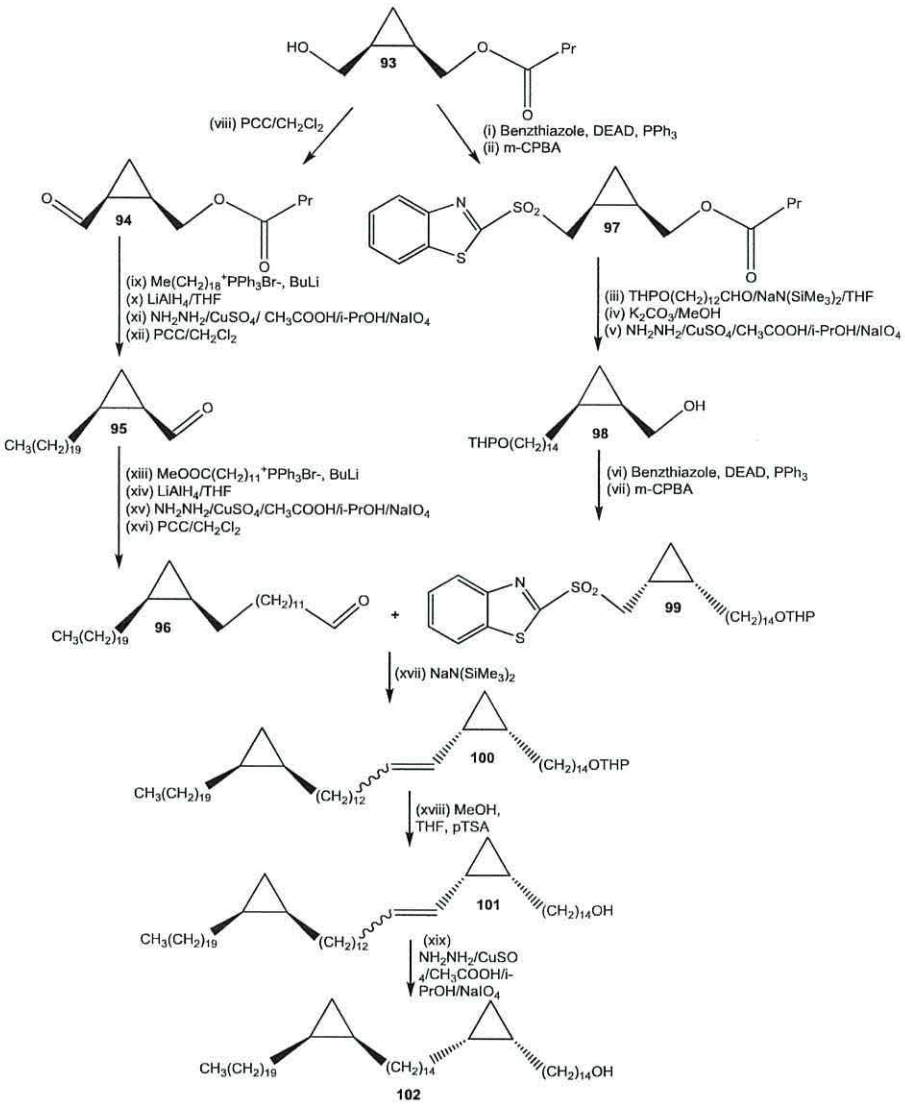
Scheme 7: The first synthetic meromycolic acid

Gensler *et al.*[211] developed an improved method for the preparation of a meromycolic acid **(92)**. This combined different fragments, where Grignard reactions were used to couple intermediates.[211] It would not be difficult to scale up this short method (**Scheme 8**). 1-Hydroxy-8-nonyne **(88)** was utilised in order to get the intermediate **(89)**. After several steps, another two intermediates **(90)** and **(91)** were obtained from this. The Grignard reagent was prepared using the alkyl bromide **(90)** for the final coupling. This was alkylated with alkyl iodide **(91)** to give compound **(87)**. Lastly, the required product **(92)** was obtained by saponification of **(87)**. Even though this method is better than the first, it nevertheless does have problems. Firstly, a (10%) yield is obtained from the final coupling. Secondly, mass spectroscopy revealed that only a small percentage (4%) of the product was the desired compound. Thirdly the product is a racemic mixture of diastereomers.



Scheme 8: The second synthesis of a meromycolic acid

Baird *et al.* documented a third approach in 2000 for the first synthesis of a single enantiomer of an analogue of meromycolic **alcohol (102)**.[212] Two key steps were involved. First was the preparation of single enantiomers of cyclopropanes.[213] This was followed by the coupling of these intermediates with no loss of stereo-chemistry. The aldehyde **(94)** was made from the anhydride of cyclopropane-*cis*-1,2-dicarboxylic acid. A Wittig reaction between this and nonadecyl triphenyl-phosphonium bromide, using *n*-butyl lithium as the base, followed by reduction of the product with lithium aluminium hydride, gave an alcohol as a mixture of *Z*- and *E*-isomers. Di-imide was used to saturate the derived alkene and this was followed by oxidation of the alcohol, which led to aldehyde **(95)**. A second Wittig reaction was used for chain extension, followed by saturation and oxidation to give the aldehyde **(96)**. A Julia reaction of sulfone **(97)** with 13-tetrahydro-pyranyloxytridecanal yielded the protected alcohol **(98)**. This was converted into sulfone **(99)**. An important aspect of this method is that the coupling reaction which was used to join the different units, secured the desired stereochemistry. The Julia reaction between aldehyde **(96)** and sulfone **(99)** gave **(100)** as a mixture of *E*- and *Z*-alkenes. The enantiomerically pure alcohol **(102)** was obtained by deprotection of the alcohol group and saturation of the derived alkene with di-imide (**Scheme 9**).



Scheme 9: The first synthesis of a single enantiomer of meromycolic alcohol

A better overall (50%) yield and control of the stereochemistry are two important advantages of this method. At different stages of the synthesis, different portions of the compounds were incorporated. Thus, this method can be applied to the synthesis of other meromycolates having varied functional groups and chain lengths. It also permits the preparation of different stereoisomers by modifying the sequence of reactions on the cyclopropyl groups, providing all the diastereoisomers of meromycolaldehydes. In line with Baird *et al.* other research has reported the preparation of meromycolate sulfones **(103, 104, 105)** (**Figure 22**).[214,215]

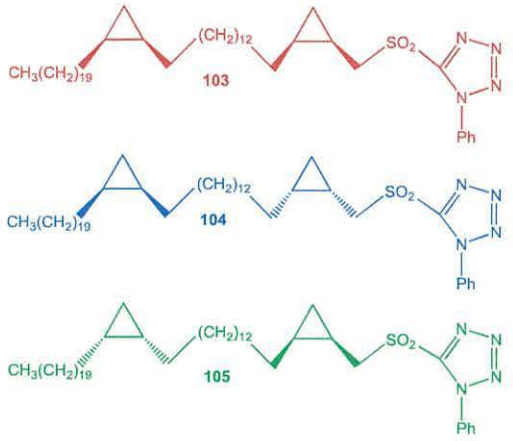
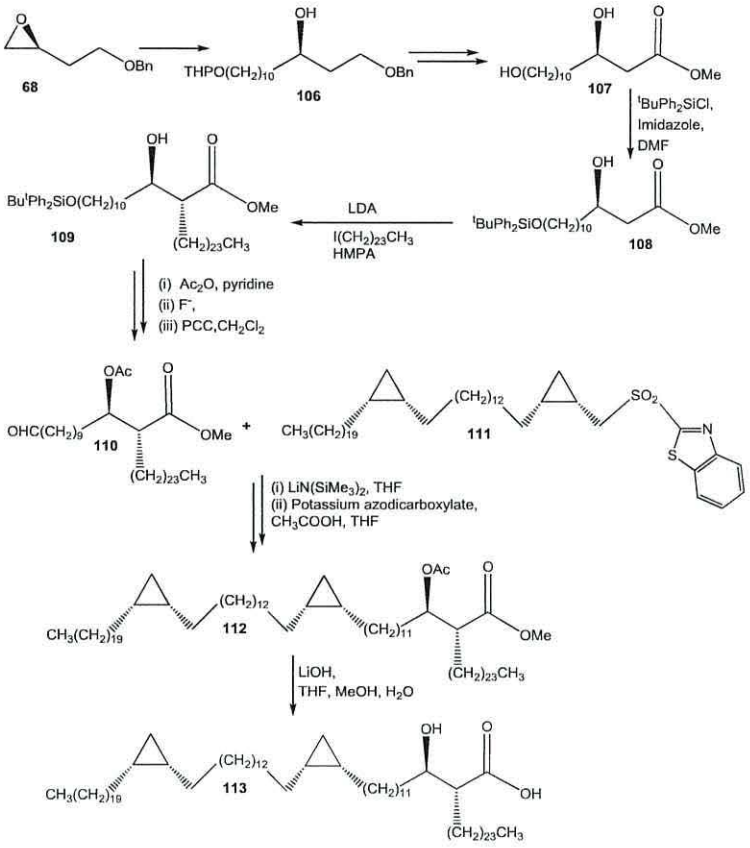


Figure 22: Meromycolate sulfones prepared by Baird *et al.*[214,215]

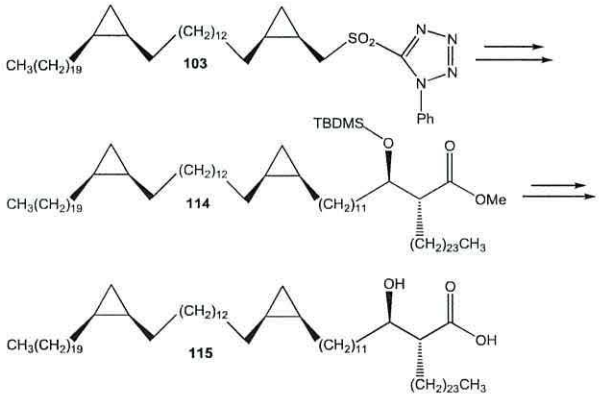
1.5.3 Synthesis of full mycolic acids

The synthesis of a single enantiomer of a major α-MA **(112)** of *M. tb* was described by Al Dulayymi *et al.* in 2005.[208] The meromycolate unit contains di-*cis*-cyclopropane rings. The acid and alcohol positions are protected in the mycolic motif. The α-alkyl-β-hydroxy acid portion of a full mycolic acid was prepared, using *R*-aspartic acid via an epoxide intermediate **(68)**. A single enantiomer of the monoprotected diol was obtained by the ring opening of the epoxide **(68)** with a Grignard reagent which was made from 9-bromononan-1-ol tetrahydropyranyl ether **(106)**. The compound was converted into diol **(107)** in a few steps (**Scheme 10**).[208] This was protected at the primary alcohol group and then alkylated at the α-carbon using 1-iodotetarcosane to provide the hydroxy ester **(109)**. Protection of the secondary alcohol in **(109)** as the acetate, followed by deprotection of the primary alcohol and subsequent oxidation led to the aldehyde **(110)**. In the final stage of the synthesis the protected aldehyde **(110)** and the dicyclopropane sulfone **(111)** were coupled in a modified Julia reaction and then treated with potassium azodicarboxylate and acid in order to give the protected α-MA **(112)**. Deprotection of **(112)** to the free mycolic acid **(113)** was not done. Nevertheless, in a recent study by Al Dulayymi *et al.*[208] the free mycolic acid **(113)** has been synthesised. However, this has yet to be published (**Scheme 10**).



Scheme 10: Synthesis of an α-mycolic acid of *M. tb*[208]

Using similar procedures (**Scheme 11**), the preparation of the enantiomer **(115)** of **(113)** from **(103)** has been reported.[215,216]



Scheme 11: Synthesis of an α-mycolic acid of *M. tb* [215,216]

In 2007, Baird *et al.*[217] reported the synthesis of three stereoisomers of a complete methoxy-MA (**116**, **117** and **118**) (**Figure 23**).[217] Also, the isolation of this type of

molecule from *M. tb* has been achieved.[218,219] The different biological effects of these acids and their stereoisomers is currently being investigated.

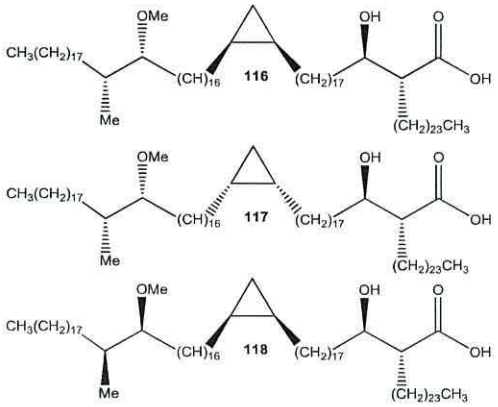


Figure 23: Synthetic methoxy-mycolic acids of *M. tb* [217]

The synthesis of ketomycolates which contain α-methyl-*trans* and *cis*-cyclopropane fragments **(119** and **120)** in order to produce a range of absolute stereochemistries and chain lengths was also undertaken, (**Figure 24**).[220]

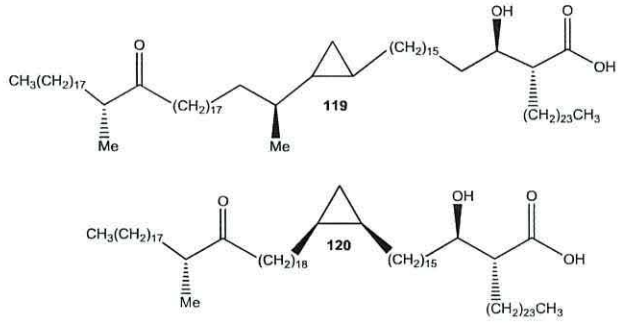


Figure 24: Synthetic keto-mycolic acids of *M. tb*[220]

A considerable contribution has been made by Baird *et al.* to the area of complete synthesis of MA. Several routes for obtaining enantiomerically pure MA have been published. Some examples are shown in **Figure 25**.[214,221,222,223,224]

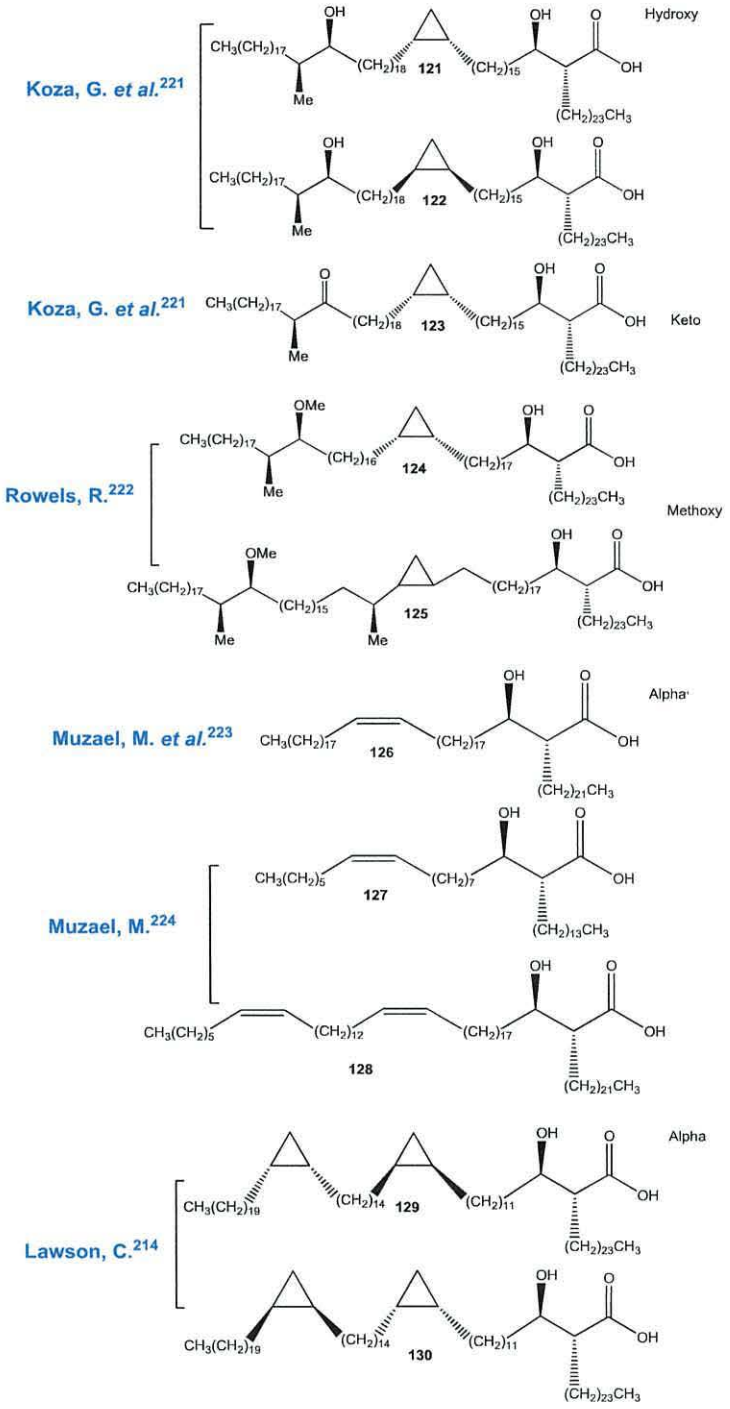
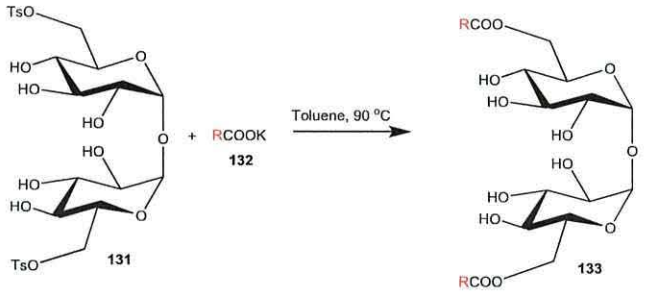


Figure 25: Synthetic mycolic acids

1.6 Synthetic TDMs

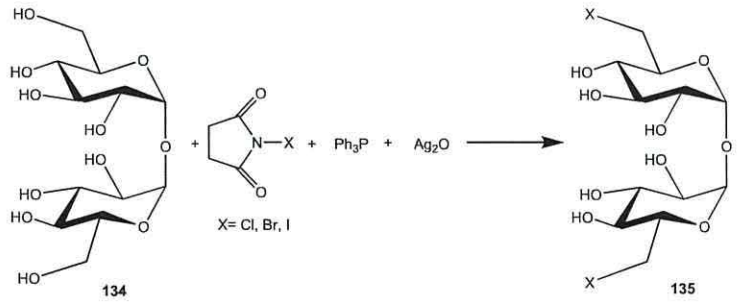
Attempts to synthesise cord factors began even before their structures were confirmed. In the 1950s, the first approach was to protect the trehalose 6,6'-dihydroxyl groups with toluenesulfonyl (tosyl) groups **(131)**, followed by nucleophilic displacement with a potassium salt of natural MA **(132)** in DMF.[225] This method gave a (14%) low yield, so it was changed by altering the solvent to toluene and adding crown ether as catalyst.[226] TDM was obtained for two potassium salts of model mycolates, C44 and C32 (**Scheme 12**). Polonsky *et al.*[226] reported that when this displacement was carried out in toluene at a low temperature -90 °C with crown ether, it formed 6,6-trehalose diester **(133)**.[22]



(RCOOK = natural mycolic acid potassium salt)

Scheme 12: The first efforts to prepare TDM [225]

Following this early attempt, a simple method for protecting the hydroxy groups of the trehalose was the focus of research in order to optimise the yield.[226] A method for the synthesis of halo-saccharides using triphenylphosphine and *N*-halogenosuccinimide in DMF was introduced by Hanessian *et al.*.[227] This method was also used to synthesise di-halo-trehalose **(135)** starting from trehalose **(134)** in one step (**Scheme 13**).[228]



Scheme 13: Synthesis of di-halo-trehalose

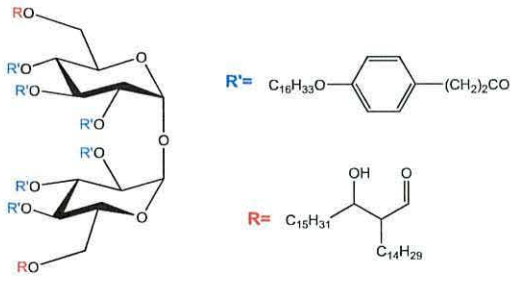
Using this method for preparing halo-trehalose **(135)**, two groups independently synthesised TDM, after protecting the secondary hydroxyl with trimethylsilyl. This was followed by refluxing with natural potassium-mycolate in HMPT.[229,230,231]

1.6.1 Recent syntheses

All the recent TDM syntheses are based on protecting the trehalose. It is then treated with MA or the potassium salts of MA.

1.6.1.1 Hexabenzyl trehalose

Protection of the hydroxy groups in trehalose with a benzyl group prevents the formation of a 3,6-anhydrotrehalose. Liav and Goren firstly made a trityl derivative to protect the 6,6'-position, **(136)**. The secondary hydroxyls were protected with benzyl groups **(137)**. Next the dityryl groups were converted into dimesyl groups **(138),** because these are better leaving groups. This was then coupled **(138)** with a potassium salt of a natural mixture of MA at 90 °C in HMPT **(139)**. The trehalose was deprotected by hydrogenation to get the TDM **(140)** (**Scheme 14**).[232,233]

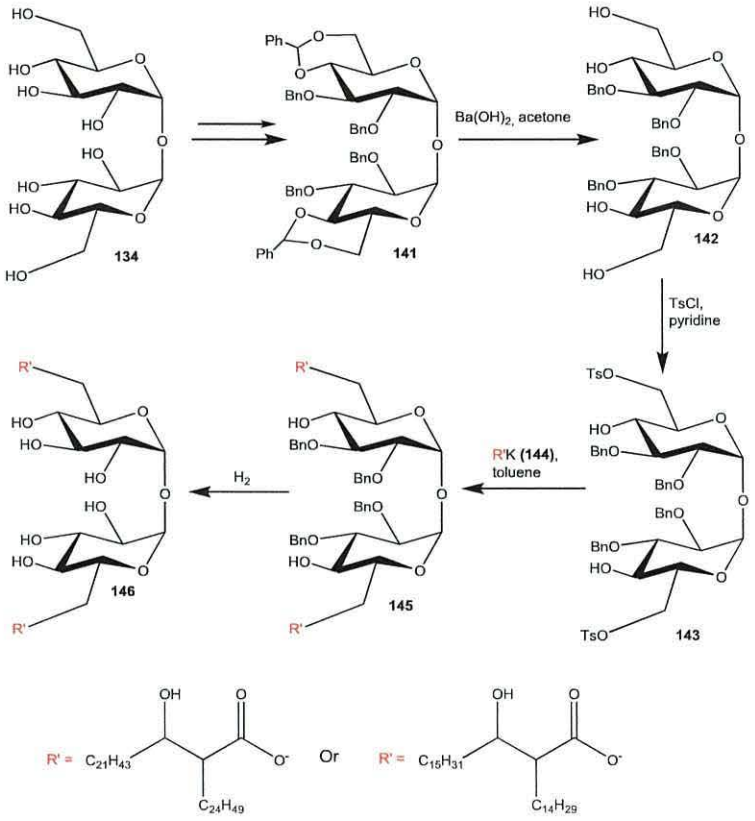


	R	R'
(136)	C-Ph_3	H
(137)	C-Ph_3	CH_2Ph
(138)	Ms	CH_2Ph
(139)	Natural mycolic acid mixture	CH_2Ph
(140)	Natural mycolic acid mixture	H

Scheme 14: Preparation of two TDMs [232,233]

1.6.1.2 Tetrabenzyl trehalose

Another way of preparing TDM is to use 2,3,2',3'-tetra-*O*-benzyl trehalose. The first step was to prepare the compound **(141)** by the benzylation of trehalose **(134)**, using sodium hydride and benzyl halide in DMSO.[234] The next step was hydrolysis of **(141)** in the 6,6'-positions, in order to get compound **(142)**. Activation of the primary hydroxyl groups at the 6,6'-positions followed, using the tosyl group. Following this was a coupling of the potassium salt of natural MA, **(144)** with 4,6,4',6'-tetrabenzyltrehalose **(143)**. Deprotection of the trehalose **(145)** using hydrogenolysis gave the TDM **(146)** (**Scheme 15**).[231,235,236]



Scheme 15: A different method for the preparation of TDM[237]

1.6.1.3 Synthesis via a Mitsunobu reaction

One of the best ways to prepare TDM is to use a Mitsunobu reaction. Bottle and Jenkins investigated a model system. They synthesised a diester of trehalose and sucrose directly with no protection of the sugar,[238] by reacting palmitic acid,

triphenylphosphine (TPP) and diisopropyl-azodicarboxylate (DIAD) in DMF with the sugar to obtain **(147)** and **(148)** in good yield, (**Figure 26**).[238]

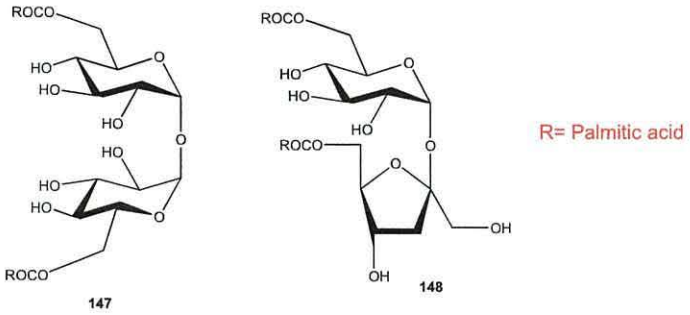
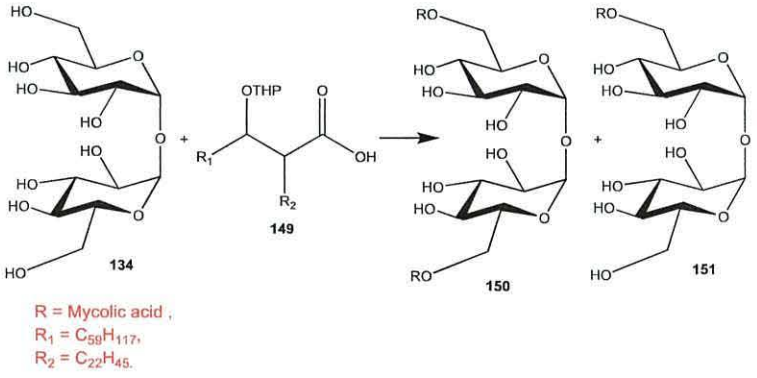


Figure 26: Coupling palmitic with trehalose and sucrose

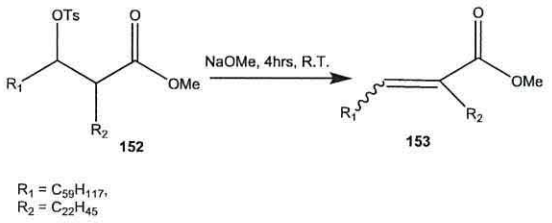
Applying this method to natural mixtures of MA did not result in the formation of TDM or TMM because the MA suffers from β-elimination in the presence of the Mitsunobu reagents. To avoid this elimination, the β-hydroxyl group was protected with tetrahydropyran (THP). A Mitsunobu esterification reaction was then performed with this protected mycolic acid **(149)** (a mixture of natural MA isolated from BCG with isomer of $R_1 = C_{59}H_{117}$, $R = C_{22}H_{45}$) and free trehalose to give a (40%) yield of TDM **(150)** and (25%) yield TMM **(151)** (**Scheme 16**).[239]



Scheme 16: Synthesis of TDM using PPh_3, DIAD, HMPT and CH_2Cl_2

Jenkins and Goren treated MA with Mitsunobu reagents in order to test for the elimination reaction in the natural mycolic acid mixtures. They also investigated the same mycolic acid after protection of the hydroxyl group with tosylate **(152)** (a good

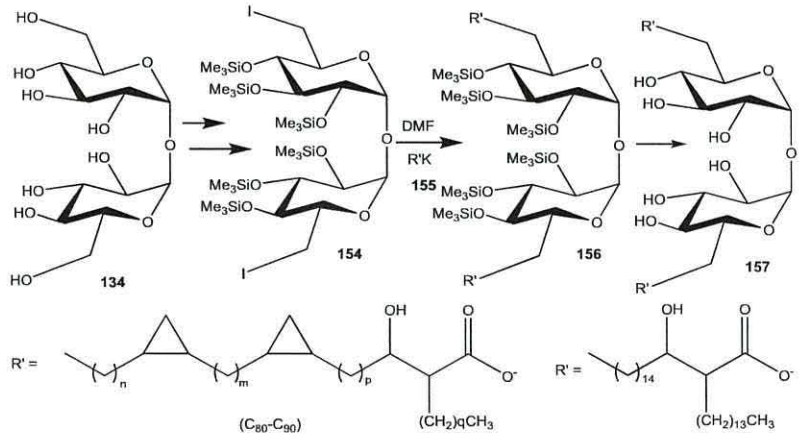
leaving group) and treatment it with sodium methoxide. The formation of the same compound **(153)** was the result of both these reactions (**Scheme 17**).[239]



Scheme 17: β-Elimination in mycolic acids after protecting the hydroxy group with tosylate[239]

1.6.1.4 Synthesis of 2,3,4,2',3',4'-Hexakis-O-(trimethylsilyl)-α,α-trehalose

The first attempts to make TDM, carried out by Tocanne and Toubiana,[229,230] involved protecting the secondary hydroxyl groups of the trehalose sugar **(134)** as trimethylsilyl ethers.[240] The primary hydroxyl in the 6,6'-position was replaced with a iodide which is a good leaving group to give **(154)**. A potassium salt of natural mixture of MA (R'K) **(155)** and the sugar **(154)** were coupled to form protected TDM **(156)**. After the trehalose sugar was deprotected from the trimethyl silyl protection groups, the free TDM **(157)** was obtained (**Scheme 18**).[229]



Scheme 18: Preparation of TDM by Toubiana *et al.* and Tocanne [229,230]

1.6.1.5 The total synthesis of TDM

Baird *et al.* prepared the first synthetic TDM which comprised a single synthetic mycolic acid of one absolute stereochemistry. This was done by using the synthetic MA in **Figure 27** and by protecting the β-hydroxyl with TBDMS. The trehalose was protected with trimethylsilyl groups and was then esterified at room temperature for one week using the Steglich coupling process (**Scheme 19**).[216]

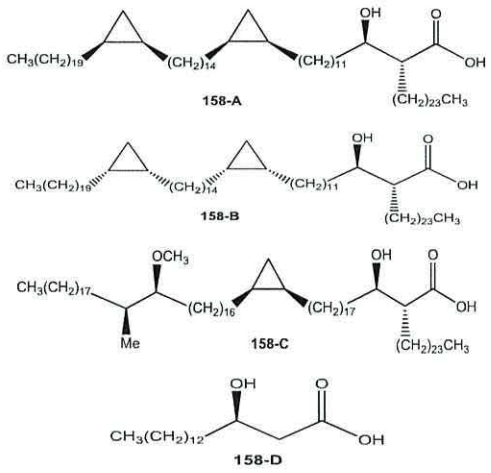
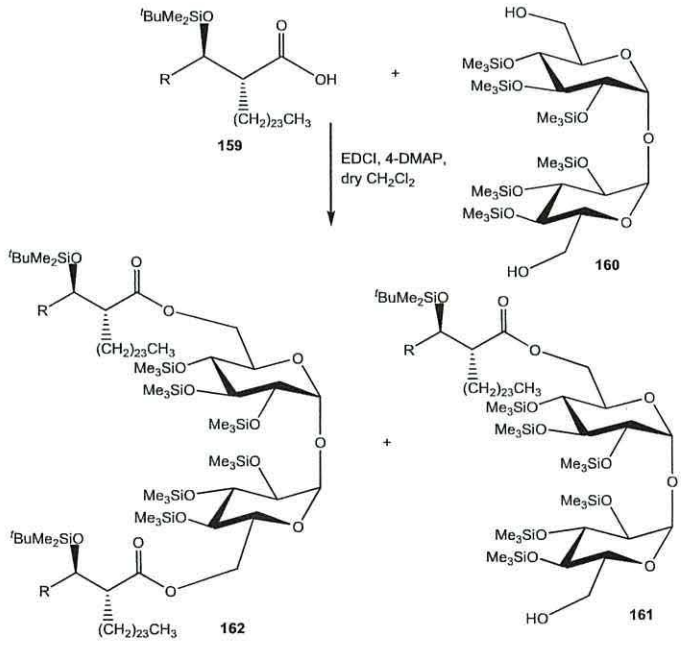
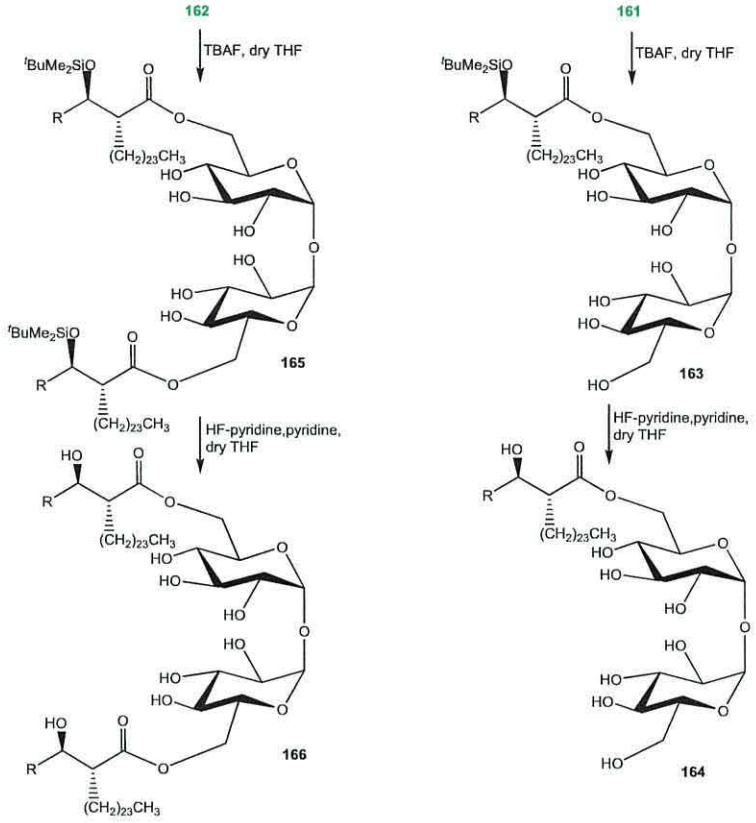


Figure 27: Synthetic mycolic acids[216]



Scheme 19: Preparation of TDM and TMM by Baird *et al.*[216]



Scheme 20: The deprotection of TDM and TMM[216]

The TDM and TMM were deprotected in two stages. Firstly the TMS- ethers were deprotected with TBAF and then the TBDMS group was deprotected with HF-pyridine (**Scheme 20**).[216]

1.6.2 Known biological activities of cord factors

Mycobacterial TDMs are interesting because of their biological activity. This has been widely researched and studies have shown that the components of mycobacteria have immune activity. TDM are needed for the survival of the mycobacteria inside macrophages.[241] TDMs are able to induce cytokine production in the host's immune system (IL-1β, IL6 and TNF) in the macrophages.[242] Early studies indicated that TDMs may be used as an adjuvant against immunological problems. In 1975, Meyer and Azuma discovered that the cell wall components have adjuvant activity.[243] Saito confirmed that mycobacterial TDM was a good adjuvant and that is able to enhance

the immune system in mice and rats through antibody production. It also causes delayed hypersensitivity.[80,244] Another role of TDM is antitumor activity and it has been used in the treatment of cancer in animals.[245,246] In addition, mice treated with TDM, had resistance against the influenza virus and bacterial species, (e.g. *Salmonella typhi* and *Salmonella tyhimurium*).[247,248] The synthetic cord factors (TDM, TMM of methoxy and α-MA) were recently tested on mouse macrophage cell wall line RAW 264.7. This was to study their activity in making cytokine and chemokine. It was discovered that the level of TNF- alpha production induced by synthetic α-TDM was three times higher than the TDM sample which had been produced commercially. The remaining synthetic TDMs and TMMs exhibited lower activity than the natural *M. tb* TDM.[216] It was also found that the level of chemokine MCP-1 production induced by α-TDM was double the production by the commercial sample. The other synthetic TDMs showed an equivalent level of MCP-1 production compared to the commercially produced TDM sample. The structure of the MA affects the biological activities of TDMs. Studies have confirmed that synthetic free MA from *M. tb* display different antigenic properties.[249,250] Many interesting biological properties are demonstrated by TDMs and mycolic aids. These include their effects of the immune system, diagnosing and controlling diseases. It is vital to understand these effects and so the enantioselective synthesis of these compounds is important.

1.7 Project aims

The work in this thesis is divided into fifth sections:

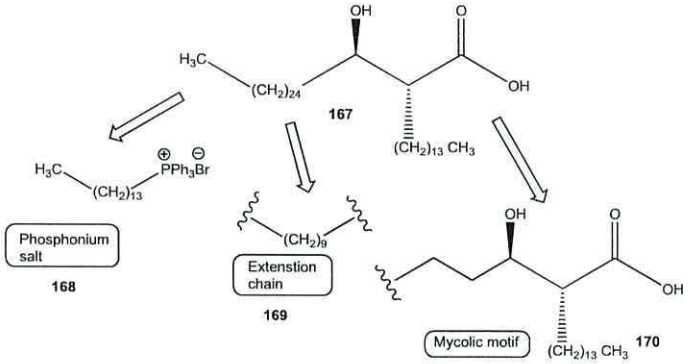
- The first section was synthesis of saturated and unsaturated MA, trehalose esters (TDM and TMM) and glucose monomycolate (GMM) present in *R. equi*
- The second section was synthesis of a methoxy MA, keto-MA and trehalose ester (TDM and TMM) present in *M. kansasii*
- The third section was synthesis of methoxy MA, and trehalose ester (TDM and TMM) present in *M. tb.*
- The fourth section was synthesis of mixed trehalose ester present in *M. kansasii* and *M. tb*.
- The fifth section was biological activity results.

2. Results and discussion

2.1 Synthesis of saturated and unsaturated MA, trehalose esters (TDM and TMM) and glucose monomycolate (GMM) present in *R. equi*

2.1.1 Synthesis of (2*R*,3*R*)-3-Hydroxy-2-tetradecyloctacosanoic acid (167)

The planned preparation of saturated mycolic acid **(167)** uses the Wittig reaction as the major step (**Scheme 21**). The first stage was the preparation of the mycolic motif **(170)**. The second stage was the preparation of 9-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)nonyl pivalate **(169)**, as a C-9 unit extension to the chain. The third stage was the coupling of a phosphonium salt **(168)** with the correct number of carbons atoms in the motif chain to prepare the full saturated mycolic acid **(167)**.

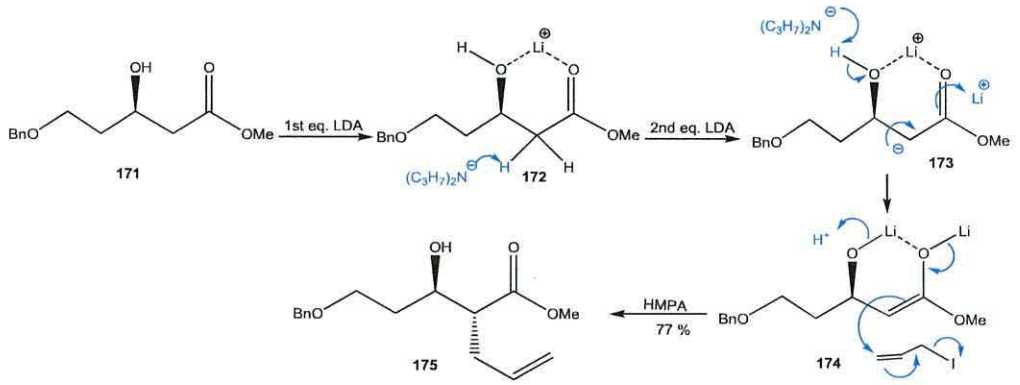


Scheme 21: Retrosynthetic plan for the synthesis of saturated MA (167)

2.1.1.1 Adding an α-alkyl chain

The Fräter-Seebach allylation is highly diastereoselective,[64,205] and was believed to be the best route for the stereocontrolled insertion of the allyl chain at the α-position of the β-hydroxy ester **(171)** to give the α-alkyl-β-hydroxy fragment in (*R*, *R*)-configuration. This short allyl chain was used because the insertion of the full alkyl group in one step has given variable results and poor (10%) yields in the past.[244]

LDA (2 mol. equiv.) was generated *in situ* from diisopropylamine and *n*-BuLi at -78 °C. This was followed by adding the β-hydroxy ester **(171)** and stirring at -65 °C for 3 h, so as to ensure the generation of a stable chelated enolate intermediate **(174)**. The mixture was stirred for 2 h at -60 to -10 °C, then re-cooled to -65 °C and allyl iodide and HMPA in dry THF was added.

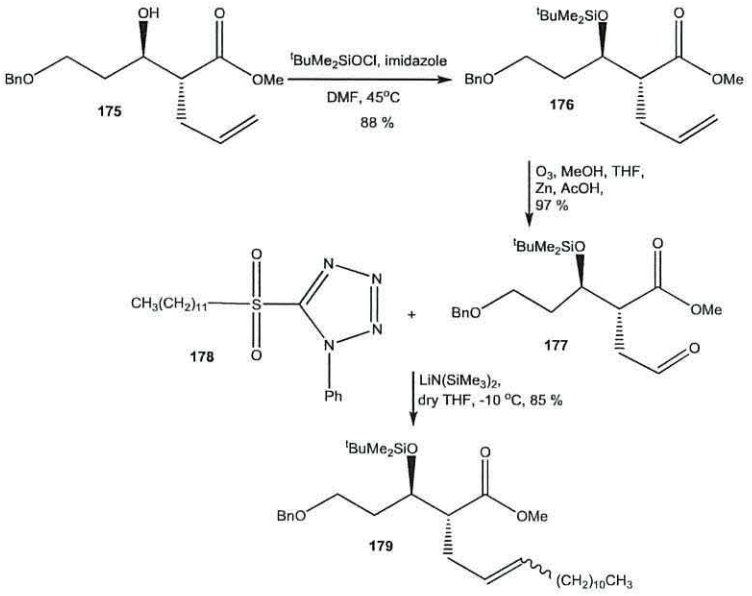


Scheme 22: The insertion of the α-allyl chain

The allyl chain was attached from the bottom face because of the steric encumbrance encountered on the top face of the six membered ring of the chelate intermediate **(174)**. This gave the *anti*–alkylated product (2*R*, 3*R*)-hydroxy ester **(175)** in (60%) yield (**Scheme 22**),[206] which gave the same proton and carbon NMR spectra as recorded in the literature.[207]

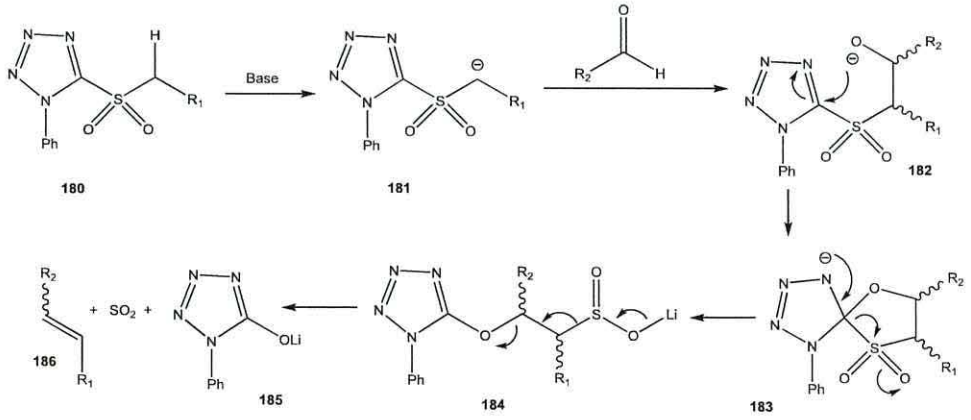
2.1.1.2 The extension of allyl chain

A standard procedure which involves the ozonlysis of the alkene to an aldehyde, and chain extension using a Julia-Kocienski reaction and hydrogenation was used to extend the allyl unit in the formation of the α-alkyl chain. The β-hydroxy-group of compound **(175)** was protected prior to oxidation of the alkene into the aldehyde, in order to give compound **(176)** (**Scheme 23**). For the protecting group, a *tert*-butyldimethylsilyl ether was selected since this is stable during the next reaction steps.[254] Compound **(176)** was oxidised to aldehyde **(177)**. The use of O_3 to oxidise the alkene instead of OsO_4, $NaIO_4$ and 2,6-lutidine as in the literature produced a better yield and also suppressed side reactions.[138] A modified Julia-Kocienski olefination of the resulting aldehyde **(177)** and a 12-carbon sulfone **(178)**, resulted the (*E*/*Z*) mixture of alkenes **(179)** (**Scheme 23**).



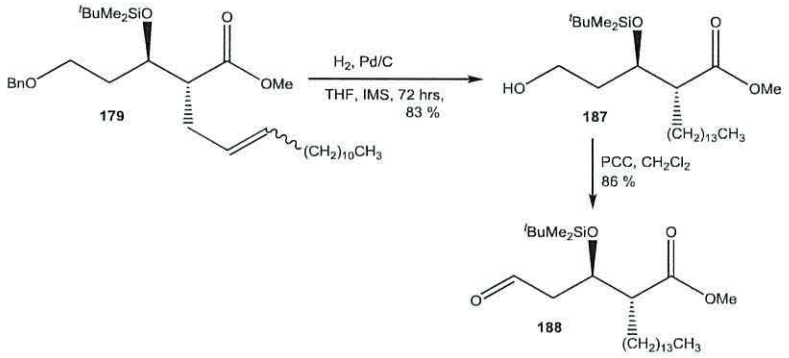
Scheme 23: The chain extension on (2*R*, 3*R*)-hydroxy ester (175)

The coupling reaction is now described in detail, since this type of chain extension is used frequently throughout this study. This reaction was first discovered by Julia *et al.* and it involves the use of a phenylsulfone and an aldehyde.[251] It was later changed slightly by Kocienski,[27,252,253] for this reason it became known as the Julia-Kocienski reaction.[27,252,253] The mechanism involves the formation of the β-alkoxysulfone **(182)** as an intermediate through reaction of the metallated sulfone **(181)** with the aldehyde. The β-alkoxysulfone **(182)** is unstable and is converted into an intermediate **(183)** by a Smiles rearrangement. This leads to the transfer of the heterocycle from sulfur to oxygen. Lastly, the lithium 1-phenyl-1-*H*-tetrazolene **(185)** and the sulfur dioxide are eliminated from **(184)** to yield the alkene **(186)** as a mixture of (*E*/*Z*)–isomers (**Scheme 24**).



Scheme 24: Mechanism of the modified Julia reaction

Debenzylation and hydrogenation of the unsaturated compound **(179)** was carried out with hydrogen gas and palladium on charcoal catalyst (10%) to yield the primary alcohol **(187)** (**See experimental 1**). The structure of compound **(187)** was confirmed primarily using ^{1}H NMR, the product no longer showing signals for the benzyl group and the olefin in its spectrum. This compound was then oxidised with PCC in order to give the aldehyde **(188)** (**See experimental 5**). The aldehyde **(188)** was different to those made before in syntheses of MA, as the α-chain carbon is shorter in **(188)** by 10 methylene groups. The formation of the aldehyde **(188)** was confirmed using ^{1}H NMR, which gave a doublet of doublets for the aldehyde proton at δ 9.80. The ^{13}C NMR spectrum offered further evidence, showing carbonyl carbons at 201.2 and 174.0 ppm for the aldehyde and carboxylic acid carbonyl groups, respectively (**Scheme 25**).

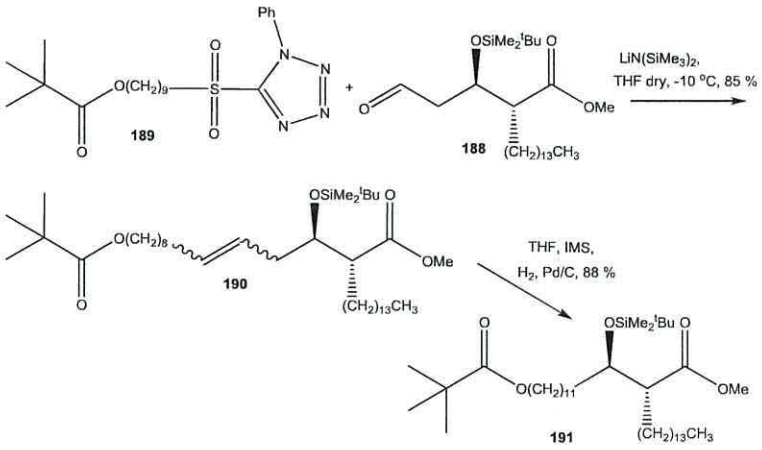


Scheme 25: Preparation of (188)

As the aldehyde **(188)** is unstable, and can undergo further oxidation to the carboxylic acid, it was used for the next stage immediately after purification.

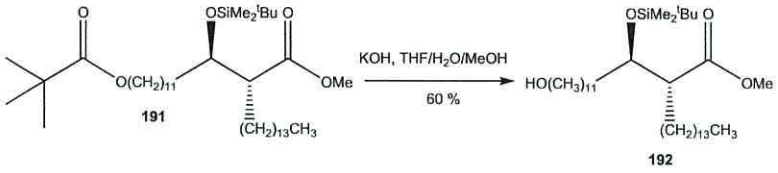
2.1.1.3 Extension of the mycolic motif chain

So as to obtain the correct chain length between the mycolic motif section and the meromycolate, the aldehyde **(188)** was coupled with **(189)** in a modified Julia reaction to give alkene **(190)** as a mixture of isomers (**Scheme 26**). The double bond was hydrogenated using hydrogen gas in the presence of palladium on carbon (10%), to give the saturated compound **(191)**. The structure of the ester **(191)** was confirmed primarily using ^{1}H NMR.



Scheme 26: Preparation of (191)

The alcohol **(192)** was prepared by deprotecting the *tert*-butyl ester **(191)** using potassium hydroxide in a mixture of THF, methanol and water at reflux. As a result, the alcohol **(192)** (**See experimental 6**) was now ready to be oxidising to the aldehyde, which could then be coupled with the meromycolate (**Scheme 27**).

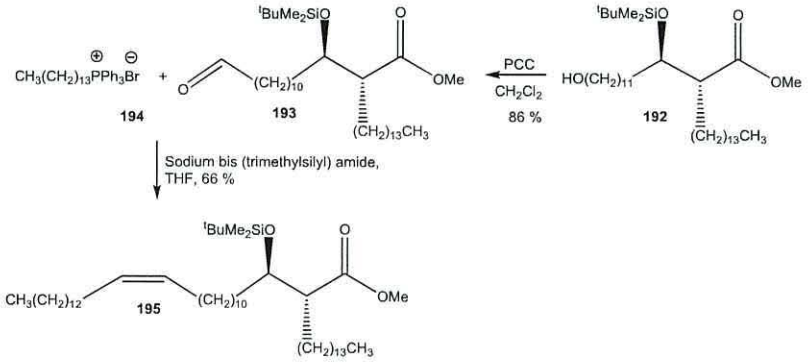


Scheme 27: Synthesis of full mycolic motif part (192)

2.1.1.4 The Wittig approach

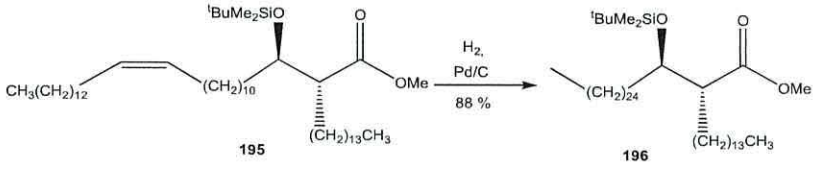
The next approach to gain the target small saturated MA **(167)**, involved a Wittig reaction. This has been reported in *R. equi*. The first step was the oxidation of alcohol **(192)** with PCC in dichloromethane at room temperature. The ^{1}H NMR spectrum of the resulting aldehyde **(193)** gave a triplet for the aldehyde proton at 9.77 ppm and the protons of the adjacent CH_2 group gave a triplet of doublets at 2.43 ppm. The ^{13}C NMR spectrum showed a signal at δ 202.9 and at 174.6 for the carbonyl groups of the ester and aldehyde, respectively. The next step was the preparation of the ylid by reacting the phosphonium salt **(194)** with sodium *bis*(trimethylsilyl)amide solution at -

78 °C, as this gives more of the *cis*-isomer **(195)** (**See experimental 8**) compared to lithium *bis*(trimethylsilyl) amide (**Scheme 28**).[258,259]



Scheme 28: Preparation of protected *cis*-alkenemycolic acid (195)

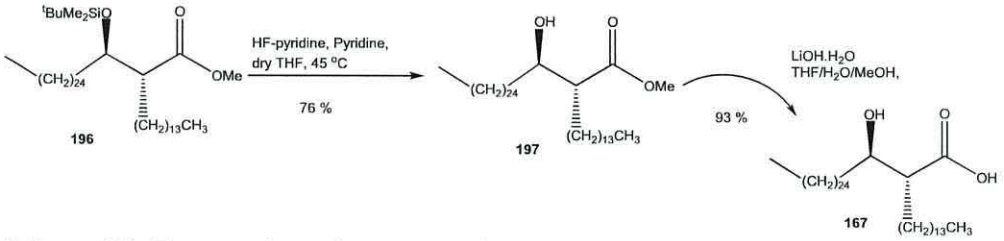
The ^{1}H NMR spectrum of the product **(195)** showed a triplet for the *cis*-alkene protons at δ 5.38. The proton at the β-chiral centre gave a multiplet between δ 3.99-3.84, while that at the α-chiral centre gave a doublet of doublets of doublets at δ 2.53. The ^{13}C NMR spectrum gave peaks at δ 129.9 and 129.8 for the alkene carbons, in addition to a carbonyl peak at δ 175.1. The IR spectrum gave absorbances at 836 and 775 cm^{-1}. The next step was the hydrogenation of *cis*-isomer **(195)** using palladium on charcoal catalyst (10%) under a hydrogen atmosphere. The formation of **(196)** (**See experimental 9**) was confirmed by the ^{1}H NMR spectrum, which showed a doublet of doublets at δ 3.91 for the β-chiral centre and a doublet of doublets of doublets at δ 2.53 for the proton at the α-chiral centre. There were no signals in the alkene region or in the ^{13}C NMR spectrum.



Scheme 29: Hydrogenation of *cis*-isomer (195)

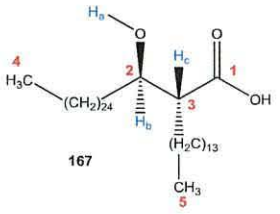
The saturated mycolic acid **(196)** was deprotected at the β-hydroxy position by stirring at 45 °C overnight with pyridine and HF-pyridine complex (**Scheme 30**). The formation of **(197)** (**See experimental 10**) was confirmed by the ^{1}H NMR spectrum,

which showed no signals for the BDMS group. The last step was the hydrolysis of the ester **(197)** to obtain the full saturated mycolic acid **(167)** using $LiOH.H_2O$. This was selected as it is a mild base compared to NaOH and KOH; furthermore, it has been reported by Yuen *et al.*[268] that it provides very good results in the hydrolysis of the esters. The hydrolysis of ester **(197)** was performed by stirring it with $LiOH.H_2O$ (15 eq.) in THF, H_2O and MeOH at 45 °C overnight (**Scheme 30**). This gave the free mycolic acid **(167)** (**See experimental 11**), which was confirmed through its analytical data being identical to those reported for the major component in the natural mixture from *R. equi.*[131]



Scheme 30: Preparation of saturated full mycolic acid (167)

The ^1H NMR spectrum of the full MA **(167)** did not show any signal corresponding to the protons of the methoxy group. This indicated that the hydrolysis had been successful. The significant peaks in the ^1H NMR spectrum were a triplet at δ 0.89 for the six protons, for the two terminal methyl groups (**Table 2**) and two multiplets between δ 2.53-2.43 and between δ 3.62-3.60 for the α and β protons respectively. The $-CH_2-$ protons of the aliphatic chains appeared as a multiplet between δ 1.57-1.45. The ^{13}C NMR spectrum gave a carbonyl signal at δ 177.9 in addition to other signals between δ 35.5-14.1 for the CH_2 and CH_3 carbons. Also, IR absorbances were seen at 3530 and 1687 cm^{-1} for the OH group and C=O group respectively.

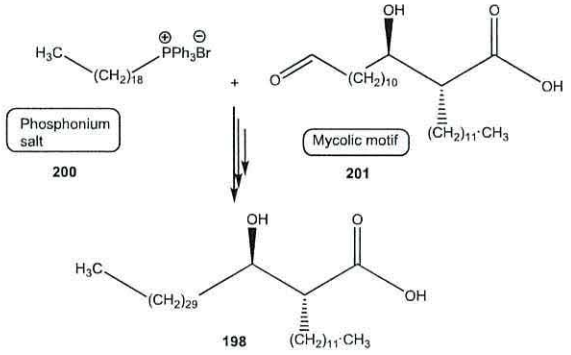


Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_a	3.84-3.66	m		1	177.9
H_b	3.62-3.60	m		2	72.1
H_c	2.53-2.43	m		3	35.5-22.6
$-(CH_2)_n-$	1.57-1.45	m		4	14.1
2 x CH_3	0.89	t	6.8	5	

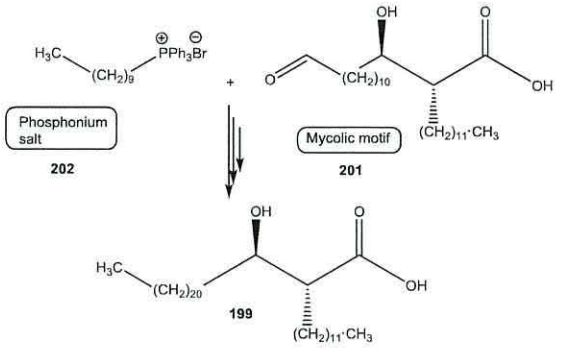
Table 2: ^{1}H and ^{13}C NMR signals for compound (167)

2.1.2 Synthesis of (2*R*,3*R*)-2-Dodecyl-3-hydroxytritriacontanoic acid (198) and (2*R*,3*R*)-2-Dodecyl-3-hydroxytetracosanoic acid (199)

Since the synthesis of the mycolic acid **(167)** was successful, the same method was applied for the synthesis of MAs **(198)** and **(199)**. The first mycolic motif **(201)** was prepared with a C-12 side chain using the same protocol as for **(193)**. Similarly, the phosphonium salts **(200)** and **(202)** were coupled to the motif chain **(201)** using a Wittig reaction to give the full saturated MA **(168)** and **(167)** respectively. This resulted in the creation of the free MAs **(198)** and **(199)**, which are identical to the major components reported in the natural mixture from *R. equi*.[131,132]



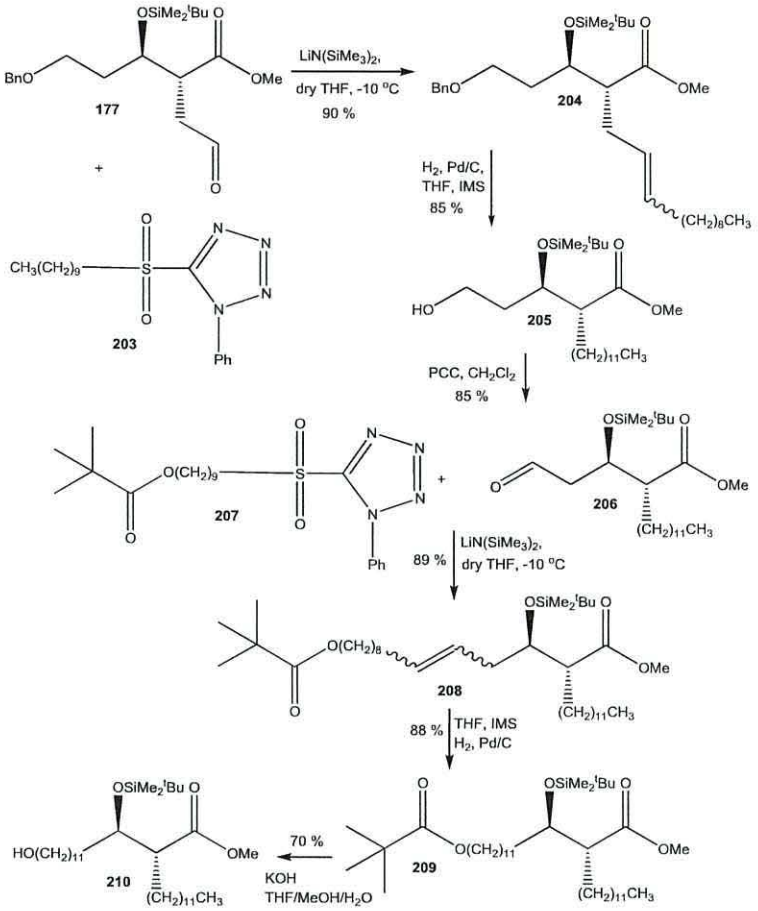
Scheme 31: Plan for the preparation of mycolic acid (198)



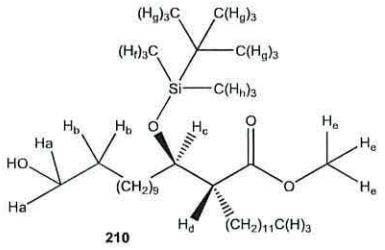
Scheme 32: Plan for the preparation of mycolic acid (199)

To prepare the mycolic motif chain **(210)** with the correct number of carbons in the side chain, O_3 was used to oxidise the alkene to the aldehyde **(177)**. Then, using a modified Julia-Kocienski olefination conducted between the resulting aldehyde **(177)** and a 10-carbon sulfone **(203)**, the alkenes **(204)** were formed. Hydrogenation and debenzylation of the alkene mixture **(204)** was carried out with hydrogen gas as described previously to give the primary alcohol **(205)**. The ^{1}H NMR of the product no longer displayed signals for either the benzyl-group or the olefin.

Oxidation of **(205)** was done using PCC gave the aldehyde **(206).** The ^{1}H NMR of this gave a doublet of doublets for the aldehyde proton at δ 9.8. The ^{13}C NMR spectrum showed signals at δ 201.2 and 174.0 for the aldehyde and carboxylic acid respectively. The aldehyde **(206)** was coupled to **(207)** using LiHMDS as the base in dry THF. The formation of the alkene **(208)** was verified by the ^{1}H NMR and the ^{13}C NMR spectra. The next step was hydrogenation to reduce the double bond using palladium on carbon (10%) as catalyst under a hydrogen atmosphere. The ^{1}H NMR spectrum showed no signals in the alkene region. The hydrogenation was followed by hydrolysis of compound **(209)** using potassium hydroxide in THF: MeOH: H_2O. The product **(210)** (**See experimental 4**) ^{1}H NMR spectrum is shown in Table **(3)**. The IR spectrum showed a broad absorbance at 3400 cm^{-1} due to the hydroxyl group.



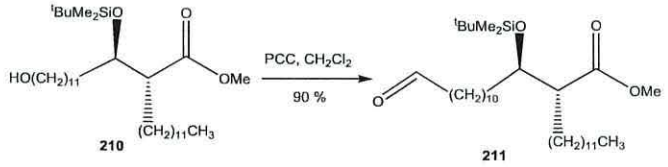
Scheme 33: Extension of the side chain of the mycolic motif to give (210)



H_x	δ	Multiplicity	Integration	J (H_z)
H_a	3.75	t	2	6.6
H_b	1.56	q	2	6.6
H_c	3.91-3.88	m	1	
H_d	2.53	ddd	1	10.9, 7.1, 3.8
H_e	3.66	s	3	-
SiMe	0.04	s	3	-
SiMe	0.02	s	3	-
^{t}Bu	0.96	s	9	-

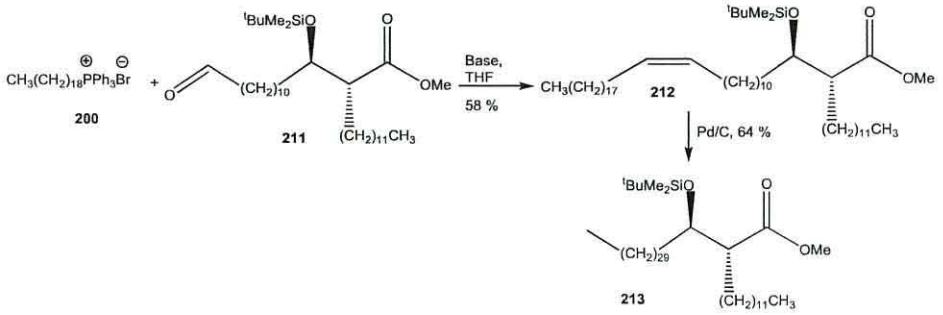
Table 3: NMR analysis for compound (210)

The next step was the formation of aldehyde **(211)** by oxidation of alcohol **(210)** with PCC in dichloromethane at room temperature. The ^{1}H NMR spectrum of the resulting aldehyde **(211)** (**See experimental 18**) gave a triplet for the aldehyde proton at δ 9.76. The ^{13}C NMR spectrum gave signals at δ 202.8 and 175.1 for the two carbonyl groups of the aldehyde and ester, respectively.



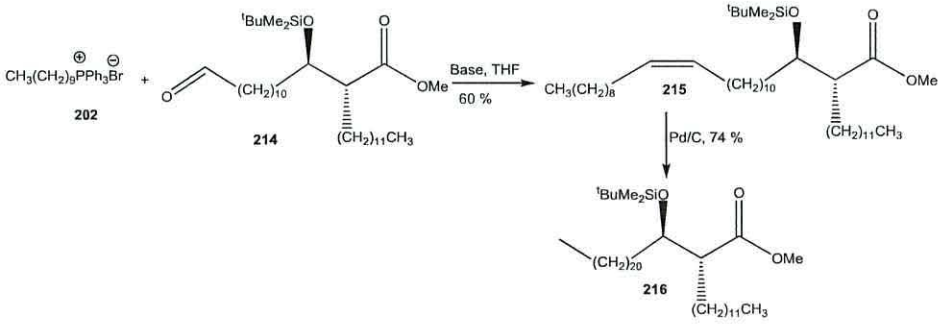
Scheme 34: Preparation of aldehyde (211)

The next step was the preparation of the ylid by reacting the phosphonium salt **(200)** with a sodium *bis*(trimethylsilyl)amide solution (2.1 M in THF) as this gives more of the *cis*-isomer **(212)** (**See experimental 19**). The addition of the base was done at -78 ^{o}C and the mixture was left to stir for 30 minutes before the aldehyde **(211)** was added to the reaction mixture to give the *cis*-isomer alkene (**Scheme 35**).



Scheme 35: Preparation of protected mycolic acid (213)

The creation of the product **(212)** was confirmed by the proton NMR spectrum which showed a triplet for the *cis* alkene protons at δ 5.38. The proton at the β-chiral centre gave a doublet of doublets at δ 3.91 while the proton at the α-chiral centre showed a doublet of doublets of doublets at δ 2.53. The ^{13}C NMR spectrum gave peaks at δ 129.9 and 129.8 for the *cis* alkene carbons, in addition to a peak at δ 175.1 for the carbonyl carbon. The next step was the hydrogenation of *cis*-isomer **(212)** using palladium on carbon (10%) and hydrogen. The ^{1}H NMR spectrum of the product **(213)** (**See experimental 21**) showed a doublet of doublets at δ 3.91 for the β-chiral centre and a doublet of doublets of doublets at δ 2.53 for the proton at the α-chiral centre. The disappearance of signals in the alkene area was also seen. The ^{13}C NMR spectrum also showed the disappearance of alkene carbons in compound **(213)**. In similar way, the saturated MA **(216)** was prepared (**Scheme 36**).

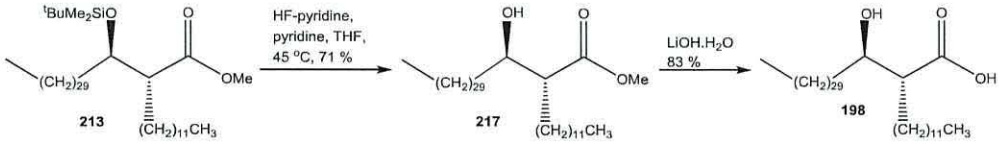


Scheme 36: Preparation of protected MA (216)

The ^{1}H NMR, ^{13}C NMR and IR data for the protected MA **(216)** (**See experimental 22**) were almost identical to those of the previously prepared protected MA **(213)**. The saturated MA **(213)** was stirred at 45 °C overnight with a mixture of pyridine and HF-pyridine complex (**Scheme 37**). The formation of **(217)** (**See experimental 23**) was

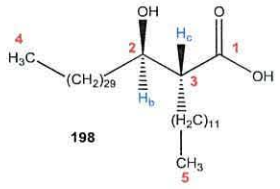
confirmed by the ^{1}H NMR spectrum which showed the disappearance of the 9H singlet at δ 0.9 and the signals belonging to the CH_3 groups bound to silicon. The last step was the hydrolysis of the ester **(217)**, in order to obtain the full saturated mycolic acid **(198)** (**See experimental 25**).

This was achieved by stirring it with $LiOH.H_2O$ (15 eq.) in $THF/H_2O/MeOH$ at 45 °C overnight (**Scheme 37**). The free mycolic acid **(198)** corresponded to the major component reported in the natural mixture from *R. equi*.[131,132] The natural MA present in *R. equi* was discussed at the introduction chapter.



