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Synthetic approaches to single enantiomers of mycolic acids

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Gianna Toschi

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Contents

Abbreviations and acronyms

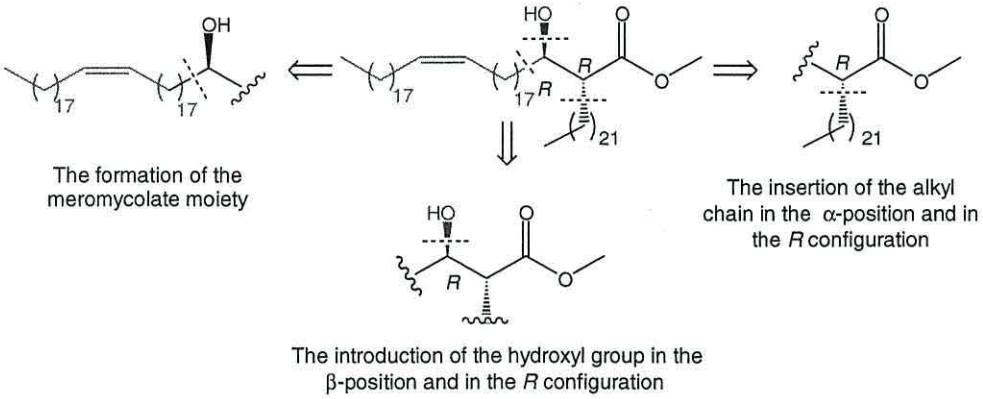
Ac	Acetyl
AG	Arabinogalactan
Alk	Alkyl
b	Broad
BCG	Bacillus Calmette-Guerin
Bn	Benzyl
BuLi	Butyllithium
CI	Chemical Ionisation
CID	Collision Induced Dissociation
Cp_2ZrCl_2	Zirconocene dichloride
CSA	Camphorsulfonic acid
d	Doublet
DEAD	Diethyl azodicarboxylate
d.e.	Diastereomeric Excess
DET	Diethyl tartrate
DHP	2,3-Dihydro-2H-pyran
$(DHQD)_2PHAL$	Hydrochinidin 1,4-phthalazinediyl diether
$(DHQ)_2PHAL$	Dihydrochinin 1,4-phthalazinediyl diether
DI	Direct Insertion
DIBAL-H	Diisobutylaluminium Hydride
dil.	Diluted
DMAC	*N,N*-Dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
EDCl	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide
e.e.	Enantiomeric Excess
EI	Electron Impact
G	Generic substituent
GC	Gas Chromatography
FAS	Fatty Acid Synthase
IR	Infra-Red
HMPA	Hexamethylphosphorotriamide
HPLC	High Performance Liquid Chromatography
Hz	Hertz
LDA	Lithium *N,N*,-Diisopropylamide
m	Multiplet
m-	*Meta*-
mmol.	millimoles

mol. eq.	Molar equivalents
mol. %	Molar percentages
mp	Melting Point
MP	*p*-Methoxyphenyl
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-Of-Flight
MCPBA	*m*-Chloroperoxybenzoic acid
MMFF	Molecular Mechanics Force Field
MS	Mass Spectroscopy
NOESY	Nuclear Overhauser Effect Spectroscopy
NMR	Nuclear Magnetic Resonance
o-	*Ortho*-
p-	*Para*-
PCC	Pyridinium Chlorochromate
petrol	Petroleum Spirit
PG	Peptidoglycan
PPTS	Pyridinium *p*-toluenesolphonate
Prot	Protected
PT	Phenyltetraazole
PTSA	*p*-Toluensulphonic acid monohydrate
Pv	Pivaloyl
q	Quartet
Red-Al	Sodium bis(2-methoxyethoxy)aluminium hydride
R_f	Retardation factor
ROESY	Rotational Nuclear Overhauser Effect Spectroscopy
s	Singlet
SAM	S-Adenosyl-L-methionine
t	Triplet
T-cells	T Lymphocytes
TBAB	Tetra-*n*-butylammonium bromide
TDM	Trehalose-6,6'-Dimycolate
TBDMS	*tert*-Butyldimethylsilyl
TBDPS	*tert*-Butyldiphenylsilyl
TEMPO	2,2,6,6-Tetramethyl-1-piperdinyloxy
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin-Layer Chromatography
Tol	Tolyl
WHO	World Health Organization

Abstract

The aim of this study was to analyse different approaches for the synthesis of one of the most frequent α^1-mycolic acids present in *Mycobacterium smegmatis*. These α-alkyl-β-hydroxylated fatty acids are major constituents of the cell envelope of this and other related organisms. They are thought to be implicated in the virulence of those species which are pathogenic, e.g. *Mycobacterium tuberculosis*.[1-5]

Many different methods were analysed for solving the three key problems encountered in the retro-synthesis of these compounds (**Scheme 1**).



Scheme 1: The three key problems.

In the preparation of the meromycolate moiety, the Julia reaction was a useful alternative to the Grignard reaction for the formation of long bifuntional chains. The double bond in the (*E*)-configuration was obtained through a Wittig reaction (**Scheme 1**).

Three methods were tested for the preparation of the β-hydroxy ester (**Scheme 1**). Two were based on either the Sharpless epoxidation, or the Sharpless dihydroxylation. In the third approach, the chiral centre was already present in the initial starting material, *D*-Aspartic acid.[6-8] Only the last two were successful.

Three approaches were also attempted for the insertion of a C_{21} chain into the α-position (**Scheme 1**) using: either the Fräter alkylation, or the regioselective and stereospecific introduction of a chain on reactive species, such as a cyclic sulphate; or an asymmetric *anti*-aldol condensation using a chiral auxiliary.[9-11]

1. Introduction

1.1 Tuberculosis and related diseases

1.1.1 Overview

Tuberculosis, a disease caused by *Mycobacterium tuberculosis,* has an ancient history and was well recognized by the time of Hippocrates, who gave an excellent clinical description of the disease.[12]

However, tuberculosis became a recognised public problem during the Industrial Revolution, when cities were overcrowded and the public health care facilities were inadequate for the number of citizens. In the 18th and 19th centuries it was the cause of 25 % of all adult deaths in European cities.[13]

Around the beginning of the 20th century, several public health measures significantly reduced the occurrence of the infection. The decline in the death rate from tuberculosis in industrialised countries clearly occurred prior to the introduction of effective chemotherapy.[12] This has been attributed to a progressive improvement in living conditions and to a reduction in the exposure to infection brought about through the segregation of persons with infectious TB.[12] In developed countries, the incidence of TB decreased even more steadily since the introduction of streptomycin, in combination with other drugs, in the 1940s. The control of the spread of this disease seemed possible at least in "Western" countries.[14]

Despite all these improvements, however, the complete disappearance of this disease was only an illusion. It always remained active in developing countries. Furthermore, during the 1980's, in developed countries such the United States, the trend of the annual rate incidence of tuberculosis began to rise again.[15]

It has been estimated that TB has killed 100 million people over the past 100 years.[16] The spread of TB in these recent years is definitely related to a new deterioration in living conditions, diet, housing and access to health care, and also to other factors only recently experienced.

A primary reason is HIV/AIDS, whose assault on immune systems makes people infected with it much more vulnerable to tuberculosis. In many countries a person co-infected with HIV and *M. tuberculosis* is 30-times more likely to become manifestly sick with TB, than someone infected with *M. tuberculosis* but HIV-negative.[17,18] In 2000, TB was the cause of 11 % of all adult AIDS deaths.[19]

In addition, there has been a simultaneous increase in cases of drug-resistant tuberculosis, which is due to an ineffective administration of antibiotic and other chemotherapeutic agents.[20] Rates of multi-drug-resistant TB are high, especially in the former Soviet Union countries, and it is a significant threat for TB control efforts.[21] The WHO estimates that up to 50 million persons worldwide may be infected with drug resistant strains of tuberculosis.[1] Another factor that helps the spread of TB is the movement of people, travellers, refugees or displaced people. In many industrialized countries, at least one-half of TB cases are among foreign-born people, in the USA nearly 40 %.[21]

The TB problem remains enormous; in 2000, there were approximately 8-9 million new cases,[21] and the disease kills about 2 million people each year.[21] Tuberculosis is estimated to be the second leading infectious cause of death in the world, after HIV/AIDS and far greater than measles and malaria.[16] Moreover, it is believed that one-third of the world population is infected with the organism that causes the disease, *M. tuberculosis*, though in the majority of the cases this will not lead to evident disease.[13]

The global incidence rate of tuberculosis is growing at approximately 0.4 %/year, but much faster in sub-Saharan Africa and in countries of the former Soviet Union.[1] The World Health Organization in 1993 took an unprecedented step and declared TB a global emergency.[21] WHO estimated that, without immediate action, 1000 million people will be newly infected, over 150 million will become ill and 36 million will die of TB between 2002 and 2020.[21]

Alongside *M. tuberculosis*, there are many other pathogenic mycobacteria: for example, *Mycobacterium canetti, Mycobacterium microti* and *Mycobacterium bovis* which are all closely related species and members of the *M. tuberculosis* complex. In particular, *M. bovis* is a causative agent of TB not only in humans but also in many other animals, the most significant being cattle. It causes worldwide annual losses to agriculture of $3 billion.[22] In the UK alone, 7000 cattle were slaughtered due to bovine tuberculosis during 1999.[23]

Moreover, there are several non-tuberculous mycobacteria (NTM) that may cause human diseases but do not cause TB. *Mycobacterium leprae* is the cause of leprosy. Over the last 17 years, the global prevalence of this disease has fallen by almost 90 % and more than 13 million patients have been cured. In 2000 leprosy was eliminated from the list of global public health problems.[24]

Buruli ulcer, a disease caused by *Mycobacterium ulcerans* had since 1980 emerged as an important cause of human suffering, making it the third most common mycobacterial infection after TB and leprosy.[25]

A particular strain, *Mycobacterium avium paratuberculosis* has been identified as a possible cause of the Chron's disease in humans,[26] and the Johne's disease in animals.[27]

Finally, other mycobacteria such as *Mycobacterium avium* complex, *Mycobacterium kansasii*, *Mycobacterium fortuitum* and *Mycobacterium chelonae* cause TB-like diseases in immune deficient people in particular in AIDS patients.[28]

1.1.2 Mycobacterial cell envelope

All the diseases described above have one common characteristic; they are caused by organisms belonging to the same genus, *Mycobacterium*, *M. tuberculosis* being probably the best-known (**Figure 1**).

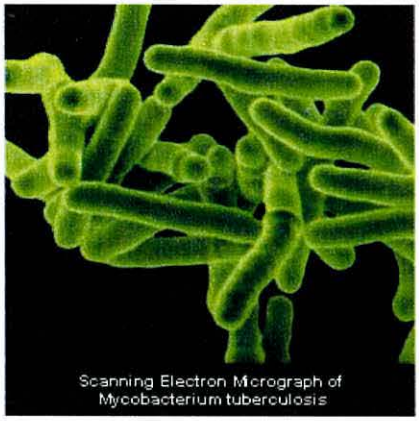


Figure 1*: M. tuberculosis (by Elizabeth Fisher copied with permission)*[29]

Part of the problem in the eradication of these infections is the high resistance of the mycobacteria to the majority of antibiotics or chemotherapeutic agents, which are effective for other common pathogenic bacteria. This resistance is thought to be related to the unique structure of the mycobacterial cell wall that is the first defence against possible toxins for the mycobacteria. In fact, the general resistance of these cells to drugs is considered to be connected with the low permeability of the mycobacterial cell wall to hydrophilic compounds.[30] An example of the protective function of this cell wall is offered by the fact that while

the majority of the micro-organisms are killed if treated with a 1 M solution of sodium hydroxide, many of *M. tuberculosis* cells remain viable and can be grown afterwards in uncontaminated cultures.[31] Moreover, it has been estimated that the *Mycobacterium chelonei* cell wall is around 5400-fold less permeable to cephalodrine, a broad spectrum semisynthetic antibiotic, than that of *Escherichia coli*.[32]

Knowledge of the organisation and of the structure of the *M. tuberculosis* cell envelope is of fundamental importance because these are factors in the pathogenesis of this disease.

Nowadays, it is known that the envelope consists of three structural components: the plasma membrane, the wall and the "capsule" as can be seen in **Figure 2.**

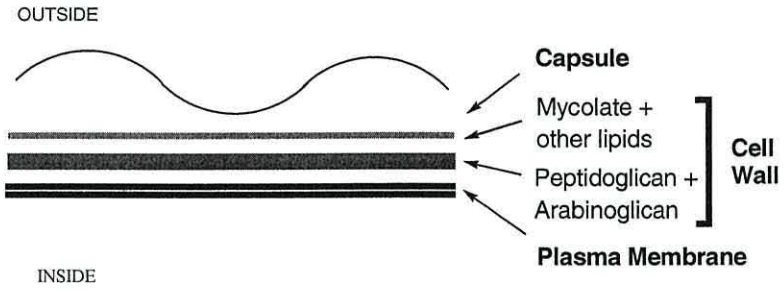


Figure 2*: Mycobacterial cell envelope*

The plasma membrane appears to be a typical bacterial membrane and it does not seem to have a particular role in the pathogenesis of the disease. The capsule-like layer is almost essentially formed by carbohydrates and proteins containing only a small quantity of lipids.[33] The cell envelopes of the mycobacteria are characterised essentially by an unusual cell wall skeleton, which has been thoroughly studied.

The lipid bilayer cell membrane of most of the Gram-positive bacteria is covered only by a porous peptidoglycan layer which does not stop most antimicrobial agents from entering in the cell (**Figure 3**). The Gram-negative bacteria are surrounded by two membranes and the outer membrane functions as an efficient permeability barrier to water soluble compounds because it contains lipopolysaccharides (LPS) and porins which are pore-forming proteins providing channels through the outer membrane. They are proteins with selective or even

specific properties concerning the diffusion of small uncharged or charged molecules (Mr < 600 Da, **Figure 3**).

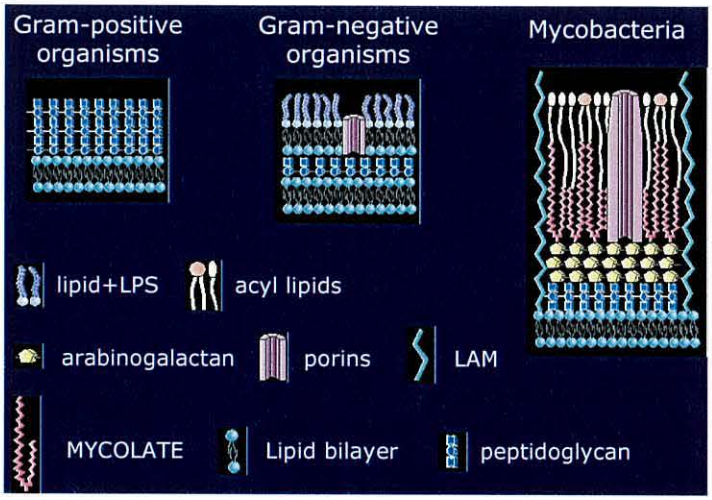


Figure 3: ***Mycobacterial cell walls (by Linda M. Stannard copied with permission)***[34]

However, the most efficient permeability barrier is the one produced by mycobacteria. This is constituted of three major macromolecules: peptidoglycan, arabinogalatan and mycolic acid, (**Figure 3**).[31]

1.1.3 The components of the cell wall

The peptidoglycan is thought to be in the innermost part of the cell wall, adjacent to the plasma membrane. It has similar structures to the ones possessed by other eucaryotic bacteria; it is composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic to which a tetrapeptide is attached. The peptide side chains cross-link the strands into a network.[35,36]

The arabinogalactan is a complex heteropolysaccharide, composed of arabinan multi-branched chains (formed by D-arabinofuranosyl units **1**) linked to a linear galactan core (constituted from D-galactofuranosyl residues **2**, **Figure 4**).[36-38]

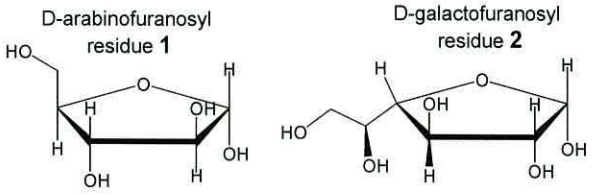


Figure 4*: The residues forming the arabinogalactan part*

Then, there are the mycolic acids (**3**), which are long chain α-alkyl, β-hydroxylated fatty acids (**Figure 5**). They esterify the majority of the terminal hydroxyl groups of the arabinofuranoside motifs.[39]

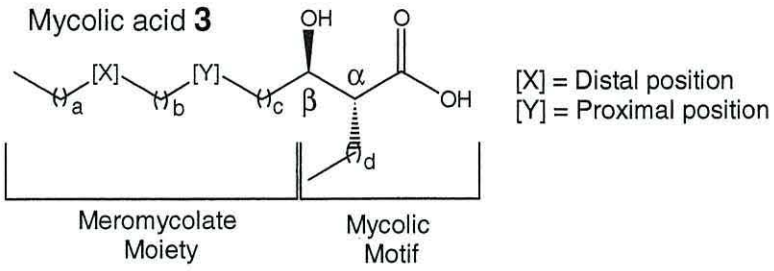


Figure 5*: A mycolic acid*

Mycolic acids were firstly defined as the major ether soluble components of the wax-like substance found in *M. tuberculosis* by Anderson *et al.*[40,41] These were subsequently characterized by Asselineau and Lederer in the 1950s.[42]

In addition to mycolic acids, which are the main constituent of the cell wall, there is a uniquely large number of different lipids: for example several different multi-methyl branched fatty acids;[43,44] lipomannan and lipoarabinomannans,[45,46] trehalose 6,6'-dimycolate and many others. The mycobacterial lipids, constituting up to 40 % of the dry weight of the cell envelope, have been the subject of numerous studies in order to determine their structure, biosynthesis and role in the virulence of the mycobacteria.[47-49]

A particularly interesting glycolipid (**4**) obtained from tubercle bacilli is called "cord factor": a trehalose, or 1α, 1α'-diglycoside, esterified at both primary alcohol groups with mycolic acids (**Figure 6**).[50]

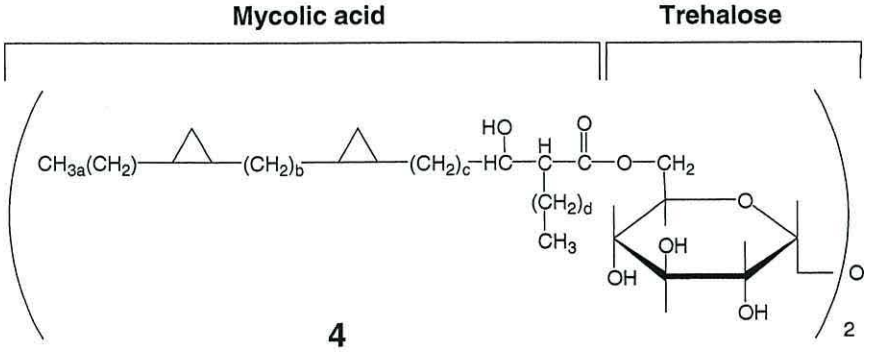


Figure 6*: Cord factor*

Cord factor is considered a "free lipid" since it can be liberated by extraction in an appropriate solvent from the cell wall, while the other mycolic acids, linked by covalent bonds to the arabinogalactan complex, can not be liberated so readily.

This glycolipid possesses a number of other biological properties, including anti-tumour,[51] granuloma formation,[52-54] and proangiogenic activities.[55,56] It is of considerable interest as it is a potent adjuvant, enhancing the immune system's response to generic antigens.[57,58]

There is evidence that mycolic acids could be one of the antigenic means for its recognition.[59] In fact, antibodies prepared against cord factors, showed greater reactivity for the types of mycolic acids contained in the glycolipids used as antigens, than other kinds of mycolic acids.[60-62] The antibodies prepared with cord factors of *M. avium* and *M. tuberculosis* were able to distinguish between these two species by recognising their different mycolic acid subclasses.[3,63,64] Trehalose 6,6'-dimycolate has, therefore, been studied for the serodiagnosis of several mycobacterial diseases giving diverse, non-optimal, results in specificity and selectivity.[4,65-67] However, all these results were obtained with polyclonal antibodies using, as antigens, all the cord factors extracted from each organism. It might be interesting to verify if the use of monoclonal antibodies formed using only one type of antigen gives more successful results. Another reason for the current interest in mycolic acids as possible antigens is that the antibody response to mycolic acids is also preserved in HIV-seropositive patients.[68]

1.1.4 The cell wall

The different components are bound to form a very complicated structure of the cell wall, which has been the subject of numerous studies. However, the presence of a large amount of lipid has made the understanding of the cell envelope organisation extremely difficult, and has raised difficult questions such as how these lipids are arranged.

Minnikin was the first to propose a significant model of the cell wall, basing its hypotheses on the chemical properties of its components.[2] The fundamental feature of this model is the observation that a large group of mycolic acids is covalently linked to the arabinogalactan complex and these are aligned in a perpendicular orientation with respect to the plasma membrane. The conformation of the mycolic acid around the C-2 branching position allows the meromycolate

chain to pack closely in parallel with the saturated alkyl side chain forming a barrier. This packing is made possible by the flexibility of the arabinogalactan molecule and its linkage unit and it is stabilised by intramolecular hydrogen bonds among the mycolic acids.[2,37] In particular, these lipids are disposed so as to form a gradient in the membrane fluidity that increases moving out from the cytoplasmic region towards the outside surface of the bacillus.[69,70]

Subsequently, X-ray diffraction studies offered experimental evidence for the existence of the second hydrophobic layer, formed by the variety of extractable lipids, which should complement the tightly packed and highly ordered mycolic acids.[28,71] This lipid bilayer could play a similar role to the one performed by the outer membrane in Gram-negative bacteria. The existence of this asymmetric lipid bilayer was subsequently proved by calorimetric and electron spin resonance analysis,[69] or by electron microscopy studies of freeze-substitution of the mycobacterial cell.[72,73]

Several other groups have analysed, each time in more detail, the complex structure of the mycolate cell wall.[28,30,31,74] For example, a few porins/pore proteins, have been recently found within the cell wall of the mycobacteria and their structures analysed.[75,76] Their discovery is of particular importance: they might be the key proteins for the uptake of hydrophilic drugs inside the mycobacterial cell.[77]

Nowadays, one of the most accredited models is the one proposed by Dimitriev *et al.*,[35] which still includes a lipid bilayer membrane. The group, however, suggested that the main chains of the arabinogalactan complex and of the pepidoglican have a perpendicular orientation with respect to the plasma membrane and not a transverse one as previously hypothesised. In particular, following this new approach the peptidoglycan strands form helices, which are cross-linked by the peptide bridges. This model illustrates even more structural reasons behind the extreme efficiency of the impermeable barrier, which is the mycobacterial cell wall.

In conclusion, as shown in **Figure 7, A**, the majority of mycolic acid residues (black) are covalently attached to the branched arabinan domain (pale blue), constituting the inner leaflet of the *M. tuberculosis* cell wall barrier. The membranous structure is completed by TDM glycolipids (green) and many other lipids (red), which form the outer leaflet.[78]

Then **Figure 7, B** shows the helical structures of the galactan (yellow) and the glycan (purple) domains. These "helices" are arranged in a grid-like structure perpendicular to the plane of the plasma membrane, cross-linked by branch peptides (blue).[78]

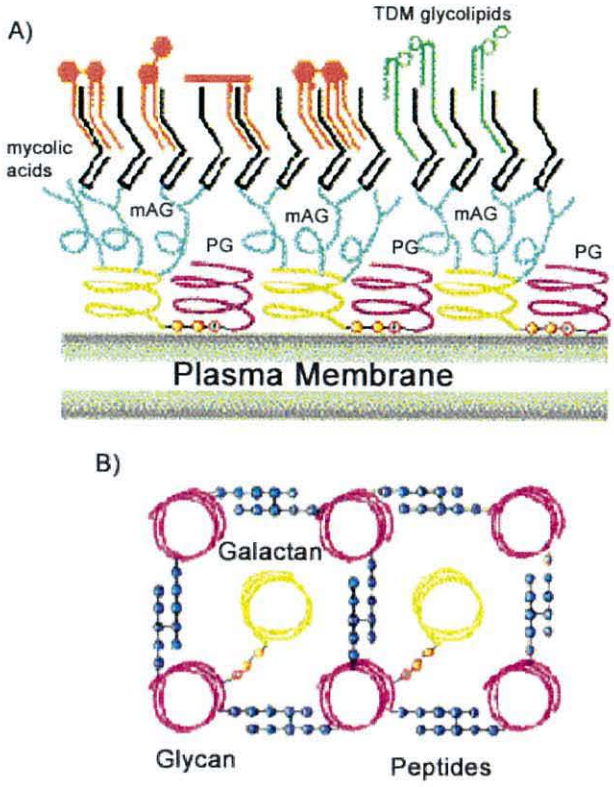


Figure 7*: Representations of some features of mycobacterial cell wall. (by Dover et al. copied with permission)*[79]

1.2. Mycolic acids

1.2.1 Overview

Mycolic acids (**3**) are characteristic of all the mycobacteria but are also present in genera other than *Mycobacterium*: in *Corynobacterium*, *Dietzia*, *Nocardia*, *Rhodococcus* and *Tsukamurella*. All of these genera are denominated "mycolata" because they contain mycolic acids.[80]

However, all these bacteria differ in the kinds of mycolic acids present in the cell envelope. Mycobacterial mycolic acids are distinguishable from those of other

genera. First of all they are usually larger (C_{70}-C_{90}), they have a larger α–branch (C_{20}-C_{25}), and contain different functionalities in the main chain (**Figure 8**).[28]

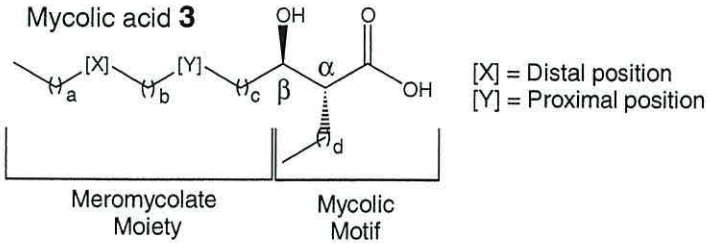


Figure 8*: A mycolic acid*

In each mycolic acid two moieties can be distinguished; the main branch is called the meromycolate moiety and the second is the mycolic motif.

The structure of the α-alkyl-β-hydroxy fatty acid portion is common to each mycolic acid, except for minor variation in the length of the chain in the α-position with respect to the carboxylic end. On the other hand, the meromycolate fraction can be differently substituted in both the proximal and the distal position, with respect to the carboxylic acid (**Figure 8**). The mycolic acids are broadly separated into classes, according to the groups present in the meromycolate moiety. The proximal or distal functional groups can be cyclopropanes, double bonds, an epoxy group, methoxyl group, carbonyl group, carboxyl group or methyl group.[2]

Two-dimensional TLC,[81-83] and subsequently GC[84] and HPLC,[85-87] in association with MS, IR and NMR techniques, have permitted the identification of several kinds of mycolic acids present in each mycobacterium. Actually, HPLC patterns are characteristic for each mycobacterium and they have been used as a rapid diagnostic tool for speciating mycobacteria.[87]

M. tuberculosis, and similar pathogenic mycobacteria, produce three major kinds of mycolic acids: cyclopropyl mycolic acids (called α) which contain two cyclopropanes generally in the *cis*-configurations (**5**);[a] keto-mycolic acids (**6a**, **6b**); and methoxy-mycolic acids (**7a**, **7b**, **Figure 9**).[88-91]

[a] The *cis* and *trans* nomenclature has been traditionally used to describe mycolic acids, It has been maintained in this Introductory chapter, while in the Results and Discussion section the more appropriate (*E*), and (*Z*)-nomenclature was preferred.

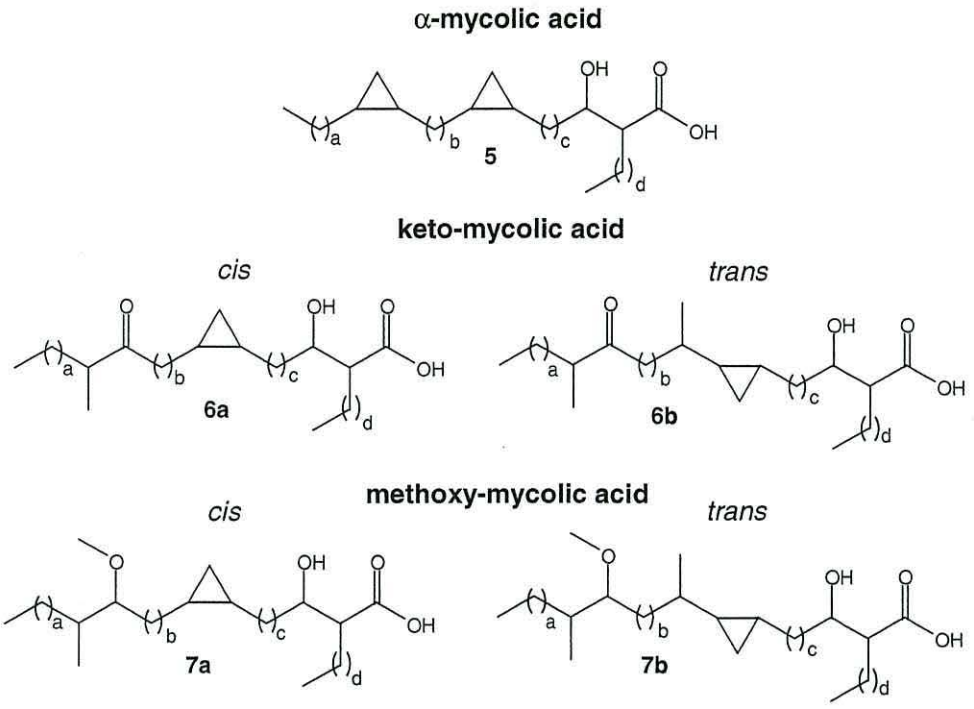


Figure 9*: Major types of mycolic acids from M. tuberculosis complex*

Other mycobacteria contain completely different sets of mycolic acids (**Figure 10**). For example *Mycobacterium smegmatis* and *Mycobacterium fortuitum* contain α^1 and α-mycolic acids (**8**, **9a**, **9b**) with either one or two double bonds, either in the *cis* or the *trans*-configuration, and epoxy-mycolic acids (**10a**, **10b**).[92-95] Finally, more different oxygenated mycolic acids have been isolated in numerous mycobacteria; these include wax esters (**11**), ω-carboxy-mycolic acids (**12**), and ω-1-methoxy-mycolic acids (**13**).[96,97]

A recent review confirmed that the most widespread of the classes of mycolic acids are the α-mycolates, with either two double bonds or two cyclopropyl rings (**5**, **9**).[80] The next most widely distributed are keto-mycolic acids (**6**), which are found in both pathogenic and saprophytic mycobacteria, in both fast and slow growing species. α^1-Mycolic acids (**8**) and wax esters (**11**) are also found extensively, while methoxy-mycolic acids (**7**) appear to be present only in pathogenic species that also produce keto-mycolic acids (**6**).[80,98] It would be interesting to understand the reasons for the existence of the methoxy-mycolic acids. In conclusion, ω-1-methoxy-mycolic acids (**13**) are specific to a very restricted number of mycobacteria.[80]

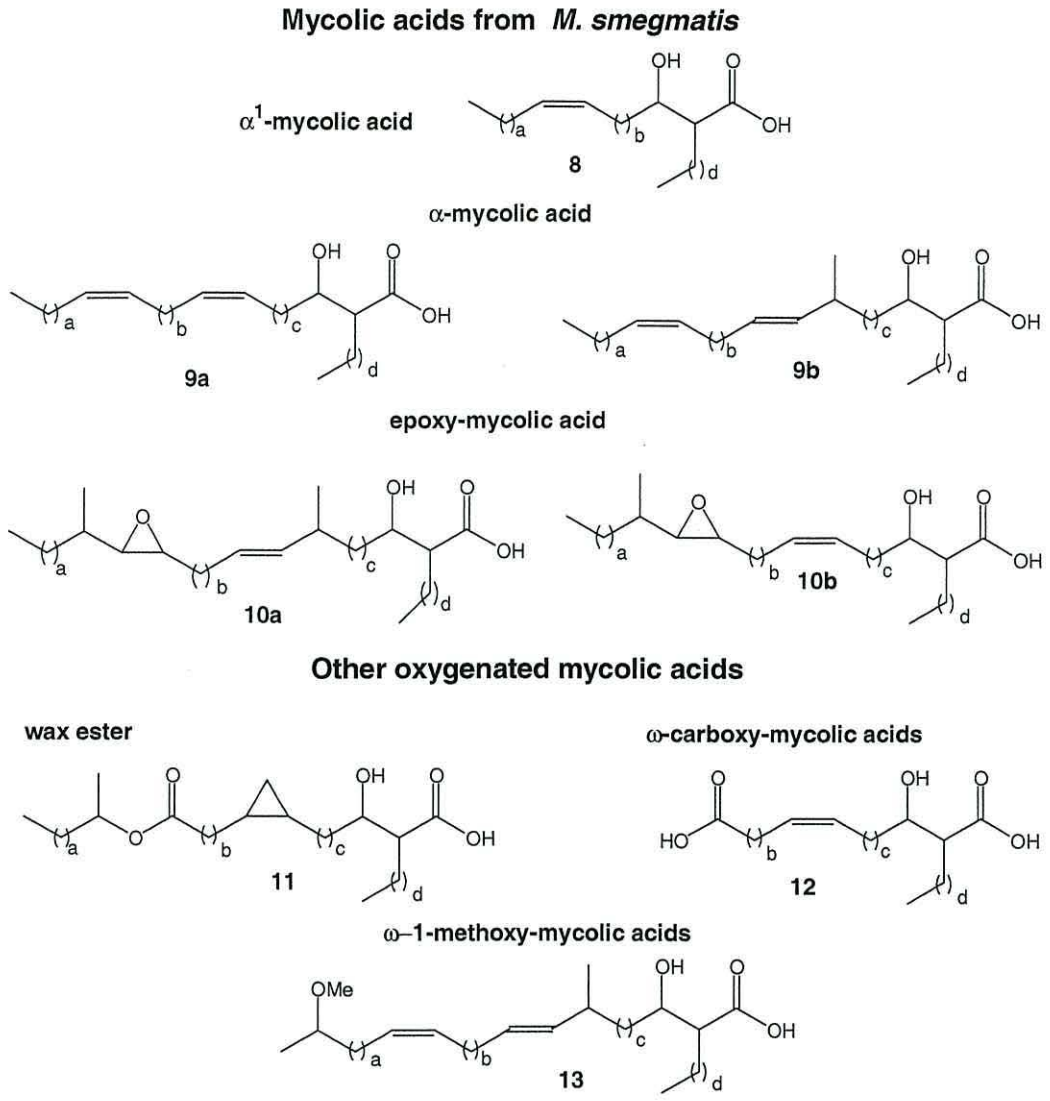


Figure 10: ***Mycolic acids from other mycobacteria***

Alongside these major types, other kinds of mycolic acids are also present in numerous mycobacteria, even if only in minor quantities. For example, in 19 strains of the *M. tuberculosis* complex there are keto, methoxy, or α–mycolic acids which have a double bond in the proximal position either in a *cis* (**15**) or a *trans*-configuration (**16**), instead of a cyclopropyl ring (**Figure 11**).[99]

A possible classification of these new kinds of lipids was presented by Watanabe *et al.*,[99] following which the acids containing in the proximal position a cyclopropane (either *cis* or *trans*) are defined **Type 1** mycolic acids, those with double bond in the *trans*-configuration are **Type 2** (**15**), and those with a *cis*-double bond are **Type 3** (**16**).

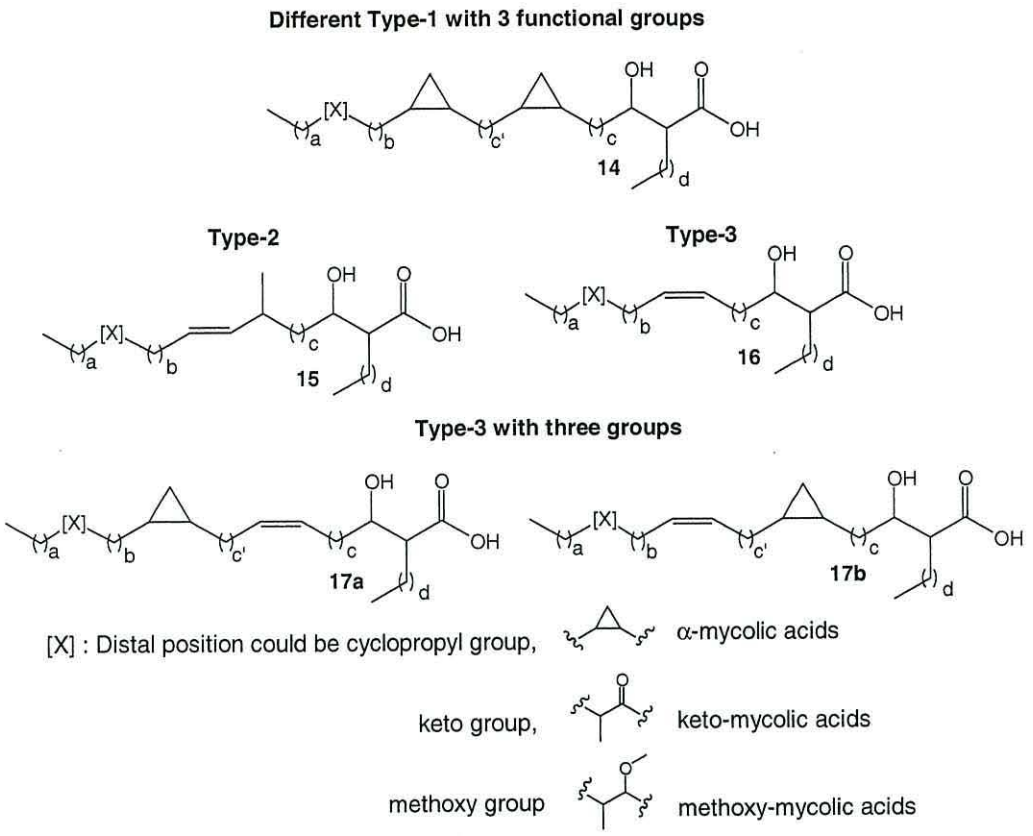


Figure 11*: Minor types of mycolic acids in slow growing mycobacteria*

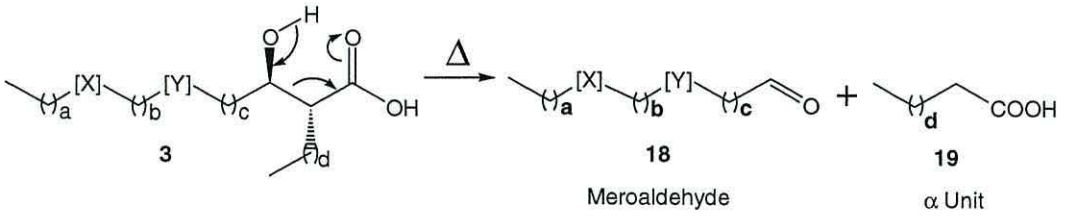
There are also other lipids, which have three different functional groups, instead of two, inside the meromycolate moiety. These mycolates with an additional functional group, have a cyclopropyl ring normally in a *cis*-configuration (**14**, **17**).[99]

1.2.2 Chain length

In all mycobacteria, there are not only different types of mycolic acids, but also different homologues for each of them. In *M. tuberculosis* alone, a family of over 500 individual mycolic acids with closely related chemical structures has been recognised.[80] These circumstances made the isolation of a single component and the determination of its real structure extremely difficult. However, mycolic acids have been the focus of constant study since their discovery. This is not only because they are unique to this type of organism but also because of their importance for the survival and virulence of the mycobacteria. The history of the elucidation of their structures has been described as being intimately related with that of the development of modern tools in analytical chemistry.[80]

The determination of the molecular weights of every homologue and particularly the location of the functional groups in each of them has been particularly challenging. Most recently, MALDI-TOF mass spectrometry has provided a rapid and highly sensitive technique for analysis of mycolic acids and other lipids.[100-102] Laval *et al.*[101] used this technique to analyse the length of the total carbon chain of the major types of mycolic acids of different mycobacteria; both pathogenic, slow-growers, such as *M. tuberculosis*, and non-pathogenic fast-growers such as *M. smegmatis*. Through this analysis structural correlations were found between α- and oxygenated mycolic acids in non-pathogenic mycobacteria: for example, between α-mycolic acids (**9**) and epoxy-mycolic acids (**10**) in *M. smegmatis*, (**Figure 10**). No such structural correlation was found between α-mycolic acids (**5**) and oxygenated mycolic acids (**6**, **7**) in pathogenic mycobacteria such as *M. tuberculosis* and *M. ulcerans* (**Figure 9**). These findings confirmed previous results which showed that, in *M. tuberculosis,* α-mycolates are normally shorter than oxygenated mycolic acids.[103,104]

Concerning the length of the internal chains, one of the first pieces of information to be discovered was the determination, by pyrolysis and mass spectrometry, of the size of the meroaldehyde (**18**) and the α-unit (**19**, **Scheme 2**).[105] Using this technique the mycolates chains were established to vary between 60 and 90 carbon units in total, and the α-unit between 22 and 26 carbons.[80,106]



Scheme 2*: Pyrolytic cleavage of mycolic acid*

Several MS techniques, in association with TLC, GC and HPLC, and various types of chemical modifications, have been used to determine the lengths of the other internal chains of different mycolic acids (**a**, **b**, **c**, **d Scheme 2**).[b] These have been estimated differently over the years, without definite results.[85,90,94,107-117]

[b] The internal chains have been named in several ways. The nomenclature employed firstly by Qureshi *et al.*[(85)] and then by Barry *et al.*[(80)] has been addopted, which described them as **a**, **b**, **c**, **d**. Other authors preferred different classifications such as: **l**, **m**, **n**, **p**,[(99)] or $\mathbf{n_1}$, $\mathbf{n_2}$, $\mathbf{n_3}$, $\mathbf{n_4}$.[(101)] respectively.

Watanabe *et al.* used MALDI spectrometry to study mycolic acids (both major and minor components) present in 19 strains of the *M. tuberculosis* complex.[99] Combining this new methodology with CID they succeeded in locating, precisely, the functional groups in the meromycolate moiety different types of mycolic acids.[102]

The methyl group next to methoxy, the keto groups, and the *trans*-cyclopropane in oxygenated mycolic acids (**6b**, **7b**), were confirmed to be nearest the ω end with respect to the functional group, whilst in **Type-2** mycolic acids (**15**), the methyl next to the *trans*-double bond was nearest the carboxyl end (**Figure 12**).

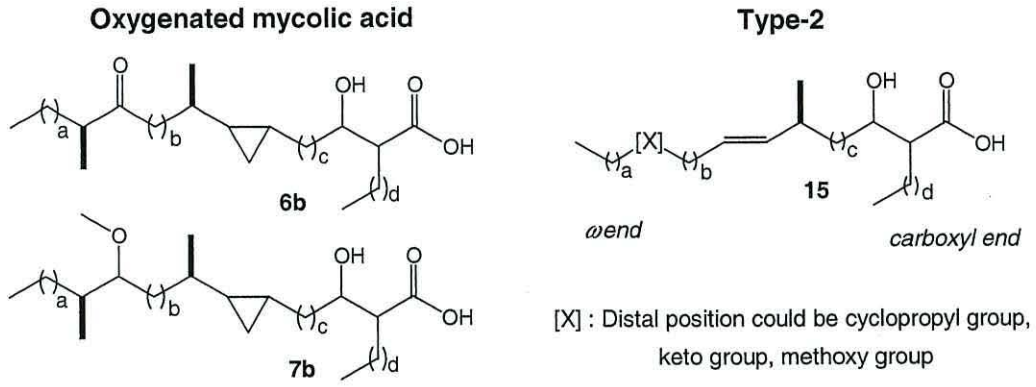


Figure 12: ***The position of the methyl group in different mycolic acids***

Moreover, analysing the location of the different functional groups, a close structural relationship was established between keto (**6**) and methoxy-mycolic acids (**7**) and between mycolic acids with a cyclopropane in the *cis*-configuration (**6a**, **7a**) and the ones with a ring in the *trans*-configuration (**6b**, **7b**, **Figure 9**).

Finally, through a detailed analysis of their structures, a close connection has been demonstrated between the major classes with a *cis*-cyclopropane in the proximal position (**20**, **Type-1**) and the minor classes with a *cis*-mono olefinic mycolic acid (**16**, **Type-3**, **Figure 13**). On the other hand, a simple and universal connection between major classes with a *trans*-cyclopropane mycolic acid (**20**, **Type 1**) and the corresponding minor ones with a *trans*-mono olefinic mycolic acid (**15**, **Type-2**) has not been found.

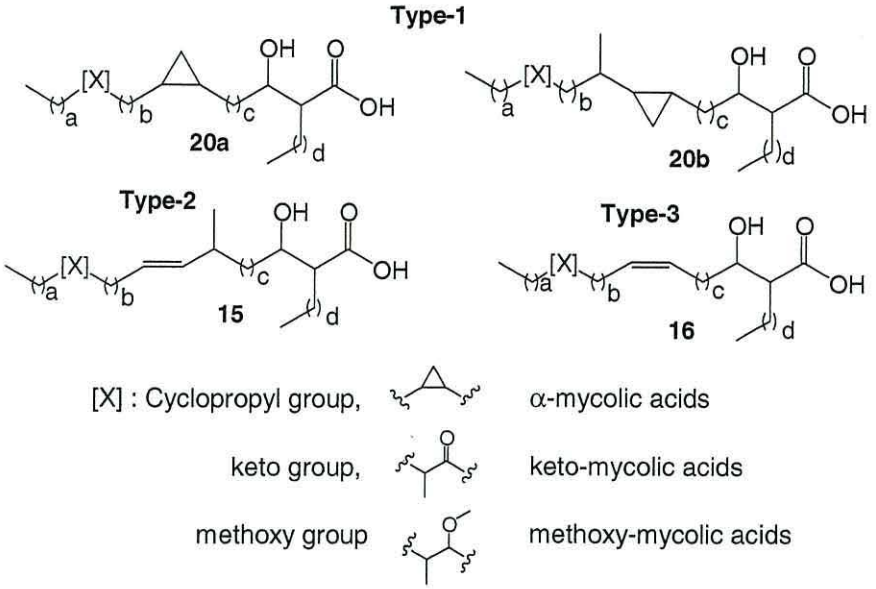


Figure 13*: Different types of mycolic acids*

They also analysed the new series of mycolic acids, which contain an additional group, usually a *cis*-cyclopropane (**14** and **17**, **Figure 14**). Some of the chains of those acids were proven to be extended by 6 to 8 carbons in comparison with the respective major components of the mycolic acids mixture. However, there is a complex and not universal correlation between them and the major kinds of mycolic acids.

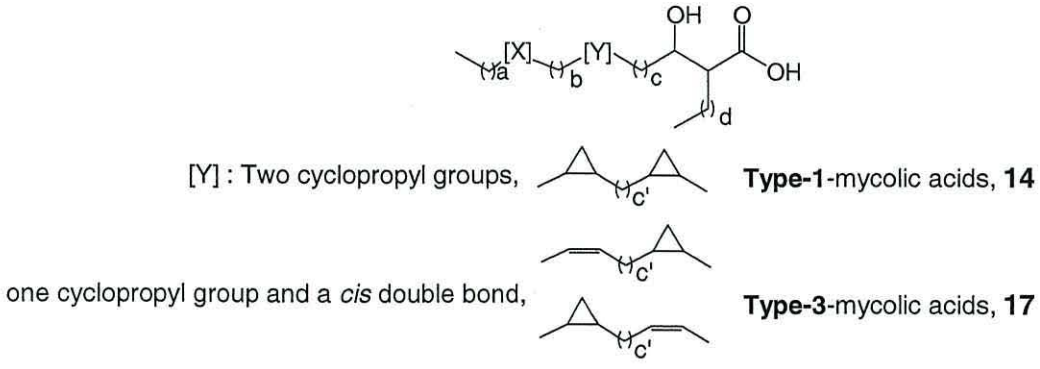


Figure 14: ***Mycolic acids with three functional groups in the meromycolate chain***

1.2.3 Stereochemistry

The stereochemistry of the chiral centres contained in mycolic acids has still not been completely clarified.

The two stereocentres in the α and β-positions relative to the carboxylic group present in all mycolic acids, have always been found when examined, to be both in the *R*-configuration, *erythro*, or better *anti*, to each other.[c]

The *(R,R)*-configuration was confirmed first for the corynomycolic acids (**21**, **Figure 15**),[121,122] which are components of the cell walls of *Corynobacteria*. These lipids have a similar structure to mycobacterial mycolates (**3**), but they have shorter chains and do not have other stereocentres in the meromycolate chain.[121,123]

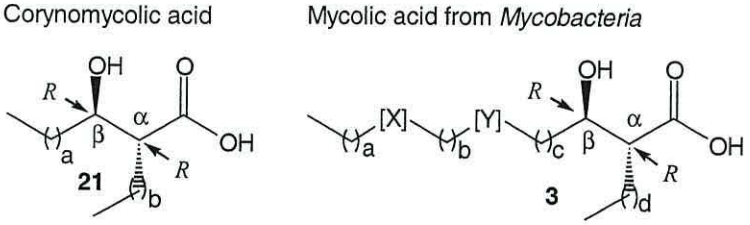


Figure 15: ***The chiral centres in the β–hydroxy fatty acid moiety***

The configuration of the two chiral centres was always found to be the same for all the mycolic acids examined from *M. tuberculosis*,[89,124] *M. fortuitum*,[93]

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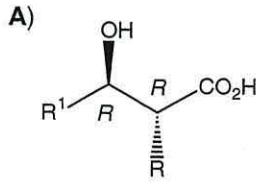
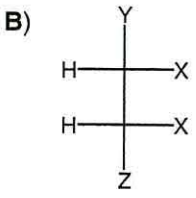
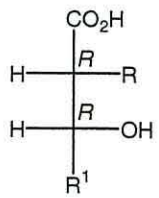
[c] In this thesis these two substituents of corynomycolic acids are defined as being in the *erythro* configuration (**a**).[(89)] This definition usually refers to molecules with one different and two identical substituents (**b**), but it had also be applied more generically to molecules with two non-hydrogen substituents on the stereocentres. A molecule in the *erythro*-configuration has the two substituents adjacent when drawn in the Fisher convention (**c**).[(118), (119)] Other authors have described the same configuration for these two stereogenic carbons in corynomycolic acids as being in the *threo*-configuration, based on the definition of Noyori *et al.*[(5), (120)] This ambiguity illustrates the problems associated with this type of designation. Therefore, these compounds would be better defined as *anti*-diastereoisomers, or, better still, as (2*R*,3*R*)-2-alkyl-3-hydroxy esters. However, sometimes in the course of this thesis, the *erythro*-prefix has been preferred for simplicity of description.



Common representation with the substituents in an *anti* configuration



The classical use of the *erythro* prefix



Fisher projection of corynomycolic acids

Mycobacterium thamnpheos,[125] *Mycobacterium marinum* and *M. ulcerans,*[126] irrespective of the other functional groups in the meromycolate moiety.

The presence of the hydroxyl group and the particular *erythro*-configuration between it and the alkyl chain has been demonstrated to be capable of altering the film molecular packing. The formation of a hydrogen bond between the hydroxyl group and the carboxylic group has a stabilising effect on the aligned form of the α-units with the meroaldehyde-units. Durand *et al.*, studied monolayers formed with corynomycolate-glycolipids derivatives.[127,128] They demonstrated that the phase transition temperature for the monolayer formed with natural *erythro*-diastereoisomers is lower than that obtained using synthetic *threo*-diastereoisomers. They also established that monolayers prepared with deoxy-analogues have a higher phase transition temperature than those obtained using the hydroxy-diastereoisomers.

Moreover, this precise stereochemistry of these two chiral centres seems to be necessary for an efficient recognition by T cells and the generation of an immune response by the host organism against the pathogenic mycobacteria, [129] and the antitumour properties of the cord factors.[130]

Very little is known instead about the chiral centres in the meromycolate chain, since their determination is particularly difficult.

Recent stereochemical studies (on *M. smegmatis* mutant genetically modified to resemble mycobacteria of the *M. tuberculosis* complex) suggested that, in the keto (**6a**) and methoxy-mycolic acid (**7a**) and in their putative precursor, the hydroxy-mycolic acid (**22**), the methyl branch adjacent to the oxygenated functions is in the (*S*)-configuration (**Figure 16**).[131] This observation has been used to confirm that the two oxygenated species are biosynthetically related.

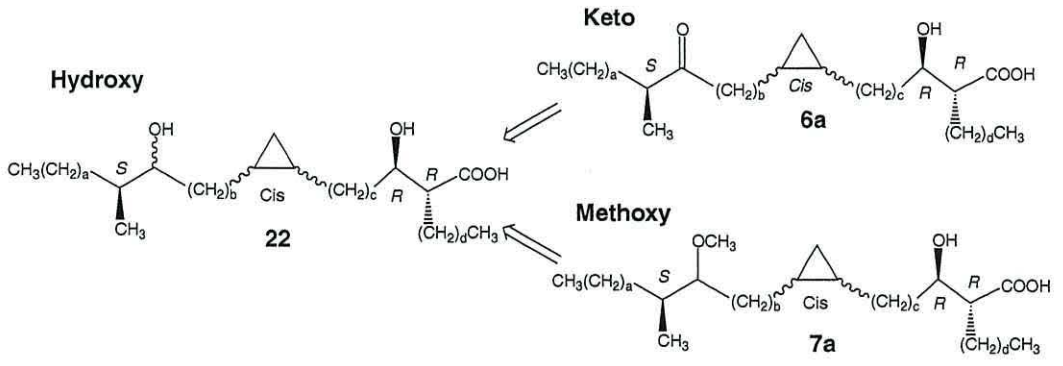


Figure 16*: The stereochemistry of some chiral centres of oxygenated mycolic acids.*

The methyl and methoxyl groups are also thought to be in the (*S*)-configuration in methoxy-mycolic acids (**7**) in *M. marinum* and *M. ulcerans*.[126] Conversely, it has been found that in the epoxy-mycolic acids (**10**) in *M. fortuitum*, the methyl groups are in the (*R*)-configuration (**Figure 17**).[93] The same configuration was also proven for the methyl branches next to *trans*-double bond (**23**) in mycolic acids of another rapid growing mycobacterium, *M. aurum*.[132]

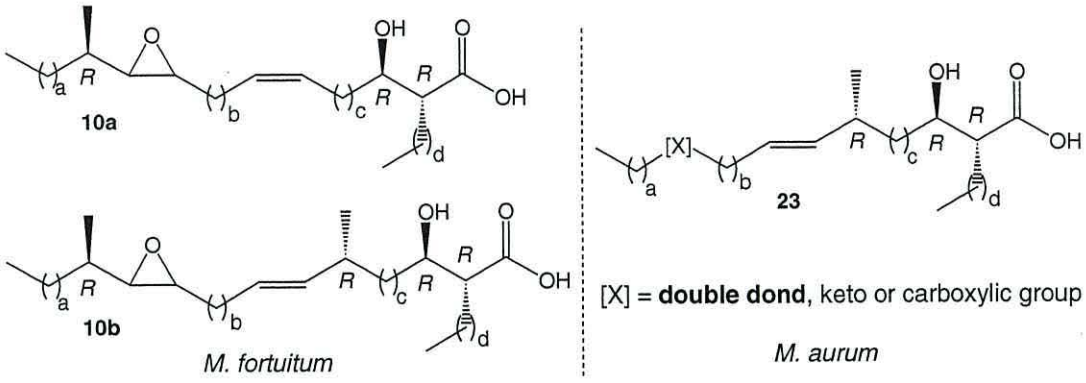


Figure 17*: The chiral centres in the epoxy-mycolic acid series*

The determination of the chiralities of this functional group has been particularly difficult. The stereochemical interpretations have been derived through comparison with a simpler, established compound, and subsequent modelling of the extent to which additional chiral centres would have changed the degree of optical rotation of the entire molecule.[126] The stereochemical results have also been obtained by fragmenting mycolic acids into smaller sub-units which could be compared with known compounds.[93]

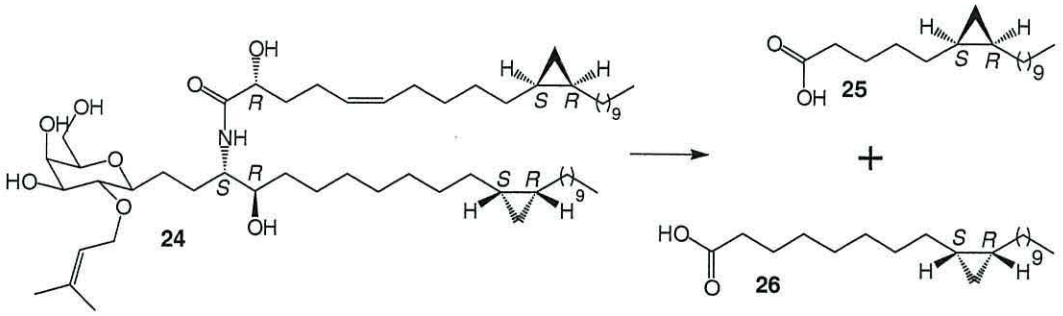
The first method appears to be speculative, while the second, obtained by degradation of mycolic acids, could allow modification of the chiral centres. Therefore, more studies seem to be needed to obtain definitive results.

Additionally, the absolute stereochemistries of the cyclopropyl centres of mycolic acids are still undefined, even if IR and ^{1}H NMR studies permit the *cis* and the *trans*-configurations to be distinguished.[88]

One of the methods used to determine the configuration of molecules with several chiral centres is the comparison between the natural compounds, or their smaller derivatives, and the respective synthetic analogues. Similar method have already been utilised to determine the configurations of the chiral centres of the cyclopropanes in other fatty acids derivatives, which were simpler than mycolic

acids. These included lactobacillic acid,[133,134] grenadamide,[135] PHYLPA[136] and Cepaciamide A.[137] The same technique was also used to determine the configurations of the methoxy group with an adjacent methyl-branch in Cystothiazole A.[138]

The determination of the absolute stereochemistries of the two cyclopropanes moieties of Plakoside A (**24**) has been particularly complex (**Scheme 3**).



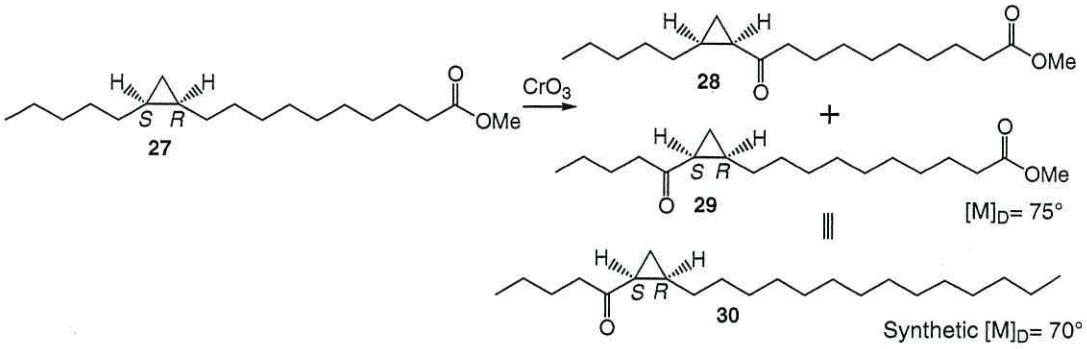
(2*S*, 3*R*, 11*R*,12*S*, 2'''*R*, 5'''*Z*, 11'''*R*, 12'''*S*) Plakoside A $[\alpha]_D$ = + 10.4 (c = 1.6,MeOH),
(2*S*, 3*R*, 11*S*,12*R*, 2'''*R*, 5'''*Z*, 11'''*S*, 12'''*R*) Plakoside A $[\alpha]^{22}_D$ = + 8.9 (c = 0.065, MeOH)
Natural Plakoside A $[\alpha]^{25}_D$ = + 7 (c =0.25, MeOH)

Scheme 3*: The absolute stereochemistry of Plakoside A*

This molecule, similarly to mycolic acids, contains two cyclopropyl rings and their absolute stereochemistry could not be proven even through preparation of the complete molecule.[139] In fact, the chemical and physical properties of Plakoside A, in particular its optical rotation, do not change significantly by modifying the absolute configuration of the cyclopropanes.[139-142] On the other hand, the exact configurations of the chiral centres of the cyclopropyl rings were shown to be critical for the immunological properties of this glycolipid.[141]

It was possible to determine the absolute configuration of each chiral centre by synthesizing the smaller derivatives (**25**, **26**) that contain only one of the cyclopropanes and comparing them with the natural analogues (**Scheme 3**).[143]

Tocanne *et al.*[144,145] and, recently, Stuart and Buist[146] have utilised comparable procedures for the determination of the absolute configurations of the cyclopropyl ring in other fatty acids (**Scheme 4**). They transformed the lipids (**27**) into the keto-derivatives (**28, 29**) so that they could more easily compare them with synthetic standards (**30**, **Scheme 4**).



Scheme 4: ***Tocanne et al. method***

The only problem with this kind of method involving derivatisation of natural compound is the risk of a modification of the configurations of the chiral centres during the process. This reservation could be completely eliminated simply by repeating the process of derivatization on a whole synthetic analogue with known stereochemistry, checking the conservation of the chiralities.

1.2.4 Roles of different mycolic acids

The distinctive cell wall and its lipids are considered to play a major role in the virulence of mycobacteria.[28,31] Mycolic acids are not only the hallmark of the cell wall of these organisms, but they are also one of their major constituents. In particular, both the length and fine structure of mycolic acids have been correlated either to the toxicity, or the intracellular survival of mycobacteria.[147-150]

The kinds of mycolic acids and their relative abundance depend upon growth conditions.[151-153] It has also been demonstrated that an alteration in the proportion and structure of mycolic acids produces significant change in the fluidity of the cell wall, changing the permeability and the virulence of the pathogenic mycobacteria.[69,154,155]

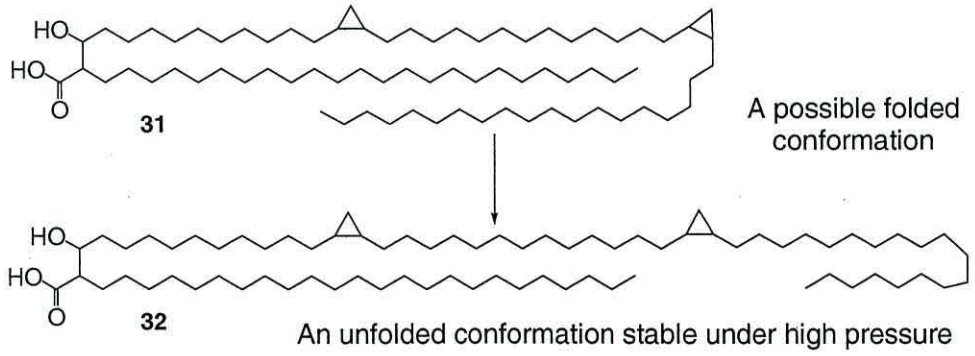
Analysing in more details the role of each mycolic acids class, in *M. tuberculosis*, the α-mycolates are predominant (about 50 % of the total).[102] One of the most interesting features of these lipids is the presence of cyclopropanes in the meromycolate chains. The necessity of this group for the pathogenesis of *M. tuberculosis* has been demonstrated.[156]

Cyclopropanation occurs in slow-growing pathogenic mycobacteria such as *M. tuberculosis*; it does not occur in environmental ones, like *M. smegmatis*.[157] Moreover, its formation in bacterial membranes should have a high energetic

cost.[158] Many researchers have investigated the reasons for this modification in several fatty acids without finding a universally accepted answer. An hypothesis is that ring creation has an effect on resistance of bacteria under oxidative stress.[158] Cyclopropane fatty acids are less sensitive than unsaturated lipids to ozonolysis and other oxidative treatments.[159]

Thus, it has been proposed that the cyclopropyl rings could also stabilise the mycolic acids of pathogenic mycobacteria in oxidative environments.[160] Mycolic acids of *M. smegmatis* with an additional cycopropane in the distal position appeared to be more stable to strongly oxidative situations.[160] However, cyclopropanes in the proximal position appear to have no significance against oxidants.[156,157]

The cyclopropane in this position might influence the rigidity of the lipid layer.[157] Studies on monolayer films of mycolic acids showed a change of conformation for the α–mycolates (from folded **31** to unfolded **32**) depending on the surface pressure applied (**Scheme 5**). This change may also affect the general packing properties of mycolic acids in mycobacterial cell wall.[161,162]



Scheme 5: ***Schematic representation of the chain unfolding process for a-mycolic acids.***

Methoxy- and keto-mycolic acids, the oxygenated series, are also critical for the virulence of mycobacteria.[147,149,163] It has been determined that slow-growing pathogenic mycobacteria can manipulate the ratios between keto and methoxy-mycolic acids in order to adapt better to the environment. Dubnau *et al.*, studying a mutant unable to produce oxygenated mycolates, verified the importance of these compounds for the permeability and fluidity of the cell wall.[147]

Interestingly, in a monolayer prepared with keto-mycolic acids, the lipids do not experience the same conformational change observed for α-mycolic acids when a

high surface pressure is applied to the monolayer.[164] This kind of mycolic acids could therefore play a important role in the determination of the permeability properties of mycobacterial cell wall.[164] In particular, it has been observed that keto-mycolic acids have an essential role in the growth of the organism within the natural host cell.[149]

Conversely, no clear role has been attributed to methoxy-mycolic acids and recent studies demonstrated that the loss of these did not effect the cell wall permeability and the resistance to antibiotics of *M. bovis* BCG even if it might influence its long-term growth *in vivo*.[165]

The *trans*-mycolic acids are less abundant, but the amount of these fatty acids is thought to be particularly important in determining many macroscopic membrane properties increasing the cell wall rigidity and influencing the drug resistance, the growth rate, and the colony morphology of the mycobacteria.[104]

Cis-olefins produce "kinks" in the carbon chain of the lipids, yet the *trans*-types do not; in this way the latter allow mycolic acids to be more tightly packed making the cell wall less fluid.[80] *Trans*-cyclopropanes have similar functions and are fundamental for the maintenance of membrane viscosity. This is confirmed by the fact that, in some mycobacterial species, the levels of *trans*-compounds rise if the mycobacterium grows under higher temperatures.[154]

It has been demonstrated that clinical strains of *M. tuberculosis* have a higher *trans*-cyclopropanated oxygenated mycolic acid content than laboratory strains. This suggests that *in vivo* growth promotes either *trans*-cyclopropane formation, or the growth of sub-populations of *M. tuberculosis* with higher *trans*-cyclopropane content.[104] This evidence is consistent with the proposal that *trans*-mycolic acids modify the macroscopic qualities of the cell wall and that their production depends on growing conditions.[166]

Mutants over-producing *trans*-substituted mycolic acids have decreased vigour; they additionally have altered proportions of keto to methoxy-mycolic acids.[104] It is important to note that these results suggest that the quantities of each of these sub-classes are interdependent. This could also lead to the hypothesis that these types of acids are biosynthetically related.

In conclusion, these results do not prove the necessity for mycobacterial virulence on one or another class of mycolic acids but they are the "starting point" in the evaluation of the role of each class for mycobacterial pathogensesis.[70]

However, an appropriate mycolate composition is a key prerequisite for mycobacterial growth and interference with this composition could be a valid strategy for the development of a new anti-mycobacteria treatment.[149] The role of each type of mycolic acid is difficult to understand because more than one mycolate functional group, or even different cellular molecules, can have similar effects on the fluidity of this membrane system.

The position of the different functional groups could also influence the macroscopic properties of the cell wall and in particular the characteristics of the mycolic acids packing.

Recently, Watanabe *et al.*[102] analysed several meromycolic acids (**33**) obtained from the corresponding mycolic acids (**3**) of different mycobacteria (**Figure 18**).

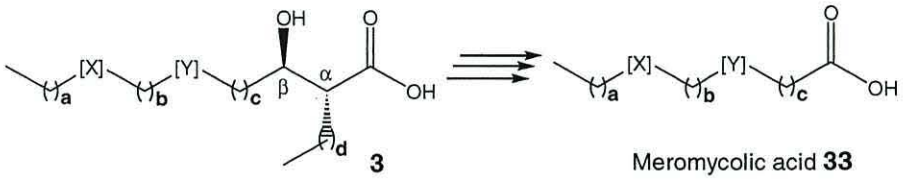


Figure 18: ***Meromycolic acid***

They showed that in homologues differing in their respective values for **a**, **b** and **c** (**Figure 18**), the sizes of **a** and **b** were often preserved in the different homologues, while that of **c** varied more. Moreover, **a** was usually longer than **c**; this observation was particularly clear in the most abundant types of mycolic acids, the α-mycolic ones. The authors did not know how this might affect the properties of the cell wall. However, they hypothesised that the carbon chain lengths could have an important role for the compactness of the mycolic acids in the cell wall and thereby influence its fluidity. A confirmation of the importance of the values of **a**, **b**, **c**, could come from the observation that several pathogenic mycobacteria have a similar pattern of values for **a, b**, **c,** while other non-pathogenic mycobacteria have a different value pattern for **a, b**, **c**.

Alongside this hypothesis, recently Hasegawa and Leblanc[162] hypothesised a fundamental function for the length of the alkyl chain in the α-position. In fact, they observed that a small alteration of the value of **d** (**Figure 18**, from 21 to 23) had a great influence on the molecular aggregation properties.

Finally, Gao *et al.* studied mutants of *M. marinum*; in these, an enzyme necessary for elongation of lipids was found to be altered.[167] This difference produced mycolic acids with the chain **c**, **Figure 18**, 2-4 carbon units shorter than those occurring naturally. Additionally, the ratios among α, keto and methoxy-mycolic acids were also changed. The authors did not reach a conclusive explanation as to whether and/or how these modifications are related, but they have shown that they are critical for cell wall impermeability and, therefore, for the survival of the mycobacterium.

In conclusion, it seems necessary to consider the roles not only of different types of mycolic acid, but also of the several isomers differing in their chain lengths within each type. This could provide a better understanding of the mechanisms of cell wall permeability, especially in relation to drug effectiveness.

1.3. Biosynthesis

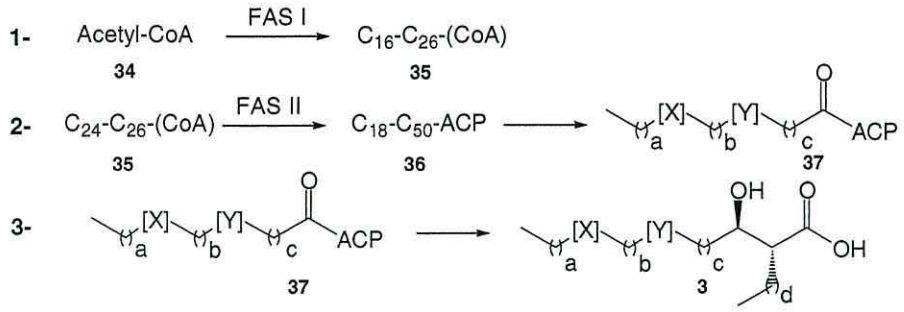
1.3.1 Overview

The biogenesis of mycolic acids has been the subject of numerous studies seeking to determine possible targets for anti-tuberculosis drugs, since mycolic acids play a fundamental role in the survival of the mycobacteria in the host.[80]

The H37Rv strain of *M. tuberculosis* contains ~ 250 genes involved in fatty acid metabolism, compared with 50 only in *E. coli*,[168] showing how essential these lipids are for the survival of the mycobacterium.

These organisms contain, simultaneously, examples of lipid biosynthetic systems from mammalian cells, as well as the common bacterial biosynthetic ones.[169] It is possible to differentiate three major steps in the biosynthesis (**Scheme 6**), even if this division is completely arbitrary and the sequence of the steps is still not completely known.[168]

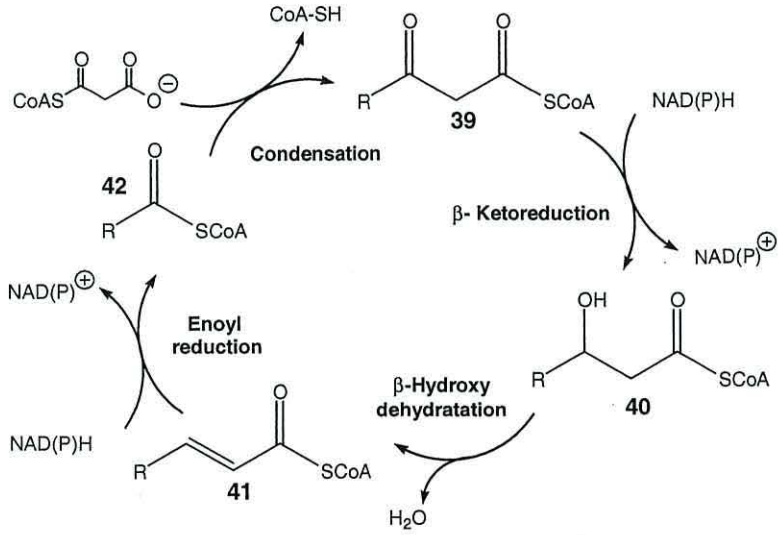
1. Synthesis of long fatty acids up to C_{26} in order to provide the main carbon framework.
2. Synthesis and modification of C_{18}-C_{50} chain to provide the meromycolic portion with different functional groups.
3. A final condensation step to give the mycolic acid.



Scheme 6: ***Biosynthesis***

1.3.2 The synthesis of long fatty acids and FAS I

Fatty acid synthesis occurs through repetitive cycles of condensation, keto reduction, dehydration and enoyl reduction to produce saturated fatty acids (**Scheme 7**).



Scheme 7: ***Elongation process***

The acyl-chains (**39**) formed in each cycle are subsequently used as substrates for other elongation processes to give the final products.

Mycobacteria possess at least two different types of enzyme system involved in long fatty acid biosynthesis: (fatty acid synthase) FAS I and FAS II.[168]

Multifunctional fatty acid synthase (FAS I) is a single polypeptide predominantly found in yeast, in all animal tissues. It creates short precursors for further processes.[170] In *M. tuberculosis* it is an unusually large system with a catalytic domain resembling a head-to-tail fusion of two yeast FAS sub-units. It also differs from other FAS systems in that it uses both NADH and NADPH,[48] and it has two

potentially active sites for the β-ketoreduction.[171] The reasons for either of these dual needs or its extraordinary dimensions, are still unknown, but one hypothesis is that these differences are due to the several tasks that it performs.[44]

In fact, it has multiple catalytic activities. In common with other FASs, it catalyses the *de novo* synthesis from acetyl-CoA by subsequent addition of other acetyl-CoA: C_2 units in order to obtain C_{16}-C_{18} fatty acids.[172] However, it also seems to be involved in an elongation process probably using malonyl-CoA, and not acetyl-CoA, to produce C_{24}-C_{26} or even longer fatty acids.[173]

FAS I produces, in a bimodal distribution, C_{16}-C_{18} and, unlike similar enzymes in mammalian cells, C_{24}-C_{26} fatty acids.[173] The rate of the fatty acid biosynthesis and the tendency to produce C_{16}-C_{18} acids is enhanced by the presence of mycobacterial *O*-methylated polysaccharides.[174] These show a high-affinity for C_{16} acyl-CoA, facilitating its dissociation from the associated protein sites, which is considered the rate-limiting step.[175] However, the effect of the polysaccharides on the enzyme *in vivo* cells is still unknown. They might play a role in determining whether FAS I would function as a *de novo* synthase or as an elongase.[48]

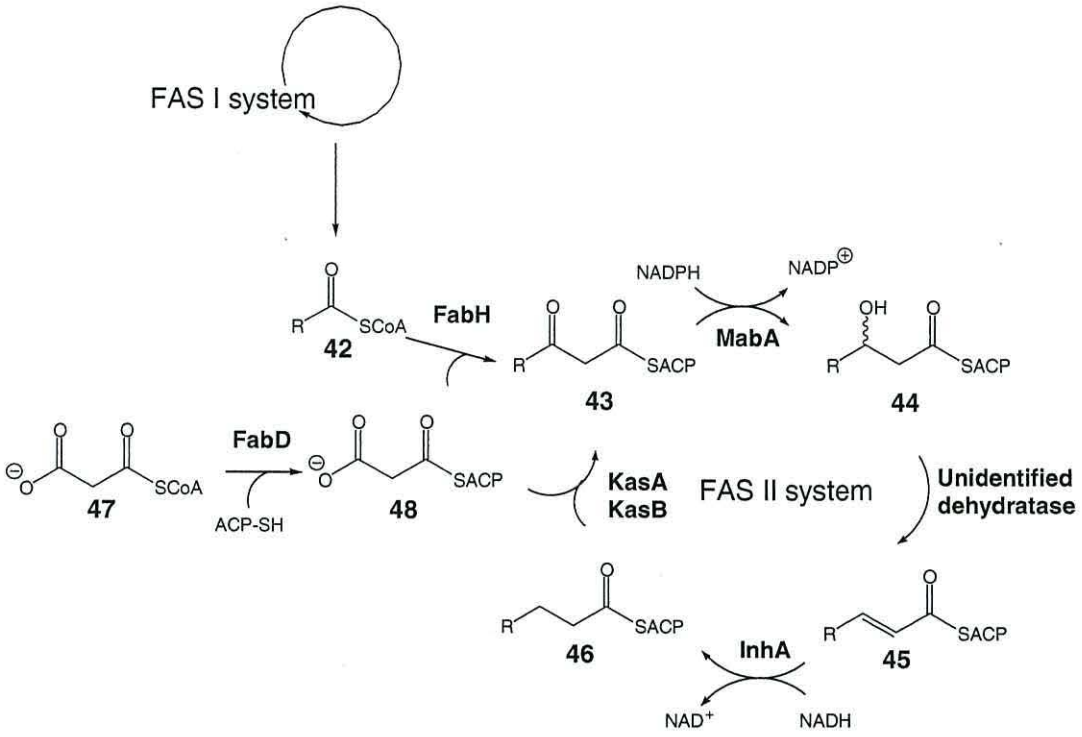
1.3.3 FAS II

The process of further elongation of the fatty acid chains in *M. tuberculosis* involves another type of system: FAS II. This type of system is usually found in prokaryote organisms, such as bacteria, and it is also encountered in plants.

FAS II, unlike FAS I, consists of several dissociated enzymes, which will be outlined later. Many of them are the targets of different anti-tuberculosis and anti-mycobacterial agents currently on the market; for example, isoniazid, (INH), one of the best known first-line drugs, is an inhibitor of this process, as well as diazaborines, ethionamide, triclosan, thiolactomycin, thiourea isoxyl and many others.[176-180] Moreover, new substances that inhibit these enzymes are currently being studied as possible chemotherapeutic agents; for example, thiolactomycin analogues, which show potency against this process.[180-182]

A major step forward in understanding the mechanism of this system came about through the extensive process that led to the deciphering of the complete genome sequence of *M. tuberculosis* in 1998.[168]

Currently, it is known that FAS II is unable to carry out *de novo* synthesis; instead it catalyses the elongation of the acyl chains formed in the FAS I to give longer chain fatty acids (from C_{24} to C_{56}, **Scheme 8**).



Scheme 8: ***The FAS II mycolic acid elongation system of M. tuberculosis***

The enzyme connecting the two systems, FAS I and FAS II, is tuberculosis FabH. It simultaneously catalyses a further condensation of a C_2 unit and the exchange into acyl carrier proteins (**Scheme 7**).[183] FAS II uses particular acyl carrier proteins, specific for long chain intermediates, up to 50 carbon units long.[184,185] This protein carries the growing acyl chain from reactive centre to reactive centre of each lipogenic enzyme. The enzymes in the system are effectively able to recognise the fatty acids as substrates only if they are linked to specific ACP.[186,187] After the above condensation, the β-ketoacyl-Acp (**43**) is reduced to β-hydroxyacyl-Acp (**44**) by a β-ketoacyl reductase (MabA) using NADPH as coenzyme (**Scheme 8**).

The β-ketoacyl reductases of different mycobacteria have been compared and they show perfect retention of the active site structure; it is suggested that, in this case, they should share similar substrate specificity. Therefore, it is likely that a single substance could inhibit all of them in several species and strains and become a

good chemotherapeutic agent for several diseases.[188] The dehydratase that catalyses the subsequent elimination of water from β-hydroxyacyl-Acp (**44**) to give enoylacyl-Acp (**45**) has not yet been identified (**Scheme 8**). The following step of the cycle has been thoroughly studied, which is the hydrogenation to give the saturated acyl-Acp (**46**).[189] The specific 2-*trans*-enoyl-acyl carrier protein reductase, InhA, that catalyses this reaction, is the main target of the first-line antituberculosis drug, isoniazid, and it appears to be critical for the production of mycolic acid and the mycobacterial survival.[178,189,190] This evidence supports the hypothesis that the production of long-chain fatty acids and mycolic acids is also vital for mycobacterial growth.[188]

The final enzymes of the cycle are two β-ketoacyl Acp synthases (KasA and KasB, **Scheme 8**). Both of these perform an activity typical of bacterial KASs: condensing an acyl-Acp (**46**, not acyl-CoAs, **42**) with a malonyl-Acp unit (**48**) previously prepared from malonyl-CoA (**47**).[185,191] However, unlike most KASs, they are more active with long chain acyl-Acp substrates than shorter acyl primers.[185] In particular, it has been suggested that KasB may be more important in the last stages of fatty acid elongation;[192] this enzyme has been proved to affect the cell wall permeability and the mycobacteria intracellular survival.[167] The termination of function of these enzymes appears to be lethal for the organism, but KasA does not seem to be inhibited by isoniazide as previously suggested.[185,193]

After being formed, the new β-ketoacyl Acp (**34**) will undergo other cycles until final completion of the desired product (**Scheme 8**).

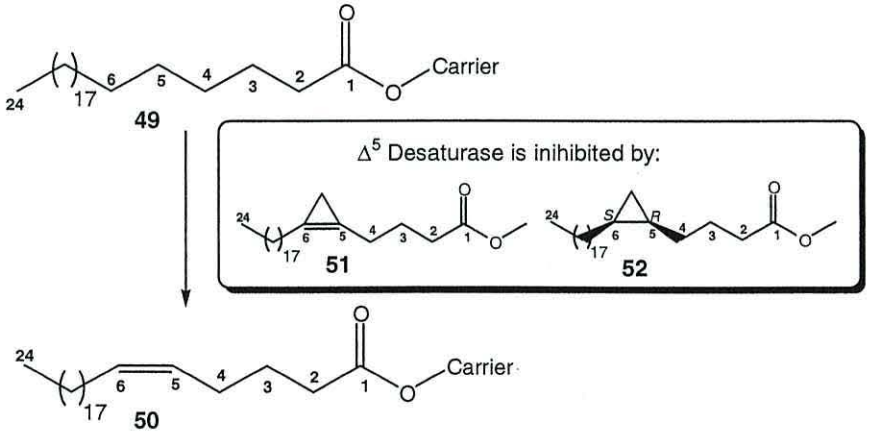
1.3.4 The desaturation

These long fatty acids also undergo processes of desaturation, cyclopropanation and other modifications that allow the formation of the extraordinary variety of functional groups present in mycolic acids. It seems likely that they occur before the final condensation between the meromycolate chain and the α-branch, but this will be better illustrated later (**Section 1.3.7**).[194] However, the order of the steps for the formation of the meromycolate chain is not yet known in detail, and there are different hypotheses.

Following the first hypothesis, initially there is a chain elongation to produce C_{48}-C_{56} saturated fatty acids; then this chain is functionalised with desaturations and cyclopropanations.[80]

A second theory proposed that the modifications of the acyl chain happen during the elongation.[80] In particular, this pathway could be obtained through two different approaches: the desaturations and the elongation could occur either simultaneously or in a step-wise fashion with interrupted cycles of additions and reductions, followed by modifications of the intermediate chain.[80]

There are no certain answers for this dilemma but there is much evidence in support of the last hypothesis. Functionalised intermediates, shorter than the full-length meromycolate, for example *cis*-tetracos-5-enoic acid (**50**, **Scheme 9**), were detected firstly in *M. phlei*, [195] and then in cell-free extracts from *M. tuberculosis*.[194]

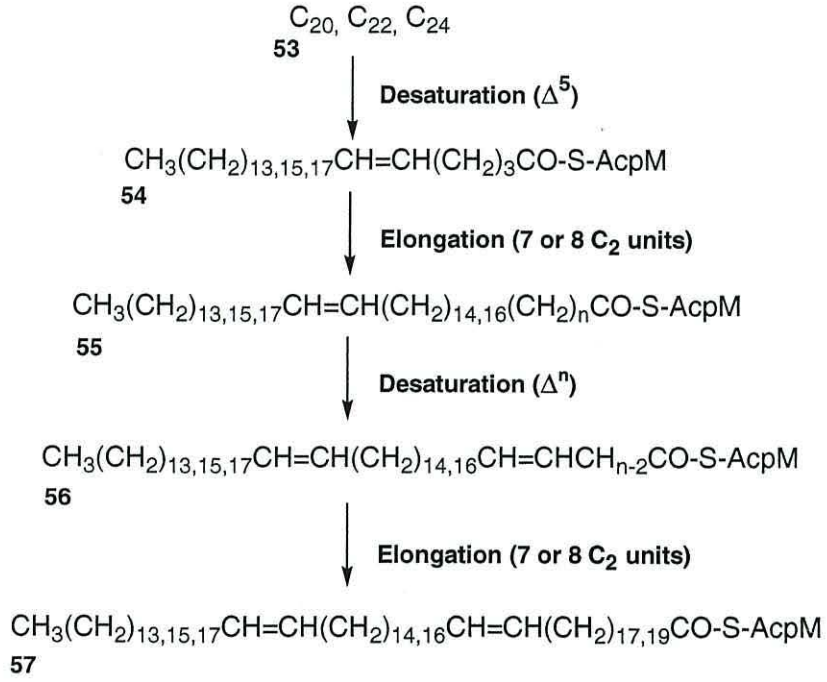


Scheme 9: *Δ⁵-Desaturase*

However, this still did not prove that the compound was the result of a mycobacterial metabolic process. This acid has also been demonstrated to be an intermediate in the α-mycolate biosynthesis.[196,197] In particular, *cis*-tetracos-5-enoic acid (**50**) can be formed from tetracosanoate (**51**) through a desaturation catalysed by a Δ^5-desaturase.[196,197] In fact, like other fatty acid desaturases, this enzyme is inhibited by $C_{24:1}$ *cis*-5-fatty acid analogues, ω-19-cyclopropene fatty acids (**51**), and ω-19-(1'*R*,2'*S*)-*cis*-cyclopropanes (**52**, **Scheme 9**).[198,199]

These compounds also inhibit the entire mycolate synthesis, showing that ω-19 *cis*-desaturase is important in the biosynthesis of mycolic acids.[199] This provides

strong evidence for the hypothesis that the meromycolate chain is obtained through cycles, each of which is composed of a desaturation followed by a series of elongation steps. This mechanism for the simple case of the synthesis of *cis*-dialkene α-mycolic acids of *M. smegmatis*, has been illustrated in **Scheme 10**.[200,201]



Scheme 10*: Presumed pathway for the biosynthesis of the meromycolate chain in M. smegmatis*

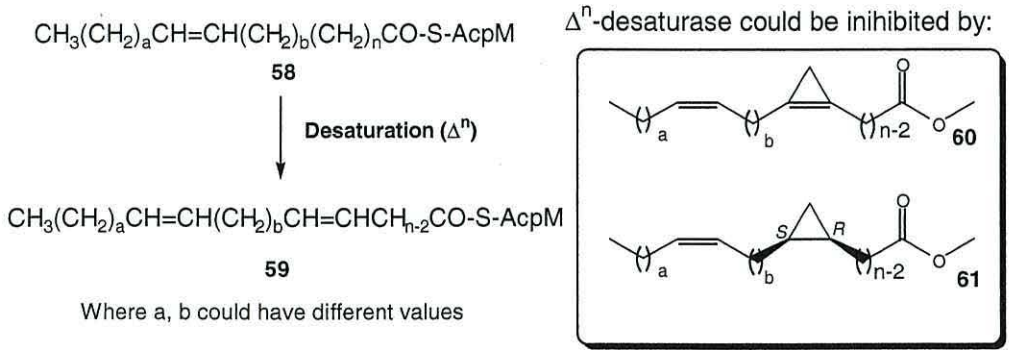
According to this pathway, an initial Δ^5-desaturation is followed by a first round of elongation steps, resulting in the addition of seven or eight C_2 units through the FAS II cycles. Then there is a second desaturation, presumably catalysed by a Δ^n-desaturase, which is followed by a second and final round of elongation (**Scheme 10**).[200,201]

The existence of this route is supported by the detection of very long β-hydroxy fatty acids (**44**, **Scheme 8**), including the complete β-hydroxymeromycolate, in a mutant of *M. smegmatis* defective in an undefinited dehydratase of FAS II.[200] These long fatty acids have the double bonds in the same positions established for the complete α-mycolic acids in *M. smegmatis*.

The major objection to this theory is that the specific enzyme that could catalyse this type of desaturation has not yet been identified in the mycobacterial genome; nothing is yet known about *in vivo* systems.[80] Moreover, according to Daffe and

Draper,[31] no-one has successfully reported a direct incorporation of labelled precursors into the final mycolic acids. Other more elaborate hypotheses have also been proposed.[202]

It would be interesting to confirm this theory by establishing if *cis*-fatty acid analogues **60** and **61**, similar to those used by Coxon *et al.*[199] (see **Scheme 9**) are inhibitors of mycolic acid biosynthesis (**Scheme 11**) identifying in this way the Δ^n-desaturase.



Scheme 11: ***Possible inhibitors of the second Δⁿ-Desaturase***

Similar pathways could also be occurring in the formation of more complex mycolic acids, such as those in *M. tuberculosis*. These lipids undergo cyclopropanation and introduction of different other functional groups.[48] However, the timing of these processes remains unclear even if there is evidence that they could happen in the last stages of the elongation process, but before the condensation with the α-unit.[194,203]

If the process of desaturation occurs alongside the elongation, then the positions of the functional groups are defined in relatively early stages of the biosynthesis, even if their completion might happen later. Therefore if different types of mycolic acids are thought to be biosynthetically closely related they should also show distinct structural correlations as will be investigated in more detail in **Section 1.5.2**.

1.3.5 Further modifications

One of the most interesting and characteristic features of mycolic acid biogenesis is the introduction of different functional groups, both cyclopropanes and oxygenated groups. Although cyclopropane synthases are present in several bacteria, in *M. tuberculosis* and other similar mycobacteria there is an elaborate

enzymatic system formed by a large family of highly homologous proteins not found in any other bacteria. In the genome sequence of H37Rv, a strain of *M. tuberculosis*, eight homologous genes have been found.[204] Despite a striking sequence conservation (they share between 50 % and 75 % identity), each member of this gene family appears to have a distinct catalytic function for which no other family member can compensate.[166] The proteins codified by these genes are *S*-adenosylmethionine dependent methyltransferases, also called cyclopropane synthases. They are responsible for cyclopropanation, oxidation and methylation of the meromycolate chains.

