

Synthesis of Reference Standards for Elucidating
Mechanisms of Anaerobic Alkane Degradation

Amy Rebecca Roberts

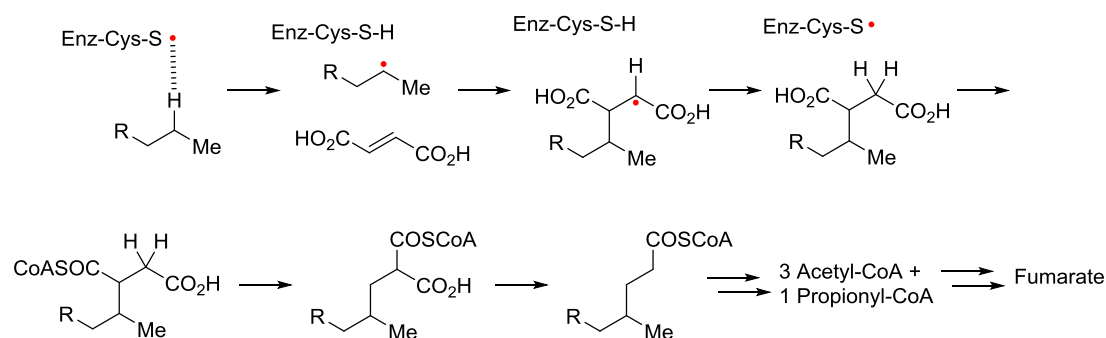


Newcastle University

2016

Abstract

Hydrocarbons are the second most prolific compound class found naturally and are used by bacteria as a source of energy in remarkable ways. Until quite recently it was believed that bacteria could only catabolise hydrocarbons in the presence of dioxygen. It has now been established that with the assistance of an electron acceptor, bacteria can break down these relatively inert compounds in anoxic environments using fumarate as a surrogate for O₂ in a process mediated by a glyceryl radical enzyme (Scheme 1).



Scheme 1-1: General scheme for alkane activation by fumarate addition (Enz-Cys. = glyceryl radical enzyme; CoA = coenzyme A).

The β -proteobacteria HxN1 is an anaerobic bacterium capable of this process and can break down hydrocarbons in the range C₆-C₈. Previous studies using (2*R*,5*R*)-, (2*S*,5*S*)- and (2*R*,5*S*)-hexane-2,5-*d*₂ showed that the initial step performed by the bacterium was the stereospecific abstraction of the *pro*-S hydrogen atom by a cysteine thiyl radical from C-2 of the alkane. The resulting hexan-2-yl radical adds to fumarate to give a stabilised radical that is quenched by re-abstraction of the hydrogen from cysteine-SH to form 2-(hexan-2-yl)succinic acid. This thesis describes an extension of the HxN1 study to the anaerobic bacterium OcN1, which degrades hydrocarbons in the range C₈-C₁₂. Efficient methods were developed for the synthesis of (2*R*,9*R*)-, (2*S*,9*S*)- and (2*R*,9*S*)-decane-2,5-*d*₂ for microbiological studies.

Naphthalene degradation occurs under anaerobic conditions either by carboxylation to 2-naphthoic acid or by methylation followed by addition of fumarate to give 2-

(naphthalen-2-yl)succinic acid. A route has been developed which yields both $^{13}\text{C}_{10}$ -naphthalene and $^{13}\text{C}_{11}$ -2-naphthoic acid to aid in metabolite analyses.

A method by which a variety of alkylsuccinates could be synthesised was initially developed [1] and optimised in the present study. This route involved the addition of HI across an alkene double bond to form an alkan-2-yl iodide. The iodide was converted into an alkyl radical that was reacted with dimethyl fumarate to afford an alkylsuccinate in a biomimetic transformation. The method was employed for the synthesis from heneicos-1-ene of the C_{21} alkylsuccinate, dimethyl 2-(heneicosan-2-yl)succinate, which was required as a reference standard for studies of pasteurised control microsomes from mud taken from the River Tyne. It was found through 2D-GC-MS analysis that a significant amount of the C-3 alkylsuccinate was formed as well as other isomers, which can be ascribed to the reversibility of the HI addition to heneicos-1-ene. An alternative synthesis was developed that circumvented the problematic step. This synthesis involved the O-methanesulfonate from heneicosan-2-ol, which was reacted with malonate anion and further alkylated to form a tricarboxylate that was subjected to Krapcho decarboxylation to yield the alkylsuccinate in good overall yield without any regioisomers. This method also allowed for control of stereochemistry.

It has been proposed that the initial hydrogen abstraction by HxN1 from hexane and fumarate addition is a concerted process rather than a stepwise pathway with a distinct hexan-2-yl radical formed. To investigate this question a six cyclopropane substrate analogues were synthesised *via* Simmons-Smith reactions of the corresponding alkenes. Unfortunately, these potential substrates proved to be toxic to the organism.

Acknowledgements

The work documented in this thesis was supported by a Deutsch Forschungsgemeinschaft (DFG) grant for which I am most thankful for the opportunity to gain such invaluable experience in this field. My incredibly patient and kind supervisor, Professor Bernard Golding has provided me with the support, guidance and advice I could have ever wanted and for that I cannot thank him enough. I would like to thank the past members of his research group who have helped and supported me throughout my research along with the past and present members of NewChem Technologies Ltd. I would also like to thank Dr Corrinne Wills for all of her help and support with NMR spectroscopy. All mass spectrometry data was conducted by the EPSRC UK National Mass Spectrometry Facility at Swansea University who's contributions are gratefully acknowledged.

A massive thank you to my parents and sister who have always been there for me no matter what and all of my amazing friends who have supported me through the process. Hopefully now we can discuss things other than my thesis!

Declaration

The work described in this thesis was performed at Newcastle University School of Chemistry in the period July 2012 to September 2015 thanks to funding provided by the Deutsch Forschungsgemeinschaft (DFG). To the best of my knowledge the work is original and acknowledgement of other work previously performed or published has been referenced and acknowledged. The work has not previously been submitted either in whole or in part, for any other degree.

Abbreviations

AIBN	Azo- <i>bis</i> -isobutyronitrile
ASS	Alkylsuccinate synthase
ATP	Adenine triphosphate
BCR	Benzoyl CoA reductase
BSS	Benzyl succinate synthase
BTEX	Benzene, toluene, ethylbenzene and xylene
d	Doublet
DHNCR	Dihydronaphthoyl CoA reductase
DiBAI	<i>Di</i> -isobutyl aluminium hydride
Diglyme	Ethyleneglycol dimethyl ether
DMeF	Dimethyl fumarate
EBDH	Ethylbenzene dehydrogenase
EPR	Electron paramagnetic resonance
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
GC-MS	Gas chromatography mass spectrometry
IR	Infrared
LC-MS	Liquid chromatography mass spectrometry
m	Multiplet
MAS	Methylalkylsuccinate synthase
MPLC	Medium pressure liquid chromatography
NCR	Naphthoyl CoA reductase
NMR	Nuclear magnetic resonance
s	Singlet
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SAM	S-adenosylmethionine
TBDPSCI	<i>Tert</i> -butyldiphenylsilyl chloride

TFA	Trifluoroacetic acid
TFE	2,2,2-Trifluoroethanol
THF	Tetrahydrofuran
THNCR	5,6,7,8-Tetrahydronaphoyl CoA reductase
TsCl	<i>p</i> -Toluenesulfonyl chloride
TTMSS	Tris(trimethylsilyl)silane
v/v	Volume to volume

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Chapter 1 Anaerobic Alkane Degradation

1.1 Introduction to Hydrocarbons

Hydrocarbons are one of the most profuse compound classes in our environment and are most abundant in anoxic milieu such as soils, sediments and deep subsurface settings.^[2] They are components of petroleum and also arise naturally from the metabolic processes of some organisms, for example, some organisms utilise biosynthetic hydrocarbons as a water barrier or as sex attractants. They make up a large fraction of crude oil, which comprises, on average, 80% saturated and aromatic hydrocarbons by mass,^[3] present in four major fractions: saturated hydrocarbons, such as hexane and octane; aromatic compounds, for example benzene and toluene; resins, for instance mastic and turpentine, and asphaltenes such as bitumen.^[4]

Hydrocarbons are vital to everyday life. Without their ready availability, transport, industry and almost every other process we have grown reliant upon in human civilisation would cease. It is estimated that there are 10^{12} tonnes of hydrocarbons formed over huge periods of time stored in worldwide reservoirs.^[2] The world as a whole annually uses an estimated 4.8 km^3 (around 30 billion barrels) of crude oil. By volume, the biggest crude oil products are fuel oil and petrol. Oil accounts for a large percentage of the World's energy consumption, with the Middle East gaining 53% of its energy from crude oil. The United States, however, is responsible for approximately 25% of all oil use worldwide.

The burning of fossil fuels as an energy source releases CO_2 , which is known to cause catastrophic damage to the environment. In 2012 it was estimated that 34 billion Mt, where $\text{Mt} = 10^9 \text{ kg}$, of CO_2 was released by burning fossil fuels, with the United States, European Union and Japan accounting for almost a third of this value^[5]. Due to the abundance of crude oil components and their wide distribution, it has been essential for Nature to develop methods of degrading these compounds *via* microorganisms to prevent their excessive accumulation^[6] (Figure 1-1).

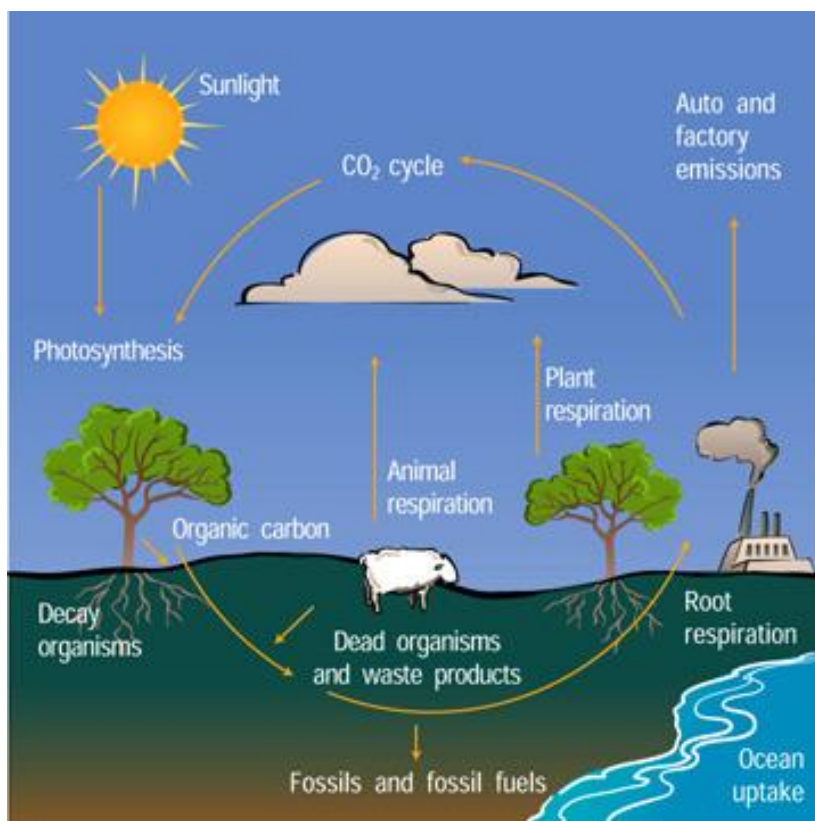


Figure 1-1: Carbon cycle diagram, taken from source.^[7]

Hydrocarbons can be sorted into four main structural types subject to the bonding features they exhibit. These groups are alkanes, alkenes, alkynes and aromatics (mono or polycyclic). The alkane, alkene and alkyne classes can be referred to as aliphatic, and these compounds can then be further defined as straight chain (*n*-alkanes), branched and cyclic aliphatic compounds.^[3] The highly apolar nature of alkanes is a direct result of their exclusive composition of C-H and C-C bonds. Due to their relative chemical inertness the introduction of functional groups is a vital feature of their metabolism to allow further processes to occur.^[8]

1.2 Anaerobic Metabolism of Hydrocarbons

Until recently it was believed that hydrocarbons could only be metabolised aerobically. This oxidative process can be accomplished by monooxygenase enzymes, which use iron-oxo species derived from an iron-haem complex and dioxygen, incorporating one oxygen atom into an alkane to form an alcohol.^[9] This intermediate can then be oxidised further to a fatty acid, which is degraded *via* the β -oxidation pathway.^[10] In the 1990s it was shown that *n*-alkanes can be metabolised

under strictly anoxic conditions using sulfate-reducing bacteria,^[11] denitrifying bacteria,^[12] under methanogenic conditions^[13] and by chlorate reducing bacteria.^[14] In recent years extensive research has been undertaken to aid our understanding of the biological mechanisms employed in the degradation of crude oil in natural reservoirs and in bioremediations of crude oil in contaminated areas, where anaerobic organisms are vital. These studies have included the isolation and characterisation of novel microorganisms capable of growing with linear, branched, cyclic and aromatic hydrocarbons as the exclusive source of carbon in the presence of an electron acceptor, such as sulfate, nitrate or chlorate^[4]

Further understanding of the mechanistic pathways of anaerobic alkane degrading enzymes could lead to improved chemical methods for introducing functional groups into alkanes, selectively producing reactive compounds such as alcohols, epoxides and carbonyl compounds. Current methods are often non-selective, expensive and use hazardous chemicals. Interest in this field is also growing due to bioremediation efforts following such environmental disasters as the Deepwater Horizon oil rig explosion, which led to the release of 780,000 m³ of crude oil into the sea (Figure 1-2).

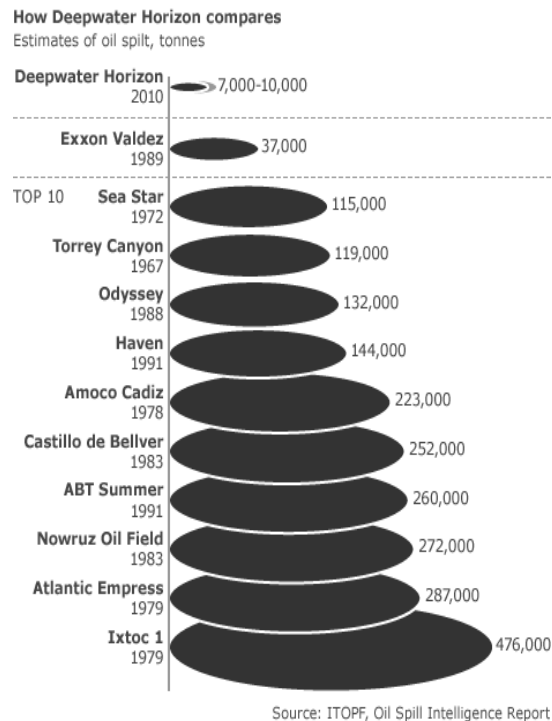


Figure 1-2: Comparison of relatively recent major oil spills in order of size measured in tonnes of oil spilt taken from source.^[15]

Accidental oil spills in the marine environment attracts much attention because of the catastrophic effects on the local environment.^[16] When oil spills in the sea, a small fraction of the lower boiling components of the crude oil will evaporate into the atmosphere and some will sit on the surface of the water in a slick causing damage to wildlife such as birds, but the majority of the spill will be pushed to the bottom of the sea in a giant plume where anaerobic organisms will break the hydrocarbons down over time (Figure 1-3).

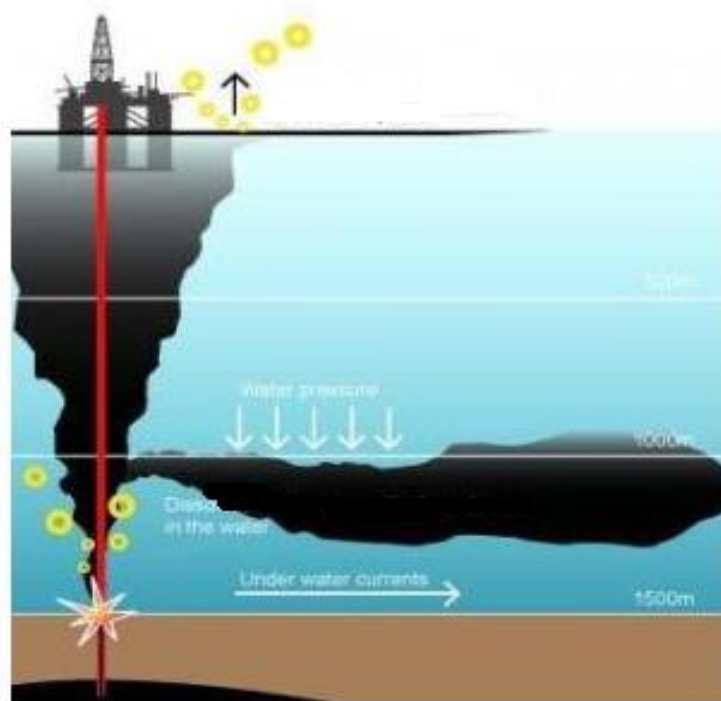


Figure 1-3: Simplified diagram of immediate distribution of oil after a spill at sea, taken from source.^[17]

Bioremediation is the use of selective microorganisms to metabolise contaminants in order to reduce their concentrations. Shorter chain alkanes ($C < 12$) are the most toxic crude oil components in aqueous environments due to their greater solubility in water^[4] They have also been shown to display negative effects on cell membranes.^{[18],[9]} Due to the low solubility of crude oil, degradation mainly occurs at the surface of the oil plumes, and as the oil is inherently low in phosphorus and nitrogen, natural microbial growth is often insubstantial.^[19]

1.3 Mechanisms of Anaerobic Hydrocarbon Activation

Two broad mechanisms of anaerobic hydrocarbon activation have been proposed: carboxylation of the C-2 position of the carbon chain and the glycy radical-mediated addition of a fumarate (**1**) molecule (Figure 1-4) primarily at C-2,^[8] the latter being the most common process occurring in anaerobic organisms.

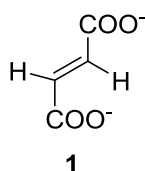
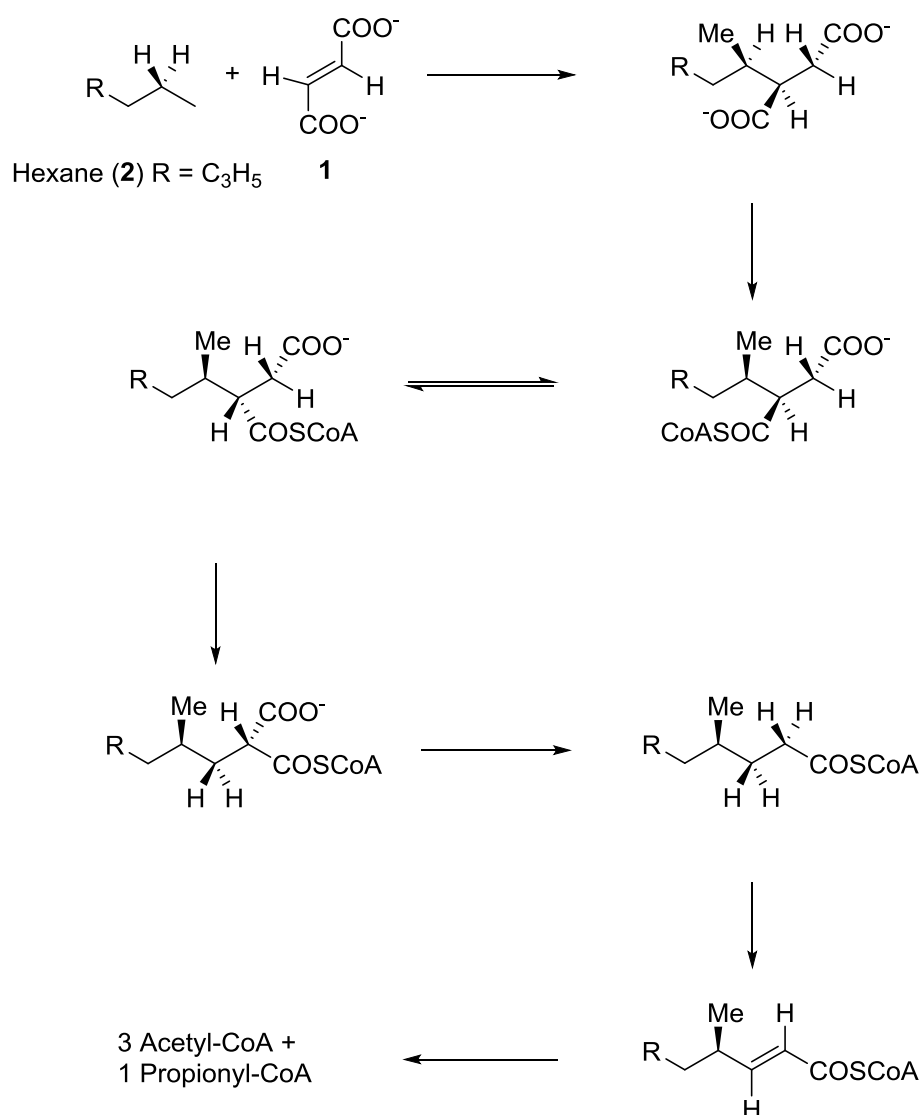


Figure 1-4: The fumarate molecule in biological systems

1.3.1 Fumarate (**1**) Activation

Owing to the apolar nature of hydrocarbons, homolytic mechanisms of substrate activation are generally employed in their metabolism. Consequently, it is common for anaerobic bacteria to selectively abstract a hydrogen atom from C-2 of the *n*-alkane using alkylsuccinate synthase (ASS) or methylalkylsuccinate synthase (MAS), both of which are glycy radical mediated enzymes (Section 1.3.1.1).^[20] This mode of attack is consistent with the relative stabilities of alkyl radicals, i.e. secondary > primary.^[21] The resulting alk-2-yl radical attacks the π bond of a fumarate molecule (**1**) to produce an alkylsuccinic acid, which is then converted into a coenzyme-A thioester. Subsequent carbon skeleton rearrangement and oxidation eventually yields fatty acid CoA esters, propionyl-CoA and acetyl-CoA in the case of hexane (**2**) degradation (Scheme 1-2).^[22]

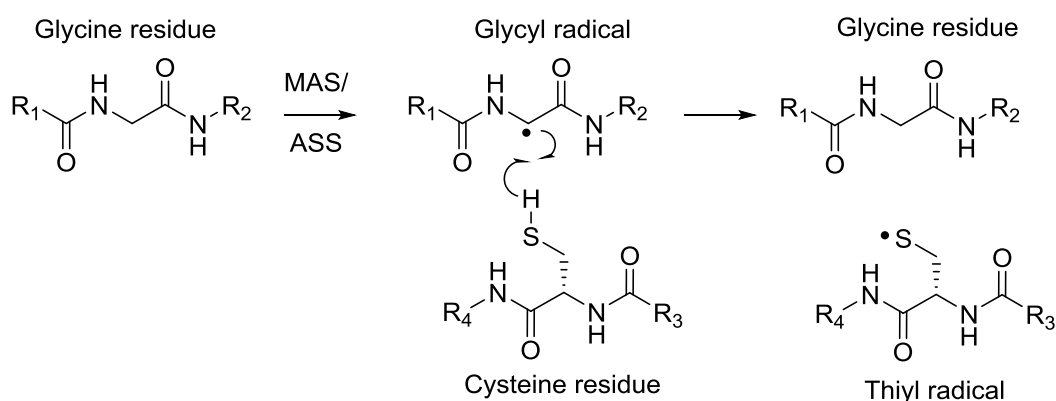


Scheme 1-2: General scheme for alkane activation by fumarate (**1**) addition.

1.3.1.1 Glycyl radical enzymes

Some proteins utilise the high reactivity of radicals to catalyse challenging reactions.^[23] Owing to the exclusive C-C and C-H σ -bond composition of hydrocarbons, glycyl radical enzymes are employed for their activation in anaerobic systems.^[20] Glycyl radicals are relatively stable storage radicals (when compared to other radical species), which are not directly involved with the catalysis, but instead are responsible for the generation of the working radical.^[23] For example, benzylsuccinate synthase (BSS) utilises a glycyl radical enzyme to form a thiyl radical, which catalyses the addition of fumarate (**1**) to the methyl group of toluene

(see Section 2.5.2). Alkylsuccinate synthase (ASS) and methylalkylsuccinate synthase (MAS) also catalyse the activation of alkanes by fumarate (1) addition, for which the sulfate reducing strain AK-01 and nitrate utilising strain HxN1, respectively, are exemplars. ASS activase, a member of the S-adenosylmethionine [SAM (3)] (Figure 1-5) superfamily (see below), is responsible for the formation of the glycy radical in active site of ASS and MAS, which, as for BSS, generates a thiyl radical from a conserved cysteine residue, which then abstracts a hydrogen from the C-2 position of a hydrocarbon activating it to fumarate (1) attack (Scheme 1-3).^[20] The conserved glycine residue is close to the C terminus of one subunit of a large dimeric apo-enzyme and can be diagnosed by a characteristic EPR signal, which is the result of a coupling interaction with the remaining hydrogen on the amino acid.^[24]



Scheme 1-3: Activation of cysteine residue by MAS/ASS enzymes *via* activation of a glycine residue of the active site.

SAM (3) is synthesised in Nature from ATP and L-methionine, and within the SAM (3) radical superfamily radicals are generated by the reductive cleavage of a C-S bond mediated by a [Fe₄S₄] cluster to produce 5'-deoxyadenosyl radicals (3•) (Figure 1-5).^[25]

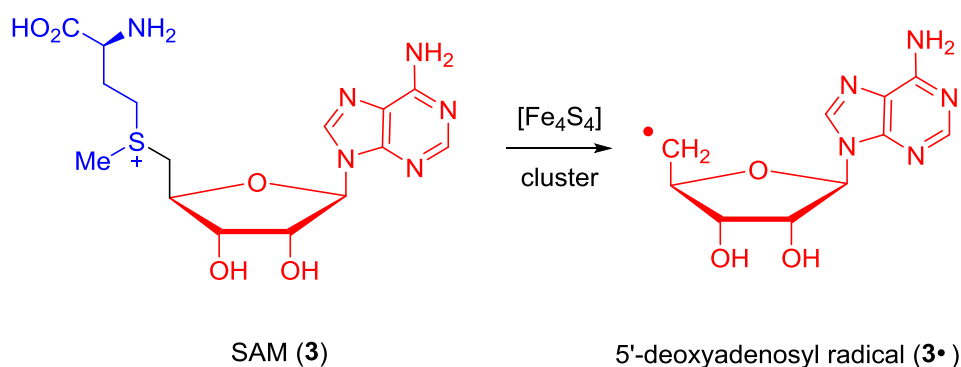
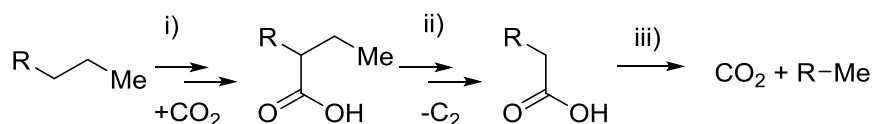


Figure 1-5: Conversion of SAM (3) (methionine moiety in blue, 5'-deoxyadenosyl in red) to a 5'-deoxyadenosyl radical (3•) catalysed by a [Fe₄S₄] cluster.

1. 3. 2 Carboxylation Activation

With regard to the carboxylation mechanism of activation of alkanes, conversion to fatty acids *via* selective addition of CO₂ from carbonate at C-3 followed by the loss of two methylene groups from the alkane chain produces C-odd fatty acids from C-even alkanes and *vice versa*. Numerous techniques have been used to elucidate the manner by which this occurs, and the current theory is shown below (Scheme 1-4).^[26] The method is further described for aromatic systems in Section 2.5.



Scheme 1-4: Postulated scheme for alkane activation by carboxylation; i) carboxylation at C-3; ii) Loss of C-1 and C-2; iii) β -elimination and further catabolism.

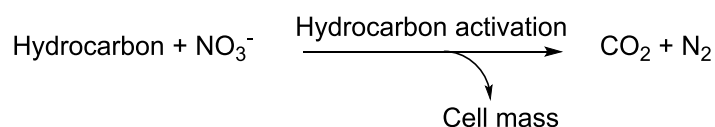
1. 4 Nitrate Utilising Microorganisms

Nitrate reducing anaerobic bacteria have been suggested as possible aids for bioremediation efforts, such as clean-up operations of oil spills in oceans and various contaminated systems such as aquifers. The metabolism of hydrocarbons by nitrate reducing bacteria was first conclusively demonstrated in 1996 using alkylbenzenes as substrates.^{[27],[4]}

Benzene, toluene, ethylbenzene and xylene (BTEX) are common components of crude oil. They have a greater solubility in water (1780, 535, 152 and 6 mg l⁻¹ respectively)^[28] than other hydrocarbons and are considered a major contaminant

with regards to groundwater. As they primarily contaminate anoxic areas, there is great interest in anaerobic bacteria capable of catabolising these substrates, along with the other components of crude oil. Bacterial strains capable of the biodegradation of these compounds under anoxic conditions with nitrate as the electron acceptor have been isolated.^{[27],[12]} The known nitrate utilising bacteria with the capability of degrading alkylbenzenes belong solely to the β -subclass of *Proteobacteria*. With regard to alkanes, nitrate utilising bacteria are known for both the β -subclass and γ -subclass of *Proteobacteria*.^[12]

Nitrates are favourable electron acceptors in aqueous environments as they are highly water soluble (1840 mgL^{-1}) and so are capable of getting to the source of the contamination quickly and easily. Reduction of nitrate is an energetically favourable process, helping drive the breakdown of the hydrocarbons in the contaminated zones (Scheme 1-5).^[4] Further knowledge of the mechanisms by which the nitrate utilising microorganisms function could increase the effectiveness of these bioremediation efforts.



Scheme 1-5: General scheme for hydrocarbon metabolism by anaerobic organisms utilising nitrate as an electron acceptor.^[3]

A study performed with the aim of determining the range of hydrocarbons that can be metabolised by denitrifying bacteria found that the growth patterns of the bacteria are biphasic. In the first phase, alkylbenzenes were primarily consumed due to the relatively higher water solubility. The second phase involved emulsification of the oil by partial adherence of the bacterial cells, allowing the less soluble alkane components to be metabolised. The exception to this situation was the denitrifying bacterium HxN1, which grew homogeneously in the aqueous medium indicating that its preferred substrate hexane (**2**) is soluble enough to diffuse through the aqueous phase. However, the study was not fully comparable with the natural conditions as the experiment optimised the hydrocarbon availability, nitrate and phosphate concentrations and vitamin supply. The study may also have excluded some bacteria

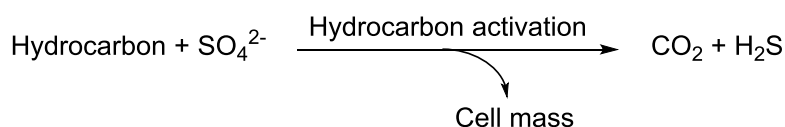
which could have competitively metabolised the hydrocarbons that persisted in the sample. It also did not accommodate for bacteria with relatively slow growth rates or a need for attachment to mineral surfaces. The study did, however, provide an indication of the capabilities of the anaerobic nitrate utilising bacteria with regard to crude oil biodegradation. Calculations were performed to estimate the anaerobically consumed fraction of crude oil in the experiment, which showed that approximately 3.1 % (by volume) of the crude oil was biodegraded by the nitrate reducing bacteria.^[12]

Examples of alkane metabolising nitrate reducing bacteria include HxN1, OcN1, and HdN1, which were all isolated from ditch sediments and preferentially metabolise hexane (**2**), octane and hexadecane respectively. In growth tests aimed at determining the degradability of alkanes under anaerobic conditions with nitrate as the sole electron acceptor using HxN1, OcN1 and HdN1 as the consumers, it was found that alkanes with chain lengths C₆-C₈, C₈-C₁₂ and C₁₄-C₂₀ were degraded, respectively, showing that the bacteria display a high degree of selectivity.^[9]

The bacterial strain associated with the preferential degradation of cyclic alkanes has not yet been identified. A study was conducted aiming to establish which nitrate utilising anaerobic bacteria were involved in the degradation of cycloalkanes using cyclohexane as the substrate. Freshwater sediments were incubated with nitrate as the electron acceptor and cyclohexane as the potential substrate in order to enrich samples of anaerobic cyclohexane degrading bacteria. Analysis of metabolites showed the presence of cyclohexylsuccinic acid, indicating the anaerobic activation of the hydrocarbon proceeds *via* the addition of fumarate (**1**). In comparison to alkanes, for which nitrate utilising bacteria are known of both the β -subclass and γ -subclass of *Proteobacteria*,^[12] the dominant bacterial strain associated with the nitrate utilising degradation of cyclohexanes appears to belong to the δ subclass.^[29] The biodegradation of alkanes under anaerobic conditions is not limited to straight chain and cyclic structures. Pristane (2,6,10,14-tetramethylpentadecane), a heavily branched, saturated terpene alkane has also been proven as a substrate for this class of microorganism.^[30]

1.5 Sulfate Utilising Microorganisms

Microorganisms that use sulfate ions as electron acceptors (Scheme 1-6) are thought to have evolved due to the natural production of sulfate and low concentrations of other electron accepting species such as oxygen, nitrate and ferric iron in hydrocarbon rich environments such as petroleum reservoirs and crude oil contaminated sediments on the ocean bed. Sulfate reduction is a major metabolic process which occurs in these environments, along with methanogenesis and fermentation. The process has caused problems in commercial oil manufacture as it releases hydrogen sulfide, which reduces the quality of the oil, is corrosive to the steel structures and is toxic to workers.^[31] Molecular hydrogen accumulates on the surface of the metal due to interactions between water and the steel. The hydrogen is then oxidised by the sulfate reducing microorganisms to produce hydrogen sulfide.^[32] Souring of the crude oil is also an issue when sulfate contaminated water is introduced to reservoirs to re-pressurise systems in secondary oil recovery.^[33]



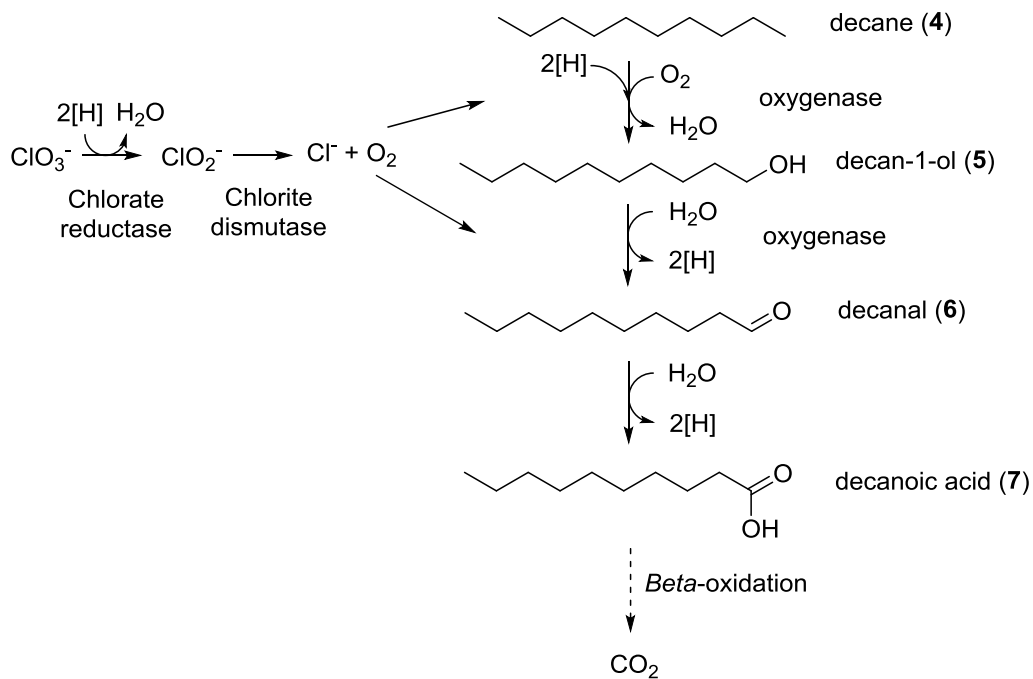
Scheme 1-6: Overall transformation for hydrocarbon metabolism by anaerobic organisms utilising sulfate as an electron acceptor.^[3]

Sulfate reducing bacteria are primarily associated with the *δ-proteobacteria* family of microorganisms, compared to nitrate reducing anaerobic bacteria which consist mostly of microorganisms belonging to the *β*- and *γ*- subclasses. Various mesophilic (i.e. growing best at ambient temperatures) forms of these sulfate reducing bacteria have been published, all of which derive from anaerobic environments with copious amounts of hydrocarbons, such as marine hydrocarbon seeps. They have been shown to biodegrade straight chain hydrocarbons of variable lengths, from C₃-C₂₀. However, very few studies have been conducted to investigate their catabolism of branched or cyclic hydrocarbons. The breakdown of ethylcyclopentane was reported to have been mediated by sulfate reducing bacteria showing that these microorganisms are capable of such processes. Some mesophilic forms have been shown to be capable of metabolising alkenes, although this is not thought to be a property of all sulfate utilising bacteria.^[4]

A study was performed to test the promiscuity of the sulfate reducing bacterium Hxd3. It was found that the bacterium was capable of growing on alkanes with chain lengths C₁₂-C₂₀, although the minimum chain length used in the growth test was C₁₂, so it could be more versatile.^[11] Another study conducted with Pnd3, which preferentially biodegrades alkanes of chain lengths C₁₄-C₁₇, alongside Hxd3, showed that metabolism by both bacteria is stimulated by the presence of α-cyclodextrins, which help deliver the hydrophobic substrate to the organisms.^[11] A further example of this class of anaerobic bacteria is AK-01 whose growth has been shown to be supported by alkanes of lengths C₁₃-C₁₈, along with 1-alkenes and primary alcohols with chain lengths C₁₅ and C₁₆. Its growth was directly dependent of sulfate reduction, and cannot be supported by different electron accepting species such as nitrate or chlorate.^[26]

1.6 Chlorate Utilising Microorganisms

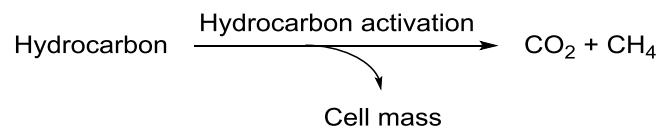
Due to their high redox potentials, chlorate and perchlorate ions make excellent electron acceptors for anaerobic organisms degrading hydrocarbons.^[4] The first reported bacterium capable of utilising chlorate as an electron acceptor was *Pseudomonas chloritidismutans* AW-1^T, which is capable of growing on alkanes of chain lengths C₇-C₁₂ under strictly anoxic conditions. In depth studies were conducted using decane (**4**) which suggested that chlorate is reduced to chlorite ([ClO₃]⁻ → [ClO₂]⁻) by a chlorate reductase enzyme followed by conversion into chlorine and molecular oxygen ([ClO₂]⁻ → Cl₂ + O₂) by chlorite dismutase enzymes. It was postulated that the molecular oxygen produced then degrades the alkanes *via* oxygenase enzymes. It was also discovered that nitrate cannot be used as an alternative electron acceptor in specific steps in the process, further indicating the use of oxygenases. Decane (**4**) is converted into decan-1-ol (**5**), and then into decanal (**6**), by chlorate dependent steps. The aldehyde is then converted to decanoic acid (**7**) *via* a chlorate independent step. The acid is further degraded by β-oxidation, ultimately to produce carbon dioxide (Scheme 1-7).^[26]



Scheme 1-7: Proposed scheme for degradation on decane (4) by AW-1^T with chlorate as the electron acceptor.

1.7 Methanogenesis of Hydrocarbons

Methanogenesis is the means by which methane and carbon dioxide are generated by anaerobic organisms during the catabolism of hydrocarbons in the absence of suitable electron accepting species such as sulfate or nitrate (Scheme 1-8).^{[14][3]} The process is generally observed in environments where such electron donating species have been depleted, such as in oil reservoirs, coal deposits, groundwater aquifers and deep sediments, and is thought to be one of the main processes responsible for the formation of heavy oil deposits and oil sands. An example of methanogenic sediment analysis is discussed in Section 1.10.^{[2],[34]}



Scheme 1-8: General scheme for hydrocarbon metabolism by anaerobic organisms to produce methane.

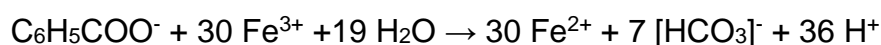
Methanogenesis is a strictly anaerobic process and oxygen has been shown to inhibit the growth of methanogenic species. The carbon source required to act as a replacement of the traditional electron donating species can come from a number of relatively small molecular weight carbon sources such as carbon dioxide and acetic acid, which are metabolites of the degradative process, allowing the methanogens to work catalytically. In terms of anaerobic respirations, it is the least thermodynamically favourable and is only generally observed after the depletion of electron donating species (A. Sherry, 2013; F. Mehboob, 2011).^{[25],[14]}

An investigation involving an enrichment culture formed from anoxic ditch sediment growing on hexadecane as the primary substrate under strictly anaerobic conditions was conducted in order to study the process of methanogenesis with regard to alkanes. Pentadecane was shown to support the culture's growth and overall methane production. However, decane (4) and hexane (2) could not support growth, showing that the bacteria in the sample display substrate specificity for pentadecane against decane (4) and hexane (2). Analysis of the gas composition in the incubation vessel found as expected that methane was the primary component. Low levels of hydrogen and acetate were found, which is anticipated as they are key metabolic substrates in the process. They are, however, transient, which prevents their accumulation, unlike methane.^{[25],[13]}

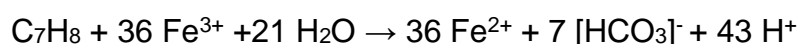
In a recent study, it was found that the methane production rate was highly dependent on the hydrocarbon source present. Cultures sourced from Chinese oil reservoirs degraded shorter hydrocarbons (C₁₀-C₃₆) readily, with a methane production rate of $76 \pm 6 \mu\text{mol/day/g}$. A microbial diversity analysis was performed which showed that similar prokaryotic bacteria are commonly associated with methanogenic communities including the Firmicutes, Proteobacteria, Bacteroidetes and Spirochaetes families. Clostridiales and Syntrophobacteriales are associated with the initial activation *via* conversion to H₂, CO₂ and acetate. The conversion of acetate to methane and CO₂ requires acetoclastic methanogens such as *Methanosarcina* and *Methanosaeta*, whilst the conversion of CO₂ and H₂ to methane is performed by hydrogenotrophic methanogens such as *Methanobacterium*.^{[2],[34]}

1.8 Ferric Iron Reduction of Hydrocarbons

Fe(III) is the most abundant electron acceptor found in anaerobic environments. It has been shown that Fe(II) accumulates in pristine contaminated aquifers which display evidence of anaerobic degradation by bacteria, setting the precedent for organic metabolism in numerous anoxic environments. It has been shown, firstly by Lovely *et al.*, that benzoate, toluene, phenol and *p*-cresol can be activated, by an unknown mechanism in the presence of Fe(III) by a pure culture of the Fe(III) reducing bacterium GS-15, an iron reducing bacteria which is more commonly known for degrading fatty acids and ethanol. It can adapt to grow on benzoate (Scheme 1-9), toluene (Scheme 1-10), phenol and *p*-cresol after a short adaptation period.^[36]



Scheme 1-9: Stoichiometric reduction of benzoate by GS-15.



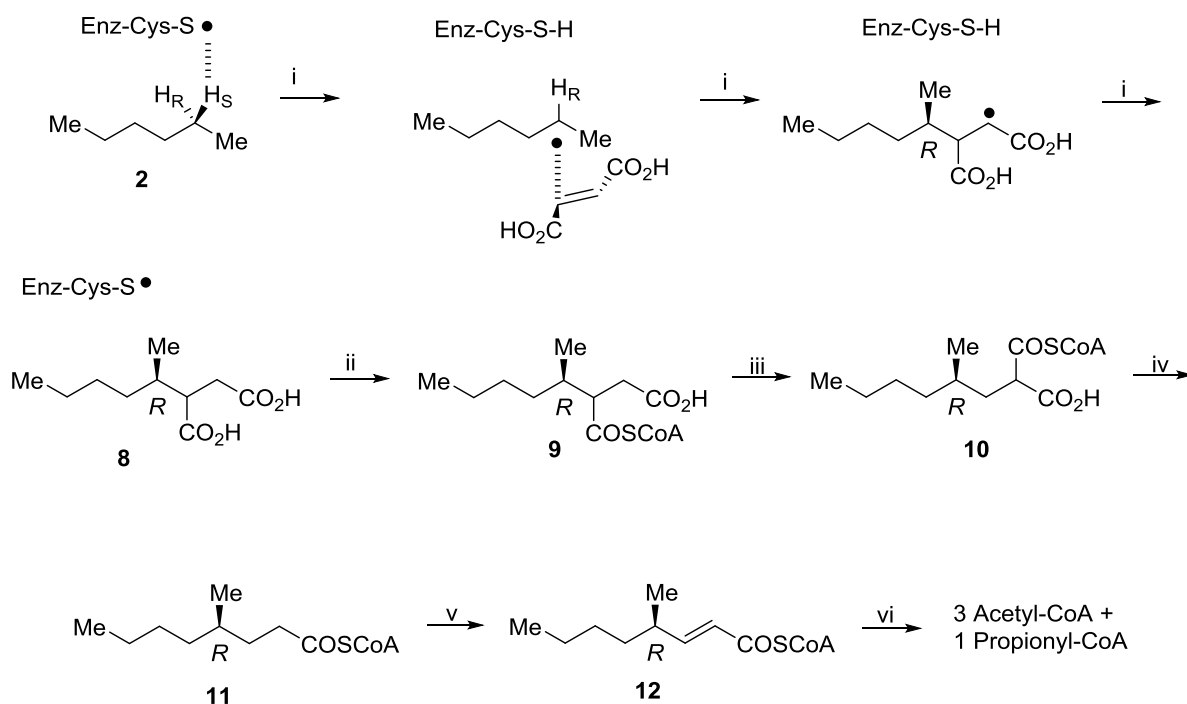
Scheme 1-10: Stoichiometric reduction of toluene by GS-15.

1.9 The Denitrifying Betaproteobacterium Strain HxN1

HxN1 derived from the bacterium *Aromatoleum* sp. has proved an excellent model organism with which to conduct mechanistic studies. In comparison to a number of other alkane metabolising anaerobic bacteria, HxN1 can be harvested easily, grows rapidly, and metabolises alkanes with relatively short chain lengths, C₆-C₈, whereas other known strains generally degrade alkanes with chain lengths C₈-C₁₈.^[8]

Previous studies have shown that strain HxN1 proceeds by the glyceryl radical hydrogen abstraction method (Section 1.3.1). Electron paramagnetic resonance (EPR) spectroscopy conducted on cells growing on hexane (**2**) indicated the presence of a glyceryl radical enzyme in the bacterial cells (Section 1.3.1.1).^[8] Analysis of the metabolites found in cells also showed the existence of 2-(hexan-2-yl)succinic acid (**8**), further validating the oxidation pathway. It was postulated that the 2-(hexan-2-yl)succinic acid (**8**) is further degraded by conversion to a coenzyme-A thioester (**9**), followed by an intramolecular rearrangement to form (2-methylmalonyl-CoA) (**10**), which undergoes decarboxylation to produce 4-methyloctanoyl-CoA (**11**). This

intermediate is broken down by dehydrogenation and β -oxidation to produce three acetyl-CoA and one propionyl-CoA molecules (Scheme 1-11).^[22]



Scheme 1-11: Postulated oxidation scheme of hexane (**2**) with the β -proteobacterium strain HxN1. i) (1-methylalkyl)succinate synthase; ii) (1-methylalkyl)succinate-CoA ligase; iii) (1-methylalkyl)succinyl-CoA epimerase; iv) (2-methylalkyl)malonyl-CoA mutase; v) (2-methylalkyl)malonyl-CoA decarboxylase; vi) 4-methylalkanoyl-CoA dehydrogenase.

The abstraction of a hydrogen from an alkane is an intrinsically difficult process. To improve understanding of this complex mechanism, a number of stereochemical studies have been performed by the Newcastle group.^[2] Metabolite analysis of cells grown on hexane (**2**) showed two diastereoisomers of the metabolite 2-(hexan-2-yl)succinic acid (**8**) present in the cell cultures. To investigate this apparent stereochemical imperfection in the hydrogen abstraction process, a culture of HxN1 was incubated with dideuterated hexane (**2**). It was shown that the deuterium is removed from the C-2 of the hexane (**2**) and transferred to the C-3 of the succinate moiety (**8**). To elucidate the stereochemistry of the two newly formed stereocentres in the 2-(hexan-2-yl)succinic acid (**8**), its 4 stereoisomers, **8a**, **8b**, **8c** and **8d** were synthesised (Figure 1-6).

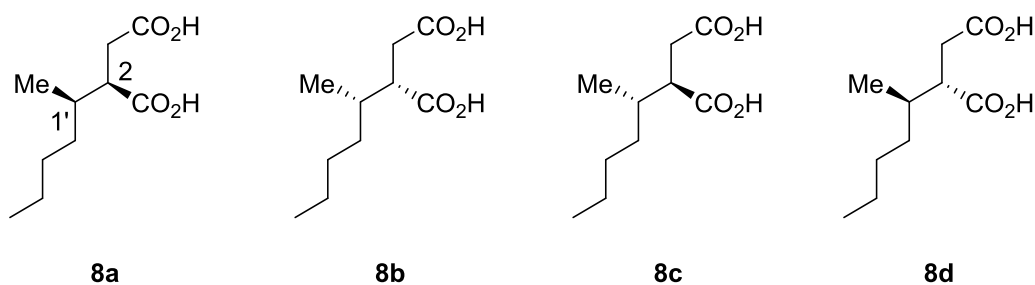


Figure 1-6: Stereoisomers of 2-(hexan-2-yl)succinic acid (**8a-d**).

GC-MS analysis of the cell cultures and the stereochemically pure standards showed an equal mixture of **8a** and **8d** present, indicating specific *R* configuration at stereocenter 1'. However, there were equal amounts of both *R* and *S* configurations at stereocenter 2. As a result of the configuration at the 1' carbon, solely (*R*)-4-methyloctanoyl-CoA (**11**) is produced downstream in the metabolic process. Building on these findings, stereochemically pure dideuterated hexanes, **2a***, **2b***, **2c***, were synthesised (Figure 1-7) for incubation experiments with HxN1.

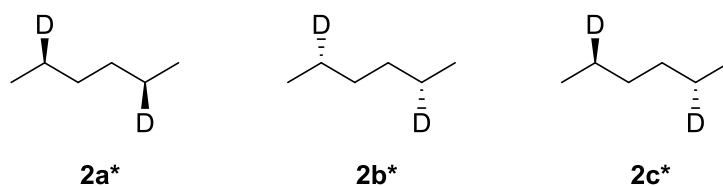
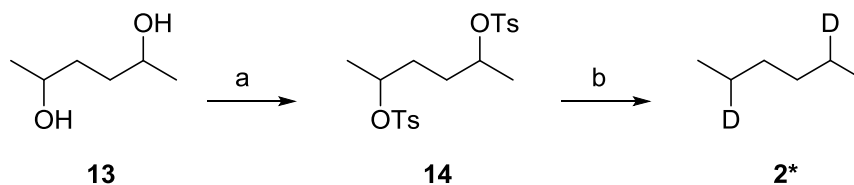


Figure 1-7: Dieuterated hexanes **2a-c***.

The synthesis was achieved by activating (*R,R*)-, (*S,S*)-, and (*R,S*)-hexane-2,5-diol (**13**) by conversion into the corresponding di-*p* toluenesulfonate (**14**) followed by reduction with LiAlD₄ in tetraglyme with inversion at each stereocenter to produce the compounds **2a-c*** (Scheme 1-12).



Scheme 1-12: Conversion of (*R,R*)-, (*S,S*)-, and (*R,S*)-hexane-2,5-diol (**13**) to stereochemically pure dideuterated hexane compounds, **2a***, **2b***, **2c***: a) *p*-toluenesulfonyl chloride, pyridine, DCM; b) LiAlD₄, tetraglyme.

Complete deuterium transfer from C-2 of the hexane (**2**) to the C-3 position of the 2-(hexan-2-yl)succinic acid (**8**) was observed with **2a***, whereas no transfer was observable with **2b***, thus indicating that the pro-S hydrogen is selectively abstracted and stereo-inversion occurred at C-2 of hexane (**2**), much like in an S_N2 type reaction, which could indicate a concerted abstraction-addition process (see Section 6-1).^[2]

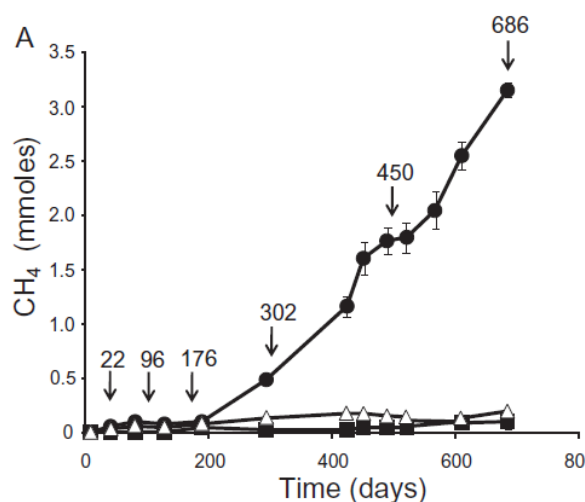
Synthesis of chemical standards of the key intermediates of this scheme are vital for providing evidence that this is indeed the mechanism by which it occurs. It is also key to indicating the stereochemical steps which take place, providing a comprehensive guide to how the bacterial strain metabolises alkanes. By synthesising substrate analogues of the hexane (**2**) we may be able to determine timescales of important steps of the degradation. Deeper understanding of the processes occurring may aid us in improved chemical methods for functionalising the relatively inert hydrocarbons, and also improve the bioremediation of hydrocarbon contaminated areas. It will also give us a deeper understanding of a relatively new and intriguing process.