Scheme 37: Preparation of saturated full MA (198)

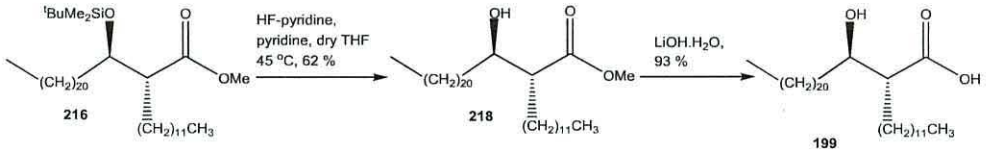
The ^{1}H NMR spectrum of acid **(198)** did not show any signal corresponding to the methoxy group. The significant peaks were a six-proton triplet at δ 0.89 for the terminal alkyl groups (**Table 4**), a multiplet between δ 2.52-2.45 for the α-proton and a doublet of triplets at δ 3.73 for the β-proton. The CH_2 protons of the aliphatic chains were seen as a multiplet between δ 1.81-1.69. The ^{13}C NMR spectrum revealed a carbonyl signal at δ 174.1, in addition to signals between δ 14.1 and 35.5 for the CH_2 and CH_3 carbons. The presence of the the -OH group and C=O groups was also confirmed by signals at 3559 and 1688 cm^{-1} in the IR spectrum.



Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_b	3.73	dt		2	72.1
H_c	2.52 –2.45	m		3	35.5 -22.6
$-(CH_2)_n-$	1.81-1.69	m		4	14.1
2 x CH_3	0.89	t	6.8	5	14.0

Table 4: ^1H and ^{13}C NMR signals for compound (198)

In similar way, the saturated mycolic acid **(199)** was prepared, (**Scheme 38**).

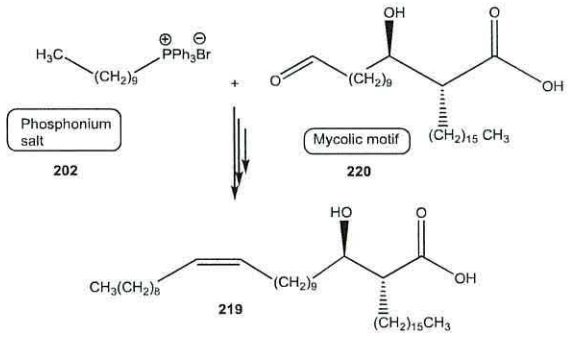


Scheme 38: Preparation of saturated full mycolic acid (199)

The ^1H NMR, ^{13}C NMR and IR data for the full saturated mycolic acid **(199)** were almost identical to those of the previously prepared full mycolic acid **(198)**.

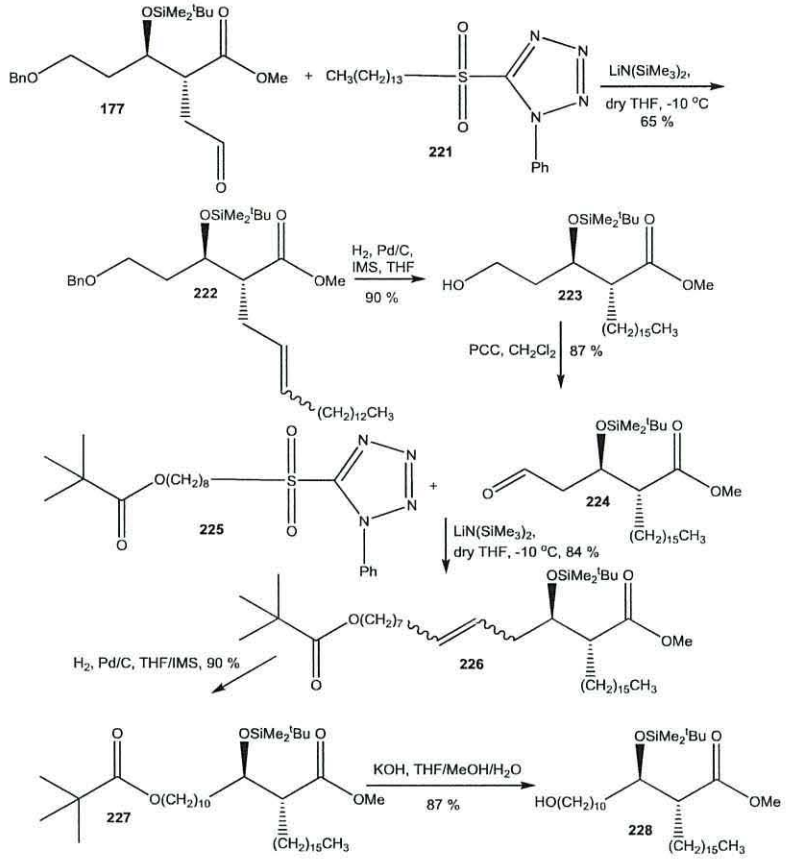
2.1.3 The synthesis of (2*R*,3*R*,*Z*)-2-Hexadecyl-3-hydroxytricos-13-enoic acid (219) as a model

As seen above, the previous synthesis of saturated mycolic acid **(167)** was successful. The first target was therefore to synthesis *cis*-MA **(219)** without the hydrogenation of the double bond formed by the Wittig reaction. This would be achieved by synthesis of the mycolic motif **(220)**, followed by linking this to the meromycolate **(202)** using similar methods to those used in the synthesis MA **(167)**.



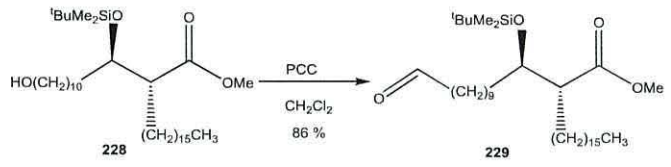
Scheme 39: Plan for the preparation of unsaturated MA as a model (219)

To prepare the mycolic motif **(228)**, with the correct number of carbons in the side chain, O_3 was used to oxidise the alkene into an aldehyde. A modified Julia-Kocienski olefination conducted between the resulting aldehyde **(177)** and the 14-carbon sulfone **(221)** resulted in the alkene mixture **(222)**. Hydrogenation and debenzylation of the mixture with hydrogen gas as described previously gave the primary alcohol **(223)**, the ^1H NMR of which no longer displayed signals for either the Bn-group or the olefin. Oxidation of the mycolic motif **(223)** using PCC gave the aldehyde **(224)**, ^1H NMR of which gave a triplet for the aldehyde proton at δ 9.81. The ^{13}C NMR spectrum provided further evidence, showing signals at δ 201.2 and 174.5 for the aldehyde and carboxylic acid carbons group, respectively. The aldehyde **(224)** was coupled to **(225)** using LiHMDS as a base, as described previously. The formation of the alkene **(226)** was verified by the ^1H NMR and ^{13}C NMR spectra. The next step was hydrogenation to reduce the double bond using palladium on carbon (10%) as the catalyst under a hydrogen atmosphere. The ^1H and ^{13}C NMR spectra of the product **(227)** showed no signals in the alkene region. The CH_2 adjacent to the oxygen showed as a triplet at δ 4.04, while the protons at the chiral centres showed a multiplet for the β-proton between δ 3.91-3.87 and a doublet of doublets of doublets at δ 2.52 for the α-proton. The hydrogenation was followed by hydrolysis of compound **(227)** using potassium hydroxide in a mixture of THF/MeOH/H_2O. The spectroscopic data for the ester **(228)** (**See experimental 38**) were almost identical to those of the previously prepared ester, the only difference being for the additional 4 × CH_2 group, measured using MALDI (**Scheme 40**).



Scheme 40: Extension of the side chain of the mycolic motif to give (228)

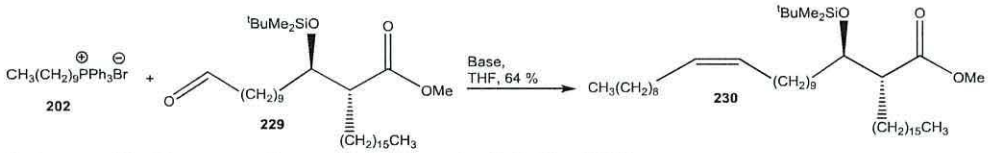
The next step was the oxidation of alcohol **(228)** with PCC in dichloromethane at room temperature. The ^{1}H NMR spectrum of the resulting aldehyde **(229)** (**See experimental 39**) gave a triplet for the aldehyde proton at δ 9.77 and the protons of the CH_2 group next to the aldehyde group gave a triplet of doublets at δ 2.42. The ^{13}C NMR spectrum gave a signal at δ 202.8 and 175.0 for the carbonyl carbons of the aldehyde and ester respectively (**Scheme 41**).



Scheme 41: Preparation of aldehyde (229)

The next step was the preparation of the ylid by reacting the phosphonium salt **(202)** with a sodium *bis*(trimethylsilyl)amide solution (2.1 M in THF) to give the *cis*-isomer

(230). The addition of the base was done at -78 °C and the mixture was left to stir for 30 minutes before the aldehyde **(229)** was added to the reaction mixture to give *cis*-isomer alkene (**Scheme 42**).

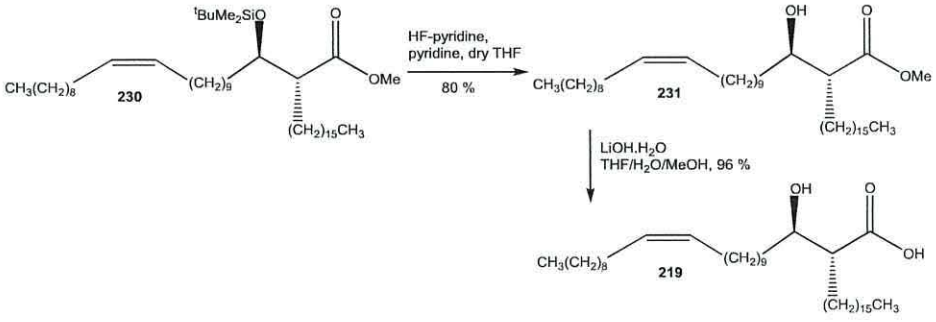


Scheme 42: Preparation of protected *cis*-MA (230)

The ^{1}H NMR spectrum of the product **(230)** (**See experimental 40**) showed a triplet for the *cis*-alkene protons at δ 5.38. The β-chiral centre gave a multiplet between δ 3.92-3.89, while the α-chiral centre showed a doublet of doublets of doublets at δ 2.55. The ^{13}C NMR spectrum gave peaks at δ 129.9 and 129.8 for the *cis*-alkene carbons, in addition to a peak at δ 175.1 for the carbonyl carbon. The IR spectrum gave absorbances at 836 and 775 cm^{-1}.

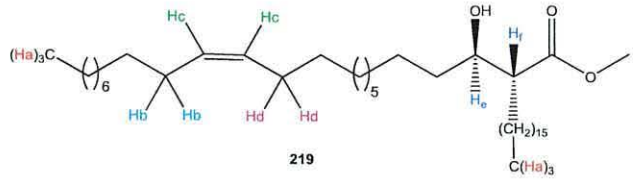
The *cis*-mycolic acid **(230)** was deprotected by stirring at 45 °C overnight with pyridine and HF-pyridine complex (**Scheme 43**). The ^{1}H NMR spectrum showed the disappearance of the 9H singlet at δ 0.84 and the signals belonging to the CH_3 groups bound to the silicon.

The last step was the hydrolysis of the ester **(231)**, in order to achieve the full unsaturated mycolic acid **(219)** (**See experimental 42**). This was achieved by stirring it with $LiOH.H_2O$ (15 eq.) in a mixture of $THF/H_2O/MeOH$ at 45 °C overnight (**Scheme 43**).



Scheme 43: Preparation of saturated full mycolic acid (219)

The ^{1}H NMR spectrum of the full MA **(219)** did not show any signal corresponding to the protons of the methoxy group. The formation of **(219)** was confirmed by the ^{1}H NMR spectrum (**Table 5**). The structure of the α-alkyl-β-hydroxy carboxylic **(219)** was further checked by the ^{13}C NMR and IR spectra.



219

Proton	δ	Multiplicity	*J* (Hz)	Integration
H_a	0.88-0.83	m	-	6
H_b	1.74-1.39	m	-	2
H_c	5.37	t	10.8	2
He	3.67-3.63	m	-	1
Hf	2.44	dt	9.15, 5.35	1

Table 5: NMR analysis for mycolic acid (219)

2.1.4 Synthesis of trehalose esters present in *R. equi*

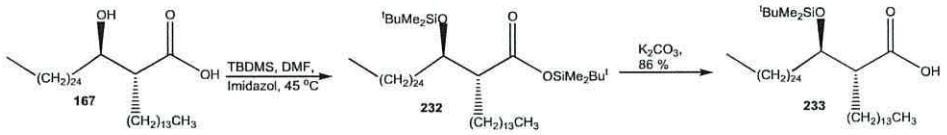
The synthesis of trehalose esters of MA in this study followed the procedure described in the Introduction. The target was to prepare sugar esters of the MAs **(167)**, **(198)**, **(199)** and **(219)**, which are identical to the major component reported in the natural mixture from *R. equi*.[131,132]

2.1.4.1 Saturated MA protection and esterification with trehalose

In order to prepare a TDM from the saturated mycolic acid **(167)**, it was necessary to protect the β-hydroxyl group as a *tert*-butyldimethylsilyl ether. The MA **(167)** was stirred with imidazole and *tert*-butyldimethylsilyl chloride in DMF for 18 h at 45 °C. By this method, the crude product was protected at both the β-hydroxyl and the carboxylic acid. To deprotect the carboxylic acid group, it was dissolved in a mixture

of THF/H_2O/MeOH and potassium carbonate was added and stirred overnight at 45 ^oC.

The formation of compound **(233)** (**See experimental 12**) was comfirmed by the ^1H NMR spectrum, which showed peaks at δ 0.14 (3H, s) and 0.13 (3H, s) for the methyl groups bound to the silicon in the protecting TBDMS group, while the protons of the *tert*-butyl group appeared as a singlet at δ 0.92 (9H) (**Scheme 44**).



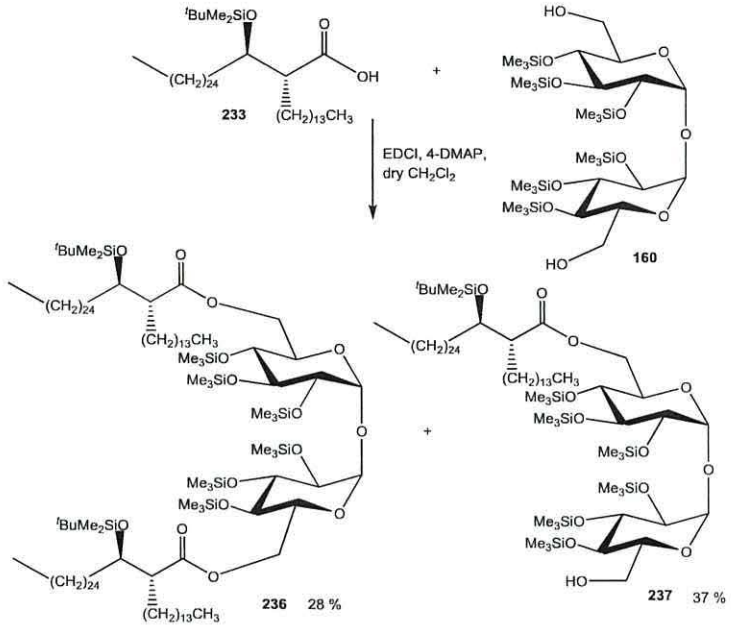
Scheme 44: Protection of the β-hydroxyl group

The ^{13}C NMR spectrum provided further evidence for the protection of the hydroxyl group, showing signals at δ -4.2 and δ -4.8 respectively for the two silicon bound methyl groups. The IR spectrum indicated a very broad absorbance at 3435 cm^{-1} for the hydroxyl group and a band for a carbonyl group at 1700 cm^{-1}.

In similar way, compounds **(234)** (**See experimental 27**) and **(235)** (**See experimental 28**) were prepared. The ^1H NMR, ^{13}C NMR and IR data for these were almost identical to those of the previously prepared ester **(233)**, the only difference being for the additional CH_2 groups, which was measured using MALDI (**Figure 28**).

Figure 28: Preparation of compounds (234) and (235)

The next step was an esterification reaction between the protected saturated mycolic acid **(233)** and protected sugar trehalose **(160)** in the presence of EDCI (1-ethyl-3(3-dimethylaminopropyl)carbodiimide) as an activating agent and DMAP (4-dimethylaminopyridine) as a catalyst, in dry CH_2Cl_2 at room temperature for 6 days (**See experimental 13**), (**Scheme 45**).



Scheme 45: Preparation of TDM and TMM

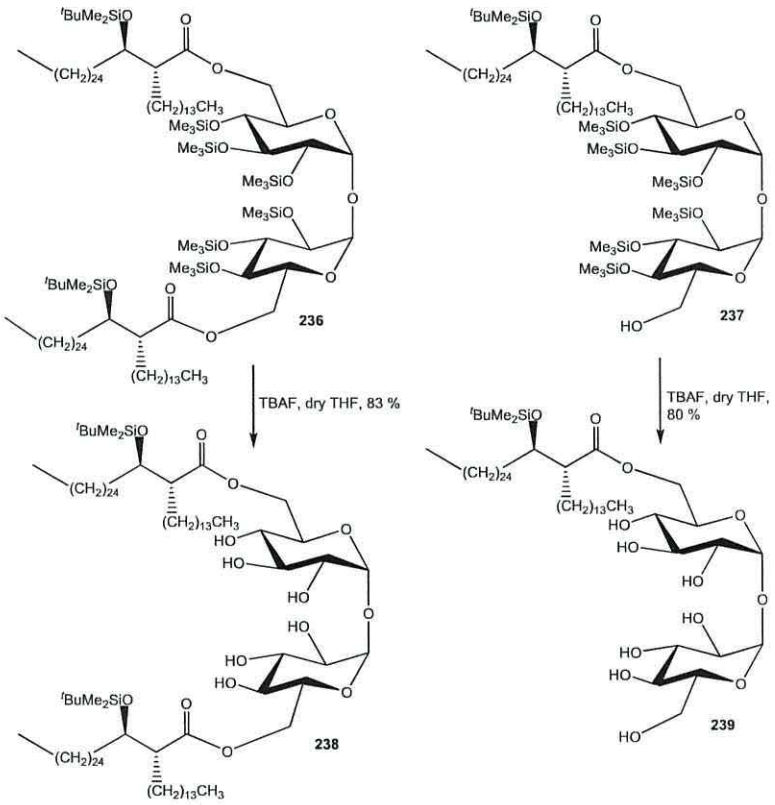
The first fraction separated by column chromatography was the trehalose-6,6'-dimycolate (TDM) **(236)**. The ^{1}H NMR spectrum for **(236)** showed H1-H1'signals in the sugar resonating at δ 4.85 with an integration of (2H). The H6-H6' protons came next in the spectrum between δ 4.67-4.40 (again 2H). The remaining sugar core signals, a total of 10 protons, could be seen from δ 4.08-3.48. The protons in the β-position of the acid came next at δ 3.38, one for each acid. The next signal resonating between δ 2.57-2.53 corresponded to the two protons in the α-position and the CH_2 within the acid chain. On the other hand the ^{13}C NMR spectrum showed the most downfield signal at δ 173.8 corresponding to the carboxylic acid, followed by the sugar core carbon signals ranging from δ 73.5-62.3. The next two signals corresponded to the β-position and α-position carbons of the acid, resonating at δ 62.3 and 51.8 respectively. The signals for the methyl carbon of the TBDMS group were seen at δ -4.4 and -4.6. MALDI MS showed an $[M+Na]^{+}$ of 2263.7, while the calculated value was 2262.7.

The second fraction was the trehalose monomycolate (TMM) **(237)**. The ^{1}H NMR spectrum showed the same pattern of signals as **(236)**; however, the sugar signals were doubled due to the lack of symmetry. The ^{13}C NMR spectrum included the C=O group resonating at δ 174.0. The sugar core carbons display signals from δ 94.5-72.7. The

remaining signals showed the same appearance as observed for the case of **(236)**. Although signals were satisfactory, they were a little bit broad, which may be due to a dilute/weak sample or the high molecular weight of the compound. MALDI MS showed an $[M+Na]^+$ of 1530.4, while the calculated value was 1530.1.

2.1.4.2 Trimethylsilyl deprotection of TDM (236) and TMM (237)

Deprotection of the trimethylsilyl groups on the sugar was achieved employing with tetra-*n*-butylammonium fluoride and dry THF under a nitrogen atmosphere to give **(238)** (83%) (**See experimental 14**) and **(239)** (80%) , (**See experimental 16**) respectively (**Scheme 46**).



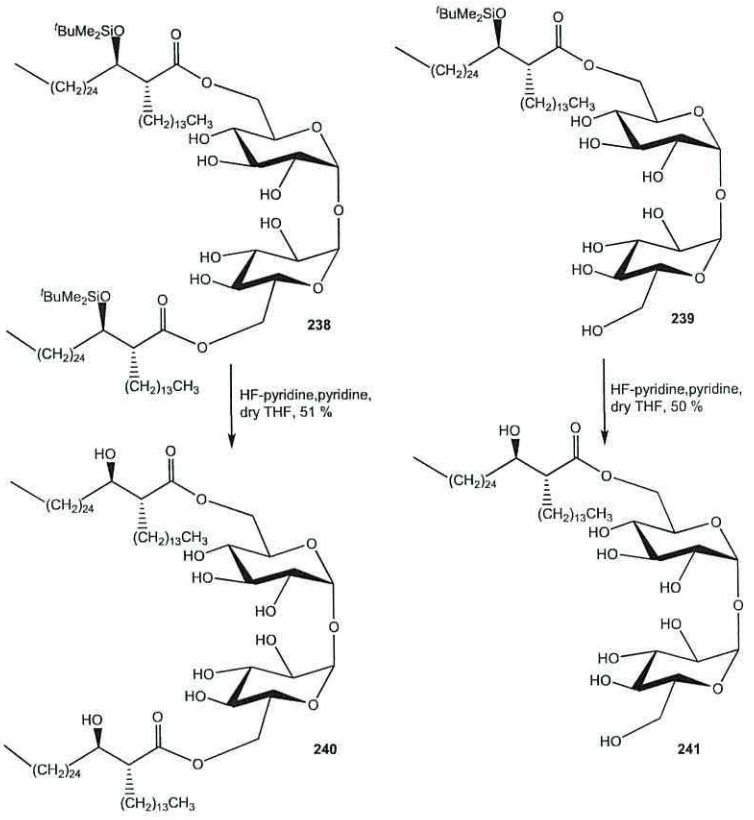
Scheme 46: The deprotection of the sugar hydroxyl groups in (236) and (237)

Compound **(238)** had lost the signals for the 54 protons corresponding to the 18 methyl groups (Me_3Si) previously resonating at δ 0.16-0.14. On the other hand, compound **(239)** had lost the protons previously resonating at δ 0.17-0.15. MALDI MS showed for **(238)** an $[M+Na]^+$ of 1831.3, while the calculated value was 1830.5. As well as

(239) MALDI MS showed an $[M+Na]^+$ of 1097.8, while the calculated value was 1097.8.

2.1.4.3 Deprotection of the TBDMS group of TDM (238) and TMM (239)

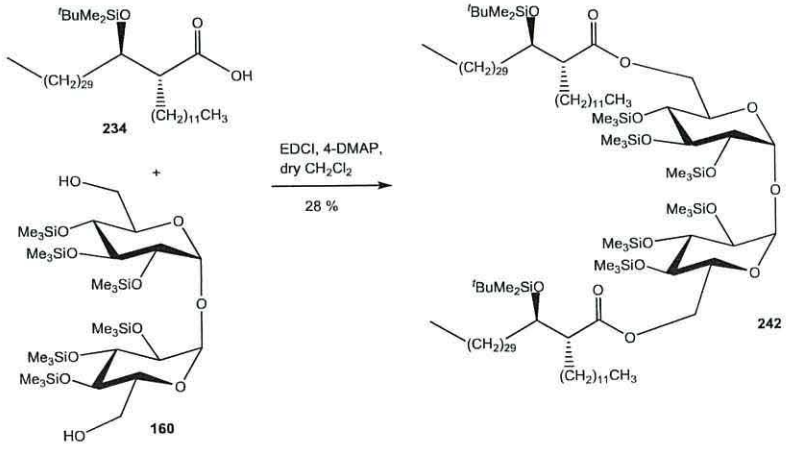
The last step was deprotection of the *t*-butyldimethylsilyl from the β-hydroxy group in **(238).** Protected TDM **(238)** was stirred at 45 °C overnight with pyridine and HF-pyridine complex, (**Scheme 50**). The formation of the resulting free TDM **(240)** (**See experimental 15**) was confirmed by the ^{1}H NMR spectrum which gave a doublet at 4.98 for the hemiacetal protons. The rest of the sugar protons resonated at δ 4.67, 4.22, 3.98-3.88, 3.76, 3.65-3.61, 3.45 and at 3.2 with two protons integrated in each signal. The protons next to the β-hydroxyl of the MA showed a multiplet at δ 3.98-3.88. The α-protons of the mycolic moiety showed a triplet of doublets at δ 2.37. The ^{13}C NMR showed the carbonyl carbon at δ 175.4 and the anomeric carbon at 94.9. The rest of the sugar carbons resonated at δ 72.5 and 71.1. MALDI MS showed for **(240)** an $[M+Na]^+$ of 1602.9, while the calculated value was 1602.3. The final step in the deprotection of TMM **(239)** was hydrolysis of the TBDMS ether using the method described above. The ^{1}H NMR spectrum of product TMM **(241)** (**See experimental 17**) gave a doublet for the hemiacetal protons at δ 5.06 and signals for the rest of the sugar protons at δ 4.61 and at δ 3.26. The MA moiety showed a doublet of doublets at δ 3.54 for the β-proton and a multiplet between δ 2.40-2.37 for the α-proton. MALDI MS showed for **(241)** an $[M+Na]^+$ of 983.6, while the calculated value was 983.7.



Scheme 47: The deprotection of the silyl groups in (238) and (239)

2.1.4.4 Esterification of MAs (234) and (235) with protected trehalose

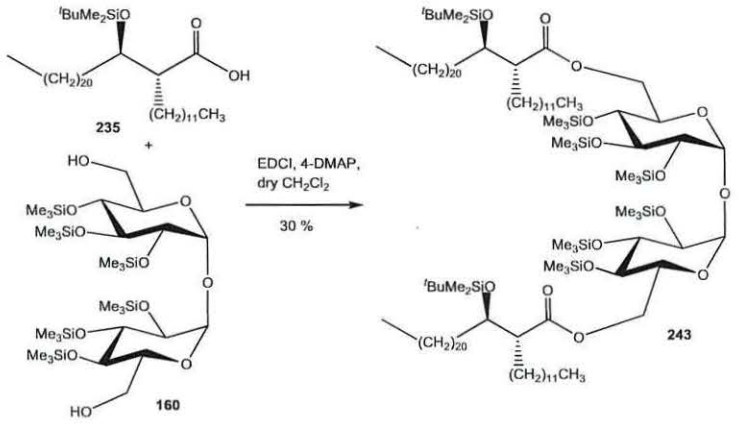
The esterification of the trehalose **(160)** with the protected β-hydroxy acid **(224)** was carried out as described in the previous esterification (**Scheme 48**).



Scheme 48: Esterification of protected trehalose with acid (234)

The major product was TDM **(242)** (28%) (**See experimental 30**). The ^{1}H NMR spectrum showed signals for five sugar protons at δ 4.85, 4.40, 4.32, 4.15 and 3.95, respectively. The signals for the CH_2 protons next to the ester oxygen appeared at δ 3.44. In addition, the trimethylsilyl groups on the sugar gave the 18H-singlets at δ 0.17-0.14. The ^{13}C NMR spectrum showed a signal at δ 173.8 corresponding to the carboxylic acid. The sugar carbon signals were seen between δ 94.3 and 70.7. Signals at δ 62.3 and 51.8 corresponded to the α-carbon and β-carbon atoms of the acid, respectively.

In the similar way, the esterification of the trehalose **(160)** with the protected β-hydroxy acid **(235)** was undertaken (**Scheme 49**).

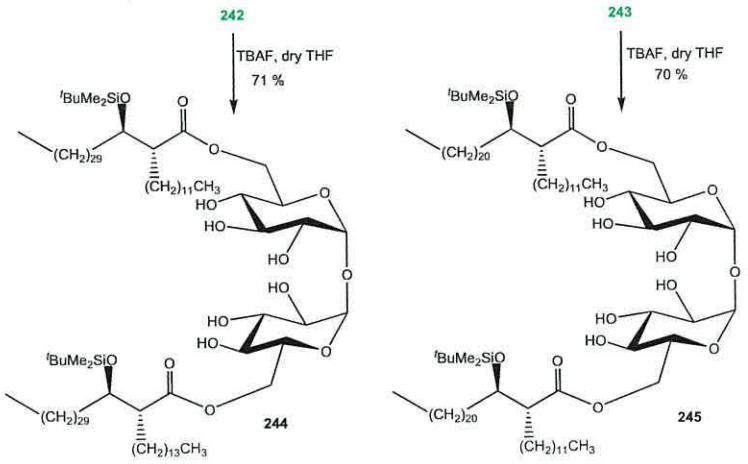


Scheme 49: Esterification of protected trehalose (160) with acid (235)

The ^{1}H NMR, ^{13}C NMR and IR data for compound **(243)** (**See experimental 29**) were almost identical to those of the previously prepared TDM **(242)**, the only difference being for the additional CH_2 group, which was measured using MALDI MS.

2.1.4.5 Removal of the trehalose protecting groups

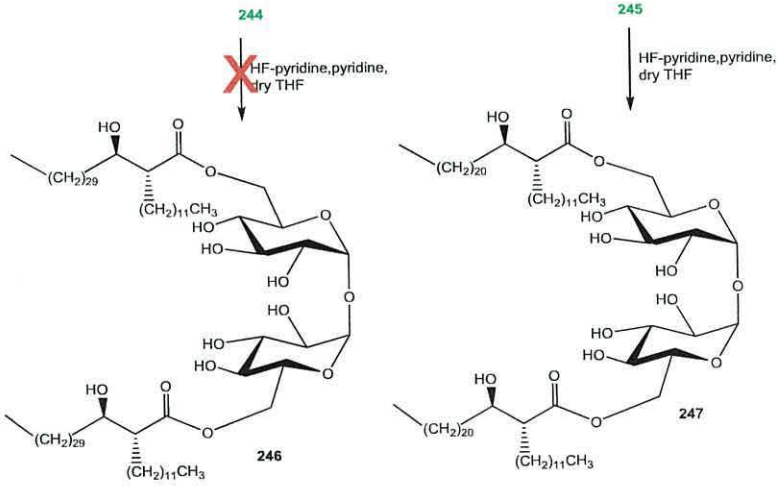
The deprotection started with the removal of the TMS groups on the sugar in compounds **(242)** and **(243)** using TBAF in dry THF. This gave a 71% yield of compound **(244)** (**See experimental 32**), and 70% yield of compound **(245)** (**See experimental 31**), respectively (**Scheme 50**). The ^{1}H NMR spectrum showed that the signals for the trimethylsilyl protecting group had disappeared confirming the successful deprotection.



Scheme 50: Desilylation of the trehalose

2.1.4.6 Deprotection of the hydroxy acid part in (244) and (245)

The second step involved deprotection of the TBDMS group on the β-hydroxy position of compounds **(244)** and **(245)** in dry THF using HF-pyridine and pyridine. Deprotection of **(245)** was successful, giving rise to TDM **(247)** with a yield of 30% (**See experimental 33**). However, the ^{1}H NMR for the crude deprotection product of **(244)**, which should have given rise to **(246)** (**See experimental 34**), instead showed a broad multiple peak for a long chain hydrocarbon and no peak for sugar protons remained (**Scheme 51**).



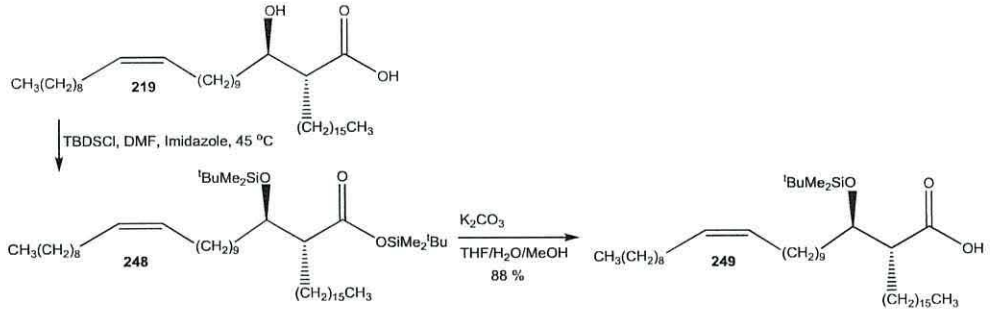
Scheme 51: Desilylation of the hydroxy acid

2.1.5 Towards the synthesis of TDM from an alkene MA

2.1.5.1 Protection of the hydroxy group of alkene MAs

In order to prepare the unsaturated TDM from the MA **(219)**, it was essential to protect the β-hydroxyl group using *tert*-butyldimethylsilyl. This was done in the same way as for compound **(233)**. The MA **(219)** was mixed with imidazole and *tert*-butyldimethylsilyl chloride in dry DMF and stirred for 18 h at 45 °C. The crude product **(248)**, protected at both the β-hydroxyl and the carboxylic acid, was dissolved in a mixture of THF/H_2O/MeOH and K_2CO_3 was added and stirred overnight at 45 °C. This deprotects the acid group while leaving the β-hydroxyl protected.

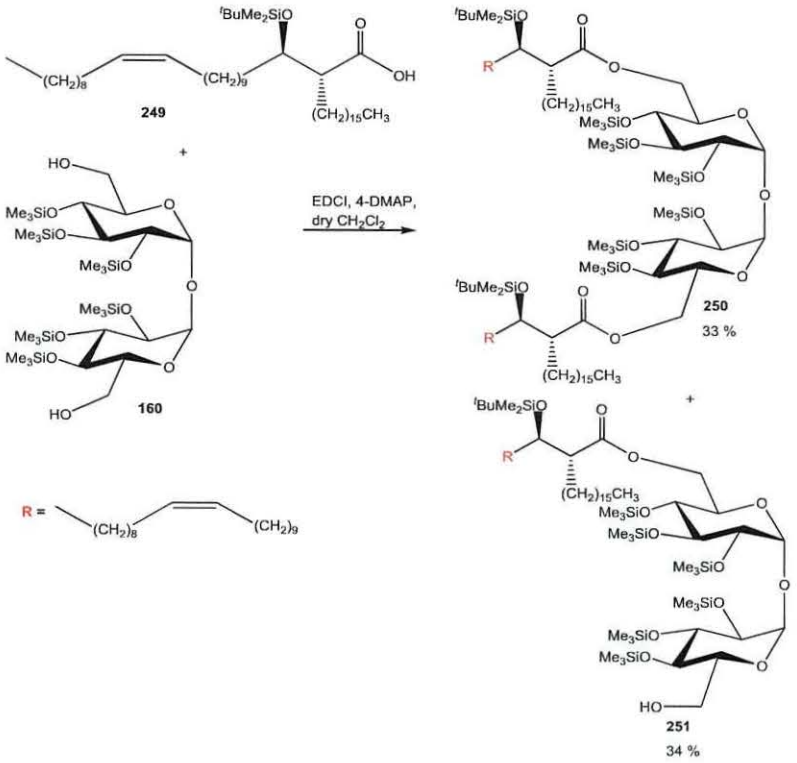
The ^{1}H NMR spectrum of **(249)** (**See experimental 43**) gave singlets for the *t*-butyl at δ 0.9, at δ 0.14 and at δ 0.13 for two CH_3 groups next to the silicon (**Scheme 52**).



Scheme 52: Protection of the β-hydroxyl group

2.1.5.2 The coupling reaction

The protected MA **(249)** was used to prepare the first unsaturated TDMs as TDM **(250)** and TMM **(251)**. The same method was employed as was used to prepare TDM **(240)** and TMM **(241)** was used. The product was separated into two fractions by column chromatography (**See experimental 44**), (**Scheme 53**).



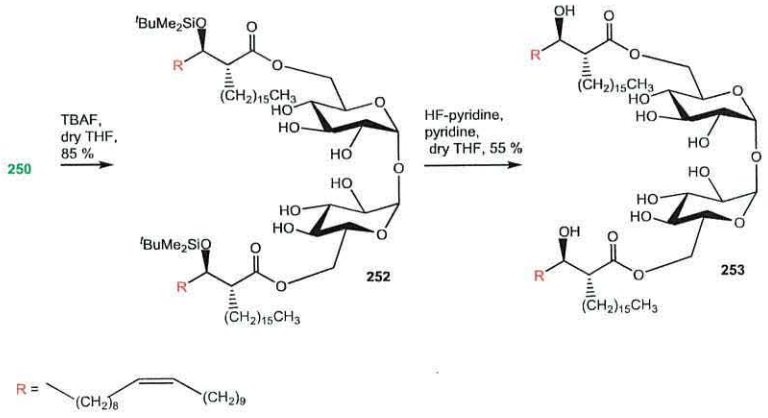
Scheme 53: Preparation of unsaturated trehalose ester

The first fraction was protected TDM **(250)**, the ^{1}H NMR spectrum of which gave a triplet at δ 5.36 for the four alkene protons, while the two hemiacetal protons gave a doublet at δ 4.84. The remainder of the sugar protons resonated between δ 4.37 and 3.38, including the two β-protons of mycolic acid component next to the silyl ether. The α-protons of the carboxylic acid of the MA showed a doublet of doublets of doublets at δ 2.56 for two protons. The trimethyl silyl ether protecting groups gave signals at δ 0.16, 0.14 and -0.01 with an integration of 18 protons for each peak. The *t*-butyl groups gave a singlet at δ 0.9 with an integration of 18 protons and the methyl groups of the TBDMS gave a broad singlet at δ -0.06. The second fraction was protected TMM **(251)**, which showed a triplet at δ 5.35 for the two alkene protons. The hemiacetal protons gave single proton doublets at δ 4.91 and at 4.84. The rest of the sugar protons resonated between δ 4.36 and 3.4. The proton at the β-position in the MA component give a doublet of triplets at δ 4.0 while the proton at the α-chiral centre gave a doublet of doublets of doublets at δ 2.57. The terminal methyl groups gave a 9H-singlet at δ 0.9. The TMS groups appeared at δ 0.17, 0.16, 0.156, 0.15 and 0.12 with an integration of nine protons for each peak and the TBDMS group gave a triplet

at δ 0.88 while the dimethyl silyl protons resonated at δ 0.06 and 0.05 with an integration of three protons per signal.

2.1.5.3 Deprotection of TDM (250)

The first deprotection step involved the use of TBAF in order to remove the TMS group from the sugar. The formation of the product **(252)** (**See experimental 45**) was confirmed by the ^{1}H NMR spectrum which showed the absence of any peak for the TMS group. The following step was the removal of the TBDMS ether from the mycolic acid of compound **(252)** using HF-pyridine and pyridine. The formation of TDM **(253)** (**See experimental 46**) was checked using ^{1}H NMR, which gave a triplet for four protons at δ 5.33 for the alkene protons, while the hemiacetal gave a doublet for two protons at δ 5.03. The remainder of the sugar protons resonated between δ 4.33 and 3.45. The protons at the β-position of the mycolic acid gave a doublet of triplets for two protons at δ 3.55. The ^{13}C NMR gave a peak for the carbonyl carbon at δ 175.4 and for the alkene carbons at δ 129.8. The hemiacetal carbon resonated at δ 94.9 and the rest of the sugar carbons resonated between δ 72.4 and 69.8 (**Scheme 54**).

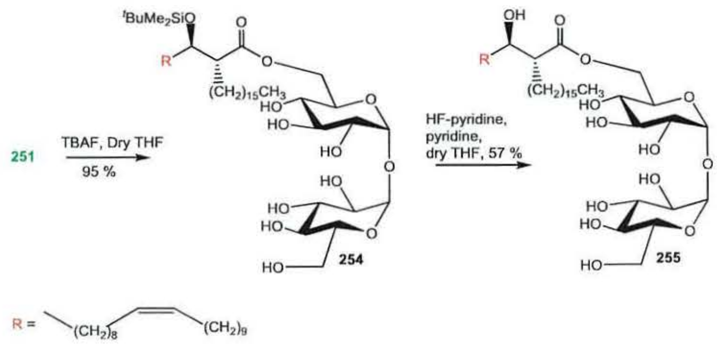


Scheme 54: Deprotection sequence

2.1.5.4 Deprotection of TMM

The deprotection of **(251)** involved firstly the use of TBAF in order to remove the silyl protecting groups on the trehalose. The ^{1}H NMR spectrum of the product **(254)** (**See experimental 47**) showed no peaks for the TMS group. The alkene protons showed a triplet δ 5.28, while the trehalose sugar protons resonated at δ 5.00, 4.65, 4.35, 4.18,

3.90, 3.70 and 3.37. The proton of the α-chiral centre contiguous to the acid showed a triplet between δ 2.53-2.31; the proton of the β-chiral centre gave a multiplet between δ 3.90-3.86 (**Scheme 55**). MALDI MS showed for **(254)** an $[M+Na]^+$ of 1053.9, while the calculated value was 1053.7 The following step was the removal of the TBDMS ether using HF-pyridine and pyridine. The ^1H NMR of the TMM **(255)** (**See experimental 48**) gave a triplet at δ 5.31 for the four alkene protons, while the hemiacetal gave a doublet for two protons at δ 5.09 and 5.02. The rest of the sugar protons resonated between δ 4.69 and 3.36. The proton at the β-position of the MAs gave a doublet of triplets for two protons at δ 3.65. The ^{13}C NMR showed the carbonyl carbon at δ 175.4 and for the alkene carbons at δ 129.8 (**Scheme 55**). MALDI MS showed for **(255)** an $[M+Na]^+$ of 939.8, while the calculated value was 939.6.

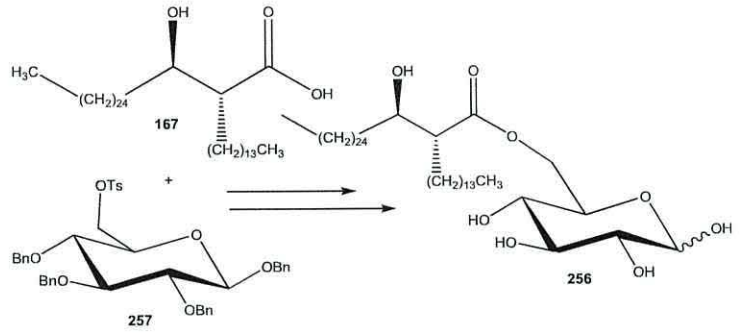


Scheme 55: Final deprotection sequence

2.1.6 Synthesis of a glucose monomycolate present in *R. equi*

2.1.6 Synthesis of glucose monomycolate (256)

In the scheme planned, the first step was the preparation of the tosylate **(257)**. The second part was the coupling of this with the unprotected mycolic acid **(167)** with the correct number of carbons atoms in the chain mycolic acid to prepare glucose monomycolate GMM **(256)**, **(Scheme 56)**.

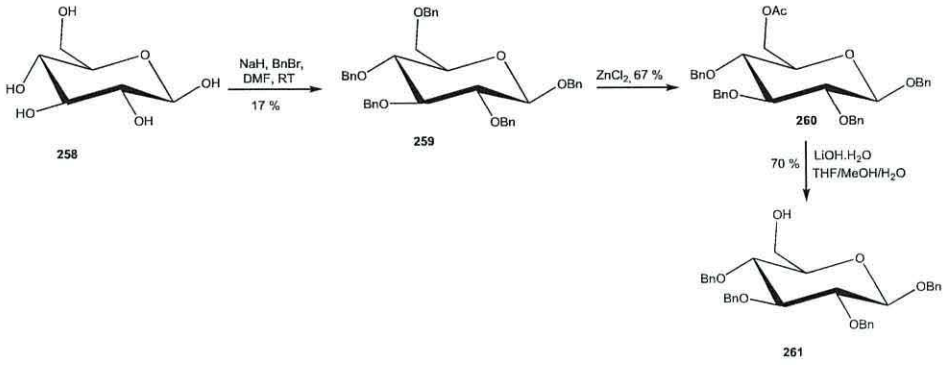


Scheme 56: Plan for the synthesis of GMM (256)

2.1.6.2 Preparation of benzyl 2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (262)

A useful three-step synthesis of benzyl 2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside, frequently used as a building block in carbohydrate chemistry, is well described. The first step required washing mineral oil stabilised NaH twice with hexane at room temperature. This was followed by adding the D-glucose **(258)** in anhydrous DMF at room temperature and stirring for 1 h, prior to its being cooled in an ice bath. Benzyl bromide was added dropwise over a period of 30 min. and after 1 h the ice bath was removed. The mixture was stirred at room temperature for 4 h, and then a second addition of the same quantities of NaH and benzyl bromide was undertaken at 0 °C. The mixture was stirred overnight in order to give product **(259),** though only in 17% yield (**See experimental 92**). The next step entailed the selective debenzylation-acetylation reaction using freshly prepared $ZnCl_2$ in HOAc-Ac_2O for 4 h at room temperature. This gave product **(260)** (67%) (**See experimental 93**). The final step was deacetylation to give product **(261)** (**See experimental 94**). This was achieved by stirring it with $LiOH.H_2O$ (15 eq.) in a mixture of THF/H_2O/MeOH at 45 °C overnight

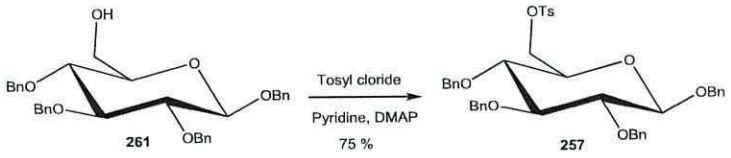
(**Scheme 57**). Compounds **(259)**, **(260)** and **(261)** gave the same spectra as in the literature.[261]



Scheme 57: Preparation of benzyl 2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (292)[261]

2.1.6.3 Tosylation of benzyl 2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (261)

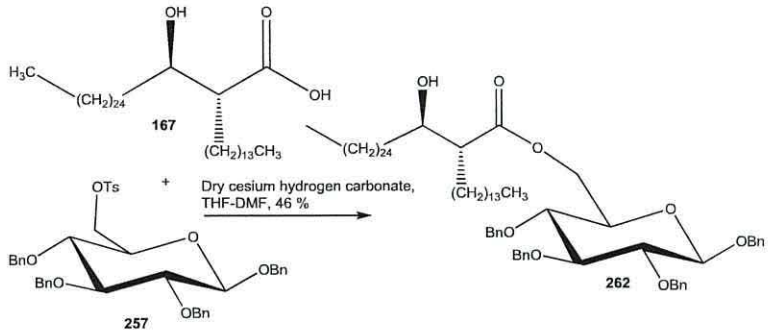
To prepare a GMM from the benzyl 2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside **(261)**, it was necessary to activate the OH group to nucleophilic attack in the coupling reaction. Compound **(261)** was added to pyridine, 4-DMAP and tosyl chloride in dichloromethane at 0 °C to give product **(257)** (**See experimental 95**) (**Scheme 58**).[262] The ^{1}H NMR spectrum showed peaks at δ 7.80 (2H, d) and 7.19 (2H, dd) for the tosyl H-Ar, and a peak at δ 2.41 (3H, s) for CH_3-tosyl.



Scheme 58: Tosylation of benzyl 2,3,4-tri-*O*-benzyl-*β*-D-glucopyranoside (261)

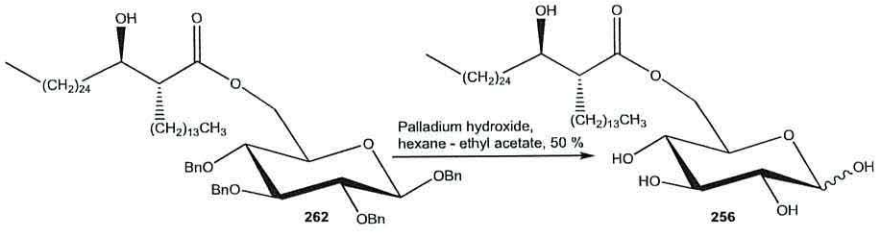
2.1.6.4 The coupling reaction

The next step was the coupling between saturated MA **(167)** and protected sugar **(257)** using dry cesium hydrogen carbonate in THF-DMF at 70 °C for 18 h. The resulting protected GMM **(262)** was obtained in 46% yield (**See experimental 96**) (**Scheme 59**).[262]



Scheme 59: Preparation of GMM

The ^{1}H NMR spectrum for compound **(262)** included a multiplet between δ 7.36-7.23 (20H) for the aromatic protons, and the benzylic methylene groups at δ 4.93 (2H), 4.88 (2H), 4.64-4.56 (2H) and 4.54-4.49 (2H). The signals for the core sugar protons included H6 at δ 4.77, H1 at δ 4.69. The β-hydroxy position showed a triplet at δ 3.65. The α-proton of the MA appeared as a multiplet between δ 2.49-2.42. The ^{13}C NMR spectrum showed a signal at δ 175.2 for the carbonyl carbon. The sugar carbon resonances included the C1 resonance at δ 102.3, C6 at δ 62.8. The final step was the deprotection of the benzyl groups of **(262)** by catalytic hydrogenation with 10% palladium hydroxide on charcoal. This gave compound **(256)** as a mixture of α and β anomers in ratio 0.4:0.6 in 50% yield (**See experimental 97**), (**Scheme 60**).



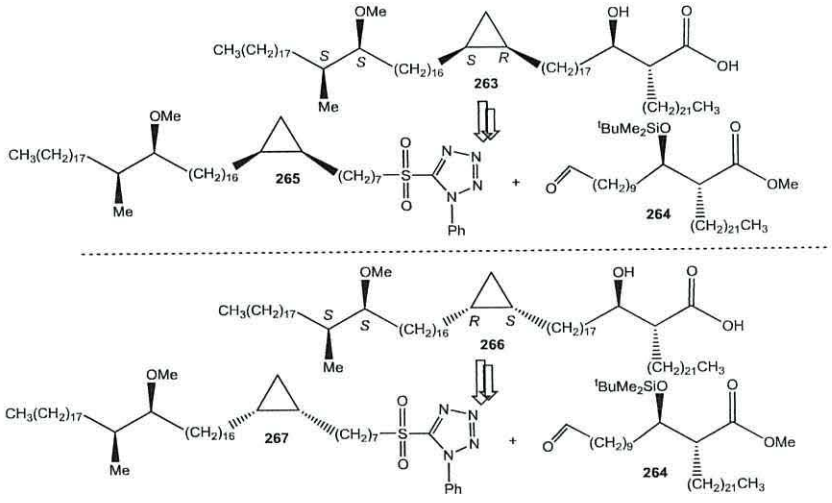
Scheme 60: Deprotection of GMM (262)

The ^{1}H NMR spectrum for compound **(256)** showed doublets at δ 5.15 (0.4H) for $H\alpha_1$ and at δ 4.51 (0.6H) for $H_{\beta 1}$. The remaining protons of the sugar component resonated between δ 4.43 (1H), 4.32 (1H), 3.67-3.57 (2H) and 3.46-3.35 (2H). The α-proton of the MA gave a multiplet at δ 2.46-2.36 (1H) and the terminal methyl groups gave a multiplet between δ 0.87-0.83 (6H). The ^{13}C NMR spectrum included the $C_{\beta 1}$ resonance at δ 96.5 and $C\alpha_1$ at 92.2. The MS showed for **(256)** an $[M+Na]^{+}$ of 821.6861, while the calculated value was 821.6846).

2.2 Synthesis of a methoxy-MA, keto-MA and trehalose ester (TDM and TMM) present in *M. kansasii*

2.2.1 Synthesis of a methoxy-mycolic acid present in *M. kansasii*

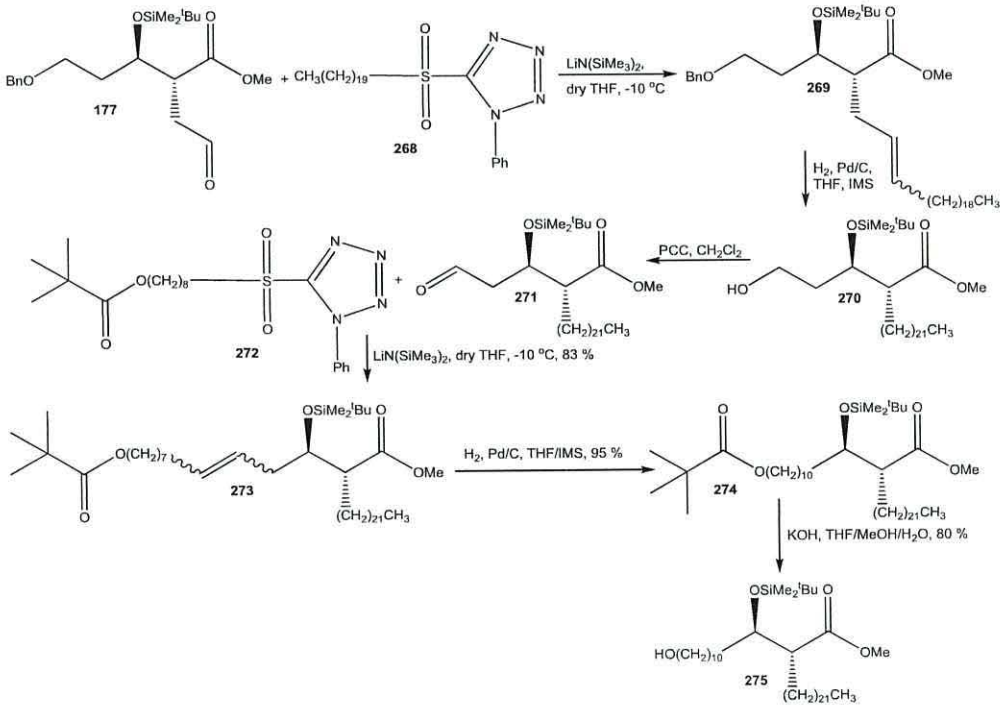
Al-Dulayymi *et al.*[217] achieved the synthesis of the full methoxy MA contained a cyclopropane ring in either the (*S,R*)-configuration **(263)** or (*R,S*)-configuration **(266)** in the proximal position, and with the distal methoxy and methyl groups in the (*S,S*)-configuration. This was coupled with trehalose to prepare TDM and TMM. These compounds differ in their α-chain length by two carbons from the corresponding compounds in *M. tb. i.e* C22 compared to C24 so that they could be used as antigens in ELISA assay so it will allow the effect of this chain length to be investigated and to check their ability to detect TB-antibodies. The methoxy MA can be divided into the mycolic motif part and the meromycolate part in order to make an explanation of the synthesis easier. In addition, the meromycolate part can be split into a *S,S*-α-methyl-methoxy unit and a *S,R-cis*-cyclopropane unit or *R,S-cis*-cyclopropane unit (**Scheme 61**).



Scheme 61: Full methoxy-mycolic acid disconnections

2.2.1.1 Synthesis of mycolic motif part

The mycolic motif chain **(275)** was prepared as previous step by coupled an aldehyde **(177)** with the sulfone **(268)** to produce the mixture of alkenes **(269)** (**Scheme 62**). Hydrogenation and debenzylation of the alkenes **(269)** was carried out with hydrogen gas as previously described to give the primary alcohol **(270)**. The structure of this was confirmed primarily using ^{1}H NMR, the product no longer showing signals for the Bn-group or the olefin. Oxidation of the mycolic motif **(270)** was done using PCC. The aldehyde **(271)** was coupled to sulphone **(272)** using LiHMDS as the base in dry THF. The formation of the alkene mixture **(273)** was verified by the ^{1}H and ^{13}C NMR spectra. The next step was hydrogenation to reduce the double bond using palladium on carbon (10%) as the catalyst under a hydrogen atmosphere. The ^{1}H NMR spectrum showed no signals in the alkene region. The hydrogenation was followed by hydrolysis of compound **(274)** using potassium hydroxide in THF/MeOH/H_2O (**Scheme 62**). The detailed spectroscopic data for **(275)** (**See experimental 50**) matched those given in the literature.[207]



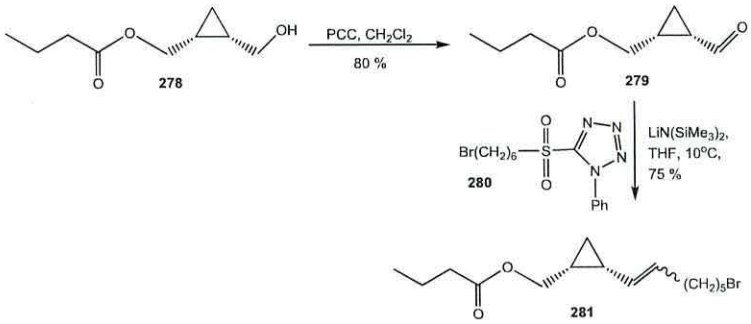
Scheme 62: Extension of the side chain of the mycolic motif to give (275)

2.2.1.2 Synthesis of the meromycolate part

Coupling the methoxy unit **(276)** with the *cis*-cyclopropane unit **(277)** in a Julia reaction is the standard method to prepare the meromycolate part required for methoxy mycolic acid **(266)**.

2.2.1.2.1 Synthesis of the proximal *cis*-cyclopropane unit (277)

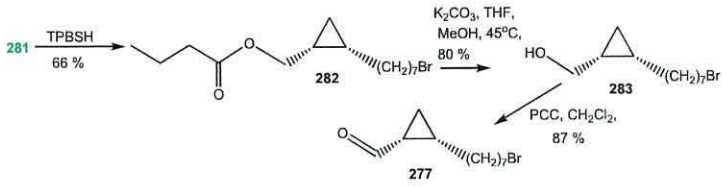
In order to extend the chain of the cyclopropane unit, the hydroxy-cyclopropyl monoester **(278)** was firstly oxidised to the aldehyde **(279)**. A typical modified Julia-Kocienski olefination using lithium *bis*(trimethylsilyl) amide as base, was performed between aldehyde **(279)** and the sulfone **(380)** to give the olefin **(381)** as a mixture of (*E/Z*) isomers (**Scheme 63**).



Scheme 63: Chain extension of the compound (279)

Ring opening of a cyclopropane can occur under the conditions employed for the hydrogenation of a double bond using palladium on carbon and hydrogen gas.[23] Hydrogenation was therefore performed by using di-imide (N_2H_2) which is a milder hydrogenation agent. It can reduce the double bond selectively in the presence of the cyclopropane ring. In this study, 2,4,6-triisopropyl-benzenesulfonyl hydrazide (TPBSH) was used. The alkene **(281)** was dissolved in THF and TPBSH was then added and heated for 3 h. This was followed by the addition of a further equivalent of TPBSH and stirring for 24 h under the same conditions. This is illustrated in **Scheme 64**. No signals for the alkene protons appeared in the ^{1}H NMR spectrum of the product **(282)**. This was deprotected using anhydrous potassium carbonate in THF and methanol at 45 °C, to give alcohol **(283)**. The resulting alcohol **(283)** was then oxidised

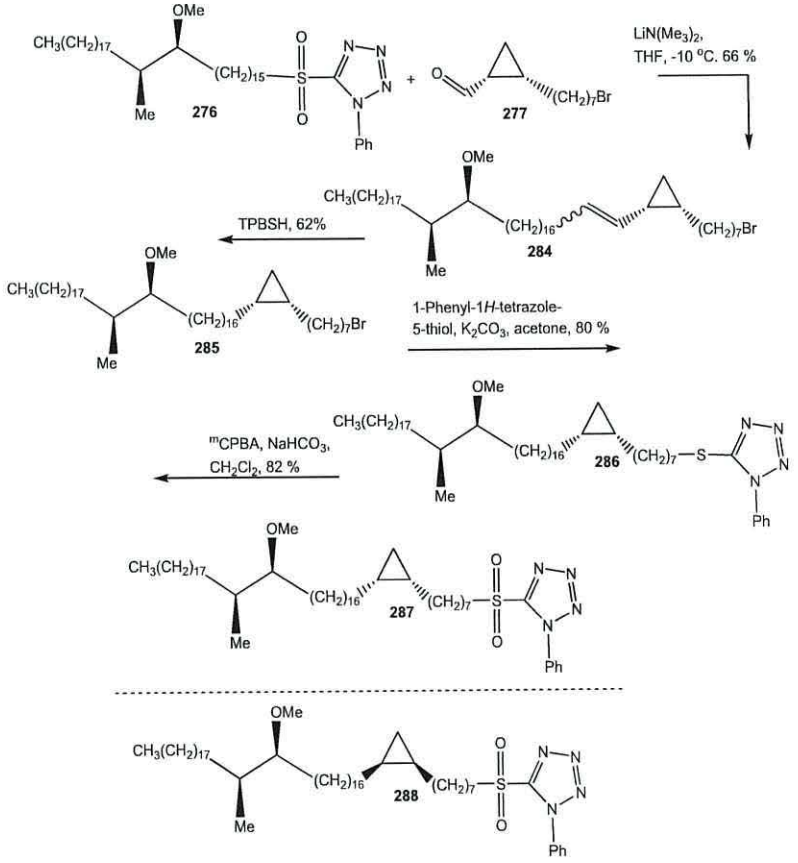
to aldehyde **(277)**. The NMR spectra of this compound were similar to those that quoted in the literature.[200,217]



Scheme 64: Synthesis of *cis*-cyclopropane unit (277)

2.2.1.2.2 Synthesis of the full meromycolates (287) and (288)

The aldehyde **(277)** was coupled with methoxy sulfone unit **(276)** in the presence of base to give alkene **(284)** as an (*E/Z*)–mixture. TPBSH was used for the hydrogenation of this mixture so as to give the compound **(285)**.



Scheme 65: Synthesis of the meromycolate intermediate (287) and (288)

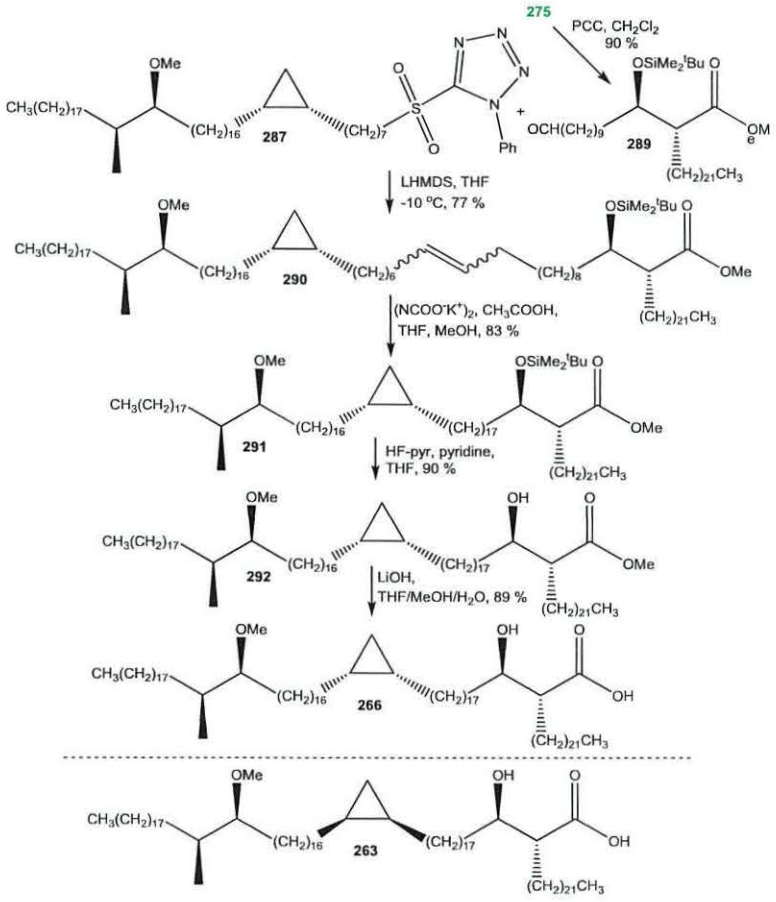
The bromo-compound was reacted with 1-phenyl-*H*-tetrazole-5-thiol in the presence of acetone and K_2CO_3 to give sulfide **(286)**. This was then oxidised to the corresponding sulfone **(287)**, using *m*-chloroperbenzoic acid in dichloromethane with sodium bicarbonate (**Scheme 65**). The spectroscopic data of this compound matched the data given in the literature.[217] The ^{1}H NMR, ^{13}C NMR and IR data for the sulfone **(288)** were identical to those of the previously prepared sulfone **(287)**, the only difference was in the stereochemistry of the cyclopropane.[217]

2.2.1.3 Final coupling to form the full methoxy mycolic acid

After preparation of the meromycolate moiety as the sulfone intermediate **(287)**, it was coupled with the corynomycolate aldehyde **(289)**, using a modified Julia-olefination. Lithium *bis*(trimethylsily)amide was used as the base in dry THF to give alkene mixture **(290)** in 77% yield. Hydrogenation of this product using di- potassium azodicarboxylate gave the saturated compound **(291)** in 83% yield. The TBDMS group was then deprotected using HF-pyridine complex to give the *cis*-cyclopropane methoxy-mycolic acid methyl ester **(292)** in 90% yield. Hydrolysis of the methyl ester was carried out using lithium hydroxide in THF, methanol and water to give the full methoxy-MA **(266)** in 89% yield (**Scheme 66**). The ^{1}H NMR spectrum of compound **(291)** showed a multiplet between δ 0.91-0.85 (9H) and two singlets at δ 0.05 (3H) and 0.03 (3H) for TBDMS group and a singlet at δ 3.66 (3H) for the methyl ester. The ^{13}C NMR spectrum showed signals at δ 30.1, 25.6, -4.5 and -5.0 for the *tert*-butyl dimethyl group, and at δ 51.0 for the methyl ester.

The ^{1}H NMR spectrum of the methoxy MA methyl ester **(292)** showed a singlet at δ 3.71 (3H) for the methyl group in the MA motif, a multiplet at δ 3.68-3.66 (1H) for the proton on the β-carbon and a multiplet at δ 2.46-2.42 (2H) for the proton on the α-carbon (CHCO, OH). The ^{1}H NMR signal for the methoxy group in the meromycolate chain appeared as a singlet at δ 3.35 and the proton next to the methoxy group appeared as a broad pentet at δ 2.96-2.95 (1H). The spectrum showed a multiplet between δ 0.66-0.64 (2H), a doublet of triplets at δ 0.57 (1H) and a broad quartet at δ -0.31 (1H) for the *cis*-cyclopropane ring protons. The ^{13}C NMR spectrum showed a carbonyl signal at δ 176.3, a signal at δ 85.5 for the carbon bearing the methoxy group

and a signal at δ 57.8 for the carbon of the methoxy group, while the β-carbon appeared at δ 72.3 and the α-carbon at δ 51.4.



Scheme 66: Synthesis of the full methoxy mycolic acids (266) and (263)

The ^{1}H NMR spectrum of the methoxy mycolic acid **(266)** (**See experimental 61**) showed a multiplet at δ 3.73-3.65 (1H) for the proton on the β-carbon, a multiplet between δ 3.0-2.8 (1H) for the CHCO proton and another multiplet between δ 2.5-2.45 (1H) for the α-carbon (CHOH). The methoxy group in the meromycolate chain appeared as a singlet at δ 3.37 (3H). The spectrum showed a multiplet between δ 0.66-0.60 (2H), a doublet of triplets at δ 0.58 (1H) and a broad quartet at δ -0.31 (1H) for the *cis*-cyclopropane ring protons. The signal in the ^{1}H NMR spectrum for the methyl ester group in the MA motif at δ 3.71 (3H) disappeared in the full mycolic acid. The ^{13}C NMR spectrum of the methoxy MA **(266)** showed a carbonyl signal at δ 173.4, a signal at δ 85.6 for the carbon bearing the methoxy group and at δ 57.6 for the carbon

of the methoxy group, while the β-carbon appeared at δ 72.2 while the α-carbon appeared at δ 50.7. The ^{1}H NMR, ^{13}C NMR and IR data for the full MA **(263)** (**See experimental 60**) were identical to those of the previously prepared MA **(266)**.

2.2.2 Synthesis of keto-MA (293)

Methoxy MA and keto MA are found in the cell wall of *M. kansasii*, and these are the major oxygenated MAs.

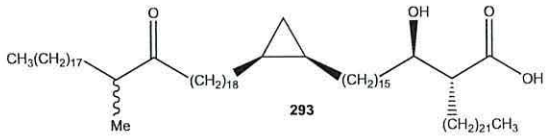
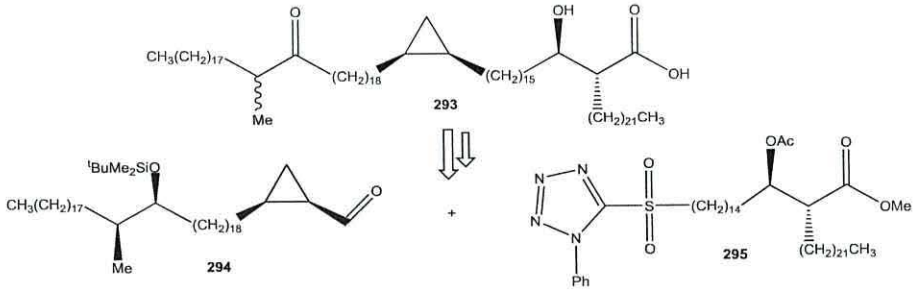


Figure 29: The target keto-MA of *M. kansasii*

Koza[210] attempted to prepare the *cis*-keto mycolic acid present in *M. tb*. It was reported that the final stage to deprotect the methyl ester and acetyl protecting groups using lithium hydroxide monohydrate, caused the epimerisation of the chiral centre next to the carbonyl to give the keto-MA present in *M. tb*.