To better understand the roles of the proteins, several of the related genes have been genetically manipulated. They could be heterogeneously expressed in bacteria that do not contain them, for example in *M. smegmatis* or in *E. coli.* These genes could also be deactivated or over-expressed in *M. tuberculosis.* Afterwards, the mutant strains have been studied for alterations in the types and distribution of their mycolic acids and for alterations in their pathogenesis. A great deal of information has been obtained which will be summarised subsequently with reference to the genes that codify the proteins (**Figure 19**):

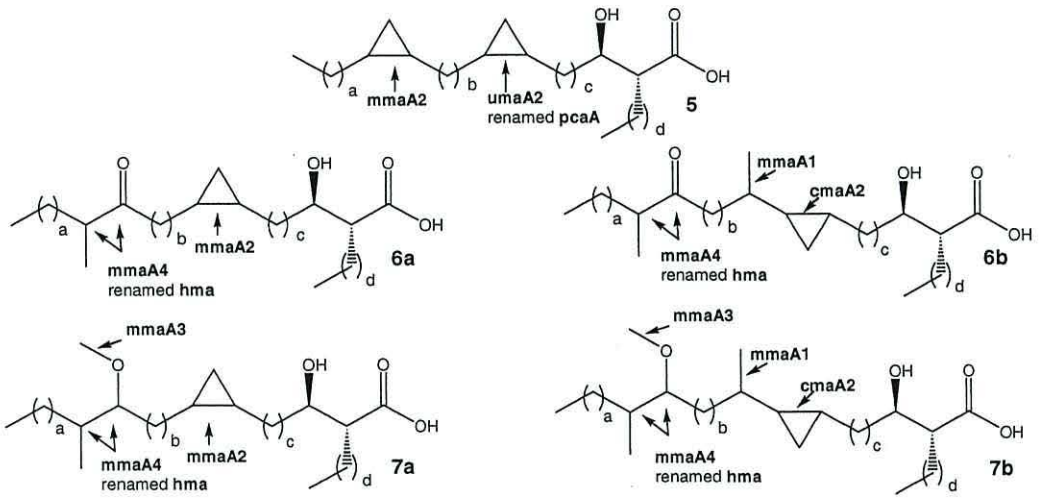
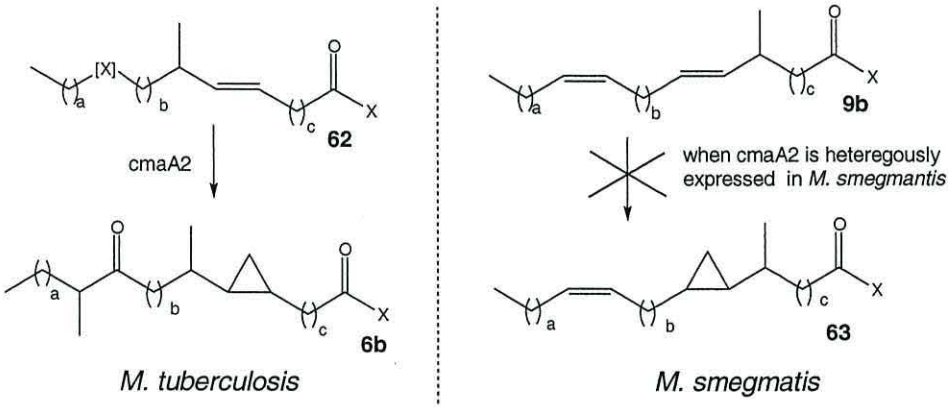


Figure 19*: The cycloclopropane synthases*

***cmaA1* (cyclopropane mycolic acid synthase 1)**: In *M. smegmatis*, this leads to the cyclopropane in the distal position.[160] Glickman has demonstrated that in *M. tuberculosis, cmaA1* is not required for mycolate modification.[204] It has been suggested that *cmaA1* may modify lipids other than mycolic acids.

***cmaA2* (cyclopropane mycolic acid synthase 2)**: In *M. tuberculosis* it encodes the protein for the formation of the *trans*-cyclopropane in the proximal position for keto and methoxy-mycolic acids (**2b**, **3b**).[166] It does not have the same function in *M. smegmatis*, where it produces the *cis*-cyclopropane in the proximal position, both in the α-mycolic acids (**1**) and in the epoxy ones (**5a**), without substrate specificity.[157] Schoeder and Barry explained this difference in behaviour, suggesting that *M. smegmatis* does not contain the correct substrate for the enzyme naturally present in *M. tuberculosis*.[205] The substrate for the cyclopropanation in *M. tuberculosis* should be *trans*-olefinic intermediate (**45**), with a methyl group nearest the ω-end with respect to the olefin, whilst the *trans*-compounds (**5b**) found in *M. smegmatis* have a methyl group nearest the carboxylic group end (**Scheme 12**). If verified, this study clearly illustrates the high substrate specificity of the enzymes.



Scheme 12: ***The position of the methyl group could be crucial for the identification of the substrate by the cyclopropane synthases.***

***umaA1* (unknown mycolic acid methyltransferase 1)**: The function of this gene is still unknown.[204]

***umaA2* (unknown mycolic acid methyltransferase 2)** also called **PcaA (proximal cyclopropanation of α-mycolate)**: In *M. tuberculosis*, this codifies the enzyme for the cyclopropanation into the proximal position of the α-mycolate (**1**). It has been proven that *umaA2* is important for the virulence and persistence of *M. tuberculosis*.[156,206]

***mmaA1* (methoxymycolic acid synthase 1)**: This gene is critical for the transition from *cis* to *trans*-double bonds introducing an allylic methyl

branch.[104,205] The related enzyme has a remarkable specificity with respect to the ω-end of the growing meroaldehyde chain.[205]

***mmaA2* (methoxymycolic acid synthase 2)**: It is responsible for the introduction of the *cis*-cyclopropane in the proximal position of both α-mycolic acids (**1**) and epoxy-mycolic acids (**5a**) in *M. smegmatis,* showing non-specific preference for the substrate.[131,163] However, in *M. tuberculosis*, *mmaA2* catalyses the distal cyclopropanation in the α-mycolates (**5**). Moreover, there is evidence that it could also introduce a *cis*-cyclopropane in the methoxy-mycolic acids (**7a**) of *M. tuberculosis*.[204] In this case as well, the different roles of the enzyme in the two mycobacteria might be explained by the fact that *M. smegmatis* does not contain the precursor normally used by the enzyme in *M. tuberculosis*.

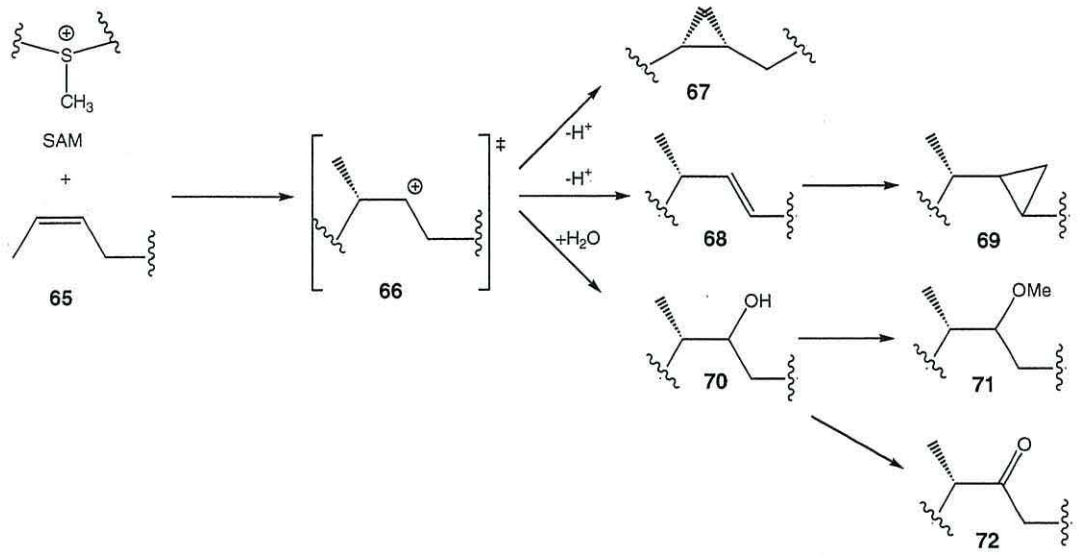
***mmaA3* (methoxymycolic acid synthase 3)**: This is required for the formation of oxygen-containing mycolate at the distal position. It codifies the enzyme for the methylation of the alcohol to give the methoxy group.[163]

***mmaA4* (methoxymycolic acid synthase 4)** recently renamed ***hma* (hydroxymycolic acid synthase)**: This is necessary for the transformation of the distal *cis*-olefin into a secondary alcohol with an adjacent methyl branch.[163,207]

1.3.6 A possible mechanism for the functionalisations

Several hypotheses proposing different mechanisms for the processes of cyclopropanation, oxygenation, and methylation have been put forward. Following a particularly interesting one, initially, there is the transfer of a methyl group from the *S*-adenosylmethionine (SAM) to the *cis*-double bond of the acid, to give a carbocation transition state, or intermediate (**Scheme 13, 66**).[208-210] This process could be stabilised by the aromatic residues present in the active-site via π-cation interaction.[211] Since it has been shown that cyclopropane fatty acids maintain the configuration of their substrate, the formation of the carbocation (**66**) has to be the slow step and then this is rapidly transformed (**Scheme 12**).[212] It can be trapped by a variety of reactions depending on the nature of the active-site residues.[158] The elimination of a proton on the methyl branch forms the *cis*-cyclopropane (**67**). The removal of a proton on the methylene group in the α-position yields allylic methyl-branched double bonds (**68**), also possible

precursors of *trans*-mycolic acids (**69**). The addition of water yields an α-alcohol (**70**) which is converted into methoxy-mycolic acids (**71**) or into keto-ones (**72**).



Scheme 13: ***The insertion of the functional groups***

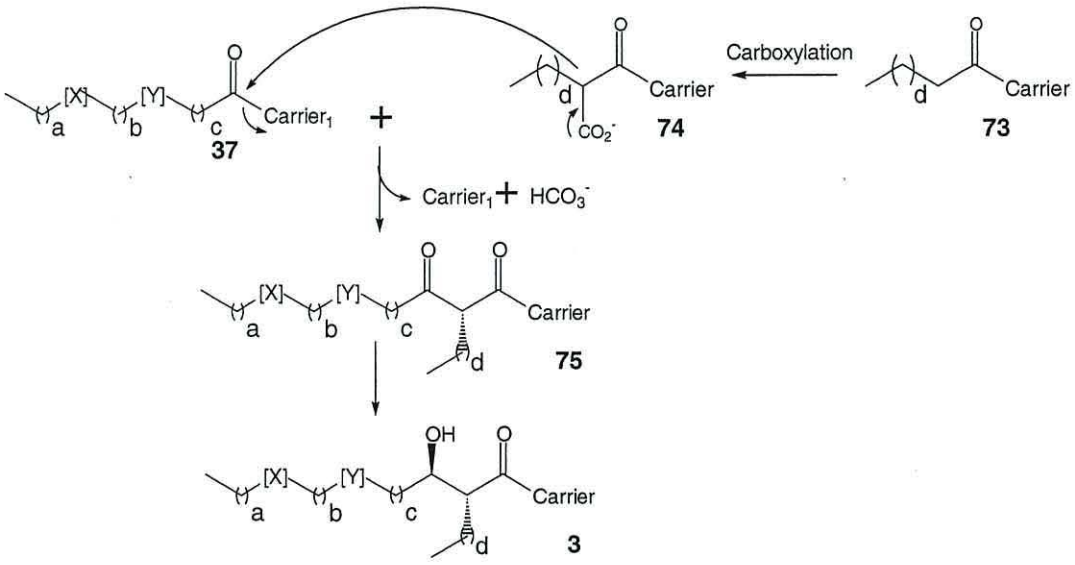
With the discovery of the cyclopropane synthases in *M. tuberculosis,* it has been demonstrated that these proteins involved in the formation of functional groups require S-adenosyl-*L*-metionine as cofactor.[213,214] The analysis of the structures of some of these proteins showed that these enzymes catalyse a methyl transfer via a carbocation mechanism similar to that already proposed for other cyclopropane synthases. Here, a bicarbonate ion could act as a general base to deprotonate the carbocation to give the final cyclopropane.[206] In addition, Dinadayala *et al.* asserted that this mechanism also leads to the creation of the oxygenated groups.[207] However, to better understand the routes followed by these enzymes, it will be important to analyse the activity of the cyclopropane synthases *in vitro*,[206] and using labelled long fatty acids as precursors.

It is important to note that this route has a significant implication. Thus, the stereochemistry of at least one carbon for all the functional groups present in the meromycolic chain has always to be in the same configuration. These observation and its implications will be explored in more detail in **Section 1.5.1**.

1.3.7 The condensation process

For the last steps of the biogenesis of the mycolic acid, different hypotheses have also been put forward. Currently, most authors believe that a Claisen condensation

occurs between the meromycolate chain (**37**) and the enolate of an α-alkyl chain precursor (**74**) to yield a β-oxo mycolate intermediate (**75, Scheme 14**).[2] The keto group of the β-oxo mycolate (**75**) is then reduced to form a complete mycolic acid (**3**), preceding its transfer to various cell wall components (**Scheme 14**).



Scheme 14: ***The Claisen condensation***

Initially, the existence of this type of mechanism was proved for shorter fatty acids, the corinomycolic acids (**21, Figure 15**).[215] This type of mechanism was also confirmed subsequently for the mycobacteria by Etemadi and Lederer.[216] They grew *M. smegmatis* in the presence of [1-^{14}C]-tetracosanoate, and the label was incorporated into the final mycolic acid. The subsequent isolation of putative meromycolate precursors in mycobacterial cultures was an important proof for the theory described above.[195,203] Others showed that these compounds could be intermediates for mycolic acids using cell-free systems for the biosynthesis of longer fatty acids.[194,201,217] The detection of an incomplete meromycolate chain, except for the presence of a β-hydroxy group, in a mycolate-deficient mutant of *M. smegmatis,* provides crucial evidence in support of this theory.[200]

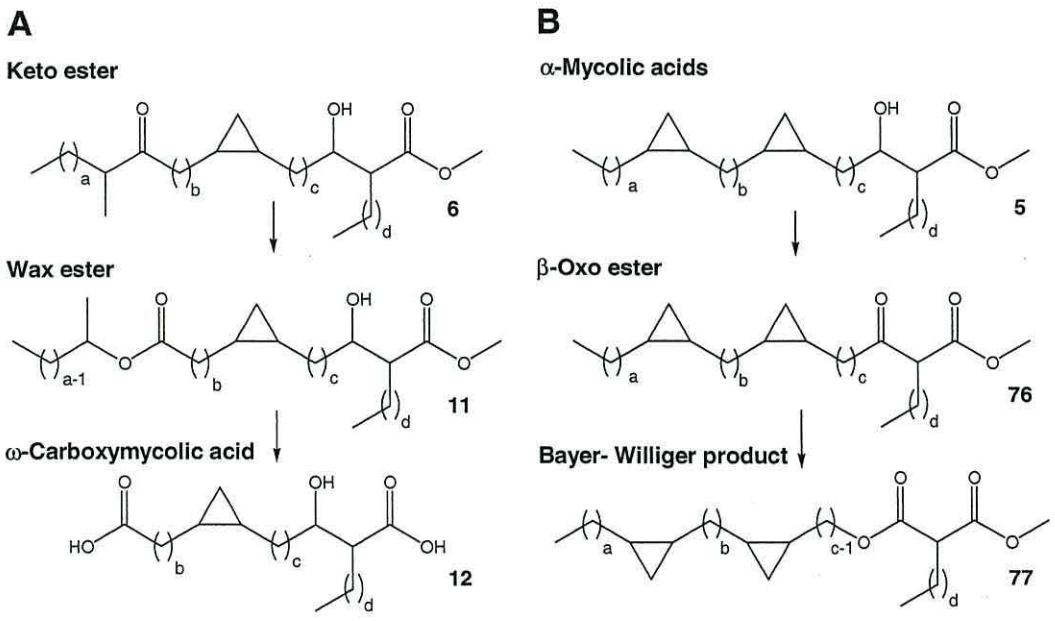
New evidence seems to suggest that the removal of a proton in the α-position with is activated through a prior carboxylation (**Scheme 14**).[218] Finally, a polyketide synthase, Pks13, involved in the condensation of two fatty acids to form mycolic acids, has been discovered.[219] This not only confirms the existence of this route, but also offers a specific target for the development of new drugs for the treatment of human infections caused by mycobacteria.

1.3.8 The catabolism of mycolic acids

Whilst there have been numerous studies about the biosynthesis of mycolic acids, very little is known regarding their catabolism.

Mycobacterial processes for the degradation of fatty acids are thought to be more common than those for their biosynthesis, because *in vivo* mycobacteria are in a lipid rich environment. Moreover, these organisms possess a very large number of genes that codify proteins for this process, illustrating how important it is for the life cycles of mycobacteria.[168] The degradation of host cell lipids is thought to be similar to the degrading processes present in other organisms, with several cycles of β-oxidations. Conversely, mycolic acids seem to be particularly resistant to degradation since they could be found in non-mummified archaeological tissues, infected with *M. tuberculosis*, which were 1400 years old.[220]

It has been suggested that the first step in the metabolism of keto-mycolic acids (**6**) was a Baeyer-Villiger reaction yielding wax-ester compounds (**11**),[221] subsequently transformed into ω-carboxy-mycolic acids (**12**, **Scheme 15**, **A**).[101]



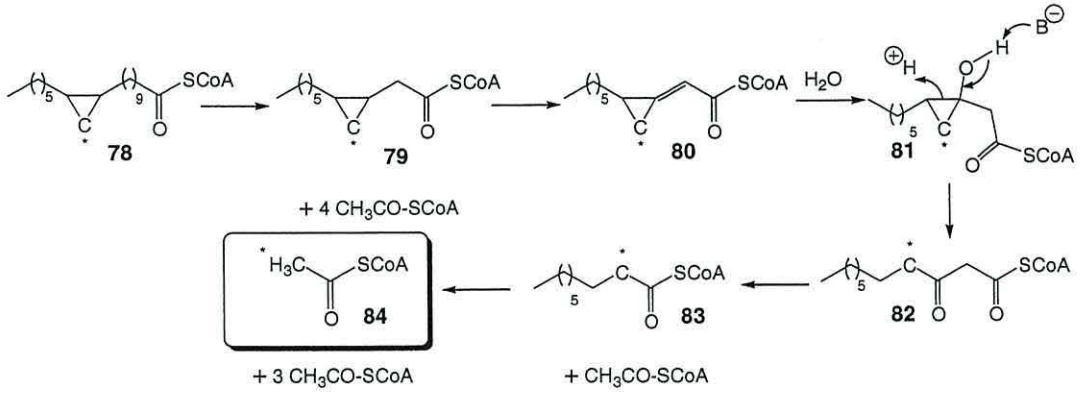
Scheme 15: ***Two possible first steps in the degradation of mycolic acids***

In a recent publication Asselineau *et al.* reported a previous study on the degradation of radio-labelled mycolates of *M. aurum*.[202] The process of oxidation of the hydroxyl group in the β-position, followed by the cleavage of the adjacent C-C bond by a Baeyer-Villiger type of reaction, was detected (**Scheme 15**, **B**).

Since this route was observed for both keto-mycolic acids (**6**) and α–mycolic acids (**5**), they concluded that the transformation of the keto-compound (**6**) into wax ester (**11**) is not essential.

The catabolism of the meromycolate chain has received very little attention, considering that it could be important for a better understanding of the properties of these acids. The degradation of the cyclopropane group is particularly interesting. In the literature, different types of ring-opening processes for this ring are reported, while functionalisations of intact cyclopropane units are almost unknown.[222] The degradation of cyclopropane fatty acids has been analysed to a very small extent. It has been reported that rat liver mitochondria can shorten the chain of these acids, but they are unable to metabolise the cyclopropane group, producing an accumulation of 3,4-methylene fatty acids.[223] However, there are other microorganisms that can degrade it. Recently, a bacterium has been discovered that can be grown using cyclopropanecarboxylate as its sole source of carbon and energy.[224]

Tipton and Al-Shatir analysed the degradation of the *cis*-11,12-[methylene-^{14}C]-methyleneoctadecanoic acid (**78**) in whole cells of *Tetrahymena pyriformis* (**Scheme 16**). They proposed a possible pathway for its metabolism which differs only marginally from the normal β-oxidation of long fatty acids. The existence of this mechanism has been supported by the detection of [2-^{14}C]-acetate (**84**).[225]



Scheme 16: ***A possible mechanism for the degradation of cyclopropane fatty acids***

On the other hand, this route contradicts the one previously proposed by Shiller and Chung for the degradation of cyclopropanecarboxylic acid.[226,227] According to their studies, there is a direct addition of water across one of the carbon to carbon single bonds of the ring, yielding γ–hydroxy-butyrate. Very recently another

group proposed another possible route for the degradation of this acid via β-hydroxybutyrate illustrating the difficulties encountered in the determination of the microbial metabolism of this kind of substance.[228]

In order to better understand the real mechanisms of the degradation of the cyclopropane group, and mycolic acids, more studies are needed.

1.4. *Mycobacterium smegmatis*

1.4.1 Overview

Mycobacterium smegmatis is a species of rapidly growing mycobacterium. For a long time, it has been considered a saprophytic environmental microorganism (an organism living on dead or decayed organic matter) and not pathogenic. Only recently have strains of *M. smegmatis* been reported to cause diseases in humans.[229,230]

M. smegmatis is mostly studied as the source for studies on mycobacterial metabolism and enzyme systems as evaluation material for the much more pathogenic *M. tuberculosis*.[231] It has been chosen for this purpose because it reproduces quickly, is easily obtainable and not highly pathogenic, even if it is considered evolutionarily distant from *M. tuberculosis*. The primary distinguishing features are the much faster growth rate of *M. smegmatis* and the fact that it does not present the same pathogenic properties described for other mycobacteria.[232] However, some studies have shown that *M. tuberculosis* and *M. smegmantis* have some genomic similarities and, therefore, *M. smegmatis* has also been proposed as an appropriate model for studying some of the properties of these mycobacteria in general.[233,234]

The structure of its cell wall and in particular its mycolic acids have been thoroughly studied. The information obtained through these studies has also given a better understanding of the role and the biosynthesis of these acids, not only in this mycobacterium but also in others, especially in *M. tuberculosis*.

1.4.2 Mycolic acids present in *M. smegmatis*

In *M. smegmatis*, as in other mycobacteria, the mycolic acids are presented as a mixture of different homologues and types. Their structures have been the object of numerous studies. Three different subclasses have been identified: α^1-mycolic acid; α-mycolic acid, either in a *cis* or *trans*-configuration;[235] and epoxy mycolic acids (**Figure 20**).[80] Etemadi *et al.* reported also the existence of **Type 2** α-mycolic acids (**85**) which contain a cyclopropyl ring and a *trans*-double bond.[117]

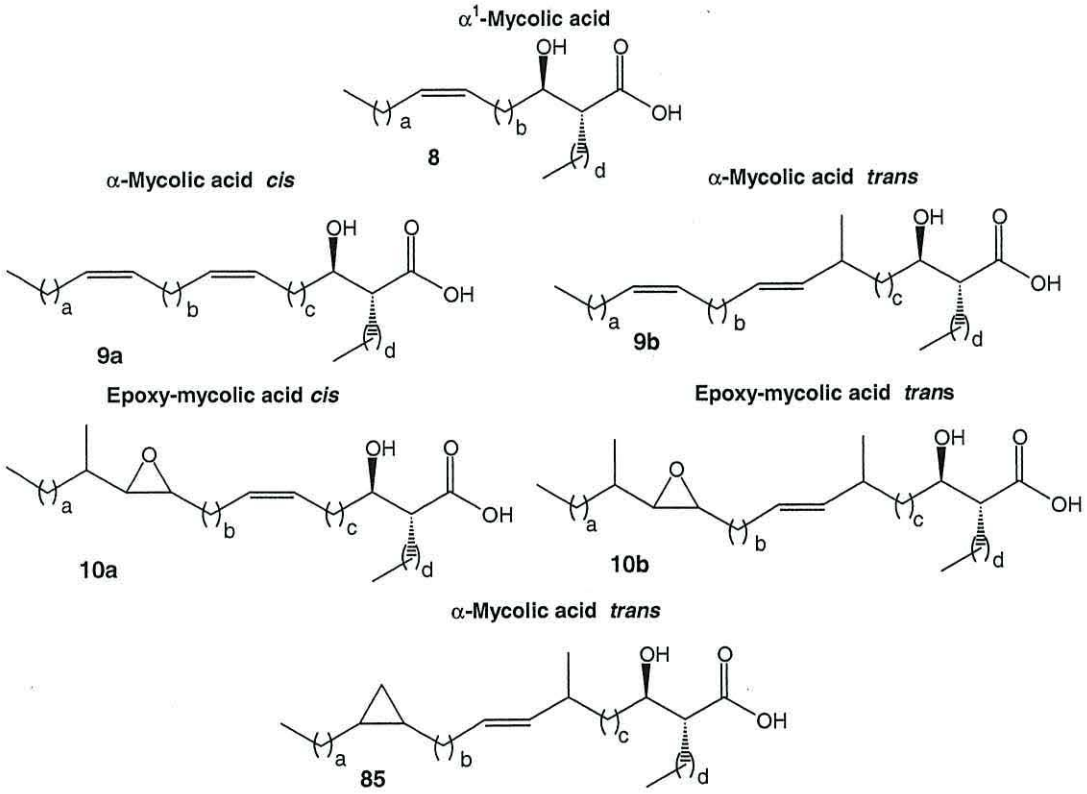


Figure 20: ***Different subclasses contained in M. smegmatis***

Other mycobacteria such as *M. chitae, M. farcinogenes, M. fortuitum, M. giae, M. peregrinum, M. senegalese*[82] contain the same characteristic pattern of mycolic acids. The similarities in the mycolic acid distribution suggest that these species might have a fairly close phylogenetic relationship.[236] There are also different homologues for each subclasses; for α-mycolates alone, 100 structural isomers have been detected (at a mole fraction of 0.1 or higher).[80]

The first two subclasses (**8, 9, Figure 20**) are the major components and they have been thoroughly studied by Gray's group in the early 1970s.[94] They separated, after derivatisation, each homologue of the major mycolic acids by reverse phase

HPLC and determined their molecular weight.[86] Subsequently, Baba *et al.* confirmed that in *M. smegmatis* α^1-mycolates (**8**) ranged from 60 to 66 carbons, while α-mycolates (**9**) ranged from 72 to 81 and epoxy-mycolates (**10**) from 73 to 81.[231] Interestingly, they discovered that the "length" isomers (homologues) of α^1-mycolates have a constant distribution in each of the eight different strains of *M. smegmatis*. This distribution was also different from the other mycobacteria that contain the same types of mycolic acids; this has, therefore, been considered a good method for the identification of the species.[231]

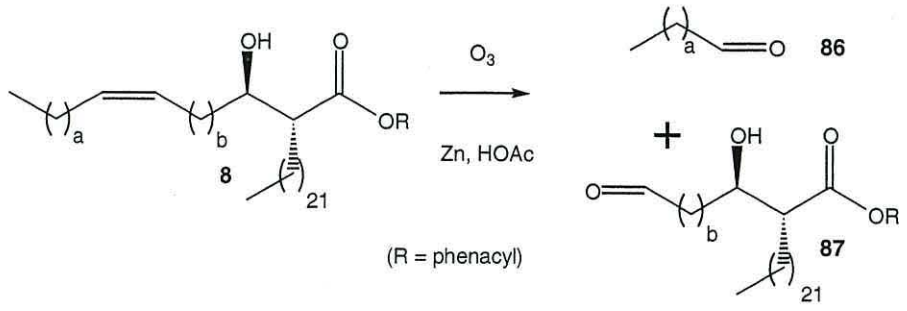
Pyrolytic GS/MS, fragmenting the mycolic acids into meroaldehyde and alpha unit, has also been useful in studying the α-branches (**Scheme 2, Section 1.2.2**).[112] Gray *et al.* found that each homologue always has an alpha unit of 24 carbons but they differed in the length of the chain of the meromycolate moiety.[94] This observation was confirmed subsequently by Kaneda and co-workers, who determined that α-units contained in α, α^1 and epoxy-mycolates are either 22 or 24 carbons in total, with a ratio 10:90.[84,106]

Gray and co-workers also identified which types of functional groups were present in the major subclasses of mycolic acids by NMR: they determined that these acids contain double bonds with both a *cis* or *trans*-configuration.[94] Another very interesting result was that this group suggested the most likely positions for the functional groups in each homologue of the major subclasses.[112]

These types of mycolic acids could be fragmented in a controlled way using either reductive or oxidative ozonolysis procedures. The fragments generated were separated and characterized by mass spectrometry. These procedures allowed both the quantification and identification of the cleavage fragments.

For example, in the case of monoalkenes, two different types of derivatives were obtained, an aldehyde (**86**) and a α-alkyl-β-hydroxylated ester (**87, Scheme 17**).

From the values and the percentages of the molecular ions obtained for each derivative, and knowing the length of the α-branch, they succeeded in identifing the most likely positions of the double bond for each homologue.



Scheme 17: ***Fragmentation of the monoalkene by ozonolysis.***

In the case of monoalkene, they found that **a** could be either **17** or **19** while **b** could be **15**, **17**, **19** and **21**. However not all the permutations were observed.[111] The major constituent of the monoalkene mycolic acids was reported to possess a structure as illustrated in **Figure 21** where **a** = **17** and **b** = **17**.[111]

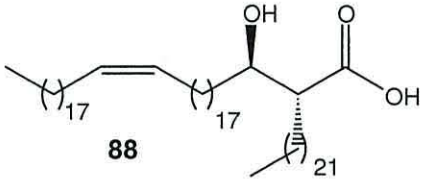


Figure 21: ***Major component of monoalkene subclass***

The same procedure was applied for the determination of the position of the double bonds in the dialkene mycolic acids. For the *cis*-dialkene-α-mycolic acid (**9a**, **Figure 20**), different combinations were observed in greater than 10 % relative abundance were **a** ranged from **13** to **17**, **b** was found to be either **10** or **12** and **c** ranged from **15** to **21**.

After fragmentation of the *trans*-dialkene-α-mycolic acids (**9b**) and analysis of the pattern of ions observed in the mass spectrum, Gray and colleagues assigned the methyl branch to the side of olefin *furthest* from the ω-end of the meromycolate chain of these acids. Therefore, the methyl group, in this case, is in the opposite position with respect to the functional group, the double bond, to the one that it has in oxygenated mycolic acids (**6b**, **7b**), but identical to the one shown in the minor classes of mycolic acids (**Type-2**, **15**) in pathogenic mycobacteria (**Figure 22**). This infers that there could be two different pathways for the insertion of the methyl group in the mycobacteria.[205]

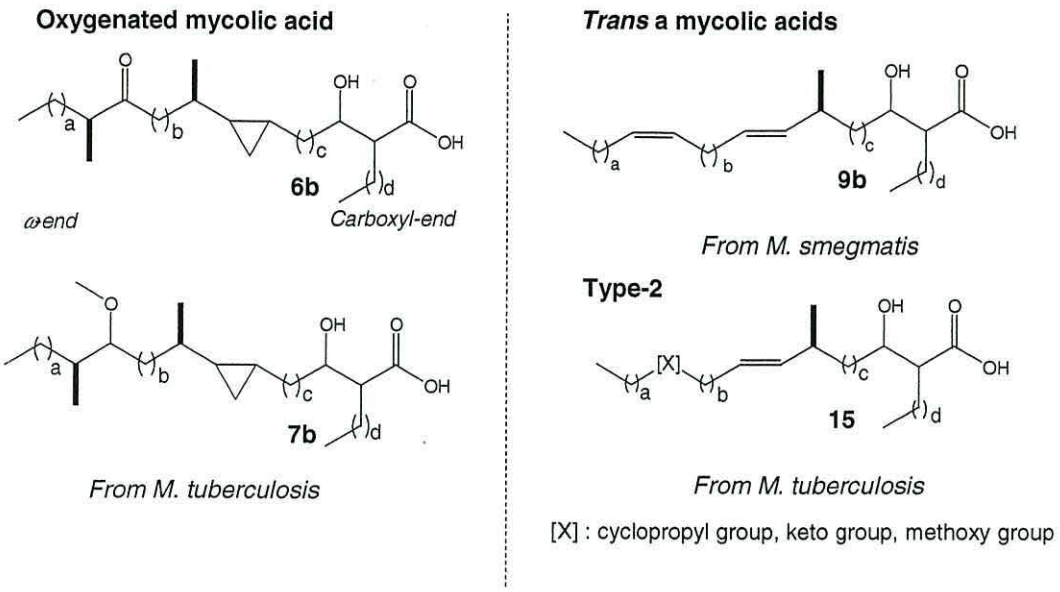
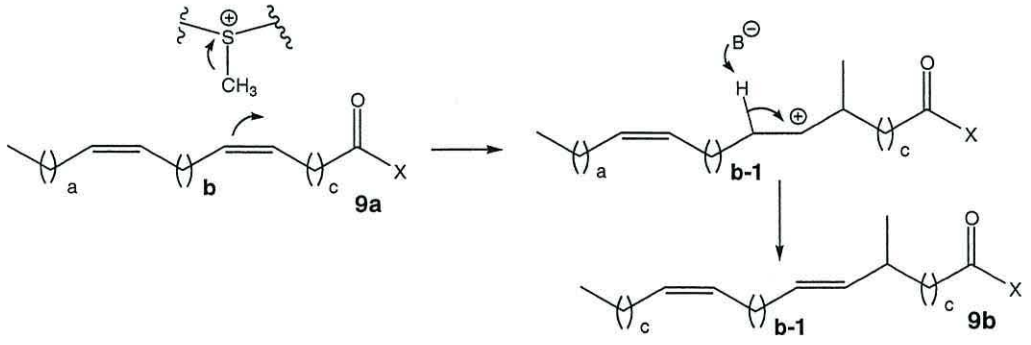


Figure 22: ***The different position of the methyl group in different types of mycolic acids***

With regard to the length of the chains present in the *trans*-mycolic acids, Gray *et al.* using similar methods used for α^1-mycolic acids, found that **a** could be **15**, **17** or **19**, while **b** ranged from **11** to **15**, and **c** varied from **16** to **20**.

These results suggested that if the *cis*-dialkene α-mycolic acids (**9a**) are precursors of the *trans*-ones (**9b**), then this must be accomplished by a route involving rearrangement of the double bond to a position one carbon *further* from the ω-end (**Scheme 18**).



Scheme 18: ***Different hypothesis to the biosynthesis of trans-α¹-mycolic acids***

In each (*cis* (**9a**)-*trans* (**9b**)) pair with an identical overall chain length, the position of the double bonds are such that the ***b-values*** of **10** and **12** predominate in the unmethylated (*cis*) homologue (**9a**), whereas ***b-values*** of **11** and **13** predominate in the methylated (*trans*) homologue (**9b**). These results seem to

contradict the generally followed biosynthetic mechanism for the conversion of *cis* into *trans*-mycolic acids by *S*-adenosylmethionine.[208] Following this mechanism, firstly there is the addition of the methyl group to the carbon-carbon double bond, followed by the deprotonation to regenerate the double bond in a position *nearer* to the ω-end (**Scheme 18**). Gray and co-workers did not suggest another likely mechanism and they specified that the conclusions obtained by their analyses are only speculative. Moreover, the method used by Gray *et al.* is not universally accepted.[102] However, these deductions highlighted, again, the problem of the *trans*-mycolic acids and their biosynthesis.

In conclusion the *trans*-double bond fatty acids may be formed by a completely different biosynthetic route from the one generally understood. It would be interesting to determine a clear answer to this dilemma by analysing the mycolic acids of the mycobacterium with new, more reliable techniques, such as MALDI-TOF/MS, or by using unambiguously labelled precursors.

Very little is known about the other mycolic acids subclass presents in *M. smegmatis*: the epoxy-mycolic acids (**10**, **Figure 20**). This type is present in some other mycobacteria that have similar lipids pattern, such as *M. fortuitum*.[82,236] The structure of the epoxy-mycolic acids in this organism has been determined both by chemical methods,[95] and by analysing the mass fragmentation.[93] It is interesting to notice that the methyl group in the α-position with respect to the epoxy group has been assigned to the side of oxirane *nearest* to the ω-end of the meromycolate chain, while the methyl group of the *trans*-mycolic acids (**9b**, **Figure 20**) is on the side of olefin *furthest* from the ω end.

The content of the mycolic acids in this mycobacterium has been demonstrated to also change depending on the conditions of growth similar to that of other mycobacteria. In *M. smegmatis*, it has been demonstrated that responses to change of temperature are: the production of shorter α^1-mycolic acids; variation in the amount of the epoxy-mycolic acids;[152] and, significantly, variation in the ratio of *cis/trans* geometry at the proximal position of mycolic acids.[154]

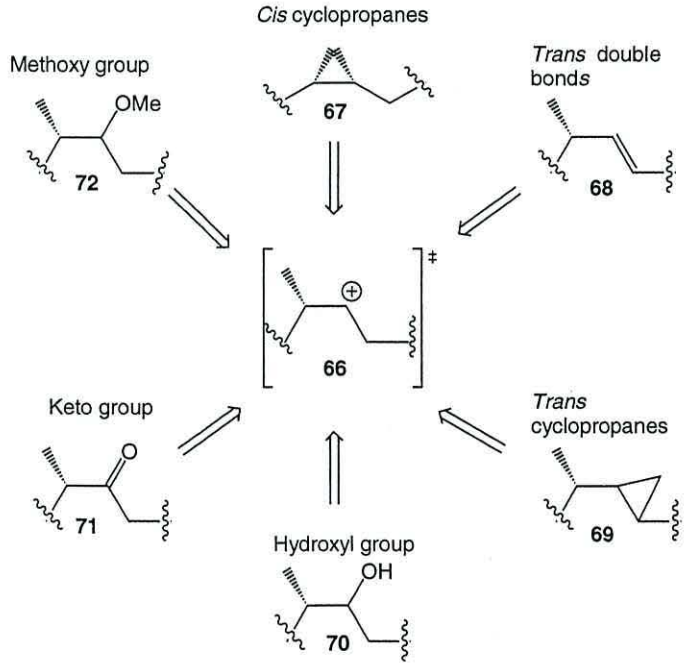
The importance of *trans*-olefins, and *trans*-substituted mycolates in general, for the fluidity and, therefore, permeability of the mycobacterial cell wall has already been illustrated (**Section 1.2.4**).[80]

1.5. Synthesis of mycolic acids

1.5.1 Links between stereochemistry and biosynthesis

The synthesis of mycolic acids presents many challenges to the organic chemist. Besides this, perhaps more esoteric aspect, however, there are also many practical reasons why such research is important.

The synthesis of these compounds will be important for the identification of the exact structures of natural mycolic acids. In particular, the configurations of the different chiral centres present in the meroaldehydes could be revealed through comparison between the natural compounds or their derivatives with the respective synthetic analogues (**Section 1.2.3**). Additionally, through a more accurate analysis of the stereochemistry of the chiral centres present in the meromycolate chain, very important information about the biosynthesis of these compounds might also be acquired.

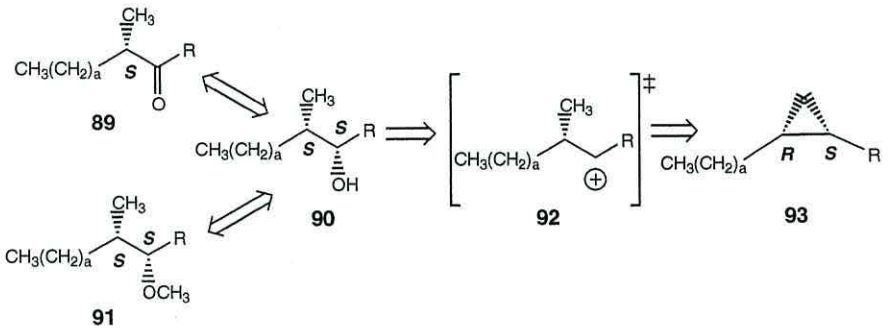


Scheme 19: ***The insertion of the functional groups***

In particular, following the theory firstly proposed by Jaureguiberry *et al.*[208] (**Section 1.3.6**) and considering the similarity of the structure of the active sites of some of the cyclopropane synthases,[206] it seems possible that all these enzymes share the same mechanism of action,[237] and possibly that the C_1 addition to the double bonds would always happen on the same face of the olefin (**Scheme 19**).

Therefore, all enzymes involved in the functionalisation of the meromycolate chain could share identical stereospecificity. It would be interesting to verify this hypothesis by analysing the stereochemistries of the several functional groups present in the mycolate chains. In fact, as a consequence of this assumption, the stereochemistry of at least one carbon in each functional group of every mycolic acid series should always be in the same configuration.

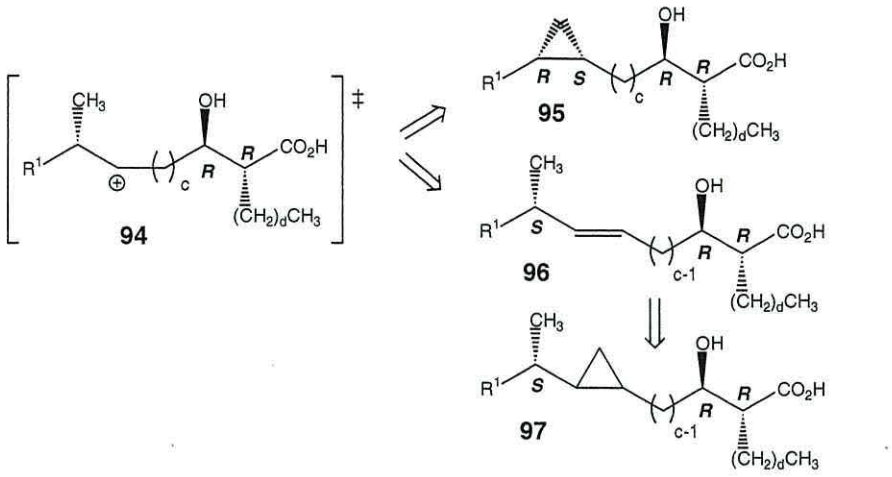
Since it is believed that the methyl branches in the oxygenated series (**89**, **90**, **91**) are in the *S*-configuration,[126,131] the methylene group in the cyclopropanes (**93**) should have the same spatial geometry and should, therefore, be in the *R*-configuration, according to the priority rule for cyclopropanes. Since the cyclopropanes are in a *cis*-configuration, the absolute stereochemistry of the other stereocentre is also fixed, it is *S* (**Scheme 20**).



Scheme 20: ***Absolute stereochemistry of cis-cyclopropanes following the hypothesis***

Following this supposition, therefore, the configuration of all the stereocentres for the functional groups in the distal position with respect to the carboxylic is fixed.

Assuming that this biosynthetic pathway is also followed for the preparation of the cyclopropanes in the proximal position of the carboxylic group, the stereochemistry of some other stereocentres could also be forecast. Firstly, the proximal *cis*-cyclopropanes (**95**) should have the same stereochemistry as the distal one (**93**). Secondly, the methyl branch in the *trans*-cyclopropane (**97**) should be in the *S*-configuration, although no prediction can be made concerning the *trans*-cyclopropane ring itself (**Scheme 21**).



Scheme 21: ***Hypothetical configurations of other chiral centres in the proximal position***

In conclusion, following these suppositions, it is possible to hypothesize the stereochemistry of the majority of the chiral centres in all the mycolic acid series. Clarification of the stereochemistry of these compounds could also be fundamental for understanding their biosynthesis, and this might lead to the identification of a new target for anti-mycobacterial drugs. Inhibitors against cyclopropane fatty acids synthases are being studied as possible anti-tuberculosis drugs.[238]

In particular, if all these processes go through similar carbocation intermediates, it would be interesting to verify if their synthetic analogues, such as the ammonium salt (**98**) or the cyclopropene (**99**) shown in **Figure 23**, could be a competitive inhibitors of these types of enzymes. These types of compound could interact with the bicarbonate ion,[206] and, via the π-cation, with the aromatic residues,[211] both present in the site. Moreover, molecules containing the amino group as a crucial element have already been successfully proposed as inhibitors of processes involving carbocation transition states;[239-241] and cyclopropenes have been proved to be inhibitors of a desaturase enzyme in the mycolic acids biosynthesis.[199]

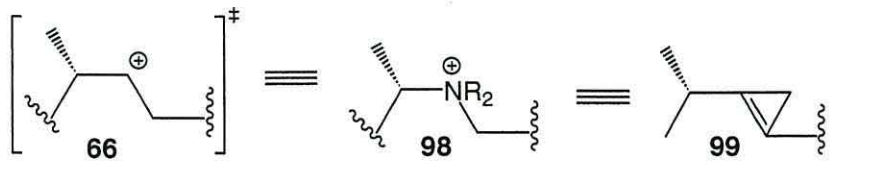


Figure 23: ***Possible inhibitors of the cyclopropane syntheses***

1.5.2 Structural information: its use to determine biosynthetic correlation

Structural information has already been used to determine the existence of biosynthetic correlations between different types of mycolic acids. For example, it has been suggested that keto and methoxy-mycolic acids derive from identical intermediates, the hydroxy-mycolic acids (**Figure 24**).[131] Determining that the methyl branch in both the hydroxy-mycolic acid and in the keto-mycolic acid is in the same *S*-configuration, in association with the presence of a perfect match between the structures of these two species in terms of size, confirmed that the two oxygenated species were biosynthetically related.

Conversely, it has been found that the methyl branch in the α-position with respect either to an epoxide or to a double bond is in the *R*-configuration both in *M. fortuitum* and *M. aurum* (**Figure 24**).[93,132]

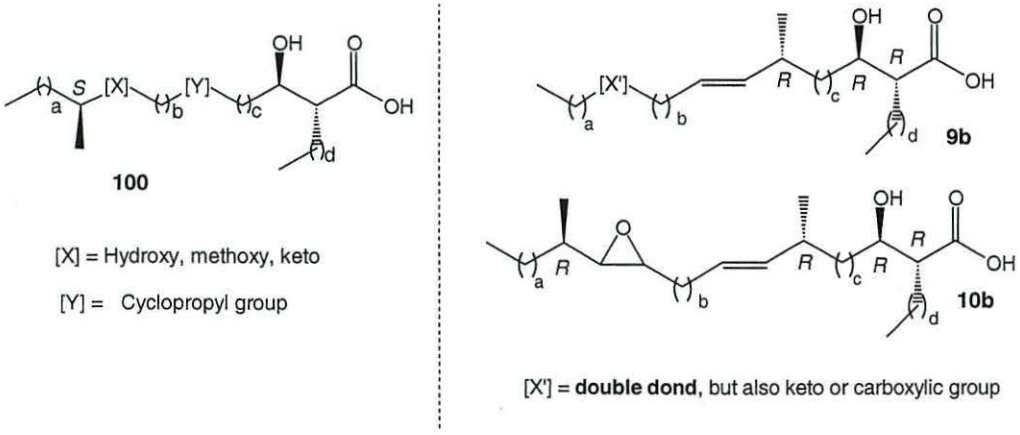


Figure 24: ***Different configurations for some chiral centres of the meromycolate moiety***

Therefore, if these conflicting structural results are both confirmed, the epoxy-mycolic acids should be formed through a different pathway from the keto and methoxy-mycolic acids.

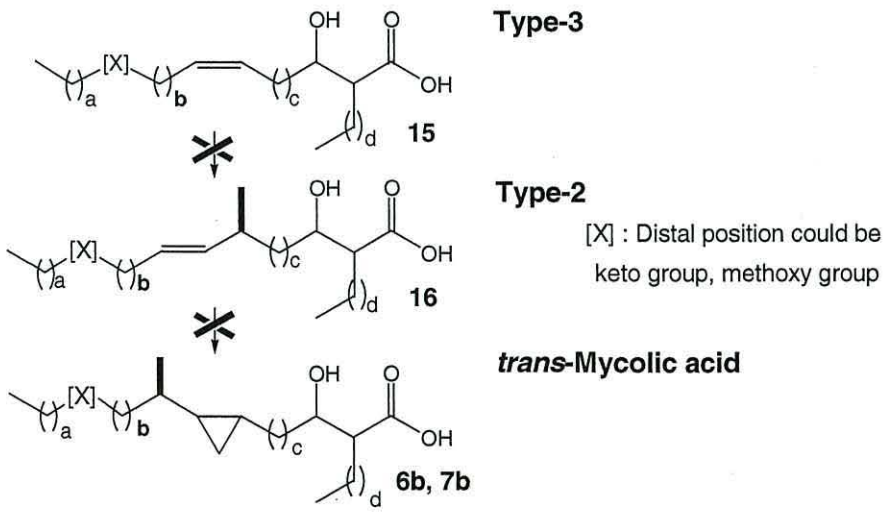
Opposite stereochemistries have also been observed for another kind of mycobacterial lipid among different species of mycobacteria, which might indicate different biosynthetic pathways. The methyl groups of C_{34} tetramethyl branched mycocerosic acid were found to be in the *R*-configuration in *M. kansasii, M. leprae, M. tuberculosis* and *M. bovis* and in the *S*-configuration in *M. marinum* and *M. ulcerans*.[43,242,243]

Structural data has already been used several times to demonstrate the lack of obvious biosynthetic correlations between different kinds of mycolic acids. For

example, Watanabe *et al.* claimed that oxygenated and α-mycolic acids were not closely related through two observations.[102] Firstly, it has been demonstrated that, within the same mycobacteria, α-mycolates and oxygenated mycolates have different total lengths. Moreover, a mycobacterium grown in different conditions, or for different periods of time, produces changes in the mycolate content, but the ratio between the α-meromycolate concentration and the oxygenated mycolate concentration does not vary.[99,102]

Secondly, it is generally believed that the process of desaturation occurs alongside the process of elongation, as illustrated above in **Section 1.3.4**.[200] This theory implies that the positions of the functional groups are defined in the relatively early stages of the biosynthesis, even if their completion might happen in a later stage. Therefore, Watanabe *et al.* concluded that if α-mycolic acids and oxygenated ones share the same precursor, this is only in the early stages of biosynthesis. In support of this, there is also evidence obtained by analysis of the functional group locations in different mycobacteria.[102] In *M. tuberculosis,* and in other pathogenic mycobacteria, a close structural relationship has been observed between the keto and the methoxy-mycolic acids, but not between the α-mycolic acids and the oxygenated ones (**Section 1.2.2**).

The structural data of Watanabe *et al.* showed also that *trans*-double bonded compounds (**Type 2**: **15**) are not closely related either to *cis*-double bonded compounds (**Type 3**: **16**, their putative precursors) or *trans*-cyclopropane-mycolic acids (**6b**, **7b**, their theoretical derivatives, **Scheme 22**).[99,102]



Scheme 22: ***The lack of correlation between different types of mycolic aicids***

Firstly, the position of the methyl group with respect to the functional group is different between **Type-2** mycolic acids (**15**) and *trans*-mycolic acids (**6b**, **7b**). Secondly, they observed the same pattern described by Gray *et al.* between *cis*-(**9a**) and *trans*-α-mycolic acids (**9b**) in *M smegmatis* (**Scheme 17, Section 1.4.2**).[94] Following the hypothesised biosynthetic pathway, the value of the internal chain **b** should diminish by 1 carbon unit in the conversion from **Type-3** (**16**) to **Type-2** (**15**), whilst an increase of one carbon unit was observed. In methoxy-mycolic acids of *M. tuberculosis, M. bovis,* and *M. bovis* BCG, the major component of **Type-3** (**16**) has **b** = **18**, and **Type-2** (**15**) has **b** = **19**, and in keto-mycolic acids of *M. tuberculosis* and *M. bovis* BCG: **Type-3** (**16**) has **b** = **18** and for **Type-2** (**15**) has **b** = **19**.

All these observations suggest the possibility of different pathways for the formation of different classes of mycolic acids. They also illustrate how structural information can be useful, if not conclusive, for a better understanding of the biosynthesis of different kinds of mycobacteria. Therefore, the preparation of synthetic analogues of the major types of mycolic acids, and their subsequent comparison with the natural occurring compounds could help to clarify the structure of the latter but, indirectly, also their biosynthesis.

1.5.3 Other reasons for preparing synthetic mycolic acids

Another reason to produce synthetic mycolic acids is that antibodies produced against different synthetic mycolic acids which have similar but not equivalent structures, could be utilized for the identification of the structure and stereochemistry of the mycolic acids in mycobacteria. If only one type of these particular antibodies recognized the mycobacterium, the synthetic mycolic acid used as the antigen to produce these competitive antibodies, should have the same structure as the natural one. The use of this type of analysis has already been utilized for a partial characterization of the structure of cord factors and their mycolic acids.[3,129]

It has also been shown that the antibodies produced by animals infected with TB were able to distinguish between different types of mycolic acids (**Section 1.2.4**).[63,64] Experiments with T cells gave more complex results, however, regarding recognition of the fine structure of the lipid moiety in cord factor.[129,244-246] In particular, in a recent paper, Grant *et al.*[245] suggested a possible reason for

the stronger recognition by T cell receptors of oxygenated mycolic acids, as opposed to α-ones. They suggested that keto and methoxy-mycolic acids fold in a way that allows the three polar functions of the lipid chain to be in proximity and to form a combinatorial epitope, which is well recognized by this kind of receptor (**Figure 25**).

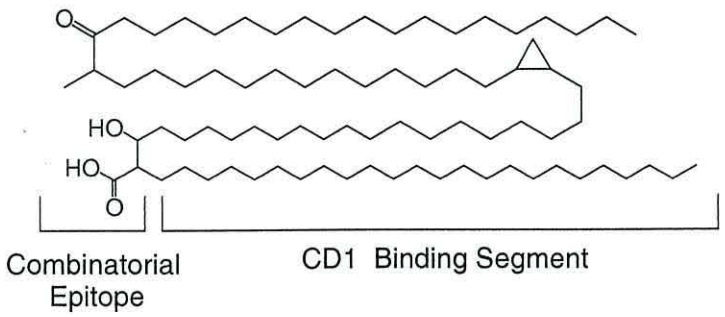


Figure 25: ***A possible folded conformation for keto-mycolic acid***

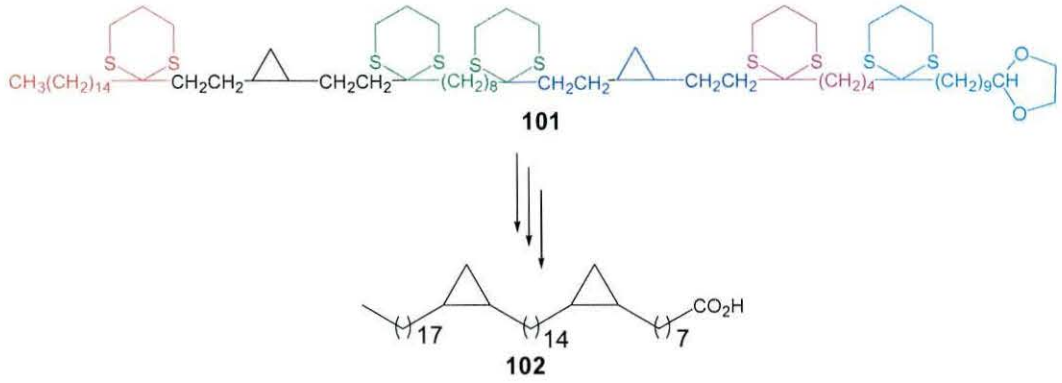
Hasegawa *et al*[161,162,164] also proposed that mycolic acids could exist in folded conformations, although this was in a completely different context, namely, when they are arranged in monolayer films. These conformations are thought to be particularly stable in keto-mycolic acids due to strong molecular interactive forces. In fact, it has been suggested that these lipids exist in a folded form even when high surface pressure is applied to the monolayer (**Scheme 5, Section 1.2.4**).

Finally, synthetic mycolic acids may be utilised for the preparation of a simple model of the multi-layer structure present in the *M. tuberculosis* cell wall. This method has already been used to determine the relationships between monolayer properties and the chemical structures of different natural types of mycolic acids (**Section 1.2.4**).[162] The functional groups in the meromycolate moiety, and the length of the α-unit, have been recognized as significantly influencing the physical properties of monolayer films of mycolic acids. The preparation of different synthetic analogues could help in the determination of the role of each particular feature of the acids in the regulation mechanisms of the drug permeability of the cell envelope.

1.5.4 Previous syntheses of mycolic acids

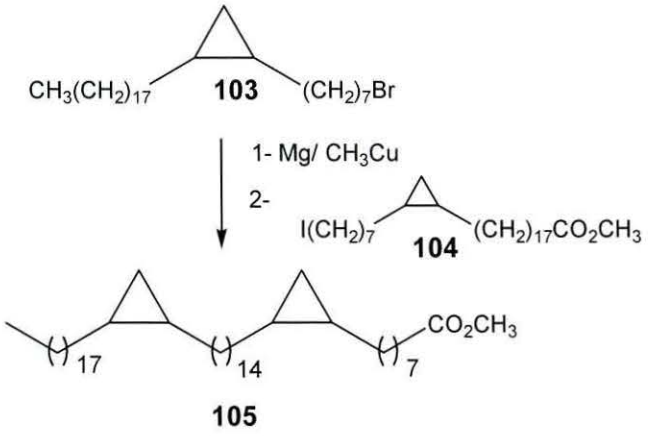
In the synthesis of mycolic acids, one problem that has to be attended to is the synthesis of its major fragment, meromycolic acid (**102**).

Gensler *et al.*[247] described a synthesis of a mixture of four stereoisomers of meromycolic acid (**102**) containing two *cis*-cyclopropanes. The coupling of different portions, indicated by different colours in **Scheme 23**, gives a structure (**101**), which has the same carbon skeleton as meromycolic acid. Finally, this is transformed into the desired product (**102**) by desulfurization and ozonolysis. The introduction of the chains at different stages provides a general method for the synthesis of this acid.



Scheme 23: ***The first Gensler approach***

Subsequently, Gensler *et al.*[248] demonstrated another method where the methyl meromycolate (**105**) is also prepared combining different portions (**Scheme 24**). This method is shorter and it could be more easily scaled up.



Scheme 24: ***The second Gensler et al. approach***

In this case the key intermediate is a Grignard reagent, which is obtained from the alkyl bromide (**103**). The subsequent reaction of it with the alkyl halide (**104**) yielded the desired product (**105**) directly. This method is an improvement over the first approach but still presents some problems. Firstly, the final coupling gives a very poor yield. In fact, the Grignard reagent not only gives the desired product (**105**) but also several others, as seen through high-resolution mass spectroscopy, and the required one is only a small part of the mixture (**Figure 26**). These results are caused by the limited solubility of the reactants (**103** and **104**). The resulting heterogeneity reduces the efficiency of the reaction because the two compounds are not able to interact. Furthermore, Gensler's method does not have any control on the stereochemistry of chiral centres of the cyclopropyl groups.

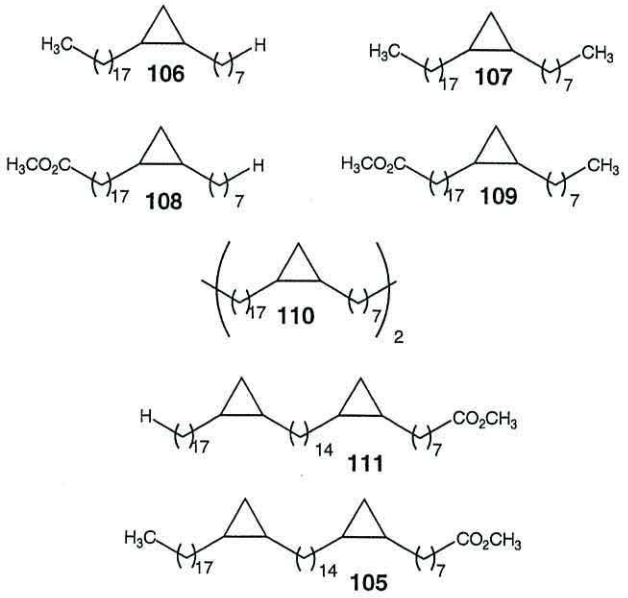
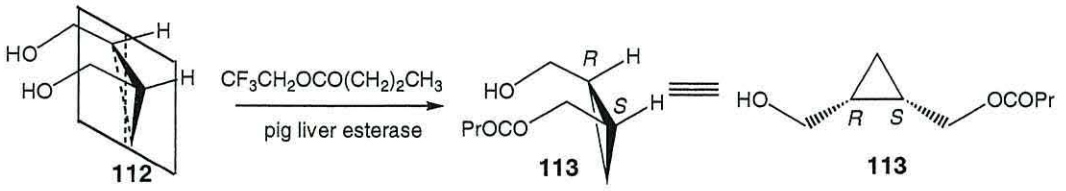


Figure 26: ***Other products***

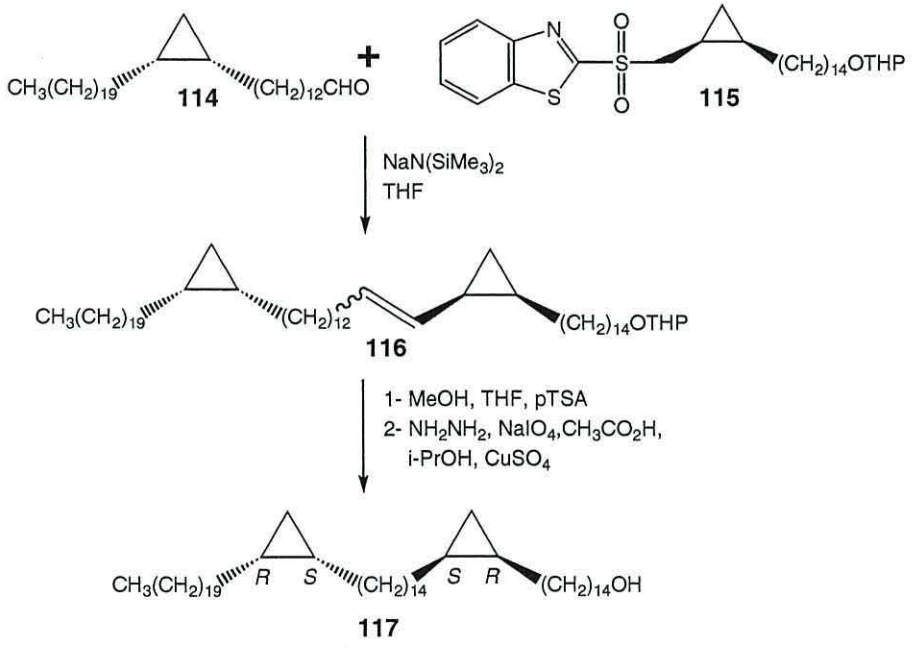
Recently, another approach for the synthesis of a single enantiomer of an analogue of meromycolic acid (**102**) was reported by Al Dulayymi *et al.*[249] There are two key steps in this method: firstly, the preparation of single enantiomers of cyclopropane building bocks followed by the coupling of these units.

Different methods have been used for the preparation of the enantiomerically pure cyclopropanes. For this purpose, the group used an enzyme, *pig liver esterase*, which can catalyse the esterification of only one of the two possible hydroxyl groups on the *meso*-compound (**112**) yielding only one enantiomerically pure compound (**113, Scheme 25**).[250,251]



Scheme 25: ***Enzyme process***

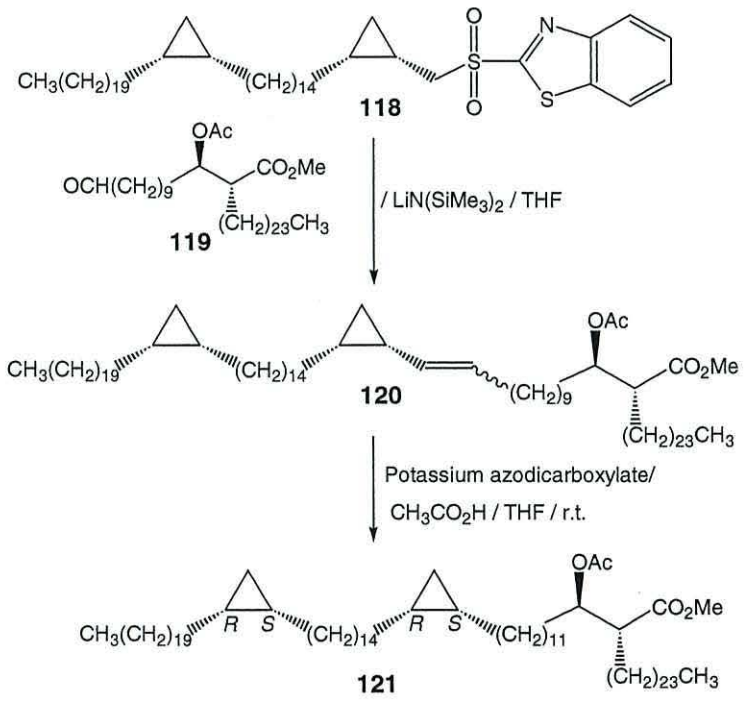
Other important features of this approach are the coupling reactions, which are used to link the different units in several stages, securing the final desired stereochemistry. For example, the condensation by a Julia reaction between (**114**) and (**115**), gives a mixture of *E* and *Z*-alkenes (**116**). The subsequent deprotection of the alcohol group and the reduction of the derived compound with di-imide, prepared *in situ* by reaction between hydrazine, sodium periodate and acetic acid,[252] gives only 14-{(1*R*,2*S*)-2-[13-((1*S*,2*R*)-2-icosylcyclopropyl)tridecyl]-cyclopropyl}-tetradecan-1-ol (**117**, **Scheme 26**).



Scheme 26*: Al Dulayymi et al. approach*

This method offers important advantages: a better overall yield and the control of the absolute stereochemistry of both the cyclopropanes. This group prepared a single enantiomer of the cyclopropane ring; its absolute stereochemistry is not modified during the course of the synthesis. This route could be easily adapted for the synthesis of other homologous meroaldehydes because the different portions

of the compound are incorporated at different stages of the synthesis. It also allows preparation of the other stereoisomers by simple modification in the sequence of reactions, which controls the position of the substituents on the cyclopropyl groups. Therefore, it is particularly useful in order to obtain all the possible diastereoisomers of meroaldehydes. Following this approach, the first entire enantiomerically pure mycolic acid has also been prepared.[253] A key step in this preparation is, once again, a Julia reaction between an analogue of meromycolic acid (**118**) with the aldehyde (**119**) yielding the desired compound (**121**, **Scheme 27**).



Scheme 27: ***An approach to the synthesis of the α-mycolic acid***

The final protected compound shows basically identical ^{1}H and ^{13}C NMR spectra to those of a mixture of α-mycolic acids extracted from *M. tuberculosis* and then protected. Moreover, the synthetic compound, which has the same configuration at the cyclopropyl-chiral centres as those hypothesised from the Jaureguiberry *et al.* [208] theory (**Section 1.5.1**) has a similar optical rotation to the sample extracted from *M. tuberculosis*. These data still do not allow the identification of the absolute configuration of the stereogenic carbon atoms in the mycolic acids, especially of those in meromycolate chains with certainty. In fact, the optical rotation probably would not show a significant change after the modification of

the stereochemistries of the cyclopropyl rings. More tests to compare the natural and synthetic samples are needed.

An important milestone for this approach remains the synthesis of molecule (**122**, **Figure 27**), which is an analogue of the corynomycolic acid (**21**). The corynomycolate analogue (**122**) possesses a long chain substituted with an alkyl group at the α-position and a hydroxy group at the β-position. Both α and β–centres are in the *R*-configuration.

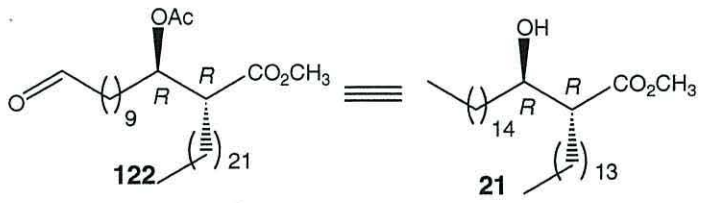
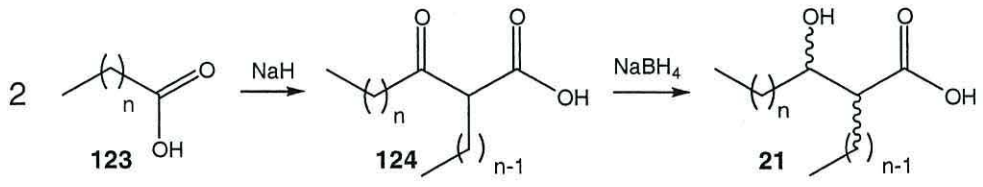


Figure 27: ***The corinomycolic acid analogue***

1.5.5 Previous syntheses of corynomycolate analogues

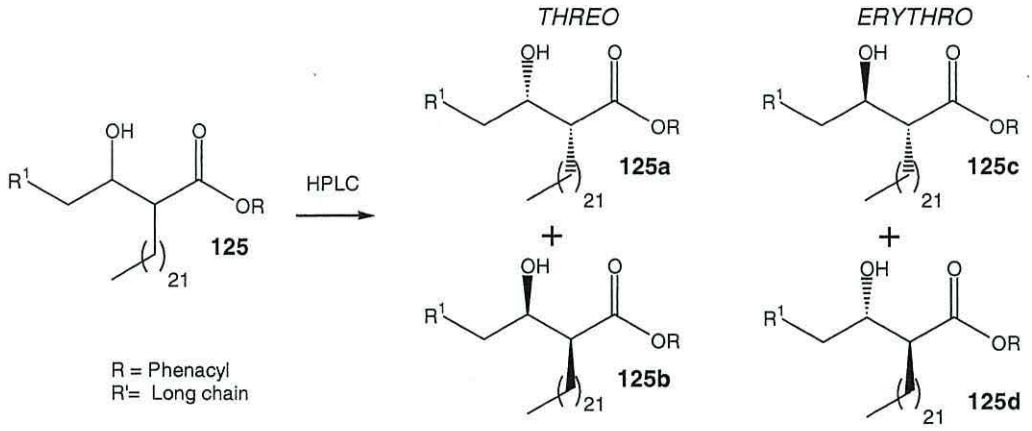
Corynomycolate analogues (**122**) or less specifically, 2-alkyl-3-hydroxycarbonyl units (**21**) have been widely studied and different methods for their preparation have been attempted. One of the first syntheses for this kind of compound was reported by Lederer *et al.* in 1952.[254] This group prepared several corynomycolate homologues by condensing two molecules of fatty acids (**123**) in the presence of sodium hydride, yielding β-keto esters (**124**), which were then reduced to give the desired compounds (**21**, **Scheme 28**). The reaction, however, was not stereoselective and mixtures of all possible four diastereoisomers were obtained.



Scheme 28: ***Method based on a Claisen condensation***

The same group, followed by others, using similar methods based on the Claisen condensation, prepared other corynomycolate analogues, but still as mixtures of diastereoisomers.[255,256]

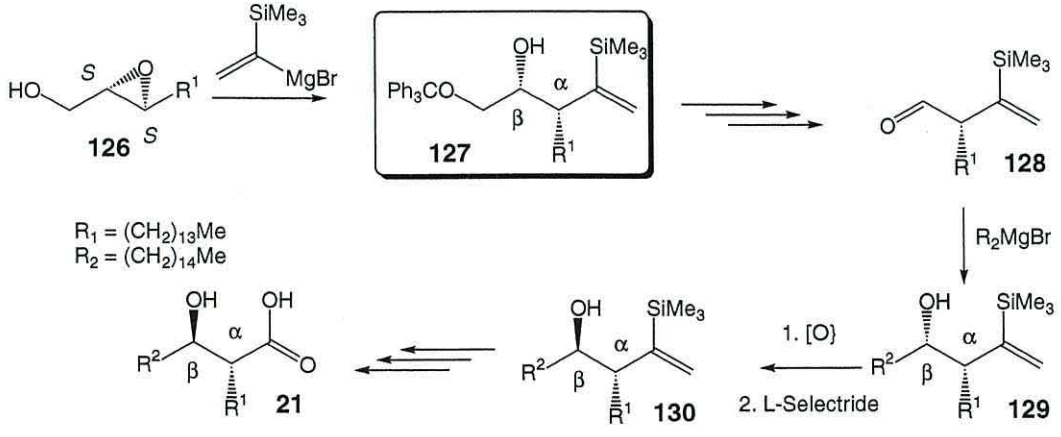
Gray's group, then described a method for separating *erythro*-phenyl-acyl esters (**125a, 125b**) and *threo* (**125c, 125d**) by HPLC (**Scheme 29**).[5] The two classes of diastereoisomers, showed different chemical physical properties, manifested through IR and ^{1}H NMR spectra. Minnikin and Polgar suggested that this was due to the fact that the *erythro*-compounds formed a much more stable six-membered intramolecularly hydrogen-bonded ring.[89]



Scheme 29: ***Separation of the four stereoisomers***

Subsequently, Utaka *et al.* reported a regioselective synthesis of the *erythro*-diastereoisomers (**125c,125d**) but still only as a racemic mixture of the two possible enantiomers.[257]

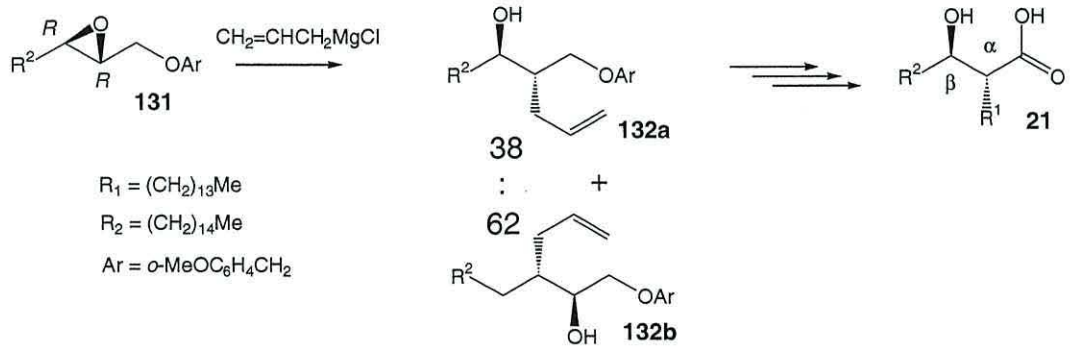
The first synthesis of an enantiomerically pure corynomycolate was described by Kitano *et al.* in 1985.[258,259] The key feature was the preparation of the optically active compound (**127**) with the correct stereochemistry in the α-position (**Scheme 30**).



Scheme 30: ***The Kitano et al. method***

This was achieved by a stereoselective ring opening of the chiral epoxide (**126**) with 1-trimethylsilylvinylmagnesium bromide. The silyl compound (**127**) was converted into the aldehyde (**128**), and then, by Grignard reaction, into the α-alkyl-β-hydroxyl derivative (**129**). Unfortunately, this molecule (**129**) had the two chiral centres in the α and β-positions in the *threo*-configuration, and not in the required *erythro*. It was, therefore, necessary to oxidise it and then stereoselectively reduce it with L-selectride to obtain the correct stereochemistry at the β-position. This α-alkyl-β-hydroxyl compound (**130**) could be then transformed in a few steps into a (+)-corynomycolate (**21**), which showed the same chemical and physical properties as the natural compound.

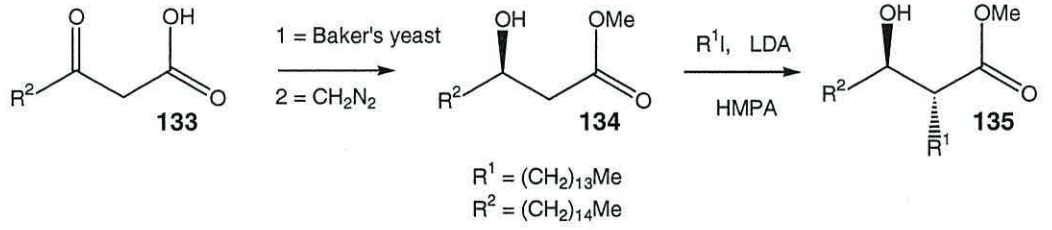
A comparable approach was proposed by Nishizawa *et al.*[260] The key step in this preparation was again a stereoselective ring opening of a chiral epoxide (**131**) but with a different kind of Grignard-reagent (**Scheme 31**). This time the reaction gave, contrarily to the previous one, the necessary *erythro*-diastereoisomer (**132a**). The small chain in the α-position was then extended in few steps to give the desired compound.



Scheme 31: ***The Nishazawa et al. approach***

Another advantage of this method was that, through simple modifications, it was possible to obtain all four possible diastereoisomers, enantiomerically pure. However, this approach still presented a drawback: the ring opening of the epoxide produced two regioisomers (**132a**, **132b**), where the one required for the synthesis was the minor one (**132a**). Moreover, their separation was achieved only through HPLC, a technique which reduces the possibility of an eventual scale up of the reaction.

Utaka *et al.*[261] described a completely different approach, in which initially there was the introduction of the hydroxyl group at the β-position by a stereoselective reduction of the β-keto ester (**133**) with Baker's yeast (**Scheme 32**).[262] The second key step of this approach is then a Fräter reaction, where the hydroxyl group in the molecule (**134**) forced the insertion of the alkyl chain with the correct configuration.[9] This method required fewer steps than the previous ones, since, through the Fräter addition, the α-alkyl-β–hydroxyl carboxilates (**135**) was directly obtained with the correct chain length and with the two chiral centres in the correct configuration.



Scheme 32: ***The Utaka et al. method***

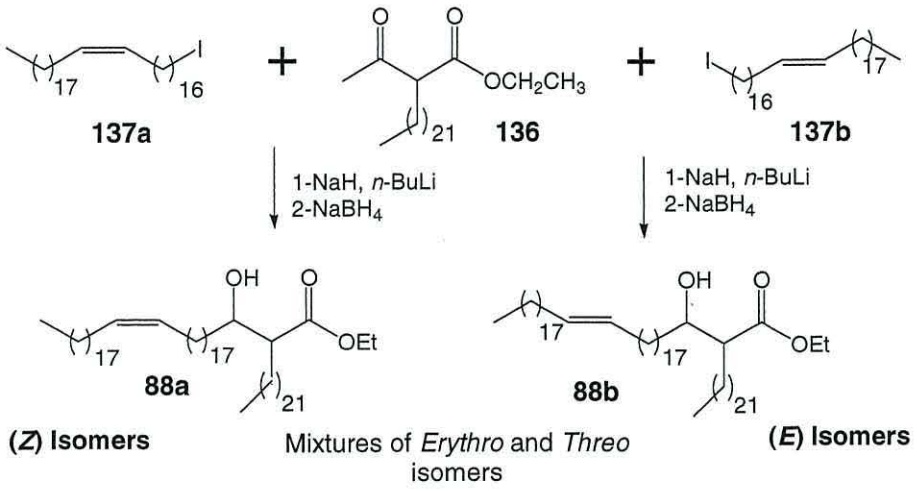
A similar approach has been used more recently by Fujisawa *et al.*[263] and by Ratovelomanana *et al.*[264] Their preparations still use the Fräter reaction for the introduction of the alkyl chain, but the previous, stereoselective reduction of the keto group was achieved using alternative methods, which were both more complex and expensive.

Another method for the introduction of this hydroxyl group into the β-position with the required chirality has been recently reported by Al Dulayymi *et al.*[253] This new method will be described in the following results and discussion chapters.

1.5.6 A previous synthesis of α^1-mycolic acids from *M. smegmatis*

Huang *et al.* synthesised the most common example of α^1-mycolic acid (**88**) of *M. smegmatis*, in order to clarify the structure of the naturally material (**Scheme 33**).[5] They prepared both *trans* (**88a**) and *cis*-(**88b**) isomers alkylating ethyl 2-docosyl-3-oxobutyrate (**136**) with either *trans*-1-iodo-17-hexatriacotene (**137a**) or *cis*-1-iodo-17-hexatracotene (**137b**) and subsequent reduction (**Scheme 33**). They did not synthesize a mixture of all the possible four. They succeeded in separating the

erythro-phenacyl ester derivatives from the *threo*-ones by HPLC. Their ^{1}H NMR analysis confirmed that *erythro*-isomers were the naturally occurring ones.[5]



Scheme 33: ***Synthesis of monoalkene mycolic acids using Gray et al. method***

The subsequent examination by ^{1}H NMR spectroscopy of the epoxide derivatives (**138a**, **938b**, **Figure 28**) of naturally occurring mycolic acids established that these were a mixture of *cis:trans* isomers (93:7). This suggested that the α^1-mycolic acids are probably present in the same ratio in *M. smegmatis*.

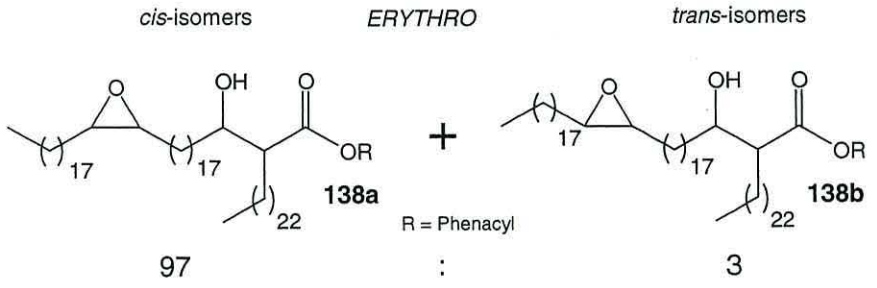


Figure 28: ***Proportion between the cis and trans- stereoisomers***

The synthesis of a single enantiomerically pure stereoisomer for this α^1-mycolic acid has not been reported; overall, only one type of mycolic acid has been synthesized in an enantiomerically pure form.[253] The scope of this chapter was to describe how their preparation is not only challenging from a chemical point of view, but also fundamental for a better understanding of the chemical structures of these acids, their properties and roles in the cell walls of the mycobacteria, and, therefore, in the pathogenesis of tuberculosis and related diseases.

2. Formation of the meromycolate moiety

2.1 The general approach

2.1.1 Aim and overview.

The target molecule of the project is an α^1-mycolic acid (**88**, **Figure 1**) which is one of the major constituent mycolic acids of *Mycobacterium smegmatis* (**Section 1.4.2**).[265]

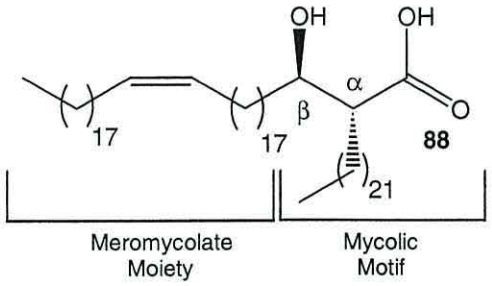


Figure 29: ***The target α^1-mycolic acid***

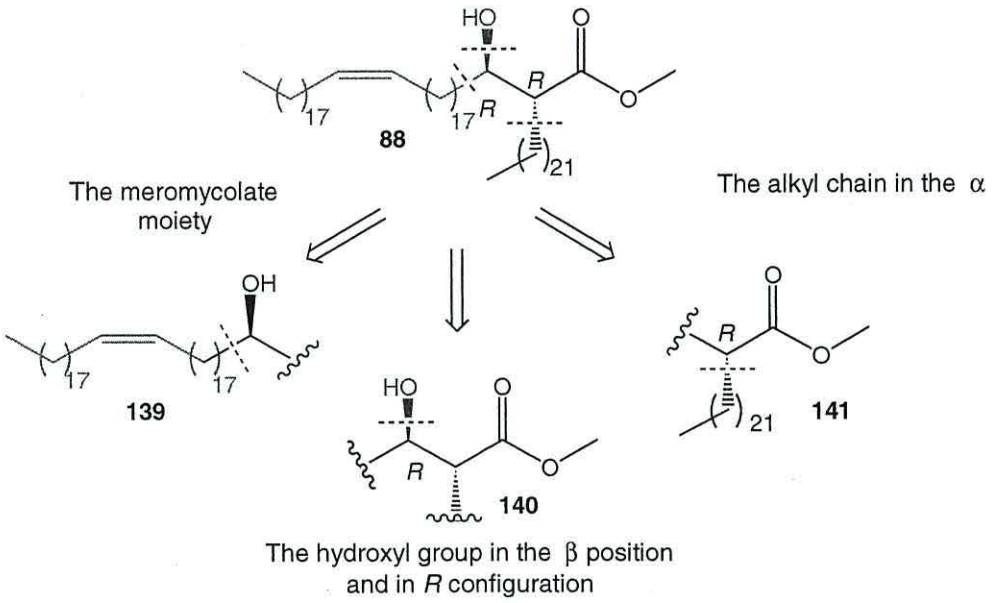
It was decided to synthesise this compound because it includes the typical structure of all mycolic acids, having a α-branched, β-hydroxy fatty acid moiety similar to that of other mycolic acids. However, it has a relatively simple meromycolate moiety, which still contains a very long chain but does not contain many functional groups such as cyclopropanes, methyl branches or methoxy- or keto-groups, which are present in other, more complex mycolic acids (**Section 1.2.1**). Therefore, it represents an ideal model for any other mycolic acids, since its synthesis permits an examination of the general difficulties encountered in the preparation of such molecules, without confronting the particular problems specific to other kinds of mycolic acid.

Moreover, *M. smegmatis*, being very different from pathogenic mycobacteria such as *M. tuberculosis*, is often used for comparison to examine similarities and differences in behaviour among the *Mycobacterium* genus, for example, regarding the biosynthesis of mycolic acids (**Section 1.3**). Therefore, the synthetic fatty acid (**88**) could be useful as a "control system" in the determination of the physical, chemical and biological properties of the different kinds of mycolic acids (**Section 1.5.3**), especially because of its simpler structure.

This α^1-mycolic acid (**88**, **Figure 29**) has already been synthesized but not enantiomerically pure (**Section 1.5.6**). The aim of this project was to analyse the

difficulties encountered in its preparation in order to find out the best solutions for the synthesis of any mycolic acids.

The preparation of this particular mycolic acid (**88**, **Figure 1**) could be achieved following any one of a large number of different methods based on the work already reported in the literature (**Section 1.5.3** and **Section 1.5.4**). In all retrosyntheses examined, the same three key problems were always found (**Scheme 34**): the formation of the long chain of the meromycolate moiety (**139**); the insertion of the β-hydroxyl group (**140**); and the introduction of the long alkyl chain in the α-position (**141**).



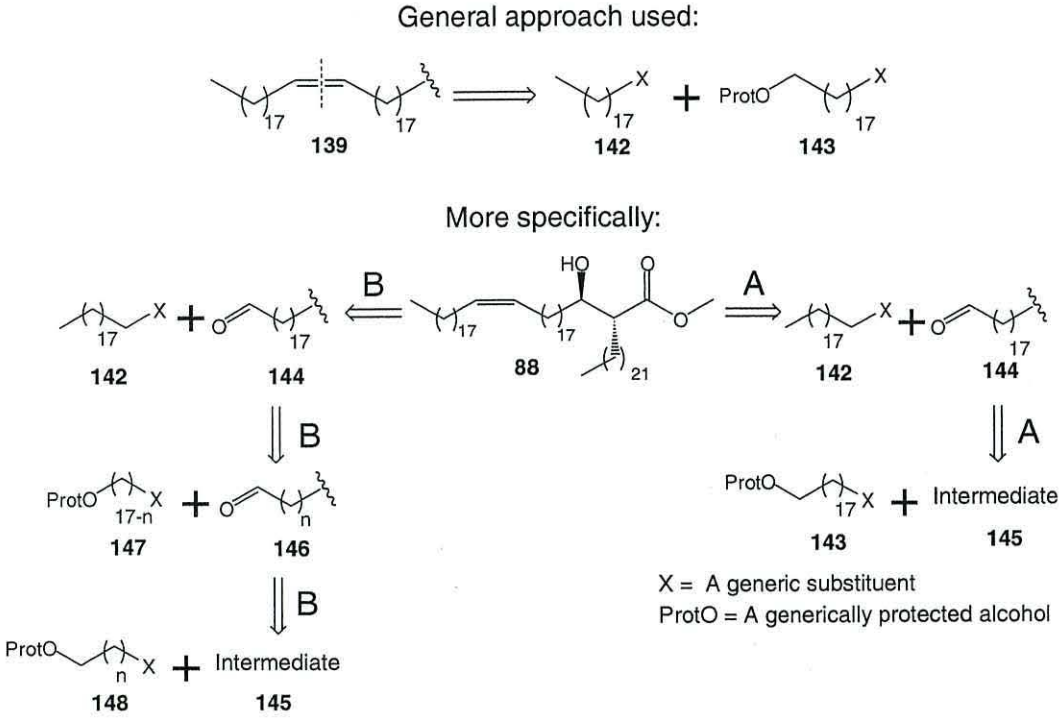
Scheme 34: ***The three key dilemmas***

In the following chapters, some of the possible approaches and problems encountered will be described, comparing the advantages and disadvantages of each of the methods utilised. The sequence for the incorporation of the different moieties was also discovered to be crucial, especially in regard to the formation of the meromycolate moiety (**139**).

The order in which the various experiments will be described below does not necessarily follow the chronological sequence in which they were attempted, as this meant switching from one to another, not always related, objective and revisiting the same problems in different circumstances, in the light of experience. The order of description has been chosen to highlight the general strategies in the synthesis.

2.1.2 The meromycolate chain.

The only functional group present in the meromycolate moiety (**139**) is a double bond in the (*Z*)-configuration. Analysing the retrosynthesis of this particular fraction of the molecule (**139**), a reasonable disconnection is at the double bond. It produces two chains, the first with only one functional group (**142**), the second with both of the ends functionalised (**143**, **Scheme 35**). More specifically, this approach includes the preparation of the aldehyde (**144**), which could be obtained though the coupling between the long chain (**143**) and a precursor of the corinomycolate moiety (**145**, **Scheme 35**, **Method A**).

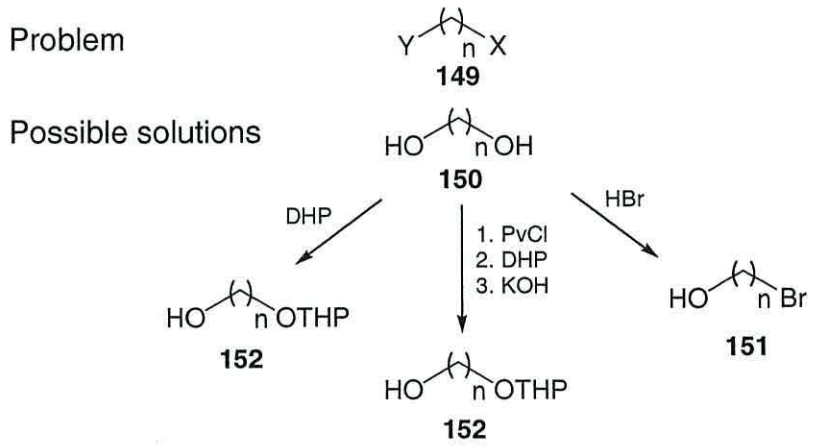


Scheme 35: ***Two possible approaches to the meromycolate moiety***

However, progressing through the synthesis, it was observed that this choice created some problems in particular reactions, where the solubility of the reagents was remarkably important (**Section 3.4.3** and **Section 4.4.3**). Another approach was considered. It involved the initial introduction of a smaller chain (**146**), which was subsequently extended to give the final compound (**144**, **Scheme 35**, **Method B**).

The best methods for the preparation chain (**143**) had to be also applicable for its completion at a later point of the synthesis, when other functional groups had already been introduced.

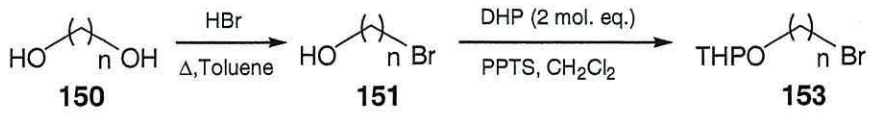
A number of methods for the formation of these long chains (**142**, **143**) were analysed. Several chains were prepared, differing both in the number of carbon units, and in the functional groups at their extremities, according to the type of reactions planned for the following steps. All the methods used began from similar starting materials, simple bifunctional diols (**150**, **Scheme 36**). It was decided to use this type of compounds since they could be bought inexpensively in a variety of different chains length, they can be easily regioselectively modified and other kinds of bifunctional chain (**149**) are not so easily accessible from commercial sources.