1. 10 River Tyne Estuarine Sediments

The degradation of hydrocarbons under anaerobic conditions utilising sulfate as an electron donor has been widely reported (see Section 1.5).^[37] Research on this process has been undertaken at Newcastle University.^{[38],[25],[39]} Findings published in 2011 focussed around libraries of 16s rRNA genes cloned from anaerobic methanogenic oil degrading microcosms grown on crude North Sea Oil inoculated with sediments from the River Tyne in Newcastle. Methanogenesis results in stoichiometric conversion of hydrocarbons to methane and carbon dioxide *via* methanogenic substrates such as acetate (see Section 1.7). Hydrocarbons can be a major contaminant of surface and shallow environments and utilising methanogenesis as a form of biodegradation could potentially be very useful. The dominant methanogens present in the samples were *Methanocalculus* spp., hydrogenotrophs from the family *Methanomicrobiales*.

The study reported that *Deltaproteobacteria* and *Syntrophaceae* from the *Smithella* genera and, more interestingly, *Gammaproteobacteria* from the genera *Marinobacter*

sp. (*Marinobacter* sp. are more commonly associated with aerobic marine environments) were enriched during the breakdown of the crude oil. *Smithella* are known for their complete degradation of crude oil alkanes to acetate and/or hydrogen in collaboration with methanogens in near short depth anaerobic environments. Alkanes of chain lengths C-7 to C-34 were the main component of the oil which was utilised by the bacteria. It was observed that almost all of the alkanes in the sample were degraded before any significant degradation of aromatic hydrocarbons could be detected. Quantitative PCR and data on alkanes were used to determine growth factors and doubling times, which were 36 days for both the *Smithella* and the *Marinobacter* sp. The growth yield of the *Smithella* [0.020 g_(cell-C)/g_(alkane-C)] was found to be consistent with other known methanogenic hydrocarbon degrading bacteria. In contrast, the growth yield for *Marinobacter* sp. was [0.0005 g_(cell-C)/g_(alkane-C)], indicating that it played a minor role in the degradation of the alkanes. *Smithella*'s growth was exponential, which directly correlated with the exponential increase in methane and decrease in alkanes (Graph 1-1).^[38]



Graph 1-1: Methane production in the methanogenic samples discussed above (page 24) over 686 days. Closed circles = methane production in crude oil amended samples. Open triangles = methane production in non crude oil amended samples.^[38]

A related study published in 2013,^[25] looking into sulfate reducing anaerobic hydrocarbon degradation, also studied 16S rRNA genes which were prepared from

DNA extracted from sulfate reducing crude oil degrading microcosms inoculated with sediments from the River Tyne. Samples were collected when they were actively sulfate reducing (day 176, $17.7 \pm 0.9 \mu\text{mol L}^{-1} \text{SO}_4^{2-} \text{ day}^{-1} \text{ g}^{-1}$ wet sediment, approx. 50 % sulfate depletion and 60 % total alkane degradation) and when the sulfate had been depleted (day 302, approx. 77 % total alkane degradation). Bacteria from the phyla *Chloroflexi*, *Firmicutes* and *Proteobacteria* (both *Gamma*- and *Delta*-subclasses) were all enriched in the samples relative to control samples. The samples did not however contain sequences of 16S rRNA from sulfate reducing bacteria previously associated with the anaerobic degradation of hydrocarbons such as *Desulfotomaculum* and *Desulfosporomusa*. Instead, *Gammaproteobacteria* was the dominant bacteria found (34 % at day 176), most closely related to *Marinobacterium* sp., along with *Peptostreptococcaceae* from the *Firmicutes* family (27 % at day 176).

Pristane, an isoprenoid associated with vegetated environments, is degraded to heptadecane ($\text{C}_{17}\text{H}_{36}$) and the ratio of the two compounds can provide an indication of the rate of hydrocarbon biodegradation. Between days 94 and 300, a significant change in the ratio was observed which coincides with the active sulfate reducing period. In control samples with added sodium molybdate (to inhibit sulfate reduction), little to no change was observed in the pristane/heptadecane ratio proving that alkane degradation occurring in the samples was reliant on sulfate reduction. After day 302, when the sulfate had been depleted, the dominant bacteria in the community was *Chloroflexi* (24 %), *Firmicutes* (20 %) and *Deltaproteobacteria* (19 %), which is more commonly associated with sulfate reducing conditions, showing that the bacterial communities are variable and are driven by hydrocarbon elimination. After complete sulfate degradation, methanogenesis commenced (Figure 1-8). The process was slow relative to the study discussed above.^[25]

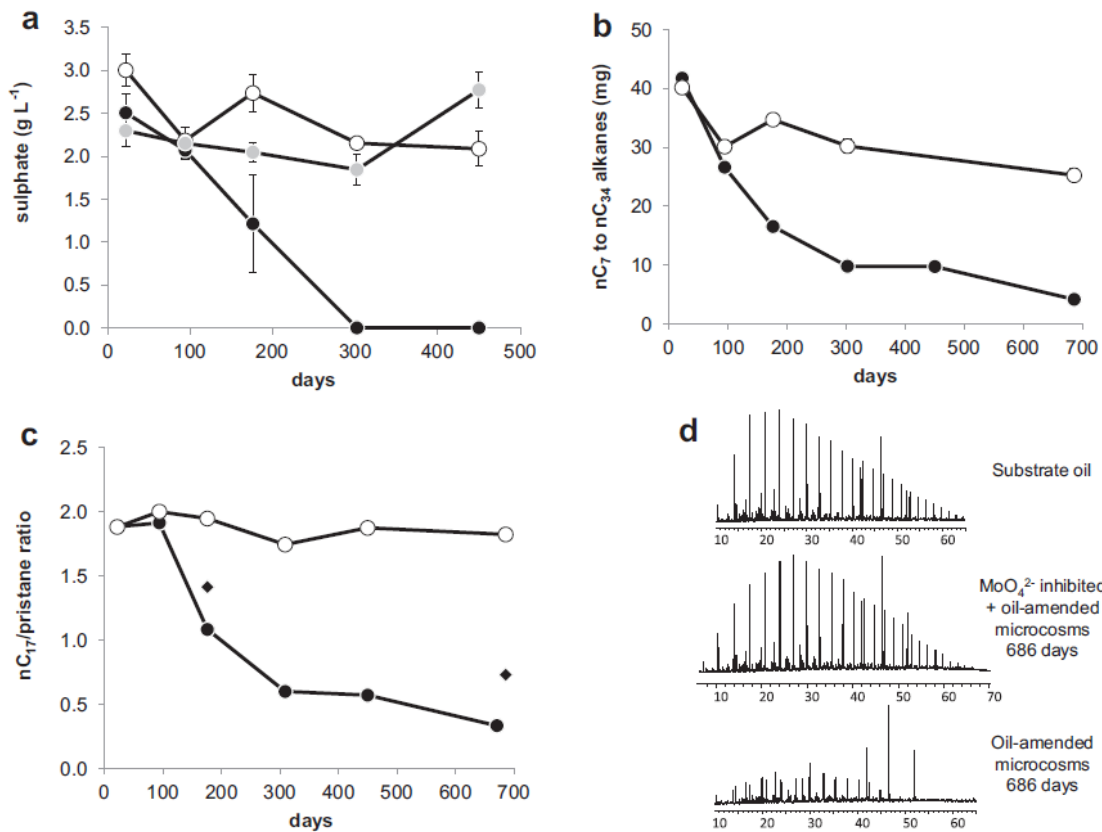


Figure 1-8: Graph a- Sulfate concentration in samples. Closed circles = oil amended samples. Open circles = oil amended samples with sodium molybdate. Grey circles = non oil amended samples.

Graph b- Depletion of alkanes. Closed circles = Oil amended samples. Open circles = oil amended samples with sodium molybdate.

Graph c- n-C₁₇ / pristane ratio. Closed circles = Oil amended samples. Open circles = oil amended samples with sodium molybdate. Black diamonds = Oil amended pasteurised samples.

Chromatograms d- Gas chromatograms of total hydrogen fractions of substrate oils, sodium molybdate inhibited samples and oil amended samples.

A further study published in 2013 provided a comparison of the alkylsuccinate concentrations detected at various time points in both the methanogenic and sulfate reducing consortia. The samples used initially contained the same quantities of North Sea Oil, nutrients and river bed sediments which contained microorganisms. One of the samples had additional sulfate to encourage sulfate reducing conditions.

Significant differences were observed between the acid metabolite profiles for the methanogenic and sulfate reducing microcosms. It is well established that under sulfate reducing conditions, alkylsuccinates are a major metabolite (see Section 1.5), and, as expected, alkylsuccinates and their respective metabolites were characterised in the sulfate reducing samples. There were not, however, significant levels of alkylsuccinates or related metabolites found in microcosms, which were under methanogenic conditions. The levels found under these conditions were concordant with those found in the control samples, suggesting that their presence was from residual sulfate in the sediment inoculum initially added to the microcosms. It was proposed that there must be a significantly different and novel method of activation for alkanes under methanogenic conditions.^[39]

As previously mentioned above, alkylsuccinates are a well-established metabolite of hydrocarbon metabolism *via* sulfate reducing microorganisms (see Section 1.3.1). Due to low levels detected in laboratory microcosm studies [e.g. approximately 0.2 – 3 nanomoles per microcosm]^[39] and in environmental studies [(e.g. approximately 10 – 170 nanomoles in 1 dm³ of contaminated groundwater samples)^[39], alkylsuccinate compounds have always been considered transient. However, much higher levels of (1-methylalkyl)succinates have been detected in microcosms (up to 650 nanomoles per microcosm), which were intended as control samples and pasteurised at 95 °C for a study aiming to look at anaerobic crude oil degradation under sulfate reducing conditions with an anoxic sediment from the River Tyne as inoculum (Figure 1-9).

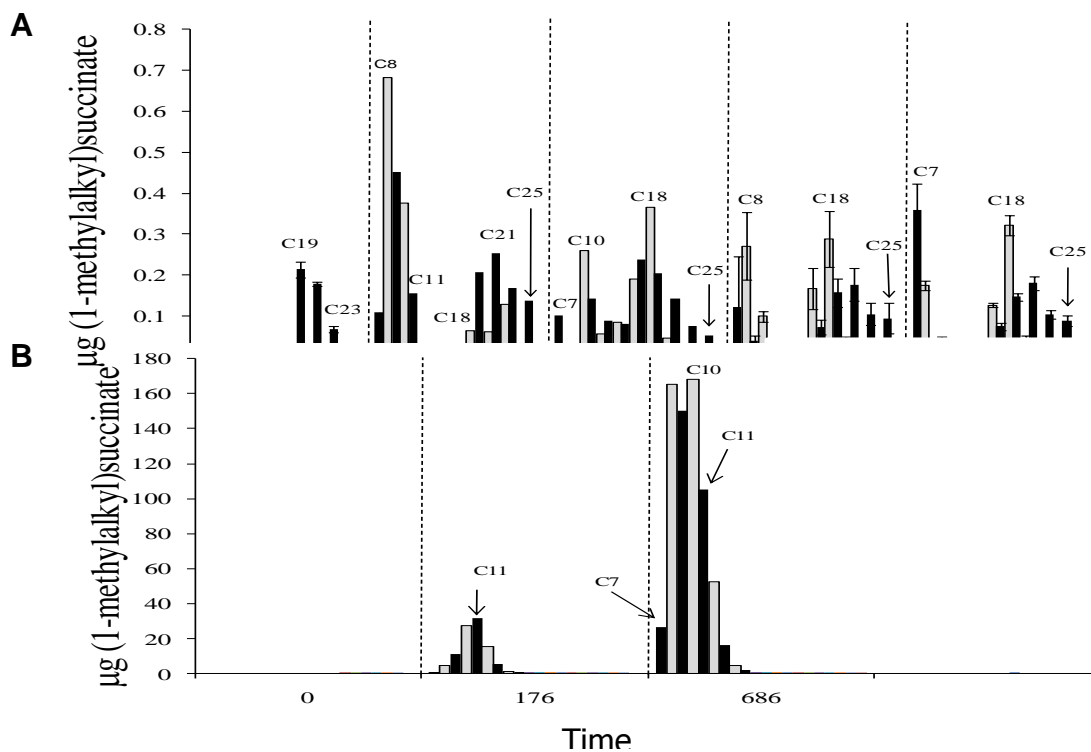


Figure 1-9: Charts depicting amounts (μg) of alkylsuccinates found in micocosms over n days. **A** shows the unpasteurised sulfate reducing micocosms. **B** shows the pasteurised samples.^[39]

A homologous series of alkylsuccinates were detected as metabolites including long chain alkylsuccinates, along with branched and cyclic alkylsuccinate metabolites. This is presumably due to thermophilic bacterial spores which have been activated due to the high temperatures during the pasteurisation. The accumulation of alkylsuccinates may be due to the denaturing of vitamin B₁₂, essential for the further processing of the alkylsuccinates, at these high temperatures. To investigate further, chemical standards of the postulated alkylsuccinates were required to substantiate these claims. Long chain alkylsuccinates have not previously been investigated in much detail, and so establishing their presence and accumulation in the pasteurised samples could lead to a deeper understanding of the downstream metabolism of alkylsuccinates in anaerobic systems (see Chapter 5).^[39]

Chapter 2 Anaerobic Degradation of Aromatic Hydrocarbons

2.1 Introduction

Owing to their natural ubiquity, hydrocarbons are a substantial growth substrate for microorganisms.^[40] Aromatic hydrocarbons mainly arise in natural environments from human interventions, such as from mineral oil spills or on former gas plant sites. However, they also arise naturally from anoxic sediments and other sources.^[41] They are abundant in ground water and aquifers, as aromatic hydrocarbons have a higher water solubility and toxicity than aliphatic hydrocarbons, methods of breaking down these compounds are of great importance.^[2] As oxygen has low water solubility (8 mg/ dm³), their catabolism in aqueous environments is often anaerobic.^[42] The hydrocarbons themselves also have limited water solubilities and are prone to adsorption on sediments; therefore, their natural attenuation is slow.^[43]

Aromatic compounds are defined by the unsaturation of a ring system and possess additional stability due to the π -electron rearrangement.^[44] They can be defined by Hückel's rule, which states that planar, fully conjugated monocyclic systems with $(4n + 2)$ π electrons have a closed shell of electrons in bonding orbitals.^[45]

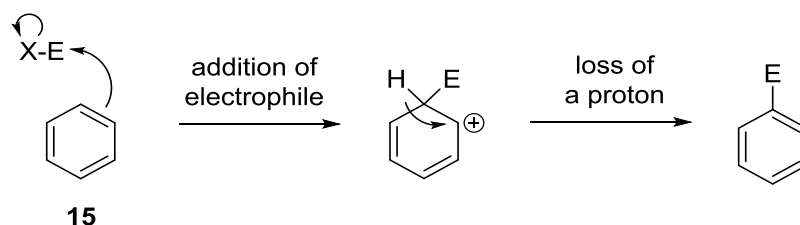
2.2 Reactivity of Aromatic Hydrocarbons

2.2.1 Benzene

Faraday was the first person to isolate benzene (**15**) in 1825 from compressed whale oil. He determined the molecular formula of benzene (**15**) by elemental composition and relative molecular mass determination.^[46] Kekulé proposed the cyclic structure of (CH)₆ in 1865 based upon reactions such as its catalytic hydrogenation, which involves the addition of six hydrogen atoms.^[47] However, as benzene (**15**) does not undergo addition reactions with HCl and HBr, it was concluded that the double bonds in benzene (**15**) differed from those in other polyene systems and the resonance structure was eventually proposed to account for this. Crystallography data shows that benzene (**15**) is a planar hexagonal system in which each C-C bond length is 1.40 pm. If the bond lengths were predicted using cyclohexatriene as a model

system, the single and double bond lengths would be expected to fluctuate between 1.33 pm and 1.54 pm.^[48]

In order to retain aromaticity, benzene (**15**) preferentially reacts *via* substitution reactions as opposed to addition reactions^[44] (Scheme 2.1).



Scheme 2-1: Electrophilic aromatic substitution of benzene (**15**) with electrophile E-X.

Benzene undergoes Friedel-Crafts alkylation and acylation reactions with highly reactive, usually cationic, electrophiles formed by the action of a Lewis acid catalyst such as AlCl_3 on an alkyl halide or acyl chloride, respectively (for an example see Chapter 4). It is also halogenated in the presence of a Lewis acid and can be sulfonated by heating with concentrated sulfuric acid. Nitration of benzene (**15**) occurs in the presence of nitric and sulfuric acid *via* a nitronium ion to form nitrobenzene.^[45]

Anaerobic organisms break down benzene (**15**) by firstly activating it by carboxylation (see Section 2.5.1). This reaction can be chemically achieved by reaction of an aromatic compound with CO_2 with the aid of a Lewis acid catalyst. Typically, this process gives poor yields due to the low electrophilicity of CO_2 and side reactions, which are caused by the catalyst.^[49] With a deeper understanding of the biological route of carboxylation for benzene (**15**), a more efficient and greener synthetic route may be possible to produce naphthoic acid.

2. 2. 2 Naphthalene (**16**)

Naphthalene (**16**) is a planar bihexagonal system with 10 sp^2 carbon atoms, with 10 π electrons in 5 molecular orbitals made up by the overlap of the 10 aromatic p orbitals on the carbon atoms (Figure 2-1).^[45] It can be sourced from coal tar and petroleum, but can also be synthesised *via* two main routes: the Haworth

synthesis^[50] (Scheme 2-2), a modernised version of which is utilised in Chapter 4, and the Diels-Alder reaction between benzo-1,4-quinone and 1,3-dienes.

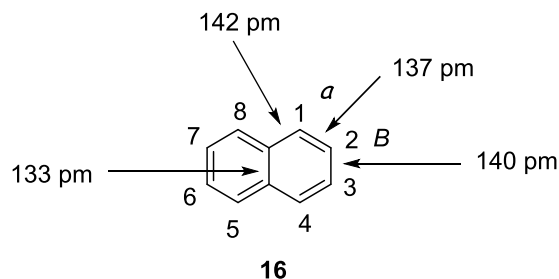
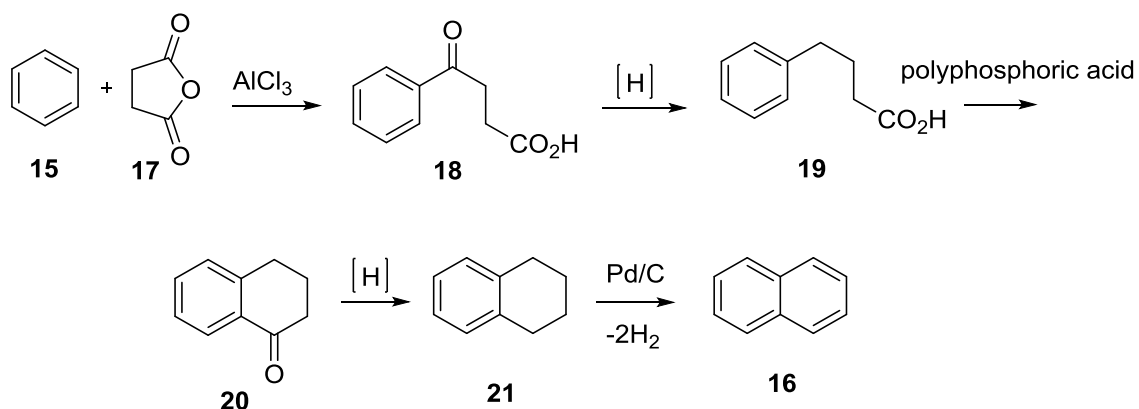


Figure 2-1: Structure of naphthalene (**16**) with bond lengths shown.



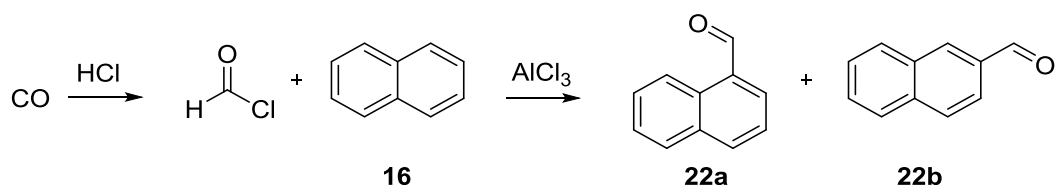
Scheme 2-2: Haworth synthesis of naphthalene (**16**).^[50]

Naphthalene (**16**) is more reactive than benzene (**15**) as it has a less of an aromatic character and therefore a lower resonance energy. The bond lengths differ as there is less delocalisation of electrons around the bicyclic structure, the two environments 1 / α and 2 / β are distinct and react differently (Figure 2-1).^[45]

Naphthalene (**16**) undergoes electrophilic substitution reactions and dependent on temperature, the substitution occurs either at the 1 or 2 position. Kinetically controlled attack on naphthalene preferentially occurs at the 1 position as this produces a lower energy transition state than attack at the 2 position. An example of this regioselectivity can be seen in Chapter 4.

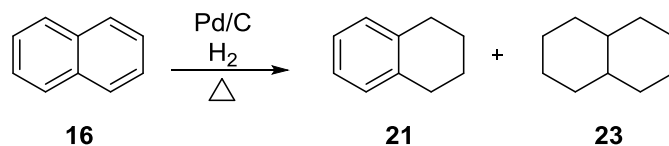
Naphthalene (**16**) undergoes halogenation reactions without catalytic assistance and can undergo Friedel-Crafts alkylations and acylations. Alkylation by alcohols and alkenes can be achieved in the presence of sulfuric or phosphoric acid catalyst.^[44]

The Gattermann-Koch reaction, a variation of the Friedel-Crafts reaction, whereby naphthalene is acylated in the presence of carbon monoxide and hydrogen chloride and a catalytic halide such as AlCl_3 , is also a useful transformation (Scheme 2-3).^{[51],[52]} A study of the reaction ranked respective halides in the following order of decreasing catalytic activity: $\text{AlBr}_3 > \text{AlI}_3 > \text{AlCl}_3 > \text{FeCl}_3$ and found that TiCl_4 , SnCl_4 , and SbCl_5 are inactive.^[52]



Scheme 2-3: Gattermann-Koch acylation of naphthalene (**1**) to produce 1- and 2-naphthaldehyde (**22a** and **b**).

Naphthalene (**16**) undergoes hydrogenation with Pd/C and H_2 at high temperatures to form tetralin (**21**) and decalin (**23**) (Scheme 2-4).^[53] The reverse dehydrogenation reaction is utilised in Chapter 4.



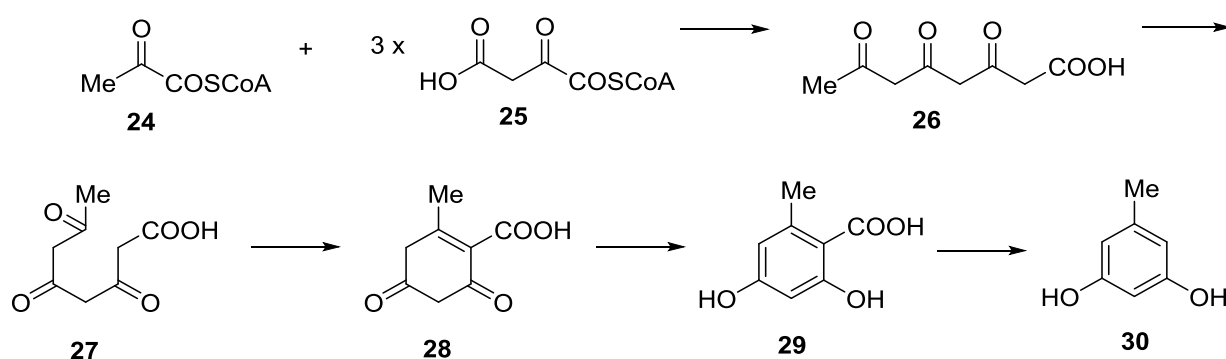
Scheme 2-4: Hydrogenation of naphthalene (**16**) to tetralin (**21**) and decalin (**23**).

2.3 Natural Routes of Formation of Aromatic Hydrocarbons

Due to the stability of aromatic compounds, Nature utilises them for many purposes. Some compounds, such as anthocyanodins and anthocyanins, give colour to plants, whilst some provide protection against predators, such as nicotinamides in the tobacco plant, and compounds such as lignin provide trees with their rigidity. Two routes to aromatic hydrocarbon compounds are known to occur biologically: the polyketide and the shikimate syntheses. Both produce compounds with high oxygen compositions due to their precursors.^{[54],[55]}

2. 3. 1 Polyketide Pathway

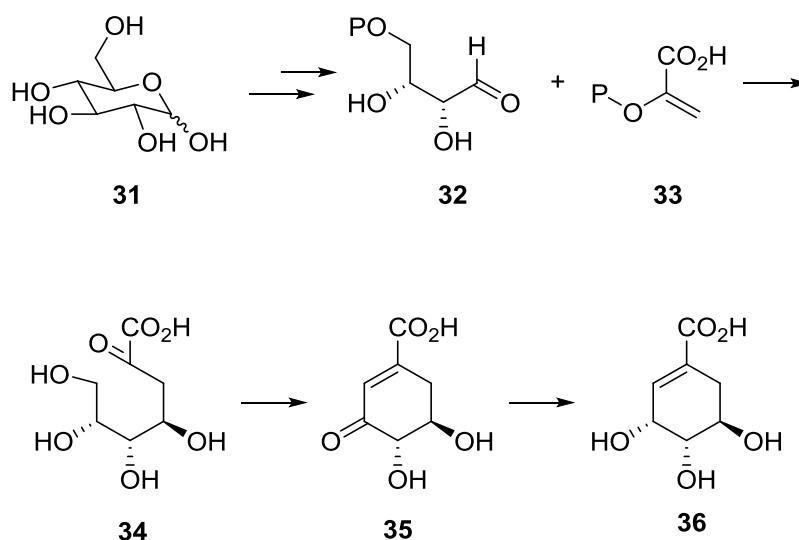
Polyketides are a secondary metabolite; i.e. compounds which serve important purposes not necessarily vital to an organism's survival.^[56] They are synthesised by decarboxylative condensations of units such as acetyl-CoA (**24**) and malonyl-CoA (**25**), which are catalysed by polyketide synthases.^[57] Orsellinic acid (**28**) is synthesised from one acetyl-CoA (**24**) and three malonyl-CoA (**25**) molecules and can go on to form orsenol (**30**)^[54] (Scheme 2-5).



Scheme 2-5: Biosynthesis of orsenol (**30**) from acetyl-CoA (**24**) and malonyl-CoA (**25**).

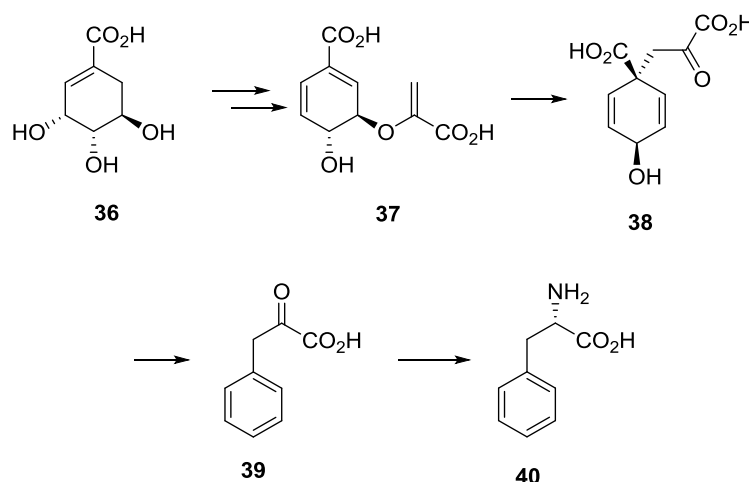
2. 3. 2 Shikimate Pathway

The shikimate pathway is a metabolic route used by many organisms to produce aromatic compounds such as the amino acids phenylalanine (**40**), tryptophan and tyrosine. These are essential dietary amino acids as animals cannot produce them naturally, and so they must consume plants to acquire them. Products of the shikimic acid pathway can be recognized by the presence of a phenylpropanoid unit, an aromatic ring joined to a three carbon atom side chain. Shikimic acid has gained popularity in recent years as it is used as a precursor to Tamiflu. One precursor of shikimic acid (**36**) is D-glucose (**31**)^[55] (Scheme 2-6).



Scheme 2-6: Biosynthetic route to shikimic acid (36) from D-glucose (31).

Shikimic acid (36) can be converted to chorismic acid (37) to prephenic acid (38), which is then transformed into the aromatic amino acids, e.g. phenylalanine (40) (Scheme 2-7).^[55]



Scheme 2-7: Conversion of shikimic acid (36) to prephenic acid (38) and phenylalanine (40).

2.4 Routes of Degradation of Aromatic Hydrocarbons

The energy barriers involved in the degradation of aromatic hydrocarbons are high due to resonance stabilisation effects. All aromatic substrates are firstly transformed

into one of three central intermediates for de-aromatisation. The process by which de-aromatisation occurs is dependent on the strength of their aromatic character.^[42] The three intermediates associated with anaerobic aromatic hydrocarbon degradation are shown in Figure 2-2:

1. Benzoyl-CoA (**41**) - this is the most common of the central intermediates. It requires a strong reducing agent to break its aromaticity. Benzoyl-CoA reductase utilises ferredoxin reduction coupled with stoichiometric electron transfer to ATP hydrolysis to achieve de-aromatisation.
2. Resorcinol (**42**) (1,3-dihydroxybenzene) - requires only ferredoxin for reduction.
3. Phloroglucinol (**43**) (1,3,5-trihydroxybenzene) – this compound has the weakest aromatic character of the proposed intermediates and can be enzymatically reduced by NADPH via its carbonyl tautomer. (R. U. Meckenstock, 2011, M. Boll, 2002).

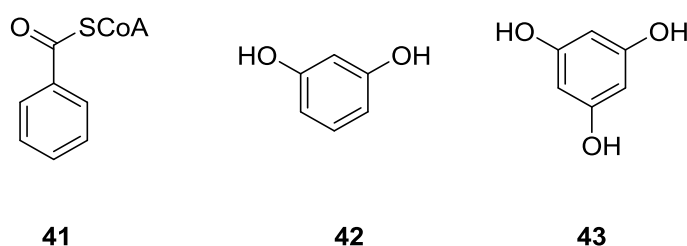
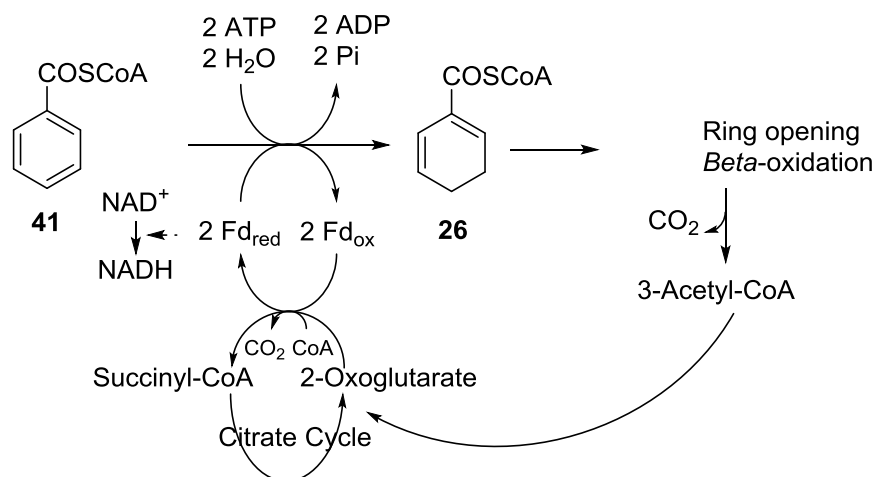


Figure 2-2: The three central intermediates associated with anaerobic aromatic hydrocarbon degradation.

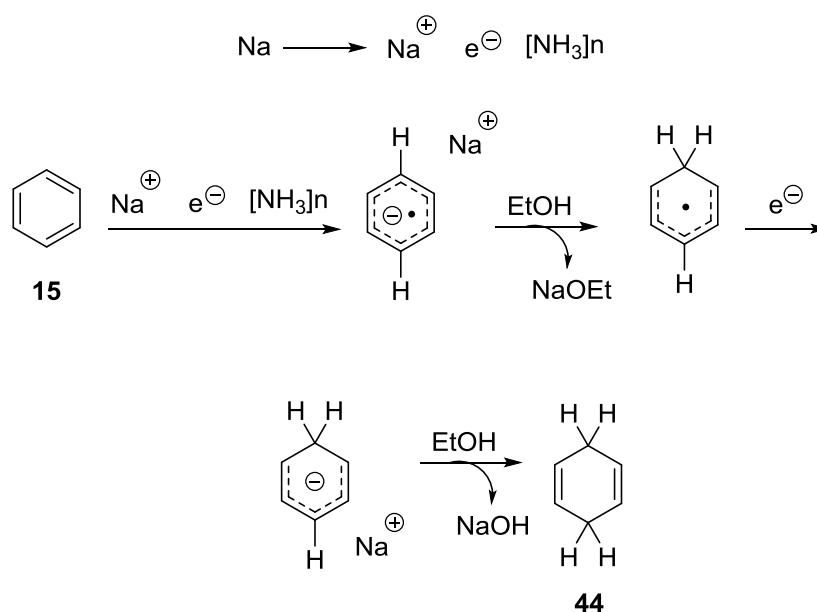
2. 4. 1 Benzoyl-CoA (**41**)

Benzoyl-CoA reductase (BCR), from the microorganism *Thauera aromatica*, has been shown to convert benzoyl-CoA (**41**) to cyclohexa-1,5-diene-1-carbonyl-CoA (**26**). BCR transfers two electrons from reduced ferredoxin to the benzoyl-CoA (**41**), coupling the process to the stoichiometric hydrolysis of ATP to ADP (Scheme 2-8).^[40]



Scheme 2-8: Conversion of benzoyl-CoA (**41**) to cyclohexa-1,5-diene-1-carbonyl-CoA (**26**) by benzoyl-CoA reductase from the microorganism *T aromatica*.

The mechanism of benzoyl-CoA reductase is analogous to that for the Birch reduction. In the latter, benzene (**15**), for example, is reduced with lithium or sodium in liquid ammonia in the presence of a proton source such as ethanol to cyclohexa-1,4-diene. When lithium or sodium dissolves in ammonia, solvated metal cations and electrons are generated. The solvated electrons reduce the benzene (**15**) to produce a radical anion, which is protonated by ethanol. The resulting pentadienyl radical can then acquire another electron and repeat the process of reduction/protonation to produce cyclohexa-1,4-diene (**44**) (Scheme 2-9).^[57] In the biochemical reduction, a thiol moiety in the active site of the enzyme catalysing the reaction was proposed to stabilise the radical intermediates. Kinetic studies performed using various substrate analogues with different electronic properties showed that the rate determining step is the first electron transfer to the ring, as in a chemical Birch mechanism.^[40]



Scheme 2-9: Birch reduction mechanism.

2.5 Aromatic Hydrocarbons

2.5.1 Anaerobic Benzene (15) Degradation

Anaerobic benzene (15) degradation has been demonstrated for all the main electron receptors associated with anaerobic hydrocarbon degradation: nitrate, sulfate, chlorate, ferric iron and fermentation coupled with methanogenesis (see Sections 1.4-1.8).

Three different degradation pathways were originally proposed for benzene (15) based on metabolite analysis: carboxylation, hydroxylation and methylation.

Generally, when elucidating enzymatic pathways, the first analytical method to be employed is metabolite analysis, which utilises gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Following these techniques, more advanced methods such as enzyme studies, proteomics and molecular studies are employed. Substrate utilisation experiments are often used, which involves exchanging a known substrate for a microorganism by a postulated metabolite, and assuming that this is a true metabolite, the culture

will be able to grow as effectively. All compounds used in such experiments must be free to diffuse through cell membranes to ensure bioavailability is not an issue.^[42]

2. 5. 1. 1 Evidence for carboxylation as the method of activation

Benzoate, the precursor to benzoyl-CoA (**41**), was found in some microbial cultures, which could indicate that direct carboxylation is the initial activation step.^{[58],[59],[60],[61],[62]} However, studies performed using ¹³C-labelled benzene (**15***) have been inconclusive. Some studies reported the carboxyl group was ¹³C-labelled indicating that it is derived from the benzene (**15***) by cellular degradative processes.^{[61],[62]} Other studies have shown that the carboxyl group came from the carbonate buffer.^{[58],[60]}

2. 5. 1. 2 Evidence for methylation as the method of activation

Metabolite analysis studies performed using ¹³C-labelled benzene (**15***) found labelled toluene (**28***) in the supernatant^[63] suggesting that the initial activation step involves the methylation of benzene (**150**) to give toluene (**28**) followed by addition of fumarate (**1**) to give benzylsuccinate, which would then be further degraded by the well-established toluene (**28**) degradation pathway (Section 2. 5. 2) leading to benzoyl-CoA (**41**). However, this seemed unlikely from metabolite analyses as benzylsuccinate or related compounds were not detected.^[42] In studies in which benzene (**15**) was exchanged for toluene (**28**) in benzene degrading cultures, it was found that some are able to grow successfully on the toluene (**28**) after a period of adaptation. This could be due to the microorganism utilising toluene (**28**) as a true metabolite, or because over the observed period of adaptation opportunistic organisms in the culture utilised the toluene (**28**). However, some cultures were unable to utilise the toluene (**28**) as a growth substrate.^{[64],[58]}

2. 5. 1. 3 Evidence for hydroxylation as the method of activation

Metabolite analysis studies also reported phenol as an intermediate and proposed hydroxylation as an initial activation step.^{[63],[65],[59]} Due to their abundance in biological systems water or O₂ are the usual precursors of the hydroxyl group. When ¹⁸O-labelled water was used in the medium, labelled phenol was found, showing that hydroxylation from water could be a possible activation step.^[65] However, a later study found no labelled phenol.^[59] In this case it was proposed that the phenol was

instead derived from a reaction with molecular oxygen and an enzyme similar to methane monooxygenase, which is used for anaerobic methane degradation. The molecular oxygen required for this oxidation may derive from a disproportionation reaction ($2\text{NO} \rightarrow \text{N}_2 + \text{O}_2$). An alternative study performed using iron and sulfide electron acceptors found that the phenol in the supernatant derived from the sampling method and not from the metabolism of benzene (**15**). Reactive oxygen species (ROS) were formed when the samples came into contact with air, which lead to the oxidation of benzene (**15**) to phenol.^{[58],[60]} It was therefore inconclusive whether phenol is a true intermediate or a product of the sampling technique.^[42] When the benzene (**15**) was exchanged for phenol, the same was found as was for toluene (**28**). Some cultures could utilise the phenol after a period of adjustment^[60] and some could not utilise it at all.^[29]

2. 5. 1. 4 Genomic studies

The genome for the benzene degrading organism, *Dechloromonas aromatic* strain RCB, has been fully sequenced. No genes were observed that were homologous to those usually associated with anaerobic aromatic hydrocarbon degradation. Instead, numerous genes which encode for monooxygenase enzymes were found. This observation could suggest a completely novel pathway of anaerobic benzene degradation from those previously postulated.^[66]

A proteogenomic study performed using an iron-reducing enrichment culture found a number of genes that encoded enzymes known to be involved in anaerobic aromatic hydrocarbon degradation. Differential protein expression experiments were then performed on the same culture, feeding it on benzene (**15**), phenol or benzoate using ferric oxide as the electron acceptor. The different proteomes for each sample were then analysed by SDS-page and either direct Edman-sequencing of excised bands or total proteomic analysis with LC-MS-MS. Two bands exclusive to the benzene sample were prominent by SDS-page. The correlating gene was homologous to phenyl phosphate carboxylase which is involved in anaerobic phenol degradation. When the sample was examined *via* a proteomic approach there were several proteins expressed which correlated with carboxylase genes including benzoate-CoA ligase. It was proposed that for this microorganism benzene (**15**) is activated by

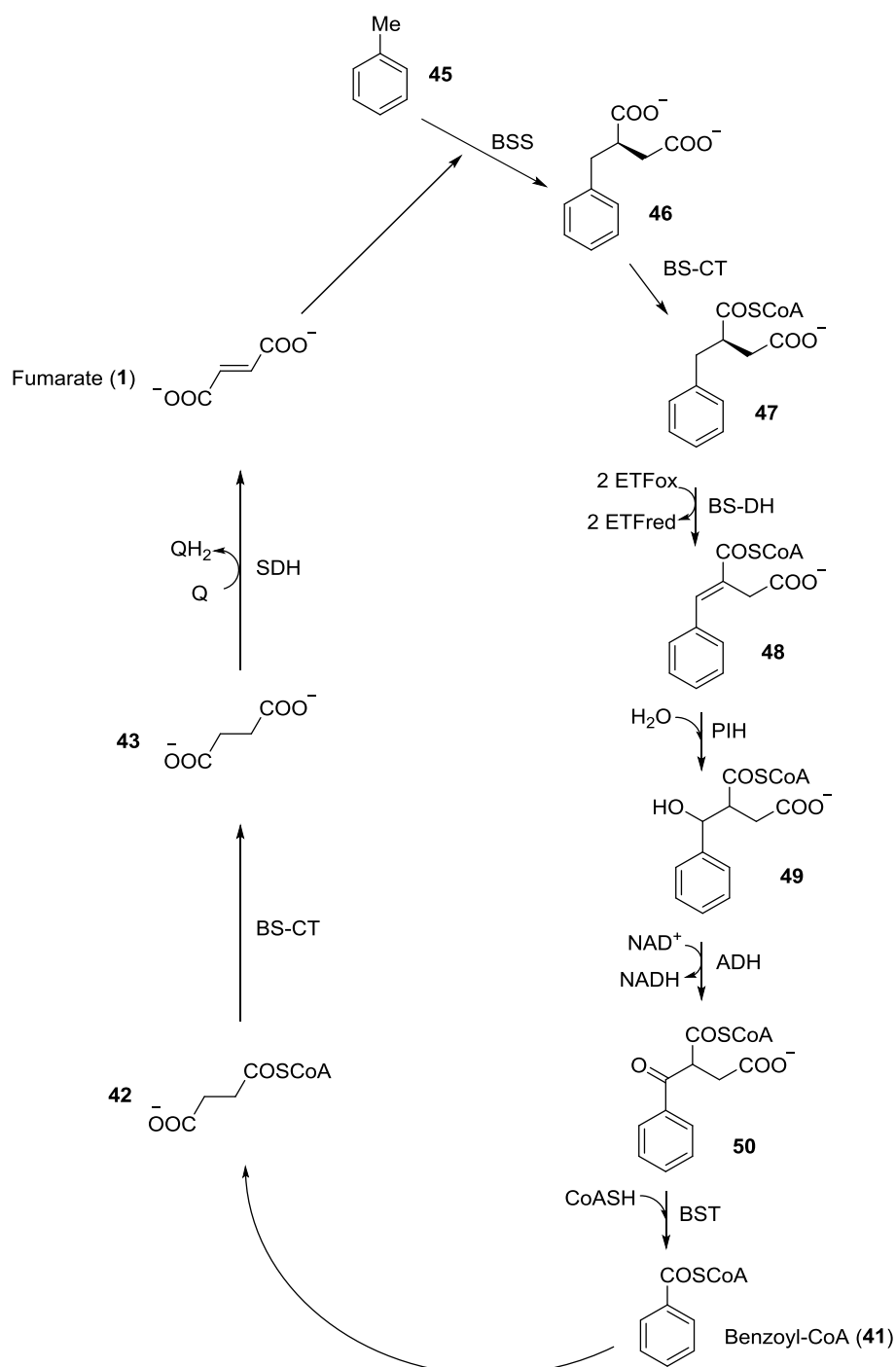
direct carboxylation catalysed by a novel enzyme called anaerobic benzene carboxylase.^[58]

2. 5. 1. 5 Accepted mechanism for benzene (15) degradation

Despite the confusion resulting from the experiments described above, it is now widely accepted that benzene (15) is activated by carboxylation to benzoate.^[67] Direct carboxylation *via* insertion into a C-H bond is mechanistically difficult due to the high C-H bond dissociation energy associated with such a process (473 kJ mol⁻¹). Presumably, the mechanism involves some kind of activation of CO₂ to enhance its electrophilicity, followed by an electrophilic substitution. The enzymes involved do not require ATP hydrolysis to activate the benzene (15). Once carboxylated, the benzene can be degraded through the established benzoyl-CoA (41) route of metabolism.^[67]

2. 5. 2 Anaerobic Toluene (45) Degradation

Toluene (45) was the first compound for which the pathway for anaerobic degradation was fully elucidated (Scheme 2-10). (*R*)-Benzylsuccinate synthase is responsible for activating toluene (45), and is a prevalent enzyme specific to toluene that has been isolated from sulfate and nitrate reducing microorganisms, phototrophic bacteria and methanogenic consortiums. A cysteinyl radical (Section 1.3.1.1) in the active site of the enzyme homolytically abstracts a hydrogen atom from the methyl group of the toluene (45) to produce a cysteine residue at the active site of the enzyme, and a benzyl radical which attacks the double bond of fumarate (1). The resulting radical can then be neutralised by re-abstracting the hydrogen from cysteine to form the product, (*R*)-benzylsuccinate (46). This step reforms the catalytic thiyl radical of the enzyme. (*R*)-Benzylsuccinate is then converted to a CoA thioester (47) by benzylsuccinate-CoA transferase and subsequent dehydrogenation by benzylsuccinyl-CoA dehydrogenase leads to (*E*)-phenylitaconyl-CoA (48).

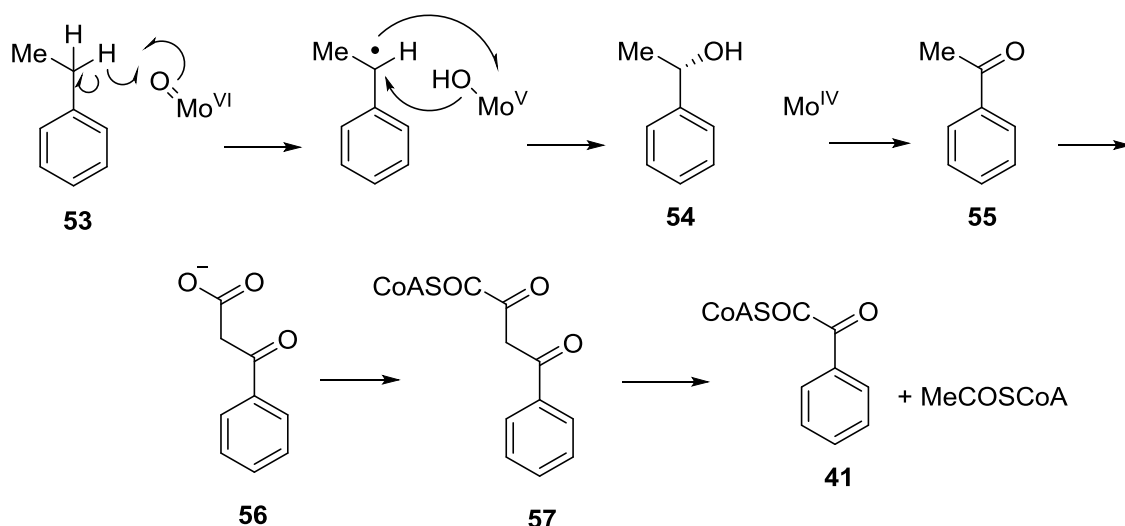


Scheme 2-10: Pathway of anaerobic toluene (45) catabolism: BSS, benzylsuccinate synthase; BS-CT, benzylsuccinate CoA transferase; BS-DH, benzylsuccinyl-CoA dehydrogenase; PIH, phenylitaconyl-CoA hydratase; ADH, alcohol dehydrogenase; BST, benzoylsuccinyl-CoA thiolase; SDH, succinate dehydrogenase. ETF indicates electron transferring flavoprotein and Q indicates quinone.^[40]

This intermediate is hydroxylated by phenylitaconyl-CoA hydratase and the resulting hydroxyl compound (**49**) is oxidised to benzoylsuccinyl-CoA (**50**) by benzoylsuccinyl-CoA thiolase and NAD⁺. Benzoyl-CoA (**41**) and succinyl-CoA (**51**) are then produced by benzoylsuccinyl-CoA thiolase. The succinyl-CoA is recycled to fumarate (**1**) by succinate dehydrogenase (Scheme 2-10).^[40] The electron carriers which are reduced in the process can be re-oxidised by anaerobic respiration of the bacteria.

2. 5. 3 Anaerobic Ethylbenzene (**53**) Degradation

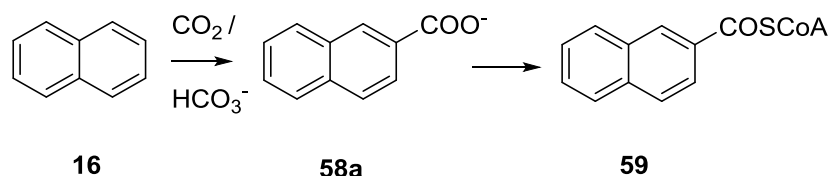
Studies have shown that instead of activation by fumarate (**1**) addition, followed by degradation *via* the succinate pathway (Section 1.3.1) as for toluene (**45**) (Section 2.6.2) and alkanes (Section 1.3.1), with the test bacterium *Aromatoleum aromaticcum* EbN1, ethylbenzene dehydrogenase (EBDH) activates ethylbenzene (**53**) by hydroxylation of the methylene carbon to yield enantiomerically pure (*S*)-1-phenylethanol (**54**). The oxygen atom of the hydroxyl group is derived from water rather than oxygen. EBDH utilises a Mo-*bis*-MGD cofactor (MoCo) in its α -subunit to form the alcohol before oxidation to the ketone (**55**) and subsequent carboxylation (**56**), conversion to the CoA-thioester (**57**) and cleavage to form the central intermediate benzoyl-CoA (**41**) (Scheme 2-11).^{[2],[68]}



Scheme 2-11: Pathway of degradation for ethylbenzene (**53**) to benzoyl-CoA (**41**).

2. 5. 4 Anaerobic Naphthalene (16) Degradation

The method of activation for anaerobic naphthalene (**16**) degradation occurs *via* selective β -carboxylation to produce 2-naphthoic acid (**58a**) (Scheme 2-12).^{[2],[67]}



Scheme 2-12: Anaerobic breakdown of naphthalene (**16**) by carboxylation activation.