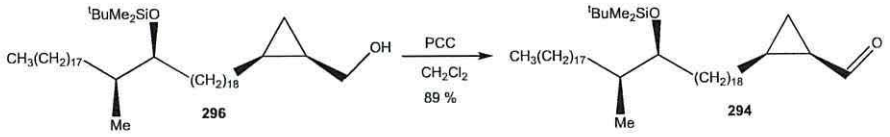
In this study, a new keto-MA present in *M. kansasii* was made. The coupling of the mycolic motif **(295)** and the meromycolate part **(294)** was successfully undertaken.



Scheme 67: Full keto-MA disconnection

2.2.2.1 Coupling to form keto-MA (293)

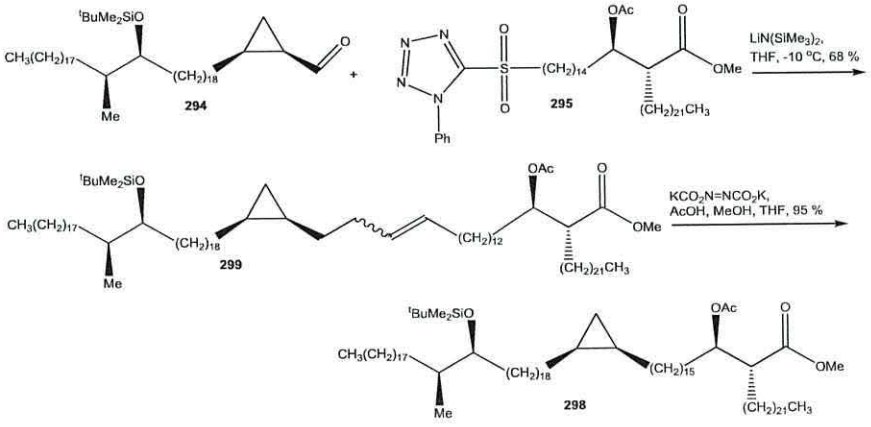
To begin with, the alcohol meromycolate **(296)**[217] was oxidised to the corresponding aldehyde **(294)** usig PCC in dichloromethane (**Scheme 68**). A doublet at δ 9.36 corresponding to the aldehyde proton was shown in the ^{1}H NMR spectrum of **(294)**. The data for this compound matched those in the literature.[220]



Scheme 68: Oxidation of the keto meromycolate

The sulfone **(295)** was coupled with the meromycolate aldehyde **(294)** in order to give alkene in an (*E/Z*) mixture as shown in Scheme **(69)**. The hydrogenation of the alkene was achieved using dipotassium azodicarboxylate, as described previously. The proton NMR of compound **(298)** did not show any signals in the alkene region. The β-proton next to the acetoxy group yielded a doublet of triplets at δ 5.10 (1H) while the proton next to the silyl group showed a multiplet between δ 3.51-3.49 (1H). The methoxy group showed as a singlet at δ 3.68 (3H) and the methyl of the (acetyl group) a singlet at δ 2.18 (3H). The α-proton displayed a doublet of doublets of doublets at δ 2.63 (1H) while a multiplet was seen between δ 0.67-0.64 (2H) for the two protons by the cyclopropane ring. One proton displayed a doublet of triplets at δ 0.57 (1H), while another proton gave a broad quartet at δ -0.32 (1H).

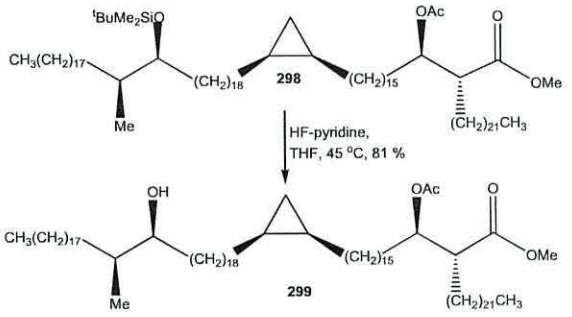
In the ^{13}C NMR spectrum, the two carbonyl carbons were observed at δ 173.6 and 170.3. There was an absence of all signals which belonged to the alkene carbons.



Scheme 69: The coupling to form the keto-MA

2.2.2.2 Deprotection of the silyl group in the meromycolate

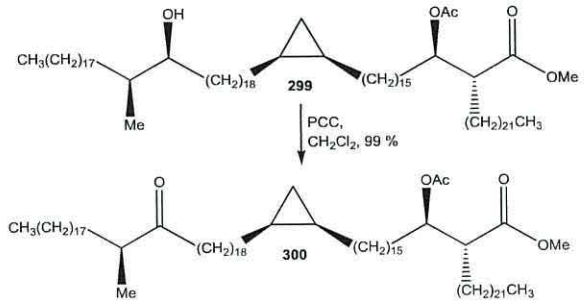
The TBDMS group was removed from compound **(298)** using HF-pyridine complex and pyridine in dry THF (**Scheme 70**). The product, **(299)**, gave a double of triplets at δ 5.10 (1H) for the proton adjacent to the acetoxy group. The proton in the α-position showed as a doublet of doublets of doublets at δ 2.63 (1H). The proton adjacent to the resulting hydroxyl group showed a multiplet between δ 3.51-3.48 (1H). A broad band at 3449 cm^{-1} for the OH-stretch was seen in the IR spectrum.



Scheme 70: Deprotection of the silyl group of (298)

2.2.2.3 Oxidation of the secondary alcohol to the ketone

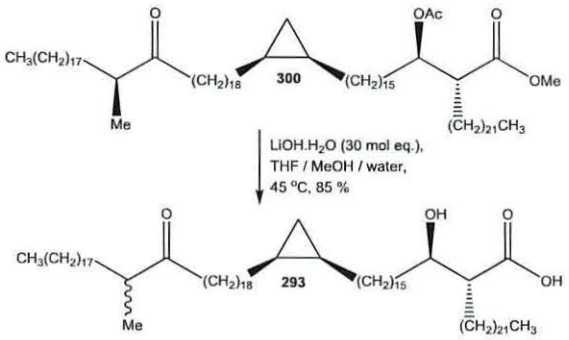
The last step for the creation of the keto-MA was oxidation. Oxidation of the hydroxy-MA **(299)** with PCC gave keto-MA **(300)** as a white solid (**Scheme 71**). The ^1H NMR spectrum showed the cyclopropane protons between δ 0.68-0.64 (2H), 0.57 (1H) and -0.32 (1H). The IR spectrum showed no O–H stretch and there was an absorbance at 1708 cm^{-1} for the C=O stretch of the ketone. The specific rotations of keto mycolates **(300)** was found to be $[\alpha]_D^{20} = +7.1$ (c 1.0, $CHCl_3$).



Scheme 71: Oxidation of the secondary alcohol to give keto-MA

2.2.2.4 The hydrolysis of the protected keto-MA

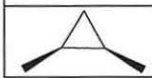
The hydrolysis of the keto-MA methyl ester to the free acid was necessary for biological testing. The acetate and methyl ester groups were deprotected using $LiOH.H_2O$ in a mixture of THF, MeOH and H_2O. The mixture was stirred at 45 °C for 18 h to obtain the free hydroxy group (**Scheme 72**). The ^1H and ^{13}C NMR data for the free keto-MA are given in (**Table 6**). The specific rotations of keto mycolates (**293**) was found to be $[\alpha]_D^{20}$ = +4.1 (c 1.0, $CHCl_3$). This compound was epimerised in order to be different form the other compounds which had been made before and see difference in biological assay.

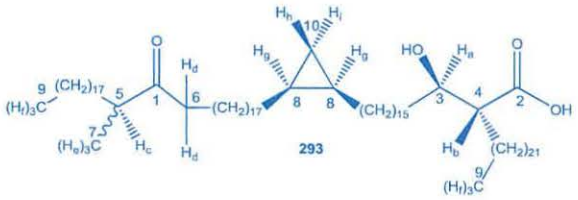


Scheme 72: Hydrolysis of the keto-MA with $LiOH.H_2O$ to give the free acid

After base hydrolysis of the protected compounds **(300)** to yield free keto-MA **(293)**, the rotation decreased from $[\alpha]_D^{20}$ = +7.1 (c 1.0, $CHCl_3$) to $[\alpha]_D^{20}$ = +4.1 (c 1.0, $CHCl_3$). The base hydrolysis reaction caused concern that it was responsible for causing the epimerization of the α-methyl ketone, as shown in **Scheme 72**. The molecular rotation of compunds **(293)** and **(300)** can be calculated from the sp. rotation, by this relationship:

$$MD = [\alpha]^{x}{}_{D} (\text{molecular weight}/100)$$

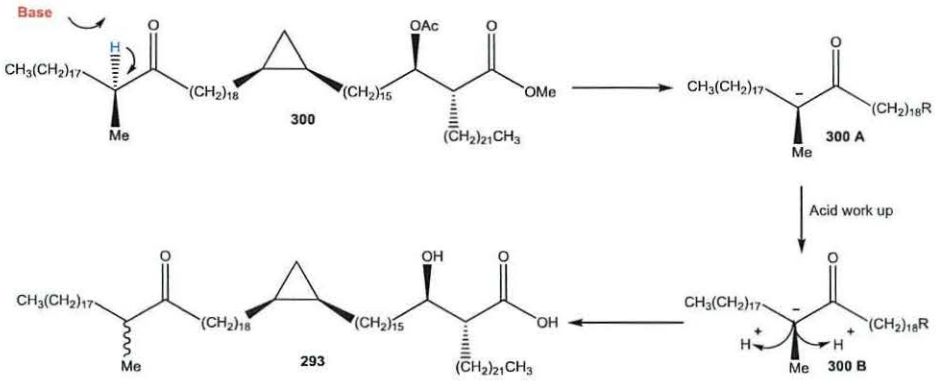
Keto Mycolic Acid	**Configuration of the α- methyl ketone**	**MD protected keto mycolic acid**	**MD deprotected keto mycolic acid**
	*S*	+90	+50



H_x	δ	Multiplicity	Integration	*J* (Hz)	C_x	δ
H_a	3.74-3.70	m	1	-	C_1	215.4
H_b	2.5	m	1	-	C_2	179.6
H_c	2.46	dt	1	5.4, 8.8	C_3	72.1
H_d	2.43 2.40	dt dt	1 1	2.0, 5.0 2.0, 5.0	C_4	50.8
$R(CH_2)_nR$	1.74-1.12	br.m	140	-	C_5	
H_e	1.05	d	3	7.7	C_6	41.1
H_f	0.9	t	6	7.5	C_7	16.4
H_g	0.69-0.64	m	2	-	C_8	15.8
H_h	0.57	dt	1	4.1, 8.2	C_9	14.10
H_i	- 0.32	br.q	1	5.5	C_{10}	10.9

Table 6: The ^1H and ^{13}C NMR analysis of the free keto-MA (293)

The reason for this could be that during the hydrolysis of the protecting groups, the base removes the acidic α-methyl ketone proton in **(300)** to give **(300 A)**. Following acid work-up, reprotonation occurs from both faces to give the epimerised keto compound **(293)**, Scheme **(72 -A)**.[270]



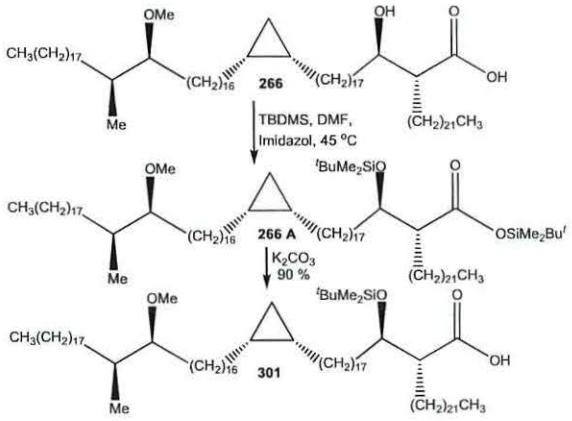
Scheme 72-A: Mechanism for the epimerisation reaction

2.2.3 Synthesis of a trehalose ester present in *M. kansasii*

The aim of this section was to synthesise a TDM and TMM which were based on a synthetic *M. kansasii* MA.

2.2.3.1 Methoxy MA protection and esterification with trehalose

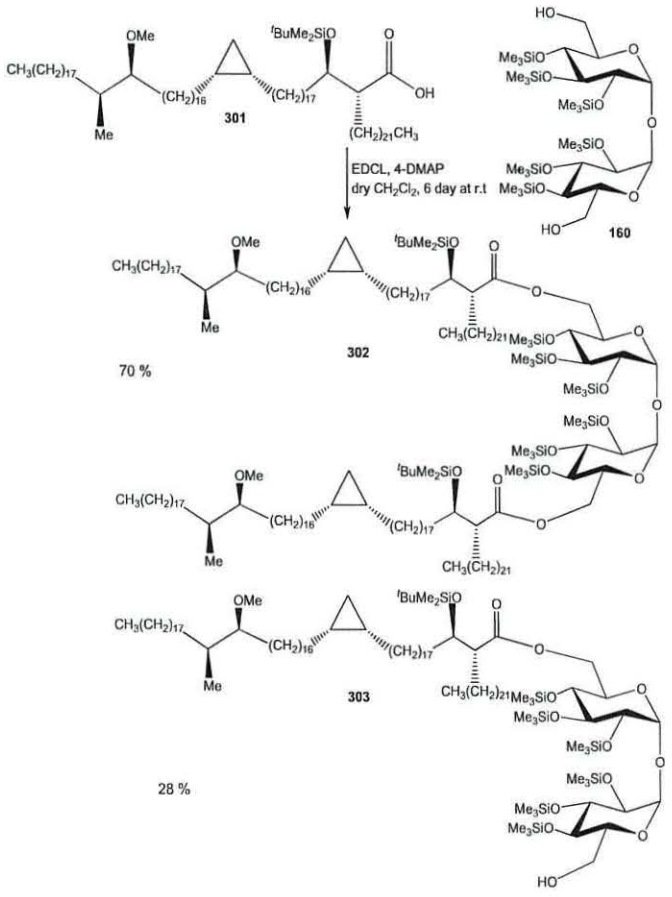
It is necessary to protect the MA in the β-hydroxy acid position as a TBDMS ether to avoid alcohol reactions. The methoxy MA **(266)**, was first converted into **(301)** (**See experimental 63**) by the standard silylation method (**Scheme 73**).



Scheme 73: Protection of methoxy-MA (266)

The ^{1}H NMR spectrum included two singlets at δ 0.16 (3H) and δ 0.14 (3H) for the methyls substituents bound to the silicon. The nine protons of the *tert*-butyl portion of protecting group were displayed as a singlet at δ 0.94. The signals in the ^{13}C NMR spectrum were also consistent with the structure of **(301)**.

Esterification of trehalose **(160)** and **(301)** was achieved using EDCI and 4-DMAP as previous step to produce products **(302-TDM)** and **(303-TMM)**, as is shown in Scheme **(74)** (**See experimental 65**).

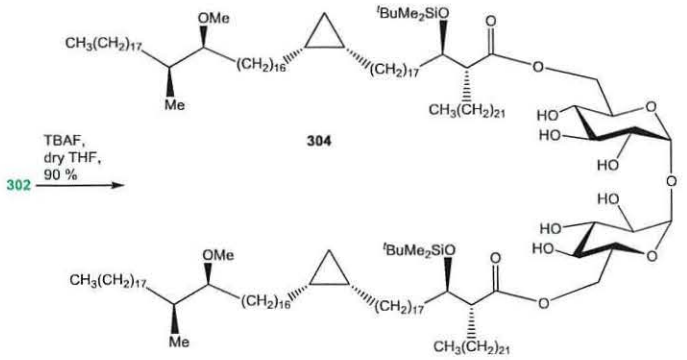


Scheme 74: Esterification of protected trehalose (160) with protected methoxy MA (301)

The first product to be obtained was TDM **(302)** in a yield of 70%. Its structure was confirmed by ^{1}H and ^{13}C NMR spectroscopy and MALDI mass spectrometry. The ^{1}H NMR spectrum for **(302-TDM)** displayed signals for the *tert*-butyl protons of the silyl protecting group as a singlet at δ 0.89 (18H). Characteristic signals were still present

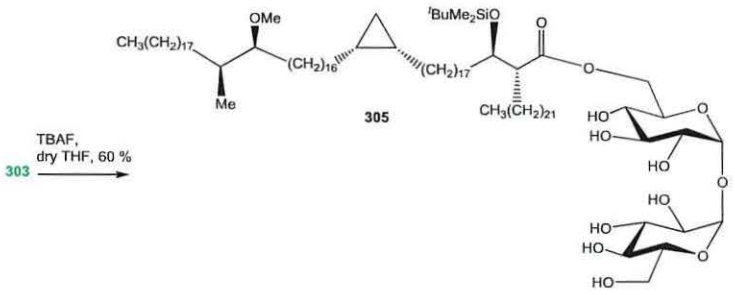
for the methoxy mycolic acid component, such as the OMe substituent which gave a signal at δ 3.34 (3H) as a singlet, and also the proton next to the β-hydroxy group which resonated at δ 3.95 the (2H) as a pentet. Similar signals to those which were observed for **(301)** were given by the cyclopropane ring protons. Signals for the six protons in the sugar core started at δ 4.85 (2H) for H1 close to the hemiacetal as a doublet, at δ 4.36 (2H) for H6 as a broad doublet, while the remaining signals were seen at δ 4.04-3.97 (2H), 3.95 (2H), 3.9 (2H), 3.53 (2H) and 3.39 (2H). The signals for the silyl protecting groups for the alcohols on the sugar core were found s a singlet at δ 0.16, 0.14 and 0.12, integrating to 18H each. The TMM **(303)** was the second fraction, obtained in a yield of 28%. The signals for the sugar protons range between δ 4.91 and δ 3.37. Here the signals were not symmetrical. It was found that the methyl signals for the protecting groups on the sugar were divided into six groups, one for each (Me_3) situated at C2-C3-C4, and which had a range of δ 0.17-0.12. In each case they corresponded to 9H and to 54 hydrogens in total. The methoxy mycolic acid displayed signals at δ 3.82 for the β-hydroxy acid and at δ 3.34 (3H) for the OMe in the ^1H NMR spectrum. The signals for the reminder of the groups did not change. However, the ^{13}C NMR spectrum displayed signals at δ 174.1 for the ester group and at δ 94.5 and 94.4 for the C1 in the sugar cores. Signals at δ -4.5 and -4.7 were displayed for the two methyl groups bound to the silicon in the $OSiMe_2{}^tBu$ group.

The next step was the deprotection of the sugars in these two products. Compounds **(302)** and **(303)** were both deprotected using TBAF in dry THF solution. This gave **(304)** (90%) (**See experimental 67**) and **(305)** (60%) (**See experimental 69**) as shown in Schemes **(75)** and **(76)**.



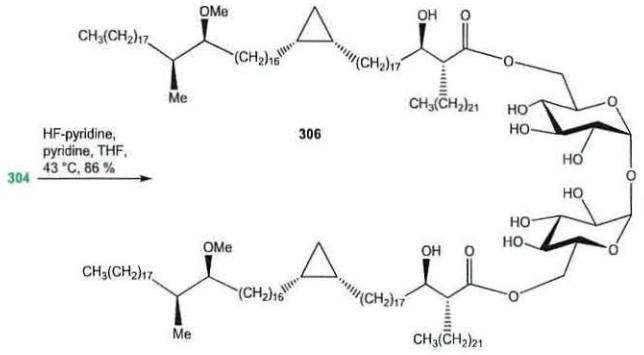
Scheme 75: Deprotection of the trehalose (302)

The ^{1}H NMR spectrum compound **(304)** had lost the signals for TMS groups. In addition, the ^{13}C NMR spectrum did not display any signals at δ 1.1, 0.9 and 0.2 which corresponded to the carbons of these groups.



Scheme 76: Deprotection of (305)

Compound **(305)** also lost the signals at δ 0.17-0.12 in the ^{1}H NMR spectrum. The next stage was the removal of the TBDMS ether protecting group from the β-position of the methoxy mycolic acid **(304)**. HF-pyridine complex and pyridine was used in dry THF solution at 45 °C overnight, as illustrated **Scheme 77**.

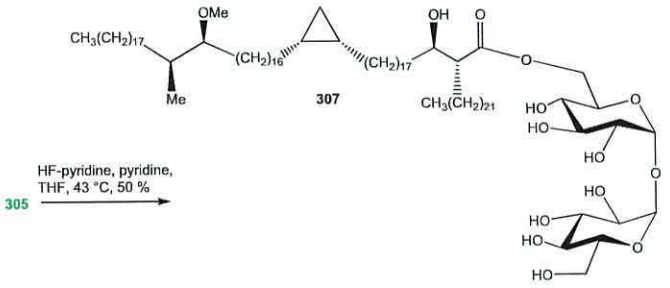


Scheme 77: Synthesis of free TDM of methoxy-MA (306)

The ^{1}H NMR spectrum established the structure of the free TDM **(306)**, (**See experimental 71**). It displayed no signals for the TBDMS group. The hemiacetal protons displayed a doublet at δ 4.96 (2H). The rest of the sugar protons resonated at δ 4.64 (2H), 4.22 (2H), 3.72 (2H), 3.62 (2H), 3.42 (2H) and 3.19 (2H), with an integration of two protons for each signal. The protons next to the β-hydroxyl of the MA showed as a broad quartet at δ 3.94. The methoxy groups of the MA yielded a

singlet at δ 3.29 (6H). A broad pentet signal showed at δ 2.93 (2H) for the protons next to the methoxy group. A multiplet occurred between δ 2.39-2.33 (2H) for the protons in the α-position adjacent to the alkyl chain of the mycolic acid. The terminal methyl groups showed as a triplet at δ 0.83 and this signal had an integration of twelve protons. The methyl next to the methoxy group showed as a doublet at δ 0.79 (6H). The protons of the cyclopropane ring showed as a multiplet between δ 0.60-0.59 (4H). Two protons were seen as a doublet of triplets at δ 0.52 and a broad quartet at δ -0.37 was displayed for another two protons. In addition, the ^{13}C NMR spectrum showed no signals belonging to the *tert*-butyldimethylsilyl group.

The method which was used for the deprotection of TDM **(304)** was deployed again in order to obtain the free TMM. The TBDMS group which was on the β-position of compound **(305)** was removed using HF-pyridine complex and pyridine in dry THF, as in Scheme **(78)**.



Scheme 78: Synthesis of free TMM of methoxy-MA (307)

The ^{1}H NMR spectrum of free TMM **(307)** (**See experimental 73**) showed no signals for the *tert*-butyl protons at δ 0.83. Nor were there signals for the protons of the two methyl groups bonded to silicon at δ 0.02 and 0.004. The hemiacetal protons showed as a singlet at δ 5.10 and 5.02. The remaining sugar protons resonated between δ 4.72 and 3.22. The proton of the methoxy-MA gave signals which were similar to those of the free TDM, but with half the number of protons. The formation of free TMM was confirmed by the ^{13}C NMR spectrum, which showed the loss of signals for the two methyl groups bonded to silicon. MALDI MS showed for **(307)** an $[M+Na]^{+}$ of 1573.3, while the calculated value was 1573.3. In a similar way, compounds **(308)** and **(309)** were prepared. The ^{1}H NMR, ^{13}C NMR and IR data for the free TDM **(308)** and

free TMM **(309)** were identical to those of the previously prepared TDM **(306)** and TMM **(307)**, the only difference being in the cyclopropane stereochemistry (**Figure 30**). The biological properties of synthetic methoxy MA are currently being studied.

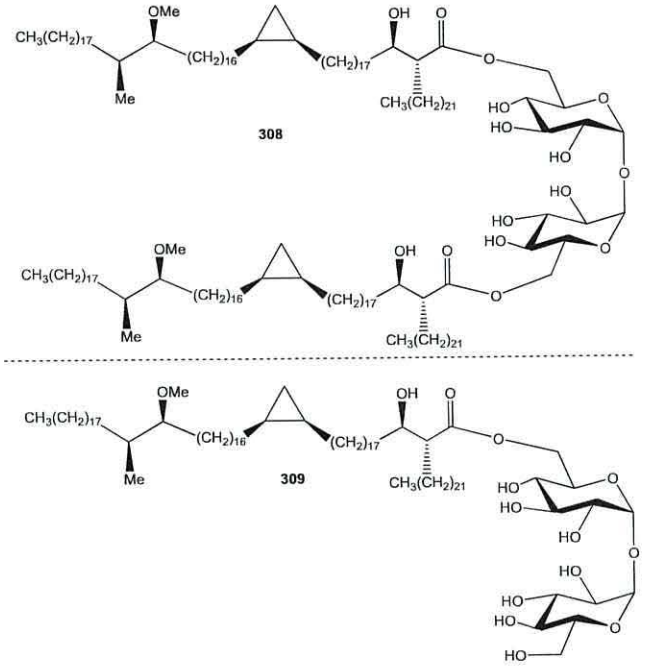
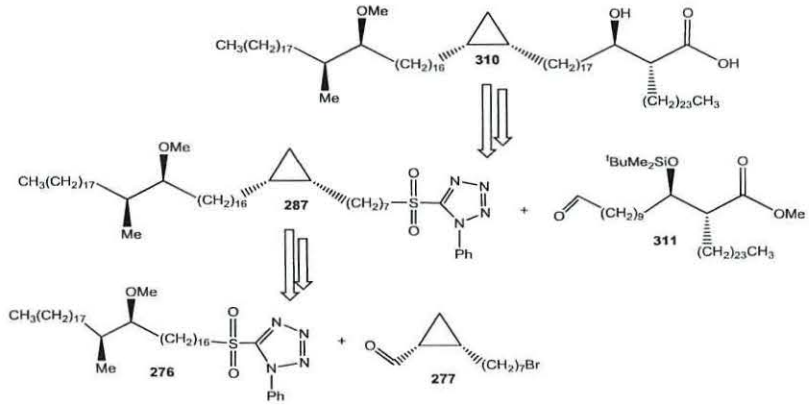


Figure 30: Synthesis of free TDM and TMM of methoxy-MA

2.3 Synthesis of methoxy MA and trehalose ester (TDM) and (TMM) present in *M. tb*

2.3.1. Synthesis of *cis*-cyclopropane methoxy-MA of *M. tb*

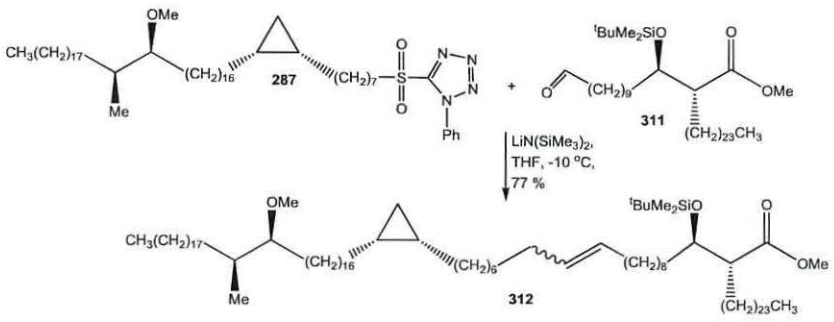
Al-Dulayymi *et al.* synthesised the full methoxy-MA **(310)** containing a proximal cyclopropane ring in the (*R*,*S*)-configuration, and with methoxy and methyl groups in the (*S*,*S*)-configuration in the distal position.[217] This would be coupled with trehalose to prepare new cord factors (TDM and TMM) so that they could be used as antigens in ELISA tests so as to check their ability to detect TB-antibodies. They would also be used to determine whether the stereochemistry of the MA in the TDM has any effect on its biological activities. The methoxy-MA can again be divided into the mycolic motif part and the meromycolate part (**Scheme 79**):



Scheme 79: Retro synthesis of the full methoxy-MA

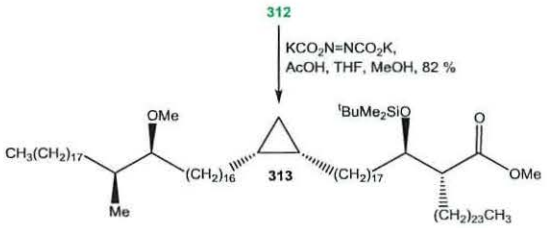
2.3.2 Final coupling to form the full methoxy-MA

In order to achieve the coupling between the mycolic motif aldehyde **(312)** and the meromycolate sulfone **(387)**, a Julia reaction was used. The alkene product **(314)** was produced as an (*E*/*Z*) mixture as illustrated in **Scheme 80**.



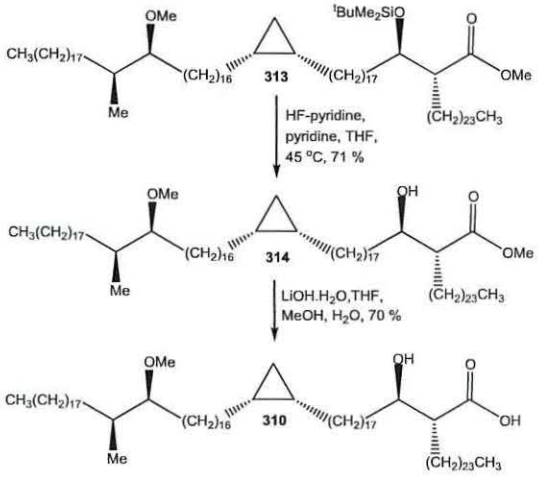
Scheme 80: The final coupling to form the methoxy-MA

The alkene **(312)** was dissolved in MeOH/THF and an excess of dipotassium azodicarboxylate was added at 0 °C under nitrogen. A solution of glacial acetic acid in the THF was slowly added over 18 h (**Scheme 81**), to ensure that the saturation of the alkene was complete, this procedure was repeated twice.



Scheme 81: Hydrogenation with dipotassium azodicarboxylate

Compound **(313)** gave the same ^{1}H and ^{13}C NMR spectra as in the literature.[210] The TBDMS group of compound **(313)** was removed using HF-pyridine and pyridine in dry THF at 45 °C for 18 h. This gave the ester **(314)** (**Scheme 82**). Using an acidic medium was not possible because of the reactivity of the cyclopropane ring.[274] The last step to obtain free methoxy-MA required the hydrolysis of the methyl ester group using lithium hydroxide monohydrate in a mixture of THF, water and methanol at 45 °C for 18 h. Compound **(310)** which resulted gave the same proton and carbon NMR spectra as in the literature.[207]



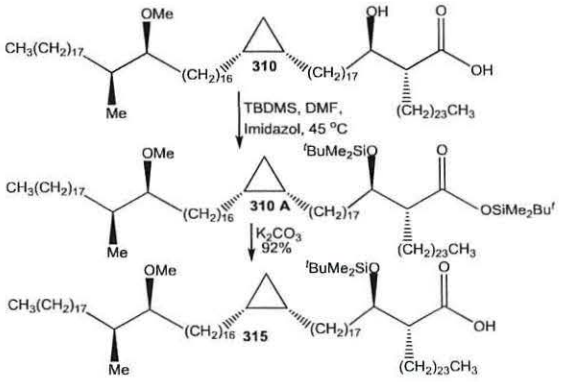
Scheme 82: Deprotection and hydrolysis to obtain the free methoxy-MA

2.3.3 Synthesis of trehalose esters present in *M.tb*

Following the literature procedure, the synthesis of new TDMs of MA **(310)** was carried out.[216] This involved the esterification of the protected MA with protected trehalose, then removal of both the protecting groups.

2.3.3.1 Protection of the secondary hydroxyl group of methoxy-MA

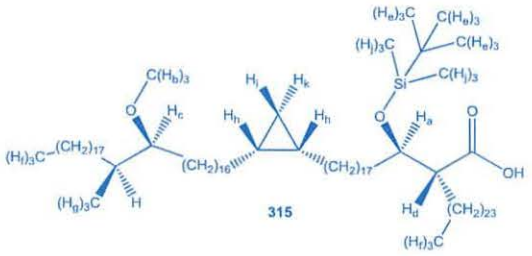
To avoid alcohol reactions, the hydroxyl group at the β-position of methoxy-MA **(310)** was protected before esterification as a TBDMS ether (**Scheme 83**).



Scheme 83: Protection of methoxy-MA (310)

The ^{1}H NMR spectrum identified the product giving a singlet at δ 0.91 (9H) for the *ter*t-butyl protons, and two singlets at δ 0.16 (3H) and at δ 0.14 (3H) for the protons of

the methyl groups of the silyl group, (**See experimental 74**). This is shown in (**Table 7**).

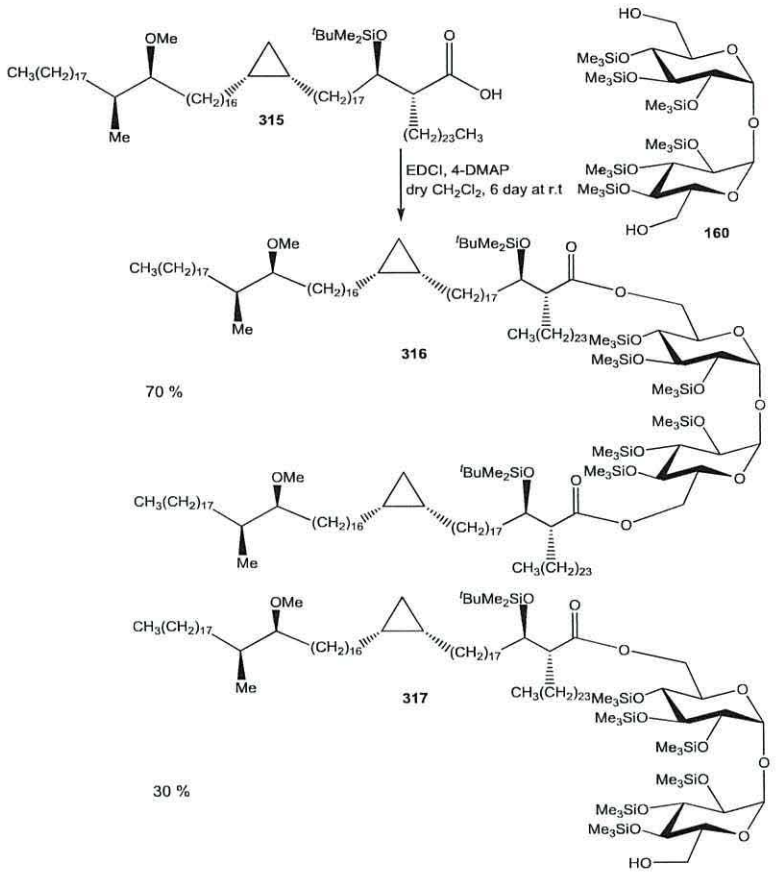


H_x	δ	Multiplicity	Integration	J (H_z)
H_a	3.87-3.82	m	1	-
H_b	3.34	s	3	-
H_c	2.96	pent.	1	4.5
H_d	2.58-2.52	m	1	-
H_e	0.91	s	9	-
H_f	0.89	t	6	6.6
H_g	0.86	m	3	-
H_h	0.67-.63	m	2	-
H_i	0.58	dt	1	4.2, 8.2
H_j	0.14, 0.16	s	2 x 3H	-
H_k	-0.31	br.q	1	5.1

Table 7: ^{1}H NMR analysis of the protected methoxy-MA (315)

2.3.3.2 Coupling of protected methoxy-MA with trehalose

The esterification reaction was done as before between protected methoxy-MA **(315)** and protected trehalose **(160)** using EDCI as an activating agent and DMAP as catalyst.[275] The first fraction was TDM **(316)** and the second fraction was TMM **(317)**, (**See experimental 75**). This is illustrated in **Scheme 84**.



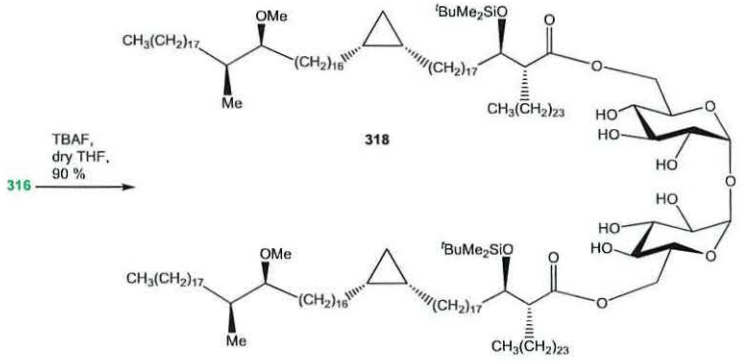
Scheme 84: Esterification of protected trehalose (160) with methoxy-MA (315)

The ^{1}H NMR spectrum of TDM **(316)** displayed a doublet at δ 4.85 (2H) for the hemiacetal protons. The remaining signals corresponded to the sugar protons and the β-hydroxyl protons at δ 4.37 (2H), 4.04-3.83 (8H), 3.53 (2H) and 3.39 (2H). The protons of the methoxy groups showed as a singlet at δ 3.34 (6H). The protons which were next to the methoxy groups were displayed as a broad pentet at δ 2.97 (2H). The α-protons of the mycolic acid showed a multiplet between δ 2.57-2.53. The TMS-groups on the sugar showed 18H-singlets at δ 0.16, 0.14 and 0.13. The twelve protons of the silicon bound methyl groups, showed as a singlet at δ 0.06. The protons of the cyclopropane ring were displayed as a multiplet between δ 0.66-0.64 for four protons and a doublet of triplets at δ 0.58 for two protons. It also showed a broad quartet at δ -0.31 for the other two protons. The ^{1}H NMR spectrum of the TMM **(317)** was more complex than the TDM due to loss of symmetry. The hemiacetal protons showed as doublets at δ 4.91 (1H) and at δ 4.84 (1H). There was an integration of one proton for each. The remaining sugar protons resonated between δ 4.63-3.37. The signals of the

methoxy-MA protons were very similar to those of the TDM **(316)**, however integrating only to half the number of protons.

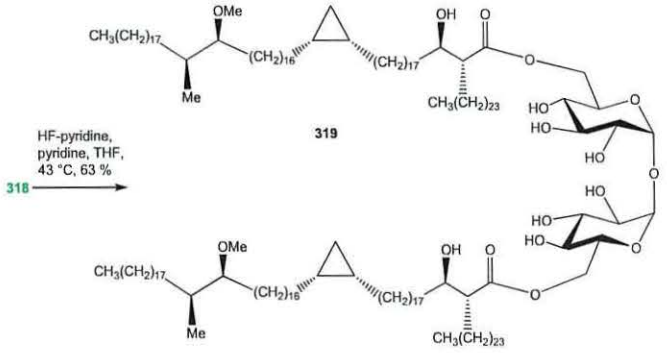
2.3.3.3 Deprotection of TDM (316)

The TMS groups were deprotected using TBAF in dry THF for 1 h at room temperature (**Scheme 85**) to give compound **(318)** (**See experimental 76**) as confirmed by the ^{1}H and ^{13}C NMR spectra which showed no TMS-signals.



Scheme 85: Deprotection of the trehalose moiety (316)

The next stage was the removal of the TBDMS ether protecting group at the β-position of the MA using HF-pyridine complex and pyridine in THF, (**See experimental 78**) (**Scheme 86**).



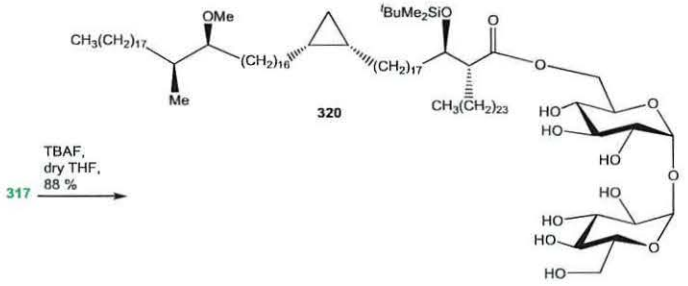
Scheme 86: Synthesis of free TDM of methoxy-MA (319)

The hemiacetal protons displayed a doublet at δ 4.96 (2H) and the rest of the sugar protons showed 2H-signals at δ 4.8, 3.34, 3.89-3.84, 3.71-3.67, 3.53 and 3.21. The protons next to the β-hydroxyl of the MA showed a broad triplet at δ 3.79. A broad

pentet appeared at δ 2.96 for the protons next to the methoxy group and a multiplet occurred between δ 2.43-2.38(2H) for the protons in the α-position of the MA. The methyl groups next to the methoxy group showed as a doublet at δ 0.83 with an integration of six protons. The cyclopropane protons showed a multiplet between δ 0.65-0.60 (4H), a doublet of triplets at δ 0.55 (2H) and a broad quartet at δ -0.33 (2H). MALDI MS gave an $[M+Na]^+$ of 2835.7 while the calculated value was 2835.6.

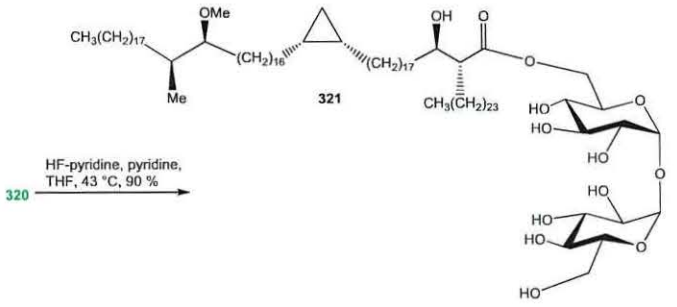
2.3.3.4 Deprotection of TMM (317)

The protecting groups of the sugar were removed by stirring compound **(317)** with TBAF in dry THF for one hour at room temperature, (**See experimental 77**) (**Scheme 87**).



Scheme 87: Deprotection of (317)

The *tert*-butyldimethylsilyl group on the β-position of compound **(320)** was then removed using HF-pyridine complex and pyridine in dry THF, as in Scheme **(88)**.



Scheme 88: Synthesis of the free TMM of methoxy-MA (321)

In the ^{1}H NMR spectrum of the free TMM **(321)** (**See experimental 79**) the hemiacetal protons showed as doublets at δ 5.12 and 5.05 while the remaining sugar protons resonated between δ 4.70 and 3.22. The signals for the methoxy-MA protons

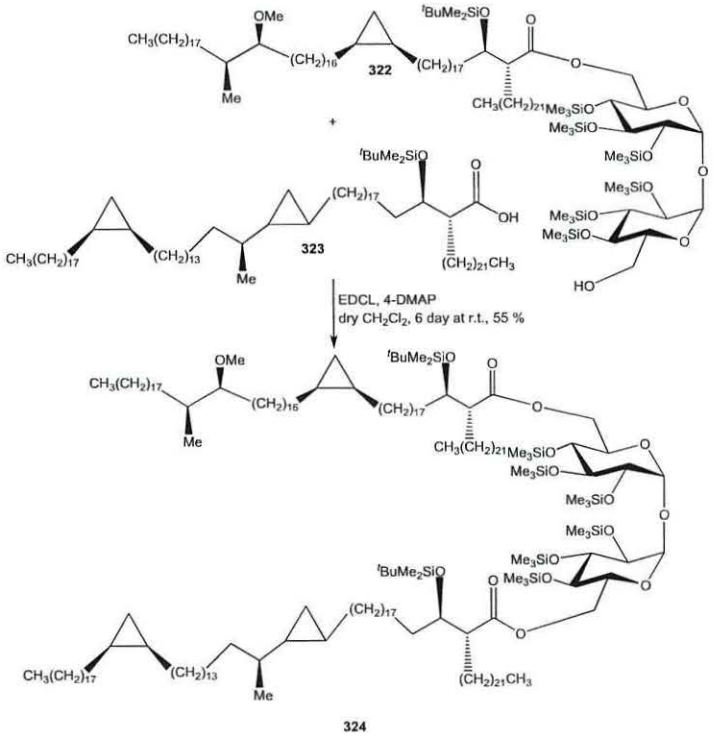
were similar to those of the free TDM but with half the number of protons. MS showed for **(321)** an $[M+Na]^+$ of 1600.3882, while the calculated value was 1600.3897.

2.4 Synthesis of mixed trehalose esters present in *M. kansasii* and *M. tb*

The aim of this section was to prepare for the first time mixed TDMs which include two different single enantiomers of MA bound to trehalose, in order to ascertain whether or not the immune activity is effect or increased. The natural TDM mixture consists of a complex mixture, thus preparing the mixed corf factors will allow their biological activity to be compared to that of those containing the same mycolic acid. It is also hoped that using these antigens will lead to an improvement in the ELISA assay. Given the potent immune signalling effects of cord factors, this research has the potential to cure, or improve the lives of many people in the future.

2.4.1 Synthesis of the first mixed TDM

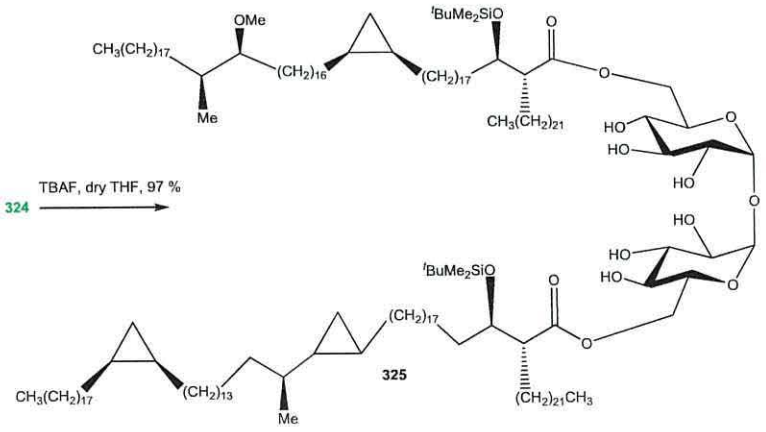
Esterification of protected mycolic acid **(323)** and protected TMM **(322)** was undertaken using EDCI as activating agent and 4-DMAP as catalyst to produce second ester bond. The reaction formed **(324)** in a yield 55% (**See experimental 86**) as is shown by **Scheme 89**.



Scheme 89: Esterification of protected TMM (322) with protected MA (323)

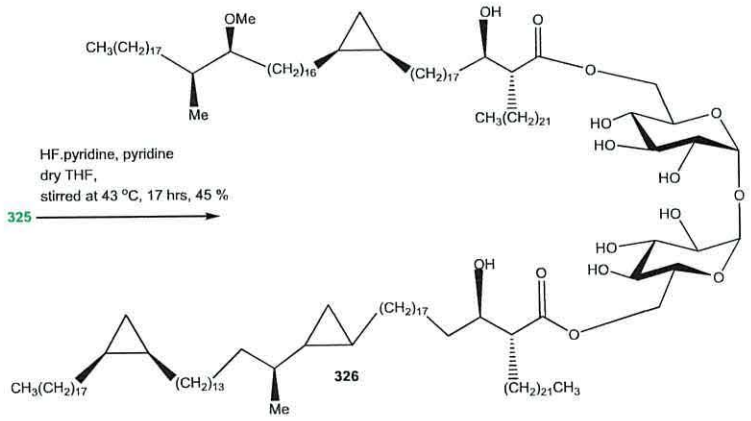
The ^{1}H NMR spectrum for TDM **(324)** showed a doublet for two acetal protons of the sugar moiety at δ 4.70. The remaining signals for the sugar protons and the β-hydroxyl protons resonated at δ 3.94 (4H), 3.85 (4H) and 3.44 (2H). The protons of the OMe resonated at δ 3.26 (3H) as a singlet while the α-protons of the mycolic acid showed as a multiplet between δ 2.49-2.45 (2H). The *t*-butyl groups gave a singlet at δ 0.78 integrating for 18 protons. The *cis*-cyclopropane ring protons showed as a multiplet for four protons between δ 0.57-0.55, a doublet of triplets for two protons at δ 0.49, and a quartet at δ -0.4. One of the protons directly bonded to the *trans*-cyclopropane ring showed as a multiplet between δ 0.40-0.32, and three remaining protons of this unite gave a multiplet between δ 0.07-0.05. The ^{13}C NMR showed the carbonyl carbon at δ 174.0, the anomeric carbon signal at δ 94.6 and the reminder of the sugar carbons between δ 73.3 and 70.5.

TDM **(324)** was stirred with TBAF in dry THF at room temperature for 1 h to give compound **(325)** in 97% yield (**See experimental 87**) (**Scheme 90**). The success of the deprotection was confirmed by the ^{1}H NMR spectrum which gave a doublet at δ 5.0 (2H) for the hemiacetal protons with the rest of the sugar protons and the β-hydroxyl protons resonating at δ 4.26 (2H), 4.17 (2H), 3.87-3.83 (4H), 3.70 (2H), 3.41 (2H) and 3.32-3.23 (2H). The MA moiety showed a multiplet between δ 2.51-2.45 (2H) for the α-protons. The *cis*-cyclopropane ring protons showed a multiplet for four protons between δ 0.61-0.65, a doublet of triplets for two protons at δ 0.49 and a quartet at δ -0.40. The *trans*-cyclopropane ring gave a multiplet between δ 0.38-0.34 for one proton, with the remaining three protons giving a multiplet between δ 0.12-0.01.



Scheme 90: Deprotection of the trehalose of (324)

The final step was to remove the TBDMS ether group from the alcohol in the β-position of the mycolic acid using HF.pyridine complex to give compound **(326)** in a yield of 45% **(See experimental 88)** **(Scheme 91)**. The creation of the free mixed TDM **(326)** was confirmed by the ^{1}H NMR spectrum, which gave for the hemiacetal protons a doublet at δ 4.96 (2H). The remainder of the sugar protons and the β-hydroxyl protons resonated at δ 4.61 (2H), 4.17 (2H), 3.97 (2H), 3.70 (2H), 3.62-3.59 (2H), 3.42 (2H) and 3.20 (2H) with two protons integrated in each signal. The α-protons of the mycolic moiety showed as a multiplet between δ 2.37-2.32 (2H). The terminal CH_3 groups gave a triplet at δ 0.81 with an integration of 12 protons while the α-methyl groups gave a doublet at δ 0.8 integrating to 6 protons. The *cis*-cyclopropane ring protons showed a four proton multiplet between δ 0.59-0.57, a doublet of triplets for two protons at δ 0.51 and a quartet at δ -0.38 for the other two protons. The *trans*-cyclopropane ring gave a multiplet between δ 0.39-0.35 for one proton and a multiplet between δ 0.15-0.01 for three protons. The ^{13}C NMR showed the carbonyl carbon at δ 175.4 while the anomeric carbon came at δ 95.1. The reminder of the sugar carbons resonated at δ 72.5 and at 69.7. The I.R spectrum showed a carbonyl band at ν_{max} 1723 cm^{-1} and an alcohol at 3468 cm^{-1}. MS showed for **(326)** an $[M+Na]^{+}$ of 2761.5985, while the calculated value was 2761.6002.



Scheme 91: Preparation of complete mixed TDM (326)

2.4.2 Synthesis of a second mixed TDM

2.4.2.1 Synthesis of keto-MA (347)

Methoxy-MA and keto-MA are found in the cell wall of *M. tb*, and they are the major oxygenated MA. It is thought that the keto-MA **(327)**, illustrated in **Figure 31**, has the same stereochemistry as the natural keto-MA.[221] In addition, it has been proved that the methyl branch next to the carbonyl group in the natural keto-MA exists in the *S*-configuration.[268]

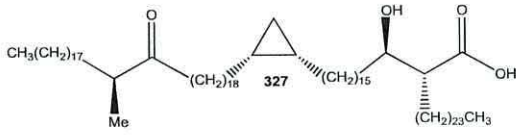
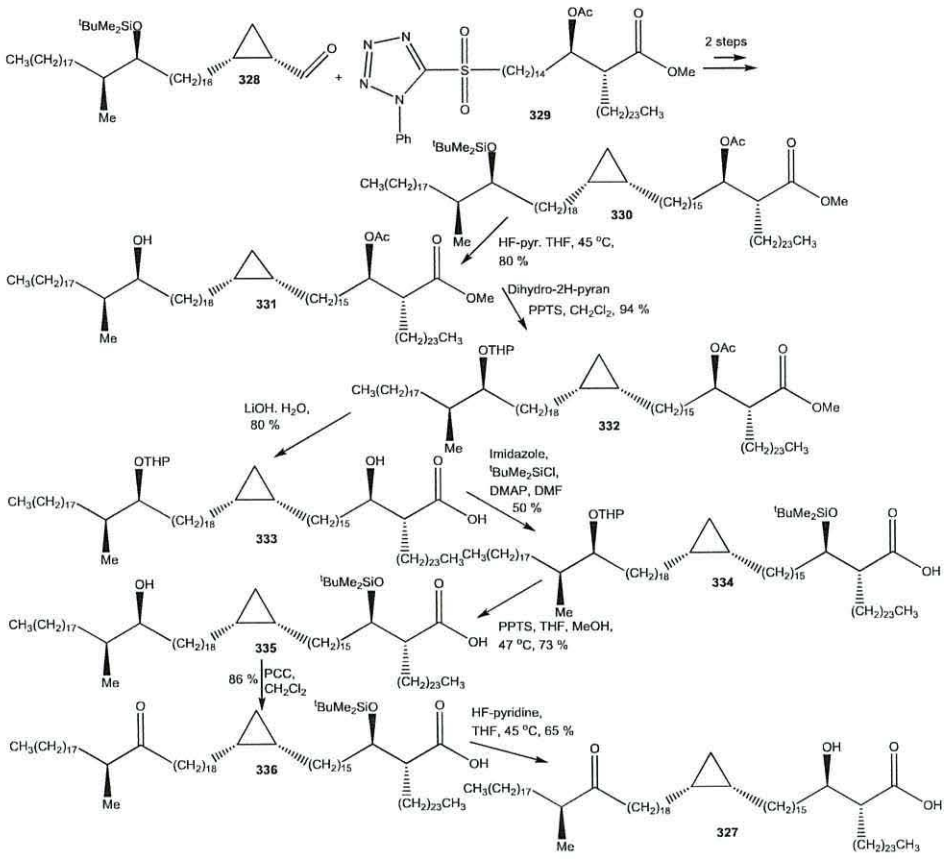


Figure 31: The keto-MA present in *M. tb*

2.4.2.2 Synthesis of a keto-MA containing a *cis*-cyclopropane (327)

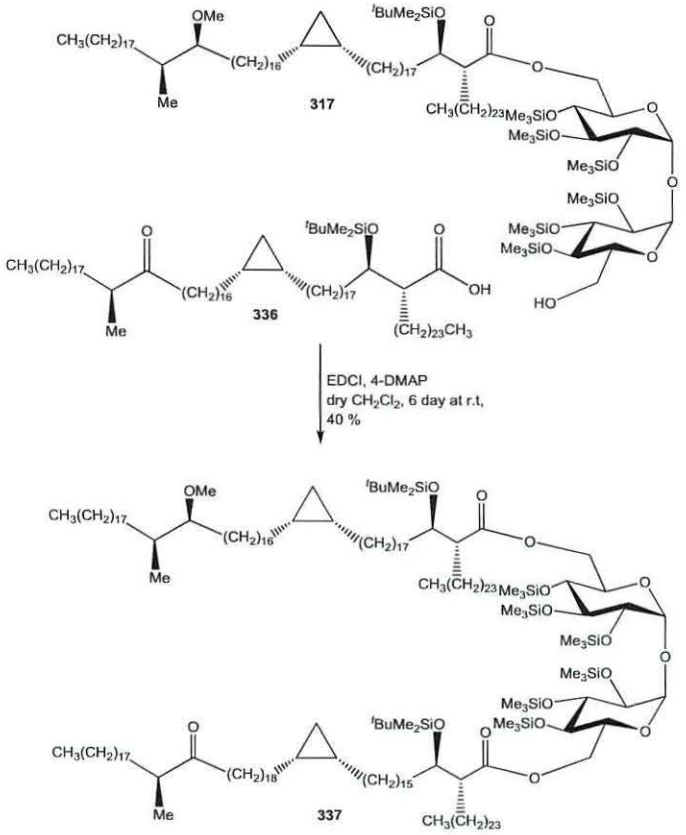
In this study, a keto-MA with the correct stereochemistry was made using the same method as Saleh.[269] The coupling of the mycolic motif part **(329)** and the meromycolate part **(328)** was undertaken, the secondary alcohols present in the two respective fragment bearing orthogonal protection. The silyl protecting group was replaced by a THP group, as this is resistant to basic media. It is also easy to remove.[254] The acetyl groups and methyl ester of **(332)** were deprotected in basic media. The next step was the re-protection of the hydroxyl group in the motif with a silyl group. Subsequently, the THP-group was deprotected and the resulting alcohol was oxidised by PCC to give ketone **(336)**. Lastly, the silyl group was removed with HF-pyridine complex to give the free keto-MA **(327)**, (**Scheme 92**).



Scheme 92: The synthesis of a keto-MA (327)[269]

2.4.2.3 Synthesis of mixed cord factors of protected keto-MA (336) and protected methoxy TMM (317)

The method that was used to prepare the mixed TDM **(326)** present in *M. kansasii* was also used to make the mixed TDM present in *M. tb.*, coupling the protected keto-MA **(336)** was reacted with protected methoxy-TMM **(317)** in dry dichloromethane in the presence of DMAP and EDCI, as in **Scheme 93**, (**See experimental 83**).

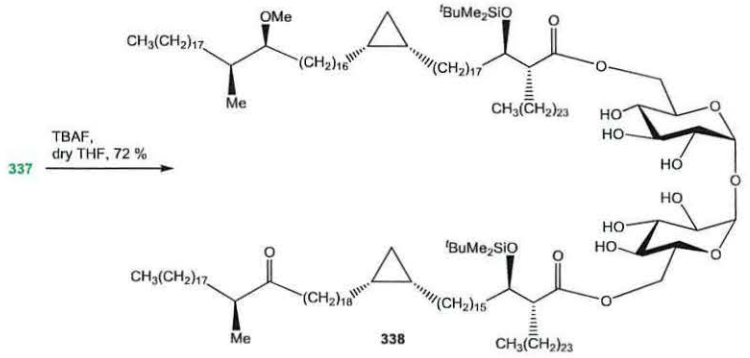


Scheme 93: Esterification of protected keto-MA (336) with protected TMM (317)

The successful formation of the mixed TDM **(337)** was confirmed by the ^{1}H NMR spectrum. The hemiacetal protons showed as a doublet at δ 4.81 (2H), while the remaining sugar protons resonated between δ 4.33 (2H), 4.00-3.94 (4H), 3.92-3.89 (2H), 3.87 (2H), 3.49 (2H) and 3.35 (2H). This included the two protons at the β-position of the motif part. The proton at the α-position relative to the carboxyl group showed a multiplet between δ 2.53-2.49. The protons of the methyl next to the carbonyl showed as a doublet at δ 1.05 and had an integration of three protons. The terminal methyl groups showed as a triplet at 0.86 (12H) and the protons of the *tert*-butyl of the protecting group of the MA showed as a singlet at 0.84 (18H). The protons of the trimethyl silyl protecting groups on the sugar, showed as singlets at δ 0.12, 0.10 and 0.09. The methyl groups bound to the silicon in the TBDMS groups of the MA showed as a singlet at δ 0.02 (12H). The cyclopropane ring protons were displayed a four proton multiplet between δ 0.62-0.6, a doublet of triplets at δ 0.42 for two protons and a broad quartet at δ -0.35 for a further two protons. The ^{13}C NMR spectrum displayed two signals at δ 215.1 and at 173.8 for the carbonyl groups of the ketone and

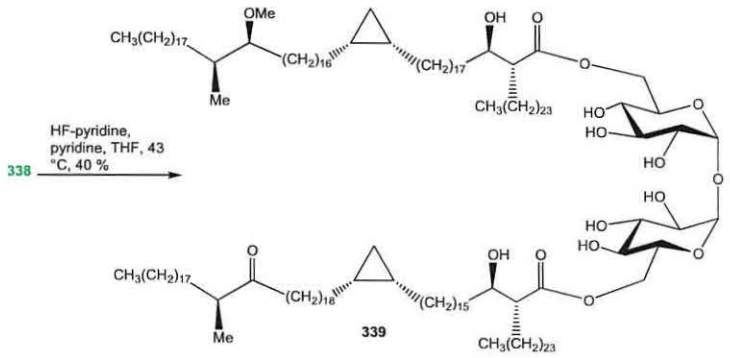
carboxylic acid respectively. The anomeric carbon signal appeared at δ 94.8. The rest of the sugar carbons showed between δ 73.5-70.7. The methyl carbon signals of the protecting groups of the sugar showed at δ 1.0, 0.9 and 0.1. In addition, the carbons of the methyl silyl groups bound to the silicon in the TBDMS groups of the MA appeared at δ -4.5 and -4.6.

The trimethylsilyl protecting groups of the TDM **(337)** were removed using TBAF in dry THF to yield **(338)**, as shown by **Scheme 94**, **(See experimental 84)** .



Scheme 94: Deprotection of the sugar protecting groups of compound (337)

After this, the TBDMS protecting groups were removed using HF-pyridine complex and pyridine resulting in a free mixed TDM **(339)** which is shown in **Scheme 95**, **(See experimental 85)**. The ^{1}H NMR spectrums of free mixed TDM **(339)** gave a doublet at δ 5.02 (2H) for the hemiacetal protons. The remaining sugar protons resonated between δ 4.71 (2H), 4.27 (2H), 3.76 (2H), 3.64 (2H), 3.49-3.47 (2H), 3.39 (2H) and 3.18 (2H), including the protons at the β-hydroxyl position. The proton at the α-position to the alkyl chain of the motif showed a multiplet between δ 2.59-2.49, with an integration of two protons. The *cis*-cyclopropane ring protons showed as a multiplet for four protons between δ 0.61-0.59, a doublet of triplets for two protons at δ 0.53 and a quartet at δ -0.36 for the other two protons. MALDI MS showed for **(339)** an $[M+Na]^{+}$ of 2822.4, while the calculated value was 2822.6.

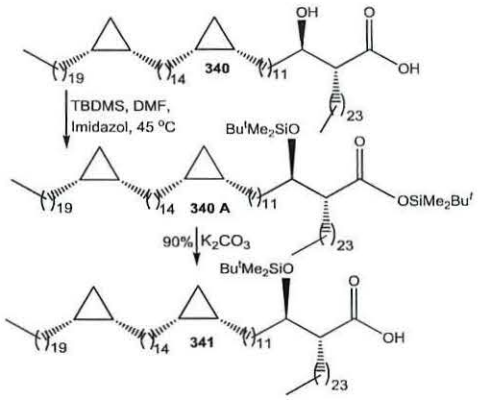


Scheme 95: Synthesis of free mixed TDM (339) of keto-MA and methoxy-MA

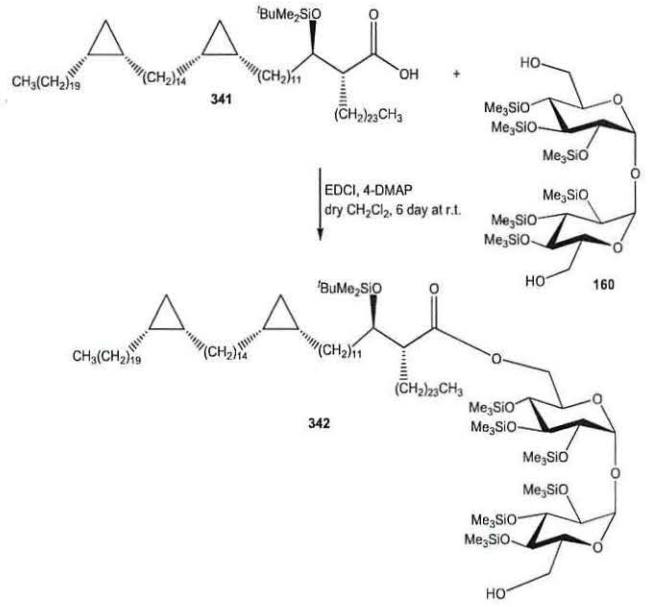
2.4.3 Synthesis of a third mixed TDM

2.4.3.1 α-MA protection and esterification with trehalose

Compound **(340)** was silylated with imidazole, TBDMS-Cl and 4-DMAP to give **(341)** (**Scheme 96**), which also gave the same proton and carbon NMR spectra as in the literature.[224] Then the α-MA **(341)** was esterified with trehalose to get the corresponding TMM using the same procedure as above (**Scheme 97**).[224]



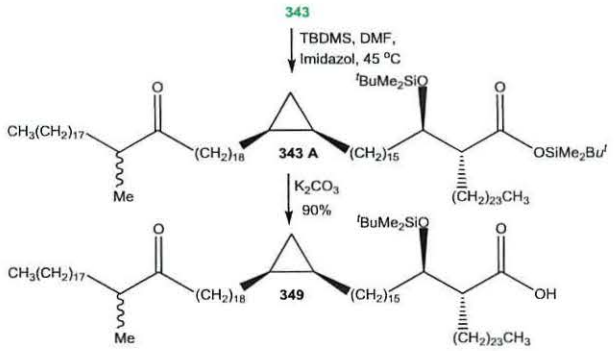
Scheme 96: Protected α-MA (341)



Scheme 97: Esterification of protected trehalose (341) with protected α-MA (160)

2.4.3.2 Protection of the secondary hydroxyl group of the keto-MA

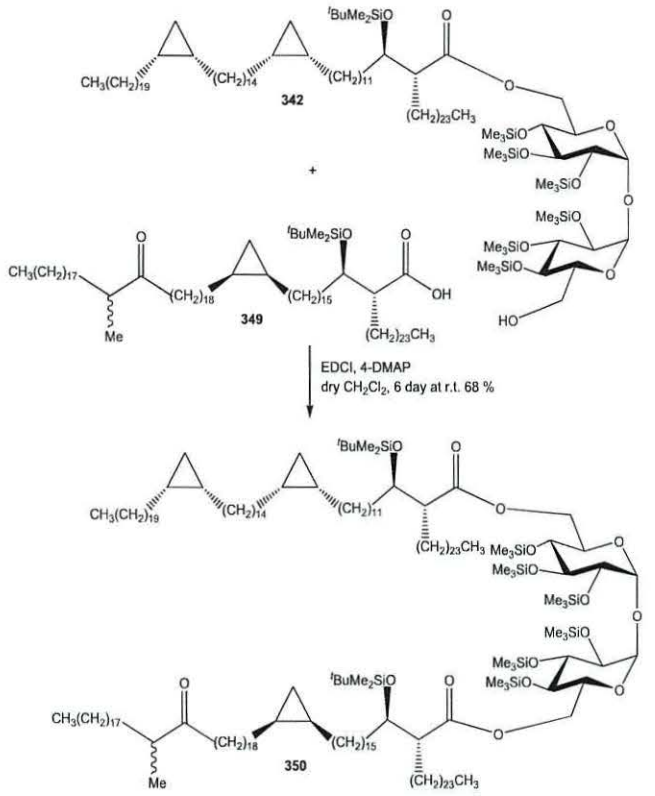
The synthesis of keto-MA **(343)** was carried out in appendices secion. In order to avoid alcohol reactions, the secondary hydroxyl group at the β-position of keto-MA **(343)** was protected as a *tert*-butyldimethylsilyl ether (**Scheme 98**).



Scheme 98: Protection of keto-MA (361)

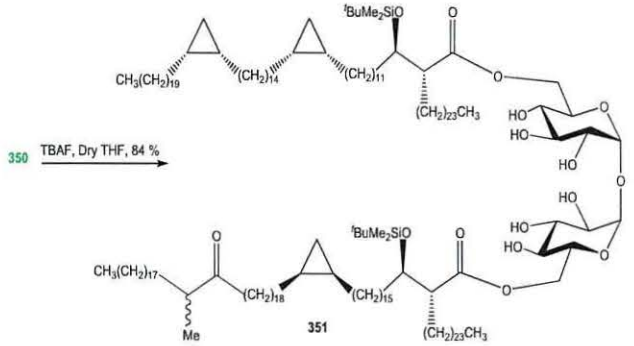
2.4.3.3 Esterification of protected MA (349) with protected TMM (342) to form mixed TDM

Esterification of TMM **(342)** and **(349)** was carried out using EDCI and 4-DMAP as before From days 4-5 the reaction began to form product **(350)** (**Scheme 99**), (**See experimental 80**).



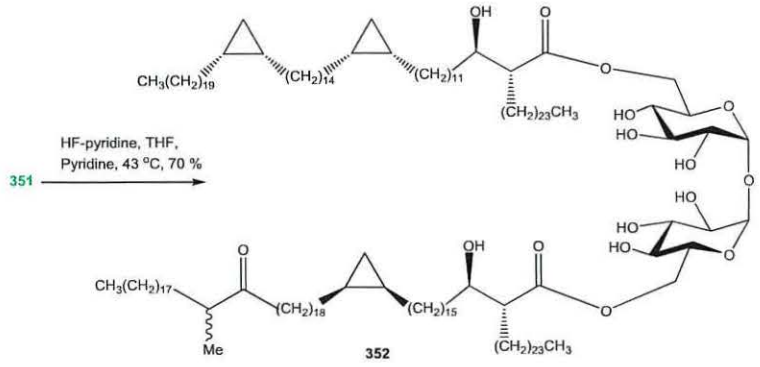
Scheme 99: Esterification of protected TMM (342) with protected keto-MA (349)

The ^{1}H NMR spectrum for TDM **(350)** showed the seven signals which corresponded to the core sugar protons, beginning with the H1 resonance at δ 4.85 (2H), and with H6 at δ 4.37 (2H) while the remaining signals appeared between δ 4.04-3.99 (4H), 3.96-3.93 (2H), 3.91 (2H) and at 3.53 (2H). The β-hydroxy protected position displayed a proton at δ 3.39 while the two protons in the α-position of the MAs appeared between δ 2.57-2.53. The twelve protons signal at δ 0.88 corresponded to the terminal methyl groups. The IR spectrum displayed absorbance characteristic for the ester at 1744 cm^{-1}. TBAF was employed in to remove the silyl protection group from the sugar core **(352)**, as shown in Scheme **(100)**, **(See experimental 81)**.