Scheme 36: ***The use of diols as starting materials***

The first problem encountered was the selective transformation of only one of the hydroxyl groups. The standard method used started with a bromination with HBr, in refluxing toluene, except for 1,5-pentanediol (**150d**) whose conversion gave better results in hexane. The bromo-alcohol (**151**) was then protected with 3,4-dihydro-2*H*-pyran (2 mol. eq.) with PPTS as the catalyst (**Table** 1).

The preparations of these compounds, and several others referred to in this thesis, have already been described in the literature, and therefore are only briefly mentioned. However, their overall yield was important for future comparisons between the different synthetic strategies.



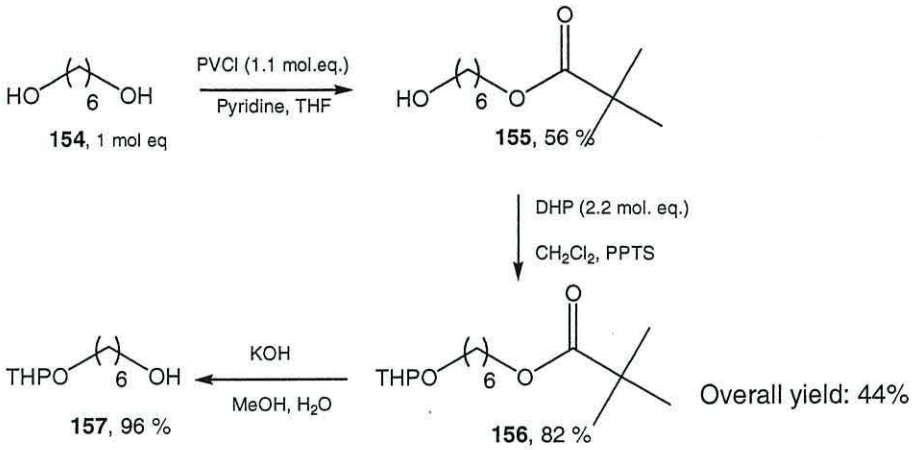
Starting Material	**150a** n = 8	**150b** n = 9	**150c** n = 12	**150d** n = 5
Bromination (yield)	**151a** 81 %	**151b** 86 %	**151c** 82 %	**151d** 42 % (toluene) 55 % (hexane)
Protection (yield)	**153a** 100 %		**153c** 94 %	**153d** 96 %
Overall (yield)	81 %		77 %	53 %

Table 1: ***Different tetrahydropyranyl derivatives prepared***

Another approach for the differentiation between the two extremities was the selective protection of only one of them. In particular, for the needs of the synthesis it was necessary to prepare tetrahydropyranyl-monoprotected alcohols (**152**, **Scheme 36**). This kind of compound could be obtained following two routes. One method included three reactions, the first of these being the protection of the diol (**154**, **Scheme 37**, **Method A**) with trimethylacetyl chloride (1.1 mol. eq.) in the presence of pyridine (1.8 mol. eq.). This protecting group was chosen since, in the literature, it was reported to be capable of discriminating between two primary alcohols having different steric environments.[266] The reaction gave a 56 % yield for the desired compound (**155**), which was then protected at the other end with 3,4-dihydro-2*H*-pyran using similar conditions to those above described. Finally, the ester group was hydrolysed in basic conditions to give an overall yield of 44 % for the desired product (**157**).

A second more direct method was attempted for 1,10-decandiol (**158**), which was immediately protected with just 1 mol. eq. of 3,4-dihydro-2*H*-pyran (**Scheme 37**, **Method B**). This approach has been reported in the literature but with contrasting results.[267,268] The use of THF as co-solvent, together with CH_2Cl_2 (1:10) proved to be crucial, since it improved the solubility of the starting material, favouring its protection, and provided a 42 % yield of the monoprotected compound (**159**) in one step.

Method A:



Method B:

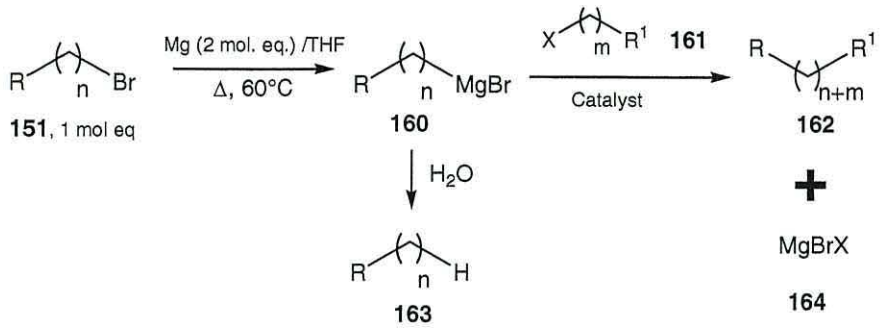
Scheme 37: ***The preparation of monoprotected alcohols***

In conclusion, bromination seemed to be the best method for differentiating between the two hydroxyl groups. However, if the bromine is not required in a later synthetic step, the direct use of dihydropyran could be a good alternative, which produced the desired compound in only one step and with a reasonable yield.

2.2. A direct approach to saturated long chains: The Grignard reaction

2.2.1 Overview

A direct approach for the formation of long chains starting from the compounds described above is based on the use of Grignard reagents (**Scheme 38**).[269]



Scheme 38: ***The Grignard reaction***

The reaction was initiated by warming the mixture, and this was kept at 60 °C for at least 2 h.[270] Quenching a small portion with water and analysing the products by GC permitted the monitoring of the formation of the organomagnesium compounds (**160**). It was possible to observe the development of a new peak, corresponding to the reduced starting material (**163**), which was formed from the Grignard reagent (**160**) and water. After completion of the reaction, the organomagnesium mixture was promptly added to a solution of the alkyl halide (**162**) in order to avoid its eventual decomposition.

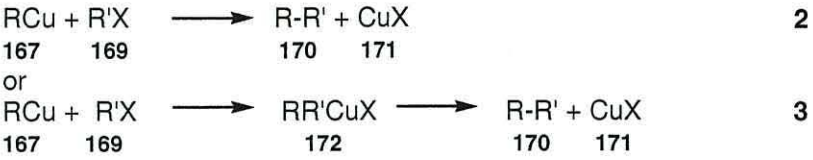
2.2.2. The use of a copper salt as catalysts

In order to obtain the above coupling in good yields the use of certain catalysts was required. Among these, Cu(I) salts (**166**) were firstly considered; specifically, the Tamura and Kochi's catalyst Li_2CuCl_4.[271] These two scientists thoroughly studied Cu(I) catalysts (**166**) and proposed a mechanism for their action (**Scheme 39**). From this it is evident that Cu(I) salts (**166**) catalyse the formation of alkylcopper species (**167**) *in situ*, which then act as an active intermediate in the coupling process.[272]

This group also showed that the presence of lithium chloride in THF enhances the thermal stability of these alkylcopper species (**167**), which otherwise would undergo disproportionation. Therefore, they suggested that low temperature and THF guaranteed the optimal conditions with this catalyst.

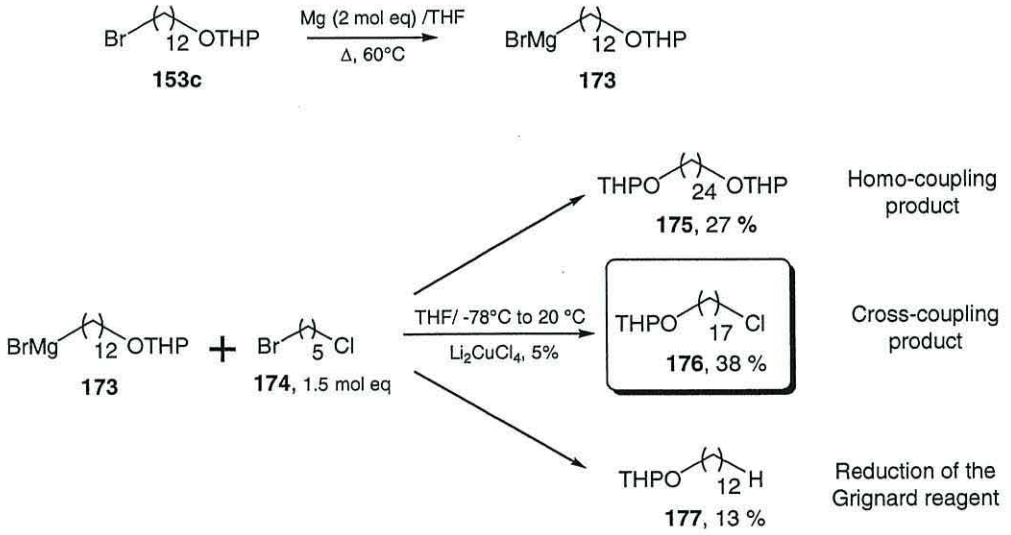
Formation of the organocupper compound

Possible mechanisms for the cross-coupling



Scheme 39: ***The possible mechanisms of the Cu(I) catalysts***

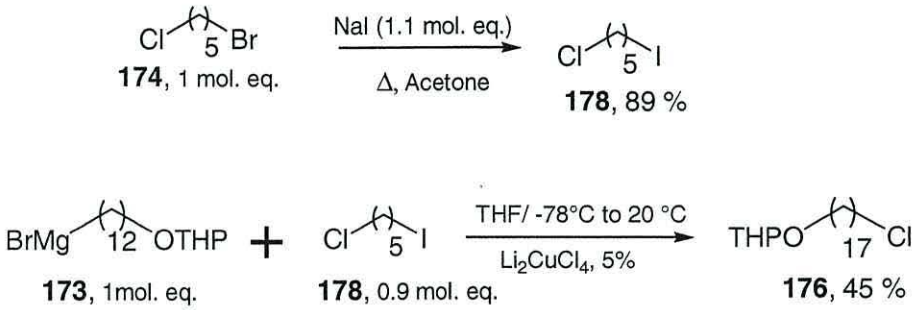
The first reaction tested was between the organomagnesium compound (**173**) obtained from 2-(12-bromododecyloxy)tetrahydropyran (**153c**, 1.0 mol. eq.) and 1-bromo-5-chloropentane (**174**, 1.5 mol. eq.), in the presence of Li_2CuCl_4 (5 mol %, **Scheme 40**). The required product 2-(17-chloroheptadecyloxy)tetrahydropyran (**176**, 38 %) was produced, along with the homo-coupling product (**175**, 27 %) and the reduced starting material (**177**, 17 %). Other by-products were never identified, but their formation cannot be excluded, since total recovery was never obtained.



Scheme 40: ***Experiments with Li_2CuCl_4 catalysts***

The reaction was repeated several times changing the conditions. The best result was obtained using Li_2CuCl_4 (5 mol %) and 1-chloro-5-iodopentane (**178**, 0.9 mol. eq.), giving the desired product (**176**) with a 45 % yield, in respect to the iodide compound (**178**, **Scheme 41**).

The alkyl iodide was previously prepared from the respective bromo-compound (**174**) by reaction with sodium iodide (1.1 mol. eq.) in acetone. The use of this derivative favoured the desired cross-coupling, since iodine is a better leaving group than bromine (**Scheme 41**).



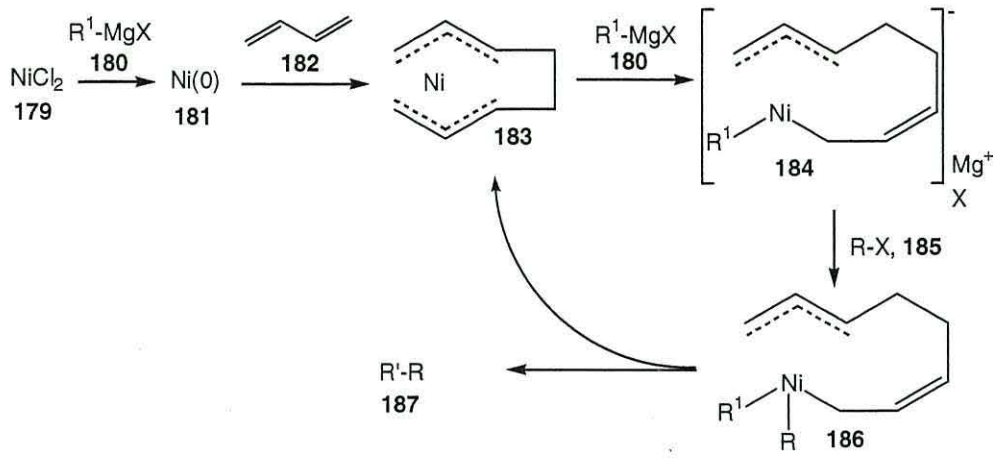
Scheme 41: ***The use of the alkyl iodide***

The yield was still rather low; a probable reason for this was the low solubility of the specific organomagnesium compound (**173**), and possibly its low reactivity with the type of catalyst employed.

It was decided to experiment the coupling with a different kind of catalyst.

2.2.3. The use of Nickel salts as catalysts

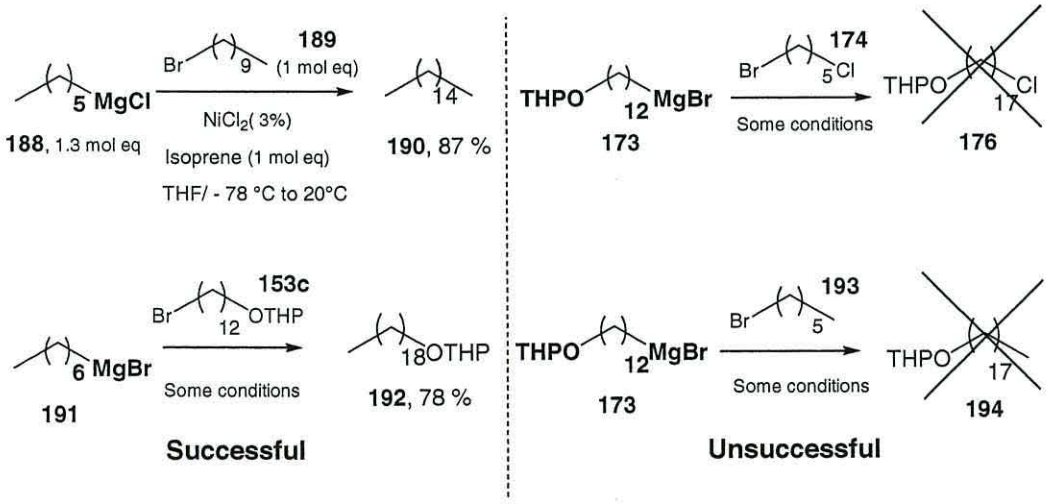
Nickel salts have been widely used for carbon-carbon bond formation by cross-coupling of Grignard reagents with organic halides that have the leaving group bonded to an sp^2 carbon.[273,274] A recent paper suggested $NiCl_2$ (**179**) in association with butadiene (**182**) as a good catalyst for the type of reactions required.[275] A possible mechanism for catalytic activity was explained, also illustrating the essential role of the dialkene compound (**182**, **Scheme 42**). Following this hypothesis, $NiCl_2$ (**179**) is reduced to Ni(0) (**181**), which then forms a complex (**183**) with two molecules of butadiene (**182**). This intermediate (**183**) is not reactive towards alkyl halides (**185**), but reacts quickly with Grignard reagent (**180**). Their coupling gives another intermediate (**184**), which has enhanced nucleophilicity towards alkyl halides (**185**). Finally, the reductive elimination on the intermediate (**186**) gives the desired product (**187**) and closes the cycle.



Scheme 42: ***The possible mechanism of the Ni(0) catalysts***

Isoprene has similar chemical properties to butadiene and it is easier to handle, since it is liquid at room temperature while butadiene is a gas. However, isoprene is less effective and, therefore, it was necessary to use it in stoichiometric quantities with respect to the coupling reagent.

Different model reactions were attempted, changing the coupling reagents, to test the efficiency of this method (**Scheme 43**). All of them were successful. The first reaction attempted was between hexylmagnesium bromide (**188**, 1.3 mol. eq.) and bromodecane (**189**, 1 mol. eq.) in THF at - 78 °C, in the presence of $NiCl_2$ (3 mol %) and isoprene (1 mol. eq.). The reaction produced a good yield of the desired compound (**190**), as did the coupling between heptylmagnesium bromide (**191**, 1.3 mol. eq.) and 2-(12-bromododecyloxy)tetrahydropyran (**153c**).

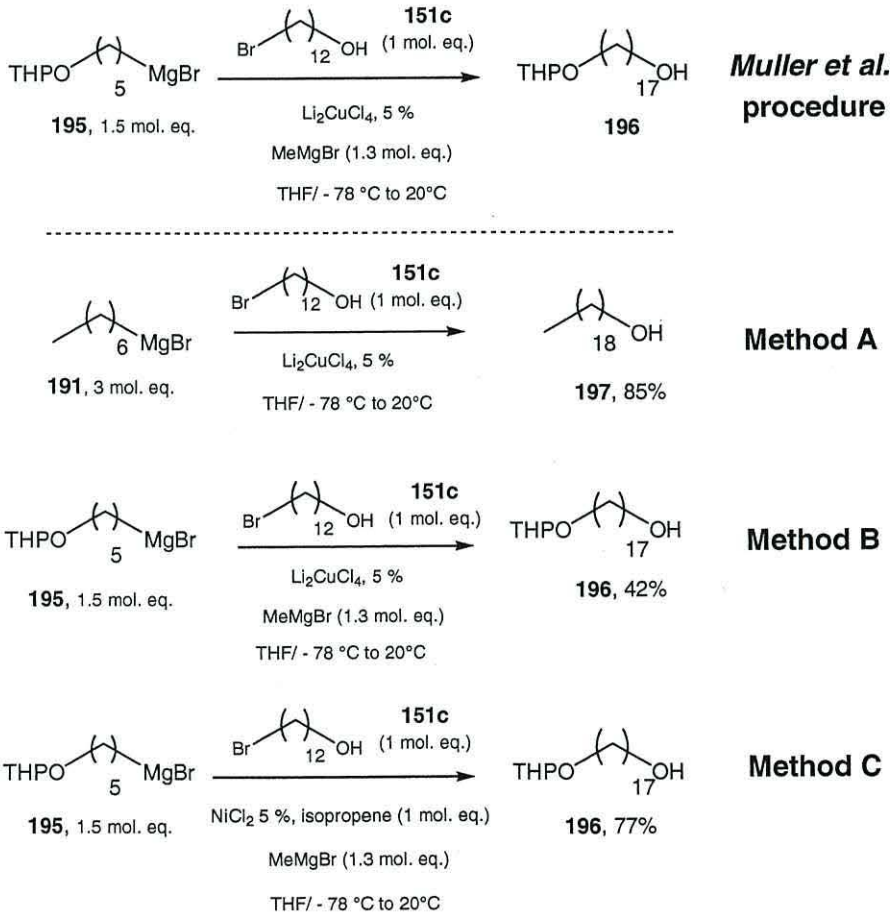


Scheme 43: ***Experiments with $NiCl_2$ and isopropene***

Nevertheless, when this new approach was applied to the necessary starting materials, the 12-(tetrahydropyran-2-yloxy)dodecyl magnesium (**173**, 1.3 mol. eq.) and 1–bromo-5-chloropentane (**174**, 1 mol. eq.), the reaction did not give the desired product (**176, Scheme 43**). Moreover, in a different experiment using the same Grignard reagent (**173**) but a simpler coupling reagent, 1-bromohexane (**193**), no products except the reduced Grignard reagent were identified.

These results suggested that the failure of this reaction could be related to the low solubility of the organomagnesium compound (**173**) in the required conditions, which made its coupling with alkyl halides difficult.

A possible answer to this problem could come from the use a smaller Grignard reagent. In particular, Müller *et al.*[276] recently described the preparation of a related compound (**196**), which was obtained from the coupling of 5-(tetrahydropyran-2-yloxy)pentyl magnesium bromide (**195**) with 12-bromododecan-1-ol (**151c, Scheme 44**).

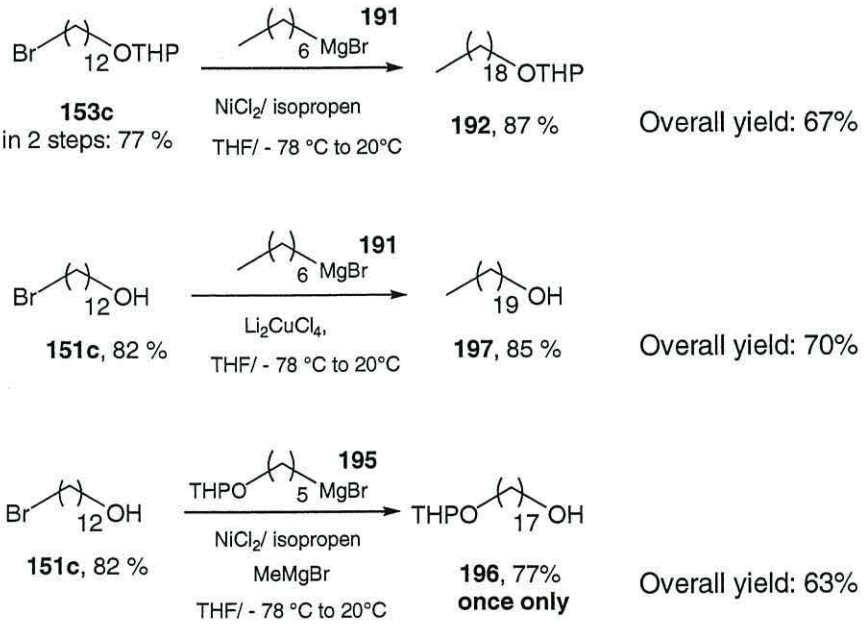


Scheme 44: ***Müller method***

Firstly, this approach was applied in the preparation of the simpler 1-nonadecanol (**197**). This was formed through the reaction between heptyl magnesium bromide (**191**, 3 mol. eq.) and 12-bromo-dodecan-1-ol (**151c**, 1 mol. eq.) using Tamura and Kochi's catalyst (5 mol %, **Scheme 44**). The presence of an unprotected hydroxyl group did not create an insoluble problem. A solution could be to increase the amount of the Grignard reagent used, since 1 mol. eq. of this would be reduced by reaction with the acidic proton of the alcohol (**Method A, Scheme 44**).[277]
Müller *et al.*[276] suggested, instead, the use of methylmagnesium bromide (1.2 mol. eq.) for quenching the unprotected hydroxyl group. Following this procedure, the reaction between the organomagnesium compound (**195**, 1.5 mol. eq.) and the alkyl halide (**151c**, 1 mol. eq.), in the presence of Li_2CuCl_4 gave only a 42 % yield of the desired product (**196, Method B, Scheme 44**). A better yield (77 %) was obtained once, when the same approach was employed, but with $NiCl_2$ and isoprene as catalysts (**Method C, Scheme 44**). However, such a good result was never repeated even after several attempts.

2.2.4. Conclusions

In conclusion, the use of Grignard reaction for the formation of long chains with only one functional group was very useful; for example, for the preparation of both the alcohol protected (**192**), and the free alcohols (**197, Scheme 45**).



Scheme 45: ***The Grignard approach***

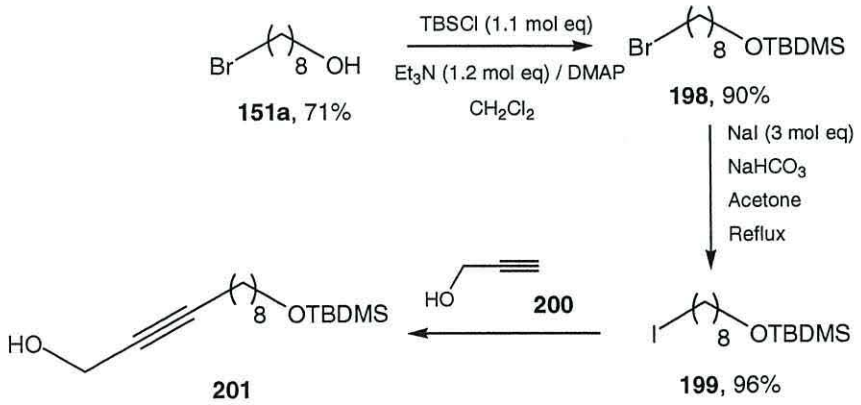
However, the use of the same approach for the preparation of long chains with both ends functionalised (**196**) did not seem to be the best route, since it did not produce consistent results (**Scheme 45**). Moreover, this method would be unlikely to find a use for completing the chains in a late stage of the preparation of α^1-mycolic acids (**Method B, Scheme 35, Section 2.1.2**). This was suggested by the difficulties already encountered in the coupling of not very complexes chains, and will be proved subsequently (**Section 3.4.6**).

2.3 Indirect methods to bifunctional long chains: the use of an alkynyl compound

2.3.1 *tert*-Butyldimethyl-silyl as protecting group

Another route tested for the formation of these chains featured, as the key reaction, the coupling between alkynyl compounds, such as prop-2-yn-1-ol (**200**), and alkyl halides. This approach does not give directly the saturated long chain but this could be obtained subsequently by hydrogenation of the triple bond. Many different examples of the use of this method are reported for the synthesis of long chains.[278]

It was interesting to test this procedure for the preparation of the required long chain with both the terminal ends functionalised. The first attempt utilised propargyl alcohol (**200**) and *tert*-butyl-(8-iodo-octyloxy)dimethylsilane (**199**, **Scheme 46**).



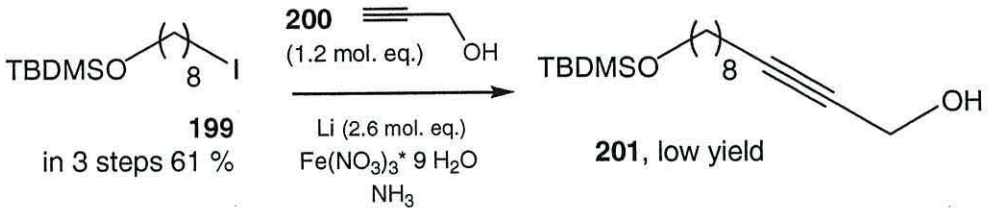
Scheme 46: ***The use of propargyl alcohol and sinalyoxy derivative***

The protected compound (**199**) had been prepared from the bromo-alcohol (**151c**). Initially, there was the protection of the hydroxyl group with *tert*-butyldimethyl-chlorosilane (1.1 mol. eq.) in the presence of triethylamine (1.2 mol. eq.), and DMAP, which was followed by conversion of the bromide into the required iodide with sodium iodide (3 mol. eq.).

All these conversions utilised standard methods. They are reported here as a synthetic strategy whose overall yield will be compared with those of other strategies.

2.3.2 The coupling reaction.

Initially, prop-2-yn-1-ol (**200**, 1.2 mol. eq.) was treated with lithium (2.6 mol. eq.) in liquid ammonia condensed at – 30 °C, in the presence of $Fe(NO_3)_3$ $9H_2O$ which is a good catalyst for the alkali metal amide formation (**Scheme 47**). It was necessary to use at least 2 mol. eq. of $LiNH_2$ for this deprotonation, since the proton of the alcohol is more acidic than the terminal alkynyl proton (pKa = 13.6 for the propargyl alcohol,[279] pKa = 25 for general terminal protons in alkynes).[280]

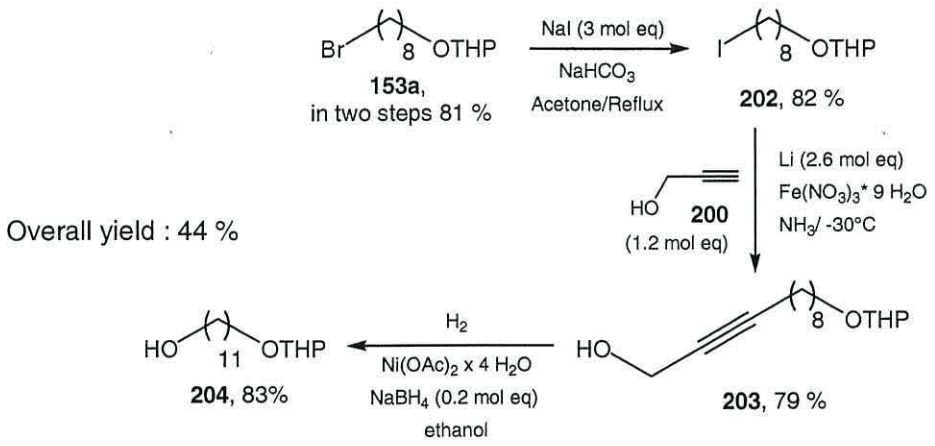


Scheme 47: ***The alkynyl coupling***

After the addition of the coupling reagent (**199**) to the mixture, this was mechanically stirred for 4 h and then the solvent was left to evaporate overnight. The coupling reaction did not produce the desired compound (**201**) in a high yield. In the ^1H NMR spectrum of the crude product, it was possible to notice several signals in the region around δ 0 corresponding to the methyl groups adjacent to the silicon atoms. This leads to the hypothesis that during the reaction the compound had lost the silyl protecting group, which was not stable enough to such basic conditions.

2.3.3 Tetrahydropyran as protecting group

It was decided to try a different protecting group for the hydroxyl group, the tetrahydropyranyl ether (**Scheme 48**), since this is considered more stable than the silanyl group to basic environments.[281] Moreover, the use of this protecting group has already been reported for this kind of reaction.[282]



Scheme 48: ***The use of propargyl alcohol and piranyloxy derivative***

The preparation of 2-(8-iodo-octyloxy)tetrahydropyran (**202**) from the bromo-compound (**153a**) was achieved using similar reactions to those previously described (**Section 2.3.1**). The coupling between this derivative (**202**) and propargyl alcohol (**200**) was attempted using the procedure illustrated before for the siloxy derivative (**Section 2.3.3**). However, this was more successful, giving the desired product (**203**) with a 79 % yield; its NMR spectra were identical with those in the literature.[283]

In order to obtain the required saturated long chain (**204**), it was then necessary to reduce the triple bond. This was achieved by reduction in ethanol under H_2 atmosphere, and in presence of nickel (II) acetate tetrahydrate (0.2 mol. eq.) and sodium borohydride (0.2 mol. eq.), which formed *in situ* nickel boride, a useful catalyst for hydrogenation reactions.[284]

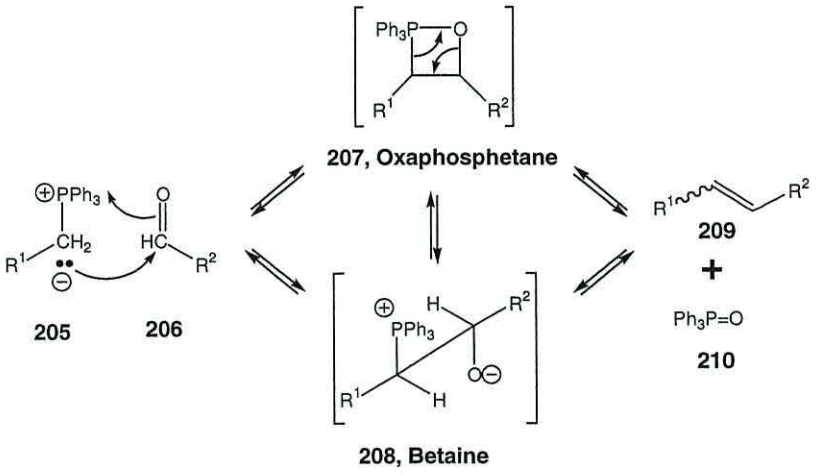
This new approach was effective and definitely more reliable with an overall yield of 44 % for the preparation of 11-(tetrahydropyran-2-yloxy)undecan-1-ol (**204**, **Scheme 48**). The spectra of the compound were consistent with those in the literature for similar compounds,[285] and they are thoroughly described in the experimental section. However if this method was to be used for the formation of

longer chains, more expensive reagents would be required, either long chain alkynes or longer diols; therefore, other more convenient methods were examined.

2.4. Indirect methods to bifunctional long chains: the Wittig reaction

2.4.1 Overview

Another indirect approach to saturated long chain employed a Wittig reaction as the key step (**Scheme 49**).[286]

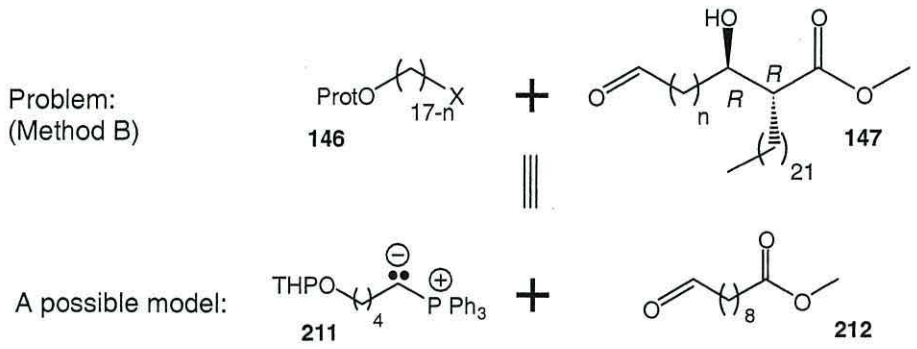


Scheme 49: ***A generic Wittig reaction***

The mechanism of the coupling between a phosphorus ylid (**205**) and an aldehyde (**206**) to give a substituted alkene (**209**) has been thoroughly studied. Currently, the most accredited theory includes the formation of a oxaphosphetane (**207**):[287] conversely there is little evidence for the existence of a betaine (**208**) as an intermediate (**Scheme 49**).[288]

This new strategy was attempted to ascertain if it would be possible to extend the chain after the introduction of other functionalities such as the terminal ester group (**Method B, Scheme 35, Section 2.1.2**).

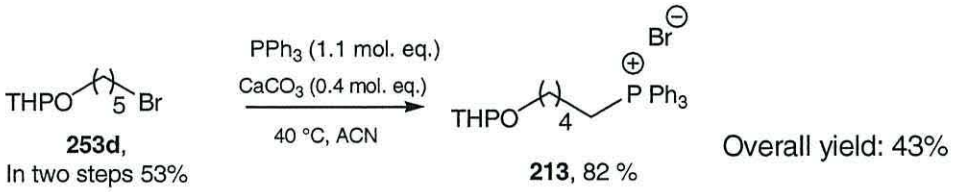
Although the model compound (**212**) used was a simple one, it had the ability to reveal if the side chain could be extended via this strategy, and if the ester would be easily hydrolysed during the reaction (**Scheme 50**).



Scheme 50: ***The Wittig approach***

2.4.2 Preparation of the phosphonium salt

It was first necessary to prepare the phosphonium salt (**213**) by treatment of the bromo-compound (**253d**) with triphenylposphine (1.1 mol. eq.) in dry acetonitrile (**Scheme 51**). The reaction was carried out in the presence of a weak base, $CaCO_3$ (0.4 mol. eq.) in order to avoid cleavage of the protecting group, which is unstable in even very weakly acid conditions, such as those produced in the course of this reaction (**Scheme 51**).[289-291]



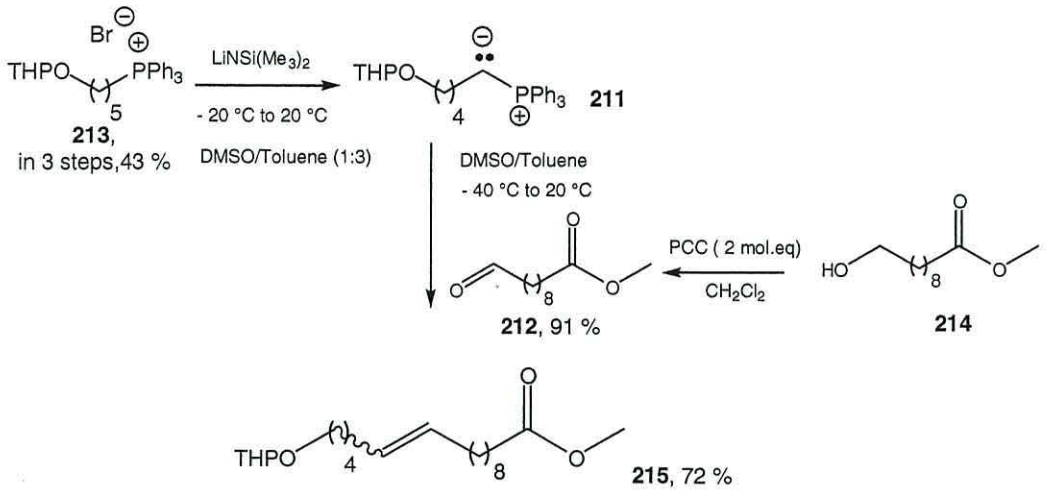
Scheme 51: ***Preparation of the phosphonium salt***

After quenching the reaction, the excess of triphenylphosphine was eliminated through several washes with a solution of petrol and ether 1:1. The compound obtained showed the same ^{1}H and ^{13}C NMR spectra as those reported in literature for triphenyl-[5-(tetrahydropyranyloxy)pentyl]-phosphonium bromide (**213**).[292]

2.4.3. The Wittig reaction itself

Initially, the phosphorus ylid (**211**) was formed by treatment of the salt (**213**) with a strong base, such as lithium bis(trimethylsilyl)amide (1.05 mol. eq.). The reaction was carried out in a mixture of DMSO and toluene (1:3) at - 20 °C (**Scheme 52**). These conditions were necessary due to the very low solubility of

the phosphonium salt (**213**). The mixture was stirred for 3 h at room temperature to ensure the complete reaction between the base and the salt.



Scheme 52: ***The use of the Wittig reaction***

Simultaneously, the aldehyde (**212**, **Scheme 52**) was prepared from the commercially available 10-hydroxydecanoic acid methyl ester (**214**) using PCC (2 mol. eq.). Although a delay in using the aldehyde (**212**) could lead to its decomposition, it was advisable to purify the compound by column chromatography, in order to eliminate any chromium salts which might disturb the Wittig reaction.

A solution of the aldehyde (**212**) in toluene was added at - 78 °C to the ylid (**211**). In normal circumstances, the mixture would be stirred overnight but, to prevent the cleavage of the ester group, the reaction was monitored by TLC and quenched after only 4 h (**Scheme 52**). The reaction gave the methyl ester (**215**) in 72 % yield based on the aldehyde (**212**). Analysing the NMR spectra of the product (**215**), the signal at δ 3.66 ($OC\underline{H}_3$) in the ^{1}H NMR and at δ 174.2 ($\underline{C}O_2CH_3$) and 51.3 ($O\underline{C}H_3$) in the ^{13}C NMR confirmed the persistence of the methyl ester, while a multiplet at δ 5.4-5.3 provided evidence for the formation of the alkene group. The new peak 1666 cm^{-1} (C=C, stretching) was further proof of the success of the reaction.

The compound (**215**) was obtained as a mixture of (*E*) and (*Z*)-stereoisomers. This was evident in the ^{13}C NMR spectrum which contained two sets of signals for the sp^2 carbons at δ 130.4 and 130.1 for the (*E*)-compound, and at δ 129.9 and 129.4

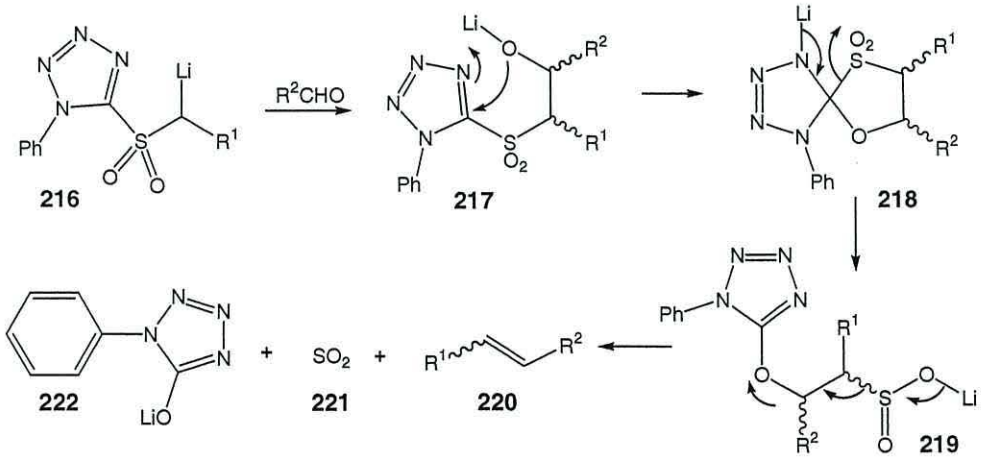
for the (*Z*)-compound. This designation of the alkene carbons was done following the approach of Bernassau and Fetizon,[293] described in detail in **Section 2.6.2.** This good result provided some reassurance of the applicability of this approach for the formation of the long chain in a later stage of α^1-mycolic acid synthesis.

2.5. Indirect methods to bifunctional long chains: the Julia reaction

2.5.1 Overview

The last method attempted for the preparation of bifunctional long chains employed a modified Julia reaction as the critical step, which produced a disubstituted alkene through a one step reaction between a heteroarylsulfone and a carbonyl compound.[294]

In the classical Julia, with simple phenylsulfones, four different steps are required in order to obtain the double bond.[295] In this version, however, the presence of an electrophilic imine-like moiety within the heterocycle (**216**) makes the intermediate β-alkoxysulfone (**217**) inherently unstable. It therefore readily undergoes a Smiles rearrangement and a spontaneous elimination of sulfur dioxide (**221**) and lithium benzothiazolone (**222**), which then leads to the desired alkene (**220**, **Scheme 53**).[294]

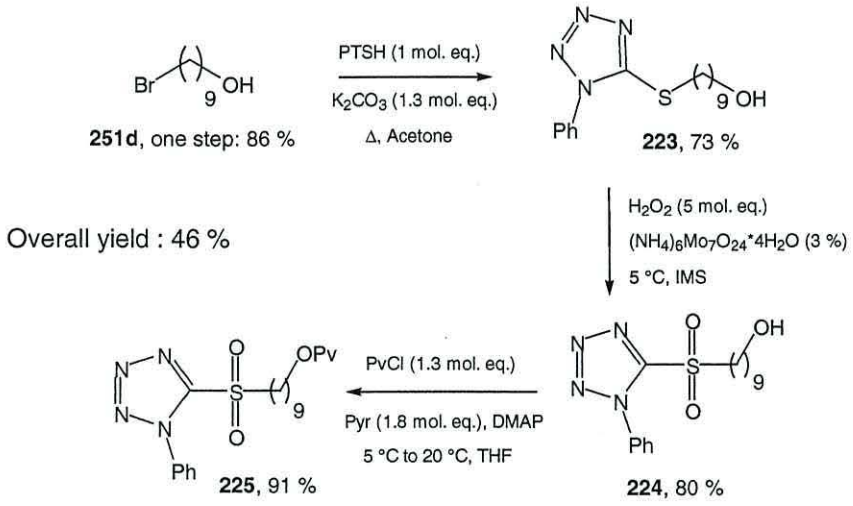


Scheme 53: ***The mechanism of the modified Julia reaction***

Among the different heterocyclic compounds suitable for this reaction, 1-phenyl-1*H*-tetrazol-5-yl sulfonyl reagent (**216**) was chosen because it was easy to prepare from commercially available materials, and it has not shown problems with self-condensation.[294, 296]

2.5.2 Preparation of the 1-phenyl-1*H*-tetrazol-5-yl sulfone.

Firstly, the phenyl-1*H*-tetrazol-5-yl-sulfanyl-derivative (**223**) was formed from 9-bromononan-1-ol (**251d**) and 1-phenyl-1*H*-tetrazole-5-thiol, using potassium carbonate (1.3 mol. eq.) to deprotonate the thiol proton (**Scheme 54**), following the method used by Roberts.[297]



Scheme 54: ***Preparation of the ester (225)***

The ^{1}H NMR of the pure compound (**223**) showed a multiplet at δ 7.55-7.50 (aromatic protons), a triplet (J = 6.6 Hz) at 3.62 ($C\underline{H}_2OH$) and a triplet (J = 7.4 Hz) at 3.36 ($C\underline{H}_2S$). The ^{13}C NMR showed a signal at δ 154.3 for the only carbon in the tetrazole ring, 4 signals for the aromatic carbons (δ 134 – 123) and signals at 62.8 ($\underline{C}H_2OH$) and 33.3 ($\underline{C}H_2S$), which confirmed the success of the reaction.

The subsequent oxidation with H_2O_2 in the presence of ammonium molybdate tetrahydrate gave the desired sulfone (**224, Scheme 54**).[298]

Interestingly, the ^{1}H NMR spectrum of this compound showed, alongside signals for the aromatic and the alcohol previously described, another anomalous signal at δ 3.73 for the two protons (**H_A and $H_{A'}$**) next to the sulfonyl group. These

displayed the typical pattern for an **AA'BB'** system, where two large substituents stop the free rotation on the C-C bond (**Figure 30**).

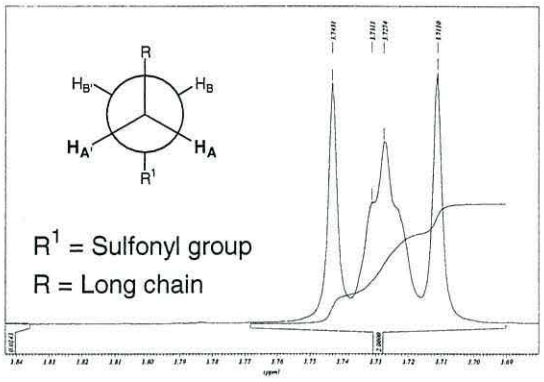


Figure 30: ***The characteristic signal of the protons (H_A and $H_{A'}$) adjacent to a sulfonyl group***

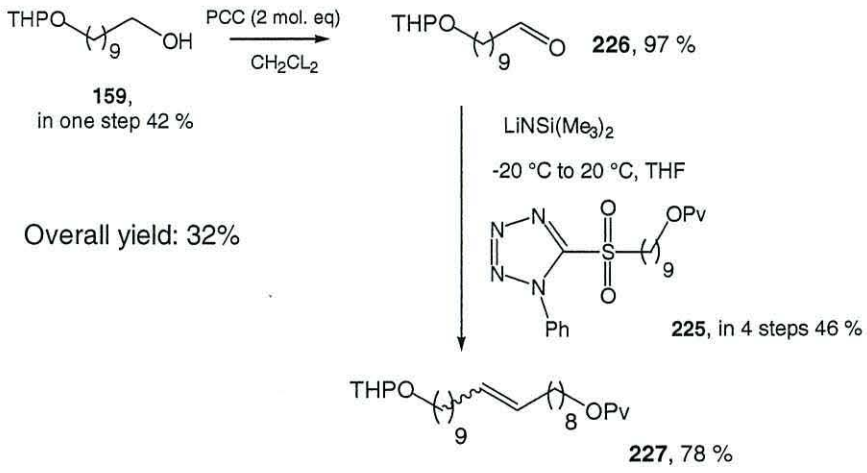
This compound (**224**) was then protected with trimethylacetyl chloride in the presence of pyridine and DMAP, to catalyse the addition of the alcohol anion to the carboxylic group (**Scheme 54**).[299] The ^{1}H NMR spectrum of the protected compound (**225**) showed a triplet (J = 6.6 Hz) at δ 4.05 for the protons adjacent to the protecting group, and a singlet at 1.20 for the protons of the three methyls of the pivaloyl group, in addition to the characteristic signal at δ 3.71 for the protons adjacent to the sulfonyl group. The ^{13}C NMR spectrum showed in addition to the signals characteristic for the sulfonyl group, a peak at δ 178.6 for the carboxyl group. The required starting material (**225**) for the Julia reaction was thus obtained in 4 steps with an overall yield of 46 %.

2.5.3 The Julia reaction.

The required aldehyde (**226**), the other coupling reagent of the Julia reaction, was prepared from the respective alcohol (**159**) using a similar method to that described in **Section 2.4.3.**

Subsequently, in the actual coupling reaction, the sulfonyl starting material (**225**) was added to the aldehyde (**226**) in the presence of a non-nucleophilic, strong base, such as lithium bis(trimethylsilyl)amide. The reaction was started at – 20 °C and subsequently allowed to reach room temperature; both the reagents were very soluble in THF (**Scheme 55**). This property was very encouraging regarding a

possible use of this kind of reaction in a later stage of the synthesis, when larger molecules would be employed and solubility was known to be a problem.[249,253]



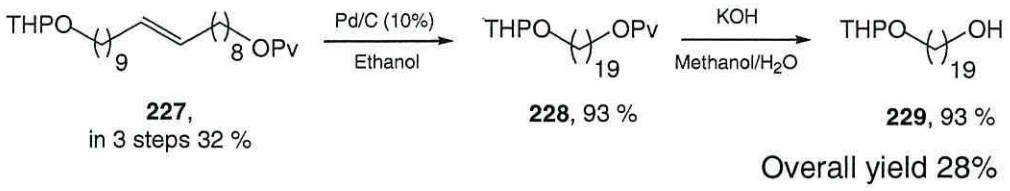
Scheme 55: ***The modified Julia reaction***

The reaction appeared to be very straightforward, giving the desired disubstituted alkene (**227**) with a 78 % yield. The product (**227**) was a mixture of (*E*) and (*Z*)-stereoismers, and had similar spectra to those of 14-(tetrahydropyran-2-yloxy)tetradec-10-enoic acid methyl ester (**215**, **Section 2.4.3**), except for the different ester groups.

The Julia reaction seemed to be preferable to the Wittig coupling, for the preparation of the long chain for several reasons. Firstly, although the preparation of the sulfonyl starting material (**225**, **Scheme 54, Section 2.5.2**) required several steps, none of them were problematic. On the other hand, the preparation of the phosponium salt (**213**, **Scheme 51**, **Section 2.4.2**) was more difficult, since the protecting group could be lost easily, and the purification of the viscous product was lengthy. Moreover, the phosphonium salt (**213**) easily decomposed; therefore, its preparation on a large scale and subsequent storage was not advisable. Secondly, the actual, modified Julia reaction (**Scheme 55**) was a more straightforward coupling reaction: not only did it give a better overall yield, but it did not present the same problems of solubility as that for the reagents observed for the Wittig reaction (**Scheme 52, Section 2.4.3**).

2.5.4 The formation of the bifunctional saturated long chain

Both the previous approaches, based on the use either of the Wittig coupling or of the modified Julia one, required the reduction of the double bond in order to obtain the required bifunctional long chain. This was achieved, in the case of the Julia product (**227**), through hydrogenation in ethanol using Pd (10 %) on carbon as a catalyst (**Scheme 56**). It was crucial to completely dissolve the starting material, as explained in more detail in **Chapter 3, Section 3.4.7**.



227, in 3 steps 32 %

228, 93 %

229, 93 %

Overall yield 28%

Scheme 56: ***Formation of the desired long chain***

The spectra of the purified compound (**228**) showed the absence of any signals for the double bond, while both the protecting groups were still present. In the ^{1}H NMR spectrum, the triplet (J = 2.9 Hz) at δ 4.56 for the acetal proton illustrated the protection with tetrahydropyran, and the singlet at δ 1.19, integrating for 9 protons, denoted the pivaloyl group. In the ^{13}C NMR spectrum, there were signals at δ 98.8 for the acetal carbon and at δ 178.6 for the carboxylic carbon of the pivaloil protection.

The subsequent step was the hydrolysis of the ester in basic conditions, which were considered safe for the other protecting group. Both NMR spectra for the purified product (**228**), still containing the characteristic signals for the tetrahydropyranyloxy protection, confirmed the success of the reaction, providing the desired product (**229**) in 5 steps with an overall yield of 28 %.

In conclusion, comparing all the four methods attempted for the formation of long chains, the Grignard reaction was definitely the most direct and the best for the preparation of monofuctionalised chains (**Scheme 45, Section 2.2.4**). However, for the preparation of bifunctionalised compounds it was not reliable and the modified Julia reaction seemed to provide a preferable alternative (**Scheme 56**).

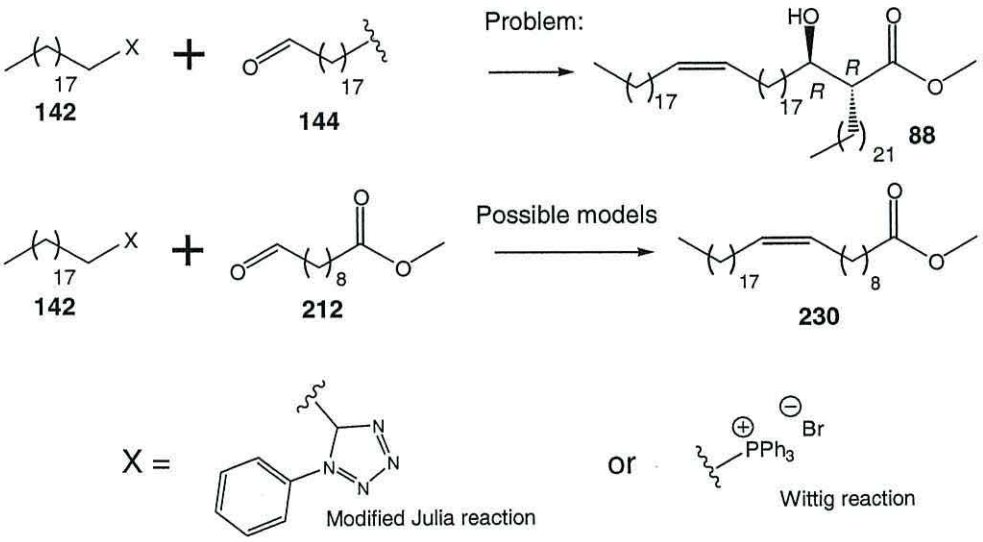
This last approach was more time consuming and the yield was reduced by the fact that it involved so many steps. However, it was consistent and could also be

used easily for a possible elongation of the chain in the later stages of the synthesis, when other functionalities already would be present.

2.6. The formation of the double bond in the meromycolate chain

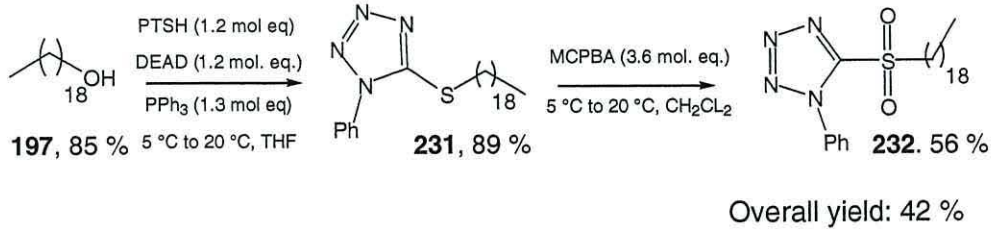
2.6.1 Overview and preparation of starting materials

In **sections 2.4** and **2.5** two methods for the formation of alkenes disubstituted with long chains have been illustrated. Models of the Julia and Wittig reactions were also attempted in order to analyse their use for the introduction of the double bond in the meromycolate chain of α^1-mycolic acids (**Scheme 57**).



Scheme 57*: Models for the formation of the meromycolate chain of α^1-mycolic acid*

First of all, it was necessary to prepare the starting materials. The sulfone (**232**) for the Julia reaction was prepared from the previously prepared alcohol (**197**) following a different method to that already described (**Section 2.5.2**).



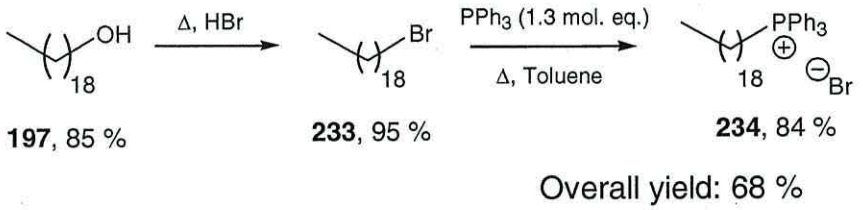
Scheme 58 : ***Preparation of the long chain 1-phenyl-1H-tetrazol-5-yl sulfone***

Initially, the alcohol (**197**) was treated with 1-phenyl-*1H*-tetrazol-5-thiol (1.2 mol. eq.) in the presence of triphenylphosphine (1.3 mol. eq.) and diethyl azodicarboxylate (1.2 mol. eq.) in a Mitzunobu reaction (**Scheme 58**).[300]

The reaction was successful, giving the desired thioether (**231**). This showed similar spectra to that previously discussed (**224**, **Section 2.5.2**). The subsequent oxidation with *m*-chloroperbenzoic acid (3.6 mol. eq.)[301] required 30 h to reach completion. The separation of the sulfone (**232**) from the unreacted peroxoic acid and the by-product benzoic acid was lengthy and problematic leading to a yield of only 56 %.

This method seemed less useful than that described in **Section 2.6.2** for several reasons. Firstly, it required more expensive reagents such as DEAD: secondly, the use of MCPBA led to a more complex purification process.

The starting material for the Wittig reaction (**234**) was also prepared from the same alcohol (**197**). This was firstly converted into the corresponding bromide (**233**) through reaction with hydrobromic acid and then into the phosphonium salt (**234**) by treatment with triphenylphosphine (**Scheme 59**).

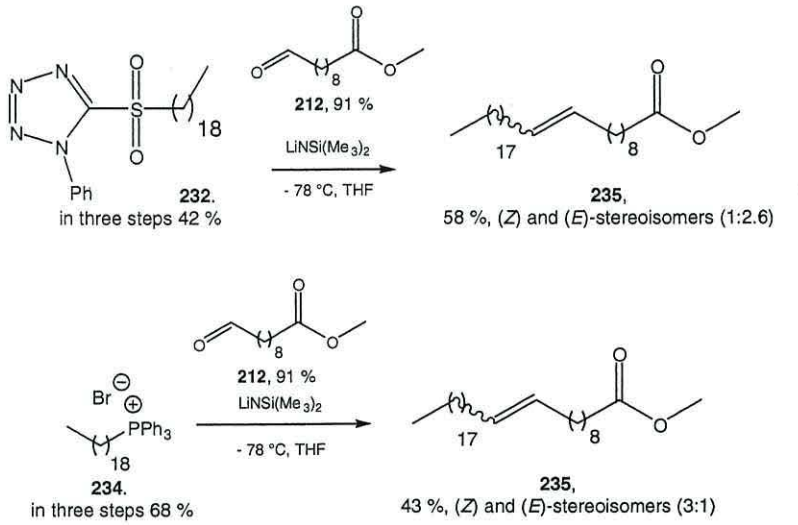


Scheme 59: ***Preparation of the long chain phosphonium salt***

The ^{13}C NMR spectrum of the phosphonium salt (**234**) showed characteristic doublets for each carbon near the phosphorus atom, due to the coupling between these atoms.[302] This feature was particularly clear in the aromatic region, where it was possible also to determine the respective coupling constants between the atoms (δ 118.5 (C adjacent to phosphorous atom, J = 85.5 Hz), 130.4 (J = 12.7 Hz). 133.7 (J = 10.1 Hz), 134.96 (J = 2.5 Hz)). Conversely, it was not so easy to identify the same pattern among the alkyl carbons due to their greater number. Similarly it was difficult to determine the coupling constants between the protons and the phosphorus atoms in the ^{1}H NMR because of the complexities of the signals.

2.6.2 The comparison between the products from Julia and Wittig reaction

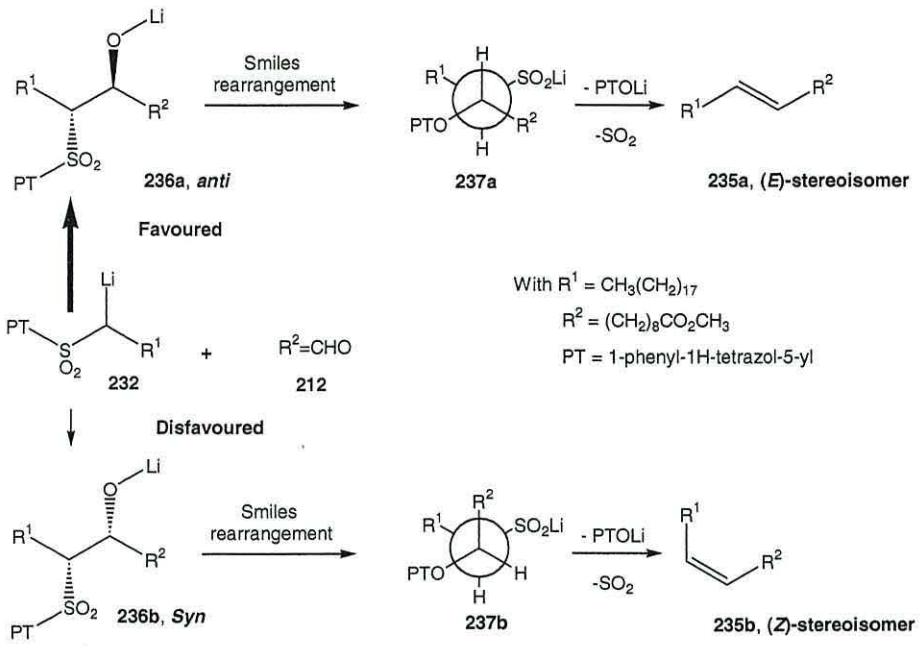
Finally, both coupling reactions were attempted, using similar procedures to those described above (**Section 2.4.3**, and **Section 2.5.3**) with the aldehyde (**212**, **Scheme 52, Section 2.4.3**) prepared in advance.



Scheme 60: ***Model reactions attempted***

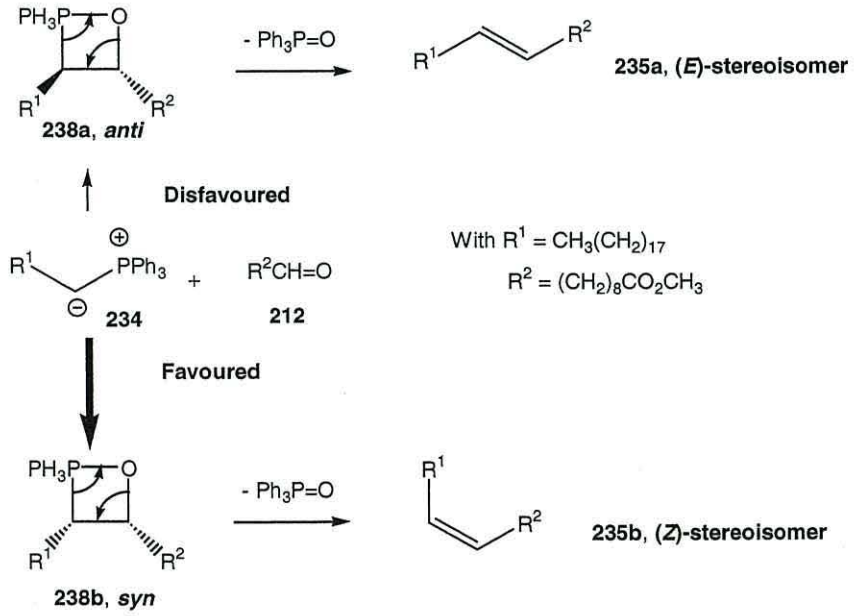
Both of the reactions gave mixtures of (*Z*) and (*E*)-stereoisomers of the desired compound (**235**, **Scheme 60**). Their separation proved to be very difficult but, using spectroscopic methods, the identification of the nature of the mixture was possible. Nevertheless, since the Julia reaction is known to yield predominantly (*E*)-alkenes, while the Wittig gives principally (*Z*)-ones, their comparison could be useful in distinguishing the two diastereoisomers in the mixture.

The reasons for the prevalence of (*E*)-alkenes (**240a**) among the mixture of products obtained from this particular modified Julia reaction have been proposed by Blakemore.[294] Analysing the mechanism of the Julia reaction he asserted that using this kind of heterocyclic sulfone, there is a kinetically controlled addition between the two reagents (**212**, **232**), which leads to the formation of *anti*-β alkoxysulfones (**236a**, **Scheme 61**).[294] The subsequent two stages of the reaction, the Smiles rearrangement and the elimination are stereospecific producing essentially the (*E*)-compounds (**235a**). Conversely, the *syn*-stereoisomer (**236b**) is formed in smaller amounts and therefore, so is the final (*Z*)-compound (**235b**).



Scheme 61: ***Reasons for the stereosectivity of the Julia reaction***

Conversely, an explanation for the prevalence of (*Z*)-compounds (**235b**) from the Wittig reaction with an unstabilised ylid (**234**) is that this process is characterised by a kinetically controlled formation of *syn*-oxaphosphetane (**238b**) by a 2+2 concerted cycloaddition (**Scheme 62**). This intermediate then undergoes a stereospecific *syn* periplanar elimination to give (*Z*)-double bonds (**235b**), and not the (*E*)-ones (**235a**).[303]



Scheme 62: ***The stereoselectivity of the Wittig reaction***

Therefore, two completely different mixtures of compounds would, in principle, be expected. Comparison between NMR spectra allowed the establishment of a method for discerning the abundance of the respective compounds.

In the literature, the values in the ^{13}C NMR corresponding to the sp^2 carbon atoms are reported to be influenced not only by the position of the unsaturation with respect to the carboxylic group, but also by the stereochemistry of the double bond.[293,304] They have also been employed in structural assignments for the determination of the nature of mixtures of (*E*) and (*Z*)-alkenes.[199]

Analysing the ^{13}C NMR of the two mixtures of products, it was possible to hypothesize that signals at δ 130.5 and 130.2 corresponded to the (*E*)-alkene, while the signals at δ 129.8 and 129.9 corresponded to the (*Z*)-alkene (**Figure 31**).

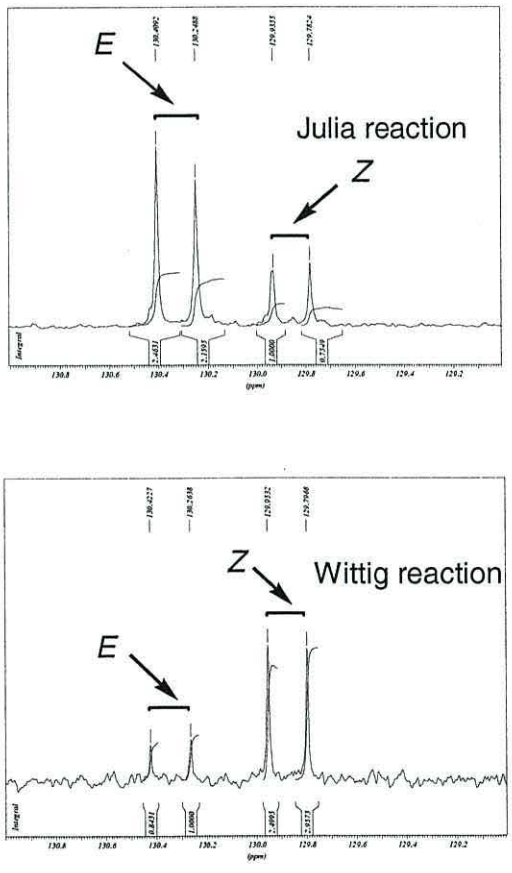


Figure 31: ***The ^{13}C NMR of the two mixtures***

This corresponded with what was found by Vieville *et al.* for similar compounds with the double bond distant from the carboxylic group.[305] These molecules have signals for the (*E*)-stereoisomer at a lower field than those for the (*Z*)-

stereoisomer.[305] In this paper, the calculated and observed values for the olefinic carbons of methyl elaidate and methyl oleate are reported (for the (*E*)-Δ^9-methyl ester they are: δ 130.4 and 130.2, and for the (*Z*)-Δ^9-methyl ester they are: δ 129.8 and 130.0). Using an inverse gated proton decoupling experiment, it was possible to integrate the different signals and to determine the ratios of the products in each reaction.[306] Using the Julia reaction, a mixture of (*E:Z*)-triacont-10-enoic acid methyl ester (2.6:1) was obtained. Following the Wittig approach, a (*E:Z*) mixture of (1:3.1) was obtained.

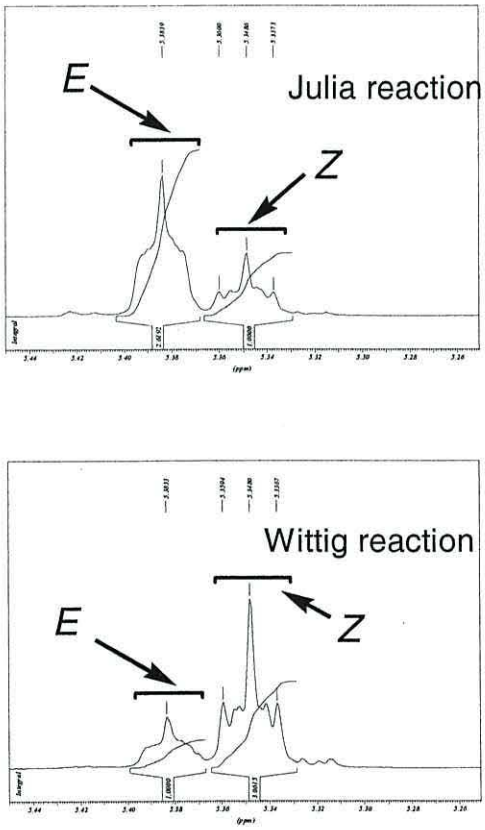
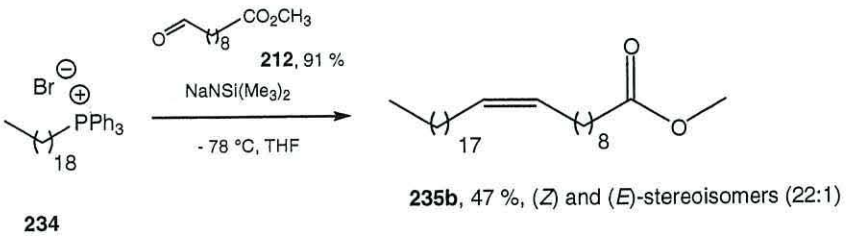


Figure 32: ***The ^{1}H NMR of the two mixtures***

Moreover, comparing these with the ratios found for the alkene protons in the ^{1}H NMR spectra, the two sets were in agreement. This led to the hypothesis that comparison between the ^{1}H NMR spectra could also give useful information regarding the ratios between (*E*) and (*Z*)-alkenes in a mixture (**Figure 32**).

The final problem to be solved was how to increase the percentage of *Z* versus *E* in the mixture of alkenes. It is known that the use of lithium reagents as base leads to the formation of complexes involving lithium halide salts which favour the

formation of the (*E*)-compound.[307,308] These do not occur when other bases, such as sodium bis(trimethylsilyl)amide, are used.[309] Repeating the reaction with the new base, the percentage of (*Z*)-compound, in the mixture of products, was notably increased (**Scheme 63**).



Scheme 63: ***The Julia reaction using sodium bis(trimethylsilyl)amide***

In the ^{13}C NMR spectrum, the signals for the (*E*)-alkene were not visible among the noise. Analising the ^{1}H NMR spectrum, the ratio between the two diastereoisomers was determined to be 22:1 (*Z*:*E*) giving an overall d.e. for the (*Z*)-compound (**235b**) of 91 % (**Figure 33**).

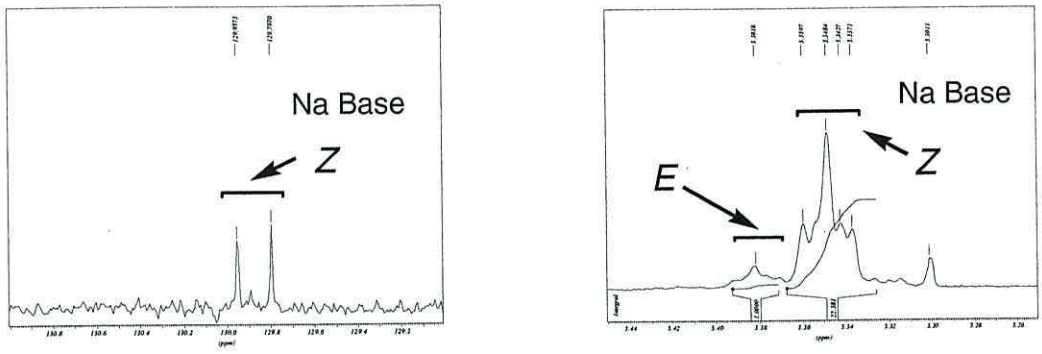


Figure 33: ***The NMR spectra of the mixture of products using the sodium base***

In conclusion, the preparation of the compound (**235**) using both Julia and Wittig reactions led to two completely different mixtures of stereoisomers. Through comparison of their NMR properties, it was possible to identify the signals for (*E*)-alkene (**235a**) and the (*Z*)-alkene (**235b**) in each mixture. These results suggested that a similar approach based on the preparation of both diastereoisomers and their comparison could also be used for characterisation of the double bond in the meroaldehyde chain. Moreover, the Wittig reaction, with sodium bis(trimethylsilyl)amide as base, supplied a good method for the preparation of (*Z*)-unsaturated compounds.

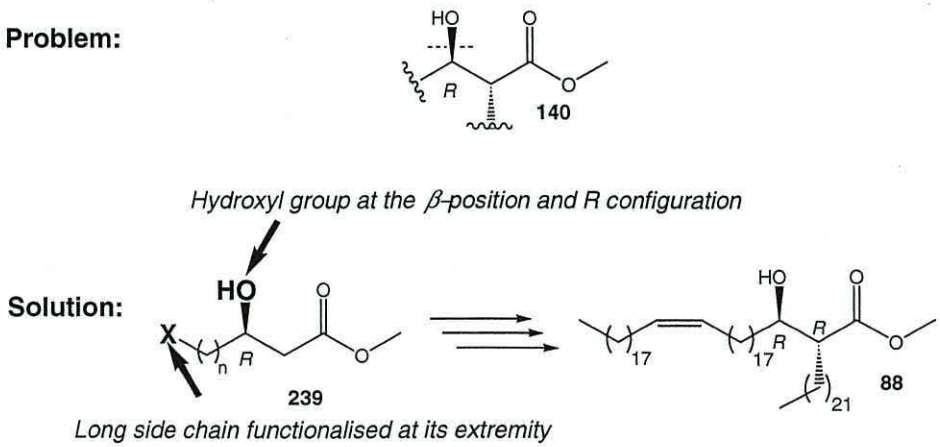
3. Introduction of the hydroxyl group

3.1 The general approach

3.1.1 Overview.

In the course of the project, the second key problem analysed was the insertion of the hydroxyl group at the β-position with respect to the carboxylic group and in the *R*-configuration (**Scheme 64**).

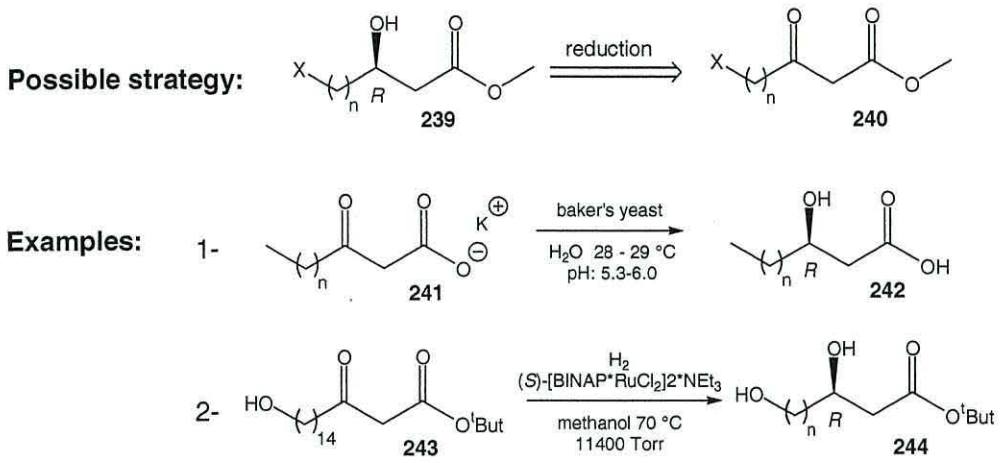
In the literature, many approaches to this kind of molecule (**140**) have been proposed. In this chapter some of them will be analysed in order to understand which could be the most suitable for the preparation of α^1-mycolic acid in particular, and mycolic acids in general. The ideal method should not only introduce the hydroxyl group with a high optical purity but it should also guarantee an easy route for the extension of the side chain in order to form the meromycolate moiety in a later stage of the synthesis. This could be achieved if the target β-hydroxy ester has a functional group at the end of its side chain which can be easily transformed (**239**, **Scheme 64**). Moreover, the perfect synthetic route should afford a good overall yield for this crucial building block (**239**), involve the fewest steps possible and use the cheapest reagents. It should be easy also to scale up to enable the production of relatively large amounts of this key intermediate.



Scheme 64: ***Two possible approaches to the meromycolate moiety***

One of the most studied methods for this kind of compound (**239**) has been the stereoselective reduction of a β-keto ester (**240**), using either yeast or chiral

catalysts (**Scheme 65, Examples 1,2**).[262,310] Neither of these two possibilities seemed particularly suitable for this preparation for reasons given below.



Scheme 65: ***A possible approach but not convenient for the preparation of α'-mycolic acid***

Reactions involving yeasts have some disadvantages, especially because they are "intrinsically messy".[311] In fact, they involve an aqueous medium that contains the cellular mass, nutrients and one or more products. Therefore, recovery of the desired product, particularly in large-scale reactions, might not be straightforward or quantitative and a lengthy purification is usually necessary.[d] Moreover, this kind of procedure has been employed for the reduction of long chain β-hydroxy esters (**241, Example 1, Scheme 65**), but not for molecules containing another functional group at the other end of the compound.[262] The presence of this new group could create serious problems in the reaction, since the enzyme might not be able to recognise the substrate.

The second methodology mentioned above, the reduction with a chiral catalyst, usually involves the use of high pressure hydrogenation and expensive catalysts; an example is illustrated in **Example 2, Scheme 65**.[312] Therefore, the implementation of the reaction would require large investment for the instrumentation and the subsequent enlargement of scale could be complex and expensive.

Having eliminated this approach based on a stereocontrolled reduction of a β-keto ester, in the rest of this chapter other three possibilities will be analysed. The

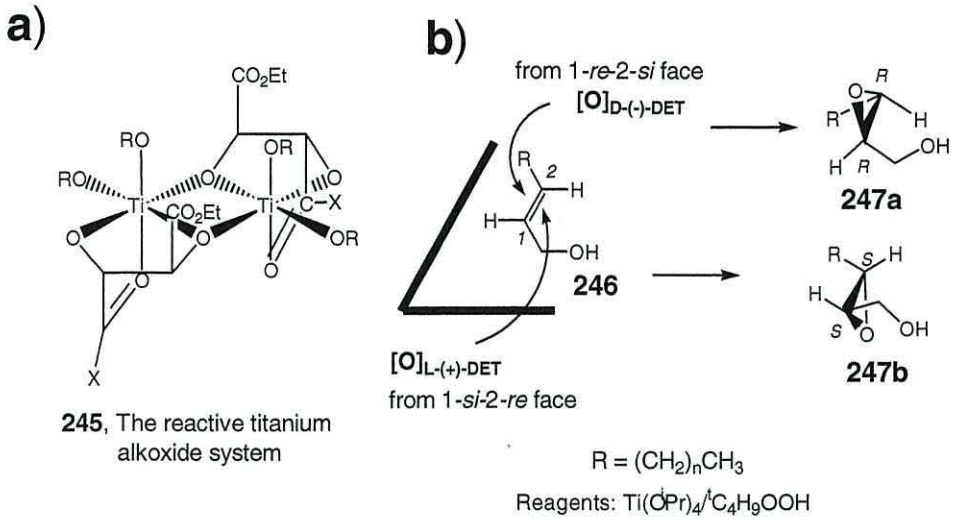
[d] This approach has been attempted on a simple model substrate but with no success. Therefore it was at once eliminated.

essence of the first two strategies was an asymmetric reaction such as the Sharpless epoxidation,[6] or the Sharpless dihydroxylation.[7] In the third route, the chiral centre was already present in the initial starting material, *D*-Aspartic acid.[8] All the methods described in this chapter use bifunctionalised long chains as starting material for the formation of the side chain of the β-hydroxy esters (**Scheme 67**, **Scheme 77** and **Scheme 87**). These starting materials contained different side chains from that required for the final target α^1-mycolic acid (**88**) because they were initially planned for the preparation of another kind of mycolic acid. However, all these strategies have been examined for the general preparation of any long β-hydroxy esters and the side chain could always be extended in later stages of the preparation of the desired compound (**88**) as shown in **Chapter 2.**

3.2 The Sharpless epoxidation method

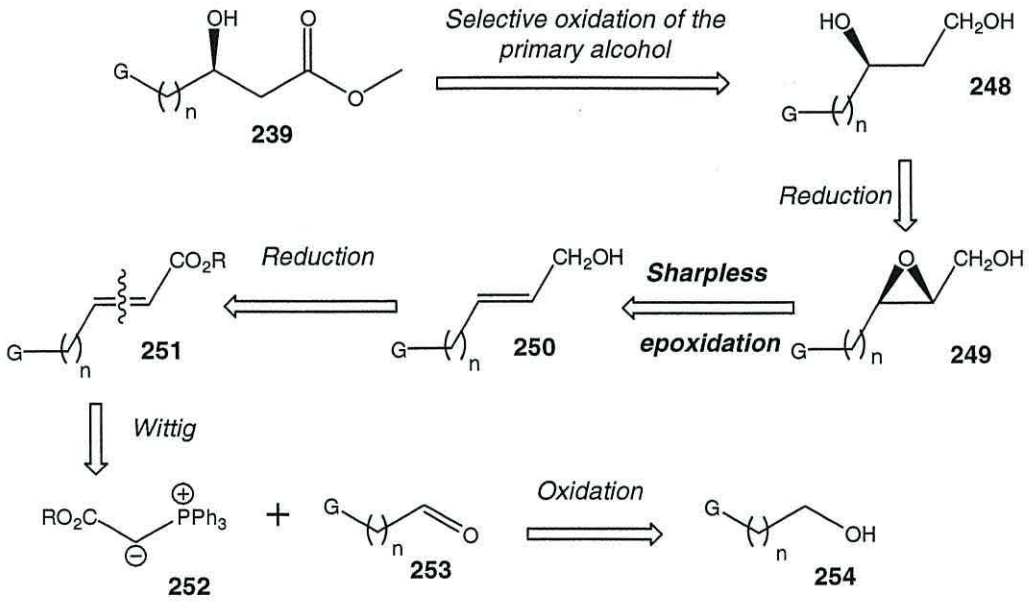
3.2.1 Overview

In assessing plausible synthetic sequences for the formation of the β-hydroxy ester containing a long, functionalised side chain (**239**), one possible method had its focal point in the Sharpless epoxidation. This reaction was reported for the first time by Katsuki and Sharpless in 1980,[6] since when it has been used for the preparation of several natural compounds.[313] It is based on the use of titanium tetraisopropoxide, which forms titanium alkoxide systems in the presence of tartaric esters. The active structure for the catalysis of the reaction is thought to be a dimer (**245**, **Scheme 66**, **a**),[314] though this structure has not been completely clarified. This kind of compound can coordinate both the allylic alcohol and the oxidant, *t*-butyl hydroperoxide, at the titanium atom, activating the nucleophilic attack of alkylperoxide onto the double bond. The stereoselectivity of the reaction is governed by the chiralty of the tartaric ester, which forces the addition of the oxidant onto only one of the two faces (**Scheme 66**, **b**). The alkoxide system formed with L-(+)-diethyl tartrate attacks from the 1-*si*-2-*re* face of an (*E*)-allylic alcohol (**246**), while that formed with D-(-)-diethyl tartrate attacks from the 1-*re*-2-*si* face (**Scheme 66**, **b**), giving two, opposite stereoisomers (**247a**, **247b**).[313,315]



Scheme 66: ***The general mechanism of the Sharpless epoxidation***

With the aim of preparing the β-hydroxy ester (**239**), the initial transformation of the retrosynthetic analysis, was the selective oxidation of the primary alcohol (**248**). More specifically, it had to be performed leaving unchanged a secondary alcohol, which was also present in the precursor (**248**, **Scheme 67**). This goal could be achieved through selective protection of the internal hydroxyl group prior to the oxidation of the terminal one.



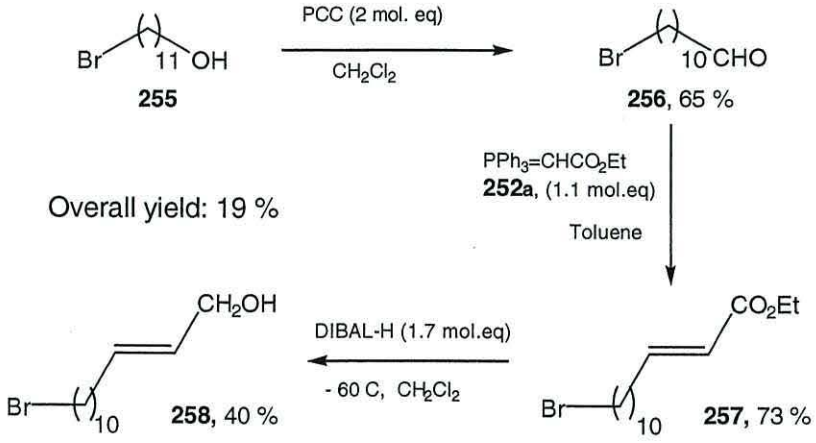
Scheme 67: ***The preparation of β-hydroxy esters using the Sharpless epoxidation approach***

The 1,3-diol (**248**) would be derived from the chiral epoxide (**249**) through regioselective reduction. The latter epoxide (**249**) could be obtained using an

asymmetric epoxidation of the allylic alcohol (**250**), which, in turn, came from the reduction of an α,β-unsaturated ester (**251**). A logical disconnection point for this compound was at the double bond. In particular, the Wittig reaction between a stabilised ylid (**252**) and an aldehyde (**253**) could lead to this (*E*)-unsaturated ester (**251**). Finally, the aldehyde (**253**) was formed through oxidation of the previously prepared bifunctional chain (**254**). Similar strategic routes were recently utilised also by Rodrigez *et al.*[316] for the preparation of β-hydroxy palmitic acid, and previously by Nemoto *et al.*[317,318] for the synthesis of amphimic acids.

3.2.2 The use of a bromo-alcohol as a bifunctional starting material

This method was initially tried using 13-bromotridec-2-en-1ol (**258**) as starting material (**Scheme 68**). The first step was the oxidation of the bromo-alcohol (**255**) using PCC (2 mol. eq.) with a similar procedure to that employed before (**Section 2.4.3**). The success of the reaction was confirmed by comparison of the NMR spectra of the product with those reported for the aldehyde (**256**).[319]



Scheme 68: ***The preparation of the allylic alcohol***

Then, a Wittig coupling of this aldehyde (**256**) with (ethoxycarbonylmethylene)-triphenylphosphorane (**252a**) in toluene at room temperature gave essentially the (*E*)-alkene (**257**). The ylid (**252a**), containing a stabilising group such as the ester, was chosen since in these conditions, it was reported to afford almost pure (*E*)-alkenes.[320]

The same kind of compound could also be obtained by a Wadsworth-Emmons reaction with phosphonates, such as methyl (diethylphosphono)acetate, which are also known to mainly give (*E*)-α,β-unsaturated ester.[307]

The ^{1}H NMR spectrum of purified (**257**) contained, for the major constituent of the alkene mixture, a double triplet at δ 6.96 (J_1 = 15.6 Hz, J_2 = 7.0 Hz) corresponding to the olefinic proton in the β-position, while the doublet at 5.8 (J =15.6 Hz) corresponded to the olefinic proton in the α-position (**Figure 34**). The coupling constant of 15.6 Hz demonstrated that the major component was the (*E*)-unsaturated ester (**257a**), (generally the coupling constant for (*E*)-alkenes is between 10 Hz and 18 Hz while for (*Z*)-alkenes it is between 6 Hz and 12 Hz).[321] The only noticeable signal of the minor constituent (**257b**) was the characteristic double triplet for the proton in the β-position at δ 6.22 with J_1 = 11.6 Hz and J_2 = 7.3 Hz. These results were also confirmed by what was reported for a similar compound (methyl (*E*)-tetradec-2-enoate showed a dt at δ 6.94 (J = 16, 7 Hz) and methyl (*Z*)-tetradec-2-enoate has a dt at 6.23 (J = 11, 7 Hz)).[322] Finally, comparing the integration of the two signals, it was possible to establish that the product was a mixture of (*E*:*Z*) esters (21.4:1). The (*E*)-compound (**257a**) was produced with a d. e. equal to 92 %.

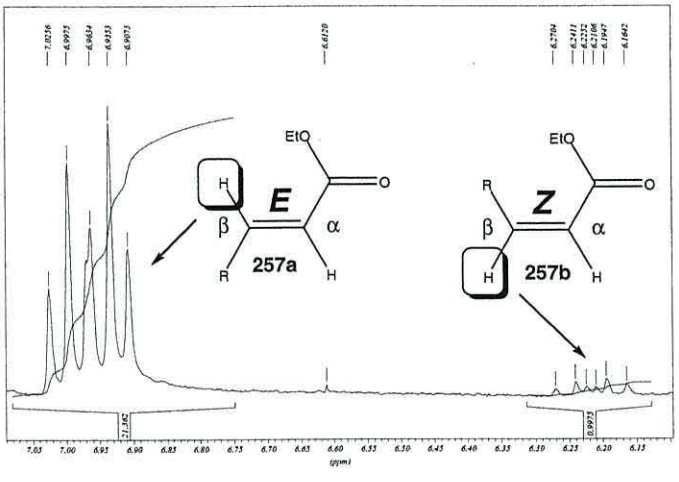


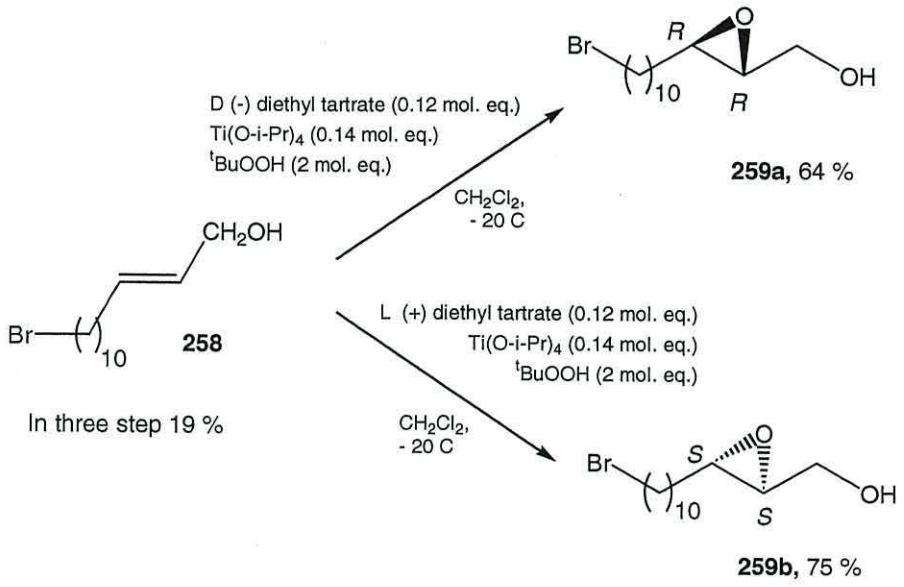
Figure 34: ***The β-protons in the two different diastereoisomers***

The ^{13}C NMR spectrum included signals at δ 168.2 for the carboxylic group, 150.9 (for $\underline{C}H{=}CHCO_2Et$) and 122.0 (for $CH{=}\underline{C}HCO_2Et$). The signals for the other stereoisomer were not detectable among the noise.