2. 5. 4. 1 Metabolite Analysis in the Elucidation of the Mechanism of Naphthalene (16) Degradation

GC-MS analysis of the supernatants of incubation experiments performed on a sulfate reducing enrichment culture capable of degrading both naphthalene (**16**) and phenanthrene found that 2-naphthoic acid (**58a**) and phenanthroic acid were the major metabolites. It was also found that when the cultures were grown with a ¹³C-labelled carbonate buffer the 2-naphthoic acid (**58a**) and phenanthroic acid were ¹³C-labelled on the carboxyl group showing that this group derived from the buffer solution / CO₂.^{[69],[41]} In substrate utilisation experiments using cultures grown on 2-naphthoic acid (**58a**), the bacterial growth matched that of control samples, which used naphthalene (**16**) as the growth substrate.^{[70],[41]}

2. 5. 4. 2 Genomic studies in the elucidation of the mechanism of naphthalene (16) degradation

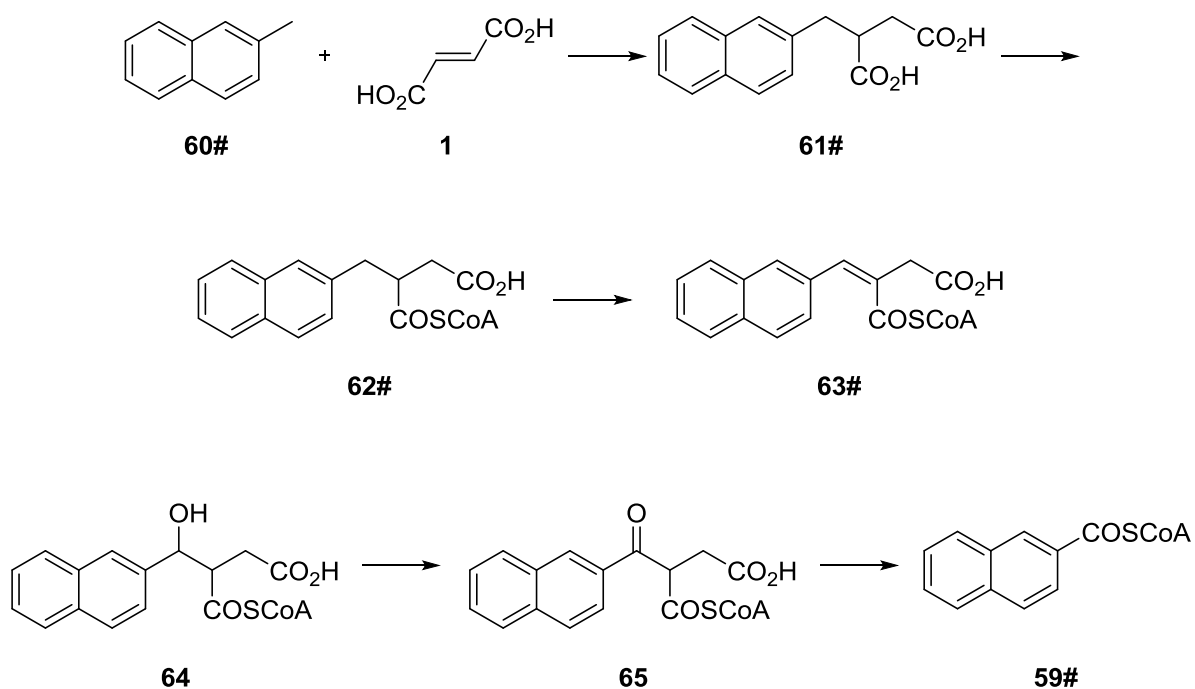
Three cultures have been isolated which are capable of catabolising naphthalene (**16**) under anaerobic conditions: NaphS2, NaphS3 and NaphS6.^[43] All are sulfate reducing and from marine sources. N47, a highly enriched sulfate reducing culture has also been produced.^[41]

Ncr is the only enzyme involved in polycyclic aromatic hydrocarbon degradation which has been isolated and characterised. It catalyses the reduction of 2-naphthoyl-CoA to 5,6,7,8-tetrahydronaphthoyl-CoA, which is a central intermediate in the ring degradation pathway proposed (see Section 2.5.6).^[71]

Two naphthalene (**16**) degrading genomes have been recently sequenced: NaphS2 is partly characterised and N47 is almost fully characterised. Protein expression profiles obtained for N47 grown with naphthalene (**16**), 2-methylnaphthalene and 2-naphthoic acid (**58a**) and the proteins which were upregulated by 2-fold or more were identified. One protein, which was more prominent in the cultures grown exclusively on naphthalene (**16**), had a 45% similarity with the α -subunit of the phenylphosphate carboxylase of EbN1 and also a 48% likeness with the α -subunit of the ABC, anaerobic benzene carboxylase enzyme, which has been proposed for the benzene degrading iron reducing culture *BF*. The authors suggested it to be naphthalene carboxylase, giving evidence that the pathway of activation is carboxylation. A similar enzyme was found to be coded for in the N47 genome, which was homologous with the α - and β - subunits of phenolphosphate carboxylase.^[72]

2. 5. 5 Anaerobic 2-Methylnaphthalene (**33**) Degradation

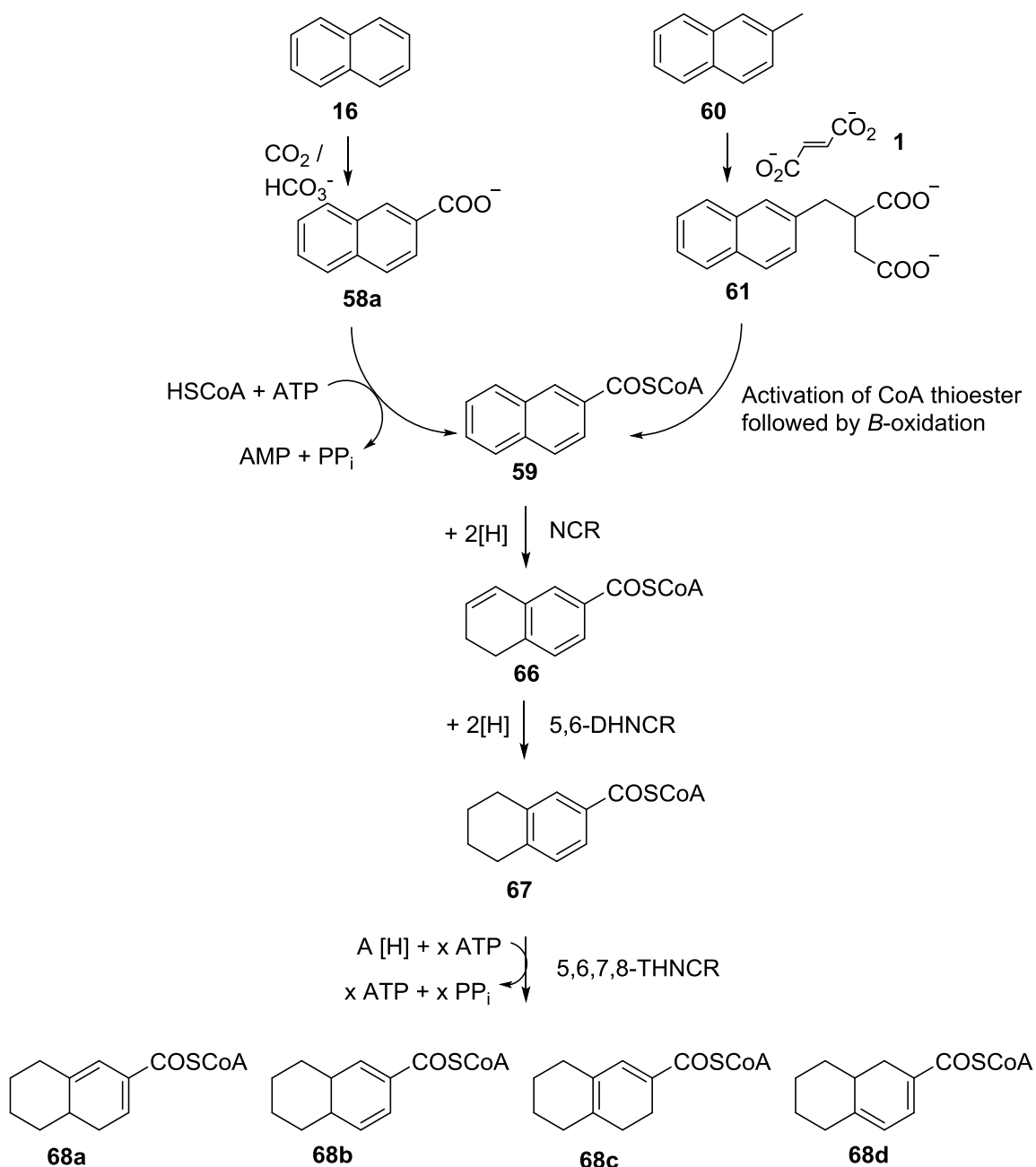
Studies have previously shown that 2-naphthoic acid (**58a**) is a prominent metabolite in anaerobic 2-methylnaphthalene (**60**) degradation, as well as in anaerobic naphthalene (**16**) degradation, indicating 2-naphthoic acid (**58a**) could be a central intermediate of both pathways, similar to benzoyl-CoA (**41**) in monocyclic anaerobic ring degradation. This study also found naphthyl-2-methylsuccinic acid (**60**) and naphthyl-2-methylenesuccinic acid to be metabolites indicating activation by fumarate (**1**) addition (see Section 1.3.1) before further degradation to 2-naphthoic acid (**58a**).^[71] A study suggested that after the addition of fumarate (**1**), the naphthyl-2-methylsuccinic acid (**61**) is converted into naphthyl-2-methyl-succinyl-CoA (**62**) (Scheme 2-13) and undergoes dehydrogenation on the methylene carbon to yield naphthyl-2-methylene-succinyl-CoA (**63**). Spontaneous addition of water across the double bond gives naphthyl-2-hydroxymethyl-succinyl-CoA (**64**), followed by further oxidation to yield naphthyl-2-oxomethyl-succinyl-CoA (**65**) and finally, a thiolitic cleavage produces 2-naphthoic acid (**58a**). This mechanism proceeds in a similar manner to toluene (**45**) metabolism.^[73]



Scheme 2-13: Proposed degradation of 2-methylnaphthalene (**60**) by fumarate (**1**) addition (# indicates the compounds which have been isolated and identified as pure samples).

2. 5. 6 2-Naphthoic Acid (58a)

Studies investigating the fate of the central 2-naphthoic acid intermediate have proposed the degradation pathways described in Scheme 2-14. The 2-naphthoyl-CoA (**59**) is reduced *via* two successive ATP independent reactions; the first by naphthoyl-CoA reductase (NCR) to form 5,6- dihydronaphthoyl-CoA (**66**), which is reduced by 5,6-dihydronaphthoyl-CoA reductase (DHNCR) to form 5,6,7,8-tetrahydronaphthoyl-CoA (**67**). These enzymes are part of the ‘old yellow enzyme’ family and utilise FAD, FMN and a [Fe₄-S₄] cluster cofactors. The 5,6,7,8-tetrahydronaphthoyl-CoA is then reduced *via* an ATP-dependent reaction catalysed by 5,6,7,8-tetrahydronaphthoyl-CoA reductase (THNCR) to form hexahydronaphthoyl-CoA (**68**), for which the exact isomeric structure is to be resolved. Further work on this pathway is discussed in Chapter 4.^[2]



Scheme 2-14: The proposed pathway of anaerobic degradation for 2-naphthoyl-CoA (59).

2.6 Overall Project Aims

The scope of the project is wide and covers many areas. It aims to deepen the current understanding on how anaerobic organisms utilise hydrocarbons as an energy source in order to potentially develop new biomimetic synthetic routes as well

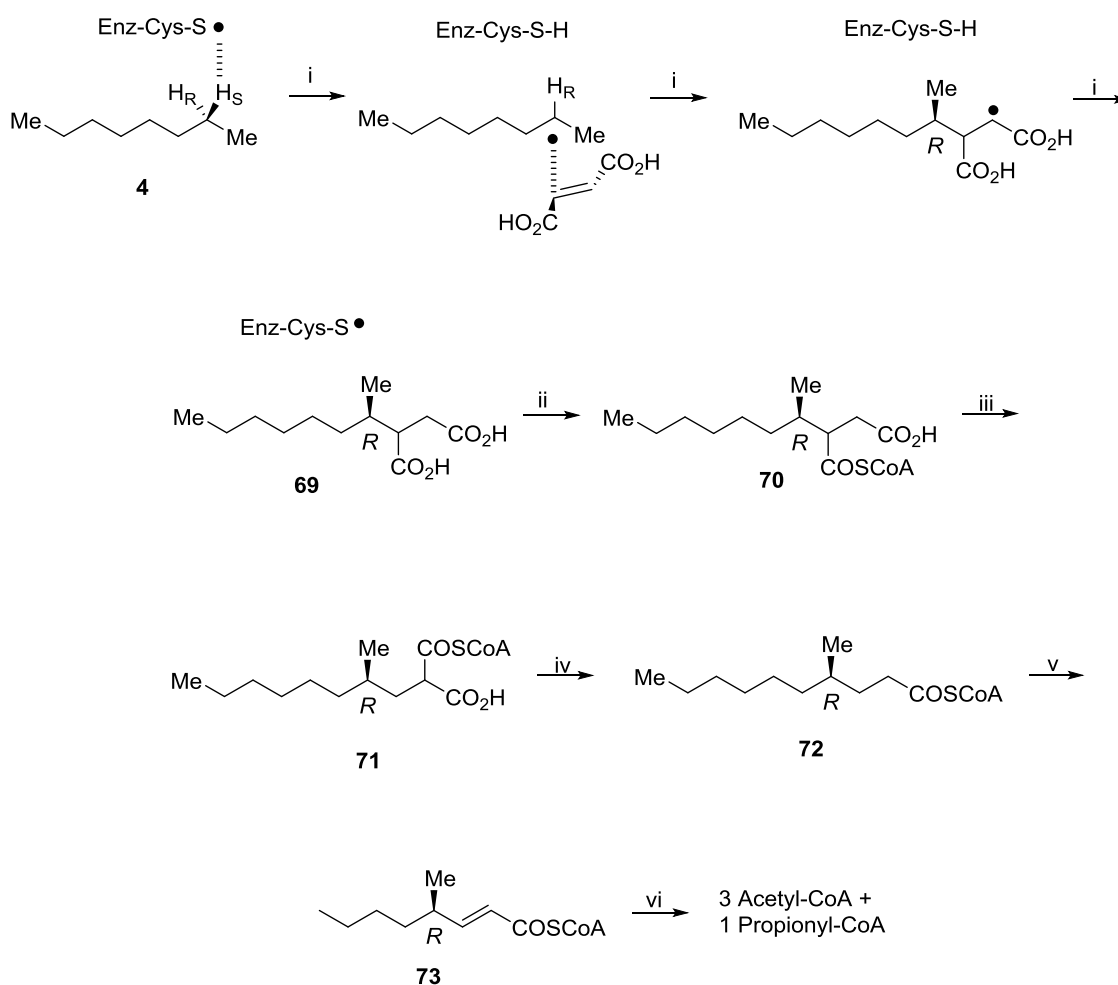
as investigate possible bioremediation methods for the clean-up of hydrocarbons in both water sources and habitats. By synthesising the substrate analogues, incubating them with cultures of specialised microorganisms and analysing the metabolites we may discover / give further evidence towards novel biodegradation pathways.

Chapter 3 Synthesis of Stereoisomeric (*S,S*)-, (*R,R*)- and (*R,S*)-

Decane- 2,9-diol Stereoisomers (4a-c)

3.1 Introduction to the of Synthesis of Stereoisomeric (*S,S*)-, (*R,R*)- and (*R,S*)-Decane- 2,9-diol Stereoisomers (4a-c)

β -proteobacterium OcN1 is capable of degrading alkanes over the range C₈-C₁₂ under anaerobic conditions (Scheme 3-1).



Scheme 3-1: Proposed transformation of decane (4) by β -proteobacterium OcN1. i) (1-methylalkyl)succinate synthase; ii) (1-methylalkyl)succinate-CoA ligase; iii) (2-methylalkyl)malonyl-CoA mutase; iv) (2-methylalkyl)malonyl-CoA decarboxylase; v) 4-methylalkanoyl-CoA dehydrogenase.

The activation method mirrors that of HxN1 with hexane (**2**), *i.e.* selective homolytic abstraction of a hydrogen atom at the C-2 position followed by fumarate (**1**) addition to a putative alkyl radical (see Section 6.1 for discussion of an alternative concerted mechanism) to produce an alkylsuccinate (see Section 1.3.1). It can be postulated that the subsequent steps for the degradation of decane (**4**) are also in accord with those suggested for HxN1 activation of hexane (**2**) (Scheme 3-1).^[22]

As discussed in Section 1.9, the previous study at Newcastle University showed, for the anaerobic bacterium HxN1, that the pro-*S* hydrogen atom is abstracted from the C-2 position of hexane (**2**). In that study, stereospecifically labelled (2,5-²H₂)hexane isomers **2a-c*** (Figure 3-3) were used as growth substrates for HxN1 and the deuterium atoms were followed through the catabolic process using GC-MS analysis.

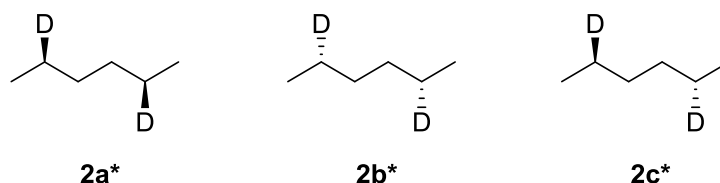


Figure 3-3: Stereospecifically dideuterated hexane isomers (**2a*-2c***)

Synthesis of the (*R,R*)-, (*S,S*)- and (*R,S*)-[2,5-²H₂]hexane stereoisomers **2a-c*** was achieved from (*R,R*)-, (*S,S*)- and (*R,S*)-2,5-hexanediols (**13**) by conversion to the corresponding di-*p*-toluenesulfonates (**14**), followed by reduction with LiAlD₄ in tetraglyme with inversion at each stereocentre to produce the dideuterated hexanes **2a-c*** (Figure 2-3). By synthesising the (*R,R*)-, (*S,S*)- and (*R,S*)-(2,9-²H₂)decane stereoisomers (**4a*-4c***) (Figure 3-1) and incubating each isomer with OcN1, it can be established whether the processes for OcN1 and HxN1 are analogous and thus whether similar enzymes are involved.

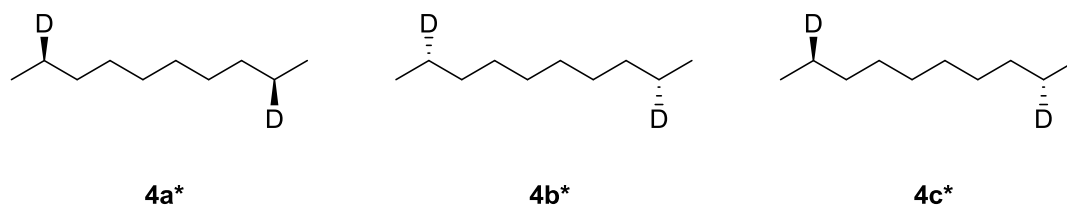


Figure 3-1: (*R,R*)-, (*S,S*)- and (*R,S*)-(2,9-²H₂)-decane stereoisomers (**4a-c***).

3.2 Project Aims

Our aim was to develop and optimise an efficient route to the (*R,R*)-, (*S,S*)- and (*R,S*)-decane-2,9-diol stereoisomers (**4a-c**) (Figure 3-2), which could undergo the same activation and reduction steps as the (*R,R*)-, (*S,S*)- and (*R,S*)-2,9-hexanediol (**13**) stereoisomers to afford the (*R,R*)-, (*S,S*)- and (*R,S*)-(2,9-²H₂)decane (**4***) stereoisomers.

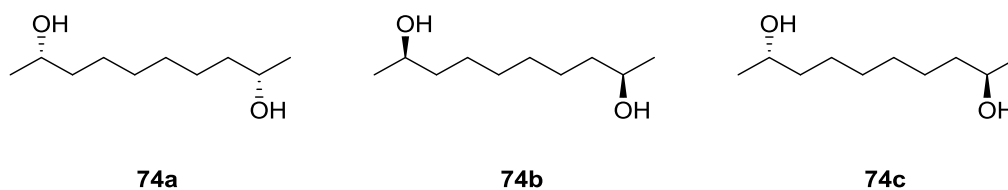


Figure 3-2: (*S,S*)-, (*R,R*)- and (*R,S*)-decane-2,9-diol stereoisomers (**74a-c**).

It was decided that for shipping purposes, the ditosylate compounds would be much more convenient end points as they were higher mass than the diol and the decane compounds (**4***) and were crystalline solids whereas the diols and decanes (**4***) were oils. Chemists within the collaborative group in Germany were happy to conduct the final conversion ready for the ultimate testing stage.

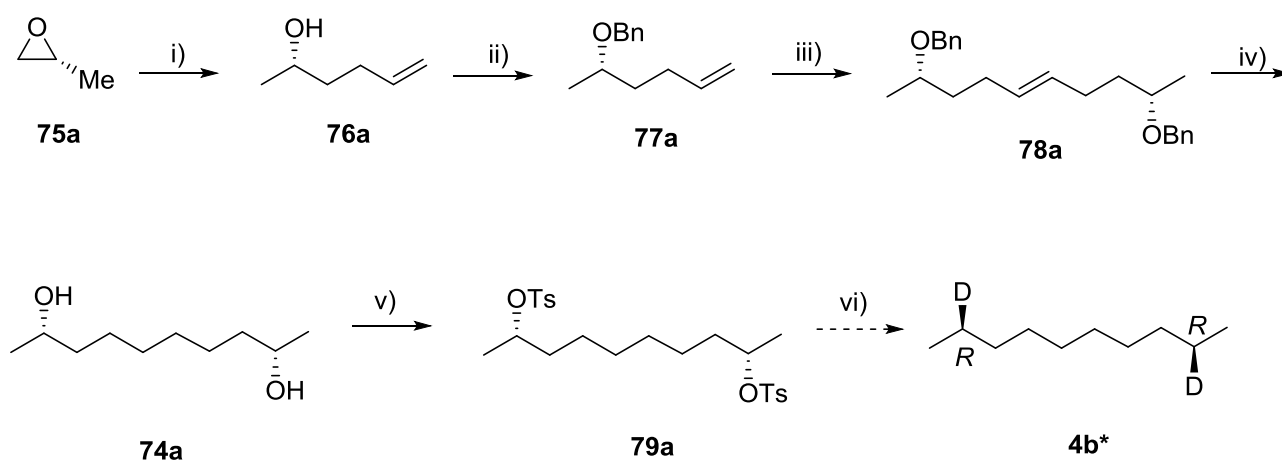
3.3 Approach for the Synthesis of (*S,S*)-, (*R,R*)- and (*R,S*)-Decane-2,9-diol Stereoisomers (**74a-c**)

The approach to the synthesis of the three stereoisomeric decane-2,9-diols (**74a-c**) involved the installation of two chiral centres. In structures which have stereocentres relatively close to one another, installation of the second stereocentre can be influenced by the first. As the decane-2,9-diols (**74**) have 6 methylene groups separating the two respective chiral centres, this directional method cannot be used. It was therefore necessary to adopt a building block approach, whereby each chiral centre was separately installed within a suitable intermediate for subsequent joining of the two chiral entities. In a previous study in our laboratory, M. Sadeghi used olefin metathesis to synthesise (*R,R*)- and (*S,S*)-decane-2,9-diols, which were converted to (*R,R*)- and (*S,S*)-(2,9-²H₂)decane stereoisomers (**4a-c***) via tosylation

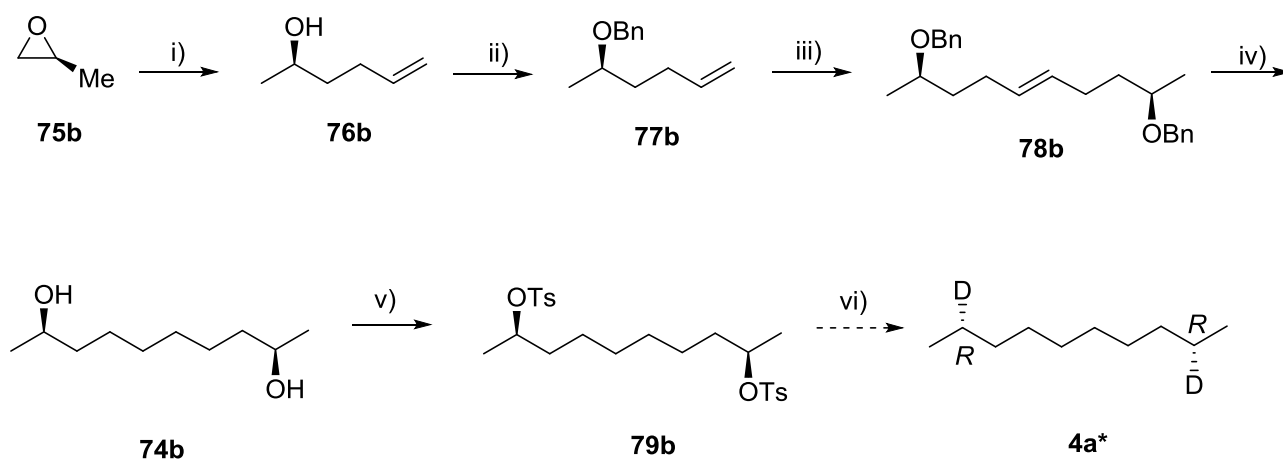
and reduction (M. Sadeghi, 2015). The aim of the present work was to optimise this method and apply it to the synthesis of all three stereoisomeric diols (**74a-c**).

3. 3. 1 (*S,S*)- and (*R,R*)-2,5-Decanediol (**74a** and **b**)

The (*R,R*)- and (*S,S*)-decane-2,9-diols (**74a** and **b**) were synthesised *via* a metathesis route (Scheme 3-2 and 3-3). Starting with stereochemically pure propylene oxide, (**75**) and performing a copper(I)-catalysed ring opening reaction with allylmagnesium bromide^[74] (i), produced hex-5-en-2-ol (**76**) with an 82 % overall yield.

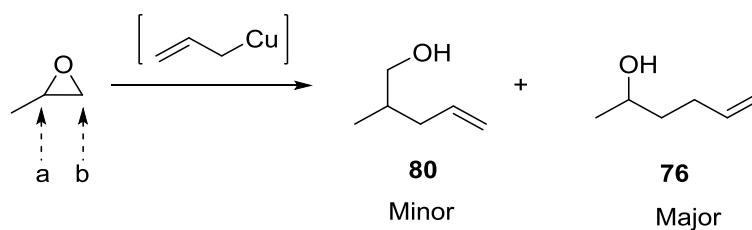


Scheme 3-2: (*R,R*)-decane-2,9-diol (**4b***) synthesis *via* metathesis route; *Reagents and conditions:* i) AllylMgBr, CuI, Et₂O, 5 h, -30 °C; ii) BnBr, NaH, TBAI, THF, 4 h, RT; iii) Grubbs catalys, 1st gen, DCM, 100 °C, 1.5 h; iv) Pd/C, H₂, MeOH, 16 h; v) *p*-TsCl, pyridine, DCM, 16 h, 0 °C – RT; vi) LiAlD₄, Et₂O.



Scheme 3-3: (*R,R*)-decane-2,9-diol (**4a***) synthesis *via* metathesis route; *Reagents and conditions:* as for Scheme 3-2.

^1H NMR spectra analysis showed that the alternative regioisomer, 2-methylpent-4-en-1-ol (**80**), produced by attack on the less favoured, more hindered CH centre of the propylene oxide was formed as a minor product (Scheme 3-4), (Figure 3-3). The major product formed is dictated primarily by steric factors for a $\text{S}_{\text{N}}2$ -like reaction.^[75]



Scheme 3-4: Regioisomeric products resulting from the copper(I)-mediated reaction with propylene oxide and allylmagnesium bromide.

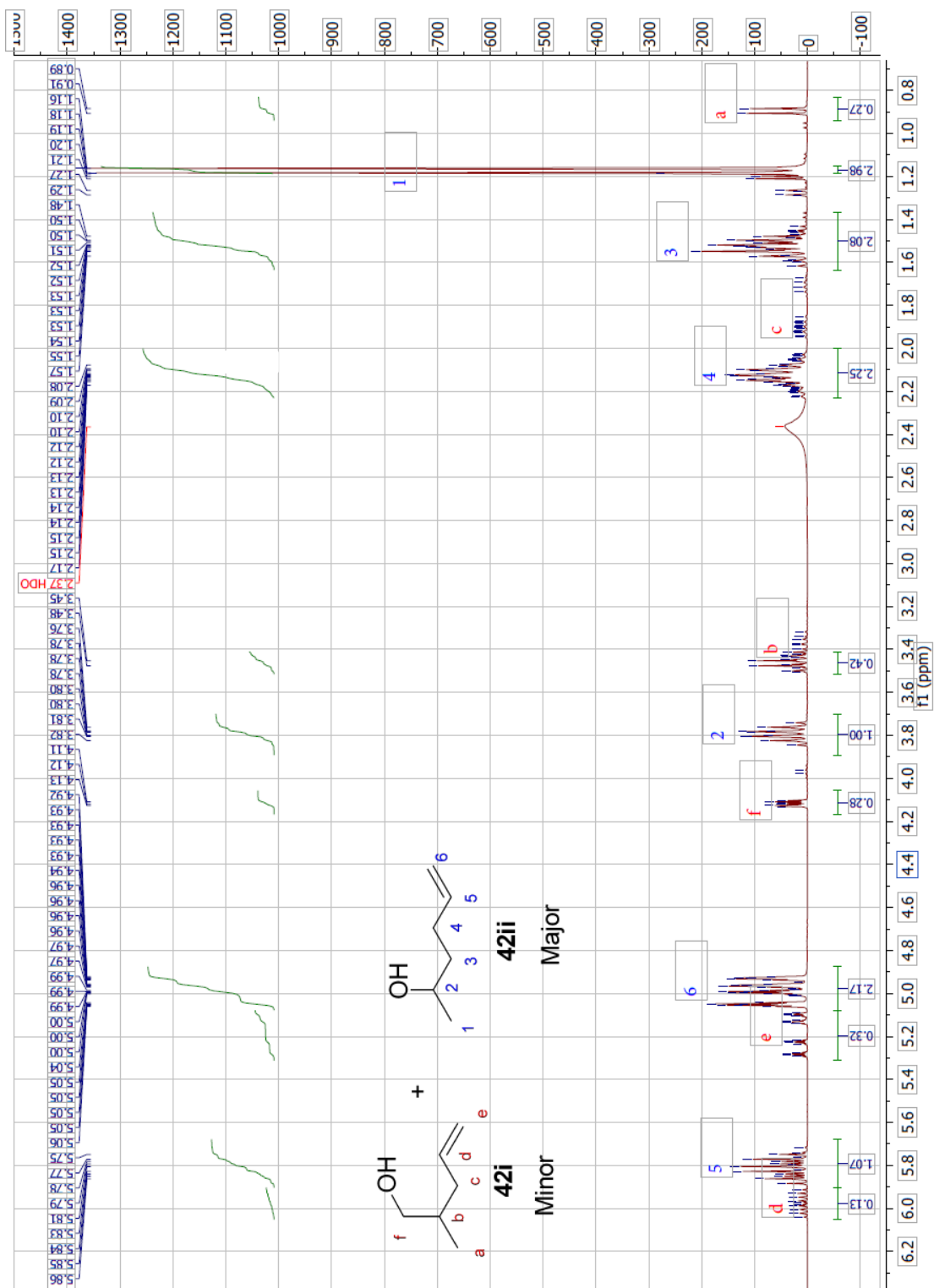
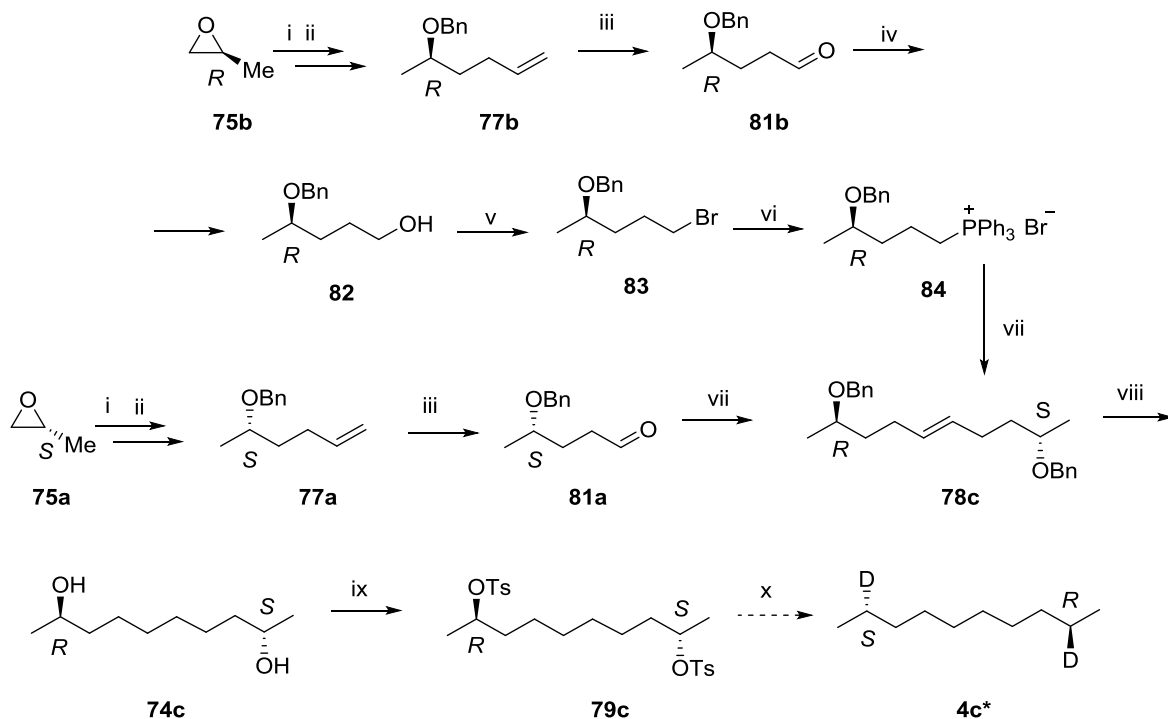


Figure 3-3: ^1H NMR spectrum of the regioisomeric products resulting from the copper(I)-mediated Grignard reaction between propylene oxide and allylmagnesium bromide/CuI.

As their chromatographic separation was difficult, the alcohols (**76** and **80**) were benzyl-protected (step ii, Schemes 3-2 and 3-3) to give [(hex-5-en-2-yl)oxy)methyl]benzene (**77**) and [(2-methylpent-4-en-1-yl)oxy)methyl]benzene,^[76] which were chromatographically separated to give the desired product in 89% yield. A metathesis reaction induced by Grubbs 1st generation catalyst (step iii, Schemes 3-2 and 3-3) was performed to link two molecules of the alkene (**77**) giving dec-5-ene-2,9-diylbis(oxy))bis(methylene))dibenzene (**78**) in yields in the range 30-60% dependent on the age of the catalyst. The reaction was carried out in DCM and heated using microwave radiation to 100 °C, with the reaction vessel being purged with N₂ every 30 min to remove the ethene produced and therefore help drive the process to completion, which required ~ 90 min.^[77] The alkene products **78** were reduced by catalytic hydrogenation.^[78] This also had the benefit of removing the benzyl protecting groups producing the decane-2,9-diols **74** in 98% yield. The next step was to activate the hydroxyl groups in order for reduction by LiAlD₄. The tosylate group was chosen rather than e.g. mesylate as it provided a chromophore enabling reduction to the decanes (**4***) to be monitored by TLC with UV detection. Tosylation was achieved in 78% yield by reacting the diol with *p*-toluenesulfonyl chloride in the presence of pyridine.^[79] The tosylates (**79**) were then shipped to our colleagues for the final transformative step.

3.3.2 (*R,S*)-Decane-2,9-diol (**74c**)

For the (*R,S*)-decane-2,9-diol (**74c**), a different route was required (Scheme 3-5).



Scheme 3-5: (*R,S*)-decane-2,9-diol (**74c**) synthesis via Wittig route; *Reagents and conditions:* i) AllylMgBr, CuI, Et₂O, 5 h, -30 °C; ii) BnBr, NaH, TBAI, THF, 4 h, RT; iii) O₃, acetone, H₂O, 5 h, 0 °C; iv) NaBH₄, MeOH, 16 h, RT; v) CBr₄, PPh₃, DCM, 16 h, 40 °C; vi) PPh₃, 16 h, 120 °C; vii) *n*-BuLi, THF, 18 h, -40 °C – 70 °C; viii) Pd/C, H₂, MeOH, 16 h; ix) *p*-TsCl, pyridine, DCM, 16 h, 0 °C – RT; x) LiAlD₄, ether.

As the two stereocentres were opposing, the metathesis route could not be used as it would give a mixture of stereochemically impure products. Instead, we had to utilise a method which would ensure that the compound had opposing stereocentres at either end. For this we decided that the synthesis of two separate moiety's, namely an aldehyde (**81a**) with *S* stereochemistry the 2 position, and a Wittig salt (**82**) with *R* stereochemistry at the 2 position, which could be linked through a simple step such as a Wittig reaction would be a favourable route. Once the Wittig step was complete the synthesis could follow an identical one as to the metathesis route. The synthesis of (*R,S*)-decane-2,9-diol (**74c**) proceeded with the same initial steps as before, i.e. the ring opening of both (*R*)- and (*S*)-propylene oxides (**75**) to give hex-5-en-2-ol (**76**), which was benzyl-protected to give (**77**) which were then chromatographically purified. The intermediate **77** was ozonolysed using O₃ in aqueous acetone at -33 °C;^[80] this method of ozonolysis was preferable to more traditional methods as it did not require reductive quenching to produce the

aldehydes **81**, as they were formed *in situ*. The method worked satisfactorily giving an average yield of 53%. Losses were observed due to the constant flow of air/O₃ through the system. From this point, the (*R*)-4-(benzyloxy)pentanal (**81b**) was reduced using NaBH₄ to give (*R*)-4-(benzyloxy)pentan-1-ol (**82**) in 80 % yield.^[80] The alcohol (**82**) was brominated in 93 % yield to (*R*)-[[5-bromopentan-2-yl)oxy]methyl]benzene (**83**) using CBr₄ and PPh₃.^[82] The bromide (**83**) was converted into the Wittig salt (*R*)-[4-(benzyloxy)pentyl]triphenylphosphonium bromide (**84**) by heating with neat PPh₃ at 120 °C for 16 h.^[83] Recrystallisation from MeCN was required to purify the salt, giving 47 % yield of pure product. The ¹³C NMR spectrum of the salt showed a resonance at δ 36.57 (d, ²J_{C-P} 15.3 Hz) assigned to the methylene group adjacent to phosphorus. The salt (**84**) was reacted with the (*S*)-4-(benzyloxy)pentanal (**49a**) in a Wittig reaction to give the (*R,S*)-[dec-5-ene-2,9-diylbis(oxy)]bis(methylene)]dibenzene (**78c**), primarily as the (*Z*)-isomer as expected for a non-stabilised ylid,^[84] which then followed the steps previously described in Section 3.3.1 (page 57), Pd/C mediated hydrogenation of the alkene to simultaneously removed the benzyl groups to give the diol (**74c**), which was converted into the ditosylate (**79c**) ready for transportation to our colleagues.

3. 3. 3 Analysis of the Ditosylate Compounds (*R,R*)-, (*S,S*)- and (*R,S*)-Decane-2,9-diyl-bis(4-methylbenzenesulfonate) (**79a-c**)

All three tosylates gave identical NMR spectra as expected for the enantiomers, but also for the *meso*-form because of the distance of separation of chiral centres (Figure 3-4).

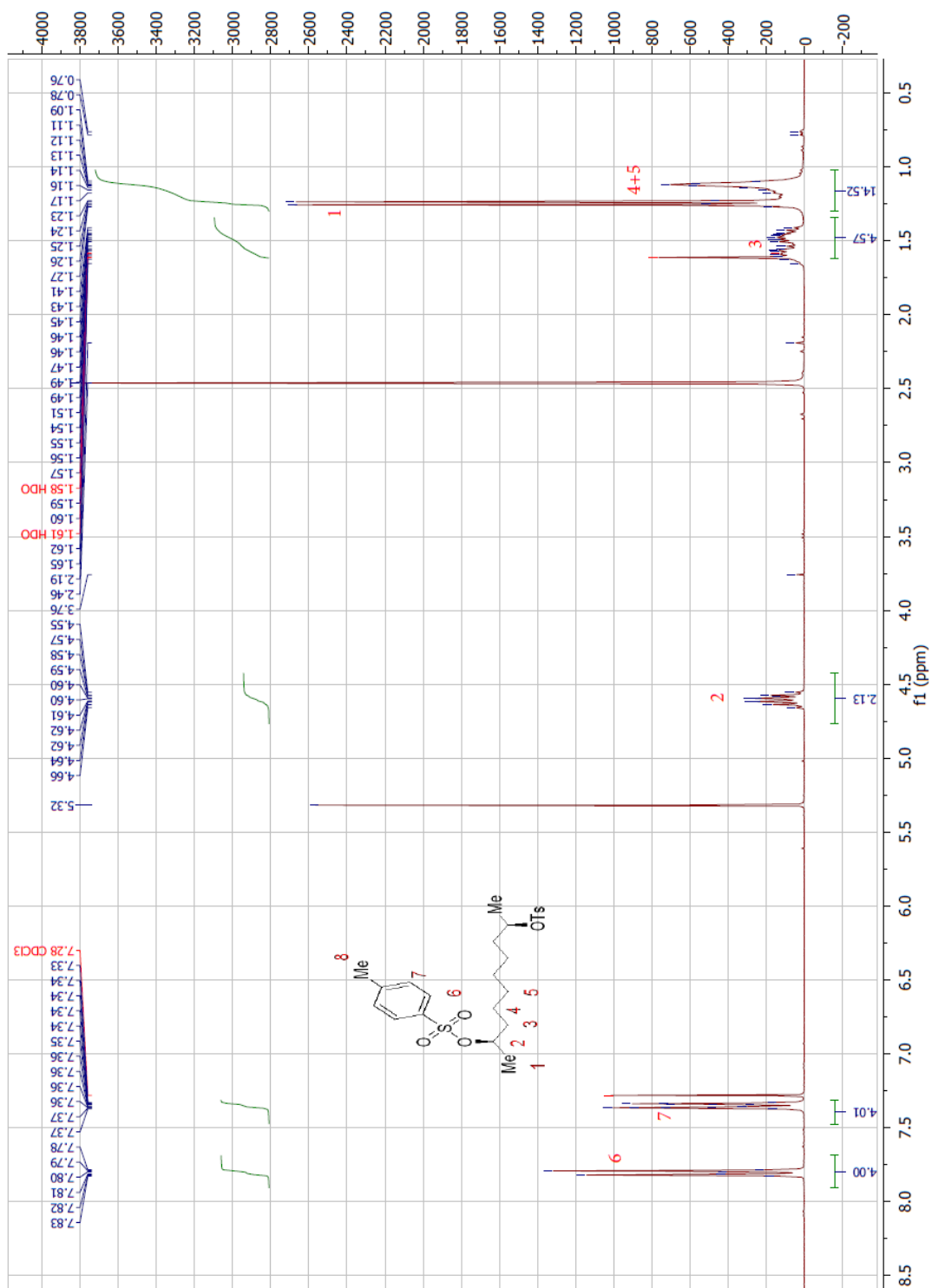


Figure 3-4: ^1H NMR for (*R,R*)- decane-2,9-diyl bis(4-methylbenzenesulfonate) (79a)

The enantiomers exhibited opposite and equal optical rotations [(*S,S*) +16.7°, (*R,R*) - 16.7°]. The mass spectra were also identical, each showing a prominent adduct with an ammonium ion at e.g. m/z 500.2122 in agreement with the calculated value (500.2135).

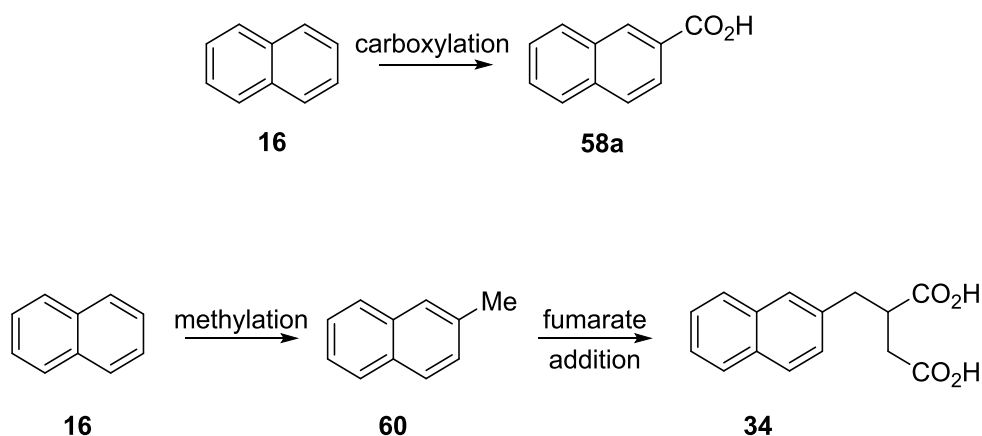
3. 4 Conclusions for the synthesis of (*S,S*)-, (*R,R*)- and (*R,S*)-Decane-2,9-diol Stereoisomers (74a-c)

Successful methods have been optimised for the synthesis of the (*S,S*)-, (*R,R*)- and (*R,S*)-decane-2,9-diol stereoisomers (**74a-c**) and conversion to their tosylates (**79a-c**) has been achieved. They have utilised well known chemical methods to develop and optimise completely novel routes to these three compounds which will hopefully provide further insight into the fumarate degradation pathway.

Chapter 4 Synthesis of $^{13}\text{C}_{10}$ -Naphthalene (**16***) and 2-Naphthoic acid (**58a**)

4.1 Introduction to the Syntheses of $^{13}\text{C}_{10}$ -Naphthalene (**16***) and $^{13}\text{C}_{11}$ -2-Naphthoic acid (**58a***)

Despite several studies, the pathway of anaerobic naphthalene (**16**) degradation has not been fully elucidated (see Section 2.6.4). Two routes of activation were initially postulated: either carboxylation to produce 2-naphthoic acid (**58a**)^{[70],[85]} or methylation to form 2-methylnaphthalene (**60**), followed by fumarate (**1**) addition to give 2-(naphthalen-2-yl) succinic acid (**61**)^[85] (Scheme 4-1). The carboxylation route is now accepted as the main route of activation.^{[67],[2]}



Scheme 4-1: Proposed routes of activation for anaerobic naphthalene (**16**) degradation.

4.2 Project Aims

The aim of the project was to synthesise $^{13}\text{C}_{10}$ -naphthalene (**16***) and $^{13}\text{C}_{11}$ -2-naphthoic acid (**58a***) (Figure 4-1) to aid in metabolite analysis studies. When trying to define an enzymatic pathway, metabolite analysis experiments using GC-MS or LC-MS are often used because of their applicability to using very small quantities.

However, whenever possible, NMR spectroscopy is employed as an aid to structure elucidation additional to MS, but this requires preferably at least one milligram for the detection of protons. On account of its low natural abundance (1.1%) ^{13}C NMRs generally require several milligrams unless ^{13}C -enrichment is utilised. The purpose of synthesising naphthalene (**16***) and 2-naphthoic acid (**58a***) fully labelled with ^{13}C was therefore to aid the identification of metabolites in a potentially complex metabolic pathway. By adding the $^{13}\text{C}_{10}$ naphthalene (**16***) to microcosms containing the naphthalene degrading bacteria, the resultant metabolic products can be traced through the catabolic process *via* GC-MS, ^1H and ^{13}C NMR spectroscopy.

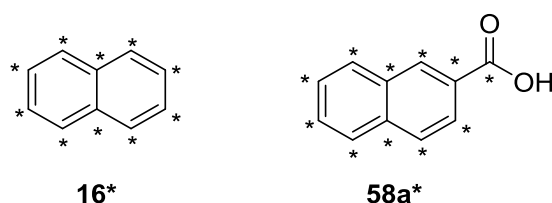
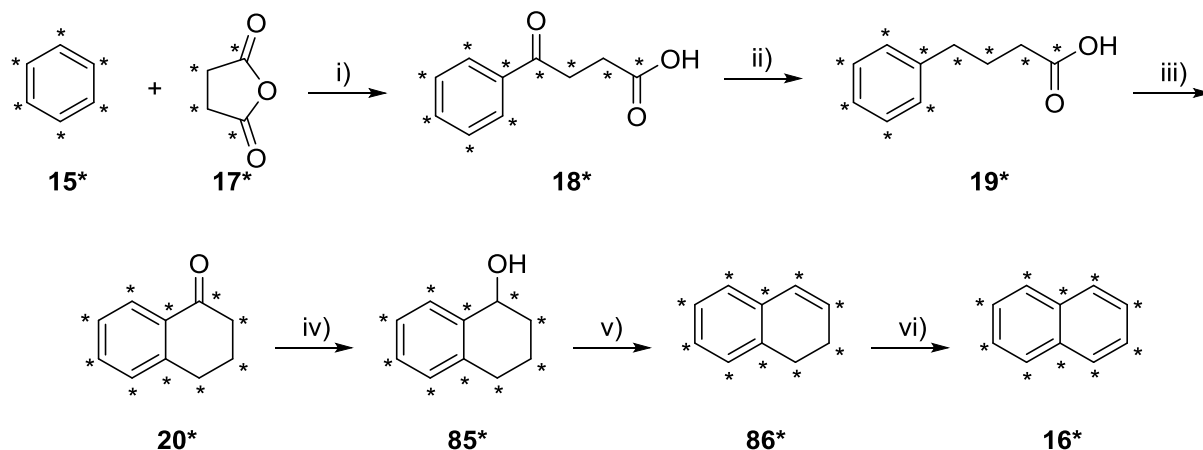


Figure 4-1: $^{13}\text{C}_{10}$ -naphthalene (**16***) and $^{13}\text{C}_{11}$ -2-naphthoic acid (**58a***).

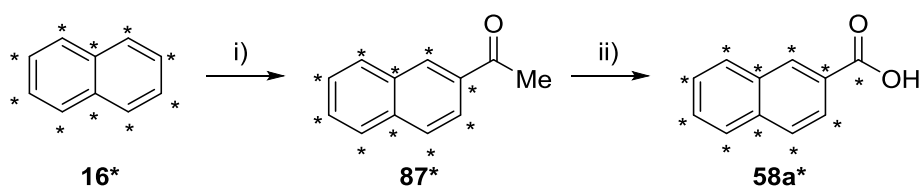
4.3 Proposed Approach to $^{13}\text{C}_{10}$ Naphthalene (**16***) and $^{13}\text{C}_{11}$ 2-Naphthoic Acid (**58a***)

The projected route to the labelled naphthalene (**16***) started from benzene (**15***) and followed the classical Haworth synthesis developed at Newcastle University in the 1930's.^[50] This method combines benzene (**15***) with succinic anhydride (**17***) *via* a Friedel-Crafts acylation. The route was attractive as both the $^{13}\text{C}_6$ -benzene (**15***) and $^{13}\text{C}_4$ -succinic anhydride (**17***) are commercially available, although both were expensive, and therefore the process was optimised using unlabelled materials. The Haworth route involves the acylation of benzene (**15**) with succinic anhydride (**17**) catalysed by AlCl_3 (i) to give 4-phenyl-4-oxobutanoic acid (**18**), which is reduced, cyclised and dehydrogenated to naphthalene (**16**). Improvements to the procedure were implemented in the present work (Scheme 4-2, shown with ^{13}C -labelling).



Scheme 4-2: i) AlCl_3 , 1,1,2,2-tetrachloroethane, 16 h, RT; ii) 10 % Pd/C, glacial acetic acid, H_2 , 48 h, 75 °C; iii) methanesulfonic acid, 90 °C, 2 h; iv) NaBH_4 , EtOH/THF, RT, 5h; v) *p*-TSA, toluene, 75 °C, 90 min; vi) 10 % Pd/C, maleic acid, 150 °C, 16 h.

For the conversion of naphthalene (**16**) into 2-naphthoic acid (**58a**), the classical method employing Friedel-Crafts acetylation to 2-acetylnaphthalene (**87**), followed by a haloform reaction was planned (Scheme 4-3, shown with ^{13}C -labelling). A potential problem with this approach was the formation of both 1- and 2- regioisomers of **87**, (addition to naphthalene can be achieved on both the 1- and 2- positions dependant on thermal vs. kinetic control factors) resulting in loss of some ^{13}C -labelled material, as well as the need for a potentially difficult separation.



Scheme 4-3: i) Acetyl chloride, AlCl_3 , nitrobenzene, 0 °C, 30 min; ii) 10 % NaOH , I_2 , KI, 60 °C, 2 h.

4. 4 Development of an Efficient Route for the Conversion of Benzene (15) into Naphthalene (16) and 2-Naphthoic Acid (58a)

4. 4. 1 Optimisation Experiments

Although the conversion of benzene (15) into naphthalene (16) and the latter into 2-naphthoic acid (58a) has been reported many times, we found that several of the reactions required extensive optimisation. All optimisation reactions were performed in triplicate to confirm reliability with average yields stated. Benzene (15) and succinic anhydride (17) were stirred with AlCl₃ in 1,1,2,2-tetrachloroethane to give 4-oxo-4-phenylbutanoic acid (18) with an average yield of 95%.^[86] Based on a literature procedure,^[87] 4-oxo-4-phenylbutanoic acid (18) in acetic acid was hydrogenated (hydrogen/palladium catalyst), initially at 75 °C. However, at this temperature over-reduction occurred, as shown by ¹H NMR analysis (Figure 4-2), from which it can be inferred that along with reduction of the keto to a methylene group as desired, the benzenoid ring was also being converted into a cyclohexane ring.