Scheme 100: Deprotection of the sugar protecting groups of compound (351)

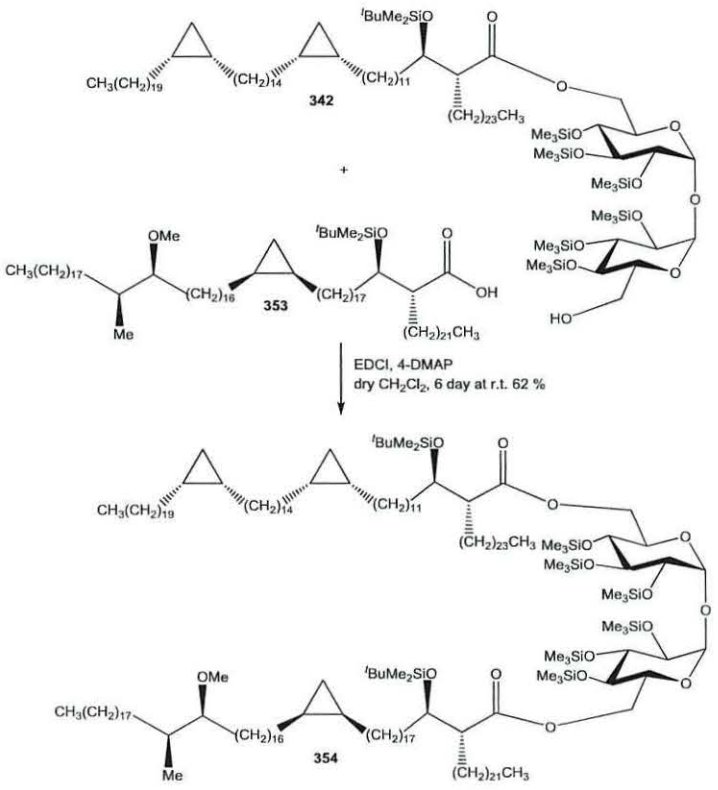
The final step was the removal of the silyl protection group from the β-hydroxy ester using HF-pyridine complex, resulting in free mixed TDM **(352)** as shown in **Scheme 101**, **(See experimental 82)**. The ^{1}H NMR spectrum of free mixed TDM **(352)** gave a doublet at δ 4.97 (2H) for the hemiacetal protons. The remaining protons of the sugar component resonated between δ 4.7 (2H), 4.29 (2H), 3.90 (2H), 3.66-3.63 (2H), 3.49 (2H) and 3.19 (2H). The proton at the β-hydroxyl position of the mycolic acid component showed as a broad triplet at δ 3.74. The protons at the α-position to the alkyl chain of the motif showed a two proton quartet at δ 2.49. The methyl group next to the ketone showed a doublet at δ 1.00 (3H) and the terminal methyl groups showed as a multiplet between δ 0.86-0.84 (12H). The cyclopropane ring gave a multiplet between δ 0.61-0.59 for six protons, a doublet of triplets for three protons at δ 0.53, and finally, a broad quartet at δ -0.36 for three protons. The removal of the silyl group was confirmed by the ^{13}C NMR spectrum, which demonstrated a loss of signals at δ -4.6 and -5.0 for the carbons of the methyl groups bound to the silicon. The optical rotation was $[\alpha]_D^{22} = +11$ (c 0.50, $CHCl_3$).



Scheme 101: Synthesis of free mixed TDM of keto MA and α-MA (352)

2.4.4 Synthesis of a fourth mixed TDM

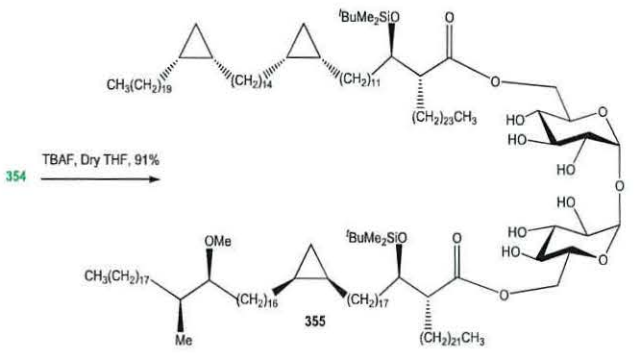
Esterification of trehalose TMM **(342)** and **(353)** was carried out using EDCI and 4-DMAP as before. A reaction time of between 4-5 days was required for the formation of product **(354)**, as shown by **Scheme 102**, (**See experimental 89**).



Scheme 102: Esterification of protected TMM (342) with protected methoxy-MA (353)

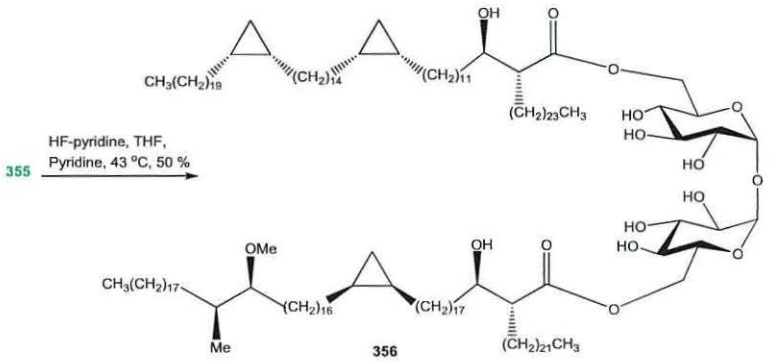
The ^{1}H NMR spectrum for TDM **(354)** showed the seven signals for the sugar protons, beginning with the H1 resonance at δ 4.85 (2H), the H6 proton at δ 4.36 (2H) and the remaining signals between δ 4.04-3.967 (4H), 3.96-3.93 (2H), 3.91 (2H) and at 3.53 (2H). The proton at the β-hydroxy protected position displayed two protons at δ 3.39 (2H); that in the α-position of the α-MA showed a signal at δ 2.57. The TMS protecting groups appeared at δ 0.16 (18H), 0.14 (18H) and 0.13 (18H). The IR spectrum displayed a characteristic band for the ester at 1744 cm^{-1}.

TBAF was again employed to remove the TMS-protection from (**Scheme 103**), (**See experimental 90**).



Scheme 103: Deprotection of the sugar protecting groups of compound (354)

The TBDMS-groups were then removed using HF-pyridine complex (**Scheme 104**), and this resulted in the free mixed TDM **(356)**, (**See experimental 91**). The ^{1}H NMR spectrum of this gave a doublet at δ 4.98 (2H) for the hemiacetal protons. The remaining protons of the sugar core resonated between δ 4.73 and 3.21. The protons at the β-hydroxyl position of the MA showed as a broad quartet at δ 3.91 (2H). The protons at the α-position to the alkyl chain of the motif showed as a multiplet between δ 2.41-2.36, with an integration of two protons. The methyl group next to the ketone, and the terminal methyl groups showed as a multiplet between δ 0.86-0.81 (15H). The cyclopropane ring gave a multiplet between δ 0.64-0.60 for six protons, a doublet of triplets for three protons at δ 0.54, and finally, a broad quartet at δ -0.35 for three protons. MALDI MS showed for **(356)** an $[M+Na]^{+}$ of 2693.2, while the calculated value was 2693.5.



Scheme 104: Synthesis of a free mixed TDM (356) of methoxy-MA and α-MA

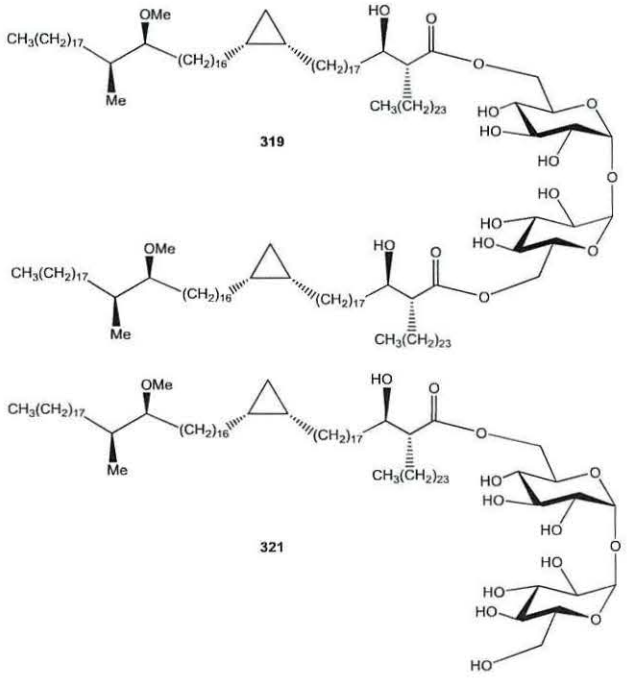
2.5 Biological activity results

2.5.1 ELISA assays

Dr. Alison Jones has carried out ELISA assays for the detection of anti-TB antibodies in both human and bovine serum samples using some of the synthetic trehalose esters discussed in this thesis. The ELISA experiment method is detailed at the end of Chapter 1. The results of these assays are now discussed.

2.5.1.1 Bovine serum samples

Twenty tuberculin skin test positive samples (naturally infected) from cattle from farms with a confirmed history of bovine TB and 16 samples from non-vaccinated young cattle, which are expected to be TB negative (all from the Veterinary Laboratories Agency (VLA)), were tested using **(319)** and **(321)** as antigens. The graph below shows the average responses of the naturally infected and non-vaccinated samples to each of the synthetic antigens along with the response to the natural bovine TDM, for comparison. All samples were run at a 1 in 20 serum dilution and anti-bovine IgG (Fc specific) secondary antibody conjugate was used.



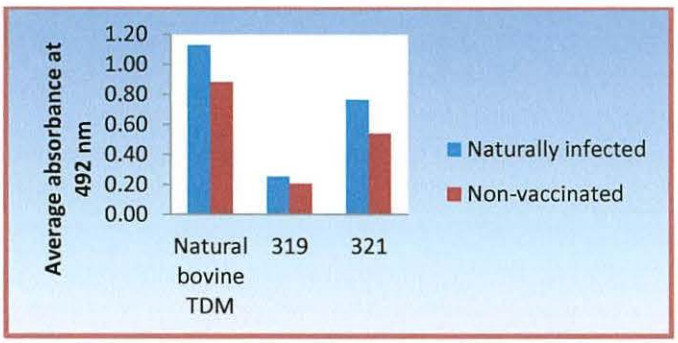


Figure 32: Responses of sets of naturally infected and non-vaccinated bovine serum samples, to natural bovine TDM, synthetic methoxy TDM (319) and TMM (321)

Figure 32 shows the average responses of the naturally infected and non-vaccinated serum samples from the VLA to the natural bovine TDM and the synthetic OMe TDM and TMM. None of the antigens show a good distinction between the two sets. However, the TDM **(319)** shows a worse distinction than TMM **(321)**. Also, the TDM shows a much lower response than the corresponding TMM.

The sensitivity and specificity values for each of the antigens with this set of serum samples were also calculated. Sensitivity is defined as the percentage of positive samples that are correctly identified, while specificity is defined as the percentage of negative samples that are correctly identified. By setting 'cut-off' values for each of the antigens and identifying all samples giving values above this 'cut-off' as positive and all those giving values below the 'cut-off' as negative, the sensitivity and specificity of each antigen could be calculated.

	Natural bovine TDM	**TDM (319)**	**TMM (321)**
Sensitivity (%)	63	26	63
Specificity (%)	56	94	38

Table 8: ELISA data natural methoxy (TDM and TMM) antigens

The best combination of sensitivity and specificity for natural TDM from *M.bovis* was 63% and 56% respectively. Although the synthetic MeO-TDM **(319)** had a high

specificity, with the chosen 'cut-off', the sensitivity was low. Changing the 'cut-off' in order to achieve a sensitivity of 53% reduced the specificity to 69%. The problem with this antigen, however, is that the absorbance values for the majority of the serum samples were very low, < 0.30 which is similar to that observed for control wells. This antigen would therefore not be a good one to use in this kind of assay for the detection of bovine TB. The MeO-TMM **(321)** showed a higher sensitivity than the corresponding TDM, the value of 63% being the same as that observed for the natural TDM. The specificity was lower at 38%. The sensitivity for this antigen compares to the values observed for other TMM, *i.e.* α- and keto, that were run with the same serum samples, which both gave values of 58%. The specificity of 38% was however much lower, the α- and keto TMMs giving values of 69% and 81% respectively. An interesting observation for the results obtained for the two methoxy-MA derived antigens, **(319)** and **(321)**, is that the TMM performs better than the TDM. Generally for other TDM/TMM pairs, *i.e.* α- and keto-, the TDM performs as well as or better than the corresponding TMM.

Two samples from cattle infected with *M. avium paratuberculosis (Map)*, one naturally and one experimentally, from Brussels were also tested using the same antigens. Both samples infected with *Map* gave high responses with all antigens (compared to the values obtained for the VLA samples). The values for **(321)** were high, those for **(319)** lower; this contrasts to other TMM/TDM pairs (α and keto), where the TDM gave a similar or higher response than the corresponding TMM.

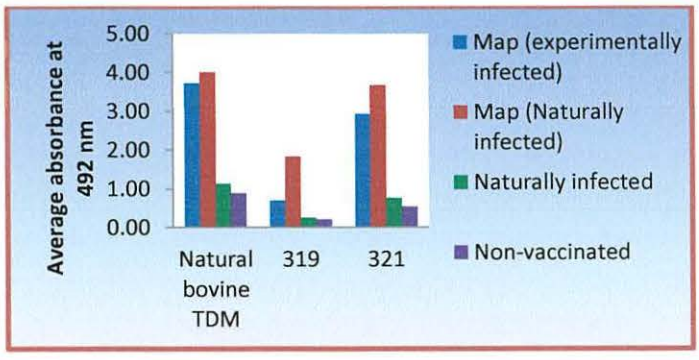


Figure 33: Absorbance of 2 individual samples infected with *Map* and average absorbance of a set of natural infected and non-vaccinated serum samples

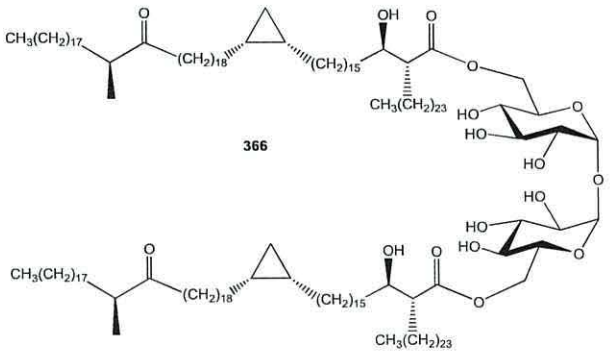
The above graph (**Figure 33**) shows the absorbance at 492 nm for the two samples infected with *Map* and the average absorbencies for the naturally infected and non-vaccinated serum samples from VLA. The two samples infected with *Map* gave a higher absorbance than either of the two sets from the VLA.

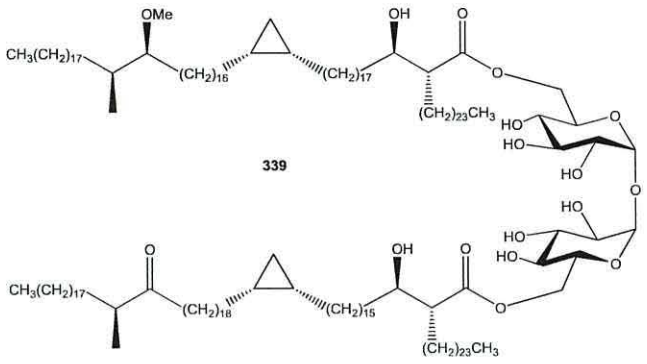
These are however values for individual samples so may not be a fair comparison. In order to determine whether this distinction between samples infected with *Map* and those infected with *M. bovis* is 'real', a larger set of samples would need to be tested.

2.5.1.2 Human serum samples

A set of 64 serum samples from the World Health Organisation (WHO) from patients from Gambia were tested using **(319)** and **(321)** as antigens. Although all the patients had been hospitalised with TB related symptoms, only 9 were diagnosed as being TB^{+}, with the other 55 being diagnosed as TB^{-}.

The graph below shows the results obtained for **(319)** and **(321)** at a 1 in 80 serum dilution. The data for another antigen **(TDM-366)**, run with the same serum samples at a 1 in 20 and 1 in 80 serum dilution, which had given the best results in a previous blind test (unpublished results within the MSB group) is shown for comparison. Anti-human IgG (Fc specific) secondary antibody conjugate was used in each case.





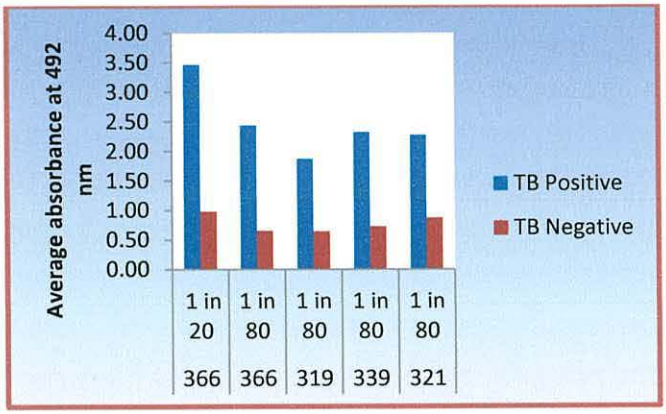


Figure 34: Average absorbance of 9 TB positive and 55 TB negative serum samples form Gambia to various antigens

As can be seen from **Figure 34**, all the antigens show a good distinction between the TB^+ and TB^- serum samples. Although the values for the samples run at a 1 in 80 serum dilution are lower than those run at a 1 in 20 dilution they show a similar distinction to that obtained for **(366)** at a 1 in 20 serum dilution.

The sensitivity and specificity values for each antigen are shown in **Table 9** below:

	(366) (1in 20)	**(366)**	**(319)**	**(339)**	**(321)**
Sensitivity (%)	89	89	78	78	78
Specificity (%)	80	78	75	65	33

Table 9: ELISA data from Keto-TDM, Methoxy-TDM and Mix-TDM antigens

- **(366)**, run with serum samples at a 1 in 20 dilution, was the antigen that gave the best results in the blind test. This was subsequently run at a dilution of 1 in 80 for comparison with other results and as can be seen above a similar sensitivity and specificity was obtained in both cases.
- All the synthetic antigens give a similar sensitivity using the 'cut-offs' chosen, however the specificities vary. Overall the TDM **(319)** gives the best result of the synthetic compounds with a sensitivity and specificity of 78 and 75% respectively. The mixed TDM **(339)** has a slightly worse specificity of 65%, while the specificity of the synthetic TMM **(321)** is low, 33%.
- None of these antigens alone reaches the minimum required cut off of 85% sensitivity and 85% specificity.

2.5.2 Biological Assays with *R. equi*

R. equi is described in detail in the Introduction. It causes chronic bronchopneumonia in young foals, (under 5 months), which have weak maternal immunity and immature immune systems. Horses carry a virulent strain almost exclusively. This remains to be explained but it is thought to be plasmids encode antigen and that the presence of a polysaccharide capsule prevents phagocytosis by the innate immune system and the cell wall mycolic acids.[272] Bacteria act as facultative intracellular pathogens. By inhibiting phagolysosomes fusion within the alveolar macrophages it is able to resist the innate immune system. Virulent strains can replicate inside the macrophages in about 6-8 h. Infection causes pyogranulomatous pneumonia. This is characterised by granuloma, which is a mass of immune cells which are made when the immune system is not able to destroy the bacteria. As the disease advances it causes necrosis and destroys the cells of the lungs, although this is true only of the virulent plasmid-positive strains. It has also been found that the disease disseminates into other areas and causes infections in the intestinal tract because of the infected sputum being ingested.[272]

2.5.2.1 Phagosome-lysosome fusion Assays

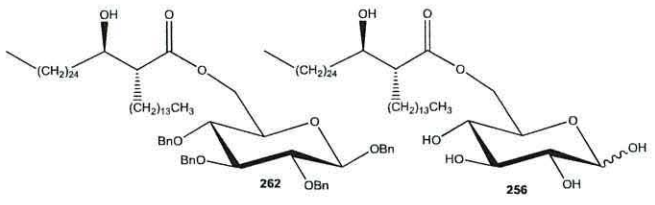
Phagosome-lysosome fusion (PLF) in lung macrophages (LMs) and peritoneal macrophages (PMs) is vital in the inactivation of macrophage-ingested micro-

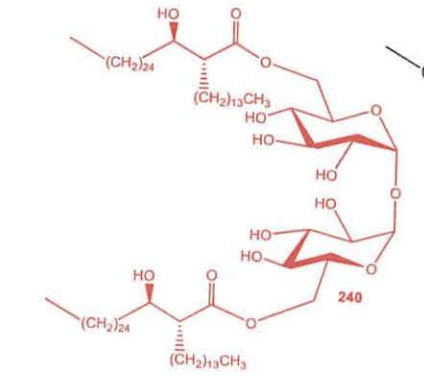
organisms. The importance of the fusion event is to maintain a healthy lung and this is shown by the fact that some pathogenic organisms are resistant to intracellular killing and the cause disease by PLF in LMs. The physiopathology of *R. equi* infection enables us to have a better understanding of the interactions between intracellular pathogens and the immune system.[273]

Cord factor is a cell wall glycolipid of *Mycobacterium*, *Rhodococcus*, *Nocardia*, and *Corynebacterium*. Observations of the effects of CF and related analogues have provided the basis for suggesting that this molecule plays a role in the persistence of the bacteria in the host cell. Indirect evidence has been provided that CF might be responsible for inhibiting fusion between adjacent membranes *in vivo*.[274] However, in order for CF to inhibit PLF *in vivo* it must be found in the phagosomal membrane. Until now, there is no evidence that this molecule is transferred from the bacterial cell, where it is synthesized, to the phagosomal membrane.[274]

Romana Icobescu in the group of Prof. Albert Haas in Bonn University has carried out PIF assays and data analysis for some synthetic trehalose esters to determine whether extension of TDM and TMM chain length beyond the *R. equi*-typical length will increase or decrease inhibitory effects on phagosome-lysosome fusion. Preliminary results are presented below.

The protocol of this assay is to coat harmless *E. coli* with various lipids then to infect them with murine macrophages and test for phagosome-lysosome fusion after 30 minutes of infection. Petrol ether (PE) is the control and the data from all experiments are shown relative to this (set as a relative 100%, which corresponds to an absolute 40-60%). The effects are clear, but not dramatic. Natural TDM (second from left) inhibits by some 40% which is the order of magnitude (**Figure 35**).





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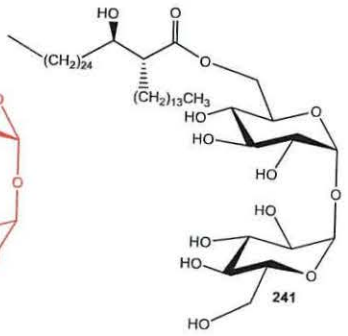

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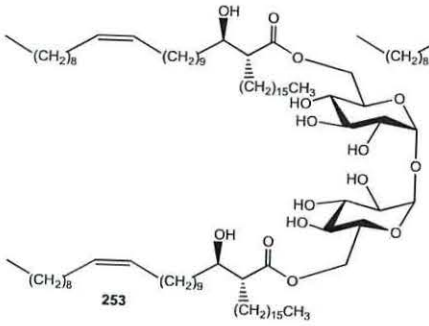

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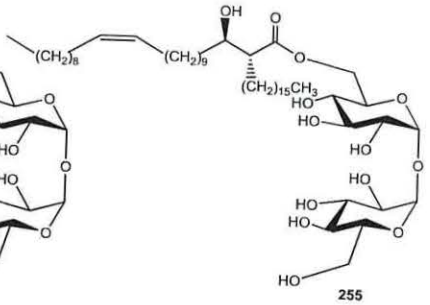

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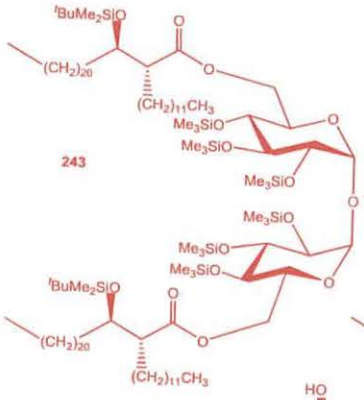

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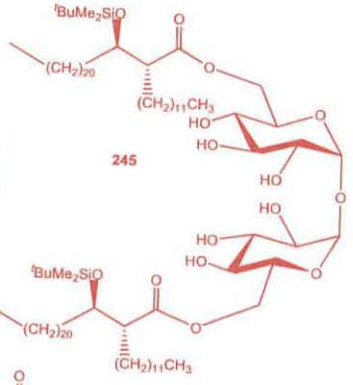

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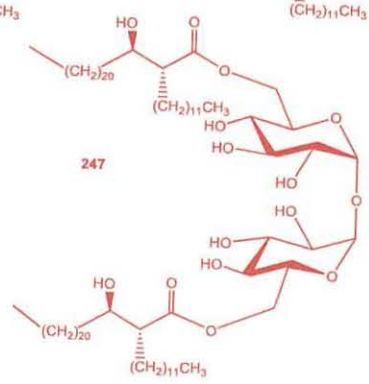

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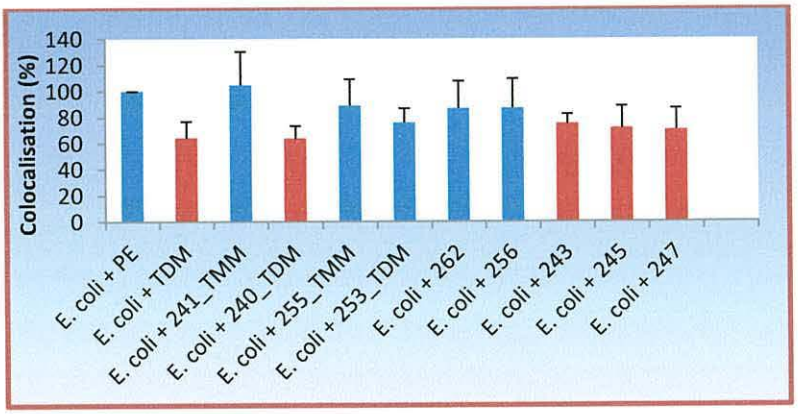


Figure 35: The results of inhibitory effects on phagosome-lycosome fusion assays to determine the effects of extension of cord factor chain length beyond the *R. equi*-typical length

Red bars show "significant" data (relative to "PE" sample). All data are from at least three experiments. Several samples were re-done in order to narrow the error further. This data is very interesting as the following is very clear:

- Extension of TDM **(240)**, **(247)** and **(253)** chain length beyond the *R. equi*-typical length does not increase the inhibitory effects on phagosome-lysosome fusion. TMM **(241)** and **(255)** or GMM **(256)** do not have an effect on the assay, regardless of chain length.

- Modification of trehalose OH groups or of the side-chain OH group by silyl groups **(243)** and **(245)** does not change the biological effects.

- Modification of GMM OH groups or of the side-chain OH group by Bn-groups **(262)** does not change the biological effects.

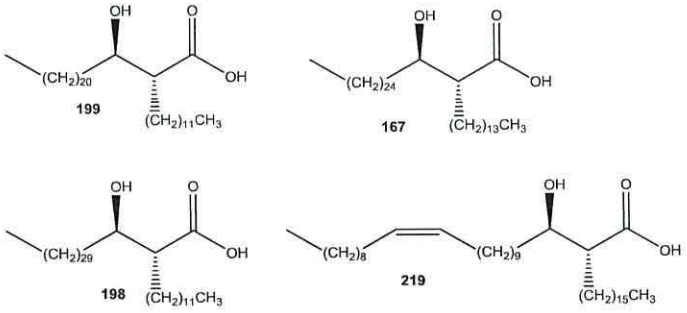
Therefore, it appears that trehalose, as a particular sugar, is not required, but rather that the number of chains and also possibly their relative positioning is important. It demonstrates a role of MA chain length in the manipulation of phagosome trafficking. However, shorter-chain mutants were partly attenuated in their virulence, allowing the conclusion that long-chain TDM can be a virulence factor.

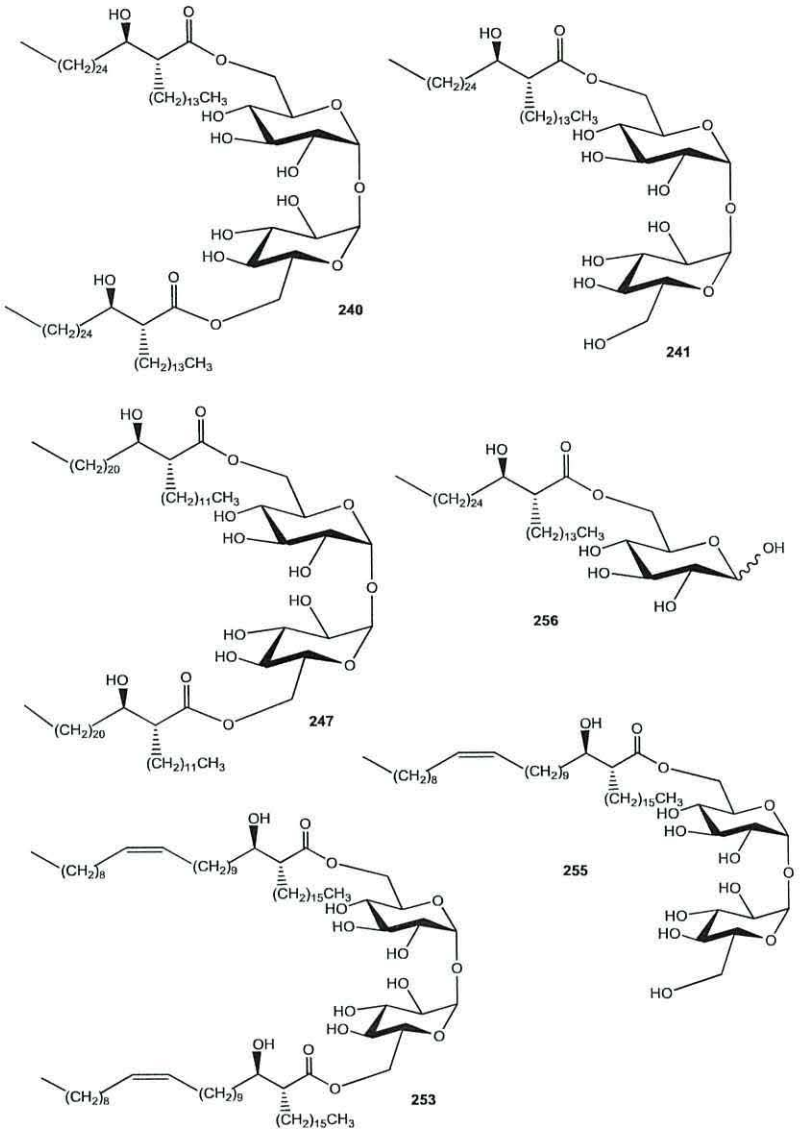
3. Conclusions

Synthetic MA, cord factors and mixed cord factors could be important in the control, detection and treatment of mycobacterial infection. They can be used in developing biosensors to detect mycobacterial disease early. Also, total synthesis of these compounds can help in the identification of the fine structures of natural MA and their absolute stereochemistry. This could be very useful in finding new anti-mycobacterial drugs.

The first part of this work were the synthesis of saturated MAs **(167)**, **(198)** and **(199)** present in *R. equi* and unsaturated mycolic acid **(219)** as a model of a molecule from *R. equi* were the first part of this project. Compound (**219**) is only a model, as the exact position of the double bond in the natural compound has not been reported in the literature.

It was however hoped that the preparation of an unsaturated compound of this kind would give some insight into its effect on the biological activity. The syntheses of their trehalose esters (cord factors) **(240, 241, 247, 253, 255** and **256)** were also achieved.



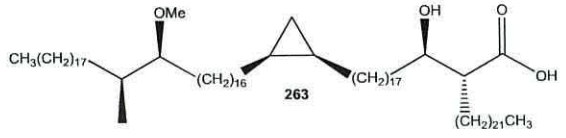


The reason for the preparation of these cord factors was to study the effect of chain length on their biological activities, specifically in phagosomes-lysosome fusion assays. Compounds were tested relative to a petrol ether (PE) control and in these initial studies it was found that the results for extension of TDMs chain length were significant and gave similar values to the natural TDM. The results also suggested that the presence of silyl groups does not have an effect on the outcome of the assay with, for example, compounds **(243)**, **(245)** and **(247)** all showing similar results. The results also suggested that TMM and GMM compounds have no effect on the assay. Future work will also involve studies of other biological effects of the cord factors present in *Nocardia* (*Nocardia* being as close to *M. tb* as to *R. equi)*. The results of these tests

will guide future synthetic targets. It would be very interesting if the inhibition, in this case, would go hand in hand with the inhibition in the macrophage, because then the surface receptors of the macrophage. It can also be seen that it would be quite likely that the effect of the lipids is in fact, through incorporation from the particle surface into the phagosome membrane.

The second part of this work were the synthesis of two stereoisomers of the key homologue of the methoxy MAs **(263)**, **(266)** and keto MA **(293)** present in *M. kansasii*, which would then be coupled to trehalose to generate the corresponding synthetic trehalose dimycolates **(306)**, **(308)**, and trehalose monomycolates **(307)**, **(309)**. These compounds differ in their α-chain length by two carbons from the corresponding compounds in *M. tb i.e.* C22 compared to C24. By preparing these compounds and then tesing them in an ELISA assay it will allow the effect of this chain length to be investigated.

The methoxy-MA was made by coupling a mycolic motif aldehyde to a methoxy meromycolate fragment by a Julia coupling reaction. A very good yield of alkenes was obtained. The alkene hydrogenation was done with di-imide as a mild hydrogenation system in order to avoid the hydrogenolysis of the cyclopropyl ring. HF-pyridine was used to remove the silyl protecting group of secondary alcohol on the β-carbon. The ester group was hydrolysed using lithium hydroxide which gave free methoxy mycolic acid **(263)** and **(266)**.



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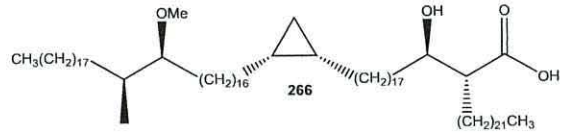
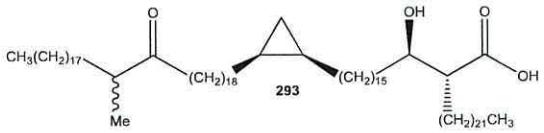


266

The synthesis of the keto-MA was done by coupling the motif part **(295)** with the meromycolate part **(294)**. This used different protecting groups. Following the hydrogenation of the alkenes, protecting and de-protecting was done. The last step was a deprotection in an acid media so, as a result, epimerisation of the methyl substituent

to the ketone group of the mycolic acid was achieved. The rotations were decreased from $[\alpha]_D^{20} = +7.1$ (c 1.0, $CHCl_3$) to $[\alpha]_D^{20} = +4.1$ (c 1.0, $CHCl_3$).

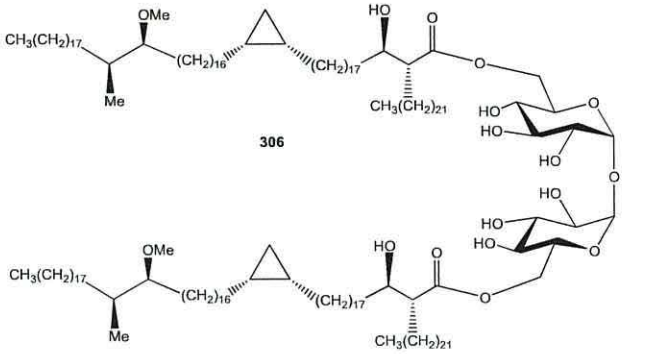


The starting materials for the preparation of four new cord factors were obtained by the completion of the synthesis of these MA. These were derivatives of MA **(263)** and **(266)**.

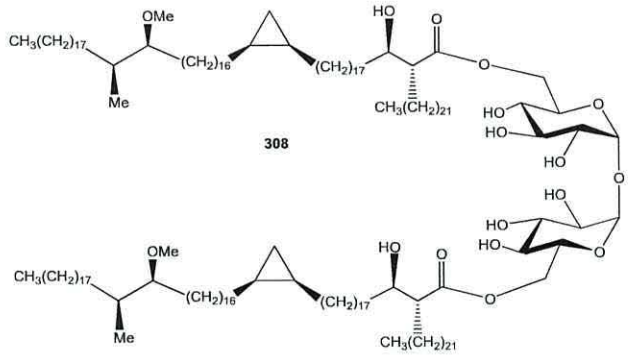
In order to synthesise the cord factors, the first stage was to protect the secondary hydroxyl groups in the trehalose sugar and MA. This was done with two different protecting groups; a trimethylsilyl group was used to protect the secondary hydroxyl groups in in the trehalose sugar and TBDMS group was used to protect the secondary hydroxyl groups in MA so as to permit selective deprotection in the synthesis later.

An esterification reaction between the trehalose and the MA followed. The result was two protected cord factors (TDM and TMM). The protecting groups were cleaved in two stages. The first stage was to deprotect the trehalose sugar using TBAF. The second stage was the deprotection of the mycolic acid using HF-pyridine complex. This was to get the free cord factors (TDM) **(306)**, **(308)** and (TMM), **(307)**, **(309)**.

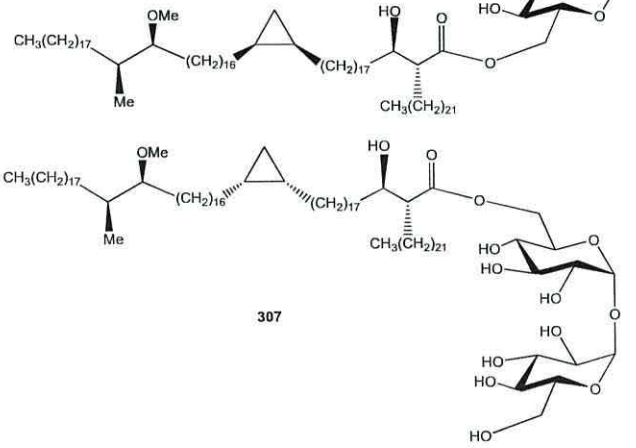
Comparison of these compounds with the corresponding C24 α-chain compounds in *M. tb*, by using them as antigens in an ELISA assay for the detection of TB, will allow the effect of the α-chain length on the assay to be investigated.



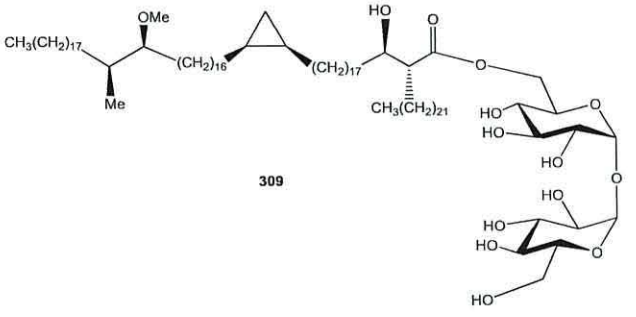
306



308

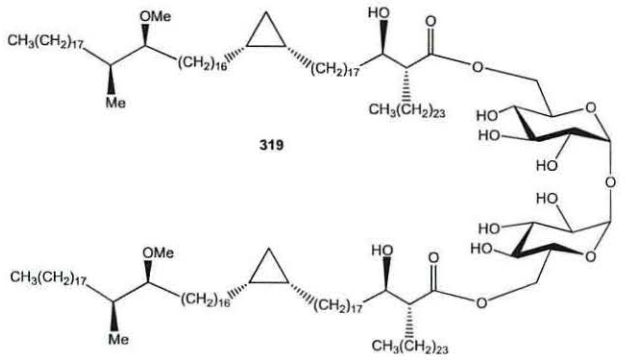


307

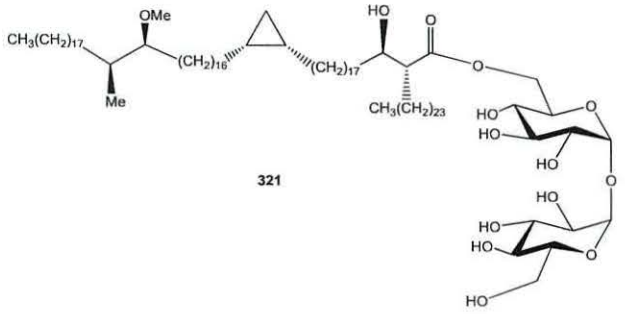


309

In similar way, the third part of this work were the synthesis of the cord factors of the *cis*-cyclopropane methoxy-MA of *M. tb* to obtain TDM **(319)** and TMM **(321)** was achieved.

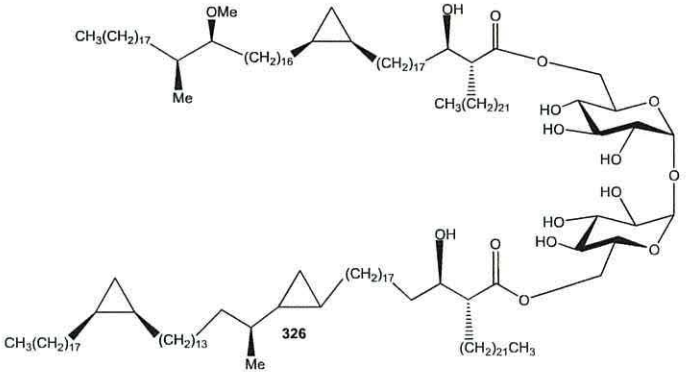


319

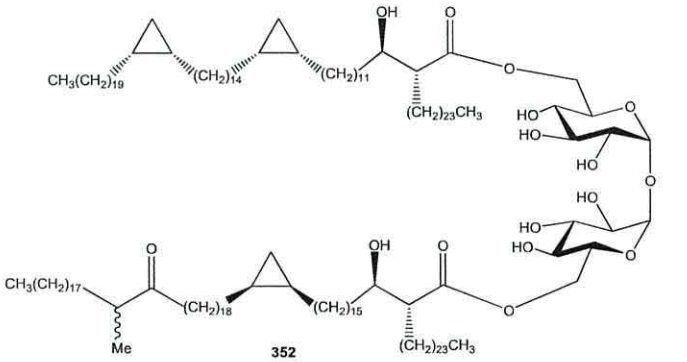


321

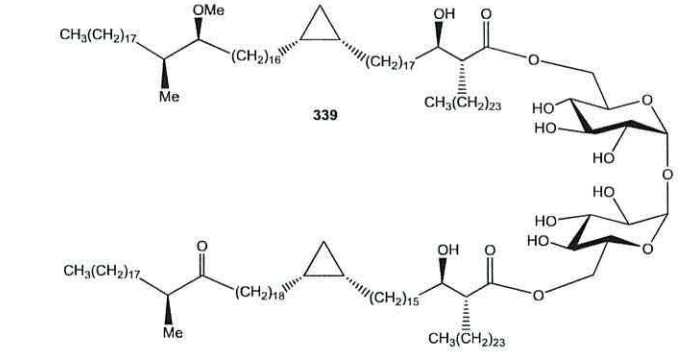
The fourth part entailed the synthesis of mixed cord factors-TDMs including two different classes of MA **(326, 339, 352** and **356)** which are known to be present in *M. kansasii* and *M. tb* respectively. Natural TDM consists of a very complex mixture of different molecules made in a biological syatem where there are many different types and homologues of MA present; unless there is a specific biological control system that introduces the same MA on both sugar rings of the TDM, it is therefore very unlikely that natural molecules contain two identical MAs. Therefore it is important to prepare compounds of containing two different MA in order to investigate their biological activity and compare them to the TDMs that contain the same MA at both positions. In order to synthesise the cord factors, the first stage was to protect the secondary hydroxyl groups in one MA as in previous examples. An esterification reaction between the TMM and the protected mycolic acid followed. The result was a protected cord factor as a mixed TDM. The protecting groups were cleaved in two stages as before, first using TBAF, then using HF.pyridine complex. This gave the free mixed cord factors **(326, 339, 352** and **356)** containing different combinations of mycolic acid classes.



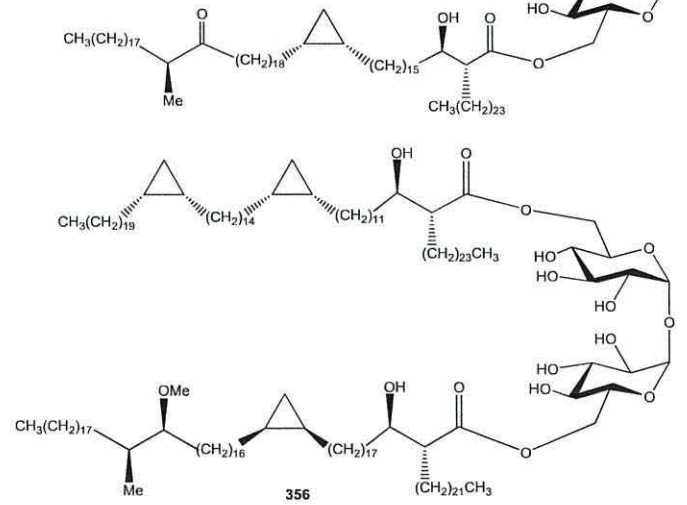
326



352



339



356

The final part of this research examined the possible application of the synthetic sugar esters as antigens in detecting TB. ELISA assays were carried out to look for antibodies responding to these compounds in both bovine and human serum samples.

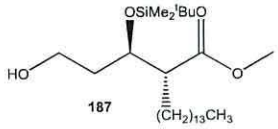
The bovine serum samples consisted of 20 naturally infected and 16 non-vaccinated (assumed to be negative) samples from the Veterinary Laboratories Agency (VLA) and the assay for these was run using the methoxy TDM **(319)** and TMM **(321)**. Neither antigen showed a good distinction between the two sets, however the TMM had a sensitivity similer to that observed for natural bovine TDM (63%). The specificity was however lower at only 38% (compared to 56% for the natural mixture). The results for the methoxy antigens were also interesting as the absorbance values observed for the TDM **(319)** with the majority of the serum samples were very low < 0.30, and also in the TDM/TMM pairs, *i.e.* α- an keto-, the TDM performs as well as or better than the TMM.

Human serum samples were also tested using the methoxy TDM **(319)** and TMM **(321)** and also the mixed cord factor **(339)** as antigens. A sub-set of samples from the WHO, which consisted of 64 samples from Gambia, were used and all 3 antigens showed a relatively good distinction between the TB^+ and TB^- samples. A sensitivity of 78% was obtained with all 3 antigens; this was slightly lower than was observed with the best synthetic antigen **(366)**, which gave a sensitivity of 89% under the same conditions. The specificities for the antigens varied and were 75%, 65% and 33% for **(319)**, **(339)** and **(321)** respectively. The value of 75% for **(319)** was comparable to that observed for the best synthetic antigen **(366)**, *i.e.* 78%. Although the mixed cord factor **339** (which contained the same MeO-mycolic acid as the TMM **(321)** and TDM **(319)** at one position and the same keto-MA as the best synthetic antigen **(366)** at the other position) performed better than the TMM **(321)**, it did not perform as well as the TDMs **(319)** and **(366)**. These initial results suggest that the assay is not improved by having TDMs with different MAs at the two positions. A larger sample set will however need to be tested with this type of antigen and also a wider range of antigens of this type need to be tested in order to determine whether they can lead to an improvement in the assay.

4. Experimental Section

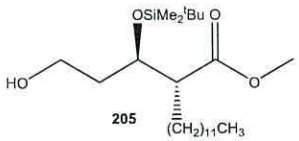
Starting materials and reagents used were purchased from Alfa Aesar, Aldrich chemicals Co and Avocado Chemical Co. Solvent. The solvents which had to be dry, e.g., ether and tetrahydrofuran were dried over sodium wire in the Chemistry laboratory, while dichloromethane and diisopropylamine were dried over calcium hydride. Organic solutions were dried over anhydrous magnesium sulfate. Silica gel (Merck 7736) and silica gel plates (Merck 7736) used for column chromatography and thin layer chromatography were obtained from Aldrich. Separated components were detected using variously UV light, I_2 and phosphomolybdic acid solution in IMS followed by charring. Triethylamine (0.1 mL) was added to silica gel (100 gm) in column chromatography to make the products stable. Solvents were removed via vacuum evaporation at 14 mmHg. Petrol was of boiling point 40-60 ^{o}C. Inert reaction conditions were carried out under a balloon of nitrogen gas. All glassware used for an anhydrous reaction was dried at 250 ^{o}C. Reactions carried out at low temperatures were cooled using a bath of IMS and liquid nitrogen. NMR spectra were recorded on a Bruker Avance 500 MHZ spectrometer or on a Bruker Advance 400 MHz spectrometer in $CDCl_3$, if not indicated differently. All chemical shifts are quoted in δ relative to the trace resonance of $CDCl_3$ (δ 7.27 ppm) for proton NMR, and (δ 77.00 ppm) for carbon NMR. Infra- red spectra were carried out on a Perkin- Elemer 1600 series FTIR spectrometer as liquid films. Data were reported as follows: chemical shift, integration, multiplicity (br, broad; s, singlet; d, doublet; t, triplet; q, quartet; pent, pentet; sext, sextet; hept, heptet, m, multiple), coupling constant. Optical rotations were recorded in $CHCl_3$ on a POLAAR 2001 Optical Activity polarimeter. Matrix Assisted Laser Desorption Ionisation (MALDI) mass spectra were obtained using a Bruker Daltonics Reflex instrument.

Experiment 1: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-propyl)hexadecanoate (187)



Lithium bis(trimethylsilyl)amide (15.1 mL, 15.9 mmol, 1.06 M) was added to a stirred solution of **(177)** (3.0 g, 7.6 mmol) and **(178)** (4.00 g, 10.6 mmol) in dry THF (50 mL) at -10 °C. The reaction turned bright yellow and was left to reach r.t. and stirred for 1 h under nitrogen atmosphere, then quenched by addition of sat. aq. NH_4Cl. The product was extracted with petrol/ethyl acetate (2:1, 3 × 50 mL), dried over $MgSO_4$, filtered and evaporated. The product was purified by column chromatography over silica gel. eluting with petrol/ethyl acetate (10:1) gave a colourless oil of *(E/Z)* as a mixture in ratio 2:1 of compound **(179)** (3.4 g, 85%). Palladium 10% on carbon (1.0 g) was added to a stirred solution of the alkene mixture **(179)** (3.4 g, 6.2 mmol) in IMS (20 mL) and THF (20 mL) under hydrogen atmosphere. Hydrogenation was carried out for one day. The mixture was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, the title compound **(187)** (2.0 g, 83%), $[\alpha]_D^{20}$ = -2.1 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 481.4; $C_{26}H_{54}NaO_4Si$ requires: 481.3}, which showed δ_H (400 MHz, $CDCl_3$): 4.24 (1H, dt, *J* 4.1, 6.6 Hz), 3.85-3.75 (2H, m), 3.67 (3H, s), 2.64 (1H, ddd, *J* 3.7, 6.8, 10.7 Hz), 1.86-1.71 (2H, m), 1.65-1.50 (2H, m), 1.27 (25H, br.s), 0.97 (9H, s), 0.91 (3H, t, *J* 6.9 Hz), 0.11 (3H, s), 0.07 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.7, 72.1, 60.4, 59.5, 51.5, 51.4, 35.1, 31.9, 29.7, 29.65, 29.6, 29.4, 29.3, 28.9, 27.8, 27.2, 25.8, 22.7, 22.3, 21.0, 17.8, 14.2, 14.1, 14.0, -4.5, -5.0; ν_{max}: 3437, 2927, 2859, 1739, 1480, 1258, 1174, 1093, 838 cm^{-1}.

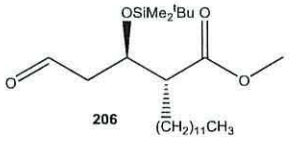
Experimental 2: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-propyl)tetradecanoate (205)



Lithium bis(trimethylsilyl)amide (15.1 mL, 15.9 mmol, 1.06 M) was added to a stirred solution of **(177)** (3.0 g, 7.6 mmol) and **(203)** (4.10 g, 11.3 mmol) in dry THF (50 mL) at -10 °C under nitrogen atmosphere. The reaction turned bright yellow, was left to

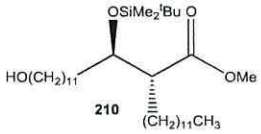
reach r.t., stirred for 1 h, then worked up and purified as above to give a colourless oil as an *E/Z*-mixture in ratio 2:1 of **(204)** (3.3 g, 90%). Palladium 10% on carbon (1.0 g) was added to a stirred solution of the alkene (3.3 g, 6.2 mmol) in IMS (20 mL) and THF (20 mL) under hydrogen atmosphere. Hydrogenation was carried out for one day. The reaction mixture was worked up and purified as before to give a colourless oil of the title compound **(205)** (2.3 g, 85%), $[\alpha]_D^{20}$ = -1.7 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 453.4 $C_{24}H_{50}NaO_4Si$ requires: 453.3}, which showed δ_H (400 MHz, $CDCl_3$): 4.22 (1H, dt, *J* 4.1, 6.6 Hz), 3.79-3.72 (2H, m), 3.67 (3H, s), 2.64 (1H, ddd, *J* 3.7, 6.8, 10.7 Hz), 1.80-1.74 (2H, m), 1.67-1.44 (2H, m), 1.29 (21H, br.s), 0.98 (9H, s), 0.92 (3H, t, *J* 6.9 Hz), 0.11 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.7, 72.1, 60.4, 59.5, 51.6, 51.4, 35.1, 31.9, 29.64, 29.6, 29.55, 29.5, 29.4, 29.3, 27.8, 27.1, 25.7, 22.7, 21.0, 17.8, 14.17, 14.1, -4.5, -5.0; ν_{max}: 3438, 2930, 2860, 1739, 1470, 1258, 1174, 1098, 839 cm^{-1}.

Experiment 3: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-3-oxopropyl)-tetradecanoate (206)



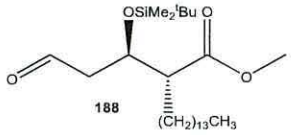
(205) (2.21 g, 5.54 mmol) in dichloromethane (10 mL) was added to a stirred suspension of PCC (2.98 g, 13.8 mmol) in dichloromethane (70 mL). During the addition a black colour appeared and the reaction mixture was stirred for 2.5 h at r.t., then petrol/ethyl acetate (10:1, 100 mL) was added. The mixture was filtered through a bed of silica gel and washed with petrol/ethyl acetate (3 × 30 mL). The combined filtrates were evaporated and the product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil **(206)** (1.8 g, 85%), $[\alpha]_D^{20}$ = -6.1 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 451.2, $C_{24}H_{48}NaO_4Si$ requires: 451.3}, which showed δ_H (400 MHz, $CDCl_3$): 9.80 (1H, dd, *J* 1.8, 2.4 Hz), 4.43 (1H, dt, *J* 6.0, 10.8 Hz), 3.68 (3H, s), 2.67 (2H, dd, *J* 1.64, 4.7 Hz), 2.62-2.59 (2H, m), 1.68-1.38 (1H, m), 1.29 (20H, br.s), 0.9 (3H, t, *J* 6.6 Hz), 0.86 (9H, s), 0.07 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 201.2, 174.0, 68.8, 52.2, 51.5, 48.0, 31.9, 29.63, 29.61, 29.6, 29.5, 29.4, 29.37, 29.3, 27.7, 27.0, 25.6, 22.6, 21.0, 17.8, 14.1, 14.0, -4.6, -4.9; ν_{max}: 2928, 2859, 1740, 1461, 1366, 1259, 1199, 1005, 837, 777 cm^{-1}.

Experiment 4: Methyl (2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-dodecyl-14-hydroxytetradecanoate (210)



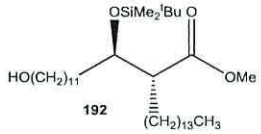
Lithium bis(trimethylsilyl)amide (8.4 mL, 8.8 mmol, 1.06 M) was added to a stirred solution of aldehyde **(206)** (1.80 g, 4.55 mmol) and **(207)** (2.58 g, 5.91 mmol) in dry THF (55 mL) at -10 °C under nitrogen atmosphere. The reaction turned bright yellow, was left to reach r.t., stirred for 1 h, then the reaction was quenched with sat. aq. NH_4Cl (50 mL). The product was extracted with petrol/ethyl acetate (5:1, 2 × 50 mL), dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give as a colourless oil as an (*E/Z)*-mixture in ratio 2:1 of **(208)** (2.6 g, 89%). Palladium 10% on carbon (0.5 g) was added to a stirred solution of the alkenes (2.6 g, 4.1 mmol) in IMS (30 mL) and THF (30 mL) under hydrogen atmosphere. Hydrogenation was carried out for 1 h. The reaction mixture was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil **(209)** (2.3 g, 88%). This (2.3, 3.6 mmol) was added to a stirred solution of potassium hydroxide (3.23 g, 57.6 mmol) dissolved in a mixture of THF:MeOH:H_2O (50:50:5, 105 mL). The mixture was refluxed at 70 °C and monitored by TLC. After 3 h, the mixture was quenched with water and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a semi-solid, the title compound **(210)** (1.4 g, 70%), $[\alpha]_D^{20}$ = -3.0 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 579.6, $C_{33}H_{68}NaO_4Si$ requires: 579.4}, which showed δ_H (400 MHz, $CDCl_3$): 3.91-3.88 (1H, m), 3.75 (2H, t, *J* 6.6 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz), 1.56 (2H, q, *J* 6.6 Hz), 1.25 (41H, s), 0.96 (9H, s), 0.91 (3H, t, *J* 6.9 Hz), 0.04 (3H, s), 0.02 (3H, s); δ_C (400 MHz, $CDCl_3$): 175.1, 73.2, 67.9, 63.0, 51.5, 51.2, 33.6, 32.7, 31.9, 29.8, 29.64, 29.6, 29.59, 29.5, 29.4, 29.3, 27.8, 27.5, 27.0, 25.8, 25.74, 25.7, 25.6, 23.6, 22.6, 17.9, 14.1, -4.3, -4.9; ν_{max}: 3400, 2930, 2859, 1742, 1644, 1466, 1254, 1180, 1074, 839 cm^{-1}.

Experiment 5: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-3-oxopropyl)-hexadecanoate (188)



(187) (2.4 g, 5.9 mmol) in dichloromethane (10 mL) was added to a stirred suspension of PCC (3.17 g, 14.7 mmol) in dichloromethane (70 mL). During the addition a black colour appeared; the mixture was stirred for 2.5 h at r.t., then worked up and purified as before to give the title compound **(188)** as a colourless oil (2.0 g, 86%), $[\alpha]_D^{20} = -6.3$ (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 456.4, $C_{26}H_{52}NaO_4Si$ requires: 456.3}, which showed δ_H (400 MHz, $CDCl_3$): 9.80 (1H, dd, *J* 2.4, 4.1 Hz), 4.43 (1H, dt, *J* 6.0, 10.8 Hz), 3.68 (3H, s), 2.66 (1H, ddd, *J* 1.5, 4.6, 6.3 Hz), 2.61 (1H, ddd, *J* 2.8, 6.3, 8.9 Hz), 2.62-2.59 (1H, m), 1.64-1.48 (2H, m), 1.25 (24H, br.s), 0.9 (3H, t, *J* 6.6 Hz), 0.86 (9H, s), 0.07 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 201.2, 174.0, 68.8, 52.2, 51.5, 48.0, 31.9, 29.67, 29.64, 29.6, 29.5, 29.4, 29.38, 29.3, 27.7, 27.0, 25.6, 22.6, 17.8, 14.1, -4.6, -4.9; ν_{max}: 2927, 2859, 1735, 1464, 1255, 1196, 1097, 1015, 837 cm^{-1}.

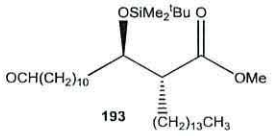
Experiment 6: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-12-hydroxy-dodecyl)hexadecanoate (192)



Lithium bis(trimethyl silyl)amide (10.1 mL, 10.7 mmol, 1.06 M) was added to a stirred solution of aldehyde **(188)** (2.12 g, 5.18 mmol) and **(189)** (2.58 g, 5.91 mmol) in dry THF (55 mL) at -10 °C under nitrogen atmosphere. The reaction turned bright yellow and allowed to reach r.t., after stirring for 1 h, the reaction mixture was worked up as before to give as a colourless oil of (*E*/*Z*) as a mixture in ratio 2:1 **(190)** (2.9 g, 85%). Palladium (10% on carbon, 0.5 g) was added to a stirred solution of the alkene **(190)** (2.9 g, 4.4 mmol) in IMS (30 mL) and THF (30 mL) under hydrogen atmosphere. Hydrogenation was carried out for 1 h, then worked up and purified as before to give a colourless oil **(191)** (2.51 g, 88%). **(191)** (2.3, 3.6 mmol) was added to a stirred solution of potassium hydroxide (3.23 g, 57.5 mmol) in a mixture of THF:MeOH:H_2O (50:50:5, 105 mL). The mixture was refluxed at 70 °C. After 3 h, the reaction mixture was worked up and purified as before to give a semi-solid of the title

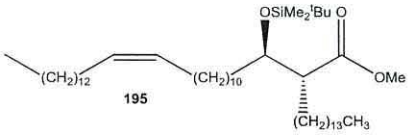
compound **(192)** (1.2 g, 60%), $[\alpha]_D^{20}$ = -3.1 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 607.6, $C_{35}H_{72}NaO_4Si$ requires: 607.5}, which showed δ_H (400 MHz, $CDCl_3$): 3.91(1H, dd, *J* 4.8, 11.5 Hz), 3.71-3.6 (2H, m), 3.5 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz), 1.53-1.25 (47H, v.br.m), 0.93 (9H, s), 0.87 (3H, t, *J* 7.0 Hz), 0.04 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.1, 73.2, 63.0, 51.5, 51.2, 33.6, 32.8, 31.9, 29.8, 29.7, 29.66, 29.65, 29.63, 29.6, 29.5, 29.43, 29.4, 29.3, 27.8, 27.4, 27.0, 25.8, 25.75, 25.7, 23.7, 22.6, 17.9, 14.1, -4.3, -4.9; ν_{max}: 3445, 2926, 2857, 1740, 1644, 1466, 1435, 1254, 1084, 837 cm^{-1}.

Experiment 7: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-12-oxo-dodecyl-)hexadecanoate (193)



(192) (1.6 g, 2.8 mmol) in dichloromethane (10 mL) was added at r.t. to a stirred suspension solution of PCC (1.5 g, 6.9 mmol) in dichloromethane (50 mL). During the addition a black colour appeared. The reaction was stirred at r.t. for 2 h. The reaction mixture was worked up and purified as before to give a colourless oil of the title compound **(193)** (1.3 g, 86%), $[\alpha]_D^{20}$ = -8.1 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 605.5, $C_{35}H_{70}NaO_4Si$ requires: 605.4}, which showed δ_H (400 MHz, $CDCl_3$): 9.77 (1H, t, *J* 1.8 Hz), 3.98-3.85 (1H, m), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.7, 7.3, 10.8 Hz), 2.45 (1H, dt, *J* 4.7, 8.3 Hz), 2.43 (1H, td, *J* 1.8, 7.4 Hz), 1.63-1.17 (44H, m), 0.99 (3H, t, *J* 7.1 Hz), 0.84 (9H, s), 0.054 (3H, s), 0.03 (3H, s); δ_C (101 MHz, $CDCl_3$): 202.9, 174.6, 73.2, 51.5, 51.2, 43.9, 33.6, 31.9, 29.8, 29.6, 29.57, 29.54, 29.5, 29.44, 29.4, 29.3, 29.1, 27.8, 27.4, 25.76, 23.7, 22.6, 22.0, 14.1, -4.3, -4.9; ν_{max}: 2925, 2854, 1745, 1465, 1372, 1249, 1048, 836, 785 cm^{-1}.

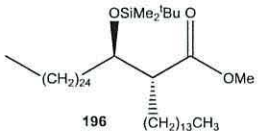
Experiment 8: Methyl (2*R*,3*R*,*Z*)-3-((*tert*-butyldimethylsilyl)oxy)-2-tetra-decyloctacos-14-enoate (195)



Sodium bis(trimethylsilyl)amide (3.6 mL, 3.8 mmol, 1.06 M in THF) was added to a stirred solution of **(194)** (1.0 g, 1.8 mmol) in dry THF (50 mL) at -78 °C under

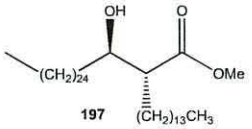
nitrogen atmosphere. The mixture was allowed to reach r.t., stirred for 30 min., cooled again to -78 °C and **(193)** (0.5 g, 0.8 mmol) in dry THF (6 mL) was added. The mixture was allowed to reach r.t. and stirred for 10 h. The reaction was quenched with sat. aq. NH_4Cl (10 mL) and the product was extracted with petrol/ethyl acetate (20:1, 4 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated. Then residue was treated with petrol/ether (1:1, 100 mL) and refluxed for 30 min. The precipitate was filtrate on celite and the filtrate was evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (40:1) then (20:1) to give the title compound **(195)** (0.4 g, 66%), $[\alpha]_D^{20}$ = -6.2 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 785.4, $C_{49}H_{98}NaO_3Si$ requires: 785.7}, which showed δ_H (400 MHz, $CDCl_3$: 5.38 (2H, t, *J* 10.8 Hz), 3.99-3.84 (1H, m), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz) , 2.13-1.94 (2H, m), 1.27 (68H, br.s), 0.88 (6H, t, *J* 6.9 Hz), 0.84 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (400 MHz, $CDCl_3$): 175.1, 129.9, 129.8, 73.2, 59.9, 51.5, 51.2, 33.6, 31.9, 29.8, 29.79, 29.77, 29.7, 29.65, 29.6, 29.5, 29.4, 29.36, 29.33, 29.32, 27.8, 27.4, 27.2, 25.7, 23.6, 22.6, 17.9, 14.1, -4.3, -4.9; ν_{max}: 2923, 2852, 1745, 1470, 1439, 1361, 1179, 836, 775, 730 cm^{-1}.

Experiment 9: Methyl (2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-tetradecyl-octacosanoate (196)



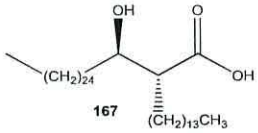
Palladium (10% on carbon, 0.2 g) was added to a stirred solution of the alkene **(195)** (0.4 g, 0.5 mmol) in IMS (10 mL) and THF (10 mL) under hydrogen atmosphere. Hydrogenation was carried out for 1 h. The reaction mixture was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compound **(196)** (0.4 g, 88%), $[\alpha]_D^{20}$ = -10 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 787.4, $C_{49}H_{100}NaO_3Si$ requires: 787.7}, which showed δ_H (400 MHz, $CDCl_3$: 3.91 (1H, dd, *J* 4.7, 11.6 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz), 1.62-1.13 (74H, m), 0.94 (6H, t, *J* 6.8 Hz), 0.90 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.1, 73.2, 51.5, 51.2, 33.6, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.8, 27.5, 25.7, 23.6, 22.6, 17.9, 14.1, -4.3, -4.9; ν_{max}: 2929, 2842, 1741, 1468, 1180, 1120, 846, 775 cm^{-1}.

Experiment 10: Methyl (2*R*,3*R*)-3-hydroxy-2-tetradecyloctacosanoate (197)



(196) (0.41 g, 0.43 mmol) was stirred in dry THF (12 mL) in a dry polyethylene vial under a nitrogen atmosphere at r.t. then pyridine (0.1 mL), followed by HF-pyridine (1.0 mL) were added and the mixture was stirred for 18 h at 45 °C. The reaction was poured slowly to a sat aq. of $NaHCO_3$ and extracted with petrol/ethyl acetate (1:1, 10 mL). The mixture was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 4 × 20 mL). The combined organic layers were washed with brine, dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound **(197)** (0.3 g, 76%), $[\alpha]_D^{20}$ = +10 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 673.4, $C_{43}H_{86}NaO_3$ requires: 673.6}, which showed δ_H (400 MHz, $CDCl_3$): 3.76 (1H, t, *J* 6.6 Hz), 3.72 (3H, s), 2.51-2.36 (1H, m), 1.79-1.39 (2H, m), 1.26 (73H, br.s), 0.89 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, CDCl3): 176.4, 77.3, 51.5, 50.9, 35.7, 31.9, 29.7, 29.65, 29.5, 29.49, 29.4, 29.3, 27.4, 25.7, 22.6, 14.1; ν_{max}: 3454, 2929, 2851, 1720, 1645, 1464, 1177, 1052, 721 cm^{-1}.