In the subsequent reduction of the ester (**257a**), the addition of the reducing agent DIBAL-H (1.7 mol. eq.) had to be conducted at – 60 °C because the reaction was extremely exothermic. The mixture was then stirred overnight at room temperature. The work-up with methanol was also conducted at – 30 °C; it gave a white emulsion, which was broken down by addition of dilute hydrochloride acid. The crude product, the allylic alcohol (**258**), was purified by column chromatography. The successful reduction of the ester was proved by analysis of ^{1}H NMR spectrum. The expected multiplet for both olefinic protons occurred at δ 5.8, with a doublet (J = 4.6 Hz) at 4.5 for the protons next to the hydroxyl group and a triplet (J = 6.7 Hz) at 3.8 (for $C\underline{H}_2Br$). The ^{13}C NMR spectrum confirmed the result because it displayed two signals for olefinic carbons at δ 133.4 and 128.8 and a signal at 63.8 (for $C\underline{H}_2OH$).

3.2.3 Sharpless epoxidation of 13-bromo-tridec-2-en-1-ol

The asymmetric epoxidation was attempted with both D (-) and L (+) tartrate in order to understand the properties of the catalyst.



Scheme 69: ***The Sharpless epoxidation of the bromo-allylic alcohol***

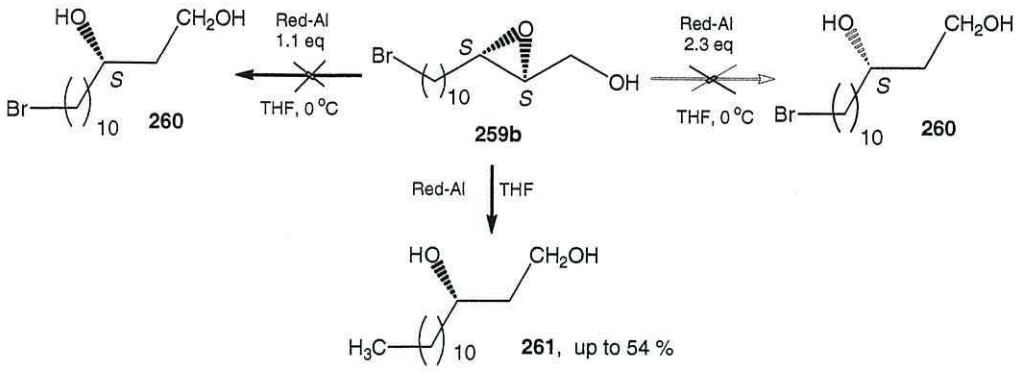
The later procedure described by Gao *et al.* was followed (**Scheme 69**).[323] *Tert*-Butyl hydroperoxide was used as an oxidizing agent in the presence of one of the diethyl tartrates (0.14 mol. eq.) and titanium tetraisopropoxide (0.12 mol. eq.). The reaction was conducted in dry dichloromethane in the presence of molecular

sieves, since this allowed it to proceed with only catalytic amounts of the titanium-tartrate complex (12 mol. %).[314] It was allowed to continue overnight at – 20 °C, though after 4.5 h the TLC showed no starting material, then quenced adding first water and subsequently a solution of sodium hydroxide, in saturated sodium chloride, to hydrolyse the diethyl tartrate. Both ^{1}H NMR spectra of the products (**259a**, **259b**) included two multiplets at δ 3.9 and 3.6 respectively attributable to the two-diastereotopic protons next to the hydroxyl group, a triplet (J = 7 Hz) at 3.4 (for $C\underline{H}_2Br$) and a multiplet at 2.9 for the two protons of the epoxide. The ^{13}C NMR spectrum exhibited the expected three signals corresponding to carbons bonded to one oxygen atom, at δ 61.6 ($\underline{C}H_2OH$), 58.4 and 55.9 (for those in the epoxide ring), while the IR spectrum showed a band at 3380 cm^{-1} for O-H stretching.

Finally, the specific rotations for both the purified compounds were measured. Using D-(-)-diethyl tartrate as catalyst, (2*R*,3*R*)[3-(10-bromo-decyl)-oxiranyl]-methanol (**259a**) was obtained with $[\alpha]_D^{19} = + 17.1$. In literature, the use of the same catalyst was reported for the synthesis of ((2*R*,3*R*)-3-dodecyl-oxiranyl)-methanol with $[\alpha]_D = + 21$. With L-(+)-diethyl tartrate, (2*S*,3*S*)[3-(10-bromo-decyl)-oxiranyl]-methanol (**259b**) with an $[\alpha]_D^{20} = - 17.5$ was obtained. In the literature the same compound was reported to have either $[\alpha]_D^{28} = - 25.5$,[324] or $[\alpha]_D = - 15.1$.[325]

3.2.4 The opening of the epoxide

Following the route described previously (**Scheme 67**), the reduction of (2*S*,3*S*)-[3-(10-bromodecyl)oxiranyl]-methanol (**259b**) was subsequently attempted utilizing Red-Al (2.3 mol. eq.) in dry THF at 0 °C (**Scheme 70**). It was reported that such conditions were selective for the preparation of 1,3-diol, as opposed to the 1,2 diol.[326] However, when attempted the reaction did not yield the desired product, the bromo-diol (**260**) because, during the reduction, the starting material also lost the terminal bromine to produce tetradecane-1,3-diol (**261**, **Scheme 70**).



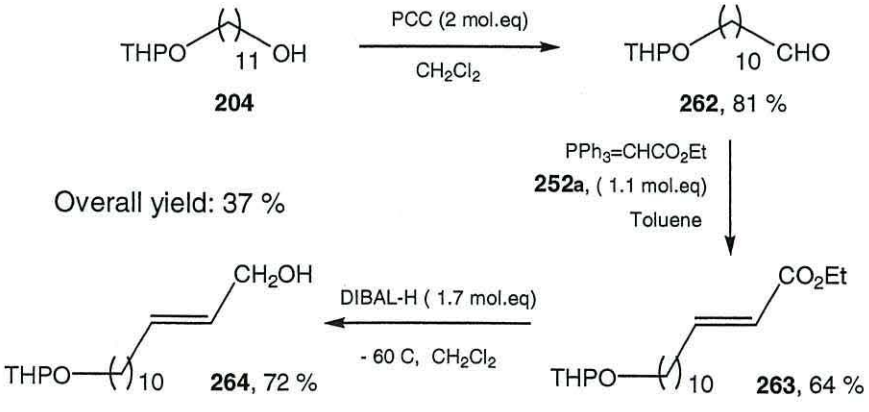
Scheme 70: ***Attempts at regioselective reduction with Red-Al***

This was demonstrated in the ^1H NMR spectrum by the absence of the triplet at δ 3.4 (for $C\underline{H}_2Br$), and the presence of a new triplet at 0.88 (J = 6.4 Hz) for the terminal methyl group. The reaction was repeated using only 1.1 mol. eq. of Red-Al but this also gave the tetradecane-1,3-diol (**261**).

It was concluded that in these conditions, the terminal bromine was reduced together with the epoxy group; for this reason its substitution with a tetrahydropyranyloxy group was considered.

3.2.5 The use of 11-(tetrahydropyran-2-yloxy)undecan-1-ol

First of all, it was necessary to prepare a new α,β-unsaturated ester which possessed a tetrahydropyranyloxy protected alcohol instead of the bromine group. Following the route already described, firstly, the alcohol (**204**) was oxidised with PCC yielding the aldehyde (**262, Scheme 71**). The success of the reaction was demonstrated by various spectra, which were compared with those reported in the literature for the same compound.[327]



Scheme 71: ***The insertion of the hydroxyl group***

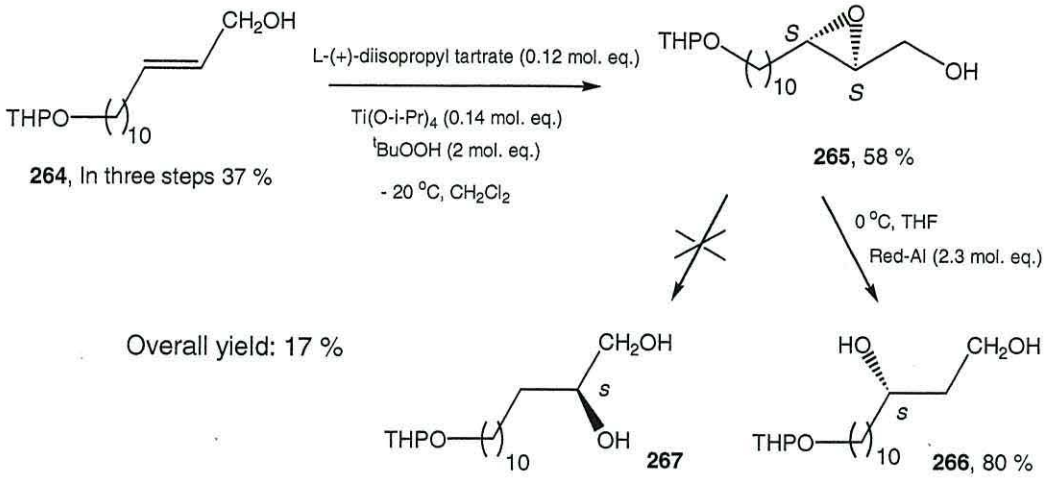
The Wittig coupling between this aldehyde (**262**) and (ethoxycarbonylmethylene)triphenylphosphorane (**252a**), utilising the procedure described previously (**Section 3.2.2**), gave the (*E*)-α,β-unsaturated ester (**263**) with a d.e. of 96 %. Its data were compared to those reported for the corresponding compound and the ^{1}H NMR spectra were identical.[328]

Finally, DIBAL-H (1.7 mol. eq.) was used once more for the next reduction of the ester (**263**) employing the procedure already described (**Section 3.2.2**). Both ^{1}H NMR and ^{13}C NMR spectra of purified (**264**) were similar to those of the allylic alcohol already illustrated (**Section 3.2.2**), except for the presence of a new set of signals characteristic of the tetrahydropyranyloxy protecting group. In the ^{1}H NMR spectrum these included a broad triplet (J = 4.3 Hz) at δ 4.57 (for the acetal proton), while in the ^{13}C NMR they were at δ 98.8 (for the acetal group), 67.2 and 62.3 (for the two carbon of the protecting group bonded to an oxygen atom).

3.2.6 Preparation of {3-[10-(tetrahydropyran-2-yloxy)decyl]-oxiranyl}-methanol

Having prepared the allylic alcohol (**264**), the next step was the Sharpless epoxidation following the procedure described above (**Section 3.2.3**). This time, however, instead of the diethyl tartrate, L-(+)-diisopropyl tartrate was used (**Scheme 72**). The use of diisopropyl instead of diethyl-tartrate, as chiral auxiliary, instead of improving the reaction, it worsened the problems in the work-up, because the new titanium tartrate complexes were more difficult to hydrolyse, forming a thicker emulsion, which was eliminated only by filtration on a celite pad.

Although, the use of the L-tartrate gave the undesired diastereoisomer, the synthesis was continued to verify the difficulties of the method. The product (**265**, **Scheme 72**) was a mixture of two diastereoisomers, because the configuration of the acetal carbon in the tetrahydropyranyl ring could not be controlled. Therefore, the specific rotation equal to - 18.5 could not easily be interpreted, even if the value was consistent with those found for (2*S*,3*S*)[3-(10-bromodecyl)oxiranyl]-methanol (**259b**, **Section 3.2.3**) and with those reported in the literature for a similar compound.[324,325]



Scheme 72: ***The Sharpless epoxidation with the pyranyloxy protection***

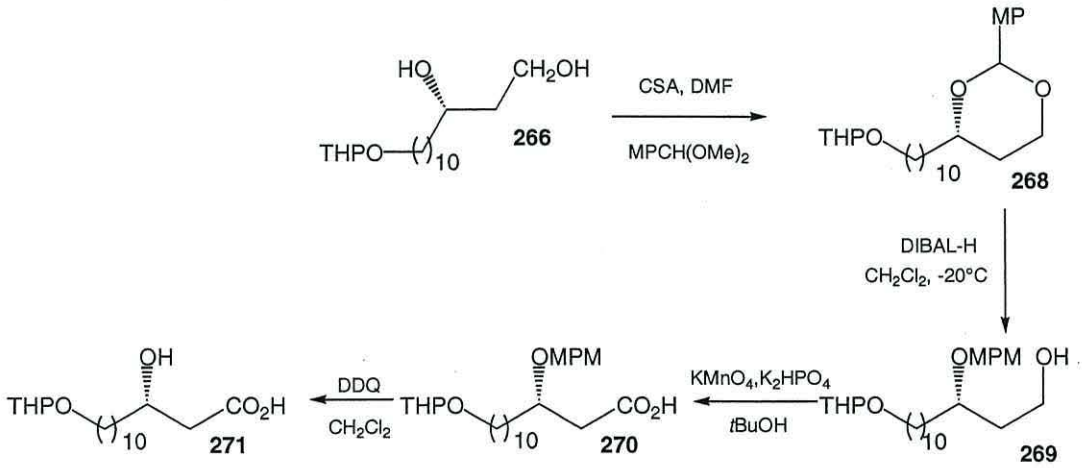
The presence of the diastereotopic protons in the protecting group made the ^{1}H NMR spectrum more complex and it was impossible to assign all of the peaks. However, a double double doublet (J_1 = 16.2 Hz, J_2 = 7.5 Hz, J_3 = 4.1 Hz) at δ 3.63 was attributable to one of the protons next to the hydroxyl group. The other proton occurred at ~ δ 3.85, coinciding with one of the protons of the tetrahydropyranyloxy ring. The spectrum also showed a multiplet at δ 2.9 corresponding to the two protons in the oxiranyl group. In the ^{13}C NMR spectrum the characteristic signals for the tetrahydropyranyloxy protection were visible, plus the signals at δ 58.6 and 56.0 for the carbon atoms of the oxirane and at 61.8 (for $\underline{C}H_2OH$).

Finally, the reduction of the epoxide (**265**) was attempted with Red-Al at 0 °C with the method employed before for the epoxide (**259b, Section 3.2.4**) but this time with more success, producing the desired compound with a yield of 80 %. The ^{1}H NMR spectrum of the product (**266**) was also very complex; it included a large multiplet in the region between δ 4 and 3.3 which was due to 4 protons of the tetrahydropyranyloxy group and to other 3 protons adjacent to the two hydroxyl groups of the molecule. The analysis of the ^{13}C NMR spectrum confirmed that the 1,3-diol (**266**) had been formed and not the 1,2-diol (**267**). It included signals at δ 72.1 (for $\underline{C}HOH$) and 61.6 (for $\underline{C}H_2OH$). In the literature, examples of ^{13}C NMR spectra for 1,3-diols are reported which showed a signal at δ 62.0 (for $\underline{C}H_2OH$) for (*S*)-tetradecane-1,3-diol,[329] and 61.4 (for $\underline{C}H_2OH$) for (*S*)-8-(tetrahydropyran-2-yloxy)octane-1,3-diol.[330] The ^{13}C NMR spectra of 1,2-diols

included signals at δ 66.83 (for $\underline{C}H_2OH$) for 1,2-hexadacanediol.[331] The difference between the peaks for the primary alcohols in the two regioisomers was significant enough to leave no doubt about the nature of the compound obtained.

3.2.7 First attempt to prepare the β-hydroxy acid (239).

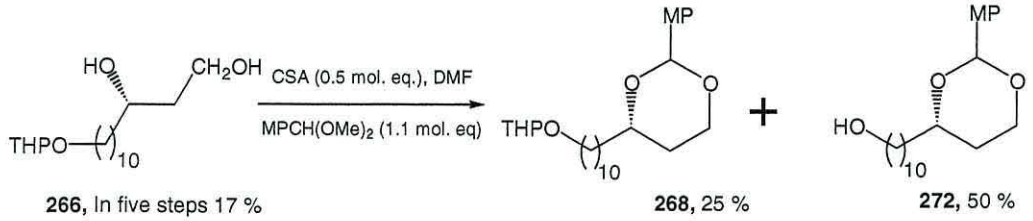
In order to obtain the β–hydroxy ester (**239**), following the approach described previously (**Scheme 67**), the next task was the oxidation of the primary alcohol of (*S*)-13-(tetrahydropyran-2-yloxy)tridecane-1,3-diol (**266**) to the corresponding carboxylic acid (**271**). The presence of a secondary alcohol in (**266**), which was also oxidable under the standard conditions used for this kind of reaction made the problem more difficult. A plausible solution could be the simultaneous protection of both the hydroxyl groups followed by the regioselective deprotection of the primary alcohol, since the latter group is usually more reactive (**Scheme 73**).



Scheme 73: ***Possible route to β-hydroxy esters***

The approach followed was based on the use of 4-methoxybenzaldehyde dimethylacetal for the protection of the 1,3-diol to form the acetal (**268**) which could be regioselectively opened with DIBAL-H,[332] and then transformed into the desired β-hydroxy acid (**271, Scheme 73**).[316]

The procedure for the protection of the diol (**266**) required the use of 4-methoxybenzaldehyde dimethylacetal (1.1 mol. eq.) in the presence of an acid such as camphor 10-sulphonic acid (0.5 mol. eq.) in a polar, but not protic solvent, in order to stabilize the different carbocation intermediates formed during the reaction (**Scheme 74**).[316]



266, In five steps 17 %

268, 25 %

272, 50 %

Overall yield 4 %

Scheme 74: ***The selective protection of the secondary alcohol***

Unfortunately, the tetrahydropyranoyl group was not stable in the acidic conditions required for the catalysis of the protection and the reaction gave a mixture of two products: the needed 2-(4-methoxyphenyl)-4-[10-(tetrahydro pyran-2-yloxy)decyl]-[1,3]-dioxane (**268**) with a yield of 25 %; and the undesired, deprotected, 10-[2-(4-methoxyphenyl)-[1,3]-dioxan-4-yl]-decan-1-ol (**272**) with a yield of 39 %.

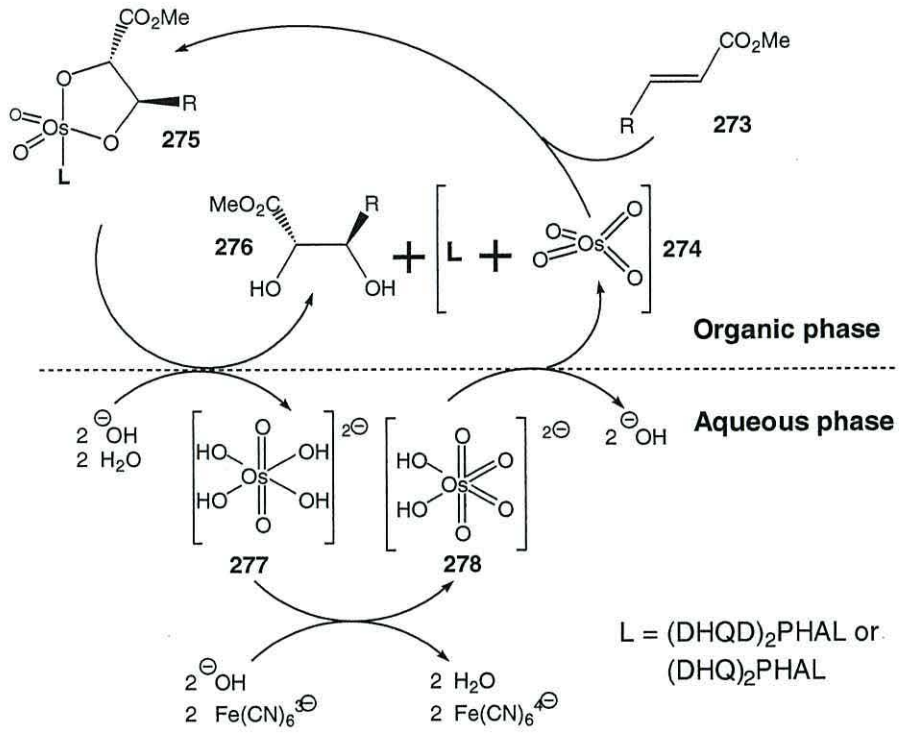
Compounds (**268**, **272**) showed similar NMR spectra to those of simpler 4-methoxyphenyl protected 1,3-diols, such as (2*S*,4*R*)-2-(4-methoxyphenyl)-4-methyl-1,3-dioxane.[333] Two doublets at about δ 7.4 and 6.9 in the ^{1}H NMR spectra of both the compound indicated the presence of a 1,4-disubstituted aromatic ring, while a singlet at δ 5.47 corresponded to the acetal proton. The only difference between the spectra was that the protected product (**268**) showed a triplet at δ 4.5 and a complex pattern for tetetrahydropyranyloxy group in the region between 3.9 and 3.3. In the ^{13}C NMR spectra of both compounds it was possible to identify four signals in the aromatic region and a signal at δ 101.0 for the acetal carbon in the α-position with respect to the aromatic ring. In the spectrum of the protected compound (**268**) the characteristic peaks of the protecting group were also displayed.

In order to complete the preparation of the β-hydroxy ester (**271**), it would have been possible to re-protect the terminal alcohol (**272**) with a different type of protecting agent and finish this synthetic route (**Scheme 67**). However, this strategy appeared to require too many steps, allowing only a very low overall yield for the final compound. Therefore, this approach was terminated in favour of others.

3.3 An approach using Sharpless dihydroxylation.

3.3.1 Overview.

The essence of the second approach examined was the Sharpless dihydroxylation. The formulation of the procedure for this asymmetric dihydroxylation resulted from the studies of Sharpless and co-workers on oxidation with OsO_4.[7] In this particular case, this oxidant is added only in catalytic concentration, because the transformation is performed in the presence of another stoichiometric co-oxidant, $K_3Fe(CN)_6$ (**Scheme 75**).[7]

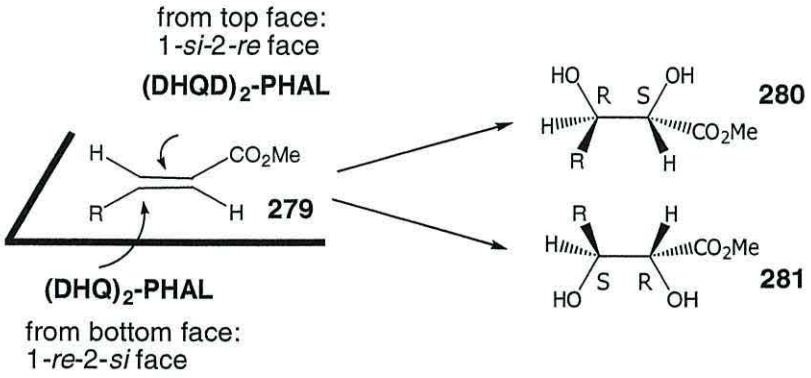


Scheme 75: ***Mechanism of the Sharpless dihydroxylation***

The reaction is conducted in a biphasic system where OsO_4 is the only oxidant present in the organic layer, while potassium ferricyanide is in the water layer as a reoxidant (**Scheme 75**). The mechanism has been thoroughly studied. Currently, one of the most accredited mechanisms includes the formation of an osmaoxatane intermediate (**275**) through the addition of osmium tetraoxide (**274**) to the double bond. This complex (**275**) then undergoes hydrolysis, giving the diol (**276**) and the osmium salt (**277**) which is finally reoxidised in two steps to its original form.

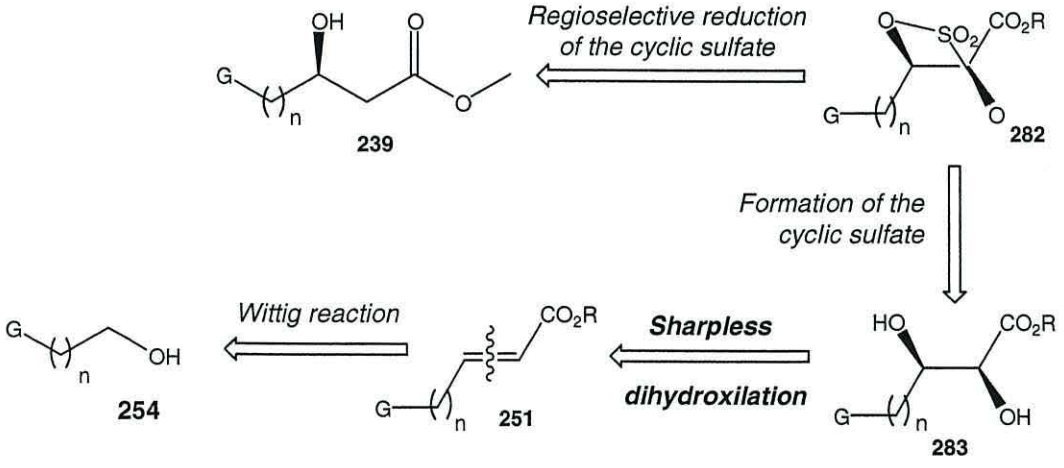
Finally, in order to obtain an asymmetric reaction Sharpless *et al.* proposed to use one of two diastereomeric ligands: either $(DHQD)_2PHAL$ or $(DHQ)_2PHAL$,

which bond the osmium atom at the reactive species (**275**) controlling the stereoselectivity of the reaction.[334] These PHAL derivatives have an ideal structure for catalysing the reaction, providing both high ligand acceleration and enantioselectivity.[334] Each of these dimers has a binding pocket that stabilises the transition state of the reaction but also forces the attacks on the olefin on only one of the two faces. In particular, $(DHQD)_2PHAL$ attacks the 1-*si*-2-*re* face of (*E*)-alkenes (**270**), permitting the acquisition of the (2*S*,3*R*)-dihydroxy compound (**280**), while $(DHQ)_2PHAL$ produces the other diastereoisomer (**281**, **Scheme 76**).



Scheme 76: ***The stereoselectivity of the dihydroxylation***

With the aim of performing a Sharpless dihydroxylation as a mean to introduce the hydroxyl group in the β-position, the cyclic sulfate (**282**) was identified as the first key target in the retrosynthesis (**Scheme 77**).



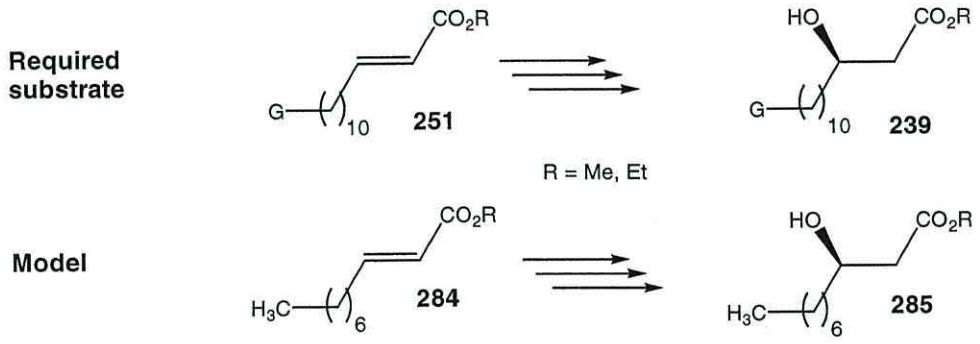
Scheme 77: ***The Sharpless dihydroxylation approach for the preparation of β–hydroxy ester***

This sulfate ester (**282**, **Scheme 77**), in fact, could be converted into the desired β-hydroxy ester through regioselctive reduction at the α-position with respect to the carboxylic group. This five-membered ring (**282**) was prepared from the 1,2-diol compound (**283**) via a cyclic sulfite intermediate. The latter (**283**) came from the α,β-unsaturated ester (**251**) through the Sharpless asymmetric dihydroxylation. Finally, the (*E*)-alkene ester (**251**) was produced by a Wittig coupling, following the same synthetic strategy employed previously (**Section 3.2.2**).

A similar strategic route has recently been utilised for the preparation of a similar compound, *R*-(+)-α-lipoic acid.[335]

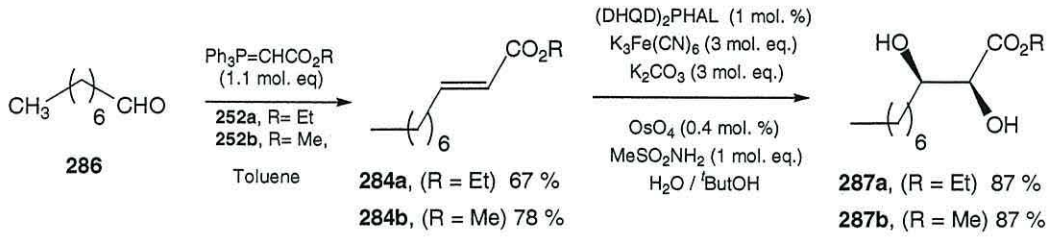
3.3.2 A possible model for the approach

Initially, this method was tried on model compounds (**284**) to verify its applicability (**Scheme 78**).



Scheme 78: ***A possible model for α,β-unsaturated ester***

The starting materials, α,β-unsaturated esters (**284a**, **284b**, **Scheme 79**), were prepared from octanal (**286**) with both (ethoxycarbonylmethylene)-triphenylphosphorane (**252a**) and the methoxy analogous (**252b**), using an identical procedure to that employed before for the Wittig coupling (**Section 3.2.2**). The use of the methyl phosphonium salt (**252b**) as the stabilised ylid was preferable, since this could be more easily purified and dried. The alkene (**284b**) was produced with a 78 % yield and no signals for the minor (*Z*)-stereoisomer were detected in the ^{1}H NMR spectrum. However, the experiments with this salt (**252b**) were conducted only in a later stage of the Ph.D. Therefore, the ethyl phosphonium salt (**252a**) was used in some of the following reactions.



Overall yield for R = Et, 58 %

Overall yield for R = Me, 68 %

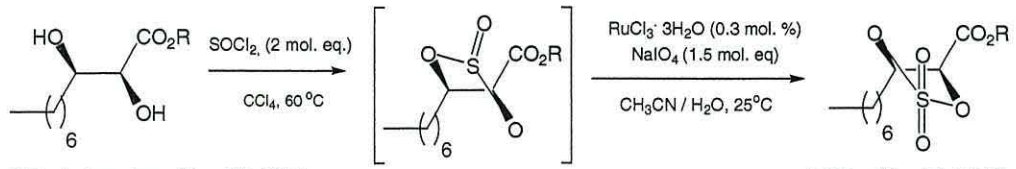
Scheme 79: ***The Sharpless dihydroxylation reaction on a model compound***

These compounds (**284a**, **284b**) were then converted into the dihydroxy compounds (**287a**, **287b**) using $(DHQD)_2PHAL$ (1 mol %) as ligand, in the presence of OsO_4 (10 mol. %) and $K_3Fe(CN)_6$ (3 mol eq.) in a mixture of ^tBuOH and H_2O (1:1). $MeSO_2NH_2$ (1 mol. eq.) was also added, since it favoured the hydrolysis of the osmium (VI) glycolate intermediate (**275**, **Scheme 75**);[336] otherwise, it would be necessary to increase the temperature from the recommended 0 °C, which would ultimately produce a decrease in the stereoselectivity of the reaction.[337]

The spectroscopic data of both products (**287a**, **287b**) were compared with those reported in the literature, and found to be similar.[338,339] In particular, the specific rotation of (2*S*,3*R*)-dihydroxydecanoic acid ethyl ester (**287a**), was found to be equal to + 9.8, (in the literature, for the same compound, the $[\alpha]_D^{25}$ was reported as + 10.1).[340] For the corresponding methyl ester (**287b**), the $[\alpha]_D^{23}$ was measured as + 10.8, (in the literature, for the same compound, the $[\alpha]_D^{25}$ was reported as + 11.23).[339]

3.3.3 The cyclic sulfate formation

The second key operation in this strategy (**Scheme 77**) was the conversion of the diols (**287a**, **287b**) into the cyclic sulfates (**289a**, **289b**). This transformation consisted of two reactions: first, the formation of the cyclic sulfites (**288a**, **288b**) using thionyl chloride; secondly, their oxidation with sodium (meta)periodate (1.5 mol. eq.) in the presence of ruthenium trichloride hydrate (0.3 mol. %, **Scheme 80**).[10]



287a, in two steps (R = Et) 58 %
287b, in two steps (R = Me) 68 %

288a, 288b

289a, (R = Et) 71 %
289b, (R = Me) 84 %

Overall yield for R = Et, 41 %
Overall yield for R = Me, 57 %

Scheme 80: ***The formation of the cyclic sulfate***

During the first reaction, HCl was produced. Since the intermediates (**288a, 288b**) were not stable in acidic conditions, it was necessary to remove the acid by refluxing the reaction mixture at 60 °C under a strong flow of nitrogen.

The purified final products (**289a, 289b**) exhibited IR spectra with two intense peaks at 1399 and at 1210 cm^{-1} characteristic of the sulfate ring.[341] Their ^{1}H NMR spectra also possessed an unusual group of signals for the protons of this ring. The proton at the α-carbon with respect to the carboxylic group appeared as a heavily tended doublet (J = 7.2 Hz) at about δ 4.85, while the H in the β-position appeared as a multiplet resembling a broad quartet at about δ 5.00 (**Figure 35**). Similar results were reported in the literature for other cyclic sulphate esters.[331,335]

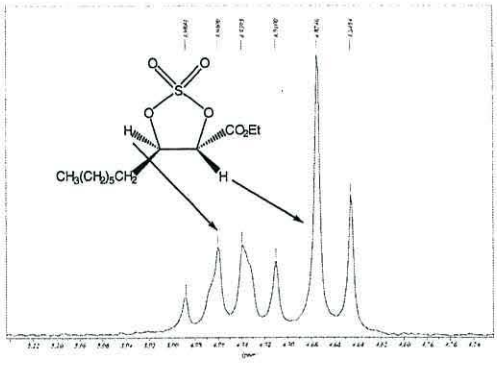
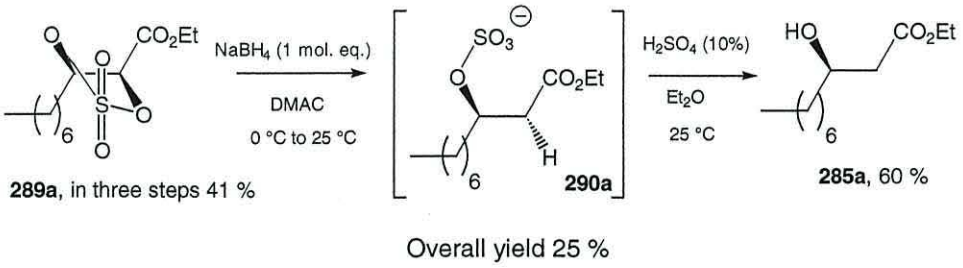


Figure 35: ***The signals of the proton in the cyclic sulphate***

Finally, the ^{13}C NMR spectra of both compounds contained two peaks in the region between δ 85 and 79 for the two carbons of the five-membered ring. The optical rotation of the methyl ester was found to be + 30.7, similar to what was reported in the literature for (4*S*,5*R*)-2,2-dioxo-5-pentadecyl-2λ^{6}-[1,3,2] dioxathiolane-4-carboxylic acid ethyl ester ($[\alpha]_D^{25} = +34.6$).[331]

3.3.4 The reduction of the cyclic sulfates

The next step was the nucleophilic addition of hydride at the α-position of the cyclic sulfate (**289a**), followed by hydrolysis of the intermediate (**290a**) in strongly acidic conditions (**Scheme 81**).[10] The reduction was performed using sodium borohydride (1 mol. eq.) in *N*,*N*-dimethylacetamide. It produced the sulfonic ester (**290a**) which was not isolated, but was directly transformed into the β-hydroxy ethyl ester (**285a**) with H_2SO_4 (10 %) in a biphasic system using ether as cosolvent.



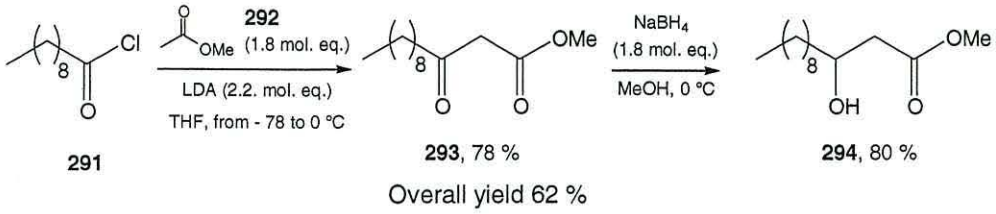
Scheme 81: ***The reduction of the cyclic sulphate***

The 3-(*R*)-hydroxy-decanoic acid ethyl ester (**285a**), obtained with a yield of 60 %, gave identical NMR spectra to those reported in the literature for the same compound and it had a very similar optical rotation (measured $[\alpha]_D^{22} = -8.2$, lit. $[\alpha]_D^{20} = -8.3$).[342]

3.3.5 Comparison of the 3-(*R*)-hydroxy-decanoic acid ethyl ester with a racemic sample

A racemic mixture of a β-hydroxy ester (**294**) had been prepared, for another use (**Section 4.2.2**) but it could also be employed in determinating of the optical purity of the 3-(*R*)-hydroxyester (**285a**).

For the production of the β-hydroxy ester (**294**), the first step was the coupling between methyl acetate (**292**, 1.8 mol. eq.) and the acyl chloride (**291**) using LDA (2.2 mol. eq.), prepared *in situ*. This was followed by the reduction of the β-keto ester (**293**) with $NaBH_4$ (1.8 mol. eq.) in methanol at 0 °C. Both reactions employed standard procedures and their respective products displayed identical NMR data to those reported in the literature.[343,344]



Scheme 82: ***Preparation of the model compound***

This compound was than used to prove that chiral GC was capable of separating a racemic mixture of β-hydroxy esters (**294**, **Figure 36**). The subsequent detection of only one signal for 3-(*R*)-hydroxy-decanoic acid ethyl ester (**285a**) was an encouraging outcome, which confirmed the stereoselectivity of the reaction.

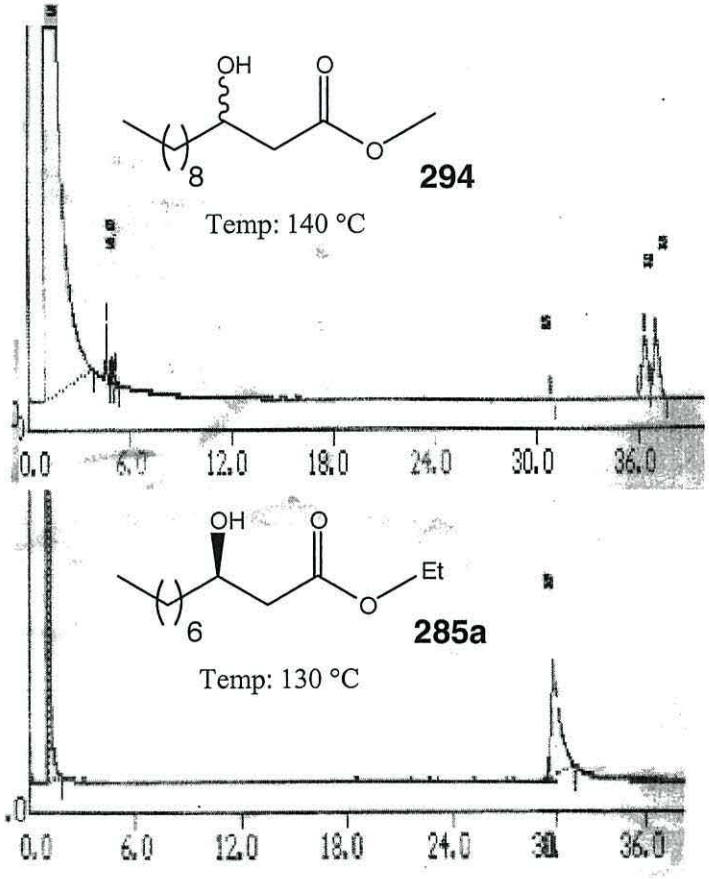
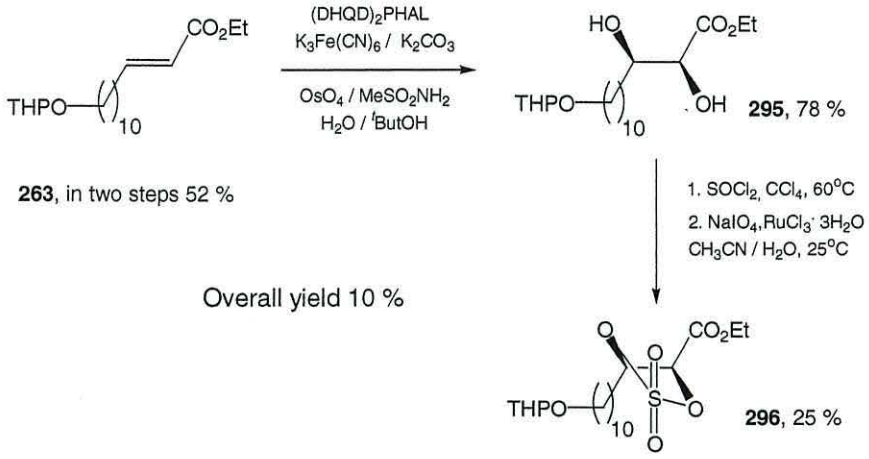


Figure 36: ***The chiral GC chromatogram for (285a) and a racemic homologue (294)***

In conclusion, this route led to the stereoselctive synthesis of the model compound in only 4 steps and with an overall yield of 25 %, a promising basis for the use of the Sharpless dihydroxylation in the preparation of the target β-hydroxy ester with both ends functionalised (**239**, **Scheme 67**).

3.3.6 The use of the pyranyloxy protection

The first reaction was the conversion of the olefin (**263**) into the dihydroxy compound (**295, Scheme 83**) following the method previously explained (**Section 3.3.1**).



Scheme 83: ***Attempted for the formation of the cyclic sulphate with the pyranyloxy protection***

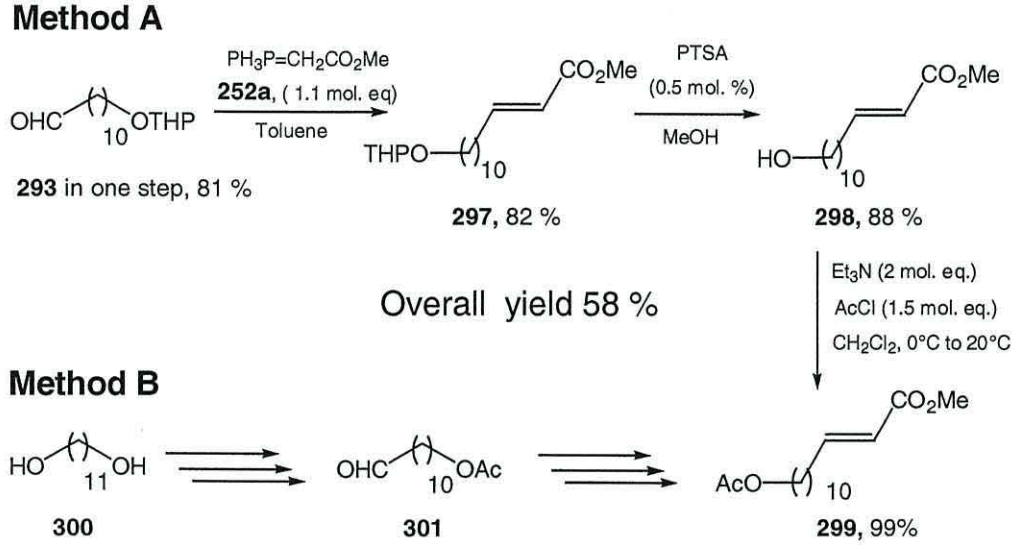
The ^{1}H NMR spectrum of the 2,3-dihydroxy-13-(tetrahydropyran-2-yloxy)tridecanoic acid ethyl ester (**295**) included the distinctive signals for the protecting group, in addition to a quartet (J = 7.0 Hz) at δ 4.23 (for $CO_2C\underline{H}_2CH_3$), and a doublet (J = 1.5 Hz) at 4.0 for the proton in the α-position with respect to the carboxylic group. A peak at δ 173.6 (for the carboxylic carbon) in the ^{13}C NMR spectrum, and the expected two peaks at δ 73.0 and 72.5 (for the carbon atoms adjacent to the two free hydroxyl groups) confirmed the formation of the desired product (**295**).

Unfortunately, the second step, which should have led to the formation of the cyclic sulfate (**296**), was not successful. In fact, the protecting group was labile in the acidic conditions produced during the reaction, providing a very low yield of (**296**). The compound was fully characterised anyway. The NMR spectra contained, in addition to the signal for the protecting group, the same distinctive multiplet for the cyclic sulfates group: at ~ δ 4.9 in the ^{1}H NMR spectrum; at δ 84.1 and 80.0 in the ^{13}C NMR spectrum.

Since the final step also required very strong acidic conditions, it was decided to replace the pyranyloxy group with another, more suitable protecting group, such as an acetate ester.

3.3.7 The use of acetate protection

In order to attempt this new approach, another α,β-unsaturated ester was prepared, 13-(tetrahydropyran-2-yloxy)tridec-2-enoic acid methyl ester (**297**, **Scheme 84**), by a Wittig reaction but with another ylid, (methoxycarbonylmethylene) triphenylphosphorane (**252a**), since it had been shown to provide better results (**Section 3.3.2**). Having obtained (**297**), this was fully characterized: its spectra were very similar to the ethyl ester previously made, except for the signals for the ester group. In this case, the methoxy group occurred as a singlet at δ 4.58 in the ^{1}H NMR spectrum and a peak at δ 51.3 in the ^{13}C NMR.



Scheme 84: ***The formation of the acetyl protected α, β-unsaturated ester***

Subsequently, it was necessary to remove the pyranyloxy protection from compound (**297**) before the asymmetric dihydroxylation in order to avoid future problems due to the presence of several hydroxyl groups. The deprotection was achieved using *p*-toluene sulphonic acid monohydrate (5 mol. %) as a catalyst.[345] The ^{1}H NMR spectrum of the purified product (**298**) included a new triplet (J = 6.7 Hz) at δ 3.61 attributable to the protons adjacent to the alcohol, while in the ^{13}C NMR, there was a new peak at δ 62.9 (for $\underline{C}H_2OH$). The signals for the stretching of the C=O and C=C bond appeared in the IR spectrum at 1726 and 1656 cm^{-1} respectively.

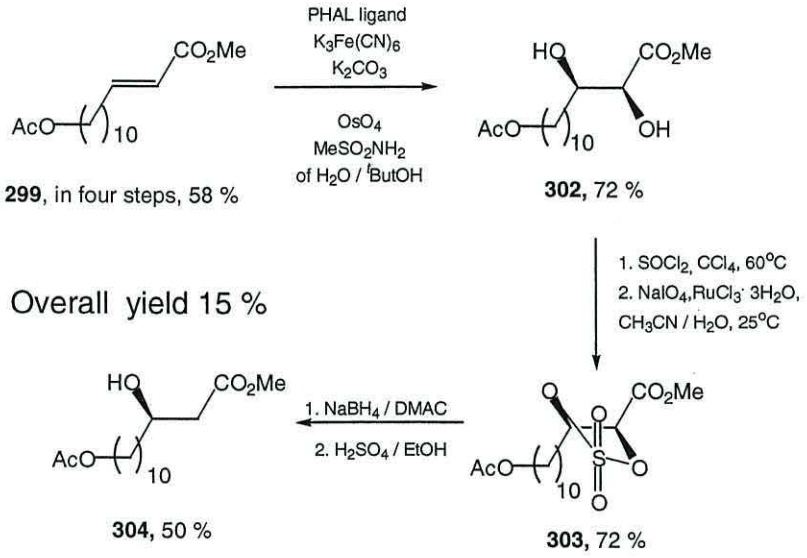
Finally, the alcohol (**298**) was treated with acetyl chloride (1.5 mol. eq.) in the presence of triethylamine (2 mol. eq.). The protection for the hydroxyl group was

proven by the presence in the ^{1}H NMR spectrum of a triplet (J = 6.7 Hz) at δ 4.04 (for $C\underline{H}_2OC{=}OCH_3$) and a singlet at 2.04 (for $CH_2OC{=}OC\underline{H}_3$). The ^{13}C NMR spectrum included two peaks in the region of the carboxyl groups at δ 171.0 and 167.1 as expected for the two different esters included in the compound (**299**).

Alternative methods for obtaining this methyl ester (**299**) might require fewer steps; for example, this compound could be prepared directly from the acetoxy-protected aldehyde (**301, Method B, Scheme 84**). However, the chosen route (**Method A**) seemed more secure, because it did not present any unresolved problems: moreover, it seemed to be reasonably efficient, giving an overall yield of 58 %.

3.3.8 The Sharpless dihydroxylation using the acetate protection

The unsaturated ester (**299, Scheme 85**) was converted into the dihydroxy ester (**302**) with the Sharpless method used before (**Section 3.3.2**).



Scheme 85: ***The formation of β-ester with a protected terminal alcohol***

The IR spectrum of the prepared diol (**302**) contained a large band at 3400 cm^{-1} (O-H stretching) and a peak at 1734 cm^{-1} (for the C=O stretching). The NMR spectra of this compound (**302**) showed similar patterns to those already discussed for the ethyl ester (**295, Scheme 83, Section 3.3.6**), except those for the different protecting group.

Having achieved the first goal, it was then possible to prepare the cyclic sulfate (**303**) using the approach illustrated above (**Section 3.3.3**). The ^{1}H NMR spectrum of this displayed the typical signals at about δ 4.9 for the two protons of the five-membered ring and two singlets at 3.88 and 2.03, for the methoxy and methyl groups, respectively. The presence of two carboxylic groups led to two peaks at δ 174.0 and 171.2 in the ^{13}C NMR spectrum.

Finally, the nucleophilic addition of sodium hydride to compound (**303**), followed by hydrolysis with sulphuric acid, gave the desired product (**304**), even if with a yield of only 50 %. The product was easily identified as the β-hydroxy ester (**304**) because it showed in the ^{1}H NMR spectrum, two double doublets for the diastereotopic protons in the α-position with respect to the carboxylate group at δ 2.53 and 2.40. They showed a reasonably large geminal coupling (J = 16.5 Hz), due to the proximity of the carboxylic acid. They also showed vicinal couplings with the neighbouring proton at the chiral centre equivalent to 3.4 Hz for the "*cis*" coupling and 8.6 Hz for the "*trans*" coupling (**Figure 37**) as shown below.

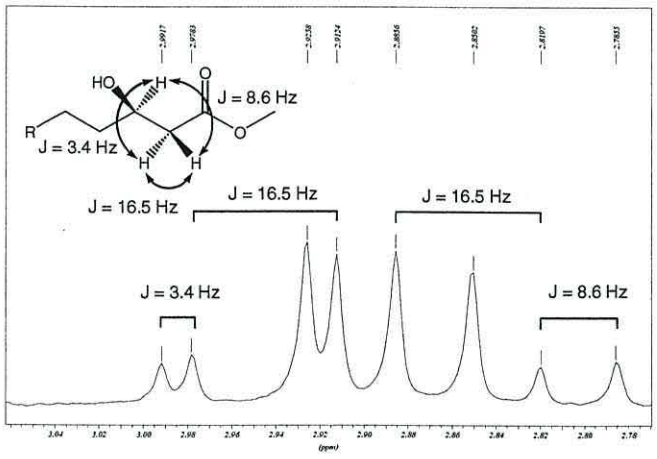


Figure 37: ***The coupling constants for the protons adjacent to the carboxylic group***

The ^{13}C NMR spectrum showed two peaks in the region of the carboxylic groups at δ 173.4 and 171.2 and three others in the region corresponding to the carbons next to an oxygen atom at δ 66.5 (for $\underline{C}HOH$), 66.1 (for $\underline{C}H_2OCO$), and 53.1 (for $O\underline{C}H_3$). The specific rotation for the compound (**304**) was measured as - 12.5 (lit. for the similar compound, (*R*)-3-hydroxy-hexadecanoic acid methyl ester, it was - 13.8).[346] Finally, in the low resolution mass spectrum, not only was there an M+1

ion, but there were also two peaks at 229 and 103 m/z, corresponding to the cleavage of the α-bonds with respect to the hydroxyl group, which is one of the most typical fragmentations for a secondary alcohol (**Figure 38**).

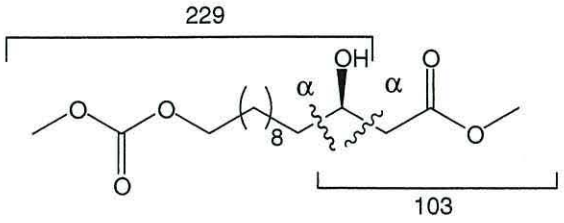


Figure 38: ***The possible fragmentation of the compound (304)***

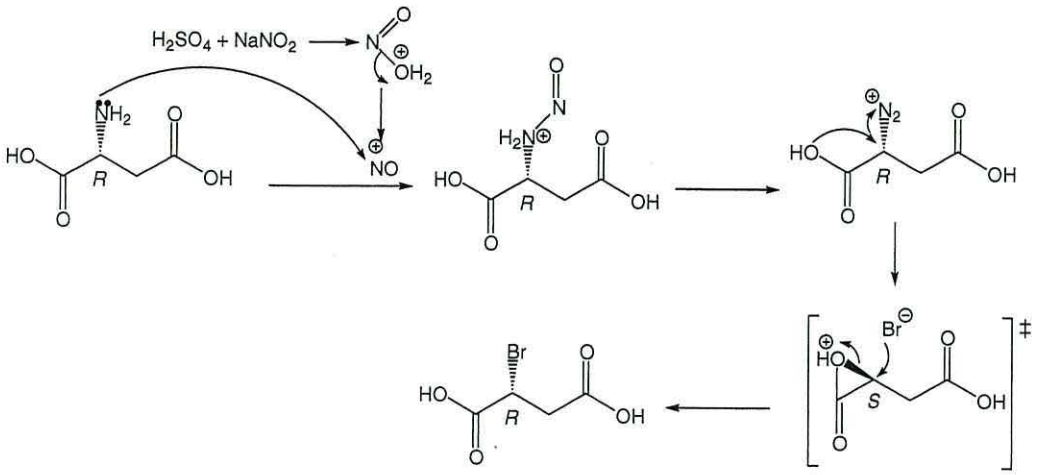
This method was successful, producing the compound in the desired optically pure configuration and with an overall yield of 15 %. However, another method was subsequently investigated, to ascertain its efficacy and productivity.

3.4 The use of a chiral starting material

3.4.1 Overview.

The final approach utilized for the formation of (*R*)-β-hydroxy esters with a long, functionalised side chain (**239**) was very different from both those previously discussed (**Section 3.2**, **Section 3.3**). In this process, the chiral centre was not obtained through asymmetric synthesis; it was present from the beginning, in the initial starting material, D-aspartic acid (**307**).

One of the crucial operations of the new synthetic strategy was the conversion of this α-amino (**307**) acid into α–bromo succinic acid (**311**) since it was the only transformation in the whole process that involved the chiral centre, which remained unaltered thereafter. This transformation was particularly interesting, since, this formal substitution of the amino group happened with retention of configuration. Its mechanism deserves more detailed analysis in order to understand the reasons for the particular stereoselectivity (**Scheme 86**).

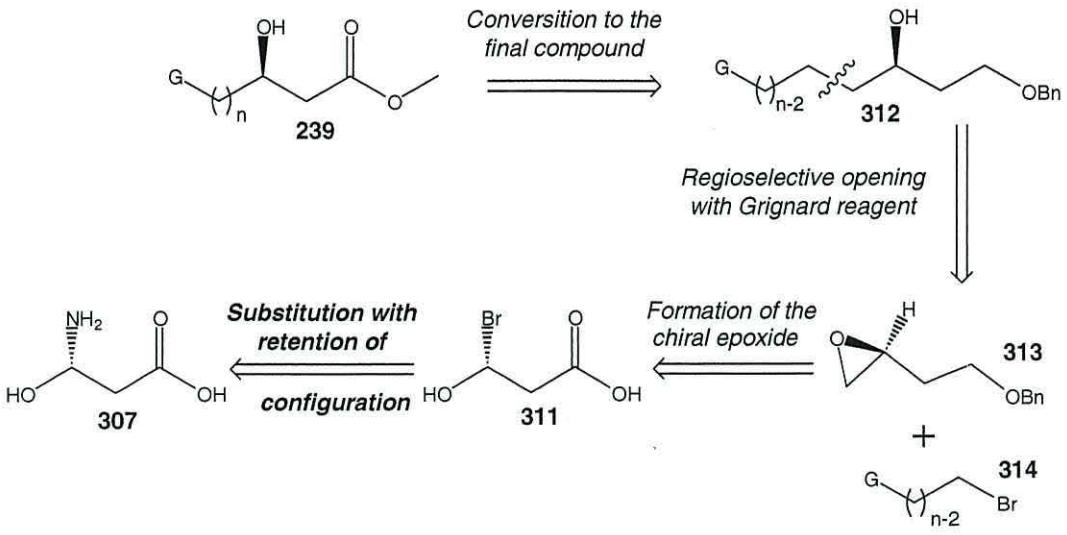


Scheme 86: ***Mechanism of the substitution with retention of configuration***

The reaction includes an initial treatment with the *in situ*-prepared nitrous acid (**305**), which leads to the formation of highly unstable species, such as the diazonium salt (**309**, **Scheme 86**). This intermediate (**309**) then undergoes a regioselective intramolecular substitution by the adjacent carboxylic acid, forming another reactive species (**310**). The subsequent nucleophilic addition of the bromine ion onto the three-membered ring leads to the final compound (**311**). Thus two attacks on the chiral atom occur, both of them characterised by a complete inversion of configuration of the centre, providing overall retention of configuration.[347]

Frick *et al.* recently developed a method based on this reaction for the preparation of (2-benzyloxyethyl)oxiranes (**313**, **Scheme 87**), which have been widely utilised as chiral building blocks in the synthesis of numerous biologically important compounds.[348] In reality, Frick *et al.*[348] employed L-aspartic acid in order to obtain the (*R*)-oxirane but, for the present synthesis, it was necessary to use the, non naturally occurring, D-amino acid (**307**), which was much more expensive. Unfortunately, to the best of our knowledge, methods to obtain the (*S*)-(2-benzyloxyethyl)oxirane (**313**) from L-aspartic acid with only one substitution and inversion of configuration are not reported.

Inspired by the above paper a new synthetic route has been proposed by Al Dulayymi *et al.* for the preparation of a similar α-alkyl-β-hydroxylated fatty acid motif of another mycolic acid.[253] Therefore, it was decided to test it for the preparation of the α^1-mycolic acid in this study.



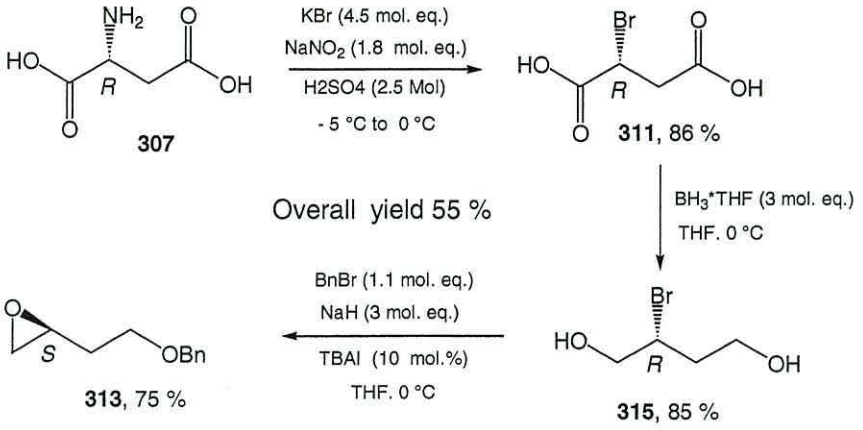
Scheme 87: ***Retrosynthesis of the third approach to β-hydroxy ester using D-aspartic acid***

In particular, analysing Al Dulayymi's strategy, the first important finding was that the precursor (**312**) could be easily converted into the final compound (**239**), with few functional group interconversions. Subsequently, an interesting disconnection could lead to the epoxide (**313**) and the alkyl bromine (**314**). In fact, this kind of compound easily arose from regioselective opening of the chiral epoxide (**313**) with a Grignard reagent. The epoxide (**313**) was formed in two steps from the β-bromine diacid (**311**) utilising the synthetic procedure proposed by Frick *et al.*[348] This involved, firstly, the simultaneous reduction of both carboxylic groups followed by an intramolecular cyclisation of the β-halo alcohol. Finally, the bromosuccinic acid (**311**) was prepared from the D-aspartic acid (**307**), as described above.

3.4.2 The preparation of a chiral epoxide

The initial challenge was the preparation of the building block (**311**) starting from D-aspartic acid (**307**).

The first step was the conversion of the amino acid (**307**) into bromosuccinic acid (**311**), which was readily achieved in 86 % yield using potassium bromine (4.5 mol. eq.) in the presence of sodium nitrite (1.8 mol. eq.) in an aqueous solution of sulfuric acid (2.5 M).



Scheme 88: ***Formation of the chiral epoxide***

For the success of the reaction, the concentration of sulfuric acid was critical. In fact, if this was too high, the salt precipitated making the reaction impossible to accomplish. Moreover, the addition of the sodium nitrite had to be conducted very slowly, maintaining the temperature below – 5 °C. Following this procedure, it was possible to control the rate of decomposition of the *in situ*-formed nitrous acid into nitrosium ion favouring the completion of the attack by the amino group onto this ion. The prepared (*R*)-(+)-bromosuccinic acid (**311**) showed the same spectral properties as those reported.[349]

The next step was the reduction of the diacid (**311**) into the diol (**315**). Among the numerous ways for the achievement of such a task, BH_3 THF (3 mol. eq.) in THF at 0 °C was chosen. The active species, diborane, was, in fact, able to reduce the two carboxylic acids into primary alcohols under mild conditions, leaving the bromine group unchanged. Bromine had already been proven to be easily reduced with other reducing reagents (**Section 3.2.4**). This chemoselectivity was achievable because the interaction between the compound and reagent, led to the formation of acyloxyboranes manifested by evolution of hydrogen, and these boron complexes had enhanced reactivity towards reduction.[350]

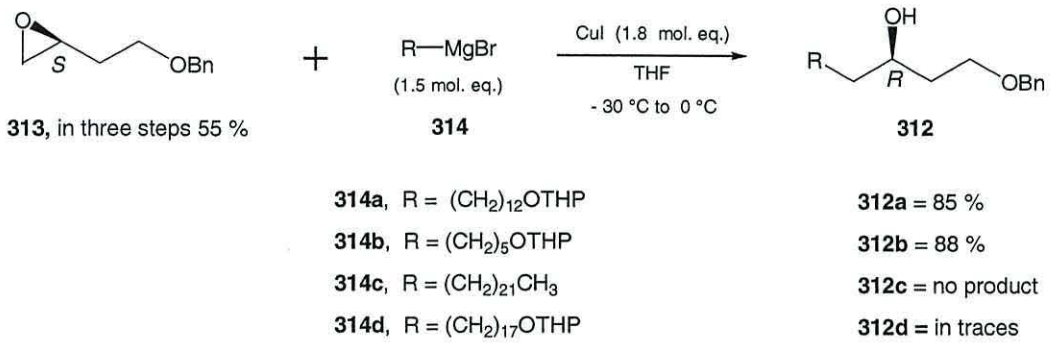
The diol (**315**) displayed similar spectral properties (NMR, IR data) to those reported for the opposite diastereoisomer, except that an $[\alpha]_D^{23}$ of + 29.2 was found, whilst in the literature for the *S* isomer $[\alpha]_D^{24}$ = - 31.9 was claimed. [348]

The oxirane (**313**) was finally obtained from this diol (**315**) by treatment with NaH (3 mol. eq.). The epoxidation was carried out in the presence of benzyl bromide (1.1 mol. eq.) in order to obtain the simultaneous protection of the other

hydroxyl group. The reaction was conducted in THF using tetrabutylammonium iodide (10 mol. %), which is a standard catalyst for the benzylation of alcohol in organic solvents, since it leads to the *in situ*-formation of benzyl iodide. The purified product (**313**) showed the same NMR spectra as those reported for the required oxirane and had a similar optical rotation.[351] A $[\alpha]_D^{21}$ = - 16.5 (c = 1.1, $CHCl_3$) was measured, while in the literature for the same compound an $[\alpha]_D^{23}$ of - 15.6 was indicated.[346]

3.4.3 The opening of the epoxide

Having achieved the preparation of this chiral building block (**313**, **Scheme 89**), the next planned synthetic operation was the regioselective opening of it by a Grignard reagent (**314a**, 1.5 mol. eq.).



Scheme 89: ***Ring opening with different Grignard reagents***

The cleavage of the epoxide bond at the less substituted carbon was the favoured reaction, since this was the most accessible position for the nucleophilic attack. The coupling was catalysed by copper (I) iodide because this salt allowed the reaction to take place under the relative mild conditions of – 30 °C.[352] The reaction was maintained at this temperature through the whole experiment in order to avoid secondary reactions. It was equally important to obtain complete reaction, since the separation of the desired product (**312a**) from starting material (**313**), if present, was not easy by column chromatography. Therefore, the reaction was continuously monitored and only quenched by addition of aq. ammonium salt solution after completion.

In the IR spectrum of the alcohol **312a**, it was possible to observe the signal for the O-H stretching at 3456 cm^{-1}. In its ^1H NMR spectrum, the expected signal for

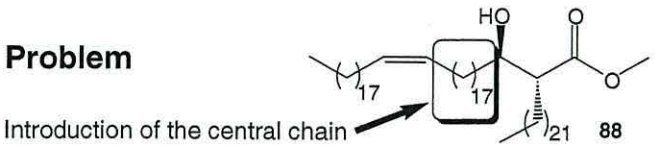
the proton next to the new hydroxyl group was not clearly identified, because it was hidden among the signals of the two protecting groups between δ 3.9 and 3.7. However, the spectrum showed the characteristic signals for both the tetrahydropyranyloxy group (already described) and the benzyloxy group (for example, a multiplet at δ 7.40-7.32 for the aromatic protons, and a singlet at 4.53 for the two benzyl protons), suggesting that the coupling has been successful. This result was confirmed by the presence in its ^{13}C NMR spectrum of not only the peaks for both protecting groups, but also a new one at δ 71.4 for the carbon adjacent to the hydroxyl group. In the literature, the secondary hydroxyl group in (*S*)-(-)-l-benzyloxy-3-hexadecanol was reported to resonate at δ 71.3.[346] Moreover, the reaction was also stereocontrolled giving an optically active compounds (**312a**) with a rotation of + 7.3, which was similar to that reported in the literature for a related compound, (*R*)-1-benzyloxy-hexadecan-3-ol ($[\alpha]_D^{23} = +$ 7.9).[346]

The same kind of reaction was repeated several times using Grignard reagents with carbon chains of different lengths. The coupling was also accomplished with 5-(tetrahydropyran-2-yloxy)pentyl magnesium bromine (**314b**) producing the secondary alcohol (**312b**) with an 88 % yield, but it was not successful when using bulkier Grignard reagents, either non-functionalised at their extremity (**314c**) or with a functional group (**314d**). Using docosanyl magnesium bromine (**314c**), the reaction did not give the desired product even in traces. When 17-(tetrahydropyran-2-yloxy)heneicosanyl magnesium bromine (**314d**) was employed, the desired product (**312d**) was obtained but in a very low yield. Although it was not completely purified, its spectral properties were consistent with those of the previously prepared (**312a**).

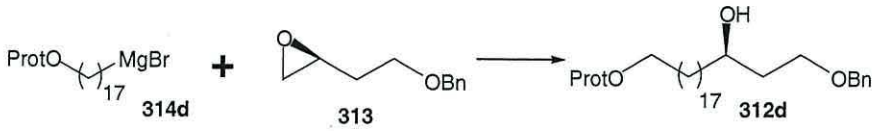
The failure of the reaction using docosanyl magnesium bromine (**314c**) suggested that it would not be possible to introduce the whole meromycolate chain at this stage of the synthesis.

Moreover, the extremely low yield for the reaction with C_{17} alkyl magnesium bromine (**314d**), when compared with the good results obtained using smaller reagents (**314a**, **314b**) prompted reflection upon the best sequence for the introduction of the central chain between the double bond and the hydroxyl group in only one stage (**Scheme 90**).

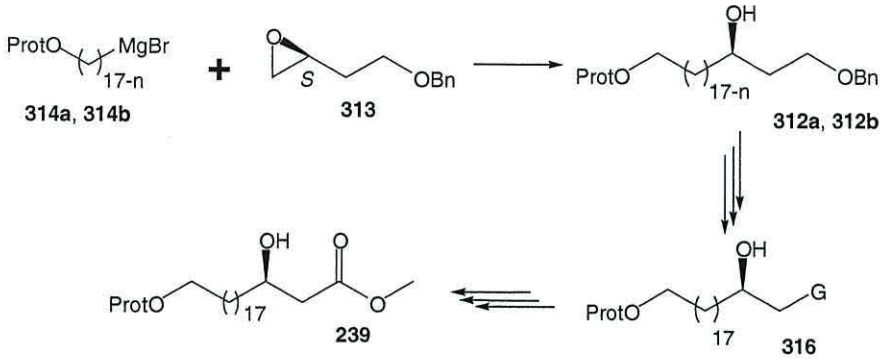
Problem



Method A: Addition of the whole chain in only one stage



Method B: Addition of a smaller chain followed by its extension



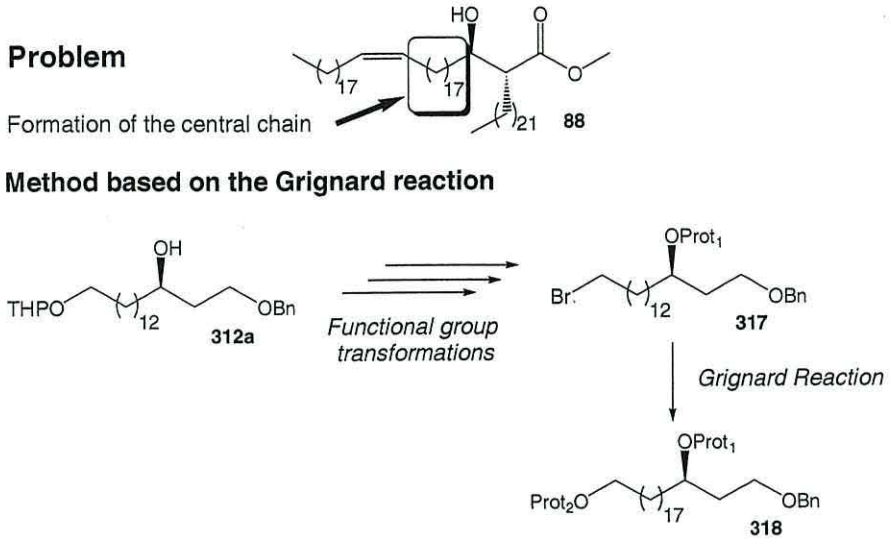
Scheme 90: ***Different sequences for the introduction of the central chain***

The introduction of the whole segment in one step would have provided a more elegant synthetic strategy (**Method A**), but this approach did not appear to be profitable when employing a Grignard reagent, as seen above. Therefore, it seemed more appropriate to introduce only a smaller part of the carbon chain and then extend it in a later phase of the α^1-mycolic acid preparation (**Method B**), which had the potential for better overall yield. The remaining questions was when and how to extend the chain?

3.4.4 An attempt to extend the side chain –the protection of the secondary alcohol

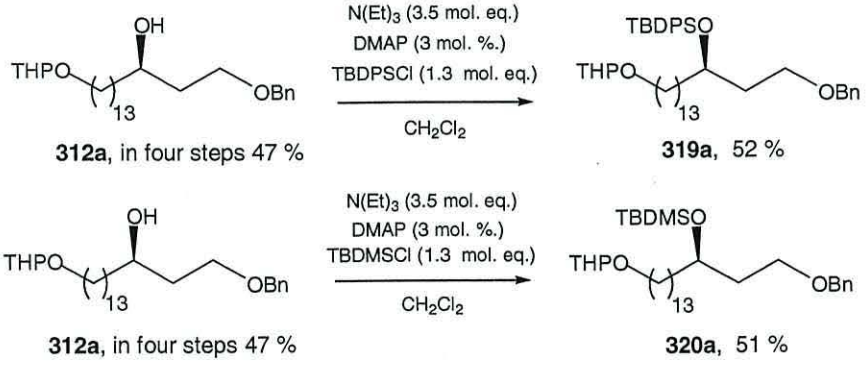
In order to extend the side chain (**Method B**, **Scheme 90**), a possibility was to use a Grignard reaction. This method has already been proved to be direct and efficient for the preparation of simple chains, although it was not reliable for the preparation of more complex chains (see **Chapter 2**). However, in order to definitely exclude its applicability for the extension of the side chain, some experiments were designed (**Scheme 91**). Firstly, a better leaving group was to be

inserted on the side chain; secondly, this substrate (**317**) was to be used to test this reaction in the production of the desired intermediate (**318**).



Scheme 91: ***Method based on the Grignard reaction for the extension of the side chain***

First of all it was necessary to protect the secondary hydroxyl group of the chiral alcohol (**312a, Scheme 92**).



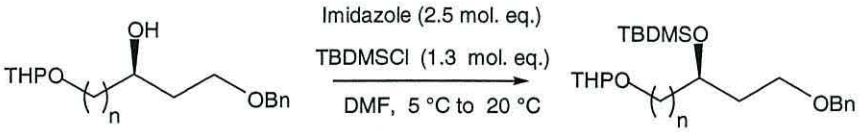
Scheme 92: ***Protection of the secondary alcohol***

The protection of this group as silyl ether using either *tert*-butyldiphenylsilylchloride (1.3 mol. eq.) or *tert*-butylmethylsilylchloride (1.3 mol. eq.) appeared to be most appropriate since both of them are reasonably stable in a wide range of situations, but can also be readily eliminated.

Initially, the protection was attempted in dry dichloromethane, using triethylamine (3.5 mol. eq.) as base and dimethylaminopyridine (3 mol. %) to drive the reaction. Both reactions gave the protected products (**319a, 320a**), as shown by their

spectral analysis. For example, both ^{1}H NMR spectra included a singlet at ~ δ 0.9 for the 9 protons of the *tert*-butyl group next to the silyl atoms; whilst the ^{13}C NMR spectra contained the peak for the quartenary carbon of the *t*-butyl group at δ 19.3 (for **319a**) or 18.0 (for **320a**). However, both conversions did not provide a satisfactory yield, even after several days.

Different conditions were therefore tested for the protection of the secondary alcohol with *tert*-butylmethylsilylchloride (1.3 mol. eq.) and imidazole (2.5 mol. eq.) as base in a polar aprotic solvent, DMF (**Scheme 93**).[353] The yield of the transformation improved to 92 % when the smaller starting material (**312b**) was employed.



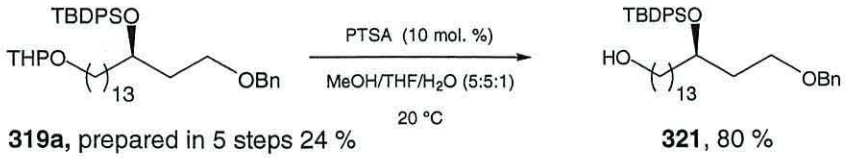
312a, n = 13 prepared in four steps 47%
312b, n = 6 prepared in four steps 48 %

320a = 87 %
320b = 92 %

Scheme 93: ***The use of different procedure for the protection of the secondary hydroxyl group***

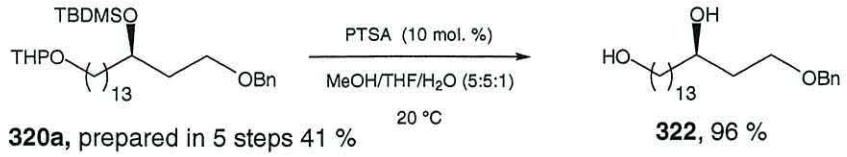
3.4.5 An attempt to extend the side chain – the introduction of a better leaving group on the side chain

The next synthetic operation was the conversion of the functional group at the extremity of the side chain into a better leaving group (**Scheme 94**).



319a, prepared in 5 steps 24 %

321, 80 %



320a, prepared in 5 steps 41 %

322, 96 %

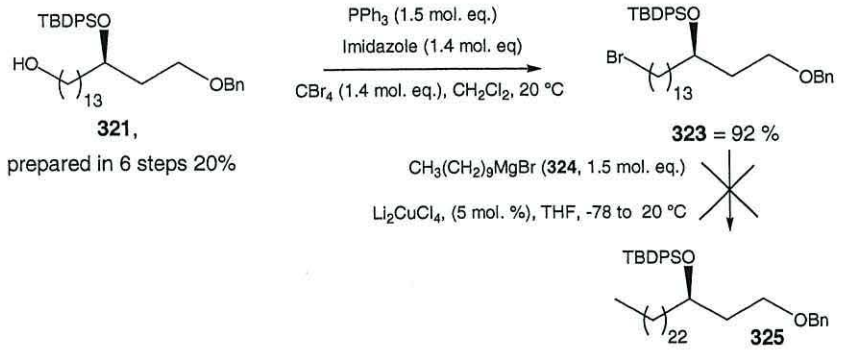
Scheme 94: ***Elimination of the tetrahydropyranyloxy protection***

The first task was the deprotection of the terminal hydroxyl group from the fully protected triols (**319a**, **320a**, **Scheme 94**), with *p*-toluenesulfonic acid

monohydrate (10 mol. %), leaving the silyl ether unaffected at the secondary hydroxyl group. The reaction was successful if the internal hydroxyl group was protected as *t*-butyldiphenyl silyl ether (**319a**), but not if it was protected as *t*-butyldimethyl silyl ether (**320a**), which was less stable under the acidic conditions employed. In this case, the diol (**322**) was obtained, as demonstrated by the spectral analysis. The NMR spectra of both compounds (**321**, **322**) showed the loss of the tetrahydropyranyloxy group by including a new triplet (J = 6.6 Hz) at ~ δ 3.63 for the two protons of the terminal hydroxyl group and at 63.2 for the corresponding carbon. In the NMR spectra (**321**), the peaks for the silyl group were still present (for example a doublet (J = 8 Hz) for the protons in the *ortho*-position at δ 7.66 of the phenyls and a singlet at 0.90 for the 3 methyls of the protecting group). In those of the product (**322**) the signals of the silyl protecting group were absent (for example, the signals for the two methyl groups next to silicon atom expected at δ 0.04 and 0.03 in the ^{1}H NMR spectrum and δ - 4.4 and - 4.7 in the ^{13}C NMR spectrum). This evidence led to the conclusion that the *t*-butyldimethylsilyl group was also lost during the transformation, producing 16-benzyloxyhexadecane-1,14-diol (**322**).

3.4.6 An attempt to extend the side chain – the Grignard reaction

Having progressed this far, the free hydroxyl group of the triol (**321**) was converted into bromine using carbon tetrabromine (1.4 mol. eq.) in the presence of triphenylphosphine (1.5 mol. eq.) and imidazole (1.4 mol. eq.), following the procedure described by Garegg and Samuelsson (**Scheme 95**).[354]

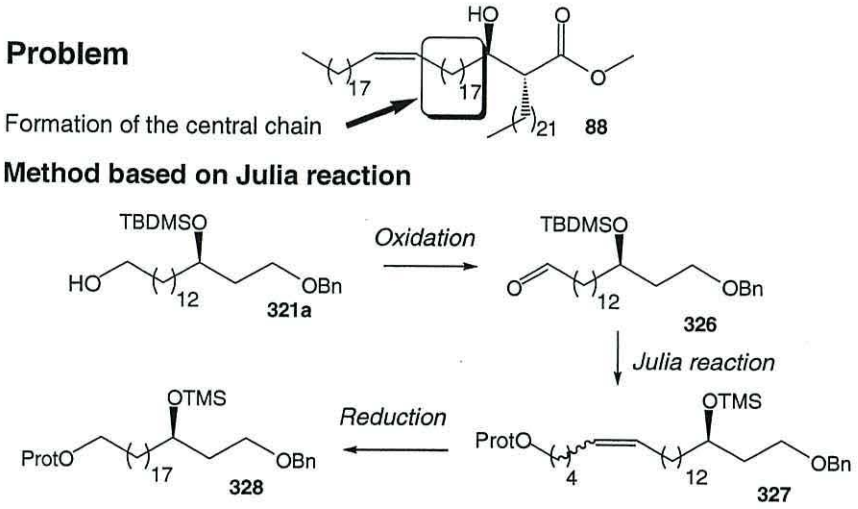


Scheme 95: ***Attempted extension of the side chain through Grignard reaction***

Comparing the ^{1}H NMR spectra of the starting material (**321**) and the product (**323**), the most significant change was the shift to a higher field of the triplet (J = 6.7 Hz), from δ 3.63 ($C\underline{H}_2OH$) to 3.43 ($C\underline{H}_2Br$), which was indicative of the success of the reaction.

Unfortunately, the subsequent Grignard coupling with decanyl magnesium bromine (**324**, 1.5 mol. eq.) using Tamura and Kochi's procedure and Li_2CuCl_4 (5 mol. %) as catalyst, did not form the desired product (**325**), only the unreacted starting material (**323**). This was probably caused by the limited solubility of starting materials (**323**, **324**), which stopped the coupling.

Since the Grignard reaction was not successful, even when the coupling molecules (**323**, **324**) were reasonably small, the possibility of its use for the extension of the side chain (**Method B**, **Scheme 90**), had to be generally excluded. A better alternative route would be a method based on the Julia reaction (**Scheme 96**).

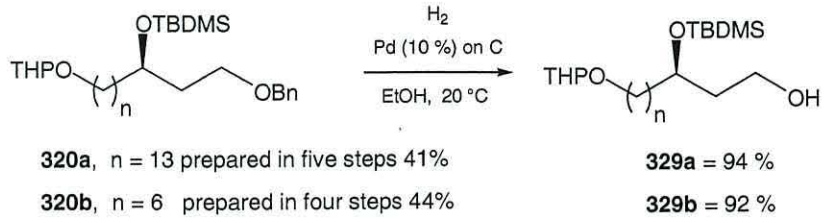


Scheme 96: ***Method based on the Julia reaction for the extension of the side chain***

It has already been demonstrated that a similar approach was very reliable in the preparation of complex long chains, see **Chapter 2**. However, by this time, it had been decided to postpone the extension of the side chain until the final stages of preparation of the α^1-mycolic acid, the reasons for which will be explained later (**Section 4.2.5**, and **Section 4.4.5**).

3.4.7 Preparation of the β–hydroxy ester

Having delayed the formation of the complete C_{17} central chain, and returning to the preparation of the β-hydroxy ester, the next step was the debenzylation of the terminal alcohol (**Scheme 97**). The hydrogenolysis was conducted in ethanol using Pd (10 %) on carbon as a catalyst.



320a, n = 13 prepared in five steps 41% — **329a** = 94 %

320b, n = 6 prepared in four steps 44% — **329b** = 92 %

Scheme 97: ***Debenzylation***

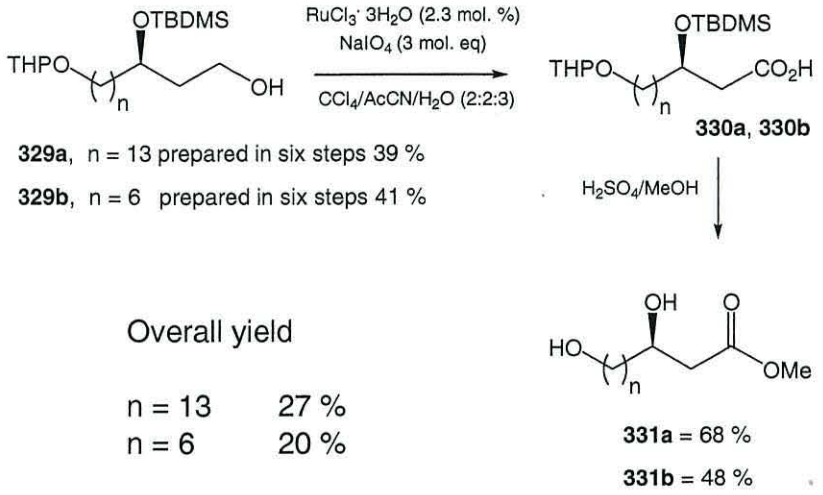
Before the addition of the catalyst it was necessary to dissolve completely the starting material (**320a**, **320b**). If the compound was not completely dissolved, the reduction of the benzyl group would not happen readily, and other side reactions could happen such as the elimination of the tetrahydropyranyl group. This type of compound is not very soluble in ethanol and so it was necessary to dilute the compound considerably (~ 0.025 M).

Both compounds (**329a**, **329b**) were fully characterized: their NMR spectra no longer suggested the presence of the benzyl group (for example a singlet at δ 4.5 for the two benzyl protons, or a peak at δ 73.2 for the benzyl carbon). Instead, signals of the terminal free alcohol were present (for example a peak at ~ δ 60.2 in the ^{13}C NMR), together with peaks for the other two protecting groups (tetrahydropyranyloxy and silyl ether).

The final steps for the production of the β-hydroxy ester, were the oxidation of the primary alcohol (**329a**, **329b**, **Scheme 98**) followed by the esterification of the acid produced (**330a**, **330b**).

The oxidation was conducted using sodium(meta)periodate (3 mol. eq.), with ruthenium(III)chloride hydrate (2.3 mol. %) as catalyst, in a mixture of three different solvents (carbon tetrachloride, acetonitrile and water, 2:2:3) following the procedure described by Carlesen *et al.*[355] Particularly important was the addition of the starting materials (**329a**, **329b**), dissolved in part of the carbon tetrachloride, which was performed very slowly over a period of 4 h so as not to

destroy the catalyst. The product (**330a, 330b**) was recovered through several extractions, since a thick black emulsion made the operation more difficult. It was not advisable to add more water in an attempt to dissolve the emulsion, because the acid (**330a, 330b**) produced was partially soluble in this solvent.



Scheme 98: ***Final oxidation to give β-hydroxy esters***

The crude product was not purified, but was immediately esterified in methanol, using a small amount of concentrated sulphuric acid as catalyst. This procedure made the formation of the methyl ester possible, as well as the deprotection of both hydroxyl groups. The desired compound (**331**) was obtained with a satisfactory yield (up to 68 %). A minor problem was the occasional occurrence of secondary reactions due to the instability of the tetrahydropyranyl group during the oxidation.

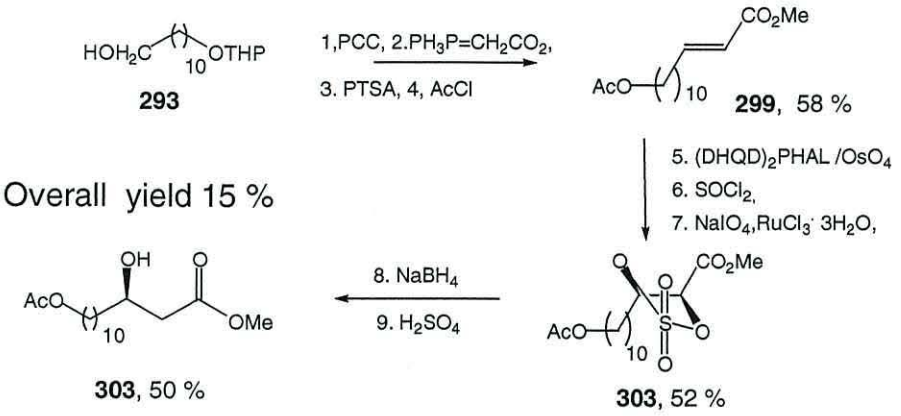
The final compounds (**331a, 331b**) showed similar spectral properties to those of the (*R*)-13-acetoxy-3-hydroxytridecanoic acid methyl ester (**304**), prepared previously (**Section 3.3.8**). In particular, in the ^{1}H NMR, two double doublets at δ 2.47 (J_1 = 16.4 Hz, J_2 = 3.7 Hz), and 2.36 (J_1 = 16.5 Hz, J_2 = 8.6 Hz,) matched the two protons in the α-position with respect to the carboxylic group. They also had a similar optical rotation. For (*R*)-3,16-dihydroxy-hexadecanoic acid methyl ester (**331a**) the $[\alpha]_D^{24}$ was - 17.6, while for (*R*)-3,9-dihydroxynonanoic acid methyl ester (**331b**) it was - 19.3. In the literature, (*R*)-3-hydroxyhexadecanoic acid methyl ester was reported to have $[\alpha]_D^{20}$ = - 13.8.[346]

Summarising the results obtained, this route permitted the stereocontrolled preparation of the β-hydroxy ester with a functional side chain in seven steps with an overall yield of 27 %.

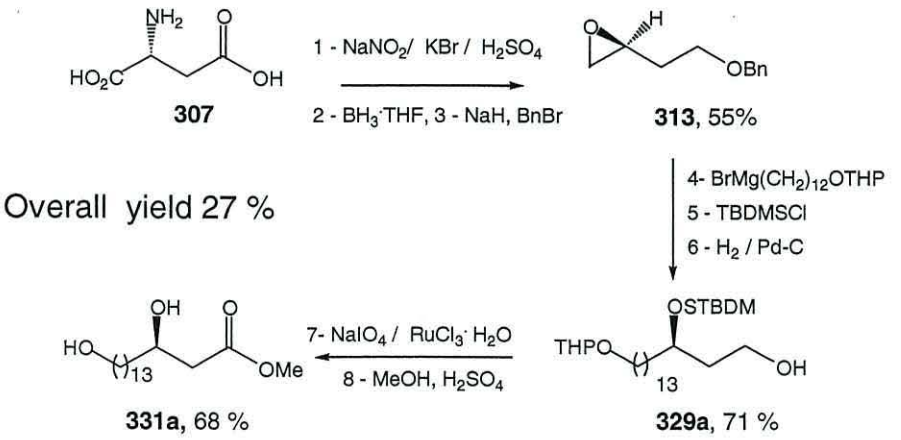
3.4.8 Conclusions

In conclusion, analysing the three different synthetic sequences for the preparation of this crucial building block: the β-hydroxy ester (**239, Scheme 64**), only two were effective (**Scheme 99**).

Method A: Based on the Sharpless Dihydroxylation



Method B: Based on the use of D-Apsartic acid



Scheme 99: ***Comparison between the two successful methods***

The first was based on the Sharpless dihydroxylation (**Method A, Scheme 99**) and it gave an overall yield of 15 % in 9 steps; the second (**Method B, Scheme 99**) used of D-aspartic acid and gave an overall yield of 27 % in 8 steps. The latter

(**Method B**, **Scheme 99**) was finally chosen for the general preparation of β-hydroxy ester, since, even though it required a more expensive starting material, it produced a better overall yield for the final compound. Moreover, this synthetic route used reactions that could be easily scaled up.

The only drawback to this approach (**Method B**, **Scheme 99**) for the preparation of the α^1-mycolic acid was that it did not allow a direct introduction of the whole central chain in one single step (**Section 3.4.3**, **Scheme 90**). However, as will be explained later, a β-hydroxy ester with a small side chain had some advantages as a building block for the preparation of any mycolic acid.