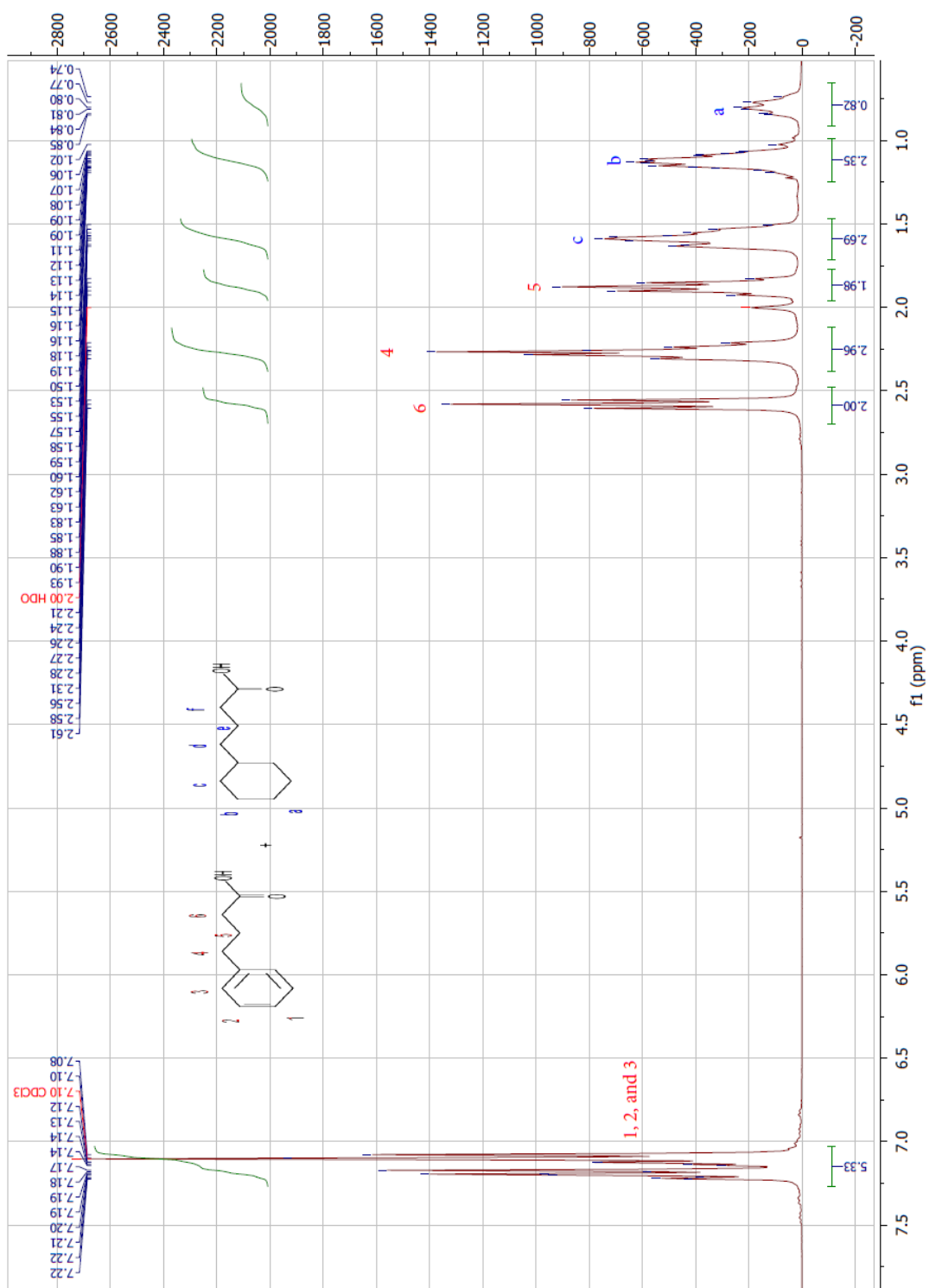


Figure 4-2: ¹H NMR of the product from reaction of 4-oxo-4-phenylbutanoic acid (**54**) in acetic acid with 5 weight % of 10% Pd/C in an atmosphere of H₂ at 75 °C for 9 h.

The reaction was repeated using 10 weight % of catalyst at RT for 16 h. After this time the starting material had fully converted to the product 4-phenylbutanoic acid (**19**) with no unwanted side products (Figure 4-3).

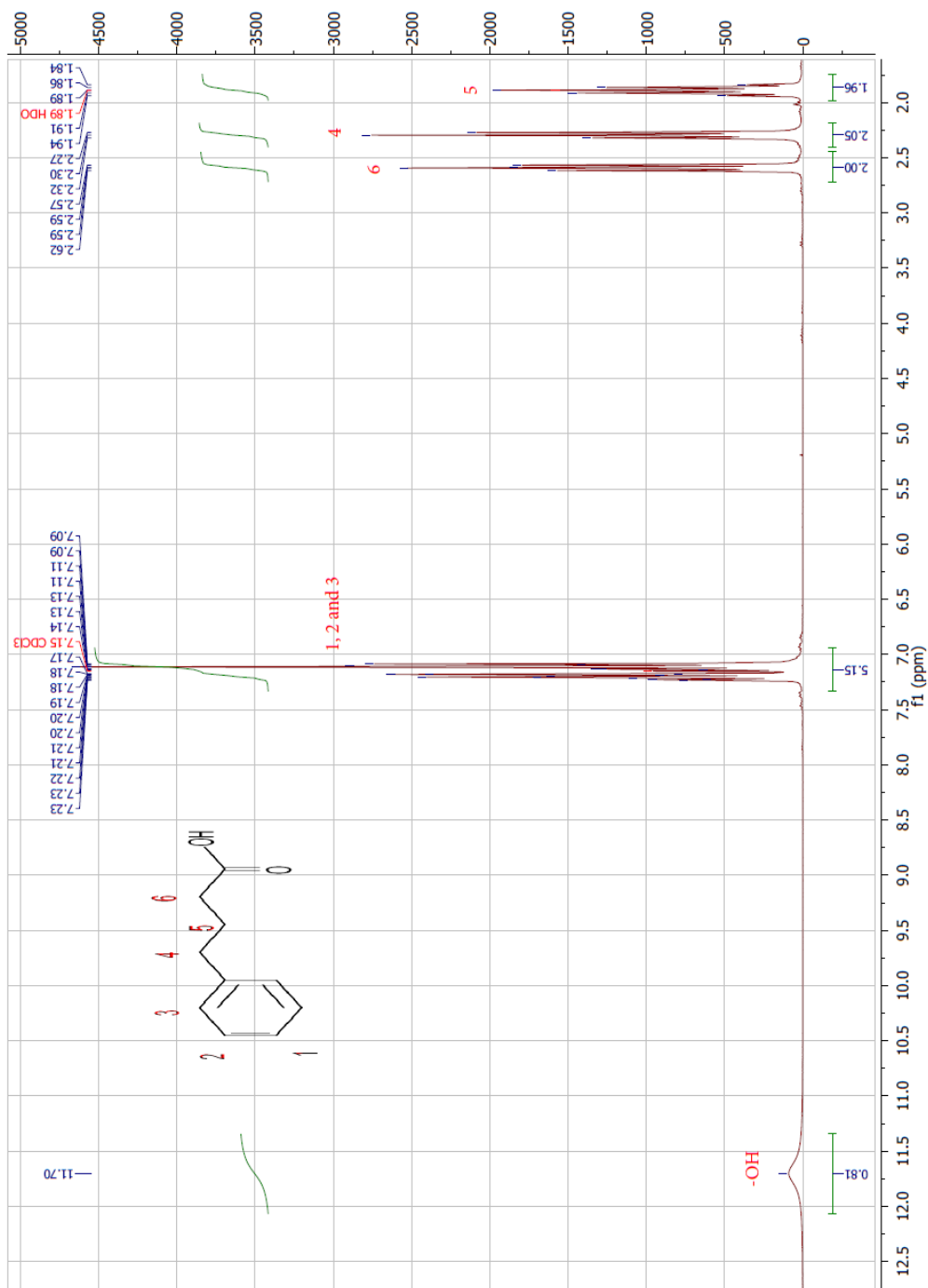


Figure 4-3: ¹H NMR of 4-phenylbutanoic acid (**19**) from reaction of 4-oxo-4-phenylbutanoic acid (**18**) in acetic acid with 10% by mass of 10 % Pd/C in an atmosphere of H₂ at room temperature for 16 h.

The 4-phenylbutanoic acid (**19**) was then cyclised with methanesulfonic acid to give 3,4-dihydronaphthalen-1(2*H*)-one (**20**) in 84 % yield.^[88] Reduction of the carbonyl group of **20** with NaBH₄ gave 1,2,3,4-tetrahydronaphthalen-1-ol (**85**) in 92 % yield,^[89] which was dehydrated in the presence of *p*-toluenesulfonic acid to afford 1,2-dihydronaphthalene (**86**).^[89]

Dehydrogenation of partially reduced aromatic compounds is a classical procedure using palladium, platinum or selenium catalysis.^[45] Thus, oxidation of (**86**) using 10 % Pd/C with no solvent gave naphthalene (**16**), but this was ~ 1:1 mixture with tetralin (**21**), which presumably arises by disproportionation of dihydronaphthalene (**86**). By raising the temperature from 150 °C to 200 °C and purging the vessel to remove H₂ the relative amount of naphthalene (**16**) was increased. We found that a dramatic improvement was achieved by adding a hydrogen acceptor, cyclopentene or maleic acid, with the latter being particularly effective because it was easily removed along with succinic acid by an aqueous base wash in the work-up of the reaction. (Table 4-1).

Conditions	% Naphthalene	% Tetralin
0.02 eq 10 % Pd/C, 150 °C, 4 h, 30 min purges	53	47
0.02 eq 10 % Pd/C, 200 °C, 4 h, 15 min purges	57	43
0.02 eq 10 % Pd/C, cyclopentane 50 °C, 4 h, 30 min purges	71	29
0.02 eq 10 % Pd/C, 5 eq maleic acid, 150 °C, 4 h, 30 min purges	92	8
0.02 eq 10 % Pd/C, 10 eq maleic acid, 150 °C, 4 h, 30 min purges	100	0

Table 4-1: Optimisation experiments to reduce the percentage of tetralin (**21**) in the dehydrogenation of (**57**). Percentages calculated by analysis of ¹H NMR.

Using 10 eq of maleic acid reduced the amount of tetralin (**21**) to zero as shown by ¹H NMR analysis. At the temperature used, 150 °C, the maleic acid melts and acts as a solvent for the reaction. The reaction was quenched with sat. NaHCO₃ whilst

the mixture was still molten, as the cooled mixture solidifies and makes work-up more difficult. Under these conditions, an average yield of 72 % was achieved.^[90] Numerous methods have been reported for the acetylation of naphthalene (**16**), with some catalysts claimed to afford very high yields of the 2-isomer (**87**). A method found in Vogel's textbook of Practical Organic Chemistry^[91] using a twofold excess of acetyl chloride and AlCl₃ at 0 °C in nitrobenzene, stirring under anhydrous conditions for 45 min, with slow addition of the AlCl₃ over this period led to the sole formation of the desired 2-acetylnaphthalene (**87**) with a 40 % yield as shown by ¹H NMR spectroscopic analysis (Figure 4-4) and comparison with commercially available reference standards. 2-Acetylnaphthalene (**87**) was reliably identifiable by singlets at δ 8.50 (CCHCCOMe) and 2.75 (CCHCCOMe).

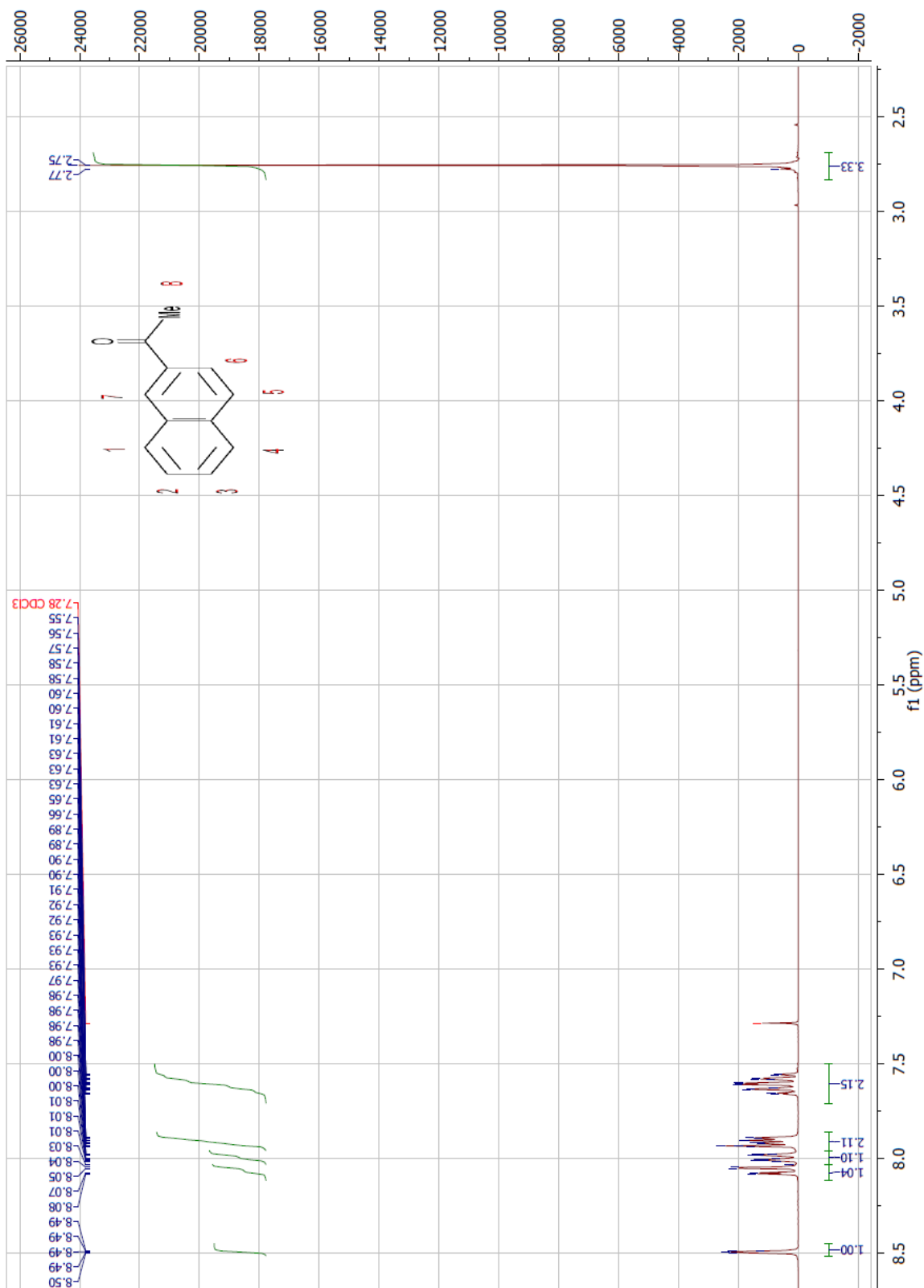


Figure 4-4: ^1H NMR spectrum of 2-acetylnaphthalene (87)

This was then converted to the 2-naphthoic acid (**58a**) utilising a haloform reaction with NaOCl and NaOH (Figure 4-5).^[92]

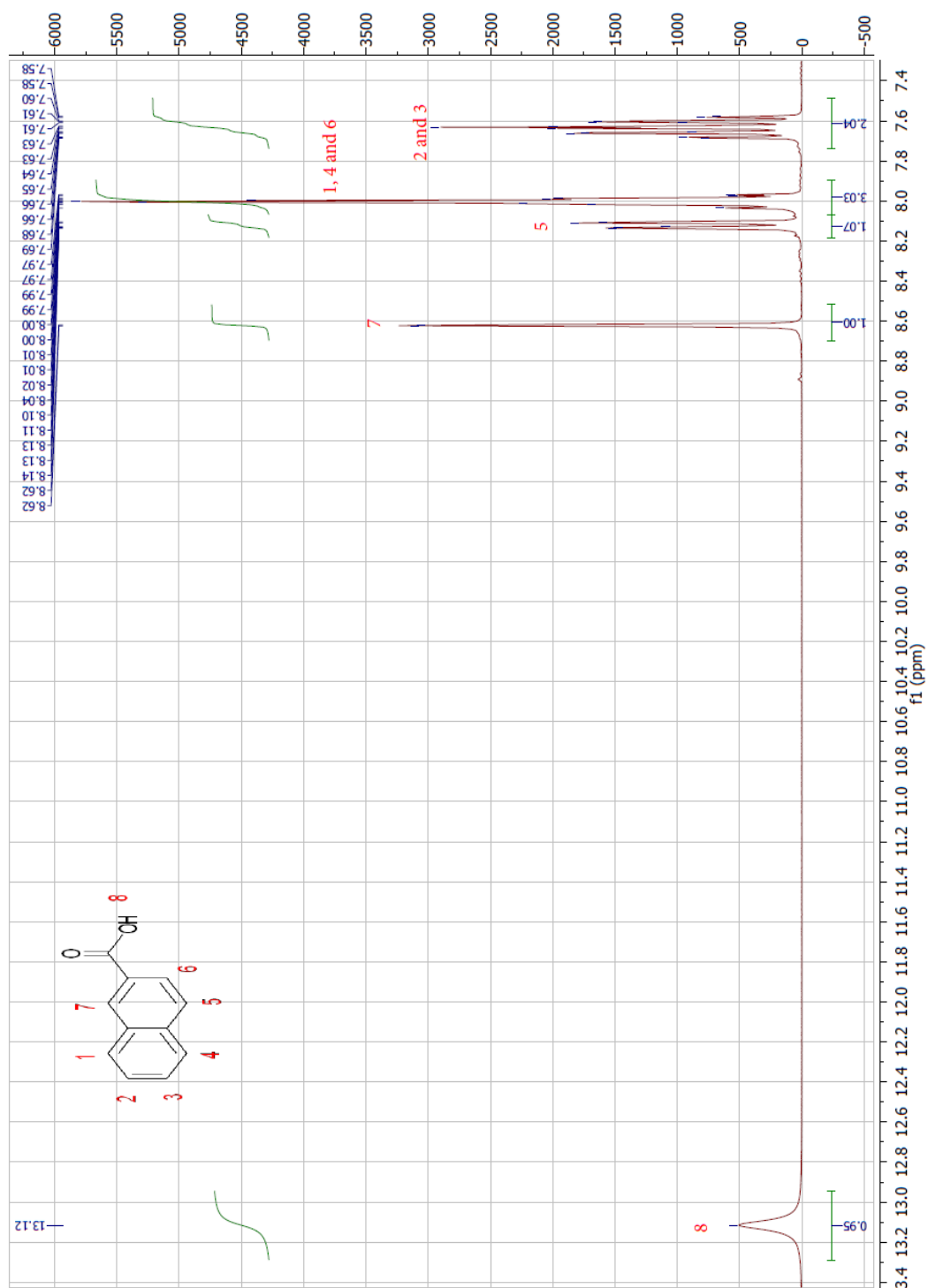


Figure 4-5: ¹H NMR spectrum of 2-naphthoic acid (**58a**)

4. 4. 2 Synthesis of $^{13}\text{C}_{10}$ Naphthalene (**16***)

The optimised synthesis of unlabelled naphthalene (**16**) described above was repeated using $^{13}\text{C}_6$ -benzene (**15***) and $^{13}\text{C}_4$ -succinic acid (**17***) to afford $^{13}\text{C}_{10}$ -naphthalene (**16***). NMR spectroscopy and MS data confirmed the structures. The ^1H NMR spectrum showed four broad multiplet peaks as two doublets, which correspond to the peaks seen in the ^1H NMR for the unlabelled naphthalene which have been split by ^{13}C coupling (Figure 4-6). The J values for these doublet of multiplets are ~ 156 Hz, which are consistent with this type of splitting. The mass spectrum of the labelled naphthalene showed a molecular ion at m/z 138.0968 (predicted mass 138.0961). Further work to convert the $^{13}\text{C}_{10}$ -naphthalene (**16***) into $^{13}\text{C}_{11}$ -2-naphthoic acid (**58a***) is underway.

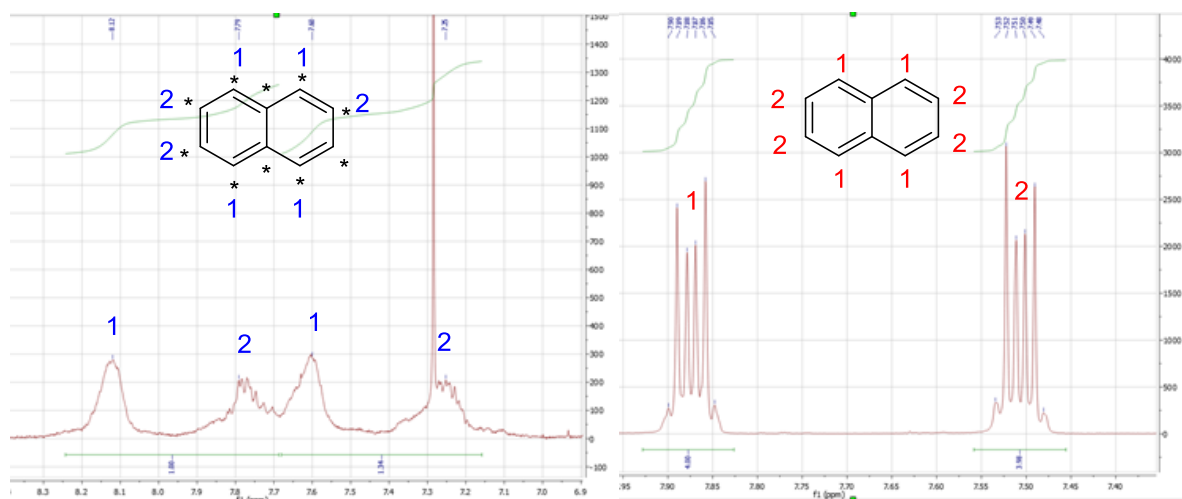


Figure 4-6: ^1H NMR spectra of $^{13}\text{C}_{10}$ -naphthalene (**16***) on the left and naphthalene (**16**) on the right.

4. 5 Conclusions for the Syntheses of $^{13}\text{C}_{10}$ -Naphthalene (**16***) and $^{13}\text{C}_{11}$ -2-Naphthoic acid (**58a***)

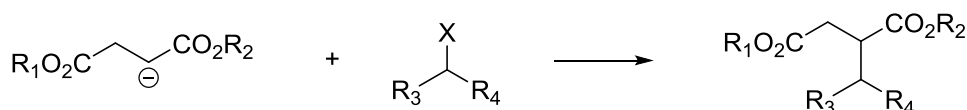
The classical Haworth route to naphthalene from benzene (**15**) and succinic anhydride (**17**) proved an excellent starting block for our synthetic scheme. Through extensive optimisation we have developed a high yielding and reliable route to naphthalene which has been applied to the labelled components. The $^{13}\text{C}_{10}$ -

naphthalene (**16***) will hopefully yield many new novel clues as to the pathway of its degradation in anaerobic microbes. We have also initiated the development of a high yielding route to $^{13}\text{C}_{11}$ -2-naphthoic acid (**58a***), however, further optimisation work must be performed before the method is applied to labelled substrates.

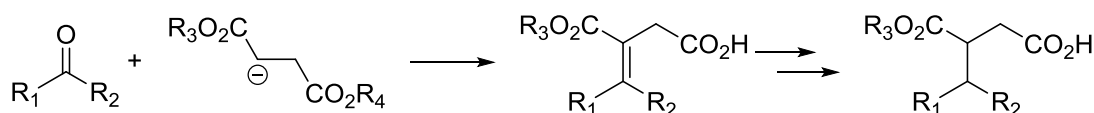
Chapter 5 Synthesis of Alkylsuccinates

5.1 Introduction to the Synthesis of Alkylsuccinates

Classical syntheses of alkylsuccinates often utilise a succinate ester as starting material, which is converted into a carbanion by deprotonation. This intermediate is either alkylated directly to an alkylsuccinate (Scheme 5-1) or is reacted with an aldehyde (Stobbe condensation) to give an alkenylsuccinate, which is reduced (Scheme 5-2).^[93]

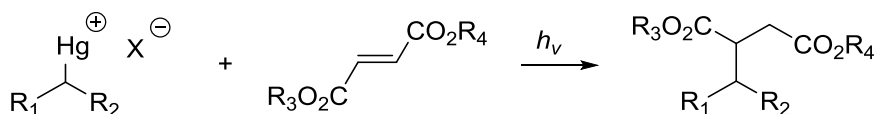


Scheme 5-1: Alkylation of a succinate carbanion to form an alkylsuccinate (X = Halogen, R = alkyl or aryl group).



Scheme 5-2: Stobbe condensation followed by reduction of double bond.

An alternative approach starts with a fumarate ester, which is reacted with an alkyl radical derived from an organomercury precursor (Scheme 5-3).^[94]



Scheme 5-3: Alkylsuccinate formation using organomercury precursor (X = Halogen).

5.2 Aims of the Syntheses of Alkylsuccinates

For the synthesis of 2-(alkan-2-yl)succinic acids [e.g. 2-(hexan-2-yl)succinic acid (**8**) Figure 5-1] a route is required that is applicable to a wide range of alkyl substituents

as there are now numerous examples of anaerobic hydrocarbon oxidation embracing alkanes with chain lengths from C₁ to at least C₅₀ (see Chapter 1).

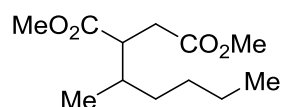


Figure 5-1: 2-(Hexan-2-yl)succinic acid (**8**)

Furthermore, 2-(alkan-2-yl)succinic acids possess two chiral centres and therefore four possible stereoisomers. Hence, any synthesis preferably enables stereocontrol, although it is now known that organisms such as HxN1 generate a ~ 1:1 mixture of two of these isomers, (2*R*,1'*R*) - and (2*S*,1'*R*)-2-(hexan-2-yl)succinic acid (**8**) (R. Jarling, 2012).^[22] In the research described below, three efficient and convenient methods were developed for the synthesis of dimethyl 2-(alkan-2-yl)succinates. The focus was on dimethyl esters because these were required as reference standards for the analysis of products from microbiological experiments.

2-(Hexan-2-yl)succinic acid (**8**) is the primary metabolite in the proposed oxidation scheme of hexane (**2**) in the *β-proteobacterium* strain HxN1 (Scheme 5-4). Synthesis of the stereoisomers of this acid would provide standard reference compounds for comparison with metabolites found in the bacterial cells. Previous work at Newcastle University^[22] showed that the first step of the catabolic process proceeds *via* the selective removal of the pro-*S* hydrogen at the C-2 position of hexane (**2**) by a glycol radical enzyme leading to two of the four diastereoisomers of 2-(hexan-2-yl)succinic acid (**8**) (Figure 5-2).

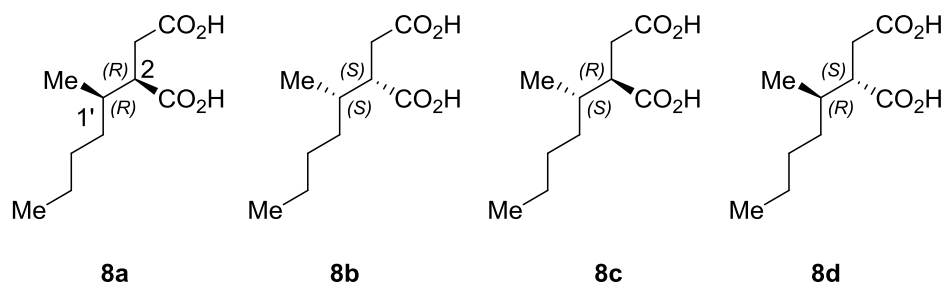


Figure 5-2: The four stereoisomers of 2-(hexan-2-yl)succinic acid (**8**) synthesised as chemical standards for comparison with metabolites of HxN1 grown on hexane (**2**).

It was also shown that the absolute configuration of the downstream 4-methyloctanoyl-CoA (**11**) is *R* (Figure 5-3).^[22]

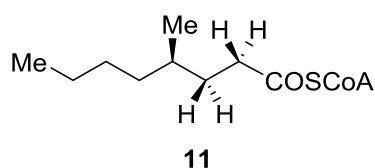


Figure 5-3: 4-methyloctanoyl-CoA (**11**) with *R*-configuration.

In addition, the four isomers of 2-(hexan-2-yl)succinic acid (**8**) were synthesised (Figure 5-4) and compared with the metabolites found in the bacterial cells using gas chromatography (GC) for analysis (M. Sadeghi, 2015; R. Jarling, 2012).^{[95],[22]}

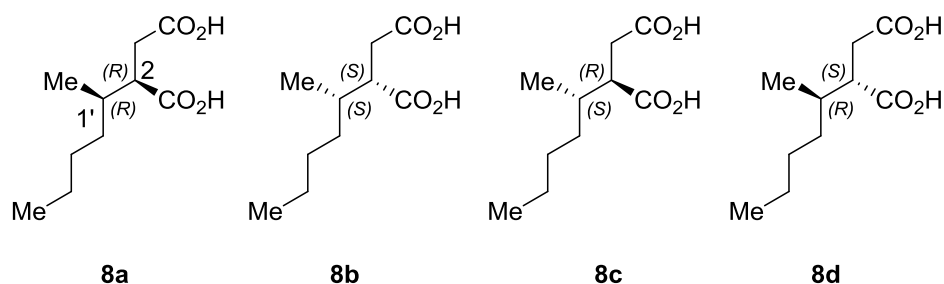


Figure 5-4: The four stereoisomers of 2-(hexan-2-yl)succinic acid (**8**) synthesised as chemical standards for comparison with metabolites of HxN1 grown on hexane (**2**).

These studies showed that whereas the stereochemistry at C-1' of 2-(hexan-2-yl)succinic acid (**8**) is exclusively (*R*), the stereochemistry at C-2 is variable, creating a mixture of (*2R,1'R*) - and (*2S,1'R*)-isomers (see Sections 1.2, 1.3.1 and 1.9). We aimed to improve the synthesis of the four stereoisomers (Figure 5-4) of the 2-(hexan-2-yl)succinic acid (**8**) for further enzymology studies. Also, the method(s) developed should be applicable to other 2-(alkan-2-yl)succinic acids to enable studies of the metabolism of e.g. henicose (C₂₁H₄₄), which is converted into 2-(henicosan-2-yl)succinate (**88**) (Figure 5-5) by fumarate (**1**) addition and is a potential metabolite of a sulfate-reducing bacteria in a control microcosm (see Section 1-10). A biomimetic route to alkylsuccinates, which involves the addition of

HI across an alkene followed by radical abstraction of the iodine and addition of dimethyl fumarate to the alkyl radical was optimised.

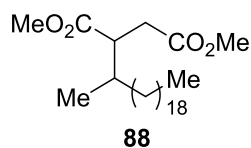


Figure 5-5: Dimethyl 2-(henicosan-2-yl) succinate (**88**).

The aforementioned microcosm was one of three samples initially intended as control microcosms, containing sediment from the River Tyne as an inoculum suspended in an aqueous nutrient medium with added sulfate and non-degraded North Sea oil as a growth substrate. The sample in question was sealed under anoxic conditions and pasteurised by heating at 95 °C in an autoclave for 2 hours, which was expected to denature the bacteria. However, when sampled, the GC-MS analysis showed the presence of many long chain alkylsuccinic acids, presumably derived from the addition of fumarate (**1**) to their hydrocarbon counterparts. The expectation was that no alkylsuccinates would be present in the sample, but unexpectedly a variety of medium and long chained alkylsuccinates were observed from day 176 (Figure 5-6). It was postulated that the high temperatures during pasteurisation destroyed any bacteria which prosper at ordinary temperatures, but spores of thermophilic bacteria present in the sample germinated. To investigate this proposal, we aimed to synthesise dimethyl 2-(henicosan-2-yl)succinate (**88**) by the previously optimised pathway in order to provide a reference compound for comparison with metabolites found in the bacterial cells.

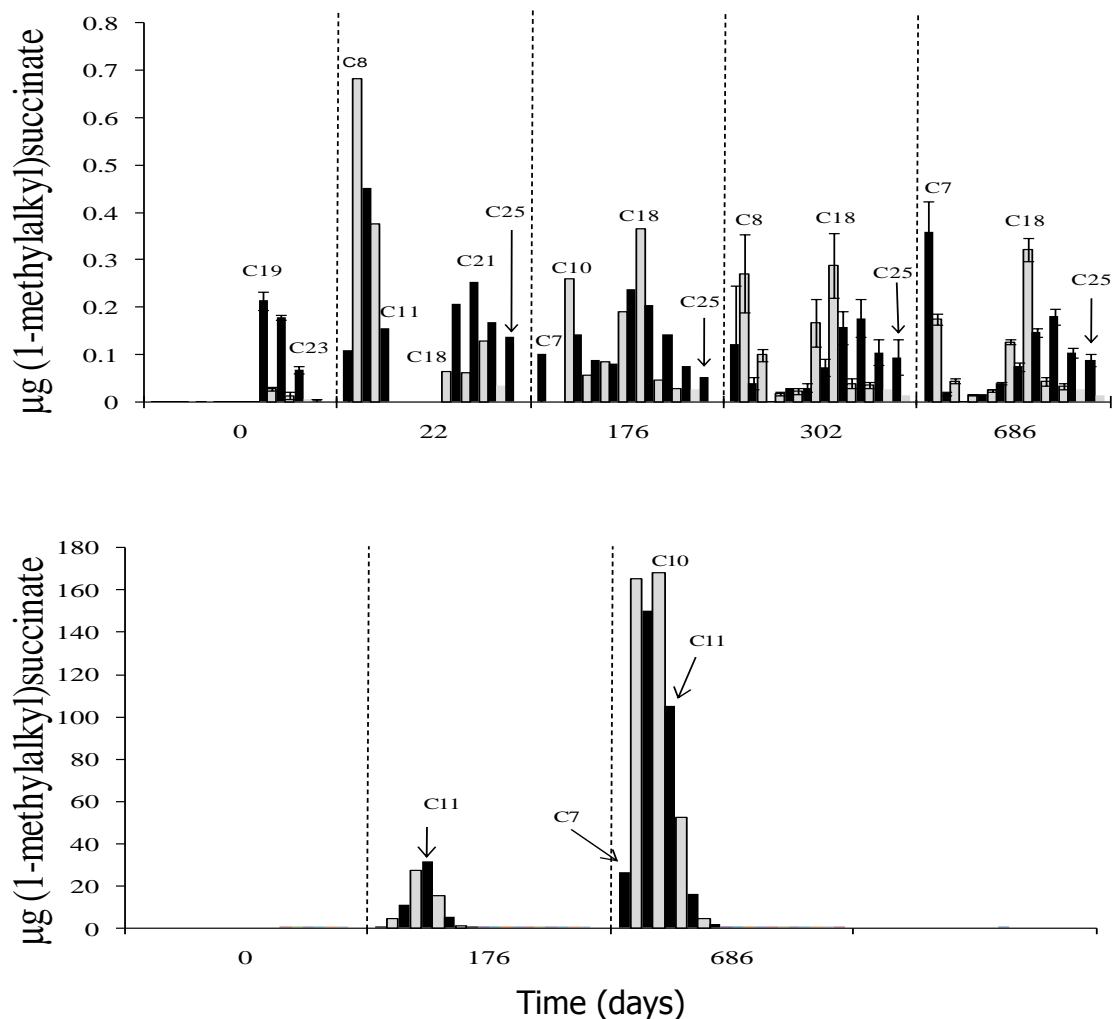


Figure 5-6: Top bar chart displays the results for the unpasteurised microcosm sample with a range of alkylsuccinate metabolites from day 0, varying to day 686. The bottom bar chart displays the results for the pasteurised sample over the same timescale.

However, when the radical addition method was used to synthesise the dimethyl 2-(hencosan-2-yl) succinate (**88**), regioisomeric products were formed. An alternative synthesis was developed utilising *O*-mesylation followed by two alkylation steps, firstly converting to a malonate then to a tricarboxylate, and finally a Krapcho decarboxylation step to yield dimethyl 2-(hencosan-2-yl) succinate (**88**). This route worked well and also proved reliable at producing both regio- and stereochemically pure products. It was therefore used to synthesise 3 more alkylsuccinates, dimethyl 2-(undecan-2-yl)succinate (**89**), dimethyl 2-(undecan-3-yl)succinate (**90**) and dimethyl 2-(undecan-6-yl)succinate (**91**) (Figure 5-7) which were used to investigate the regioselectivity of the enzymes addition of fumarate (**1**) to undecane.

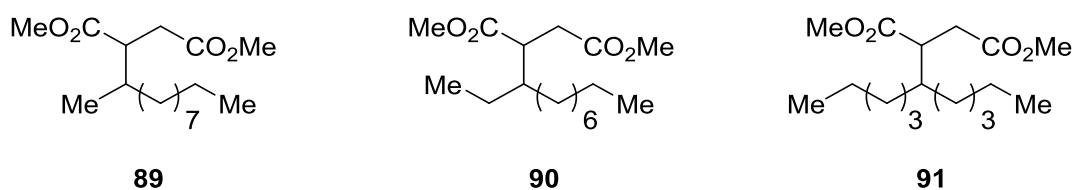
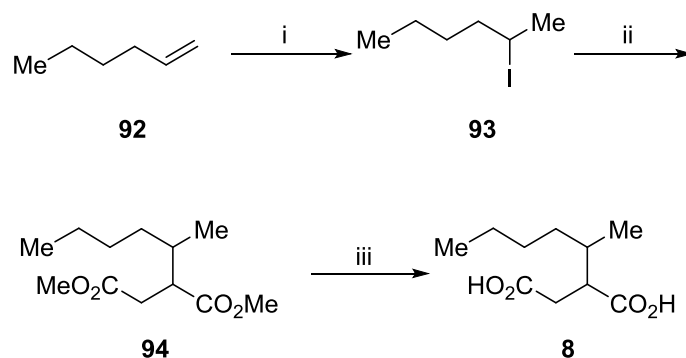


Figure 5-7: Dimethyl 2-(undecan-2-yl)succinate (**89**), dimethyl 2-(undecan-3-yl)succinate (**90**) and dimethyl 2-(undecan-6-yl)succinate (**91**).

5.3 Methodology for the Synthesis of 2-(Hexan-2-yl)succinic acid (**3**)

Initially, the previously employed method for producing 2-(hexan-2-yl)succinic acid (**3**) was utilised, but with the intention of optimising this method. This method involved the conversion of hex-1-ene (**60**) into 2-iodohexane (**61**) using hydrogen iodide^[96] and a subsequent biomimetic radical addition to dimethyl fumarate giving dimethyl 2-(hexan-2-yl)succinate (**62**)^[97]. The diester could be further hydrolysed to produce 2-(hexan-2-yl)succinic acid (**3**) as a ~ 1:1 mixture of the (2*R*,1'*S*/2*S*,1'*R*)- and (2*R*,1'*R*/2*S*,1'*S*)-diastereoisomers (Scheme 5-4).^[98] The method was developed to allow variability of the alkyl substituent for application to other enzymatic pathways. The substrate tolerance with a radical reaction means that the developed method can be used to synthesise primary-, secondary- and tertiary-alkylsuccinic acids dependent on the alkyl iodide used. As well as being an attractive route to numerous succinic acids, the method can also be used to synthesise maleic and fumaric acids by addition to alkynes as opposed to alkenes.^[1]



Scheme 5-4: Synthesis of 2-(hexan-2-yl)succinic acid (**3**). *Reagents and conditions:* i) KI, phosphoric acid, 80 °C, 48 h; ii) Dimethyl fumarate, *tris*(trimethylsilyl)silane (TTMSS), azo-*bis*-isobutyronitrile (AIBN), α,α,α -trifluorotoluene, N₂, 105 °C, 12 h; iii) LiOH, THF, water, N₂, RT, 12 h

The (2*R*,1'*S*/2*S*,1'*R*)-diastereoisomers of 2-(hexan-2-yl)succinic acid (**8**) selectively crystallised out of solution containing the diastereoisomer mixture. This result was established by analysis of the crystal structure (Figure 5-8).

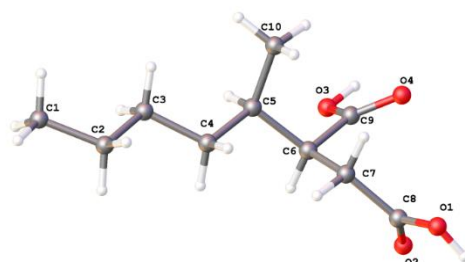


Figure 5-8: X-ray crystal structure of crystalline 2-(hexan-2-yl)succinic acid (**8**) (only (2*S*,1'*R*)-diastereoisomer shown).^[1]

The method shown in Scheme 5-4 worked well and proved to be reliable. The iodination reaction proceeded with an average yield of 52% to give the product (**93**) as a yellow-brown oil with no purification required, the colour was attributed to small amounts of iodine in the sample. The ¹H NMR shows a clear doublet at δ 1.88, $J = 6.7$ Hz, relating to the methyl group adjacent to the carbon iodide bond (Figure 5-9).

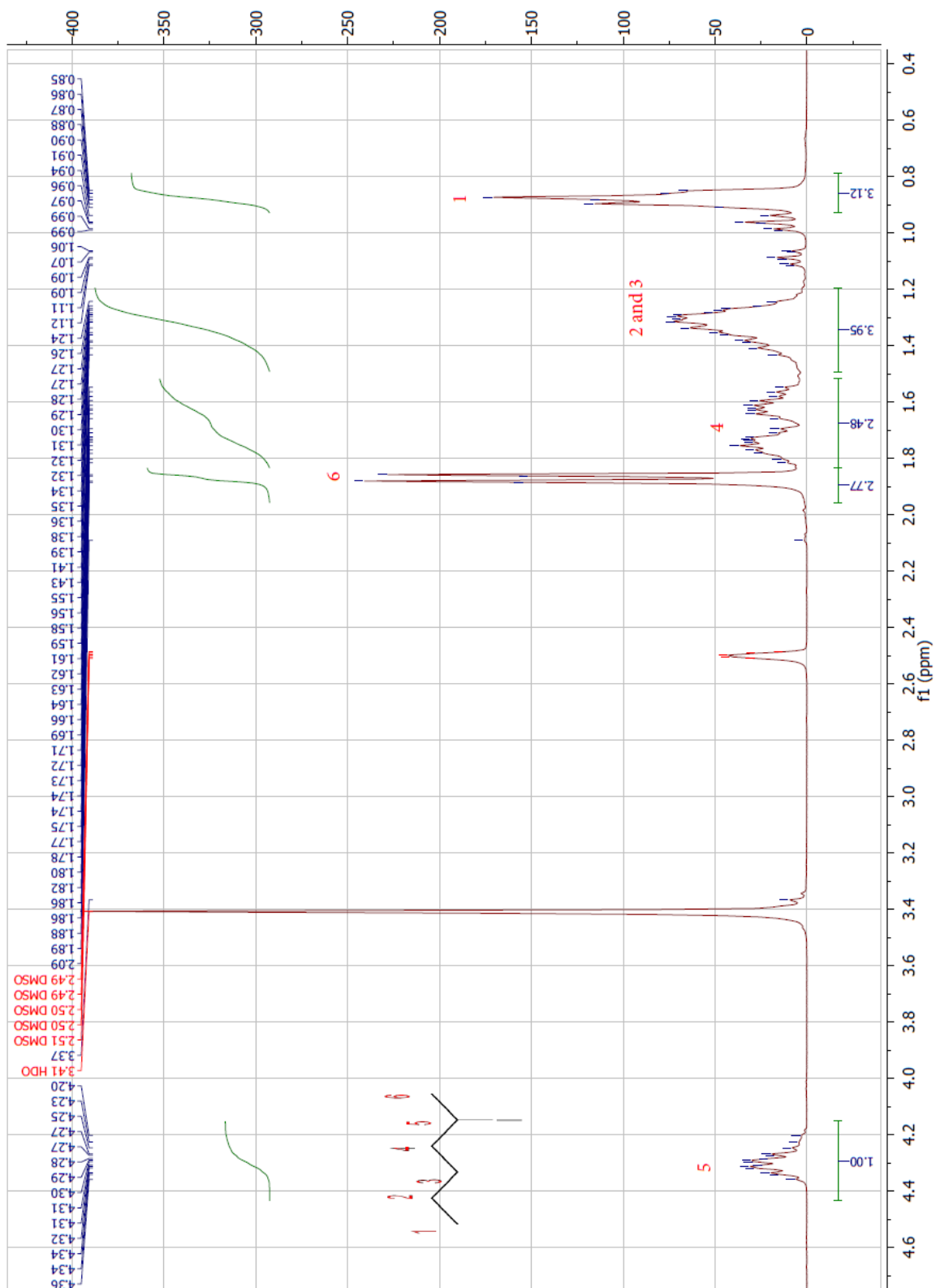


Figure 5-9: ¹H NMR spectrum of 2-iodohexane (93).

The generation of the hex-2-yl radical produced from the 2-iodohexane (**93**) and the azo-*bis*-isobutyronitrile (AIBN)] and its addition to dimethyl fumarate was investigated under a variety of conditions to try to optimise the method (Table 5-1). The reactions were monitored by liquid chromatography-mass spectrometry (LC-MS) to determine the extent of the reaction at set time points. It was found that reaction 4 (Table 5-1) gave the best results. Increase of the dimethyl fumarate (shown in table as DMeF) for rows 1-6 shows that increasing the amount of dimethyl fumarate increases reaction rate to a point (around 2.5-3 equivalents, reaction 3 and 4) past this point there is no increase. We therefore set the optimum value as 3 eq. For AIBN, variation in values (rows 7-12) shows a similar pattern. The lowest concentration at the point of maximum rate is 0.15 eq (reaction 4). When the value of tris(trimethylsilyl)silane is altered, rows 13-15, not much change is observed, it was therefore decided as it is difficult to separate from the product using chromatography that a low amount of 1.25 equivalents would be used. Altering the volume of trifluorotoluene (rows 16-19) also did not seem to affect reaction rates significantly and 10 ml/mmol was used. Reaction 4, shown in bold in the table, was decided to be the best approach for the synthesis and these values were decided upon as the standard amount for the radical addition method.

Reaction	Molar Eq.			Volume (mmol/mL) Trifluorotoluene	% Conversion after 4h
	DMeF	AIBN	TTMSS		
1	1.5	0.15	1.25	10	85
2	2	0.15	1.25	10	89
3	2.5	0.15	1.25	10	95
4	3	0.15	1.25	10	98
5	4	0.15	1.25	10	99
6	5	0.15	1.25	10	98
7	3	0.05	1.25	10	81
8	3	0.1	1.25	10	89
9	3	0.2	1.25	10	97
10	3	0.3	1.25	10	96
11	3	0.4	1.25	10	98
12	3	0.5	1.25	10	97
13	3	0.15	1	10	96
14	33	0.15	1.5	10	97
15	3	0.15	2	10	98
16	3	0.15	1.25	5	92
17	3	0.15	1.25	15	94
18	3	0.15	1.25	20	93
19	3	0.15	1.25	25	94

Table 5-1: Optimisation of the synthesis of dimethyl 2-(hexan-2-yl) succinate (**8**).

When performed for synthesis rather than for kinetic analysis, the radical addition reaction using the above conditions proceeded with an average yield of 76% to give the product **94** as a clear oil. However, multiple columns were required to fully separate the product from silyl by-products. The final hydrolysis step required a relatively long stirring period, generally taking around 96 h to complete, and the 2-(hexan-2-yl)succinic acid (**8**) product required no further purification after work up

and was obtained with an average 80% yield as a clear oil. Vapour diffusion recrystallisation of the diastereomeric mixture from ethyl acetate and petrol yielded the pure (2*R*,1'*S*/2*S*,1'*R*)--stereoisomer as white crystals in 24% yield (Figures 5-10 and 5-11).

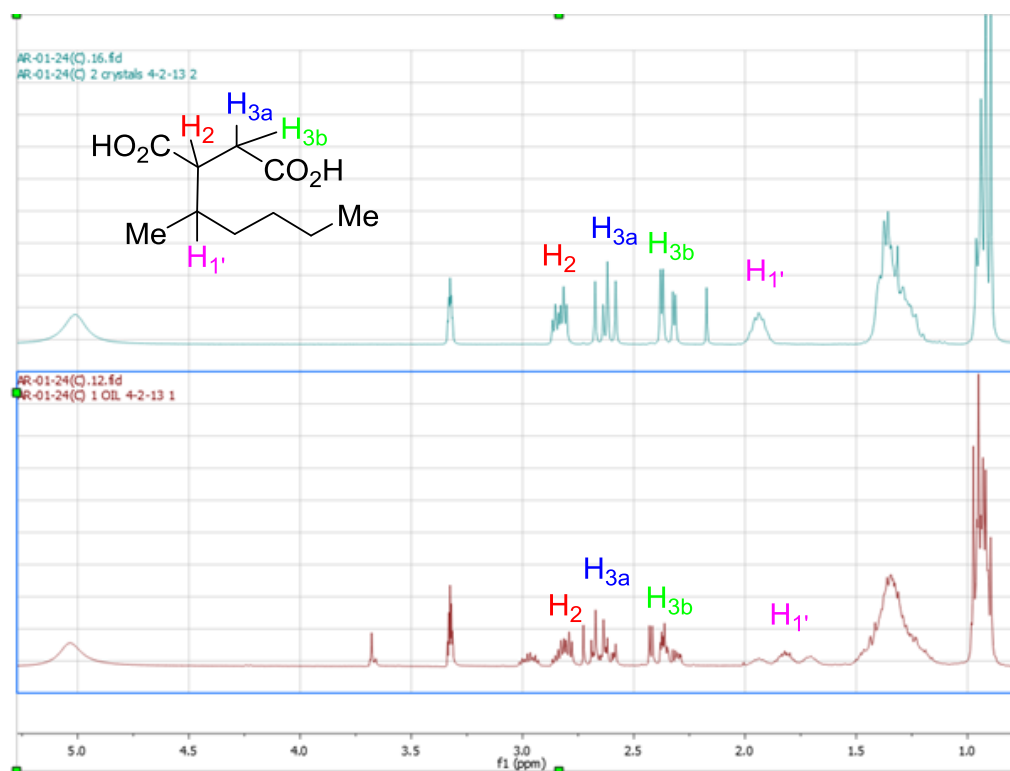


Figure 5-10: ¹H NMR spectra of pure (2*R*,1'*S*/2*S*,1'*R*)- 2-(hexan-2-yl)succinic acid (**3**) (top spectrum) and a mixture of the diastereoisomers (bottom spectrum).

The ¹H NMR spectrum for the mixture of diastereoisomers shows a series of peaks in the 1.5-3 ppm region in which clear differences can be observed between the two diastereoisomers when compared to the ¹H NMR spectrum for the pure of diastereoisomer sample. For the pure diastereomer sample, the set of peaks relating to the succinic acid moiety show interesting coupling patterns (Figure 5-11). The ¹H NMR spectrum for the mixture of diastereoisomers showed a series of peaks in the 1.5-3 ppm region in which clear differences can be observed between the two diastereoisomers when compared to the ¹H NMR for the pure of diastereoisomer

sample. For the pure diastereomer sample (Figure 5-11), the proton H_2 showed vicinal coupling to H_{3a} , H_{3b} and $H_{1'}$, producing a double double doublet, which due to overlap of some peaks is an apparent doublet of triplets at δ 2.84 [$J = 3.6$ Hz (vicinal to H_{3b}), 4.5 Hz (vicinal to $H_{1'}$) and 10.9 Hz (vicinal to H_{3a})]. The proton H_{3a} shows vicinal coupling to H_2 and geminal coupling to H_{3b} . This produces a double doublet splitting pattern at δ 2.63 [$J = 10.9$ Hz (vicinal to H_2) and 16.7 Hz (geminal to H_{3b})]. A double doublet at δ 2.35 was also observed for proton H_{3b} [3.6 Hz (vicinal to H_2) and 16.7 Hz (geminal to H_{3a})].

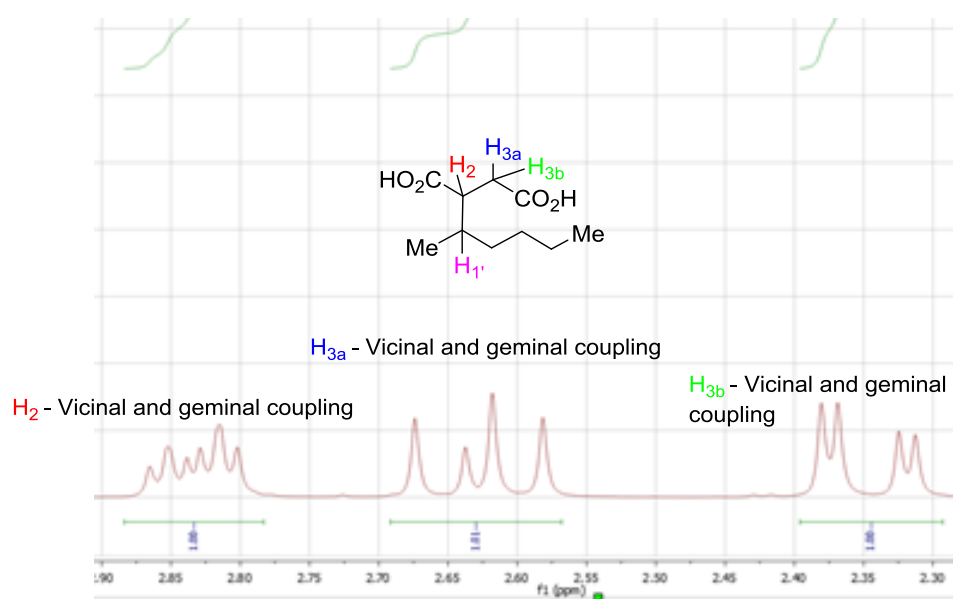


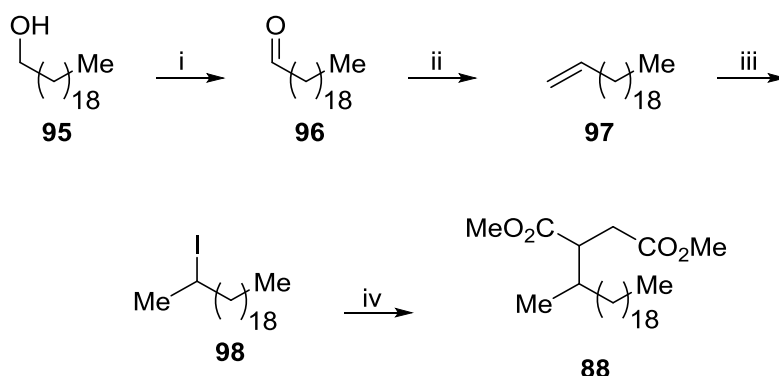
Figure 5-11: Coupling patterns observed for succinic acid moiety of pure (2*R*,1'*S*/2*S*,1'*R*)- 2-(hexan-2-yl)succinic acid (**8**).