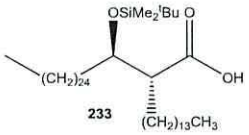
Experiment 11: (2*R*,3*R*)-3-Hydroxy-2-tetradecyloctacosanoic acid (167)



Lithium hydrogen monohydrate (0.3 g, 6.7 mmol) was added at r.t. to a stirred solution of methyl **(197)** (0.31 g, 0.43 mmol) in THF (12 mL), water (2 mL), MeOH (1 mL). The mixture was stirred for 18 h at 45 °C then dissolved in warmed petrol/ethyl acetate 5:1 (50 mL) and acidified with 5% HCl until pH 1-2. The organic layers were separated and the aq. layer then re-extracted with (3 × 50 mL). The combined organic layers were dried, evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:2) to give the title compound **(167)** (0.27 g, 93%), $[\alpha]_D^{20}$ = +12 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 659.4, $C_{42}H_{84}NaO_3$ requires: 659.6}, which showed δ_H (400 MHz, $CDCl_3$): 3.84-3.66 (1H, m), 3.62-3.60 (1H, m), 2.53-2.43 (1H, m), 1.57-1.45 (2H, m), 1.42-1.38 (2H, m), 1.45-1.14 (71H, m), 0.89 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 177.9, 72.1, 60.4, 50.6,

35.5, 31.9, 29.7, 29.6, 29.5, 29.49, 29.4, 29.3, 27.3, 25.7, 22.6, 21.0, 14.1; ν_{max}: 3530, 2928, 2882, 1687, 1472, 1385, 1206, 965, 720 cm^{-1}.

Experiment 12: (2*R*,3*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-tetradecyloctacosanic acid (233)



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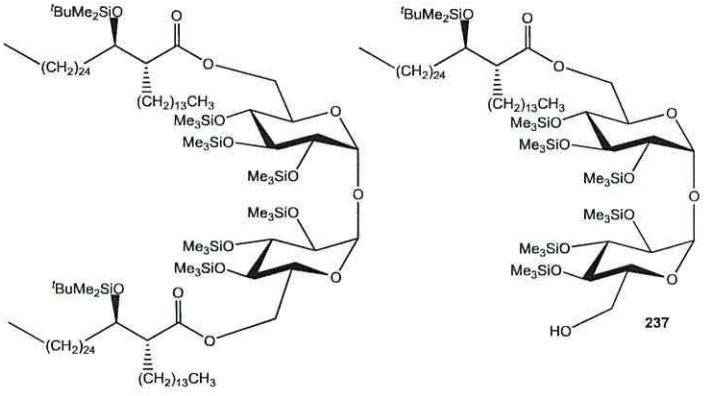
Imidazole (0.26 g, 3.82 mmol) was added to a stirred solution of **(167)** (0.27 g, 0.38 mmol) in dry DMF (2 mL) and dry toluene (3 mL) at r.t. followed by the addition of *tert*-butyldimethylsilylchloride (0.6 g, 3.9 mmol) and 4-DMAP (0.05 g, 0.41 mmol). The reaction mixture was stirred at 70 °C for 18 h. The solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate 5:1 (100 mL) and water (10 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (3 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue **(232)**. The residue **(232)** was dissolved in THF (6 mL), water (2 mL), and methanol (1 mL), and to this was added potassium carbonate (0.2 g, 1.4 mmol). The reaction mixture was stirred at 45 °C for 3 h. The mixture was diluted with petrol/ethyl acetate 10:1, (40 mL) and water (3 mL) then acidified with potassium hydrogen sulphate to pH 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 4 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate 10:1 to give the title compound **(233)** (0.2 g, 86%), $[\alpha]_D^{20}$ = +8.2 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 773.4, $C_{48}H_{98}NaO_3Si$ requires: 773.7}, which showed δ_H (400 MHz, $CDCl_3$): 3.90-3.80 (1H, m), 2.53 (1H, ddd, *J* 3.3, 5.7, 9.2 Hz), 1.70 (2H, dt, *J* 7.1, 10.4 Hz), 1.53-1.49 (2H, m), 1.26 (71H, br.s), 0.92 (9H, s), 0.90-0.79 (6H, t, *J* 7.1 Hz), 0.14 (3H, s), 0.13 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.5, 73.6, 50.1, 35.6, 31.9, 29.8, 29.7, 29.65, 29.6, 29.5, 29.46, 29.4, 29.39, 29.3, 27.4, 25.7, 25.0, 22.6, 17.9, 14.1, -4.2, -4.8; ν_{max}: 3435, 2927, 2858, 1700, 1638, 1463, 1070, 886, 775 cm^{-1}.

Experiment 13: ((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-Oxybis(3,4,5tris((trimethylsilyl)-oxy)tetrahydro-2*H*-pyran-6,2-diyl))bis(methylene)(2*R*,2'*R*,3*R*,3'*R*)-bis(3-((*tert*-butyldimethylsilyl)oxy)-2-tetradecyloctacosanoate (236) and ((2*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4*S*,6*R*)-6-(hydroxymethyl)-3,4,5tris((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)-3,4,5-tris((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl(2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-tetradecylocosanoate (237)



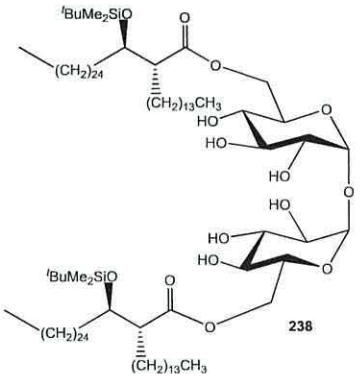
236 237

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.3 g, 1.4 mmol) and 4-DMAP (0.1 g, 1.2 mmol) were added to a stirred solution of **(233)** (0.22 g, 0.29 mmol) and **(160)** (0.14, 0.18 mmol) and powdered 4 Å molecular sieves in dry dichloromethane (4 mL) at r.t. under a nitrogen atmosphere. The mixture was stirred for 6 days at r.t., then diluted with dichloromethane (5 mL) and silica gel (1 g) was added. The mixture was evaporated under reduced pressure to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the first fraction **(236)** as a colourless thick oil (0.1 g, 28%), $[\alpha]_D^{22}$ = +80 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2263.7, $C_{126}H_{262}NaO_{15}Si_8$ requires: 2262.7}, which showed δ_H (400 MHz, $CDCl_3$): 4.85 (2H, d, *J* 3.0 Hz), 4.67 (1H, br.dd, *J* 2.4, 11.0 Hz), 4.53-4.40 (1H, m), 4.08-3.95 (4H, m), 3.88-3.84 (4H, m), 3.56-3.48 (2H, m), 3.38 (2H, dt, *J* 3.0, 9.3 Hz), 2.57-2.53 (2H, m), 1.64-1.30 (6H, m), 1.26 (142H, br.s), 0.9 (18H, s), 0.88-0.85 (12H, t, *J* 7.0 Hz), 0.16 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.06 (3H, s), 0.05 (3H, s), 0.04 (3H, s), 0.03 (3H, s); δ_C (101 MHz, $CDCl_3$): 173.8, 94.8, 73.5, 73.4, 72.8, 71.8, 70.7, 62.3, 51.8, 33.4, 31.9, 29.85, 29.8, 29.7, 29.68, 29.6, 29.5, 29.3, 28.1, 26.2, 25.9, 25.8, 25.1, 22.6, 18.0, 14.1, 1.0, 0.98, 0.94, 0.1, -4.2, -4.4, -4.5, -4.6; ν_{max}: 2926, 2857, 1750, 1607, 1493, 1413, 1251, 1076, 1080, 686 cm^{-1}.

The second fraction was the **(237)** as a colourless thick oil (0.1 g, 37%), $[\alpha]_D^{22}$ = + 45 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1530.4, $C_{78}H_{166}NaO_{13}Si_7$ requires:

1530.1}, which showed δ_H (400 MHz, $CDCl_3$): 4.92 (1H, d, *J* 3.1 Hz), 4.85 (1H, d, *J* 3.1 Hz), 4.35 (1H, dd, *J* 2.1, 11.8 Hz), 4.08 (1H, dd, *J* 2.1, 11.8 Hz), 4.02-3.81 (2H, m), 3.76-3.63 (4H, m), 3.56-3.46 (2H, m), 3.39-3.33 (2H, m), 2.56 (1H, ddd, *J* 3.2, 5.3, 9.2 Hz), 1.72 (1H, dd, *J* 5.5, 7.3 Hz), 1.68-1.54 (2H, m), 1.53-1.36 (4H, m), 1.26 (69H, br.s), 0.91 (9H, s), 0.89-0.85 (6H, t, *J* 7.3 Hz), 0.17 (18H, s), 0.16 (18H, s), 0.15 (18H, s), 0.06 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.0, 94.5, 94.3, 73.4, 73.3, 72.87, 72.8, 72.7, 71.9, 71.4, 70.7, 62.4, 61.6, 51.8, 33.4, 31.9, 29.8, 29.78, 29.7, 29.6, 29.5, 29.3, 28.1, 26.3, 25.8, 24.8, 22.6, 18.0, 14.1, 1.05, 1.0, 0.9, 0.8, 0.1, 0.04, -4.4, -4.6; ν_{max}: 3610, 2935, 2855, 1745, 1493, 1607, 1493, 1076, 1050, 1016, 874, 686 cm^{-1}.

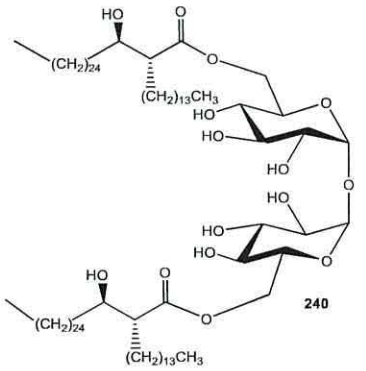
Experiment 14: ((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-(Oxybis(3,4,5-trihydroxy tetra-hydro-2*H*-pyran-6,2-diyl))bis(methylene)(2*R*,2'*R*,3*R*,3'*R*)-bis(3-((*tert*-butyldimethylsilyl)oxy)-2-tetradecyloctacosanoate) (238)



Tetrabutylammonium fluoride (0.2 mL, 0.2 mmol, 1.0 M) was added to a stirred solution of TDM **(236)** (0.16 g, 0.07 mmol) in dry THF (15 mL) at 5 °C under a nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 1 h, then evaporated to give a residue, which was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(238)** as a colourless thick oil (0.1 g, 83%), $[\alpha]_D^{22}$ = +45 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1831.3, $C_{108}H_{214}NaO_{15}Si_2$ requires: 1830.5}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.06 (2H, d, *J* 3.6 Hz), 4.67-4.60 (2H, br.dd, *J* 2.2, 11.8 Hz), 4.33 (2H, dd, *J* 2.3, 12.2 Hz), 4.21 (2H, dd, *J* 2.5, 11.9 Hz), 3.94-3.85 (4H, m), 3.76 (2H, m), 3.46 (2H, dt, *J* 2.6, 9.7 Hz), 2.56-2.49 (2H, m), 1.91-1.77 (2H, m), 1.53-1.41 (4H, m), 1.21 (148H, v.br.s), 0.90 (12H, t, *J* 6.9 Hz), 0.87 (18H, s), 0.01 (6H, s), -0.01 (6H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.2, 93.4, 73.2, 73.0, 71.6, 70.2, 69.9,

62.8, 51.5, 33.5, 31.8, 29.7, 29.64, 29.6, 29.5, 29.2, 27.7, 26.9, 25.7, 25.6, 24.1, 22.5, 17.8, 13.9, -4.5, -4.9; ν_{max}: 3378, 2927, 2850, 1745, 1467, 1076, 846 cm^{-1}.

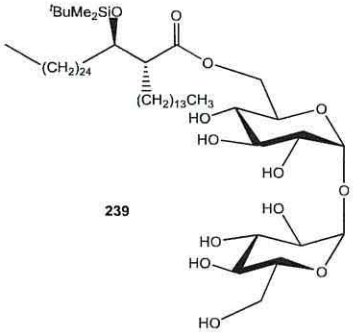
Experiment 15: ((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-Oxybis(3,4,5-trihydroxy tetrahydro-2*H*-pyran-6,2-diyl))bis(methylene)(2*R*,2'*R*,3*R*,3'*R*)-bis(3-hydroxy-2-tetradecyloctacosanoate) (240)



A dry polyethylene vial equipped with an acid proof rubber septum was charged with **(238)** (0.09 g, 0.05 mmol) and pyridine (0.1 mL) in dry THF (10 mL) and stirred at r.t. under nitrogen. HF-pyridine (0.8 mL) was added at 5 °C. The mixture was stirred at 43 °C for 17 h, then neutralised by slowly pouring the mixture into sat. aq. sodium hydrogen carbonate (10 mL) until no more carbon dioxide was liberated. The product was extract with $CHCl_3$ (3 × 25 mL), dried and evaporated to give a white solid. This was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(240)** (0.04 g, 51%), $[\alpha]_D^{22}$ = +32 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1602.9, $C_{96}H_{186}O_{15}Na$ requires: 1602.3}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.98 (2H, d, *J* 3.4 Hz), 4.67 (2H, br.d, *J* 10.6 Hz), 4.22 (2H, t, *J* 8.8 Hz), 3.98-3.88 (2H, m), 3.76 (2H, t, *J* 9.3 Hz), 3.65-3.61 (2H, m), 3.45 (2H, dd, *J* 2.8, 9.7 Hz), 3.2 (2H, t, *J* 8.1 Hz), 2.37 (2H, td, *J* 4.8, 9.6 Hz), 1.55-1.45 (4H, m), 1.27 (152H, v.br.s), 0.84 (12H, t, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.4, 94.9, 72.5, 72.3, 71.2, 71.1, 69.8, 64.4, 52.1, 34.6, 31.8, 29.6, 29.59, 29.5, 29.4, 29.3, 29.2, 27.1, 25.0, 22.5, 13.9; ν_{max}: 3382, 2920, 2850, 1726, 1476, 1376, 1078 cm^{-1}.

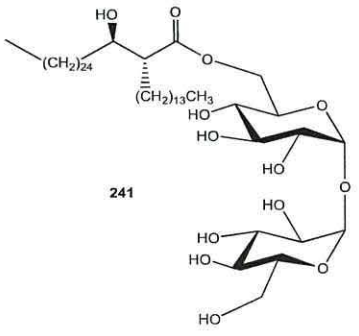
Experiment 16: ((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl(2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-tetradecyloctacosanoate

(239)



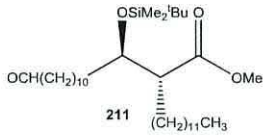
Tetrabutylammonium fluoride (0.3 mL, 0.3 mmol, 1.0 M) was added to a stirred solution of TMM **(237)** (0.14 g, 0.09 mmol) in dry THF (15 mL) at 5 °C under a nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 3 h, then evaporated to give a residue, which was purified by column chromatography eluting with $CHCl_3$/MeOH (5:1) to give the title compound **(239)** (0.08, 80%), $[\alpha]_D^{19}$ = +10 (c 0.50, $CHCl_3$) {Found $[M+Na]^+$: 1097.8229, $C_{60}H_{118}NaO_{13}Si$ required 1097.8239}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.04 (1H, d, *J* 3.3 Hz), 4.46 (1H, d, *J* 1.2 Hz), 4.24 (1H, dd, *J* 3.5, 11.0 Hz), 4.11 (1H, br.d, *J* 11.2 Hz), 3.95-3.73 (1H, m), 3.72-3.59 (2H, t, *J* 6.6 Hz), 3.47 (1H, d, *J* 9.8 Hz), 3.36-3.26 (1H, d, *J* 9.0 Hz), 3.20 (6H, t, *J* 6.6 Hz), 2.54-2.45 (1H, m), 1.97 (1H, d, *J* 6.0 Hz), 1.81 (6H, t, *J* 4.7 Hz), 1.31-0.94 (74H, m), 0.87 (6H, t, *J* 5.7 Hz), 0.83 (9H, s), -0.01 (3H, s), -0.03 (3H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.0, 93.5, 93.4, 73.1, 72.9, 72.6, 72.1, 71.5, 70.6, 70.1, 69.9, 62.6, 61.9, 51.6, 33.4, 31.7, 29.6, 29.57, 29.55, 29.5, 29.2, 27.6, 26.8, 25.5, 24.1, 22.5, 17.8, 13.9, -4.6, -5.0; ν_{max}: 3388, 2926, 2844, 1751, 1468, 1085, 840 cm^{-1}.

Experiment 17: ((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl(2*R*,3*R*)-3-hydroxy-2-tetradecyloctacosanoate (241)



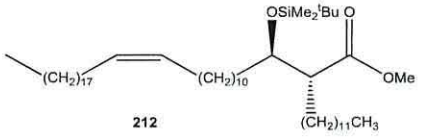
A dry polyethylene vial equipped with an acid proof rubber septum was charged with **(239)** (0.08 g, 0.07 mmol) and pyridine (0.1 mL) in dry THF (10 mL) and stirred at r.t. under nitrogen. HF-pyridine (0.8 mL) at 5 °C was added. The mixture was stirred at 43 °C for 17 h, then neutralized by triethyl amine drop wise until pH 5. The product was evaporated to give a residue which was purified by chromatography eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(241)** as a syrup (0.035 g, 50%), $[\alpha]_D^{21}$ = +31 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 983.6, $C_{54}H_{104}NaO_{13}$ requires: 983.7}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.11 (1H, d, *J* 2.5 Hz), 5.06 (1H, d, *J* 2.5 Hz), 4.61 (1H, d, *J* 10.8 Hz), 4.14 (1H, br.t, *J* 7.4 Hz), 4.07 (1H, br.t, *J* 6.7 Hz), 3.91 (4H, br.pent., *J* 9.1 Hz), 3.68-3.66 (2H, m), 3.59 (1H, dd, *J* 2.1, 9.6 Hz), 3.54 (1H, dd, *J* 2.1, 9.5 Hz), 3.38-3.26 (2H, m), 2.40-2.37 (1H, m), 1.55 (1H, d, *J* 8.2 Hz), 1.48-1.33 (2H, m), 1.24 (79H, br.s), 0.86 (6H, t, *J* 6.6 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.4, 94.2, 72.8, 72.6, 72.3, 71.5, 71.4, 71.0, 70.2, 64.1, 62.2, 52.3, 34.7, 31.8, 29.67, 29.63, 29.6, 29.5, 29.38, 29.3, 29.2, 27.2, 25.1, 22.5, 13.9; ν_{max}: 3376, 2926, 2845, 1741, 1468, 1075, 849 cm^{-1}.

Experiment 18: Methyl (2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-dodecyl-14-oxotetradecanoate (211)



(210) (1.2 g, 2.2 mmol) in dichloromethane (10 mL) was added at r.t. to a stirred suspension solution of PCC (1.2 g, 5.5 mmol) in dichloromethane (50 mL). During the addition a black colour appeared. The reaction was stirred at r.t. for 2 h. The reaction mixture was worked up and purified as before to give a colourless oil of the title compound **(211)** (1.0 g, 90%), $[\alpha]_D^{22}$ = -7.2 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 577.2, $C_{33}H_{66}NaO_4Si$ requires: 577.4}, which showed δ_H (400 MHz, $CDCl_3$): 9.76 (1H, t, *J* 1.8 Hz), 3.90 (1H, dt, *J* 4.7, 7.0 Hz), 3.65 (3H, s), 2.52 (1H, ddd, *J* 3.7, 7.2, 10.9 Hz), 2.45(1H, dt, *J* 4.7, 8.3 Hz), 2.41 (1H, td, *J* 1.8, 7.4 Hz), 1.66-1.58 (2H, m), 1.56-1.12 (38H, m), 0.90 (3H, t, *J* 7.1 Hz), 0.84 (9H, s), 0.03 (3H, s), 0.01 (3H, s); δ_C (101 MHz, $CDCl_3$): 202.8, 175.1, 73.1, 60.3, 51.5, 51.1, 43.8, 33.5, 32.6, 31.8, 29.7, 29.63, 29.61, 29.54, 29.5, 29.46, 29.4, 29.35, 29.32, 29.1, 27.79, 27.4, 25.79, 25.71, 23.62, 22.6, 22.0, 21.0, 17.9, 14.1, 14.0, -4.4, -4.9; ν_{max}: 2925, 2843, 1745, 1465, 1372, 1249, 1168, 1048, 836, 765 cm^{-1}.

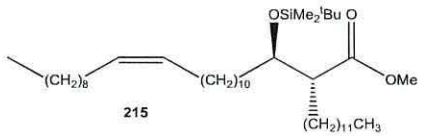
Experiment 19: Methyl (2*R*,3*R*,*Z*)-3-((*tert*-butyldimethylsilyl)oxy)-2-dodecyl-triacont-14-enoate (212)



212

Sodium bis(trimethylsilyl)amide (3.8 mL, 3.8 mmol, 1.0 M in THF) was added to a stirred solution of **(200)** (1.12 g, 1.83 mmol) in dry THF (50 mL) at -78 °C under nitrogen atmosphere. The mixture was allowed to reach r.t., stirred for 30 min., cooled again to -78 °C and **(211)** (0.5 g, 0.9 mmol) in dry THF (6 mL) was added. The mixture was allowed to reach r.t. and stirred for 10 h. The reaction was quenched with sat. aq. NH_4Cl (10 mL) and the product was extracted with petrol/ethyl acetate (20:1, 4 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated. Then residue was treated with added petrol/ether (1:1, 100 mL) and reflexed for 30 min. The precipitate was filtrate on celite and the filtrate was evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (40:1) then (20:1) to give the title compound **(212)** (0.4 g, 58%), $[\alpha]_D^{22}$ = -9.2 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 827.3, $C_{52}H_{104}NaO_3Si$ requires: 827.7}, which showed δ_H (400 MHz, $CDCl_3$: 5.38 (2H, t, *J* 10.8 Hz), 3.91 (1H, dd, *J* 4.8, 11.5 Hz), 3.61 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz), 2.07-1.98 (2H, m), 1.59-1.15 (74H, br.s), 0.93 (6H, t, *J* 6.9 Hz), 0.85 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.1, 129.9, 129.8, 73.2, 51.5, 51.2, 36.3, 34.1, 33.6, 32.6, 31.9, 29.8, 29.78, 29.7, 29.67, 29.66, 29.6, 29.5, 29.4, 29.35, 29.3, 29.1, 28.9, 28.8, 27.8, 27.5, 27.2, 25.8, 25.7, 23.6, 22.69, 22.6, 22.3, 17.9, 14.2, 14.1, 14.0, 8.8, -4.3, -4.9; ν_{max}: 2924, 2853, 1745, 1468, 1179, 1120, 836, 775, 720, 695 cm^{-1}.

Experiment 20: Methyl (2*R*,3*R*,*Z*)-3-((*tert*-butyldimethylsilyl)oxy)-2-dodecyl-tetracos-14-enoate (215)

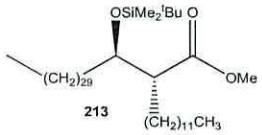


215

Sodium bis(trimethylsilyl)amide (3.8 mL, 3.8 mmol, 1.0 M in THF) was added to a stirred solution of **(202)** (0.8 g, 1.8 mmol) in dry THF (50 mL) at -78 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. stirred for 30 min., cooled again to -78 °C and **(214)** (0.5 g, 0.9 mmol) in dry THF (6 mL) was added. The

mixture was allowed to reach r.t. and stirred for 10 h. The reaction mixture was worked up and purified as before to give the title compound **(215)** (0.3 g, 60%), $[\alpha]_D^{22}$ = +5.5 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 701.3, $C_{43}H_{86}NaO_3Si$ requires: 701.6}, which showed δ_H (400 MHz, $CDCl_3$): 5.38 (2H, t, *J* 10.8 Hz), 3.91 (1H, dd, *J* 4.8, 11.6 Hz), 3.67 (3H, s), 2.55 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz) , 2.07-1.97 (2H, m), 1.70-1.17 (56H, m), 0.90 (6H, t, *J* 6.8 Hz), 0.84 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.1, 129.9, 129.8, 73.2, 51.5, 51.2, 33.6, 32.6, 31.9, 29.8, 29.78, 29.7, 29.67, 29.64, 29.62, 29.6, 29.58, 29.5, 29.4, 29.34, 29.3, 28.8, 28.5, 27.8, 27.4, 27.2, 26.7, 26.0, 25.9, 25.7, 23.6, 22.6, 17.9, 14.2, 14.1, -4.3, -4.9; ν_{max}: 2926, 2855, 1745, 1468, 1179, 1120, 836, 775, 720, 695 cm^{-1}.

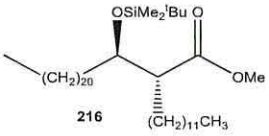
Experiment 21: Methyl (2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-dodecyl-triacontanoate (213)



213

Palladium (10% on carbon, 0.2 g) was added to a stirred solution of the alkene **(212)** (0.37 g, 0.45 mmol) in IMS (10 mL) and THF (10 mL) under hydrogen atmosphere. Hydrogenation was carried out for 1 h. The reaction mixture was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compound **(213)** (0.25 g, 64%), $[\alpha]_D^{23}$ = -15 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 829.7, $C_{52}H_{106}NaO_3Si$ requires: 829.7}, which showed δ_H (400 MHz, $CDCl_3$): 3.91 (1H, dd, *J* 4.7, 11.6 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz), 1.61-1.16 (80H, m), 0.94 (6H, t, *J* 6.8 Hz), 0.91 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.1, 73.2, 51.5, 51.2, 33.6, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.8, 27.5, 25.7, 23.6, 22.6, 17.9, 14.1, -4.3, -4.9; ν_{max}: 2928, 2854, 1741, 1458, 1437, 1179, 1130, 836, 775, 721, 696 cm^{-1}.

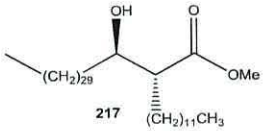
Experiment 22: Methyl (2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-dodecyl-tetracosanoate (216)



216

Palladium (10% on carbon, 0.2 g) was added to a stirred solution of the alkene **(215)** (0.27 g, 0.45 mmol) in IMS (10 mL) and THF (10 mL) under hydrogen atmosphere. Hydrogenation was carried out for 1 h. The reaction mixture was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compound **(216)** (0.2 g, 74%), $[\alpha]_D^{22}$ = +9.2 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 703.6, $C_{43}H_{88}NaO_3Si$ requires: 703.6}, which showed δ_H (400 MHz, $CDCl_3$): 3.91 (1H, dd, *J* 4.7, 11.6 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.2, 10.9 Hz) , 1.61-1.17 (62H, m), 0.94 (6H, t, *J* 6.8 Hz), 0.90 (9H, s),0.05 (3H, s), 0.03 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.1, 73.2, 59.9, 51.5, 51.2, 45.7, 33.6, 32.5, 31.9, 29.8, 29.7, 29.67, 29.6, 29.5, 29.48, 29.4, 29.3, 27.8, 27.5, 25.9, 25.7, 23.6, 22.6, 17.9, 14.2, 14.1, -4.3, -4.9; ν_{max}: 2926, 2855, 1745, 1468, 1437, 1179, 1120, 836, 775, 725, 695 cm^{-1}.

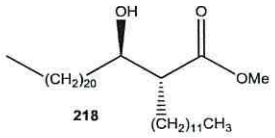
Experiment 23: Methyl (2*R*,3*R*)-2-dodecyl-3-hydroxytritriacontanoate (217)



217

(213) (0.25 g, 0.31 mmol) was stirred in dry THF (12 mL) in a dry polyethylene vial under a nitrogen atmosphere at r.t. then pyridine (0.1 mL) followed by HF-pyridine (1.0 mL) were added and the mixture was stirred for 18 h at 45 °C. The reaction was poured slowly to a sat. solution of $NaHCO_3$ and extracted with petrol/ethyl acetate (1:1, 10 mL). The mixture was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 4 × 20 mL). The combined organic layers were washed with brine, dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound **(217)** (0.15 g, 71%), $[\alpha]_D^{21}$ = +12 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 715.7, $C_{46}H_{92}NaO_3$ requires: 715.6}, which showed δ_H (400 MHz, $CDCl_3$): 3.77 (1H, t, *J* 6.6 Hz), 3.72 (3H, s), 2.48-2.39 (1H, m), 1.71 (1H, dt, *J* 8.7, 13.2 Hz), 1.65-1.54 (2H, m), 1.27 (78H, br.s), 0.89 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 176.2, 72.2, 60.3, 51.4, 50.9, 35.6, 31.9, 29.69, 29.65, 29.63, 29.61, 29.6, 29.57, 29.55, 29.53, 29.5, 29.4, 29.35, 29.3, 27.4, 25.7, 22.6, 21.0, 14.18, 14.1; ν_{max}: 3446, 2925, 2854, 1720, 1644, 1474, 1377, 1195, 1052, 722 cm^{-1}.

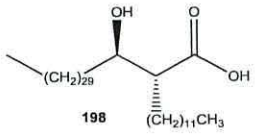
Experiment 24: Methyl (2*R*,3*R*)-2-dodecyl-3-hydroxytetracosanoate (218)



218

(216) (0.2 g, 0.3 mmol) was stirred in dry THF (10 mL) in a dry polyethylene vial under a nitrogen atmosphere at r.t. then pyridine (0.1 mL) and HF-pyridine (1.0 mL) were added and the mixture was stirred for 18 h at 45 °C. The reaction was poured slowly on to sat. solution of $NaHCO_3$ (10 mL) and extracted with petrol/ethyl acetate (1:1, 10 mL). The mixture was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 4 × 20 mL). The combined organic layers were washed with brine, dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound **(218)** (0.11 g, 62%), $[\alpha]_D^{22}$ = +9.1 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 589.6, $C_{37}H_{72}NaO_3$ requires: 589.5}, which showed δ_H (400 MHz, $CDCl_3$): 3.75 (1H, t, *J* 6.5 Hz), 3.72 (3H, s), 2.48-2.39 (1H, m), 1.71 (1H, dt, *J* 8.7, 13.2 Hz), 1.65-1.54 (2H, m), 1.27 (58H, br.s), 0.89 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, CDCl3): 176.2, 72.2, 60.3, 51.4, 50.9, 50.8, 35.6, 31.9, 29.69, 29.64, 29.62, 29.58, 29.56, 29.54, 29.52, 29.5, 29.40, 29.34, 29.3, 27.4, 25.7, 22.6, 21.0, 14.1, 14.0; ν_{max}: 3444, 2925, 2854, 1720, 1645, 1464, 1377, 1195, 1167, 1052, 721 cm^{-1}.

Experiment 25: (2*R*,3*R*)-2-Dodecyl-3-hydroxytritriacontanoic acid (198)

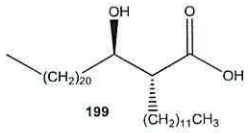


198

Lithium hydroxide monohydrate (0.11 g, 2.62 mmol) was added at r.t. to a stirred solution of **(217)** (0.13 g, 0.18 mmol) in THF (10 mL), water (1 mL), MeOH (0.5 mL). The mixture was stirred for 18 h at 45 °C. The mixture was dissolved in warmed petrol/ethyl acetate 5:1 (50 mL) and acidified with 5% HCl until pH 1-2. The organic layer was separated and the aqueous layer was re-extracted with (3 × 50 mL). The combined organic layers were dried, evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:2) to give the title compound **(198)** (0.1 g, 83%), $[\alpha]_D^{23}$ = +15 (c 0.50, $CHCl_3$) {Found $[M+Na]^+$: 701.6777, $C_{45}H_{90}NaO_3$ requires: 701.6787}, which showed δ_H (400 MHz, $CDCl_3$): 3.73 (1H, dt, *J* 4.6, 9.2 Hz), 3.62-3.59 (1H, m), 2.52-2.45 (1H, m), 1.81-1.69 (2H, m),

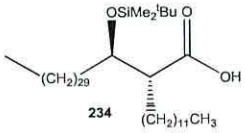
1.68-1.58 (2H, m), 1.29 (77H, br.s), 0.89 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 174.1, 72.1, 50.6, 35.5, 31.9, 29.7, 29.6, 29.5, 29.49, 29.4, 29.3, 27.3, 25.7, 22.6, 14.1, 14.0; ν_{max}: 3559, 2929, 2842, 2361, 1688, 1462, 1375, 965, 725 cm^{-1}.

Experiment 26: (2*R*,3*R*)-2-Dodecyl-3-hydroxytetracosanoic acid (199)



Lithium hydroxide monohydrate (0.13 g, 3.09 mmol) was added at r.t. to a stirred solution of **(218)** (0.11 g, 0.19 mmol) in THF (10 mL), water (1 mL), MeOH (0.5 mL). The mixture was stirred for 18 h at 45 °C. The reaction mixture was worked up and purified as before to give the title compound **(199)** (0.1 g, 93%), $[\alpha]_D^{23}$ = +8.9 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 575.9, $C_{36}H_{72}NaO_3$ requires: 575.5}, which showed δ_H (400 MHz, $CDCl_3$): 3.72 (1H, d, *J* 4.6 Hz), 3.62-3.58 (1H, m), 2.46 (1H, dd, *J* 5.4, 14.2 Hz), 1.73 (1H, dt, *J* 8.4, 16.5 Hz), 1.63 (1H, td, *J* 5.9, 14.1 Hz), 1.57-1.44 (2H, m), 1.44-1.18 (59H, br.s), 0.84-0.82 (6H, m); δ_C (101 MHz, $CDCl_3$): 180.3, 72.1, 50.9, 35.4, 31.9, 29.7, 29.67, 29.6, 29.59, 29.5, 29.45, 29.4, 29.3, 27.33, 25.7, 22.6, 14.1, 14.0; ν_{max}: 3549, 2928, 2845, 1690, 1462, 1375, 1206, 965, 720 cm^{-1}.

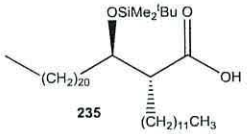
Experiment 27: (2*R*,3*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-dodecyltritria-contanoic acid (234)



Imidazole (0.1 g, 1.4 mmol) was added to a stirred solution of **(198)** (0.11 g, 0.14 mmol) in dry DMF (1 mL) and dry toluene (2 mL) at r.t. followed by the addition of *tert*-butyldimethylsilyl (0.22 g, 1.46 mmol) and 4-DMAP (0.01 g, 0.08 mmol). The reaction mixture was stirred at 70 °C for 18 h. The solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate 5:1 (100 mL) and water (10 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (3 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue. The residue was dissolved in THF (1.6 mL) then was added tetra butylammonium hydroxide 40% (1 mL), (0.1 mL from Bu_4NOH in 0.9 mL H_2O). The reaction mixture was stirred at room

temperature for 15 min.The mixture was diluted with petrol/ethyl acetate 10:1, (40 mL) and brine (3 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 4 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate 10:1 to give the title compound **(234)** (0.1 g, 86%), $[\alpha]_D^{22}$ = +12 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 815.2, $C_{51}H_{104}NaO_3Si$ requires: 815.7}, which showed δ_H (400 MHz, $CDCl_3$): 3.86 (1H, dd, *J* 5.8, 10.0 Hz), 2.56-2.50 (1H, m), 1.67 (2H, ddd, *J* 6.8, 11.6, 15.0 Hz), 1.61-1.44 (2H, m), 1.37-1.18 (77H, br.s), 0.92 (9H, s), 0.9-0.79 (6H, t, *J* 7.1 Hz), 0.13 (3H, s), 0.12 (3H, s); δ_C (101 MHz, $CDCl_3$): 176.5, 73.6, 60.3, 50.4, 37.0, 35.3, 32.7, 31.9, 29.7, 29.65, 29.6, 29.5, 29.49, 29.45, 29.4, 29.3, 27.4, 25.7, 25.5, 24.8, 22.6, 21.0, 19.7, 17.9, 14.1, -4.2, -4.9; ν_{max}: 3435, 2927, 2856, 1709, 1658, 1463, 1254, 1074, 836, 778 cm^{-1}.

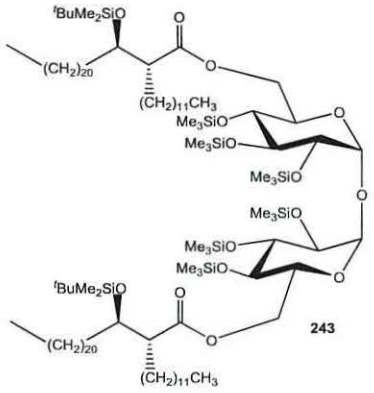
Experiment 28: (2*R*,3*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-dodecyltetra-cosanoic acid (235)



235

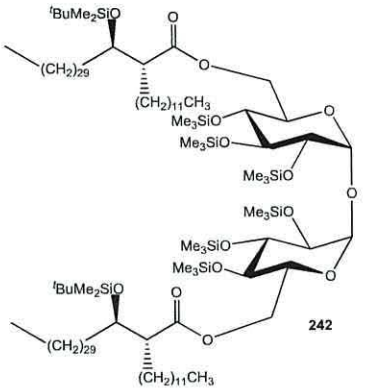
Imidazole (0.12 g, 1.76 mmol) was added to a stirred solution of **(199)** (0.1 g, 0.2 mmol) in dry DMF (1 mL) and dry toluene (2 mL) at r.t. followed by the addition of *tert*-butyldimethylsilylchloride (0.27 g, 1.79 mmol) and 4-DMAP (0.02 g, 0.16 mmol). The reaction mixture was stirred at 70°C for 18 h. then the reaction mixture was worked up and purified as before to give the title compound **(235)** (0.1 g, 83%), $[\alpha]_D^{24}$ = +6.4 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 689.3, $C_{42}H_{86}NaO_3Si$ requires: 689.6}, which showed δ_H (400 MHz, $CDCl_3$): 3.87 (1H, dd, *J* 5.2, 10.7 Hz), 2.53 (1H, dt, *J* 4.8, 9.7 Hz), 1.71-1.59 (2H, m), 1.59-1.42 (2H, m), 1.37-1.18 (58H, br.s), 0.94 (9H, s), 0.92-0.79 (6H, t, *J* 6.9 Hz), 0.12 (3H, s), 0.10 (3H, s); δ_C (101 MHz, $CDCl_3$): 177.4, 73.5, 50.6, 35.0, 31.9, 29.7, 29.68, 29.65, 29.6, 29.56, 29.5, 29.48, 29.4, 29.3, 29.0, 27.5, 25.7, 24.6, 22.6, 17.9, 14.1, -4.3, -4.9; ν_{max}: 3435, 2926, 2855, 1709, 1638, 1466,1254, 1075, 836, 778 cm^{-1}.

Experiment 29: ((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-Oxybis(3,4,5-tris((tri-methylsilyl)oxy)tetrahydro-2*H*-pyran-6,2-diyl))bis(methylene)2*R*,2'*R*, 3*R*,3'*R*)-bis(3-((*tert*-butyldimethylsilyl)oxy)-2-dodecyltetracosanoate) (243)



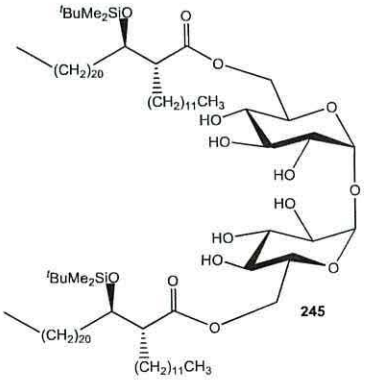
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimidehydrochloride (0.11 g, 0.57 mmol) and 4-DMAP (0.06 g, 0.48 mmol) were added to a stirred solution of **(235)** (0.11 g, 0.15 mmol) and **(160)** (0.06 g, 0.07 mmol) and powdered 4 Å molecular sieves in dry dichloromethane (4 mL) at r.t. under nitrogen atmosphere. The mixture was stirred for 6 days at r.t., then the reaction mixture was diluted with dichloromethane (5 mL) and silica gel (1.0 g) was added. The mixture was evaporated under reduced pressure to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compound as **(243)** (0.09 g, 30%), $[\alpha]_D^{23}$ = +60 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2094.4, $C_{114}H_{238}NaO_{15}Si_8$ requires: 2094.5}, which showed δ_H (400 MHz, $CDCl_3$): 4.85 (2H, d, *J* 3.0 Hz), 4.37 (2H, br.d, *J* 10.1 Hz), 4.06-3.95 (4H, m), 3.88-3.86 (4H, m), 3.53 (2H, t, *J* 8.9 Hz), 3.38 (2H, dd, *J* 3.0, 9.3 Hz), 2.59-2.52 (2H, m), 1.64-1.30 (6H, m), 1.29-1.10 (136H, br.s), 0.9 (18H, s), 0.88-0.85 (12H, t, *J* 7.0 Hz), 0.16 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.06 (12H, br.s); δ_C (101 MHz, $CDCl_3$): 173.8, 94.8, 73.5, 73.4, 72.82, 71.8, 70.7, 62.4, 62.3, 51.8, 33.4, 31.9, 29.85, 29.8, 29.7, 29.67, 29.6, 29.5, 29.3, 28.1, 25.8, 22.7, 14.1, 1.0, 0.9, 0.1, -4.4, -4.6; ν_{max}: 2925, 2855, 1748, 1607, 1493, 1251, 1076, 686, 828 cm^{-1}.

Experiment 30: ((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-Oxybis(3,4,5-tris((tri methyl-sily)oxy)tetrahydro-2*H*-pyran-6,2-diyl))bis(methylene)(2*R*,2'*R*, 3*R*,3'*R*)-bis(3-((*tert*-butyldimethylsilyl)oxy)-2-dodecyltritriacontanoate) (242)



EDCI (0.09 g, 0.46 mmol) and 4-DMAP (0.06 g, 0.48 mmol) were added to a stirred solution of **(234)** (0.11 g, 0.12 mmol) and **(160)** (0.05, 0.06 mmol) and powdered 4 Å molecular sieves in dry dichloromethane (3 mL) at r.t. under a nitrogen atmosphere. The mixture was stirred for 6 days at r.t. Then the reaction mixture was worked up and purified as before to give the title compound **(242)** (0.08 g, 28%), $[\alpha]_D^{24} = +80$ (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2346.1, $C_{132}H_{274}NaO_{15}Si_8$ requires: 2346.8}, which showed δ_H (400 MHz, $CDCl_3$): 4.85 (2H, d, *J* 2.9 Hz), 4.40-4.32 (2H, m), 4.15-3.95 (4H, m), 3.88-3.81 (4H, m), 3.73-3.65 (2H, m), 3.44 (2H, dt, *J* 3.0, 9.3 Hz), 2.59-2.52 (2H, m), 1.75-1.07 (164H, m), 0.90 (18H, s), 0.88-85 (12H, t, *J* 7.0 Hz), 0.17 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.06 (12H, br.s); δ_C (101 MHz, $CDCl_3$): 173.8, 94.3, 73.5, 73.4, 73.3, 72.88, 72.8, 72.7, 71.9, 71.8, 71.4, 70.7, 62.3, 61.6, 51.8, 33.4, 31.9, 29.8, 29.7, 29.6, 29.5, 28.1, 26.2, 25.9, 25.8, 25.7, 25.1, 22.7, 14.1, 1.2, 0.9, -4.5, -4.6; ν_{max}: 2925, 2856, 1750, 1610, 1452, 1403, 1076, 1050, 686, 835 cm^{-1}.

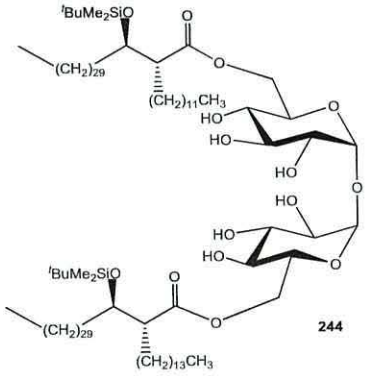
Experiment 31: (Oxybis(3,4,5-trihydroxytetrahydro-2*H*-pyran-6,2-diyl)) bis-((methylene)(2*R*,2'*R*,3*R*,3'*R*)-bis(3-((*tert*-butyldimethylsilyl)oxy)-2-doecyl-tetracosanoate) (245)



Tetrabutylammonium fluoride (0.1 mL, 0.1 mmol, 1.0 M) was added to a stirred

solution of **(243)** (0.1 g, 0.5 mmol) in dry THF (5 mL) at 5 °C under a nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 1 h. The reaction mixture was evaporated to give a residue, which was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(245)** (0.5 g, 70%), $[\alpha]_D^{24}$ = +30 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1662.6, $C_{96}H_{190}NaO_{15}Si_2$ requires: 1662.3}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.06 (2H, d, *J* 3.4 Hz), 4.67-4.66 (2H, br.dd, *J* 2.2, 11.1 Hz), 4.33 (2H, dd, *J* 3.7, 11.9 Hz), 4.22 (2H, d, *J* 10.9 Hz), 3.89 (2H, dt, *J* 9.4, 14.9 Hz), 3.76 (2H, m), 3.47 (2H, dd, *J* 3.3, 9.7 Hz), 3.33 (2H, t, *J* 9.4 Hz), 2.56-2.50 (2H, m), 1.93-1.78 (2H, m), 1.56-1.06 (128H, m), 0.86 (12H, t, *J* 7.0 Hz), 0.83 (18H, s), 0.01 (6H, s), -0.004 (6H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.2, 93.3, 73.3, 73.2, 72.8, 71.6, 70.2, 69.9, 67.9, 62.8, 51.6, 34.7, 33.5, 32.5, 31.8, 30.7, 29.7, 29.65, 29.6, 29.59, 29.57, 29.5, 29.29, 29.2, 28.3, 27.7, 27.5, 26.9, 25.7, 25.6, 25.5, 24.6, 24.2, 22.6, 20.9, 18.7, 18.0, 17.8, 14.1, 13.9, 1.0, -4.5, -4.9; ν_{max}: 3377, 2927, 2850, 1735, 1465, 1076, 835 cm^{-1}.

Experiment 32: ((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-Oxybis(3,4,5-trihydroxy-tetrahydro-2*H*-pyran-6,2-diyl))bis(methylene)(2*R*,2'*R*,3*R*,3'*R*)-bis(3-((*tert*-butyldimethylsilyl)oxy)-2-dodecyltritriacontanoate) (244)

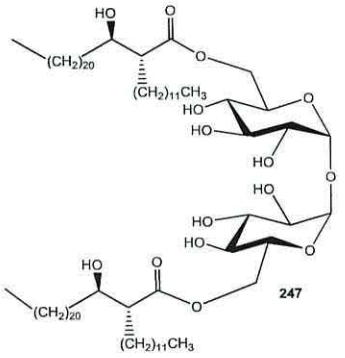


244

Tetrabutylammonium fluoride (0.1 mL, 0.1 mmol, 1.0 M) was added to a stirred solution of **(242)** (0.08 g, 0.03 mmol) in dry THF (5 mL) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 1 h. Then the reaction mixture was worked up and purified as before to give the title compound **(244)** (0.43 g, 71%), $[\alpha]_D^{24}$ = +50 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1914.5, $C_{114}H_{226}NaO_{15}Si_2$ requires: 1914.6}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.05 (2H, d, *J* 3.5 Hz), 4.67-4.6 (2H, m), 4.33 (2H, dd, *J* 4.0, 12.1 Hz),

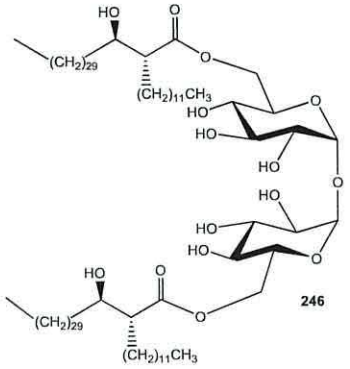
4.20 (2H, d, *J* 10.5 Hz), 3.97-3.83 (4H, m), 3.78 (2H, t, *J* 9.3 Hz), 3.46 (2H, dd *J* 3.5, 9.7 Hz), 2.61-2.47 (2H, m), 1.93-1.78 (2H, m), 1.64-0.93 (164H, m), 0.93 (12H, t, *J* 6.90 Hz), 0.79 (18H, s), 0.01 (6H, s), -0.01 (6H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 93.4, 73.4, 73.2, 72.9, 71.7, 70.2, 69.9, 67.9, 62.8, 51.6, 37.0, 34.7, 33.5, 32.6, 32.5, 31.86, 31.8, 29.9, 29.7, 29.68, 29.6, 29.58, 29.5, 29.4, 29.3, 29.2, 28.3, 27.7, 27.5, 27.3, 26.9, 25.7, 25.6, 25.5, 25.4, 24.7, 24.5, 24.2, 22.6, 22.5, 19.6, 18.8, 17.8, 14.31, 14.0, 11.3, 1.0, -3.8, -4.5, -5.0; ν_{max}: 3377, 2927, 2859, 1745, 1468, 1076, 846 cm^{-1}.

Experiment 33: ((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-Oxybis(3,4,5-trihydroxy-tetrahydro-2*H*-pyran-6,2-diyl))bis(methylene)(2*R*,2'*R*,3*R*,3'*R*)-bis(2-dodecyl-3-hydrox-ytetracosanoate) (247)



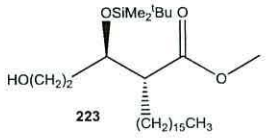
A dry polyethylene vial equipped with an acid proof rubber septum was charged with **(245)** (0.04 g, 0.02 mmol) and pyridine (0.05 mL) in dry THF (7 mL) and stirred at r.t. under nitrogen. HF-pyridine (0.6 mL) was added at 5 °C. The mixture was stirred at 43 °C for 17 h., then neutralized by triethylamine dropwise until pH 5. The product was evaporated to give a white solid. This was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(247)** (0.01 g, 30%), $[\alpha]_D^{22}$ = +25 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1434.3, $C_{84}H_{162}NaO_{15}$ requires: 1434.1}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.97 (2H, d, *J* 3.6 Hz), 4.68 (2H, d, *J* 10.6 Hz), 4.28-4.21 (2H, m), 3.91 (2H, dd, *J* 8.0, 11.9 Hz), 3.76 (2H, t, *J* 9.1 Hz), 3.65-3.61 (2H, m), 3.47 (2H, dd, *J* 3.5, 9.8 Hz), 3.19 (2H, t, *J* 9.6 Hz), 2.37 (2H, ddd, *J* 4.7, 7.9, 10.1 Hz), 1.55 (2H, t, *J* 18.2 Hz), 1.11 (130H, v.br.s), 0.83 (12H, t, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.3, 94.7, 72.4, 71.1, 71.0, 69.7, 64.1, 52.2, 51.5, 45.8, 34.5, 31.7, 30.5, 29.48, 29.46, 29.4, 29.3, 29.2, 29.1, 27.1, 25.5, 25.0, 22.4, 13.8; ν_{max}: 3372, 2922, 2851, 1717, 1466, 1386, 1088 cm^{-1}.

Experiment 34: ((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-Oxybis(3,4,5-trihydroxy-tetrahydro-2*H*-pyran-6,2-diyl))bis(methylene)(2*R*,2'*R*,3*R*,3'*R*)-bis(2-dodecyl-3-hydroxytritriacontanoate) (246)



A dry polyethylene vial equipped with an acid proof rubber septum was charged with **(244)** (0.038 g, 0.021 mmol) and pyridine (0.05 mL) in dry THF (7 mL) and stirred at r.t. under nitrogen. HF-pyridine (0.6 mL) was added at 5 °C. The mixture was stirred at 43 °C for 17 h. 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 extract with $CHCl_3$ (3 × 25 mL), dried and evaporated to give a white solid. This was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(246)** but the ^1H NMR of the crude showed a broad multiple peak for a long chain hydrocarbon and no peak for sugar protons.

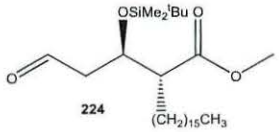
Experimental 35: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-propyl)octadecanoate (223)



Lithium bis(trimethylsilyl)amide (7.0 mL, 7.4 mmol, 1.06 M) was added to a stirred solution of **(177)** (1.5 g, 3.8 mmol) and **(221)** (2.0 g, 4.9 mmol) in dry THF (10 mL) at -10 °C. The reaction turned bright yellow and was left to reach r.t. and stirred for 1 h under nitrogen atmosphere. The reaction mixture was quenched by addition of sat. aq. NH_4Cl. The product was extracted with petrol/ethyl acetate (2:1, 3 × 50 mL), dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography over silica gel, eluting with petrol/ethyl acetate (10:1) gave a colourless oil of (*E/Z*) as a mixture in ratio 2:1 of **(222)** (1.3 g, 65%). Palladium 10%

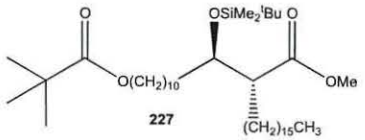
on carbon (0.5 g) was added to a stirred solution of alkene mixture **(222)** (1.3 g, 2.3 mmol) in IMS (20 mL) and THF (20 mL) under hydrogen atmosphere. Hydrogenation was carried out for one day. The mixture was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil of the title compound as white sold **(223)** (1.0 g, 90%), $[\alpha]_D^{22}$ = -1.8 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 509.7, $C_{28}H_{58}NaO_4Si$ requires: 509.4}, which showed δ_H (400 MHz, $CDCl_3$): 4.25 (1H, dt, *J* 4.2, 6.7 Hz), 3.85-3.77 (2H, m), 3.66 (3H, s), 2.67-2.63 (1H, m), 1.60-1.50 (2H, m), 1.50-1.40 (2H, m), 1.27 (29H, br.s), 0.87 (3H, t, *J* 7.6 Hz), 0.86 (9H, s), 0.1 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.9, 64.6, 60.3, 59.4, 51.5, 35.2, 29.67, 29.64, 29.6, 29.5, 29.4, 29.3, 27.8, 27.5, 25.6, 22.6, 21.1, 17.9, 17.8, 14.1, 14.0, -4.5, -4.9; ν_{max}: 3435, 2926, 2854, 1739, 1470, 1258, 1174, 1093, 856 cm^{-1}.

Experiment 36: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-3-oxo-propyl)octadecanoate (224)



(223) (1.1 g, 2.1 mmol) in dichloromethane (10 mL) was added to a stirred suspension solution of PCC (1.1 g, 5.1 mmol) in dichloromethane (50 mL) at r.t. During the addition a black colour appeared and the reaction mixture was stirred for 2.5 h at r.t., then petrol/ethyl acetate (10:1) was added. The mixture was filtered through a bed of silica gel and washed with petrol/ethyl acetate (3 × 30 mL). The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of the title compound **(224)** (0.87 g, 87%), $[\alpha]_D^{20}$ = -5.8 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 507.9, $C_{28}H_{56}NaO_4Si$ requires: 507.3}, which showed δ_H (400 MHz, $CDCl_3$): 9.81 (1H, t, *J* 2.5 Hz), 4.44 (1H, dt, *J* 6.0, 11.8 Hz), 3.68 (3H, s), 2.67 (2H, dd, *J* 1.6, 4.7 Hz), 2.64-2.56 (1H, m), 1.60-1.50 (2H, m), 1.26 (28H, br.s), 0.9 (3H, t, *J* 6.6 Hz), 0.84 (9H, s), 0.08 (3H, s), 0.07 (3H, s); δ_C (101 MHz, $CDCl_3$): 201.2, 174.5, 68.8, 52.2, 51.5, 48.0, 31.9, 29.68, 29.65, 29.6, 29.53, 29.5, 29.38, 29.3, 27.7, 27.0, 25.87, 25.8, 22.5, 20.4, 17.8, 14.2, -4.6, -4.9; ν_{max}: 2926, 2855, 1745, 1464, 1362, 1265, 1196, 1097, 1005, 837, 778 cm^{-1}.

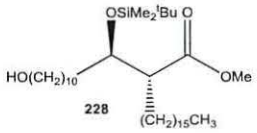
Experiment 37: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-11-(pivaloyloxy)undecyl)octadecanoate (227)



227

Lithium bis(trimethylsilyl)amide (3.3 mL, 3.5 mmol, 1.06 M) was added to a stirred solution of aldehyde **(224)** (0.87 g, 1.81 mmol) and **(225)** (0.98 g, 2.32 mmol) in dry THF at -10 °C. The reaction turned bright yellow and was left to reach r.t. and stirred for 1h under a nitrogen atmosphere. The reaction was quenched by addition of sat. aq. NH_4Cl. The product was extracted with petrol/ethyl acetate (5:1, 2 × 50 mL), dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give a colourless of (*E*/*Z*) as a mixture in ratio 2:1 **(226)** (1.1 g, 84%). Palladium 10% on carbon (0.2 g) was added to a stirred solution of alkene mixture **(226)** (1.1 g, 1.6 mmol) in IMS (20 mL) and THF (20 mL) under hydrogen. Hydrogenation was carried out for 1 h. The solution was filtered over a bed of celite and the solvent was evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, the title compound **(227)** (1.0 g, 90%), $[\alpha]_D^{23}$ = -4.2 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 705.2, $C_{41}H_{82}NaO_5Si$ requires: 705.5}, which showed δ_H (400 MHz, $CDCl_3$): 4.04 (2H, t, *J* 6.6 Hz), 3.91 (1H, m), 3.66 (3H, s), 2.52 (1H, ddd, *J* 3.8, 6.9, 10.7 Hz), 1.65-1.43 (2H, m), 1.31-1.20 (46H, v.br.s), 1.18 (9H, s), 0.89 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$): 178.6, 175.0, 64.4, 51.5, 51.1, 50.2, 38.7, 33.6, 31.9, 29.7, 29.67, 29.66, 29.63, 29.6, 29.5, 29.44, 29.4, 29.3, 29.1, 29.0, 28.6, 27.8, 27.4, 27.1, 25.9, 25.8, 25.7, 23.7, 22.6, 22.5, 17.9, 14.0, 11.3, -4.3, -4.9; ν_{max}: 2927, 2855, 1732, 1463, 1362, 1254, 1156, 856, 774 cm^{-1}.

Experiment 38: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-11-hydroxy-undecyl)octadecanoate (228)

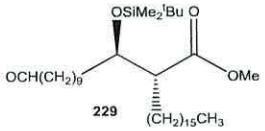


228

(227) (1.0 g, 1.4 mmol) was added to a stirred solution of potassium hydroxide (1.21 g,

21.3 mmol) dissolved in a mixture of THF:MeOH:H_2O (50:50:5, 105 mL). The mixture was refluxed at 70 °C. After 3 h, the reaction was quenched with water and extracted with ethyl acetate (3 × 100 mL), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give a semi-solid, the title compound **(228)** (0.7 g, 87%), $[\alpha]_D^{20}$ = -3.0 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 621.1, $C_{36}H_{74}NaO_4Si$ requires: 621.5}, which showed δ_H (400 MHz, $CDCl_3$): 3.91-3.89 (1H, m), 3.66 (3H, s), 3.63 (2H, t, *J* 6.6 Hz), 2.55 (1H, ddd, *J* 3.8, 6.9, 10.7 Hz), 1.58 (2H, q, *J* 6.6 Hz), 1.25 (47H, s), 0.92-0.83 (12H, m), 0.04 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.3, 73.1, 63.0, 62.9, 51.5, 51.2, 33.6, 32.7, 31.5, 29.7, 29.67 (br.), 29.65, 29.6, 62.5, 29.56, 29.5, 29.4, 29.3, 27.8, 27.4, 26.8, 25.7, 25.6, 23.6, 22.6, 17.9, 14.0, -4.4, -4.9; ν_{max}: 3435, 2928, 2867, 1743, 1644, 1466, 1366, 1254, 1074, 836 cm^{-1}.

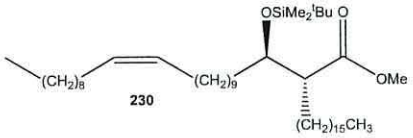
Experiment 39: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-11-oxo-undecyl)octadecanoate (229)



229

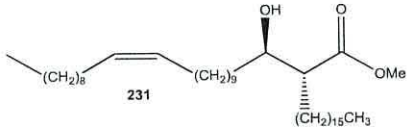
(228) (0.7 g, 1.1 mmol) in dichloromethane (5 mL) was added at r.t. to a stirred solution of PCC (0.6 g, 2.9 mmol) in dichloromethane (50 mL). During the addition a black colour appeared, and then the reaction mixture was stirred at r.t. for 2 h. The reaction mixture was worked up and purified as before to give a colourless oil, the title compound **(239)** (0.6 g, 86%), $[\alpha]_D^{20}$ = -7.1 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 619.0, $C_{36}H_{72}NaO_4Si$ requires: 619.5}, which showed δ_H (400 MHz, $CDCl_3$): 9.77 (1H, t, *J* 1.8 Hz), 3.91-3.85 (1H, m), 3.66 (3H, s), 2.54 (1H, ddd, *J* 3.6, 7.3, 11.0, Hz), 2.44 (2H, td, *J* 1.6, 7.3, Hz), 1.64 (2H, q, *J* 6.9 Hz), 1.25 (44H, br.s), 0.90 (3H, t, *J* 7.0 Hz), 0.85 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$): 202.8, 175.0, 73.1, 51.5, 51.2, 43.9, 33.8, 31.9, 31.5, 29.67, 29.66, 29.63, 29.62, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 27.8, 27.4, 25.7, 23.6, 22.6, 22.0, 17.9, 14.1, -4.3, -4.9; ν_{max}: 2924, 2873, 1745, 1465, 1372, 1167, 1058, 836, 775 cm^{-1}.

Experiment 40: Methyl (2*R*,3*R*,*Z*)-3-((*tert*-butyldimethylsilyl)oxy)-2-hexadecyltricos-13-enoate (230)



Sodium bis(trimethylsilyl)amide (2.8 mL, 2.8 mmol, 1.0 M in THF) was added to a stirred solution of **(202)** (0.7 g, 1.4 mmol) in dry THF (20 mL) at -78 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 30 min., then cooled again to -78 °C and **(229)** (0.4 g, 0.7 mmol) in dry THF (5 mL) was added. The mixture was allowed to reach r.t. and stirred for 10 h. The reaction was quenched with sat. aq. NH_4Cl (10 mL) and the product was extracted with petrol/ethyl acetate (20:1, 4 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated. Then residue was treated with petrol/ether (1:1, 100 mL) and reflexed for 30 min. The precipitate was filtered on celite and the filtrate was evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (40:1) to give the title compound **(230)** (0.3 g, 64%), $[\alpha]_D^{20}$ = -5.4 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 743.4, $C_{46}H_{92}NaO_3Si$ requires: 743.6}, which showed δ_H (400 MHz, $CDCl_3$): 5.38 (2H, t, *J* 10.8 Hz), 3.92 (1H, m), 3.66 (3H, s), 2.55 (1H, ddd, *J* 3.8, 7.2, 11.1 Hz) , 2.03-1.97 (4H, m), 1.27 (60H, br.s), 0.90 (6H, t, *J* 6.8 Hz), 0.84 (9H, s), 0.05 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.1, 129.9, 129.8, 73.2, 51.5, 51.2, 33.6, 31.9 , 29.8, 29.77, 29.7, 29.6, 29.58, 29.5, 29.4, 29.36, 29.3, 27.8, 27.5, 27.2, 25.7, 23.6, 22.6, 17.9, 14.1, -4.3, -4.9; ν_{max}: 2923, 2852, 1741, 1468, 1437, 1361, 1179, 1120, 836, 775, 720, 695 cm^{-1}.

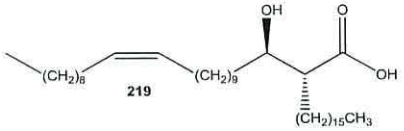
Experiment 41: Methyl (2*R*,3*R*,*Z*)-2-hexadecyl-3-hydroxytricos-13-enoate (231)



(230) (0.45 g, 0.62 mmol) was stirred in dry THF (20 mL) in a dry polyethylene vial under nitrogen atmosphere at r.t. then pyridine (0.1 mL) and HF-pyridine (0.7 mL) were added and the mixture was stirred for 18 h at 45 °C. The reaction was poured slowly on to sat. aq. solution of $NaHCO_3$ (10 mL) and extracted with petrol/ethyl acetate (1:1, 10 mL). The mixture was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 4 × 20 mL). The combined organic layers were

washed with brine, dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) gave the title compound **(231)** as a colourless oil (0.3 g, 80%), $[\alpha]_D^{20}$ = +7.2 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 629.3, $C_{40}H_{78}NaO_3$ requires: 629.5}, which showed δ_H (400 MHz, $CDCl_3$): 5.37 (2H, t, *J* 10.8 Hz), 3.71 (3H, s), 3.67-3.63 (1H, m), 2.45 (1H, dt, *J* 5.3, 9.1 Hz), 2.05 (2H, q, *J* 6.3 Hz), 1.75-1.67 (2H, m), 1.53-1.44 (2H, m), 1.27 (59H, br.s), 0.88-0.83 (6H, m); δ_C (101 MHz, $CDCl_3$): 176.2, 129.9, 129.7, 72.7, 60.3, 51.4, 50.9, 35.6, 31.9, 31.79, 29.7, 29.66, 29.63, 29.60, 29.5, 29.49, 29.48, 29.44, 29.4, 29.3, 29.2, 28.9, 27.4, 27.17, 27.1, 25.7, 22.6, 22.6, 14.1, 14.0; ν_{max}: 3445, 2925, 2864, 1720, 1655, 1465, 1377, 1052, 721 cm^{-1}.

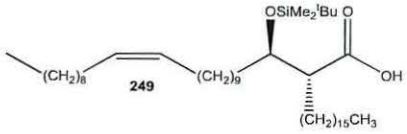
Experiment 42: (2*R*,3*R*,*Z*)-2-Hexadecyl-3-hydroxytricos-13-enoic acid (219)



219

Lithium hydroxide monohydrate (0.3 g, 6.7 mmol) was added at r.t. to a stirred solution of **(231)** (0.26 g, 0.42 mmol) in THF (7 mL), water (1.5 mL), MeOH (0.7 mL). The mixture was stirred for 18 h at 45 °C. The mixture was dissolved in warmed petrol/ethyl acetate 5:1 (50 mL) and acidified with 5% HCl until pH 1-2. The organic layer was separated and the aqueous layer was re-extracted (3 × 50 mL). The combined organic layers were dried, evaporated. The crude product was purified by column chromatography with warm eluting petrol/ethyl acetate (7:2) to give the title compound **(219)** (0.24 g, 96%), $[\alpha]_D^{22}$ = +11 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 615.1, $C_{39}H_{76}NaO_3$ requires: 615.5}, which showed δ_H (400 MHz, $CDCl_3$): 5.37 (2H, t, *J* 10.8 Hz), 3.67-3.63 (1H, m), 2.44 (1H, dt, *J* 5.3, 9.1 Hz), 2.05 (4H, q, *J* 6.3 Hz), 1.74-1.39 (2H, m), 1.27 (60H, br.s), 0.88-0.83 (6H, m); δ_C (101 MHz, $CDCl_3$): 180.5, 129.9, 129.7, 60.4, 51.0, 35.3, 31.9, 31.7, 29.73, 29.7, 29.69, 29.67, 29.65, 29.6, 29.5, 29.49, 29.4, 29.3, 29.2, 28.9, 27.3, 27.2, 27.1, 25.6, 22.67, 22.6, 14.4; ν_{max}: 3540, 2918, 2842, 2365, 1687, 1462, 1365, 965, 719 cm^{-1}.

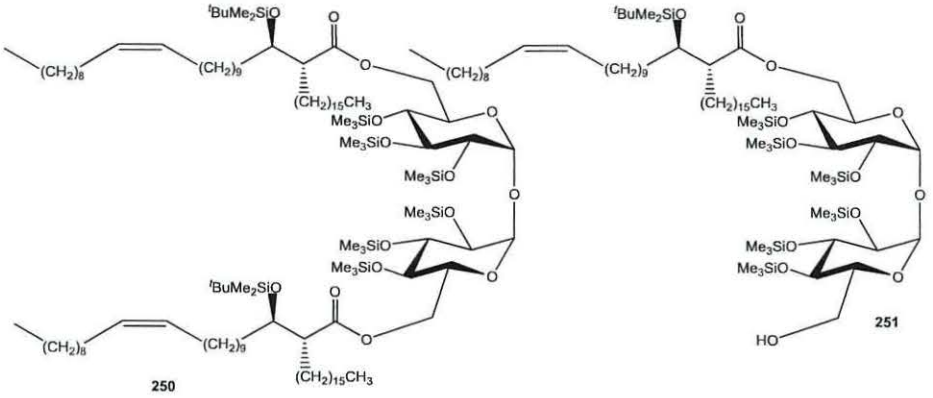
Experiment 43: (2*R*,3*R*,*Z*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-hexadecyltricos-13-enoic acid (249)



Imidazole (0.26 g, 3.82 mmol) was added to a stirred solution of **(219)** (0.23 g, 0.38 mmol) in dry DMF (2 mL) and dry toluene (3 mL) at r.t. followed by the addition of *tert*-butyldimethylsilylchloride (0.58 g, 3.85 mmol) and 4-DMAP (0.05 g, 0.41 mmol). The reaction mixture was stirred at 70 °C for 18 h. Then the solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate 5:1 (100 mL) and water (10 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (3 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue **(248)**. The residue **(248)** was dissolved in THF (5 mL), water (2 mL), and methanol (1 mL), and to this was added potassium carbonate (0.21 g, 1.41 mmol). The reaction mixture was stirred at 45 °C for 3 h. The mixture was diluted with petrol/ethyl acetate 10:1, (40 mL) and water (3 mL) then acidified with potassium hydrogen sulfate to pH 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 4 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate 10:1 to give the title compound **(249)** as colourless oil (0.24 g, 88%), $[\alpha]_D^{20}$ = +7.0 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 729.3, $C_{45}H_{90}NaO_3Si$ requires: 729.6}, which showed δ_H (400 MHz, $CDCl_3$): 5.38 (2H, t, *J* 10.8 Hz), 3.84 (1H, dt, *J* 5.9, 9.8 Hz), 2.54 (1H, dt, *J* 4.3, 9.3 Hz), 2.03 (4H, q, *J* 6.5 Hz), 1.73-1.46 (4H, m), 1.62-1.60 (1H, m), 1.56-1.52 (2H, m), 1.45-1.42 (1H, m), 1.25 (53H, br.s), 0.9 (9H, s), 0.89 (6H, t, *J* 7.5 Hz), 0.14 (3H, s), 0.13 (3H, s); δ_C (101 MHz, $CDCl_3$): 177.7, 129.9, 129.7, 73.5, 50.7, 31.9, 29.73, 29.7, 29.69, 29.65, 29.6, 29.5, 29.4, 29.3, 28.9, 25.7, 25.68, 25.6, 24.4, 22.68, 22.6, 18.1, 17.9, 14.1, -4.3, -4.9; ν_{max}: 3450, 2927, 2845, 1710, 1638, 1463, 1070, 836, 774 cm^{-1}.