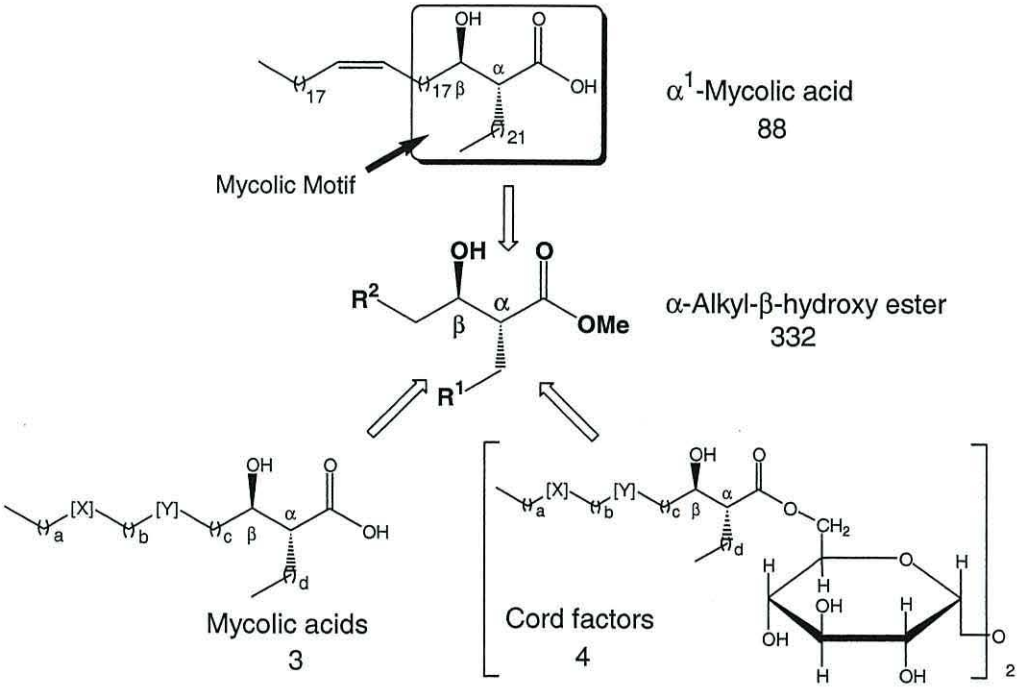
Finally, analysing, retrospectively, the procedure used during the course of the preparation, the major complication related to the debenzylation reaction, which was not always reliable, due to the low solubility of the starting materials. It would be interesting to verify if changing the solvent could solve the problems encountered. Another minor difficulty was the partial loss of the tetrahydropyranyl group in the final oxidation. Therefore, this reaction might be improved by changing this protecting group, to the pivaloyl group, for example.

4. Formation of the α-alkyl-β-hydroxy ester

4.1 The general approach

4.1.1 Overview.

The final problem of this project was the preparation of (2*R*,3*R*)-α-alkyl-β-hydroxy esters, where the two chiral centres are in the *anti*-configuration with respect to each other. This chemical structure constitutes the mycolic motif of the α^1-mycolic acid (**88**), the target compound of this project (**Scheme 100**). However, a similar structural motif is common to all of the mycolic acids (**3**). Therefore, the preparation of α-branched, β-hydroxy esters is fundamental in the synthesis of any mycolic acid and their derivatives, such as cord factor (**Section 1.5**).



Scheme 100: ***The α^1- mycolic acid final target of this project***

Moreover, this structural motif is also a feature of other natural compounds of completely different nature. For example, this structure is found in Marimastat (**333**, **Figure 39**), a molecule currently on trial as an active agent for the treatment of cancer.[356] This feature is also recognisable as part of the spiroketal ring of calcimycin (**334**),[357] one of the polyether ionophore antibiotics,[358] and it forms the β-lactone of (-)-tetrahydrolipstatin (**335**) an anti-obesity agent of microbial

origins.[359] However, the most varied class of naturally occurring substances containing *anti*-3-hydroxy-2-methylcarbonyl units in their structural framework are the polyketide family. Since this group includes numerous antibiotic, immunosuppressive and anti-tumor agents, as well as other bioactive materials of widespread importance in chemotherapy, it has been thoroughly studied and several attempts at the preparation of some of its members are reported.[360,361] One of the best known examples of a polyketide with this structural motif is Miyakolide (**336**), a marine metabolite (**Figure 39**).[362]

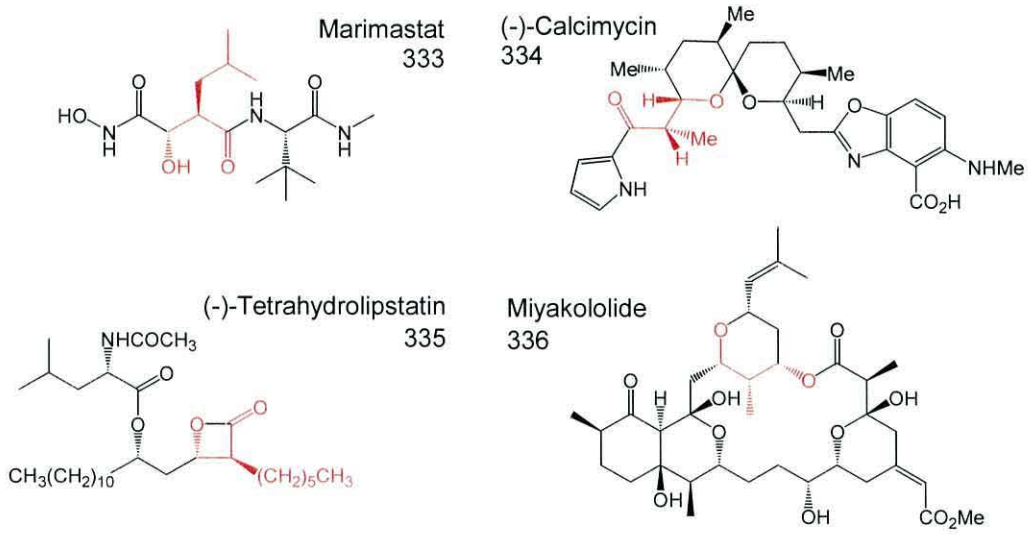
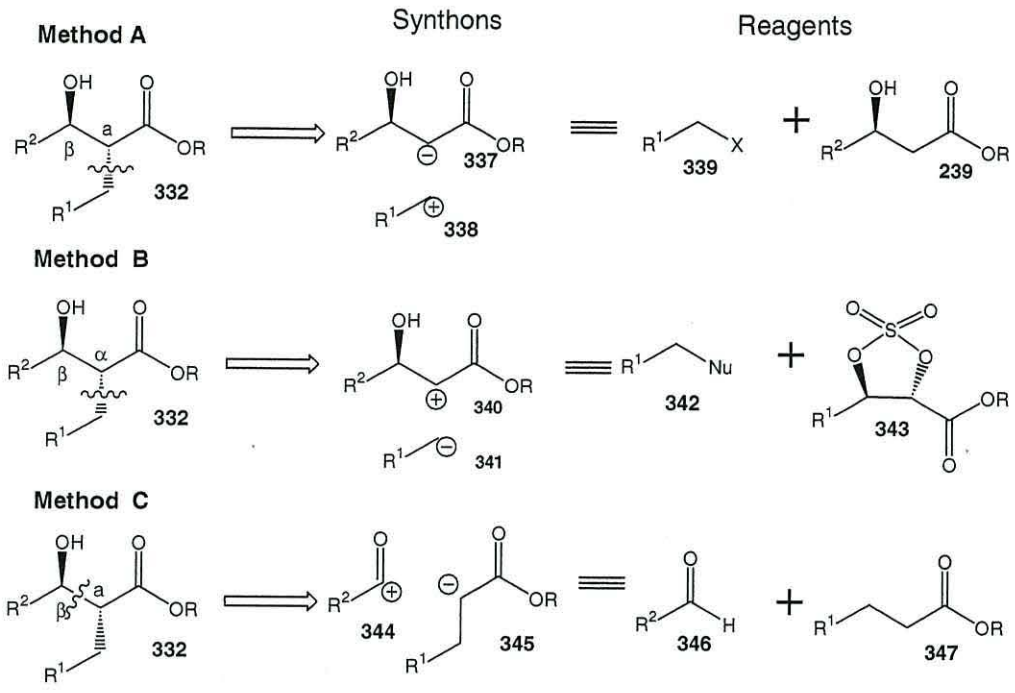


Figure 39: ***Other natural compounds containing the anti-β-hydroxy-α-alkylcarbonyl motif***

An immense number of methods have been used for the preparation of *anti*-3-hydroxy-2-alkylcarbonyl units. In this chapter, illustrative examples of three completely different approaches will be described and analysed (**Scheme 101**).

In the first method, the electrophilic carbon centre is on the long carbon chain, while the nucleophilic one is at the α-position with respect to a carboxylic group. The alpha alkyl chain is inserted through the coupling between an enolate and an alkyl-bromine (**Method A**). The second approach is based on the introduction of the alkyl chain as a nucleophile on a reactive species, such as the α,β-dihydroxy ester cyclic sulphate (**343**, **Method B**). In the third route, the disconnection is not at the alkyl chain but adjacent to the hydroxyl group of the mycolic acid, and an *anti*-aldol reaction leads to the simultaneous introduction of both the β-hydroxyl group and the α-alkyl chain with the correct stereochemistry (**Method C**).

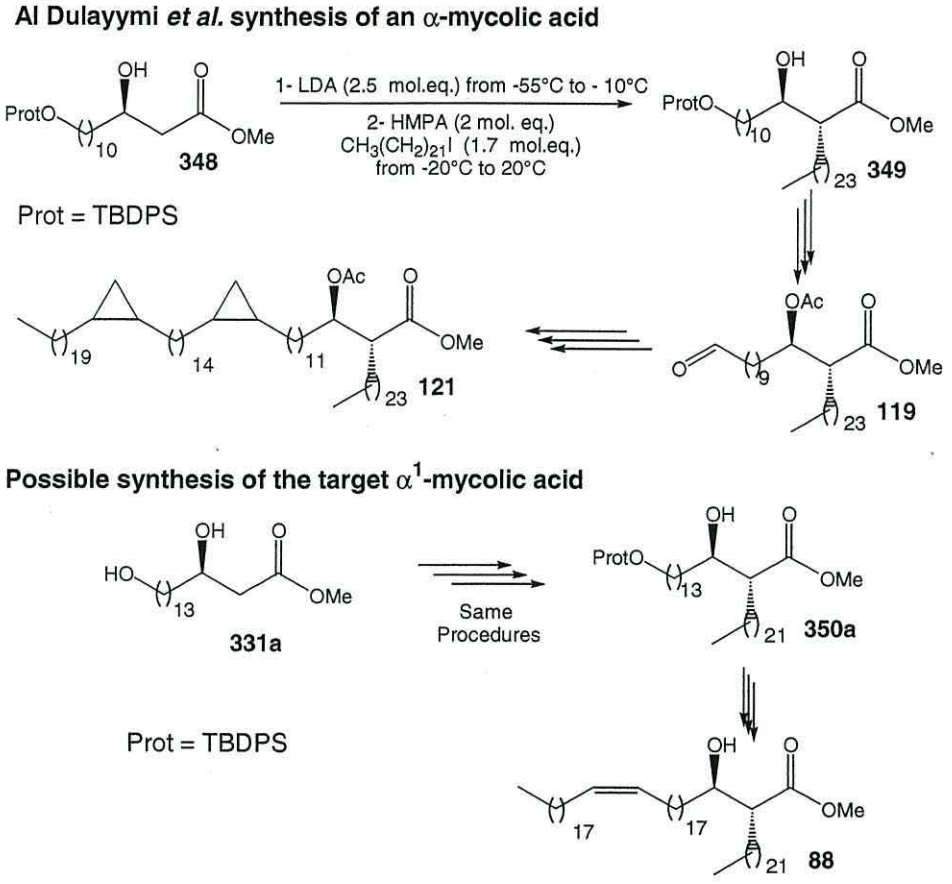


Scheme 101: ***The retrosynthetic analysis of the three different approaches analysed***

4.2 The use of the Fräter alkylation

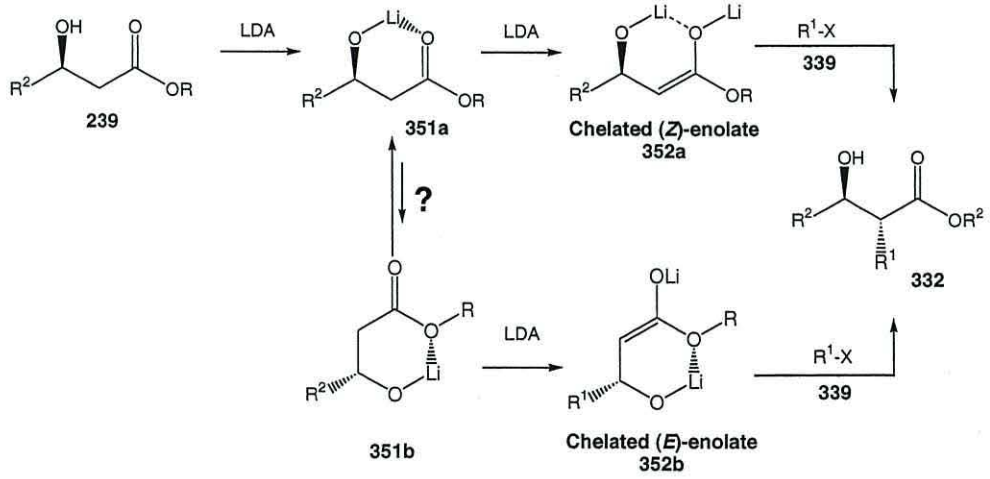
4.2.1 Overview.

For the preparation of *anti*-3-hydroxy-2-methylcarbonyl units (**332**), key intermediates in the synthesis of any mycolic acid, the most widely utilized method is based on the use of the Fräter alkylation.[9,363,364] This approach has been employed by Utaka *et al.*[261] for the preparation of the corynomycolates (**Section 1.5.5**), and recently Al Dulayymi *et al.*[253] utilised a similar strategy for the synthesis of the first example of an enantiomerically pure mycolic acid (**Section 1.5.4**). Therefore, it seemed advisable to employ it for the preparation of the corynomicolate analogue (**350a**, **Scheme 102**), which was a crucial intermediate in the preparation of the desired α^1-mycolic acid (**88**, **Scheme 102**).



Scheme 102: ***The Al Dulayymi et al. approach***

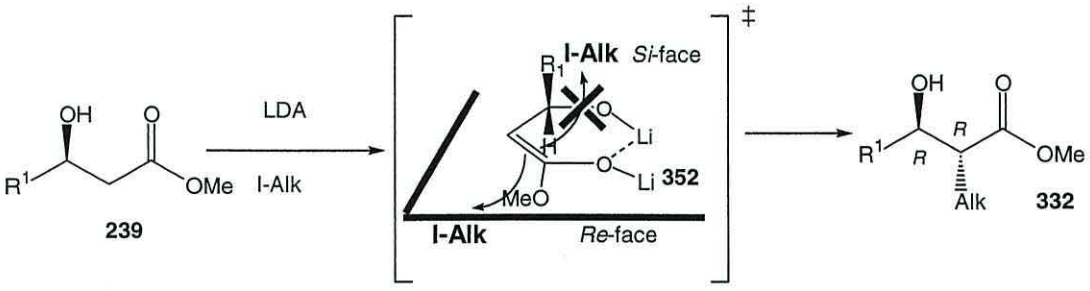
This method requires as a substrate a β-hydroxy ester (**239**, **Scheme 103**). Different synthetic strategies for the preparation of this kind of compound were previously compared, illustrating the advantages and difficulties of each of them (**Chapter 3**).



Scheme 103: ***The formation of a chelated enolate***

Fräter *et al.*[9,363,364] demonstrated that this kind of substrate (**239**, **Scheme 103**) is ideal for a stereocontrolled insertion of the alkyl chain at the α-position. The hydroxyl group in the β-position with respect to the carboxylic group induces the compound, when treated with a Li-base, to form stable metal ion chelated structures (**352**). In particular, if the compound (**239**) is treated with LDA (2 mol. eq.), it primarily forms the chelated (*Z*)-enolate (**352a**). In reality, the geometry of the enolate is not so important. However, the formation of a chelated species is fundamental.[365]

In fact, the six-membered ring transition state (**352**) with a chain on the *Si*-face could allow an addition from the other face (*Re*-face). Therefore, this steric effect ensures a good level of diastereoselection (10:1) for the *anti*-addition (**Scheme 104**).

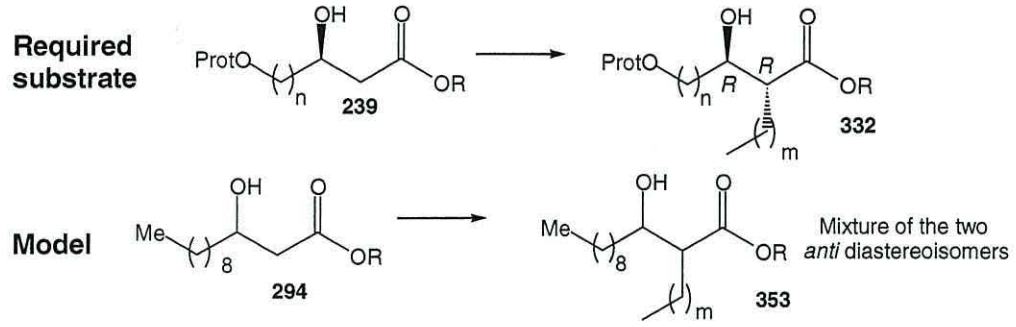


Scheme 104: ***The mechanism of the anti-addition***

If the β-hydroxy ester is not chiral, the two *anti*-diastereoisomers, among the four stereoisomers possible, are formed. If the substrate is chiral (**239**), for example in the *R*-configuration, only one stereoisomer, such as (2*R*,3*R*)-α-alkyl-β-hydroxy ester (**332**), is obtained through this asymmetric reaction (**Scheme 104**).

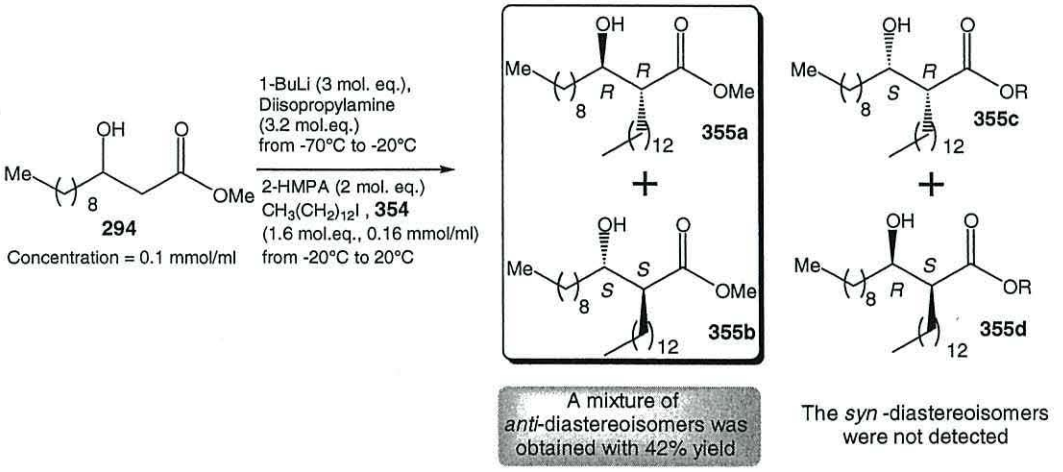
4.2.2 Model reaction.

To become familiar with this type of reaction a model compound was initially utilised. The β-hydroxy ester (**294**, **Scheme 105**), which did not have any functional group at the end of the side chain was chosen, making the actual coupling easier. Moreover, the preparation of racemic compounds was also much easier.



Scheme 105: ***The model reaction***

The preparation of the substrate (**294**) has already been described in **Section 3.3.5**, where it was used as a standard for Chiral GC. Its chelate enolate was obtained by treating it (**294**) with *in situ*-prepared LDA (3 mol. eq.). In order to ensure the formation of this intermediate, the temperature was raised from the initial - 78 °C to - 20 °C, then the enolate was coupled with 1-iododecane (**354**, 1.6 mol. eq.) in the presence of HMPA (2 mol. eq.) as an additive for the coupling. The mixture was stirred overnight and sat. aq. ammonium chloride was added, since it limited the amount of emulsion formed during the quenching.

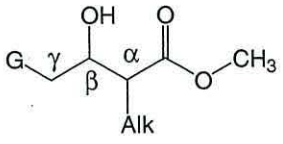


Scheme 106: ***The first attempt of the Fräter reaction***

The reaction was successful, giving a 42 % yield of the coupling product, but four different diastereoisomers could hypothetically have been obtained (**355a**, **355b**, **355c**, **355d**). This condensation is known to give the product almost purely in an *anti*-configuration (**355a**, **355b**). For example, Utaka *et al.*[262] reported, for a similar Fräter alkylation, an *anti/syn* ratio equal of 94:6; nevertheless, it was necessary to verify the absence of the *syn*-products (**355c**, **355d**) in the final

mixture. It was possible to do this through comparison of chemical and physical properties of the purified product, with the data reported in the literature for the different diastereoisomers.

Firstly, a TLC in chloroform of the purified product showed only one spot with an R_f of 0.44. In the literature, the R_f of the *anti*-distereoisomers in the same conditions was reported to be 0.42, whilst the *syn*-diastereoisomers (R,S and S,R), under the same conditions, had an R_f of 0.55.[129,366] Moreover, the ^{13}C NMR spectrum showed only one peak at δ 176.2, in the region of the carboxylic carbons, a peak at 72.3 for the secondary alcohol, and one at 51.5 for the methoxy group. The signal at δ 50.9 corresponded to the α-carbon, while that at 35.7 represented the carbon in the γ-position with respect to the carboxylic group and in the α-position relative to the hydroxyl group. These values perfectly matched those listed in the literature for a racemic mixture of *anti* diastereoisomers, methyl (2*RS*,3*RS*)-3-hydroxy-2-tetradecyloctadecanoates.[367] The racemic mixture of the other diastereoisomers, the methyl (2*RS*,3*SR*)-3-hydroxy-2-tetradecylocta decanoates, was reported to have a slightly different pattern of signals in the ^{13}C NMR spectrum (**Table 2**).



Atom	δ for product	δ for *anti*-hydroxy-2-tetradecyloctadecanoates	δ for *syn*-hydroxy-2-tetradecyloctadecanoates
C=O	176.2	176.2	176.1
C_α	50.9	50.9	51.0
C_β	72.3	72.3	72.1
Cγ	**35.7**	**35.7**	**34.3**
OCH_3	51.5	51.5	51.6

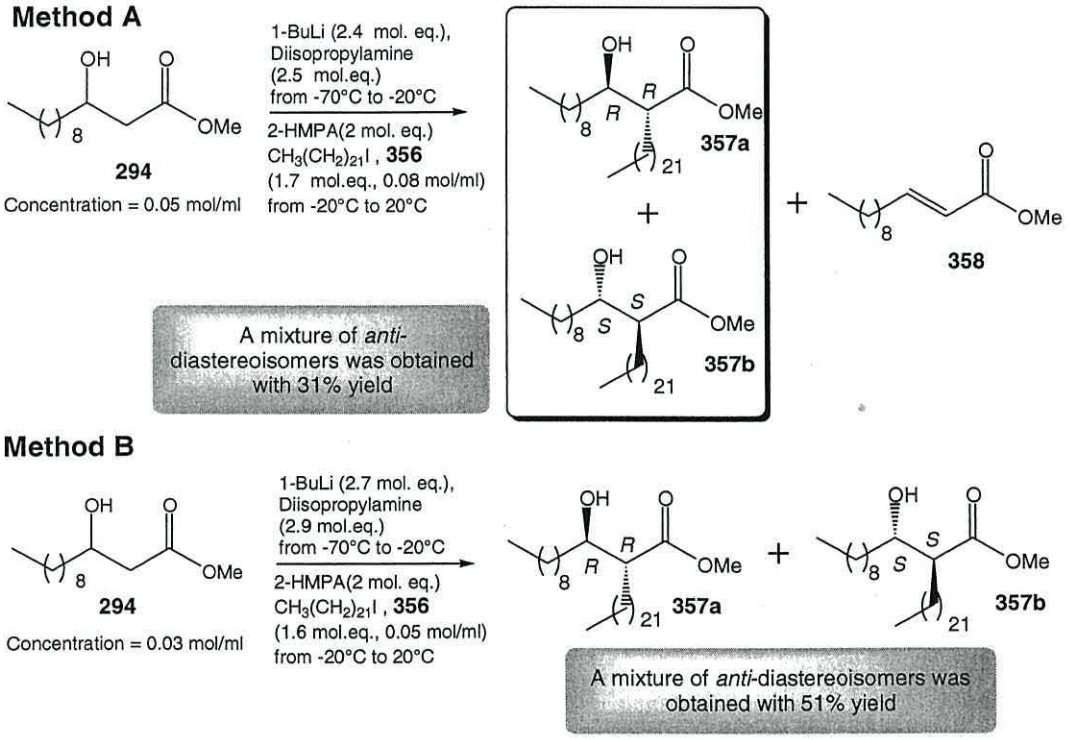
Table 2: ***The differences between anti and syn-diastereoisomers in the ^{13}C NMR spectrum***

The values for each carbon of the two kinds of diastereoisomers are not very different. However, the detection of only one set of signals in the carbon spectrum

of the product, which was also completely identical to that of another *anti*-β-hydroxy-α-alkyl ester, together with the other evidence previously examined, confirmed the formation of a mixture of only *anti*-diastereoisomers (**355a**, **355b**), as predicted.

The ^{1}H NMR spectrum of the product was also analysed. It included an expected singlet at δ 3.71 (for the methoxy group), a multiplet at 3.66 (for the hydroxyl group C<u>H</u>OH) and another multiplet at 2.44 (for two protons; that in the α-position, and that of the hydroxyl group).

Following a similar procedure, the reaction was repeated with a longer alkyl chain, the previously prepared 1-iododocosane (**356**, **Scheme 107**).



Scheme 107: ***The use of a long alkyl iodide***

In the first attempt, the desired product was obtained with only a 30 % yield, together with α,β-unsaturated ester (**358**), which was found to have identical NMR spectra to those reported in the literature for dodec-2-enoic acid methyl ester, which was formed by dehydration of the β-hydroxy ester (**294**).

The yield of the coupling was improved to 51 % by significantly increasing the amount of the solvent (~ 66 %, **Method B**, **Scheme 107**). By diluting the starting materials (**294**, **356**), it was possible to improve their solubility; these would

otherwise form a very viscous solution at very low temperature, thereby inhibiting the coupling. The product displayed a very similar R_f (0.45), and NMR spectra to those found previously for 2-(1-hydroxy-decyl)-tetradecanoic acid methyl ester (**355a**, **355b**) proving the formation of a mixture of *anti*-diastereoisomers (**357a**, **357b**).

4.2.3 Analysis of the proton in the α-position by ^{1}H NMR (500 MHz)

The most interesting result was obtained after treating this final product (**357**) with D_2O and analysing t is by ^{1}H NMR (500 MHz). The previous multiplet at δ 2.44 was transformed into a broad double triplet with J_1 = 9.3 Hz, J_2 = 5.3 Hz, (**Figure 40**).

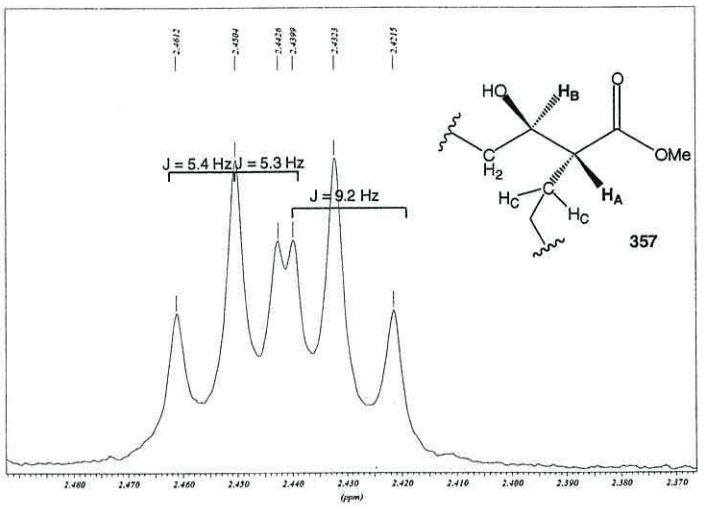


Figure 40: ***The double triplet corresponding to H_A after the addition of D_2O***

The proton (H_A) coupled with the proton (H_B) next to the hydroxyl group at δ 3.66 and the first two protons in the long alkyl chain (Hc, Hc^1). Irradiating proton (H_B), the signal of the proton in α-position was simplified to a double doublet with J_1 = 8.9 Hz, and J_2 = 5.3 Hz (**Figure 41**).

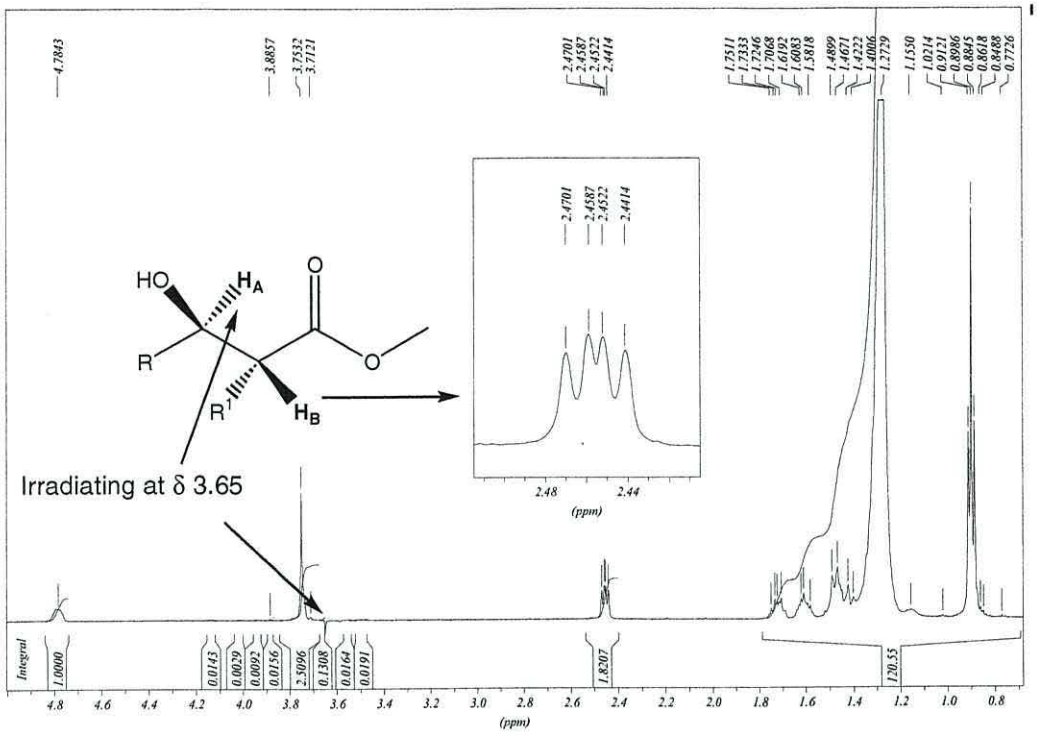
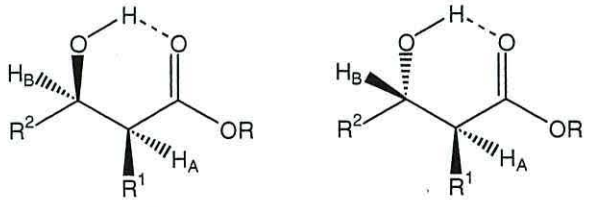


Figure 41: ***The double doublet at δ 2.45 corresponding to H_B obtained by irradiating at δ 3.65***

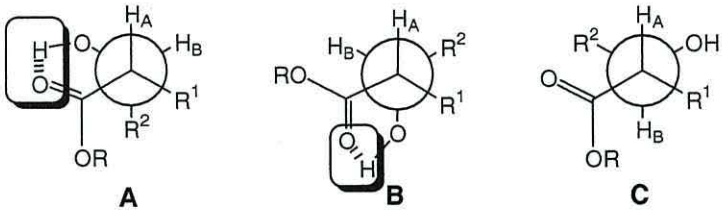
Through extrapolation, it was deduced that the coupling constant ($J_{A,B}$) between the H_A and H_B should be approximately 5.3 Hz. This value was similar to those reported for a similar *anti*-diastereoisomer, e.g. *anti*-3-hydroxy-2,4-dimethylpentanoic acid ($J_{A,B}$ = 5.5 Hz), while the respective *syn*-diastereosimers displayed a $J_{A,B}$ of 3.2 Hz.[368] S*yn* and *anti*-diastereoisomers of this kind of compound show different coupling constants because they have different more stable conformations and their observed $J_{A,B}$ is a weighted combination of the coupling constants of each conformation. Heathcock, analysing these different conformations, explained that the existence of a hydrogen bond between the hydroxyl group and the carboxylic group makes **A** and **B** conformations the most stable ones for *syn*-3-hydroxy-2-alkylcarbonyl units (**361**, **Figure 42**).[368] Instead, for the *anti*-diastereoismer, the most stable conformations are **A'** and **B'** (**362**, **Figure 42**).

In the two most stable conformations (**A**, **B**) of the *syn*-compounds (**361**), the H_A and H_B are always in *gauche*-relationship to each other. The *anti*-compounds (**362**) have one stable conformation (**A'**) with the two protons in an *anti*-relationship, and another one (**B'**) where they are in a *gauche*-relationship. It is

has been demonstrated that when H_A and H_B are in a *gauche*-relationship they have a smaller coupling constant than when they are in an *anti*-relationship. Therefore, the *syn*-diastereoisomers (**361**) should have a smaller coupling constant than the *anti*-diastereoisomers (**362**).

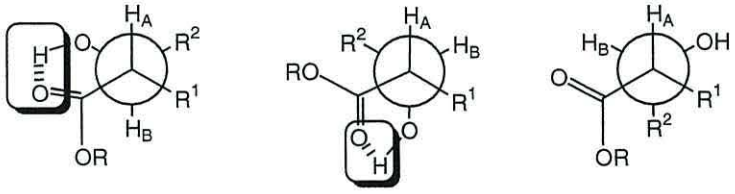


361, *Syn* **362**, *Anti*



A **B** **C**

Conformations for *syn* β-hydroxy-α-alkyl esters, 361



A' **B'** **C'**

Conformations for *anti* β-hydroxy-α-alkyl esters, 362

Figure 42: ***The different conformations of the anti and syn-diastereoismers***

This theory, however, is true as long as the substituents (R^1, R^2) are not too bulky, otherwise the conformation where these two substituents are in an *anti*-relationship to each other would be the most stable (**C**, and **B'**, for the *syn*-molecules and *anti*-respectively). In this case, the coupling constant in the *anti*-diasteroisomer (**362**) is smaller than that of the *syn* (**361**); for example for 2-*tert*-butyl-3-hydroxy-4,4-dimethyl-pentanoic acid, $J_{A,B}$ *syn* = 10 Hz and $J_{A,B}$ *anti* = 0 Hz.[368]

To the best of our knowledge, the values for this coupling constant ($J_{A,B}$) in *syn* and *anti*-α-alkyl-β-hydroxy esters with long chains, such as the mycolic acids, have never been analysed.

The success in the determination of the $J_{A,B}$ values for this compound with two long chains is likely to be important in the search for the most stable conformations of corynomycolic and other mycolic acids.

Moreover, it is known that some properties of these acids are influenced by the spatial positions of the substituents at these two chiral centres (**Section 1.2.3**). Therefore, an analysis of the most stable conformations could also help to explain the role of these two groups in determining the characteristics of the mycolic acids. This was not the aim of this project, so this was not further investigated, but it would be interesting to verify the values of this coupling constant, $J_{A,B}$, for some mycolic acids, and their respective diastereoisomers in order to understand which conformations are the most stable for each compound.

4.2.4 The use of the Fräter coupling on functionalised β-hydroxy esters

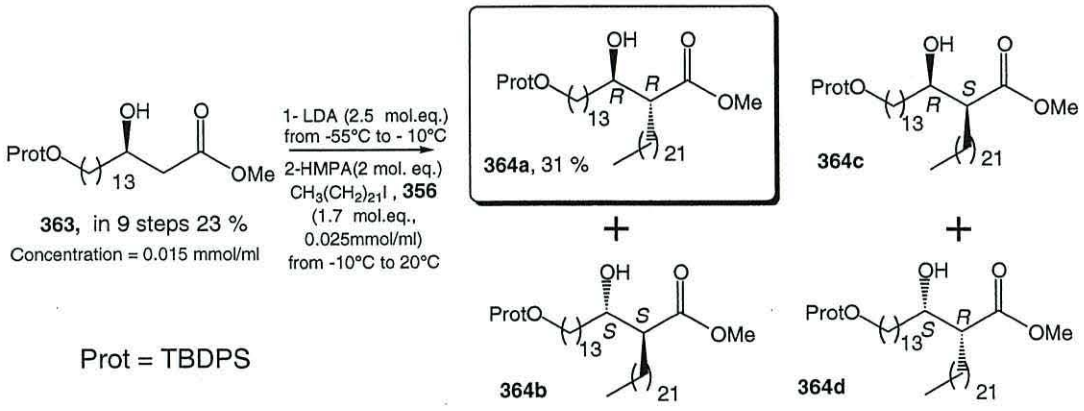
After having tested the Fräter coupling with model reactions, the method was applied to the real substrate (**331a**), a β-hydroxy ester with the side chain functionalised. The first task was the selective protection of the primary hydroxyl group of the diol (**331a**), previously prepared (**Section 3.4.7**). Still following the approach described by Al Dulayymi *et al.*,[253] the alcohol (**331a, Scheme 108**) was treated with *tert*-butyldiphenylsilylchloride (1.3 mol. eq.) employing a similar procedure to that previously described for the protection of the alcohol (**312a, Scheme 92, Section 3.4.4**).

Scheme 108: ***Protection with tert-butyldiphenylsilylchloride***

The desired product (**363**) was obtained with an 86 % yield. Its spectral properties corresponded to a molecule containing both the silyl protecting group and a β-hydroxy ester unit. The presence of the protecting group was showed, in the ^{1}H NMR spectrum, by signals for the aromatic rings (two multiplets at δ 7.7-7.6 and 7.4-7.3) and by a singlet for the *tert*-butyl group (at δ 1.0). The presence of the β-hydroxy ester was indicated by a large band at 3471 cm^{-1} and a strong peak at

1734 cm^{-1} in the IR spectrum. In the ^1H NMR spectrum, a singlet at δ 3.73 indicated the presence of the methoxy group and two double doublets (at 2.52 and 2.39) were indicative of the two protons next to both the carboxylic group and the hydroxyl group. Finally, in the ^{13}C NMR spectrum, peaks at δ 173.5 and 68.0 corresponded to the carboxyl carbon and the secondary hydroxyl group respectively.

The Fräter alkylation between the latter compound (**363**) and iododocosane (**356**) proved very challenging and several different conditions were tested (See experimental section). The best results were obtained when a very large amount of solvent was used, which allowed the starting materials to be almost completely dissolved; the final concentration for the β-hydroxy ester (**363**) was 0.15 mmol/ml, while that for the alkyl iodide (**356**) was 0.17 mmol/ml. Moreover, increasing of the temperatures for both stages (**Scheme 109**) also improved the solubility of the starting materials (**363**, **356**), favouring their coupling.



Scheme 109: ***The Fräter alkylation on a tert-butyldiphenylsilyl protected β-hydroxy ester***

In these conditions, the reaction gave the desired product (**364a**), but this was obtained with a maximum yield of only 31 % after several attempts. Moreover, its formation was completely unpredictable: in fact, the coupling often did not happen at all.

The ^1H NMR spectrum of the desired product (**364a**), treated with D_2O still included the signals for the protecting group (for example the multiplets in the aromatic region). It also showed clear indications of the success of the coupling, including a multiplet at δ 3.66 (for C<u>H</u>OH). The broad double triplet at δ 2.44 for the proton on the α-carbon had very similar coupling constants (J_1 = 9.5 Hz and J_2

= 5.5 Hz) to those found previously for the same proton in ester (**357**, **Figure 40**, **Section 4.2.3**). This suggested the presence of an *anti*-diastereoisomer. This assumption was confirmed by analysis of the ^{13}C NMR spectrum showing peaks at δ 176.3 (for C=O), 72.3 (for $\underline{C}HOH$), 51.5 (for $O\underline{C}H_3$), 50.9 (for $\underline{C}H(CH_2)_{11}CH_3$) and 35.7 (for $\underline{C}H_2$ in the γ position) which were identical to those found for the methyl *anti*-(2*RS*,3*SR*)-3-hydroxy-2-tetradecyloctadecanoates.[367] Finally, its optical rotation of + 4.7 was similar to that found for the (2*R*, 3*R*)-corinomycolic acid methyl ester ($[\alpha]_D^{23} = + 5.7$).[264]

4.2.5 Possible reasons for the low yield.

A clear cause of the poor yield (31 %, **Scheme 109**) for the Fräter alkylation with the functionalised β-hydroxy ester (**363**) and the long alkyl iodide (**356**) was the low solubility of these starting materials, but possibly this was not the only reason. Through a more accurate analysis of the spectral characteristics of the *tert*-butyldiphenylsilyl protected β-hydroxy ester (**363**), with a ROESY experiment, some unexpected results were obtained, which could lead to a different interpretation.

This technique, similarly to a NOESY experiment, measures the correlation of signals using the nuclear Overhauser effect. The major difference between the two spectroscopic techniques is that, in the ROESY spectrum, through rotation of the frame, the values of the NOE enhancements are always positive. In both of them, however, the presence of cross peaks in the 2D spectrum indicates a spatial proximity of the protons. They therefore provide crucial information about the geometry of the molecules. This technique is particularly useful for the determination of the stable conformations of the molecules in solution. The NOE signal is affected by several variables, but it rapidly diminishes when increasing the internuclear separation of the two protons in resonance. It falls off with $1/r^{-6}$, where r is the distance between the protons producing the cross-peak. A rough but useful guide is provided by Williams and Fleming: large cross-peaks correspond to an r (distance between the protons) of 2.0-2.5 Å; medium cross-peaks correspond to an r of 2.0-3.0 Å; small cross peaks correspond to an r of 2.0-5.0 Å.[369]

Analysing the starting material of this Fräter alkylation (**363**, **Scheme 109**), the

ROESY spectrum showed a cross-peak of medium intensity between the protons of the methoxy group (δ 3.7) and the aromatic protons (between δ 7.8 and δ 7.2, **Figure 43**) indicating a proximity between these two groups.

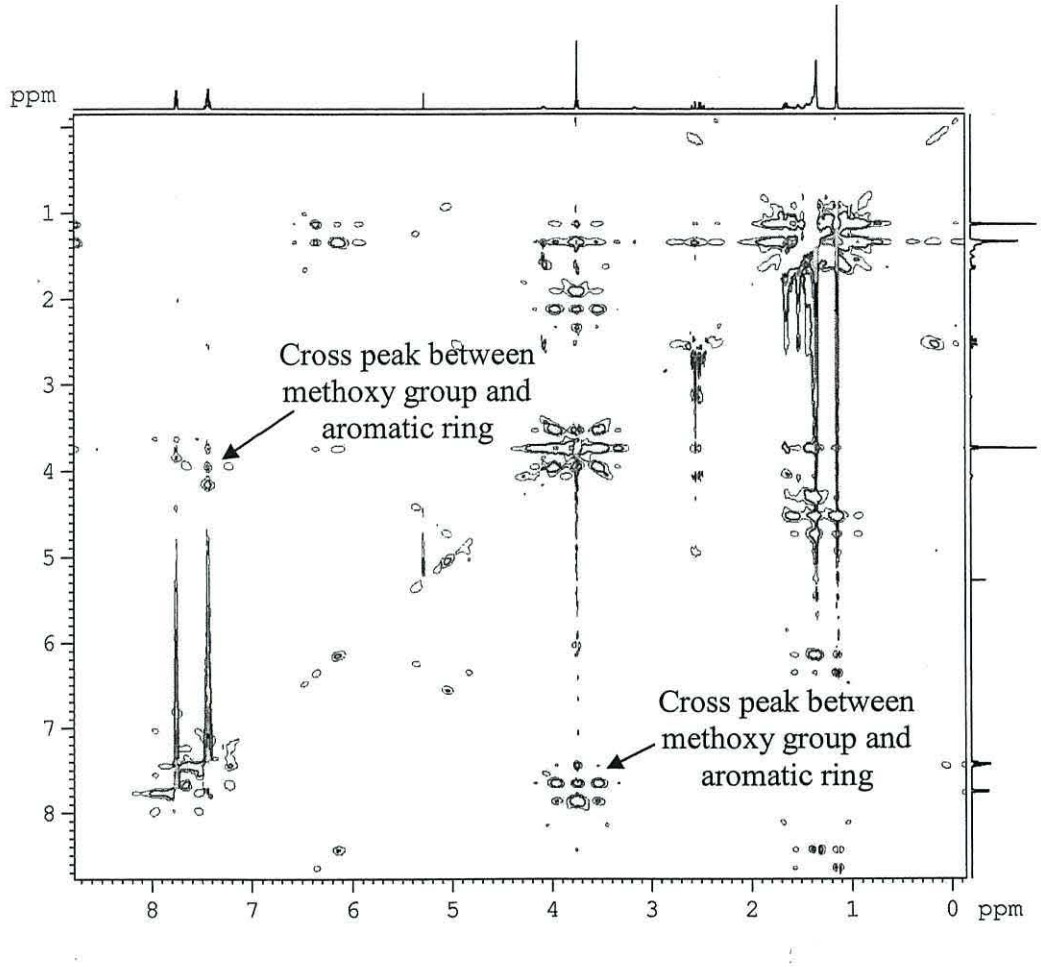


Figure 43: ***The ROESY NMR of the tert-butyldiphenylsilyl protected β-hydroxy ester, 363***

A possible explanation for this result was that the molecule in solution was in a U-shaped conformation. In fact, in this case, the two groups are close enough in space to give this type of cross-peak. A representation of this conformation is illustrated in **Figure 44**. The atoms in red are oxygens with their lone pairs in pink; the silicon atom is in purple. This picture shows the proximity of a benzene ring (a representative proton is emphasized in black) and the methoxy group (still with one proton highlighted in black), and how this is likely to result in the improbability of an attack on the two protons in the α-position (yellow) since they are partially hidden by the skeleton of the molecule.

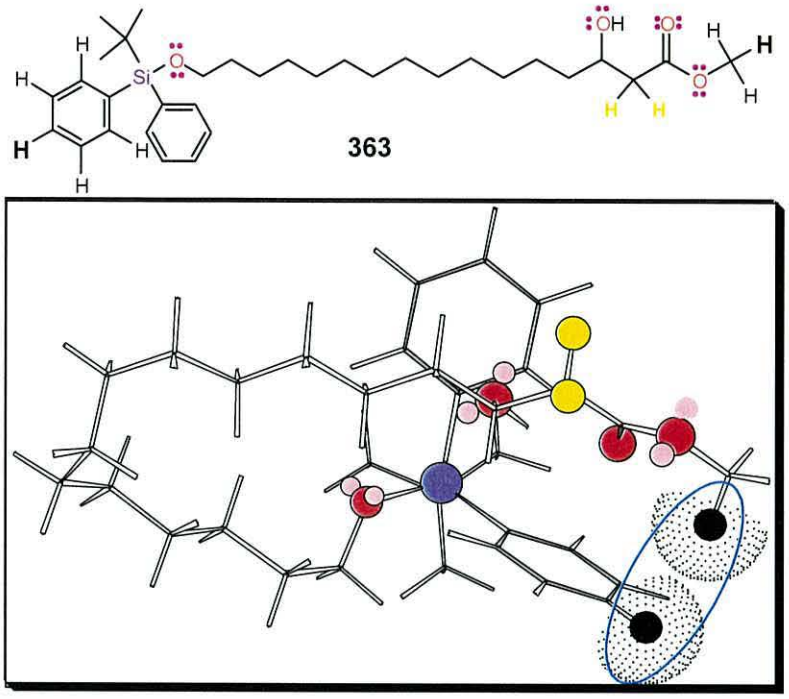


Figure 44: ***A possible conformation which could explain the ROESY interaction***

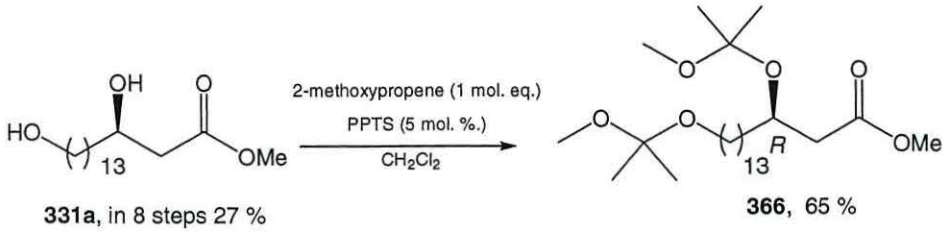
To confirm this theory, Fabio Costiniti, an ERASMUS student in the Chemistry Department of Bangor, made some preliminary experiments of conformer distributions using a Molecular Mechanics Force Field program (MMFF) (Spartan '02 for Macintosh v1.0.4e) (**Appendix 1**). These experiments confirmed that the most stable conformations for the *tert*-butyldiphenylsilyl protected β-hydroxy ester (**363**) showed the side chain twisted on itself, with the two extremities next to each other. Through other experiments, using a similar program, the model compound (**294**) was shown to have a minimum of energy when the side chain was almost straight and not coiled on itself (**Appendix 2**). Similar conformers with straight side chains were demonstrated to have minimum of energy in another β-hydroxy ester (**365a**, **Scheme 111**, **Section 4.2.6**) with the terminal hydroxy group differently protected (**Appendix 2**).

An explanation for the stability of this U-shaped conformation in the case of the *tert*-butyldiphenylsilyl protected compound (**363**) can be found in the formation of π-interactions between the lone pairs on the oxygen atoms in the carboxylic group and the benzyl rings of the protecting group, which stabilise this particular conformation. The existence of this kind of binding interaction has already been proven through *ab initio* calculations for similar molecules, e.g. water and

benzene.[370,371] Moreover, it has been also demonstrated that carbanions in the presence of an aromatic ring can give similar anion binding π-interactions.[372]
These results cannot be considered conclusive: however they do suggest possible explanations. If during the reaction, this U-shape remains the most stable conformer, at least for the starting material and possibly also for the enolate, then it seems unlikely that the alkylation could take place. The reactive centre would be too hidden to permit, firstly the deprotonation of the protons, and then the coupling of the enolate with the alkyl chain. This hypothesis could then explain the poor results obtained when the Fräter addition was tried on this particular starting material, the *tert*-butyldiphenylsilyl protected β-hydroxy ester (**363**).

4.2.6 The use of different protecting groups.

In order to overcome the low efficiency of this reaction, one solution could be to modify the protecting group. The ideal protecting group must not contain any substituent that could interact with the oxygen's lone pairs, and it should also be small, in order not to decrease the solubility of the starting materials.
One possible approach was to protect the hydroxyl group as an acetal with 2-methoxy-propene (1 mol. eq.).[373] Unfortunately, this transformation, which was carried out in acid conditions (*p*-piridinum-toluensolphonate, 5 mol. %) was not regioselective and protected both the hydroxyl groups presents in the molecule (**366, Scheme 110**).



331a, in 8 steps 27 %

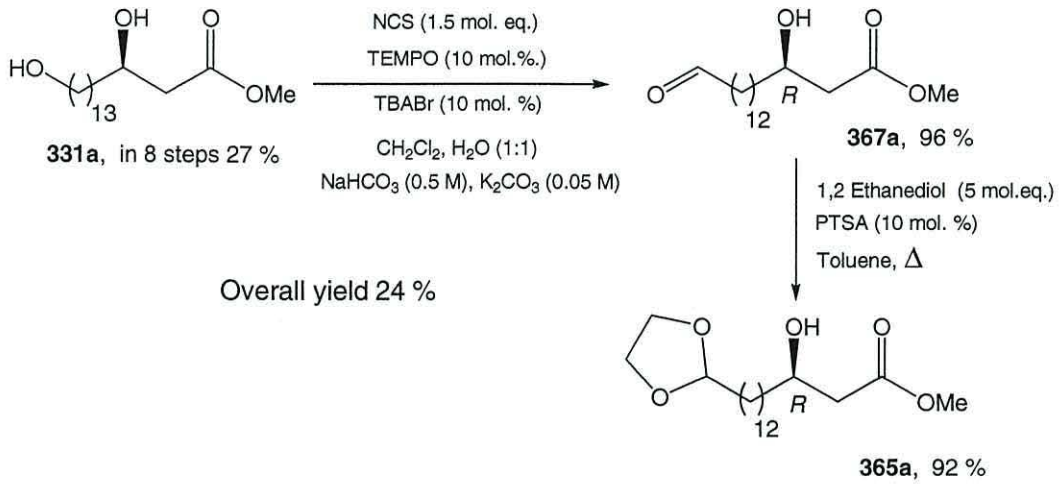
366, 65 %

Scheme 110: ***An Attempt to protect the β-hydroxy ester with 2-methoxy-propene***

The formation of this compound (**366**) was proved as follows. The IR spectrum did not show the band for the hydroxyl group. The ^{1}H NMR spectrum showed two singlets at 3.17 and 3.16 for the methoxy of the protecting unit, each of them integrating for 3 protons. Another two singlets at δ 1.31 and 1.30, each of them integrating for 6 protons, corresponded to the other two methyls in the protecting

group. In the ^{13}C NMR spectrum, two sets of peaks for the protecting group, at δ 100.8 and 99.6 (for acetal carbons) and at 49.0 and 48.2 (for the methoxy) confirmed this hypothesis.

An alternative approach employed selective oxidation with TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy), which is a known oxidant for primary alcohols alone,[374] and protection of the obtained aldehyde (**367**) as an acetal (**368**, **Scheme 111**).



Scheme 111: ***Another attempt to protect the hydroxyl group as an acetal***

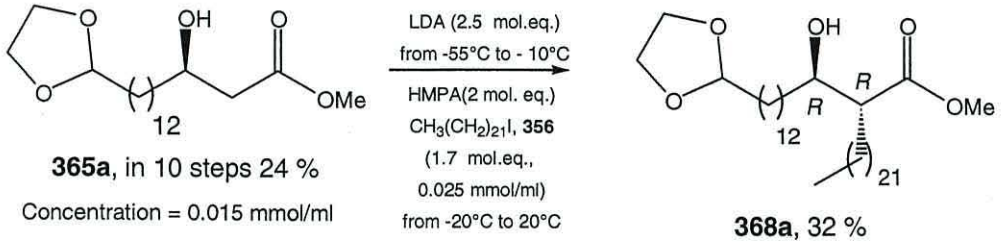
For the oxidation, the procedure described by Einhorn *et al.* was used.[375] Following this, the expensive TEMPO (10 mol. %) was used only in a catalytic amount, while the stoichiometric oxidant was the inexpensive *N*-chloro-succinimide (1.5 mol. eq.). The reaction was carried out in a biphasic system, using dichloromethane and a water solution of $NaHCO_3$ (0.5 M) and K_2CO_3 (0.05 M). TBABr (10 mol. %) was also added as a phase transfer agent. This showed itself to be an efficient method for the selective oxidation of a primary alcohol, giving the required aldehyde with 96 % yield.

In the IR spectrum of the compound (**367a**), the two peaks at 1734 and 1716 cm^{-1} suggested the presence of two carboxylic groups. In the ^1H NMR spectrum, the broad triplet at δ 9.76 (J = 1.9 Hz) confirmed the existence of an aldehyde, while the multiplet at δ ~ 4.0 confirmed the non-oxidation of the secondary hydroxyl group. Finally, in the ^{13}C NMR spectrum the expected peaks for the two different quaternary carbons were at δ 202.9 and 173.4, with the signal at 68.0 corresponding to the carbon of the hydroxyl group.

The next step was the preparation of the acetal (**365a**), which was achieved following the procedure described by Al Dulayymi *et al.*[376] This method required the use of a large excess of 1,2-ethandiol (5 mol. eq.) at reflux with a Dean Stark apparatus in order to eliminate the water produced. The protection was enhanced by the presence of an acid catalyst such as *p*-toluensulphonic acid monohydrate (10 mol. %). The protected compound (**365a**) was recovered and fully characterized. The most significant peak in the ^{1}H NMR spectrum was a triplet (J = 4.8 Hz) at δ 4.85 which demonstrated the existence of an acetal proton, while the two multiplets at ~ 3.9 and at ~ 3.8 corresponded to the two methylene groups in the five membered ring. The ^{13}C NMR spectrum showed, in addition to the signals for the β-hydroxy ester (δ 173.4 for the carboxyl group and 68.0 for the hydroxyl group) a peak at 104.7 for the acetal carbon. Finally, the ROESY spectrum of this compound (**365a**) did not show evidence for the existence of the U-shaped conformation. The presence of a linear side chain was also confirmed by the preliminary analysis by Fabio Costiniti of the compound (**365a**).

The subsequent Fräter coupling provided encouraging outcomes (**Scheme 112**). Using the same conditions as before but with this newly prepared starting material (**365a**, **Scheme 112**), the desired compound (**368a**) was obtained with slightly better yields, but, more importantly, the reaction appeared to be more reliable.

These results supported the proposal that success of the reaction is favoured when the starting material is in an almost straight conformation.



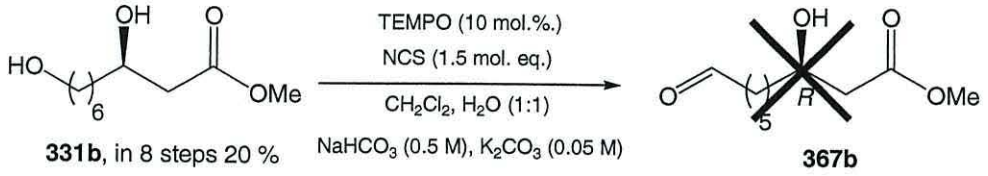
Scheme 112: ***The Fräter alkylation with the acetal protection***

The final product (**368a**) was characterised: the carbon NMR showed the same pattern described above for the *anti*-diastereosimer (δ 176.2 (for C=O), 72.3 (for CHOH), 51.5 (OCH_3), 51.0 (the carbon in the α-position) and 35.7 (the carbon in the γ-position). It also included signals for the protecting group; for example, at δ

104.7 (for the acetal carbon) and at 64.8 (for the two methylene carbon in the five membered ring).

The proton NMR of the product (**368a**) included a triplet (J = 4.8 Hz) for the acetal proton at δ 4.85 and a multiplet at ~ 2.45 for the proton in the α-position. Finally, the optical rotation of + 4.7 suggested the preparation of the desired (2*R*, 3*R*) 2-(13-[1,3]dioxolan-2-yl-1-hydroxytridecyl)tetracosanoic acid methyl ester.

With the aim of improving the yield, a final attempt was made using the same method, but with a smaller side chain (**Scheme 113**). In particular, it was decided to use 3,9-dihydroxynonanoic acid methyl ester (**331b**), whose synthesis had already been described (**Section 3.4**). Unfortunately, the oxidation of this compound (**331b**) with TEMPO gave a viscous polymer, insoluble in the most readily available organic solvents. The reasons for this polymerisation were not investigated, but might be related to a higher reactivity of the smaller compound.



331b, in 8 steps 20 % **367b**

Scheme 113: ***An attempt to use a smaller chain***

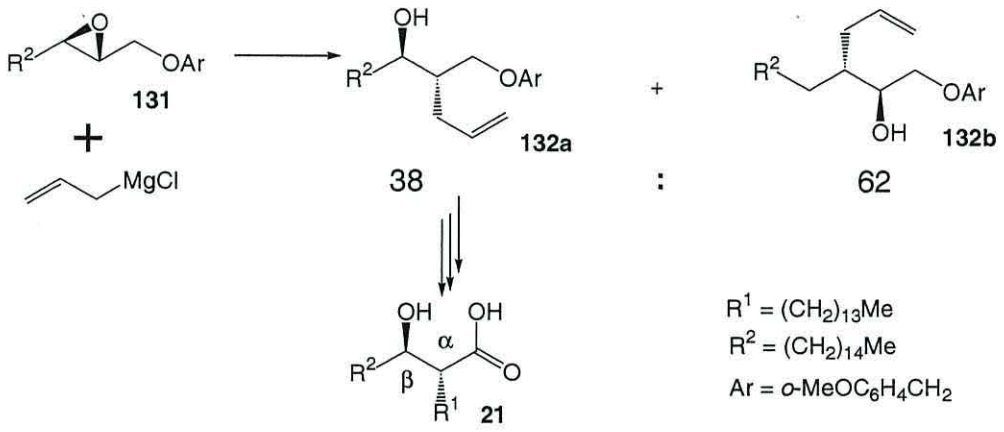
Therefore, it was deduced that a β-hydroxy ester (**365a**) with a medium side chain and a small non-aromatic protecting group was the optimal key intermediate for this synthetic strategy based on the use of the Fräter alkylation.

In conclusion, after several attempts, a possible method for the preparation of corinomycolate analogues was determined. It did not give a very good overall yield (only 8 % from the initial starting material the *D*-aspartic acid) but the α-alkyl-β-hydroxy ester (**368a**) was produced with a good enantiomeric excess and the final Fräter alkylation was no longer so unpredictable.

4.3 The use of a cyclic sulphate

4.3.1 Overview.

The formation of a carbon-carbon bond through reaction between a Grignard reagent and a reactive species had already been used for the formation of this structural motif. For example, the reaction between the epoxide (**131, Scheme 114**) and an allylic Grignard as used by Nishizawa *et al.*[260] in the preparation of the corinomycolic acid (**Section 1.5.5**)[260]

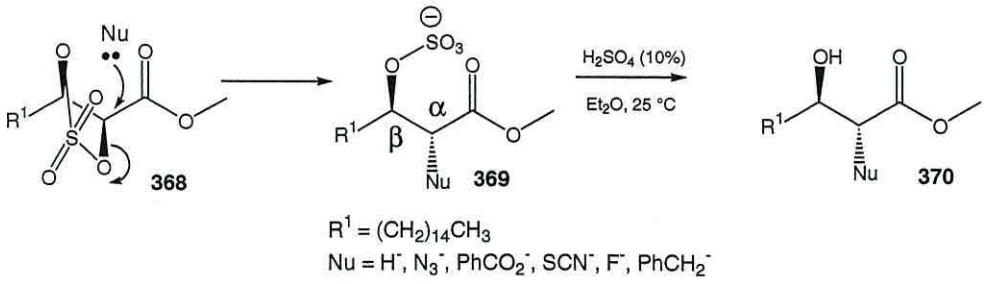


Scheme 114: ***The Nishizawa et al. method***

Analysing this synthetic strategy, the major weakness was that the Grignard reagent did not show any preference for the required α-position, during the coupling with the epoxide (**131, Scheme 114**).

Furthermore, several examples of nucleophilic couplings on α,β-epoxyesters occurring at either the α- or the β-position, are reported in the literature, where the regioselectivity really was influenced by the conditions of the reactions. [377-379]

Gao *et al.*[10] proposed an interesting synthetic alternative to the use of an epoxy substrate; they used α,β-dihydroxy ester cyclic sulfate (**370, Scheme 115**).



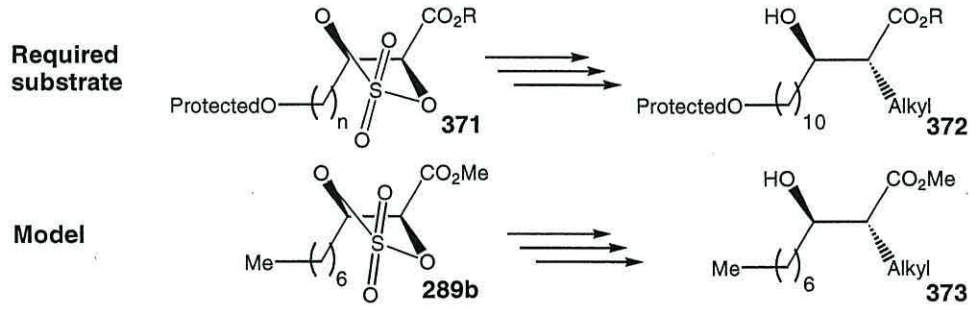
Scheme 115: ***The mechanism for the nucleophilic substitution on cyclic sulfate intermediates***

This group can similarly undergo nucleophilic substitutions, but addition is almost exclusively at the α-position. Finally, the ring opening proceeds through an S_N2 mechanism and a complete inversion of configuration at the reacting centre, thereby providing the desired configurations (**370**).[380]

In more recent years, different kinds of cyclic sulphates have been reported as being reactive toward S_N2 reactions,[331] even when carbanions were employed as nucleophiles.[381-383] Therefore, even if examples of the addition of any alkyl chain onto α,β-dihydroxy ester cyclic sulfate could not be found, it was interesting to test the applicability of this approach, firstly for the preparation of α^1-mycolic acid, and, more generally, for α-alkyl-β-hydroxy esters.

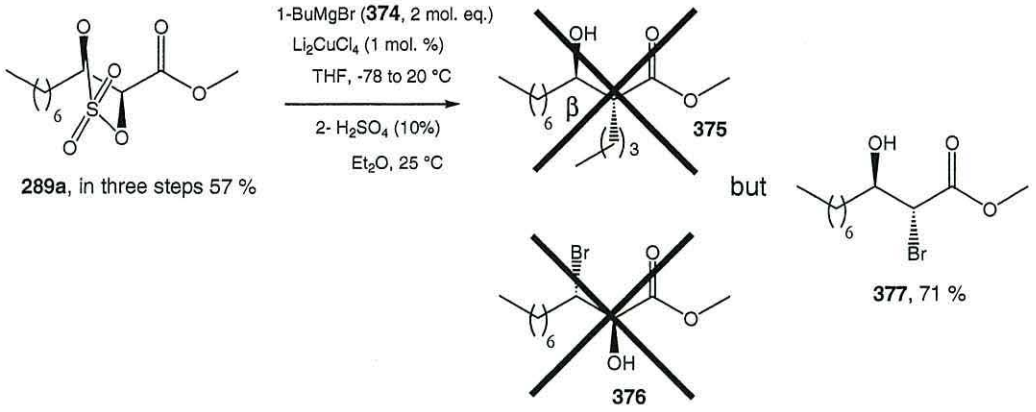
4.3.2 Attempts at the addition of carbon nucleophiles onto a model compound

Initially, the possibility of a carbon nucleophilic attack on this kind of substrate was tested on the model compound (**289b**, **Scheme 116**), which had already been prepared for previous studies for the preparation of β-hydroxy ester (**Section 3.3.3**).



Scheme 116: ***The use of a model compound***

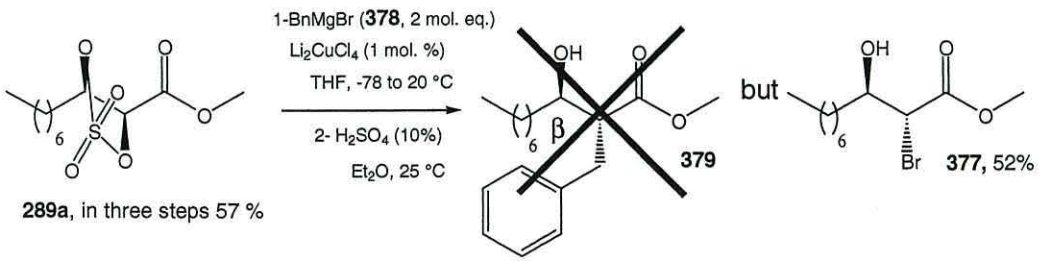
The first attempt used *n*-butyl magnesium bromide (**374**, 2 mol. eq.) as the nucleophile. This compound was added to the cyclic sulfate (**289a**, **Scheme 117**) at – 78 °C, followed by addition the catalyst Li_2CuCl_4 (1 mol. %). The product of this coupling was not purified, but it was immediately treated with sulphuric acid in ether.



Scheme 117: ***The use of BuMgBr as the carbanion***

The process was not successful. Instead it gave 2-bromo-3-hydroxy-decanoic acid methyl ester (**377**). The attack at the α-position by the bromide ion was demonstrated by comparison of spectroscopic data of the purified compound with those reported in the literature for 2-bromo-3-hydroxy-decanoic acid methyl ester,[339] and those of 2-hydroxy-3-bromohexanoic acid methyl ester.[384] For example, in the ^{1}H NMR spectrum of the prepared compound, a doublet (J = 7.6 Hz) at δ 4.14 (for C<u>H</u>Br) and a double triplet (J_1 = 7.6Hz, J_2 = 2.7 Hz) at δ 4.00 (for C<u>H</u>OH) were very similar to the signals reported in the literature for the α-bromo-β-hydroxy ester (a doublet with a coupling constant of 8 Hz at δ 4.09–4.13 and a multiplet at δ 3.97).[339] However, the ^{1}H NMR spectrum of the α-hydroxy-β-bromo ester was reported to have a doublet (J = 3.1 Hz) at δ 4.38 and a multiplet at δ 4.25-4.12, therefore at a much higher field.[384]

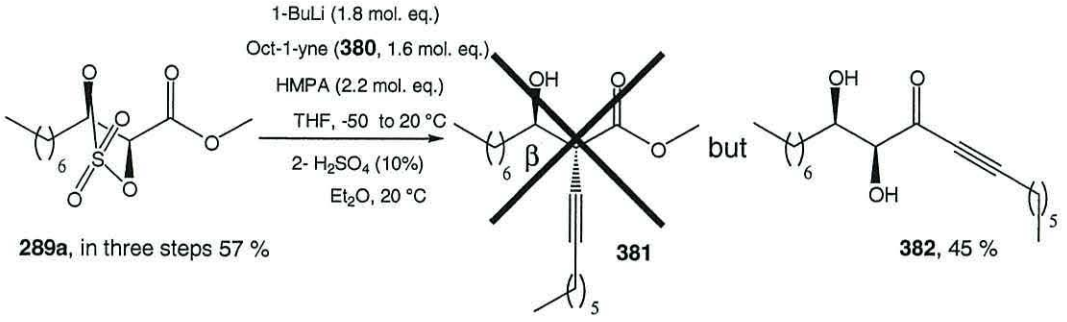
The failure of this reaction was therefore attributed to the inability of the carbanion to attack the α-position of α,β-dihydroxy ester cyclic sulfates, whereas other nucleophiles, such the bromide ion, are able to do so. In order to overcome to this problem, different carbon nucleophiles were tested (**Scheme 118**).



Scheme 118: ***The use of BnMgBr as the nucleophile***

The reaction was repeated using the same procedure, but this time using benzyl magnesium bromine (**378**, 2 mol. eq.), since it had been reported that the benzyl carbanion was a successful nucleophile for another kind of cyclic sulfate. [10] However, this coupling did not give better results and only the 2-bromo-3-hydroxy-decanoic acid methyl ester (**377**, **Scheme 118**) was identified after purification of the crude product.

Another attempt was made employing an alkyne anion as the nucleophile (**Scheme 119**). Examples of successful nucleophilic attacks by this kinds of compound on different kind of cyclic sulfate have also been reported in the literature.[385,386]



Scheme 119: ***The use of an alkyne as nucleophile***

In this case, the carbanion was formed *in situ* by the reaction of oct-1-yne (**380**, 1.6 mol. eq.) with BuLi (1.8 mol. eq.). This time, the coupling was helped by the presence of HMPA (2.2.mol. eq.). However, even in this case, the reaction did not give the desired product (**381**). Instead, 10,11-dihydroxyoctadec-7-yn-9-one (**382**) was probably recovered, through its identification was not complete. The ^{1}H NMR spectrum showed a doublet (J = 3.4 Hz) at δ 4.40 for the proton in the α-position with respect to the carboxylic group, a broad double triplet (J_1 = 9.5 Hz, J_2 = 3.1 Hz) at δ 4.07 for the proton in the β-position and a triplet (J = 7.3 Hz) at 2.41 for the two protons next to the alkyne group. From the analysis of the ^{13}C NMR and DEPT spectra, the peak at δ 186.7 was assigned as corresponding to the carboxylic quaternary carbon, and those at 101.6 and 78.5 to the two sp^3 quaternary carbons, while the signals at 81.8 and 72.9 corresponded to the two carbons next to the two hydroxyl groups. The IR spectrum confirmed the presence of a carbonyl group with a strong band at 1666 and of the alkyne group with a sharp peak at 2214 cm^{-1}.

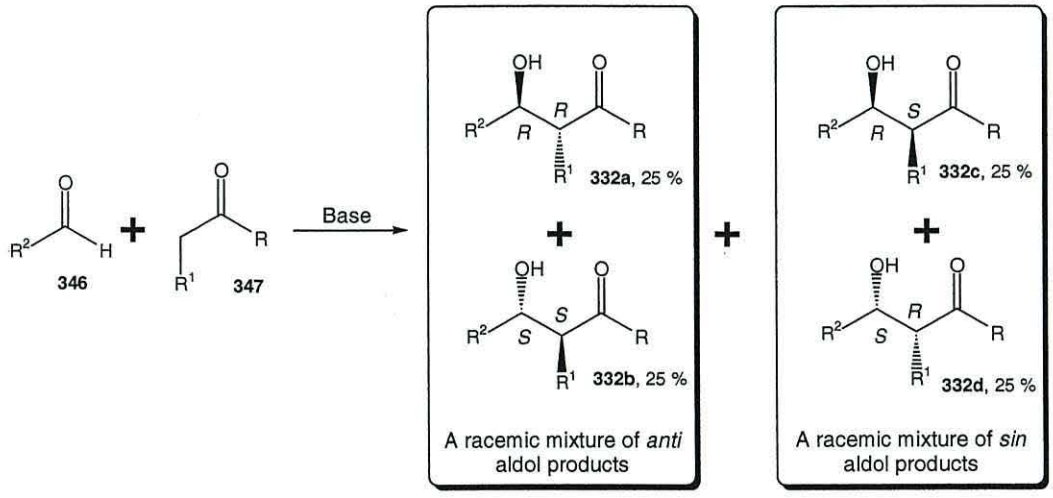
Since none of the examples attempted were successful, this approach was discarded and another one was examined.

4.4 The use of a stereospecific aldol reaction

4.4.1 Overview

One of the most common methods for the formation of a β-hydroxy-α-alkyl carbonyl unit (**332a**, **332b**, **332c**, **332d**) is based on the use of a stereospecifc aldol reaction.

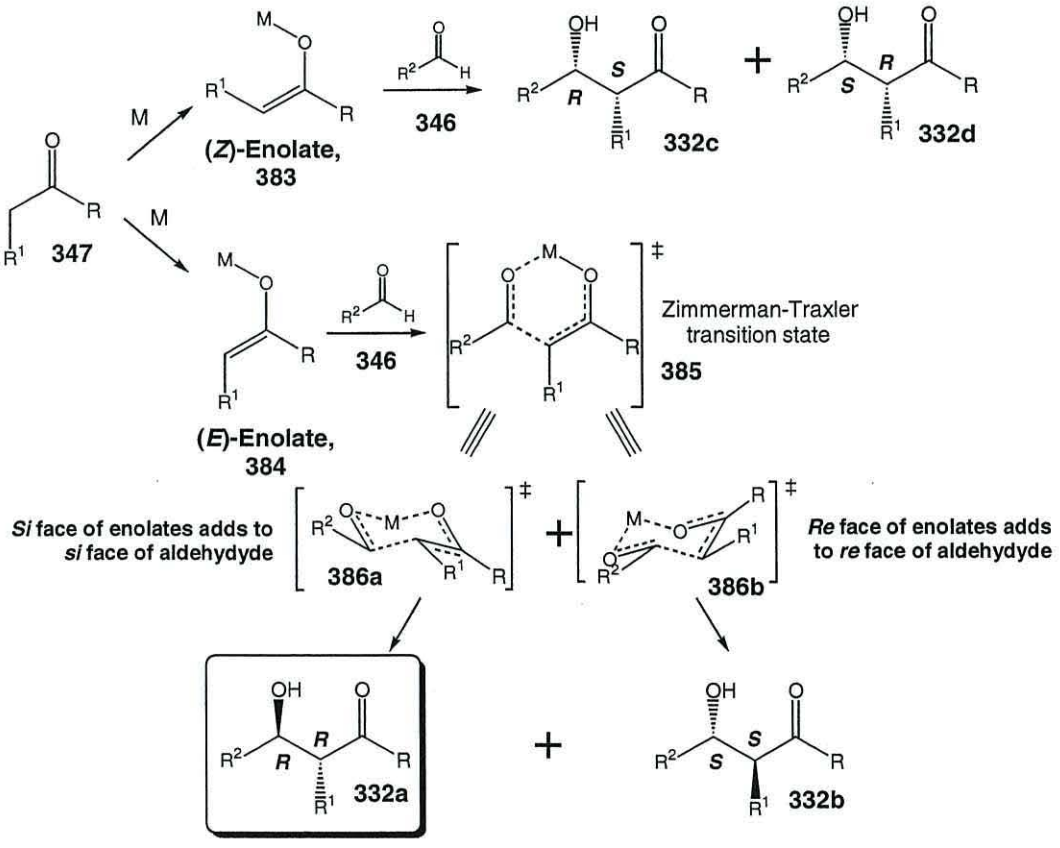
During this kind of coupling two new stereocenters are created from two prochiral carbon atoms; thus, four possible diastereoisomeric products can be formed. In the simplest case, when no chiral starting materials or reagents are employed, the products are two racemic diastereoisomers, usually referred to as the *syn* and the *anti*-aldol products (**332a**, **332b**, **332c**, **332d**, **Scheme 120**).[387]



Scheme 120: ***The different aldol products***

Analysing its mechanism, it has been demonstrated that many aldol reactions, including those with lithium as the promoter, proceed through an ordered transition state, often called the Zimmerman-Traxler transition state (**385**). In these cases, the metal ion is bonded to the oxygen of the enolate, but it also co-ordinates the oxygen of the aldehyde, forming a six membered ring. It has also been demonstrated that, if there is an initial formation of the (*E*)-enolate (**384**),

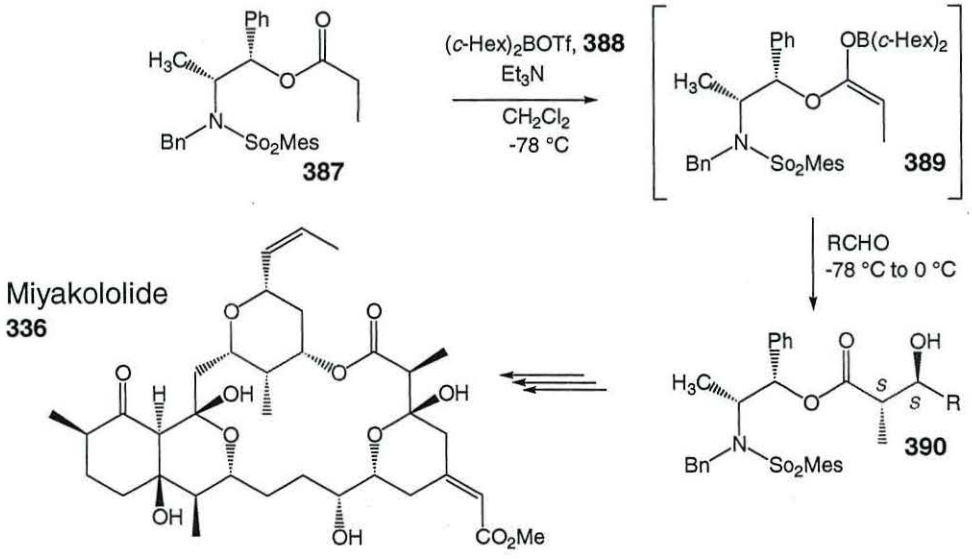
through the formation of a Zimmerman-Traxler transition state, this would give *anti*-aldol products (**332a**, **332b**,**Scheme 121**), while, through a similar mechanism the (*Z*)-enolate (**383**) would give *syn*-aldol products (**332c**, **332d**).[387] One crucial problem in the achievement of a stereospecific aldol reaction is the control of the enolate geometry. In particular, for the aim of this project, it was necessary to form the *anti*-aldol and, thereby the (*E*)-enolate (**384**). A second one is to restrain the attack from only one face of the enolate formed to one side of the aldehyde, in order to obtain only one of the possible two enantiomers of the racemic mixture. In the present case, the (2*R*,3*R*)-3-hydroxy-2-alkyl carbonyl unit (**332a**) had to be produced and, therefore, the attack from both the *si*-faces (**386a**) would be optimal.



Scheme 121: ***Mechanism of the aldol condensation***

Analysing possible solutions to both these two difficulties, different procedures could be used to achieve the desired diastereofacial selectivity; the use of a chiral substrate, or a chiral enolate, or a chiral auxiliary.[388] The controlled formation of the (*E*)-enolate (**384**) has created many more problems, nevertheless. Several

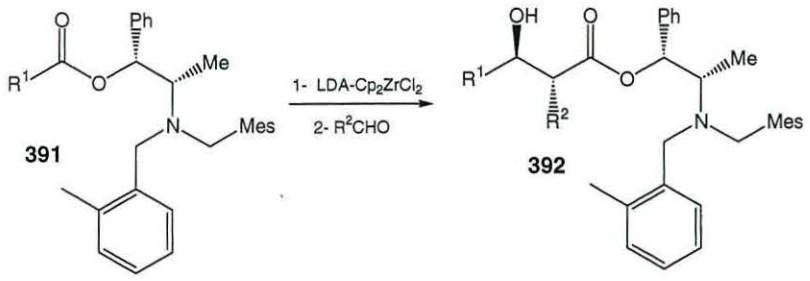
"successful" methods for the selective formation of the (*Z*)-enolate (**383**) and therefore the *syn*-β-hydroxy-α-alkyl carbonyl structural motif have been reported. However, approaches for the direct formation of the *anti*-counterparts have met only limited success. The procedures described usually employ very expensive chiral catalysts which cannot be fully recovered, or reagents that are not commercially available. As a result, even if these procedures provide *anti*-aldols with high enantioselectivities, they are rarely employed in natural product synthesis. For example, diastereofacial selectivity could be easily obtained though the formation of boron enolates,[389] but these reagents are either very expensive or are not available from commercial sources. The use of dialkylboron reagent (**388**) has recently been used by Yoshimitsu *et al.*[362] for the synthesis of a similar structural motif (**390**) in Miyakolide (**336**), a marine polyketyde (**Scheme 122**). This research group demonstrated that dicyclohexylboron triflate (**388**), in the presence of a norephedrine derivative as chiral auxiliary (**387**), could lead to the production of the (*E*)-enolate followed by the (2*S*,3*S*)-3-hydroxy-2-methyl carbonyl unit (**390**, **Scheme 122**).[390]



Scheme 122: ***The Yoshimitsu et al. approach***

Subsequently, Kurosu and Lorca developed a new method using a similar chiral auxiliary, but without employing a boron enolate.[11] They used LDA, which has been proven to give mostly (*E*)-enolate, together with Cp_2ZrCl_2 and a (-)-N,N-dibenzylnorephedrine derivative (**391**), which enhanced both the

diastereoselectivity and diastereofacial selectivity. They managed to obtain various (2*R*,3*R*)-3-hydroxy-2-alkyl derivatives (**392**) with high chemical yields, enantiomeric excess greater than 98 % and an *anti/syn* selectivity up to 15:1 (**Scheme 123**).



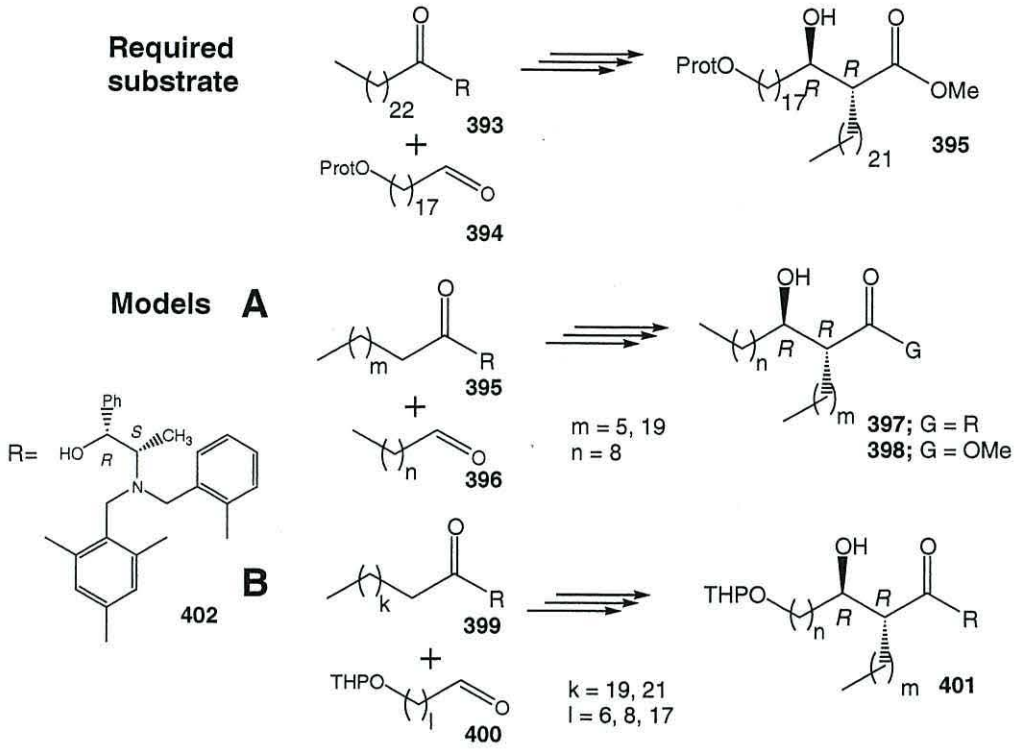
Scheme 123: ***The Kuruso and Lorca approach***

They proved the advantages of this new method over those utilised before. In fact, it provided an extremely high degree of diastereofacial selectivity using commercially available reagents. Moreover, the use of this particular norephedrine derivative made the purification of *anti* and *syn*-diastereomers easier. Finally, following their procedure, it was possible to recover a large amount of the auxiliary. The same authors tested this methodology, with excellent results, for the synthesis of the lipophilic side chain of polyoxypeptins, which are anticancer agents.[391]

In the course of this project, it was decided to verify if this new approach could be used in the preparation of the corinomycolate moiety of mycolic acids.

4.4.2 Preparation of the chiral auxiliary

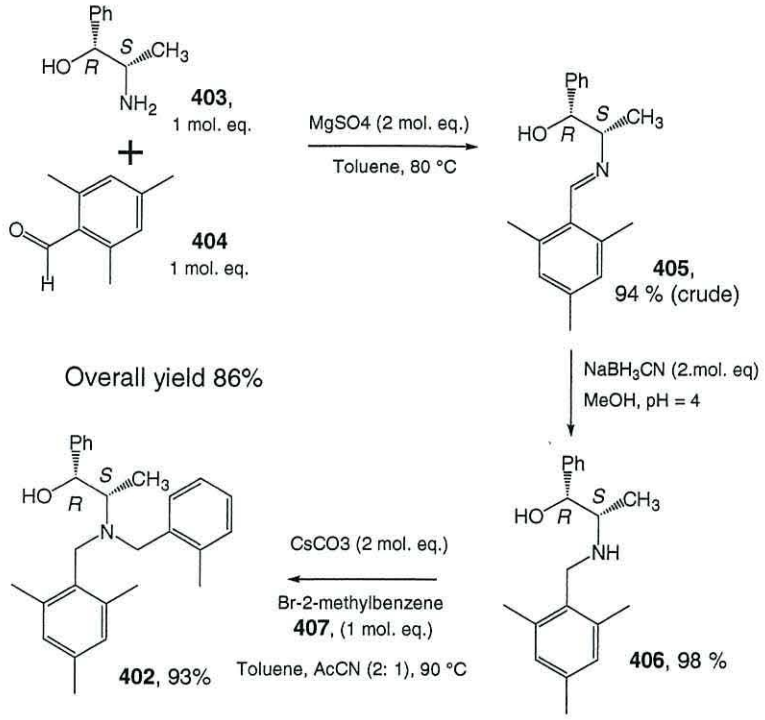
The authors of this new procedure did not report its use with starting materials with long chains or with more than one functional group in these chains, for example a protected hydroxyl group. Due to the limited time available, it was decided to concentrate on studying the limits of this new approach through different model reactions, instead of directly attempting to prepare the corinomycolate analogues (**395**) necessary for the preparation of the α^1-mycolic acid (**88**), the initial target of this project (**Scheme 124**).



Scheme 124: ***Different models attempted***

Initially, it was necessary to prepare the chiral auxiliary (**402**). The first step was the coupling between 2,4,6-trimethylbenzaldehyde (**403**, 1 mol. eq.) and (-)-norephedrine (**404**, 1.8 mol. eq.) in dry toluene at 80 °C using magnesium sulphate (2 mol. eq.) for the elimination of the water produced (**Scheme 125**). The imide (**405**) was not purified, so as to avoid its decomposition, but it was immediately reduced with sodium cyanoborohydride (2 mol. eq.). This compound (**406**) was then joined to 1-bromomethyl-2-methylbenzene (**407**, 1 mol. eq.) in the presence of a base, such as caesium carbonate (2 mol. eq.). All the reactions employed the procedures described in the article of Kuruso and Lorca,[11] except in the initial transformation. It was noticed that the use of identical amounts for both starting materials (**403**, **404**), led to an easier purification of the subsequent amine (**406**).

The products of each reaction displayed identical NMR data to those reported in the literature.[11] In conclusion, the preparation of this chiral auxiliary (**402**, **Scheme 125**) was straightforward. It also appeared to be easy to scale up for future preparation of an eventual intermediate for the preparation of mycolic acids.



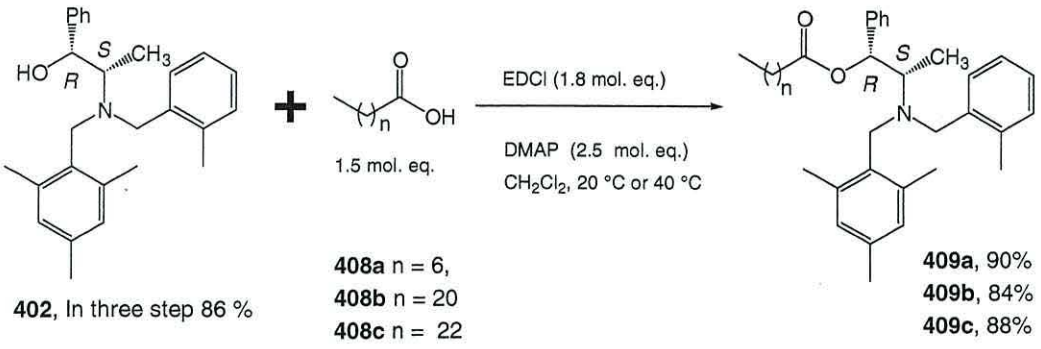
Scheme 125: ***Preparation of the chiral auxiliary***

4.4.3 The esterification process.

The next transformation was the esterification of different fatty acids with this chiral auxiliary compound (**Scheme 126**). The first acid chosen (**408a**) had a relatively short chain, even thought it was longer than that reported in the literature (**Scheme 124**). This was in order to prove the success of the reaction without problems of low solubility or reactivity, very often present when bigger molecules are employed. The second was a longer fatty acid (**408b**), which was very similar to the one necessary for the preparation of the target α^1-mycolic acid, but it was cheap and available from commercial sources. Therefore, this seemed the best test before progressing to the use of tetracosanoic acid (**408c**), the acid required for the preparation of the desired α^1-mycolate, which had to be prepared by oxidation of the corresponding alcohol using the Jones reagent.[392]

All the esterifications were achieved using similar procedures to that described in the paper of Kuruso and Lorca.[11] However, an excess of acid (**408**, 1.5 mol. eq.) was necessary in order to complete of the reaction. The process was helped by the use of the water soluble coupling reagent, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (1.8 mol. eq.) in conjunction with DMAP (2.5 mol. eq.). The excesses of EDCl and urea analogue, produced in the course of the reaction, were

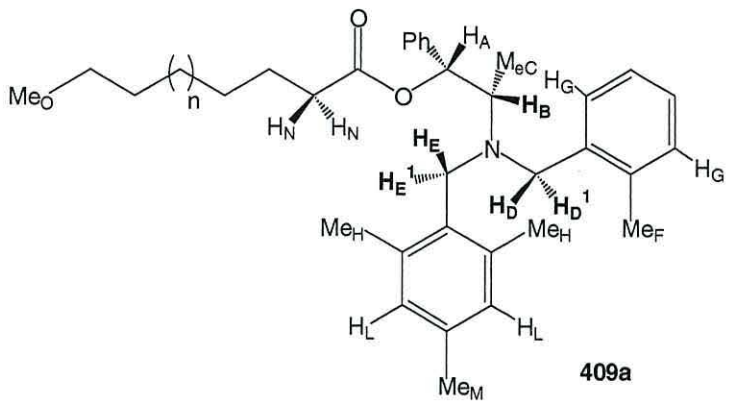
subsequently eliminated by washing with water, while the excess of acid was eliminated by subsequent washes with aq. sat. sodium bicarbonate. When the transformation was carried out with longer chains it was also necessary to reflux the solution to 40 °C to dissolve the acid completely (**Scheme 126**).