5. 4 Synthesis of Dimethyl 2-(henicosan-2-yl) Succinate (**63**): Radical

Addition Method

The C_{21} alkene, henicos-1-ene (**97**), is not commercially available. The synthesis of **88** was performed by oxidising commercially available icosan-1-ol (**95**) with pyridinium chlorochromate (PCC) to icosanal (**96**)^[99], which was converted to henicos-1-ene (**97**) by a Wittig reaction^[100]. The alkene was reacted with HI in the manner previously described for hex-1-ene (**92**) to give (supposedly – see below) 2-iodohenicosane **98**. This material was combined with dimethyl fumarate using the

biomimetic method (i.e. *via* an intermediate heneicosan-2-yl radical), which was expected to give dimethyl 2-(heneicosan-2-yl)succinate (**88**) (Scheme 5-5).



Scheme 5-5: Synthesis of dimethyl 2-(heneicosan-2-yl)succinate (**88**). i) PCC, DCM, RT, 3 h; ii) KO^tBu, THF, MePPh₃Br, RT, 16 h; iii) KI, phosphoric acid, 80 °C, 48 h; iv) Dimethyl fumarate, *tris*(trimethylsilyl)silane (TTMSS), azo-*bis*-isobutyronitrile (AIBN), α,α,α-trifluorotoluene, N₂, 105 °C, 12 h.

5.5 Analysis of the Putative Dimethyl 2-(heneicosan-2-yl)-succinate (**88**)

The route to **88** (Scheme 5-5) initially appeared to work well despite issues experienced with the substrate due to its physical properties. The oxidation step proceeded in 65% yield, with losses occurring in the work-up. Similar observations were made with the Wittig step, which gave an average 68% yield. The iodination and radical addition steps occurred with similar yields to those found before, 59% and 71%, respectively. Analysis of the product dimethyl 2-(heneicosan-2-yl) succinate (**88**) by two-dimensional (2D) GC-MS showed, instead of a single peak, five closely clustered peaks (Figure 5-12).

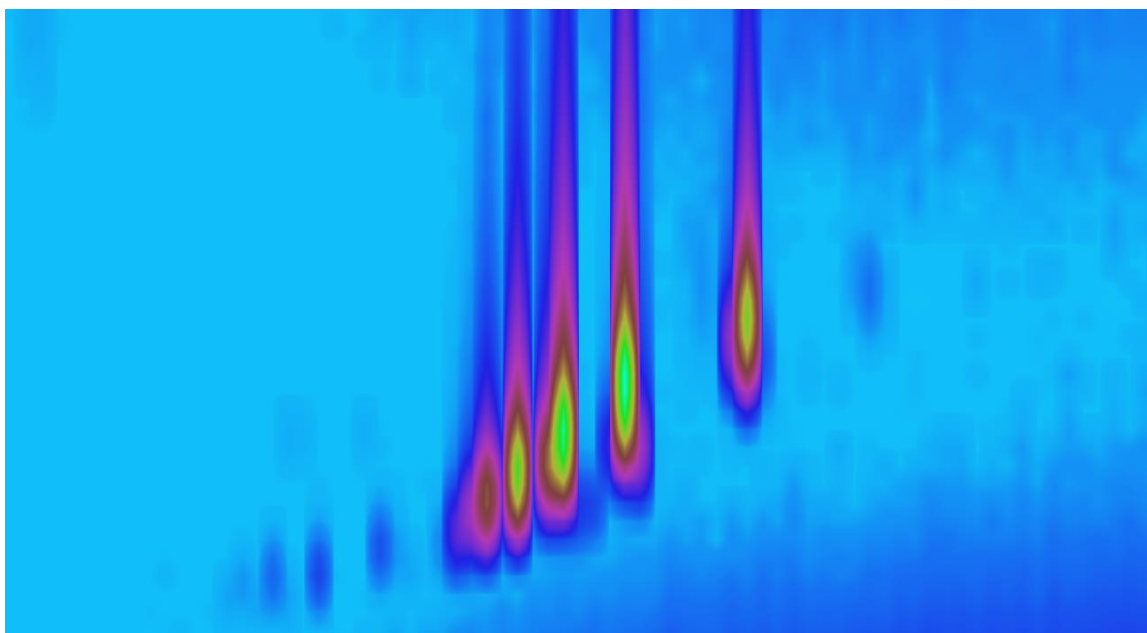
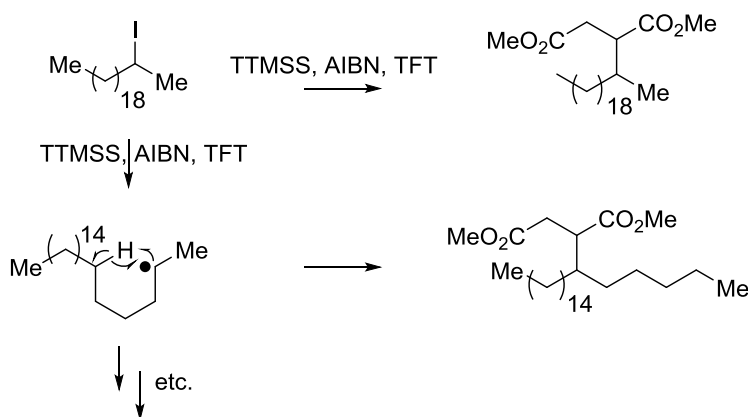


Figure 5-12: Total ion current (TIC) chromatogram for the dimethyl 2-(henicosan-2-yl)succinate (**88**) sample prepared *via* the radical addition method.

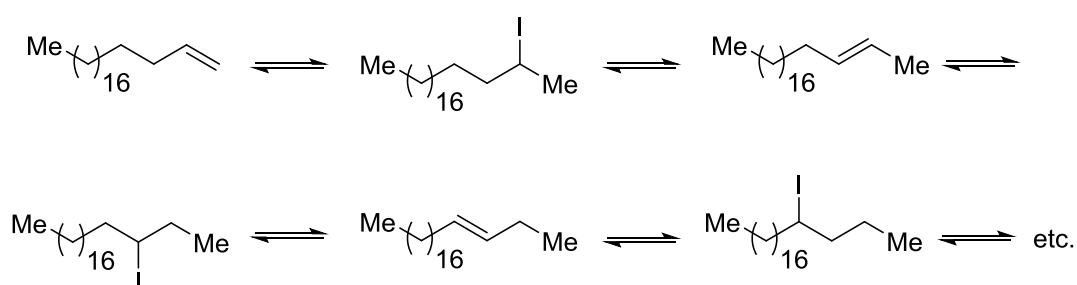
Two possible explanations for this observation are;

1. Multiple 1,5-hydrogen atom shifts during the biomimetic radical addition step producing a series of alkylsuccinate isomers (Scheme 5-6). The probability of each successive isomer decreases as the radical shifts along the chain. This process will afford a number of distinctive (henicosan-2-yl)-succinate isomers (i.e. dimethyl 2-(henicosan-2-yl)succinate, dimethyl 6-(henicosan-2-yl)succinate, dimethyl 10-(henicosan-2-yl)succinate etc).



Scheme 5-6: Rearrangement of the henicosan-2-yl radical via 1,5-hydrogen atom shifts.

2. Reversibility of the addition of HI to the alkene producing isomeric iodoalkanes (Scheme 5-7). As henicos-2-ene is more stable than henicos-1-ene, this isomer will be preferentially formed from 2-iodohenicosane. Addition of HI to henicos-2-ene will afford 2- and 3-iodohenicosane. Subsequent eliminations/re-additions of HI will move the iodine atom along the chain. The resulting distribution of iodohenicosanes will depend on the time of reaction and the relative stabilities of the compounds, which is expected to be similar. This process can, in principle, lead to all possible (henicosan-2-yl)-succinate isomers.



Scheme 5-7: Potential reversible addition of HI across the alkene.

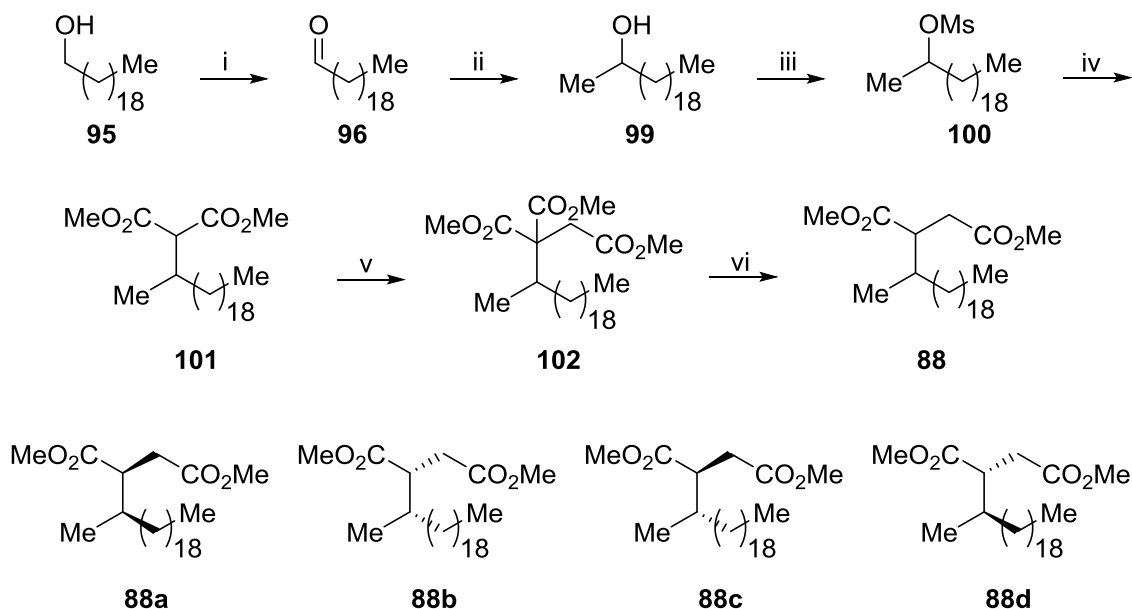
Given the problematical issues with the iodination of henicos-1-ene (**97**) in the synthesis of dimethyl 2-(henicosan-2-yl)succinate (**88**), two alternative routes to the latter compound were developed.

It is assumed that the dimethyl 2-(henicosan-2-yl)succinate (**88**) cannot be obtained pure in the same manner as dimethyl 2-(hexan-2-yl)succinate (**94**) as the long hydrocarbon chain of the dimethyl *n*-(henicosan-2-yl)succinates makes the R_f values for the regioisomers much more similar than those for the dimethyl *n*-(hexan-2-yl)succinates as they are chemically more similar. The multiple columns required to purify the dimethyl 2-(hexan-2-yl)succinate (**94**) from the silyl impurities may have inadvertently separated any regioisomers out of the mixture.

5.6 Alkylsuccinates *via* a Krapcho Degradation

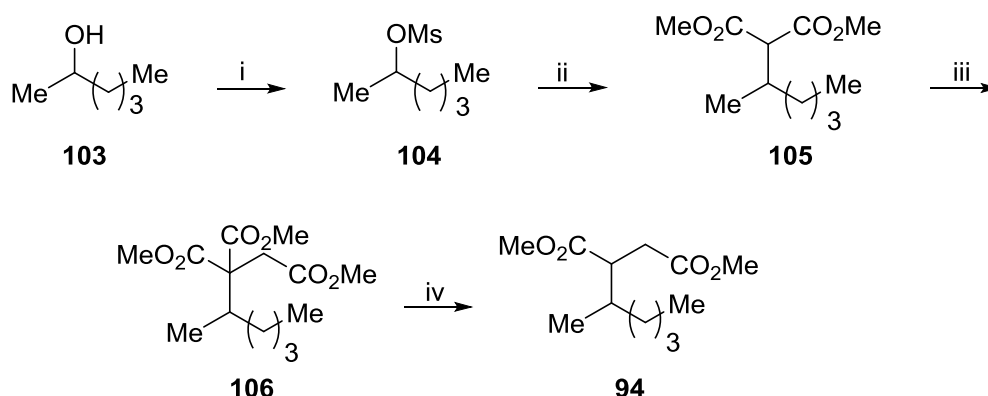
5.6.1 Aim and Proposed Approach for the Krapcho Synthesis

Owing to the formation of isomers when dimethyl 2-(hencicosan-2-yl) succinate (**88**) was synthesised according to Scheme 5-5, an alternative route circumventing the problematic HI addition (Scheme 5-8) was developed from hencicosan-2-ol (**99**). The commercially available icosan-1-ol (**95**) was oxidised to icosan-1-al (**96**),^[101] which was reacted with methylmagnesium bromide to produce hencicosan-2-ol (**99**).^[102] The alcohol was converted into its mesylate **100**, which was reacted with the anion of dimethyl malonate to give alkylmalonate **101**^[103] that was further alkylated with methyl bromoacetate. The resulting triester (**102**) was subjected to a Krapcho-type decarboxylation^[104] to afford the desired dimethyl alkylsuccinate as a mixture of racemic diastereoisomers (**88a-d**). A similar route was employed by Bian *et al* 2015, whereby the alkyl halide and tricarboxylate fragments were synthesised separately and combined to produce an alk-2-yl tricarboxylate, which underwent a modified Krapcho decarboxylation in the presence of D₂O to produce β -d₂-labelled alkylsuccinates.^[105]



Scheme 5-8: Alternative pathway for the synthesis of dimethyl 2-(hencicosan-2-yl)succinate (**88**). i) PCC, DCM, RT, 3 h; ii) 3 M MeMgBr, Et₂O, THF, RT, 16 h; iii) Et₃N, MeSO₂Cl, DCM, 0 °C, 1.5 h; iv) NaH, dimethyl malonate, DME, 85 °C, 5 h; v) NaH, methyl bromoacetate, 48 h; vi) Bu₄NOAc, DMF, μ W 130 °C, 30 min.

This method was initially trialled using hexan-2-ol (**103**) (Scheme 5-9) as dimethyl 2-(hexan-2-yl)succinate (**94**) had already been prepared high purity (see page 71 for a possible explanation for this).



Scheme 5-9: Alternative pathway for the synthesis of dimethyl 2-(hexan-2-yl)succinate (**94**). i) Et₃N, MeSO₂Cl, DCM, 0 °C, 1.5 h; ii) NaH, dimethyl malonate, DME, 85 °C, 5 h; iii) NaH, methyl bromoacetate, 48 h; iv) Bu₄NOAc, DMF, μW 130 °C, 30 min.

5. 6. 2 Synthesis of Alkylsuccinic Acids Utilising the Krapcho de-alkylation

5. 6. 2. 1 Dimethyl 2-(hexan-2-yl)succinate (**94**)

Mesylation of hexan-2-ol (**103**) proceeded with an overall yield of 89% under standard conditions. The conversion of **104** to the alkylmalonate **105** also occurred with no issues, with an average yield of 58%. Alkylation of **105** gave the triester **106**, which was subjected to Krapcho de-alkylation with concomitant decarboxylation. As described^[106] this reaction is usually performed by heating an alkylmalonate dimethyl or diethyl ester with lithium or sodium chloride in dimethyl sulfoxide (DMSO) at ~ 150 °C. An alternative thermal procedure utilises tetrabutylammonium acetate in dimethylformamide (DMF)^{[104],[105]}. The mechanism of the reaction involves S_N2 attack by the halide or acetate on one of the carbo(m)ethoxy groups liberating an alkylmalonate monoester, which undergoes decarboxylation. We have found that the reaction occurs smoothly with microwave (μW) heating at 130 °C within 30 min and gave the desired product, dimethyl 2-(hexan-2-yl)succinate (**94**), in 48% yield after purification (see Scheme 5-9). This modified method is easier to perform and the purification steps are much simpler compared to the scheme shown in Section 5.3. It

also has the advantage that the stereochemistry at one of the product stereogenic centres can be controlled, by using a single enantiomer of the starting secondary alcohol, whereas this is not the case with the radical addition method as this proceeds through a planar alkyl radical.

5. 6. 3 Dimethyl 2-(henicosan-2-yl)succinate (**88**)

Icosanal (**96**), prepared as described above in Scheme 5-8 was efficiently converted into henicosan-2-ol (**99**) by reaction with methylmagnesium bromide. This intermediate was mesylated as before, with a much longer reaction time needed to achieve full conversion to the mesylate (**100**) (48 h as opposed to 1.5 h, yield, 98%). The mesylate (**100**) was converted into the malonate (**101**) as before, again requiring more time to achieve full conversion (16 h as oppose to 5 h, 60% yield,). It was important to achieve complete conversion in these reactions as separation of the products from starting materials was unachievable owing to their similar R_f values. The subsequent formation of the triester (**102**) proceeded in excellent yield (89%). The final Krapcho degradation to dimethyl 2-(henicosan-2-yl)succinate (**88**) was accomplished in a higher yield than for (**94**) (71% versus 48%) by using a longer reaction time (Figure 5-13). Overall, the newly developed method seems a more attractive route to dimethyl alkylsuccinates than the radical addition method.

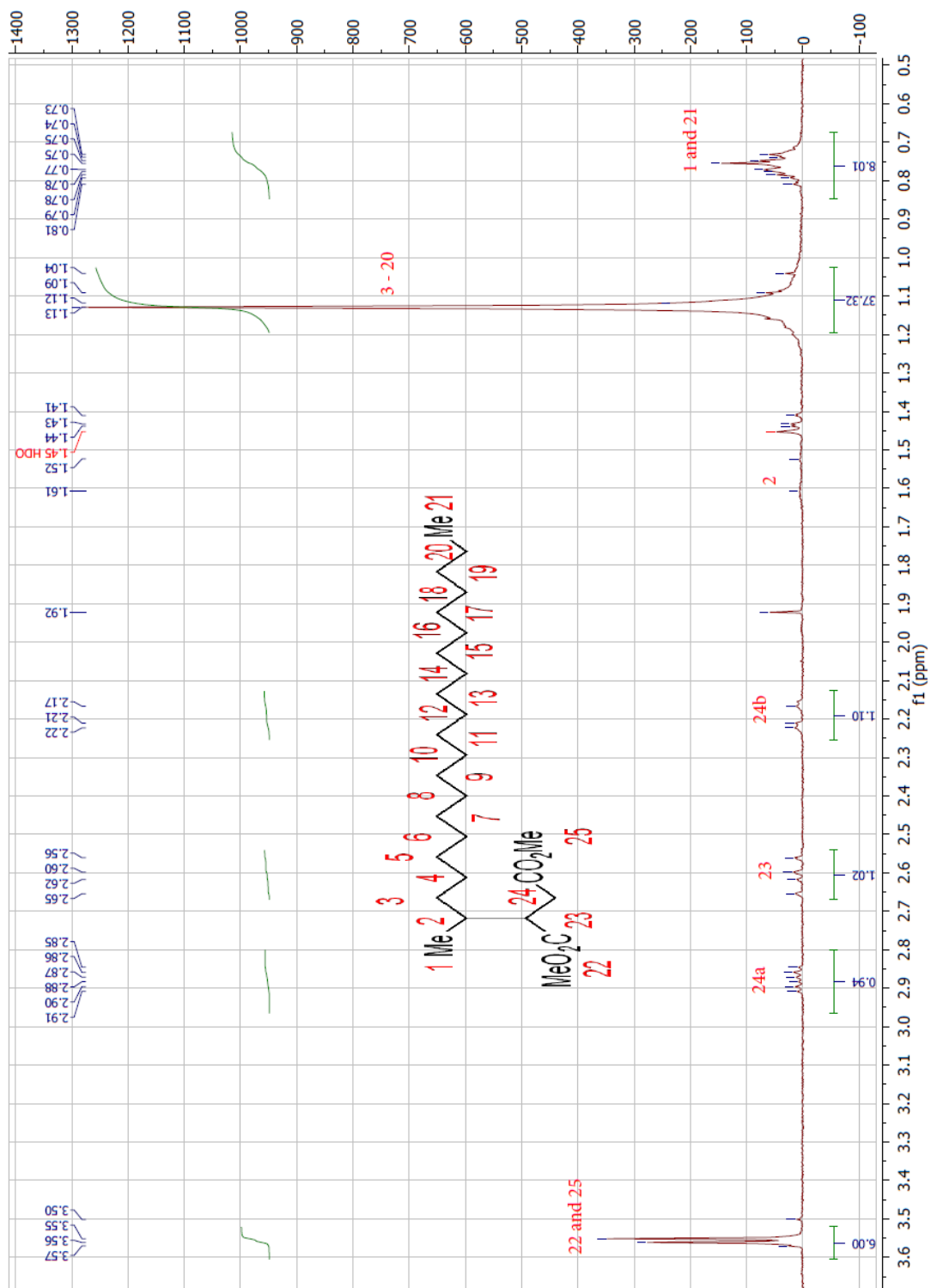


Figure 5-13: ^1H NMR of 2-(henicosan-2-yl)succinate (**88**) synthesised *via* the Krapcho decarboxylation method.

The ^1H NMR of the 2-(henicosan-2-yl)succinate (**88**) shows two doublets relating to the Me group closest to the succinate, one for each diastereoisomer, and a triplet in the methyl region which is indicative of the desired regioisomer. 2D GC-MS of the synthetic 2-(henicosan-2-yl)succinate (**88**) (Figure 5-14) also showed only one peak as opposed to the previously observed five peaks (Figure 5-12), further showing that the Krapcho decarboxylation method produces a single regioisomer of the desired product (Figure 5-14). The 2D GC-MS showed co-elution of the 2-(henicosan-2-yl)succinate (**88**) standard synthesised *via* the Krapcho decarboxylation with the peak postulated to be the C_{21} succinate in the pasteurised sample, indicating that the sample contained long chained alkyl succinates such as 2-(henicosan-2-yl)succinate (**63**).

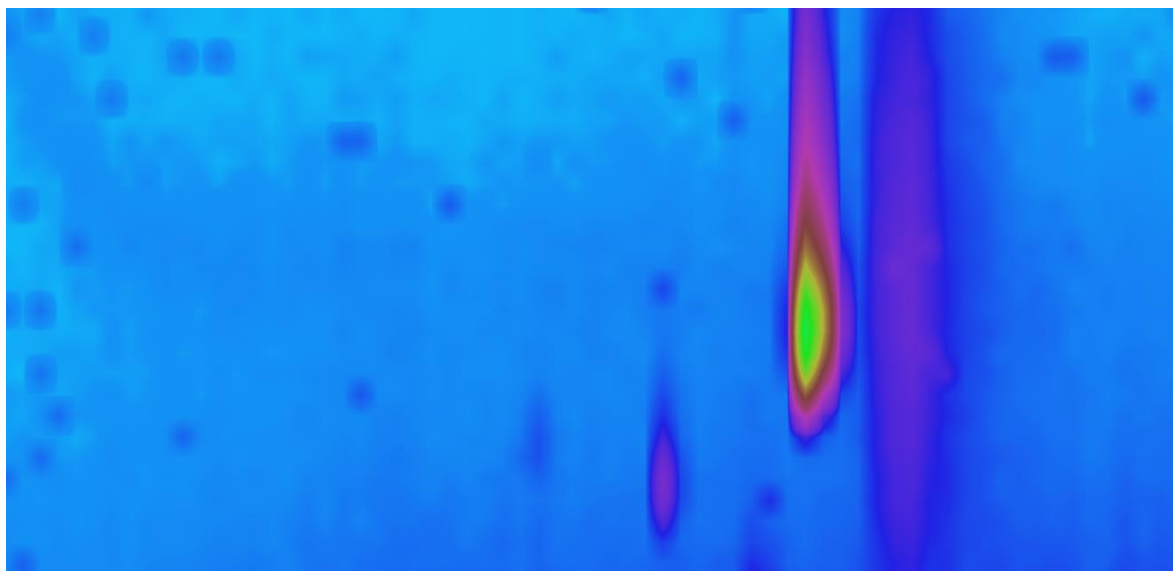


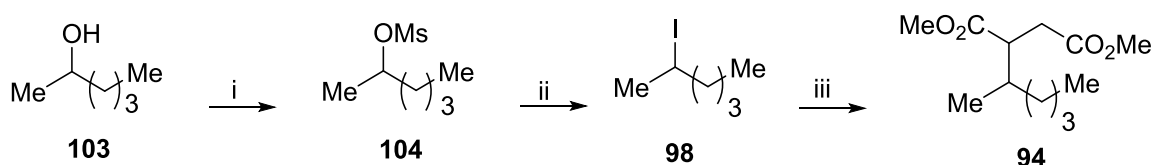
Figure 5-14: Partial total ion current (TIC) chromatogram for dimethyl 2-(henicosan-2-yl)succinate (**88**) prepared *via* the Krapcho method.

5.7 Improving the Radical Methodology

5.7.1 Synthesis of 2-Iodoalkanes via Precursor Mesylates

Reaction of a 2-hydroxyalkane *O*-mesylate with iodide ion to form a 2-iodoalkane is an $\text{S}_{\text{N}}2$ displacement avoiding the rearrangement observed when alkenes are reacted with hydrogen iodide (see Section 5-4). The radical addition method should

now afford a single alkylsuccinate (e.g. Scheme 5-10). The conversion from mesylate (**104**) to iodide (**98**) proceeded in an average yield of 78%. However, the issues with purification were still evident in the radical addition step. Overall, the method utilising a Krapcho degradation is still the most synthetically efficient route to alkylsuccinates. Furthermore, the Krapcho degradation method enables control of stereochemistry at one stereogenic centre, which is not possible via an intermediate alkan-2-yl radical.



Scheme 5-10: Synthesis of 2-(hexan-2-yl)succinate (**94**) via hexan-2-ol O-mesylate (**104**); i) Et₃N, MeSO₂Cl, DCM, 0 °C, 1.5 h; ii) LiI, MeCN, RT, 16 h; iii) Dimethyl fumarate, *tris*(trimethylsilyl)silane (TTMSS), azo-*bis*-isobutyronitrile (AIBN), α,α,α-trifluorotoluene, N₂, 105 °C, 12 h.

5. 8 Syntheses of Dimethyl 2-(undecan-2-yl)succinate (**89**), Dimethyl 2-(undecan-3-yl)succinate (**90**) and Dimethyl 2-(undecan-6-yl)succinate (**91**)

Also found in the control microcosm described in Sections 1-10 and 5-2 were C₁₁ chain succinates. When the C₂₁ chain succinate was synthesised by the radical addition pathway, the C-3 regioisomer of the succinate was found along with the desired C-2. This C-3 regioisomer co-eluted with succinates found in the biological sample. It was therefore postulated that the bacteria were not only converting the C₂₁ chain alkane to an alk-2-ylsuccinic acid, but also to an alk-3-ylsuccinic acid. To investigate this further we aimed to synthesise the C₁₁ chain succinic acid regioisomers: dimethyl 2-(undecan-2-yl)succinate (**89**), dimethyl 2-(undecan-3-yl)succinate (**90**) and dimethyl 2-(undecan-6-yl)succinate (**91**) (Figure 5-15).

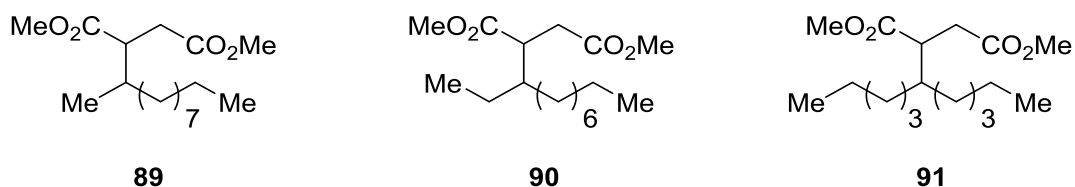
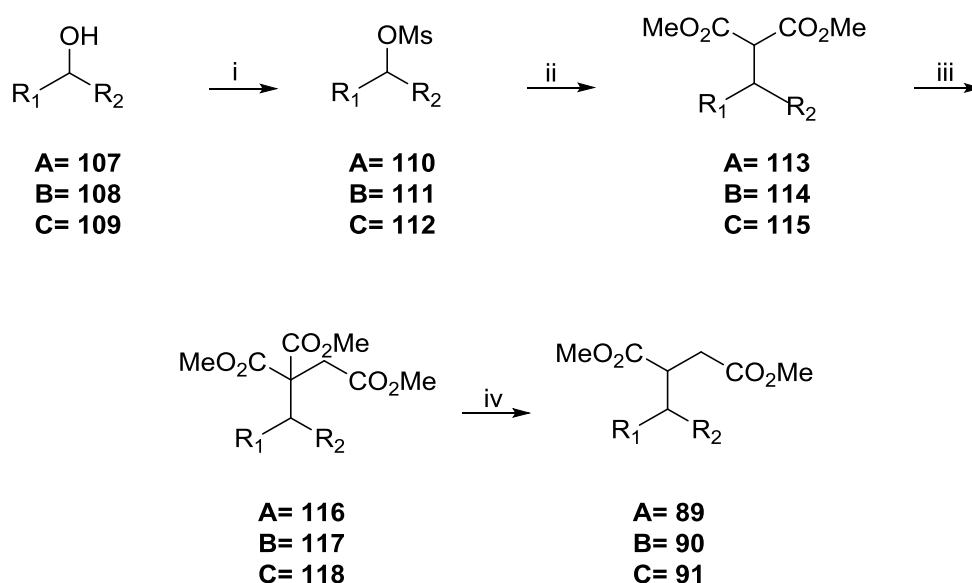


Figure 5-15: Dimethyl 2-(undecan-2-yl)succinate (**89**), dimethyl 2-(undecan-3-yl)succinate (**90**) and dimethyl 2-(undecan-6-yl)succinate (**91**).

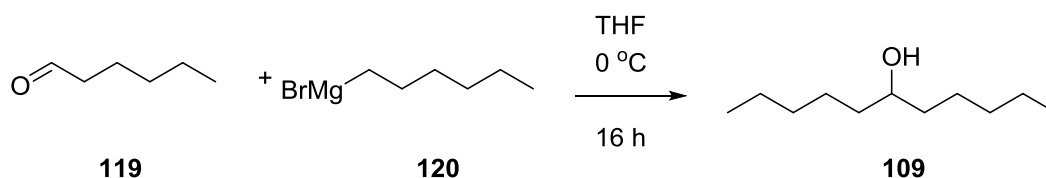
Routes that included the Krapcho degradation (Section 5-4) were used to synthesise the compounds (Scheme 5-11).



- A: Dimethyl 2-(undecan-2-yl)succinate (**89**) R₁ = Me R₂ = (CH₂)₈Me
 B: Dimethyl 2-(undecan-3-yl)succinate (**90**) R₁ = Et R₂ = (CH₂)₇Me
 C: Dimethyl 2-(undecan-6-yl)succinate (**91**) R₁ = (CH₂)₄Me R₂ = (CH₂)₄Me

Scheme 5-11: Synthetic plan for dimethyl 2-(undecan-2-yl)succinate (**89**), dimethyl 2-(undecan-3-yl)succinate (**90**) and dimethyl 2-(undecan-6-yl)succinate (**91**).

Undecan-2-ol (**107**) and undecan-3-ol (**108**) were both commercially available, but undecan-6-ol (**109**) required synthesis. This was carried out *via* a Grignard reaction between hexanal (**119**) and pentylmagnesium bromide (**120**) (Scheme 5-12).^[102]



Scheme 5-12: Synthesis of undecan-6-ol (**109**) from pentylmagnesium bromide (**120**) and hexanal (**119**).

5. 8. 1 Results and Discussion

5. 8. 1. 1 Dimethyl 2-(undecan-2-yl)succinate (**89**)

The mesylate (**110**), malonate (**113**) and tricarboxylate (**116**) were all made in good yields (97%, 73% and 80% respectively). The Krapcho degradation proceeded with an average yield of 65 % to afford dimethyl 2-(undecan-2-yl)succinate (**89**).

5. 8. 1. 2 Dimethyl 2-(undecan-3-yl)succinate (**90**)

As before, the mesylate (**111**), malonate (**114**) and tricarboxylate (**117**) were all obtained in good yields (96%, 86% and 76% respectively) and the Krapcho degradation proceeded with an average yield of 67% to yield dimethyl 2-(undecan-2-yl)succinate (**90**).

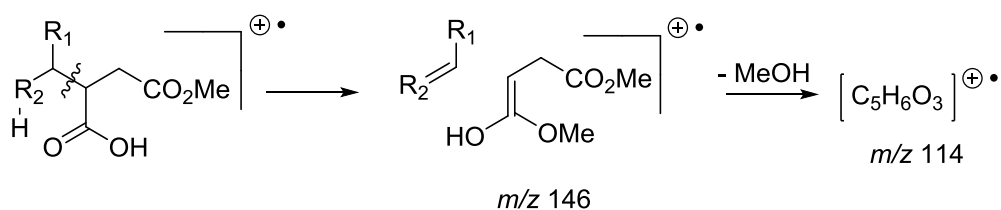
5. 8. 1. 3 Dimethyl 2-(undecan-6-yl)succinate (**91**)

The Grignard synthesis of undecan-6-ol (**109**) (Scheme 5-12) went well with a yield of 95%. Again, the mesylate (**112**), malonate (**115**) and tricarboxylate (**118**) were all made in good yield (81%, 90% and 75% respectively) and the Krapcho degradation proceeded with an average yield of 72% to afford dimethyl 2-(undecan-2-yl)succinate (**91**).

5. 8. 1. 4 ¹H NMR analysis of dimethyl 2-(undecan-2-yl)succinate (**89**), dimethyl 2-(undecan-3-yl)succinate (**90**) and dimethyl 2-(undecan-6-yl)succinate (**91**)

GC-MS analysis of dimethyl alkylsuccinic acids produces highly characteristic fragmentation patterns due to the McLafferty rearrangement. Fragments can be

observed at m/z 114 and 146. These can be attributed to the rearrangement shown in Scheme 5-13.^{[107],[108]}



Scheme 5-13: McLafferty rearrangement in electron ionisation mass spectrometry of dimethyl alkylsuccinates to produce characteristic m/z 146 ion (derived 114 also shown).

Dimethyl 2-(undecan-6-yl)succinate (**91**) (Figure 5-16) has a single pair of enantiomers, which gives rise to relatively simple ^1H (Figure 5-17).

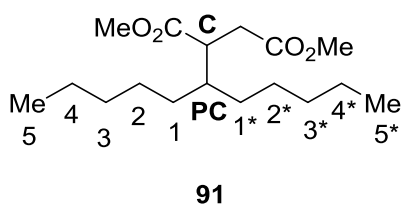


Figure 5-16: Dimethyl 2-(undecan-6-yl)succinate (**91**), **C** = chiral centre, **PC** = prochiral centre.

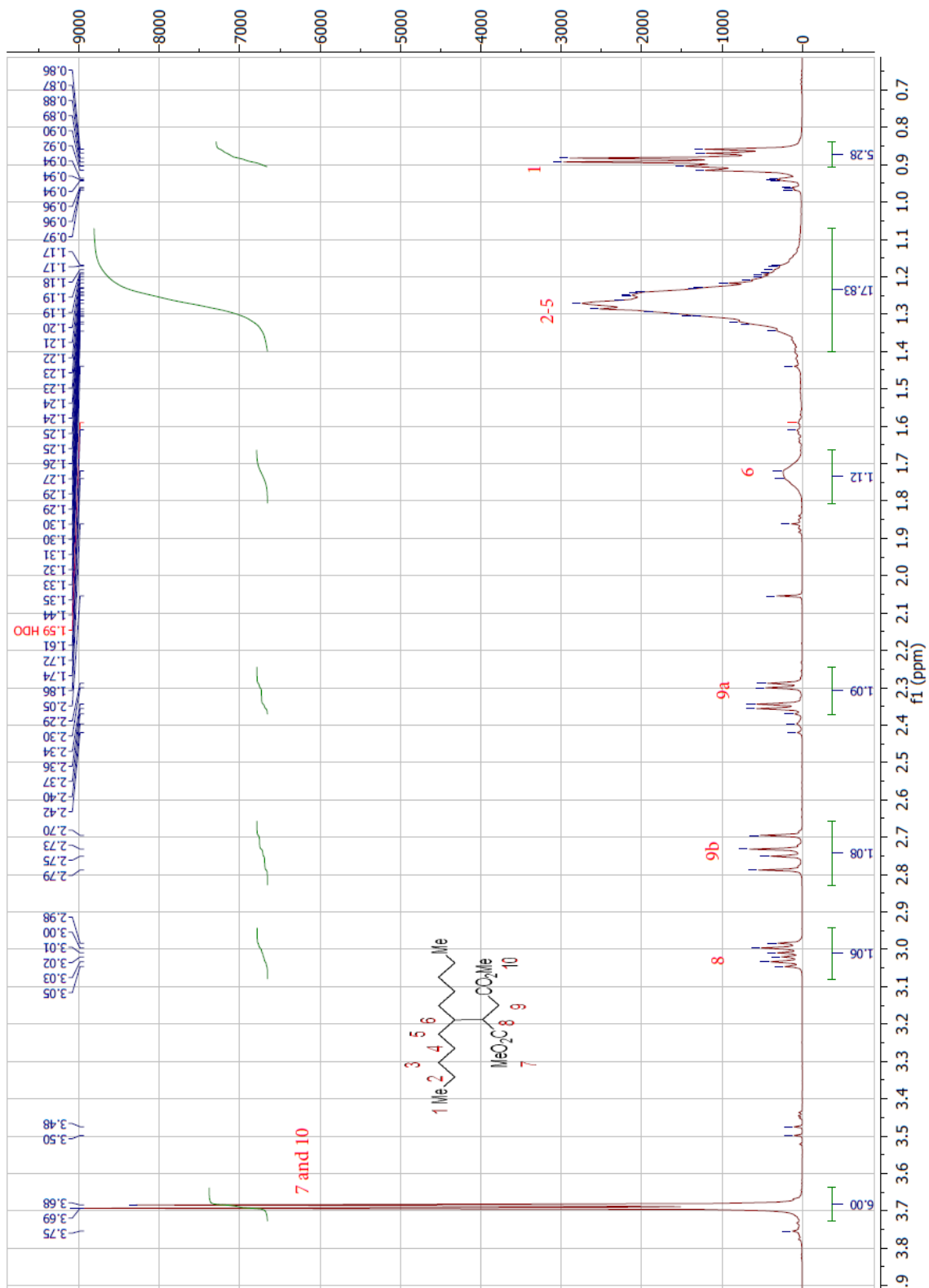


Figure 5-17: ^1H NMR of dimethyl 2-(undecan-6-yl)succinate (91).

For the ^1H NMR (Figure 5-17), the methyl groups 5 and 5* appear as two overlapping triplets at δ 0.88-0.92. The ^{13}C NMR shows 17 resonances as expected and resolves both methyl groups as separate peaks at δ 14.04 and 14.16. GC-MS analysis gave a peak at retention time 30.890 min, with fragment ions m/z 114 [$\text{C}_5\text{H}_6\text{O}_3$] $^+$, 146 [$\text{C}_6\text{H}_{10}\text{O}_4$] $^+$, 227 [$\text{C}_{14}\text{H}_{28}\text{O}_2$] $^+$ and 269 [$\text{C}_{16}\text{H}_{28}\text{O}_2$].

Dimethyl 2-(undecan-3-yl)succinate (**90**) (Figure 5-18) has two chiral centres and two diastereoisomers are present in the sample.

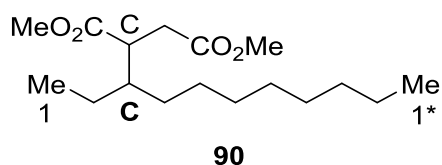


Figure 5-18: Dimethyl 2-(undecan-3-yl)succinate (**90**), **C** = chiral centre.

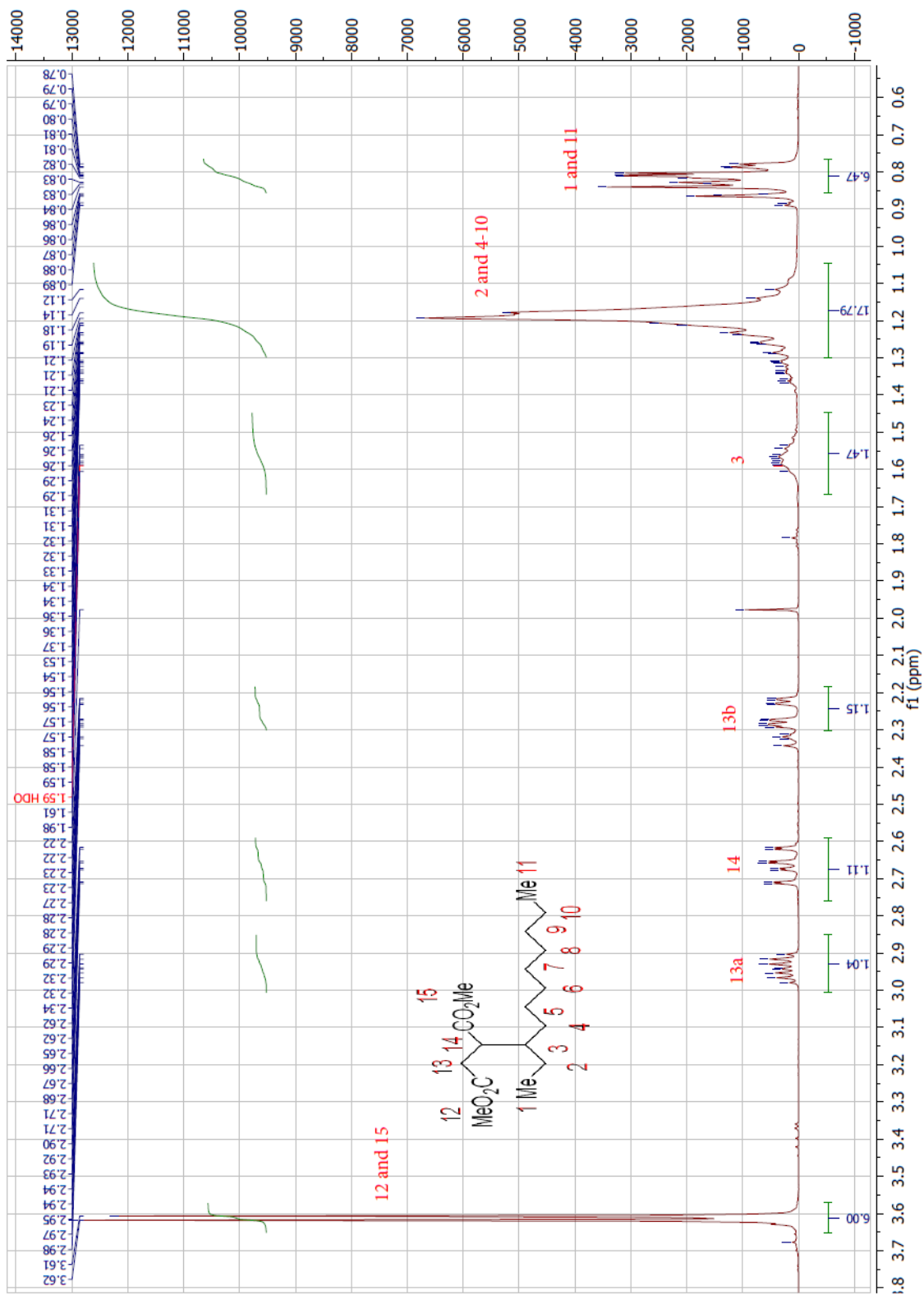


Figure 5-19: ^1H NMR of dimethyl 2-(undecan-3-yl)succinate (90).

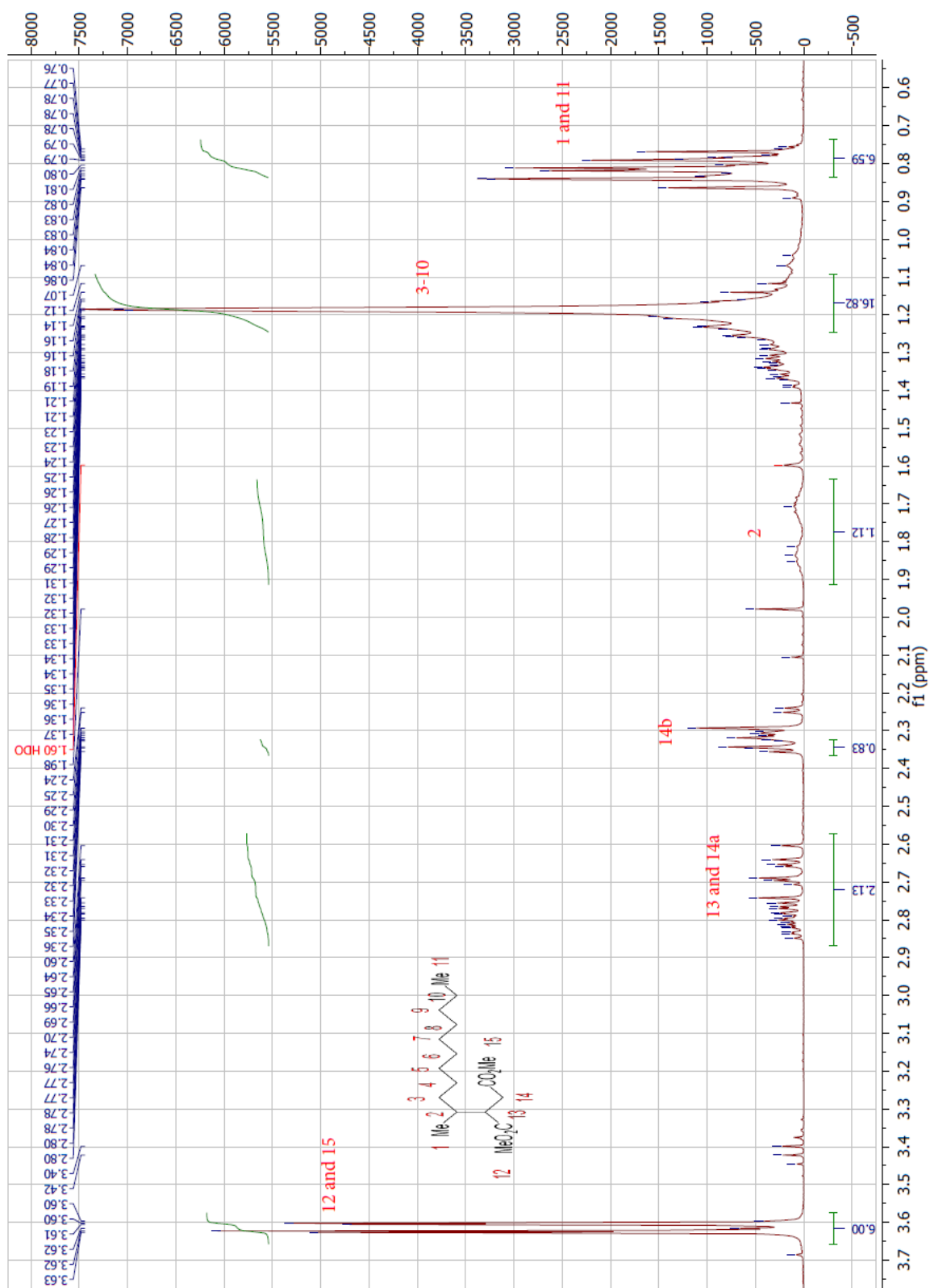


Figure 5-21: ^1H NMR of dimethyl 2-(undecan-3-yl)succinate (89).

The methyl region at δ 0.75-0.87 of the ^1H NMR spectrum (Figure 2-21) is simpler than for compound **90** (Figure 5-19), as expected because one methyl is adjacent to a CH rather than a CH_2 . Both resonances of the methyl region around 1 ppm are distinguishable as separate doublets at due to their proximity to the chiral centre. The resonances associated with methyl region 1' showed as only one distinguishable triplet as there is relatively large separation from the chiral centre. Similarly, the ^{13}C spectrum exhibits two resolved peaks for methyl region 1 at δ 16.23 and 16.90 but only one for methyl region 1' at δ 14.14. GC-MS analysis gave a peak at retention time 32.673 min, with fragment ions m/z 114 [$\text{C}_5\text{H}_6\text{O}_3$] $^+$, 146 [$\text{C}_6\text{H}_{10}\text{O}_4$] $^+$, 227 [$\text{C}_{14}\text{H}_{28}\text{O}_2$] $^+$ and 269 [$\text{C}_{16}\text{H}_{28}\text{O}_2$].

5.9 Conclusions the Synthesis of Alkylsuccinates

Three synthetic routes to secondary alkylsuccinates have been developed. The first involves a biomimetic radical addition of fumarate (**1**) to an alkyl radical derived from a secondary alkyl halide (Scheme 5-4). This route works well for short chain alkylsuccinates as regioisomers that may accompany the desired product are more easily resolved by chromatography, although multiple columns were required to remove silyl by-products. A disadvantage of this method is that the stereochemistry of the product cannot be controlled.

The second method retains this biomimetic radical addition step but removes the risk of regioisomer formation during preparation of the required haloalkane by using a mesylate to form this precursor (Scheme 5-11). This step also does not allow for any stereocontrol because the conversion of mesylate into iodoalkane with iodide ($\text{S}_{\text{N}}2$) can be followed by racemisation of the iodoalkane via reaction with iodide. This method also suffers from the difficult product purification as for the first method after the radical addition. If the secondary alcohol starting material, is not commercially available (especially for longer chain alcohols) it can be produced by Grignard reaction.

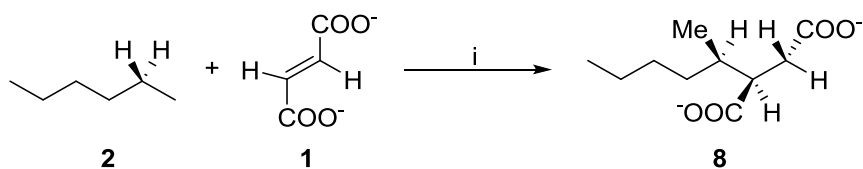
The third method involved a Krapcho dealkylation/decarboxylation of a tricarboxylate ester to form a dimethyl alkylsuccinate (Scheme 5-9). This method removes the radical addition step and, therefore, avoids the unfavourable purification step. It also allows for control of stereochemistry at one stereogenic centre, which is

advantageous when the samples are being utilised as chemical standards. All considered, the third route is the most attractive route in terms of ease and avoidance of complications.

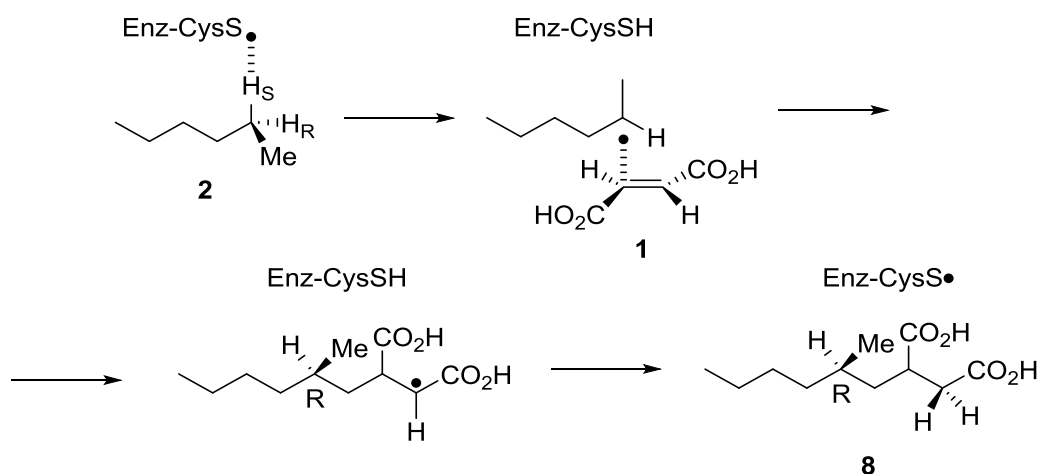
Chapter 6 Synthesis and Microbiological Examination of Dialkylcyclopropanes Substrate Analogues

6.1 Introduction to the Synthesis of the Dialkylcyclopropanes Substrate Analogues

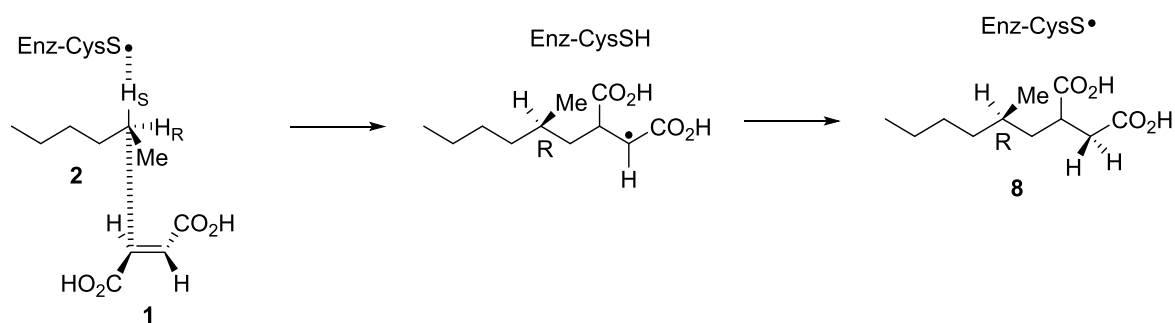
The degradation of hexane (**2**) by the proteobacterium HxN1 requires addition of fumaric acid (**1**) to the C-2 position of the alkane chain to give 2-(hexan-2-yl)succinic acid (**3**) (Scheme 6-1). As discussed in Sections 1-9 and 5-1, this process could occur either by a stepwise (Scheme 6-2) or a concerted (Scheme 6-3) mechanism.^[22]



Scheme 6-1: First step of the catabolic process associated with HxN1 with hexane (**2**) as the substrate. *i*) (1-Methylalkyl)succinate synthase.