Experiment 44: (2*R*,2'*R*,3*R*,3'*R*,13*Z*,13'*Z*)-((2*R*,2'*R*,3*R*,3'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,-6'*R*)-6,6'-Oxybis(3,4,5-*tris*((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-6,2-diyl))bis (methylene)bis(3-((*tert*-butyldimethylsilyl)oxy)-2-hexadecyltricos-13-enoate) (250) and (2*R*,3*R*,*Z*)-((2*R*,3*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4*S*,5*R*,6*R*)-6-(hydroxy-methyl)-3,4,5-

tris((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)-3,4,5-tris((tris((trimethyl-silyl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl-3-((*tert*-butyldimeth-ylsilyl)oxy)-2-hexadecyltricos-13-enoate (251)

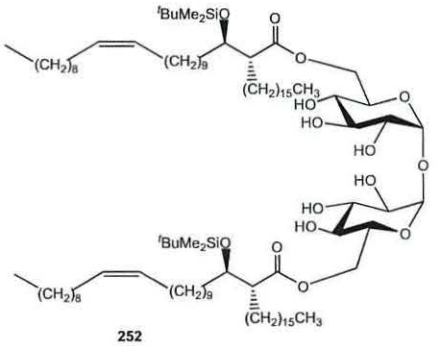


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.3 g, 1.4 mmol) and 4-DMAP (0.15 g, 1.22 mmol) were added to a stirred solution of **(249)** (0.22 g, 0.31 mmol) and **(160)** (0.14 g, 0.18 mmol) and powdered 4 Å molecular sieves in dry dichloromethane (4 mL) at r.t. under a nitrogen atmosphere. The mixture was stirred for 6 days at r.t., the reaction mixture was diluted with dichloromethane (5 mL) and silica gel (1.0 g) was added. The mixture was evaporated under reduced pressure to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the first fractions **(250)** as a colourless thick oil (0.2 g, 33%), $[\alpha]_D^{20}$ = +90 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2174.7, $C_{120}H_{246}NaO_{15}Si_8$ requires: 2174.6}, which showed δ_H (400 MHz, $CDCl_3$): 5.35 (4H, t, *J* 10.8 Hz), 4.84 (2H, d, *J* 3.0 Hz), 4.37 (2H, br.d, *J* 9.8 Hz), 4.20-4.01 (4H, m), 3.87-3.72 (4H, m), 3.53 (2H, t, *J* 9.0 Hz), 3.38 (2H, dd, *J* 2.9, 9.2 Hz), 2.56 (2H, ddd, *J* 3.4, 9.5, 13.4 Hz), 2.04 (8H, q, *J* 4.8 Hz), 1.15-1.06 (120H, m), 0.90 (18H, s), 0.88 (12H, t, *J* 3.8 Hz), 0.16 (18H, s), 0.14 (18H, s), -0.013 (18H, s), -0.06 (12H, br.s); δ_C (101 MHz, $CDCl_3$): 173.8, 129.8, 129.6, 94.8, 73.5, 73.4, 72.8, 71.8, 70.7, 62.3, 51.8, 41.3, 33.4, 31.9, 29.8, 29.79, 29.7, 29.6, 29.57, 29.5, 29.36, 29.3, 29.0, 28.1, 27.6, 27.2, 26.2, 25.9, 25.8, 25.6, 25.1, 22.69, 22.6, 20.4, 19.4, 18.0, 14.3, 14.1, 11.4, 1.0, 0.9, 0.1, -4.5, -4.6; ν_{max}: 2925, 2855, 1750, 1607, 1493, 1452, 1413, 1252, 1076, 1050, 686, 825 cm^{-1}.

The second fractions **(251)** as a colourless thick oil (0.13 g, 34%), $[\alpha]_D^{21}$ = +42 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1486.1, $C_{75}H_{158}NaO_{13}Si_7$ requires: 1485.9}, which showed δ_H (400 MHz, $CDCl_3$): 5.35 (2H, t, *J* 10.7 Hz), 4.91 (1H, d, *J* 3.1 Hz), 4.84

(1H, d, *J* 2.8 Hz), 4.36 (1H, dd, *J* 2.3, 11.8 Hz), 4.09 (1H, dd, *J* 7.6, 11.9 Hz), 4.01 (1H, dt, *J* 2.2, 6.3 Hz), 3.95-3.93 (3H, m), 3.84 (1H, td, *J* 4.7, 8.2 Hz), 3.76-3.65 (2H, m), 3.50 (2H, dt, *J* 5.7, 8.9 Hz), 3.43 (1H, dd, *J* 3.1, 9.1 Hz), 3.40 (1H, dd, *J* 2.7, 9.2 Hz), 2.57 (1H, ddd, *J* 3.2, 5.3, 9.2 Hz), 2.02 (4H, br. q, *J* 6.8 Hz), 1.26 (70H, s), 0.91 (9H, s), 0.88 (6 H, t, *J* 6.0 Hz), 0.18 (9H, s), 0.17 (9H, s), 0.16 (9H, s), 0.156 (9H, s), 0.15 (9H, s), 0.12 (9H, s), 0.061 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$): 173.9, 129.8, 129.6, 94.5, 94.3, 72.87, 72.8, 72.7, 71.9, 71.4, 70.7, 62.4, 61.6, 51.8, 41.3, 33.4, 31.9, 29.72, 29.7, 29.59, 29.5, 29.36, 29.3, 29.0, 28.1, 27.6, 27.2, 26.3, 25.8, 24.8, 22.68, 22.6, 20.4, 18.0, 14.3, 14.1, 1.05, 1.0, 0.9, 0.8, 0.1, 0.0, -4.4, -4.6; ν_{max}: 3619, 2927, 2855, 1744, 1490, 1607, 1493, 1453, 1403, 1251, 1176, 1050, 1006, 874, 748, 688 cm^{-1}.

Experiment 45: (2*R*,2'*R*,3*R*,3'*R*,13*Z*,13'*Z*)-((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-6,6'-Oxy-bis(3,4,5-trihydroxytetrahydro-2*H*-pyran-6,2-diyl)bis(methylene)-bis(3((*tert*-butyldimethylsilyl)oxy)-2-hexadecyltricos-13-enoate) (252)

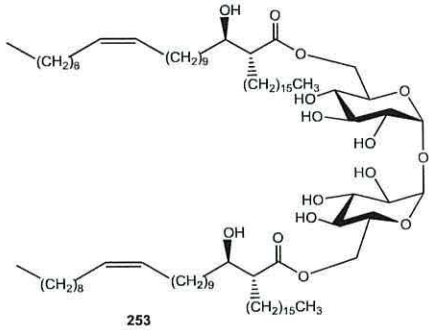


252

Tetrabutylammonium fluoride (0.3 mL, 0.3 mmol, 1.0 M) was added to a stirred solution of **(250)** (0.2 g, 0.1 mmol) in dry THF (15 mL) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 1 h, then the reaction mixture was diluted with $CHCl_3$ (50 mL) and evaporated to give a residue, which was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(252)** as a colourless thick oil (0.13 g, 86%), $[\alpha]_D^{20}$ = +42 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1742.1, $C_{102}H_{198}NaO_{15}Si_2$ requires: 1742.4}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.33 (4H, t, *J* 10.3 Hz), 5.03 (2H, d, *J* 3.1 Hz), 4.33 (2H, br.d, *J* 11.0 Hz), 4.25 (2H, d, *J* 8.1 Hz), 3.97 (2H, dd, *J* 6.9, 10.8 Hz), 3.79 (2H, t, *J* 9.0 Hz), 3.72-3.60 (4H, m), 3.49 (2H, dd, *J* 2.7, 9.1 Hz), 2.36 (2H, ddd, *J* 5.0, 8.2, 11.6 Hz), 1.98 (4H, q, *J* 6.6 Hz), 1.20 (130H, v.br.s), 0.85 (18, s), 0.81 (12H, t, *J* 6.6 Hz), 0.08 (6H, s), 0.01 (6H, s); δ_C (101 MHz, $CDCl_3$ + few drops of

CD_3OD): 175.4, 129.7, 129.5, 94.8, 72.5, 71.2, 71.1, 69.7, 64.3, 34.6, 31.7, 29.5, 29.46, 29.4, 29.3, 29.2, 29.1, 27.1, 27.07, 27.0, 25.7, 25.4, 25.0, 22.5, 18.2, 13.9, -3.9, -6.0; ν_{max}: 3386, 2927, 2859, 1735, 1456, 1086, 866 cm^{-1}.

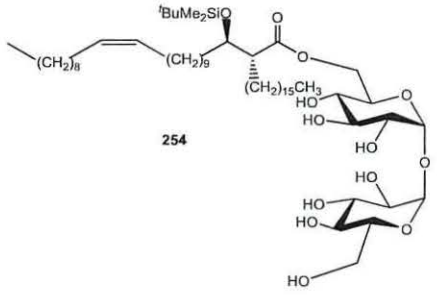
Experiment 46: (2*R*,2'*R*,3*R*,3'*R*,13*Z*,13'*Z*)-((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-6,6'-Oxy -bis(3,4,5-trihydroxytetrahydro-2*H*-pyran-6,2diyl))bis(methylene)-bis(2-hexa-decyl-3-hydroxytricos-13-enoate) (253)



253

A dry polyethylene vial equipped with an acid proof rubber septum was charged with **(252)** (0.11 g, 0.06 mmol) and pyridine (0.1 mL) in dry THF (10 mL) and stirred at r.t under nitrogen. HF-pyridine (0.3 mL) at 5 °C was added. The mixture was stirred at 43 °C for 17 h., then neutralized by triethylamine drop wise until pH 5. The product was evaporated to give a residue which was purified by chromatography eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(253)** as a syrup (0.05 g, 55%), $[\alpha]_D^{21}$ = +30 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1515.7, $C_{90}H_{170}O_{15}Na$ requires: 1515.2}. which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.33 (4H, t, *J* 10.7 Hz), 5.03 (2H, d, *J* 3.1 Hz), 4.33 (2H, br.d, *J* 11.0 Hz), 4.20 (2H, d, *J* 9.1 Hz), 3.97 (2H, dd, *J* 6.9, 10.8 Hz), 3.79 (2H, t, *J* 8.8 Hz), 3.72-3.60 (2H, m), 3.55 (2H, dt, *J* 3.0, 8.0 Hz), 3.45 (2H, dd, *J* 2.8, 9.1 Hz), 2.36 (2H, ddd, *J* 5.05, 8.2, 11.6 Hz), 1.96 (4H, q, *J* 6.6 Hz), 1.20 (132H, v.br.s), 0.85-0.81(12H, m); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.4, 129.8, 129.7, 94.9, 72.4, 72.3, 71.3, 71.0, 69.8, 64.5, 52.0, 34.6, 31.84, 31.8, 29.7, 29.68, 29.63, 29.6, 29.54, 29.53, 29.5, 29.39, 29.3, 29.28, 29.26, 29.2, 27.2, 27.15, 27.12, 25.0, 22.6, 14.0; ν_{max}: 3392, 2920, 2850, 1716, 1466, 1376, 1078 cm^{-1}.

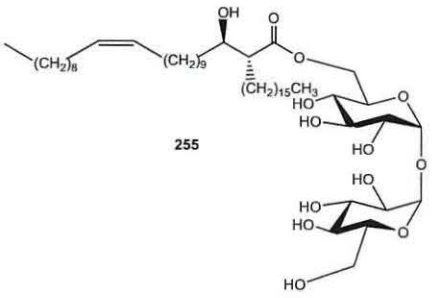
Experiment 47: (2*R*,3*R*,*Z*)-((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl-3-((*tert*-butyldimethylsilyl)oxy)-2-hexadecyltricos-13-enoate (254)



254

Tetrabutylammonium fluoride (0.3 mL, 0.3 mmol, 1.0 M) was added to a stirred solution of **(251)** (0.12 g, 0.08 mmol) in dry THF (5 mL) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 3 h, then the reaction mixture was evaporated to give a residue, which was purified by column chromatography eluting with $CHCl_3$/MeOH (5:1) to give the title compound **(254)** as a colourless thick oil (0.09 g, 95%), $[\alpha]_D^{19}$ = +7.9 (c 0.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1053.9, $C_{57}H_{110}NaO_{13}Si$ required 1053.7}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.28 (2H, t, *J* 10.8 Hz), 5.00 (1H, d, *J* 3.8 Hz), 4.65 (1H, d, *J* 3.8 Hz), 4.35 (1H, dd, *J* 3.7, 11.7 Hz), 4.18 (1H, br.d, *J* 12.3 Hz), 3.90-3.75 (3H, m), 3.70 (2H, t, *J* 6.1 Hz), 3.37 (1H, d, *J* 8.2 Hz), 3.30 (2H, d, *J* 8.5 Hz), 3.26 (2H, t, *J* 12.5 Hz), 3.21-3.13 (1H, m), 2.53-2.31 (1H, m), 2.28 (1H, t, *J* 8.1 Hz), 1.82-1.20 (70H, m), 0.87 (6H, t, *J* 6.0 Hz), 0.83 (9H, s), 0.0 (3H, s), -0.02 (3H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 174.5, 129.8, 129.7, 93.4, 73.1, 72.2, 72.0, 70.8, 70.1, 62.8, 62.1, 58.7, 51.8, 33.3, 31.88, 31.8, 29.8, 29.7, 29.69, 29.65, 29.6, 29.59, 29.55, 29.51, 29.3, 29.29, 29.2, 27.7, 27.1, 27.1, 26.4, 25.7, 24.4, 23.9, 22.64, 22.6, 19.6, 17.9, 14.07, 13.6, -4.5, -4.8; ν_{max}: 3388, 2926, 2844, 1745, 1469, 1075, 849 cm^{-1}.

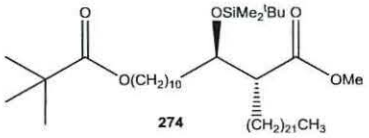
Experiment 48: (2*R*,3*R*,*Z*)-((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,-4*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-tetrahydro-2*H*-pyran-2-yl)methyl-2-hexadecyl-3-hydroxytricos-13-enoate (255)



255

A dry polyethylene vial equipped with an acid proof rubber septum was charged with **(254)** (0.08 g, 0.07 mmol) and pyridine (0.1 mL) in dry THF (10 mL) and stirred at r.t. under nitrogen. HF-pyridine (0.3 mL) at 5 °C was added. The mixture was stirred at 43 °C for 17 h, then neutralized by triethylamine drop wise until pH 5. The product was evaporated to give a residue which was purified by chromatography eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(255)** as a syrup (0.04 g, 57%), $[\alpha]_D^{20}$ = +30 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 939.8, $C_{51}H_{96}NaO_{13}$ requires: 939.6}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.31 (2H, t, *J* 10.8 Hz), 5.09 (1H, d, *J* 2.9 Hz), 5.02 (1H, d, *J* 3.0 Hz), 4.69 (1H, dd, *J* 3.7, 11.7 Hz), 4.24 (1H, br.d, *J* 12.3 Hz), 3.9-3.8 (3H, m), 3.65 (2H, t, *J* 6.1 Hz), 3.50 (1H, d, *J* 8.2 Hz), 3.48 (1H, d, *J* 8.5 Hz), 3.36 (2H, t, *J* 12.5 Hz), 3.31-3.10 (2H, m), 2.53-2.31 (1H, m), 2.28 (1H, t, *J* 8.0 Hz), 1.64-1.21 (71H, m), 0.87 (6H, t, *J* 6.3 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.4, 129.7, 129.7, 94.1, 72.5, 72.4, 72.2, 71.3, 71.2, 70.7, 70.0, 63.9, 61.9, 58.6, 52.3, 46.4, 45.8, 34.5, 31.8, 29.7, 29.64, 29.6, 29.56, 29.5, 29.49, 29.4, 29.3, 29.28, 29.25, 29.2, 29.1, 27.0, 25.0, 23.7, 23.1, 22.5, 19.5, 16.8, 13.9, 13.4; ν_{max}: 3388, 2917, 2864, 1745, 1468, 1075, 849 cm^{-1}.

Experiment 49: (*R*)-2-[(*R*)-1-(*tert*)-Butyldimethylsilanyloxy)-11-(2,2-di-methyl-propionyloxy)undecyl]tetracosanoic acid methyl ester (274)

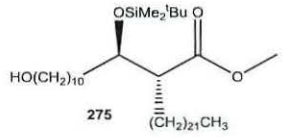


274

Lithium bis(trimethylsilyl)amide (13.9 mL, 14.7 mmol, 1.06 M) was added dropwise to a stirred solution of **(271)** (4.5 g, 7.5 mmol) and **(272)** (4.1 g, 9.7 mmol, 1.3 mol. equiv.) in dry THF (100 mL) at -15 °C. The mixture was then stirred for 3 h at r.t., when TLC analysis indicated completion of the reaction, sat. aq. ammonium chloride (50 mL) and petrol/ethyl acetate (1:1, 200 mL) were added. The aqueous layer was re-

extracted with petrol/ether (1:1, 2 × 50 mL) and the combined organic extracts washed with brine (50 mL), dried and evaporated to give yellow oil. The crude product was purified by column chromatography eluting with petrol/ether (20:1) to give a colourless oil, **(273)** (5.0 g, 83%) as a mixture in ratio (2:1). Palladium (10% on carbon, 0.25 g) was added to a stirred solution of (*E*/*Z*) as a mixture in ratio (2:1) **(273)** (5.0 g) in IMS (25 mL) and THF (25 mL) under hydrogen atmosphere. 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 the title compound **(274)** as a colourless oil (4.9 g, 95%), $[\alpha]_D^{22}$ = -3.2 (c 1.0, $CHCl_3$) {MALDI Found $[M+Na]^+$: 789.1 $C_{47}H_{94}NaO_5Si$ requires: 789.6}, which showed δ_H (400 MHz, $CDCl_3$): 4.02 (2H, t, *J* 7.0 Hz), 3.95-3.86 (1H, m), 3.76 (3H, s), 2.53-2.48 (1H, m), 1.66-1.24 (60H, m), 1.2 (9H, s), 0.87 (3H, t, *J* 6.9 Hz), 0.84 (9H, s) 0.04 (3H, s), 0.02 (3H, s); δ_C (101 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, 29.6, 29.57, 29.5, 29.46, 29.4, 29.3, 29.2, 28.6, 27.8, 27.5, 27.2, 25.9, 25.8, 25.79, 23.7, 22.7, 17.9, 14.1, -4.4, -4.9; ν_{max}: 2943, 2876, 1743, 1467, 1154 cm^{-1}.

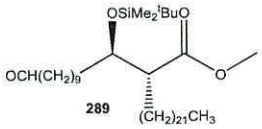
Experiment 50: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-hydroxy-undecyl]tetracosanoic acid methyl ester (275)



Ester **(274)** (9.38 g, 11.6 mmol) in THF (50 mL) was added to a stirred solution of potassium hydroxide (9.71 g, 172 mmol, 15 mol. equiv.) in THF (140 mL), methanol (140 mL) and water (14 mL). The mixture was heated to 70 °C for 2 h, then cooled to r.t. and quenched with water (10 mL) and the aqueous layer was extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were dried and evaporated and the crude product purified by column chromatography eluting with petrol/ether (5:2) to give the title compound **(275)** as a colourless oil (6.6 g, 80%), $[\alpha]_D^{23}$ = -5.1 (c 1.0, $CHCl_3$) [MALDI Found $[M+Na]^+$: 721.2; $C_{42}H_{86}NaO_4Si$ requires: 721.6], which showed δ_H (400 MHz, $CDCl_3$): 3.90-3.84 (1H, m), 3.60 (3H, s), 3.58 (2H, t, *J* 7.0 Hz), 2.50 (1H, ddd, *J* 3.8, 7.0, 11.1 Hz), 1.60-1.20 (61H, m), 0.92 (3H, t *J* 7.0 Hz), 0.85 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.1, 73.2, 63.1, 51.6, 51.2,

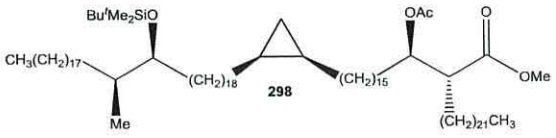
33.7, 32.8, 31.9, 29.8, 29.7, 29.6, 29.58, 29.5, 29.42, 29.4, 29.3, 27.9, 27.5, 25.8, 25.7, 23.8, 22.7, 18.0, 14.1, -4.4, -4.9; ν_{max}: 3323, 2943, 2854, 1753, 1434 cm^{-1}.

Experiment 51: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-oxoundecyl]tetracosanoic acid methyl ester (289)



(275) (6.6 g, 9.2 mmol) in dichloromethane (50 mL) was added to a stirred suspension of PCC (5.10 g, 23.1 mmol, 2.5 mol. equiv.) in dichloromethane (400 mL) at r.t. The mixture was stirred vigorously for 2 h. The mixture was diluted with petrol/ethyl acetate (5:1) (100 mL) and filtered through a bed of silica gel. The filtrate was evaporated to give a residue which was purified by column chromatography eluting with petrol/ether (10:1) to give the title compound **(289)** as a colourless oil (5.8 g, 90%), $[\alpha]_D^{22}$ = -4.2 (c 1.0, $CHCl_3$) [MALDI Found $[M+Na]^+$: 719.3; $C_{42}H_{84}NaO_4Si$ requires: 719.5], which showed δ_H (500 MHz, $CDCl_3$): 9.8 (1H, t, *J* 2.5 Hz), 3.90-3.86 (1H, m), 3.61 (3H, s), 2.5 (1H, ddd, *J* 4.3, 7.2, 11.1 Hz), 2.45 (2H, dt, *J* 2.2, 7.5 Hz), 1.70 (2H, m), 1.54-1.21 (56H, m), 0.9 (3H, t, *J* 7.7 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C (126 MHz, $CDCl_3$): 202.8, 175.1, 73.2, 51.6, 51.2, 43.9, 33.7, 31.9, 29.8, 29.7, 29.6, 29.59, 29.5, 29.4, 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}.

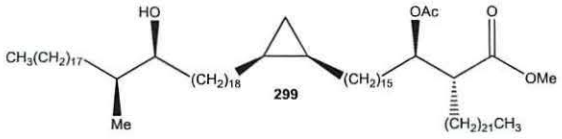
Experiment 52: (*R*)-2-((*R*)-1-Acetoxy-16-{(1*R*,2*S*)-2-[(19*S*,20*S*)-19-(*tert*-butyldimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}hexadecyl)tetracosanoicacid methyl ester (298)



Lithium *bis*-(trimethylsilyl)amide (3.6 mL, 3.7 mmol, 1.06 M) was added to a stirred solution of ester **(295)** (2.2 g, 2.4 mmol) and aldehyde **(294)** (1.7 g, 2.2 mmol) in dry THF (110 mL) at -10 °C under nitrogen atmosphere. The reaction mixture was allowed to reach r.t. and stirred for 2 h, then sat. aq. ammonium chloride (30 mL) and ethyl acetate were added. The organic phase was separated and the water layer was extracted

with petrol/ethyl acetate (5:1, 3 × 100 mL). The combined organic layers were dried and the solvent was evaporated. Chromatography eluting with petrol/ethyl acetate (30:1) gave a colourless oil of a mixture of *(E/Z)* isomers in ratio (4:1) **(297)** (2.1 g, 68%). Dipotassium azodicarboxylate (2.10 g, 10.3 mmol) was added to a stirred solution of the above alkenes mixture **(297)** (2.1 g, 1.5 mmol) in THF (60 mL) and methanol (10 mL) at 5 °C under nitrogen, resulting in a yellow suspension. A solution of glacial acetic acid (5 mL) and THF (5 mL) was added dropwise over 48 h, after a white precipitate had formed. The mixture was cooled to 0 °C and poured slowly into sat. aq. ammonium chloride, extracted with petrol/ethyl acetate (5:1, 3 × 80 mL) and the combined organic layers were washed with water (50 mL) and evaporated. The procedure was repeated. Chromatography eluting with petrol/ether (20:1) gave a white semi-solid of title compound **(298)** (2.0 g, 95%), $[\alpha]_D^{22}$ = -6.6 (c 1.0, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1404.3, $C_{91}H_{180}NaO_5Si$ requires: 1404.3}, which showed δ_H (500 MHz, $CDCl_3$): 5.10 (1H, dt, *J* 4.0, 8.1 Hz), 3.68 (3H, s), 3.51-3.49 (1H, m), 2.63 (1H, ddd, *J* 4.3, 6.5, 10.7 Hz), 2.18 (3H, s), 1.68-1.14 (142H, v.br.m), 1.07-1.02 (1H, m), 0.91- 0.88 (15H, including a singlet at 0.84), 0.81 (3H, d, *J* 6.7 Hz), 0.67-0.64 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.3 Hz), 0.03 (3H, s), 0.02 (3H, s), -0.32 (1H, br.q, *J* 5.2 Hz); δ_C (126 MHz, $CDCl_3$): 173.6, 170.3, 75.9, 74.1, 51.5, 49.6, 37.7, 33.6, 32.5, 31.9, 31.7, 30.2, 30.0, 29.9, 29.72 (v.br.), 29.7, 29.6, 29.5, 29.46, 29.44, 29.4, 28.7, 28.1, 27.7, 27.5, 26.0, 25.9, 25.0, 22.7, 21.0, 18.2, 15.8, 14.4, 14.1, 10.9, -4.2, -4.0; ν_{max}: 2924, 2853, 1747, 1465, 1372, 1236 cm^{-1}.

Experiment 53: (*R*)-2-{(*R*)-1-Acetoxy-16-[(1*R*,2*S*)-2-((19*S*,20*S*)-19-hydroxy-20-methyl-octatriacontyl)-cyclopropyl]-hexadecyl}tetracosanoicacid-methyl ester (299)



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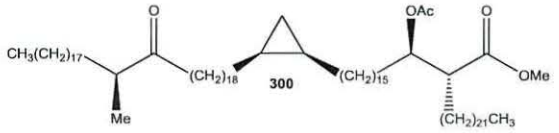
The silyl ether **(298)** (2.0 g, 1.3 mmol) was dissolved in dry THF (15 mL) in a dry polyethylene vial under nitrogen at r.t. and stirred. Then pyridine (0.9 mL) and HF-pyridine (1.2 mL) were added at 5 °C and the mixture was stirred for 17 h at 5 °C. The reaction mixture was poured slowly to sat. aq. $NaHCO_3$ until no more carbon dioxide was liberated. The mixture was extracted with petrol/ethyl acetate (1:1, 3 × 50 mL). The combined organic layers were washed with brine and dried. The solvent was

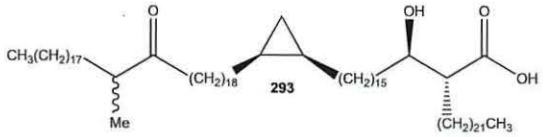
evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give the title compound **(299)** as a white solid (1.5 g, 81%), m.p.: 47-48 °C, $[\alpha]_D^{21}$ = -9.2 (c 1.0, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1290.2, $C_{85}H_{166}NaO_5$ requires: 1290.2}, which showed δ_H (500 MHz, $CDCl_3$): 5.10 (1H, dt, *J* 3.9, 8.0 Hz), 3.68 (3H, s), 3.51-3.48 (1H, m), 2.63 (1H, ddd, *J* 4.3, 6.8, 10.7 Hz), 2.04 (3H, s), 1.66-1.12 (147H, v.br.m), 0.9 (3H, t, *J* 7.0 Hz), 0.86 (3H, d, *J* 7.7 Hz), 0.67-0.64 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), -0.32 (1H, br.q, *J* 5.4 Hz); δ_C (126 MHz, $CDCl_3$): 173.7, 170.3, 75.2, 74.1, 51.5, 49.6, 38.2, 34.5, 33.4, 31.9, 31.7, 30.2, 27.0, 29.8, 29.7 (v.br.), 29.6, 29.57, 29.47, 29.44, 29.4, 29.36, 28.7, 28.1, 27.5, 27.4, 26.3, 25.0, 22.7, 21.0, 15.8, 14.1, 13.6, 10.9; ν_{max}: 3449, 2918, 2850, 1743, 1470, 1374, 1238, 1022 cm^{-1}.

Experiment 54: (*R*)-2-{(*R*)-1-Acetoxy-16-[(1*R*,2*S*)-2-((*S*)-20-methyl-19-oxo-octatriacontyl)-cyclopropyl]-hexadecyl}-tetracosanoic acid methyl ester (300)



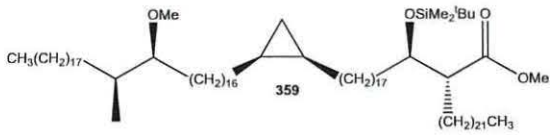
The alcohol **(299)** (0.5 g, 0.3 mmol) in CH_2Cl_2 (30 mL) was added to a stirred solution of PCC (0.25 g, 1.15 mmol) in CH_2Cl_2 (10 mL) at r.t. during the addition a black colour appeared. The mixture was stirred for 3 h at r.t. The mixture was diluted with ethyl acetate (40 mL) and petrol (40 mL) then filtered through a bed of silica gel. The solvent was evaporated and the product was purified by column chromatography eluting with petrol/ether (10:1) to give a white solid, the title compound **(300)** (0.5 g, 99%), m.p.: 49-50 °C, $[\alpha]_D^{20}$ = +7.1 (c 1.0, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1288.1, $C_{85}H_{164}NaO_5$ requires: 1288.2}, which showed δ_H (500 MHz, $CDCl_3$): 5.1 (1H, dt, *J* 3.9, 8.1 Hz), 3.68 (3H, s), 2.63 (1H, ddd, *J* 4.3, 6.9, 10.7 Hz), 2.50 (1H, m), 2.43 (1H, dt, *J* 7.3, 14.7 Hz), 2.4 (1H, dt, *J* 7.3, 14.7 Hz), 2.03 (3H, s), 1.68-1.14 (140H, v.br.m), 1.05 (3H, d, *J* 7.7 Hz), 0.89 (6H, t, *J* 7.6 Hz), 0.68-0.64 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), -0.32 (1H, br.q, *J* 5.2 Hz); δ_C (126 MHz, $CDCl_3$): 215.1, 173.6, 170.3, 74.1, 51.5, 49.6, 46.3, 41.1, 33.1, 31.9, 31.7, 30.2, 29.8, 29.7 (v.br.), 29.65, 29.63, 29.6, 29.56, 29.5, 29.49, 29.46, 29.44, 29.4, 29.3, 28.7, 28.1, 27.5, 27.3, 25.0, 23.7, 22.7, 21.1, 16.4, 15.8, 14.1, 10.9; ν_{max}: 2919, 2850, 1740, 1708, 1471, 1376, 1241, 1166, 1020 cm^{-1}.

Experiment 55: (*R*)-2-{(*R*)-1-Hydroxy-16-[(1*R*,2*S*)-2-((*S*)-20-methyl-19-oxo-octatriacontyl)-cyclopropyl]-hexadecyl}-tetracosanoic acid (293)



Lithium hydroxide monohydrate (0.24 g, 5.71 mmol) was added to a stirred solution of the acetyl protected methyl ester **(300)** (0.51 g, 0.42 mmol) in THF (15 mL), methanol (1.5 mL) and water (2 mL) at r.t. The mixture was stirred at 45 °C for 18 h. It was cooled to r.t. and a mixture of petrol/ether (1:1, 10 mL) and then sat aq. NH_4Cl (10 mL) was added and the mixture was acidified with 5% HCl until pH 1. Further petrol/ether (1:1, 20 mL) were added and the organic layer was separated. The aq. layer was re-extracted with petrol/ether (1:1, 2 × 20 mL). The combined organic layers were washed with water (15 mL), dried and the solvent was evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (7:2) to give a white solid, title compound **(293)** (0.4 g, 85%), m.p.: 70-72 °C; $[\alpha]_D^{20}$ = +4.1 (c 1.0, $CHCl_3$) {MS Found $[M+Na]^+$: 1232.2197, $C_{82}H_{160}NaO_4$ requires: 1232.2294}. This showed; δ_H (500 MHz, $CDCl_3$): 3.74-3.70 (1H, m), 2.51 (1H, m), 2.46 (1H, dt, *J* 5.4, 8.8 Hz), 2.43 (1H, dt, *J* 2.0, 5.0 Hz), 2.40 (1H, dt, *J* 2.0, 5.0 Hz), 1.75-1.12 (140H, v.br.m), 0.9 (3H, d, *J* 7.7 Hz), 0.86 (6H, t, *J* 7.5 Hz), 0.69-0.64 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), -0.32 (1H, br.q, *J* 5.5 Hz); δ_C (126 MHz, $CDCl_3$): 215.4, 179.6, 72.1, 50.8, 46.3, 41.1, 35.5, 33.0, 31.9, 30.2, 29.7 (v.br.), 29.66, 29.6, 29.52, 29.5, 29.46, 29.4, 29.36, 29.3, 28.7, 27.3, 25.7, 23.7, 22.7, 16.4, 15.8, 14.1, 10.9; ν_{max}: 3285, 2919, 2850, 1707, 1470, 1377, 1204, 1019 cm^{-1}.

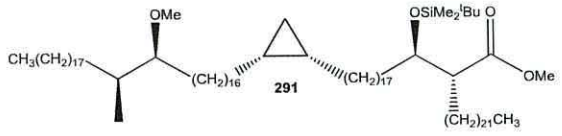
Experiment 56: (*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}-tetracosanoic acid methyl ester (359)



Lithium *bis*(trimethylsilyl)amide (4.3 mL, 4.5 mmol, 1.06 M) was added dropwise to a stirred solution of **(289)** (2.0 g, 2.8 mmol) and **(357)** (2.7 g, 3.0 mmol) in dry THF (80 mL) under nitrogen at -12 °C. The reaction was exothermic and the temperature rose to -5 °C resulting in dark orange solution. The mixture was allowed to reach r.t. and

stirred for 2 h, then cooled to 0 °C and quenched with sat. aq. ammonium chloride (25 mL). The product was extracted with petrol/ethyl acetate (1:1, 3 × 60 mL). The combined organic extracts were dried and evaporated, the crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil **(358)** (3.0 g, 78%) as mixture in ratio of (2;1). Dipotassium azodicarboxylate (8.10 g, 43.0 mmol) was added with stirring to above alkene mixture **(358)** (3.0 g, 2.1 mmol) in dry THF (55 mL) and methanol (55 mL) at 10 °C under nitrogen, resulting in a yellow suspension. A solution of glacial acetic acid (10 mL) in dry THF (10 mL) was added dropwise over 16 h, after that a white precipitate had formed. The mixture was cooled to 0 °C and poured slowly into satd. aq. $NaHCO_3$ (50 mL). The product was extracted with petrol/ether (1:1, 3 × 50 mL). The combined organic layers were washed with water (100 mL), dried and evaporated to give a thick oil, which solidified slowly; however, the ^1H NMR spectra showed that there was still starting material left. The procedure was repeated twice for another 24 h and the crude product was purified by column chromatography eluting with petrol/ether (20:1) to give a colourless oil, the title compound **(359)** (2.5 g, 83%), $[\alpha]_D^{20}$ = -4.2 (c 1.2, $CHCl_3$) [MALDI Found $[M+Na]^+$: 1376.2; $C_{90}H_{180}NaO_4Si$ requires: 1376.3]; which showed δ_H (400 MHz, $CDCl_3$): 3.92-3.89 (1H, m), 3.66 (3H, s), 3.34 (3H, s), 2.96-2.94 (1H, m), 2.55 (1H, ddd, *J* 4.0, 7.0, 11.1 Hz), 1.67-1.09 (143H, m), 0.90-0.84 (18H, including a doublet integrating to 3H, a triplet integrating to 6H and a singlet integrating to 9H), 0.71-0.67 (2H, m), 0.60 (1H, dt, *J* 3.7, 7.6 Hz), 0.05 (3H, s), 0.02 (3H, s), -0.32 (1H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 85.3, 73.1, 57.6, 51.5, 51.1, 35.3, 33.6, 32.3, 31.9, 30.4, 30.2, 30.1, 29.88, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.6, 27.7, 27.5, 27.4, 26.1, 25.7, 23.6, 22.6, 18.0, 15.7, 14.7, 14.1, 10.9, -4.4, -5.0; ν_{max}: 2924, 2853, 1741, 1465, 1099 cm^{-1}.

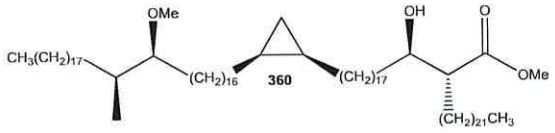
Experiment 57: (*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*S*,2*R*)-2((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl)-tetracosanoic acid methyl ester (291)



Lithium bis(trimethylsilyl)amide (2.0 mL, 2.1 mmol, 1.06 M) was added dropwise to a stirred solution of **(289)** (1.2 g, 1.3 mmol) and **(287)** (0.9 g, 1.3 mmol) in dry THF (30

mL) under nitrogen at -12 °C. The reaction was allowed to reach r.t. and stirred for 2 h. Then the reaction mixture was worked up and purified as before to give a colourless oil of **(290)** (1.45 g, 77%) as mixture in ratio (1:3). Hydrogenation was carried out with dipotassium azocarboxylate as before and the crude product was purified as before to give a colourless oil, title compound **(291)** (1.4 g, 83%), $[\alpha]_D^{22}$ = -4.1 (c 1.2, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1376.25; $C_{90}H_{180}NaO_4Si$ requires: 1376.3548}, which showed δ_H (400 MHz, $CDCl_3$): 3.93-3.90 (1H, m), 3.66 (3H, s), 3.35 (3H, s), 2.97-2.95 (1H, m), 2.55 (1H, ddd, *J* 4.0, 7.0, 11.2 Hz), 1.65-1.09 (143H, m), 0.91-0.85 (18H, m, including a doublet integrating to 3H, a triplet integrating to 6H and a singlet integrating to 9H), 0.66-0.64 (2H, m), 0.6 (1H, dt, *J* 3.7, 7.6 Hz), 0.05 (3H, s), 0.03 (3H, s), -0.31 (1H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 85.3, 73.2, 57.5, 51.4, 51.0, 35.2, 33.5, 32.2, 31.8, 30.4, 30.2, 30.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.6, 27.7, 27.5, 27.4, 26.0, 25.6, 23.6, 22.5, 17.8, 15.6, 14.8, 14.0, 10.8, -4.5, -5.0; ν_{max}: 2927, 2854, 1717, 1466, 1093 cm^{-1}.

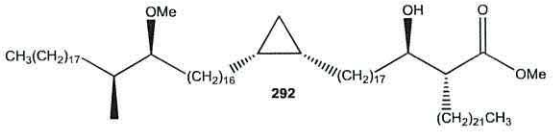
Experiment 58: (*R*)-2-{(*R*)-1-Hydroxy-18-[(1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]ctadecyl}tetracosanoic acid-methyl ester (360)



A dry polyethylene vial equipped with a rubber septum was charged with **(359)** (2.0 g, 1.4 mmol) and pyridine (2 mL) in dry THF (50 mL) and stirred at r.t. under nitrogen. It was added HF-pyridine (6 mL). The mixture was stirred at 43 °C for 17 h, the mixture was neutralised by slowly pouring the mixture into sat. aq. sodium hydrogen carbonate (50 mL) until no more carbon dioxide was liberated. The product was extract with petrol/ethyl acetate (5:2, 3 × 50 mL), dried and evaporated to give a white solid. This was purified by column chromatography eluting with petrol/ethyl acetate (10:1), (5:1) to give a white solid, the title compound **(360)** (1.5 g, 83%), m.p.: 59-61 °C, $[\alpha]_D^{23}$ = -1.1 (c 1.2, $CHCl_3$) [MALDI Found $[M+Na]^+$: 1262.2; $C_{84}H_{166}NaO_4$ requires: 1262.2], which showed δ_H (400 MHz, $CDCl_3$): 3.72 (3H, s), 3.68-3.64 (1H, m), 3.34 (3H, s), 2.97-2.94 (1H, br.pent, *J* 4.4 Hz), 2.46-2.41 (2H, m, including OH-group), 1.74-1.12 (143H, m), 0.90-0.83 (9H, including t, *J* 7.0 Hz, for 2 x CH_3, d, *J* 7.0 Hz, for $CHCH_3$), 0.68-0.64 (2H, m), 0.57 (1H, dt, *J* 4.1, 8.2 Hz), -0.31 (1H, br.q, *J* 5.11 Hz); δ_C (101

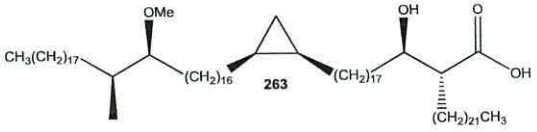
MHz, $CDCl_3$): 176.2, 85.4, 72.3, 57.7, 51.5, 50.9, 35.7, 35.3, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.74, 29.7, 29.64, 29.6, 29.58, 29.56, 29.55, 29.5, 29.4, 29.3, 28.7, 27.5, 27.4, 26.2, 25.7, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}: 3524, 2920, 2850, 1711, 1470 cm^{-1}.

Experiment 59 : (*R*)-2-((*R*)-1-Hydroxy-18-[(1*S*,2*R*)-2((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl)-tetracosanoic acid-methyl ester (292)



A dry polyethylene vial equipped with a rubber septum was charged with **(291)** (1.2 g, 0.8 mmol) in dry THF (25 mL) under nitrogen at 0 °C. Pyridine (1.6 mL) and HF-pyridine (1 mL) were added and the mixture was stirred for 18 h at 43 °C. The reaction mixture was worked up and purified as before to give a white solid, the title compound **(292)** (0.9 g, 90%), m.p.: 62-65 °C, $[\alpha]_D^{26}$ = -1.4 (c 1.0, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1262.2, $C_{84}H_{166}NaO_4$ requires: 1262.2}, which showed δ_H (400 MHz, $CDCl_3$): 3.71 (3H, s), 3.68-3.66 (1H, m), 3.35 (3H, s), 2.96-2.95 (1H, br.pent, *J* 4.4 Hz), 2.46-2.42 (2H, m, including OH-group), 1.74-1.12 (143H, m), 0.90-0.84 (9H, including t, *J* 7.0 Hz, for 2 x CH_3 and d, *J* 7.0 Hz, for $CHCH_3$), 0.66-0.64 (2H, m), 0.57 (1H, dt, *J* 4.1, 8.2 Hz), -0.31 (1H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 176.3, 85.5, 72.3, 57.8, 51.4, 50.9, 35.7, 35.3, 32.4, 31.9, 30.5, 30.2, 30.0, 29.95, 29.9, 29.7, 29.61, 29.6, 29.58, 29.56, 29.55, 29.5, 29.4, 29.3, 28.7, 27.5, 27.4, 26.2, 25.7, 22.7, 15.7, 14.8, 14.0, 10.9; ν_{max}: 3524, 2920, 2850, 1711, 1470 cm^{-1}.

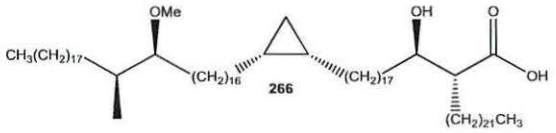
Experiment 60: (*R*)-2-{(*R*)-1-Hydroxy-18-[(1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}tetracosanoic acid (263)



Lithium hydroxide monohydrate (0.71 g, 16.6 mmol) was added to a stirred solution of **(360)** (1.5 g, 1.1 mmol) in THF (30 mL), methanol (2 mL) and water (1 mL) at r.t. The mixture was stirred at 43 °C for 24 h. It was cooled to r.t. quenched with satd. ammonium chloride (5 mL), diluted with petrol/ethyl acetate (5:1) (3 × 30 mL) and then acidified with 5% HCl. The organic layer was separated and the aqueous layer

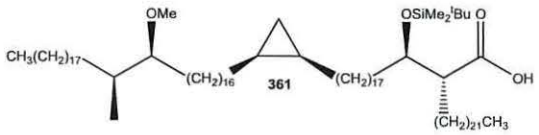
was re-extracted with petrol/ethyl acetate (5:1) (2 × 50 mL). The combined organic layers were dried, evaporated and purified by chromatography on silica eluting with warm petrol/ethyl acetate (7:2) to give title compound **(263)** as a white solid (1.1 g, 78%), m.p.: 65-67 °C, $[\alpha]_D^{23}$ = -1.9 (c 1.0, $CHCl_3$) {Found $[M+Na]^+$: 1248.2558; $C_{83}H_{164}NaO_4$ requires: 1248.2461}, which showed δ_H (400 MHz, $CDCl_3$): 3.73-3.72 (1H, m), 3.36 (3H, s), 3.0-2.8 (1H, m), 2.48-2.46 (1H, m), 1.76-1.73 (1H, m), 1.65-1.63 (2H, m), 1.53-1.12 (142H, m), 0.91-0.86 (9H, including t, *J* 6.61 Hz, for 2 x CH_3 and a doublet *J* 6.62, for $CHCH_3$), 0.65 (2H, m), 0.58 (1H, dt, *J* 3.7, 7.9 Hz), -0.32 (1H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$): 173.3, 85.6, 72.1, 57.7, 50.7, 35.6, 35.3, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.7, 29.66, 29.6, 29.5, 29.4, 29.3, 28.7, 27.6, 27.3, 26.1, 25.7, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}: 3396, 2922, 2851, 1707, 1467 cm^{-1}.

Experiment 61: (*R*)-2-[(*R*)-1-Hydroxy-18-[(1*S*,2*R*)-2((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl]-tetracosanoic acid (266)



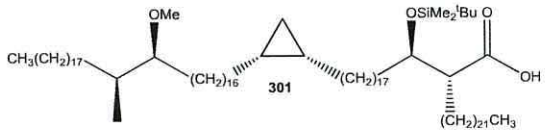
Lithium hydroxide monohydrate (0.4 g, 9.5 mmol) was added to a stirred solution of **(292)** (0.91 g, 0.72 mmol) in THF (15 mL), methanol (1.5 mL) and water (2 mL) at r.t. The mixture was stirred at 43 °C for 18 h. The reaction mixture was worked up and purified as before to give the title compound as a white solid **(266)** (0.6 g, 89%), $[\alpha]_D^{21}$ = -1.2 (c 1.0, $CHCl_3$) {MS Found $[M+Na]^+$: 1248.2558; $C_{83}H_{164}NaO_4$ requires: 1248.2461}, which showed δ_H (400 MHz, $CDCl_3$): 3.73-3.65 (1H, m), 3.37 (3H, s), 3.0-2.8 (1H, m), 2.51-2.45 (1H, m), 1.75-1.72 (1H, m), 1.64-1.61 (2H, m), 1.53-1.12 (142H, m), 0.90-0.85 (9H, including t, *J* 6.6 Hz, for 2 x CH_3 and a doublet *J* 6.6 Hz, for $CHCH_3$), 0.66-0.60 (2H, m), 0.58 (1H, dt, *J* 3.7, 7.9 Hz), -0.31 (1H, br.q, *J* 5.0 Hz); δ_C (400 MHz, $CDCl_3$): 173.4, 85.6, 72.2, 57.6, 50.7, 35.5, 35.4, 32.3, 32.0, 30.5, 30.3, 30.0, 29.9, 29.7, 29.66, 29.6, 29.5, 29.4, 29.37, 28.7, 27.5, 27.3, 26.2, 25.7, 22.7, 15.8, 14.9, 14.2, 11.0; ν_{max}: 3419, 2927, 2854, 1702, 1466 cm^{-1}.

Experiment 62: (*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methyl-hexatriacontyl)cyclopropyl]octadecyl}-tetracosanoic acid (361)



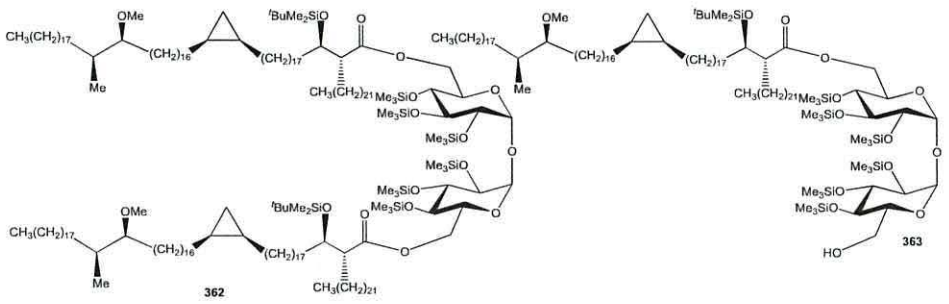
Imidazole (1.11 g, 16.3 mmol) was added to a stirred solution of **(263)** (2.0 g, 1.6 mmol) in dry DMF (6 mL) and dry toluene (10 mL) at r.t. followed by the addition of *tert*-butyldimethylsilylchloride (2.41 g, 16.0 mmol) and 4-DMAP (0.12 g, 0.81 mmol). The reaction mixture was stirred at 70 °C for 24 h and at r.t. for 8 h. The solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate (10:1, 100 mL) and water (50 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 2 × 30 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue. The residue was dissolved in THF (20 mL), water (4 mL), and methanol (4 mL), to this was added potassium carbonate (0.72 g, 5.22 mmol). The reaction mixture was stirred at 45 °C for 18 h. The mixture was diluted with petrol/ethyl acetate (10:1, 50 mL) and water (10 mL) then acidified with potassium hydrogen sulfate to pH 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (2 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (20:1), (10:1) to give title compound **(361)** as colourless oil (1.9 g, 90%), $[\alpha]_D^{19}$ = -0.7 (c 1.5 , $CHCl_3$) {MALDI Found $[M+Na]^+$: 1362.3; $C_{89}H_{178}O_4SiNa$ requires: 1362.3}; which showed δ_H (400 MHz, $CDCl_3$): 3.86-3.8 (1H, m), 3.35 (3H, s), 2.97 (1H, br.pent, *J* 4.5 Hz), 2.53 (1H, m), 1.75-1.05 (144H, m), 0.93 (9H, s), 0.9 (6H, t, *J* 5.5 Hz), 0.85 (3H, m), 0.68-0.63 (2H, m), 0.59 (1H, dt, *J* 5.1, 10.0 Hz), 0.16 (3H, s), 0.14 (3H, s), -0.31 (1H, br.q, *J* 6.0 Hz); δ_C (101 MHz, $CDCl_3$): 177.2, 85.5, 73.6, 57.7, 50.0, 35.3, 35.0, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.78 (very broad), 29.7, 29.56 29.53, 29.5, 29.42, 29.4, 29.0, 28.7, 27.6, 27.5, 26.1, 25.7, 24.6, 22.6, 17.9, 15.7, 14.8, 14.1, 10.9, -4.3, -4.9; ν_{max}: 3471 (broad, OH for the carboxylic group), 2924, 2853, 1744, 1464, 1360, 1300, 1237, 1099, 1048, 939, 836 cm^{-1}.

Experiment 63: (*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methyl-hexatriacontyl)cyclopropyl]octadecyl}-tetracosanoic acid (301)



Imidazole (0.24 g, 3.52 mmol) was added to a stirred solution of **(266)** (0.45 g, 0.35 mmol) in dry DMF (2 mL) and dry toluene (3.5 mL) at r.t. followed by the addition of *tert*-butyldimethylsilylchloride (0.5 g, 3.5 mmol) and 4-DMAP (0.04 g, 0.32 mmol). The reaction mixture was stirred at 70 °C for 24 h at r.t. The reaction mixture was worked up and purified as before to give a colourless oil residue. The residue was dissolved in THF (8 mL), water (1.5 mL), and methanol (1.3 mL), to this was added potassium carbonate (0.1 g, 1.2 mmol). The reaction mixture was stirred at 45 °C for 18 h. The reaction mixture was worked up and purified as before to give the title compound **(301)** as colourless oil (0.39 g, 90%), $[\alpha]_D^{19}$ = -0.7 (c 1.5, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1362.3; $C_{89}H_{178}O_4SiNa$ requires: 1362.3}, which showed δ_H (400 MHz, $CDCl_3$): 3.85-3.82 (1H, m), 3.35 (3H, s), 2.97 (1H, br.pent, *J* 4.5 Hz), 2.55-2.51 (1H, m), 1.63-1.06 (144H, m), 0.94 (9H, s), 0.9 (6H, t, *J* 5.5 Hz), 0.86 (3H, m), 0.65-0.63 (2H, m), 0.58 (1H, dt, *J* 5.0, 7.1 Hz), 0.16 (3H, s), 0.14 (3H, s), -0.31 (1H, br.q, *J* 6.0 Hz); δ_C (101 MHz, $CDCl_3$): 177.2, 85.4, 73.7, 57.7, 50.0, 35.2, 35.1, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.72, 29.7, 29.56, 29.53, 29.5, 29.43, 29.4, 29.0, 28.7, 27.5, 27.4, 26.1, 25.7, 25.1, 22.6, 17.9, 15.7, 14.8, 14.1, 10.9, -4.2, -4.1; ν_{max}: 3471 (broad, OH for the carboxylic group), 2924, 2853, 1744, 1464, 1360, 1300, 1237, 1099, 1048, 939, 836 cm^{-1}.

Experiment 64: 6,6'-bis-O-(*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]-octadecyl}-tetracosanoic-2,3,4,2',3',4',-hexakis-O-(trimethylsilyl)-α,α'-trehalose (362) and 6-O-(*R*)-2-{(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-18-[(1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)-cyclopropyl]-octadecyl}-tetracosanoic-2,3,4,2',3',4'-hexakis-O-(trimethylsilyl)-α,α'-trehalose (363)

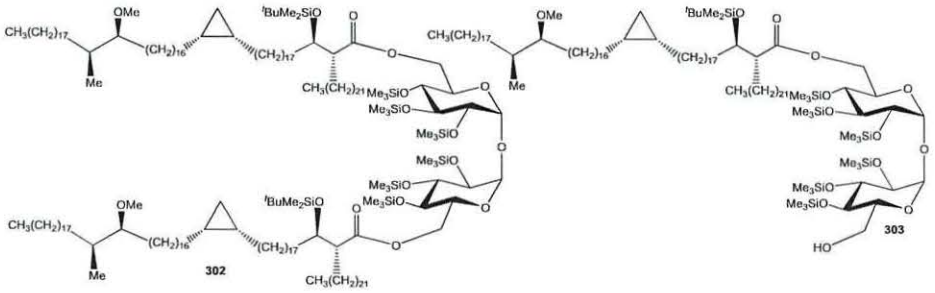


EDCI (0.9 g, 4.6 mmol) and 4-DMAP (0.5 g, 4.0 mmol) were added to a stirred solution of **(361)** (1.5 g, 1.1 mmol) and **(160)** (0.45 g, 0.58 mmol) and powdered 4 Å molecular sieves in dry dichloromethane (20 mL) at r.t. under nitrogen. The mixture was stirred for 6 days at r.t. The mixture was then diluted with dichloromethane (5 mL) and filtered. The filtrate was evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with petrol/ethyl acetate 20:1, 10:1 to give the first fraction as a colourless thick oil **(362)** (0.75 g, 62%); $[\alpha]_D^{23}$ = +20 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3442.7; $C_{208}H_{422}NaO_{17}Si_8$ requires: 3441.0}; which showed δ_H (400 MHz, $CDCl_3$): 4.85 (2H, d, *J* 3.9 Hz), 4.36 (2H, br.d, *J* 12.7 Hz), 4.04-3.97 (2H, m), 3.95 (2H, br.pent, *J* 4.2 Hz), 3.93 (2H, br.q, *J* 5.0 Hz), 3.9 (2H, t, *J* 9.0 Hz), 3.53 (2H, t, *J* 11.1 Hz), 3.39 (2H, dd, *J* 3.4, 11.6 Hz), 3.34 (6H, s), 2.96 (2H, br.pent, *J* 5.1 Hz), 2.56 (2H, m), 1.63-1.61 (4H, m), 1.52-1.01 (282H, m), 0.9 (12H, t, *J* 7.0 Hz), 0.89 (18H, s), 0.84 (6H, d, *J* 7.0 Hz), 0.66-0.63 (4H, br.m), 0.57 (2H, dt, *J* 4.5, 9.5 Hz), 0.16 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.06 (12H, s), -0.32 (2H, br.q, *J* 6.2 Hz); δ_C (101 MHz, $CDCl_3$): 173.8, 94.8, 85.4, 73.5, 73.4, 72.8, 71.8, 70.7, 62.4, 57.7, 51.9, 35.4, 33.7, 32.4, 31.9, 30.5, 30.2, 30.0, 29.98, 29.9, 29.7 (very broad), 29.6, 29.5, 29.4, 28.7, 28.1, 27.6, 26.2, 25.8, 25.2, 22.7, 18.0, 15.8, 14.9, 14.1, 10.9, 1.1, 0.9, 0.2, -4.5, -4.6; ν_{max}: 2925, 2854, 1734, 1494, 1251.6, 1077, 1050, 907, 825 cm^{-1}.

The second fraction was **(363)** (0.36 g, 30%), $[\alpha]_D^{23}$ = +43 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2118.8, $C_{119}H_{246}NaO_{14}Si_7$ requires: 2118.6}; which showed δ_H (400 MHz, $CDCl_3$): 4.91 (1H, d, *J* 3.6 Hz), 4.84 (1H, d, *J* 3.7 Hz), 4.36 (1H, dd, *J* 2.1, 14.5 Hz), 4.09 (1H, dd, *J* 5.1, 14.5 Hz), 4.01 (1H, m), 3.94 (1H, m), 3.91 (2H, dt, *J* 6.6, 9.0 Hz), 3.82 (1H, br.td, *J* 3.4, 9.5 Hz), 3.70-3.65 (2H, m), 3.50 (2H, m), 3.43 (1H, m), 3.37 (1H, m), 3.34 (3H, s), 2.96 (1H, br.pent, *J* 5.3 Hz), 2.57-2.54 (1H, m), 1.72 (1H, m), 1.62-1.56 (2H, m), 1.51-1.06 (144H, m), 0.90 (3H, t, *J* 7.0 Hz), 0.88 (9H, s), 0.85

(3H, d, *J* 7.0 Hz), 0.66-0.64 (2H, m), 0.57 (1H, dt, *J* 4.9, 9.9 Hz), 0.17 (9H, s), 0.16 (9H, s), 0.15 (9H, s), 0.15 (9H, s), 0.14 (9H, s), 0.12 (9H, s), 0.058 (3H, s), 0.054 (3H, s), -0.32 (1H, br.q, *J* 5.9 Hz); δ_C (101 MHz, $CDCl_3$): 174.1, 94.5, 94.4, 85.4, 73.43, 73.4, 72.9, 72.8, 72.7, 72.0, 71.4, 70.7, 62.4, 61.7, 57.7, 51.8, 35.4, 33.4, 32.4, 31.9, 30.5, 30.2, 30.0, 29.93, 29.9, 29.8, 29.7, 29.6 (v.br.), 29.5, 29.4, 28.7, 28.1, 27.6, 26.4, 26.2, 25.8, 24.8, 22.7, 18.0, 15.8, 14.9, 14.1, 10.8, 1.2, 1.1, 1.0, 0.9, 0.8, 0.2, 0.03, -4.5, -4.7; ν_{max}: 3436, 2926, 2856, 1733, 1494, 1252, 1215, 1076, 907 cm^{-1}.

Experiment 65: 6,6'-bis-O-(*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)-cyclopropyl]-octadecyl}-tetracosanoic-2,3,4,2',3',4',-hexakis-O-(trimethylsilyl)-α,α'-trehalose (302) and 6-O-(*R*)-2-{(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-18-[(1*S*,2*R*)-2-((17*S*,18*S*)-17-meth-oxy-18-methylhexatriacontyl)-cyclopropyl]-octadecyl}-tetracosanoic-2,3,-4,2',3'-,4'-hexakis-O-(trimethylsilyl)-α,α'-trehalose (303)

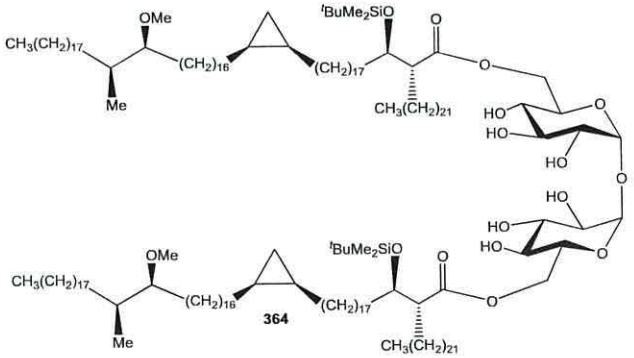


EDCI (0.14 g, 0.77 mmol) and 4-DMAP (0.08 g, 0.67 mmol) were added to a stirred solution of **(301)** (0.33 g, 0.24 mmol) and **(160)** (0.07 g, 0.09 mmol) and powdered 4 Å molecular sieves in dry dichloromethane (3.5 mL) at r.t. under nitrogen. The mixture was stirred for 6 days at r.t. The mixture was worked up and purified as before to give the first fraction as a colourless thick oil as **(302)** (0.23 g, 70%), $[\alpha]_D^{21}$ =+20 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3442.7, $C_{208}H_{422}O_{17}Si_8Na$ requires: 3441.0}, which showed δ_H (400 MHz, $CDCl_3$): 4.85 (2H, d, *J* 3.9 Hz), 4.36 (2H, br.d, *J* 12.7 Hz), 4.04-3.97 (2H, m), 3.95 (2H, br.pent, *J* 4.2 Hz), 3.93 (2H, br.q, *J* 5.0 Hz), 3.9 (2H, t, *J* 9.0 Hz), 3.53 (2H, t, *J* 11.1 Hz), 3.39 (2H, dd, *J* 3.4, 11.6 Hz), 3.34 (6H, s), 2.96 (2H, br.pent, *J* 5.1 Hz), 2.56 (2H, m), 1.63-1.61 (4H, m), 1.52-1.01 (282H, m), 0.90 (12H, t, *J* 7.0 Hz), 0.89 (18H, s), 0.84 (6H, d, *J* 7.0 Hz), 0.66-0.63 (4H, br.m), 0.57 (2H, dt, *J* 4.5, 9.5 Hz), 0.16 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.06 (12H, s), -

0.32 (2H, br.q, *J* 6.2 Hz); δ_C (101 MHz, $CDCl_3$): 173.8, 94.8, 85.4, 73.5, 73.4, 72.8, 71.7, 70.7, 61.3, 57.7, 51.9, 35.3, 33.5, 32.3, 31.9, 30.5, 30.2, 30.0, 29.98, 29.9, 29.7 (v.br.), 29.6, 29.5, 29.4, 28.7, 28.1, 27.6, 26.2, 25.8, 25.2, 22.7, 18.0, 15.8, 14.9, 14.1, 10.9, 1.1, 0.9, 0.2, -4.5, -4.6; ν_{max}: 2925, 2854, 1734, 1494, 1251.6, 1077, 1050, 907, 825 cm^{-1}.

The second fraction was **(303)** (0.1 g, 28%); $[\alpha]_D^{21}$ = +44 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2118.7; $C_{119}H_{246}O_{14}Si_7Na$ requires: 2118.7}, which showed δ_H (400 MHz, $CDCl_3$): 4.91 (1H, d, *J* 3.6 Hz), 4.84 (1H, d, *J* 3.7 Hz), 4.36 (1H, dd, *J* 2.1, 8.0 Hz), 4.09 (1H, dd, *J* 5.1, 9.0 Hz), 4.0 (1H, m), 3.94 (1H, m), 3.9 (2H, dt, *J* 6.6, 9.0 Hz), 3.82 (1H, br.td, *J* 3.4, 9.5 Hz), 3.70-3.65 (2H, m), 3.5 (2H, m), 3.43 (1H, m), 3.37 (1H, m), 3.34 (3H, s), 2.96 (1H, br.pent, *J* 5.3 Hz), 2.57-2.54 (1H, m), 1.72 (1H, m), 1.62-1.56 (2H, m), 1.51-1.06 (144H, m), 0.9 (3H, t, *J* 7.0 Hz), 0.88 (9H, s), 0.85 (3H, d, *J* 7.0 Hz), 0.66-0.64 (2H, m), 0.57 (1H, dt, *J* 4.9, 9.8 Hz), 0.17 (9H, s), 0.16 (9H, s), 0.15 (9H, s), 0.15 (9H, s), 0.14 (9H, s), 0.12 (9H, s), 0.058 (3H, s), 0.054 (3H, s), -0.32 (1H, br.q, *J* 5.9 Hz); δ_C (101 MHz, $CDCl_3$): 174.1, 94.5, 94.4, 85.4, 73.5, 73.4, 72.9, 72.8, 72.7, 72.0, 71.4, 70.7, 62.4, 61.7, 57.7, 51.8, 35.4, 33.4, 32.4, 31.9, 30.5, 30.2, 30.0, 29.93, 29.9, 29.8, 29.7, 29.6 (v.br.), 29.5, 29.4, 28.7, 28.1, 27.6, 26.4, 26.2, 25.8, 24.8, 22.7, 18.0, 15.8, 14.9, 14.1, 10.8, 1.1, 1.0, 0.9, 0.8, 0.2, 0.03, -4.5, -4.7; ν_{max}: 3436, 2926, 2856, 1733, 1494, 1252, 1215, 1076, 907 cm^{-1}.

Experiment 66: 6,6'-bis-O-(*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*R*,-2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}-tetracosanoic-α,α'-trehalose (364)



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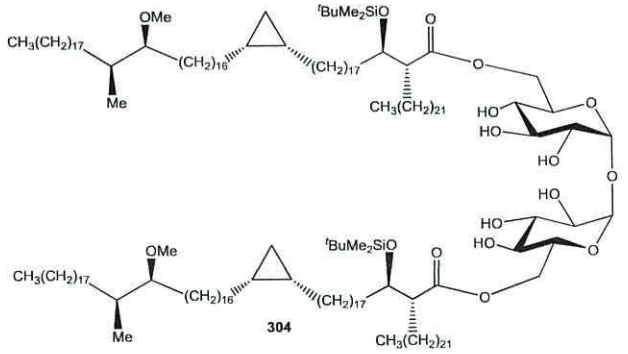
Tetrabutylammonium fluoride (0.3 mL, 0.3 mmol, 1.0 M) was added to a stirred solution of **(362)** (0.36 g, 0.10 mmol) in dry THF (10 mL) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 1 h. The reaction was

cooled to 5 °C and quenched with sat. aq. sodium bicarbonate (3 mL) then diluted with cold $CHCl_3$ (50 mL). The organic layer was separated and the aqueous layer was re-extracted with $CHCl_3$ (2 × 50 mL). The combined organic layers were washed with brine (50 mL), dried and evaporated to give a residue, which was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH 10:1 to give the title compound as a colourless thick oil **(364)** (0.27 g, 87%), $[\alpha]_D^{23}$ = +10 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3009.9; $C_{190}H_{376}NaO_{17}Si_2$ requires: 3009.7}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.91 (2H, d, *J* 3.0 Hz), 4.13 (2H, br.m), 4.1 (2H, br. m), 3.96-3.92 (2H, m), 3.78 (2H, br.t, *J* 13.4 Hz), 3.66-3.58 (2H, m), 3.38-3.18 (8H, including a singlet for the methoxy groups resonated at 3.30), 2.90-2.83 (2H, m), 2.45-2.34 (2H, m), 1.5-1.05 (292H, m), 0.80 (12H, m), 0.78 (18H, s), 0.73 (6H, m), 0.54-0.46 (4H, m), 0.41 (2H, dt, *J* 5.2, 9.6 Hz), -0.10 (6H, s), -0.12 (6H, s), -0.4 (2H, br.q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.0, 93.3, 85.5, 73.1, 72.9, 71.6, 70.2, 70.0, 62.8, 57.4, 51.5, 35.2, 33.6, 32.3, 31.9, 31.2, 30.4, 30.3, 30.2, 30.1, 30.0, 29.87, 29.82, 29.74, 29.7, 29.6, 29.53, 29.5, 29.41, 29.4, 29.3, 28.7, 27.8, 27.6, 27.0, 26.0, 25.8, 25.7, 24.2, 22.7, 18.2, 15.7, 14.7, 14.2, 10.9, -4.4, -4.8; ν_{max}: 3436, 2925, 2853, 1734, 1456, 1252, 1100, 1078, 992, 874, 825, 760, 720, 672 cm^{-1}.