Scheme 126: ***The esterification reaction***

All three reactions provided the desired compounds, which showed very similar spectroscopic characteristics.

The ^{1}H NMR spectra of the products (**409a**, **409b**, **409c**) were very complex. In particular, in this section only that of compound (**409a**) will be described (**Table 3**). As expected, the four protons ($\mathbf{H_D}$, $\mathbf{H_D}^1$, $\mathbf{H_E}$ $\mathbf{H_E}^1$) next to both the nitrogen and the aromatic rings, gave two different kinds of signal, depending on their spatial interactions. The double doublet (J_1 = 13.3 Hz, J_2 = 2.5 Hz) at δ 3.74 corresponded to one proton next to the mesytal group and another proton next to the methyl benzene ($\mathbf{H_D}$, $\mathbf{H_E}$), while a triplet (J = 12 Hz) at δ 3.59 represented the other two protons next to these two groups, but on the opposite face of the plane ($\mathbf{H_D}^1$, $\mathbf{H_E}^1$).



409a

Groups	Multiplicity	δ (J in H_Z)
Me_O	t	0.88 (6.6)
Me_C	m	1.7-1.6
Me_M	s	2.10
Me_H	s	2.14
Me_F	s	2.27
H_N	t	2.33 (7.4)
H_B	m	3.22
H_D, H_E	t	3.59 (12.0)
H_D^1, H_E^1	dd	3.74 (13.3, 2.5)
H_A	d	6.07 (6.0)
H_L	s	6.78
H_G	dd	6.93 (9.7, 1.6)
Other aromatic protons	m	7.3-7.0
Other aliphatic protons	m	1.3-1.2

Table 3: ***The most significant peaks in the ^1H NMR spectrum of the ester, 409a.***[e]

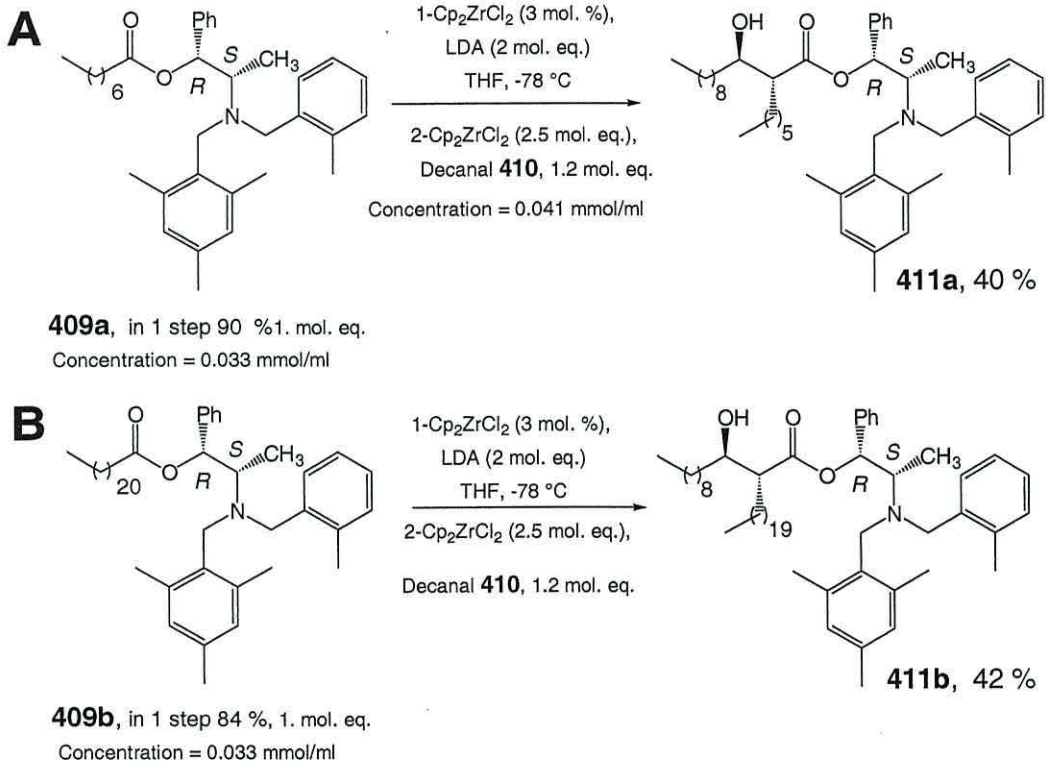
In the ^{13}C NMR spectrum, alongside the 14 different peaks for the different aromatic carbons, there were signals for the carboxylic group at δ 172.9 (C=O), a peak at 76.5 for the ester carbon and a peak at 34.8 for the methylene next to the carboxylic group. This spectrum also showed three different peaks corresponding to the carbons next to the nitrogen atom at δ 56.5 ($N\underline{C}HCH_3$), 51.3 ($N\underline{C}H_2$) and 47.4 ($N\underline{C}H_2$).

[e] It was not possible to determine by ROESY NMR, the spatial geometry of $\mathbf{H_D}$, $\mathbf{H_E}$ and $\mathbf{H_D^1}$, $\mathbf{H_E^1}$. They are illustrated in this way only to show that they are opposite to each others.

In the IR spectrum, medium bands at 3062 and 1612 cm^{-1} indicated the presence of aromatic protons, while a large band at 1735 cm^{-1} provided evidence of the ester group.

4.4.4 Use of the aldol condensation with long chain aldehydes

Having prepared the different ester substrates, the subsequent operation was their aldol condensation with a simple aldehyde (**410, Scheme 127**).



Scheme 127: ***The aldol condensation using a non-functionalised long chain***

The first couplings attempted employed compounds which did not contain any other functional groups, starting from small side chains (**409a, A, Scheme 127**), and then increasing their sizes (**409b, B, Scheme 127**).

The selective generation of the (*E*)-enolates of the various substrates (**409**) was obtained using LDA (2.5 mol. eq.) in the presence of Cp_2ZrCl_2 (0.3 mol. eq.) which was known to favour *anti/syn* selectivity. This process was then followed by transmetallation of the intermediates with Cp_2ZrCl_2 (2.5 mol. eq.) and then their aldolisation with the different aldehydes (1.2 mol. eq.).

None of these reactions reached completion. They were quenched by adding a diluted solution of HCl when variations in the proportions between the starting

materials and products with time were no longer noticeable using the TLC.

The examination of the TLC and of the ^{1}H NMR spectra of the crude products (**411a**, **411b**) treated with deuterated water suggested that mixtures of two different diastereoisomers were obtained. For example, for (**411b**), two doublets were visible in the ^{1}H NMR spectra corresponding to proton ($\mathbf{H_A}$) adjacent both to the oxygen and a phenyl ring (**Figure 45**), the major at δ 6.01 (J = 8.2 Hz) and smaller one at 6.03 (J = 6.7 Hz) (with a ratio up to 9:1); the same proton ($\mathbf{H_A}$) in the starting material resonated at δ 6.07 (J = 6.0 Hz). In a TLC with petrol ether (9:1), the two diastereosimers appeared as two spots at R_f = 0.66 for the major component of the mixture and at R_f = 0.59 for the other one.

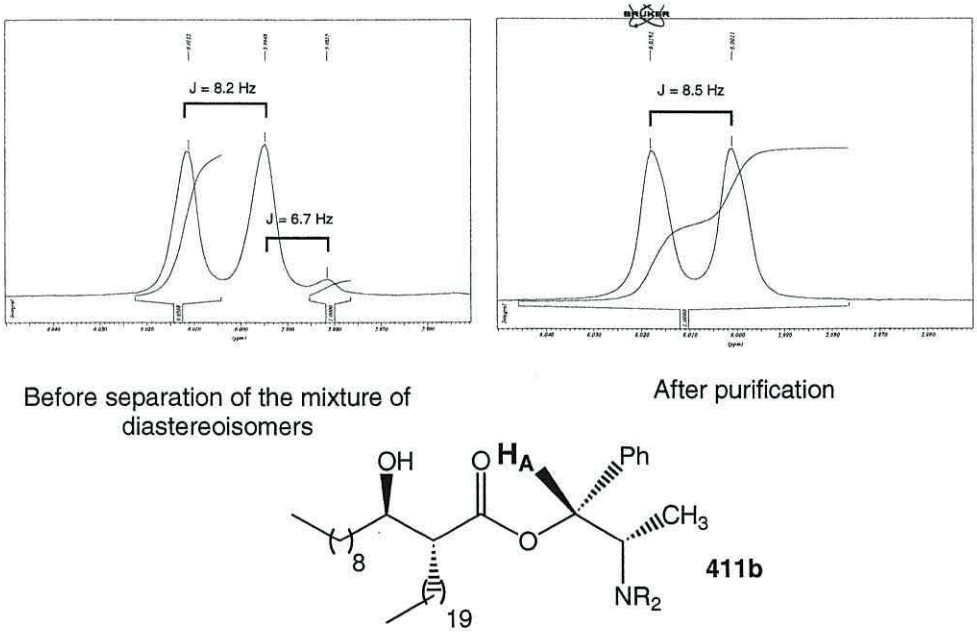


Figure 45: ***The different doublets for the proton next to the phenyl group and an oxygen atom***

It was possible to isolate the major component of the mixture by column chromatography.

Since both products (**411a**, **411b**) showed very similar chemical and spectroscopic characteristics, only those of (**411a**) are described in this section. Its IR spectrum displayed another band at ~ 3500 cm^{-1} for the hydroxyl group, when compared with the previously described starting material (**409a**). Its ^{1}H NMR spectrum, in addition to the doublet (J= 8.2 Hz) at δ 6.01 and the peaks for the chiral auxiliary, showed a multiplet at δ 3.60 for the proton next to the hydroxyl group and a double triplet (J_2 = 8.9 and J_1 = 5.2 Hz) at 2.38 for the proton in the α-position

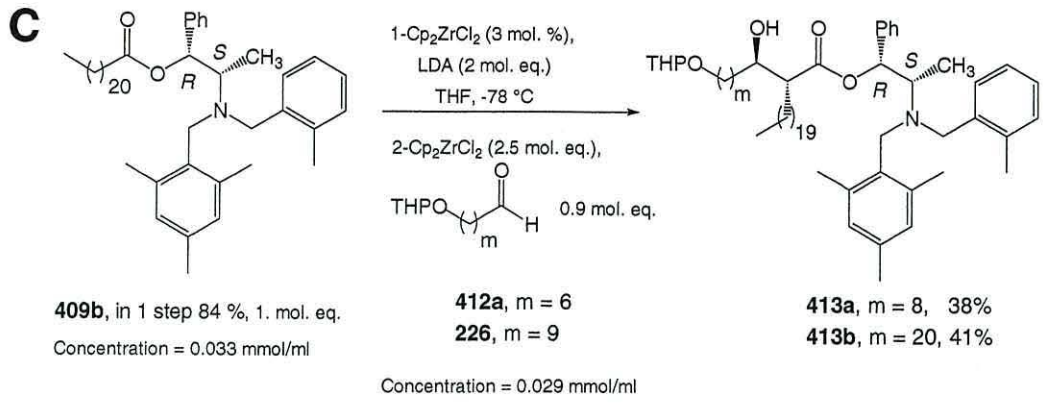
relative to the carboxylic group. The fact that there was only one set of signals for the product with the chiral auxiliary in place is the best evidence of the optical purity. Instead its optical rotation ($[\alpha]_D^{24}$ = - 3.8) does not give any evidence about its stereochemistry because the values reported in the literature for similar compound are very variable.[393] Its absolute stereochemistry could only be confirmed later, after eliminating the chiral auxiliary and then comparing the corinomycolate analogue obtained with the spectral values of the natural compound.

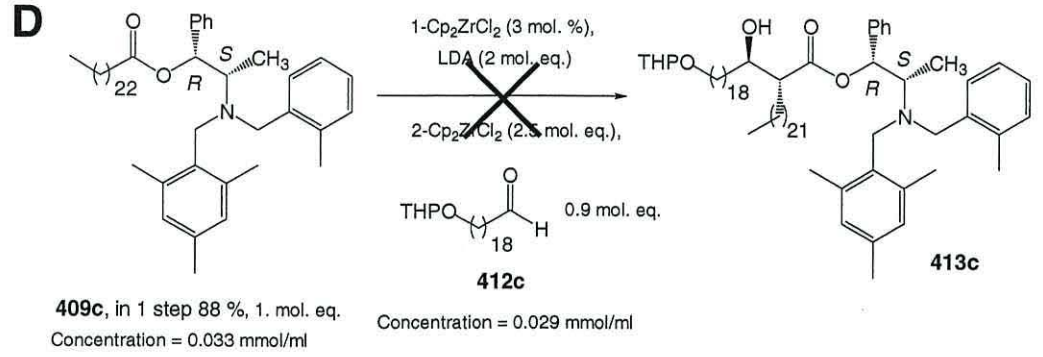
The ^{13}C NMR spectrum of the products (**411**) was very similar to that previously reported for the ester starting materials (**409**, **Section 4.4.2**); the most interesting peaks were at δ 72.2 (for the new hydroxyl group) and at 50.9 (for the carbon in the α-position). This confirmed the success of the aldol reaction.

4.4.5 The tetrahydropyranyloxy protection

Other attempts were subsequently carried out using previously prepared aldehydes that contained a hydroxyl group protected with tetrahydropyranyloxy group at the other end of the chain (**412a**, **226**, **412c**, **Scheme 128**). A similar procedure to that already described (**Section 4.4.3**) was employed, except for the amount of the aldehyde utilised. The use of a smaller quantity of the aldehyde (0.9 mol. eq.) made the purification of the final product easier, since the two have very similar R_f values.

Moreover, since the protecting group is labile in acid conditions, the aldol condensations could not be quenched with acid, and sat. aq. ammonium chloride was preferred. Its use, however, led to the formation of a thick emulsion, which could only be eliminated by filtration through a small pad of celite. The desired aldol products were always obtained, except when very long chain starting materials (**409c** and **412c**) were employed (**Experiment D**, **Scheme 128**). In this case, large amounts of the two starting materials were recovered. The most probable reason for this failure was the limited solubility of such large molecules at low temperatures.





Scheme 128: ***Other aldol reactions***

This conclusion is not definitive; more experiments need to be done, changing the conditions. However, it does suggest that whole meromycolate chains probably cannot be directly introduced in one step, using this method.

This is a similar conclusion to that previously arrived at for another method attempted (**Section 3.4.3**) which shows the difficulties in forming C-C bonds when very long molecules are utilised.

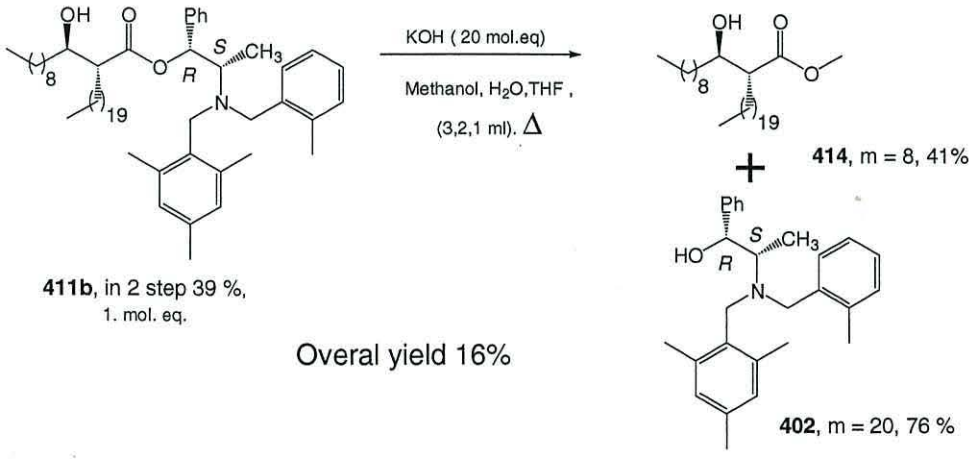
The products obtained (**413a**, **413b**) showed similar spectral properties to those described above for products of the aldol condensation with aldehydes containing long straight un-functionalised chains (**411**). For example, in the ^{1}H NMR spectrum of the purified product (**413a**), only one doublet (J = 8.2 Hz) was noticeable at δ 6.01 corresponding to the first proton of the ester group next to the aromatic ring ($\mathbf{H_A}$). The double triplet (J_1 = 8.9 Hz, J_2 = 5.4 Hz), at 2.37 for the proton in the α-position with respect to the carboxylic group, and its respective peak for the C in the same position in the ^{13}C NMR spectrum at δ 50.9, provided evidence of the success of the aldol condensation. The NMR spectra also proved the retention of the tetrahydropyranyloxy protecting group; for example the triplet

(J = 4.1 Hz) at δ 4.57 in the ^{1}H NMR spectrum and the peak in the ^{13}C NMR at δ 98.9 both corresponding to the acetal group.

4.4.6 The hydrolysis of the chiral auxiliary.

The final hydrolysis of the chiral auxiliary could only be achieved using strong basic conditions (KOH, in refluxed methanol) due to the presence of a very large ester group (**Scheme 129**). The reaction was tested only with a simple model compound, **411b**.

In order to avoid epimerisation of the proton in the α-position, which is slightly acidic, the reaction was conducted employing a large amount of water, as suggested by Minnikin.[394] The reaction was quenched with a dilute solution of HCl and the crude product was then treated with diazomethane. Both the chiral auxiliary (**402**) and (2*R*,3*R*)-2-(1-hydroxy-decyl)-docosanoic acid methyl esters (**414**) were recovered and fully characterised. The former (**402**) showed identical spectroscopic data to those illustrated in the literature for this compound,[11] while the latter showed the typical spectra of a β-hydroxy-α-alkyl ester, which have already been described above (**Section 4.2.2** and **Section 4.2.3**).



Scheme 129: ***The final hydrolysis***

In particular, it was possible to confirm the production of the required diastereoisomer alone. A TLC in chloroform of the purified product (**414**) showed a similar R_f (0.45) to that of (+)-corinomycolate (R_f = 0.42).[129] Moreover, in the ^{13}C NMR spectrum, the pattern of the signals at δ 176.3 (for C=O), 72.3 (for the carbon in the β-position), 51.5 (for the methoxy group), 50.9 (for the carbon in the

α-position) and 35.7 (for the carbon in the position γ), indicated the presence of an *anti*-diastereoisomer (**Section 4.2.2**). The ^{1}H NMR spectrum of the product (having treated it with D_2O) showed a singlet a δ 3.72 relating to the methoxy group, a multiplet at δ 3.66 for the proton next to the hydroxyl group and a double triplet (J_1 = 9.2 Hz, J_2 = 5.4 Hz) at δ 2.44 for the proton in the α-position (**Section 4.2.3**). The optical rotation of + 5.1 confirmed the formation of a (2*R*,3*R*)-3-hydroxy-2-alkyl ester, since the (2*R*,3*R*)-corinomycolate has an $[\alpha]_D^{23}$ of + 5.7.[264]

The overall yield for the formation of the β-hydroxy-α-alkyl ester (**414**, **Scheme 129**), was still only 16 %, but the outcome of the final reaction (currently 41 %) could definitely be improved by optimising the conditions for the hydrolysis of the ester.

In conclusion, these results, although they are preliminary because they were obtained only from model reactions, showed how this method could be an interesting alternative to the Fräter alkylation. This new approach seemed to be able to produce enantiomerically pure (2*R*,3*R*)-2-alkyl-3-hydroxy ester in fewer steps and probably with a better overall yield. It could be used for the formation of the target corinomycolate analogues with a high enantiomeric excess.

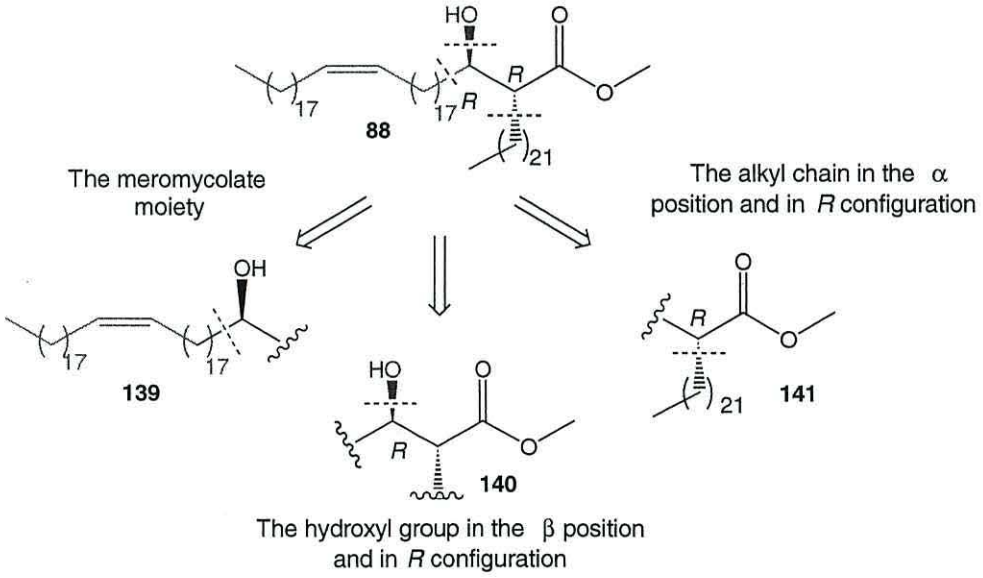
5. Conclusions

5.1.1 Overview

The aim of this study was to analyse different approaches for the synthesis of an α^1-mycolic acid (**88, Scheme 130**) presents in *Mycobacterium smegmatis*, a species which has often been used in studies for understanding the properties of this kind of organism.

This mycolic acid (**88**) had a simpler structure than most others, but the same difficulties common to the preparation of all types of mycolic acids were still encountered in its synthesis. Therefore, it was the ideal prototype in order to learn the best method for the preparation of all these fatty acids.

Three key steps of its preparation were analysed: the formation of the meromycolate chain (**139**); the introduction of the hydroxyl group in the β-position in an *R*-configuration (**140**); finally, the insertion of a C-21 chain in the α-position in *R*-configuration (**141, Scheme 130**).



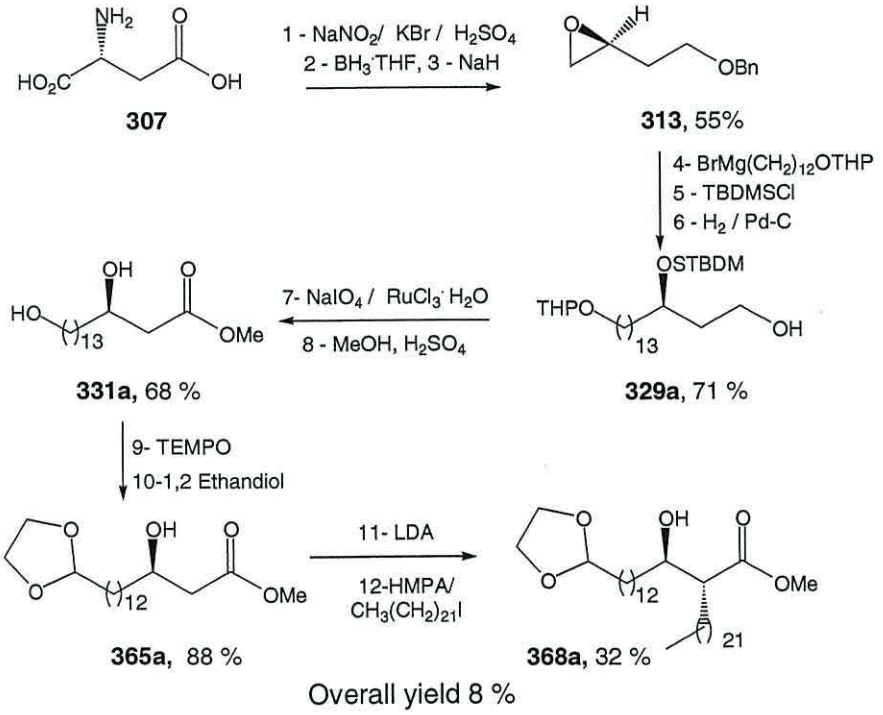
Scheme 130: ***The three different key steps***

Initially, different methods for the formation of the long chain were analysed (**139, Scheme 130**). The use of the Grignard coupling was determined to be the best method for the formation of a long, mono-functionalised chain, while a Julia reaction was preferable if a bi-functionalised chain had to be formed. For the introduction of the double bond into the meromycolate chain, a Wittig reaction

was a good method for the preparation of (*Z*)-unsaturated compounds with a good diastereomeric excess (**Chapter 2**).

Subsequently, three methods were tested for the preparation of the β-hydroxy ester (**140**, **Scheme 130**). Two of them were successful, one based on the use of the Sharpless dihydroxylation and one employing a chiral starting material, D-aspartic acid; the later approach was chosen because it required fewer steps and produced a better yield (**Chapter 3**).

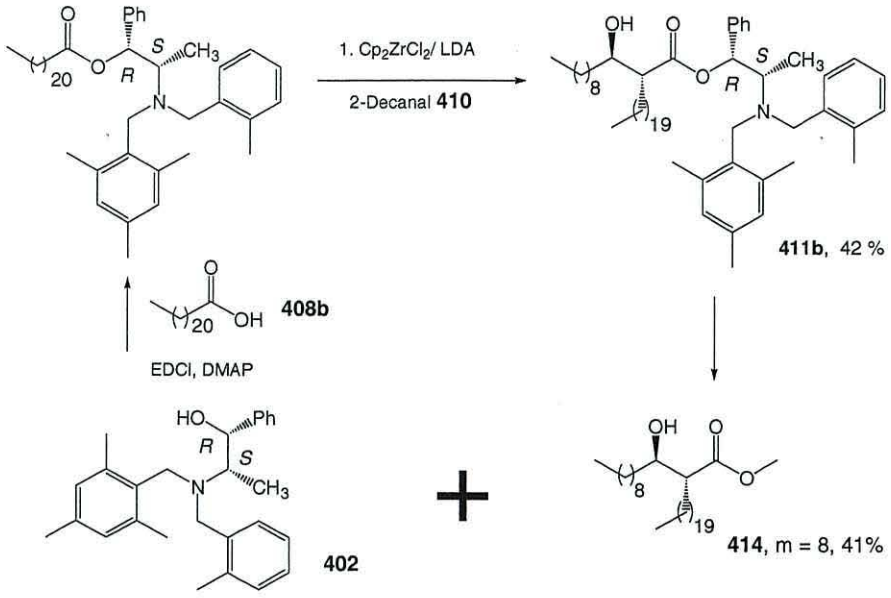
Finally, different approaches were analysed for the insertion of the alkyl chain (**141**, **Scheme 130**). The use of a Fräter coupling with a β-hydroxy ester, with a medium length side chain and a small non-aromatic protecting group was demonstrated to be a reliable method for the preparation of this kind of compound (**Chapter 4**). In particular, the desired β-hydroxy-α-alkyl ester (**368a**) was formed from the *D*-asparitc acid (**307**) in 11 steps with an overall yield of 8 % (**Scheme 131**).



Scheme 131: ***The preparation of the corinomycolate moiety through Dulayymi et al. method***

However, recently, a promising new method had been investigated. It was based on the use of a stereospecific aldol condensation. This approach was only tested in the production of a simple model compound (**414**). However, it gave interesting

results, producing the desired compound (**414**), optically pure and with an overall yield of 16 % in only three steps (**Chapter 4**). Moreover, it permitted the recovery of the chiral auxiliary (**402**). More tests remains to be done but this could constitute an interesting alternative to the Fräter alkylation for the general production of mycolic acids.



Scheme 132: ***New method for the preparation of β-hydroxy-α-alkyl esters***

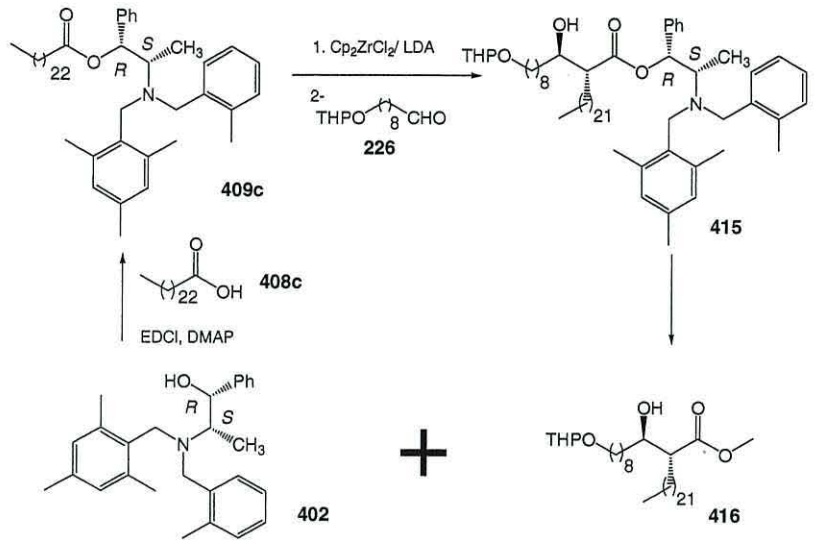
In conclusion, at end of this study the target molecule was not achieved. However, interesting synthetic approaches were investigated, determining the advantages and disadvantages of each of them in the preparation of not only the target molecule, but all mycolic acids.

5.1.2 Future work

In the light of the results achieved, further approaches could be tested in order to obtain the desired compound.

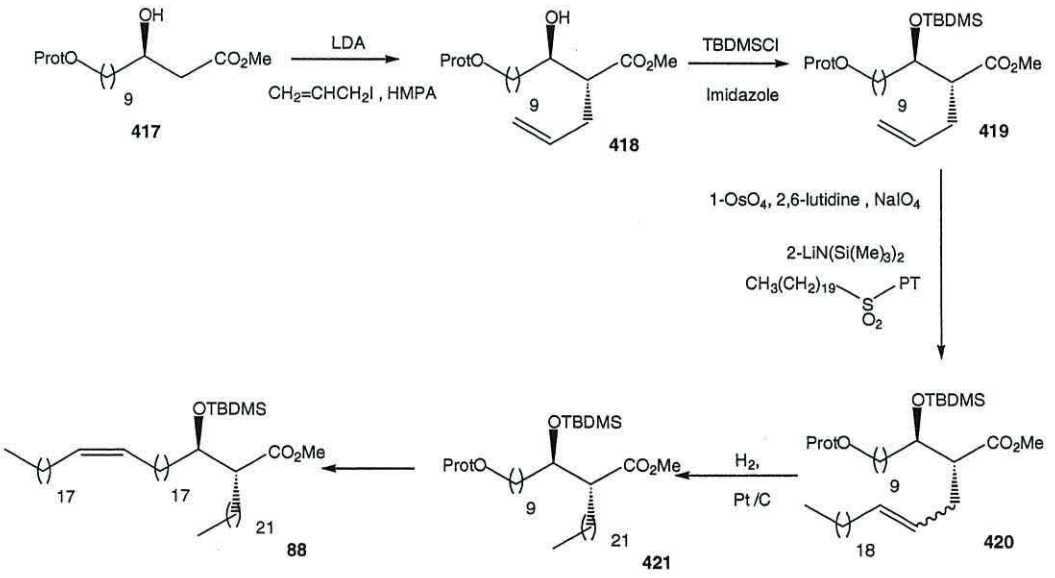
The major difficulty encountered in the synthesis of the target molecule was the introduction of the alkyl chain in the α-position in the *R*-configuration.

One possibility that should be investigated would be to use the aldol-condensation method with the real compound in order to verify if this strategy is as successful as it was in the tests with the model compounds (**Scheme 133**).



Scheme 133: ***The aldol condensation method for the preparation of the mycolic motif***

A second interesting alternative would be to reattempt the Fräter alkylation, but with a smaller and more reactive iodide compound, such as allyl iodide, which should favour this difficult coupling (**Scheme 134**). After having protected the secondary alcohol (**418**), an OsO_4-$NaIO_4$ oxidation could transform the alkene into the corresponding aldehyde. Then, a Julia reaction, followed by the reduction of the double bond, could lead to the formation of the desired long chain (**421**, **Scheme 134**). This strategy has already been demonstrated to be a very successful method for extending alkyl chains.



Scheme 134: ***A new Fräter alkylation approach for the preparation of the mycolic motif***

Having prepared the mycolic motif (**421**), the preparation of the final α^1-mycolic acid (**17**) should be relatively straightforward, following the methods described previously. This compound could then be tested to determine the physical and biological properties of mycolic acids in general, hopefully giving interesting new results.

6 Experimental section

6.1 General considerations

All chemicals were purchased from Lancaster Synthesis Ltd., Aldrich Chemical Co. Ltd., or Avocado Chemical Co. Ltd. Diethyl ether and THF were distilled over sodium and benzophenone under nitrogen, while dichloromethane was distilled over calcium hydride. Organic solutions were dried over anhydrous magnesium sulphate and bulk solvents were removed at 14 mm Hg; residual traces of solvent were finally removed at 0.1 mmHg. All glassware used in anhydrous reactions was dried for not less than 5 h in a 250 °C oven.

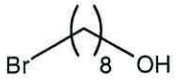
Column chromatography was conducted under medium pressure using silica gel from *BDH* (particle size 33-70 μm); thin layer chromatography (TLC) was carried out on pre-coated Kieselgel 60 F254 (Art. 5554; Merck) plates. Routine gas liquid chromatography (GLC) was performed using a temperature programmable Hewlett-Packard (Agilent) 5890 Gas Chromatograph with manual injection. The carrier gas was 5.0 grade helium with a column head pressure of 100Kpa supplied by Air Products plc. The column was Rtx-5 supplied by Restek Corporation. The phase thickness was 25 μm. Column internal diameter was 0.31 mm and the length was 15 m. Chiral GLC, was conducted using an isothermal Perkin Elmer Sigma 4 instrument fitted with a 25 m, 0.25 mm internal diameter heptakis (3-*O*-acetyl-2-*O*-methyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodestryn capillary column. Helium was employed as carrier gas; the column was fitted with a Perking Flame ionization detector. Melting points were measured using a Gallenkamp melting point apparatus. Optical rotations were measured as solutions in chloroform of known concentration using a Polar 2001 automatic polarimeter. Infra-red spectra were recorded as KBr disc (solid) or thin films on NaCl windows or using a Perkin Elmer 1600 series FT-IR spectrometer. NMR spectra were recorded either on a Bruker AC 250 spectrometer with 5 mm Dual probe or on a Bruker Advance 500 spectrometer with 5 mm BBO probe as solutions in deaturated chloroform ($CDCl_3$) if not differently indicated. All chemical shifts were quoted in δ relative to the trace resonance of protonated chloroform (δ 7.27 ppm), and $CDCl_3$ (δ 77.0 ppm). Low resolution mass spectra using electron impact (EI) were measured at 70 eV on Hewlett-Packard (Agilent) 5970 quadrupole mass selective detector where the Gas Chromatograph was Hewlett-Packard (Agilent) 5890 Gas

Chromatograph with a 5973 autosampler. It contained a Rtx-5 column supplied by Restek corporation. The phase thickness is 25 μm. Column internal diameter was 0.25 mm and the length is 25 m.

Elemental analysis (C, H, N) was performed with a Carlo-Erba Model 1106 CHN analyser with a precision of 0.2 % for each element. Accurate mass spectra were obtained through a Waters (Micromass) GCT time of flight (TOF) mass spectrometer. The system was controlled by MassLynx version 4 software. Spectra were acquired at 70 ev using electron ionisation. Or by Chemical ionisation (NH_3, CH_4). Precise mass determination on pure compounds was measured to better than 5 ppm precision above a mass of 300 Da; samples were introduced by Direct Insertion Probe programmable up to 550 °C. For very large compounds an LC Agilent 1100 system with quaternary pumping system, degasser, diode array detector, and autosampler, and is capable of isocratic or gradient elution was used. Thy system was controlled by Agilent Chemstation or Bruker Hystar software depending upon which of the following mass spectrometers it was connected to, a Bruker MicroTOF time of flight mass spectrometer with ESI or APCI source.

6.2 Experiments

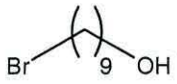
Experiment 1: 8-Bromo-octan-1-ol (151a)



1,8-Octandiol (**150a**, 25 g, 171 mmol) was dissolved in toluene (300 ml) and aqueous hydrobromic acid (30 ml, 48 % w.w.) was added. The mixture was refluxed for two days monitoring the reaction with TLC. The mixture was then cooled to room temperature, the organic layer was separated and the solvent was removed. The residue, a brown oil, was dissolved in dichloromethane (600 ml) and washed with sodium bicarbonate (300 ml). The aqueous layer was re-extracted with dichloromethane (3 x 150 ml). The combined organic layers were dried and the solvent evaporated to give the crude product which was purified by column chromatography eluting with petrol and ether (5:1) to give 8-bromo-

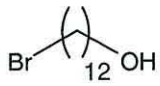
octan-1-ol (**151a**) as pale yellow oil, (29 g, 81 %), The NMR spectra of the compound obtained were identical to those reported.[395]

Experiment 2: 9-Bromononan-1-ol (151b)



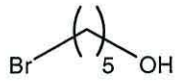
The procedure used in **Experiment 1** was repeated in order to convert 1,9-nonandiol (**150b**, 25 g, 156 mmol) into 9-bromononan-1-ol (**150b**, 30 g, 86 %), whose NMR spectra were identical to the ones reported.[396]

Experiment 3: 12-Bromododecan-1-ol (151c)



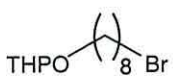
The procedure used in **Experiment 1** was repeated in order to convert 1,12-dodecandiol (**150c**, 25 g, 124 mmol) into 12-bromododecan-1-ol (**151c**, 27 g, 82 %). whose NMR spectra corresponded to the ones reported.[397]

Experiment 4: 5-Bromopentan-1-ol (151d)



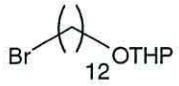
The procedure used in **Experiment 1** was repeated, but using hexane instead of toluene as solvent, in order to convert the 1,5-pentandiol (**150d**, 25 g, 240 mmol) into 5-bromopentan-1-ol (**151d**, 17 g, 42 %), whose ^{1}H ^{13}C NMR and IR spectra were identical to the ones reported.[398]

Experiment 5: 2-(8-Bromo-octyloxy)tetrahydropyran (153a)



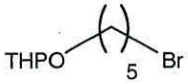
3,4-Dihydro-2*H*-pyran (33.2 g 394 mmol) and then pyridinium *p*-toluenesulfonate (1.9 g, 7.8 mmol) were added to a solution of 8-bromo-octan-1-ol (**151a**, 33 g, 157 mmol) in dry dichloromethane (100 ml). The reaction was followed by TLC; after 2 h, it was quenched by adding sat. aq. sodium bicarbonate (50 ml). The aqueous layer was extracted with dichloromethane (3 x 20 ml) and the combined organic layers were dried. The solvent was removed to give 2-(8-bromo-octyloxy)tetrahydropyran (**153a**, 46 g, 100 %) as a pale yellow oil which was used without other purification. Its NMR spectra were identical to those reported.[399]

Experiment 6: 2-(12-Bromododecyloxy)tetrahydropyran (153c)



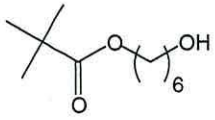
The procedure used in **Experiment 5** was repeated in order to convert 12-bromododecan-1-ol (**151c**, 20 g, 75 mmol) into 2-(12-Bromododecyloxy)tetrahydropyran (**153c**, 25 g, 94 %), whose NMR spectra corresponded to the ones reported.[400]

Experiment 7: 2-(5-Bromopentyloxy)tetrahydropyran (153d)



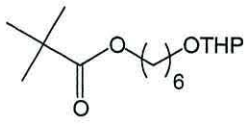
The procedure used in **Experiment 5** was repeated in order to convert 5-bromopentan-1-ol (**151d**, 15 g, 89 mmol) into 2-(5-bromo pentyloxy)tetrahydropyran (**153d**, 21.5 g, 96 %), whose NMR spectra were identical to the ones reported.[401]

Experiment 8: 2,2-Dimethylpropionic acid 6-hydroxyhexyl ester (155)



Trimethylacetyl chloride (13.2 g, 13.5 ml, 110 mmol) was added to a solution of 1,6-hexanediol (**154**, 11.8 g, 100 mmol) and pyridine (14.2 g, 14.5 mml, 180 mmol) in dry THF (100 ml) at 5 °C. Within a few minutes, pyridine hydrochloride separated out as a white solid. The mixture was stirred overnight at room temperature. Then the reaction was quenched with dil. HCl (2 N, 200 ml). The product was extracted with dichloromethane (3 x 300 ml) and the combined organic layers were washed with dil. HCl (2 N, 100 ml) and sat. aq. sodium bicarbonate (100 ml), dried and the solvent evaporated. The crude product was then purified by column chromatography eluting with petrol: ether (7:3) to give 2,2-dimethylpropionic acid 6-hydroxyhexyl ester (**155**, 11.3 g, 56 %). The final compound showed the same NMR properties as those reported.[402]

Experiment 9: 2,2-Dimethylpropionic acid 6-(tetrahydropyran-2-yloxy)hexyl ester (156)



The procedure in **Experiment 5** was repeated only using (2,2-dimethylpropionic acid 6-hydroxyhexyl ester (**155**, 3 g, 14.8 mmol) to give *2,2-dimethylpropionic acid 6-(tetrahydropyran-2-yloxy)hexyl ester* (**156**, 3.5 g, 82 %).

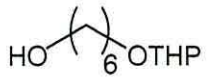
Found $_{(TOF\ MS)}$ $(M+K)^+$: 325.1760; $C_{16}H_{30}KO_4$ requires: 325.1781.

ν_{max}/cm^{-1}: 2934, 2867, 1730, 1480, 1461, 1397, 1383, 1365, 1352, 1322, 1284, 1200, 1157, 1078, 1035.

$\delta_{H\ (500\ MHz)}$: 4.52 (1H, t, J = 2.9 Hz, OCHO) 4.00 (2H, t, J = 6.7 Hz CH_2OCO) 3.83-3.77 (1H, m,), 3.72-3.67 (1H, m), 3.46-3.42 (1H, m), 3.36-33.30 (1H, m), 1.9-1.3 (14H, m), 1.14 (9H, s $(CH_3)_3$).

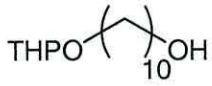
$\delta_{C\ (125.8\ MHz)}$: 178.4 (C=O), 98.7 (O<u>C</u>HO), 67.3 (CH_2OTHP), 64.2 (<u>C</u>H_2O), 62.1 (CH_2OTHP), 38.6 (<u>C</u>$(CH_3)_3$), 30.6 (<u>C</u>H_2), 29.5 (<u>C</u>H_2), 28.4 (<u>C</u>H_2), 27.0 (<u>C</u>H_3), 25.8 (<u>C</u>H_2), 25.7 (<u>C</u>H_2), 25.4 (<u>C</u>H_2), 19.5 (<u>C</u>H_2).

Experiment 10: 6-(Tetrahydropyran-2-yloxy)hexan-1-ol (157)



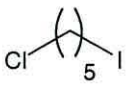
2,2-Dimethylpropionic acid 6-(tetrahydropyran-2-yloxy)hexyl ester (**156**, 3.2 g, 11 mmol) was added to a solution of KOH (1.8 g, 33 mmol) in methanol (40 ml) and water (5 ml). The mixture was refluxed overnight, then worked up by evaporating the solvent. The residue was dissolved in water (50 ml) and extracted by dichloromethane (3 x 75 ml). The combined organic layers were dried and evaporated to give the crude product which was purified by column chromatography eluting with petrol and ether (7:3) to give 6-(tetrahydropyran-yloxy)hexan-1-ol (**157**, 2.1 g, 96 %) which showed the same NMR spectra that those reported.[403]

Experiment 11: 10-(Tetrahydropyran-2-yloxy)decan-1-ol (159)



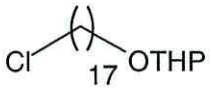
1,10-Decandiol (**158**, 10g, 57 mmol) was dissolved in dichloromethane (200 ml) and THF (20 ml). 3,4 dihydropyran (4.8 g, 57 mmol) and pyridinium *p*-toluene-sulphonate (5.7 mmol) were added to the solution. The reaction was stirred for 6 h when TLC still showed starting material but a majority of mono-protected and a small amount of diprotected diol. The reaction was quenched by adding sodium bicarbonate (60 ml). The organic layer was separated and the water layer was extracted with dichloromethane (3 x 60 ml). The combined organic layers were dried over magnesium sulphate and the solvent was evaporated to give the crude product which was purified by column chromatography eluting with petrol and ether (9:1) to give 10-(tetrahydropyran-2-yloxy)decan-1-ol (**159**, 6.2 g, 42 %) which showed the same ^{1}H NMR and ^{13}C NMR spectra to those described.[404,405]

Experiment 12: 1-Chloro-5-iodopentane (178)



To a solution of 1-bromo-5-chloropentane (**174**, 12.8 g, 69 mmol) in acetone (150 ml) was added sodium iodide (11.4 g, 76 mmol). The mixture was stirred at 60 °C and the reaction was followed by GC. After 5 h, the mixture still contained a small amount of starting material but the reaction was worked up in order to avoid the formation of the diiodo-compound. The solvent was evaporated to give a solid which was dissolved in dichloromethane (50 ml) and washed with water (50 ml). The water layer was extracted with dichloromethane (2 x 15 ml) and the organic layers were combined, dried and evaporated. Eliminating the solvent gave the crude product which was purified by column chromatography eluting with only petrol to give 1-chloro-5-iodopentane (**178**, 14.3 g, 89 %). The compound showed the same proton NMR and IR to those reported.[406]

Experiment 13: 2-(17-Chloroheptadecyloxy)tetrahydropyran (176)



A) Magnesium shavings (0.67 g, 28 mmol) were suspended in dry THF (10ml) under nitrogen and 2-(12-bromododecyloxy)tetrahydropyran (**153c**, 5 g, 14.3 mmol), dissolved in dry THF (15 ml) was added over a period of 1 h. In order to start the reaction, the mixture was warmed. The temperature was kept around 60 °C during the addition by heating. After the completion of the addition the mixture was refluxed for 2 h, when GC showed the absence of starting material. The mixture was cooled to – 10 °C and 1-bromo-5-chloropentane (**174**, 4 g, 21.5 mmol) dissolved in dry THF (20 ml) was added over a 5 min. The mixture was cooled to – 60 °C and a solution of Li_2CuCl_4 (7 ml, 0.7 mmol, 0.1 M in THF) was added. The reaction was warmed to room temperature and stirred overnight, then quenched with sat. aq. ammonium chloride (20 ml) followed by addition of dichloromethane (30 ml). The mixture was stirred for 0.5 h, the two layers were separated and the aqueous layer was washed with dichloromethane (3 x 20 ml). The combined organic layers were dried and the solvent evaporated to give a liquid. The starting material and 1-bromo-tridecane, a by-product of the reaction, were eliminated by distillation under reduced pressure to give a pale yellow solid which was purified by column chromatography eluting with petrol and ether

(10:1) to give *2-(17-chloroheptadecyloxy)tetrahydropyran* (**176**, 2 g, 38 %) and *1,24-bis(2-tetrahydropyranyloxy)tetracosane* (**175**, 2 g, 27 %), 2-dodecyloxy-tetrahydropyran (**177**, 0.5g, 13 %) which showed the same ^{1}H NMR to that reported,[407] and other by-products.

B) Repeating the same reaction as in (**A**), only using 2 mol % of Li_2CuCl_4, gave a similar mixture of products. After purification it was possible to obtain the desired product (**176**) with a yield of 25 %.

C) Repeating the same reaction as in (**A**), without using of Li_2CuCl_4, gave a similar mixture of products. After purification it was possible to obtain the desired product (**176**) with a yield of only 10 % after 3 days.

D) Repeating the same reaction as in (**A**), but with 1-chloro-5-iodopentane (1.5 mol. eq.) as coupling reagent, gave a similar mixture of products. After purification it was possible to obtain the desired product with a yield of 42 % (**176**) but still not completely purified.

E) Repeating the same reaction as in (**A**), but using 5 mol % of Li_2CuCl_4 and 1-chloro-5-iodopentane (1.5 mol. eq.) as coupling reagent, gave a similar mixture of products. After purification it was possible to obtain the desired product pure (**176**) with a yield of 45 %.

For *2-(17-chloroheptadecyloxy)tetrahydropyran*

Found C: 69.9, H: 11.4, required for $C_{22}H_{43}ClO_4$: C: 70.46, H: 11.56.

ν_{max}/cm^{-1}: 2926, 2854, 1465, 1366, 1353, 1322, 1284, 1260, 1200, 1184, 1132.

$\delta_{H\ (250\ MHz)}$: 4.56 (1H, t, J = 3.6 Hz, OC<u>H</u>O), 3.90-3.78 (1H, m), 3.67 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.6 Hz) 3.5-3.4 (3H, m including t for 2H, J = 6.7 Hz C<u>H</u>$_2$Cl), 3.33 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.7 Hz) 1.9-1.2 (36H, m).

$\delta_{C\ (62.9\ MHz)}$: 98.8, (O<u>C</u>HO), 67.6 (CH_2OTHP), 62.3 (CH_2OTHP), 44.8 32.6, 30.7, 29.7, 29.6, 29.6, 29.5, 29.4, 28.8, 26.8, 26.3, 25.5, 22.5, 19.6.

For *1,24-bis(2-tetrahydropyranyloxy)tetracosane*

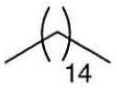
Found C: 75.5, H: 12.7, required for $C_{34}H_{66}O_4$: C: 75.78, H: 12.34.

ν_{max}/cm^{-1}: 2926, 2854, 1465, 1353, 1323, 1260, 1200, 1135, 1076, 1032.

$\delta_{H\ (250\ MHz)}$: 4.56 (2H, t, J = 3.6 Hz, OC<u>H</u>O), 3.90-3.78 (2H, m), 3.67 (2H, dt, J_1 = 9.5 Hz, J_2 = 6.6 Hz), 3.5-3.4 (2H, m), 3.33 (2H, dt, J_1 = 9.5 Hz, J_2 = 6.6 Hz) 1.9-1.2 (56H, m).

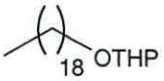
$\delta_{C\ (62.9\ MHz)}$: 98.8, (O<u>C</u>HO), 67.6 (CH_2OTHP), 62.3 (CH_2OTHP), 30.7 29.7, 29.7, 29.6, 29.5, 29.0, 26.3, 25.5, 22.5, 20.4, 19.6.

Experiment 14: Hexadecane (190)



The Grignard reagent (**188**) obtained from 1-chlorohexane (5 g, 42 mmol), was prepared with the same method described in **Experiment 13**. This was added to a solution of 1-bromodecane (**189**, 7 g, 32 mmol) in dry THF (20 ml) at – 20 °C. The reaction mixture was cooled to - 78 °C and isoprene (2.17 g, 32 mmol) and $NiCl_2$ (0.125 g, 1 mmol) were added. The reaction was warmed to room temperature and stirred overnight. The reaction was then quenched with sat. aq. ammonium chloride (20 ml) followed by addition of dichloromethane (30 ml). The mixture was stirred for 10 min and the two layers were separated. The aqueous layer was washed with dichloromethane (3 x 20 ml). The combined organic layers were dried and the solvent was evaporated to give the crude product which was purified by column chromatography eluting with petrol and ether (10:1) to give hexadecane (**190**, 6.3 g, 87 %) which showed the same proton NMR, and IR and MS spectra as those reported.[408-410]

Experiment 15: 2-Nonadecyloxytetrahydropyran (192)



The procedure used in **Experiment 14** was repeated in order to couple 1-bromoheptane (3 g, 16.7 mmol) with 2-(12-bromododecyloxy)tetrahydropyran (**153c**, 3.9 g, 11.2 mmol) to give *2-nonadecyloxytetrahydropyran* (**92**, 3.2 g, 78 %).

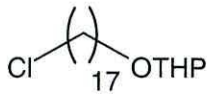
Found $_{(TOF\ MS)}$ $(M+Na)^+$: 391.3551; $C_{24}H_{48}O_2Na$ requires: 391.3552.

ν_{max}/cm^{-1}: 2924, 2853, 1466, 1352, 1322, 1260, 1200, 1120, 1079, 1034.

$\delta_{H\ (250\ MHz)}$: 4.57 (1H, t, J = 3.6 Hz, OC<u>H</u>O), 3.9-3.8 (1H, m), 3.73 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.7 Hz) 3.5-3.4 (1H, m) 3.4 (1H, dt, J_1 = 9.4 Hz, J_2 = 6.6 Hz) 1.9-1.2 (40H, m), 0.88 (3H, t, J = 7Hz, $CH_2C\underline{H}_3$).

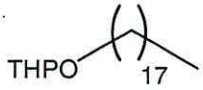
$\delta_{C\ (62.9\ MHz)}$: 98.8, (O<u>C</u>HO), 67.6 (CH_2O THP), 62.2 (CH_2O THP), 31.9, 30.7, 29.7, 29.7, 29.6, 29.5, 29.3, 29.2, 26.2, 25.5, 22.7, 19.6, 14.1(<u>C</u>H_3).

Experiment 16: Attempted preparation of 2-(17-chloroheptadecyloxy) tetrahydropyran (176)



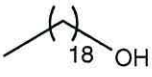
The procedure used in **Experiment 14** was repeated in order to couple 2-(12-bromododecyloxy)tetrahydropyran (**153c**, 3.5 g, 10 mmol) with 1-bromo-5-chloropentane (**174**, 1.43 g, 7.7 mmol). The reaction gave a complex mixture of by-products, which contained the desired product (**176**) only in traces.

Experiment 17: 2-Octadecyloxytetrahydropyran (194)



The procedure used in **Experiment 14** was repeated in order to couple 2-(12-bromododecyloxy)tetrahydropyran (**153c**, 3.75 g, 11 mmol) and 1-bromohexane (**193**, 2.4 g, 14.3 mmol). The reaction produced only reduced starting material 12-dodecyloxytetrahydropyran (**177,** 1.3 g, 45 %) which showed the same ^{1}H NMR to that reported.[407]

Experiment 18: Nonadecan-1-ol (197)



The procedure used in **Experiment 13 E** was repeated in order to couple 12-bromododecan-1-ol (**151c**, 5.32 g; 20 mmol) with the Grignard reagent (**191**)

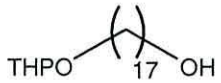
obtained from 1-bromoheptane (10.8 g, 60 mmol) and Mg (1.92 g, 80 mmol) in the presence of a solution of Li_2CuCl_4 (10 ml, 1 mmol, 0.1 M in THF) in THF (80 ml). The residue was purified by crystallisation from dichloromethane to give nonadecan-1-ol (**197**, 5.6 g, 98 %) as a white solid that showed the same ^1H NMR spectrum as that reported,[411] and a similar mp.[412]

mp: 59 - 62 °C, lit. 62 °C.[412]

$\delta_{H\ (500\ Mhz)}$: 3.67 (2H, t, J = 6.6 Hz, $C\underline{H}_2OH$), 1.67 (2H, m, $C\underline{H}_2CH_2OH$), 1.4-1.1 (33H, m), 0.88 (3H, t, J = 6.8 Hz, $C\underline{H}_3$).

$\delta_{C\ (125.8\ MHz)}$: 63.1 ($\underline{C}H_2OH$), 32.8 ($\underline{C}H_2CH_2OH$), 31.9 ($\underline{C}H_2$), 29.7 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 25.7 ($\underline{C}H_2$), 25.7 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 14.1 ($\underline{C}H_3$).

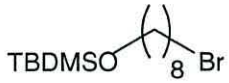
Experiment 19: 17-(Tetrahydropyran-2-yloxy) heptadecan-1-ol (196)



A) The Grignard reagent from 2-(5-bromopentyloxy)tetrahydropyran (*195*, 3 g, 12 mmol, 1.5 mol. eq.) was prepared has described in **Experiment 13**. A solution of methyl magnesium chloride (3.3 ml. 10 mmol, 3 M in THF), was added over 5 min at - 25 °C to a solution of 12-bromododecan-1-ol (**151c**, 2.13 g, 8 mmol) in dry THF (15 ml), followed by the addition of a solution of Li_2CuCl_4 (1.6 mml, 0.16 mmol, 0.1 M in THF). After 15 min, the above Grignard solution was added over 10 min to this solution. The reaction was warmed to room temperature and stirred for 3 days, then quenched with sat. aq. ammonium chloride (20 ml) followed by the addition of dichloromethane (30 ml). The reaction mixture was stirred for 10 min and the two layers were separated. The aqueous layer was washed with dichloromethane (3 x 20 ml). The combined organic layers were dried and the solvent was evaporated to give the crude product which was purified by column chromatography eluting with petrol and ether (5:2) to give 17-(tetrahydropyran-2-yloxy)heptadecan-1-ol (**196**, 1.2 g, 42 %), which showed ^1H NMR and ^{13}C NMR spectra identical to those reported.[413]

B) A solution of methyl magnesium chloride (3.3 ml. 10 mmol, 3 M in THF) was added over a period of 5 min to a solution of 1-bromododecan-1-ol (**151c**, 2.13 g, 8 mmol) in dry THF (15 ml) at – 25 °C followed by the addition of the Grignard reagent (**195**) prepared from 2-(5-bromopentyloxy)tetrahydropyran (3.75 g, 15 mmol, 1.5 mol. eq.) using the method described above. The mixture was cooled to - 78 °C and isoprene (681 mg, 10 mmol) and $NiCl_2$ (40 mg, 0.3 mmol) were added. The mixture was warmed to room temperature and stirred overnight. Then the reaction was quenched with sat. aq. ammonium chloride (20 ml) followed by addition of dichloromethane (30 ml). The mixture was stirred for 10 min and the two layers were separated. The aqueous layer was washed with dichloromethane (3 x 20 ml). The combined organic layers were dried and the solvent was evaporated to give the crude product which was purified by column chromatography eluting with petrol and ether (3:2) to give 17-(tetrahydropyran-2-yloxy)heptadecan-1-ol (**196**, 2.2 g, 77 %), identical by NMR and IR to that above.

Experiment 20: (8-Bromo-octyloxy)*tert*-butyldimethylsilane (198)



Triethylamine (16.8 ml, 120 mmol) was added dropwise at 0 °C to a solution of bromo-octan-1-ol (**151a**, 21 g, 100 mmol) in dry dichloromethane (100 ml) under nitrogen. After stirring for 10 min, *tert*-butyldimethylchlorosilane (15.9 g, 106 mmol) was added followed by the addition of 4-dimethylaminopyridine (130 mg). The reaction mixture was then stirred for 16 h at room temperature followed by quenching with water (25 ml). The aqueous layer was extracted with dichloromethane (3 x 15 ml) and the combined organic layer were dried and concentrated to give the crude product. This was purified by column chromatography eluting with petrol and ethyl acetate (9:1) to give (8-bromo-octyloxy)*tert*-butyldimethylsilane (**198**, 29 g, 90 %) whose NMR and IR spectra were consistent with the data reported.[414]

Experiment 21: *tert*-Butyl(8-iodo-octyloxy)dimethylsilane (199)

(8-Bromo-octyloxy)*tert*-butyldimethylsilane (**198**, 29 g, 90 mmol) was added to a stirred suspension of sodium iodide (40.5 g, 269 mmol) in acetone (500 ml) at room temperature. After the addition of sodium bicarbonate (7.9 g, 94 mmol), the reaction mixture was refluxed for 2.5 h. The solvent was then evaporated and the residue was dissolved in dichloromethane (500 ml) and washed with water (200 ml). The aqueous layer was extracted with dichloromethane (3 x 50 ml) and the combined organic layers were dried and concentrated. The crude product was purified by column chromatography eluting with petrol and ether (9:1) to give *tert*-butyl(8-iodo-octyloxy)dimethylsilane (**199**, 31.9 g, 96 %). The compound had already been reported in several publications, however none of them describe any physical or chemical data.[415-417]

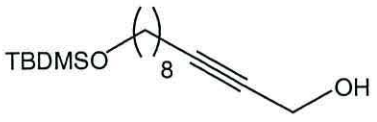
Found $_{(TOF\text{-}MS)}$ $(M+H)^+$: 371.1257; $C_{14}H_{32}IOSi$ requires: 371.1267.

ν_{max}/cm^{-1}: 2928, 2855, 1471, 1386, 1360, 1254, 1195, 1101, 1005.

$\delta_{H\ (250\ MHz)}$: 3.60 (2H, t, J = 6.4 Hz, C$\underline{H}_2$OSi), 3.20, (2H, t, J = 7.0 Hz, C$\underline{H}_2$I), 1.9-1.2 (13H, m), 0.90 (9H, s (C$\underline{H}_3$)$_3$CSi), 0.05 (6H, s, (C$\underline{H}_3$)$_2$Si).

$\delta_{C\ (62.9\ MHz)}$: 63.2 (C$\underline{H}_2$OSi), 33.5, 32.8, 30.4, 29.2, 28.5, 26.0, 25.7, 18.4 ($\underline{C}$(CH$_3$)$_2$), 7.3 ($\underline{C}$H$_2$I), -5.3 ($\underline{C}$H$_3$).

Experiment 22: 11-(*tert*-Butyldimethylsilanyloxy)undec-2-yn-1-ol (201)



After liquid ammonia (300 ml) was decanted into a three neck round bottom flask surrounded by a liquid nitrogen methylated spirit condenser protected by a soda lime guard tube, lithium wire (0.15 g) was added in 0.2 cm portions to achieve to a dark blue solution. Then ferric nitrate (0.2 g) was added and the solution was stirred for 5 min until it lost the colour. After the slow addition, over 30 min, of lithium wire (1.6 g, 225 mmol) in 0.5 cm portions, the mixture was stirred for a

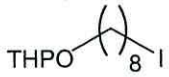
further 20 min, followed by addition over 20 min of a solution of prop-2-yn-1-ol (**200**, 5.72 g, 102 mmol) in dry ether (15 ml). After stirring for another 0.5 h, a solution of the alkyl iodide (**199**, 31.5 g, 85 mmol) in dry ether (50 ml) was added over 20 min. The reaction was subsequently stirred for 5 h keeping the condenser at - 60 °C and then the condenser was removed to allow the evaporation of the liquid ammonia overnight. The reaction was quenched by slowly adding diluted (10 %) sulphuric acid until pH = 7. The compound was extracted in ether (3 x 150 ml). The combined organic layers were dried and concentrated to give a residue, which was a mixture of the desired compound *11-(tert-butyldimethyl silanyloxy)undec-2-yn-1-ol* (**201**) and other unknown by-products. The product was not purified and the yield was not determined.

Found $_{(TOF\ MS)}$ $(M+H)^+$: 299.2380 $C_{17}H_{35}OSi$ requires: 299.2406.

m/z: 297 $(M-H)^+$, 242 $(M-C(CH_3)_3)^+$, 131 $(Si(CH_3)_2C(CH_3)_3)^+$, 185.

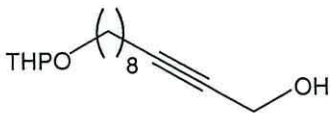
$\delta_{H\ (250\ MHz)}$ (in the spectrum of the crude among other signals): 4.25 (2H, m, $C\underline{H}_2OH$), 3.57, (2H, t, J = 7.0 Hz, $C\underline{H}_2OSi$), 2.2-2.0 (1H, O$\underline{H}$), 1.9-1.2 (13H, m), 0.90 (9H, s $(C\underline{H}_3)_3CSi$), 0.1 (6H, s, $(C\underline{H}_3)_2Si$).

Experiment 23: 2-(8-Iodo-octyloxy)tetrahydropyran (202)



The same approach described in **Experiment 21** was used in order to transform 2-(8-bromo-octyloxy)tetrahydropyran (**153**, 46 g, 157 mmol) into 2-8-iodo-octyloxy)tetrahydropyran (**202**, 44 g, 82 %). The product was purified by column chromatography using as eluting solvent petrol and ether (5:1). The compound showed proton and carbon NMR spectra identical to those reported.[418]

Experiment 24: 11-(Tetrahydropyran-2-yloxy)undec-2-yn-1-ol (203)



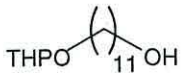
The same approach, described in **Experiment 22** was utilised in order to convert 2-(8-iodo-octyloxy)tetrahydropyran (**202**, 44 g, 129 mmol) into 11-(tetrahydropyran-2-yloxy)undec-2-yn-1-ol (**203**, 27 g, 79 %), which was purified by column chromatography eluting with petrol and ether (5:2). The NMR spectra of the compound obtained were identical to the ones reported.[283]

m/z: 269 $(M+1)^+$, 237 $(M-CH_3OH)^+$, 185 $(M-THP)^+$, 85 $(THP)^+$, 55.

ν_{max}/cm^{-1}: 3417, 2932, 2854, 2225, 1453, 1352, 1323, 1260, 1200, 1184, 1135, 1119, 1076, 1023.

$\delta_{H\ (250\ MHz)}$: 4.57 (1H, t, J = 3.4 Hz, OC<u>H</u>O), 4.2 (2H, m, CH_2OH), 3.9-3.7 (1H, m) 3.67 (1H, dt, J_1 = 9.5 Hz, J_2 = 7.0 Hz), 3.5-3.4 (1H, m), 3.33 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.8 Hz), 2.65 (1H, OH), 2.2-2.1 (2H, m) 1.8-1.2 (20H, m).

Experiment 25: 11-(Tetrahydropyran-2-yloxy)undecan-1-ol (204)



Nickel (II) acetate tetrahydrate (4.82 g, 11 mmol) was dissolved in absolute ethanol (100 ml) and the mixture was put under hydrogen after having evacuated the flask and refilling with hydrogen several times. Then a solution of sodium borohydride (0.73 g, 11 mmol) in absolute ethanol (40 ml) was added and the reaction was stirred for 0.5 h. Finally, a solution of the alkyne (**203**, 26 g, 97 mmol) in absolute ethanol (80 ml) was added dropwise and left under hydrogen until no more was absorbed. The mixture was diluted with diethyl ether (250 ml) and filtered on a mixture of celite and silica. The filtrate was concentrated and the residue was dissolved in dichloromethane (200 ml), washed with water (100 ml), dried and evaporated to give the product which was purified by column chromatography eluting with petrol and ether (5:2) to produce 11-(tetrahydropyran-2-yloxy)undecan-1-ol (**204**, 22 g, 83 %); this has been previously reported,[419] but no spectral properties were given.

Found C: 70.7, H: 11.7, required for $C_{16}H_{32}O_3$ C: 70.54, H: 11.84.

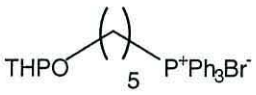
m/z: 273 $(M+H)^+$, 255 $(M-H_2O)^+$, 189 $(M-THP)^+$, 101 $(OTHP)^+$, 85 $(THP)^+$.

ν_{max}/cm^{-1}: 3382, 2925, 2853, 1465, 1352, 1322, 1260, 1200, 1121, 1077, 1033.

$\delta_{H\ (250\ MHz)}$: 5.35 (1H, s, O<u>H</u>), 4.57 (1H, t, J = 3.9 Hz, OC<u>H</u>O), 3.9-3.8 (1H, m), 3.72 (1H, dt, J_1 = 9.5 Hz, J_2 = 7.0 Hz), 3.62 (2H, t, J = 6.5 Hz, C<u>H</u>$_2$OH), 3.5-3.4 (1H, m), 3.37 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.8 Hz), 1.8-1.2 (26H, m).

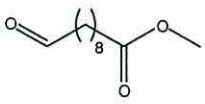
$\delta_{C\ (62.9\ MHz)}$: 98.8, (O<u>C</u>HO), 67.7 (CH_2OTHP), 62.9 (CH_2OH), 62.3 (CH_2OTHP), 32.8, 30.7, 29.7, 29.5, 29.5, 29.5, 29.4, 29.4, 26.2, 25.7, 25.5, 19.7.

Experiment 26: Triphenyl-[5-(tetrahydropyran-yloxy)pentyl]-phosphonium; bromide (213)



2-(5-Bromopentyloxy)tetrahydropyran (**153d**, 3.5 g, 14 mmol) was added to a solution of tryphenylphosphine (3.93 g, 15 mmol) and potassium carbonate (0.76 g, 5.6 mmol) in dry acetonitrile (60 ml), previously dried through stirring on anhydrous potassium carbonate for a day. The mixture was refluxed for 2 days then cooled, filtered and the solvent removed by evaporation. The product was washed with dry diethyl ether (2 x 50 ml) to give triphenyl-[5-(tetrahydropyranyloxy)pentyl]-phosphonium bromide (**213**, 5 g, 82 %) which was utilized without further purification. It showed the same NMR spectra as those reported.[292]

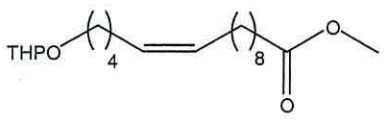
Experiment 27: 10-Oxodecanoic acid methyl ester (212)



A solution 10-hydroxydecanoic acid methyl ester (**214**, 1 g, 5 mmol) in dichloromethane (20 ml) was added to a solution of PCC (2.5 g, 10 mmol) in dichloromethane (200 ml). The mixture was stirred for 2 h when TLC showed no starting material. The reaction was quenched by diluting with ether (500 ml) and filtered on a pad of celite and silica. The solvent was evaporated to give crude 10-oxodecanoic acid methyl ester, which was purified by column chromatography

using petrol and ether (10:1). The product (**212**, 0.9 g, 91 %) showed the same NMR, IR spectra as the ones reported.[420]

Experiment 28: 14-(Tetrahydropyran-2-yloxy)tetradec-10-enoic acid methyl ester (215)



To a solution of triphenyl-[5-(tetrahydropyranyloxy)pentyl]-phosphonium bromide (**213**, 5 g, 9.7 mmol) in dry DMSO (25 ml) and dry toluene (75 ml), lithium bis(trimethylsilyl)amide (1.0 M in hexanes, 10.5 mmol, 10.5 ml) was added at – 20 °C under argon. The solution was slowly warmed to room temperature and stirred for 3 h, then cooled again to – 40 °C and a solution of 10-oxo-decanoic acid methyl ester (**212**, 1 g, 4.9 mmol) in dry toluene added. The mixture was slowly allowed to reach room temperature and stirred for 4 h then quenched with sat. aq. ammonium chloride and extracted with dichloromethane (3 x 100 ml). The combined organic layers were dried and the solvent was evaporated to give the crude product which was purified by column eluting with petrol and ether (4:1) to give *14-(tetrahydropyran-2-yloxy)tetradec-10-enoic acid methyl ester* (**215**, 1.3 g, 72 %).

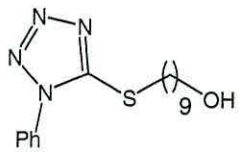
Found C: 70.1, H: 10.4; $C_{20}H_{36}O_4$ requires: C: 70.55, H: 10.66.

ν_{max}/cm^{-1}: 3004, 2928, 2855, 1742, 1666, 1459, 1438, 1353, 1323, 1260, 1200, 1173, 1138, 1120, 1077, 1034.

$\delta_{H\ (500\ MHz)}$: 5.4-5.3 (2H, m, C<u>H</u>=C<u>H</u>), 4.55 (1H, t, J = 1.4 Hz), 3.9-3.8 (1H, m), 3.73 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.9 Hz), 3.66 (3H, s, OC<u>H</u>$_3$), 3.52-3.46 (1H, m), 3.38 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.9 Hz), 2.28 (2H, t, J = 3.5 Hz, C<u>H</u>$_2$CO$_2$CH$_3$), 2.1-1.9 (4H, dm, C<u>H</u>$_2$CH=CHC<u>H</u>$_2$), 1.8-1.2 (26H, m).

$\delta_{C\ (125.8\ MHz)}$: 174.2 (<u>C</u>O$_2$CH$_3$), 130.4 (CH=CH, *E*), 130.1 (CH=CH, *E*), 129.9 (CH=CH, *Z*), 129.4 (CH=CH, *Z*), 98.7 (O<u>C</u>HO), 67.7 (CH$_2$OTHP), 62.3 (CH$_2$OTHP), 51.3 (O<u>C</u>H$_3$), 34.0 (<u>C</u>H$_2$), 30.7 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.3 (<u>C</u>H$_2$), 29.2 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 29.0 (<u>C</u>H$_2$), 27.1 (<u>C</u>H$_2$), 26.9 (<u>C</u>H$_2$), 26.3 (<u>C</u>H$_2$), 25.4 (<u>C</u>H$_2$), 24.9 (<u>C</u>H$_2$), 19.7 (<u>C</u>H$_2$).

Experiment 29: 9-(1-Phenyl-1*H*-tetrazol-5-ylsulfanyl)nonan-1-ol (223)



Potassium carbonate (8 g, 58 mmol) was added to a solution of 9-bromononan-1-ol (**251b**, 10 g, 45 mmol) and 1-phenyl-1*H*-tetrazole-5-thiol (12.5 g, 45 mmol) in acetone (300 ml). The mixture was vigorously stirred and refluxed at (60 °C) overnight. The inorganic salts were filtered off and washed well with acetone. The solvent was evaporated and the residue was dissolved in water (300 ml) and extracted with dichloromethane (3 x 300 ml). The combined organic layers were dried, evaporated. The crude product was columned with petrol and ether (10:1) to give *9-(1-phenyl-1H-tetrazol-5-ylsulfanyl)nonan-1-ol* (**223**, 10.5 g, 73 %).

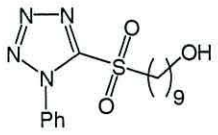
Found $_{(EI)}$ M^+ 320.1681; $C_{16}H_{24}N_4OS$ requires: 320.1671.
$(M+H)^+$: 321.1747; $C_{16}H_{25}N_4OS$ requires: 321.1749.

ν_{max}/cm^{-1}: 3414, 2925, 2853, 1654, 1596, 1499, 1458, 1387, 1243, 1073.

$\delta_{H\ (500\ Mhz)}$: 7.55-7.50 (5H, m, aromatic), 3.62 (2H, t, J = 6.6 Hz, $C\underline{H}_2OH$), 3.37 (2H, t, J = 7.4 Hz, $C\underline{H}_2S$), 2.0 (1H, m, O<u>H</u>), 1.8 (2H, m, $C\underline{H}_2CH_2S$), 1.56 (2H, m, $C\underline{H}_2CH_2OH$), 1.53 (2H, m), 1.4-1.1 (9H, m).

$\delta_{C\ (125.8\ MHz)}$: 154.3 (C=N heterocyclic), 133.6 (C-N aromatic), 129.9 (CH aromatic), 129.7 (CH aromatic), 123.8 (CH aromatic), 62.8 ($\underline{C}H_2O$), 33.3 ($\underline{C}H_2S$), 32.6 ($\underline{C}H_2CH_2OH$), 29.3 ($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 28.6 ($\underline{C}H_2$), 28.1($\underline{C}H_2$), 25.8 ($\underline{C}H_2$).

Experiment 30: 9-(1-Phenyl-1*H*-tetrazole-5-sulfonyl)nonan-1-ol (224)



Ammonium molybdate tetrahydrate (11.5 g, 1 mmol) was dissolved in stages in aq. H_2O_2 (35% w/w, 15.6 ml, 150 mmol). Small amounts of the solution were added slowly to 9-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-nonan-1-ol (**223**, 10 g, 31 mmol) in IMS (200 ml) while maintaining the temperature below 5 °C. The

mixture was stirred at room temperature overnight then the solvent was evaporated to give a yellow residue. This was dissolved in water (150 ml) and the product was extracted with dichloromethane (3 x 150 ml). The combined organic layers were dried and evaporated to give the crude product which was purified by column chromatography eluting with petrol and ether (1:1) to give *9-(1-phenyl-1H-tetrazoel-5-sulfonyl)nonan-1-ol* (**224**, 8.7 g, 80 %) which showed:

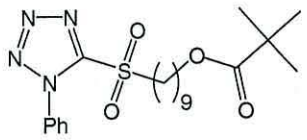
Found $_{(EI)}$ M^+: 352.1562; $C_{16}H_{24}N_4O_3S$ requires: 352.1569 and $(M+H)^+$: 353.1640; $C_{16}H_{25}N_4O_3S$ requires: 353.1647).

ν_{max}/cm^{-1}: 3394, 2929, 2855, 1595, 1498, 1463, 1340, 1142, 1050.

$\delta_{H\ (500\ MHz)}$: 7.68 (2H, m, aromatic), 7.60 (3H, m, aromatic), 3.73 (2H, m, $C\underline{H}_2SO_2$), 3.60 (2H, t, J = 6.0 Hz, $C\underline{H}_2OH$), 1.90 (2H, m, $C\underline{H}_2CH_2S$), 1.72 (1H, m, O<u>H</u>), 1.53 (2H, m, $C\underline{H}_2CH_2OH$) 1.47 (2H, m), 1.4-1.2 (9H, m).

$\delta_{C\ (125.8\ MHz)}$: 153.3 (C=N heterocyclic), 133.1 (C-N aromatic), 131.3 (CH aromatic), 129.7 (CH aromatic), 125.2 (CH aromatic), 62.8 ($\underline{C}H_2O$), 55.8 ($\underline{C}H_2S$), 32.6 ($\underline{C}H_2CH_2OH$), 29.1 ($\underline{C}H_2$), 29.0 ($\underline{C}H_2$), 28.7 ($\underline{C}H_2$), 28.0($\underline{C}H_2$), 25.6 ($\underline{C}H_2$), 21.9 ($\underline{C}H_2CH_2S$).

Experiment 31: 2,2-Dimethylpropionic acid 9-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-nonyl ester (225)



Trimethylacetyl chloride (3.3 g, 27.6 mmol) was added to a solution of 9-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-nonan-1-ol (**224**, 7.5 g, 21.3 mmol) and DMAP (0.2 g) and pyridine (3 g, 38 mmol) in dry THF (50 ml) at 5 °C. Within a few minutes pyridine hydrochloride separated out as a white solid. The mixture was stirred overnight at room temperature. The reaction was quenched with aq. HCl (2 N, 75 ml). The product was extracted with dichloromethane (3 x 100 ml) and the combined organic layers were washed with aq. HCl (2 N, 50 ml), dried and the solvent evaporated. The crude product was then purified by chromatography

eluting with petrol and ether (10:1): to give *2,2-dimethyl-propionic acid 9-(1-phenyl-1H-tetrazole-5-sulfonyl)-nonyl ester* (**225**, 8.6 g, 91 %).

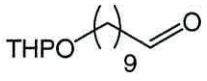
Found $_{(EI)}$ $(M+H)^+$: 437.2219; $C_{21}H_{33}N_4O_4S$ requires: 437.2223.

ν_{max}/cm^{-1}: 2934, 2867, 1730, 1480, 1461, 1397, 1383, 1365, 1352, 1322, 1284, 1200, 1157, 1078, 1035.

$\delta_{H\ (500\ MHz)}$:, 7.68 (2H, m, aromatic), 7.60 (3H, m, aromatic), 4.05(2H, t, J = 6.6 Hz CH_2OCO), 3.71 (2H, m, $C\underline{H}_2SO_2$), 2.00-1.90 (2H, m, $C\underline{H}_2CH_2S$), 1.65-1.53 (2H, m, $C\underline{H}_2CH_2OH$) 1.55-1.45 (2H, m,), 1.4-1.2 (8H, m), 1.20 (9H, s $(CH_3)_3$).

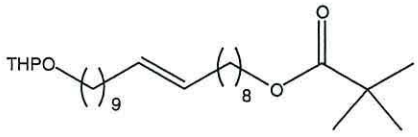
$\delta_{C\ (125.8\ MHz)}$: 178.6 (C=O), 153.5 (C=N heterocyclic), 133.0 (C-N aromatic), 131.5 (CH aromatic), 129.7 (CH aromatic), 125.2 (CH aromatic), 64.3 ($\underline{C}H_2O$), 56.0 ($\underline{C}H_2S$), 38.6 ($\underline{C}(CH_3)_3$), 29.0 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 28.5 ($\underline{C}H_2$), 28.1 ($\underline{C}H_2$), 27.2 ($\underline{C}H_3$), 25.8 ($\underline{C}H_2$), 21.9 ($\underline{C}H_2CH_2S$).

Experiment 32: 10-(Tetrahydropyran-2-yloxy)decanal (226)



The procedure in **Experiment 27** was utilised with 10-(tetrahydropyran-2-yloxy)decan-1-ol (**159**, 1 g, 3.8 mmol) to give 10-(tetrahydropyran-2-yloxy)decanal (**226**, 0.95 g, 97 %) which showed the same ^1H and ^{13}C NMR spectra as those reported.[421]

Experiment 33: 2,2-Dimethylpropionic acid 19-tetrahydropyran-2-yloxy) nonadec-9-enyl ester (227)



Lithium bis(trimethylsilyl)amide (13 mmol, 13 ml, 1 M, in hexanes) was added at - 20 °C to a solution of 10-(tetrahydropyran-2-yloxy)-decanal (**226**, 1.7 g, 6.65 mmol) and 2,2-dimethylpropionic acid 9-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-nonyl ester (**225**, 3.5 g, 8 mmol) in dry THF (50 ml) under nitrogen. The reaction

was stirred at room temperature for 24 h then quenched by the addition of sat. aq. ammonium chloride (20 ml). The compound was extracted with ethyl acetate (3 x 50 ml). The combined organic layers were dried and concentrated to give the crude product. This was purified by column chromatography eluting with petrol and ether (10:0.5), to give *2,2-dimethylpropionic acid 19-tetrahydropyran-2-yloxy)nonadec-9-enyl ester* (in a mixture of (*E*) and (*Z*)-stereoisomer, 2.7:1) (**227**, 2.9 g, 78 %).

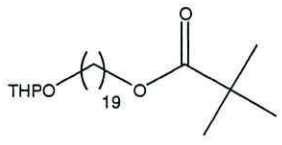
Found $_{(CI)}$ $(M+H)^+$: 467.4117; $C_{29}H_{55}N_4O_4$ requires: 467.4110.

ν_{max}/cm^{-1}: 2924, 2851, 1730, 1538, 1480, 1463, 1397, 1383, 1365, 1352, 1322, 1284, 1200, 1156, 1121, 1079, 1033.

$\delta_{H\ (500\ MHz)}$: 5.38 (2H, m, C<u>H</u>=C<u>H</u>), 4.56 (1H, t, J = 2.9 Hz, OC<u>H</u>O), 4.03 (2H, t, J = 6.6 Hz C<u>H</u>$_2$OCO), 3.85-3.80 (1H, m, CH_2OTHP), 3.71 (1H, dt,J_1 = 9.5 Hz, J_2 = 6.5 Hz, CH_2OTHP), 3.5-3.4 (1H, m, CH_2OTHP), 3.36 (1H, dt,J_1 = 9.5 Hz, J_2 = 6.5 Hz, CH_2OTHP), 1.8-1.3 (36H, m), 1.18 (9H, s $(CH_3)_3$).

$\delta_{C\ (125.8\ MHz)}$: 178.5 (C=O), 130.3 (CH=CH, *E*), 130.2 (CH=CH, *E*), 129.9 (CH=CH, *Z*), 129.7 (CH=CH, *Z*), 98.7 (O<u>C</u>HO), 67.7 (CH_2OTHP), 64.4 (<u>C</u>H_2O), 62.3 (CH_2OTHP), 38.6 (<u>C</u>$(CH_3)_3$), 32.5 (<u>C</u>H_2), 30.7 (<u>C</u>H_2), 29.7 (<u>C</u>H_2), 29.6 (<u>C</u>H_2), 29.5 (<u>C</u>H_2), 29.4 (<u>C</u>H_2), 29.3 (<u>C</u>H_2), 29.1 (<u>C</u>H_2), 29.0 (<u>C</u>H_2), 28.5 (<u>C</u>H_2), 27.2 (<u>C</u>H_3), 26.2 (<u>C</u>H_2), 25.8 (<u>C</u>H_2), 25.5 (<u>C</u>H_2), 19.7 (<u>C</u>H_2).

Experiment 34: 2,2- Dimethyl-propionic acid 19-tetrahydropyran-2-yloxy)nonadecyl ester (228)



Using a similar methodology to the one described in **Experiment 25**, 9-(1-phenyl-1H-tetrazole-5-sulfonyl)nonyl ester (**227**, 2.8 g, 6 mmol) was completely dissolved in ethanol (200 ml) then Pd on C (10 %, 0.7g) was added. The mixture was stirred under hydrogen. The reaction was quenched and the product purified

as described previously to give *2,2- dimethylpropionic acid 19-tetrahydropyran-2-yloxy)nonadecyl ester* (**228**, 2.6 g, 93 %).

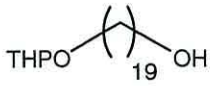
Found $_{(TOF\ MS)}$ $(M+Na)^+$: 491.4076; $C_{29}H_{56}O_4Na$ requires: 491.4071.

ν_{max}/cm^{-1}: 2917, 2848, 1728, 1467, 1365, 1284, 1200, 1156, 1120, 1078, 1034.

$\delta_{H\ (500\ MHz)}$: 4.56 (1H, t, J = 2.9 Hz, OC<u>H</u>O), 4.03 (2H, t, J = 6.6 Hz C<u>H</u>$_2$OCO), 3.85-3.80 (1H, m, of the OTHP group), 3.71 (1H, dt,J_1 = 9.5 Hz J_2 = 6.9 Hz, CH_2OTHP), 3.51-3.45 (1H, m, CH_2OTHP), 3.36 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.5 Hz, CH_2OTHP), 1.85-1.75 (1H, m), 1.74-1.65 (1H, m), 1.60-1.20 (38H, m) 1.19 (9H, s $(CH_3)_3$).

$\delta_{C\ (125.8\ MHz)}$: 178.6 (C=O), 98.8 (O<u>C</u>HO), 67.7 (CH_2OTHP), 64.4 (<u>C</u>H$_2$OCO), 62.3 (CH_2OTHP), 38.6 (<u>C</u>$(CH_3)_3$), 30.8 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.2 (<u>C</u>H$_2$), 28.6 (<u>C</u>H$_2$), 27.2 (<u>C</u>H$_3$), 26.2 (<u>C</u>H$_2$), 25.8 (<u>C</u>H$_2$), 25.5 (<u>C</u>H$_2$), 19.6 (<u>C</u>H$_2$).

Experiment 35: 19-(Tetrahydropyran-2-yloxy)nonadecan-1-ol (229)



The procedure in **Experiment 10** was used to hydrolyse 2,2-dimethylpropionic acid 19-tetrahydropyran-2-yloxy)nonadecyl ester (**228**, 2.5 g, 5.3 mmol) to give, after purification by column chromatography eluting with petrol and ether (7:1), *19-(tetrahydropyran-2-yloxy)nonadecan-1-ol* (**229**, 1.9 g, 93 %).

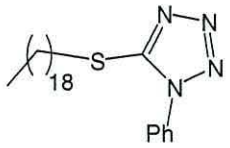
Found $_{(TOF\ MS)}$ $(M+Na)^+$: 407.3477; $C_{24}H_{48}O_3Na$ requires: 407.3479.

ν_{max}/cm^{-1}: 3379, 2920, 2850, 1466, 1352, 1322, 1200, 1136, 1076, 1031.

$\delta_{H\ (500\ MHz)}$: 4.56 (1H, t, J = 3.2 Hz, OC<u>H</u>O), 3.85-3.75 (1H, m, of the OTHP group), 3.71 (1H, dt, J_1 = 9.7 Hz, J_2 = 6.9 Hz, CH_2OTHP), 3.55 (2H, t, J = 6.7 Hz C<u>H</u>$_2$OH), 3.51-3.45 (1H, m, CH_2OTHP), 3.36 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.7 Hz CH_2OTHP), 2.53 (1H, m, O<u>H</u>), 1.85-1.75 (1H, m), 1.74-1.65 (1H, m), 1.60-1.20 (32H, m).

$\delta_{C\ (125.8\ MHz)}$: 98.6 (O<u>C</u>HO), 67.6 (CH_2OTHP), 62.7 (<u>C</u>H$_2$O), 62.1 (CH_2OTHP), 32.7 (<u>C</u>H$_2$), 31.8 (<u>C</u>H$_2$), 30.6 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_3$), 29.4 (<u>C</u>H$_2$), 29.2 (<u>C</u>H$_2$), 26.1 (<u>C</u>H$_2$), 25.7 (<u>C</u>H$_2$), 25.4 (<u>C</u>H$_2$), 22.5 (<u>C</u>H$_2$), 19.5 (<u>C</u>H$_2$).

Experiment 36: 5-Nonadecylsulfanyl-1-phenyl-*1H*-tetrazole (231)



Diethyl azocadicarboxylate (1.46 g, 8.4 mmol) in dry THF was added to a stirred solution of 1-nonadecanol (**197**, 2 g, 7 mmol), triphenylphosphine (2.4 g, 9.1 mmol) and 1-phenyl-*1H*-tetrazol-5-thiol (1.5 g, 8.4 mmol) in dry THF at 5 °C under nitrogen. The mixture was allowed to reach room temperature and stirred for 24 h, then the solvent was evaporated and the residue was dissolved in ether and petrol and stirred for 15 min. The precipitate was filtered off through a pad of celite and the filtrate was evaporated to give a white residue which was purified by column chromatography eluting with petrol/ ether (5:1) to give 5-*nonadecylsulfanyl-1-phenyl-1H-tetrazole* (**231**, 2.6 g, 89 %).

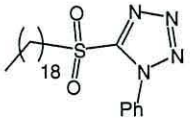
Found C: 73.3, H: 9.8; $C_{33}H_{52}O_4Si$ requires: C: 73.28, H: 9.69.

ν_{max}/cm^{-1}: 2914, 2849, 1594, 1497, 1471, 1390, 1274, 1247, 1089, 1074, 1014.

$\delta_{H\ (500\ MHz)}$: 7.55-7.45 (5H, m, aromatic), 3.36 (2H, t, J = 7.2 Hz, C$\underline{H}_2$S), 1.8 (2H, m), 1.4 (32H, br m), 0.85 (3H, t, J = 6.0 Hz, C$\underline{H}_3$).