Scheme 6-2: Stepwise hydrogen atom abstraction and fumarate (**1**) addition.



Scheme 6-3: Concerted hydrogen atom abstraction and fumarate (**1**) addition.

This activation step is performed by methylalkylsuccinate synthase (MAS), and the hydrogen cleaved from the C-2 position is transferred back onto the 2-(hexan-2-yl)succinic acid with stereochemical inversion. This indicates a concerted mechanism, where the fumarate (**1**) and the active radical of the enzyme are on opposite sides of the hydrocarbon.^[2] Ab initio molecular orbital calculations indicate that this pathway is of marginally lower energy than the stepwise route.^[109]

6.2 Project Aims

A series of substrate analogues (Figure 6-1) were synthesised to help determine which process is occurring in the metabolism of hexane (**2**) by HxN1

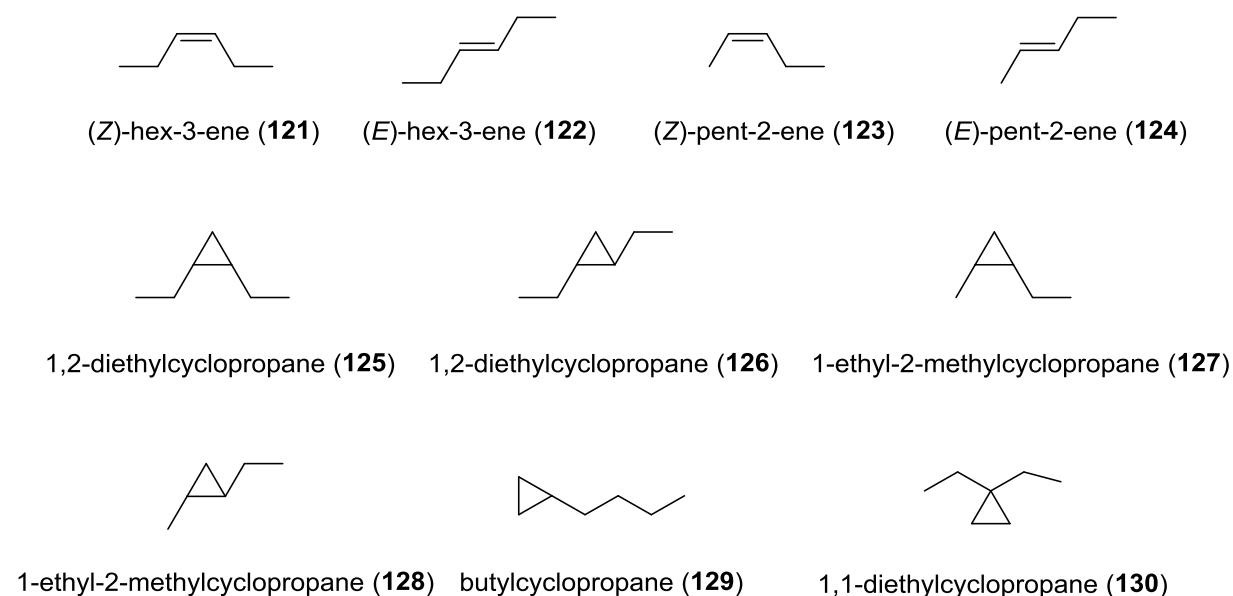
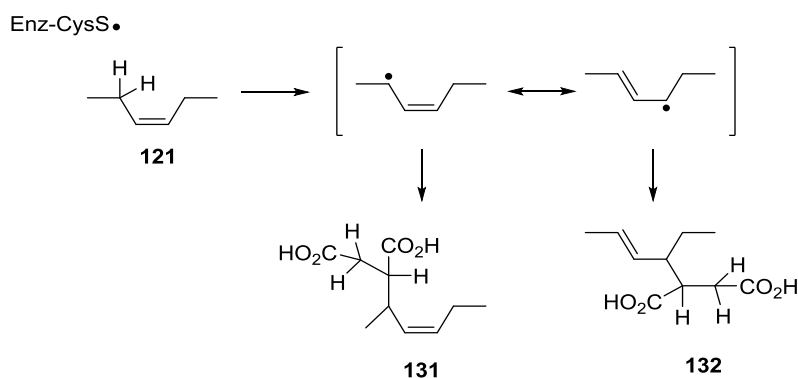
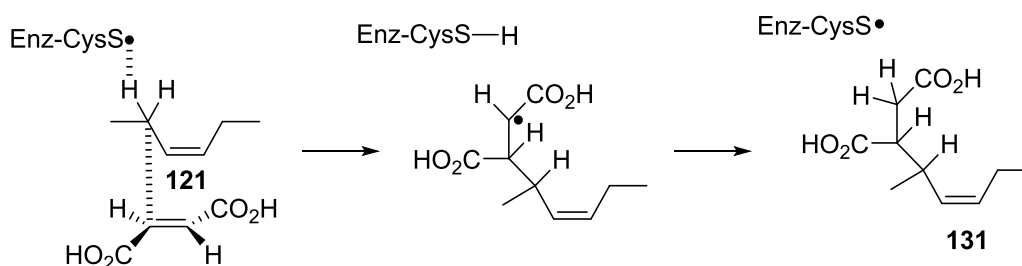


Figure 6-1: Substrate analogues **121-130**.

With the alkenes (**121-124**) (Figure 6-1), if the fumarate (**1**) activation proceeds *via* a stepwise mechanism the radical formed by the hydrogen abstraction would be resonance stabilised (Scheme 6-4) and a mixture of regioisomeric alkenes (**131** and **132**) could arise. If, however, the activation proceeds *via* a concerted process the product of the fumarate (**1**) addition (**131**) would be expected to retain its initial *E/Z* configuration (Scheme 6-5).

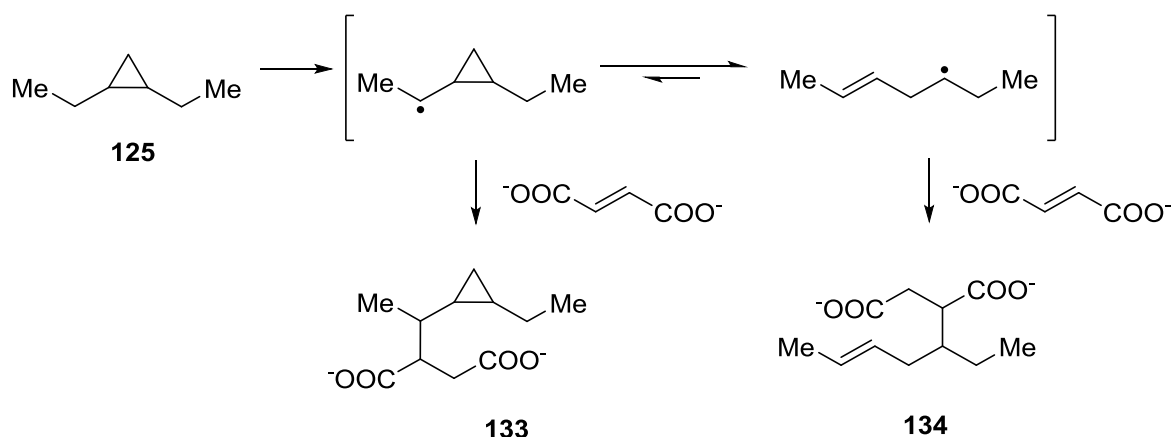


Scheme 6-4: Possible products from utilisation of hex-3-ene (**121**) as substrate analogue in HxN1.



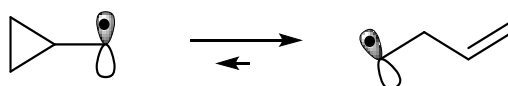
Scheme 6-5: Alkene product with retention of regiochemistry.

With regards to the cyclopropane compounds (**125-130**) the initial hydrogen abstraction in the stepwise mechanism would produce a cyclopropylcarbinyl radical intermediate, which may or may not ring open to form a but-3-enyl radical, depending on the lifetime of the radical intermediate and the rate of capture of this radical by fumarate (**1**) (Scheme 6-6).



Scheme 6-6: Cyclopropylcarbinyl and but-3-enyl radicals produced by the stepwise reaction mechanism if cyclopropane substrate analogue is used.

The cyclopropylcarbinyl to but-3-enyl radical ring opening mechanism (Scheme 6-7) is one of the fastest unimolecular conversions known with a rate constant of $6.1 \times 10^8 \text{ s}^{-1}$ at 353 K.^[110] The reverse reaction has a rate constant of 10^3 s^{-1} and so the butenyl radical is the dominant species at equilibrium.



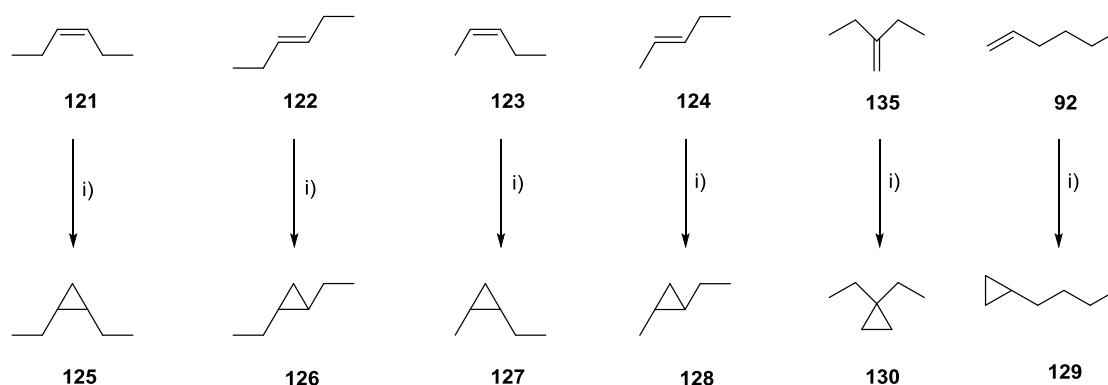
Scheme 6-7: Cyclopropylcarbinyl radical to but-3-enyl radical ring opening conversion.

If incubated with the β -*proteobacteria* HxN1 the metabolites of these compounds would provide information on the timescale of the initial step of the metabolism as well as which process is occurring. If the concerted mechanism was proved to be the favoured mechanism, the product would retain the cyclopropane ring (e.g. **133**). If however the stepwise mechanism was followed either a product containing a carbon-carbon double bond (e.g. **134**) or a mixture of products may be observed, some retaining the cyclopropane ring and some containing a carbon-carbon double bond. If the latter is the case, the ratio of the products could give information about how long-lived is the initially formed radical. This 'radical clock' approach has previously been successfully used to help determine lifetimes of radical intermediates in other systems, including cytochrome P450-catalysed reactions on hydrocarbons (Atkinson *et al.*, 1994).

We aimed to synthesise the cyclopropanes **125-130** to allow for the biological assays to be performed, which were intended to provide clues towards the nature of the primary step of the metabolism of *n*-alkanes occurring in HxN1.

6. 2. 1 Synthesis of Dialkylcyclopropanes using the Simmons-Smith Reaction

The alkenes **121-124** are all commercially available, so their synthesis was not required. They did however provide suitable precursors to the cyclopropanes **125-130**. The transformation of the alkenes to the corresponding cyclopropanes was to be achieved in one step *via* a Simmons-Smith reaction. This involved refluxing the chosen alkene in diethyl ether with zinc-copper couple and diiodomethane (Scheme 6-8).¹¹⁰ Due to the volatility of both the alkene and the cyclopropanes, careful control of reflux temperature and nitrogen flow were required, and gentle distillation of the product to separate it from diethyl ether was necessary for purification.



Scheme 6-8: Synthetic route to cyclopropanes **125-130**. i) Et₂O, Zn/Cu, diiodomethane, 9 hours reflux, 16 hours room temperature, N₂.

All of the cyclopropanes were successfully prepared. Due to the similarity in the boiling points of the products and the diethyl ether solvent, total separation of the products from the solvent *via* distillation proved to be difficult. We attempted to remove the diethyl ether by passing a gentle stream of dry nitrogen over the samples but this resulted in comparable loss of sample and of diethyl ether. Owing to these issues, the yields of the reaction ranged between 4 and 20%. This was deemed to be due to loss of both reagent and product in the reaction vessel whilst under reflux, and loss during the distillation. To try to counteract this loss, the reaction was also performed at room temperature but no product was observed. Successful purification of the cyclopropanes was achieved by distillation performed on an automated

distillation machine, followed by secondary distillation using conventional distillation techniques. The compounds were characterised by ^1H (Figures 6-2 and 6-3) and ^{13}C NMR analysis.

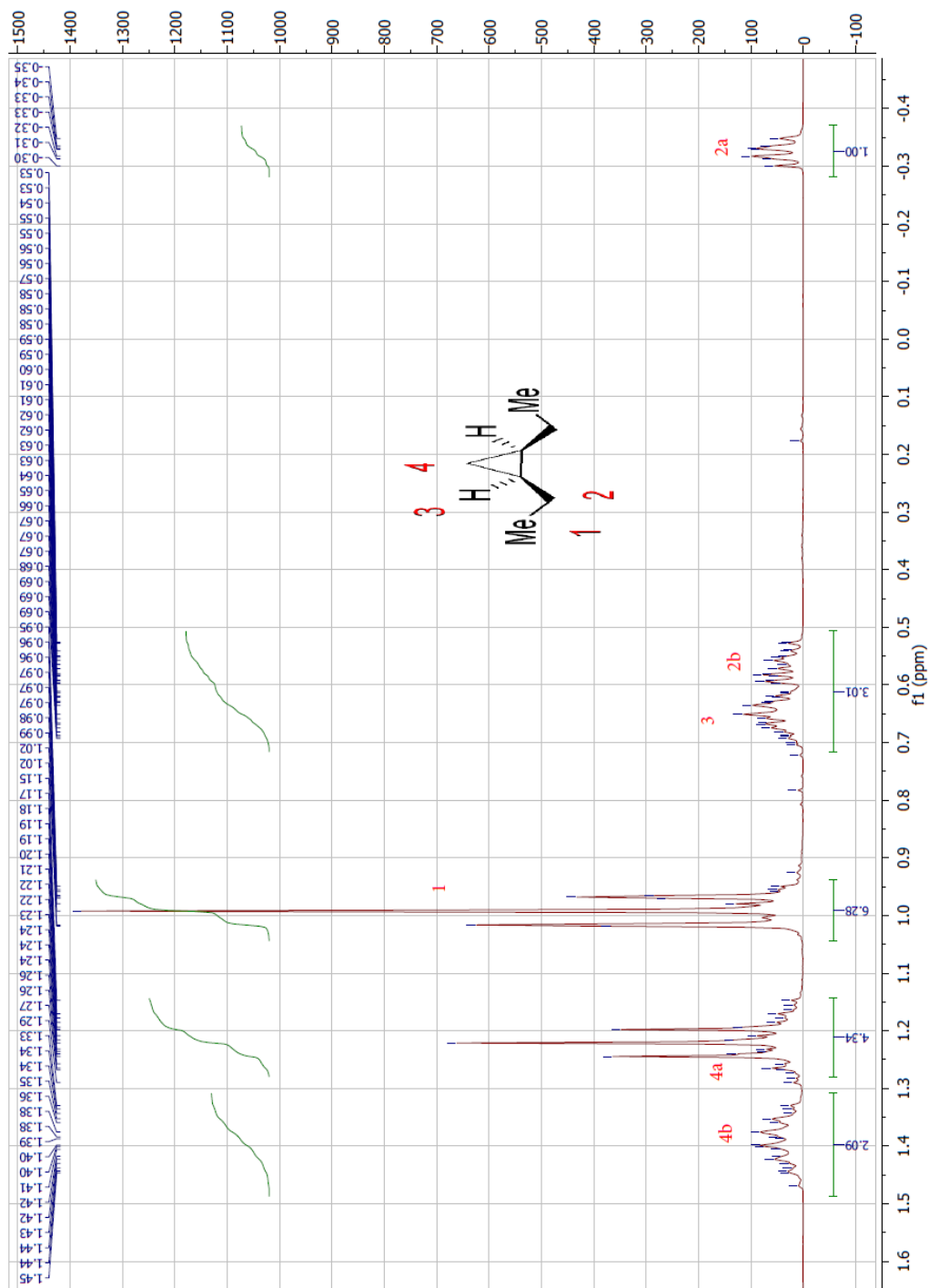


Figure 6-2: ^1H NMR of the *cis*-1,2-diethylcyclopropane (125) stereoisomer.

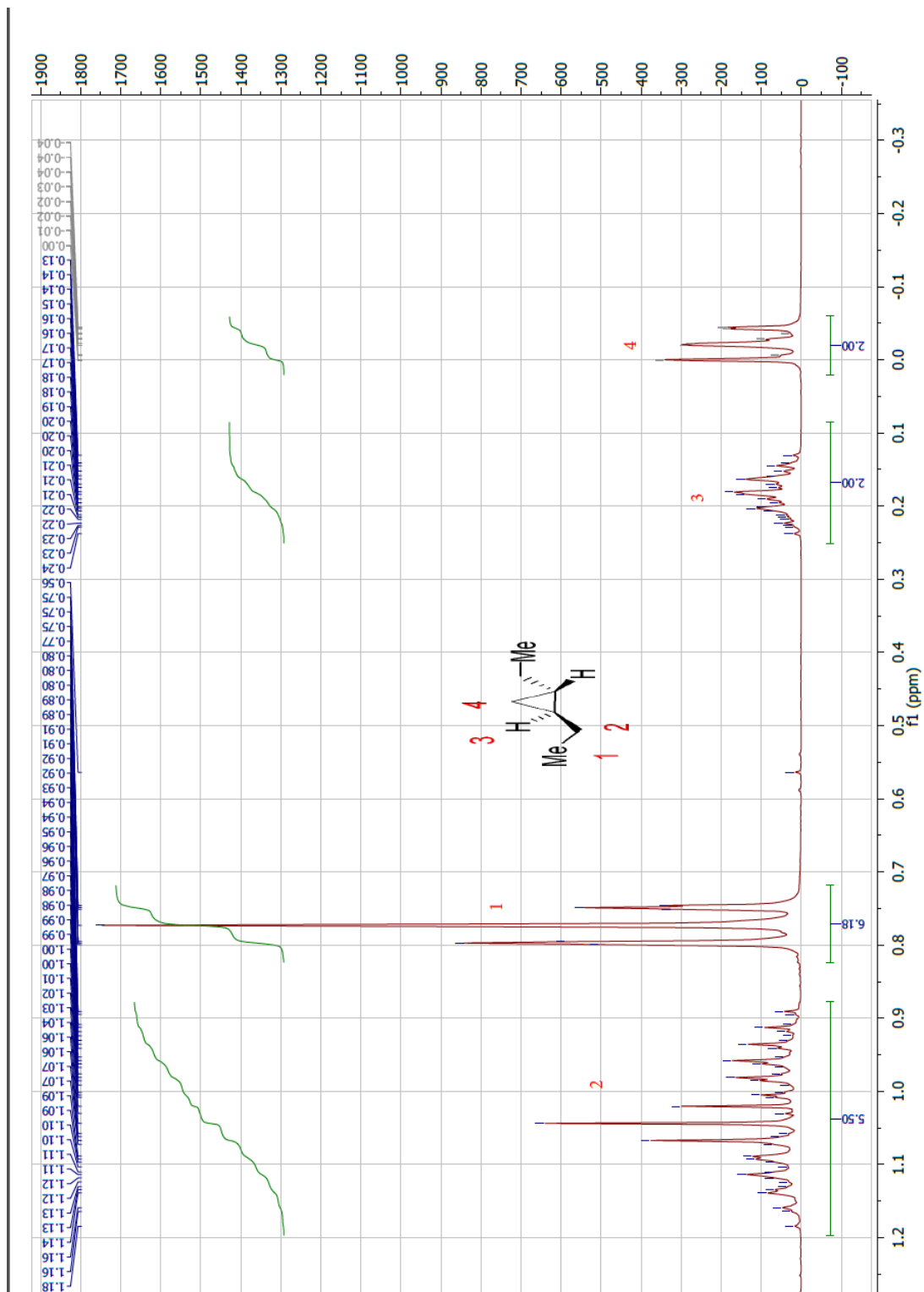


Figure 6-3: ^1H NMR of the *trans*-1,2-diethylcyclopropane (**126**) stereoisomer.

The cyclopropane compounds were identifiable by methylene peaks below 0 ppm.

6. 2. 2 Incubation Experiments with the Cyclopropanes

Incubation experiments with HxN1 were performed with the cyclopropanes **125-130**. The compounds were provided as a mixture with known growth substrates for HxN1; hexane (**2**) for the C₆ cyclopropanes **127** and **128**, and heptane for the C₇ cyclopropanes **125**, **126**, **129** and **130**. It was observed that the growth of these systems was weak in comparison to control systems in which no cyclopropanes were added, indicating that these substrate analogues have a toxic effect on the organism. The samples were analysed by GC-MS according to previously optimised conditions, and although low levels of the metabolite products from the degradation of hexane (**2**) and heptane were detected, no metabolites associated with the degradation of the cyclopropane compounds were detected, further indicating toxicity. This toxicity could be attributed to the compounds acting as an irreversible inhibitors by covalently binding at the enzyme active site. This process could occur via hydrogen atom abstraction from the substrate analogue to give a radical species that fails to engage in the normal catalytic pathway and instead, reacts with amino acid residues. Validation of this proposal requires identification of modified fragments from the protein. Experiments with the alkene compounds 93-96 are yet to be performed.

6. 3 Concluding thoughts to Synthesis of the Dialkylcyclopropanes

Substrate Analogues

Overall, the synthesis of these novel substrate analogues of hexane (**2**) were achieved through a difficult and low yielding process. Despite many attempts to optimise the reactions, the similarity in boiling point between the compounds and the ethereal solvent were not overcome. Despite this, microbiological experiments did yield results which we can utilise. The compounds are deemed to be toxic to the organisms, which suggests that they are binding as irreversible inhibitors to the active site of the enzyme in question. Further experiments with the alkene analogues (**121-124**) will help establish further information on this process.

Chapter 7 Experimental

7.1 Introduction to Experimental

7.1.1 Chemicals and Solvents

All chemicals were acquired from either Sigma-Aldrich, Alfa Aesar or Apollo Scientific. All anhydrous solvents used in the reactions were from Sigma-Aldrich SureSeal™ bottles which were stored under dry N₂, or were of HPLC grade acquired from Fischer Scientific. All chemicals and solvents were used as purchased. Where petrol was used it was the petroleum ether fraction which boils between 40-60 °C. Any solvents used in work up procedures were of HPLC grade. Where room temperature (RT) is stated, it refers to between 20 and 25 °C.

7.1.2 Chromatography

Thin layer chromatography (TLC) was conducted on Merck silica gel 60F₂₅₄ coated alumina sheets using varying ratios of solvents as stated. The plates were visualised by using short wave (254 nm) ultraviolet light, iodine vapour or by potassium permanganate solution. Medium pressure chromatography (MPC) was performed on Merck Geduran Si 60 (40-63 μm) silica gel. Elutions were performed with the solvent mixtures stated.

7.1.3 Analytical Techniques

Infra-red (IR) spectra were acquired using a Varian 800 FT-IR Scimitar series infrared spectrometer with neat sample and are stated in cm⁻¹. ¹H NMR spectra were recorded using Bruker Advance 300 MHz or 700 MHz spectrometers. ¹³C spectra were recorded at 75 MHz on the same spectrometers. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS) as standard. Multiplicities are specified by s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet) where no specific splitting pattern was observed. Any peaks labelled br were broad and may not be of the splitting pattern expected. Any combinations of the aforementioned multiplicities have been included for example dd, referring to a doublet of doublets. Any measurable coupling constants (*J*) have been stated in

Hertz (Hz). Optical rotations ($[\alpha]_{20/D}$) were recorded on an ADP 440 polarimeter and measured as a 1% solution in chloroform, calibrated using pure 1% sucrose in ethanol solution.

7. 1. 4 Distillations

Distillations were either performed traditionally using glass apparatus or were performed on an automated distillation apparatus, the Spaltröhr™ column MMS 155 with intermediate receiver at atmospheric pressure. This device is capable of automatically distilling mixtures under atmospheric pressure or under high vacuum, and all parameters can be set before and during distillation.

7. 1. 5 Safety Considerations

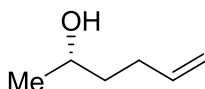
COSHH risk assessments were performed prior to experiments being conducted and appropriate control measures put in place. Additionally, Good Chemical Practice was adhered to for all laboratory work and compliance with the School of Chemistry's Health and Safety Policy maintained throughout.

7.2 Stereoisomeric (*S,S*)-, (*R,R*)- and (*R,S*)-2,9-decanediols (43a-c)

7.2.1 General Procedure A: Hex-5-en-2-ols (45)^[74]

Copper (I) iodide (0.14 eq) was dried at 200 °C for 5 min before dilution with Et₂O (3 mL / 1 mmol) at RT and cooling to -30 °C. To this, allylmagnesium bromide (1M in Et₂O, 1.4 eq) and propylene oxide (1 eq) in Et₂O was added dropwise and stirred at -30 °C for 5 h. The reaction was quenched with sat. aq. NH₄Cl (1.5 mL / 1 mmol) and warmed to RT. After extraction with Et₂O (3 x 100 mL) the combined organic fractions were washed with brine, dried over MgSO₄ and concentrated under reduced pressure.

7.2.1.1 (*S*)-Hex-5-en-2-ol (45a)^[112]



Copper (I) iodide (2.28 g, 12 mmol), Et₂O (250 mL), allylmagnesium bromide (1M in Et₂O, 120 mL, 120 mmol) and propylene oxide (5.00 g, 86 mmol). Product as a clear oil (7.02 g, 70 mmol, 81 %). *R_f* = 0.41, petrol-diethyl ether, 6:4 (v/v).

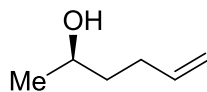
¹H NMR (300 MHz, CDCl₃) δ 1.16 (3H, d, *J* = 6.0 Hz, CH₃CH), 1.41-1.61 (2H, m, CH₃CHCH₂), 2.12 (2H, dddd, *J* = 15.0, 8.2, 6.7 and 3.3 Hz, CH₂CHCH₂CH₂), 3.76 (1H, dq, *J* = 12.3 and 6.0 Hz, CH₃CH), 4.93 (1H, ddt, *J* = 10.2, 3.3 and 1.7 Hz, CH_{CIS}CH_{TRANS}CHCH₂), 5.01 (1H, dd, *J* = 17.0 and 1.7 Hz, CH_{CIS}CH_{TRANS}CHCH₂), 5.80 (1H, ddt, *J* = 17.0, 10.2 and 6.7 Hz, CH_{CIS}CH_{TRANS}CHCH₂);

¹³C NMR (75 MHz, CDCl₃) δ 15.0 (CH₃CH), 29.9 (CH₂CHCH₂CH₂), 38.4 (CH₂CHCH₂CH₂), 67.8 (CH₃CH), 114.5 (CH₂CHCH₂CH₂), 138.4 (CH₂CHCH₂CH₂);

IR (cm⁻¹) 1205, 1304, 1374, 1415, 1451, 1498, 1641, 2929, 2969, 3078, 3338;

[α]_D²⁰ +15.2°, *c* = 1% in chloroform, lit. value = +14.0°, *c* = 1% in chloroform, 94 % ee.

7. 2. 1. 2 (R)-Hex-5-en-2-ol (45b)^[113]



Copper (I) iodide (4.50 g, 23.8 mmol), Et₂O (500 mL), allylmagnesium bromide (1M in Et₂O, 240 mL, 240 mmol) and propylene oxide (10.1 g, 170 mmol). Product as a clear oil (14.8 g, 148 mmol, 87 %). *R_f* = 0.39, petrol-diethyl ether, 6:4 (v/v).

¹H NMR (300 MHz, CDCl₃) δ 1.2 (3H, d, *J* = 6.0 Hz, CH₃CH), 1.44-1.66 (2H, m, CH₃CHCH₂), 2.15 (2H, dttd, *J* = 13.4, 7.9 and 6.4 Hz, CH₂CHCH₂CH₂), 3.76 (1H, h, *J* = 6.0 Hz, CH₃CH), 4.96 (1H, ddd, *J* = 10.1, 2.2 and 1.7 Hz, CH_{CIS}CH_{TRANS}CHCH₂), 5.04 (1H, dq, *J* = 17.2 and 1.7 Hz, CH_{CIS}CH_{TRANS}CHCH₂), 5.68 (1H, ddt, *J* = 17.2, 10.1 and 6.4 Hz, CH_{CIS}CH_{TRANS}CHCH₂);

¹³C NMR (75 MHz, CDCl₃) δ 15.0 (CH₃CH), 23.3 (CH₂CHCH₂CH₂), 40.9 (CH₂CHCH₂CH₂), 67.7 (CH₃CH), 114.5 (CH₂CHCH₂CH₂), 138.4 (CH₂CHCH₂CH₂);

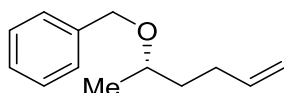
IR (cm⁻¹) 1207, 1307, 1374, 1416, 1451, 1641, 2874, 2930, 2971, 3078, 3344;

[α]_D²⁰ -15.6°, c = 1% in chloroform.

7. 2. 2 General Procedure B: [(Hex-5-en-2-yloxy)methyl]benzenes (46) [76]

Hex-5-en-2-ol (1 eq) and tetrabutylammonium iodide (0.015 eq) were dissolved in THF (1.4 mL / 1 mmol) and cooled to 0 °C before NaH (60 % in mineral oil, 4 eq) was added portion-wise. Benzyl bromide (1.4 eq) was added and stirred at RT for 16 h. The volume was halved under reduced pressure and neutralised with HCl (2 M) before extraction with Et₂O (3 x 50 mL), the combined organic extracts were washed with brine and dried over MgSO₄ before concentration under reduced pressure to give the crude product as a brown oil, which was purified by MPC, eluting with petrol – dichloromethane, gradient of 9:1- 1:1 over 2.5 L (v/v).

7. 2. 2. 1 (S)-[(Hex-5-en-2-yloxy)methyl]benzene (46a) [114]



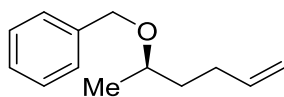
Hex-5-en-2-ol (7.00 g, 70 mmol), tetrabutylammonium iodide (380 mg, 1.0 mmol), THF (100 mL), NaH (60 % in mineral oil, 11.23 g, 280 mmol) and benzyl bromide (11.66 mL, 98 mmol). Title product as a clear oil (11.32 g, 59 mmol, 85 %). R_f = 0.40, petrol-DCM, 9:1 (v/v).

¹H NMR (300 MHz, CDCl₃) δ 1.24 (3H, d, J = 6.1 Hz, CH₃CH), 1.56 (1H, dddd, J = 13.7, 9.0, 6.6 and 5.3 Hz, CH₃CHCHH), 1.74 (2H, dddd, J = 13.7, 9.0, 7.0 and 6.1 Hz, CH₃CHCHH), 2.06-2.33 (2H, m, CH₂CHCH₂CH₂), 3.58 (1H, ddd, J = 9.0, 6.1 and 6.1 Hz, CH₃CH), 4.48 (1H, d, J = 11.7 Hz, CHCHHAr), 4.59 (1H, d, J = 11.7 Hz, CHCHHAr), 4.92-5.15 (2H, m, CH₂CH₂CHCH₂), 5.85 (1H, ddt, J = 6.6, 10.2, 16.9 Hz, CH₂CH₂CHCH₂), 7.08-7.54 (5H, m, Ar);

¹³C NMR (75 MHz, CDCl₃) δ 19.5 (CH₃CH), 29.7 (CH₂CHCH₂CH₂), 36.0 (CH₂CHCH₂CH₂), 73.4 (CH₂Ar), 76.6 (CH₃CH), 114.3 (CH₂CHCH₂CH₂), 127.2 (*para*-Ar), 127.3 (*ortho*-Ar), 128.3 (*meta*-Ar), 138.6 (CH₂C), 138.3 (CH₂CHCH₂CH₂);

IR (cm⁻¹) 1206, 1240, 1342, 1373, 1453, 1496, 1640, 1740, 2862, 2930, 2971, 3065; $[\alpha]_D^{20}$ +17.4°, c = 1% in chloroform, lit. value = +17.0°, c = 1.1% in chloroform.

7. 2. 2. 2 (R)-[(Hex-5-en-2-yloxy)methyl]benzene (46b)^[115]



Hex-5-en-2-ol (17.70 g, 176 mmol), tetrabutylammonium iodide (1.01 g, 2.6 mmol), THF (250 mL), NaH (60 % in mineral oil, 28.16 g, 708 mmol) and benzyl bromide (30.00 mL, 246 mmol). Title product as a clear oil (26.98 g, 142 mmol, 81 %). $R_f = 0.41$, petrol-DCM, 9:1 (v/v).

^1H NMR (300 MHz, CDCl_3) δ 1.26 (3H, d, $J = 6.1$ Hz, CH_3CH), 1.59 (1H, dddd, $J = 13.7, 9.3, 6.6$ and 5.3 Hz, CH_3CHCHH), 1.68-1.83 (2H, m, CH_3CHCHH), 2.2 (2H, dddt, $J = 16.9, 9.1, 6.6$ and 1.4 Hz, $\text{CH}_2\text{CHCH}_2\text{CH}_2$), 3.59 (1H, ddd, $J = 9.3$ and 6.1 Hz, CH_3CH), 4.48 (1H, d, $J = 11.7$ Hz, CHCHHAr), 4.61 (1H, d, $J = 11.7$ Hz, CHCHHAr), 5.00 (1H, dd, $J = 10.2$ and 1.7 Hz, $\text{CH}_2\text{CH}_2\text{CHCH}_{\text{CIS}}\text{H}_{\text{TRANS}}$), 5.04-5.11 (1H, dd, $J = 17.2, 1.7$ Hz, $\text{CH}_2\text{CH}_2\text{CHCH}_{\text{CIS}}\text{H}_{\text{TRANS}}$), 5.87 (1H, ddt, $J = 17.2, 10.2$ and 6.6 Hz, $\text{CH}_2\text{CH}_2\text{CHCH}_2$), 7.10-7.61 (5H, m, Ar);

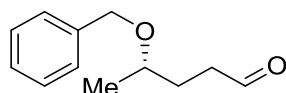
^{13}C NMR (75 MHz, CDCl_3) δ 19.5 (CH_3CH), 33.4 ($\text{CH}_2\text{CHCH}_2\text{CH}_2$), 37.9 ($\text{CH}_2\text{CHCH}_2\text{CH}_2$), 72.2 (CH_2Ar), 74.4 (CH_3CH), 116.7 ($\text{CH}_2\text{CHCH}_2\text{CH}_2$), 127.5 (*para*-Ar), 128.2 (*ortho*-Ar), 128.2 (*meta*-Ar), 138.6 (CH_2C), 139.3 ($\text{CH}_2\text{CHCH}_2\text{CH}_2$);

IR (cm^{-1}) 1205, 1239, 1343, 1373, 1453, 1496, 1640, 1740, 2863, 2930, 2972, 3065; $[\alpha]_{\text{D}}^{20} -17.9^\circ$, $c = 1\%$ in chloroform, lit. value = -15.7° , $c = 1.1\%$ in chloroform.

7. 2. 3 General Procedure C: (Benzyloxy)pentanals (49)^[80]

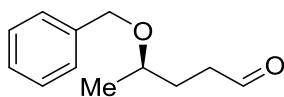
[(Hex-5-en-2-yloxy)methyl]benzene (1 eq) was dissolved in an acetone-water solution (acetone / water 95:5 v/v with an overall concentration of 0.15 M of alkene), cooled to 0 °C and ozone was bubbled through the solution for 5 h. It was then warmed to RT and quenched with NH₄Cl until no darkening of KI paper was observed. It was then diluted with water (10mL) and extracted with DCM (3 x 10 mL). The combined organic extracts were then washed with brine, dried over MgSO₄ and concentrated under reduced pressure to give the crude product as a clear oil. This was purified by MPC, eluting with petrol-diethyl ether 6:4.

7. 2. 3. 1 (S)-4-(Benzyloxy)pentanal (49a)



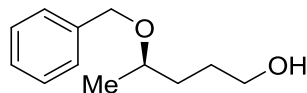
[(Hex-5-en-2-yloxy)methyl]benzene (250 mg, 1.3 mmol), acetone-water solution (acetone / water 95:5 v/v with an overall concentration of 0.15 M of alkene. Title product as a clear oil (133 mg, 0.70 mmol, 53 %). $R_f = 0.50$, petrol-Et₂O 6:4 (v/v). ¹H NMR (300 MHz, CDCl₃) δ 1.25 (3H, d, $J = 6.1$ Hz, CH₃CH), 1.78-1.97 (2H, m, CH₃CHCH₂), 2.56 (2H, dt, $J = 7.0$ and 1.7 Hz, CH₃CHCH₂CH₂), 3.52 (1H, tq, $J = 11.6$ and 6.1 Hz, CH₂CHCH₂), 4.33 (1H, d, $J = 11.5$ Hz, CHCHHAr), 4.65 (1H, d, $J = 11.5$ Hz, CHCHHAr), 7.07-7.59 (5H, m, Ar), 9.78 (1H, t, $J = 1.7$ Hz, CHO); ¹³C NMR (75 MHz, CDCl₃) δ 20.0 (CH₃CH), 29.6 (CH₂CHCH₂CH₂), 39.7 (CH₂CHCH₂CH₂), 70.9 (CH₂Ar), 76.4 (CH₃CH), 127.4 (*para*-Ar), 127.7 (*ortho*-Ar), 128.2 (*meta*-Ar), 138.9 (CH₂C), 201.5 (CHO); IR (cm⁻¹) 1205, 1275, 1342, 1375, 1453, 1496, 1602, 1721, 2724, 2836, 2868, 2944, 2970, 3031, 3064; $[\alpha]_D^{20} +20.6^\circ$, $c = 1\%$ in chloroform.

7. 2. 3. 2 (R)-4-(Benzyloxy)pentanal (49b)



[(Hex-5-en-2-yloxy)methyl]benzene (250 mg, 1.3 mmol), acetone-water solution (acetone-water 95:5 v/v with an overall concentration of 0.15 M of alkene. Title product as a clear oil (153 mg, 0.8 mmol, 61 %). $R_f = 0.48$, petrol-Et₂O 6:4 (v/v). ¹H NMR (300 MHz, CDCl₃) δ 1.24 (3H, d, $J = 6.1$ Hz, CH₃CH), 1.83-1.91 (2H, m, CH₃CHCH₂), 2.55 (2H, dt, $J = 7.0$ and 1.7 Hz, CH₃CHCH₂CH₂), 3.56 (1H, tq, $J = 11.7$ and 6.1 Hz, CH₂CHCH₂), 4.42 (1H, d, $J = 11.5$ Hz, CHCHHAr), 4.59 (1H, d, $J = 11.5$ Hz, CHCHHAr), 7.26-7.40 (5H, m, Ar), 9.77 (1H, t, $J = 1.7$ Hz, CHO); ¹³C NMR (75 MHz, CDCl₃) δ 19.3 (CH₃CH), 29.2 (CH₂CHCH₂CH₂), 39.9 (CH₂CHCH₂CH₂), 70.4 (CH₂Ar), 76.6 (CH₃CH), 127.4 (*para*-Ar), 127.5 (*ortho*-Ar), 128.2 (*meta*-Ar), 138.8 (CH₂C), 201.5 (CHO); IR (cm⁻¹) 1204, 1275, 1342, 1375, 1453, 1496, 1602, 1720, 2724, 2836, 2868, 2944, 2970, 3031, 3064; $[\alpha]_D^{20} -20.3^\circ$, $c = 1\%$ in chloroform.

7. 2. 4 (*R*)-4-(Benzyloxy)pentan-1-ol (50)^[81]



To a solution of (*R*)-4-(benzyloxy)pentanal (820 mg, 4.3 mmol) in MeOH (30 mL) at 0 °C, NaBH₄ (813 mg, 21.5 mmol) was added portionwise and stirred for 16 h at RT.

The volume of the solution was reduced by half under reduced pressure and acidified to pH 2 with HCl (1 M, 2 mL) and extracted with Et₂O (3 x 40 mL). The combined organics were washed with brine, dried over MgSO₄ and concentrated under reduced pressure to give the title product as a clear oil (668 mg, 3.4 mmol, 80 %). *R_f* = 0.25, petrol-DCM, 9:1 (v/v).

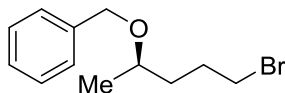
¹H NMR (300 MHz, CDCl₃) δ 1.25 (3H, d, *J* = 6.1 Hz, CH₃CH), 1.57-1.75 (4H, m, CH₃CHCH₂CH₂), 2.15-2.25 (1H, br. s, CH₂OH), 3.54-3.66 (1H, m, CH₂CHCH₂), 4.47 (1H, d, *J* = 11.6 Hz, CHCHHAr), 4.62 (1H, d, *J* = 11.6 Hz, CHCHHAr), 7.25-7.40 (5H, m, Ar);

¹³C NMR (75 MHz, CDCl₃) δ 19.3 (CH₃CH), 28.7 (CH₂CHCH₂CH₂), 33.1 (CH₂CHCH₂CH₂), 62.4 (CH₂OH), 74.7 (CH₂Ar), 76.5 (CH₃CH), 127.3 (*para*-Ar), 127.5 (*ortho*-Ar), 128.2 (*meta*-Ar), 138.9 (CH₂C);

IR (cm⁻¹) 1204, 1275, 1342, 1375, 1453, 1496, 1602, 1720, 2724, 2836, 2868, 2944, 2970, 3031, 3064;

[α]_D²⁰ -17.2°, *c* = 1% in chloroform.

7. 2. 5 (*R*)-{[(5-Bromopentan-2-yl)oxy]methyl}benzene (51)^[82]



(*R*)-(Benzyloxy)pentan-1-ol (2.00 g, 10 mmol) was dissolved in DCM (30 mL) and CBr₄ (3.76 g, 1.1 mmol) was added and cooled to 0 °C before CPh₃ (2.96 g, 11.3 mmol) was added portionwise. This was stirred for 16 h at RT before a mixture of petrol and Et₂O 3:1 (v/v) (20 mL) was added and filtered through a short pad of silica to give the title product as a clear oil (2.58 g, 10 mmol, 93 %). *R*_f = 0.64, petrol-DCM, 9:1 (v/v).

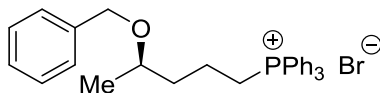
¹H NMR (300 MHz, CDCl₃) δ 1.28 (3H, d, *J* = 6.1 Hz, CH₃CH), 1.56-1.82 (2H, m, CH₃CHCH₂CH₂), 1.84-2.23 (2H, m, CH₃CHCH₂CH₂), 3.45 (2H, t, *J* = 6.7 Hz, CH₂Br), 3.59 (1H, tq, *J* = 11.7 and 6.1 Hz, CH₃CH) 4.47 (1H, d, *J* = 11.7 Hz, CHCHHAr), 4.62 (1H, d, *J* = 11.7 Hz, CHCHHAr), 7.30-7.44 (5H, m, Ar);

¹³C NMR (75 MHz, CDCl₃) δ 19.5 (CH₃CH), 28.9 (CH₂CHCH₂CH₂), 35.2 (CH₂CHCH₂CH₂), 62.4 (CH₂OH), 70.3 (CH₂Ar), 73.9 (CH₃CH), 127.3 (*para*-Ar), 127.51 (*ortho*-Ar), 128.2 (*meta*-Ar), 139.1 (CH₂C);

IR (cm⁻¹) 1241, 1340, 1373, 1453, 1496, 1737, 2867, 2868, 2968;

[α]_D²⁰ -23.5°, *c* = 1% in chloroform.

7. 2. 6 (*R*)-(4-(Benzyloxy)pentyl)triphenylphosphonium bromide (52)^[83]



(*R*)-{[(5-Bromopentan-2-yl)oxy]methyl}benzene (100 mg, 0.4 mmol) and CPh_3 (140 mg, 0.5 mmol) were stirred at 120 °C for 16 h. It was then cooled and diluted with DCM (1 mL) and sonicated until a solution formed. Petrol (3 mL) was added and the solution was filtered to give the crude product as a brown powder. This was purified by recrystallisation from MeCN to give the pure product as a white crystalline solid (97 mg, 0.2 mmol, 47%). MP = 189 °C.

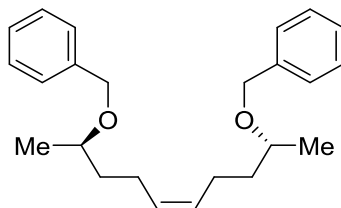
^1H NMR (300 MHz, CDCl_3) δ 1.16 (3H, d, $J = 6.1$ Hz, CH_3CH), 1.66-1.82 (2H, m, $\text{CH}_3\text{CHCH}_2\text{CH}_2$), 1.90-2.10 (2H, m, $\text{CH}_3\text{CHCH}_2\text{CH}_2$), 3.55-3.37 (2H, m, CH_2PPh_3), 3.80-3.95 (1H, m, CH_3CH), 4.31-4.34 (1H, d, $J = 11.6$ Hz, CHCHHAr), 4.52-4.56 (1H, d, $J = 11.6$ Hz, CHCHHAr), 6.99-7.15 (15 H, *P-Ar*), 7.16-7.25 (5H, m, Ar);

^{13}C NMR (75 MHz, CDCl_3) δ 18.7 (d, $J = 4$ Hz, $\text{CH}_2\text{CHCH}_2\text{CH}_2$), 19.28 (CH_3CH), 36.57 (d, $J = 15$ Hz, CH_2PPh_3), 35.2 (CH_2CHCH_2), 70.0 (CH_2Ar), 73.8 (CH_3CH), 118.5 (*P-C*), 127.1 (*C-para-Ar*), 127.5 (*C-ortho-Ar*), 128.1 (*C-meta-Ar*), 130.3 (d, $J = 12$ Hz, *P-ortho-Ar*), 133.7 (d, $J = 10$ Hz, *P-meta-Ar*), 134.8 (*para-Ar*) 139.1 (CH_2C);

^{31}P NMR (75 MHz) δ 36.5 (d, $^2J_{\text{C-P}}$ 15 Hz);

IR (cm^{-1}) 1220, 1324, 1343, 1400, 1438, 1486, 1587, 2870, 2987, 3010, 3040.

7. 2. 7 (*R,S*)-[(Dec-5-ene-2,9-diylbis(oxy))]bis(methylene)dibenzene (47c)^[84]



n-BuLi (7.9 mL, 12.7 mmol) was added dropwise to THF (30 mL) at -40 °C and the (*R*)-4-(Benzyloxy)pentyltriphenylphosphonium bromide was added and the solution stirred at RT for 2 h. To this, (*R*)-4-(Benzyloxy)pentanal (2.5 g, 12.7 mmol) was added and the solution as refluxed at 70 °C for 16 h before cooling, diluting with Et₂O (20 mL) and filtering through a bed of silica. The filtrate was dried over MgSO₄ and concentrated to afford the product as a clear oil (3.57 g, 10.0 mmol, 80 %) *R_f* = 0.52, DCM.

¹H NMR (300 MHz, CDCl₃) δ 1.21-1.27 (6H, m, 2 x CHCH₃), 1.42-1.53 (2H, m, 2 x CH₃CHCHH), 1.61-1.79 (2H, m, 2 x CH₃CHCHH), 2.21 (4H, ddd, *J* = 16.2, 9.1, 3.4 and 1.5 Hz, 2 x CH₃CHCH₂CH₂), 3.57 (2H, ddt, *J* = 8.7, 6.2 and 4.6 x CH₃CHCH₂), 4.48 (2H, d, *J* = 11.7 Hz, 2 x CHH-Ar), 4.60 (2H, d, *J* = 11.7 Hz, 2 x CHH-Ar), 5.35-5.49 (2H, m, CH₂CHCH₂), 7.07-7.54 (10H, m, 2 x Ar);

¹³C NMR (75 MHz, CDCl₃) δ 19.6 (2 x CH₃), 28.6 (2 x CH₂CH), 36.6 (2 x CH₃CH₂CH₂), 70.3 (2 x OCH₂), 74.3 (2 x CHO), 127.4 (2 x *para*-Ar), 127.6 (2 x *ortho*-Ar), 128.3 (*meta*-Ar), 130.1 (2 x alkene CH);

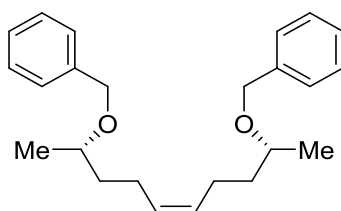
IR (cm⁻¹) 1209, 1312, 1347, 1371, 1462, 1489, 2867, 2925, 2963, 3025, 3062;

[α]_D²⁰ -0.3°, c = 1% in chloroform.

7. 2. 8 General Procedure D: (*R,S*)-[(Dec-5-ene-2,9-diylbis(oxy))bis(methylene)] dibenzenes (47a and b)^[77]

[(Hex-5-en-2-yloxy)methyl]benzene (1 eq) and Grubbs catalyst first generation (0.05 eq) were dissolved in DCM (2.5 mL / 1 mmol) and heated to 100 °C for 1.5 h using microwave heating, flushing the vial with N₂ every 30 min. This was then concentrated under reduced pressure to give the crude product as a purple solid which was purified using MPC, eluting with DCM to give the title compound.

7. 2. 8. 1 (*S,S*)-[Dec-5-ene-2,9-diylbis(oxy)]bis(methylene)dibenzene (47b)



[(Hex-5-en-2-yloxy)methyl]benzene (500 mg, 2.6 mmol), Grubbs catalyst first generation (110 mg, 0.1 mmol), DCM (10 mL). Title compound as a clear oil (600 mg, 1.7 mmol, 65 %). $R_f = 0.5$, DCM.

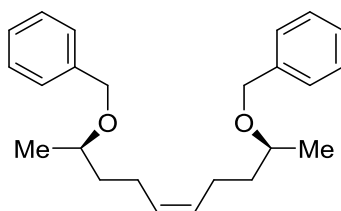
¹H NMR (300 MHz, CDCl₃) δ 1.23 (6H, d, $J = 6.1$ Hz, 2 x CHCH₃), 1.42-1.56 (2H, m, 2 x CH₃CHCHH), 1.62-1.76 (2H, m, 2 x CH₃CHCHH), 2.05-2.20 (4H, m, 2 x CH₃CHCH₂CH₂), 3.48-3.60 (2H, m, 2 x CH₃CHCH₂), 4.47 (2H, d, $J = 11.7$ Hz, 2 x CHH-Ar), 4.59 (2H, d, $J = 11.7$ Hz, 2 x CHH-Ar), 5.39-5.43 (2H, m, CH₂CHCHCH₂), 7.25-7.40 (10H, m, 2 x Ar);

¹³C NMR (75 MHz, CDCl₃) δ 19.6 (2 x CH₃), 28.6 (2 x CH₂CH), 36.5 (2 x CH₃CH₂CH₂), 70.3 (2 x OCH₂), 74.3 (2 x CHO), 127.3 (2 x *para*-Ar), 127.6 (2 x *ortho*-Ar), 128.3 (*meta*-Ar), 130.1 (2 x alkene CH);

IR (cm⁻¹) 1205, 1306, 1341, 1373, 1453, 1496, 2860, 2929, 2967, 3028, 3064;

$[\alpha]_D^{20} +31.3^\circ$, $c = 1\%$ in chloroform.

7. 2. 8. 2 (R,R)-[(Dec-5-ene-2,9-diylbis(oxy))bis(methylene)]dibenzene (47a)



[(Hex-5-en-2-yloxy)methyl]benzene (500 mg, 2.6 mmol), Grubbs catalyst first generation (110 mg, 0.1 mmol) and DCM (10 mL). Title compound as a clear oil (540 mg, 2.5 mmol, 59 %). $R_f = 0.48$, DCM.

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 1.21 (6H, d, $J = 6.1$ Hz, 2 x CHCH_3), 1.44-1.59 (2H, m, 2 x CH_3CHCHH), 1.60-1.74 (2H, m, 2 x CH_3CHCHH), 2.03-2.13 (4H, m, 2 x $\text{CH}_3\text{CHCH}_2\text{CH}_2$), 3.50-3.60 (2H, m, 2 x CH_3CHCH_2), 4.47 (2H, d, $J = 11.7$ Hz, 2 x CHH-Ar), 4.60 (2H, d, $J = 11.7$ Hz, 2 x CHH-Ar), 5.39-5.44 (2H, m, $\text{CH}_2\text{CHCHCH}_2$), 7.28-7.38 (10H, m, 2 x Ar);

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 19.6 (2 x CH_3), 28.6 (2 x CH_2CH), 36.5 (2 x $\text{CH}_3\text{CH}_2\text{CH}_2$), 70.3 (2 x OCH_2), 74.3 (2 x CHO), 127.39 (2 x *para*-Ar), 127.6 (2 x *ortho*-Ar), 128.3 (*meta*-Ar), 130.1 (2 x alkene CH);

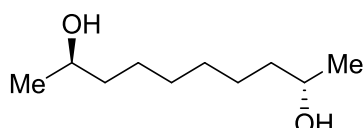
IR (cm^{-1}) 1204, 1308, 1341, 1372, 1450, 1496, 2861, 2930, 2965, 3027, 3065;

$[\alpha]_{\text{D}}^{20} -31.5^\circ$, $c = 1\%$ in chloroform.