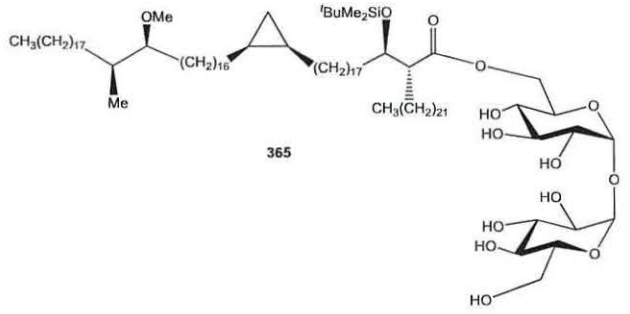
Experiment 67: 6,6'-bis-O-(*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*S*,-2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}-tetracosanoic-α,α'-trehalose (304)



Tetrabutylammonium fluoride (0.2 mL, 0.2 mmol, 1.0 M) was added to a stirred solution of **(302)** (0.23 g, 0.06 mmol) in dry THF (10 mL) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 1 h. The reaction mixture was worked up and purified as before to give the title compound **(304)** as a

colourless thick oil (0.17 g, 90%), $[\alpha]_D^{23}$ = +11 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3009.9; $C_{190}H_{376}O_{17}Si_2Na$ requires: 3009.7}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.01 (2H, d, *J* 3.0 Hz), 4.28 (2H, br.dd, *J* 5.9, 15.7 Hz), 4.2 (2H, br.d, *J* 13.6 Hz), 3.89-3.84 (2H, m), 3.72 (2H, br.t, *J* 11.4 Hz), 3.44-3.43 (2H, m), 3.38-3.20 (8H, including a singlet for the methoxy groups resonated at 3.3), 2.92-2.91 (2H, m), 2.50-2.46 (2H, m), 1.5-1.03 (292H, m), 0.81 (12H, m), 0.79 (18H, s), 0.78 (6H, m), 0.57-0.51 (4H, m), 0.49 (2H, dt, *J* 5.2, 9.6 Hz), 0.008 (6H, s), -0.02 (6H, s), -0.40 (2H, br.q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.0, 93.3, 85.5, 73.1, 72.9, 71.6, 70.3, 70.0, 62.8, 57.6, 51.6, 35.2, 33.6, 32.3, 31.9, 31.2, 30.4, 30.3, 30.2, 30.1, 30.0, 29.87, 29.8, 29.75, 29.7, 29.6, 29.56, 29.5, 29.47, 29.4, 29.3, 28.7, 27.8, 27.6, 27.0, 26.0, 25.8, 25.7, 24.2, 22.7, 18.2, 15.7, 14.7, 14.2, 10.9, -4.4, -4.7; ν_{max}: 3436, 2925, 2853, 1734, 1456, 1252, 1100, 1078, 992, 874, 825, 760, 720, 672 cm^{-1}.

Experiment 68: 6-O-(*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*R*,2*S*)-2-((-17*S*,18*S*)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl]-octadecyl}tetra-cosanoic-α,α'-trehalose (365)

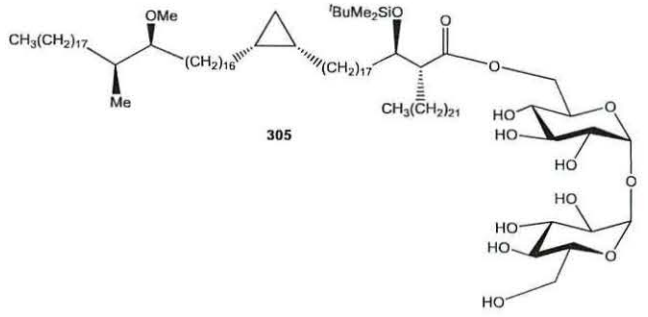


365

Tetrabutylammonium fluoride (0.3 mL, 0.3 mmol, 1.0 M) was added to a stirred solution of **(363)** (0.20 g, 0.09 mmol) in dry THF (7 mL) at 5 °C under nitrogen. The mixture was allowed to reach r.t. then stirred for 1 h. The reaction mixture was worked up and purified as before to give the title compound as a colourless syrup **(365)** (0.13 g, 82%), $[\alpha]_D^{24}$ = +27 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1686.9; $C_{101}H_{198}NaO_{14}Si$ requires: 1686.4], which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.01 (2H, d, *J* 3.2 Hz), 4.32-4.24 (2H, m), 3.94 (1H, br.d, *J* 12.7 Hz), 3.90 (3H, br.t, *J* 10.5 Hz), 3.82-3.80 (2H, br.m), 3.69 (1H, br.d, *J* 8.5 Hz), 3.50 (2H, br.d, *J* 11.6 Hz), 3.37 (2H, br.d, *J* 10.8 Hz), 3.3 (3H, s), 2.94 (1H, br.pent, *J* 5.2 Hz), 2.50 (1H, m), 1.59-1.02 (150, m), 0.87 (6H, t, *J* 8.1 Hz), 0.83 (9H, s), 0.8 (3H, d, *J* 6.1 Hz), 0.62-

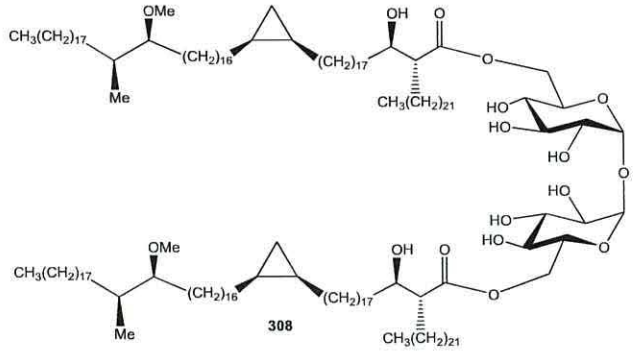
0.60 (2H, m), 0.54 (1H, dt, *J* 4.9, 9.5 Hz), 0.02 (3H, s), -0.00 (3H, s), -0.35 (1H, br.q, *J* 6.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 93.5, 93.4, 85.5, 73.1 (broad), 72.9, 72.6, 72.1, 71.6, 70.7, 70.1, 69.8, 62.7, 62.0, 57.3, 51.5, 35.1, 33.4, 29.7, 29.65, 29.6, 29.54, 29.5, 29.2, 28.5, 27.5, 27.3, 26.8, 26.0, 25.5, 24.0, 22.5, 17.7, 15.5, 14.6, 13.8, 10.6, -4.7, -5.1; ν_{max}: 3400, 2926, 2854, 1716, 1464, 1078, 824, 760 cm^{-1}.

Experiment 69: 6-O-(*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-18-[(1*S*,2*R*)-2-(-(17*S*,18*S*)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl]-octadecyl}-tetracosanoic-*α*,*α*'-trehalose (305)



Tetrabutylammonium fluoride (0.2 mL, 0.2 mmol, 1.0 M) was added to a stirred solution of **(303)** (0.09 g, 0.04 mmol) in dry THF (6 mL) at 5 °C under nitrogen. The mixture was allowed to reach r.t. and then stirred for 1 h. The reaction mixture was worked up and purified as before to give the title compound as a colourless syrup **(305)** (0.04 g, 60%), $[\alpha]_D^{24}$ = +28 (c 1.00, $CHCl_3$) {Found Maldi $[M+Na]^+$: 1686.1; $C_{101}H_{198}NaO_{14}Si$ requires: 1686.4}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.0 (2H, d, *J* 3.2 Hz), 4.32-4.24 (2H, m), 3.94 (1H, br.d, *J* 12.7 Hz), 3.9 (3H, br.t, *J* 10.5 Hz), 3.82-3.80 (2H, br.m), 3.69 (1H, br.d, *J* 8.5 Hz), 3.5 (2H, br.d, *J* 11.6 Hz), 3.35 (2H, br.d, *J* 10.8 Hz), 3.30 (3H, s), 2.94 (1H, br.pent, *J* 5.2 Hz), 2.54-2.49 (1H, m), 1.5-1.02 (150, m), 0.86 (6H, t, *J* 8.1 Hz), 0.83 (9H, s), 0.8 (3H, d, *J* 6.0 Hz), 0.62-0.55 (2H, m), 0.54 (1H, dt, *J* 4.9, 9.5 Hz), 0.02 (3H, s), -0.004 (3H, s), -0.35 (1H, br.q, *J* 6.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 93.5, 93.4, 85.5, 73.1 (broad), 72.9, 72.6, 72.1, 71.6, 70.7, 70.2, 69.9, 62.7, 62.0, 57.5, 51.6, 35.2, 33.5, 29.68, 29.65, 29.6, 29.53, 29.5, 29.2, 28.6, 27.6, 27.4, 26.9, 26.0, 25.6, 24.2, 22.6, 17.8, 15.6, 14.7, 13.9, 10.8, -4.6, -5.0; ν_{max}: 3400, 2926, 2854, 1716, 1464, 1078, 824, 760 cm^{-1}.

Experiment 70: ((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-Oxybis(3,4,5-trihydroxytetra-hydrdro-2*H*-pyran-6,2-diyl))bis(methylene)(2*R*,2'*R*)-*bis*(2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)-octadecyl)tetrac-osanoate) (308)

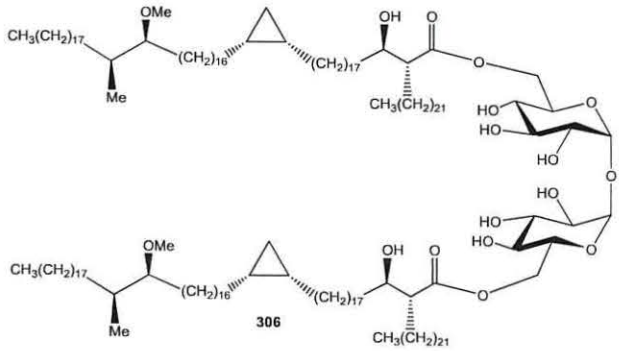


308

A dry polyethylene vial equipped with a rubber septum was charged with **(364)** (0.27 g, 0.09 mmol) and pyridine (0.40 mL) in dry THF (20 mL) was stirred at r.t. under nitrogen atmosphere. HF-pyridine (2 mL) was then added. The mixture was stirred at 43 °C for 17 h, then the reaction mixture was cooled to r.t. and slowly poured it into sat. aq. sodium bicarbonate until no more CO_2 was liberated. The product was extracted with chloroform (3 × 50 mL), then the combined organic layers were dried and evaporated to give a residue which was purified by column chromatography eluting with $CHCl_3$/MeOH 10:1 to give the title compound as a syrup **(308)** (0.016 g, 70%), $[\alpha]_D^{23}$ = +31 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2779.6; $C_{178}H_{346}NaO_{17}$ requires: 2779.6}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.0 (2H, d, *J* 3.5 Hz), 4.80 (2H, br.d, *J* 13.6 Hz), 4.34 (2H, br.t, *J* 12.1 Hz), 3.87 (2H, br.q, *J* 11.9 Hz), 3.78 (2H, t, *J* 8.1 Hz), 3.69 (2H, br. m), 3.53 (2H, dd, *J* 4.4, 11.8 Hz), 3.28 (6H, s), 3.21 (2H, t, *J* 11.9 Hz), 2.95 (2H, br.pent, *J* 5.0 Hz), 2.43-2.37 (2H, m), 1.58-1.05 (294H, m), 0.87 (12H, t, *J* 8.1 Hz), 0.83 (6H, d, *J* 8.9 Hz), 0.64-0.62 (4H, m), 0.56 (2H, dt, *J* 4.7, 9.7 Hz), -0.33 (2H, br.q, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.2, 94.6, 85.4, 72.4, 71.2, 71.1, 69.7, 64.1, 57.4, 52.2, 35.2, 34.5, 32.2, 31.7, 30.3, 30.1, 30.0, 29.7, 29.62, 29.6, 29.57, 29.55, 29.5, 29.4, 29.3, 29.2, 29.1, 28.5, 27.3, 27.2, 25.9, 25.0, 22.5, 15.5, 14.4, 13.7, 10.6; ν_{max}: 3436, 2922, 2852, 1721, 1466, 1098, 734 cm^{-1}.

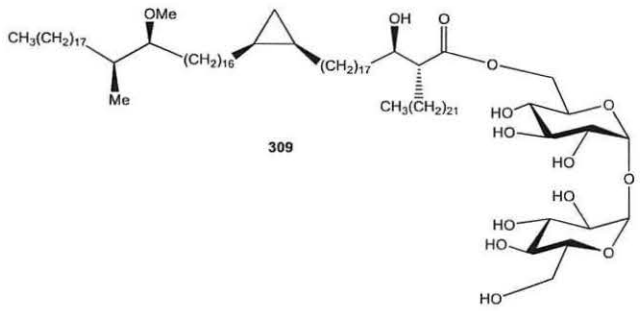
Experiment 71: ((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-Oxybis(3,4,5-trihydroxytetra-hydro-2*H*-pyran-6,2-diyl))bis(methylene)(2*R*,2'*R*)-bis(2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)-

octadecyl)tetracosanoate) (306)



A dry polyethylene vial equipped with a rubber septum was charged with **(304)** (0.17 g, 0.05 mmol) and pyridine (0.2 mL) in dry THF (20 mL) was stirred at r.t. under nitrogen. HF-pyridine (1 mL) was added. The mixture was stirred at 43 °C for 17 h. The reaction mixture was worked up and purified as before to give the title compound as a syrup **(306)** (0.13 g, 86%), $[\alpha]_D^{23}$ = +31 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2779.6; $C_{178}H_{346}O_{17}Na$ requires: 2779.6], which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.96 (2H, d, *J* 4.0 Hz), 4.64 (2H, br.d, *J* 13.3 Hz), 4.22 (2H, br.t, *J* 12.8 Hz), 3.94 (2H, br.q, *J* 9.2 Hz), 3.72 (2H, t, *J* 11.4 Hz), 3.62 (2H, br.m), 3.42 (2H, dd, *J* 4.4, 12.5 Hz), 3.29 (6H, s), 3.19 (2H, t, *J* 11.9 Hz), 2.93 (2H, br.pent, *J* 5.3 Hz), 2.39-2.33 (2H, m), 1.57-1.02 (294H, m), 0.83 (12H, t, *J* 8.1 Hz), 0.79 (6H, d, *J* 8.9 Hz), 0.60-0.59 (4H, m), 0.52 (2H, dt, *J* 4.8, 9.7 Hz), -0.37 (2H, br.q, *J* 6.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.4, 94.9, 85.5, 72.4, 71.1, 71.1, 69.7, 64.4, 57.4, 52.2, 35.2, 34.6, 32.2, 31.7, 30.8, 30.5, 30.0, 29.7, 29.65, 29.6, 29.58, 29.55, 29.5, 29.4, 29.3, 29.2, 29.0, 28.5, 27.3, 27.1, 25.9, 25.1, 22.5, 15.6, 14.6, 13.9, 10.7; ν_{max}: 3436, 2922, 2852, 1721, 1466, 1098, 734 cm^{-1}.

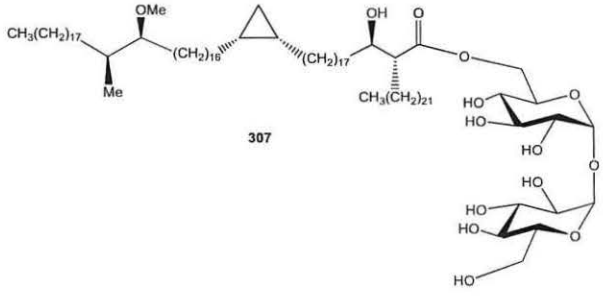
Experiment 72: ((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylh-exatriacontyl)cyclopropyl)octadecyl)tetracosanoate (309)



309

A dry polyethylene vial equipped with a rubber septum was charged with **(365)** (0.13 g, 0.07 mmol) and pyridine (0.2 mL) in dry THF (15 mL) and stirred at r.t. under nitrogen atmosphere. HF-pyridine (1 mL) was added. The mixture was stirred at 43 °C for 17 h., then it was worked up and purified as before to give a title compound **(309)** as a syrup (0.09 g, 73%), $[\alpha]_D^{23}$ = +49 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1573.3, $C_{95}H_{184}O_{14}Na$ requires: 1573.3}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.97 (1H, br.d, *J* 4.2 Hz), 4.93 (1H, br.d, *J* 4.2 Hz), 4.49 (1H, br.d, *J* 10.1 Hz), 4.08-4.06 (1H, br.m), 4.01-3.97 (1H, br.m), 3.94-3.89 (4H, m), 3.73-3.66 (2H, m), 3.6 (1H, br.d, *J* 7.9 Hz), 3.5 (1H, br.d, *J* 7.5 Hz), 3.37-3.27 (4H, including a singlet (OMe) resonated at 3.31), 3.22 (1H, br.t, *J* 12.2 Hz), 2.88-2.85 (1H, m), 2.32-2.27 (1H, m), 1.77-1.73 (2H, m), 1.45-1.02 (149H, m), 0.76 (6H, t, *J* 7.7 Hz), 0.72 (3H, d, *J* 8.3 Hz), 0.63-0.52 (2H, m), 0.44 (1H, dt, *J* 4.7, 10.2 Hz), -0.44 (1H, br.q, *J* 6.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.3, 94.2, 85.5, 72.3 (broad), 72.3, 71.3, 71.1, 71.0, 70.7, 70.5, 70.0, 64.0, 62.0, 57.4, 52.3, 35.0, 34.4, 32.1, 31.7, 30.4, 30.1, 29.96, 29.9, 29.7, 29.65, 29.6, 29.5, 29.3, 29.2, 28.5, 27.2, 27.0, 26.0, 25.2, 22.9, 15.4, 14.4, 13.7, 10.5; ν_{max}: 3400, 2924, 2853, 1721, 1466, 993 cm^{-1}.

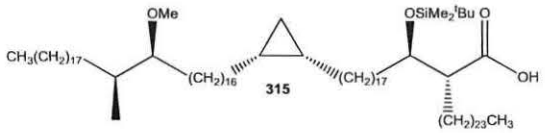
Experiment 73: ((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)-methyl(2*R*)-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhex-atriacontyl)cyclopropyl)octadecyl)tetracosanoate (307)



307

A dry polyethylene vial equipped with a rubber septum was charged with **(305)** (0.04 g, 0.02 mmol) and pyridine (0.15 mL) in dry THF (10 mL) and stirred at r.t. under nitrogen atmosphere. HF-pyridine (0.7 mL) was then added. The mixture was stirred at 43 °C for 17 h, then it was worked up and purified as before to give the title compound **(307)** as a syrup (0.02 g, 50%), $[\alpha]_D^{23}$ = +49 (c 1.00, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1573.3; $C_{95}H_{184}O_{14}Na$ requires: 1573.3}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.11 (1H, br.s), 5.02 (1H, br.s), 4.72 (1H, br.d, *J* 10.1 Hz), 4.26 (1H, br.s), 3.97-3.92 (1H, br.m), 3.88-3.76 (4H, m), 3.65-3.61 (2H, m), 3.51 (1H, br.d, *J* 4.0 Hz), 3.49 (1H, br.d, *J* 4.1 Hz), 3.31-3.27 (4H, including a singlet (OMe) resonated at 3.3), 3.22 (1H, br.t, *J* 12.2 Hz), 2.96-2.95 (1H, m), 2.37 (1H, m), 1.58-1.57 (2H, m), 1.35-1.0 (149H, m), 0.86 (6H, t, *J* 7.6 Hz), 0.83 (3H, d, *J* 8.7 Hz), 0.62-0.58 (2H, m), 0.55 (1H, dt, *J* 4.5, 10.5 Hz), -0.34 (1H, br.q, *J* 4.5 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.4, 94.5, 85.5, 72.5 (broad), 72.5, 72.3, 71.4, 71.2, 71.0, 70.8, 70.0, 64.2, 62.0, 57.5, 52.3, 35.2, 34.6, 32.2, 31.8, 30.4, 30.1, 29.83, 29.8, 29.7, 29.65, 29.6, 29.5, 29.3, 29.2, 28.6, 27.4, 27.2, 26.0, 25.1, 22.5, 15.6, 14.7, 13.9, 10.7; ν_{max}: 3400, 2924, 2853, 1721, 1466, 993 cm^{-1}.

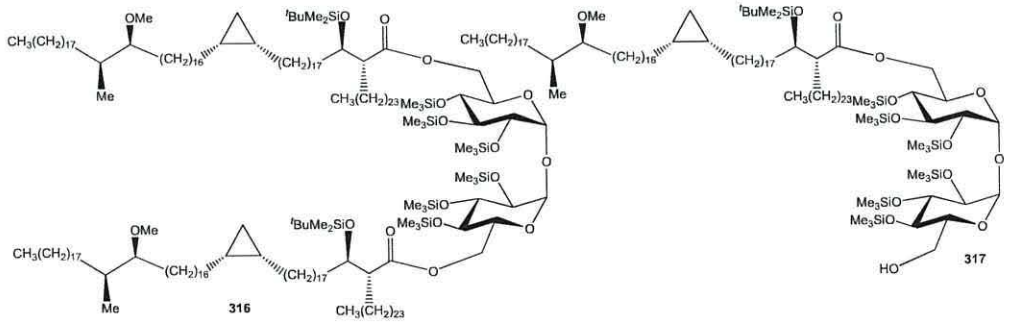
Experiment 74: (*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-18-((1*S*,2*R*)-2-((17*S*,-18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoic-acid (315)



Imidazole (0.27 g, 3.97 mmol) was added to a stirred solution of **(310)** (0.51 g, 0.39 mmol) in dry DMF (2 mL) and dry toluene (3.5 mL) at r.t. followed by the addition of *tert*-butyldimethylsilylchloride (0.61 g, 4.00 mmol) and 4-DMAP (0.048 g, 0.391 mmol). The reaction mixture was stirred at 70 °C for 24 h, and at r.t. The solvent was removed under high vacuum and the residue was diluted with petrol/ ethyl acetate (10:1, 100 mL) and water (50 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 2 × 30 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue. The residue was dissolved in THF (8 mL), water (1.3 mL), and methanol (1.5 mL), then potassium carbonate (0.24 g, 1.73 mmol) was added. The reaction mixture was stirred at 45 °C for 18 h. The mixture was diluted with petrol/ethyl acetate (10:1,

50 mL) and water (10 mL) then acidified with potassium hydrogen sulfate to pH 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (2 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (20:1), (10:1) to give the title compound **(315)** as a colourless oil (0.5 g, 92%). $[\alpha]_D^{24}$ = -1.0 (c 1.0, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1390.2; $C_{91}H_{182}O_4SiNa$ requires:1390.3}, which showed δ_H (400 MHz, $CDCl_3$): 3.87-3.82 (1H, m), 3.34 (3H, s), 2.96 (1H, br.pent, *J* 4.5 Hz), 2.58-2.52 (1H, m), 1.73-1.10 (148H, m), 0.91 (9H, s), 0.89 (6H, t, *J* 6.6 Hz), 0.86 (3H, m), 0.67-0.63 (2H, m), 0.58 (1H, dt, *J* 4.2, 8.2 Hz), 0.16 (3H, s), 0.14 (3H, s), -0.31 (1H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 177.2, 85.4, 73.7, 57.7, 50.1, 35.2, 35.1, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.72, 29.71, 29.7, 29.54, 29.5, 29.45, 29.4, 29.0, 28.7, 27.5, 27.4, 26.1, 25.7, 25.1, 22.6, 18.0, 15.7, 14.8, 14.1, 10.9, -4.2, -4.1; ν_{max}: 3676 (broad, OH for the carboxylic group), 2925, 2853, 1708, 1465, 1361, 1301, 1254, 1098, 1050, 907, 835 cm^{-1}.

Experiment 75: (*S*,*S*,*R*,*S*,*R*,2*R*,2'*R*)-((2*R*,2'*R*,3*R*,3'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-6,6'-Oxybis(3,4,5*tris*((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-6,2diyl))bis(methylene)-bis(2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate) (316) and (*R*)-((2*R*,3-*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4*S*,5*R*,6*R*)-6-(hydroxymethyl)-3,4,5tris((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)-3,4,5-tris((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-18-((1*S*,2*R*)-2-((17-((17*S*,1-8*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)-hexacosanoate (317)



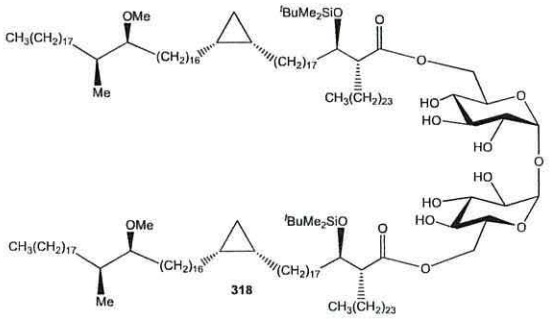
EDCI (0.29 g, 1.51 mmol) and 4-DMAP (0.16 g, 1.31 mmol) were added to a stirred solution of **(315)** (0.50 g, 0.36 mmol) and **(160)** (0.16 g, 0.22 mmol) and powdered 4 Å

molecular sieves in dry dichloromethane (5 mL) at r.t. under nitrogen. The mixture was stirred for 6 days at r.t. The mixture was then diluted with dichloromethane (5 mL). The mixture was evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with petrol/ethyl acetate 50:1, 20:1, 10:1 to give, the first fraction **(316)** as a colourless thick oil (0.25 g, 70%), $[\alpha]_D^{20}$ = +17 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3496.4; $C_{212}H_{430}NaO_{17}Si_8$ requires: 3496.0}, which showed δ_H (400 MHz, $CDCl_3$): 4.85 (2H, d, *J* 2.9 Hz), 4.37 (2H, br.d, *J* 10.4 Hz), 4.04-3.97 (4H, m), 3.95-3.88 (4H, m), 3.53 (2H, t, *J* 8.8 Hz), 3.39 (2H, dd, *J* 2.4, 8.9 Hz), 3.34 (6H, s), 2.97 (2H, br.pent, *J* 4.0 Hz) 2.57-2.53 (2H, m), 1.64-1.58 (4H, m), 1.52-1.01 (290H, m), 0.89 (12H, br.t, *J* 6.5 Hz), 0.88 (18H, s), 0.85 (6H, d, *J* 6.8 Hz), 0.66-0.64 (4H, br.m), 0.58 (2H, dt, *J* 3.8, 7.7 Hz), 0.16 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.06 (12H, s), -0.31 (2H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 173.8, 94.8, 85.4, 73.5, 73.4, 72.8, 71.8, 70.7, 62.3, 57.7, 51.8, 35.3, 33.4, 32.3, 31.9, 30.4, 30.2, 30.0, 29.9, 29.7, 29.5, 29.3, 28.7, 28.1, 27.5, 26.1, 26.0, 25.8, 25.6, 25.1, 22.6, 18.0, 15.7, 14.8, 14.1, 10.9, 1.9, 1.0, 0.9, 0.1, -4.5, -4.6; ν_{max}: 2925, 2854, 1734, 1494, 1251.6, 1077, 1050, 907, 825 cm^{-1}.

The second fraction was **(317)** (0.16 g, 30%), $[\alpha]_D^{19}$ = +45 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2147.2; $C_{121}H_{250}NaO_{14}Si_7$ requires: 2147.7}, which showed δ_H (400 MHz, $CDCl_3$): 4.91 (1H, d, *J* 3.0 Hz), 4.84 (1H, d, *J* 2.9 Hz), 4.36 (1H, dd, *J* 2.0, 11.8 Hz), 4.09 (1H, dd, *J* 4.0, 11.8 Hz), 4.01-3.97 (1H, m), 3.95-3.87 (4H, m), 3.86 (1H, t, *J* 3.4 Hz), 3.83 (1H, t, *J* 3.3 Hz), 3.71-3.67 (1H, m), 3.51-3.45 (2H, m), 3.44-3.37 (2H, m), 3.34 (3H, s), 2.97 (1H, br.pent, *J* 4.1 Hz), 2.58-2.53 (1H, m), 1.73-1.70 (1H, m), 1.64-1.59 (2H, m), 1.39-1.26 (148H, m), 0.90-0.84 (15H, including s at 0.88), 0.66-0.64 (2H, m), 0.58 (1H, dt, *J* 3.8, 7.6 Hz), 0.17 (9H, s), 0.16 (9H, s), 0.15 (9H, s), 0.14 (9H, s), 0.12 (9H, s), 0.09 (9H, s), 0.07 (3H, s), 0.06 (3H, s), -0.31 (1H, br.q, *J* 5.01 Hz); δ_C (101 MHz, $CDCl_3$): 174.0, 94.5, 94.3, 85.4, 73.4, 73.3, 72.87, 72.81, 72.7, 71.9, 71.4, 70.7, 62.4, 61.6, 57.7, 51.8, 35.3, 33.4, 32.3, 32.0, 30.4, 30.2, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.3, 28.7, 28.1, 27.5, 26.3, 26.1, 25.8, 24.8, 22.6, 18.0, 15.7, 14.8, 14.1, 10.9, 1.9, 1.05, 1.01, 0.9, 0.8, 0.1, 0.0, -4.4, -4.6; ν_{max}: 3436, 2926, 2856, 1733, 1494, 1252, 1215, 1076, 907 cm^{-1}.

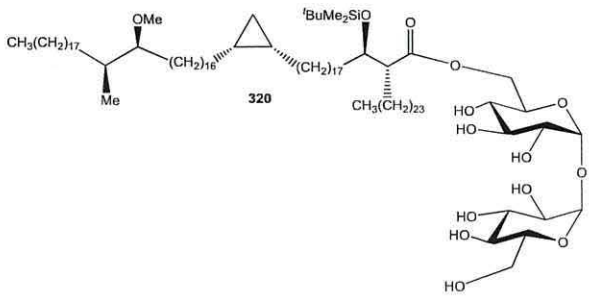
Experiment 76:(*S*,*S*,*R*,*S*,*R*,2*R*,2'*R*)-((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-6,6'-Oxybis(3,4,5-trihydroxytetrahydro-2*H*-pyran-6,2-diyl))bis(methylene)bis(2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexa-

triacontyl)cyclopropyl)octadecyl)hexacosanoate) (318)



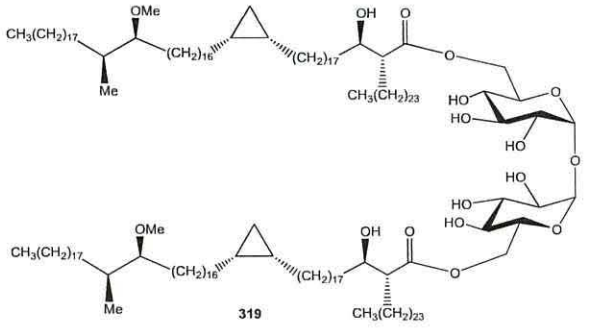
Tetrabutylammonium fluoride (0.2 mL, 0.2 mmol, 1.0 M) was added to a stirred solution of **(316)** (0.23 g, 0.06 mmol) in dry THF (10 mL) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 1 h. The reaction was cooled to 5 °C and quenched with sat. aq. sodium bicarbonate (3 mL) then diluted with cold $CHCl_3$ (50 mL). The organic layer was separated and the aqueous layer was re-extracted with $CHCl_3$ (2 × 50 mL). The combined organic layers were washed with brine (50 mL), dried and evaporated to give a residue, which was purified by column chromatography on silica eluting with $CHCl_3$/MeOH 10:1 to give title compound **(318)** as a colourless thick oil (0.2 g, 90%), $[\alpha]_D^{20}$ = +12 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3063.8; $C_{194}H_{382}NaO_{17}Si_2$ requires: 3063.8}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.26 (2H, d, *J* 3.2 Hz), 4.32 (2H, br.dd, *J* 3.7, 11.9 Hz), 4.20 (2H, br.d, *J* 11.7 Hz), 3.92-3.86 (4H, m), 3.76 (2H, br.t, *J* 9.6 Hz), 3.46 (2H, dd, *J* 3.5, 9.6 Hz), 3.35-3.33 (2H, m), 3.29 (6H, s), 2.92-2.90 (2H, m), 2.52-2.48 (2H, m), 1.32-1.09 (298H, m), 0.86-0.84 (12H, m), 0.83 (18H, s), 0.81-0.79 (6H, m), 0.61-0.58 (4H, m), 0.53 (2H, dt, *J* 3.76, 7.48 Hz), 0.00 (6H, s), -0.02 (6H, s), -0.36 (2H, br.q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 93.5, 85.5, 73.1, 73.0, 72.5, 71.6, 70.2, 69.8, 67.8, 62.8, 57.5, 51.6, 35.2, 33.5, 32.2, 31.8, 30.3, 30.1, 29.8, 29.7, 29.6, 29.5, 29.2, 28.6, 27.6, 27.4, 27.1, 26.9, 25.9, 25.7, 25.6, 25.46, 25.43, 24.1, 22.5, 17.8, 15.6, 14.6, 13.9, 10.7, -4.6, -5.0; ν_{max}: 3436, 2925, 2853, 1734, 1456, 1252, 1100, 1078, 992, 874, 825, 760, 720, 672 cm^{-1}.

Experiment 77: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (320)



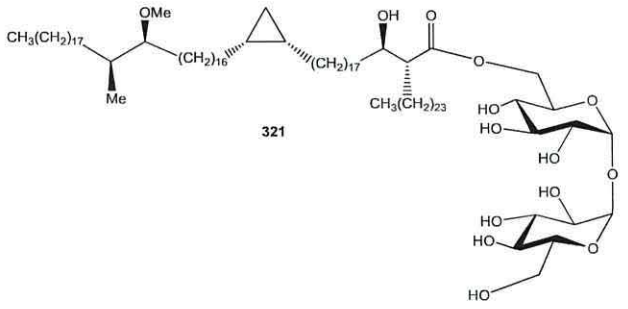
Tetrabutylammonium fluoride (0.04 mL, 0.04 mmol, 1.00 M) was added to a stirred solution of **(317)** (0.03 g, 0.01 mmol) in dry THF (6 mL) at 5 °C under nitrogen. The mixture was allowed to reach r.t. then stirred for 1 h. The mixture was evaporated and purified by column chromatography on silica eluting with $CHCl_3$/MeOH 10:1 to give the title compound **(320)** as a colourless syrup (0.02 g, 88%), $[\alpha]_D^{20}$ = +30 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 1714.6; $C_{103}H_{202}NaO_{14}Si$ requires: 1714.4}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.03 (2H, d, *J* 3.1 Hz), 4.27-4.21 (2H, m), 3.92 (1H, br.d, *J* 9.7 Hz), 3.85 (3H, br.t, *J* 8.4 Hz), 3.79-3.76 (2H, br.m), 3.65 (1H, br.q, *J* 5.5 Hz), 3.46 (2H, br.d, *J* 9.9 Hz), 3.32 (2H, br.d, *J* 9.7 Hz), 3.28 (3H, s), 2.97-2.90 (1H, m), 2.51-2.46 (1H, m), 1.27-1.19 (150, m), 0.82 (6H, t, *J* 6.4 Hz), 0.79 (9H, s), 0.77 (3H, d, *J* 4.9 Hz), 0.59-0.57 (2H, m), 0.51 (1H, dt, *J* 3.8, 7.7 Hz), -0.01 (3H, s), -0.03 (3H, s), -0.38 (1H, br.q, *J* 4.9 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 93.5, 93.3, 85.3, 72.9, 72.7, 72.3, 71.9, 71.3, 70.4, 70.0, 69.6, 62.4, 61.7, 57.3, 51.7, 51.3, 34.9, 33.2, 31.9, 31.5, 30.1, 29.8, 29.6, 29.5, 29.48, 29.4, 29.36, 29.35, 29.3, 29.2, 29.0, 28.3, 27.4, 27.1, 26.6, 25.7, 25.3, 24.8, 23.8, 22.3, 19.6, 15.4, 14.4, 13.7, 13.1, 10.5, -4.6, -5.0; ν_{max}: 3400, 2926, 2854, 1716, 1464, 1078, 824, 760 cm^{-1}.

Experiment 78: (*S*,*S*,*R*,*S*,*R*,2*R*,2'*R*)-((2*R*,2'*R*,4*S*,4'*S*,5*R*,5'*R*,6*R*,6'*R*)-6,6'-Oxybis(3,-4,5-trihydroxytetrahydro-2*H*-pyran-6,2-diyl))bis(methylene)bis(2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)-octadecyl)hexacosanoate) (319)



A dry polyethylene vial equipped with a rubber septum was charged with **(318)** (0.17 g, 0.05 mmol) and pyridine (0.15 mL) in dry THF (15 mL) was stirred at r.t. under nitrogen atmosphere. HF-pyridine (1 mL) was then added. The mixture was stirred at 43 °C for 17 h, then it was neutralized by pouring it slowly into sat. aq. sodium bicarbonate until no more CO_2 was liberated. The product was extracted with chloroform (3 × 50 mL), then the combined organic layers were dried and evaporated to give a residue which was purified by column chromatography eluting with $CHCl_3$/MeOH 10:1 to give title compound **(319)** as a syrup (0.1 g, 63%), $[\alpha]_D^{23} = +27$ (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2835.7; $C_{182}H_{354}NaO_{17}$ requires: 2835.6}; which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.0 (2H, d, *J* 3.0 Hz), 4.8 (2H, br.d, *J* 11.8 Hz), 4.34 (2H, br.t, *J* 8.3 Hz), 3.89-3.84 (2H, m), 3.79 (2H, t, *J* 10.0 Hz), 3.71-3.67 (2H, br.m), 3.53 (2H, dd, *J* 2.3, 11.2 Hz), 3.32 (6H, s), 3.21 (2H, t, *J* 9.5 Hz), 2.96 (2H, br.pent, *J* 4.2 Hz), 2.43-2.38 (2H, m), 1.39-1.10 (302H, m), 0.88 (12H, t, *J* 6.6 Hz), 0.83 (6H, d, *J* 6.8 Hz), 0.65-0.60 (4H, m), 0.55 (2H, dt, *J* 3.8, 7.6 Hz), -0.33 (2H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.3, 94.9, 85.5, 72.4, 72.3, 71.2, 71.0, 70.8, 69.76, 69.7, 64.3, 57.4, 52.2, 36.9, 35.1, 34.5, 32.5, 32.2, 31.7, 30.3, 30.0, 29.8, 29.79, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.5, 27.3, 27.1, 26.9, 25.9, 25.0, 22.5, 19.5, 15.6, 14.6, 13.8, 10.7; ν_{max}: 3436, 2922, 2852, 1721, 1466, 1098, 734 cm^{-1}.

Experiment 79: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (321)

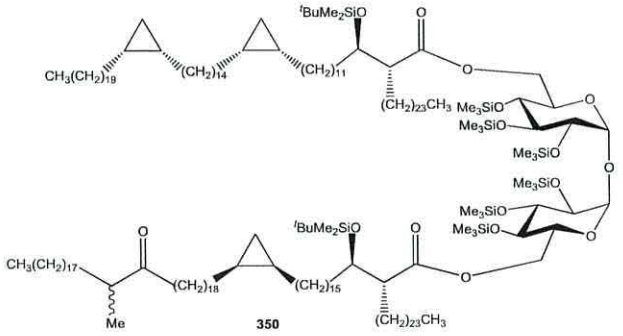


321

A dry polyethylene vial equipped with a rubber septum was charged with **(320)** (0.02 g, 0.01 mmol) and pyridine (0.05 mL) in dry THF (6 mL) and stirred at r.t. under nitrogen atmosphere. HF-pyridine (0.7 mL) was then added. The mixture was stirred at 43 °C for 17 h, then neutralized by triethyl amine drop wise until pH 5. The product was

evaporated to give a residue which was purified by column chromatography eluting with $CHCl_3$/MeOH 10:1 then 1:1 to give the title compound **(321)** as a syrup (0.01 g, 90%), $[\alpha]_D^{20}$ = +46 (c 0.50, $CHCl_3$) {MS Found $[M+Na]^+$: 1600.3882; $C_{97}H_{188}NaO_{14}$ requires: 1600.3897}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.12 (1H, br.s), 5.05 (1H, br.s), 4.70 (1H, br.d, *J* 10.3 Hz), 4.21-4.20 (1H, m), 3.90-3.84 (4H, br.m), 3.66-3.62 (4H, m), 3.54-3.51 (2H, m), 3.33-3.30 (4H, including a singlet (OMe) resonated at 3.32), 2.96-2.92 (1H, m), 2.41-2.23 (1H, m), 1.62-1.54 (2H, m), 1.37-1.09 (153H, m), 0.86 (6H, t, *J* 6.6 Hz), 0.82 (3H, d, *J* 6.8 Hz), 0.63-0.61 (2H, m), 0.55 (1H, dt, *J* 3.8, 7.5 Hz), -0.33 (1H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$+ few drops of CD_3OD): 175.4, 94.5, 85.5, 72.53 (br.), 72.5, 72.3, 71.4, 71.2, 71.0, 70.8, 70.0, 64.2, 62.0, 57.5, 52.3, 35.2, 34.6, 32.2, 31.8, 30.4, 30.1, 29.86, 29.8, 29.7, 29.64, 29.6, 29.5, 29.3, 29.2, 28.6, 27.4, 27.2, 26.0, 25.1, 22.5, 15.6, 14.7, 13.9, 10.7; ν_{max}: 3400, 2924, 2853, 1721, 1466, 993 cm^{-1}.

Experiment 80: (2*R*)-((2*R*,3*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4*S*,5*R*,6*R*)-6-((((*R*)-2-((*R*)-1-(-(*tert*-Butyldimethylsilyl)oxy)-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoyl)oxy)methyl)-3,4,5-tris((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)3,4,5-tris((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-16-((1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)hexacosanoate (350)

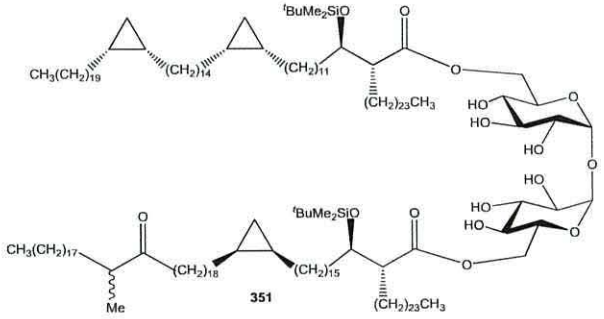


350

EDCI (0.03 g, 0.15 mmol) and 4-DMAP (0.02 g, 0.16 mmol) were added to a stirred solution of **(342)** (0.11 g, 0.05 mmol) and **(349)** (0.20 g, 0.14 mmol) and powdered molecular sieves 4 Å in dry dichloromethane (4 mL) at r.t. under nitrogen atmosphere. The mixture was stirred for 6 days at r.t. then the mixture was diluted with dichloromethane (5 mL) and mixed with silica gel (2 g) then the solvent was evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (30:1, 10:1) to give the title compound **(350)**

as a colourless oil (0.11 g, 68%); $[\alpha]_D^{20}$ = +18 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3363.8; $C_{204}H_{410}NaO_{16}Si_8$ requires: 3363.9}, which showed δ_H (400 MHz, $CDCl_3$): 4.85 (2H, d, *J* 2.9 Hz), 4.37 (2H, br.d, *J* 10.1 Hz), 4.04-3.99 (4H, m), 3.96-3.93 (2H, m), 3.91(2H, t, *J* 8.9 Hz), 3.53 (2H, t, *J* 8.9 Hz), 3.39 (2H, dd, *J* 3.1, 9.3 Hz), 2.57-2.53 (2H, m), 2.50 (1H, pent, *J* 6.8 Hz), 2.39 (2H, dt, *J* 1.2, 7.2 Hz), 1.38-1.14 (278H, m), 1.05 (3H, d, *J* 6.9 Hz), 0.90-0.86 (30H, including 2 x *tert*-butyl groups and 4 x terminal CH_3 groups), 0.66-0.64 (6H, br.m), 0.58 (3H, dt, *J* 3.8, 7.6 Hz), 0.16 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.06 (12H, s), -0.31 (3H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 215.1, 173.8, 94.8, 73.5, 73.4, 72.8, 71.8, 70.7, 62.3, 51.8, 46.3, 41.1, 33.4, 33.0, 31.9, 30.2, 29.9, 29.8, 29.74, 29.71, 29.7, 29.6, 29.5, 29.4, 29.37, 29.3, 28.7, 28.1, 27.3, 26.2, 25.9, 25.8, 25.7, 25.1, 23.7, 22.6, 18.0, 16.3, 15.7, 14.1, 10.9, 1.0, 0.9, 0.1, -4.5, -4.6; ν_{max}: 2923, 2853, 1744, 1465, 1251, 1099, 1010, 899, 839 cm^{-1}.

Experiment 81: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4*S*,6*R*)-6-((((*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoyl)oxy)methyl)-3,4,5-trihydroxytetrahydro-2*H*-pyr-an-2-yl)oxy)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-yl)methyl-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-16-((1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cycloprop-yl)hexadecyl)hexacosanoate (351)

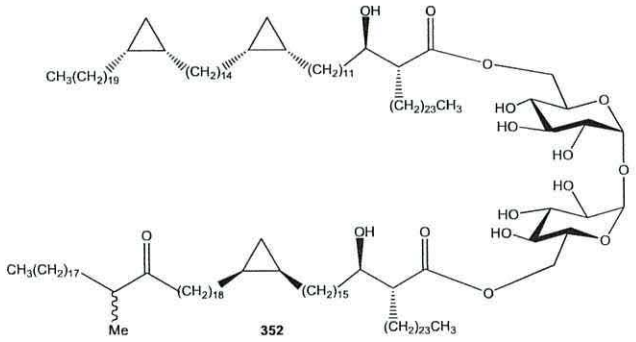


351

Tetrabutylammonium fluoride (0.2 mL, 0.2 mmol, 1.0 M) was added to a stirred solution of **(350)** (0.11 g, 0.03 mmol) in dry THF (10 mL) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and then it was stirred for 20 min. The solvent was evaporated and the residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(351)** as a colourless thick oil (0.06 g, 84%), $[\alpha]_D^{20}$ = +12 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2931.2; $C_{186}H_{362}NaO_{16}Si_2$ requires: 2931.6}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.05 (2H, d, *J* 3.5 Hz), 4.33 (2H, br.dd, *J* 4.2, 12.3

Hz), 4.2 (2H, br.dd, *J* 4.1, 10.6 Hz), 3.93-3.85 (4H, m), 3.76 (2H, br.t, *J* 9.2 Hz), 3.47 (2H, dd, *J* 3.52, 9.6 Hz), 3.31 (2H, t, *J* 9.5 Hz), 2.55-2.50 (2H, m), 2.48 (1H, pent, *J* 6.8 Hz), 2.39 (2H, t, *J* 7.1 Hz), 1.42-1.07 (286H, m), 1.01 (3H, d, *J* 6.9 Hz), 0.86 (12H, t, *J* 6.7 Hz), 0.82 (18H, s), 0.62-0.59 (6H, m), 0.54 (3H, dt, *J* 3.7, 7.8 Hz), 0.01 (6H, s), -0.01 (6H, s), -0.35 (3H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 216.0, 175.1, 93.4, 73.1, 72.9, 71.6, 70.1, 70.0, 62.8, 51.5, 46.2, 41.0, 33.5, 32.9, 31.8, 30.1, 29.7, 29.6, 29.5, 29.41, 29.4, 29.34, 29.3, 29.1, 28.6, 27.6, 27.2, 27.0, 25.6, 25.4, 24.1, 23.6, 22.5, 17.8, 16.2, 15.6, 13.9, 10.7, -4.6, -5.0; ν_{max}: 3401, 2926, 2854, 1724, 1466, 1215, 1093, 1078, 991, 929, 836, 757, 736, 669 cm^{-1}.

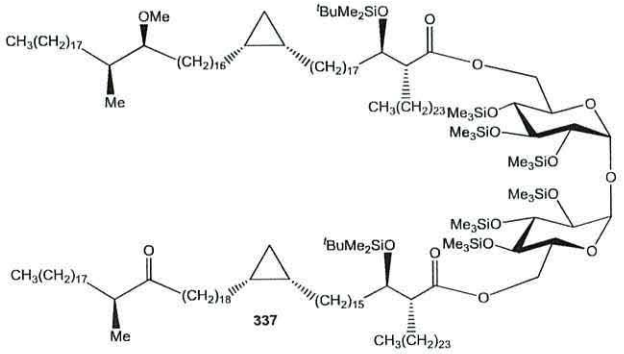
Experiment 82: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-((((2*R*)-2-((1*R*)-1-hydroxy-16-((1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)hexacosanoyl)oxy)methyl)tetrahydro-2*H*-pyran-2-yl)o-xy)tetrahydro-2*H*-pyran-2-yl)methyl-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (352)



A dry polyethylene vial equipped with a rubber septum was charged with **(351)** (0.06 g, 0.02 mmol) and pyridine (0.05 mL) in dry THF (12 mL) was stirred at r.t. under nitrogen. HF-pyridine (0.3 mL) was added. The mixture was stirred at 43 °C for 15 h. Then it was neutralized by pouring it slowly into sat. aq. sodium bicarbonate until no more CO_2 was liberated. The product was extracted with chloroform (3 × 50 mL), then the combined organic layers were dried and evaporated to give a residue which was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give the title compound **(352)** (0.04 g, 70%), $[\alpha]_D^{10}$ = +31 (c 0.50, $CHCl_3$) {MS Found $[M+Na]^+$: 2704.5255; $C_{174}H_{334}NaO_{16}$ requires: 2704.5219}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.97 (2H, d, *J* 3.0 Hz), 4.72 (2H, br.d, *J* 12.1 Hz), 4.29 (2H, br.t, *J* 9.8 Hz), 3.90 (2H, br.t, *J* 11.5 Hz), 3.74 (2H, t, *J* 9.4 Hz), 3.66-3.63 (2H, m), 3.49 (2H, dd, *J* 2.8, 8.9 Hz), 3.19 (2H, t, *J* 9.4 Hz), 2.49 (2H, br.q, *J* 6.8 Hz), 2.40-2.36 (3H, m), 1.64-1.00 (286H, m), 1.00

(3H, d, *J* 6.9 Hz), 0.84 (12H, m), 0.61-0.59 (6H, m), 0.53 (3H, dt, *J* 3.9, 7.6 Hz), -0.36 (3H, br.q, *J* 4.9 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 216.3, 175.3, 94.7, 72.4 (br.), 71.0 (br.), 69.6 (br.), 64.0, 52.2, 46.1, 41.0, 34.5, 32.8, 31.9, 31.7, 30.0, 29.4, 29.37, 29.34, 29.3, 29.26, 29.2, 29.1, 29.0, 28.5, 27.0, 26.1, 25.0, 23.4, 23.1, 22.4, 16.0, 15.5, 13.8, 10.7, 10.6; ν_{max}: 3368, 2920, 2851, 1727, 1467, 1101, 720 cm^{-1}.

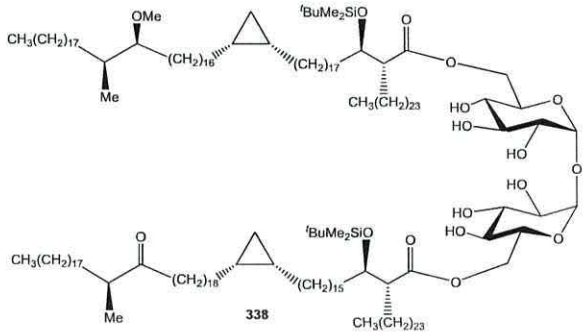
Experiment 83: (*R*)-((2*R*,3*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4*R*,5*R*,6*R*)-6-((((*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxoocatriacontyl)-cyclopropyl)hexadecyl)hexacosanonyl)oxy)methyl)-3,4,5tris((trimethylsilyl)oxy)-tetrahydro-2*H*-pyran-2-yl)methyl-3,4,5-tris((trimethylsilyl)oxy))tetrahydro-2*H*-pyran-2-yl)methyl2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (337)



EDCI (0.05 g, 0.26 mmol) and 4-DMAP (0.02 g, 0.16 mmol) were added to a stirred solution of **(336)** (0.11 g, 0.07 mmol) and **(317)** (0.15 g, 0.07 mmol) powdered molecular sieves 4 Å in dry dichloromethane (3 mL) at r.t. under nitrogen atmosphere. The mixture was stirred for 6 days at r.t. then the mixture was then diluted with dichloromethane (5 mL) and mixed with silica gel (1 g) then the solvent was evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (20:1, 10:1) to give a colourless thick oil, the title compound **(337)** (0.06 g, 40%), $[\alpha]_D^{20}$ = +20 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3480.9; $C_{211}H_{426}NaO_{17}Si_8$ requires: 3480.8}, which showed δ_H (400 MHz, $CDCl_3$): 4.81 (2H, d, *J* 2.9 Hz), 4.33 (2H, br.d, *J* 10.0 Hz), 4.00-3.94 (4H, m), 3.92-3.89 (2H, m), 3.87 (2H, t, *J* 8.8 Hz), 3.49 (2H, t, *J* 8.9 Hz), 3.35 (2H, dd, *J* 2.9, 9.3 Hz), 3.3 (3H, m), 2.93 (1H, br.pent, *J* 4.1 Hz), 2.53-2.49 (2H, m), 2.46 (1H, t, *J* 6.8 Hz), 2.39 (2H, dt, *J* 1.2, 7.2 Hz), 1.34-1.13 (288H, m), 1.01 (3H, d, *J* 6.9 Hz), 0.86 (12H, t, *J* 7.1 Hz), 0.84 (18H, s), 0.8 (6H, d, *J* 7.0 Hz), 0.62-0.6 (4H, br.m), 0.54 (2H, dt, *J* 3.8, 7.5 Hz), 0.12 (18H, s), 0.10 (18H, s), 0.09 (18H, s), -0.02

(12H, s), -0.35 (2H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$): 215.1, 173.8, 94.8, 85.4, 73.5, 73.4, 72.8, 71.8, 70.7, 62.3, 57.7, 51.8, 46.3, 41.1, 35.3, 33.4, 33.0, 32.3, 31.9, 30.4, 30.2, 30.0, 29.9, 29.8, 29.77, 29.7, 29.66, 29.6, 29.5, 29.4, 29.34, 29.3, 28.7, 28.1, 27.5, 27.3, 26.2, 26.1, 25.9, 25.8, 25.1, 23.7, 22.6, 18.0, 16.3, 15.7, 14.8, 14.1, 10.9, 1.0, 0.9, 0.1, -4.5, -4.6; ν_{max}: 2924, 2853, 1744, 1464, 1251, 1099, 1047, 872, 842 cm^{-1}.

Experiment 84: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4*S*,6*R*)-6-((((*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriacontyl)-cyclopropyl)hexadecyl)hexacosanoyl)oxy)methyl)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-yl)oxy)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-yl)methyl-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (338)

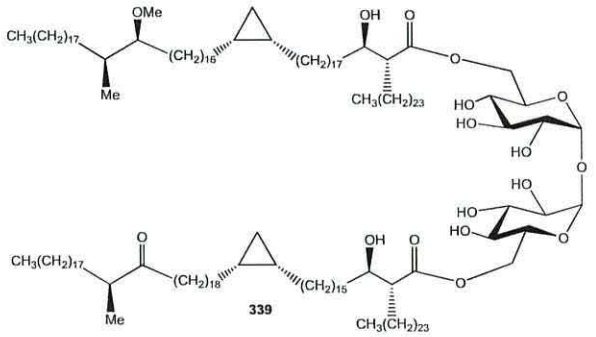


338

Tetrabutylammonium fluoride (0.1 mL, 0.1 mmol, 1.0 M) was added to a stirred solution of **(337)** (0.06 g, 0.02 mmol) in dry THF (3 mL) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 15 min. The reaction mixture was worked up and purified as before to give title compound **(338)** as a colourless thick oil (0.04 g, 72%), $[\alpha]_D^{20}$ = +11 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3053.6; $C_{193}H_{384}NaO_{17}Si_2$ requires: 3053.8}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.04 (2H, d, *J* 3.5 Hz), 4.34 (2H, br.dd, *J* 4.4, 12.1 Hz), 4.21 (2H, br.dd, *J* 4.6, 12.2 Hz), 3.91-3.84 (4H, m), 3.75 (2H, br.t, *J* 9.2 Hz), 3.46 (2H, dd, *J* 3.5, 9.6 Hz), 3.35-3.27 (5H, including s for the methoxy groups resonated at 3.3), 2.94 (1H, br.pent, *J* 4.4 Hz), 2.54-2.49 (2H, m), 2.47 (1H, br.t, *J* 6.8 Hz), 2.38 (2H, br.t, *J* 7.2 Hz), 1.56-1.05 (300H, m), 1.00 (3H, d, *J* 6.9 Hz), 0.85 (12H, t, *J* 6.9 Hz), 0.81 (18H, s), 0.79 (3H, d, *J* 6.9 Hz), 0.76 (3H, d, *J* 6.5 Hz), 0.61-0.59 (4H, m), 0.53 (2H, dt, *J* 3.7, 7.5 Hz), 0.0 (6H, s), 0.01 (6H, s), -0.36 (2H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 216.0, 175.2, 93.4, 85.5, 73.1, 73.0, 71.6, 70.1, 69.8, 62.8, 57.5, 51.5, 46.2, 41.0, 35.2, 33.5, 32.9, 32.2, 31.8, 30.3, 30.1, 29.8, 29.6,

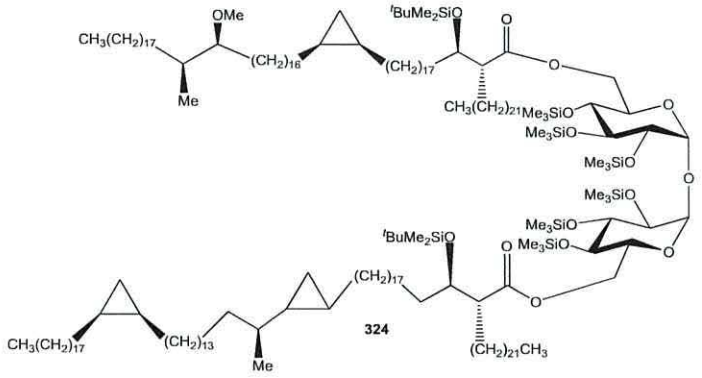
29.5, 29.4, 29.34, 29.3, 29.1, 28.6, 27.6, 27.4, 27.2, 26.9, 25.9, 25.7, 25.6, 24.1, 23.5, 22.5, 17.8, 16.2, 15.6, 14.6, 13.9, 10.7, -4.6, -5.0; ν_{max}: 3523, 2918, 2850, 1701, 1467, 1215, 1093, 1050, 908, 874, 824, 759, 736, 669 cm^{-1}.

Experiment 85: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-((((*R*)-2-((*R*)-1-hydroxy-16-((1*S*,2*R*)-2-((*S*)-20-methyl-19-oxooctatriaconty)cyclopropyl)hexadecyl)hexacosanoyl)oxy)methyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (339)



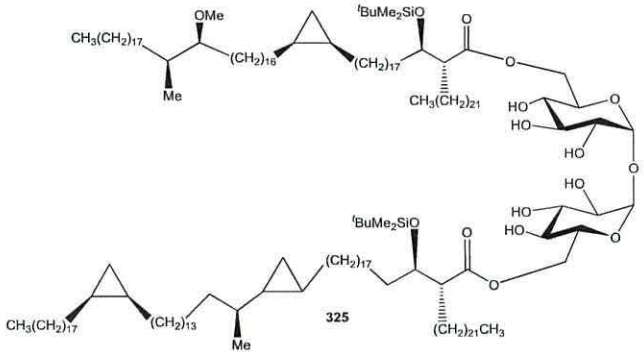
A dry polyethylene vial equipped with a rubber septum was charged with **(338)** (0.04 g, 0.01 mmol) and pyridine (0.1 mL) in dry THF (3 mL) was stirred at r.t. under nitrogen. HF-pyridine (0.3 mL) was added. The mixture was stirred at 43 °C for 17 h, then the reaction mixture was worked up and purified as before to give the title compound **(339)** as a syrup (0.01 g, 40%), $[\alpha]_D^{15}$ = +30 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2822.4; $C_{181}H_{353}NaO_{17}$ requires: 2822.6}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.02 (2H, d, *J* 3.7 Hz), 4.71 (2H, br.d, *J* 10.9 Hz), 4.27 (2H, br.t, *J* 12.1 Hz), 3.76 (2H, br.q, *J* 8.2 Hz), 3.64 (2H, t, *J* 8.0 Hz), 3.49-3.47 (2H, br.m), 3.39 (2H, dd, *J* 2.1, 13.6 Hz), 3.35 (3H, s), 3.18 (2H, m), 2.94 (1H, br.pent, *J* 3.4 Hz), 2.54-2.49 (2H, m), 2.47-2.40 (1H, m), 2.38 (2H, br.t, *J* 7.2 Hz), 1.54-1.06 (299H, m), 1.0 (3H, d, *J* 6.9 Hz), 0.85-0.80 (18H, m), 0.61-0.59 (4H, m), 0.53 (2H, dt, *J* 3.9, 8.4 Hz), -0.36 (2H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 216.3, 175.2, 94.5, 85.5, 73.0, 72.5, 72.4, 71.1, 70.9, 69.7, 63.9, 57.4, 52.3, 51.5, 46.1, 40.9, 35.1, 34.5, 32.8, 32.1, 31.7, 30.2, 30.0, 29.7, 29.6, 29.5, 29.4, 29.3, 29.27, 29.24, 29.2, 29.1, 29.0, 28.5, 27.2, 27.1, 27.0, 25.8, 25.5, 25.0, 23.4, 22.4, 16.0, 15.5, 14.5, 13.7, 10.6; ν_{max}: 3369, 2920, 2851, 1727, 1467, 1101, 720 cm^{-1}.

Experiment 86: (2*R*)-((2*R*,3*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4*S*,5*R*,6*R*)-6-((((*R*)-2-((*R*)-1-((-*tert*-Butyldimethylsilyl)oxy)-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriaconyl)cyclopropyl)octadecyl)tetracosanoyl)oxy)methyl)-3,4,5-tris((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)-3,4,5-tris((trimethylsilyl)oxy)-tetrahydro-2*H*-pyran-2-yl)methyl-2-((1*R*)-1-((*tert*-Butyl-dimethyl-silyl)oxy)-19-(2-((*S*)-16-((1*R*,2*S*)-2-octadecylcyclopropyl)hexadecan-2-yl)cyclopropyl)nonadecyl)-tetracosanoate (324)



EDCI (0.05 g, 0.26 mmol) and 4-DMAP (0.02 g, 0.16 mmol) were added to a stirred solution of **(323)** (0.11 g, 0.08 mmol) and **(322)** (0.15 g, 0.07 mmol) and powdered molecular sieves 4 Å in dry dichloromethane (3 mL) at r.t. under nitrogen atmosphere. The mixture was stirred for 6 days at r.t. then the reaction mixture was worked up and purified as before to give the title compound **(324)** as a colourless thick oil (0.11 g, 55%), $[\alpha]_D^{20}$ = +24 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3422.5; $C_{208}H_{420}NaO_{16}Si_8$ requires: 3422.0}, which showed δ_H (400 MHz, $CDCl_3$): 4.70 (2H, d, *J* 2.7 Hz), 4.26 (2H, br.d, *J* 10.7 Hz), 3.95 (4H, br.t, *J* 12.1 Hz), 3.85 (4H, br.q, *J* 4.6 Hz), 3.44 (2H, t, *J* 8.8 Hz), 3.32-3.27 (2H, m), 3.26 (3H, s), 2.90 (1H, br.pent, *J* 4.9 Hz), 2.49-2.45 (2H, m), 1.28-1.08 (284H, m), 0.80-0.75 (36H, including 2 x *tert*-butyl groups as s, 4 x terminal CH_3 groups as t at 0.79 and d for 2 x CH_3), 0.57-0.55 (4H, br.m), 0.49 (2H, dt, *J* 4.7, 10.0 Hz), 0.40-0.32 (1H, m), 0.07-0.05 (3H, m), 0.07 (18H, s), 0.058 (18H, s), 0.054 (18H, s), -0.028 (12H, s), -0.40 (2H, br.q, *J* 4.9 Hz); δ_C (101 MHz, $CDCl_3$): 174.0, 94.6, 85.5, 73.3, 73.2, 72.6, 71.6, 70.5, 62.4, 57.4, 51.5, 37.9, 37.2, 35.1, 34.3, 33.3, 32.1, 31.7, 30.2, 30.0, 29.8, 29.7, 29.5, 29.2, 29.1, 28.5, 27.8, 27.3, 27.0, 26.1, 25.9, 25.8, 25.7, 25.6, 24.8, 22.4, 19.4, 18.4, 17.8, 15.5, 14.5, 13.8, 10.6, 10.2, 0.8, 0.6, -0.0, -4.7, -4.9; ν_{max}: 2924, 2853, 1737, 1493, 1252, 1077, 1045, 898, 842 cm^{-1}.

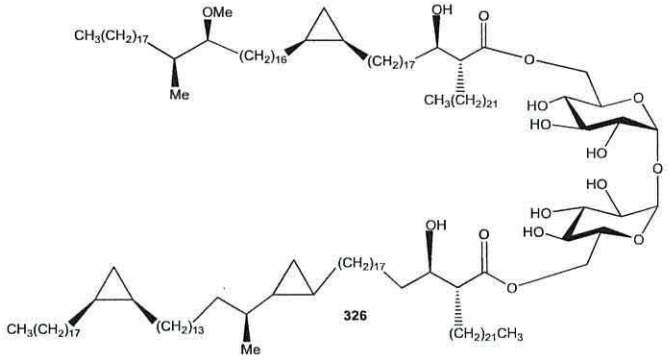
Experiment 87: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4*S*,6*R*)-6-((((*R*)-2-((*R*)-1-((*tert*-Butyldimethyl-silyl)oxy)-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriaconyl)cyclopropyl)octadecyl)tetracosanoyl)oxy)methyl)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-yl)-3,4,5,-trihydroxytetrahydro-2*H*-pyran-2-yl)methyl-2-((1*R*)-1-((*tert*-butyl-dimethyl-silyl)oxy)-19-(2-((*S*)-16-((1*R*,2*S*)-2-octadecylcyclopropyl)-hexadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoate (325)



Tetrabutylammonium fluoride (0.1 mL, 0.1 mmol, 1.0 M) was added to a stirred solution of **(325)** (0.11 g, 0.03 mmol) in dry THF (10 mL) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 1 h. The reaction mixture was worked up and purified as before to give the title compound **(324)** as a colourless thick oil (0.09 g, 97%), $[\alpha]_D^{22}$ = +12 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2989.9; $C_{190}H_{372}NaO_{16}Si_2$ requires: 2989.7}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.0 (2H, d, *J* 2.9 Hz), 4.26 (2H, br.dd, *J* 4.0, 12.0 Hz), 4.17 (2H, br.d, *J* 11.1 Hz), 3.87-3.82 (4H, m), 3.70 (2H, br.t, *J* 9.4 Hz), 3.41 (2H, dd, *J* 3.0, 9.4 Hz), 3.32-3.23 (5H, including a singlet for the methoxy groups resonated at 3.2), 2.91-2.88 (1H, m), 2.51-2.45 (2H, m), 1.46-0.97 (290H, m), 0.79-0.76 (36H, including 2 x *tert*-butyl groups as a singlet, 4 x terminal CH_3 groups as triplet at 0.77 and a doublet for 2 x CH_3), 0.61-0.56 (4H, m), 0.49 (2H, dt, *J* 3.7, 8.2 Hz), 0.38-0.34 (1H, m), 0.12-0.01 (3H, m), 0.034 (6H, s), -0.05 (6H, s), -0.40 (2H, br.q, *J* 4.4 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 93.3, 85.5, 73.1, 71.5, 70.2, 69.8, 62.8, 57.4, 51.5, 37.9, 37.2, 35.1, 34.3, 33.4, 32.1, 31.7, 30.3, 30.0, 29.8, 29.7, 29.6, 29.5, 29.1, 28.5, 27.6, 27.3, 27.0, 26.8, 25.9, 25.8, 25.6, 25.5, 24.0, 22.4, 19.4, 18.4, 17.7, 15.5, 14.5, 13.8, 10.6, 10.2, -4.7, -5.1; ν_{max}: 3523, 2918, 2850, 1701, 1467, 1215, 1093, 1050, 908, 874, 824, 759, 736, 669 cm^{-1}.

Experiment 88: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-((*R*)-1-hydroxy-19-(2-((*S*)-16-((1*R*,2*S*)-2-octadecylcyclopropyl)hexa-

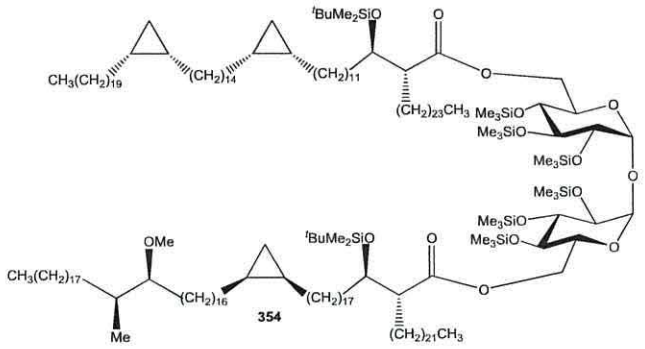
decane-2-yl)cyclopropyl)nonadecyl)tetracosanoyl)oxy)methyl)tetrahydro-2*H*-pyran-2-yl)-tetrahydro-2*H*-pyran-2-yl)methyl-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatricontyl)cyclopropyl)octadecyl)tetracosanoate (326)



A dry polyethylene vial equipped with a rubber septum was charged with **(325)** (0.08 g, 0.02 mmol) and pyridine (0.15 mL) in dry THF (10 mL) was stirred at r.t. under nitrogen. HF-pyridine (0.6 mL) was added. The mixture was stirred at 43 °C for 17 h, then the mixture was worked up and purified as before to give the title compound **(326)** as a colourless oil (0.03 g, 45%), $[\alpha]_D^{23}$ = +30 (c 0.50, $CHCl_3$) {MS Found $[M+Na]^+$: 2761.5985; $C_{178}H_{344}NaO_{16}$ requires: 2761.6002}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.96 (2H, d, *J* 2.2 Hz), 4.61 (2H, br.dd, *J* 1.8, 11.7 Hz), 4.17 (2H, br.t, *J* 8.0 Hz), 3.97 (2H, br.q, *J* 8.9 Hz), 3.70 (2H, t, *J* 8.3 Hz), 3.62-3.59 (2H, br.m), 3.45 (2H, dd, *J* 2.2, 9.4 Hz), 3.35 (3H, s), 3.20 (2H, t, *J* 9.2 Hz), 2.93-2.90 (1H, m), 2.37-2.32 (2H, m), 1.48-1.03 (292H, m), 0.83-0.77 (18H, m, 4 x terminal CH_3 groups as t at 0.81 and a d for 2 x CH_3), 0.59-0.57 (4H, m), 0.51 (2H, dt, *J* 4.2, 8.1 Hz), 0.39-0.35 (1H, m), 0.15-0.01 (3H, m) -0.38 (2H, br.q, *J* 4.3 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.4, 95.1, 85.5, 72.5, 72.3, 71.4, 71.0, 69.7, 67.8, 64.5, 57.5, 52.1, 38.0, 37.3, 35.2, 34.6, 34.3, 32.2, 31.8, 30.3, 30.1, 29.9, 29.8, 29.7, 29.67, 29.6, 29.58, 29.5, 29.4, 29.3, 29.2, 28.6, 27.4, 27.2, 27.1, 26.0, 25.9, 25.6, 25.4, 25.0, 22.5, 19.5, 18.5, 15.6, 14.6, 13.9, 10.7, 10.3; ν_{max}: 3468, 2925, 2854, 17235, 1459, 1076, 735 cm^{-1}.