$\delta_{C\ (125.8\ MHz)}$: 154.3 (C=N heterocyclic), 133.6 (C-N aromatic), 129.9 (CH aromatic), 129.6 (CH aromatic), 123.7 (CH aromatic), 33.2 (C$\underline{H}_2$S), 32.4 (C$\underline{H}_2$), 32.1 (C$\underline{H}_2$), 32.1 (C$\underline{H}_2$), 30.2 (C$\underline{H}_2$), 30.1 (C$\underline{H}_2$), 30.1 (C$\underline{H}_2$), 30.0 (C$\underline{H}_2$), 29.9(C$\underline{H}_2$), 29.8 (C$\underline{H}_2$), 29.6 (C$\underline{H}_2$), 29.5 (C$\underline{H}_2$), 29.2 (C$\underline{H}_2$), 29.1 (C$\underline{H}_2$), 29.1(C$\underline{H}_2$), 23.2 (C$\underline{H}_2$), 23.1 (C$\underline{H}_2$), 23.0 (C$\underline{H}_2$), 14.6 (C$\underline{H}_3$).

Experiment 37: 5-(Nonadecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (232)



A solution of 5-nonadecylsulfanyl-1-phenyl-*1H*-tetrazole (**231**, 2 g, 4.5 mmol) in dichloromethane (60 ml) was cooled to 0° C and MCPBA (55 %, 5.6 g, 16 mmol) was added in small portions over 0.5 h. The reaction was allowed to warm to

room temperature and stirred for 30 h before quenching by adding sat. aq. $NaHCO_3$. The compound was extracted with dichloromethane (3 x 100 ml) and the organic layers were washed with sat. aq. of $NaHCO_3$ (2 x 75 ml), dried and evaporated. The crude product was purified by column chromatography eluting with petrol and ether (10:1) to give 5-(nonadecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**232**, 1.2 g, 56 %).

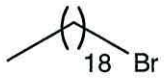
Found $_{(EI)}$ M^+: 476.3181; $C_{26}H_{44}O_2N_4S$ requires: 476.3185.

ν_{max}/cm^{-1}: 2917, 2849, 1498, 1471, 1346, 1216, 1152, 1016.

$\delta_{H\ (500\ MHz)}$: 7.68 (2H, m, aromatic), 7.60 (3H, m, aromatic), 3.72 (2H, m, $C\underline{H}_2S$), 1.95 (2H, m, $C\underline{H}_2CH_2S$), 1.4-1.2 (32H, m), 0.85 (3H, t, J = 6.0 Hz, $C\underline{H}_3$).

$\delta_{C\ (125.8\ MHz)}$: 153.5 (C=N heterocyclic), 133.1 (C-N aromatic), 131.3 (CH aromatic), 129.7 (CH aromatic), 125.1 (CH aromatic), 56.0 ($\underline{C}H_2S$), 31.9 ($\underline{C}H_2$), 29.7 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 28.9 ($\underline{C}H_2$), 28.2 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 21.9 ($\underline{C}H_2$), 14.1 ($\underline{C}H_3$).

Experiment 38: 1-Bromo-nonadecane (233)



Nonadecan-1-ol (**197**, 10 g, 35 mmol) was dissolved in aqueous hydrobromic acid (50 ml, 48 % w. w.) and refluxed for 32h, when TLC showed the absence of starting material. After cooling the mixture, sat. aq. sodium bicarbonate (20 ml) was added very slowly and it was extracted with dichloromethane (3 x 150 ml). The combined organic layers were dried and evaporated to give the crude product which was purified by column chromatography eluting with petrol ether. 1-bromo nonadecane (**233**, 1.6 g, 95 %) was obtained with a similar mp that reported.[422]

mp: 33-35 °C; lit. 37 °C.[422]

$\delta_{H\ (500\ Mhz)}$: 3.42 (2H, t, J = 7.4 Hz, $C\underline{H}_2Br$), 1.86 (2H, m, $C\underline{H}_2CH_2Br$), 1.4-1.1 (32H, m) 0.89 (3H, t, J = 7.0 Hz, $C\underline{H}_3$).

$\delta_{C\ (125.8\ MHz)}$: 34.0 ($\underline{C}H_2Br$), 32.9 ($\underline{C}H_2$- CH_2Br), 31.9 ($\underline{C}H_2$), 31.8 ($\underline{C}H_2$), 29.7 ($\underline{C}H_2$), 29.7 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.6($\underline{C}H_2$), 29.5 ($\underline{C}H_2$),

29.4 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 28.2 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 22.6 ($\underline{C}H_2$), 14.1 ($\underline{C}H_3$).

Experiment 39: Nonadecyltriphenylphosphonium bromide (234)

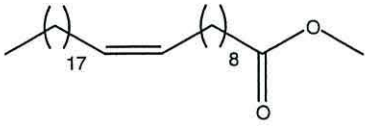
Triphenylphosphine (2.8 g, 10.7mmol) was added to a solution of 1-bromononadecane (**233**, 2.85 g, 8.2 mmol) in dry toluene (80ml), and refluxed for 5 days. The solvent was evaporated to give a brown solid, which was washed with a mixture of petrol and ether (1:1, 30 ml) to give nonadecyltriphenylphosphonium bromide (**234**, 4.2 g, 84 %). This has already been prepared but its spectral properties have not been described.[249]

Found $_{(EI)}$ M^+ (not considering Br): 529.3966; $C_{37}H_{55}P$ requires: 529.3963.

$\delta_{H\ (500\ MHz)}$: 8.0-7.5 (15H, m, aromatic), 3.73 (2H, m, $C\underline{H}_2Br$), 2.3-2.2 (2H, m, $C\underline{H}_2CH_2Br$), 2.0-1.8-1.1 (23H, br m), 0.88 (3H, t, J = 6 Hz, $C\underline{H}_3$).

$\delta_{C\ (125.7\ MHz)}$: 134.96 ($\underline{C}H$, aromatic, d, J = 2.5 Hz), 133.7 ($\underline{C}H$, aromatic, d, J = 10.1 Hz), 130.4 ($\underline{C}H$, aromatic, d, J = 12.7 Hz), 118.5 ($\underline{C}$, aromatic, d, J = 85.5 Hz), 31.9 ($\underline{C}H_2$), 30.5 ($\underline{C}H_2$, d, J = 15.1 Hz), 29.7 ($\underline{C}H_2$), 29.6($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 29.3 (CH_2, d, J = 20.1 Hz), 29.2 ($\underline{C}H_2$), 23.05 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 14.1 ($\underline{C}H_3$).

Experiment 40: Triacont-10-enoic acid methyl ester (235)



A) Using a similar procedure to the one described in **Experiment 33**, 10-oxodecanoic acid methyl ester (**212**, 0.5 g, 2.47 mmol) and 5-(nonadecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**232**, 1.5 g, 3.2 mmol) were coupled together. The temperature was maintained at - 78 °C. The crude product was purified by column chromatography eluting with petrol and ether (10:0.5) to give *triacont-10-*

enoic acid methyl ester (**235**, 640 mg, 58 %) as mixture of the (*Z*) and (*E*)-stereoisomers (1:2.6).

B) Using a similar procedure described in **Experiment 28**, lithium bis(trimethylsilyl)amide (1 M in hexanes, 4.8 mmol, 4,8 ml) was added at - 40 ° C to a solution of nonadecyltriphenylphosphonium bromide (**234**, 2g, 3.3 mmol) in dry THF (30 ml) under nitrogen. The bright red solution was stirred at room temperature for 1 h then 10-oxodecanoic acid methyl ester (**212**, 0.5 g, 2.47 mmol) was added at – 78° C. The solution was stirred at this temperature for 2 h than quenched with sat. aq. ammonium chloride (20 ml) and extracted with ethyl acetate (3 x 100 ml). The organic layers were dried and evaporated to give the crude product which was purified by column chromatography eluting with petrol to give a mixture of (*Z*) and (*E*)-stereoisomers (3.0:1) of triacont-10-enoic acid methyl ester (**235**, 500 mg, 43 %).

C) The procedure in (**B**) was repeated using sodium bis(trimethylsilyl)amide (1 M M, in THF, 4.8 mmol, 4,8 ml) as a base instead than lithium bis(trimethylsilyl)amide. A mixture of the (*Z*) and (*E*)-stereoisomers (22:1) of triacont-10-enoic acid methyl ester (**235**, 540 mg, 47 %) was obtained.

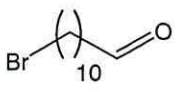
Found $_{(EI)}$ M^+: 450.4421; $C_{30}H_{58}O_2$ requires: 450.4437.

ν_{max}/cm^{-1}: 2924, 2952, 1740, 1467, 1438, 1407, 1367, 1255, 1204, 1177, 1065.

$\delta_{H\ (500\ MHz)}$: 5.38 (2H, m, C<u>H</u>=C<u>H</u>, *E*), 5.35 (2H, m, C<u>H</u>=C<u>H</u>, *Z*), 3.67 (3H, s, OC<u>H</u>$_3$), 2.30 (1H, t J = 7.6 Hz, C<u>H</u>$_2$CO$_2$CH$_3$), 2.01 (4H, m, C<u>H</u>$_2$CH$_2$=CH$_2$C<u>H</u>$_2$, *Z*), 1.96 (4H, m, C<u>H</u>$_2$CH$_2$=CH$_2$C<u>H</u>$_2$, *E*), 1.65-1.55 (2H, m, C<u>H</u>$_2$CH$_2$CO$_2$CH$_3$), 1.2-1.0 (42H, m), 0.88 (3H, t, J = 6.6 Hz, C<u>H</u>$_3$).

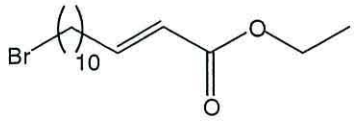
$\delta_{C\ (125.8\ MHz)}$: 174.3 (C=O), 130.4 (CH=CH, *E*), 130.2 (CH=CH, *E*), 129.9 (CH=CH, *Z*), 129.9 (CH=CH, *Z*), 51.4 (O<u>C</u>H$_3$), 34.1 (<u>C</u>H$_2$CO), 32.5 (<u>C</u>H$_2$), 31.9 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_2$), 29.2 (<u>C</u>H$_2$), 29.2 (<u>C</u>H$_2$), 27.2 (<u>C</u>H$_2$), 27.2 (<u>C</u>H$_2$), 24.9 (<u>C</u>H$_2$), 22.7 (<u>C</u>H$_2$), 14.0 (CH$_3$).

Experiment 41: 11-Bromoundecanal (256)



Using the procedure described in **Experiment 29**, 11-bromoundecan-(1)-ol (**255**, 26 g, 103 mmol) was converted into 11-bromoundecanal (**256**, 16.5 g, 65%), which was a colourless oil with NMR and IR spectra consistent with literature values.[319]

Experiment 42: (*E*)-13-Bromotridec-2-enoic ethyl ester (257)



The 11-bromoundecanal (**256**, 16.5 g, 66 mmol) was dissolved in toluene (20 ml) and then added to a suspension of (ethoxycarbonylmethylene) triphenylphosphorane (**252a**, 25.4 g, 73 mmol) in toluene (100 ml). The precipitate disappeared after 1 h; however the mixture was stirred overnight. The solvent was evaporated to give a white solid which was partially dissolved using a warm solution petrol and ether (1:1, 300 ml). The mixture was filtered and the precipitate washed with the same solution (150 ml). The combined organic layers were dried and the solvent was evaporated to give pale yellow oil. The crude product was purified by column eluting with petrol and ether (5:1) in order to obtain (*E*)-13-bromotridec-2-enoic ethyl ester (**257**, 17 g, 73 %) with a d. e. = 92 %. The synthesis of this compound has already been described but not its spectral properties.[423]

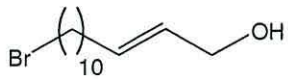
m/z: 319 $(M+1)^+$, 273 $(M\text{-}OEt)^+$, 230, 141.

ν_{max}/cm^{-1}: 2927, 2854, 1720, 1654, 1464, 1366, 1265, 1178, 1129, 1095, 1043.

$\delta_{H\ (250\ MHz)}$ 6.96 (1H, dt, J_1 = 15.7 Hz, J_2 = 7.0 Hz, for the *E* C<u>H</u>=CHCO$_2$Et), 6.22 (1H, dt, J_1 = 11.6 Hz, J_2 = 7.3 Hz, for the *Z* C<u>H</u>=CHCO$_2$Et), 5.81 (1H, d, J = 15.8 Hz, CH=C<u>H</u>CO$_2$Et), 4.19 (2H, quintet, J = 7.0 Hz, OC<u>H$_2$</u>CH$_3$), 3.41 (2H, t, J = 7.0 Hz, C<u>H$_2$</u>Br), 2.3- 2.1 (2H, m), 1.85 (2H, m), 1.5-1.2 (17H, m).

$\delta_{C\ (62.9\ MHz)}$: 168.3 (<u>C</u>O$_2$Et), 150.9 (<u>C</u>H=CHCO$_2$Et), 122.0 (CH=<u>C</u>HCO$_2$Et), 61.6, 35.5, 34.3, 33.7, 30.9, 30.6, 30.2, 29.6, 29.5, 15.8.

Experiment 43: (*E*)-13-Bromotridec-2-en-1-ol (258)



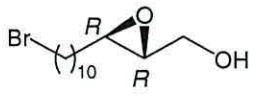
A solution of DIBAL-H (297 ml, 297 mmol, 1 M in toluene) was added to a stirred solution of (*E*)-13-bromo-tridec-2-enoic ethyl ester (**257**, 54 g, 170 mmol) in dry dichloromethane (250 ml) at -60 °C under nitrogen. The temperature rose to - 36 °C during the addition. The mixture was stirred overnight at room temperature, and then quenched by adding sat. aq. ammonium chloride (30 ml) at - 30 °C. The mixture was allowed to reach room temperature and stirred for 0.5 h. Subsequently, the mixture was cooled to 0 °C and hydrochloric acid (18 %) was added until it became a clear solution. The aqueous layer was extracted with dichloromethane (3 x 150 ml) and the combined organic layers were dried. The evaporation of the solvent yielded a pale yellow oil, which was purified by column chromatography eluting with petrol and ether (5:2) to give (*E*)-13-bromotridec-2-en-1-ol (**258**, 18.7 g, 40 %). The compound has been reported in the literature but not its spectral properties:[423]

ν_{max}/cm^{-1}: 3364, 2925, 2853, 1670, 1466, 1437, 1346, 1301, 1265, 1241, 1209 1187, 1087, 1058, 1035, 1017, 1001.

$\delta_{H\ (250\ MHz)}$: 5.8-5.5 (2H, m, C<u>H</u>=C<u>H</u>), 4.07 (2H, d, J = 4.6 Hz, CH=CHC<u>H</u>$_2$OH), 3.40 (2H, t, J = 6.7 Hz, C<u>H</u>$_2$Br), 2.1-1.9 (2H, m), 1.85 (2H, m), 1.6-1.2 (15H, m).

$\delta_{C\ (62.9\ MHz)}$: 133.4 (<u>C</u>H=CHCH$_2$OH), 128.8 (CH=<u>C</u>HCH$_2$OH), 63.8 (<u>C</u>H$_2$OH), 34.0, 32.8, 32.2, 29.4, 29.4, 29.1, 29.1, 28.7, 28.1, 27.4, 22.6.

Experiment 44: (*2R,3R*)-[3-(10-Bromodecyl)oxiranyl]-methanol (259a)

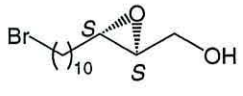


Titanium tetraisopropoxide in dry dichloromethane (3.4 M, 0.32 ml, 1.1 mmol) was added to a stirred solution of D-(-)-diethyl tartrate (0.22 ml, 1.3 mmol) in dry dichloromethane (100 ml) under nitrogen at - 20 °C in the presence of molecular sieves 4 A (0.3 g, 0.12 mol. wt.). The mixture was stirred at - 20 °C for 0.5 h then

tert-butyl hydroperoxide in dry dichloromethane (3 M, 5.32 ml, 18.1 mmol) was added dropwise and the mixture stirred for another 0.5 h. To this solution, 13-bromotridec-2-en-1-ol (**258**, 2.5 g, 9.1 mmol) in dry dichloromethane (20 ml), was added dropwise. After stirring at the same temperature for 4.5 h, the ^{1}H NMR spectrum of the crude product showed the absence of the starting material. However the reaction was left at – 20 °C overnight in the freezer as was suggested in the literature,[323] then quenched with water (10 ml) and allowed to reach room temperature. After the mixture had been stirred for 50 min a solution of sodium hydroxide (30 %) in sat. aq. sodium chloride (6 ml) was added. After stirring the mixture for a further 0.5 h, the phases were separated and the aqueous layer was extracted with dichloromethane (3 x 15 ml). The combined organic layers were dried and evaporated to give a thick oil, which was crystallised from petrol to give (2*R*,3*R*)-[3-(10-bromodecyl)oxiranyl]-methanol (**259a**) as a white solid (1.7 g, 64 %). This compound has already been described but not its chemical or physical properties.[423]

mp:	40-43 °C.
Found $_{(TOF\ MS)}$	$(M+Na)^+$ 315.0905; $C_{13}H_{25}BrO_2Na$ requires: 315.0936.
ν_{max}/cm^{-1}:	3381, 2919, 1461,1376, 1297, 1236, 1211, 1187, 1082, 1049, 1023.
$\delta_{H\ (250\ MHz)}$:	4.0-3.8, (1H, m, C<u>H</u>$_2$OH) 3.7-3.5 (1H, m, C<u>H</u>$_2$OH), 3.41 (2H, t, J = 6.9 Hz, C<u>H</u>$_2$-Br), 3.0-2.9 (2H, m, epoxide ring), 1.9-1.7 (2H, m), 1.6-1.2 (17H, m).
$\delta_{C\ (62.9\ MHz)}$:	61.6 (<u>C</u>H$_2$OH), 58.4 (epoxide ring), 56.0 (epoxide ring), 34.1 (<u>C</u>H$_2$Br), 32.8, 31.5, 29.4, 29.3, 28.7, 28.1, 25.9, 25.7.
$[\alpha]_D^{19}$ =	+ 17.1 (c = 1.2, $CHCl_3$), lit. for ((2*R*, 3*R*)-3-dodecyl-oxiranyl)-methanol $[\alpha]_D$ = + 21 (c = 1.57, $CHCl_3$).[424]

Experiment 45: (*2S,3S*)-[3-(10-Bromodecyl)oxiranyl]-methanol (259b)



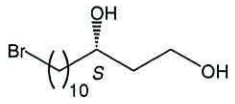
The procedure described in **Experiment 44** was repeated to transform 13-bromotridec-2-en-1-ol (**258**, 13.5 g, 48.6 mmol) into (2*S*,3*S*)[3-(10-bromodecyl)oxiranyl]-methanol (**259b**, 10.7 g, 75 %). The only difference was

that instead of employing D-(-)-diethyl tartrate, L-(+)-diethyl tartrate was used. This compound showed the same physical and spectral properties as the other stereoisomer except it has an opposite specific rotation.

mp: 42-43 °C.

$[\alpha]_D^{19}$ = - 17.5 (c = 0.9 mg, $CHCl_3$), lit. for (2*S*,3*S*)-[3(10-bromodecyl)-oxiranyl]-methanol $[\alpha]_D^{28}$ = - 25.5 (c = 1, $CHCl_3$)[324] and $[\alpha]_D$ = - 15.1 (c = 1, $CHCl_3$).[325]

Experiment 46: Attempted preparation of (*3S*)-13-bromotridecane-1, 3-diol (260)

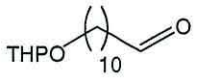


A) A 3.2 M solution of Red–Al (2.5 ml, 7.8 mmol) was added dropwise at 0 °C to a stirred solution of the epoxide (**259b**, 1 g, 3.4 mmol) in dry THF (10 ml) under nitrogen. After stirring for 5 h, the reaction was quenched by adding methanol (2 ml) dropwise at 0 °C, and subsequently sat. aq. sodium chloride (2 ml), ether (25 ml) and magnesium sulphate (5 g). The mixture was stirred at room temperature for 30 min and then filtered. The filtrate was concentrated and the residual oil was purified by chromatography eluting with petrol and ethyl acetate (5:2) to give tetradecane-1, 3-diol (**261**, 0.4 g, 41 %) with an ^1H NMR spectrum identical to the one reported in the literature.[425]

B) The same method utilised in (**A**) was repeated to hydrolyse (2*S*, 3*S*)-[3-(10-bromodecyl)oxiranyl]-methanol (**259b**, 0.5 g, 1.7 mmol) but employing only (0.6 ml, 1.90 mmol) of the solution of Red-Al. The reaction again gave tetradecane-1, 3-diol (**261**, 0.2 g, 54 %).

$\delta_{H\ (250\ MHz)}$: 4.0-3.8, (2H, m, C<u>H</u>$_2$OH), 3.7-3.6 (1H, m, C<u>H</u>–OH), 1.6-1.2 (22H, m), 0.88 (3H, t, J = 6.4 Hz, C<u>H</u>$_3$).

Experiment 47: 11-(Tetrahydropyran-2-yloxy)undecanal (262)



The approach described in **Experiment 27** was used in order to convert 11-(tetrahydropyran-2-yloxy)undecan-1-ol (**204**, 22 g, 0.81 mmol) into 11-(tetrahydropyran-2-yloxy)undecanal (**262**, 17.8 g, 81 %) which was purified by chromatography eluting with petrol and ethyl acetate (5:1). The compound showed the same IR spectrum to the one reported in the literature.[327]

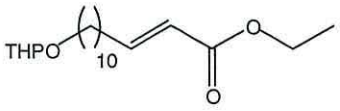
m/z: 271 $(M+1)^+$, 199, 101 $(OTHP)^+$, 85 $(THP)^+$.

ν_{max}/cm^{-1}: 2927, 2854, 1709, 1465, 1352, 1260, 1200, 1120, 1077, 1033.

$\delta_{H\ (250\ MHz)}$: 9.76 (1H, t, J = 1.8 Hz, C<u>H</u>O), 4.57 (1H, t, J = 3.7 Hz, OC<u>H</u>O), 3.9-3.8 (1H, m), 3.72 (1H, dt, J_1 = 9.4 Hz, J_2 = 6.7 Hz), 3.5-3.4 (1H, m), 3.37 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.8 Hz), 2.41 (2H, dt, J_1 = 7.2 Hz, J_2 = 1.8 Hz, C<u>H</u>$_2$CHO), 1.8-1.2 (24H, m).

$\delta_{C\ (62.9\ MHz)}$: 202.9 (<u>C</u>HO), 98.8, (O<u>C</u>HO), 67.6, 62.3, 43.9, 30.8, 29.7, 29.5, 29.4, 29.3, 29.3, 29.1, 29.0, 26.2, 25.5, 22.5, 22.0, 19.7.

Experiment 48: (*E*)-13-(tetrahydropyran-2-yloxy)tridec-2-enoic acid ethyl ester (263)



11-(Tetrahydropyran-2-yloxy)undecanal (**262**, 13 g, 48 mmol) was converted into the desired α,β-unsaturated ester (**263**) using the method described in **Experiment 42**. However, the crude product was purified by chromatography eluting with petrol and ethyl acetate (5:1). The (*E*)-13-(tetrahydropyran-2-yloxy)tridec-2-enoic acid ethyl ester was obtained as pale oil (**263**, 10 g, 64 %) (d. e. = 96 %), which showed the same ^{1}H NMR as those reported.[328]

Found C: 70.1, H: 10.5, required for $C_{20}H_{36}O_4$: C: 70.55, H: 10.66.

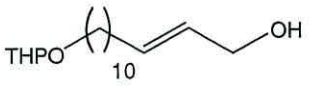
m/z: 339 $(M-H)^+$, 311, 239 $(M-OTHP)^+$, 101 $(OTHP)^+$, 85 $(THP)^+$.

ν_{max}/cm^{-1}: 2927, 2854, 1722, 1654, 1465, 1366, 1351, 1264, 1182, 1121, 1078.

$\delta_{H\ (250\ MHz)}$: 6.96 (1H, dt, J_1 = 15.6 Hz, J_2 = 7.0 Hz, C<u>H</u>=CHCO$_2$Et), 5.80 (1H, dt, J_1 = 15.6 Hz, J_2 = 1.5 Hz CH=C<u>H</u>CO$_2$Et), 4.57 (1H, t, J = 3.7 Hz, OC<u>H</u>O), 4.18 (2H, q, J = 7.1 Hz, OC<u>H$_2$</u>CH$_3$), 3.9-3.8 (1H, m), 3.73 (1H, dt, J_1 = 9.8 Hz, J_2 = 6.7 Hz), 3.5-3.4 (1H, m), 3.37 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.8 Hz), 2.2-2.1 (2H, m), 1.8-1.2 (21H, m).

$\delta_{C\ (62.5\ MHz)}$: 166.8 (<u>C</u>H$_3$O), 149.5 (<u>C</u>H=CHCO$_2$Et), 121.2 (CH=<u>C</u>HCO$_2$Et) 98.8, (O<u>C</u>HO), 67.6, 62.3, 60.1, 32.2, 30.8, 29.7, 29.5, 29.4, 29.3, 28.0, 26.2, 25.5, 19.7, 14.3.

Experiment 49: (*E*)-13-(Tetrahydropyran-2-yloxy)tridec-2-en-1-ol (264)



The approach described in **Experiment 43** was utilised for the successful conversion of 13-(tetrahydropyran-2-yloxy)tridec-2-enoic acid ethyl ester (**263**, 9.5 g, 28 mmol) into (*E*)-13-(tetrahydropyran-2-yloxy)tridec-2-en-1-ol (**264**, 6 g, 72 %) which was purified by column chromatography eluting with petrol and ethyl acetate (5:1). It showed the same IR spectrum to that reported .[327]

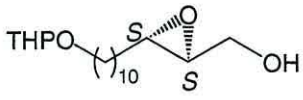
m/z: 297 $(M-1)^+$, 199 $(M-THP)^+$, 101 $(OTHP)^+$, 85 $(THP)^+$.

ν_{max}/cm^{-1}: 3378, 2925, 2853, 1670, 1465, 1439, 1351, 1322, 1260, 1200, 1183, 1136, 1120, 1077, 1022.

$\delta_{H\ (250\ MHz)}$: 5.8-5.5 (2H, m, C<u>H</u>=C<u>H</u>), 4.58 (1H, b s, OC<u>H</u>O), 4.07 (2H, d, J = 4.3 Hz, CH=CHC<u>H$_2$</u>OH), 3.9-3.8 (4H, 4m), 2.1-2.0 (2H, m), 1.8-1.2 (19H, m).

$\delta_{C\ (62.9\ MHz)}$: 133.5 (<u>C</u>H=CHCH$_2$OH), 128.8 (<u>C</u>H=CHCH$_2$OH), 98.8, (O<u>C</u>HO), 67.6 (CH$_2$OTHP), 63.8 (<u>C</u>H$_2$OH), 62.3 (CH$_2$OTHP), 32.2, 30.8, 29.7, 29.5, 29.5, 29.4, 29.1, 29.1, 26.2, 25.5, 19.7, 18.8.

Experiment 50: (*2S, 3S*)-{3-[10-(Tetrahydropyran-2-yloxy)decyl]-oxiranyl}-methanol (265)



Following the approach described in **Experiment 44,** 13-(tetrahydropyran-2-yloxy)tridec-2-en-1-ol (**264**, 6 g, 20 mmol) was transformed into {3-[10-tetrahydropyran-2-yloxy)decyl]-oxiranyl}-methanol (**265**). The only difference was the use of L-(+)-diisopropyl tartrate instead of L-(+)-diethyl tartrate. This caused many problems during the work up. After the addition of the solution of sodium chloride, it was possible to separate the two phases only by filtration on a celite pad. The aqueous layer was extracted with dichloromethane and the combined organic layers were dried and evaporated. The crude product was purified by chromatography eluting with petrol and ethyl acetate (5:2) to give (2*S*, 3*S*)-{3-[10-tetrahydropyran-2-yloxy)decyl]-oxiranyl}-methanol (**265**, 3.5 g, 58%) as a thick yellow oil. Its synthesis has already been described but not its chemical physical properties.[426]

Found $_{(TOF\ MS)}$ $(M+Na)^+$: 337.2326; $C_{18}H_{34}NaO_4$ requires: 337.2355.

m/z: 315 $(M+1)^+$, 255, 231, 85 $(THP)^+$.

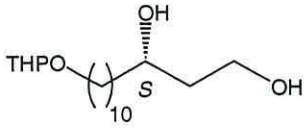
ν_{max}/cm^{-1}: 3452, 2924, 2853, 1740, 1465, 1371, 1352, 1322, 1239, 1200, 1183, 1119, 1077, 1033.

$\delta_{H\ (250\ MHz)}$: 4.57 (1H, t, J = 3.7 Hz, OC<u>H</u>O), 4.0-3.3 (6H, m, including a ddd, J_1 = 16.2 Hz, J_2 = 7.5 Hz, J_3 = 4.1 Hz, C<u>H</u>$_2$OH), 2.9-2.8 (2H, m), 1.9-1.2 (23H, m).

$\delta_{C\ (62.9\ MHz)}$: 98.7 (O<u>C</u>HO), 67.6 (CH_2OTHP), 62.2 ((CH_2OTHP), 61.8 (<u>C</u>H_2OH), 58.6 (epoxide ring), 56.0 (epoxide ring), 31.5, 30.7, 29.7, 29.5, 29.4, 29.4, 29.3, 26.1, 25.9, 25.4, 19.6.

$[\alpha]_D^{19}$ = - 18.5 (lit. for ((2*S*, 3*S*)-3-undecyl-oxiranyl)-methanol an $[\alpha]_D^{28}$ = - 25.5 (c = 1, $CHCl_3$)[324] was reported and $[\alpha]_D$ = - 15.1 (c = 1, $CHCl_3$).[325]

Experiment 51: (*S*)-13-(Tetrahydropyran-2-yloxy)tridecane-1,3-diol (266)



{3-[10-Tetrahydropyran-2-yloxy)decyl]-oxiranyl}methanol (**265**, 2 g, 6.3 mmol) was hydrolysed using the method described in **Experiment 46** (**A**) and gave a

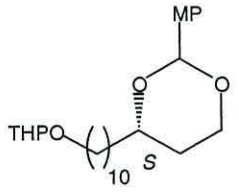
crude product, which was purified by chromatography eluting with petrol and ethyl acetate (3:2) to probably give *13-(tetrahydropyran-2-yloxy)tridecane-1,3-diol* (**266**, 1.6 g, 80%), which was not fully analysed but promptly used for the following step.

ν_{max}/cm^{-1}: 3440, 2925, 2852, 1465, 1352, 1322, 1260, 1200, 1183, 1121, 1076, 1032.

$\delta_{H\ (250\ MHz)}$: 4.56 (1H, t, J = 3.7 Hz, OC<u>H</u>O), 3.9-3.2 (7H, m), 2.8-2.5 (2H, m) 1.8-1.2 (22H, m).

$\delta_{C\ (62.9\ MHz)}$: 98.8, (O<u>C</u>HO), 72.1 (<u>C</u>HOH), 67.7 (CH_2O THP), 62.3 (CH_2O THP), 61.6, 38.2, 27.8, 30.7, 29.7, 29.6, 29.4, 26.2, 25.4, 19.6.

Experiment 52: (3*S*)-2-(4-methoxyphenyl)-4-[10-(tetrahydropyran-2-yloxy) decyl]-[1,3]-dioxane (268)



The 1,3-diol (**266**, 0.9 g, 2.8 mmol) was dissolved in dry DMF (8 ml) followed by the addition of 4-methoxybenzaldehyde dimethylacetal (0.58 g, 3.2 mmol) in dry DMF (3 ml) and CSA (0.3 g, 1.4 mmol) was added. The mixture was stirred overnight, then quenched with water (10 ml) and extracted with ethyl acetate (3 x 25 ml). The combined organic layers were washed with water (20 ml), dried and the solvent was removed to give the crude product which was a mixture of two compounds which were purified by chromatography eluting with petrol and ethyl acetate (5:2). This gave *2-(4-methoxyphenyl)-4-[10-(tetrahydropyran-2-yloxy) decyl]-[1,3]-dioxane* (**268**, 300 mg, 25 %), and probably *10-[2-(4-methoxy-phenyl)-[1,3]-dioxan-4-yl]-decan-1-ol* (**272**, 380 mg, 39 %).

For *2-(4-methoxyphenyl)-4- [10-(tetrahydropyran-2-yloxy)decyl]-[1,3]-dioxane*:

Found $_{(TOF\ MS)}$ $M^+ + H^+$: 435.3088; $C_{26}H_{43}O_5$ requires: 435.3110.

ν_{max}/cm^{-1}: 2927, 2854 1699, 1685, 1600, 1578, 1511, 1465, 1396, 1364, 1314, 1302, 1259, 1216, 1160, 1109, 1074, 1032.

$\delta_{H\ (250\ MHz)}$: 7.42 (2H, d, J = 8.5 Hz, aromatic), 6.90 (2H, d, J = 8.5 Hz, aromatic), 5.47 (1H, s, $CH_2OC\underline{H}OCH_2$), 4.56 (1H, t, J = 3.7 Hz, $OC\underline{H}O$), 4.26 (1H, b dd, J_1 = 5.5 Hz, J_2 = 11.8 Hz, $C\underline{H}O$), 3.9-3.7 (7H a singolet at δ3.8 for 3H $OC\underline{H}_3$), 3.55-3.45 (1H, m, CH_2OTHP), 3.36 (1H, m, CH_2OTHP), 1.6-1.2 (27H, m).

$\delta_{C\ (62.9\ MHz)}$: 159.8 ($\underline{C}OCH_3$ aromatic), 131.6 ($\underline{C}C$ aromatic), 127.3 ($\underline{C}H$ aromatic), 113.5 ($\underline{C}H$ aromatic), 101.0 ($\underline{C}HO_2$), 98.8, ($O\underline{C}HO$), 77.2 ($\underline{C}HO$), 67.7 (CH_2OTHP), 67.0 (CH_2O), 62.3 (CH_2OTHP), 55.2 ($O\underline{C}H_3$), 36.0, 30.8, 29.7, 29.6, 26.2, 25.5, 25.0, 19.3.

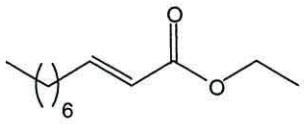
For *10-[2-(4-methoxy-phenyl)-[1,3]-dioxan-4-yl]-decan-1-ol* (which was not completely characterized):

ν_{max}/cm^{-1}: 3480, 2924, 2853, 1654, 1636, 1617, 1458, 1375, 1247, 1167, 1080.

$\delta_{H\ (250\ MHz)}$: 7.42 (2H, d, J = 8.5 Hz, aromatic), 6.90 (2H, d, J = 8.5 Hz, aromatic), 5.47 (1H, s, $CH_2OC\underline{H}OCH_2$), 4.26 (1H, dd, J_1 = 5.5 Hz, J_2 = 11.8 Hz,), 3.79 (1H, dt, J_1 = 1.8 Hz, J_2 = 12.0 Hz,), 3.80 (3H, s, $OC\underline{H}_3$), 3.60 (2H, m), 1.6-1.2 (25H, m).

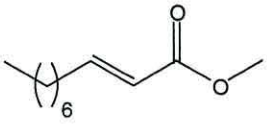
$\delta_{C\ (62.9\ MHz)}$: 159.8 ($\underline{C}OCH3$ aromatic), 131.6 ($\underline{C}C$ aromatic), 127.3 ($\underline{C}H$ aromatic), 113.5 ($\underline{C}H$ aromatic), 101.0 ($\underline{C}HO_2$), 77.2 ($\underline{C}HO$), 67.0 (CH_2O), 62.9 (CH_2OH), 55.2 ($O\underline{C}H_3$), 36.0, 32.7, 31.3, 29.5, 29.3, 24.9.

Experiment 53: (*E*)-Dec-2-enoic acid ethyl ester (284a)



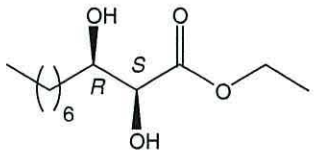
Octanal (**286**, 5g, 39mmol) was converted into the (*E*)-dec-2-enoic acid ethyl ester (**284a**, 5.2, 67 %) (d.e. = 96 %) using the method described in **Experiment 42.** The NMR spectra of the compound were identical to those reported.[427]

Experiment 54: (*E*)-Dec-2-enoic acid methyl ester (284b)



Octanal (**286**, 5g, 38.9 mmol) was converted into (*E*)-dec-2-enoic acid methyl ester (**284a**, 5.6g, 78 %) with (methoxycarbonylmethylene) triphenylphosphorane using the method described in the **Experiment 42 .** The NMR spectra correspond with those reported, and the signals for the (*Z*)-diastereoisomer were not seen.[428]

Experiment 55: (2*S*,3*R*)-2,3-Dihydroxydecanoic acid ethyl ester (287a)

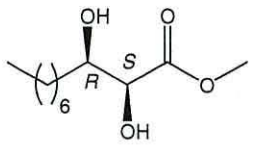


The $(DHQD)_2PHAL$ ligand (63 mg, 0.08 mmol), $K_3Fe(CN)_6$ (7.92 g, 24 mmol), K_2CO_3 (3.36 g, 24 mmol) and a solution 2.5 % of OsO_4 in *tert*-butyl alcohol (320 µl, 0.032 mmol) were dissolved in a 1:1 mixture of water and *tert*-butyl alcohol (80 ml) at room temperature. Then $MeSO_2NH_2$ (760 mg, 8 mmol) was added and the mixture, vigorously stirred, was cooled to 0 °C when (*E*)-dec-2-enoic acid ethyl ester (**284a**, 1.5 g, 8 mmol) was added. The reaction was maintained at this temperature and monitored by TLC. After completion, the reaction was worked up by addition of sodium sulphite (12 g), then the mixture was warmed to room temperature and stirred for 1 h and extracted with dichloromethane (3 x 20 ml); the organic layers were washed with a 2N solution of KOH (20 ml), dried and concentrated to give the crude product. This was purified by chromatography using petrol and ethyl acetate (5:2), to give (2*S*,3*R*)-2,3-dihydroxydecanoic acid ethyl ester 2,3-dihydroxydecanoic acid ethyl ester (**287a**, 1.6 g, 87 %). This white solid showed the same NMR and IR spectra and similar optical rotation to those reported.[338]

mp: 42-45, (Lit. mp: 41-42).[338]

$[\alpha]_D^{19}$ = + 9.8 (c = 1.1, $CHCl_3$), (lit. $[\alpha]_D^{24}$ = +11.4 (c = 0.57, $CHCl_3$)[338] and $[\alpha]_D^{25}$ = + 10.1 (c = 1.4, $CHCl_3$).[340]

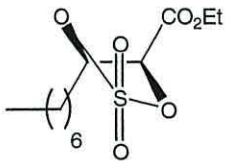
Experiment 56: (2*S*,3*R*), 2,3-(Dihydroxydecanoic acid methyl ester (287b)



The method described in **Experiment 55** was used to convert the ester (**284b,** 1.5g, 8.0 mmol) into (2*S*,3*R*), 2, 3-dihydroxydecanoic acid methyl ester (**287b**, 87 %). It showed similar spectral properties and optical rotation to those reported.[339]

$[\alpha]_D^{23}$ = + 10.8 (c = 1.3, $CHCl_3$), (lit. $[\alpha]_D^{25}$ = + 11.23 (c = 1.0, $CHCl_3$).[339]

Experiment 57: (4*S*,5*R*)-5-Heptyl–2,2-dioxo-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid ethyl ester (289a)



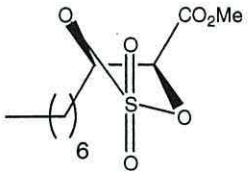
In a two neck round bottomed flask equipped with a reflux condenser and topped with a drying tube, the 2,3-dihydroxydecanoic acid ethyl ester (**287a**, 400 mg, 1.7 mmol) was dissolved in CCl_4 (10 ml) under nitrogen. Thionyl chloride (0.2 ml, 3.4 mmol) was added and the mixture was refluxed for 1 h. After cooling, the solution was diluted with CH_3CN (7 ml) and ruthenium trichloride hydrate (1 mg) and $NaIO_4$ (0.7g, 2.6 mmol) were added followed by water (10 ml). The mixture was stirred at room temperature for 20 min then diluted by adding ether (25 ml). The water layer was extracted with ether (2 x 15 ml). The organic layers were washed with water (15 ml), sat. aq. sodium bicarbonate (2 x 15 ml) and brine (15 ml) and dried. The solvent was evaporated to give crude *5-heptyl-2,2-dioxo-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid ethyl ester* (**289a**, 360 mg, 71 %), which was utilised without other purification.

ν_{max}/cm^{-1}: 2928, 2856, 1769, 1744, 1466, 1399, 1302, 1210, 1028.

$\delta_{H\ (250\ MHz)}$: 5.0-4.8 (2H, m, C<u>H</u>OSO$_2$OC<u>H</u>, including a d at δ 4.85, J = 7.2 Hz, C<u>H</u>CO), 4.33 (2H, quintet, J = 7.3 Hz, OC<u>H$_2$</u>CH$_3$), 2.1-2.0 (2H, m), 2.1-1.6 (15H, m), 0.88 (3H, t, J = 7.8 Hz, C<u>H$_3$</u>).

$\delta_{C\ (62.9\ MHz)}$: 164.8 (<u>C</u>=O), 84.2 (<u>C</u>HOC=O), 79.9 (CH$_2$<u>C</u>HO), 63.2 (O<u>C</u>H2CH3), 32.9, 31.5, 29.2, 28.7, 24.7, 22.5, 13.9 (<u>C</u>H$_3$).

Experiment 58: (4*S*,5*R*)-5-Heptyl-2,2-dioxo-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid methyl ester (289b)



The procedure of **Experiment 57** was utilised in order to convert the dihydroxy ester (**287b**, 5 g, 22.9 mmol) into *(4S,5R)-5-Heptyl-2,2-dioxo-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid methyl ester* (**289b,** 5.4 g, 84 %) after purification by chromatography eluting with petrol and ether (5:1).

Found C: 47.0, H: 7.5, required for $C_{11}H_{20}O_6S$: C: 47.13, H: 7.19.

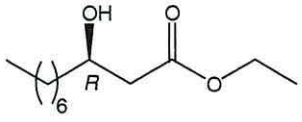
ν_{max}/cm^{-1}: 2927, 2853, 1773, 1742, 1460, 1395, 1309, 1210, 1023.

$\delta_{H\ (250\ MHz)}$: 5.0-4.8 (2H, m, C<u>H</u>OSO$_2$OC<u>H</u>, including a doublet at δ 4.87, J = 7.2 Hz, C<u>H</u>CO), 3.99 (3H, s, OC<u>H$_3$</u>), 2.1-2.0 (2H, m), 2.1-1.6 (15H, m), 0.88 (3H, t, J = 7.8 Hz CH$_2$C<u>H$_3$</u>);

$\delta_{C\ (62.9\ MHz)}$: 165.3 (<u>C</u>=O), 84.1 (<u>C</u>HOC=O), 79.8 (<u>C</u>HOCH$_2$), 53.6 (O<u>C</u>H$_3$), 32.9, 31.5, 29.2, 28.7, 24.7, 22.5, 13.9.

$[\alpha]_D^{19}$ = + 30.7 (c = 0.9, $CHCl_3$), (lit. $[\alpha]^{25}_D$ = + 34.6 for (4*S*, 5*R*)-2,2-dioxo-5-pentadecyl-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid ethyl ester (3.12, $CHCl_3$).[331]

Experiment 59: (*R*)-3-Hydroxydecanoic acid ethyl ester (285a)



The cyclic sulphate (**289a**, 250 mg. 0.86 mmol) was dissolved in DMAC (10 ml) and $NaBH_4$ (33 mg, 0.86 mmol) was added at 0 °C. After stirring the mixture at room temperature for 1 h, this was concentrated by distillation under high vacuum. Then ether (5 ml) and a 10 % solution of H_2SO_4 (3 ml) were added to the residue. The addition of sulphuric acid had to be done carefully because the reaction was very exothermic. The mixture was stirred vigorously for further 6 h. After separating the two layers, the water layer was extracted with ether (4 x 20 ml). The combined organic layers were washed with water and brine dried and concentrated. The crude product was purified by chromatography eluting with petrol and ethyl acetate (5:1). (*R*) 3-hydroxydecanoic acid ethyl ester (**285a**, 110 mg, 60 %) was obtained. It showed the same NMR spectra and similar optical rotation to those reported.[342]

m/z: 215 $(M-1)^+$, 153$(M-OEt)^+$, 117 $(M-(CH_3(CH_2)_6)^+$, 71.

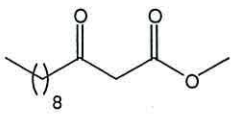
ν_{max}/cm^{-1}: 3460, 2925, 2854, 1740, 1437, 1336, 1172.

$\delta_{H\ (250\ MHz)}$: 4.12 (2H, q, J = 7.0 Hz $OC\underline{H}_2CH_3$), 4.1-3.9 (1H, m, $C\underline{H}OH$), 3.1-3.0 (1H, b s, $O\underline{H}$), 2.47 (1H, dd, J_1 = 3.7 Hz, J_2 = 16.3 Hz, $COC\underline{H}_2CHOH$), 2.36 (1H, dd, J_1 = 8.6 Hz, J_2 = 16.2 Hz, $COC\underline{H}_2CHOH$), 1.7-1.0 (14H, m) 0.85 (3H, t, J = 7.0 Hz, $C\underline{H}_3$).

$\delta_{C\ (62.9\ MHz)}$: 173.0: ($\underline{C}$=O), 68.0 ($\underline{C}$HOH), 60.6 ($O\underline{C}H_2CH_3$), 41.3 ($CHOC\underline{H}_2CO$), 36.5, 31.7, 29.4, 29.2, 25.4, 22.6, 14.1 ($\underline{C}H_3$).

$[\alpha]_D^{22}$ = - 9.2, (c = 0.9, $CHCl_3$), (lit. $[\alpha]_D^{20}$ = - 10 for (c = 1.2, $CHCl_3$).[342]

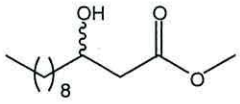
Experiment 60: 3-Oxododecanoic acid methyl ester (293)



Butyllithium (70 ml, 112.5 mmol) was added dropwise at – 40 °C to a solution of anhydrous diisopropylamine (13.6 g, 135 mmol) in dry THF (60 ml) under nitrogen. The mixture was stirred for 1 h at room temperature. Subsequently, it was cooled to – 78 °C and a solution of methyl acetate (**292**, 6 g, 81 mmol) in dry THF (10 ml) was added at a rate that allowed the temperature to remain below – 65 °C, then the mixture was stirred at – 78 °C for 10 min. Finally, a solution of

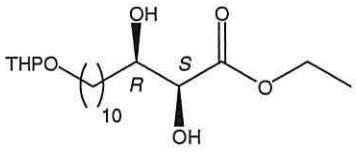
decanoyl chloride (**291**, 8.5g, 44.7 mmol) in dry THF (10 ml) was added dropwise. The reaction was stirred at room temperature for two days, then quenched with dilute hydrochloric acid (10 %) until pH = 5. The aqueous layer was extracted with dichloromethane (3 x 300 ml). The combined organic layers were dried and the solvent evaporated to give the crude product which was purified by chromatography eluting with petrol and ether (2:1) to produce 3-oxododecanoic acid methyl ester (**293**, 8 g, 78 %), whose ^{1}H NMR and IR spectra were identical to the those reported.[343]

Experiment 61: (*R,S*)-3-Hydroxydodecanoic acid methyl ester (294)



The 3-oxododecanoic acid methyl ester (**293**, 1 g, 4.3 mmol) was dissolved in methanol (15 ml) and the solution cooled to 0 °C and treated with sodium borohydride (0.3 g, 6.9 mmol). The mixture was stirred for 10 h and then at room temperature for 3.5 h, then quenched by the addition of water (30 ml). The mixture was then extracted with ether (3 x 15 ml). The combined organic layers were dried and concentrated to give the crude product which was purified by chromatography eluting with petrol and ether (1:1) to obtain a racemic mixture of (*R,S*)-3-hydroxydodecanoic acid methyl ester (0.78 g, 80 %) (**95**), which had the same ^{1}H NMR spectrum to that reported.[344]

Experiment 62: (2*S*,3*R*), 2,3-Dihydroxy-13-(tetrahydropyran-2-yloxy) tridecanoic acid ethyl ester (295)

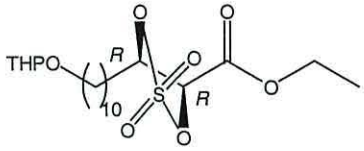


The procedure used in the **Experiment 55** was repeated for the dihydroxylation of 13-(tetrahydropyran-2-yloxy)tridec-2-enoic acid ethyl ester (**263**, 3.5g, 11.2 mmol). The crude was purified by chromatography eluting with petroleum and

ethyl acetate (5:2) to give the *(2S,3R)-2,3-dihydroxy-13-(tetrahydropyran-2-yloxy)tridecanoic acid ethyl ester* (**295**, 3.02 g, 78 %).

Found	C: 64.8, H: 10.0, required for $C_{14}H_{26}O_3$ C: 64.14, H: 10.23;
m/z:	374 M^+, 341, 273 $(M\text{- OTHP})^+$ 85 $(THP)^+$.
ν_{max}/cm^{-1}:	3440, 2926, 2855, 1739, 1466, 1367, 1201, 1137, 1078, 1032.
$\delta_{H\ (250\ MHz)}$:	4.52 (1H, t, J = 2.8 Hz, OC<u>H</u>O), 4.23 (2H, q, J = 7.0 Hz, OC<u>H</u>$_2$), 4.0 (1H, d, J = 1.5 Hz, HOC<u>H</u>CO), 3.9-3.8 (2H, m), 3.73 (1H, dt, J_1 = 9.8 Hz, J_2 = 6.7 Hz, CH_2OTHP), 3.5-3.4 (2H, m), 3.37 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.8 Hz, CH_2OTHP), 2.5-2.4 (1H, b s, O<u>H</u>), 1.7-1.0 (27H, m).
$\delta_{C\ (62.9\ MHz)}$:	173.6 (<u>C</u>=O), 98.8 (O<u>C</u>HO), 73.0 (<u>C</u>HOH), 72.5 (<u>C</u>HOH), 67.6 (<u>C</u>H_2OTHP), 62.3 (<u>C</u>H_2OTHP), 62.0 (O<u>C</u>H_2CH_3), 37.8, 30.8, 29.7, 29.5, 29.4, 26.2, 25.7, 25.5, 19.6.

Experiment 63: (4*S*,5*R*)-2,2-Dioxo-5-[10-(tetrahydropyran-2-yloxy)decyl]-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid ethyl ester (296)

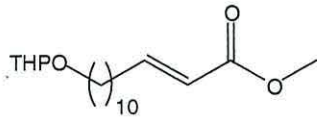


In order to obtain the cyclic sulphate (**296**), the approach described in **Experiment 57** was used. It gave the desired product *(4S,5R)-2,2-dioxo-5-[10-(tetrahydropyran-2-yloxy)decyl]-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid methyl ester* in a low yield (**296**, 25 %).

Found	C: 55.4, H: 8.1, required for $C_{20}H_{36}O_8S$ C: 55.02, H 8.31.
m/z:	420 $(M\text{-O})^+$, 392 $(M\text{-OCH}_2CH_2)^+$, 334, 101 $(OTHP)^+$,
ν_{max}/cm^{-1}:	3436 2924, 2854, 1740, 1466, 1399, 1301, 1209, 1120, 1024.
$\delta_{H\ (250\ MHz)}$:	5.0-4.8 (2H, m, C<u>H</u>OSO$_2$OC<u>H</u>, including a d at δ 4.83, J = 7.3 Hz, C<u>H</u>CO) 4.52 (1H, t, J = 2.8 Hz, OC<u>H</u>O), 4.23 (2H, q, J = 7.0 Hz, OC<u>H</u>$_2$), 3.9-3.8 (1H, m), 3.73 (1H, dt, J_1 = 9.8 Hz, J_2 = 6.7 Hz), 3.5-3.4 (1H, m), 3.37 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.8 Hz), 1.7-1.0 (27H, m).

$\delta_{C\ (62.9\ MHz)}$: 176.8 (C=O), 98.8 (OCHO), 84.1 (CHOC=O), 80.0 ($CHOCH_2$), 67.6 (CH_2OTHP), 63.3 (OCH_2CH_3), 62.3 (CH_2OTHP), 32.9, 30.7, 29.7, 29.4, 29.4, 29.3, 29.1, 28.8, 26.2, 25.5, 24.7, 19.7.

Experiment 64: (*E*)-13-(Tetrahydropyran-2-yloxy)tridec-2-enoic acid methyl ester (297)



The method described in **Experiment 42** was used to convert 11-(tetrahydropyran-2-yloxy)undecanal (**293**, 17.6 g, 65 mmol) into (**297**). The crude product was purified by chromatography eluting with petrol and ethyl acetate (5:1) to give *(E)-13-(tetrahydropyran-2-yloxy)tridec-2-enoic acid methyl ester* (**297**, 17.5 g, 82 %).

Found: C: 69.9, H: 10.2, required for $C_{19}H_{34}O_4$: C: 69.90, H: 9.92.

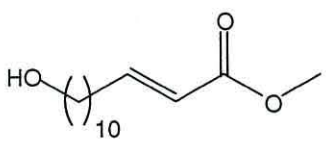
m/z: 326 M^+, 311 $(M\text{-}CH_3)^+$, 241 $(M\text{-}THP)^+$, 225 $(M\text{-}OTHP)^+$, 101 $(OTHP)^+$, 85 $(THP)^+$.

ν_{max}/cm^{-1}: 2924, 2853, 1727, 1658, 1454, 1435, 1351, 1322, 1270, 1200, 1173, 1136, 1121, 1078, 1034.

$\delta_{H\ (250\ MHz)}$: 6.97 (1H, dt, J_1 = 15.6 Hz, J_2 = 7.0 Hz, CH=CHCO$_2$Me), 5.82 (1H, dt, J_1 = 15.6 Hz, J_2 = 1.5 Hz CH=CHCO$_2$Me), 4.58 (1H, t, J = 3.7 Hz, OCHO), 3.73 (3H, s, OCH_3), 3.9-3.8 (1H, m), 3.73 (1H, dt, J_1 = 9.8 Hz, J_2 = 6.7 Hz), 3.5-3.4 (1H, m), 3.37 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.8 Hz), 2.2-2.1 (2H, m), 1.8-1.2 (22H, m).

$\delta_{C\ (62.9\ MHz)}$: 167.2 (C=O), 149.8 (CH=CHCO$_2$Me), 120.8 (CH=CHCO$_2$Me), 98.8 (OCHO), 67.7 (CH_2OTHP), 62.3 (CH_2OTHP), 51.3 (OCH_3), 32.2, 30.8, 29.7, 29.5, 29.4, 29.3, 29.1, 29.0, 28.0, 26.2, 25.5, 19.7.

Experiment 65: (*E*)-13-Hydroxytridec-2-enoic acid methyl ester (298)



(*E*)-13-(Tetrahydropyran-2-yloxy)tridec-2-enoic acid methyl ester (**297**, 5g, 15.3 mmol) was dissolved in methanol (75 ml) and PPTS (0.15 g, 076 mmol) was added. The mixture was stirred overnight. The reaction was quenched by adding water (5 ml) and the mixture was extracted with dichloromethane (3 x 75 ml). The combined organic layers were dried and concentrated to give the crude product which was purified by chromatography eluting with petrol and ether (5:2) giving *(E)-13-hydroxytridec-2-enoic acid methyl ester* (**298**, 3.26 g, 88 %).

Found $_{(TOF\ MS)}$ $(M+H)^+$: 243.1951; $C_{14}H_{27}O_3$ requires: 243.1960.

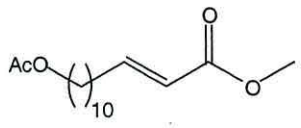
m/z: 243 $(M+1)^+$, 210 $(M-CH_3OH)^+$, 164, 81, 55.

ν_{max}/cm^{-1}: 3417, 2927, 2854, 1726, 1656, 1436, 1272, 1201, 1176, 1043.

$\delta_{H\ (250\ MHz)}$: 6.96 (1H, dt, J_1 = 15.6 Hz, J_2 = 7.0 Hz, C<u>H</u>=CHCO$_2$Me), 5.82 (1H, d, J = 15.6 Hz, CH=C<u>H</u>CO$_2$Me), 3.70 (3H, s, OC<u>H</u>$_3$), 3.61 (2H, t, J = 6.7 Hz, C<u>H</u>$_2$OH), 2.3-2.1 (2H, m), 1.8-1.2 (17H, m).

$\delta_{C\ (62.9\ MHz)}$: 167.2 (<u>C</u>=O), 149.8 (C<u>H</u>=CHCO), 120.7(CH=C<u>H</u>CO), 62.9 (CH_2OH), 51.3 (OCH_3), 32.7, 32.1, 29.5, 29.3, 28.9, 27.9, 25.7, 22.5.

Experiment 66: (*E*)-13-Acetoxytridec-2-enoic acid methyl ester (299)



The alcohol (**298**, 3g, 12.4 mmol) was dissolved in dry dichloromethane (80 ml) under nitrogen and triethylamine (2.5 g, 24.8 mmol) was added. After cooling to 0 °C, acetyl chloride (1.5g, 19.0 mmol) was added and the solution was stirred overnight. The reaction was quenched with water (150 ml) and extracted with dichloromethane (3 x 50 ml). The combined organic layers were washed with a dil.hydrochloric acid (50 ml, 2 N), dried and the solvent evaporated to give the crude product. This was purified by chromatography eluting with petrol and ether (5:1). The desired product *(E)-13-acetoxy-tridec-2-enoic acid methyl ester* (**299**, 3.5 g, 99 %) was obtained.

Found C: 67.7, H: 9.9, required for $C_{16}H_{28}O_4$: C: 67.57, H: 9.92

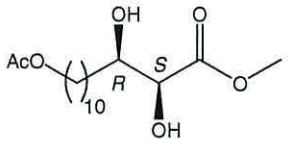
m/z: 285 $(M+1)^+$, 252 $(M-CH_3OH)^+$, 210 , 164, 135, 81, 55.

ν_{max}/cm^{-1}: 2927, 2854, 1739, 1726, 1657, 1548, 1530, 1435, 1366, 1241, 1040.

$\delta_{H\ (250\ MHz)}$: 6.96 (1H, dt, J_1 = 15.6 Hz, J_2 = 7.0 Hz, C<u>H</u>=CHCO$_2$Me), 5.82 (1H, d, J = 15.6 Hz, CH=C<u>H</u>CO$_2$Me), 4.04 (2H, t, J = 6.7 Hz, C<u>H</u>$_2$OCO), 3.73 (3H, s, OC<u>H</u>$_3$), 2.2-2.1 (2H, m), 2.04 (3H, s, C<u>H</u>$_3$CO), 1.8-1.2 (16H, m).

$\delta_{C\ (62.9\ MHz)}$: 171.0 (<u>C</u>=O), 167.1 (<u>C</u>=O), 149.5 (<u>C</u>H=CHCO$_2$Me), 120.8 (CH=<u>C</u>HCO$_2$Me), 64.5 (<u>C</u>H$_2$OCO), 51.3 (OCH3), 32.1, 29.4, 29.3, 29.3, 29.2, 29.0, 28.6, 27.9, 25.8, 20.9.

Experiment 67: (3*R*,2*S*)-13-Acetoxy-2,3-dihydroxytridecanoic acid methyl ester (302)



The approach described in **Experiment 55** was utilised for the conversion of (*E*)-13-acetoxytridec-2-enoic acid methyl ester (**299**, 9.5 g, 28 mmol) into an oil which was purified by chromatography eluting with petrol and ethyl acetate (5:1) to give *(3R,2S)-13-acetoxy-2,3-dihydroxytridecanoic acid methyl ester* (**302**, 6 g, 72 %).

Found C: 60.7, H: 9.6, required for $C_{16}H_{30}O_6$: C: 60.36, H: 9.50.

m/z: 315 $(M-3H)^+$, 259 $(M-CO_2Me)^+$, 229, 90.

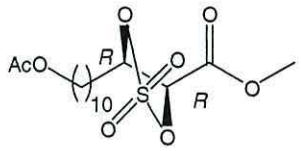
ν_{max}/cm^{-1}: 3 442, 2933, 2853, 1736, 1468, 1368, 1251, 1134, 1044.

$\delta_{H\ (250\ MHz)}$: 4.2-4.0 (5H, m, including a t at δ 4.02, J = 6.7 Hz, C<u>H</u>OHCO and C<u>H</u>$_2$OCO), 3.9-3.8 (1H, m, CH$_2$C<u>H</u>OH), 3.78 (3H, s, OC<u>H</u>$_3$), 3.4-3.3 (1H, b s, OH), 2.5-2.4 (1H, b s, OH), 2.01 (3H, s, C<u>H</u>$_3$CO), 1.6-1.0 (12H, m).

$\delta_{C\ (62.9\ MHz)}$: 174.0 (<u>C</u>=O), 171.2 (<u>C</u>=O), 73.2 (<u>C</u>HOH), 72.4 (<u>C</u>HOH), 64.6 (<u>C</u>H$_2$OCO), 52.6 (O<u>C</u>H$_3$), 33.5, 29.4, 29.2, 28.5, 25.8, 25.6, 20.9.

$[\alpha]_D^{22}$ = + 9.7 (c = 1.0 in $CHCl_3$).

Experiment 68: (4*S*,5*R*)-5-(10-Acetoxydecyl)-2,2-dioxo-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid methyl ester (303)



The approach described in **Experiment 57** was used in order to convert 13-acetoxy-2,3-dihydroxytridecanoic acid methyl ester (**302**, 500mg, 1.58 mmol) into *(4S,5R)-5-(10-acetoxydecyl)-2,2-dioxo-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid methyl ester* (**303,** 430 mg, 72 %) after purification by column chromatography eluting with petrol and ether (5:2).

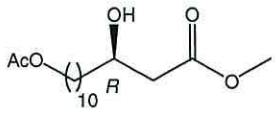
Found C: 50.7, H: 7.4, required for $C_{16}H_{28}O_8S$: C: 50.51, H: 7.42.

ν_{max}/cm^{-1}: 2929, 2855, 1777, 1738, 1462, 1396, 1366, 1244, 1210, 1039.

$\delta_{H\ (250\ MHz)}$: 5.0-4.8 (2H, m, C<u>H</u>OSO$_2$OC<u>H</u>, including a d at δ 4.86, J = 7.1 Hz, C<u>H</u>CO), 4.04 (2H, t, J = 6.7 Hz, C<u>H</u>$_2$OCO), 3.78 (3H, s, OC<u>H</u>$_3$), 2.0-1.9 (5H, m including at δ 2.03 s, 3H for OCH_3), 1.6-1.0 (17H, m).

$\delta_{C\ (62.9\ MHz)}$: 171.3 (<u>C</u>=O), 165.4 (C=O), 84.1 (<u>C</u>HOCO), 79.9 (<u>C</u>HOCO), 64.6 (<u>C</u>H2OCO), 53.4 (O<u>C</u>H$_3$), 32.9, 29.3, 29.2, 29.1, 28.8, 28.5, 25.8, 24.8, 24.7, 21.0.

Experiment 69: (*R*)-13-Acetoxy-3-hydroxytridecanoic acid methyl ester (304)



The procedure used in **Experiment 59** was repeated in order to convert the cyclic sulphate (**303**, 0.378g, 1 mmol) into *(R)-13-acetoxy-3-hydroxytridecanoic acid methyl ester* (**304**, 150 mg, 50 %):

Found C: 63.9, H: 9.9, required for $C_{16}H_{30}O_5$: C: 63.55, H: 10.00;

m/z: 303 $(M+1)^+$, 269, 229 $(CH_3(CH_2)_{10}CHOH)^+$, 103 $(CHOHCH_2CO_2Me)^+$, 71, 61.

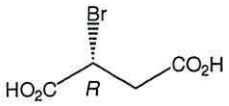
ν_{max}/cm^{-1}: 3453, 2926, 2854, 1736, 1630.4, 1438, 1365, 1242, 1040.

$\delta_{H\ (250\ MHz)}$: 4.12 (2H, t, J = 6.7 Hz, C<u>H</u>$_2$OCO), 4.1-4.0 (1H, m, C<u>H</u>OH), 3.78 (3H, s, COOC<u>H</u>$_3$), 2.64 (1H, b s, O<u>H</u>), 2.53 (1H, dd, J_1 = 16.2 Hz, J_2 = 3.5 Hz, COC<u>H</u>$_2$CHOH), 2.40 (1H, dd, J_1 = 16.5 Hz, J_2 = 8.6 Hz, COC<u>H</u>$_2$CHOH), 2.12 (3H, s, C<u>H</u>$_3$), 1.7-1.0 (20H, m).

$\delta_{C\ (62.9\ MHz)}$: 173.4 (<u>C</u>=O), 171.2 (<u>C</u>=O), 66.5 (<u>C</u>HOH), 66.1 (<u>C</u>H$_2$OCO), 53.1 (O<u>C</u>H$_3$), 42.7 (CHOH<u>C</u>H$_2$CO), 38.0, 30.9, 30.7, 30.0, 27.4, 27.3, 24.0, 22.4.

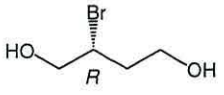
$[\alpha]_D^{19}$ = - 12.5 (c = 1.0 in $CHCL_3$) (lit. for (*R*)-3-hydroxyhexadecanoic acid methyl ester $[\alpha]_D^{20}$ = - 13.8 (c = 1.48 in $CHCl_3$).[346]

Experiment 70: (*R*)-Bromosuccinic acid (311)



KBr (100 g, 840 mmol) was added to a solution of D-aspartic acid (**307**, 25g, 188 mmol) in a 2.5 M solution of sulphuric acid (500 ml) stirred by a mechanical stirrer. The solution was then cooled at -5 °C and $NaNO_2$ (23.8 g, 340 mmol) in water (40 ml) was added over 75 min maintaining the temperature constantly below 0 °C. The resulting dark yellow mixture was stirred for 4 h at -5 °C and then extracted with ethyl acetate (4 x 300 ml). The combined organic layers were dried, filtered and concentrated to give a white solid, (*R*)-bromosuccinic acid (**311**, 32 g, 86 %), which was used without further purification. The product had physical and chemical properties corresponding to the data in the literature.[349]

Experiment 71: (*R*)-2-Bromo-1,4-butanediol (315)

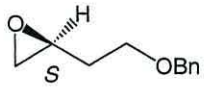


A solution of BH_3·THF (450 ml, 450 mmol, 1 M in THF) was added to a mechanically stirred suspension of (*R*)- bromosuccinic acid (**311**, 30 g, 151 mmol) in THF (250 ml) at 0 °C. The addition was completed over a period of 1 h then the mixture was stirred at room temperature for 5 h. The excess borane was quenched with a solution of THF and H_2O (1:1, 100 ml) followed by addition of K_2CO_3 (80 g). This mixture was stirred for 0.5 h then filtered, and the solid residue was

washed with ether (3 x 100 ml). The combined liquid layers were concentrated to give a mixture. The compound was re-dissolved in ether and filtered. The filtrate was dried, and the solvent evaporated to give a crude oil which was purified by chromatography eluting with dichloromethane and acetone (1:1) to give (*R*)-2-bromo-1,4-butanediol (**315**, 21 g, 85 %). The compound had the same NMR spectra as reported for the enantiomer and a nearly opposite optical rotation.[348]

$[\alpha]_D^{23}$ = + 29.2 (c = 0.9, $CHCl_3$). Lit. for (*S*)-isomer $[\alpha]_D^{24}$ = - 31.9 (c = 1.52, $CHCl_3$).[348]

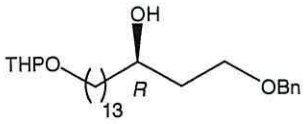
Experiment 72: (*S*)-(2-benzyloxyethyl)oxirane (313)



To a suspension of sodium hydride (15 g, 368 mmol) in dry THF under nitrogen was added at - 10 °C a solution of (*R*)-2-bromo-1,4-butanediol (**315**, 20 g, 120 mmol) in dry THF (20 ml). The addition was completed in 5 min and the mixture was stirred for another 0.5 h. Then benzyl bromide (22.2 g, 130 mmol) and TBAI (4.4 g, 11.8 mmol) were added. After 5 min, the mixture was brought to room temperature and stirred for further 1 h. The reaction was quenched by addition of sat. aq. ammonium chloride (100 ml). The compound was extracted with ethyl acetate (3 x 200 ml) then the combined organic layers were dried, filtered and evaporated to give the crude product, which was purified by chromatography eluting with petrol and ether (5: 2) to give (*S*)-(2-benzyloxyethyl) oxirane (**313**, 16.5 g, 75 %). The product had a ^1H NMR spectrum identical to that reported and a similar optical rotation: [351]

$[\alpha]_D^{21}$ = - 16.5 (c = 1.1, $CHCl_3$). Lit. $[\alpha]_D^{23}$ = - 15.6 (c = 5.1, $CHCl_3$).[346]

Experiment 73: (*R*)-1-Benzyloxy-16-(tetrahydropyran-2-yloxy)hexadecan-3-ol (312a)



A solution of 2-(12-bromododecyloxy)tetrahydropyran (**314a**, 4 g, 11.4 mmol) in THF (15 ml) was added dropwise to a suspension of magnesium turnings (731 mg, 30 mmol) in THF (5 ml) under nitrogen over a period of 0.5 h. The mixture was refluxed for 2 h, then cooled to room temperature and added dropwise to a stirred solution of purified copper iodide (144 mg, 0.76 mmol) in dry THF (15 ml) at - 30 °C. After 10 min, a solution of (*S*)-(2-benzyloxyethyl) oxirane (**313**, 1.4 g, 7.6 mmol) in tetrahydrofuran (10 ml) was added dropwise. The mixture was stirred for 3 h at - 30 °C, when TLC still showed the presence of starting material, then warmed to -15 °C for 1 h when TLC showed no starting material was left. The mixture was quenched with sat. aq. ammonium chloride (10 ml) and then allowed to reach room temperature. The product was extracted with dichloromethane (3 x 25 ml); the combined organic layers were washed with water (10 ml), dried and the solvent evaporated to give a colourless oil which was purified by chromatography eluting with petrol and ether (5:3) to give *(R)-1-benzyloxy-16-(tetrahydropyran-2-yloxy)hexadecan-3-ol* (**312a**, 2.8 g, 85 %).

Found C: 73.9, H: 11.1, required for $C_{28}H_{48}O_4$: C: 74.95, H: 10.78.

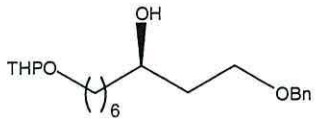
ν_{max}/cm^{-1}: 3456, 3030, 2924, 2853, 1496, 1454, 1364, 1322, 1260, 1200, 1184, 1118, 1078, 1029.

$\delta_{H\ (250\ MHz)}$: 7.40-7.32 (5H, m, aromatic), 4.58 (1H, b, t, J = 3.6 Hz, OC<u>H</u>O), 4.53 (2H, s, C<u>H</u>$_2$OPh), 3.9-3.7 (5H, m), 3.52-3.48 (1H, m), 3.40-3.35 (1H, dt J_1 = 9.5 Hz, J_2 = 6.6 Hz, CH_2OTHP), 2.9-2.8 (1H, m, O<u>H</u>), 1.98-1.78 (2H, m), 1.75-1.23 (29H, m).

$\delta_{C\ (62.9\ MHz)}$: 138 (aromatic), 128.5 (aromatic), 127.7 (aromatic), 127.6 (aromatic), 98.8 (O<u>C</u>HO), 73.3 (O<u>C</u>H$_2$Ph), 71.4 (<u>C</u>HOH), 69.1 (<u>C</u>H$_2$OBn), 67.7 (<u>C</u>H$_2$OTHP), 62.3 (CH_2OTHP), 37.4, 36.4, 30.8, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 26.2, 25.7, 25.6, 25.5, 19.7.

$[\alpha]_D^{23}$ = + 7.3 (c = 1.1, $CHCl_3$) lit. for (*R*)-1-benzyloxy-hexadecan-3-ol $[\alpha]_D^{23}$ = + 7.9 (c = 2.97, $CHCl_3$).[346]

Experiment 74: (*R*)-1-Benzyloxy-9-(tetrahydropyran-2-yloxy)-nonan-3-ol (312b)



The procedure used in **Experiment 73** was repeated using 2-(5-bromo-pentyloxy)-tetrahydropyran (**314b**, 4.5 g, 1.8 mmol) and 2-(2-benzyl-ethyl)-oxirane (**313**, 1.8 g, 1 mmol) in order to obtain *(R)-1-benzyloxy-9-(tetrahydropyran-2-yloxy)nonan-3-ol* (**312b**, 3.1 g, 88 %).

Found $_{(EI)}$ M^+: 350.2465; $C_{21}H_{34}O_4$ requires: 350.2450.

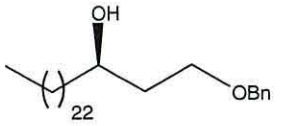
ν_{max}/cm^{-1}: 3464, 3062, 3029, 2934, 2856, 1496, 1454, 1364, 1322, 1260, 1200, 1184, 1120, 1077, 1028.

$\delta_{H\ (250\ MHz)}$: 7.40-7.32 (5H, m, aromatic), 4.57 (1H, b t, J = 3.5 Hz, OC<u>H</u>O), 4.53 (2H, s, C<u>H</u>$_2$OPh), 3.9-3.82 (1H, m, CH$_2$OTHP), 3.82-3.75 (1H, m, C<u>H</u>OH), 3.75 3.65 (2H, m, C<u>H</u>$_2$OBn), 3.65-3.58 (1H, m, CH$_2$OTHP), 3.52-3.45 (1H, m, CH$_2$OTHP), 3.40-3.35 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.5 Hz, 1H, CH$_2$OTHP), 2.9-2.8 (1H, m, O<u>H</u>), 1.9-1.75 (2H, m), 1.75-1.65 (4H, m), 1.65-1.3 (12H, m).

$\delta_{C\ (62.9\ MHz)}$: 137.9 (aromatic), 128.4 (aromatic), 127.6 (aromatic), 127.6 (aromatic), 98.7 (O<u>C</u>HO), 73.2 (O<u>C</u>H$_2$Ph), 71.2 (<u>C</u>HOH), 69.1 (<u>C</u>H$_2$OBn), 67.5 (CH$_2$OTHP), 62.2 (CH$_2$OTHP), 37.3, 36.3, 30.7, 29.6, 29.4, 26.1, 25.5, 19.6.

$[\alpha]_D^{24}$ = + 8.0 (c = 1.1, $CHCl_3$).

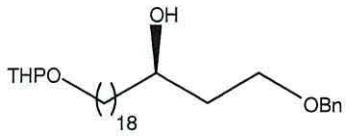
Experiment 75: Attempted preparation of (*R*)-1-Benzyloxyhexacosan-3-ol (312c)



The procedure of **Experiment 73** was repeated using (*S*)-(2-benzyloxyethyl) oxirane (**313**, 1 g, 0.56 mmol), and 1-bromodocosane (4.21 g, 1.12 mmol) to form the Grignard reagent (**314c**). However the coupling reaction did not give the

desired product but several by-products including the starting material (**313**), which showed the same spectral properties described above.

Experiment 76: (*R*)-1-Benzyloxy-21-(tetrahydropyran-2-yloxy)heneicosan-3-ol (312d)



The procedure used in **Experiment 73** was repeated using the Grignard reagent (**314d**) from 2-(17-bromoheptadecyloxy)tetrahydropyran (1g, 2.86 mmol) in order to obtain *(R)-1-benzyloxy-21-(tetrahydropyran-2-yloxy)heneicosan-3-ol* (**312d**, 400 mg) in a poor yield. It was not possible to completely purify this even after a column.

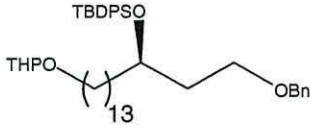
Found $_{(TOF\ MS)}$ $(M-H)^+$: 503.4100; $C_{32}H_{55}O_4$ requires: 503.4139.

ν_{max}/cm^{-1}: 3456, 3030, 2924, 2853, 1496, 1454, 1364, 1322, 1260, 1200, 1184, 1118, 1078, 1029.

$\delta_{H\ (250\ MHz)}$ (crude product): 7.40-7.32 (5H, m, aromatic), 4.58 (1H, b, t, J = 3.6 Hz, OC<u>H</u>O), 4.53 (2H, s, C<u>H</u>$_2$OPh), 3.92-3.85 (1H, m), 3.85-3.77 (1H, m,), 3.77-3.69 (2H, m), 3.69-361 (1H, m), 3.5-3.1 (3H, m), 1.98-1.78 (2H, m), 1.75-1.23 (other H, m).

$\delta_{C\ (62.9\ MHz)}$ (crude product): 137.7 (aromatic), 128.5 (aromatic), 127.8 (aromatic), 127.7 (aromatic), 98.8 (O<u>C</u>HO), 73.3 (O<u>C</u>H$_2$Ph), 72.0 (<u>C</u>HOH), 69.1 (<u>C</u>H$_2$OBn), 67.7 (CH$_2$OTHP), 62.3 (CH$_2$OTHP), 37.5, 35.8 and others.

Experiment 77: (*R*)-[1-(2-Benzyloxyethyl)-14-(tetrahydropyran-2-yloxy) tetradecyloxy]-*tert*-butyldiphenylsilane (319a)



Triethylamine (867 mg, 8.6 mmol) was added to a stirred solution of (*R*)-1-benzyloxy-16-(tetrahydropyran-2-yloxy)hexadecan-3-ol (**312a**, 1.1 g, 2.5 mmol) in dry dichloromethane (15 ml) under nitrogen. The solution was stirred for 10 min then *tert*-butyldiphenylsilylchloride (875 mg, 3.2 mmol) in dry dichloromethane were added, followed by of DMAP (30 mg, 0.25 mmol) in dichloromethane (1 ml). The solution was stirred for 72 h and then quenched with water (10 ml). The organic layer was separated and the aqueous layer re-extracted with dichloromethane (2 x 50 ml). The combined organic layers were dried and the solvent evaporated to give a pale yellow oil which was purified by chromatography eluting with petrol and ether (5:1) to give *(R)-[1-(2-benzyloxy ethyl)-14-(tetrahydropyran-2-yloxy)tetradecyloxy]-tert-butyldiphenylsilane* (**319a**, 1.2 g, 71 %).

Found C: 77.0, H: 9.8; required for $C_{44}H_{66}O_4Si$ C: 76.92, H: 9.68.

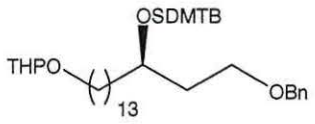
ν_{max}/cm^{-1}: 3070, 2925, 2854, 1654, 1589, 1464, 1247, 1362, 1322, 1260, 1200, 1111, 1034.

$\delta_{H\ (250\ MHz)}$: 7.66 (4H, d J = 8 Hz, aromatic in *ortho*), 7.4-7.2 (11H, m, aromatic), 4.57 (1H, t, J = 3.2 Hz, OC<u>H</u>O), 4.35 (2H, d, J 2.5 Hz, OC<u>H</u>$_2$Ph), 3.93-3.83 (2H, m, including C<u>H</u>OSi), 3.73 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.9 Hz), 3.52-3.46 (3H, m, including C<u>H</u>$_2$OBn), 3.38 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.9 Hz), 1.8-1.0 (42H, m).

$\delta_{C(62.9\ MHz)}$: 138.2 (aromatic), 135.9 (aromatic), 134.6 (aromatic), 134.5 (aromatic), 129.4 (aromatic), 129.3 (aromatic), 128.2 (aromatic), 127.5 (aromatic), 127.4 (aromatic), 127.3 (aromatic), 127.3 (aromatic), 98.8 (O<u>C</u>HO), 72.7(O<u>C</u>H$_2$Ph), 70.8 (<u>C</u>HOSi), 67.6 (CH$_2$OTHP), 67.2 (<u>C</u>H$_2$OBn), 62.3 (CH$_2$OTHP), 36.9, 30.8, 29.7, 29.6, 29.6, 29.5, 29.4, 27.0, 26.0, 26.2, 25.5, 24.7, 19.6 , 19.3 (C(C<u>H</u>$_3$)$_3$).

$[\alpha]_D^{22}$ = - 5.3 (c = 1.1, $CHCl_3$).

Experiment 78: (*R*)-[1-(2-Benzyloxyethyl)-14-(tetrahydropyran-2-yloxy) tetradecyloxy]-*tert*-butyldimethylsilane (320a)



A) The procedure of **Experiment 77** was repeated using *tert*-butyldimetylsilylchloride as protecting agent. The reaction was stirred for one week. *(R)-[1-(2-benzyloxyethyl)-14-(tetrahydropyran-2-yloxy)tetradecyloxy]-tert-butyldimethylsilane* (**320a**) was obtained in a 52 % yield.

B) Imidazole (4.2g, 61mol) was added to a solution of (*R*)-1-benzyloxy-16-(tetrahydropyran-2-yloxy)hexadecan-3-ol (**312a**, 11 g, 24.5 mmol) in dry DMF (60ml) at 5° C followed by the addition of *tert*-butyldimethylsilylchloride (4.8 g, 32 mmol). The mixture was stirred at the same temperature for further 15 min, then at room temperature overnight. When TLC showed no starting material, the mixture was quenched with water (300 ml) and the product was extracted with dichloromethane (3 x 200ml). The combined organic layers were washed with water (100 ml), dried and evaporated to give an oil, which was purified by chromatography eluting with petrol and ether (9:1) to give *(R)-[1-(2-benzyloxy ethyl)-14-(tetrahydropyran-2-yloxy)tetradecyloxy]-tert-butyldimethylsilane* (**320a**, 12 g, 87 %).

Found C: 72.6 H: 11.3; $C_{33}H_{60}O_4Si$ requires: C: 72.21, H: 11.02.

ν_{max}/cm^{-1}: 3364, 3070, 2926, 2853, 1589, 1463, 1427, 1361, 1188, 1111, 938.

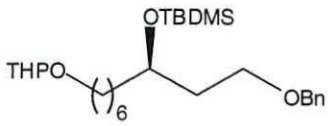
$\delta_{H\ (250\ MHz)}$: 7.4-7.2 (5H, m, aromatic), 4.57 (1H, t, J 3.5 Hz, OC<u>H</u>O), 4.52 (1H, d, J = 11.9 Hz, OC<u>H</u>$_2$Ph) 4.46 (1H, d, J = 11.9 Hz, OC<u>H</u>$_2$Ph), 3.9-3.8 (2H, m, including C<u>H</u>OSi) 3.73 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.8 Hz), 3.6-3.45 (3H, m including t, 2H at δ 3.54 J = 6.4 Hz C<u>H</u>$_2$OBn), 3.37 (1H, dt, J_1 = 9.7 Hz, J_2 = 6.7 Hz), 1.9-1.2 (32H, m) 0.87 (9H, s, C(C<u>H</u>$_3$)$_3$), 0.040 (3H, s, SiC<u>H</u>$_3$), 0.032 (3H, s, SiC<u>H</u>$_3$).

$\delta_{C\ (62.9\ MHz)}$: 138.6 (aromatic), 128.3 (aromatic), 127.6 (aromatic), 127.4 (aromatic), 98.8 (O<u>C</u>HO), 72.9 (O<u>C</u>H$_2$Ph), 69.5 (<u>C</u>HOSi), 67.7 (<u>C</u>H$_2$OBn), 67.6 (CH$_2$OTHP), 62.3 (CH$_2$OTHP), 37.5, 36.9, 30.7,

29.8, 29.7, 29.6, 29.5, 26.2, 25.9, 25.5, 25.0, 19.6, 18.0 ($C(\underline{C}H_3)_3$), - 4.4 ($Si\underline{C}H_3$), - 4.7 ($Si\underline{C}H_3$).

$[\alpha]_D^{22}$ = - 5.9 (c = 1.5, $CHCl_3$).

Experiment 79: (*R*)-[1-(2-Benzyloxyethyl)-7-(tetrahydropyran-2-yloxy) heptyloxy]-*tert*-butyldimethylsilane (320b)



The procedure of **Experiment 78 B** was repeated using 1-benzyloxy-9-(tetrahydropyran-2-yloxy)nonan-3-ol (**312b**, 16 g, 46 mmol). The reaction was stirred overnight to give *(R)-[1-(2-benzyloxyethyl)-7-(tetrahydropyran-2-yloxy)heptyloxy]-tert-butyldimethylsilane* (**320b**, 17 g, 80 %).

Found $_{(EI)}$ M^+: 464.3338; $C_{27}H_{48}O_4Si$ requires: 465.3400.

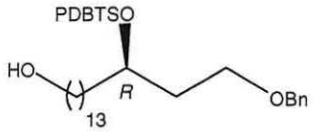
ν_{max}/cm^{-1}: 3029, 2931, 2854, 1461, 1360, 1322, 1254, 1200, 1120, 1078, 1034.

$\delta_{H\ (500\ MHz)}$: 7.30-7.25 (3H, m, aromatic),7.25-7.15 (2H, m, aromatic), 4.54 (1H, m, $OC\underline{H}O$), 4.48 (1H, d, J = 11.7 Hz, $OC\underline{H}_2Ph$), 4.42 (1H, d, J = 11.7 Hz, $OC\underline{H}_2Ph$),3.85-3.75 (2H, m, including $C\underline{H}OSi$) 3.67 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.8 Hz), 3.5-3.4 (3H, m including t at δ 3.48, J = 6.9 Hz ,2H $C\underline{H}_2OBn$), 3.31 (1H, dt, J_1 = 9.7 Hz, J_2 = 6.7Hz), 1.9-1.2 (19H, m) 0.87 (9H, s, $C(C\underline{H}_3)_3$), 0.0070 (3H, s, $SiC\underline{H}_3$), 0.0001 (3H, s, $SiC\underline{H}_3$)

$\delta_{C\ (125.8\ MHz)}$: 138.6 (aromatic quartenary), 128.3 (aromatic), 127.6 (aromatic), 127.4 (aromatic), 98.8 ($O\underline{C}HO$), 72.9 ($O\underline{C}H_2Ph$), 69.4 ($\underline{C}HOSi$), 67.6 ($\underline{C}H_2OBn$), 67.2 (CH_2OTHP), 62.3 (CH_2OTHP), 37.5 (CH_2), 36.9 (CH_2), 32.7 (CH_2), 30.7 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 26.2 (CH_2), 25.9 (CH_2), 25.0 (CH_2), 22.5 (CH_2), 19.6 (CH_2), 18.0 ($C(C\underline{H}_3)_3$), - 4.4 ($Si\underline{C}H_3$), - 4.7 ($Si\underline{C}H_3$).

$[\alpha]_D^{22}$ = - 4.7 (c = 1.2, $CHCl_3$).

Experiment 80: (*R*)- 16-Benzyloxy-14-(*tert*-butyldiphenylsilanyloxy)-hexadecan-1-ol (321)



PTSA (28 mg, 0.15 mmol) was added to a stirred solution of (*R*)-2-[16-benzyloxy-14-2,2-diethyl-1,1-diphenylpropoxy)hexadecyloxy]-tetra hydropyran (**319a**, 1 g, 1.5 mmol) in a solution of methanol (5 ml), tetrahydrofuran (5 ml) and water (1 ml). The mixture was stirred overnight. When TLC showed no starting material left, the mixture was quenched with sat. aq. sodium bicarbonate (20 ml). The product was extracted with dichloromethane (3 x 25 ml); the combined organic layers were dried and the solvent evaporated to give a colourless oil which was purified by column chromatography eluting with petroleum and ether (5:2) to give *(R)-16-benzyloxy-14-(tert-butyldiphenyl silanyloxy)hexadecan-1-ol* (**321**, 800 mg, 80 %)

Found C: 78.2 H: 9.8; $C_{39}H_{58}O_3Si$ requires: C: 77.69, H: 9.70.

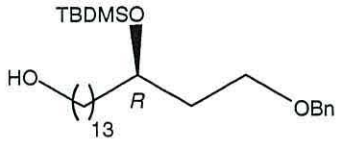
ν_{max}/cm^{-1}: 3364, 3070, 2926, 2853, 1589, 1463, 1427, 1361, 1188, 1111.

$\delta_{H\ (250\ MHz)}$: 7.66 (4H, d J = 8 Hz, aromatic in *ortho*), 7.4-7.2 (11H, m., aromatic), 4.35 (2H, d, J 2.5 Hz, $OC\underline{H}_2Ph$), 3.9 (1H, quintet, J = 5.7 Hz, $C\underline{H}OSi$), 3.63 (2H, t, J = 6.6 Hz, $C\underline{H}_2OH$), 3.49 (2H, m, $C\underline{H}_2OBn$), 1.79 (2H, quintet, J = 6.3 Hz, $C\underline{H}_2CH_2OBn$), 1.56 (2H, quintet, J = 7.6 Hz, $C\underline{H}_2CH_2OH$) 1.5-1.0 (31H, m, including singlet at δ 0.90 for 9H $C(C\underline{H}_3)_3$).

$\delta_{C\ (62.9\ MHz)}$: 138.5 (aromatic), 135.9 (aromatic), 134.6 (aromatic), 134.5 (aromatic), 129.4 (aromatic), 129.4 (aromatic), 128.2 (aromatic), 127.5 (aromatic), 127.4 (aromatic), 127.3 (aromatic), 127.3 (aromatic), 72.7 ($O\underline{C}H_2Ph$), 70.8 ($\underline{C}HOSi$), 67.2 ($\underline{C}H_2OBn$), 63.2 ($\underline{C}H_2OH$), 36.9, 36.3, 32.7, 29.6, 29.6, 29.5, 29.5, 29.4, 27.0, 25.7, 24.7, 19.3 ($C(C\underline{H}_3)_3$).

$[\alpha]_D^{22}$ = - 6.3 (c = 1.05, $CHCl_3$).

Experiment 81: Attempted preparation of 16-benzyloxy-14-(*tert*-butyldimethylsilanyloxy)hexadecan-1-ol



The procedure used in **Experiment 80** was repeated using (*R*)-[1-(2-benzyloxyethyl)14-(tetrahydropyran-2-yloxy)tetradecyloxy]–*tert*butyldimethyl silane (**320a**, 0.56 g, 1 mmol). However the reaction gave *16-benzyloxyhexadecane-1,14-diol* (**322**, 350 mg, 96 %).

Found C: 76.0, H: 11.5; $C_{23}H_{40}O_3$ requires: C: 75.77, H: 11.06.

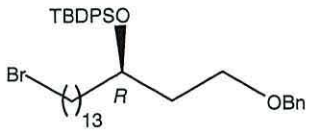
ν_{max}/cm^{-1}: 3444, 2925, 2854, 1454, 1374, 1249, 1094.

$\delta_{H\ (250\ MHz)}$: 7.3-7-2 (5H, b s, aromatic), 4.5 (2H, s, $OC\underline{H}_2Ph$), 3.8-3-6 (6H, m, including a triplet at δ 3.6 J = 6.6 Hz, 2H, $C\underline{H}_2OH$), 1.75 (2H, b t, J = 5.6 Hz, $C\underline{H}_2CH_2OH$), 1.5-1.0 (23H, m).

$\delta_{C\ (62.9\ MHz)}$: 137.8 (aromatic), 128.4 (aromatic), 127.6 (aromatic), 127.5 (aromatic), 73.2 ($O\underline{C}H_2Ph$), 71.3 ($\underline{C}HOH0$), 69.2 ($\underline{C}H_2OBn$), 62.9 ($\underline{C}H_2OH$), 37.4, 36.4, 32.8, 29.7, 29.6, 29.4, 25.7, 25.6.

$[\alpha]_D^{21}$ = + 5.7 (c = 1.3, $CHCl_3$).