7. 2. 9 General Procedure E: Decane-2,9-diols (43)^[78]

(Z)-[(Dec-5-ene-2,9-diy]bis(oxy)]bis(methylene)dibenzene (1 eq) was dissolved in MeOH (10 mL / mmol) and 10 % Pd/C (10 % by mass) was added and the system was put under a positive pressure of hydrogen. It was stirred for 48 h, filtered through celite and washed with DCM before concentration under reduced pressure.

7. 2. 9. 1 (R,S)-Decane-2,9-diol (43c)



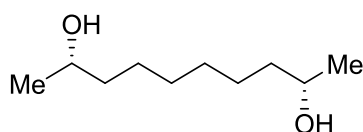
(Z)-[(Dec-5-ene-2,9-diy]bis(oxy)]bis(methylene)dibenzene (100 mg, 0.3 mmol), MeOH (3 mL) and 10 % Pd/C (100 mg). Product as a clear oil (520 mg, 3.0 mmol, 98 %). $R_f = 0.27$, petrol-EtOAc, 3:7 (v/v).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 1.17 (6H, d, $J = 6.2$ Hz, 2 x CHCH_3), 1.26-1.51 (12H, m, 6 x CH_2), 3.74-3.86 (2H, m, 2 x CHCH_3);

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 23.4 (2 x Me), 25.6 (2 x CHCH_2CH_2), 29.5 (2 x $\text{CHCH}_2\text{CH}_2\text{CH}_2$), 39.2 (CHCH_2), 68.1 (2 x CH);

$[\alpha]_{\text{D}}^{20} +0.5^\circ$, $c = 1\%$ in chloroform.

7. 2. 9. 2 (S,S)-Decane-2,9-diol (43b)



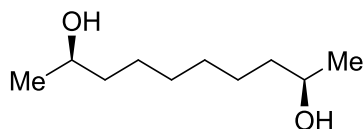
(Z)-[(Dec-5-ene-2,9-diy]bis(oxy)]bis(methylene)dibenzene (1.27 g, 3.6 mmol), MeOH (35 mL) and 10 % Pd/C (1.27 g). Product as a clear oil (633 mg, 3.6 mmol, 100 %). $R_f = 0.30$, petrol-EtOAc, 3:7 (v/v).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 1.19 (6H, d, $J = 6.2$ Hz, 2 x CHCH_3), 1.25-1.50 (12H, m, 6 x CH_2), 3.73-3.85 (2H, m, 2 x CHCH_3);

^{13}C NMR (75 MHz, CDCl_3) δ 23.4 (2 x Me), 25.6 (2 x CHCH_2CH_2), 29.5 (2 x $\text{CHCH}_2\text{CH}_2\text{CH}_2$), 39.2 (CHCH_2), 68.12 (2 x CH);

$[\alpha]_{\text{D}}^{20} +28.8^\circ$, $c = 1\%$ in chloroform.

7. 2. 9. 3 (R,R)-Decane-2,9-diol (43a)



(Z)-[(Dec-5-ene-2,9-diylbis(oxy))]bis(methylene)dibenzene (3.57 g, 10.0 mmol), MeOH (100 mL) and 10 % Pd/C (3.57 mg). Product as a clear oil (1.74 g, 10.0 mmol, 100 %). $R_f = 0.26$, petrol-EtOAc, 3:7 (v/v).

^1H NMR (300 MHz, CDCl_3) δ 1.18 (6H, d, $J = 6.2$ Hz, 2 x CHCH_3), 1.25-1.43 (12H, m, 6 x CH_2), 3.69-3.86 (2H, m, 2 x CHCH_3);

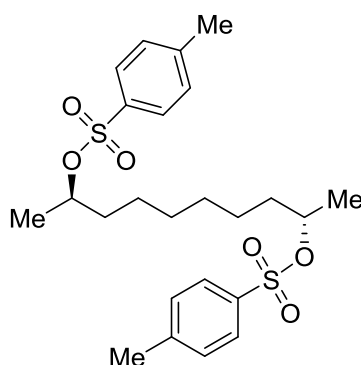
^{13}C NMR (75 MHz, CDCl_3) δ 23.3 (2 x Me), 25.7 (2 x CHCH_2CH_2), 29.4 (2 x $\text{CHCH}_2\text{CH}_2\text{CH}_2$), 39.2 (CHCH_2), 68.1 (2 x CH);

$[\alpha]_{\text{D}}^{20} -28.1^\circ$, $c = 1\%$ in chloroform.

7. 2. 10 General Procedure F: Decane-2,9-diyl bis(4-methylbenzenesulfonates) (48)^[79]

Decane-2,9-diol (1 eq) was dissolved in DCM (4 mL / 1 mmol) and cooled to 0 °C before pyridine (4 eq) and *p*-toluene sulfonyl chloride (3 eq) were added. The solution was stirred at RT for 16 h before dilution with DCM (10 mL) and water (5 mL), the organic fraction was washed with HCl (2 M, 5 mL) and NaHCO₃ (5 mL), dried over MgSO₄ and concentrated under reduced pressure to give the crude product. This was purified by MPC, eluting with DCM.

7. 2. 10. 1 (*R,S*)- Decane-2,9-diyl bis(4-methylbenzenesulfonate) (48c)



Decane-2,9-diol (512 mg, 3.0 mmol), DCM (13 mL), pyridine (0.95 mL, 11.9 mmol) and *p*-toluene sulfonyl chloride (1.68 g, 8.8 mmol). Title product as a white crystalline solid (740 mg, 1.5 mmol, 51 %). $R_f = 0.53$, petrol-DCM, 9:1 (*v/v*). MP 176-178 °C.

¹H NMR (300 MHz, CDCl₃) δ 1.07-1.18 (8H, m, 4 x CH₂), 1.26 (6H, d, $J = 6.3$ Hz, 2 x CH₃CH), 1.38-1.72 (4H, m, 2 x CH₃CHCH₂), 2.51 (6H, s, 2 x Ar-CH₃), 4.49-4.74 (2H, m, 2 x CH₃CH), 7.42 (4H, d, $J = 8.5$ Hz, *meta*-Ar) 7.91 (4H, d, $J = 8.5$ Hz, *ortho*-Ar);

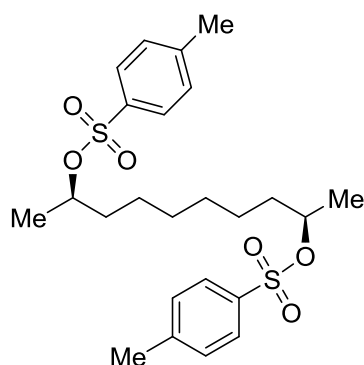
¹³C NMR (75 MHz, CDCl₃) δ 20.6 (2 x Ar-CH₃), 21.3 (2 x CH₃CH), 24.6 (2 x CHCH₂CH₂), 28.8 (2 x CHCH₂CH₂CH₂), 36.4 (2 x CH₃CHCH₂), 80.2 (CH₃CH), 127.5 (*ortho*-Ar), 129.5 (*meta*-Ar), 135.2 (2 x CCH₃), 144.1 (S-C);

IR (cm⁻¹) 1210, 1355, 1464, 1493, 1599, 2851, 2925, 2947, 2989;

$[\alpha]_D^{20} -0.6^\circ$, $c = 1\%$ in chloroform,

HRMS FTMS $m/z = 500.2131$, $[M+NH_3] C_{24}H_{34}O_6S_2NH_4$, pred $m/z = 500.2135$

7. 2. 10. 2 (R,R)- Decane-2,9-diyl bis(4-methylbenzenesulfonate) (48a)



Decane-2,9-diol (633 mg, 3.6 mmol), DCM (13 mL), pyridine (1 mL, 13.4 mmol) and *p*-toluene sulfonyl chloride (2.1 g, 10.9 mmol). Title product as a white crystalline solid (1.48 g, 3.06 mmol, 84 %). $R_f = 0.51$, petrol-DCM, 9:1 (v/v). MP 178-179 °C.

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 1.05-1.16 (8H, m, 4 x CH_2), 1.25 (6H, d, $J = 6.3$ Hz, 2 x CH_3CH), 1.36-1.71 (4H, m, 2 x CH_3CHCH_2), 2.51 (6H, s, 2 x Ar- CH_3), 4.48-4.73 (2H, m, 2 x CH_3CH), 7.43 (4H, d, $J = 8.2$ Hz, *meta*-Ar), 7.93 (4H, d, $J = 8.2$ Hz, *ortho*-Ar);

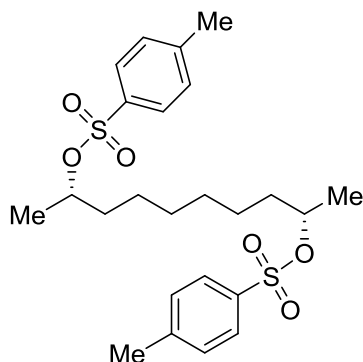
$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 20.6 (2 x Ar- CH_3), 21.3 (2 x CH_3CH), 24.6 (2 x CHCH_2CH_2), 28.8 (2 x $\text{CHCH}_2\text{CH}_2\text{CH}_2$), 36.4 (2 x CH_3CHCH_2), 80.2 (CH_3CH), 127.6 (*ortho*-Ar), 129.6 (*meta*-Ar), 135.2 (2 x CCH_3), 144.1 (S-C);

IR (cm^{-1}) 1211, 1354, 1465, 1494, 1598, 2850, 2923, 2941, 2983;

$[\alpha]_{\text{D}}^{20} -16.3^\circ$, $c = 1\%$ in chloroform.

HRMS FTMS $m/z = 500.2130$, $[\text{M}+\text{NH}_3] \text{C}_{24}\text{H}_{34}\text{O}_6\text{S}_2\text{NH}_4$, pred $m/z = 500.2135$

7. 2. 10. 3 (S,S)- Decane-2,9-diyl bis(4-methylbenzenesulfonate) (48b)



Decane-2,9-diol (1.74 g, 10 mmol), DCM (50 mL), pyridine (3.22 mL, 40 mmol) and *p*-toluene sulfonyl chloride (5.7 g, 30 mmol). Title product as a white crystalline solid (3.8 g, 7.9 mmol, 79 %). $R_f = 0.53$, petrol-DCM, 9:1 (v/v). MP 178.93 °C.

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 1.09-1.15 (8H, m, 4 x CH_2), 1.24 (6H, d, $J = 6.3$ Hz, 2 x CH_3CH), 1.40-1.60 (4H, m, 2 x CH_3CHCH_2), 2.46 (6H, s, 2 x Ar- CH_3), 4.60 (2H, tq, $J = 12.5$ and 6.3, 2 x CH_3CH), 7.35 (4H, d, $J = 8.2$ Hz, *meta*-Ar), 7.81 (4H, d, $J = 8.2$ Hz, *ortho*-Ar);

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 20.6 (2 x Ar- CH_3), 21.3 (2 x CH_3CH), 24.6 (2 x CHCH_2CH_2), 28.8 (2 x $\text{CHCH}_2\text{CH}_2\text{CH}_2$), 36.4 (2 x CH_3CHCH_2), 80.2 (CH_3CH), 127.6 (*ortho*-Ar), 129.5 (*meta*-Ar), 135.2 (2 x C CH_3), 144.1 (S-C);

IR (cm^{-1}) 1211, 1341, 1463, 1495, 1598, 2857, 2927, 2973;

$[\alpha]_{\text{D}}^{20} +16.7^\circ$, $c = 1\%$ in chloroform.

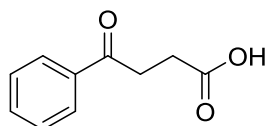
HRMS FTMS $m/z = 500.2122$, $[\text{M}+\text{NH}_3]$ $\text{C}_{24}\text{H}_{34}\text{O}_6\text{S}_2\text{NH}_4$, pred $m/z = 500.2135$

7.3 Synthesis of Naphthalene $^{13}\text{C}_{10}$ (16*) and 2-naphthoic acid $^{13}\text{C}_{11}$ (58a*)

7.3.1 General Procedure G: 4-Oxo-4-phenylbutanoic acids (54)^[86]

Benzene (1 eq) was dissolved in 1,1,2,2-tetrachloroethane (1.8 mL / 1 mmol) and succinic anhydride (1 eq) was added. The suspension was cooled to 0 °C and aluminium chloride (4.7 eq) was added portion-wise and stirred overnight at RT. It was then poured over ice and 1M HCl, extracted into Et₂O and the combined organics washed with water and dried over MgSO₄ before concentration under vacuum to give the title compound.

7.3.1.1 4-Oxo-4-phenylbutanoic acid (54)^[116]

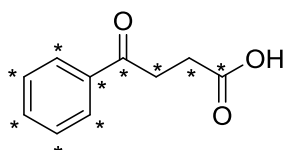


Benzene (1.15 mL, 12.8 mmol), 1,1,2,2-tetrachloroethane (24 mL), succinic anhydride (1.3 g, 12.8 mmol) and aluminium chloride (8.04 g, 60.1 mmol). Title compound as a brown solid (2.05g, 11.5 mmol, 90 %). MP 116-117 °C. Literature value 117-119 °C.

¹H NMR (300 MHz, CDCl₃) δ 2.90 (2H, t, *J* = 6.5 Hz, COCH₂CH₂CO₂H), 3.35 (2H, t, *J* = 6.5 Hz, COCH₂CH₂CO₂H), 7.47-7.53 (2H, m, *meta* Ar), 7.58-7.63 (1H, m, *para* Ar), 8.00-8.03 (2H, m, *ortho* Ar);

¹³C NMR (75 MHz, CDCl₃) δ 28.0 (CH₂CO₂H), 33.2 (CH₂CH₂CO₂H), 128.1 (*meta*-Ar), 128.6 (*ortho*-Ar), 133.3 (*para*-Ar), 136.4 (CCO), 178.5 (CO₂OH), 197.8 (CCO); IR (cm⁻¹) 1207, 1238, 1294, 1347, 1399, 1447, 1594, 1681, 2589, 2670, 2771, 2923, 3028.

7.3.1.2 $^{13}\text{C}_{10}$ 4-Oxo-4-phenylbutanoic acid (54*)



$^{13}\text{C}_6$ Benzene (1.15 mL, 12.8 mmol), 1,1,2,2-tetrachloroethane (24 mL), $^{13}\text{C}_4$ succinic anhydride (1.3 g, 12.8 mmol) and aluminium chloride (8.04 g, 60.1 mmol). Title compound as a brown solid (2.055g, 11.5 mmol, 90 %). MP 118-119 °C.

^1H NMR (300 MHz, CDCl_3) δ 2.85 (2H, dm, $J_{\text{C}^{13}\text{-H}} = 135$ Hz, $\text{COCH}_2\text{CH}_2\text{CO}_2\text{H}$), 3.35 (2H, dm, $J_{\text{C}^{13}\text{-H}} = 132$ Hz, $\text{COCH}_2\text{CH}_2\text{CO}_2\text{H}$), 7.53 (2H, dm, $J_{\text{C}^{13}\text{-H}} = 189$ Hz, *meta* Ar), 7.60 (1H, dm, $J_{\text{C}^{13}\text{-H}} = 159$ Hz, *para* Ar), 8.02 (2H, dm, $J_{\text{C}^{13}\text{-H}} = 153$ Hz, *ortho* Ar);

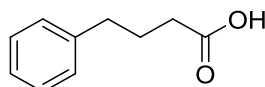
^{13}C NMR (75 MHz, CDCl_3) δ 27.5-28.1 (m, $\text{CH}_2\text{CO}_2\text{H}$), 32.9-33.7 (m, $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 126.8-128.9 (*ortho*- and *meta*-Ar), 132.4-133.1 (*para*-Ar), 135.7-136.4 (CCO), 177.8-178.3 (CO_2OH), 196.1-196.9 (CCO);

IR (cm^{-1}) 1232, 1363, 1377, 1486, 1523, 1675, 2572, 2785, 2936, 3042.

7. 3. 2 General Procedure H: 4-Phenylbutanoic acids (55)^[87]

Benzoylpropionic acid (1 eq) and 10 % Pd/C (10 % by mass) were dissolved in acetic acid (2.6 mL/ 1 mmol) and stirred in an atmosphere of H₂ for 16 h. The mixture was then filtered through Celite and evaporated under reduced pressure to give a yellow oil. Traces of acetic acid were removed azeotropically with toluene yield the title compound.

7. 3. 2. 1 4-Phenylbutanoic acid (55)^[117]



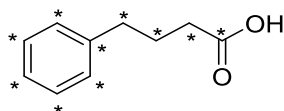
Benzoylpropionic acid (2 g, 11.5 mmol), 10 % Pd/C (200 mg) and acetic acid (30 mL) Product as a yellow solid (1.86 mg, 11.3 mmol, 98 %). MP 47-48 °C. Literature value 47-47 °C.

¹H NMR (300 MHz, CDCl₃) δ 1.88 (2H, tt, *J* = 7.5 Hz, CH₂CH₂CO₂H), 2.29 (2H, t, *J* = 7.5 Hz, CH₂CH₂CH₂CO₂H), 2.59 (2H, t, *J* = 7.5 Hz, CH₂CO₂H), 7.05-7.25 (5H, m, Ar);

¹³C NMR (75 MHz, CDCl₃) δ 26.2 (CH₂CH₂CO₂H), 33.4 (CH₂CH₂CH₂CO₂H), 35.0 (CH₂CO₂H), 126.1 (*para*-Ar), 128.4 (*ortho*-Ar), 128.5 (*meta*-Ar), 141.2 (α-Ar), 180.2 (CO₂H);

IR (cm⁻¹) 1203, 1276, 1342, 1408, 1435, 1496, 1603, 1686, 2602, 2689, 2953, 3022.

7. 3. 2. 2 ¹³C₁₀ 4-Phenylbutanoic acid (55*)



¹³C₁₀ Benzoylpropionic acid (2.33 g, 13.0 mmol), 10 % Pd/C (233 mg) and acetic acid (30 mL). Products as a solid (1.57 g, 9.5 mmol, 74 %). MP 51-54 °C.

^1H NMR (300 MHz, CDCl_3) δ 1.89 (2H, dm, $J_{\text{C}^{13}\text{-H}} = 129$ Hz, $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 2.31 (2H, dm, $J_{\text{C}^{13}\text{-H}} = 120$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 2.55 (2H, dm, $J_{\text{C}^{13}\text{-H}} = 156$ Hz, $\text{CH}_2\text{CO}_2\text{H}$), 7.17 (5H, dm, $J_{\text{C}^{13}\text{-H}} = 192$ Hz, Ar);

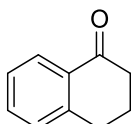
^{13}C NMR (75 MHz, CDCl_3) δ 25.7-26.6 (t, $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 32.5-33.7 (m, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 34.4-35.0 (m, $\text{CH}_2\text{CO}_2\text{H}$), 125.1-126.7 (m, *para*-Ar), 126.7-128.1 (m, *meta*- and *ortho*-Ar), 139.9-141.8 (m, α -Ar), 178.8-179.5 (q, CO_2H);

IR (cm^{-1}) 1253, 1349, 1468, 1486, 1646, 1689, 2645, 2675, 2942, 3035.

7. 3. 3 General Procedure I: 3,4-Dihydronaphthalen-1(2H)-ones (56)^[88]

4-Phenylbutanoic acid (1 eq) was dissolved in methanesulfonic acid (2 mL/ 1 mmol) and heated at 90 °C for 4 h before dilution in ice water (50 mL) and extraction with Et₂O (3 x 50 mL). The combined organic extracts were washed with brine, dried over MgSO₄ and concentrated under reduced pressure to afford the title product.

7. 3. 3. 1 Dihydronaphthalen-1(2H)-one (56)^[132]



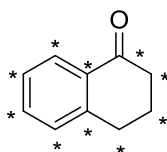
4-Phenylbutanoic acid (1.00 g, 6.1 mmol), methanesulfonic acid (12 mL). Product as a brown oil (770 mg, 5.3 mmol, 86 %).

¹H NMR (300 MHz, CDCl₃) δ 1.82-2.04 (2H, m, COCH₂CH₂), 2.35-2.53 (2H, m, CCH₂), 2.76 (2H, t, *J* = 6.1 Hz, COCH₂), 6.97-7.17 (2H, m, CH₂CCH), 7.28 (1H, td, *J* = 7.5 and 1.5 Hz, CH₂CCHCH and COCCHCH), 7.83 (1H, ddd, *J* = 7.5, 1.5 and 0.6 Hz, COCCH)

¹³C NMR (75 MHz, CDCl₃) δ 20.3 (COCH₂CH₂), 20.9 (COCH₂CH₂CH₂), 29.7 (COCH₂), 128.6 (CH₂CCH), 134.9 (COCCHCH), 138.8 (COCCH), 139.1 (CH₂CCHCH), 177.0 (CO);

IR (cm⁻¹) 1225, 1285, 1323, 1454, 1600, 1679, 1868, 2943.

7. 3. 3. 2 ¹³C₁₀ Dihydronaphthalen-1(2H)-one (56*)



4-Phenylbutanoic acid (1.57 g, 9.50 mmol) and methanesulfonic acid (18 mL).

Products as a brown oil (1.03 mg, 7.05 mmol, 95 %).

^1H NMR (300 MHz, CDCl_3) δ 1.83-2.24 (2H, dm, $J_{\text{C}13-\text{H}} = 123$ Hz COCH_2CH_2), 2.26-2.78 (2H, dm, $J_{\text{C}13-\text{H}} = 156$ Hz, CCH_2), 2.66-3.08 (2H, dm, $J_{\text{C}13-\text{H}} = 126$ Hz, COCH_2), 6.57-7.14 (2H, dm, $J_{\text{C}13-\text{H}} = 171$ Hz, CH_2CCH), 6.92-7.66 (1H, dm, $J_{\text{C}13-\text{H}} = 222$ Hz, CH_2CCHCH and COCCHCH), 7.64-8.22 (1H, dm, $J_{\text{C}13-\text{H}} = 174$ Hz, COCCH);

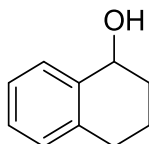
^{13}C NMR (75 MHz, CDCl_3) δ 23.2 (t, COCH_2CH_2), 29.7-30.3 (m, $\text{COCH}_2\text{CH}_2\text{CH}_2$), 38.5-39.2 (m, COCH_2), 125.7-129.4 (m, CH_2CCH and COCCHCH), 131.5-133.4 (m, COCCH), 145.8 (p, CH_2CCHCH), 178.3 (t, CO);

IR (cm^{-1}) 1235, 1272, 1349, 1426, 1615, 1648, 1892, 2936.

7. 3. 4 General Procedure J: 1,2,3,4-tetrahydronaphthalen-1-ols (57)^[89]

3,4-Dihydronaphthalen-1(2H)-one (1 eq) was dissolved in EtOH (1 mL/ 1 mmol) and THF (1 mL/ 1 mmol) and NaBH₄ (1 eq) was added portion-wise. The solution was stirred at RT for 16 h and concentrated under reduced pressure. The resultant solid was dissolved in 1 M HCl (15 mL) and Et₂O (15 mL) and the aqueous layer was further extracted with Et₂O (2 X 15 mL) and the combined organics were washed with NaHCO₃ (30mL), dried over MgSO₄ and concentrated under reduced pressure to give the product.

7. 3. 4. 1 1,2,3,4-tetrahydronaphthalen-1-ol (57)^[118]



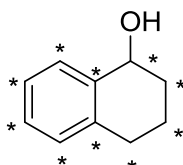
3,4-Dihydronaphthalen-1(2H)-one (770 mg, 5.25 mmol), EtOH (5 mL), THF (5 mL) and NaBH₄ (200 mg, 5.25 mmol). Product as a yellow oil (709 mg, 4.82 mmol, 92 %).

¹H NMR (300 MHz, CDCl₃) δ 1.56-2.09 (4H, m, CHOHC₂H₂CH₂), 2.58-3.04 (2H, m, COHC₂H₂), 4.70-4.91 (1H, m, CHOH), 7.06-7.15 (1H, m, CH₂CCH), 7.16-7.25 (2H, m, CH₂CCHCH₂), 7.39-7.49 (1H, m, CHOCCCH);

¹³C NMR (75 MHz, CDCl₃) δ 18.8 (CHOHCH₂CH₂), 29.2 (CCH₂), 32.3 (CHOHCH₂), 68.2 (CHOH), 126.2 (CHOHCCH₂), 127.6 (CH₂CCH₂), 128.6 (CHOHCCH), 129.0 (CH₂CCH), 137.1 (CH₂C), 139.2 (CHOHC);

IR (cm⁻¹) 1270, 1451, 2934, 3326.

7. 3. 4. 2 ¹³C₁₀ 1,2,3,4-tetrahydronaphthalen-1-ol (57*)



3,4-Dihydronaphthalen-1(2H)-one (780 mg, 5.35 mmol), EtOH (5 mL), THF (5 mL) and NaBH₄ (210 mg, 5.35 mmol). Product as a yellow oil (660 mg, 4.50 mmol, 84 %).

¹H NMR (300 MHz, CDCl₃) δ 1.45-2.20 (4H, dm, $J_{C13-H} = 225$ Hz, CHOHC $\overline{H}H$ C $\overline{H}H$ CH₂), 2.56-2.98 (2H, dm, $J_{C13-H} = 126$ Hz, COHC $\overline{H}H$ C $\overline{H}H$), 4.57-5.05 (1H, dm, $J_{C13-H} = 144$ Hz CHOH), 6.87-7.19 (1H, dm, $J_{C13-H} = 96$ Hz, CH₂C $\overline{C}H$), 6.96-7.49 (2H, dm, $J_{C13-H} = 159$ Hz, CH₂C $\overline{C}H$ C $\overline{H}H$), 7.38-7.70 (1H, dm, $J_{C13-H} = 96$ Hz, CHOCC $\overline{C}H$);

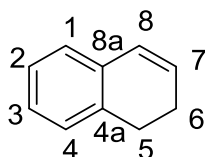
¹³C NMR (75 MHz, CDCl₃) δ 18.7 (t, CHOHCH₂CH₂), 27.6 (t, CCH₂), 32.3 (t, CHOHCH₂), 68.1 (t, CHOH), 125.3-126.2 (m, CHOHC $\overline{C}H$ CH, CH₂C $\overline{C}H$ CH, CHOHC $\overline{C}H$ and CH₂C $\overline{C}H$), 136.7-138.5 (CH₂C and CHOHC);

IR (cm⁻¹) 1263, 1271, 1289, 1412, 1483, 2864, 2936, 3359.

7. 3. 5 General Procedure K: 1, 2-Dihydronaphthalenes (58)^[89]

1, 2, 3, 4-tetrahydronaphthalene-1-ol (1 eq) was dissolved in anhydrous toluene (3 mL/ 1 mmol) and *p*-toluenesulfonic acid monohydrate (0.1 eq) was added. The solution was heated at 75 °C for 1.5 h, cooled, filtered through MgSO₄ and concentrated under reduced pressure to afford the pure product.

7. 3. 5. 1 1, 2-Dihydronaphthalene (58)^[133]



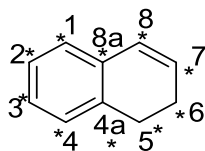
1, 2, 3, 4-tetrahydronaphthalene-1-ol (500 mg, 3.37 mmol), anhydrous toluene (10 mL) and *p*-toluenesulfonic acid monohydrate (83 mg, 0.43 mmol) to afford the pure product as a light brown oil (357 mg, 2.70 mmol, 81 %). *R_f* = 0.85 eluent petrol-EtOAc 8.:2 (v/v).

¹H NMR (300 MHz, CDCl₃) δ 2.44 (2H, tdd, *J* = 9.8, 5.7 and 2.6 Hz, H-6), 2.92 (2H, t, *J* = 5.7 Hz, H-5), 6.15 (1H, dt, *J* = 9.8 and 4.4 Hz, H-7), 6.59 (1H, dt, *J* = 4.4 and 2.6 Hz, H-8), 6.95-7.47 (4H, m, H 1-4);

¹³C NMR (75 MHz, CDCl₃) δ 23.3 (C-6), 27.6 (C-5), 125.4 (C-7), 125.4, 126.5, 127.6, 128.3, 128.7, 129.1, 135.5 (C-4a);

IR (cm⁻¹) 1278, 1326, 1452, 1484, 2829, 2883, 2932, 3032.

7. 3. 5. 2 ¹³C₁₀1, 2-Dihydronaphthalene (58*)



1, 2, 3, 4-tetrahydronaphthalene-1-ol (780 mg, 5.27 mmol), anhydrous toluene (15 mL) and *p*-toluenesulfonic acid monohydrate (100 mg, 0.53 mmol) to afford the pure

product as a light brown oil (500 mg, 3.85 mmol, 73 %). $R_f = 0.82$ eluent petrol-EtOAc 8.:2 (v/v).

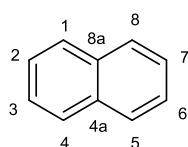
$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 2.18-2.61 (2H, dm, $J_{\text{C}13\text{-H}} = 129$ Hz, H-6), 2.61-3.04 (2H, dm, $J_{\text{C}13\text{-H}} = 129$ Hz, H-5), 5.81-6.28 (2H, dm, $J_{\text{C}13\text{-H}} = 141$ Hz, H-7 and 8), 6.88-7.43 (4H, dm, $J_{\text{C}13\text{-H}} = 165$ Hz, H-1-4);

IR (cm^{-1}) 1267, 1311, 1394, 1437, 1475, 1491, 2826, 2872, 2945, 3028.

7. 3. 6 General Procedure L: Naphthalenes (16)^[90]

1, 2-Dihydronaphthalene (1 eq), maleic acid (10 eq) and 10 % palladium on carbon (10 % by mass) were stirred at 150 °C for 16 h before dilution with water (4 mL) and petrol (4 mL). The mixture was filtered through Celite and the fractions separated. The aqueous fraction was washed with petrol (3 x 4 mL) and the combined organic fractions were dried over MgSO₄ before concentration under reduced pressure to give a white crystalline solid.

7. 3. 6. 1 Naphthalene (16)^[131]



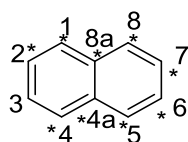
1, 2-Dihydronaphthalene (492 mg, 3.84 mmol), maleic acid (3.50 g, 30.70 mmol) and 10 % palladium on carbon (50 mg). Product as a white crystalline solid (354 mg, 2.76 mmol, 72 %). $R_f = 0.72$ eluent petrol-EtOAc 9.5.:0.5 (v / v). Melting point = 81 °C. Literature value 81-82 °C.

¹H NMR (300 MHz, CDCl₃) δ 7.48-7.54 (4H, m, H-2, 3, 6, 7), 7.85-7.90 (4H, m, H-1, 4, 5, 8);

¹³C NMR (75 MHz, CDCl₃) δ 125.8 (C-2, 3, 6, 7), 127.8 (C1, 4, 5, 8), 134.0 (C-4a, 8a);

IR (cm⁻¹) 1211, 1245, 1272, 1389, 1504, 1593, 1738, 1789, 1858, 1909, 1952, 2965, 3050.

7. 3. 6. 2 Naphthalene-¹³C₁₀ (16*)



1, 2-Dihydronaphthalene-10 ¹³C (492 mg, 3.84 mmol), maleic acid (3.5 g, 30.70 mmol) and 10 % palladium on carbon (50 mg). Product as a white crystalline solid

(354 mg, 2.76 mmol, 72 %). $R_f = 0.69$ eluent petrol-EtOAc 9.5.:0.5 (v/v). Melting point = 79 °C.

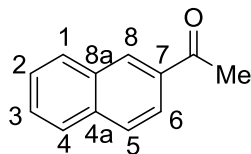
^1H NMR (300 MHz, CDCl_3) δ 7.25-7.60 (4H, dm, $J_{\text{C}13\text{-H}} = 156$ Hz H-2, 3, 6, 7), 7.79-8.12 (4H, dm, $J_{\text{C}13\text{-H}} = 156$ Hz, H-1, 4, 5, 8);

^{13}C NMR (75 MHz, CDCl_3) δ 125.3-126.5 (C-2, 3, 6, 7), 127.3-128.4 (C1, 4, 5, 8), 132.2-133.4 (C-4a, 8a);

IR (cm^{-1}) 1209, 1246, 1271, 1385, 1509, 1593, 1737, 1789, 1856, 1901, 1952, 2966, 3050.

HRMS TOF MS EI+ $m/z = 138.0961$, PPM 0.7, Form $^{13}\text{C}_{10}$

7. 3. 7 1-(Naphthalen-2-yl)ethan-1-one^{[119],[91]}

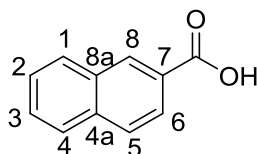


Naphthalene (0.32 g, 2.54 mmol) was dissolved in nitrobenzene (1.5 mL) and acetyl chloride (0.39 mL, 5.50 mmol) was added and the solution cooled to 0 °C before portion-wise addition of AlCl₃ (0.74 g, 5.50 mmol). The solution was stirred at 0 °C for 1 h. The flask was placed under vacuum for 5 min, brought to standard pressure and ice (1 g) was added along with nitrobenzene (2 mL) and brine (5 mL). The organic fraction was dried over MgSO₄ and purified by MPC, eluting with petrol-EtOAc (95:5) to afford the product as a white powder (0.173 g, 40 %). MP = 50-51 °C, literature value 52-54 °C.

¹H NMR (300 MHz, CDCl₃) δ) 2.75 (3H, s, Me), 7.50-7.67 (2H, m, H-6, 7 and 8), 7.88-7.95 (2H, m, H-1 and 4). 7.99 (1H, d, *J* = 8.0, H-5), 8.06 (1H, d, *J* = 8.0, H-6), 8.50 (1H, s, H-8);

¹³C NMR (75 MHz, CDCl₃) δ 27.7 (Me), 123.9 (C-6), 126.8 (C-6), 127.8 (C-5), 128.4 (C-8a), 128.5 (C-4a), 129.5 (C-2), 130.2 (C-3), 132.5 (C-4), 134.5 (C-1), 135.6 (C-7), 136.8 (C-8), 198.1 (C=O).

7. 3. 8 2-Naphthoic acid (58a)^{[134], [120]}



NaOH (0.2 g, 5.00 mmol) was dissolved in water (10 mL) and bleach (4.7 % NaOCl, 32 mL, 20.00 mmol) and heated to 55 °C before addition of 1-(naphthalen-2-yl)ethan-1-one (0.5 g, 2.93 mmol) and the reaction stirred for 2 hours. The solution was cooled to RT and 1 M Na₂SO₄ (25 mL) and conc. HCl (3 mL) were added to form a milky suspension. This was filtered to produce the product as a white powder (500 mg, 2.90 mmol, 99 %). MP = 183-184 °C, literature value 185-186 °C.

¹H NMR (300 MHz, CDCl₃) δ 7.37-7.81 (2H, m, H-2 and 3), 7.86-8.08 (3H, m, H-1, 4 and 6), 8.06-8.20 (1H, m, H-5), 8.62 (1H, s, H-8), 13.12 (1H, s, -OH) ;

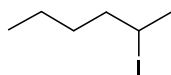
¹³C NMR (75 MHz, CDCl₃) δ 125.6 (C-6), 127.3 (C-5), 128.1 (C-8a), 128.5 (C-4a), 128.7 (C-2), 128.8 (C-3), 129.7 (C-4), 130.1 (C-1), 132.6 (C-7), 135.4 (C-8), 167.9 (C=O).

7.4 Synthesis of 2-(Hexan-2-yl)succinic acid (3)

7.4.1 General Procedure O: Iodination of C1-alkenes^[96]

Alk-1-ene (1 eq) was added to KI (3 eq) in 85% phosphoric acid (4 eq) and heated under reflux for 48 h. The resulting mixture was cooled to 0°C and diluted with Et₂O and H₂O. The aqueous layer was extracted into Et₂O and the combined organic layers were washed with 10% aq. Na₂SO₃ and brine, dried over Na₂SO₄ and concentrated under reduced pressure to give the title compound.

7.4.1.1 2-Iodoheptane (61)^[121]



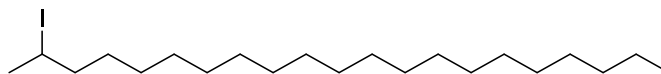
Hex-1-ene (2.00 g, 2.95 mL, 23.70 mmol), KI (11.83 g, 71.30 mmol) in 85% phosphoric acid (11.04 g, 6.55 mL, 95.70 mmol). Title compound as a yellow oil (2.48 g, 11.70 mmol). $R_f = 0.45$ eluent petrol-EtOAc 8:2 (v/v).

¹H NMR (300 MHz, DMSO-*d*₆) δ 0.83-0.91 (3H, m, CH₃CH₂), 1.20-1.48 (4H, m, CH₃CH₂CH₂), 1.50-1.68 (1H, m, CH₂CHHCHI), 1.70-1.81 (1H, m, CH₂CHHCHI), 1.88 (3H, d, $J = 6.8$ Hz, CHICH₃), 4.00-4.20 (1H, m, CH₂CHICH₃);

¹³C NMR (75 MHz, DMSO-*d*₆) δ 13.4 (CH₃CH₂), 21.8 (CH₃CH₂CH₂), 28.7 (CHICH₃), 31.3 (CH₂CHICH₃), 33.1 (CH₃CH₂CH₂), 41.8 (CH₂CH₂CHI);

IR (cm⁻¹) 2958, 2926, 2871, 1456, 1377, 1225.

7. 4. 1. 2 2-Iodohenicosane (67)



Henicos-1-ene (676 mg, 2.3 mmol), KI (1.15 g, 7 mmol) in 85 % phosphoric acid (3 mL). Title compound as a brown oil (578 mg, 1.36 mmol, 59 %). $R_f = 0.92$ eluent petrol.

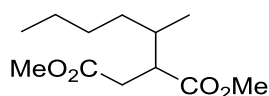
^1H NMR (300 MHz, CDCl_3) δ 0.84-0.95 (3H, m, CH_2CH_3), 1.27 (34H, s, $(\text{CH}_2)_{17}\text{CH}_3$), 1.79-1.89 (2H, m, CHICH_2), 1.94 (3H, d, $J = 6.9$ Hz, CH_3CHI), 4.21 (1H, dq, $J = 6.9$ and 13.5 Hz, CH_3CHI);

^{13}C NMR (75 MHz, CDCl_3) δ 14.1 (CH_2CH_3), 22.7, CH_2CH_3), 28.7 ($\text{CHICH}_2\text{CH}_2\text{CH}_2$), 28.9 (CH_3CHI), 29.3 (CH_3CHI), 29.6 [$(\text{CH}_2)_{14}\text{CH}_3$], 31.0 (CHCH_2CH_2), 31.4 (CHICH_2); IR (cm^{-1}) 1301, 1377, 1464, 1862, 2851, 2921.

7. 4. 2 General Procedure P: Radical Fumarate Addition^[97]

To a solution of 2-iodoalkane (**1** eq) in α,α,α -trifluorotoluene (10 mL / 1 mmol), under anhydrous conditions, tris(trimethylsilyl)silane (1.25 eq), dimethyl fumarate (3 eq) and AIBN (0.15 eq) were successively added. The mixture was heated at reflux for 4 h and concentrated under vacuum to give a crude oil. The crude oil was purified *via* MPC eluting with petrol – EtOAc (8:2, v/v) to afford the product as a colourless oil (832 mg, 3.61 mmol, 76 %). R_f 0.61 (petrol-EtOAc, 8:2, v/v).

7. 4. 2. 1 Dimethyl 2-(hexane-2-yl)succinate (**62**)^[22]



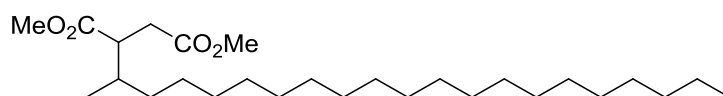
2-iodohexane (1.00 g, 4.72 mmol) in α,α,α -trifluorotoluene (47 mL), under anhydrous conditions, tris(trimethylsilyl)silane (1.46 g, 1.81 mL, 5.88 mmol), dimethyl fumarate (2.01 g, 13.90 mmol) and AIBN (0.39 g, 2.38 mmol). The crude oil was purified *via* MPC eluting with petrol – EtOAc (8:2, v/v) to afford the product as a colourless oil (832 mg, 3.61 mmol, 76 %). R_f 0.61 (petrol-EtOAc, 8:2, v/v).

¹H NMR (300 MHz, CDCl₃) δ 0.69-0.83 (12H, m, 2 x CH₃CH₂ and CHCH₃), 0.98-1.28 (12H, m, 2 x CH₃CH₂CH₂CH₂), 1.60-1.72 (1H, m, CH₂CHCH₃), 1.72-1.86 (1H, m, CH₂CHCH₃), 2.15-2.32 (2H, m, 2 x CH₃O₂CCHHCH), 2.54-2.66 (2H, m, 2 x CH₃O₂COCHHCH), 2.68-2.82 (2H, m, 2 x CH₃O₂CCHHCHCO₂CH₃), 3.56 (12H, dd, $J = 6.2$ and 1.2 Hz, 2 x CH₃O₂CCHHCHCO₂CH₃);

¹³C NMR (75 MHz, CDCl₃) δ 13.8 (2 x CH₃CH₂), 16.1 and 16.6 (2 x CH₃CH), 22.5 (2 x CH₃CH₂), 29.2 and 29.3 (2 x CH₂CH₂CH₂), 33.1 and 33.4 (2 x CH₂CO₂), 33.9 (2 x CH₂CH₂CH), 34.6 and 35.1 (2 x CH), 45.9 and 46.1 (2 x CHCO₂), 51.1 and 51.2 (2 x CH₂CO₂CH₃), 51.2 and 51.3 (CHCO₂CH₃), 172.5 and 172.6 (2 x CH₂CO₂), 174.6 and 174.7 (2 x CHCO₂);

IR (cm⁻¹) 2948, 1815, 1756, 1437, 1352, 1255.

7. 4. 2. 2 Dimethyl 2-(hencosan-2-yl)succinate (**63**)



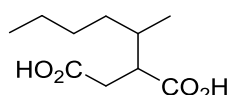
2-iodohexane (579 mg, 1.37 mmol) in α, α, α -trifluorotoluene (13 mL), tris(trimethylsilyl)silane (0.52 mL, 1.71 mmol), AIBN (112 mg, 0.68 mmol) and dimethyl fumarate (590 mg, 4.10 mmol). The crude oil was purified by MPC on silica, eluting with petrol – EtOAc (9:1, v/v) to afford the product as a white solid (428 mg, 0.97 mmol, 71 %). R_f 0.61 (petrol-EtOAc, 8:2, v/v).

^1H NMR (300 MHz, CDCl_3) δ 0.64-0.84 (6H, m, CH_3CH and CH_2CH_3), 1.01-1.23 [36H, m, $(\text{CH}_2)_{18}\text{CH}_3$], 2.11-2.26 (1H, m, CH_3CH), 2.61 (1H, dd, $J = 16.8$ and 11.3 Hz, $\text{MeO}_2\text{CCHCH}_2\text{CO}_2\text{Me}$), 2.88 (2H, d, $J = 11.3$ Hz, $\text{MeO}_2\text{CCHCH}_2\text{CO}_2\text{Me}$), 3.52 (3H, s, $\text{MeO}_2\text{CCHCH}_2\text{CO}_2\text{Me}$), 3.60 (3H, s, $\text{MeO}_2\text{CCHCH}_2\text{CO}_2\text{Me}$);

^{13}C NMR (75 MHz, CDCl_3) δ 14.1 (CH_2CH_3), 16.2 and 16.9 (CH_3CH), 22.7 (CH_2CH_3), 27.1 and 27.2 ($\text{CH}_3\text{CHCH}_2\text{CH}_2$), 29.3 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 29.6 ($\text{CHCH}_2\text{CH}_2\text{CH}_2(\text{CH}_2)_{12}$), 29.7 ($\text{CHCH}_2\text{CH}_2\text{CH}_2$), 31.3 and 31.9 ($\text{MeO}_2\text{CCHCH}_2\text{CO}_2\text{Me}$), 33.3 and 33.9 (CH_3CHCH_2), 34.4 and 34.6 (CH_3CH), 45.7 and 46.1 ($\text{MeO}_2\text{CCHCH}_2\text{CO}_2\text{Me}$), 51.6 and 61.8 ($\text{MeO}_2\text{CCHCH}_2\text{CO}_2\text{Me}$), 174.7 and 175.16 ($\text{MeO}_2\text{CCHCH}_2\text{CO}_2\text{Me}$);

IR (cm^{-1}) 1254, 1379, 1437, 1467, 1734, 2850, 2918.

7. 4. 3 2-(Hexan-2-yl)succinic acid (**3**)^[98]



A solution of LiOH (126 mg, 5.30 mmol) in H_2O (7.5 mL) was added dropwise to dimethyl-2-(hexane-2-yl)succinate (150 mg, 0.65, 1 eq) in THF (7.5 mL). The mixture was stirred at RT for 96 h. The THF was removed under reduced pressure and the remaining aqueous solution was acidified using 5 M HCl and extracted using EtOAc (4 x 5 mL). The organic extracts were combined, washed with brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure to give the title compound as a clear oil (113 mg, 0.56 mmol, 86%).

^1H NMR (300 MHz, CHCl_3) δ 0.81-0.97 (12H, m, 2 x CH_3CH_2 and CHCH_3), 1.08-1.48 (12H, m, 2 x $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2$), 1.79-1.91 (1H, m, CH_2CHCH_3), 1.93-2.03 (1H, m, CH_2CHCH_3), 2.31-2.46 (2H, m, 2 x HO_2CCHHCH), 2.66-2.81 (2H, m, 2 x $\text{HO}_2\text{COCHHCH}$), 2.82-3.04 (2H, m, 2 x $\text{HO}_2\text{CCHHCHCO}_2\text{H}$), 1.20-1.40 (2H, br s, $\text{HO}_2\text{CCHHCHCO}_2\text{H}$);

^{13}C NMR (75 MHz, CDCl_3) δ 14.2 and 14.3 (2 x CH_3CH_2), 16.7 and 17.3 (2 x CH_3CH), 23.7 and 23.7 (2 x CH_3CH_2), 30.6, 30.6, 32.7 and 33.1 (2 x CH_2CH_2), 34.8 and 35.1 (2 x CH_3CH), 35.9 and 36.2 (2 x CH_2CO_2), 47.3 and 47.6 (2 x CHCO_2), 176.0 and 176.2 (2 x CH_2CO_2), 178.2 and 178.3 (2 x CHCO_2);

Crystallisation of the crude product from EtOAc – petrol by vapour diffusion gave the single (*R,S/S,R*) diastereoisomer.

^1H NMR (300 MHz, MeOD) δ 0.88-0.99 (6H, m, CH_3CH_2 and CHCH_3), 1.18-1.46 (6H, m, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2$), 1.86-1.99 (1H, m, CH_2CHCH_3), 2.35 (1H, dd, $J = 3.6$ Hz and 16.8 Hz, HO_2CCHHCH), 2.63 (1H, dd, $J = 11.0$ Hz and 16.7 Hz, $\text{HO}_2\text{COCHHCH}$), 2.83 (1H, ddd, $J = 3.6$ Hz, 4.5 Hz and 10.9 Hz, $\text{HO}_2\text{CCHHCHCO}_2\text{H}$), 1.30 (2H, br s, $\text{HO}_2\text{CCHHCHCO}_2\text{H}$);

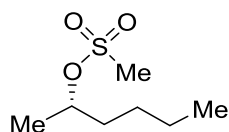
^{13}C NMR (75 MHz, CDCl_3) δ 14.2 (CH_3CH_2), 16.6 (CH_3CH), 23.7 (CH_3CH_2), 30.6 and 32.7 (CH_2CH_2), 35.1 (CH_3CH), 35.8 (CH_2CO_2), 47.2 (CHCO_2), 176.2 (CH_2CO_2), 178.2 (CHCO_2);

IR (cm^{-1}) 2927, 2849, 1702, 1421, 1388, 1345, 1305, 1246.

7. 4. 4 General Procedure Q: O-mesylation of Alcohols

The alcohol (1 eq) was dissolved in DCM and cooled to 0 °C before dropwise addition of Et₃N (1.3 eq) and methanesulfonyl chloride (1.3 eq). The solution stirred for 90 min, quenched with NaHCO₃, diluted with DCM and the organic phase was washed with 1M HCl and water, dried over MgSO₄ and concentrated to afford the product.

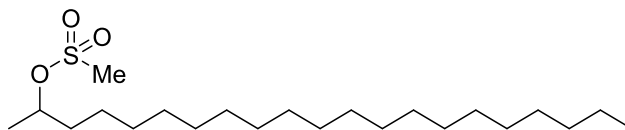
7. 4. 4. 1 (S)-Hexan-2-yl methanesulfonate (73)



(S)-Hexan-2-ol (500 mg, 4.90 mmol), DCM (2 mL), Et₃N (0.90 mL, 6.35 mmol) and methanesulfonyl chloride (0.50 mL, 6.35 mmol). To afford (S)-hexan-2-yl methanesulfonate as a yellow oil (776 mg, 4.30 mmol, 89 %). *R*_f = 0.41, petrol-diethyl ether 6:4 (v/v).

¹H NMR (300 MHz, CDCl₃) δ 0.88-0.92 (3H, t, *J* = 6.9 Hz, CH₂CH₃), 1.30-1.38 (4H, m, CH₂CH₂CH₃), 1.39-1.41 (3H, d, *J* = 6.3 Hz, CH₃CH), 1.50-1.80 (2H, m, CHCH₂), 2.99 (3H, s, SCH₃), 4.72-4.83 (1H, dqd, *J* = 5.4, 6.3 and 7.3 Hz, CH₃CH).

7. 4. 4. 2 Henicosan-2-yl methanesulfonate (69)

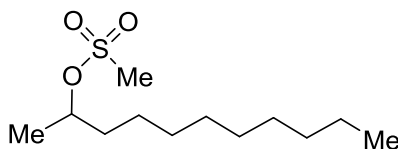


Henicosan-2-ol (4.27 g, 13.9 mmol), DCM (40 mL), Et₃N (2.5 mL, 17.9 mmol) and methanesulfonyl chloride (1.4 mL, 17.9 mmol). To give the title product as a yellow oil (5.26 g, 13.6 mmol, 98 %).

¹H NMR (300 MHz, CDCl₃) δ 0.81 (3H, t, *J* = 6.9 Hz, CH₂CH₃), 1.19 (34H, s, (CH₂)₁₇CH₃), 1.34 (3H, d, *J* = 6.3 Hz, CH₃CH), 1.44-1.71 (2H, m, CHCH₂), 2.91 (3H, s, OSO₂Me), 4.71 (1H, dt, *J* = 6.3 and 7.1 Hz);

¹³C NMR (75 MHz, CDCl₃) δ 14.2 (CH₂CH₃), 21.65 (CH₃CH), 22.68 (CH₂CH₃), 25.16 (CHCH₂CH₂), 29.65 (CH₂CH₂CH₂CH₃), 29.70 [(CH₂)₁₃CH₃], 31.92 (CH₂CH₂CH₃), 36.68 (CHCH₂), 39.37 (OSO₂Me), 68.10 (CH₃CH).

7. 4. 4. 3 Undecan-2-yl methanesulfonate (82)

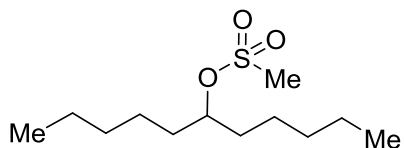


Undecan-2-ol (500 mg, 2.9 mmol), DCM (2 mL), Et₃N (0.53 mL, 3.8 mmol) and methanesulfonyl chloride (0.3 mL, 3.8 mmol). To afford undecan-2-yl methanesulfonate as a yellow oil (705 mg, 2.8 mmol, 97 %). *R*_f = 0.54, petrol-diethyl ether 6:4 (v/v).

¹H NMR (300 MHz, CDCl₃) δ 0.79-0.83 (3H, pseudo triplet, CH₂CH₃), 1.18-1.21 (14H, m, CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 1.34-1.36 (3H, d, *J* = 6.3 Hz, CH₃CH), 1.47-1.67 (2H, m, CHCH₂), 2.93 (3H, s, SO₂Me), 4.67-4.77 (1H, sextet, *J* = 6.3 Hz, CH);

¹³C NMR (75.5 MHz, CDCl₃) δ 14.1 (CH₂CH₃), 21.2 (CH₃CH), 22.7 (CH₂CH₃), 25.2 (CH₂CH₂CH₃), 29.3, 29.5, 29.5, 29.6 (CH₂CH₂CH₂CH₂CH₂CH₂CH₃), 31.9 (CHCH₂CH₂), 36.7 (CHCH₂), 38.6 (SO₂Me), 80.5 (CH₃CH).

7. 4. 4. 4 Undecan-6-yl methanesulfonate (84)

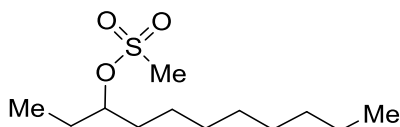


Undecan-6-ol (5 g, 30 mmol), DCM (20 mL), Et₃N (5.5 mL, 39 mmol) and methanesulfonyl chloride (3 mL, 39 mmol). To afford a yellow oil (6.02 g, 24 mmol, 81 %). *R*_f = 0.57, petrol-diethyl ether 6:4 (v/v).

¹H NMR (300 MHz, CDCl₃) δ 0.78-0.88 (3H, m, 2 x CH₃), 1.17-1.38 (12H, m, 2 x CH₃(CH₂)₃), 1.57-1.66 (4H, m, 2 x CHCH₂), 2.92 (3H, s, SO₂Me), 4.59-4.67 (1H, p, *J* = 6.1 Hz, CH);

¹³C NMR (75.5 MHz, CDCl₃) δ 14.0 (2 x CH₂CH₃), 22.48 (2 x CH₂CH₃), 24.62 (2 x CH₂CH₂CH₃), 31.55 (2 x CHCH₂CH₂), 34.40 (CHCH₂), 38.68 (SO₂Me), 70.22 (CH).

7. 4. 4. 5 Undecan-3-yl methanesulfonate (83)



Undecan-3-ol (4 g, 23 mmol), DCM (25 mL), Et₃N (4.2 mL, 30 mmol) and methanesulfonyl chloride (2.33 mL, 30 mmol). To afford undecan-2-yl methanesulfonate as a yellow oil (3.77 g, 15.2 mmol, 66 %). *R_f* = 0.51, petrol-diethyl ether 6:4 (v/v).

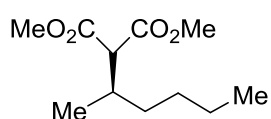
¹H NMR (300 MHz, CDCl₃) δ 0.78-0.85 (3H, pseudo triplet, CHCH₂CH₃), 0.86-1.00 (3H, t, *J* = 7.4 Hz CH₂CH₂CH₃) 1.17-1.27 (12H, m, (CH₂)₆CH₃), 1.55-1.75 (4H, m, CH₃CH), 2.93 (3H, s, SO₂Me), 4.48-4.70 (1H, pentet, *J* = 5.9 Hz, CH);

¹³C NMR (75.5 MHz, CDCl₃) δ 9.27 (CH₂CH₂CH₃) 14.11 (CHCH₂CH₃), 22.65 (CH₂CH₂CH₃), 25.00 (CH₂CH₂CH₃), 27.39 (CH₂CH₂ CH₂CH₂CH₂CH₃), 29.22, 29.40, 29.42 [(CH₂)₃(CH₂)₃CH₃], 31.83 (CHCH₂CH₃), 33.93 (CHCH₂CH₂), 38.65 (SO₂Me), 85.42 (CH₃CH₂CH).

7. 4. 5 General Procedure R: Malonation of Mesylates^[103]

To a solution of NaH, 60 % in mineral oil (1.1 eq) in 1,2-dimethoxyethane under an atmosphere of dry N₂, dimethyl malonate (1.1 eq) was added dropwise. This stirred for 30 min before the alk-2-yl methanesulfonate (1 eq) was added dropwise and the solution heated at 85 °C for 16 h. It was then diluted with Et₂O and NH₄Cl, the aqueous phase extracted with Et₂O and the combined organic extracts were washed with water, dried over MgSO₄ and concentrated to afford the crude product.

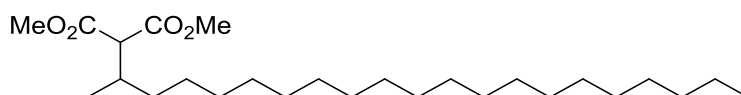
7. 4. 5. 1 (R)-Dimethyl (R)-2-(hexan-2-yl)malonate (74)^[122]



NaH, 60 % in mineral oil (200 mg, 5.6 mmol), 1,2-dimethoxyethane (3.5 mL), dimethyl malonate (0.64 mL, 5.6 mmol) and (S)-hexan-2-yl methanesulfonate (770 mg, 5.25 mmol). This was purified by MPC on silica eluting with petrol - diethyl ether (8:2, v/v) to attain the title compound as a clear oil (540 mg, 2.5 mmol, 58 %). *R*_f = 0.39 petrol-diethyl ether 8:2 (v/v).

¹H NMR (300 MHz, CDCl₃) δ 0.81-0.91 (3H, m, CH₂CH₃), 0.97 (3H, d, *J* = 6.8 Hz, CH₃CH), 1.14-1.42 (6H, m, CHCH₂CH₂CH₂CH₃), 2.16-2.30 (1H, m, CH₃CH), 3.26 (1H, d, *J* = 8.11 Hz, CH₃O₂CH), 3.72 (6H, s, CO₂CH₃), 3.73 (6H, s, CO₂CH₃);
¹³C NMR (75.5 MHz, CDCl₃) δ 13.7 (CH₂CH₃), 16.8 (CH₃CH), 22.5 (CH₂CH₃), 28.9 (CH₂CH₂CH₃), 33.4 (CHCH₂), 33.9 (CH₃CH), 51.8 (2 x CO₂CH₃), 57.4 (CHCO₂CH₃), 169.2 (2 x CO₂CH₃).

7. 4. 5. 2 Dimethyl 2-(henicosan-2-yl)malonate (70)



NaH, 60 % in mineral oil (660 mg, 27.50 mmol), 1,2-dimethoxyethane (40 mL), dimethyl malonate (2.00 mL, 17.40 mmol) and henicosan-2-yl methanesulfonate

(5.36 g, 13.90 mmol). To give the product as a white solid (3.52 g, 8.34 mmol, 60%).

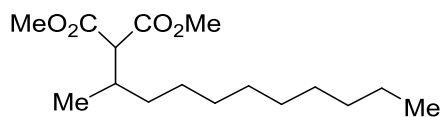
R_f = 0.69 eluent DCM.

^1H NMR (300 MHz, CDCl_3) δ 0.82-0.95 (3H, m, CH_2CH_3), 0.98 (3H, d, J = 6.8 Hz, CH_3CH), 1.27 (36H, s, $(\text{CH}_2)_{18}\text{CH}_3$), 2.17-2.38 (1H, m, CH_3CH), 3.29 (1H, d J = 8.2 Hz, MeO_2CCH), 3.75 (3H, s, MeO_2CCH), 3.75 (3H, s, CHCO_2Me);

^{13}C NMR (75 MHz, CDCl_3) δ 14.5 (CH_2CH_3), 15.6 (CH_3CH), 22.7 (CH_2CH_3), 26.6 ($\text{CH}_3\text{CHCH}_2\text{CH}_2$), 28.8 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 29.6 [$(\text{CH}_2)_{14}\text{CH}_3$], 33.3 (CH_3CH), 34.7 (CH_3CHCH_2), 51.6 ($\text{MeCO}_2\text{CHCO}_2\text{Me}$), 57.6 ($\text{MeCO}_2\text{CHCO}_2\text{Me}$), 169.3 ($\text{MeCO}_2\text{CHCO}_2\text{Me}$);

IR (cm^{-1}) 1342, 1435, 1465, 1755, 2850, 2916.

7. 4. 5. 3 Dimethyl-2-(undecan-2-yl)malonate (85)

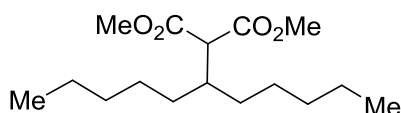


NaH, 60 % in mineral oil (245 mg, 6.10 mmol), 1,2-dimethoxyethane (4 mL), dimethyl malonate (0.80 mL, 6.60 mmol), undecan-2-yl methanesulfonate (1.28 g, 5.10 mmol). This was purified by MPC on silica eluting with petrol - diethyl ether (8:2, v/v) to attain the title compound as a clear oil (1.10 g, 3.70 mmol, 72 %). $R_f = 0.45$ petrol-diethyl ether 8:2 (v/v).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 0.87-0.89 (3H, pseudo t, CH_2CH_3), 0.98 (3H, d, $J = 6.8$ Hz, CH_3CH), 1.15-1.40 (16H, m, $\text{CHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 2.20-2.30 (1H, m, CH_3CH), 3.28 (1H, d, $J = 8.2$ Hz, $\text{CH}_3\text{O}_2\text{CH}$), 3.74 (3H, s, CO_2CH_3), 3.75 (3H, s, CO_2CH_3);

$^{13}\text{C NMR}$ (75.5 MHz, CDCl_3) δ 14.1 (CH_2CH_3), 16.9 (CH_3CH), 22.7 (CH_2CH_3), 26.81 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 29.3 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 29.5, 29.6, 29.8 ($\text{CH}_2\text{CH}_2\text{CH}_2$ $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 31.9 ($\text{CH}_3\text{CHCH}_2\text{CH}_2$), 33.5 (CH_3CHCH_2), 34.3 (CH_3CH), 52.2, 52.3 (2 x CO_2CH_3), 57.5 (CHCO_2CH_3), 169.2, 169.4 (2 x CO_2CH_3);
IR (cm^{-1}) 1236, 1434, 1736, 1855, 2925.

7. 4. 5. 4 Dimethyl 2-(undecan-6-yl)malonate (87)



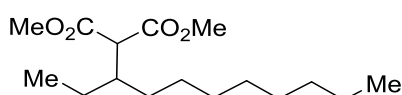
NaH, 60 % in mineral oil (1.16 g, 29.30 mmol), 1,2-dimethoxyethane (18 mL), dimethyl malonate (3.50 mL, 31.36 mmol) and undecan-6-yl methanesulfonate (6.02 g, 24.22 mmol). Purified by MPC on silica eluting with petrol - diethyl ether (8:2, v/v) to attain the title compound as a clear oil (7.75 g, 21.60 mmol, 90 %). $R_f = 0.47$ petrol-diethyl ether 8:2 (v/v).

^1H NMR (300 MHz, CDCl_3) δ 0.88 (6H, t, $J = 6.8$ Hz, 2 x CH_2CH_3), 1.12-1.30 (16H, m, 2 x $\text{CH}_3(\text{CH}_2)_4$), 2.08 (1H, dtt, $J = 5.8$ and 1.3 Hz, CH), 3.37 (1H, d, $J = 5.8$ Hz, $\text{CH}_3\text{CO}_2\text{CH}$), 3.65 (6H, s, 2 x CO_2CH_3);

^{13}C NMR (75.5 MHz, CDCl_3) δ 14.0 (2 x CH_3), 22.5 (2 x CH_2CH_3), 26.1 (2 x $\text{CH}_2\text{CH}_2\text{CH}_3$), 30.9 (2 x $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 31.9 (2 x CHCH_2), 38.3 (CH_2CH), 52.2 (2 x CO_2CH_3), 55.1 (CHCO_2CH_3), 169.5 (2 x CO_2CH_3);

IR (cm^{-1}) 1241, 1439, 1728, 1843, 2924, 2982.

7. 4. 5. 5 Dimethyl 2-(dodecan-3-yl)malonate (86)



NaH, 60 % in mineral oil, (730 mg, 15.20 mmol), 1,2-dimethoxyethane (12 mL), dimethyl malonate (2.34 mL, 19.70 mmol) and undecan-2-yl methanesulfonate (3.70 g, 15.20 mmol). To afford the crude dimethyl (R)-2-(undecan-2-yl)malonate. Purified by MPC silica eluting with petrol - diethyl ether (8:2, v/v) to attain the title compound as a clear oil (4.69 g, 13.10 mmol, 86 %). $R_f = 0.45$ petrol-diethyl ether 8:2 (v/v).

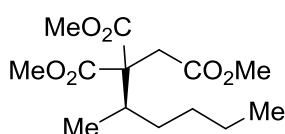
^1H NMR (300 MHz, CDCl_3) δ 0.86-0.91 (6H, m, 2 x CH_2CH_3), 1.25-1.30 (16H, br s, $\text{CH}_2\text{CH}(\text{CH}_2)_7$), 2.05-2.16 (1H, m, CH_2CH), 3.45 (1H, d, $J = 7.9$ Hz, $\text{CH}_3\text{O}_2\text{CH}$), 3.73 (6H, s, CO_2CH_3);

^{13}C NMR (75.5 MHz, CDCl_3) δ 10.7 $[(\text{CH}_2)_2\text{CH}_3]$, 14.0 ($\text{CH}_3\text{CH}_2\text{CH}$), 22.6 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 23.4 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 25.1 ($\text{CH}_2(\text{CH}_2)_2$), 26.4 ($\text{CH}_2(\text{CH}_2)_3$), 26.8 ($\text{CH}_2(\text{CH}_2)_4$), 29.2 ($\text{CH}_2(\text{CH}_2)_5$), 30.3 ($\text{CH}_3\text{CH}_2\text{CH}$), 31.8 (CHCH_2CH_2), 33.4 (CH_2CH), 52.2, 52.3 (2 x CO_2CH_3), 54.9 (CHCO_2CH_3), 169.5, 169.9 (2 x CO_2CH_3); IR (cm^{-1}) 1242, 1379, 1434, 1461, 1736, 2855, 2952.

7. 4. 6 General Procedure S: Tricarboxylate Formations

NaH, 60 % in mineral oil (1.1 eq), was dissolved in 1,2-dimethoxyethane and cooled to 0 °C. To this, the dimethyl-2-(alk-2-yl)malonate (1 eq) was added dropwise and left to stir for 3h at RT. Methylbromoacetate (1.1 eq) was then added dropwise and the solution stirred for a further 48h at RT. It was then poured over a mixture of 2 M HCl in ice, diluted with water and extracted with DCM. The combined organic extracts were dried over MgSO₄ and concentrated to afford the crude product.

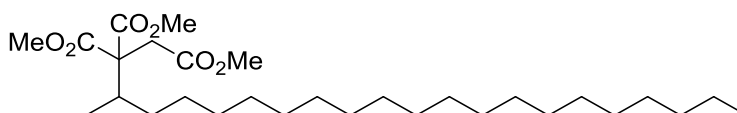
7. 4. 6. 1 Trimethyl (*R*)-3-methylheptane-1,2,2-tricarboxylate (75)



NaH, 60 % in mineral oil (70 mg, 1.85 mmol), 1,2-dimethoxyethane (10 mL), dimethyl (*R*)-2-(hexan-2-yl)malonate (400 mg, 1.75 mmol) and methylbromoacetate (0.18 mL, 1.85 mmol). Purified by MPC on silica eluting with petrol – ethyl acetate (5:1, v/v) to attain the title compound as a clear oil (373 mg, 1.29 mmol, 70 %). *R_f* = 0.45 petrol-diethyl ether 5:1 (v/v).

¹H NMR (300 MHz, CDCl₃) δ 0.88 (3H, t, *J* = 6.8 Hz, CH₂CH₃), 0.95 (3H, d, *J* = 6.8 Hz, CH₃CH), 1.05-1.45 (6H, m, CHCH₂CH₂CH₂CH₃), 2.05-2.15 (1H, m, CH₃CH), 2.91 (2H, s, CH₂CO₂CH₃), 3.68 (3H, s, CH₂CO₂CH₃), 3.76 (6H, s, 2 x CO₂CH₃);
¹³C NMR (75.5 MHz, CDCl₃) δ 13.7 (CH₂CH₃), 14.9 (CH₃CH), 22.4 (CH₂CH₃), 30.1 (CH₂CH₂CH₃), 31.9 (CH₂CO₂CH₃), 38.7 (CH₃CH), 51.4 (CH₂CO₂CH₃), 52.0 (2 x CHCO₂CH₃), 60.2 (CHCO₂CH₃), 170.6 (2 x CHCO₂CH₃), 171.2 (CH₂CO₂CH₃).

7. 4. 6. 2 Trimethyl 3-methyldocosane-1,2,2-tricarboxylate (71)



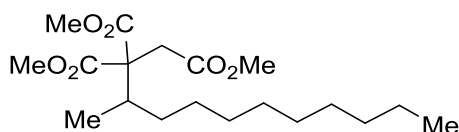
NaH (104 mg, 2.60 mmol) in 1,2-dimethoxyethane (3 mL), dimethyl 2-(henicosan-2-yl)malonate (1 mg, 2.40 mmol) in DME (3 mL), methyl bromoacetate (0.24 mL, 2.60 mmol). Purified by MPC on silica eluting with DCM to attain the title compound as a clear oil (1.01 mg, 2.11 mmol, 89 %). $R_f = 0.69$ DCM.

^1H NMR (300 MHz, CDCl_3) δ 0.79-0.82 (3H, m, CH_3CH_2), 0.88 (3H, d, $J = 6.8$ Hz, CH_3CH), 1.18-1.33 (36H, m, $(\text{CH}_2)_{18}$), 1.97-2.05 (1H, m, CH_3CH), 2.83 (2H, s, $\text{CH}_2\text{CO}_2\text{Me}$), 3.60 (3H, s, $\text{CH}_2\text{CO}_2\text{Me}$), 3.68 (6H, s, 2 x CO_2Me);

^{13}C NMR (75.5 MHz, CDCl_3) δ 14.1 (CH_2CH_3), 15.0 (CH_3CH), 22.7 (CH_2CH_3), 29.3 (CHCH_2CH_2), 29.5 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 29.6 [$(\text{CH}_2)_{13}\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$], 29.7 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 31.9 (CH_3CHCH_2), 37.5 (CH_3CH), 38.9 ($\text{CH}_2\text{CO}_2\text{Me}$), 51.3 (CHCO_2Me), 51.8 ($\text{CH}_2\text{CO}_2\text{Me}$), 52.4 (CCO_2Me), 165.1 (CCO_2Me), 170.9 ($\text{CH}_2\text{CO}_2\text{Me}$);

IR (cm^{-1}) 1355, 1401, 1435, 1464, 1725, 2849, 2915.

7. 4. 6. 3 Trimethyl-3-methyldodecane-1,2,2-tricarboxylate (88)



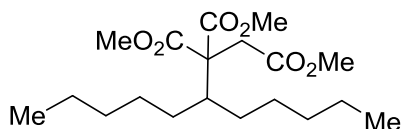
NaH (70 mg, 1.80 mmol) in 1,2-dimethoxyethane (2 mL), dimethyl-2-(undecan-2-yl)malonate (460 mg, 1.60 mmol) in 1,2-dimethoxyethane (2 mL), methylbromoacetate (0.15 mL, 1.70 mmol). Purified by MPC on silica eluting with petrol – EtOAc (5:1, v/v) to attain the title compound as a clear oil (482 mg, 1.30 mmol, 80 %). $R_f = 0.52$ petrol- EtOAc 5:1 (v/v).

^1H NMR (300 MHz, CDCl_3) δ 0.86-0.92 (3H, pseudo t, CH_2CH_3), 0.96 (3H, d, $J = 6.8$ Hz, CH_3CH), 1.24-1.32 (16H, m, CH $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 2.04-2.16 (1H, m, CH_3CH), 2.92 (2H, s, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.69 (3H, s, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.77 (6H, s, 2 x CO_2CH_3);

^{13}C NMR (75.5 MHz, CDCl_3) δ 14.1 (CH_2CH_3), 15.0 (CH_3CH), 21.0 (CH_2CH_3), 27.9 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 29.3 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 29.5 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 31.9 (CHCH_2CH_2), 32.2 (CHCH_2), 37.5 ($\text{CH}_2\text{CO}_2\text{CH}_3$), 38.9 (CH_3CH), 52.4 ($\text{CH}_2\text{CO}_2\text{CH}_3$), 52.4 (2 x CHCO_2CH_3), 60.2 (CCO_2CH_3), 170.9 (2 x CHCO_2CH_3), 171.6 ($\text{CH}_2\text{CO}_2\text{CH}_3$);

IR (cm^{-1}) 1215, 1237, 1274, 1356, 1372, 1435, 1737, 2855, 2925, 2953.

7. 4. 6. 4 Trimethyl 3-pentyl-octane-1,2,2-tricarboxylate (90)



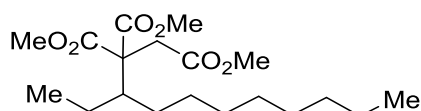
NaH, 60 % in mineral oil, (230 mg, 5.70 mmol) in 1,2-dimethoxyethane (6.5 mL), dimethyl-2-(undecan-2-yl)malonate (2 mg, 5.20 mmol) in DME (6.5 mL) methylbromoacetate (0.57 mL, 5.70 mmol) Purified by MPC on silica eluting with petrol – EtOAc (5:1, v/v) to attain the title compound as a clear oil (1.39 mg, 3.87 mmol, 75 %). $R_f = 0.54$ petrol- EtOAc 5:1 (v/v).

^1H NMR (300 MHz, CDCl_3) δ 0.85-0.89 (6H, pseudo t, 2 x CH_2CH_3), 1.18-1.42 (16H, m, 2 x $\text{CH}(\text{CH}_2)_4\text{CH}_3$), 1.82-1.88 (1H, m, CH), 2.88 (2H, s, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.67 (3H, s, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.74 (6H, s, 2 x CO_2CH_3);

^{13}C NMR (75.5 MHz, CDCl_3) δ 14.0 (2 x CH_2CH_3), 24.6 (2 x CH_2CH_3), 28.9 (2 x $\text{CH}_2\text{CH}_2\text{CH}_3$), 32.1 (2 x $\text{CH}_2(\text{CH}_2)_2\text{CH}_3$), 38.2 (CH_2CH), 44.76 (CH_2CH), 51.8 (CHCCH_2), 52.4 (CCH₂), 60.37, 60.56 (3 x CO_2Me), 171.1 (2 x CHCO_2CH_3), 171.6 ($\text{CH}_2\text{CO}_2\text{CH}_3$);

IR (cm^{-1}) 1245, 1286, 1368, 1439, 1731, 2835, 2955, 2963.

7. 4. 6. 5 Trimethyl 3-ethylundecane-1,2,2-tricarboxylate (89)



NaH, 60 % in mineral oil, (230 mg, 5.70 mmol) in 1,2-dimethoxyethane (6.5 mL), dimethyl-2-(undecan-2-yl)malonate (1.5 mg, 5.20 mmol) in DME (6.5 mL), methylbromoacetate (0.57 mL, 5.70 mmol). Purified by MPC on silica eluting with petrol – EtOAc (5:1, v/v) to attain the title compound as a clear oil (1.43 mg, 3.98 mmol, 76 %). $R_f = 0.43$ petrol- EtOAc 5:1 (v/v).

^1H NMR (300 MHz, CDCl_3) δ 0.63-0.72 (6H, m, 2 x CH_2CH_3), 0.99-1.06 (16H, m, $\text{CH}_2\text{CH}(\text{CH}_2)_7\text{CH}_3$), 1.54-1.64 (1H, m, CH), 2.67 (2H, s, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.45 (3H, s, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.53 (6H, s, 2 x CO_2CH_3);

^{13}C NMR (75.5 MHz, CDCl_3) δ 13.6 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 14.1 (CHCH_2CH_3), 22.6 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 24.8 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 29.2 ($\text{CH}_2(\text{CH}_2)_2\text{CH}_3$), 29.3 ($\text{CH}_2(\text{CH}_2)_3\text{CH}_3$), 29.9 ($\text{CH}_2(\text{CH}_2)_4\text{CH}_3$), 31.7 ($\text{CH}_2(\text{CH}_2)_5\text{CH}_3$), 31.8 (CHCH_2CH_2), 38.2 (CH_2CH), 46.3 (CH_2CH), 51.8 (CHCCH_2), 52.4 (CCH_2), 60.3, 60.5 (3 x CO_2Me) 171.1, 171.1 (2 x CHCO_2CH_3), 171.6 ($\text{CH}_2\text{CO}_2\text{CH}_3$);

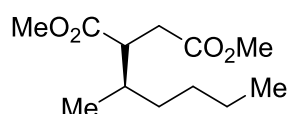
IR (cm^{-1}) 1238, 1272, 1287, 1359, 1432, 1439, 1738, 2828, 2952, 2967.

7. 4. 7 General Procedure T: Krapcho Decarboxylation Synthesis of

Succinates^[104]

The trimethyl-3-methylalkane-1,2,2-tricarboxylate (1 eq) and tetrabutylammonium acetate (3.7 eq) were dissolved in DMF and heated by microwave at 130 °C for 30 min before dilution with water and extraction with Et₂O. The combined organic fractions were dried over MgSO₄ and concentrated to give the crude product.

7. 4. 7. 1 Dimethyl (S)-2-[(R)-hexan-2-yl]succinate (62)^[22]



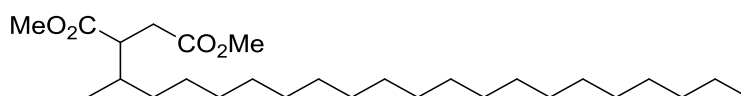
Trimethyl (R)-3-methylheptane-1,2,2-tricarboxylate (100 mg, 0.35 mmol), tetrabutylammonium acetate (387 mg, 1.30 mmol), DMF (2.5 mL). Purified by MPC on silica, eluting with petrol – ethyl acetate (8:2, v/v) to attain the title compound as a clear oil (37 mg, 0.16 mmol, 46 %). *R_f* = 0.61 petrol-diethyl ether 8:2 (v/v).

¹H NMR (300 MHz, CDCl₃) δ 0.69-0.83 (12H, m, 2 x CH₃CH₂ and CHCH₃), 0.98-1.28 (12H, m, 2 x CH₃CH₂CH₂CH₂), 1.60-1.72 (1H, m, CH₂CHCH₃), 1.72-1.86 (1H, m, CH₂CHCH₃), 2.15-2.32 (2H, m, 2 x CH₃O₂CCHHCH), 2.54-2.66 (2H, m, 2 x CH₃O₂COCCHHCH), 2.68-2.82 (2H, m, 2 x CH₃O₂CCHHCHCO₂CH₃), 3.56 (12H, dd, *J* = 6.2 and 1.2 Hz, 2 x CH₃O₂CCHHCHCO₂CH₃);

¹³C NMR (75 MHz, CDCl₃) δ 13.8 (2 x CH₃CH₂), 16.1 and 16.6 (2 x CH₃CH), 22.5 (2 x CH₃CH₂), 29.2 and 29.3 (2 x CH₂CH₂CH₂), 33.1 and 33.4 (2 x CH₂CO₂), 33.9 (2 x CH₂CH₂CH), 34.6 and 35.1 (2 x CH), 45.9 and 46.1 (2 x CHCO₂), 51.1 and 51.2 (2 x CH₂CO₂CH₃), 51.2 and 51.3 (CHCO₂CH₃), 172.5 and 172.6 (2 x CH₂CO₂), 174.6 and 174.7 (2 x CHCO₂);

IR (cm⁻¹) 2948, 1815, 1756, 1437, 1352, 1255.

7. 4. 7. 2 Dimethyl 2-(hencosan-2-yl)succinate (63)



Trimethyl 3-methyldocosane-1,2,2-tricarboxylate (250 mg, 0.52 mmol), tetrabutylammonium acetate (582 mg, 1.92 mmol), DMF (4 mL). Purified by MPC eluting with DCM to afford the title product as a colourless oil (157 mg, 0.37 mmol, 71 %). $R_f = 0.43$ DCM.

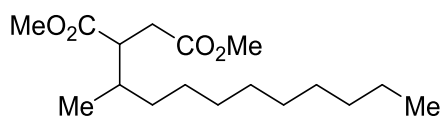
^1H NMR (300 MHz, CDCl_3) δ 0.77-0.84 (6H, m, CH_2CH_3 and CH_3CH), 1.18 (16H, s, $(\text{CH}_2)_{18}$), 1.68-1.86 (1H, m, CH_3CH), 2.29-2.85 (3H, m, $\text{CHCH}_2\text{CO}_2\text{Me}$), 3.60 (3H, d, $J = 0.9$ Hz, CO_2Me), 3.63 (3H, d, $J = 1.3$ Hz, CO_2Me);

^{13}C NMR (75.5 MHz, CDCl_3) δ 14.1 (CH_2CH_3), 16.2 and 16.8 (CH_3CH), 22.7 (CH_2CH_3), 27.1 and 27.2 (CHCH_2CH_2), 29.3 ($\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 29.6 [$(\text{CH}_2)_{12}\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$], 29.7 ($\text{CHCH}_2\text{CH}_2\text{CH}_2$), 30.6 ($\text{CH}_2\text{CH}_2\text{CH}_3$), 31.9 ($\text{CH}_2\text{CO}_2\text{Me}$), 33.3 and 33.9 (CH_3CHCH_2), 34.4 and 34.5 (CH_3CH), 45.6 and 46.0 (CHCHCH_2), 51.5 and 51.7 (2 x CO_2Me), 174.7 and 175.1 (2 x CO_2Me);

IR (cm^{-1}) 1379, 1437, 1467, 1734, 1850, 2918.

GC-MS retention time 47.866 min fragments ions m/z 114 [$\text{C}_5\text{H}_6\text{O}_3$] $^{+\cdot}$, 146 [$\text{C}_6\text{H}_{10}\text{O}_4$] $^{+\cdot}$, 367 [$\text{C}_{24}\text{H}_{48}\text{O}_2$] $^{+\cdot}$, and 409 [$\text{C}_{26}\text{H}_{49}\text{O}_3$].

7. 4. 7. 3 Dimethyl 2-(undecan-2-yl)succinate (76)



Trimethyl-3-methyldodecane-1,2,2-tricarboxylate (315 mg, 0.90 mmol), tetrabutylammonium acetate (980 mg, 3.30 mmol), DMF (6 mL). Purified by MPC on silica, eluting with petrol – EtOAc (5:1, v/v) to attain the title compound as a clear oil (173 mg, 0.60 mmol, 65 %). R_f = 0.65 petrol-EtOAc 5:1 (v/v).

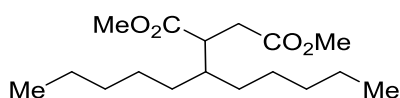
^1H NMR (300 MHz, CDCl_3) δ 0.75-0.87 (m, 6H, CH_3CH_2 and CHCH_3), 1.15-1.25 (m, 16H, 2 x $\text{CH}_3(\text{CH}_2)_8$), 1.65 (1H, m, CH_2CHCH_3), 2.31-2.36 (1H, m, CHHCO_2Me), 2.60-2.85 (2H, m, $\text{CHCHHCO}_2\text{Me}$), 3.60 (3H, d, J = 1.2 Hz, CO_2Me), 3.62 (3H, d, J = 1.2 Hz, CO_2Me);

^{13}C NMR (75.5 MHz, CDCl_3) δ 14.1 (CH_2CH_3), 16.2 and 16.9 (CH_3CH), 27.1 and 27.2 (CH_3CHCH_2), 29.2, 29.3, 29.5, 29.6, 31.9, 33.3, 33.9 ($\text{CH}_3(\text{CH}_2)_7$), 34.3 and 34.5 (CH_3CH), 35.1 ($\text{CH}_2\text{CO}_2\text{Me}$), 45.6 and 46.0 (CH_3CHCH), 51.5 ($\text{CH}_2\text{CO}_2\text{Me}$), 51.7 (CHCO_2Me), 174.7 (CHCO_2Me), 175.5 ($\text{CH}_2\text{CO}_2\text{Me}$);

IR (cm^{-1}) 1350, 1436, 1734, 2854, 2925.

GC-MS retention time 32.673 min, with fragment ions m/z 114 [$\text{C}_5\text{H}_6\text{O}_3$] $^+$, 146 [$\text{C}_6\text{H}_{10}\text{O}_4$] $^+$, 227 [$\text{C}_{14}\text{H}_{28}\text{O}_2$] $^+$ and 269 [$\text{C}_{16}\text{H}_{28}\text{O}_2$].

7. 4. 7. 4 Dimethyl 2-(undecan-6-yl)succinate (78)



Trimethyl 3-pentyloctane-1,2,2-tricarboxylate (800 mg, 2.22 mmol), tetrabutylammonium acetate (2.50 g, 8.25 mmol), DMF (15 mL). Purified by MPC on silica, eluting with petrol – EtOAc (5:1, v/v) to attain the title compound as a clear oil (479 mg, 1.60 mmol, 72 %). R_f = 0.87 petrol-EtOAc 5:1 (v/v).

^1H NMR (300 MHz, CDCl_3) δ 0.88-0.92 (6H, m, 2 x CH_2CH_3), 1.15-1.35 (16H, m, 2 x $\text{CH}_3(\text{CH}_2)_4$), 1.71-1.76 (1H, m, CH_2CHCH_2), 2.33 (1H, dd, J = 16.8 and 3.7 Hz,

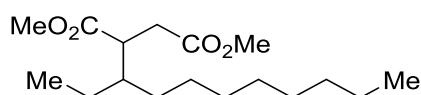
CHCHHCO₂Me), 2.75 (1H, dd, *J* = 16.8 and 11.1 Hz, CHCHHCO₂Me), 2.01-3.04 (ddd, 1H, *J* = 11.1, 3.7 and 1.8 Hz, CHCHHCO₂Me), 3.69 and 3.73 (2 x 3H, 2 x s, 2 x CO₂Me);

¹³C NMR (75.5 MHz, CDCl₃) δ 14.0 and 14.1 (2 x CH₂CH₃), 22.5 and 22.5 (2 x CH₃CH₂), 26.8 and 26.9 (CH₃CH₂CH₂), 31.0 and 31.4 (2 x CH₃(CH₂)₂CH₂), 31.7 and 31.8 (2 x CH₃(CH₂)₃CH₂), 31.9 (CH₂CH CH₂), 39.7 (CH₂CO₂Me), 43.3 (CHCH₂CO₂Me), 51.7 and 51.6 (2 x CO₂Me), 173.1 (CHCO₂Me), 175.2 (CH₂CO₂Me);

IR (cm⁻¹) 1339, 1435, 1734, 2858, 2928.

GC-MS retention time 30.890 min, with fragment ions *m/z* 114 [C₅H₆O₃]⁺, 146 [C₆H₁₀O₄]⁺, 227 [C₁₄H₂₈O₂]⁺ and 269 [C₁₆H₂₈O₂].

7. 4. 7. 5 Dimethyl 2-(undecan-3-yl)succinate (77)



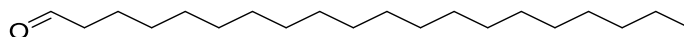
Trimethyl 3-ethylundecane-1,2,2-tricarboxylate (800 mg, 2.20 mmol), tetrabutylammonium acetate (2.50 g, 8.30 mmol), DMF (15 mL). Purified by MPC on silica, eluting with petrol – EtOAc (5:1, *v/v*) to attain the title compound as a clear oil (442 mg, 1.5 mmol, 67 %). *R*_f = 0.71 petrol-EtOAc 5:1 (*v/v*).

¹H NMR (300 MHz, CDCl₃) δ 0.86-0.90 (6H, m, 2 x CH₂CH₃), 1.22-1.31 (16H, m, CH₃CH₂CH(CH₂)₇), 1.63-1.68 (1H, m, CH₂CHCH₂), 2.33 (1H, dd, *J* = 16.8 and 3.7 Hz, CHCHHCO₂Me), 2.74 (1H, dd, *J* = 16.8 and 11.1 Hz, CHCHHCO₂Me), 3.02 (ddd, 1H, *J* = 11.1, 3.7 and 1.9 Hz, CHCHHCO₂Me), 3.68 and 3.69 (2 x 3H, 2 x s, 2 x CO₂Me);

¹³C NMR (75.5 MHz, CDCl₃) δ 11.81[(CH₂)₂CH₃], 14.11 (CHCH₂CH₃), 22.67, 24.14, 27.11, 29.29, 30.64, 30.93, 31.84, 31.88 (CH₃CH₂CH(CH₂)₇), 31.98 (CH₂CHCH₂), 41.47 (CH₂CO₂Me), 43.08 (CHCH₂CO₂Me), 51.67 and 51.76 (2 x CO₂Me), 173.14 (CHCO₂Me), 175.27 (CH₂CO₂Me);

IR (cm⁻¹) 1225, 1361, 1435, 1734, 2855, 2925.

7. 4. 8 Icosanal (65)^{[101],[123]}



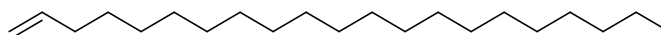
Eicosanol (3 g, 10 mmol) in DCM (20 mL) was added to a suspension of pyridinium chlorochromate (3.25 g, 15 mmol) in DCM (20 mL) to give a black solution which was stirred for 2.5 h before dilution with Et₂O (20 mL) and filtration through Celite and silica. The flask was washed with Et₂O (3 x 5 mL) and the combined filtrates were concentrated under reduced pressure to give the title product as a white solid (1.93 g, 6.52 mmol, 65 %). *R*_f = 0.86 eluent 9:1 petrol-EtOAc (*v/v*). MP 50-51 °C, literature value 50-51 °C.

¹H NMR (300 MHz, CDCl₃) δ 0.87-0.92 (3H, m, CH₃), 1.25-1.35 (32H, m, CH₃(CH₂)₁₆), 1.64 (2H, dt, *J* = 7.3 and 1.8 Hz, CH₂CHO), 2.44 (2H, tt, *J* = 7.3 and 1.9 Hz, CH₂CH₂CHO), 9.78 (1H, t, *J* = 1.8 Hz, CHO);

¹³C NMR (75 MHz, CDCl₃) δ 13.8 (CH₃), 22.1 (CH₃CH₂), 22.5 (CH₂CH₂CH₃), 29.1, 29.2, 29.3, 29.4, 29.5, 29.5 (CH₃(CH₂)₁₄), 31.8 (CHOCH₂CH₂), 43.7 (CHOCH₂), 202.1 (CHO);

IR (cm⁻¹) 1372, 1392, 1411, 1471, 1703, 1788, 1751, 2848, 2914, 2953.

7. 4. 9 Henicos-1-ene (66)^{[124],[125]}



Methyltriphenylphosphonium bromide (3.96 g, 11.10 mmol) in THF (24 mL) was added dropwise to a solution of KO^tBu in THF (10 mL) at 0 °C stirred for 30 min. To this, a solution of icosanal (3.00 g, 10.10 mmol) in THF (40 mL) was added stirred for a further 16h at RT. It was concentrated under reduced pressure, diluted with water (50 mL) and extracted with petrol (3 x 50 mL) before addition of MgSO₄ and filtration through silica and Celite to give the title product as a white solid (2.00 g, 6.80 mmol, 68 %). $R_f = 0.5$ eluent petrol. MP = 30-31 °C, literature value 32.3 °C.

¹H NMR (300 MHz, CDCl₃) δ 0.83-0.93 (3H, m, CH₃), 1.26-1.30 (34H, m, CH₃(CH₂)₁₇), 2.02-2.09 (2H, m, CHCH₂), 4.95 (1H, dd, $J = 16.9$ and 1.6 Hz, CHCH_{CIS}H_{TRANS}), 5.01 (1H, dd, $J = 10.1$ and 1.6 Hz, CHCH_{CIS}H_{TRANS}), 5.84 (1H, ddt, $J = 16.9, 10.1$ and 2.3 Hz, CHCH_{CIS}H_{TRANS});

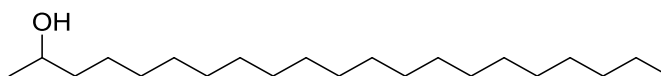
¹³C NMR (75 MHz, CDCl₃) δ 13.8 (CH₃), 22.5 CH₃CH₂, 28.9, 29.0, 29.2, 29.4, 29.5, 29.5 (CH₃CH₂(CH₂)₁₅), 31.8 (CHCH₂CH₂), 33.6 (CHCH₂CH₂), 113.9 (CH₂CH₂CHCH₂), 139.12 (CH₂CH₂CHCH₂);

IR (cm⁻¹) 1392, 1411, 1471, 1642, 1703, 2751, 2849, 2914, 2955, 3079.

7. 4. 10 General Procedure U: Wittig Reactions^[102]

Alkylmagnesium bromide (2 eq) was added dropwise to aldehyde (1 eq) in specified solvent at 0 °C and stirred at RT for 16 h before quenching with NH₄Cl and extraction into DCM. The combined organics were washed with 1M Na₂SO₄ and 1M HCl before drying over MgSO₄ and concentrating under reduced pressure to give the title product.

7. 4. 10. 1 Henicosan-2-ol (68)^[126]



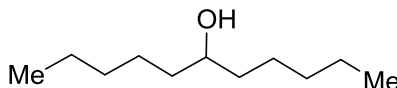
Eicosanal (500 mg, 1.69 mmol), THF (30 mL), 3 M solution of MeMgBr in Et₂O (56 mL, 3.4 mmol). Product as a white solid (467 mg 1.43 mmol, 86 %). *R_f* = 0.56 eluent petrol. MP = 61-62 °C, literature value 59.2 – 60.2 °C.

¹H NMR (300 MHz, CDCl₃) δ 0.82-0.93 (3H, m, CH₂CH₃), 1.15 (3H, d, *J* = 7.0 Hz, CH₃OH), 1.19-1.24 (34H, m, (CH₂)₁₇CH₃), 1.73-1.84 (2H, m, CH₂COH), 3.61-3.73 (1H, m, CHOH), 4.74 (1H, br s, CHOH);

¹³C NMR (75 MHz, CDCl₃) δ 14.1 (CH₂CH₃), 23.3 (CH₃CH₂), 22.7 (CH₂CH₃), 25.1 (CHCH₂CH₂), 29.3 (CH₂CH₂CH₂CH₃), 29.6 (CH(CH₂)₁₃), 29.9 (CHCH₂CH₂CH₂), 31.9 (CH₂CH₂CH₃), 40.0 (CHCH₂), 68.1 (CH₃CHOH);

IR 1346, 1378, 1463, 2848, 2915, 2956, 3321 (cm⁻¹).

7. 4. 10. 2 Undecan-6-ol (81)^[127]

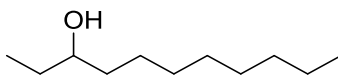


Pentylmagnesium bromide, 2 M in Et₂O (30 mL, 60 mmol), hexanal (3.60 mL, 30 mmol), THF (50 mL). To give a clear oil (4.9 g, 28 mmol, 95 %). *R_f* = 0.40 petrol-EtO₂ 6:4 (v/v).

¹H NMR (300 MHz, CDCl₃) δ 0.78-0.86 (6H, m, 2 x CH₃), 1.17-1.30 (12H, m, 2 x CH₃(CH₂)₃), 1.32-1.39 (4H, m, CH(CH₂)₂), 3.47-3.52 (1H, m, CH);

^{13}C NMR (75.5 MHz, CDCl_3) δ 14.0 (2 x CH_3), 22.6 (2 x CH_3CH_2), 25.3 (2 x $\text{CH}_3\text{CH}_2\text{CH}_2$), 31.9 (2 x $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2$), 37.4 (CH_2CHCH_2), 72.0 (CH);
IR (cm^{-1}) 1341, 1378, 1461, 2858, 2927, 3351.

7. 4. 10. 3 Undecan-3-ol (80)^[128]



Ethylmagnesium bromide, 3 M in Et_2O (32 mL, 70 mmol), nonanal (5.00 g, 35 mmol), THF (100 mL) To give a clear oil (4.05 g, 23 mmol, 67 %). R_f = 0.43 petrol- EtO_2 6:4 (v/v).

^1H NMR (300 MHz, CDCl_3) δ 0.78-0.86 (3H, m, CHCH_2CH_3), δ 0.86 (3H, t, J = 7.54 Hz, CH_2CH_3) 1.15-1.28 (12H, m, $\text{CH}_3(\text{CH}_2)_6$), 1.30-1.48 (4H, m, CH_2CHCH_2 , 3.40-3.49 (1H, m, CH);

^{13}C NMR (75.5 MHz, CDCl_3) δ 9.9 (CHCH_2CH_3), 14.1 (CH_2CH_3), 22.6 $\text{CH}_3\text{CH}_2\text{CH}_2$), 25.6 ($\text{CH}_3\text{CH}_2\text{CH}_2$), 29.3-30.1 ($\text{CHCH}_2(\text{CH}_2)_4$), 31.9 (CHCH_2CH_2), 36.9 ($\text{CH}_3\text{CH}_2\text{CH}$), 73.3 (CH);

IR (cm^{-1}) 1346, 1352, 1372, 1469, 2879, 2998, 3345.

7.5 Syntheses of Cyclopropane Compounds

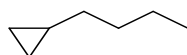
7.5.1 Preparation of Zinc/Copper Couple^[110]

Zn powder (10 g) was stirred rapidly in 1 M HCl (8 mL) for 1 min. The liquid was decanted off and the process repeated a further 3 times with 1 M HCl (3 x 8 mL), 5 times with distilled H₂O (5 x 20 mL), twice with 2 % aqueous CuSO₄ solution (2 x 15 mL) and a further 5 times with distilled H₂O (5 x 20 mL). After this the couple was washed 4 times with EtOH (4 x 20 mL) and 5 times with Et₂O (5 x 20 mL). The EtOH and Et₂O washings were decanted directly onto a Buchner funnel to prevent loss. The remaining couple was filtered and dried over phosphorous pentoxide for 16 h before use.

7.5.2 General Procedure V: Simmons-Smith Conversion of Alkenes to Cyclopropanes^[110]

I₂ (50 mg) was added to a stirred suspension of the Zn/Cu couple (1.75 g) in anhydrous Et₂O (9 mL) under N₂. This was stirred for 15 min at RT before a mixture of the alkene (23 mmol) and diiodomethane (2.01 mL, 6.67 g, 25 mmol) was added. The suspension was refluxed for 9 h before cooling to RT and stirred for 16 h. The remaining couple was removed by filtration and the solid washed with Et₂O (2 x 2 mL). The filtrate was washed with NH₄Cl (2 x 4 mL), NaHCO₃ (4 mL) and distilled H₂O (4 mL) before being dried over MgSO₄. The prepared cyclopropanes were purified *via* distillation using diglyme (4 mL) as a chaser solvent.

7. 5. 2. 1 Butylcyclopropane (101)^[129]



Hex-1-ene (2.56 mL, 2.00 g, 23 mmol) used. 2 fractions obtained from distillation, the first relating to the Et₂O (36 °C), the second relating to the product (103 °C) as a clear oil (350 mg, 3.56 mmol, 15%).

¹H NMR (300 MHz, CHCl₃) δ 0.20-0.27 (2H, m, CHHC^HHCHCH₂, trans), 0.59-0.67 (2H, m, CHHC^HHCHCH₂, cis), 0.82-0.97 (1H, m, CH₂CH), 1.11-1.18 (3H, m, CH₂CH₃), 1.39-1.48 (2H, m, CHCH₂CH₂), 1.51-1.68 (4H, m, CH₃CH₂CH₂CH₂CH);

¹³C NMR (75 MHz, CDCl₃) δ 4.5, 11.1, 14.2, 22.7, 27.2, 34.6;

IR (cm⁻¹) 1378 (CH₃ symmetrical deformation), 1461 (R₃C-H deformations), 2855, 2921, 2958, 3001 and 3076 (R₃C-H stretch).

7. 5. 2. 2 (1*R*,2*R*)/(1*S*,2*S*)-1,2-Diethylcyclopropane (**98**)^[130]



(*E*)-Hex-3-ene (2.95 mL, 2.00 g, 23 mmol) used. 2 fractions obtained from distillation, the first relating to the Et₂O (36 °C), the second relating to the product (93 °C), as a clear oil (316 mg, 3.21 mmol, 13%).

¹H NMR (300 MHz, CHCl₃) δ 0.37-0.43 (2H, m, CHCH₂CH), 0.54-0.67 (2H, m, CHCH₂CH), 1.15-1.23 (3H, m, CH₃CH₂CHCHCH₂CH₃), 1.30-1.62 (4H, m, CH₃CH₂CHCHCH₂CH₃);

¹³C NMR (75 MHz, CDCl₃) δ 11.2 (CHCH₂CH), 13.3 (CH₃CH₂CHCHCH₂CH₃), 20.4 (CH₂CHCHCH₂), 27.1 (CH₃CH₂CHCHCH₂CH₃);

IR (cm⁻¹) 1299, 1376 (CH₃ symmetrical deformation), 1461 (R₃C-H deformations), 2874, 2923, 2959 and 3062 (R₃C-H stretch).

7. 5. 2. 3 (1*R*,2*S*)-1,2-Diethylcyclopropane (97)^[131]



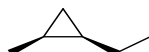
(*Z*)-Hex-3-ene (2.95 mL, 2.00 g, 23 mmol) used. 2 fractions obtained from distillation, the first relating to the Et₂O solvent used (36 °C), the second relating to the product (101 °C), as a clear oil (243 mg, 2.89 mmol, 12%).

¹H NMR (300 MHz, CHCl₃) δ - 0.9 (1H, ddd, *J* = 5.3, 5.1 and 3.8 Hz, CHCH_{TRANS}HCH), 0.75-0.83 (1H, m, CHCH_{CIS}CH), 0.84-0.94 (2H, m, CHCH₂CH), 1.22 (3H, t, *J* = 7.3 Hz, Me), 1.36-1.53 (2H, m, CH₃CH₂CHCHCH₂CH₃), 1.55-1.71 (2H, m, CH₃CH₂CHCHCH₂CH₃);

¹³C NMR (75 MHz, CDCl₃) δ 10.4 (CHCH₂CH), 13.4 (CH₃CH₂CHCHCH₂CH₃), 17.9 (CH₂CHCHCH₂), 21.7 (CH₃CH₂CHCHCH₂CH₃);

IR (cm⁻¹) 1302 and 1377 (CH₃ symmetrical deformation), 1465 (R₃C-H deformations), 2873, 2932, 2960, 2992 and 3061 (R₃C-H stretch).

7. 5. 2. 4 (1*R*,2*S*)-1-Ethyl-2-methylcyclopropane (99)



(*Z*)-pent-2-ene (2.48 mL, 1.61 g, 23 mmol) used. 2 fractions obtained from distillation, the first relating to the Et₂O solvent used (36 °C), the second relating to the product (62 °C), as a clear oil (727 mg, 8.64 mmol, 12%).

¹H NMR (300 MHz, CHCl₃) δ -0.13 - -0.05 (1H, m, CHCH_{TRANS}HCH), 0.76-0.93 (2H, m, CH₃CHCH₂CHCH₂CH₃), 0.94-1.10 (1H, m, CHCH_{CIS}HCH), 1.18-1.31 (6H, m, CH₃CHCH₂CHCH₂CH₃), 1.43-1.62 (2H, m, CHCH₂CH₃);

¹³C NMR (75 MHz, CDCl₃) δ 9.3 (MeCH), 12.8 (CH₂Me), 14.1 (CHCH₂CH), 15.1 (CHCH₂Me), 17.6 (CH₂CMe), 21.6 (MeCH);

IR (cm⁻¹) 1307 and 1384 (CH₃ symmetrical deformation), 1455 (R₃C-H deformations), 2874, 2961, 2992, 2992 and 3062 (R₃C-H stretch).

7. 5. 2. 5 (1*R*,2*R*)/(1*S*,2*S*)-1-Ethyl-2-methylcyclopropane (100)



(*E*)-Pent-2-ene (2.48 mL, 1.61 g, 23 mmol) used. 2 fractions obtained from distillation, the first relating to the Et₂O solvent used (36 °C), the second relating to the product (69 °C), as a clear oil (331 mg, 3.2 mmol, 4.7%).

¹H NMR (300 MHz, CHCl₃) δ 0.31-0.44 (2H, m, CHCH₂CH), 0.52-0.72 (2H, m, CHCH₂CH), 1.18 (3H, t, *J* = 7.3 Hz, CH₂CH₃), 1.25 (3H, d, *J* = 5.8 Hz, CHCH₃), 1.34-1.54 (2H, m, CH₂CH₃);

¹³C NMR (75 MHz, CDCl₃) δ 12.7 (CH₂CHCH₃), 13.3 (CH₂CH₃), 15.0 (CHCH₂CH), 18.8 (CH₂CHCH₂), 21.8 (CHCH₂CH₃), 24.1 (CH₃CH);

IR (cm⁻¹) 1304 and 1377 (CH₃ symmetrical deformation), 1454 (R₃C-H deformations), 2874, 2927, 2957, 2995, 3063 (R₃C-H stretch).

7. 5. 2. 6 1,1-Diethylcyclopropane (102)^[132]



3-Methylenepentane (2.81 mL, 1.93 g, 23 mmol) used. 2 fractions were obtained from distillation, the first relating to the Et₂O solvent used (36 °C), the second relating to the product (92 °C), as a clear oil (821 mg, 836 mmol, 12.5%).

¹H NMR (300 MHz, CHCl₃) δ 0.43 (4H, s, CH₃CH₂CCH₂CH₂CH₂CH₃), 1.12 (6H, t, *J* = 7.4 Hz, CH₃CH₂CCH₂CH₂CH₂CH₃), 1.51 (4H, q, *J* = 7.5 Hz, CH₃CH₂CCH₂CH₂CH₂CH₃);

¹³C NMR (75 MHz, CDCl₃) δ 11.1 (CH₃CH₂CCH₂CH₂CH₂CH₃), 15.0 (CH₃CH₂CCH₂CH₂CH₂CH₃), 21.2 (CH₃CH₂CCH₂CH₂CH₂CH₃), 28.2 (CH₃CH₂CCH₂CH₂CH₂CH₃);

IR (cm⁻¹) 1302 and 1375 (CH₃ symmetrical deformation), 1460 (R₃C-H deformations), 2875, 2963 and 3069 (R₃C-H stretch).

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