Experiment 89: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4*S*,6*R*)-6-((((*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriaconyl)cyclopropyl)octadecyl)tetracosanoyl)oxy)methyl)-3,4,5-tri((trimethyl-

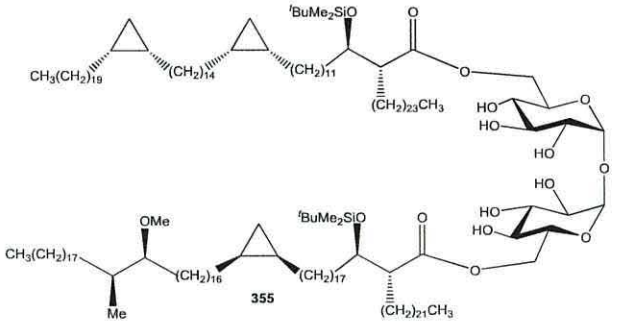
silyl)oxy)tetrahydro-2*H*-pyran-2-yl)oxy)-3,4,5-tris((trimethylsilyl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (354)



EDCI (0.03 g, 0.15 mmol) and 4-DMAP (0.02 g, 0.16 mmol) were added to a stirred solution of **(342)** (0.11 g, 0.05 mmol) and **(353)** (0.06 g, 0.04 mmol) and powdered molecular sieves 4 Å in dry dichloromethane (2 mL) at r.t. under nitrogen atmosphere. The reaction mixture was stirred for 6 days at r.t. then the reaction mixture was then diluted with dichloromethane (5 mL) and mixed with silica gel (2 g) then the solvent was evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (30:1, 10:1) to give the title compound as a colourless oil **(354)** (0.1 g, 62%), $[\alpha]_D^{20}$ = +20 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 3359.4; $C_{203}H_{418}NaO_{16}Si_8$ requires: 3359.9}, which showed δ_H (400 MHz, $CDCl_3$): 4.85 (2H, d, *J* 3.0 Hz), 4.36 (2H, br.d, *J* 10.1 Hz), 4.04-3.97 (4H, m), 3.96-3.93 (2H, m), 3.91 (2H, br.t, *J* 8.8 Hz), 3.53 (2H, t, *J* 8.9 Hz), 3.39 (2H, dd, *J* 2.9, 9.2 Hz), 3.34 (3H, s), 2.97 (1H, br.pent, *J* 4.2 Hz), 2.57 (2H, pent, *J* 3.5 Hz), 1.64-1.13 (285H, m), 0.90-0.84 (33, including a singlet at 0.9 for 2 x *tert*-butyl groups, a doublet at 0.88, *J* 6.92 for a 1 x CH_3 group and a triplet at 0.86 for the terminal methyl groups), 0.67-0.64 (6H, br.m), 0.58 (3H, dt, *J* 3.8, 7.6 Hz), 0.16 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.06 (12H, s), -0.31 (3H, br.q, *J* 5.0 Hz); δ_C (101 MHz, $CDCl_3$): 173.8, 94.8, 85.4, 73.5 (broad), 73.4, 72.8, 71.8, 70.7, 62.3, 57.7, 51.8, 35.3, 33.4, 32.3, 31.9, 30.5, 30.2, 30.0, 29.9, 29.8, 29.74, 29.7, 29.6, 29.5, 29.3, 28.7, 28.1, 27.5, 26.2, 26.1, 25.9, 25.8, 25.1, 22.7, 18.0, 15.7, 14.8, 14.1, 10.9, 1.0, 0.9, 0.1, -4.5, -4.6; ν_{max}: 2923, 2853, 1744, 1465, 1251, 1099, 1010, 899, 839 cm^{-1}.

Experiment 90: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-6-(((2*R*,3*R*,4S,6*R*)-6-((((*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoyl)oxy)methyl)-3,4,5-trihydroxy-

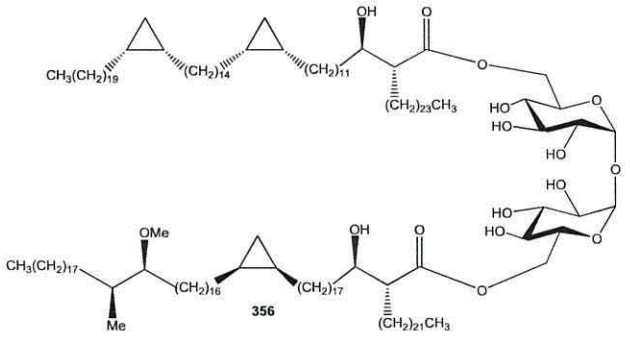
tetrahydro-2*H*-pyran-2-yl)oxy)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-yl)-methyl-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (355)



Tetrabutylammonium fluoride (0.2 mL, 0.2 mmol, 1.0 M) was added to a stirred solution of **(354)** (0.11 g, 0.03 mmol) in dry THF (10 mL) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach r.t. and then it was stirred for 20 min. The reaction was worked up and purified as before to give the title compound **(355)** as a colourless thick oil (0.08 g, 91%), $[\alpha]_D^{15}$ = +12 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2923.2; $C_{185}H_{366}NaO_{16}Si_2$ requires: 2923.7}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.02 (2H, d, *J* 3.2 Hz), 4.29 (2H, br.d, *J* 11.1 Hz), 4.20 (2H, br.d, *J* 10.9 Hz), 3.91 (2H, br.d, *J* 9.5 Hz), 3.87 (2H, br.q, *J* 4.7 Hz), 3.80 (2H, br. t, *J* 9.2 Hz), 3.45 (2H, dd, *J* 2.9, 9.6 Hz), 3.32-3.28 (5H, including s for the methoxy groups at 3.2), 2.93-2.90 (1H, m), 2.52-2.47 (2H, m), 1.57-0.91 (287H, m), 0.84-0.77 (33H, including s at 0.84 for 2 x *tert*-butyl groups, a d at 0.81, *J* 6.9 for the methyl group and t at 0.79 for the terminal methyl groups), 0.60-0.57 (6H, m), 0.52 (3H, dt, *J* 3.8, 7.7 Hz), -0.007 (6H, s), -0.02 (6H, s), -0.37 (3H, br.q, *J* 4.7 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 93.4, 85.5, 73.1, 72.9, 71.6, 70.2, 69.8, 62.8, 57.5, 51.5, 35.2, 33.5, 32.2, 31.7, 30.3, 30.0, 29.8, 29.7, 29.6, 29.58, 29.5, 29.4, 29.2, 28.5, 27.6, 27.3, 26.8, 25.9, 25.7, 25.6, 24.1, 22.5, 17.8, 15.6, 14.6, 13.9, 10.7, -4.6, -5.0; ν_{max}: 3401, 2926, 2854, 1724, 1466, 1215, 1093, 1078, 991, 929, 836, 757, 736, 669 cm^{-1}.

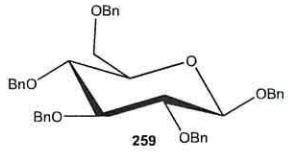
Experiment 91: (2*R*)-((2*R*,4*S*,5*R*,6*R*)-3,4,5-Trihydroxy-6-(((2*R*,3*R*,4*S*,6*R*)-3,4,5-trihydroxy-6-((((*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoyl)oxy)methyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecy)icosylcyclopropyl)-

tetradecyl)cyclopropyl)dodecyl)hexacosanoate (356)



A dry polyethylene vial equipped with a rubber septum was charged with **(355)** (0.08 g, 0.02 mmol) and pyridine (0.1 mL) in dry THF (12 mL) was stirred at r.t. under nitrogen atmosphere. HF-pyridine (0.8 mL) was then added. The mixture was stirred at 43 °C for 15 hs, then the reaction was worked up and purified as before to give the title compound **(356)** as a colourless thick oil (0.04 g, 50%), $[\alpha]_D^{20}$ = +32 (c 0.50, $CHCl_3$) {MALDI Found $[M+Na]^+$: 2693.2; $C_{173}H_{336}NaO_{16}$ requires: 2693.5}, which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.98 (2H, br.d, *J* 2.4 Hz), 4.73 (2H, br.d, *J* 10.8 Hz), 4.28 (2H, br.t, *J* 8.8 Hz), 3.91 (2H, br.q, *J* 11.8 Hz), 3.49 (2H, t, *J* 11.3 Hz), 3.37-3.36 (2H, br.m), 3.53 (2H, dd, *J* 3.8, 11.7 Hz), 3.31 (3H, s), 3.21 (2H, t, *J* 9.2 Hz), 2.95 (2H, br.pent, *J* 4.2 Hz), 2.41-2.36 (2H, m), 1.31-1.22 (286H, m), 0.86-0.81 (15H, including d at 0.85 for the methyl group and t at 0.83 for terminal methyl groups), 0.62-0.60 (6H, m), 0.54 (3H, dt, *J* 3.9, 7.7 Hz), -0.35 (3H, br.q, *J* 4.8 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.4, 95.0, 85.5, 72.5, 72.3, 71.1, 69.8, 64.6, 57.5, 52.0, 35.2, 34.6, 32.2, 31.8, 30.7, 30.3, 30.1, 29.87, 29.8, 29.7, 29.63, 29.6, 29.3, 29.2, 28.6, 27.4, 27.2, 26.0, 25.6, 25.0, 22.5, 15.6, 14.7, 13.9, 10.8; ν_{max}: 3368, 2920, 2851, 1727, 1467, 1101, 720 cm^{-1}.

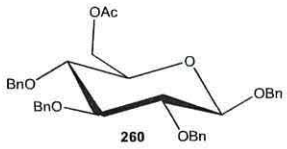
Experimental 92: (2*R*,3*R*,4*S*,5*R*,6*R*)-2,3,4,5-Tetrakis(benzyloxy)-6-((benzyloxy)-methyl)tetrahydro-2*H*-pyran (259)



NaH (8.0 g of 60% dispersion in mineral oil, 20 mmol) was washed twice with hexane at r.t. then a suspension of D-glucose **(258)** (10.0 g, 55.5 mmol) in anhydrous DMF (300 mL) was added at r.t. The suspension was stirred for 1 h then cooled in an ice bath. Benzyl bromide (23.11 mL, 187.2 mmol) was added drop wise over a 30 min

period, and after 1 h the ice bath was removed. After stirring at r.t. for 4 h, then the second addition of the same quantities of NaH and benzyl bromide were added consecutively at 0 °C. The reaction mixture was stirred overnight for 18 h, and then (30 mL) of anhydrous MeOH was added slowly to react with the excess of the NaH. DMF was removed under reduced pressure at 55 °C. The residue was dissolved in CH_2Cl_2 (200 mL) and washed with water and brine, dried ($MgSO_4$) filtered and evaporated to give a yellow oil, Flash chromatography on silica gel eluting with petrol/ethyl acetate (9:1, 5:2) yielded a light yellow solid. Recrystallization from MeOH to give the title compound **(259)** as a white solid (6.0 g, 17%), m.p.: 83-86 °C: *lit* 83-86 °C; $[\alpha]_D^{21}$ = -10 (c 1.00, $CHCl_3$) *lit.* $[\alpha]_D^{20}$ = -9.1[261] {MALDI Found $[M+Na]^+$: 653.1, $C_{41}H_{42}NaO_6$ requires: 653.2}, which showed δ_H (400 MHz, $CDCl_3$):7.40-7.28 (23H, m), 7.18 (2H, dd, *J* 2.2, 7.2 Hz), 5.00-4.92 (3H, m), 4.84-4.51 (8H, m), 3.78 (1 H, dd, *J* 1.9, 10.8 Hz), 3.72 (1H, dd, *J* 4.8, 10.8 Hz), 3.69-3.59 (2 H, m), 3.54 (1 H, t, *J* 8.30 Hz), 3.51-3.46 (1H, m); δ_C (101 MHz, $CDCl_3$): 138.4, 138.1, 128.38, 128.36, 128.3, 128.1, 127.9, 127.8, 127.75, 127.7, 127.6, 127.5, 102.6, 84.7, 82.3, 77.9, 75.6, 74.98, 74.92, 74.8, 73.4, 71.1, 68.9.[261]

Experimental 93: ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-Tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl acetate (260)

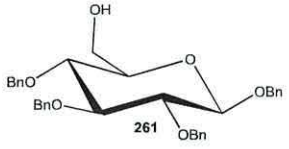


260

Zinc chloride (4.31 g, 31.6 mmol) has been dried under high vacuum for 1 h, and then acetic acid-acetic anhydride (1:1, 30 mL) were added. The mixture was cooled down to -5 °C, and then a solution of **(259)** (4.0 g, 6.3 mmol) in acetic acid–acetic anhydride (1:1, 20 mL) was added drop wise. The reaction mixture was stirred at r.t. for 4 h, and then ice water (180 mL) was added. The resulting light-brown precipitate was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and the filtrate was evaporated. The residue was purified by flash chromatography on silica gel eluting with petrol/ethyl acetate (5:1) gave the title compound **(260)** as a white solid (2.5 g, 67%): m.p.: 116–117 °C, *lit.* 116–117 °C; $[\alpha]_D^{21}$ = -3.2 (c 1.0, $CHCl_3$) *lit.* $[\alpha]_D^{20}$ = -2.9[261] {MALDI Found $[M+Na]^+$: 605.5, $C_{36}H_{38}NaO_7$ requires: 605.2}; which showed δ_H (400 MHz, $CDCl_3$): 7.39-7.25 (20H, m) 5.00-4.93 (3H , m), 4.87 (1H, d, *J* 10.8 Hz), 4.80 (1H, d, *J* 10.9 Hz), 4.74 (1H, d, *J* 10.9 Hz), 4.67 (1H, d, *J* 11.9 Hz), 4.58 (1H,

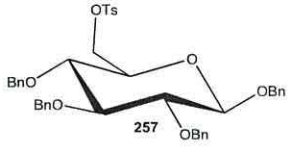
d, *J* 10.8 Hz), 4.52 (1H, d, *J* 7.8 Hz), 4.38 (1H, dd, *J* 2.0, 11.9 Hz), 4.26 (1H, dd, *J* 4.7, 11.9 Hz), 3.68 (1H, t, *J* 8.8 Hz), 3.59-3.49 (3H, m), 2.07 (3H, s); δ_C (101 MHz, $CDCl_3$): 170.7, 138.4, 138.2, 137.7, 137.1, 128.49, 128.44, 128.4, 128.3, 128.1, 128.09, 128.0, 127.9, 127.8, 127.7, 102.4, 84.6, 82.2, 75.7, 75.0, 74.89, 72.87, 71.2, 63.1, 20.9.[261]

Experimental 94: ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-Tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methanol (261)



Lithium hydroxide monohydrate (1.20 g, 25.7 mmol) was added to the stirred solution of **(260)** (1.0 g, 1.7 mmol) in THF (50 mL), water (4 mL), MeOH (2 mL). The mixture was stirred for 18 h at 45 °C. The mixture was dissolved in warmed petrol/ethyl acetate 5:1 (50 mL) and an aqueous solution of $KHSO_4$ was added until the water layer was pH 1-2. The organic phase was separated and the aqueous layer was re-extracted with ethyl acetate (2 × 100 mL) and then combined organic layers were dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give the title compound **(261)** (0.8 g, 70%), m.p.: 104-106 °C, *lit.* 104–106 °C; $[\alpha]_D^{24}$ = -11 (c 1.00, $CHCl_3$), *lit.* $[\alpha]_D^{20}$ = -9.2[261] {MALDI Found $[M+Na]^+$: 563.4, $C_{34}H_{36}NaO_6$ requires: 563.2}; which showed δ_H (400 MHz, $CDCl_3$); 7.39-7.29 (20H, m), 4.98-4.94 (1H, m), 4.92 (1H, d, *J* 4.7 Hz), 4.87 (1H, d, *J* 11.2 Hz), 4.82 (1H, d, *J* 10.9 Hz), 4.72 (1H, dd, *J* 11.4, 13.8 Hz), 4.65 (1H, d, *J* 10.9 Hz), 4.58 (1H, d, *J* 7.8 Hz), 3.88 (1H, ddd, *J* 2.7, 5.6, 11.9 Hz), 3.71 (2H, m), 3.58 (1H, t, *J* 9.3 Hz), 3.50 (1H, dd, *J* 7.9, 9.1 Hz), 3.37 (1H, ddd, *J* 2.8, 4.7, 9.6 Hz); δ_C (101 MHz, $CDCl_3$): 138.4, 138.2, 137.7, 137.1, 128.5, 128.44, 128.4, 128.3, 128.1, 128.09, 128.0, 127.9, 127.8, 127.7, 102.4, 84.6, 82.2, 75.7, 75.0, 74.89, 72.8, 71.2, 63.1.[261]

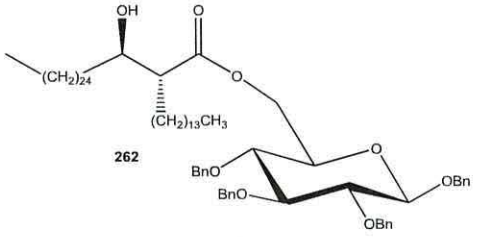
Experimental 95: ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-Tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl-4-methy4-methylbenzenesulfonate (257)



Pyridine (5 mL), 4-DMAP (0.1 g, 0.8 mmol) and tosyl chloride (1.3 g, 9.2 mmol) were sequentially added to a stirred solution of **(261)** (1.0 g, 1.8 mmol) in dichloromethane

(20 mL) at 0 °C. The mixture was warmed to r.t. and was stirred overnight. The mixture was diluted with CH_2Cl_2 and hydrolyzed with HCl (10 mL, 3 M). The organic phase was separated and the aqueous layer was re-extracted with CH_2Cl_2 (2 × 100 mL), then combined organic layers were washed with saturated solution of $NaHCO_3$ and brine. The combined organic layers were dried, evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give the title compound **(257)** (0.9 g, 75%); $[\alpha]_D^{20}$ = -4.6 (c 1.0, $CHCl_3$) *lit* $[\alpha]_D^{20}$ = -4.1[262] {MAL-DI Found $[M+Na]^+$: 717.3, $C_{41}H_{42}NaO_8S$ requires: 717.2}, Which showed δ_H (400 MHz, $CDCl_3$): 7.80 (2H, d, *J* 8.3 Hz), 7.37-7.26 (20H, m), 7.19 (2H, dd, *J* 2.5, 6.9 Hz), 4.93 (2H, dd, *J* 3.5, 10.9 Hz), 4.85 (2H, t, *J* 11.1 Hz), 4.76 (1H, d, *J* 10.9 Hz), 4.70 (1H, d, *J* 10.9 Hz), 4.59 (1H, d, *J* 11.9 Hz), 4.51 (1H, d, *J* 10.8 Hz), 4.46 (1H, d, *J* 7.8 Hz), 4.45-4.44 (1H, m), 4.27 (1H, dd, *J* 4.8, 10.5 Hz), 3.62 (1H, t, *J* 8.6 Hz), 3.53-3.41 (3H, m), 2.41 (3H, s); δ_C (101 MHz, $CDCl_3$): 144.8, 138.2, 138.1, 137.5, 137.0, 132.8, 129.7, 128.5, 128.4, 128.39, 128.3, 128.1, 128.08, 128.0, 127.97, 127.9, 127.85, 127.8, 127.7, 127.6, 102.1, 84.4, 81.9, 75.6, 74.9, 74.8, 72.6, 71.0, 68.6, 21.6.[262]

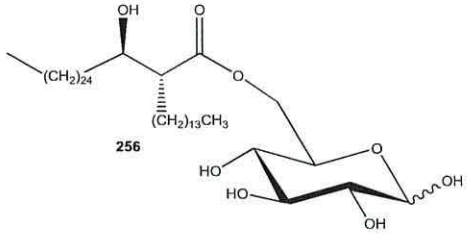
Experimental 96: ((2*R*,4*S*,5*R*,6*R*)-3,4,5,6-Tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl-(2*R*,3*R*)-3-hydroxy-2-tetradecyloctacosanoate (262)



(167) (0.04 mg, 0.06 mmol) was dissolved in THF/DMF (1:1, 2 mL) at r.t., then warm up very gently until all mycolic acid been dissolved. Dry cesium hydrogen carbonate (0.1 mg, 0.5 mmol) was added to a stirred solution and the mixture left at r.t. for 1 h. Tosylate **(257)** (0.04 mg, 0.05 mmol) was added. The mixture was stirred at 70 °C for 18 h, then the reaction was quenched with water (7 mL). The product was extracted with CH_2Cl_2 (3 × 60 mL), dried over $MgSO_4$, filtered and evaporated. The crude product was purified by chromatography over silica gel, eluting with petrol/ethyl acetate (4:1) to give the title compound **(262)** (0.035 mg, 46%). {MS Found $[M+Na]^+$: 1181.8737; $C_{76}H_{118}NaO_8$ requires: 1181.8724}, which showed δ_H (400 MHz, $CDCl_3$): 7.36-7.23 (20H, m), 4.93 (2H, dd, *J* 3.5, 11.0 Hz), 4.88 (2H, d, *J* 12.0 Hz), 4.77 (1H, d, *J* 10.9 Hz), 4.69 (1H, d, *J* 10.9 Hz), 4.64-4.56 (2H, m), 4.54-4.49 (2H, m), 4.23-4.18

(1H, m), 3.65 (2H, t, *J* 8.8 Hz), 3.53-3.45 (3H, m), 2.49-2.42 (1H, m), 1.78-1.38 (6H, m), 1.37-1.13 (69H, m), 0.87 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 175.2, 138.3, 138.2, 137.7, 137.1, 128.5, 128.44, 128.4, 128.3, 128.1, 128.0, 127.9, 127.87, 127.8, 127.6, 102.3, 84.5, 82.2, 77.8, 75.7, 75.1, 74.9, 72.8, 72.3, 71.1, 62.8, 51.2, 35.6, 31.9, 29.69, 29.65, 29.63, 29.59, 29.5, 29.4, 29.3, 27.5, 25.8, 22.6, 21.0, 14.1; ν_{max}: 3539, 2918, 2842, 2364, 1687, 1506, 1462, 1375, 1206, 1035, 738 cm^{-1}.

Experimental 97: ((2*R*,4*S*,5*R*)-3,4,5,6-Tetrahydroxytetrahydro-2*H*-pyran-2-yl)-methyl-(2*R*,3*R*)-3-hydroxy-2-tetradecyloctacosanoate (256)



(262) (0.03 mg, 0.02 mmol) was dissolved in a mixture of hexane and ethyl acetate (1 mL of a 1:1 mixture) and treated under a hydrogen atmosphere with a catalytic amount of 10% palladium hydroxide on charcoal. The mixture was stirred for 18 h at r.t. and the residue was filtered and washed with $CHCl_3$ and evaporated to give the title compound **(256)** as a mixture α:β in ratio 0.4:0.6 as semi solid (0.01 mg, 50%), $[\alpha]_D^{24}$ = 8.1 (c 1.0, $CHCl_3$) {MS Found $[M+Na]^+$: 821.6861; $C_{48}H_{94}NaO_8$ requires: 821.6846}; the mixture showed δ_H (400 MHz, $CDCl_3$): 5.15 (0.4H, d, *J* 3.8 Hz (H α_1)), 4.51 (0.6H, d, *J* 7.7 Hz (H β_1)), 4.43 (1H, dd, *J* 3.71, 12.6 Hz), 4.32 (1H, dd, *J* 5.61, 11.71 Hz), 3.67-3.57 (2H, m), 3.46-3.35 (2H, m), 3.24-3.21 (1H, m), 2.46-2.36 (1H, m), 2.31-2.24 (1H, m), 1.62-1.15 (78H, m), 0.87-0.83 (6H, m); δ_C (α, β isomers) (101 MHz, $CDCl_3$): 175.0, 96.5, 92.2, 74.4, 73.5, 73.4, 72.3, 72.1, 70.3, 69.0, 63.4, 52.6, 52.5, 37.2, 36.8, 34.8, 33.4, 32.5, 31.7, 29.8, 29.5, 29.45, 29.4, 29.3, 29.2, 29.1, 29.0, 27.7, 27.2, 26.8, 25.2, 25.1, 24.2, 22.4, 22.3, 19.4, 18.9, 14.1, 13.8, 11.1; ν_{max}: 3501, 2918, 2845, 1730, 1462, 1206 cm^{-1}.

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6. Appendix

6.1 Synthesis of keto-MA present in *M. tb* (343)

Keto-MA are found in the cell wall of *M. tb* and they are the major oxygenated MA.

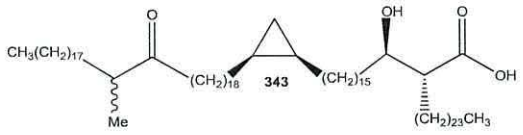
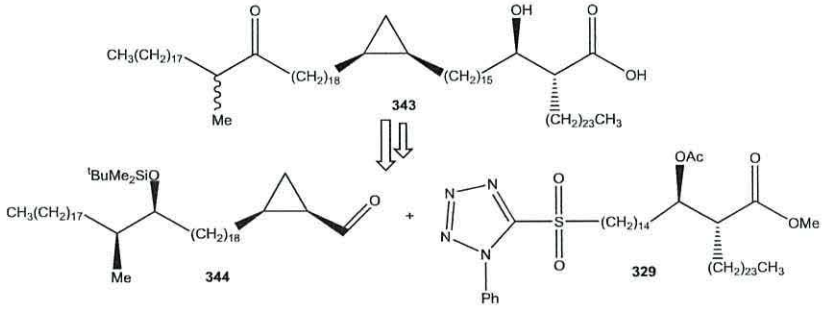


Figure 36: The keto-MA of derived from *M. tb*

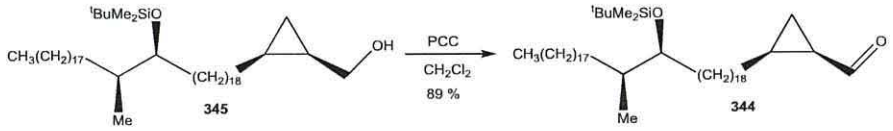
In this study, a keto-MA present in *M. tb* was made by a known method. The coupling of the mycolic motif part **(329)** and the meromycolate part **(344)** was undertaken.



Scheme 105: Full keto-MA disconnection

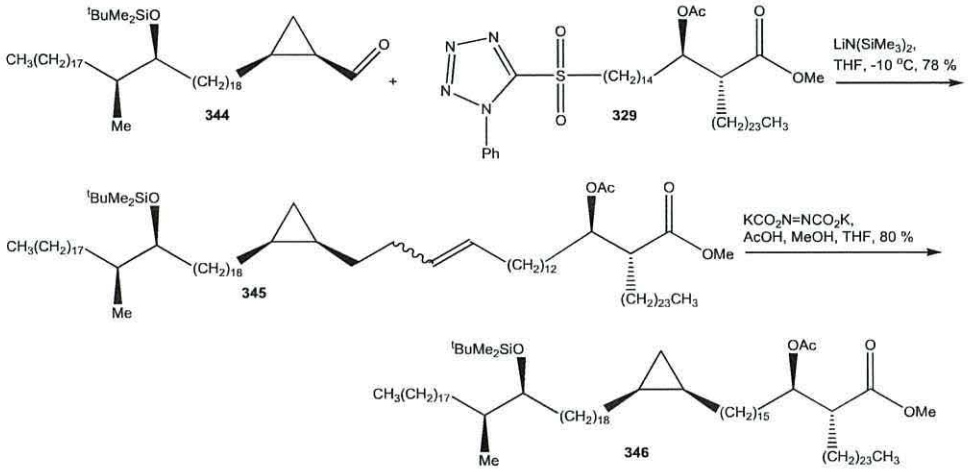
6.1.1 Coupling to form keto-MA (343)

To begin with, the alcohol meromycolate **(345)** was oxidized to the corresponding aldehyde **(344)**, using PCC in dichloromethane solution, as in **Scheme 106**. The spectroscopic data of this compound matched the data given in the literature.[217]



Scheme 106: Oxidation of the keto-meromycolate

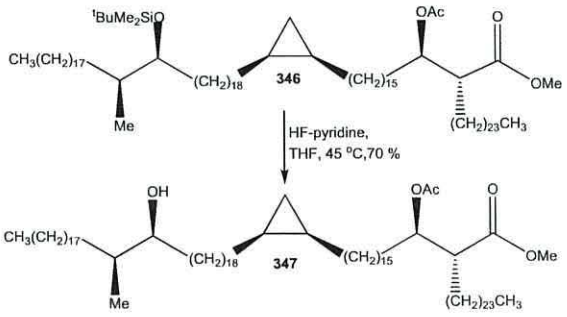
Sulfone **(329)** was coupled with meromycolate aldehyde **(344)** to give alkene **(345)** in an (*E/Z*) mixture as shown in Scheme **(107)**. The hydrogenation of the alkene was achieved as described previously using dipotassium azodicarboxylate.



Scheme 107: The coupling to form keto-MA

6.1.2 Deprotection of the silyl group in meromycolate

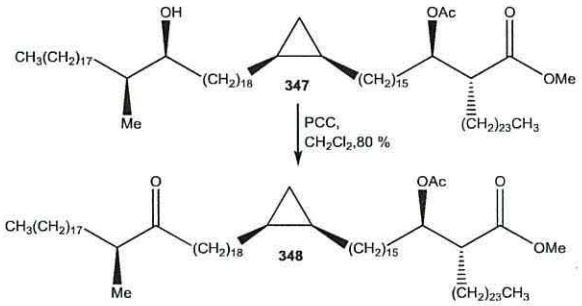
The silyl protecting group was removed from compound **(346)**, using HF-pyridine complex and pyridine in dry THF solution, as shown in Scheme **(108)**.



Scheme 108: Deprotection of the silyl group of (346)

6.1.3 Oxidation of the secondary alcohol to form a ketone

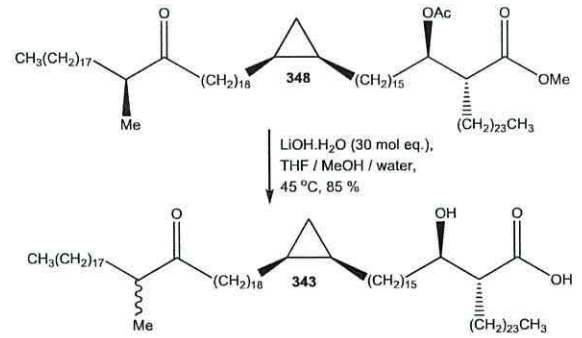
Oxidation of the hydroxy-MA **(347)** with PCC gave keto-MA **(348)** as a white solid (Scheme **109)**.



Scheme 109: Oxidation of the secondary alcohol to give the protected keto-MA

6.1.4 The hydrolysis of the keto-MA

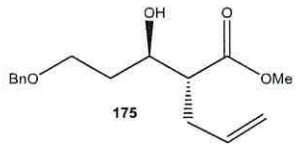
The hydrolysis of the keto-MA methyl ester to the free acid was necessary for biological testing. The acetate and methyl ester groups were deprotected using $LiOH.H_2O$ in a mixture of THF, MeOH and H_2O. The mixture was stirred at 45 ^oC for 18 h to obtain the free hydroxy acid in Scheme **(110)**. The reason for epimerised keto compound **(343)** was explained in chapter result and discussion.[270]



Scheme 110: Hydrolysis of the keto-MA with $LiOH.H_2O$ to give the free acid[270]

6.2 Experimental Section

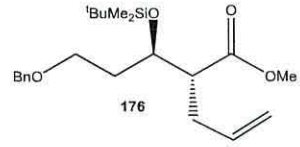
(*R*)-2-((*R*)-3-Benzyloxy-1-hydroxy-propyl)-pent-4-enoic acid methyl ester (175)[207]



175

Diisopropylamine (10.61 g, 104.8 mmol) was dissolved in dry THF (60 mL) and cooled to -20˚C. BuLi (44.0 mL, 66.0 mmol, 1.5M) was added between -20 °C to -10 °C, then stirred to +16 ˚C for 20 min. then re-cooled to -65 ˚C and **(171)** (10.0 g, 41.9 mmol) in dry THF (10 mL) was added and the mixture was stirred at - 45 ˚C for 1 h, - 25 ˚C for 40 min. and then at -20 ˚C to -10 ˚C for 20 min. It was re-cooled to -65 ˚C and allyl iodide (5.77 mL, 62.8 mmol) in dry THF (15 mL) and HMPA (7.27 mL, 83.9 mmol) were added and the mixture was stirred at - 45 ˚C for 1 h, - 45 ˚C to - 20 ˚C for 30 min and then -20 ˚C to -10 ˚C for 30 min. sat. aq. NH_4Cl (70 mL) was added and the product was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried and evaporated. The crude product was purified by column chromatograph eluting with petrol/ethyl acetate (2:1) to give the title compound **(175)** as a colourless oil (9.0 g, 77%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[207]

(*R*)-2-[(*R*)-3-Benzyloxy-1-(*tert*-butyldimethylsilanyloxy)propyl]-pent-4-enoic-acid methyl ester (176)[207]

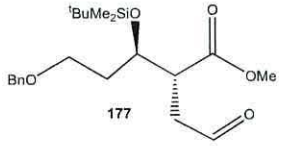


176

Imidazole (22.55 g, 331.2 mmol) was added to a stirred solution of **(175)** (37.10 g, 132.2 mmol) in dry DMF (100 mL) at r.t. and stirred for 30 min. the mixture was then cooled to 0˚C and *tert*-butyldimethylchlorosilane (26.11 g, 172.4 mmol) in dry DMF (10 mL) was added. The cooling bath was removed and the reaction mixture was stirred at 45 ˚C for 20 h. DMF was removed by flash distillation under high vacuum. Water (1000 mL) was added and the product was extracted with dichloromethane (3 × 400 mL). The combined organic layers were washed with water (200 mL), dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give the title compound **(176)** as a colorless oil (46.1 g,

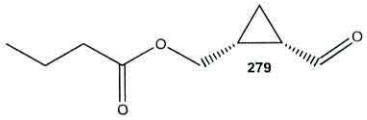
88%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[207]

(2*R*,3*R*)-5-Benzyloxy-3-(*tert*-butyldimethylsilanyloxy)-2-(oxoethyl)pentanoic-acid methyl ester (177)[207]



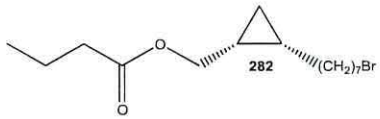
The alkene **(176)** (10.0 g, 25.5 mmol) was weighed in a dry three necked round bottom flask and then dry THF (100 mL) with MeOH (100 mL) added and cooled to -70 °C. O_3 was added and stirred for 10 min. Reaction mixture allowed to warm to -34 °C. Acetic acid (25 mL) and water (1 mL) were added to reaction after remove O_3 from reaction and Zn (2 g) was added slowly to reaction until gray colour disappear and t. going up, $NaHCO_3$ (200 mL) was added slowly and the mixture was stirred for 1 h then added water (200 mL) and extracted with petrol/ethyl acetate (1:1, 3 × 100 mL), dried and the solvent was evaporated. Crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give the title compound **(177)** as a colourless oil (9.7 g, 97%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[207]

(1*S*,2*R*)-1-Butyryloxymethyl-2-formyl-cyclopropane (279)[217]



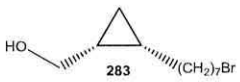
(1*S*,2*R*)-2-hydroxymethyl-cyclopropyl-methylbutyrate **(278)** (10.1 g, 58.1 mmol) in dichloromethane (50 mL) was added in portions to a stirred solution of PCC (1.08 g, 5.02 mmol) in dichloromethane (30 mL) at room temperature. The mixture was stirring vigorously for 2 h. It was poured in petrol/ethyl acetate (10:1, 500 mL), filtered through a pad of silica and the solvent evaporated. The crude product was evaporated and purified by chromatography eluting with petrol/ethyl acetate (10:1) to give the title comound **(279)** as a colourless oil (8.0 g, 80%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

((1*R*,2*S*)-2-(7-Bromoheptyl)cyclopropyl butyrate (282)[217]



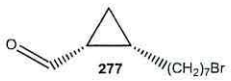
The procedure used in Experiment **1** was repeated in order to couple **(279)** (8.20 g, 47.0 mmol) and **(280)** (22.7 g, 61.1 mmol) using lithium bis (trimethylsilyl) amide (76.5 mL, 81.1 mmol, 1.06 M) in dry THF (200 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick oil of **(281)** (6.0 g, 75%) as a mixture. Hydrogenation was carried out with dipotassium azodicarboxylate (3.51 g, 18.1 mmol), which was added to a stirred solution of the above alkenes mixture **(281)** (6.10 g, 18.9 mmol) in THF (60 mL) and methanol (20 mL) at 5 °C. A solution of glacial acetic acid (20 mL) in THF (20 mL) was added dropwise over a period of two days. Further portions dipotassium azodicarboxylate (2.5 g) and glacial acetic acid (10 mL) were added and the mixture was stirred overnight. This mixture was added slowly to a sat. aq. $NaHCO_3$ and the product was extracted with petrol/ethyl acetate (1:1, 5 × 80 mL) and the combined organic layers were washed with water (200 mL) dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound **(282)** as yellow oil (4.0 g, 66%), which showed δ_H (500MHz, $CDCl_3$), δ_C (125MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

[(1*R*,2*S*)-2-(7-Bromoheptyl)cyclopropyl]methanol (283)[217]



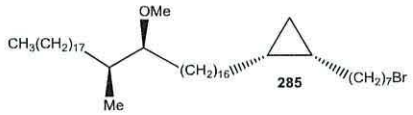
Anhydrous potassium carbonate (4.51 g, 32.6 mmol) was added to a stirred solution of **(282)** (4.21 g, 12.5 mmol) in methanol (30 mL) and THF (20 mL) at room temperature. After 2 h at 45 °C, it was diluted with water (150 mL) and ether (100 mL). The aqueous layer was re-extracted with ether (2 × 50 mL). The combined organic layers were washed with brine, dried and evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give the title compound **(283)** as a colourless oil (2.5 g, 80%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

(1*R*,2*S*)-2-(7-Bromoheptyl)cyclopropane carbaldehyde (277)[217]



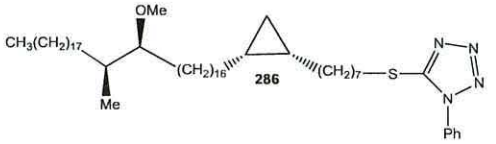
The procedure used in Experiment **3** was repeated in order to to oxidise **(283)** (2.51 g, 10.0 mmol) using PCC (5.45 g, 25.2 mmol) in dichloromethane (200 mL). The product was purified by chromatography with petrol/ethyl acetate (10:1) to give the title compound **(277)** as a colourless oil (2.1 g, 87%) which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

(1*S*,2*R*)-1-(7-Bromoheptyl)-2-(17*S*,18*S*)-17-methoxy-18-methyl-hexatriacontenyl)-cyclopropane (285)[217]



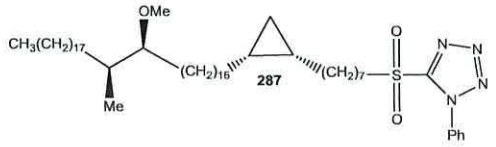
The procedure used in Experiment **1** was repeated in order to couple **(277)** (2.0 g, 8.1 mmol) and **(276)** (6.1 g, 8.2 mmol) using lithium bis (trimethylsilyl) amide (11.5 mL, 12.2 mmol, 1.06 M) in dry THF (80 mL). The crude prodect was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil of **(284)** (4.0 g, 66%) as a alkenes mixture. Hydrogenation was carried out with dipotassium azodicarboxylate (3.51 g, 18.1 mmol), which was added to a stirred solution of the above alkenes mixture **(284)** (4.0 g, 5.2 mmol) in THF (60 mL) and methanol (20 mL) at 5 °C. A solution of glacial acetic acid (20 mL) in THF (20 mL) was added dropwise over a period of two days. Further portions dipotassium azodicarboxylate (2.0 g) and glacial acetic acid (5 mL) were added and the mixture was stirred overnight. This mixture was added slowly to a sat. aq. $NaHCO_3$ and the product was extracted with petrol/ethyl acetate (1:1, 6 × 80 mL) and the combined organic layers were washed with water (200 mL) dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compound **(285)** as a viscous colourless oil (2.5 g, 62%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

5-{7-[(1*S*,2*R*)-2-((17*S*,18*S*)-17-Methoxy-18-methylhexatriacontenyl)cyclopropyl]-heptylsulfanyl}-1-phenyl-1*H*-tetrazole (286)[217]



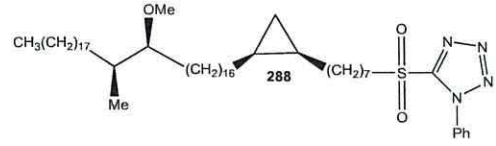
1-Phenyl-*1H*-tetrazole-5-thiol (0.4 g, 2.1 mmol) and **(285)** (1.4 g, 1.8 mmol) in THF (5 mL) and anhydrous potassium carbonate (0.95 g, 6.87 mmol) in acetone (25 mL) were mixed at r.t. then the mixture was vigorously stirred for 18 h. Water (50 mL) was added to the mixture and the product was extracted with dichloromethane (1 × 50 mL, 1 × 100 mL). The combined organic layers were washed with brine (2 × 50 mL), dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound **(286)** as a semi-solid (1.2 g, 80%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

5-{7-[(1*S*,2*R*)-2-((17*S*,18*S*)-17-Methoxy-18-methylhexatriacontenyl)cyclopropyl]-heptylsulfonyl}-1-phenyl-1*H*-tetrazole (287)[217]



m-Chloroperbenzoic acid (0.8 g, 4.9 mmol) in dichloromethane (15 mL) was added at 0 °C to a stirred solution of **(286)** (1.2 g, 1.3 mmol) and $NaHCO_3$ (0.5 g, 5.9 mmol) in dichloromethane (25 mL) and stirred at r.t. for 20 h. The mixture was quenched by addition of a sat. aq. NaOH (30 mL) and extracted with dichloromethane (4 × 50 mL). The combined organic layers were washed with water (100 mL), dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give the title compound **(287)** as a white solid (0.8 g, 82%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

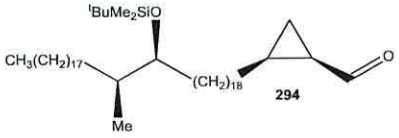
5-{7-[(1*R*,2*S*)-2-((17*S*,18*S*)-17-Methoxy-18-methylhexatriacontenyl)cyclopropyl]-heptylsulfonyl}-1-phenyl-1*H*-tetrazole (288)[217]



m-Chloroperbenzoic acid (1.0 g, 5.8 mmol) in dichloromethane (15 mL) was added at 0 °C to a stirred solution of 5-{7-[(1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexa-

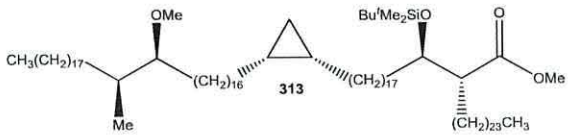
triacontenyl)cyclopropyl]heptylsulfanyl}-1-phenyl-1*H*-tetrazole (1.5 g, 1.7 mmol) and $NaHCO_3$ (0.8 g, 9.5 mmol) in dichloromethane (25 mL) and stirred at r.t. for 20 h. The mixture was quenched by addition of a sat. aq. NaOH (30 mL) and extracted with dichloromethane (2 × 30 mL). The combined organic layers were washed with water (100 mL), dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give the title compund **(288)** as a white solid (0.9 g, 80%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

(1*R*,2*S*)-2-[(19*S*,20*S*)-19-(*tert*-butyldimethylsilanyloxy)-20-methyl-octatriacontyl]-cyclopropanecarbaldehyde (294)[217]



(296) (1.9 g, 2.5 mmol) in dichloromethane (40 mL) was added in portions to a stirred solution of PCC (1.6 g, 7.6 mmol) in dichloromethane (60 mL) at r.t. and stirred vigorously for 2 h. It was poured in petrol/ethyl acetate (10:1, 100 mL), filtered through a pad of silica gel and the solvent evaporated. The crude product was purified by chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound **(294)** as colourless oil (1.7 g, 89%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

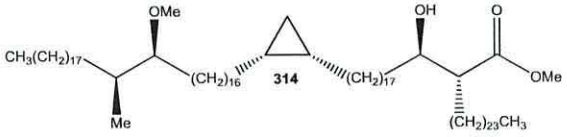
(*R*)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*S*,2*R*)-2-(17*S*,18*S*)-17-methoxy-18-methylhexatricontyl)cyclopropyl]octadecyl}hexacosanoic acid methyl ester (313)[217]



Lithium bis(trimethylsilyl)amide (2.5 mL, 2.7 mmol, 1.06 M) was added dropwise to a stirring solution of **(311)** (1.7 g, 2.1 mmol) and **(287)** (1.5 g, 1.8 mmol) in dry THF (50 mL) at -10 °C under nitrogen. The reaction turned bright yellow and was left to reach r.t. and stirred for 1 h under nitrogen. The reaction was quenched by adding sat. aq. NH_4Cl. The product was extracted with petrol/ethyl acetate (10:1) (3 x 100 mL), dried over $MgSO_4$, filtered and the solvent evaporated. The crude product was purified by column chromatography over silica gel, eluting solvent with petrol/ethyl acetate (20:1)

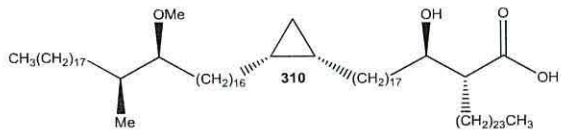
to give the mixture of alkene **(312)** as a colourless oil (1.7 g, 77%). Hydrogenation was carried out with dipotassium azodicarboxylate (2.10 g, 10.3 mmol), which was added to a stirred solution of the above alkenes **(312)** (1.7 g, 1.2 mmol) in THF (30 mL) and methanol (7 mL) at 5 °C. A solution of glacial acetic acid (5 mL) in THF (5 mL) was added dropwise over a period of two days. Further portions dipotassium azodicarboxylate (1 g) and glacial acetic acid (1 mL) were added and the mixture was stirred overnight. This mixture was added slowly to a sat. aq. $NaHCO_3$ and the product was extracted with petrol/ethyl acetate (1:1, 3 × 80 mL) and the combined organic layers were washed with water (100 mL) dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compund **(313)** as a colourless oil (1.4 g, 82%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

(*R*)-2-{(*R*)-1-Hydroxy-18-[(1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatricontyl)cyclopropyl]octadecyl}hexacosanoic acid methyl ester (314)[217]



(313) (1.4 g, 1.0 mmol) was dissolved in dry THF (20 mL) in a dry polyethylene vial under nitrogen at 0 °C. Pyridine (0.1 mL) and HF-pyridine (1.2 mL) were added and the mixture was stirred at 45 °C for 18 h. The mixture was added slowly to sat. aq. $NaHCO_3$ (20 mL). The product was extracted with petrol/ethyl acetate (5:1, 3 × 50 mL) and the combined organic extracts were dried, filtered and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compund **(314)** as a white solid (1.0 g, 71%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

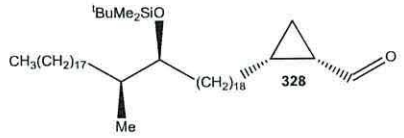
(*R*)-2-{(*R*)-1-Hydroxy-18-[(1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatricontyl)cyclopropyl]octadecyl}hexacosanoic acid (310)[217]



Lithium hydroxide monohydrate (0.51 g, 11.9 mmol) was added to a stirred solution of **(314)** (1.0 g, 0.8 mmol) in THF (13 mL), methanol (1 mL) and water (0.5 mL) at r.t.

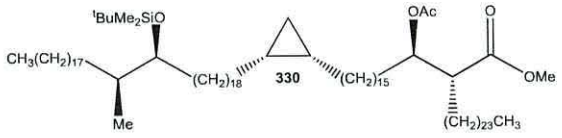
The mixture was stirred at 45 °C for 24 h. The reaction was acidified with 5% HCl and the product was extracted with petrol/ethyl acetate (5:1) (2 × 50 mL), dried and evaporated to give a white solid. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:2) gave the title compund **(310)** as a white solid (0.7 g, 70%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

(1*S*,2*R*)-2-[(19*S*,20*S*)-19-(*tert*-butyldimethylsilanyloxy)-20-methyl-octatriacontyl]-cyclopropanecarbaldehyde (328)[217]



(1*S*,2*R*)-2-[(19*S*,20*S*)-19-(*tert*-butyldimethylsilanyloxy)-20-methyloctatriacontyl]cyclo-propyl}methanol (1.0 g, 1.1 mmol) in dichloromethane (10 mL) was added in portions to a stirred solution of PCC (0.7 g, 3.3 mmol) in dichloromethane (20 mL) at r.t. then stirred vigorously for 2 h. It was poured in petrol/ethyl acetate (10:1, 100 mL), filtered through a pad of silica and the solvent evaporated. The crude product was purified by chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound **(328)** as a colourless oil (0.8 g, 88%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[217]

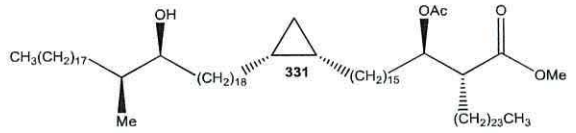
Methyl (*R*)-2-((*R*)-1-Acetoxy-16-{(1*S*,2*R*)-2-[(19*S*,20*S*)-19-(*tert*-butyldimethyl-silanyloxy)-20-methyloctatriacontyl]cyclopropyl}hexdecyl)hexacosanoate (330)[269]



The procedure used in Experiment **1** was repeated in order to couple **(328)** (1.7 g, 2.2 mmol) and **(329)** (2.2 g, 2.5 mmol) using lithium bis(trimethylsilyl)amide (3.5 mL, 3.7 mmol, 1.06 M) in dry THF (30 mL) at -10 °C. The crude prodect was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil as mixture of alkene (2.10 g, 65%). Hydrogenation was carried out using dipotassium azodicarboxylate (2.10 g, 10.3 mmol) which was added to a stirred solution of the above alkenes (2.1 g, 1.4 mmol) in THF (30 mL) and methanol (10 mL) and a solution of glacial acetic acid (10 mL) and THF (10 mL) was added as before. The crude

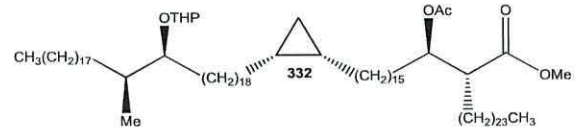
product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compund **(330)** as a white semi-solid (1.5 g, 71%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[269]

(*R*)-2-((*R*)-1-Acetoxy-16-{(1*S*,2*R*)-2-[(19*S*,20*S*)-19-hydroxy-20-methylocatriacontyl]cyclopropyl}hexadecyl)hexacosanoic acid methyl ester (331)[269]



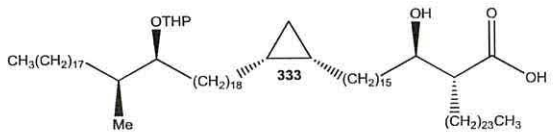
The procedure used in Experiment **24** was repeated using **(330)** (0.8 g, 0.5 mmol) pyridine (0.1 mL) and HF-pyridine (1.0 mL) in dry THF (20 mL). The product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compund **(331)** as a white semi-solid (0.7 g, 80%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[269]

(*R*)-2-((*R*)-1-Acetoxy-16-{(1*S*,2*R*)-2-[(19*S*,20*S*)-19-(tetrahydropyran-2-yloxy)octatriacontyl]cyclopropyl}hexadecyl)hexacosanoic acid methyl ester (332)[269]



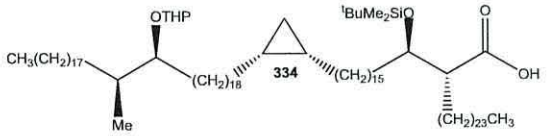
Pyridinium-*p*-toluene sulfonate (0.04 g, 0.15 mmol) in dry dichloromethane (8 mL) was added with stirring of **(331)** (0.5 g, 0.4 mmol) and freshly distilled dihydro-2*H*-pyran (0.3 mL, 3.8 mmol) in dry dichloromethane (20 mL) at r.t. under nitrogen. After 3 h, the reaction was quenched with a sat. aq. $NaHCO_3$ (10 mL), extracted with dichloromethane (4 × 50 mL) and the combined organic layers were dried and evaporated. The product was purified by chromatography eluting with petrol/ethyl acetate (10:1) to give the title compund **(332)** as a white semi-solid (0.5 g, 94%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[269]

(*R*)-2-((*R*)-1-Hydroxy-16-{(1*S*,2*R*)-2-[(19*S*,20*S*)-19-(tetrahydropy-ran-2-yloxy)-octatriacontyl]cyclopropyl}hexadecyl)hexacosanoic acid (333)[269]



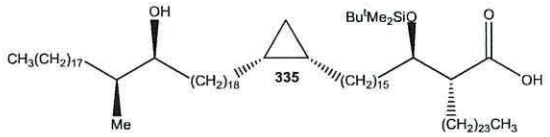
The procedure in Experiment **25** was repeated to hydrolyse **(332)** (0.5 g, 0.4 mmol) using lithium hydroxide monohydrate (0.2 g, 5.5 mmol) in THF (20 mL), methanol (1.5 mL) and water (2 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compund **(333)** as a white semi solid (0.38 g, 80%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[269]

(*R*)-2-((*R*)-1-(*tert*-butyldimethylsilanyloxy)-16-{(1*S*,2*R*)-2-[(19*S*,20*S*)-19-(tetrahydropyran-2-yloxy)octatriacontyl]cyclopropyl}hexadecyl)hexacosanoic-acid(334)[269]



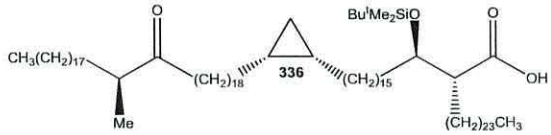
Imidazole (0.2 g, 2.8 mmol) was added with stirring to **(333)** (0.38 g, 0.28 mmol) in dry DMF (2 mL) and dry toluene (3 mL) at r.t. followed by the addition of tertbutyldimethylsilyl chloride (0.43 g, 2.85 mmol) and 4-DMAP (0.03 g, 0.24 mmol). The reaction mixture was stirred at 70 °C for 18 h at r.t. The solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate (10:1) (30 mL) and sat. aq. $NaHCO_3$ (10 mL). The organic layer was separated, and the aqueous layer was re-extracted with petrol/ethyl acetate (4 × 20 mL). The combined organic layers were washed with water, dried and evaporated. The residue was dissolved in THF (6 mL), water (1 mL) and methanol (0.5 mL); to this was added a potassium carbonate (0.15 g, 1.08 mmol). The reaction mixture was stirred at 45 °C for 6 h. The mixture was diluted with petrol/ethyl acetate (10:1, 40 mL) and water (1 mL), then acidified with potassium hydrogen sulfate to pH 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (2 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compund **(334)** as a white semi-solid (0.2 g, 50%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[269]

(*R*)-2-{(*R*)-1-(*tert*-butyldimethylsilanyloxy)-16-[(1*S*,2*R*)-2-[(19*S*,20*S*)-19-hydroxy-20-methyloctatriacontyl)cyclopropyl]hexadecyl}hexacosanoic acid (335)[269]



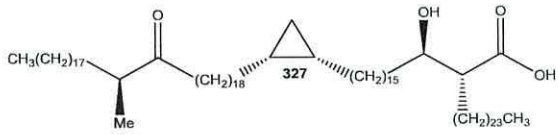
Pyridinium-*p*-toluenesulfonate (0.3 g, 1.2 mmol) was added of **(334)** (0.2 g, 0.1 mmol) in THF (7 mL), MeOH (0.5 mL) and stirred at 47 °C for 7 h. Sat. aq. sodium bicarbonate (0.5 mL) was added and the product was extracted with petrol/ethyl acetate (5 × 25 mL, 1:1). The combined organic layers were dried and evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title comound **(335)** as a white semi solid (0.14 g, 73%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[269]

(*R*)-2-{(*R*)-1-(*tert*-butyldimethylsilanyloxy)-16-[(1*S*,2*R*)-2-((*S*)-20-methyl-19-oxo-20-octatriacontyl)cyclopropyl]hexadecyl}-hexacosanoic acid (336)[269]



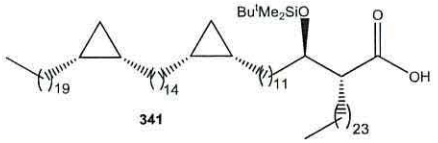
The procedure used in Experiment **3** was repeated in order to oxidise of **(335)** (0.24 g, 0.17 mmol) using PCC (0.15 g, 0.69 mmol) in dichloromethane (15 mL). The crude product was purified by chromatography eluting with petrol/ethyl acetate (10:1) to give the titel compound **(336)** as a white semi-solid (0.2 g, 86%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[269]

(*R*)-2-{(*R*)-1-Hydroxy-16-[(1*S*,2*R*)-2-((*S*)-20-methyl-19-oxo-20-octatriacontyl)-cyclopropyl]hexadecyl}hexacosanoic acid (327)[269]



The procedure used in Experiment **23** was repeated using **(336)** (0.10 g, 0.08 mmol), pyridine (0.1 mL) and HF.pyridine (0.5 mL) in dry THF (10 mL) The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compund **(327)** as a white solid (0.07 g, 65%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[269]

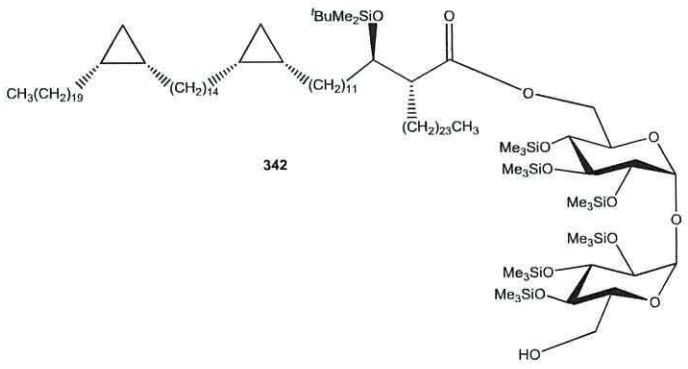
(*R*)-2-((*R*)-1-(*tert*-butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic acid (341)[224]



341

Imidazole (0.2 g, 0.3 mmol) was added to a stirred solution of **(339)** (0.3 g, 0.2 mmol) in dry DMF (2 mL) and dry toluene (3 mL) at r.t. followed by the addition of *tert*-butyldimethylsilylchloride (0.6 g, 4.0 mmol) and 4-DMAP (0.05 g, 0.41 mmol). The reaction mixture was stirred at 70 °C for 24 h. The solvent was removed under reduced vacuum and the residue was diluted with petrol/ethyl acetate 10:1 (11 mL) and water (5 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (5:1, 2 × 40 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue. The residue was dissolved in THF (8 mL), water (1.5 mL), and methanol (1.2 mL), to this was added potassium carbonate (0.2 g, 1.4 mmol). The reaction mixture was stirred at 45 °C overnight. The mixture was diluted with petrol/ethyl acetate (10:1, 20 mL) and water (2 mL) then acidified with $KHSO_3$ to pH 2. The organic layer was separated and the aqueous layer was re extracted with petrol/ethyl acetate (5:1, 2 × 50 mL). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (20:1), (10:1) to give the title compound **(341)** as a colourless oil (0.3 g, 90%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[224]

6-O-(*R*)-2-((*R*)-1-(*tert*-butyldimethylsilanyloxy)-12-{(1*R*,2*S*)-2-[14-((1*R*,2*S*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-2,3,4,2',3',4'-hexakis-O-(trimethylsilyl)-α,α'-trehalose (342)[224]

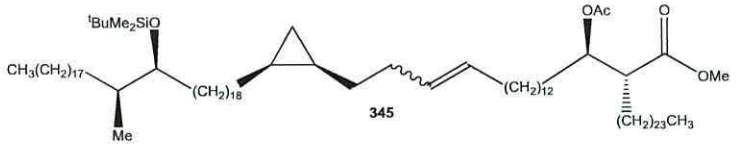


342

(EDCI) (0.3 g, 1.5 mmol) and 4-DMAP (0.16 g, 1.31 mmol) were added to a stirred solution of **(341)** (0.30 g, 0.24 mmol) and **(160)** (0.15 g, 0.19 mmol) powdered 4 Å

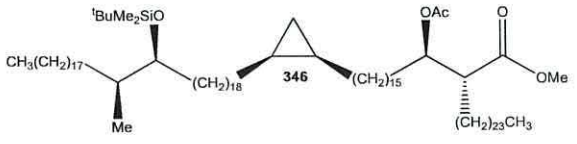
molecular sieves in dry dichloromethane (4 mL) at r.t. under nitrogen atmosphere. The mixture was stirred for 6 days at r.t. then diluted with dichloromethane (5 mL) and filtered. The filtrate was evaporated under reduced pressure to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compound **(342)** as colourless oil (0.24 g, 57%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[224]

(*R*)-2-((*E/Z*)-(*R*)-1-Acetoxy-16-{(1*R*,2*S*)-2-[(19*S*, 20*S*)-19-(*tert*-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-hexadec-15-enyl)hexa-cosanoicacid methyl ester (345)[270]



(329) (2.2 g, 2.5 mmol) was dissolved in dry THF (30 mL) and a solution of **(344)** (1.7 g, 2.3 mmol) in dry THF (70 mL) was added at r.t. This solution was cooled to -12 °C and lithium bis(trimethylsilyl) amide (3.4 mL, 3.6 mmol, 1.06 M) was added at -12 °C. The solution was allowed to reach r.t. and stirred for 2 h. Petrol/ethyl acetate (100 mL) and sat. aq. ammonium chloride (100 mL) were added. The organic phase was separated and water layer was extracted with petrol/ether (20:1, 3 × 100 mL). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ether (20:1) to give the title compound **(345)** as a mixture of *E/Z* isomers in ratio 4:1 and as a colourless oil **(345)** (2.50 g, 78%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[270]

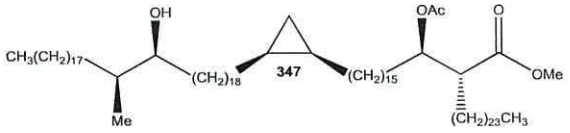
(*R*)-2-((*R*)-1-Acetoxy-16-{(1*R*,2*S*)-2-[(19*S*,20*S*)-19-(*tert*-butyl-dimethylsilanyl-oxy)-20-methyloctatriacontyl]cyclopropyl}-octadec-yl)-hexacosanoic acid methyl ester (346)[270]



Dipotassium azodicarboxylate (4.10 g, 20.2 mmol) was added to a stirred solution of **(345)** (2.5 g, 1.7 mmol) in THF (50 mL) and methanol (10 mL) at 5 °C. A solution of glacial acetic acid (10 mL) and THF (5 mL) was prepared and half of this solution was added dropwise at 5 °C and the mixture was stirred at r.t. for 2 h. The other half of the

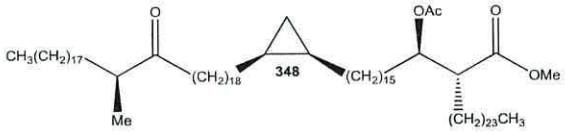
glacial acetic acid solution was added dropwise at r.t and the mixture was stirred at r.t. for 18 h. Further dipotassium azodicarboxylate (2.0 g) was added and glacial acetic acid (10 mL) was added and the mixture was stirred another 18 h. and then the reaction was quenched by slow addition to sat. aq. NH_4Cl (100 mL). The organic component was extracted with petrol/ethyl acetate (1:1, 3 × 80 mL) and the combined organic layers were washed with water (50 mL), dried and evaporated. The procedure was repeated and the product was purified by column chromatography, eluting with petrol/ ether (20:1) to give the title compound **(346)** as a white semi-solid (2.0 g, 80%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[270]

(*R*)-2-((*R*)-1-Acetoxy-18-{(1*R*,2*S*)-2-[(19*S*,20*S*)-19-hydroxy-20-methyloctatria-contyl]-cyclopropyl}-octadecyl)-hexacosanoic acid methyl ester (347)[270]



(346) (0.8 g, 0.5 mmol) was dissolved in dry THF (12 mL) in a dry polyethylene vial under argon at r.t and stirred. Pyridine (0.1 mL) and HF-pyridine (1.0 mL) was added and the mixture was stirred for 17 h at 40 °C. The reaction was diluted with petrol/ether (1:1, 70 mL) and neutralized with sat.aq. $NaHCO_3$ until no more carbon dioxide was liberated. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 × 50 mL). The combined organic layers were washed with brine and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ether (5:2) to give the title compound **(347)** as a white solid (0.5 g, 70%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[270]

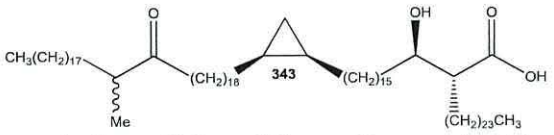
(*R*)-2-{(*R*)-1-Acetoxy-16-[(1*R*,2*S*)-2-((*S*)-20-methyl-19-oxo-octatriacontyl)-cyclopropyl]-hexadecyl}-hexacosanoic acid methyl ester (348)[270]



(347) (0.5 g, 0.4 mmol) was dissolved in dichloromethane (10 mL) and added in portions to a stirred solution of PCC (0.2 g, 1.1 mmol) in dichloromethane (10 mL) at r.t. The mixture was stirred for 3 h at r.t., then diluted with ether (10 mL) and filtered through a bed of silica gel. The solvent was evaporated and the crude product was

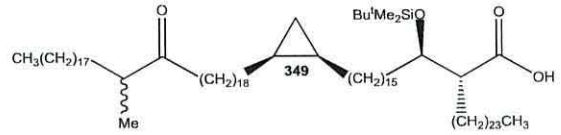
purified by column chromatography eluting with petrol/ether (5:1) to give the title compound **(348)** as a white solid (0.4 g, 80%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[270]

(*R*)-2-{(*R*)-1-Hydroxy-16-[(1*R*,2*S*)-2-((*S*)-20-methyl-19-oxo-octatriacontyl)-cyclopropyl]hexadecyl}-hexacosanoic acid (343)[270]



Lithium hydroxide monohydrate (0.2 g, 5.7 mmol) was added to a stirred solution of **(348)** (0.51 g, 0.38 mmol) in THF (15 mL), methanol (1.5 mL) and water (2 mL) at r.t., then stirred at 45 °C for 18 h. It was cooled to r.t. and a mixture of petrol/ethyl acetate (1:1, 20 mL) was added and the mixture was acidified with 5% HCl until pH 1. Further petrol/ethyl acetate (5:2, 40 mL) was added and the organic layer was separated. The aq. layer was re-extracted with petrol/ethyl acetate (5:2, 4 × 20 mL). The combined organic layers were washed with water (20 mL), dried and evaporated. The product was purified by column chromatography eluting with petrol/ ethyl acetate (7:2) to give the title compound **(343)** as a white solid (0.4 g, 85%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[270]

(*R*)-2-{(*R*)-1-(*tert*-butyldimethylsilanyloxy)-16-[(1*R*,2*S*)-2-((*S*)-20-methyl-19-oxo-20-octatriacontyl)cyclopropyl]hexadecyl}hexacosanoic acid (349)[270]



Imidazole (0.11 g, 1.61 mmol) was added to a stirred solution of **(343)** (0.21 g, 0.16 mmol) in dry DMF (0.5 mL) and dry toluene (1 mL) at r.t. followed by the addition of *tert*-butyldimethylsilylchloride (0.24 g, 1.59 mmol) and 4-DMAP (0.01 g, 0.08 mmol). The reaction mixture was stirred at 70 °C for 24 h. The solvent was removed under reduced vacuum and the residue was diluted with petrol/ethyl acetate 10:1 (20 mL) and water (5 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (5:1, 2 × 40 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue. The residue was dissolved in THF (2 mL), water (0.5 mL) and methanol (0.3 mL) to this was added potassium carbonate (0.15 g, 1.08 mmol). The reaction mixture was stirred at 45 °C

overnight. The mixture was diluted with petrol/ethyl acetate (10:1, 11 mL) and water (2 ml) then acidified with $KHSO_3$ to pH 2. The organic layer was separated and the aqueous layer was re extracted with petrol/ethyl acetate (5:1, 2 × 50 mL). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound **(349)** as a colourless oil (0.18 g, 90%), which showed δ_H (500MHz, $CDCl_3$), δ_C (126MHz, $CDCl_3$), ν_{max} identical to the literature.[270]