Experiment 82: (*R*)-[1-(2-Benzyloxyethyl)-14-bromotetradecyloxy]-*tert*-butyldiphenylsilane (323)



To a 0 °C solution of (*R*)-16-benzyloxy-14-(*tert*-butyldiphenylsilanyloxy)-hexadecan-1-ol (**321**, 500 mg, 0.85 mmol), imidazole (81 mg, 1.2 mmol) and triphenylphosphine (340 mg. 1.28 mmol) in dry dichloromethane (20 ml) was added carbon tetrabromide (400 mg, 1.2 mmol) under nitrogen. After 5 min, the mixture was brought to room temperature and stirred for 2 h. The reaction was then quenched with 10 % aq. Na_2SO_3 (20 ml) and extracted with dichloromethane (3 x 20 ml). The combined organic layers were dried and concentrated. The

residue was purified by chromatography eluting with petroleum and ether (10:0.5) to give *(R)-[1-(2-benzyloxyethyl)-14-bromotetradecyloxy]-tert-butyl diphenylsilane* (**323**, 510 mg, 92 %).

Found C: 70.6 H: 8.9; $C_{39}H_{57}BrO_2Si$ requires: C: 70.35, H: 8.63.

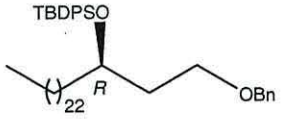
ν_{max}/cm^{-1}: 3070, 2925, 2853, 1681, 1650, 1588, 1556, 1462, 1427, 1360, 1258, 1110.

$\delta_{H\,(250\,MHz)}$: 7.66 (4H, d, J = 8 Hz, H in *ortho* with respect to the P), 7.4-7.2 (11H, m., aromatic), 4.35 (2H, d, J 2.5 Hz, $OC\underline{H}_2Ph$), 3.9 (1H, quintet, J = 5.7 Hz, $C\underline{H}OSi$), 3.49 (2H, m, $C\underline{H}_2OBn$), 3.43 (2H, t, J = 6.7 Hz, $C\underline{H}_2Br$), 1.9-1.7 (2H, m) -1.81 (4H, q, J = 6.62 Hz, $C\underline{H}_2CH_2OBn$) 1.5-1.0 (31H, m).

$\delta_{C\,(62.9\,MHz)}$: 138.6 (aromatic), 135.9 (aromatic), 134.6 (aromatic), 134.5 (aromatic), 129.4 (aromatic), 129.4 (aromatic), 128.2 (aromatic), 127.5 (aromatic), 127.4 (aromatic), 127.4 (aromatic), 72.7 ($O\underline{C}H_2Ph$), 70.8 ($\underline{C}HOSi$), 67.2 ($\underline{C}H_2OBn$), 36.9, 36.3, 33.9, 32.8, 29.6, 29.5, 28.7, 28.1, 27.0, 24.7, 19.4 ($C(C\underline{H}_3)_3$).

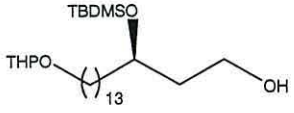
$[\alpha]_D^{22}$ = - 6.1 (c = 1.2, $CHCl_3$).

Experiment 83: Attempted preparation of [1-(2-benzyloxyethyl) tetracosyloxy]-*tert*-butyldiphenylsilane (325)



The procedure used in **Experiment 13 E** was repeated in order to couple (*R*)-[1-(2-benzyloxyethyl)-14-bromotetradecyloxy]-*tert*-butyldiphenylsilane (**323**, 400 mg, 0.7 mmol) with the Grignard reagent (**324**) obtained from 1–bromodecane (220 mg, 1 mmol). After purification by column chromatography only starting material (**325**, 300 mg) was obtained.

Experiment 84: 3-(*tert*-Butyldimethylsilanyloxy)-16-(tetrahydropyran-2-yloxy)hexadecan-1-ol (329a)



Using a similar procedure to that described in **Experiment 36**. (*R*)-[1-(2-benzyloxyethyl)-14-(tetrahydropyran-2-yloxy)tetradecyloxy]-*tert*-butyldimethyl silane (**320a**, 7.5 g, 13.3 mmol) was reduced to give *(R)-3-(tert-butyldimtehylsilanyloxy)-16-(tetrahydropyran-2-yloxy)hexadecan-1-ol* (**329a**, 5.9 g, 94 %), which had been purified by chromatography eluting with petrol and ether (3:1).

Found C: 68.3, H: 12.2; $C_{27}H_{56}O_4Si$ requires: C: 68.59, H: 11.94.

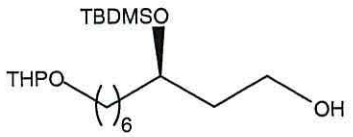
ν_{max}/cm^{-1}: 3437, 2925, 2854, 1464, 1440, 1407, 1360, 1322, 1255, 1200, 1184, 1137, 1120, 1077, 1033.

$\delta_{H\ (250\ MHz)}$: 4.57 (1H, b t, J 4.3 Hz), 4.0-3.8 (3H, m), 3.8-3.7 (2H, m), 3.6-3.5 (1H, m), 3.36 (1H, dt, J_1 = 9.8 Hz, J_2 = 6.7 Hz), 2.6 (1H, b s), 2.0 1.7 (2H, m), 1.7-1.4 (10H, m), 1.4-1.2 (20H, b s) 0.87 (9H, s, $C(C\underline{H}_3)_3$), 0.069 (3H, s, $SiC\underline{H}_3$), 0.054 (3H, s, $SiC\underline{H}_3$).

$\delta_{C\ (62.9\ MHz)}$: 98.7 ($O\underline{C}HO$), 71.8 ($\underline{C}HOSi$), 67.6 (CH_2OTHP), 62.2 (CH_2OTHP), 60.1 ($\underline{C}H_2OH$), 37.6, 36.8, 30.7, 29.7, 29.7, 29.5, 26.2, 25.7, 25.4, 25.3, 19.6, 17.9 ($C(C\underline{H}_3)_3$), - 4.5 ($Si\underline{C}H_3$), - 4.8 ($Si\underline{C}H_3$).

$[\alpha]_D^{20}$ = - 12.2 (c = 1.5, $CHCl_3$).

Experiment 85: (*R*)-3-(*tert*-Butyldimethylsilanyloxy)-9-(tetrahydropyran-2-yloxy)nonan-1-ol (329b)



The procedure in **Experiment 36** was repeated using (*R*)-[1-(2-benzyloxyethyl)-7-(tetrahydropyran-2-yloxy)heptyloxy]-*tert*-butyl dimethylsilane (**320b**, 7.5 g, 16 mmol). The reaction was stirred overnight to give *(R)-9-benzyloxy-7-(tert-butyldimethylsilanyloxy)nonan-1-ol* (**329b**, 5.5 g, 92 %).

Found $_{(EI)}$ M^+: 374.2869, and $C_{20}H_{42}O_4Si$ requires: 374.2852.

ν_{max}/cm^{-1}: 3446, 2927, 2856, 1470, 1365, 1207, 1186, 1075, 1033.

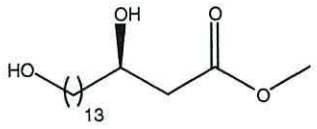
$\delta_{H\ (500\ MHz)}$: 4.56 (1H, m, $OC\underline{H}O$), 4.0-3.8 (3H, m, $C\underline{H}OSi$), 3.85-3.75 (2H, m) 3.55-3.45 (1H, m, CH_2OTHP), 3.4-3.3 (1H, m, CH_2OTHP), 2.5 (1H,

m, O<u>H</u>), 1.9-1.8 (2H, m), 1.8-1.5 (10H, m), 1.4-1.2 (6H, m), 0.88 (9H, s, C(C<u>H</u>$_3$)$_3$), 0.07 (3H, d, J = 3.2 Hz, SiC<u>H</u>$_3$), 0.06 (3H, s, SiC<u>H</u>$_3$) 0.054 (3H, s, SiC<u>H</u>$_3$) .

$\delta_{C\ (125.8\ MHz)}$: 98.8 (O<u>C</u>HO), 71.8 (<u>C</u>HOSi), 67.5 (CH_2OTHP), 62.3 (CH_2OTHP), 60.2 (<u>C</u>H_2OH), 37.7 (CH_2), 36.7 (CH_2), 30.8 (CH_2), 30.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 26.2 (CH_2), 25.8 (CH_2), 25.4 (CH_2), 25.2 (CH_2), 19.6 (CH_2), 17.9 (C(C<u>H</u>$_3$)$_3$), -4.5 (Si<u>C</u>H$_3$), - 4.8 (Si<u>C</u>H$_3$).

$[\alpha]_D^{22}$ = - 15 (c = 1.1, $CHCl_3$).

Experiment 86: (*R*)-3,16-Dihydroxyhexadecanoic acid methyl ester (331a)



A solution of (*R*)-1-hydroxy-3-*tert*-butyldimethylsilyloxy-13-tetrahydro pyranyloxytridecane (**329a**, 3 g; 6.35 mmol) in carbon tetrachloride (15 ml) was added over a period of 4 h at 20 °C with stirring to a solution of sodium(meta)periodate (4.33 g; 0.02 mol) and ruthenium(III)chloride hydrate (130 mg; 0.6 mol) in acetonitrile (30 ml), carbon tetrachloride (15 ml) and water (40 ml). The mixture was stirred overnight, and then diluted with dichloromethane (200 ml). The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (3 x 150 ml). The combined organic layers were dried and filtered through a pad of celite, and the solvent was evaporated to give a residue. This was dissolved in methanol (50 ml) and concentrated sulphuric acid (2 ml). The mixture was refluxed for 6 h, then cooled to room temperature and stirred overnight. The solvent was evaporated to give a residue, which was dissolved in dichloromethane (250 ml) and washed with a sat. aq. sodium bicarbonate (30 ml). The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (3 x 150 ml). The combined organic layers were dried and the solvent evaporated to give a brown precipitate. The crude product was purified by chromatography eluting with petroleum and ethyl acetate (1:1) to give *(R)-3,16-dihydroxyhexadecanoic acid methyl ester* (**331a**, 1.3 g; 68 %) and *(R)-3-hydroxyhexadecanedioic acid dimethyl ester* (100 mg, 4.8 %).

For *(R)-3,16-dihydroxyhexadecanoic acid methyl ester*:

Found C: 67.8, H: 11.4; $C_{17}H_{34}O_4$ requires: C: 67.51, H: 11.33.

ν_{max}/cm^{-1}: 3437, 2928, 2854, 1729, 1463, 1439, 1374, 1334, 1300, 1251, 1175, 1112, 1092, 1049, 1020.

$\delta_{H\ (250\ MHz)}$: 3.96 (1H, m, C<u>H</u>OH), 3.66 (3H, s, COOC<u>H</u>$_3$), 3.57 (2H, t, J = 6.4 Hz, C<u>H</u>$_2$OH), 2.47 (1H, dd, J_1 = 3.7 Hz, J_2 = 16.2 Hz, COC<u>H</u>$_2$CHOH), 2.36 (1H, dd, J_1 = 16.5 Hz, J_2 = 8.5 Hz, COC<u>H</u>$_2$CHOH), 1.6-1.1 (26H, m).

$\delta_{C\ (62.9\ MHz)}$: 173.4 (<u>C</u>=O), 67.1 (<u>C</u>HOH), 62.7 (<u>C</u>H$_2$OH), 51.6 (O<u>C</u>H$_3$), 41.1 (CHOH<u>C</u>H$_2$CO), 36.5, 32.7, 30.7, 29.5, 25.7, 25.4.

$[\alpha]_D^{24}$ = - 17.6 (c = 1, $CHCl_3$).

For *(R)-3-hydroxyhexadecanedioic acid dimethyl ester*:

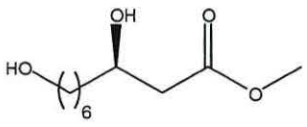
Found C: 65.4, H: 10.4; $C_{18}H_{34}O_5$ requires: C: 65.42, H: 10.37.

ν_{max}/cm^{-1}: 3582, 2928, 2855, 1727, 1462, 1439, 1365, 1334, 1256, 1224, 1202, 1176, 1092, 1045.

$\delta_{H\ (250\ MHz)}$: 3.99 (1H, m, C<u>H</u>OH), 3.70 (3H, s, COOC<u>H</u>$_3$), 3.65 (3H, s, COOC<u>H</u>$_3$), 2.93 (1H, b s, OH), 2.50 (1H, dd, J_1 = 16.5 Hz, J_2 = 3.4 Hz, COC<u>H</u>$_2$CHOH), 2.36 (1H, dd, J_1 = 16.2 Hz, J_2 = 8.5 Hz, COC<u>H</u>$_2$CHOH), 1.6-1.1 (22H, m).

$\delta_{C\ (62.9\ MHz)}$: 174.3 (<u>C</u>=O), 173.5 (<u>C</u>=O), 70.0 (<u>C</u>HOH), 51.7 (<u>C</u>H$_2$OH), 51.4 (O<u>C</u>H$_3$), 41.1 (CHOH<u>C</u>H$_2$CO), 36.5, 34.0, 29.5, 29.4, 29.2, 29.1, 25.4, 24.9.

Experiment 87: (*R*)-3,9-Dihydroxynonanoic acid methyl ester (331b)



The procedure in **Experiment 86** was repeated using (*R*)-3-(tert-butyldimethylsilanyloxy)-9-(tetrahydropyran-2-yloxy)nonan-1-ol (**329b**, 2.7 g, 7.2 mmol) to give *(R)-3,9-dihydroxynonanoic acid methyl ester* (**331b**, 0.7 g, 48 %).

Found $_{(EI)}$ M^+: 205.1449, and $C_{10}H_{21}O_4$ requires: 205.1440.

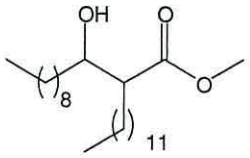
ν_m/cm^{-1}: 3385, 2934, 2859, 1732, 1439, 1288, 1198, 1172, 1054.

$\delta_{H\ (500\ MHz)}$: 3.96 (1H, m, C<u>H</u>OH), 3.66 (3H, s, COOC<u>H</u>$_3$), 3.57 (2H, t, J = 6.5 Hz, C<u>H</u>$_2$OH), 2.9 (2H, O<u>H</u>), 2.47 (1H, dd, J_1 = 16.2 Hz, J_2 = 3.7 Hz, COC<u>H</u>$_2$CHOH), 2.36 (1H, dd, J_1 = 16.5 Hz, J_2 = 8.6 Hz, COC<u>H</u>$_2$CHOH), 1.7-1.2 (10H, m).

$\delta_{C\ (125.8\ MHz)}$: 173.4 (<u>C</u>=O), 67.9 (<u>C</u>HOH), 62.6 (<u>C</u>H$_2$OH), 51.6 (<u>C</u>H$_3$), 41.1 (CHOH<u>C</u>H$_2$CO), 36.3 (<u>C</u>H$_2$), 32.4 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 25.5 (<u>C</u>H$_2$), 25.3 (<u>C</u>H$_2$).

$[\alpha]_D^{24}$ = - 19.3 (c = 1.0, $CHCl_3$).

Experiment 88: 2-(1-Hydroxydecyl)tetradecanoic acid methyl ester (355a and 355b)



Butyllithium (4.4 ml; 6.6 mmol, 1.5 M solution in hexanes) was added to a stirred solution of diisopropylamine (1.02 g; 10.6 mmol) in dry THF (8.5 ml) at - 40 °C. The temperature was slowly increased to - 20 °C over about 1.5 h, then the solution of lithium diisopropylamide was cooled to - 70 °C and 3-hydroxydodecanoic acid methyl ester (**294**, 0.5 g; 2.2 mmol, final concentration 0.1 mmol/ml) in dry THF (5 ml) was added dropwise maintaining the temperature below - 60° C. After that the reaction was allowed to reach - 20 °C, then a mixture of 1-iododecane (1.1 g; 3.6 mmol, final concentration 0.16 mmol/ml), and HMPA (0.8 g; 4.4 mmol) in THF (8 ml) was added. The mixture was slowly allowed to reach room temperature and stirred for 24 h. The reaction was quenched with ammonium chloride solution (15 ml) and extracted with ethyl acetate (3 x 50 ml). The combined organic layers were washed with water (2 x 20 ml), dried and evaporated to give a residue, which was purified by chromatography on silica eluting with petrol and ethyl acetate (9:1) to give *2-(1-hydroxydecyl)tetradecanoic acid methyl ester* (**355a** and **355b**, 400 mg; 46 %).

Found C: 75.0, H: 12.5, $C_{25}H_{50}O_3$ requires: C: 75.32, H: 12.54.

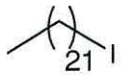
ν_{max}/cm^{-1}: 3471, 2924, 2854, 1724, 1472, 1458, 1380, 1170.

$\delta_{H\,(250\,MHz)}$: 3.71 (3H, s, $OC\underline{H}_3$), 3.66 (1H, m, $C\underline{H}OH$), 2.44 (2H, including $C\underline{H}(CH_2)_{11}CH_3$ and $O\underline{H}$), 1.7-1.2 (38H, m), 0.87 (6H, t, J = 6.7 Hz, $C\underline{H}_3$).

$\delta_{C\,(62.9\,MHz)}$: 176.4 ($\underline{C}O_2CH_3$), 72.3 ($\underline{C}HOH$), 51.5 ($O\underline{C}H_3$), 50.9 ($\underline{C}H(CH_2)_{11}CH_3$), 35.7, 31.9, 29.6, 29.5, 29.5, 29.4 29.3, 29.3, 27.4, 25.7, 22.7, 14.1 ($\underline{C}H_3$).

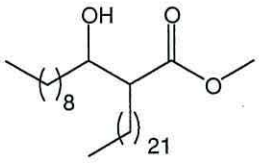
R_f = 0.44 ($CHCl_3$)

Experiment 89: 1-Iododocosane (356)



The procedure described in **Experiment 23** was used to convert 1-bromodocosane (10g, 25.6 mmol) into 1-iododocosane (**356**, 11g, 98%). The compound showed the same NMR spectra to those reported.[5]

Experiment 90: 2-(1-Hydroxydecyl)tetracosanoic acid methyl ester (357a and 357b)



A) The procedure used in **Experiment 88** was repeated only in much more dilute conditions:

butyllithium (3.52 ml; 5.28 mmol, 1.5 M solution in hexanes),

diisopropylamine (555 mg; 5.5 mmol) in dry THF (10 ml),

3-hydroxydodecanoic acid methyl ester (**294**, 0.5 g; 2.2 mmol, final concentration 0.05 mmol/ml) in dry THF (15 ml),

1-iododocosane (**356**, 1.6 g; 3.75 mmol, final concentration 0.08 mmol/ml),

HMPA (760 mg, 4. 4 mmol) in dry THF (20 ml).

After purification by column chromatography, *2-(1-hydroxydecyl)tetracosanoic acid methyl ester* was obtained (350 mg, 30 %) and dodec-2-enoic acid methyl

ester which showed the same ^{1}H NMR, IR spectra as those reported in the literature.[429]

B) The procedure in (**A**) was utilised but in even more dilute conditions

butyllithium (3.6 ml; 5.5 mmol, 1.5M solution in hexanes),

diisopropylamine (0.66 g; 6.5 mmol) in dry THF (20 ml),

3-hydroxydodecanoic acid methyl ester (**294**, 0.5 g; 2.2 mmol, final concentration 0.03 mmol/ml) in dry THF (25 ml),

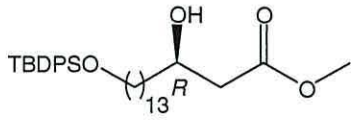
1-iododocosane (**356**, 1.6 g; 3.75 mmol, final concentration 0.05 mmol/ml),

HMPA (760 mg, 4.4 mmol) in dry THF (30 ml).

2-(1-Hydroxydecyl)tetracosanoic acid methyl ester was obtained with a higher yield (600 mg, 51 %).

Found	C: 78.2, H: 13.3; $C_{35}H_{70}O_3$ requires: C: 78.00, H: 13.09.
ν_{max}/cm^{-1}:	3545, 2927, 2854, 1716, 1466, 1378, 1170, 1111.
$\delta_{H\ (250\ MHz)}$:	3.71 (3H, s, $OC\underline{H}_3$), 3.66 (1H, m, C<u>H</u>OH), 2.44 (2H, including b dt, J_1 = 9.3 Hz, J_2 = 5.3 Hz, $C\underline{H}(CH_2)_{21}CH_3$ and O<u>H</u>), 1.7-1.2 (58H, m), 0.88 (6H, b t, J = 6.7 Hz).
$\delta_{C\ (62.5\ MHz)}$:	176.3 ($\underline{C}O_2CH_3$), 72.3 (<u>C</u>HOH), 51.5 ($O\underline{C}H_3$), 50.9 ($\underline{C}H(CH_2)_{21}CH_3$), 41.3, 35.7, 31.9, 29.7, 29.6, 29.4, 29.3, 29.0, 27.4, 25.7, 22.7, 22.6, 14.2 ($\underline{C}H_3$). 14.1 ($\underline{C}H_3$).
R_f =	0.45 ($CHCl_3$)

Experiment 91: 16-(*tert*-Butyldiphenylsilanyloxy)-3-hydroxyhexadecanoic acid methyl ester (363)



A similar procedure to that described in **Experiment 77** was used to convert (*R*)-3,16-dihydroxy-hexadecanoic acid methyl ester (**331a**, 1.3 g; 4.3 mmol) into *16-(tert-butyldiphenylsilanyloxy)-3-hydroxyhexadecanoic acid methyl ester* (**363**, 2.0 g; 86 %).

Found C: 73.3, H: 9.8; $C_{33}H_{52}O_4Si$ requires: C: 73.28, H: 9.69.

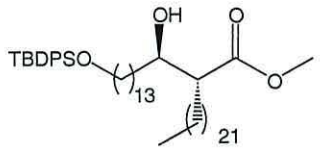
ν_{max}/cm^{-1}: 3471, 3071, 3049, 2927, 2954, 1734, 1472, 1458, 1436, 1428, 1388, 1362, 1303, 1172, 1111, 1007.

$\delta_{H\ (500\ MHz)}$: 7.7-7.6 (4H, m, aromatic in *ortho*), 7.4-7.3 (6H, m, aromatic), 4.02 (1H, m, C<u>H</u>OH), 3.73 (3H, s, OC<u>H</u>$_3$), 3.66 (2H, t, J = 6.4 Hz, C<u>H</u>$_2$OSi), 2.52 (1H, dd, J_1 = 16.5 Hz, J_2 = 3.4 Hz, COC<u>H</u>$_2$CHOH), 2.39 (1H, dd, J_1 = 16.5 Hz, J_2 = 8.5 Hz, COC<u>H</u>$_2$CHOH), 1.6-1.2 (23H, m) 1.0 (9H, s, C(C<u>H</u>$_3$)$_3$).

$\delta_{C\ (125.8\ MHz)}$: 173.5 (C=O), 135.5 (<u>C</u>H aromatic), 134.1 (<u>C</u>Si aromatic), 129.5 (<u>C</u>H, aromatic), 127.5 (<u>C</u>H, aromatic), 68.0 (<u>C</u>HOH), 64.0 (<u>C</u>H$_2$OSi), 51.7 (O<u>C</u>H$_3$), 41.1 (CHOH<u>C</u>H$_2$CO), 36.5 (<u>C</u>H$_2$), 32.5 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.3 (<u>C</u>H$_2$), 26.8 (C<u>C</u>H$_3$), 25.7 (<u>C</u>H$_2$), 25.4 (<u>C</u>H$_2$), 19.2 (<u>C</u>(CH$_3$)$_3$).

$[\alpha]_D^{24}$ = - 10.6 (c = 0.97, $CHCl_3$).

Experiment 92: 2- [14-(*tert*-butyldiphenylsilanyloxy)-1-hydroxytetradecyl) tetracosanoic acid methyl ester (364a)



The procedure used in **Experiment 90 C** was repeated utilising:

A) butyllithium (0.6 ml; 0.88 mmol, 1.5 M solution in hexanes),
diisopropylamine (94 mg; 0.925 mmol) in dry THF (5 ml),
16-(*tert*-butyldiphenylsilanyloxy)-3-hydroxyhexadecanoic acid methyl ester (**363**, 0.2 g; 0.37 mmol, final concentration 0.025 mmol/ml) in dry THF (4 ml),
1-iododocosane (**356**, 274 mg; 0.63 mmol, final concentration 0.042 mmol/ml),
HMPA (0.740 mmol, 130 mg) in dry THF (6 ml),

After purification by column chromatography, *16-(tert-butyldiphenylsilanyloxy) hexadec-2-enoic acid methyl ester* (40 mg, 21 %) was obtained.[430,431]

B) butyllithium (0.9 ml, 0.925 mmol, 1.25 M solution in hexanes),

diisopropylamine (90 mg; 0.88 mmol) in dry THF (5 ml),

16-(*tert*-butyldiphenylsilanyloxy)-3-hydroxyhexadecanoic acid methyl ester (**363**, 0.2 g; 0.37 mmol, final concentration 0.025 mmol/ml) in dry THF,

1-iododocosane (**256**, 274 mg; 0.63 mmol, final concentration 0.042 mmol/ml),

HMPA (0.75 mmol, 130 mg) in dry THF (6 ml).

A mixture of compounds was obtained not including the desired one but incorporating *16-tertbutyldiphenylsilanyloxy)-3-hydroxyhexadecanoic acid docosyl ester* (95 mg, 31 %) and *16-(tertbutyldiphenylsilanyloxy)hexadec-2-enoic acid methyl ester* (20 mg, 10 %).

C) LDA (0.93mmol, 0.6 ml, 1.5 M) in dry THF (8 ml),

16-(*tert*-butyldiphenylsilanyloxy)-3-hydroxyhexadecanoic acid methyl ester (**363**, 200 mg; 0.37 mmol, final concentration 0.015 mmol/ml) in THF (7 ml),

The addition was done at – 55 °C. Then the temperature was brought to – 10 °C over a period of 2 h. Thenthe following were added:

1-iododocosane (**256**, 274 mmg, 0.63 mmol, final concentration 0.025 mmol/ml),

HMPA (0.740 mmol, 130 mg) in dry THF (10 ml),

The mixture was allowed to reach room temperature over period of 24 h, then the reaction was worked up following the procedure described in **Experiment 90**. The crude product was purified by column chromatography to give *2-[14-(tert-butyldiphenylsilanyloxy)-1-hydroxytetradecyl)tetracosanoic acid methyl ester* (**364**, 67 mg, 31 %).

For *16-tert-butyl-diphenylsilanyloxy)-3-hydroxyhexadecanoic acid docosyl ester*:

Found C: 77.7, H: 11.4; $C_{54}H_{94}O_4Si$ requires: C: 77.64, H: 11.34.

ν_{max}/cm^{-1}: 3545, 2927, 2954, 1716, 1466, 1378, 1170, 1111.

$\delta_{H\ (500\ MHz)}$: 7.7-7.6 (4H, m, aromatic in *ortho*), 7.4-7.3 (6H, m, aromatic protons), 4.10 (2H, t, J = 6.7 Hz, $OC\underline{H}_2(CH_2)_{20}CH_3$), 4.01 (1H, m, C<u>H</u>OH), 3.66 (2H, t, J = 6.4 Hz, $C\underline{H}_2OSi$), 2.52 (1H, dd, J_1 = 16.5 Hz, J_2 = 3.4 Hz, $COC\underline{H}_2CHOH$), 2.39 (1H, dd, J_1 = 16.5 Hz, J_2 = 8.8 Hz,

$COC\underline{H}_2CHOH$), 1.6-1.2 (65H, m) 1.01 (9H, s, $C(C\underline{H}_3)_3$), 0.88 (3H, b t, J = 6.5 Hz, $C\underline{H}_3$).

$\delta_{C\ (125.8\ MHz)}$: 173.2 ($\underline{C}O_2CH_3$), 135.6 ($\underline{C}$ aromatic), 134.2 ($\underline{C}$Si aromatic), 129.5 ($\underline{C}$H aromatic), 127.5 ($\underline{C}$H aromatic), 68.0 ($\underline{C}$HO), 64.8 ($O\underline{C}H_2(CH_2)_{20}CH_3$), 64.0 ($\underline{C}H_2OSi$), 41.2 ($CHOC\underline{H}_2CO$), 36.4 ($CH_2$), 32.6 (CH2) 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.0 ($CH_2$), 27.4 ($CH_2$), 26.8 ($\underline{C}H_3$) 25.9 ($CH_2$), 25.8 ($CH_2$), 25.5 ($CH_2$), 22.7 ($CH_2$), 22.6 ($CH_2$), 19.2 ($\underline{C}(CH_3)_3$), 14.2 ($\underline{C}H_3$).

For *16-(tert-butyldiphenylsilanyloxy)hexadec-2-enoic acid methyl ester:*

Found C: 78.0, H: 13.1; $C_{35}H_{70}O_3$ requires: C: 78.00, H: 13.09).

ν_{max}/cm^{-1}: 3545, 2927, 2954, 1716, 1466, 1378, 1170, 1111, 907.

$\delta_{H\ (250\ MHz)}$: 7.7-7.6 (4H, m, aromatic in *ortho*), 7.4-7.3 (6H, m, aromatic), 7.01 (1H, dt, J_1 = 15.7 Hz, J_2 = 7.0 Hz, $C\underline{H}$=CHCO), 5.85 (1 H, dt, J_1 = 15.7 Hz, J_2 = 1.2 Hz, CH=$C\underline{H}$CO), 3.73 (3H, s, $OC\underline{H}_3$), 3.65 (2H, t, J = 6.4 Hz, $C\underline{H}_2OSi$), 2.2 (2H, dq, J_1 = 7.0 Hz, J_2 = 1.2 Hz, CH_2CH=CH) 1.7-1.2 (21H, m), 1.05 (9H, s, $C(C\underline{H}_3)_3$), 0.88 (3H, b t, J = 6.7 Hz, $C\underline{H}_3$).

$\delta_{C\ (62.9\ MHz)}$: 167.2 ($\underline{C}O_2CH_3$), 149.8 ($\underline{C}$H=CHCO), 135.6 ($\underline{C}$H aromatic), 134.2 ($\underline{C}$Si aromatic), 129.5 ($\underline{C}$H aromatic), 127.5 ($\underline{C}$H aromatic), 120. 7 ($\underline{C}$H=CHCO), 63.9 ($\underline{C}H_2OSi$), 51.3 ($O\underline{C}H_3$), 32.5, 32.2, 29.7, 29.6, 29.4, 29.1, 29.0, 27.9, 36.8, 19.1.

For *2-[14-(tert-butyldiphenylsilanyloxy)-1-hydroxytetradecyl)tetracosanoic acid methyl ester*:

Found C: 77.5, H: 11.1; $C_{55}H_{96}O_4Si$ requires: C: 77.77, H: 11.39.

ν_{max}/cm^{-1}: 3442, 3070, 2929, 2954, 1711, 1658, 1589, 1548, 1513, 1469, 1427, 1390, 1361, 1113.

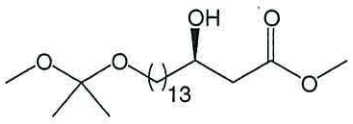
$\delta_{H\ (500\ MHz)}$: 7.7-7.6 (4H, m, aromatic), 7.4-7.3 (6H, m, aromatic), 3.71 (3H, s, $OC\underline{H}_3$), 3.66 (1H, m, $C\underline{H}OH$), 3.65 (2H, t, J = 6.4 Hz, $C\underline{H}_2OSi$), 2.44 (1H, b dt, J_1 = 9.5 Hz, J_2 = 5.5 Hz, $C\underline{H}(CH_2)_{21}CH_3$), 2.25 (1H, m, $O\underline{H}$), 1.7-1.2 (66H, m), 1.08 (9H, s, $C(C\underline{H}_3)_3$), 0.87 (3H, t, J = 6.7 Hz, CH_3).

$\delta_{C\ (125.8\ MHz)}$: 176.3 ($\underline{C}O_2CH_3$), 135.6 ($\underline{C}$H, aromatic), 134.2 ($\underline{C}$Si, aromatic), 129.4 ($\underline{C}$H, aromatic), 127.5 ($\underline{C}$H, aromatic), 72.3 ($\underline{C}$HOH), 64.0 (CH_2OSi), 51.5 ($O\underline{C}H_3$), 50.9 ($\underline{C}H(CH_2)_{11}CH_3$), 35.7 ($\underline{C}H_2$), 32.6 ($\underline{C}H_2$), 31.9

($\underline{C}H_2$), 29.7 ($\underline{C}H_2$), 29.7 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.5($\underline{C}H_2$), 29.4($\underline{C}H_2$), 29.3($\underline{C}H_2$), 27.4 ($\underline{C}H_2$), 26.9 ($3\underline{C}H_3$), 25.7 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 19.2 ($Si\underline{C}(CH_3)_3$, 14.1 ($\underline{C}H_3$).

$[\alpha]_D^{23}$ = + 4.7 (c = 1.1, $CHCl_3$).

Experiment 93: Attempted preparation of 3-hydroxy-16-(1-methoxy-1-methylethoxy)hexadecanoic acid methyl ester



PPTS (8 mg; 0.033 mmol) was added to a stirred solution of (*R*)-3,16-dihydroxy-hexadecanoic acid methyl ester (**331a**, 200 mg; 0.67 mmol) and 2-methoxypropene (47.5 mg, 0.67 mmol) in dry dichloromethane (15 ml). The mixture was stirred for 0.5 h. The mixture was quenched with water (20 ml) and the organic layer was separated. The water layer was extracted with dichloromethane (3 x 150 ml). The combined organic layers were dried and the solvent was evaporated to give the crude product which was purified by column chromatography eluting with petrol and ether (10:1) to give *3,16-bis-(1-methoxy-1-methylethoxy)hexadecanoic acid methyl ester* (**366**, 190 mg, 65 %).

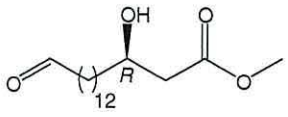
Found C: 67.4, H: 11.3; $C_{25}H_{50}O_6$ requires: C: 67.22, H: 11.28.

ν_{max}/cm^{-1}: 2989, 2924, 2952, 1740, 1467, 1438, 1407, 1367, 1255, 1204, 1177, 1065.

$\delta_{H\ (500\ MHz)}$: 4.1 (1H, m, C$\underline{H}$OH), 3.63 (3H, s, $CO_2C\underline{H}_3$), 3.34 (2H, t, J = 6.9 Hz, $C\underline{H}_2O$), 3.17 (3H, s, $OC\underline{H}_3$), 3.16 (3H, s, $OC\underline{H}_3$), 2.55 (1H, dd, J_1 = 15.0 Hz, J_2 = 6.0 Hz, $COC\underline{H}_2CHOH$), 2.41 (1H, dd, J_1 = 15.2 Hz, J_2 = 6.0 Hz, $COC\underline{H}_2CHOH$), 2.13 (1H, m, O$\underline{H}$), 1.6-1.4 (2H, m), 1.31 (6H, s, $C(C\underline{H}_3)_2$), 1.30 (6H, s, $C(C\underline{H}_3)_2$), 1.3-1.1 (16H, m).

$\delta_{C\ (125.8\ MHz)}$: 172.1 (C=O), 100.8 (O$\underline{C}$OCH), 99.6 ($O\underline{C}OCH_2$), 68.3 ($\underline{C}$HO), 60.6 ($\underline{C}H_2O$), 51.3 ($O\underline{C}H_3$), 49.0 (2 $O\underline{C}H_3$), 48.2 (2 $O\underline{C}H_3$), 40.7 ($CO\underline{C}H_2CHOH$), 35.7 ($\underline{C}H_2CHOH$), 30.0 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 26.3 ($\underline{C}H_2$), 25.1 ($\underline{C}H_3$), 25.1 ($\underline{C}H_3$), 24.8 (2 $\underline{C}H_3$).

Experiment 94: 3-Hydroxy-16-oxohexadecanoic acid methyl ester (367)



N-Chlorosuccinimide (66 mg, 0.5 mmol) was added to a vigorously stirred solution of the alcohol (**331a**, 100 mg, 0.33 mmol), TEMPO (5.2 mg, 0.033 mmol), TBABr (9.4 mg, 0.033 mmol) in dichloromethane (20 ml) and an aqueous solution (5 ml) of $NaHCO_3$ (0.5 M) and K_2CO_3 (0,05 M) at room temperature. Stirring was maintained for 5 h when TLC showed no starting material. The organic layer was separated, and the aqueous phase was extracted with dichloromethane. The organic layers were dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ether (1:1) to give *3-hydroxy-16-oxohexadecanoic acid methyl ester* (**367a**, 95 mg, 96 %).

Found $_{(EI)}$ M^+: 300.2311; $C_{17}H_{32}O_4$ requires: 300.2301.

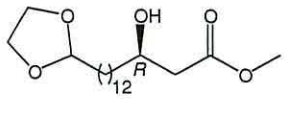
ν_{max}/cm^{-1}: 3380, 2912, 2846, 1734, 1716, 1691, 1540, 1508, 1378, 1463, 1438, 1308, 1173, 1113, 1088, 1014.

$\delta_{H\ (500\ MHz)}$: 9.76 (1H, b t, J = 1.9 Hz, C<u>H</u>=O), 4.05-3.95 (1H, m, C<u>H</u>OH), 3.70 (3H, s, OC<u>H</u>$_3$), 2.89 (1H, b s, O<u>H</u>), 2.51 (1H, dd, J_1 = 16.4 Hz, J_2 = 3.1 Hz, COC<u>H</u>$_2$CHOH), 2.45-2.35 (3H, including 1H, dd, J_1 = 16.6 Hz, J_2 = 8.9 Hz, COC<u>H</u>$_2$CHOH and at δ 2.41 2H, m, C<u>H</u>$_2$CO), 1.62 (2H, quintet, J = 7.25, C<u>H</u>$_2$CH$_2$C=O), 1.5-1.2 (20H, m).

$\delta_{C\ (125.8\ MHz)}$: 202.9 (<u>C</u>H=O), 173.4 (<u>C</u>O$_2$Et), 68.0 (<u>C</u>HOH), 51.7 (O<u>C</u>H$_3$), 43.9 (<u>C</u>H$_2$CH=O), 41.1 (CHOH<u>C</u>H$_2$CO), 36.5 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_2$), 29.3 (<u>C</u>H$_2$), 29.3 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 25.4 (<u>C</u>H$_2$), 22.0 (<u>C</u>H$_2$).

$[\alpha]_D^{24}$ = - 10.6 (c = 0.97, $CHCl_3$).

Experiment 95: 15-[1,3]-Dioxolan-2-yl-3-hydroxypentadecanoic acid methyl ester (365a)



1,2-Ethanediol (0.18 ml, 3.24 mmol) was added to a stirred solution of 3-hydroxy-16-oxohexadecanoic acid methyl ester (**367a**, 200 mg, 0.66 mmol) and PTSA (0.012 g, 0.065 mmol) in toluene (25 ml). The mixture was refluxed for 2 h using a Dean Stark apparatus to eliminate any water produced, then cooled to room temperature and the reaction quenched with water (5 ml) and sat. aq. sodium bicarbonate (5 ml). The product was extracted with ether (3 x 25 ml), and the combined organic layers were dried and concentrated. The residue was purified by column chromatography eluting with petrol and ether (1:1) to give *15-[1,3]-dioxolan-2-yl-3-hydroxypentadecanoic acid methyl ester* (**365a**, 220 mg, 96 %).

Found $_{(EI)}$ M^+: 344.2568; $C_{19}H_{36}O_5$ requires: 344.2563.

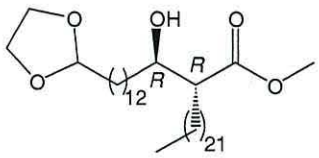
ν_{max}/cm^{-1}: 3506, 2920, 2850, 1732, 1542, 1507, 1466, 1437, 1411, 1362, 1140.

$\delta_{H\ (500\ MHz)}$: 4.85 (1H, t, J = 4.8 Hz, OC<u>H</u>O), 4.1 (1H, m, C<u>H</u>OH), 3.95-3.75 (2H, m, OC<u>H</u>$_2$CH$_2$O), 3.85-3.75 (2H, m, OC<u>H</u>$_2$CH$_2$O), 3.64 (3H, s, OC<u>H</u>$_3$), 2.52 (1H, dd, J_1 = 16.4 Hz, J_2 = 3.2 Hz, COC<u>H</u>$_2$CHOH), 2.42 (1H, dd, J_1 = 16.0 Hz, J_2 = 9.0 Hz, COC<u>H</u>$_2$CHOH), 1.8-1.2 (22H, m) 0.85 (3H, t, J = 6.0 Hz, C<u>H</u>$_3$).

$\delta_{C\ (125.8\ MHz)}$: 173.4 (<u>C</u>=O), 104.7 (O<u>C</u>HO), 68.0 (<u>C</u>HOH), 64.8 (O<u>C</u>H$_2$CH$_2$O), 51.6 (O<u>C</u>H$_3$), 41.2 (CHOH<u>C</u>H$_2$CO), 36.6 (<u>C</u>H$_2$), 33.9 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_2$), 24.0 (<u>C</u>H$_2$).

$[\alpha]_D^{24}$ = - 11.7 (c = 1.03, $CHCl_3$).

Experiment 96: 2-(13-[1,3]-Dioxolan-2-yl-1-hydroxytridecyl)tetracosanoic acid methyl ester (368a)



The procedure described in **Experiment 90 C** was repeated using similar concentrations, in order to couple 15-[1,3]-dioxolan-2-yl-3-hydroxypentadecanoic acid methyl ester (**365a**, 220 mg, 0.63 mmol, final concentration 0.015 mol. eq.) with 1-iododocosane (**356**, 470 mg, 1.1 mmol, final concentration 0.025 mol. eq.). The crude product was purified by column chromatography eluting with petrol

and ether (5:2) to give *2-(13-[1,3]-dioxolan-2-yl-1-hydroxytridecyl)tetracosanoic acid methyl ester* (**368a**, 130 mg, 32 %).

Found $_{(EI)}$ M^+: 652.6021; $C_{41}H_{80}O_5$ requires: 652.6009.

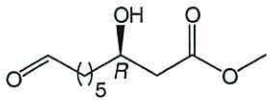
ν_{max}/cm^{-1}: 2916, 2848, 1710, 1464, 1168.

$\delta_{H\ (500\ MHz)}$: 4.85 (1H, t, J = 4.8 Hz OC<u>H</u>O), 3.95-3.75 (2H, m, OC<u>H</u>$_2$CH$_2$O), 3.85-3.75 (2H, m, OC<u>H</u>$_2$CH$_2$O), 3.64 (3H, s, OC<u>H</u>$_3$), 3.59 (1H, m, C<u>H</u>OH), 2.5-2.4 (1H, m, C<u>H</u>(CH$_2$)$_{21}$CH$_3$), 1.6-1.2 (67H, m), 0.88 (3H, t, J = 7.0 Hz, C<u>H</u>$_3$).

$\delta_{C\ (125\ MHz)}$: 176.2 (C=O), 104.7 (O<u>C</u>HO), 72.3 (<u>C</u>HOH), 64.8 (O<u>C</u>H$_2$CH$_2$O), 51.5 (O<u>C</u>H$_3$), 51.0 (<u>C</u>HCO), 35.7 (<u>C</u>H$_2$), 33.9 (<u>C</u>H$_2$), 31.9 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_2$), 29.3 (<u>C</u>H$_2$), 28.9 (<u>C</u>H$_2$), 27.4 (<u>C</u>H$_2$), 25.7 (<u>C</u>H$_2$), 24.1 (<u>C</u>H$_2$), 22.7 (<u>C</u>H$_2$), 15.1 (CH$_3$).

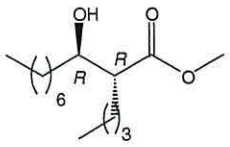
$[\alpha]_D^{24}$ = + 4.7 (c = 1.06, $CHCl_3$).

Experiment 97: Attempted preparation of 3-hydroxy-9-oxononanoic acid methyl ester (367b)



The procedure in **Experiment 94** was repeated in order to oxidise 3, 9-dihydroxy-nonanoic acid methyl ester (**331b**, 0.4 g, 2 mmol). The reaction was not successful producing a polymer insoluble in most common organic solvents.

Experiment 98: Attempted preparation (2*R*,3*R*)-2-butyl-3-hydroxydecanoic acid methyl ester (375)



The cyclic sulfate (**289a**, 300 mg. 1 mmol) was dissolved in THF (10 ml) and a solution of the butyl magnesium bromide (**374**, 1.2 ml, 2 mmol, 1.75 M in THF)

was added at - 78 °C under nitrogen followed by Li_2CuCl_4 (0.1 ml, 0.01 mmol, 0.1 M in THF). The mixture was warmed to room temperature and stirred for 3 h. The solution was concentrated and a 10 % solution of H_2SO_4 (5 ml) and ether (5 ml) were added to the residue. The mixture was stirred vigorously for 6 h. The water layer was extracted with ether (3 x 20 ml). The combined organic layers were washed with water and brine, dried and concentrated. The crude product was purified by column chromatography eluting with petrol and ethyl acetate (5:1). The reaction did not give the desired product; instead 2-bromo-3-hydroxydecanoic acid methyl ester (**377**, 200 mg, 71 %) was obtained, which showed the same NMR spectra as those reported.[339]

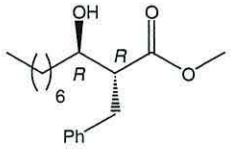
Found C: 47.147, H: 7.845, required for $C_{11}H_{21}BrO_3$: C: 46.99, H: 7.53.

ν_{max}/cm^{-1}: 3475, 2925, 2855, 1743, 1459, 1438, 1400, 1377, 1356, 1285, 1198, 1150, 1089, 1066, 1026.

$\delta_{H\ (250\ MHz)}$: 4.14 (1H, d, J = 7.6 Hz, CHBrCO), 4.00 (1H, dt, J_1 = 7,6 Hz, J_2 = 2.7 Hz, CHOH), 3.86 (3H, s, OCH_3), 2.6-2.5 (1H, br s, OH), 1.7-1.0 (12H, m), 0.88 (3H, t, J = 7.0 Hz, CH_3).

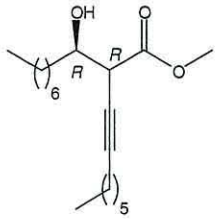
$\delta_{C\ (62.9\ MHz)}$: 169.8 (C=O), 72.4 (COH), 53.0 (CHOH), 47.9 (CHBr), 33.4, 31.7, 29.2, 29.1, 25.2, 22.6, 14.0 (CH_3).

Experiment 99: Attempted preparation of 2-benzyl-3-hydroxydecanoic acid methyl ester (379)



The procedure in **Experiment 98** was repeated using as nucleophile benzyl magnesium bromide (**378**). The desired product (**379**) was not obtained but instead 2-bromo-3-hydroxydecanoic acid methyl ester (**377**) was again isolated with a yield of 52 %.

Experiment 100: Attempted preparation of 2-(1-hydroxyoctyl)dec-3-ynoic acid methyl ester (381)



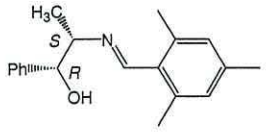
Buthyllithium (1.2 ml, 1.8 mmol, 1.5 M solution in hexanes) was added to a solution of oct-1-yne (**380**, 177 mg, 1.6 mmol) in dry THF (15 ml) at – 50 °C. The mixture was stirred for 1 h, then a solution of the cyclic sulphate (**289a**, 300 mg, 1 mmol) was added follow by HMPA (0.37 ml, 2.2 mmol) and the solution was stirred for 4 h. Then, hydrolysis employing sulphuric acid was performed as in **Experiment 98**. The desired product (**381**) was not obtained. The reaction probably produced 10,11-dihydroxyoctadec-7-yn-9-one, (**382**, 140 mg, 45 %).

ν_{max}/cm^{-1}: 2928, 2858, 2214, 1666, 1467, 1381, 1242, 1377, 1356, 1285, 1198, 1150, 1089, 1066, 1026.

$\delta_{H\ (250\ MHz)}$: 4.40 (1H, d, J = 3.4 Hz, CHOHCO), 4.07 (1H, b dt, J_1 = 9.5 Hz, J_2 = 3.1 Hz, CHOH) 3.2-2.8 (1H, b s, OH), 2.41 (2H, t, J = 7.3 Hz, $CH_2C{\equiv}C$), 1.7-1.1 (24H, m), 0.88 (3H, t, J = 7.0 Hz, CH_3).

$\delta_{C\ (62.9\ MHz)}$: 186.7 (C=O), 101.6 (C≡CCO), 81.8 (CHOHCO), 78.5 (C≡CCO), 72.9 (CHOHCHOHCO), 31.8, 31.2, 31.1, 29.4, 29.1, 28.5, 28.1, 27.1, 25.8, 22.6, 22.4, 19.2, 14.0 (CH_3).

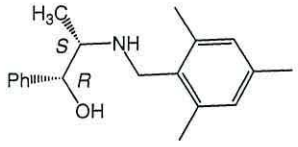
Experiment 101: (1*R*,2*S*)-1-Phenyl-2-[(2,4,6-trimethylbenzylidene)amino]-propan-1-ol (405)



2,4,6-Trimethylbenzaldehyde (**404**, 2.94 g, 20 mmol) was added to a solution of magnesium sulphate (4.8 g, 40 mmol) and (-)-norephedrine (**403**, 3 g, 20 mmol) in dry toluene (80 ml). The mixture was stirred at 80 °C for 30 h then filtered. The filtrate was evaporated to give the crude product, (1*R*,2*S*)-1-phenyl-2-[(2,4,6-

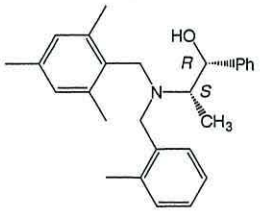
trimethylbenzylidene)amino]-propan-1-ol. (**405**, 5.3 g, 94 %) which showed identical NMR spectra to those reported.[11] It was utilised without further purification.

Experiment 102: (1*R*,2*S*)-1-Phenyl-2-(2,4,6-trimethylbenzylamino)propan-1-ol (406)



Sodium cyanoborohydride (2.4 g, 38 mmol) was added to a solution of (1*R*,2*S*)-1-phenyl-2-[(2,4,6-trimethylbenzylidene)amino]-propan-1-ol (**405**, 5.3 g, 19 mmol) in methanol (60 ml). During the addition the pH was maintained at 4 by adding acetic acid in small portions. The solution was stirred for 4 h, when the NMR showed the absence of starting material. The reaction was quenched with sat. aq. sodium bicarbonate (80 ml). The product was extracted with dichloromethane (3 x 200 ml). The combined organic layers were washed with sat. aq. sodium bicarbonate (1 x 75 ml), dried and concentrated to give the crude product, which was purified by column chromatography eluting with petrol and ether (1:1). 1-Phenyl-2-(2,4,6-trimethylbenzylamino)propan-1-ol (**406**, 5.2 g, 98 %) was obtained, which showed the same NMR spectra as those reported.[11]

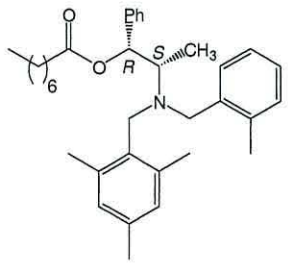
Experiment 103: (1*R*,2*S*)-2-[(2-Methylbenzyl)-(2,4,6-trimethylbenzylamino)-1-phenylpropan-1-ol (402)



Caesium carbonate (3.4 g, 10 mmol) was added to a solution of (1*R*, 2*S*)-1-phenyl-2-(2,4,6-trimethylbenzylamino)propan-1-ol (**406**, 1.4 g, 5 mmol) and 1-bromomethyl-2-methylbenzene (**407**, 1 g, 5 mmol) in dry toluene (40 ml) and dry acetonitrile (20 ml). The mixture was stirred for 20 h at 90 °C. After cooling the

mixture, the solvent was evaporated to give a white solid. The residue was dissolved in water (40 ml) and extracted with dichloromethane (3 x 100 ml); the combined organic layers were dried and concentrated. The crude product was purified by column chromatography eluting with petrol and ether (9:1) to give (1*R*,2*S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzylamino)-1-phenylpropan-1-ol (**402**, 1.8 g, 93 %) which showed the same NMR spectra as the ones reported.[11]

Experiment 104: Octanoic acid (1*R*,2*S*)-2-[(2-methylbenzyl)-(2,4,6-trimethyl benzylamino)-1-phenylpropyl ester (409a)



(1*R*,2*S*)-2-[(2-Methylbenzyl)-(2,4,6-trimethylbenzylamino)-1-phenylpropan-1-ol (**402**, 1 g, 2.6 mmol) was dissolved in dry dichloromethane (15 ml) at room temperature under nitrogen. To this solution was added octanoic acid (**408a**, 0.375 g, 2.6 mmol) in dry dichloromethane (5 ml), followed by EDCl (600 mg, 3.1 mmol) and DMAP (800 mg, 6.5 mmol). The reaction was monitored by TLC; after stirring the reaction for 24 h starting material (**402**) was still present. More octanoic acid (0.19 g, 1.3 mmol) was added followed by EDCl (300 mg, 1.6 mmol). After 32 h the reaction was quenched with water and extracted with dichloromethane (3 x 50 ml). The combined organic layers were washed with sat. aq. sodium bicarbonate, dried and concentrated. The residue was columned eluting with petrol and ether (10:0.5) to give *octanoic acid (1R,2S)-2-[(2-methyl benzyl)-(2,4,6-trimethylbenzylamino)-1-phenylpropyl ester* (**409a**, 1.2 g, 90 %).

Found $_{(EI)}$ $(M-H)^+$: 512.3548; $C_{35}H_{46}O_2N$ requires: 512.3529.

ν_{max}/cm^{-1}: 3062, 2954, 2926, 2856, 1735, 1612, 1582, 1494, 1457, 1377, 1245, 1163, 1116, 1046, 1012.

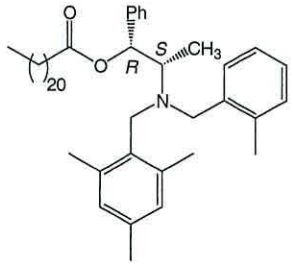
$\delta_{H\ (500\ MHz)}$: 7.3-7.0 (7H, m, aromatic), 6.93 (2H, dd, J_1= 9.7 Hz, J_2 = 1.6 Hz, aromatic in Tol), 6.78 (2H, s, aromatic in Mes), 6.07 (1H, d, J = 6 Hz, C<u>H</u>OCO), 3.74 (2H, dd, J_1 = 13.3 Hz, J_2 = 2.5 Hz, 1H of NC<u>H</u>Mes

and 1H of NC<u>H</u>$_2$Tol), 3.59 (2H, t, J = 12 Hz, 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.22 (1H, m, NC<u>H</u>CH$_3$), 2.33 (2H, t, J = 7.4 Hz, C<u>H</u>$_2$CO), 2.27 (3H, s, C<u>H</u>$_3$Ph) 2.14 (6H, s, two methyls in *ortho* of Mes group), 2.10 (3H, s, a methyl in *para* of Mes group), 1.7-1.6 (3H, m, CHC<u>H</u>$_3$), 1.3-1.2 (10H, m), 0.88 (3H, t, J = 6.6 Hz).

$\delta_{C\ (125.8\ MHz)}$: 172.9 (C=O), 139.9 (aromatic), 138.5 (aromatic), 137.4 (aromatic), 137.1 (aromatic), 136.3 (aromatic), 131.7 (aromatic), 130.7 (aromatic), 130.1 (aromatic), 129.0 (aromatic), 128.0 (aromatic), 127.3 (aromatic), 127.0 (aromatic), 126.9 (aromatic), 125.3 (aromatic), 76.5 (<u>C</u>OH), 56.5 (N<u>C</u>HCH$_3$), 51.3 (N<u>C</u>H$_2$ aromatic), 47.4 (N<u>C</u>H$_2$ aromatic), 34.8 (C<u>H</u>$_2$CO), 31.7 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 28.9 (<u>C</u>H$_2$), 24.9 (<u>C</u>H$_2$), 22.6 (<u>C</u>H$_2$), 20.9 (<u>C</u>H$_3$), 20.2 (<u>C</u>H$_3$), 19.2 (<u>C</u>H$_3$), 14.0 (<u>C</u>H$_3$), 9.3 (<u>C</u>H$_3$).

$[\alpha]_D^{24}$ = - 7.8 (c = 1.1, $CHCl_3$).

Experiment 105: Docosanoic acid (1*R*,2*S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester (409b)



The procedure used in **Experiment 104** was repeated using docosanoic acid (**408b**, 1 g, 3 mmol) and the chiral auxiliary (**402**, 0.775 g, 2 mmol). It was necessary to reflux the mixture at 40 °C for 24 h. *Docosanoic acid (1R,2S)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester* (**409b**, 1.2 g, 84 %) was obtained.

Found $_{(CI)}$ M^+: 709.5795 $C_{49}H_{75}O_2N$ requires: 709.5798.

ν_{max}/cm^{-1}: 2922, 2852, 1735, 1612, 1458, 1376, 1239, 1163, 1048, 1012.

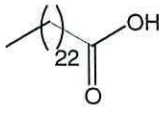
$\delta_{H\ (500\ MHz)}$: 7.3-7.0 (7H, m, aromatic), 6.93 (2H, d, J = 6.3 Hz, aromatic in Tol), 6.79 (2H, s, aromatic in Mes), 6.09 (1H, d, J = 6.3 Hz, C<u>H</u>OCO), 3.75 (2H, m, 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.60 (2H, t, J = 12.6

Hz, 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.23 (1H, m, NC<u>H</u>CH$_3$), 2.34 (2H, t, J = 7.6 Hz, C<u>H</u>$_2$CO), 2.28 (3H, s, C<u>H</u>$_3$Ph), 2.16 (6H, s, two methyls in *ortho* of Mes group), 2.15 (3H, s, a methyl in *para* of Mes group), 1.7-1.6 (3H, m, CHC<u>H</u>$_3$), 1.4-1.2 (42H, m), 0.93 (3 H, t, J = 6.6 Hz).

$\delta_{C\ (125.8\ MHz)}$: 172.8 (C=O), 139.9 (aromatic), 138.4 (aromatic), 137.3 (aromatic), 137.0 (aromatic), 136.2 (aromatic), 131.6 (aromatic), 130.7 (aromatic), 130.1 (aromatic), 129.0 (aromatic), 128.0 (aromatic), 127.3 (aromatic), 127.0 (aromatic), 126.8 (aromatic), 125.3 (aromatic), 76.5 (<u>C</u>OH), 56.5 (N<u>C</u>HCH$_3$), 51.2 (N<u>C</u>H$_2$-aromatic), 47.4 (N<u>C</u>H$_2$-aromatic), 34.7 (C<u>H</u>$_2$CO), 31.9 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_2$), 29.2 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 24.9 (<u>C</u>H$_2$), 22.7 (<u>C</u>H$_2$), 20.9 (<u>C</u>H$_3$), 20.1 (<u>C</u>H$_3$), 19.2 (<u>C</u>H$_3$), 14.1 (<u>C</u>H$_3$), 9.2 (<u>C</u>H$_3$).

$[\alpha]_D^{23}$ = - 6.1 (c = 1.1, $CHCl_3$).

Experiment 106: Tetracosanoic acid (408c)

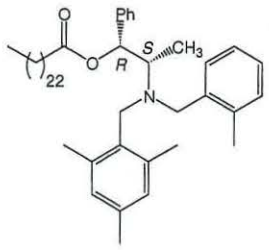


A solution of Jones reagent (2.2 ml, 2.6 M, 5.6 mmol) was added to tetracosan-1-ol (1.7 g, 4.8 mmol)[f] in acetone (500 ml). The mixture was stirred a room temperature for 1 h and a TLC still showed starting material. More Jones reagent (3 ml, 7.8 mmol) was added and the mixture refluxed for 3 h. The mixture was stirred at room temperature overnight when a TLC showed no starting material. The solvent was evaporated to give a residue, which was dissolved in water (100 ml) and extracted with warm dichloromethane (4 x 300 ml). The combined organic layers were dried and concentrated to give a yellowish solid which was re-crystallised from methanol (200 ml) and water (20 ml) to give a white solid, tetracosanoic acid (**408c**, 1.6 g, 91 %) which showed the same ^{1}H NMR spectrum[432] and IR spectrum[433] as the ones in the literature.

mp = 83-85 °C (lit. 83.7 – 84.1).[434]

[f] Kindly given by E. Roberts.

Experiment 107: Tetracosanoic acid (1*R*,2*S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester (409c)



The procedure used in **Experiment 104** was repeated using tetracosanoic acid (**408c**, 1.5 g, 4 mmol). It was necessary to reflux at 40 °C for 24 h. *Tetracosanoic acid (1R,2S)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester* (**409c**, 2.7 g, 88 %) was obtained.

Found $_{(CI)}$ M^+: 737.6078 $C_{51}H_{79}O_2N$ requires: 737.6111.

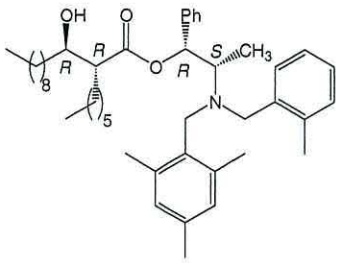
ν_{max}/cm^{-1}: 2920, 2852, 1735, 1612, 1458, 1376, 1239, 1163, 1046.

$\delta_{H\ (500\ MHz)}$: 7.3-7.0 (7H, m, aromatic), 6.93 (2H, d, J = 6.3 Hz, aromatic in Tol), 6.79 (2H, s, aromatic in Mes), 6.08 (1H, d, J = 6.0 Hz, C<u>H</u>OCO), 3.75 (2H, m, 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.60 (2H, t, J = 12.4 Hz, 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.23 (1H, m, NC<u>H</u>CH$_3$), 2.34 (2H, t, J = 7.6 Hz, C<u>H</u>$_2$CO), 2.28 (3H, s, C<u>H</u>$_3$Ph), 2.16 (6H, s, two methyls in *ortho* of Mes group), 2.15 (3H, s, methyl in *para* of Mes group), 1.7-1.6 (3H, m, CHC<u>H</u>$_3$), 1.4-1.2 (46H, m), 0.93 (3 H, t, J = 6.6 Hz).

$\delta_{C\ (125.8\ MHz)}$: 172.8 (C=O), 139.9 (aromatic), 138.4 (aromatic), 137.3 (aromatic), 137.0 (aromatic), 136.2 (aromatic), 131.6 (aromatic), 130.7 (aromatic), 130.1 (aromatic), 129.0 (aromatic), 128.0 (aromatic), 127.3 (aromatic), 127.0 (aromatic), 126.8 (aromatic), 125.3 (aromatic), 76.5 (<u>C</u>OH), 56.5 (N<u>C</u>HCH$_3$), 51.2 (N<u>C</u>H$_2$-aromatic), 47.4 (N<u>C</u>H$_2$-aromatic), 34.7 (C<u>H</u>$_2$CO), 31.9 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_2$), 29.2 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 24.9 (<u>C</u>H$_2$), 22.7 (<u>C</u>H$_2$), 20.9 (<u>C</u>H$_3$), 20.8 (<u>C</u>H$_3$), 19.2 (<u>C</u>H$_3$), 14.1 (<u>C</u>H$_3$), 9.2 (<u>C</u>H$_3$).

$[\alpha]_D^{20}$ = - 6.3 (c = 1.2, $CHCl_3$).

Experiment 108: (2*R*,3*S*) 2-Hexyl-3-hydroxydodecanoic acid (1*R*,2*S*) 2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amin]-1-phenylpropyl ester (411a)



A THF solution (2 ml) of Cp_2ZrCl_2 (43 mg, 0.11 mmol), was added at – 78 °C to a stirred solution of LDA (0.8 mmol, 0.67 ml, 1.2 M, in heptane/tetrahydrofuran/ethylbenzene), in THF (4 ml) under nitrogen. After 0.5 h, octanoic acid (*1R, 2S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzylamino)-1-phenylpropyl ester (**409a**, 200 mg, 0.4 mmol, final concentration 0.033 mmol/ml), in THF (2 ml), was added. The mixture was stirred for 3 h and Cp_2ZrCl_2 (360 mg, 1 mmol), in THF (4 ml) was added. After an additional 0.5 h at – 78 °C, decanal (**410**, 80 mg, 0.5 mmol, final concentration 0.041 mmol/ml), in THF (2 ml) was added to the reaction. The mixture was stirred at – 78 °C for 3 h then the temperature was allowed to rise very slowly to – 40 °C. After 9 h, the reaction was quenched with 1 N HCl (10 ml). The mixture was extracted with ether (3 x 50 ml) and the combined organic layers were dried and the solvent evaporated. The residue was purified by column chromatography eluting with petrol and ether (10:1). The product was obtained as a mixture (150 mg, 58 %) of two diastereoisomers of (**411a**): *erythro* and *threo* (10:1, the ratio between the stereoisomers was established by analysis of the mixture by ^{1}H NMR). It was possible to partially separate the mixture obtaining *(2R,3R)-3-hydroxy-2-hexyldodecanoic acid 2-[(2-methylbenzyl)-(2,4,6-trimethyl-benzyl)amin]-1-phenyl propyl ester* (**411a**, 105 mg, 40 %), while the minor isomer was never completely purified.

The major isomer, *(2R,3R)-3-hydroxy-2-hexyldodecanoic acid 2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amin]-1-phenylpropyl ester* (**411a**) showed the following:

Found $_{(CI)}$ $(M-H)^+$ 668.5063 and $C_{45}H_{66}O_3N$ 668.5043.

ν_{max}/cm^{-1}: 3519, 3064, 3033, 2954, 2925, 2855, 1715, 1613, 1582, 1495, 1485, 1458, 1378, 1307, 1265, 1241, 1164, 1130, 1092, 1049, 1011.

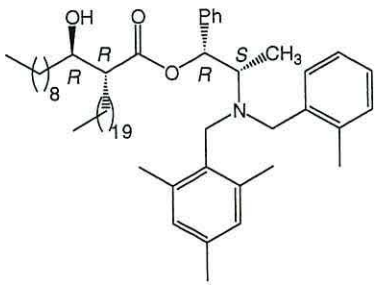
$\delta_{H\ (500\ MHz)}$: 7.3-7.0 (7H, m, aromatic), 6.91 (2H, d, J = 7.6 Hz, aromatic in Tol), 6.74 (2H, s, aromatic in Mes), 6.01 (1H, d, J = 8.2 Hz, C<u>H</u>OCO), 3.68 (2H, t, J = 13.6 Hz, 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.60 (1H, m, C<u>H</u>OH), 3.52 (2H, dd, J_1 = 12.4 Hz, J_2 = 2.6 Hz 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.33 (1H, m, NC<u>H</u>CH$_3$), 2.45 (1H, d, J = 8.2 Hz, O<u>H</u>, disappeared with D_2O), 2.38 (1H, dt, J_1 = 8.9 Hz, J_2 = 5.2 Hz), 2.26 (3H, s, C<u>H</u>$_3$Ph), 2.07 (3H, s, Me), 2.02 (6H, s, Me), 1.7-0.9 (29H, m), 0.88 (6H, J = 6.6 Hz).

$\delta_{C\ (125.8\ MHz)}$: 175.1 (C=O), 139.2 (aromatic), 138.5 (aromatic), 137.4 (aromatic), 136.7 (aromatic), 136.2 (aromatic), 131.4 (aromatic), 130.9 (aromatic), 130.1 (aromatic), 128.9 (aromatic), 127.9 (aromatic), 127.9 (aromatic), 127.7 (aromatic), 126.9 (aromatic), 125.3 (aromatic), 77.0 (<u>C</u>HOCO), 72.2 (C<u>H</u>OH), 55.5 (N<u>C</u>HCH$_3$), 50.9 (N<u>C</u>H$_2$ aromatic), 50.9 (C<u>H</u>-alk), 47.2 (N<u>C</u>H$_2$ aromatic), 35.6 (<u>C</u>H$_2$), 31.9 (<u>C</u>H$_2$), 31.5 (<u>C</u>H$_2$), 29.8 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.3 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 26.9 (<u>C</u>H$_2$), 25.8 (<u>C</u>H$_2$), 22.7 (<u>C</u>H$_2$), 22.4 (<u>C</u>H$_2$), 20.8 (<u>C</u>H$_3$), 20.2 (<u>C</u>H$_3$), 19.2 (<u>C</u>H$_3$), 14.1 (<u>C</u>H$_3$), 14.0 (<u>C</u>H$_3$), 9.8 (<u>C</u>H$_3$).

Rf = 0.66 (petrol: ether 9:1)

$[\alpha]_D^{24}$ = - 3.8 (c = 0.8, $CHCl_3$).

Experiment 109: (2*R*,3*R*)- 2-(1-Hydroxydecyl)docosanoic acid (1*R*,2*S*) 2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester (411b)



The procedure in **Experiment 108** was repeated using for each compound similar concentrations, with docosanoic acid (1*R*,2*S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylnenzyl)amino]-1-phenylpropyl ester (**409b**, 350 mg, 0.5 mmol). A mixture of desired *anti*-diastereoisomer and the *syn*, (270 mg, 63%) was obtained ina ratio as 9:1, which was established by analysis of the mixture by ^{1}H NMR. It was possible to separate only the major isomer *(2R,3R)-2-(1-hydroxydecyl)docosanoic acid (1R,2S)-2-[(2-methylbenzyl)-(2,4,6-trimethyl benzyl)amino]-1-phenylpropyl ester* (**411b**, 180 mg, 42 %) from the mixture.

Found $_{(TOF\ MS)}$ $(M+H)^+$: 866.7358; $C_{59}H_{96}NO_3$ requires: 866.7390.
$(M+Na)^+$: 888.7179; $C_{59}H_{95}NO_3Na$ requires: 888.7210.
$(M+K)^+$: 904.6917; $C_{59}H_{95}NO_3K$ requires: 904.6949.

ν_{max}/cm^{-1}: 3519, 3064, 3033, 2954, 2925, 2855, 1715, 1613, 1582, 1495, 1485, 1458, 1378, 1307, 1265, 1241, 1164, 1130, 1092, 1049, 1011.

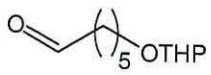
$\delta_{H\ (500\ MHz)}$: 7.3-7.0 (7H, m, aromatic), 6.91 (2H, d, J = 7.6 Hz, aromatic in Tol), 6.74 (2H, s, aromatic in Mes), 6.01 (1H, d, J = 8.2 Hz, C<u>H</u>OCO), 3.68 (2H, t, J = 13.6 Hz, 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.60 (1H, m, C<u>H</u>OH), 3.52 (2H, dd, J_1 = 12.4 Hz, J_2 = 2.6 Hz 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.33 (1H, m, NC<u>H</u>CH$_3$), 2.45 (1H, d, J = 8.2 Hz, O<u>H</u>, disappeared with D_2O), 2.38 (1H, dt, J_1 = 8.9 Hz, J_2 = 5.2 Hz), 2.26 (3H, s, C<u>H</u>$_3$Ph), 2.07 (3H, s, Me), 2.02 (6H, s, Me), 1.7-0.9 (57H, m), 0.88 (6H, J = 6.6 Hz).

$\delta_{C\ (125.8\ MHz)}$: 175.1 (C=O), 139.2 (aromatic), 138.5 (aromatic), 137.4 (aromatic), 136.7 (aromatic), 136.2 (aromatic), 131.4 (aromatic), 130.9 (aromatic), 130.1 (aromatic), 128.9 (aromatic), 127.9 (aromatic), 127.9 (aromatic), 127.7 (aromatic), 126.9 (aromatic), 125.3 (aromatic), 77.0 (<u>C</u>HOCO), 72.2 (C<u>H</u>OH), 55.5 (N<u>C</u>HCH$_3$), 50.9 (N<u>C</u>H$_2$ aromatic), 50.9 (C<u>H</u>-alk), 47.2 (N<u>C</u>H$_2$ aromatic), 35.6 (<u>C</u>H$_2$), 31.9 (<u>C</u>H$_2$), 31.5 (<u>C</u>H$_2$), 29.8 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.5 (<u>C</u>H$_2$), 29.3 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 26.9 (<u>C</u>H$_2$), 25.8 (<u>C</u>H$_2$), 22.7 (<u>C</u>H$_2$), 22.4 (<u>C</u>H$_2$), 20.8 (<u>C</u>H$_3$), 20.1 (<u>C</u>H$_3$), 19.2 (<u>C</u>H$_3$), 14.1 (<u>C</u>H$_3$), 14.0 (<u>C</u>H$_3$), 9.8 (<u>C</u>H$_3$).

R_f = 0.66 (petrol: ether 9:1)

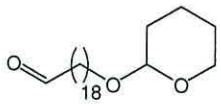
$[\alpha]_D^{24}$ = - 2.6 (c = 0.9, $CHCl_3$).

Experiment 110: 6-(Tetrahydropyran-2-yloxy)hexanal (412a)



The procedure in **Experiment 27** was utilised with 6-(tetrahydropyran-2-yloxy) hexan-1-ol (0.7 g, 3.4 mmol) to give 6-(tetrahydropyran-2-yloxy)hexanal (**412a**, 0.6 g, 88 %) which showed the same NMR spectra as in the literature.[403]

Experiment 111: 19-(Tetrahydropyran-2-yloxy)nonadecanal (412c)



The procedure used in **Experiment 27** was repeated to convert 19-(tetrahydropyran-2-yloxy)nonadecan-1-ol (**229**, 1g, 2.6 mmol) into *19-(tetrahydropyran-2-yloxy)nonadecanal* (**412c**, 0.9g, 91 %).

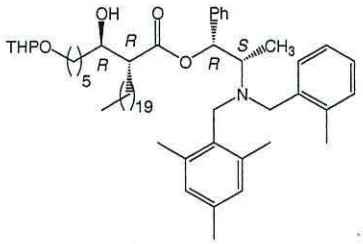
Found $_{(TOF\ MS)}$ $(M+Na)^+$: 405.3323; $C_{24}H_{46}NaO_3$ requires: 405.3345.

ν_{max}/cm^{-1}: 2920, 2852, 1735, 1612, 1458, 1376, 1239, 1163, 1046.

$\delta_{H\ (500\ MHz)}$: 9.76 (1H, t, J = 1.9 Hz, C<u>H</u>=O), 4.56 (1H, t, J = 4.6 Hz, OC<u>H</u>O), 3.9-3.85-3.75 (1H, m, CH_2OTHP), 3.72 (1H, dt, J_1 = 9.5 Hz, J_2 = 6.9 Hz, CH_2OTHP) 3.55-3.45 (1H, m CH_2OTHP), 3.36 (1H, dt, J_1 = 9.7 Hz, J_2 = 6.7 Hz, CH_2OTHP), 2.41 (2H, dt, J_1 = 7.3Hz, J_2 = 1.9 Hz), 1.8-1.2 (3H, m), 0.88 (3H, t, J = 6.9 Hz).

$\delta_{C\ (125.8\ MHz)}$: 202.9 (CH=O), 98.8 (O<u>C</u>HO), 67.7 (CH_2OTHP), 62.3 (CH_2OTHP), 46.2 (CH_2), 43.9 (CH_2), 30.7 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.5 (CH_2), 22.0 (CH_2), 19.7 (CH_2).

Experiment 112: (*2R,3R*) 2-[1-Hydroxy-6-(tetrahydropyran-2-yloxy)hexyl]-docosanoic acid (*1R,2S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester (413a)



The procedure in **Experiment 108**, was used to couple 6-(tetrahydro pyranyloxy)hexanal (**412a**, 47 mg, 0.25 mmol, 0.029 mmol/ml) and docosanoic acid (1*R*,2*S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)-amino]-1-phenyl-propyl ester (**409b**, 200 mg, 0.28 mmol, 0.033 mmol/ml) to give *(2R,3R)-2-[1-hydroxy-6-(tetrahydropyran-2-yloxy)hexyl]-docosanoic acid (1R,2S)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester* (**413a**, 98 mg, 38 %).

Found $_{(TOF\ MS)}$ $(M+Na)^+$: 932.7118; $C_{60}H_{95}NO_5Na$ requires: 932.7109.

ν_{max}/cm^{-1} 3482, 2924, 2852, 1719, 1612, 1464, 1378, 1255, 1164, 1033.

$\delta_{H\ (500\ MHz)}$: 7.3-7.0 (7H, m, aromatic), 6.91 (2H, d, J = 7.6 Hz, aromatic in Tol), 6.73 (2H, s, aromatic in Mes), 6.01 (1H, d, J = 8.2 Hz, C<u>H</u>OCO), 4.57 (1H, t, J = 4.1 Hz, OC<u>H</u>O), 3.9-3.8 (1H, m, CH_2OTHP group), 3.7-3.64 (3H, including t, J = 13.0 Hz, 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.60 (1H, m, C<u>H</u>OH), 3.53-3.45 (3H, including dd, J_1 = 12.4 Hz, J_2 = 4.5 Hz for 1H of NC<u>H</u>Mes and 1H of NC<u>H</u>$_2$Tol), 3.33 (2H, m, NC<u>H</u>CH_3 and 1H of CH_2OTHP group), 2.45 (1H, dd, J_1 = 8.2 Hz, J_2 = 3.8 Hz, O<u>H</u>), 2.37 (1H, dt, J_1 = 5.4 Hz, J_2 = 8.9 Hz), 2.25 (3H, s, C<u>H</u>$_3$Ph), 2.07 (3H, s, Me), 2.01 (6H, s, Me), 1.7-1 (55H, m), 0.88 (3H, t, J = 6.6 Hz).

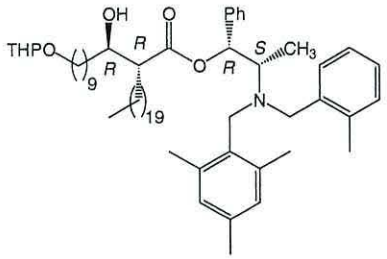
$\delta_{C\ (125.8\ MHz)}$: 175.0 (C=O), 139.2 (aromatic), 138.5 (aromatic), 137.5 (aromatic), 136.8 (aromatic), 136.2 (aromatic), 131.4 (aromatic), 130.9 (aromatic), 130.1 (aromatic), 128.9 (aromatic), 127.9 (aromatic), 127.9 (aromatic), 127.7 (aromatic), 126.9 (aromatic), 125.3 (aromatic), 98.9 (O<u>C</u>HO), 77.0 (<u>C</u>HOCO), 72.1 (C<u>H</u>OH), 67.8 (CH_2OTHP group), 62.6 (CH_2OTHP group), 55.4 (N<u>C</u>HCH_3), 51.0 (N<u>C</u>H_2 aromatic), 50.9 (C<u>H</u>-alk), 47.4 (N<u>C</u>H_2 aromatic), 35.5 (<u>C</u>H_2),

31.9 ($\underline{C}H_2$), 30.7 ($\underline{C}H_2$), 29.8 ($\underline{C}H_2$), 29.7 ($\underline{C}H_2$), 29.7 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 27.0 ($\underline{C}H_2$), 26.1 ($\underline{C}H_2$), 26.1 ($\underline{C}H_2$), 25.6 ($\underline{C}H_2$), 25.5 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 20.8 ($\underline{C}H_3$), 20.1 ($\underline{C}H_3$), 19.7 ($\underline{C}H_2$), 19.2 ($\underline{C}H_3$), 14.1 ($\underline{C}H_3$), 9.8 ($\underline{C}H_3$).

R_f = 0.72 (petrol: ether 9:1)

$[\alpha]_D^{22}$ = - 2.1 (c = 0.9, $CHCl_3$).

Experiment 113: (*2R,3R*)-2-[1-Hydroxy-10-(tetrahydropyran-2-yloxy)decyl]-docosanoic acid (*1R,2S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester (413b)



The procedure in **Experiment 108**, was used with 9-(tetrahydropyranyloxy)decanal (**226**, 130 mg, 0.51 mmol) and docosanoic acid (1*R*, 2*S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester (**409b**, 400 mg, 0.56 mmol) to give *(2R,3R)-2-[1-hydroxy-10-(tetrahydropyran-2-yloxy)decyl]-docosanoic acid (1R,2S)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester* (**413b**, 220 mg, 41 %).

Found $_{(TOF\ MS)}$ $(M+H)^+$: 966.7915; $C_{64}H_{104}NO_5$ requires: 966.7915.
$(M+Na)^+$: 932.7118; $C_{60}H_{95}NO_5Na$ requires: 932.710).

ν_{max}/cm^{-1}: 3582, 2923, 2852, 1729, 1612, 1464, 1378, 1162, 1033.

$\delta_{H\ (500\ MHz)}$: 7.3-7.0 (7H, m, aromatic), 6.91 (2H, d, J = 7.3 Hz, aromatic in Tol), 6.73 (2H, s, aromatic in Mes), 6.01 (1H, d, J = 8.2 Hz, C$\underline{H}$OH), 4.57 (1H, t, J = 4.1 Hz, OC$\underline{H}$O), 3.9-3.8 (1H, m, CH_2OTHP group), 3.7-3.64 (3H, including t, J = 13.0 Hz, 1H of NC$\underline{H}$Mes and 1H of NC$\underline{H}_2$Tol), 3.60 (1H, m, C$\underline{H}$OH), 3.53-3.45 (3H, including dd, J_1 = 12.4 Hz, J_2 = 4.5 Hz for 1H of NC$\underline{H}$Mes and 1H of NC$\underline{H}_2$Tol), 3.33 (2H, m, NC$\underline{H}$$CH_3$ and 1H of CH_2OTHP group), 2.45 (1H, dd, J_1 = 8.2 Hz, J_2 = 3.8 Hz, O$\underline{H}$), 2.37 (1H, dt, J_1 = 8.8 Hz, J_2 = 5.3 Hz), 2.25 (3H,

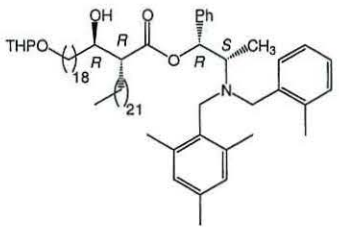
s, C<u>H</u>$_3$Ph), 2.07 (3H, s, Me), 2.01 (6H, s, Me), 1.7-1 (63H, m), 0.88 (3 H, J = 6.6 Hz).

$\delta_{C\ (125.8\ MHz)}$: 175.0 (C=O), 139.2 (aromatic), 138.5 (aromatic), 137.5 (aromatic), 136.8 (aromatic), 136.2 (aromatic), 131.4 (aromatic), 130.9 (aromatic), 130.1 (aromatic), 128.9 (aromatic), 128.0 (aromatic), 127.9 (aromatic), 127.8 (aromatic), 126.9 (aromatic), 125.3 (aromatic), 98.9 (O<u>C</u>HO), 77.0 (<u>C</u>HOCO), 72.2 (C<u>H</u>OH), 67.7 (CH_2OTHP), 62.4 (CH_2OTHP group), 55.8 (N<u>C</u>HCH$_3$), 51.0 (N<u>C</u>H$_2$ aromatic), 50.9 (C<u>H</u>-alk), 47.4 (N<u>C</u>H$_2$ aromatic), 35.5 (<u>C</u>H$_2$), 31.9 (<u>C</u>H$_2$), 30.7 (<u>C</u>H$_2$), 29.8 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.7 (<u>C</u>H$_2$), 29.6 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_2$), 29.4 (<u>C</u>H$_2$), 29.3 (<u>C</u>H$_2$), 27.0 (<u>C</u>H$_2$), 26.1 (<u>C</u>H$_2$), 26.1 (<u>C</u>H$_2$), 25.6 (<u>C</u>H$_2$), 25.5 (<u>C</u>H$_2$), 22.7 (<u>C</u>H$_2$), 20.8 (<u>C</u>H$_3$), 20.1 (<u>C</u>H$_3$), 19.7 (<u>C</u>H$_2$), 19.2 (<u>C</u>H$_3$), 14.1 (<u>C</u>H$_3$), 9.8 (<u>C</u>H$_3$).

R_f = 0.72 (petrol: ether 9:1)

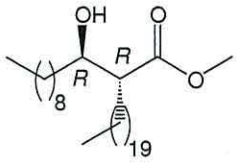
$[\alpha]_D^{22}$ = - 1.5 (c = 0.9, $CHCl_3$).

Experiment 114: Attempted preparation of (*2R,3R*)-2-[1-Hydroxy-19-(tetrahydropyran-2-yloxy)nonadecyl]-tetracosanoic acid (*1R,2S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester (413c)



The procedure in **Experiment 108** and similar concentrations, were used to couple 19-(tetrahydropyranyloxy)nonadecanal (**412c**, 187 mg, 0.49 mmol) and tetracosanoic acid (1*R*,2*S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester (**409c**, 400 mg, 0.54 mmol). After purification only the two starting materials (**412c**, **409c**) were recovered.

Experiment 115: (2*R*,3*R*)-2-(1-Hydroxydecyl)docosanoic acid methyl ester (414)



A solution of potassium hydroxide (0.5 g, 12.8 mmol) in water (5ml) was added to (2*R*,3*R*)-2-(1-hydroxydecyl)docosanoic acid (1*R*,2*S*)-2-[(2-methylbenzyl)-(2,4,6-trimethylbenzyl)amino]-1-phenylpropyl ester (**411b**, 50 mg, 0.06 mmol), in methanol (3 ml) and THF (1 ml). The mixture was refluxed for 18 h then cooled, quenched with dil. hydrochloric acid (5 ml, HCl, 2 M), and extracted with ether (4 x 20 ml). The combined organic layers were dried and the solvent evaporated to give a residue which was treated with diazomethane and then purified by column chromatography eluting with petrol to give 2-[(2-methylbenzyl)-(2,4,6-trimethyl benzylamino)-1-phenylpropan-1-ol (**402**, 17 mg, 76 %) and *(2R,3R) 2-(1-hydroxydecyl)docosanoic acid methyl ester* (**414**, 12 mg, 41 %).

Found $_{(EI)}$ $(M-H_2O)^+$: 492.4925; $C_{33}H_{64}O_2$ requires: 492.4906.

Found $_{(TOF\ MS)}$ $(M+H)^+$: 511.5090; $C_{33}H_{67}O_3$ requires: 511.5085.

ν_{max}/cm^{-1}: 3545, 2927, 2954, 1716, 1466, 1378, 1170, 1111, 907.

$\delta_{H\ (500\ MHz)}$: 3.72 (3H, s, $OC\underline{H}_3$), 3.66 (1H, m, $C\underline{H}OH$), 2.44 (1H, dt, J_1 = 9.2 Hz, J_2 = 5.4 Hz, and $C\underline{H}(CH_2)_{19}CH_3$) 2.34 (1H, d, J = 8.5 Hz, $O\underline{H}$), 1.75-1.65 (1H, m, $CHC\underline{H}_2(CH2)_{18}CH_3$), 1.65-1.55 (1H, m, $CHC\underline{H}_2(CH2)_{18}CH_3$), 1.7-1.2 (52H, m), 0.88 (6H, t, J = 6.7 Hz).

$\delta_{C\ (125.8\ MHz)}$: 176.3 ($\underline{C}O_2CH_3$), 72.3 ($\underline{C}HOH$), 51.5 ($O\underline{C}H_3$), 50.9 ($\underline{C}H(CH_2)_{11}CH_3$), 35.7 ($CH_2$), 31.9 ($CH_2$), 29.7 ($CH_2$), 29.6 ($CH_2$), 29.5 ($CH_2$), 29.5 ($CH_2$), 29.5 ($CH_2$), 29.4 ($CH_2$), 29.3 ($CH_2$), 29.0 ($CH_2$), 27.4 ($CH_2$), 26.4 ($CH_2$), 25.7 ($CH_2$), 22.7 ($CH_2$), 14.1 ($\underline{C}H_3$).

R_f = 0.45 ($CHCl_3$)

$[\alpha]_D^{23}$ = + 5.1 (c = 0.5, $CHCl_3$).

7. References

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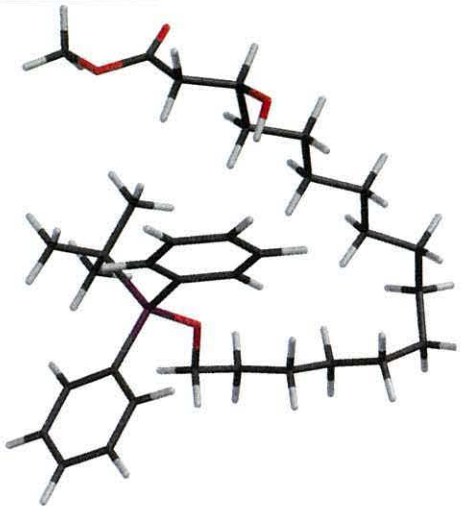
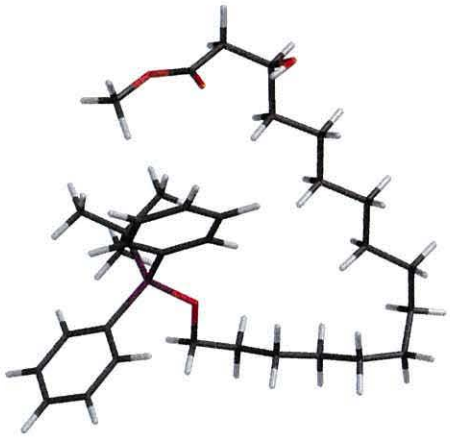
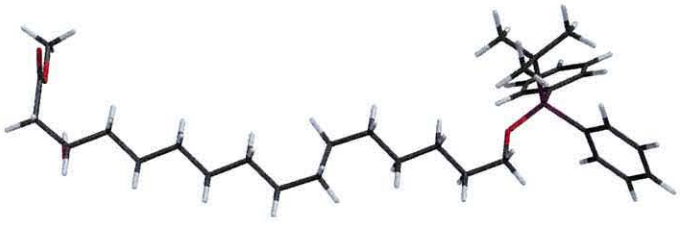
Appendix 1

Conformer distribution with Molecular Mechanics Force Field (MMFF):

Spartan '02 for Macintosh v1.0.4e

Costiniti Fabio

The β-hydroxy ester (**363**, **Figure 45, Section 4.2.5**) was analysed. The following are some of the most stable conformations. The average distance between the protons of the methyl group and those of a phenyl group are also indicated for each conformer.

Conformer in a minimum of energy. Average distance: **8,29 Å**	
Conformer at **3.47 KJ / mol** Average distance: **3,98 Å**	
Conformer at **13.06 KJ / mol** Average distance: **20,4 Å**	

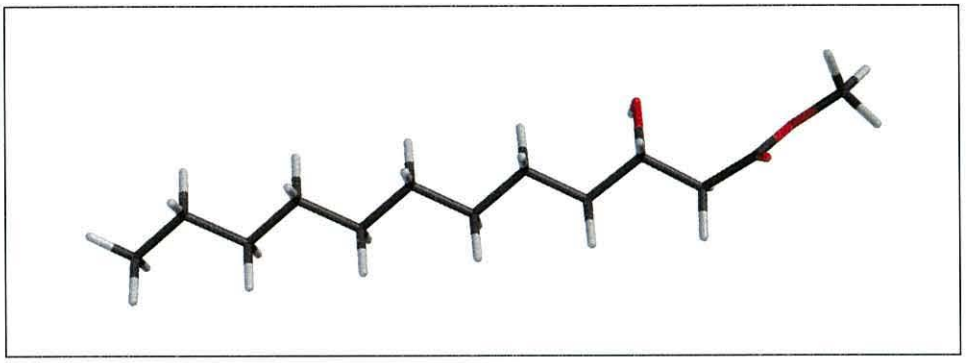
Appendix 2

Conformer at a minimum energy for different other molecules:

Spartan '02 for Macintosh v1.0.4e

Costiniti Fabio

The most stable conformation of the model compound (**294**, **Scheme 106**, **Section 4.2.2**) was established to be:



The most stable conformation of the β-hydroxy ester protected as acetal (**365a**, **Scheme 113**, **Section 4.2.6**) was established to be: